The evolution of exoskeleton moulting in Trilobita

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Abstract

Moulting is the process of replacing an exoskeleton. The exoskeleton provides protection but must be periodically moulted for growth, development, and repair. Moulting is therefore intrinsic to ontogeny, but is risky and energetically-expensive, meaning the morphology and behaviours of an animal are also important. Ecdysozoa (e.g. Euarthropoda, Nematoida) are united by moulting, but Euarthropoda (e.g. insects, crustaceans) have particularly tough, usually reinforced, exoskeletons. Correspondingly they have a high preservation potential and exceptional fossil record. Amongst arthropods, the extinct trilobites were extraordinarily abundant and diverse, with an unparalleled fossil record. Both trilobite carcasses and moults are abundant, and when correctly identified these moults inform on many aspects of evolutionary history. Trilobites were both extremely inter- and intraspecifically variable in their moulting compared to other euarthropods. By examining trilobite moults we can reconstruct and contrast moulting behaviours to other groups to explore the ways in which moulting has affected trilobite evolutionary history.

This thesis illustrates the importance of moulting to the evolution of trilobite, and euarthropod, life history. First, I provide a review of published information on trilobite moulting, and discuss the importance of accurate moult identification. I then present a broad-scale database of trilobite behavioural and morphological data, which is used to quantitatively answer "how variable was trilobite moulting behaviour, and is this linked to differences in morphology?" Case studies then explore the detailed moulting information available from Konservat-Lagerstätten (focusing on the early Cambrian Emu Bay Shale); the association between moulting and morphology through trilobite ontogenetic history (by describing the development of *Dalmanitina*); and show that the process of moulting has been conserved throughout euarthropod evolution (by describing a complete moulting sequence of *Symphysurus*). The manuscripts constituting this thesis demonstrate the trilobite fossil record of moulting is an important source of information that can inform on the evolutionary history of development, behaviour, ecology, and morphology in this influential group.

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Abbreviations

Institutional

CGS	Czech Geological Survey, Prague, Czech Republic
GSC	Canadian Museum of History, Ottawa, Canada
MCZ	Museum of Comparative Zoology, Cambridge, USA
MGL	Musée Géologie Lausanne, Switzerland
NHMUK	Natural History Museum, London, UK
NMNH	Smithsonian National Museum of Natural History, Washington, USA
NMP	National Museum, Prague, Czech Republic
OUMNH	Oxford University Museum of Natural History, Oxford, UK
PMU	Uppsala University Museum of Natural History, Uppsala, Sweden
ROM	Royal Ontario Museum, Toronto, Canada
SAM	South Australian Museum, Adelaide, Australia
USNM	Smithsonian National Museum of Natural History, Washington, USA
YPM	Yale Peabody Museum of Natural History, New Haven, USA

Statistical

CI	95% Confidence Interval
ANOVA	Analysis of Variance

Palaeontological

Ma	Million years ago
CT scan	Computed tomography scan
LCU	Lower Cephalic Unit
P(n)	Protaspid Stage (n) during trilobite development
M(n)	Meraspid Stage (n) during trilobite development
H(n)	Holaspid Stage (n) during trilobite development

Chapter 1: Introduction and literature review

This chapter features a general introduction to exoskeleton moulting, a discussion of its importance, and a full literature review of the published fossil record of trilobite moulting. A number of terms both novel and from the literature relevant to the contents of this thesis are also defined. I discuss the necessary background within which the scientific research comprising this thesis is placed, state the importance of research into the evolution of moulting, and outline the contents of the rest of this thesis.

Much of this chapter is comprised of edited and updated excerpts from a published literature review (Daley and Drage, 2016). All sections of the article used here pertain to trilobites (and are parts originally written by H.B.D.); the original article was co-authored by H.B.D. and A.C.D. Additional sections have been added that are important for a complete literature review of trilobite moulting and also key for understanding the rest of this thesis.

Daley, A. C. and Drage, H. B. 2016. The fossil record of ecdysis, and trends in the moulting behaviour of trilobites. *Arthropod Structure & Development* 45, 71–96.

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Abstract

Moulting, the process of shedding an exoskeleton, is one of the key characters uniting euarthropods and a number of smaller phyla in the Ecdysozoa. The euarthropod fossil record, particularly of trilobites, eurypterids and decapod crustaceans, yields abundant information on moulting as moults are frequently preserved, although most current focus in the field is predominantly descriptive and lacks a broader evolutionary perspective. Moulting is important to study because it is a necessary recurrent and risky endeavor in the life histories of all Ecdysozoa, and particularly euarthropods owing to their heavily reinforced exoskeletons. Trilobites are an excellent study group with which to explore moulting in the fossil record because they have the most abundant and global moult record, and they have a unique link between behavioural and morphological flexibility and moulting.

Here, I review the literature on the fossil record of moulting in trilobites, synthesising research on the behaviour, evolutionary trends, and phylogenetic significance of moulting throughout the Palaeozoic. Rare examples of comprehensive or quantitative studies examining the evolutionary impact of moulting in trilobites reveal extensive and ubiquitous variability in their moulting behaviours, in comparison to other euarthropod groups. These also indicate a number of potential taxonomic, temporal, and morphological signals in this moulting variability. The available body of descriptive literature, and the enormous moult fossil record of trilobites, suggest that it is feasible to extract broad-scale evolutionary information from the fossil record of moulting. This abundant source of data is presented in full, as well as related aspects of trilobite biology, ecology, and behaviour.

What is exoskeleton moulting?

Ecdysis, the act of moulting an exoskeleton, is one of the key characters uniting arthropods, nematodes, tardigrades, and a number of smaller phyla in the aptly named group Ecdysozoa (Fig. 1; Aguinaldo et al., 1998; Telford et al., 2008). Ecdysozoans include both the most abundant and the most diverse animal groups on the planet today, representing over 80% of total animal biodiversity. They have been key components of ecosystems since the early Cambrian, when trilobites and various soft-bodied Burgess Shale-type ecdysozoans including other arthropods, lobopodians, priapulids and palaeoscolecids dominated marine animal communities (Budd and Jensen, 2000), and have remained important throughout the whole of the Phanerozoic. Understanding the processes and behaviours of moulting in the fossil record is important because this life history strategy would have imparted constraints on morphology throughout the evolution of modern animal groups. It also allows us to access information on growth and development in deep time, which can help resolve the affinity of enigmatic taxa early in the evolution of animals. This is particularly important because many of these Cambrian fossils are early diverging stem lineage taxa that are critically important for breaking up long branches, helping us to resolve current ambiguous relationships in modern taxa (e.g. Legg et al., 2012; Fig. 1). Additionally, studying moulting in the fossil record can provide insight into understanding ecology because moults, along with ichnofossils, give the most detailed examples of direct evidence of behaviour preserved in the fossil record.

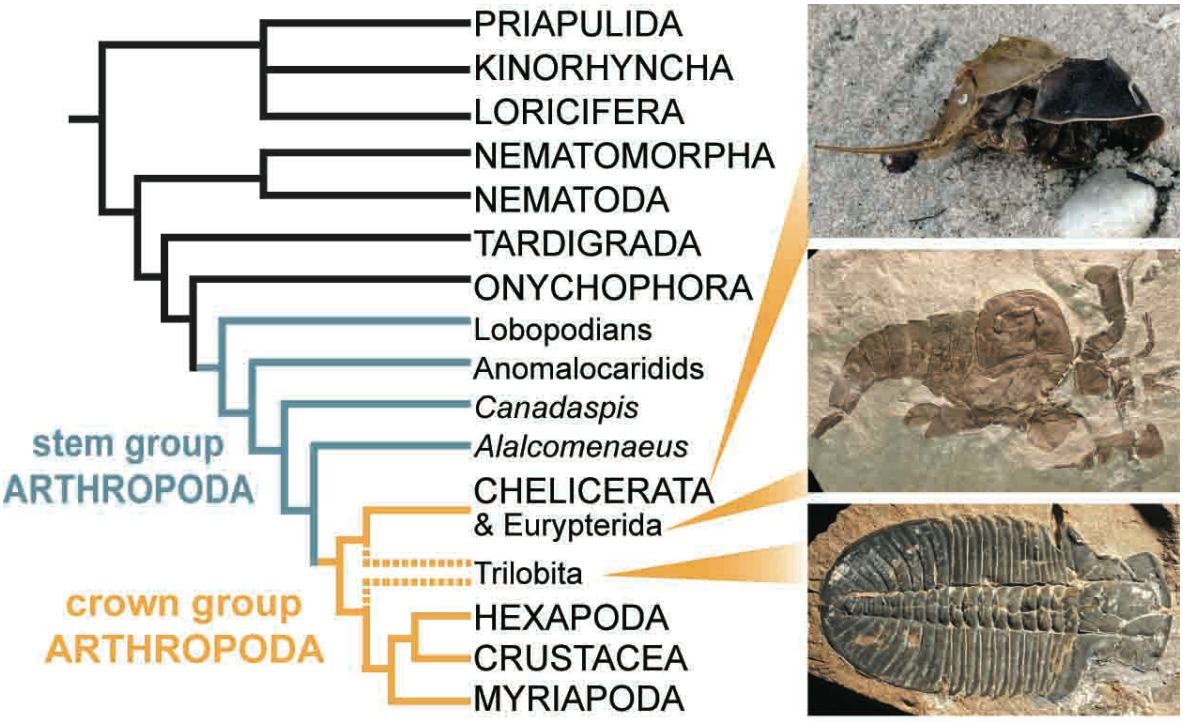


Figure 1: Generalised phylogeny of ecdysozoans, united by the presence of an exoskeleton and moulting, with extant phyla in uppercase and fossil groups/taxa in lowercase. Phylogeny based on Legg et al. (2013), Rota-Stabelli et al. (2013), and Dunn et al. (2014). Images from top: horseshoe crab (*Limulus polyphemus* Linnaeus 1758) in the process of moulting (Wikimedia Commons); eurypterid (*Eurypterus lacustris* Harlan 1834) moult configuration (YPM 208303, J. Utrup); moulted trilobite (*Ogygopsis klotzi* Rominger 1887) axial shield (OUMNH AT.205). Trilobites are considered to lie in one of the two indicated positions; as the 'Mandibulata' (Hexapoda, Crustacea, and Myriapoda; Scholtz and Edgecombe, 2005), or with the Chelicerata (Legg et al., 2013). Photograph scales: ~20 cm length for *L. polyphemus*; ~10 cm full length for *E. lacustris*; and full length of ~8 cm for *O. klotzi*.

For both extant and extinct taxa, moulting is best known for the euarthropods. Euarthropoda includes many extremely diverse modern groups (including the crustaceans, insects, spiders and scorpions, and horseshoe crabs), and large extinct groups (stem lineage groups like radiodontans, and crown groups like trilobites). The euarthropod external cuticle is a chitinous exoskeleton that provides protection from mechanical injury and

desiccation, while also giving strong structural support for muscle attachment and locomotion (Moussian, 2013). This rigid exoskeleton is moulted multiple times during normal growth and development, at which time regeneration and repair of damage may also occur. In extant taxa, moulting is a relatively rapid process, with actual exuviation and subsequent hardening of the new exoskeleton taking only a brief period of time (minutes to hours generally, highly variable between taxa, but little known for groups not reared in laboratory-environments; see Skinner, 1962; Sekiguchi et al., 1988; Shuster and Sekiguchi, 2004). The brevity of this physical process is reflected in the fossil record by the rarity of specimens that are preserved mid-moult (e.g. *Marrella splendens* Walcott 1912, García-Bellido and Collins, 2004; *Sirilorica carlsbergi* Peel 2010, Peel et al., 2013; *Alacaris mirabilis* Yang et al. 2018, 2019; Appendix 3) or during the immediate post-exuviation stage when the exoskeleton is not yet fully hardened (e.g. Whittington, 1990; Drage et al., accepted; Chapter 7). During this process the arthropod is unable to move to escape threats (and is vulnerable to desiccation if it has a terrestrial lifestyle), leading to an evolutionary constraint for exuviation to occur as rapidly as possible and behavioural adaptations that minimise risk (e.g. mass moulting behaviour and seeking shelter during moulting).

The full process of moulting, as determined by examining extant arthropods, begins with a complex series of biological interactions long before the actual act of exuviation takes place (Fig. 2). The exoskeleton consists of cuticle that was secreted by underlying epidermal cells, to which it remains attached during its functional lifetime (Moussian, 2013). The process of moulting starts with the separation of the cuticle from the epidermis, referred to as apolysis, after which the epidermal cells undergo a round of cell division to increase surface area, followed by the secretion of a new layer of cuticle, which remains soft until the old cuticle is shed (Nijhout, 2013). A moulting fluid is secreted between the old and new cuticles, and in some taxa (e.g. most crustaceans) is involved with digestion

and reabsorption of the old exoskeleton (Skinner, 1962; Luquet and Marin, 2004). At some point, all structures associated with the old cuticle (e.g. muscle attachment, sensory neurons, circulatory system, etc.) must be transferred to the new cuticle (Ewer, 2005). The old cuticle is then split open along specific lines of weakness, usually by movement of the body or through an increase in internal pressure, so that the arthropod can emerge and the new cuticle can begin to harden (Fig. 2). In all arthropods this process is controlled by the steroid hormones ecdysone and 20-hydroxyecdysone (Krishnakumaran and Schneiderman, 1970; Nijhout, 2013), which are synthesised from ingested cholesterols using enzymes encoded by the 'Halloween' genes, although little is known about the control of moulting for other ecdysozoans (Schumann et al., 2018). There is great variation in the number of moulting episodes within the phylum, with some taxa requiring many moults to reach adult size, while others can reach the same size in fewer moults by growing in larger increments at each moult (Nijhout, 2013). Based on research mostly with *Drosophila melanogaster* Meigen 1830, it has been determined that biochemical control of moulting involves the 'ecydsone' cassette (Hyde et al., 2018). This is stimulated by the cyclical production of ecdysone, and is responsible, at least in Pancrustacea, for the upregulation of a suite of genes responsible for physiological changes during moulting, such as release of the new cuticle and tissue reorganisation (Hyde et al., 2018). Given moulting is the most obvious synapomorphy of the Ecdysozoa, it is extraordinary that more is not known about its process in modern groups. Even this basic process differs greatly across Euarthropoda, being complicated by aspects such as metamorphosis and timings of sexual maturity (Hyde et al., 2018).

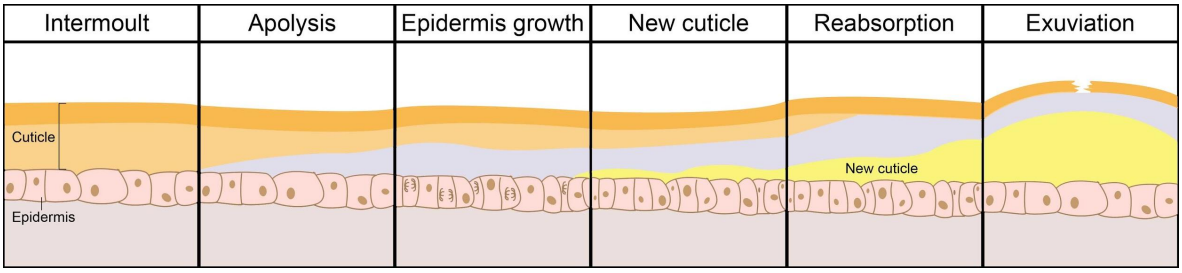


Figure 2: The complete exoskeleton moulting process in extant, and presumably extinct, Euarthropoda. From left to right: the intermoult stage, with a normal exoskeleton and epidermis; apolysis, where the old exoskeleton and epidermis separate, leaving empty space between them (in grey); cell growth of the epidermis and secretion of moulting fluids into the space between epidermis and old exoskeleton; the new cuticle is secreted; enzymes in the moulting fluids cause digestion and reabsorption of the old exoskeleton; the old exoskeleton ruptures and is exuviated, and the new exoskeleton expands.

Most palaeontological data that exist for euarthropod moulting are derived from the trilobites (Fig. 3), eurypterids (sea scorpions), and crustaceans. This is largely because these groups have uniquely thick and robust exoskeletons often reinforced with additional materials, with correspondingly high preservation potential. Research on moulting tends to be descriptive and focused on particular specimens or assemblages, with only a few studies examining the fossil record of ecdysis as a whole. Henningsmoen (1975) describes in detail the characteristic modes of moulting observed in different groups of trilobites, but does not examine the evolutionary significance of these trends. A broader look at such evolutionary trends is taken by Brandt (2002), who examines the link between trilobite moulting method and geological survivorship of taxa. A brief treatment of the fossil record of moulting is used by Valentine and Collins (2000) in conjunction with molecular phylogenetic information to make broad inferences on the process of evolution within Ecdysozoa, but this focuses mostly on developmental data in modern taxa and does not

succinctly summarise or integrate the broad record known from fossils. A more fossil-based perspective is provided by Speyer (1990), who evaluates the reliability of the fossil record for understanding moulting behaviour in a wide variety of moulting groups, including trilobites (based heavily on Henningsmoen, 1975), decapod crustaceans, insects, and spiders. An informative and substantial body of descriptive research focused specifically on evidence of moulting in the arthropod fossil record has been accumulating since these reviews. I will bring together this information on moulting in trilobites (e.g. Fig. 3) to examine their patterns of moulting and briefly compare these to other Euarthropoda, with the aim of setting the scene for the remainder of this thesis on the current knowledge of this major morphological innovation.

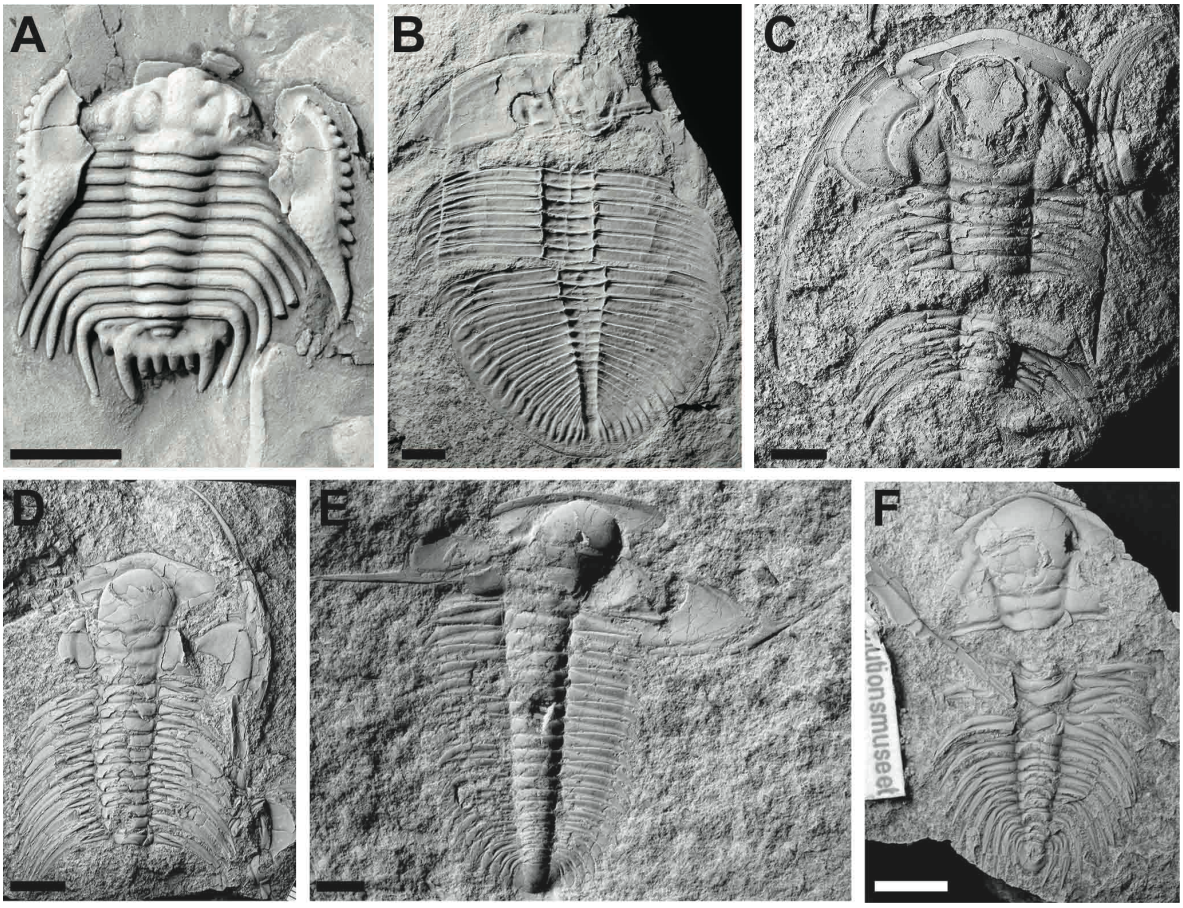


Figure 3: Different moulting behaviours preserved as trilobite exuviae (moulted exoskeletons). A: *Acidaspis coronata* Salter 1853 from the Silurian Wenlock Shale in Malvern, UK, facial sutures

opened and displaced slightly backwards (OUMNH C.17494). B: *Ogygiocarella debuchii* Brongniart 1822 from the Ordovician Llandeilo Series in Meadowtown, Shropshire, with displaced cephalon and break through the thorax (OUMNH B.263). C-F: Cambrian trilobites from Jämtland, Sweden. C: *Acadoparadoxides* sp. Šnajdr 1957 with left facial suture opened, crushed glabella, and middle thoracic segments displaced and rotated 90^{o} over the right side of the cephalon (PMU 28736). D: *Acadoparadoxides* sp. with left free cheek missing and right free cheek inverted (PMU 25995). E: *Eccaparadoxides* sp. Šnajdr 1957 showing both free cheeks inverted (PMU 25636). F: *Acadoparadoxides* sp. with right free cheek missing, left free cheek inverted, cranidium displaced, and disrupted thoracic segments (PMU 25690). Scale bars 5 mm in A, C, E, and F, and 10 mm in B and D.

Moulting in Euarthropoda

Identifying moults in the fossil record

Describing patterns of ecdysis in the fossil record necessitates the ability to distinguish preserved exoskeleton moults from carcasses. Only in extremely rare cases have specimens been preserved mid-moult (e.g. García-Bellido and Collins, 2004; Peel et al., 2013; Yang et al., 2019; Appendix 3; Fig. 4C) with both the moult and the exiting animal in close association. In the vast majority of cases, individual specimens must be examined to determine if they are empty moults or carcasses. Important aspects to consider when identifying moults include the locations of 'gape sutures', planes of weakness where the exoskeleton splits to produce an opening through which the individual exits; movements of the animal to facilitate opening of the gape sutures; the direction of exuviation (anteriorly or posteriorly, any lateral movements); the orientation during exuviation (dorsal- or ventral-up); and the final configuration of exoskeleton segments that may be preserved in the fossil record. Interpretations of moulting behaviour can only be made from definite exuviae (moults), so as not to introduce misleading signals on, for

example, the locations of putative gape sutures identified from fragmented carcasses. Physical disturbance of carcasses (e.g. from scavenging, decay, currents, reworking, or compaction) can lead to preservation of disarticulated assemblages, and distinguishing these from true empty moults requires consideration of the morphology, and taphonomic and depositional environment of the fossil. The procedure for identifying moults depends on the taxonomic group, owing to the broad morphological and behavioural variation within Euarthropoda. This is more difficult for some groups, particularly those with suture lines that close following exuviation (e.g. modern *Limulus*; Sekiguchi et al., 1988; Vrazo and Braddy, 2011), but may be aided by consideration of the taphonomic aspects detailed below.

Numerous criteria have been suggested for identifying fossil moults, based on observations from fossil material in previous studies (Henningsmoen, 1975; Whittington, 1990; Brandt, 1993; Braddy, 2001; Tetlie et al., 2008; Ebbestad et al., 2013). At least Criteria 1 and/or 2 below are crucial for identifying a moulted exuvia. Criterion 3 may provide additional evidence for identifying moults, but only if Criteria 1 and/or 2 have been met and the depositional environment and preservational processes considered (Criterion 4). Criteria 4-6 provide further support and are dependent on the preservation and cuticle type (soft-part; mineralised).

Criteria for identifying moults in the fossil record:

1. Presence of exuvial suture lines showing evidence of having been opened. Criteria 1 and/or 2 are crucial for identification of a fossil moult specimen.
2. Repeated configurations of specific disarticulated exoskeletal parts (for examples see Figs 3-7; Drage et al., 2018a; Chapter 5). Criteria 1 and/or 2 are crucial for identification of a fossil moult specimen.

3. Disturbance of mineralised exoskeletal features in a systematic, repeated and characteristic way. This may include flattening or distortion of elements (Braddy, 2001); superimposition of dorsal and ventral surfaces; telescoping of segments (Braddy, 2001); and possibly crushing of the glabella (Figs 2C, 4B) (Ebbestad et al., 2013). The exact nature of the disturbance may vary between different assemblages and types of fossils. Identification of these types of features may assist with recognising fossil moults in taxa where suture lines close after exuviation, for example scorpions where the relative positioning of anatomical features is thought to allow for identification of fossil moults even without suture lines (McCoy and Brandt, 2009). Generally, Criterion 3 would not be enough on its own to conclusively identify a moult assemblage, but may provide additional evidence if Criteria 1 and/or 2 are met and if Criterion 4 has been considered.
4. Consideration of contextual information, including biostratinomic data, palaeoenvironmental conditions, and preservational processes. Evidence indicating a quiet depositional environment with low energy conditions and a low degree of transportation is preferred for moulting assemblages, as it would preclude post-depositional disarticulation that could cause carcasses to look like empty moults. Avoiding heavily reworked and/or time averaged deposits also reduces the chance of incorrectly identifying carcasses as moults. This type of information is not enough on its own to identify a moult, but would provide supporting evidence for moults identified using Criteria 1, 2, and/or 3.
5. Lack of clear internal carcass features, including: the midgut and/or gut contents (as some arthropods moult part of the gut with the exuviae); musculature; and the nervous or circulatory system. This criterion is only applicable at localities known to have soft-part preservation, so is not diagnostic on its own for the arthropod taxa

that comprise the vast majority of the fossil record of moulting, including trilobites, eurypterids and crustaceans. Only rarely are these taxa found with soft-part preservation (e.g. Whittington, 1990) and so a lack of internal organs in arthropod taxa does not necessarily indicate a moult unless minimally Criteria 1 and/or 2 are also met. Criterion 5 is more relevant when discussing the ecdysis fossil record of lobopodians, loriciferans, priapulids and stem-lineage arthropods without a reinforced exoskeleton.

6. Lack of evidence of behaviours associated with body fossils, such as predation and scavenging. For eurypterids and crustaceans at least, moult assemblages are thought to be better preserved than carcasses, because they would not be a target for scavenging and predatory activities (Clarke and Ruedemann, 1912; Feldmann and Tshudy, 1987; Braddy, 2001). Again, this criterion is not enough on its own to identify a moult assemblage, but provides supporting evidence for specimens that minimally meet Criteria 1 and/or 2.

Preservation potential must also be considered when investigating the moult fossil record. Moults in animals with thickened exoskeletons, such as Trilobita, may have increased preservation potential compared to carcasses (due to presumably decreased incidence of scavenging). This could result in a bias to moults in the fossil record of these groups, exacerbating inflation already present from the fact that one individual produces numerous moults during its lifetime (but only one carcass). This bias is also dependent on additional moulting behaviours. Specifically, some arthropod taxa consume their moults entirely, while others reabsorb exoskeletal material for energy conservation (Brandt, 2002). Consequently, moults of some clades may be more fragile (for example eurypterids; Vrazo et al., 2014), with decreased preservation potential compared to their respective

carcasses. Further, body sections in segmented ecdysozoans are more likely to become disarticulated in the moult as connective tissue (integument) may be lacking. However, the opposite could also be true; preserved carcasses could appear to be moults (based on criterion 3 above) due to the presence of exoskeletal distortion following the rapid decay of soft connective tissue (Hegna, 2012). Factors such as these may decrease the likelihood of moult preservation, but the lack of published experimental taphonomic (decay) data comparing moults and carcasses compounds this knowledge gap (although see Hegna, 2012). A number of criteria must therefore be considered when attempting to justify a moult identification, and decisions must be rooted within specific contextual knowledge of the clade and depositional environment.

Moulting in non-euarthropod Ecdysozoa

The fossil record of moulting consists mainly of specimens belonging to the Arthropoda, or falling on the stem lineage to this phylum, however the smaller ecdysozoan phyla Priapulida and Loricifera also have preserved evidence of moulting. Only sub-fossil Holocene moults of tardigrades have been described (Gibson et al., 2007), and the ecdysozoan phyla Nematoda, Nematomorpha, and Kinorhyncha have poor or absent fossil records for which no moulting assemblages have yet been found. Despite the sparse fossil record also for the Loricifera, a convincing example of a mid-moult individual of *Sirilorica pustulosa* Peel 2010 was described by Peel et al. (2013) from the early Cambrian Sirius Passet, which mirrors the moulting behaviours of extant loriciferans (e.g. *Rugiloricus* Higgins and Kristensen 1986). Conway Morris (1977) suggested a few specimens of the priapulid worm *Ottoia prolifica* Walcott 1911 that may represent moults based on specimens consisting only of the anterior proboscis (which is detached during moulting in extant priapulids). Further, the lack of interior anatomical features and

wrinkled texture of one specimen of *Eximipriapulus globocaudatus* Ma et al. 2014 from the early Cambrian Chengjiang led to it being described as a moulted exoskeleton (Ma et al., 2014). The moulting behaviours of lobopodians have also been discussed in the literature, with these being thought to fall within Panarthropoda but likely as stem members of different groups (Euarthropoda, Tardigrada, or Onychophora). For example, the enigmatic *Hallucigenia* Conway Morris 1977 lobopodian was found to retain moulted sclerites (much like ostracods) through examination of multi-layered claws and spines (Caron et al., 2013; Smith and Ortega-Hernández, 2014). Other lobopodians likely did not retain successive sclerite moults, with those of *Microdictyon* Bengtson, Matthews and Missarzhevsky 1986 being preserved as a large sclerite underlying a smaller one thought about to be moulted (Chen et al., 1995), and this was probably usual for lobopodians (Zhang and Aldridge, 2007; Topper et al., 2013; Fig. 4B).

Moulting in non-trilobite Euarthropoda

A reasonably extensive fossil record of moulting exists for the Crustacea, as they have heavily reinforced (chitin-protein matrix; Raabe et al., 2005) exoskeletons, much like the Trilobita. Some crustacean groups, in particular the bivalved Ostracoda and spinicaudatan branchiopods, demonstrate unusual moulting behaviours; in this case retaining unshed moults during development to produce a multi-layered carapace (Orr et al., 2008; Olempska, 2012), as for the peripheral sclerites of *Hallucigenia*. The record of decapod crustaceans is particularly abundant owing to their heavily calcified exoskeletons, however less so for the crayfish/shrimp than the lobsters and crabs because the former tend to consume their exuviae after moulting to conserve resources (Bishop, 1986). Lobster moult fossils are usually preserved in the ‘Open Moult Position’ (Glaessner, 1969), with the carapace (head shield) separating from the abdomen at the posterior and usually at a

medial suture, then becoming displaced at a 90° angle to the abdomen as the individual exuviates up and forwards (*Glyphea* Von Meyer 1835*, Protaxius* Beurlen 1930*,* Glaessner, 1969; *Thalassina* Latreille 1806, Murray and Hanley, 1986; *Oncoparia* Bernhauer 1936*, Linuparis* White 1847*, Hoploparia* McCoy 1849*,* Bishop and Williams, 1986; *Oosterinkia* Klompmaker and Fraaije, 2011; *Meyeria* McCoy 1849*,* Feldmann et al. 1995; González-León et al., 2014; Fig. 4E). This configuration is also formed in crab moults when the carapace disarticulates at the posterior and sides, and is pushed up and forwards, although unlike lobsters the individual exuviates posteriorly (see Glaessner, 1969; Bishop, 1972, 1986; Vega et al. 2005). Only two moulting configurations have been described as preserved for decapods, with the second, the 'Overturned-Carapace Position' being a variant on the Open Moult Position. This has the carapace displaced further, to rest upside down (the crab *Dakoticancer* Rathburn 1917, Bishop 1972; the lobsters *Pseudoglyphea* Oppel 1861*, Clytiopsis* Bill 1914, Klompmaker and Fraaije, 2011). However, it is possible that some fossil crab specimens with the sternum displaced also represent moults, based on their size and cuticle thickness (*Dakoticancer*, Vega and Feldmann, 1991; *Carcineretes* Withers 1922, Vega et al., 2001).

The fossil record of chelicerate moulting is reasonably abundant in large part due to descriptions of eurypterid (sea scorpion) exuviae. Eurypterid fossils are very often described as moults unless they present obvious internal structures (midgut, musculature, etc.; Braddy, 2001; Tetlie et al., 2008; Daley and Drage, 2016). Tetlie et al. (2008) suggested that eurypterids and modern horseshoe crabs potentially moulted in the same way. This involves opening of a suture around the anterolateral margins of the head shield, anterior exuviation of the individual, and then effective closure of the suture as the dorsal part of the head shield settles back *in situ* over the ventral parts (e.g. Fig. 4D). This means that horseshoe crab moults are often impossible to identify in the fossil record (Shuster and

Sekiguchi, 2004), which could also be the case for some eurypterids (Tetlie et al., 2008), although they may also simply represent carcasses. More reliable eurypterid moult fossils (e.g. *Eurypterus remipes* De Kay 1825) show a moulting process in which the marginal suture opens and the head shield, usually with the first body segment, is disarticulated (Tetlie et al., 2008, fig. 10; Fig. 4A). For the other major groups of chelicerates, scorpions tend to moult in the same way as that described for *E. remipes* and horseshoe crabs (Tetlie et al., 2008), but spiders (both extant and extinct taxa show the same behaviours; Petrunkevitch, 1942; Selden et al., 1991) moult like crabs (are usually found in the Overturned-Carapace Position), as described above. The moult fossil records of these groups are particularly sparse due to their terrestrial nature, although McCoy and Brandt (2009) attempted to identify fossil scorpion moults based on comparisons to extant chelicerate moult characteristics.

A number of stem euarthropod groups, usually from Cambrian Konservat-Lagerstätten, have also provided information on early moulting behaviours in the group. This includes the best preserved mid-moult euarthropod (*Marrella splendens* from the Burgess Shale, García-Bellido and Collins, 2004; Fig. 4C), specimens of fuxianhuiid euarthropods that appear to show either the Open Moult or Overturned-Carapace Positions (Yang et al., 2019; Appendix 3), and other stem-lineage taxa interpreted as mass moulting surfaces (*Canadaspis* Briggs 1978 and *Alalcomenaeus* Simonetta 1970 from the Burgess Shale, Haug et al., 2013).

In contrast to what is known for these other groups, trilobite moulting is extremely variable. This is true both between orders, which although they show a reasonably conserved body plan do have key morphological differences, but also between closely-related species and individuals of the same species (see Chapters 3-5). The isopod crustaceans likely represent the group of arthropods that moult in the most similar way to

trilobites, presumably because of their reasonably close exoskeletal morphology, although to date only one mode of moulting has been described. This is a biphasic mode, in which the posterior part of the exoskeleton is moulted usually several days before the fragmentary anterior components, and it is thought that fossil isopods likely moulted in the same way (Wieder and Feldmann, 1989; Feldmann, 2009). Although preliminary, work on fossil isopods by Hyžny et al. (2013) suggests they may bear even more similarity to trilobite moults. In taxonomic studies, individual trilobites are often distinguished an empty moults or carcasses, and this has provided a wealth of descriptive information on the ecdysial modes of trilobites and their growth and development. The different modes of trilobite moulting behaviour, and the resultant fossil moult configurations that these produce, are described below, followed by a full literature review of published descriptions on trilobite moulting to date.

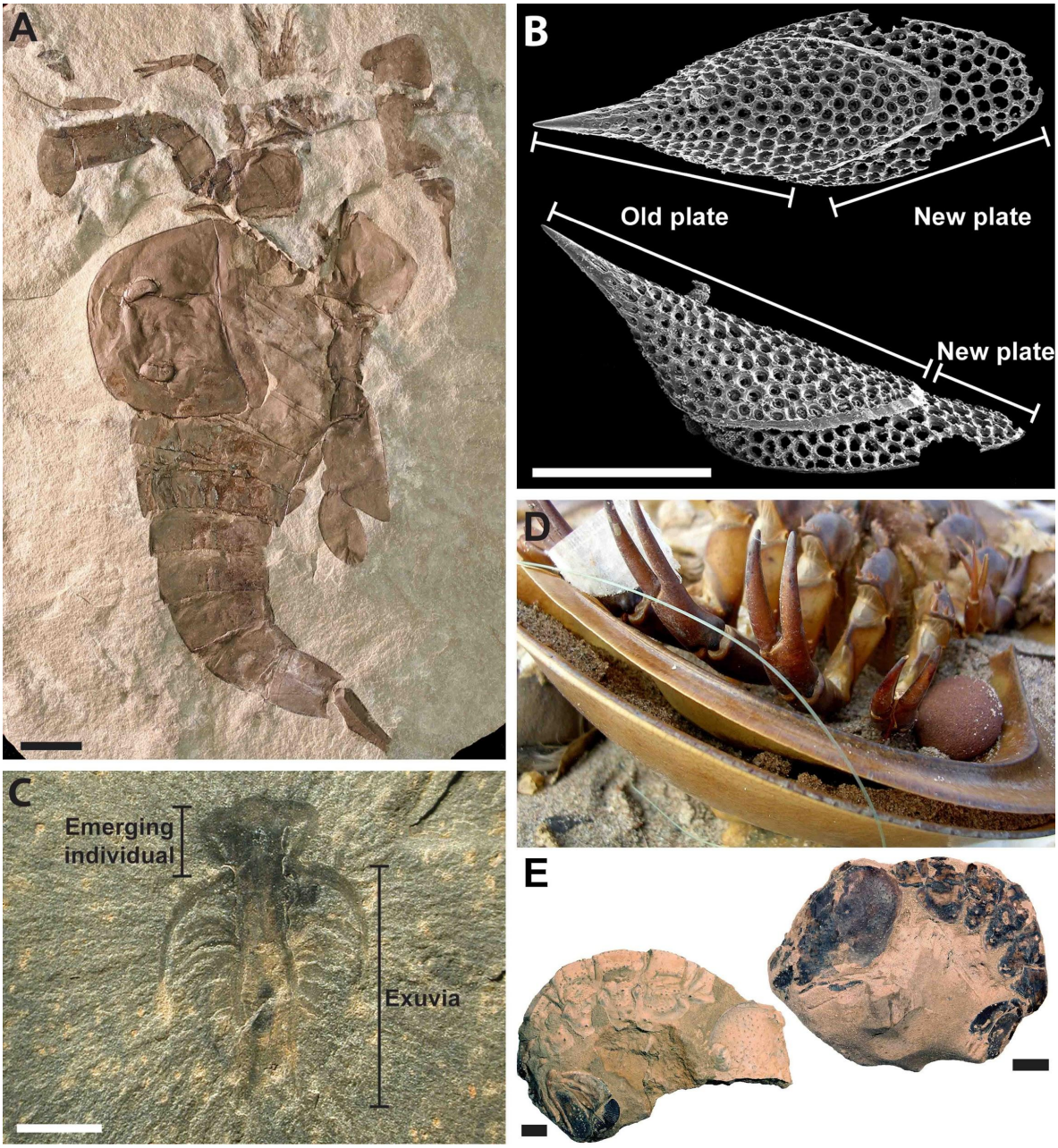


Figure 4: Moulting in ecdysozoans. A: Moult of the euarthropod eurypterid *Eurypterus lacustris* (YPM 208303) from the Silurian Williamville Formation, Ridgemount Quarry, Ontario. The exuvial gape has been created between the first and second tergites. Photo: J. Utrup. B: Conjoined plates of the lobopodian *Onychodictyon* sp. Hou, Ramsköld and Bergström 1991 from the lower Cambrian of North Greenland (from Topper et al., 2013). C: The euarthropod *Marrella splendens* from the middle Cambrian Burgess Shale, British Columbia, preserved mid-moult (from García-Bellido and Collins, 2004). D: Empty moult of the extant horseshoe crab *Limulus polyphemus* from North America (CC BY licensed, no scale). The exuvial gape is clearly visible at the marginal

suture between the dorsal and ventral halves of the carapace. E: Moults of *Meyeria magna* McCoy 1849 from the Lower Greensand of the Atherfield Clay, Isle of Wight (OUMNH K.755 and K.576) preserved in the Open Moult Position, with the carapace displaced from the abdomen. Scale bars 10 mm for A, 500 μm for B, 5 mm for C and E.

The fossil record of moulting in trilobites

The majority of literature published on ecdysis in the fossil record concerns patterns of moulting in Class Trilobita. This group was extremely long-lived and diverse, living from the early Cambrian until their disappearance at the end-Permian mass extinction, following a drawn-out decline (Brandt, 2002). In addition to being geologically successful and geographically diverse, trilobites filled varied ecological niches, including as predators, scavengers, and filter-feeders. Trilobites have been variously interpreted as related to the mandibulates, chelicerates, and as stem lineage arthropods (see Budd and Telford, 2009; Edgecombe, 2010, for a review), but regardless of their exact phylogenetic affinity, they are important as early arthropods owing to their unparalleled abundance in Palaeozoic marine environments. Their thick exoskeletons had a high preservation potential, providing researchers with extensive collections of material with which to investigate biostratigraphy, biogeography, and their morphology, development and ecology. Trilobites were a key component of ancient marine ecosystems, with a complex evolutionary history shaped by their ability to moult. Understanding their ecdysial behaviours is central to the study of the early evolution of Euarthropoda and its evolution for the next 540 million years (Henningsmoen, 1975).

Trilobite moult configurations (Figs 3, 5), the repeated patterns of fragmented exuviae preserved in the fossil record (see moult identification Criterion 2), demonstrate that the ecdysial behaviours employed by trilobites were highly varied. These behaviours

have been reconstructed using the consistent patterns of disarticulated elements observed in empty moult configurations because no specimens have yet been found preserved midway through the act of moulting. Three modes of moulting are recognised from the fossil record of trilobites, indicating extensive flexibility in this behaviour (Fig. 5). These are the 'Sutural Gape', 'Marginal Suture', and 'Salter's' modes of moulting (see Fig. 5; Table 1). The first involves the opening of cephalic sutures, which depending on morphology may be comprised of the facial sutures connecting the librigenae (free cheeks) to the cranidium (most commonly), the rostral suture connecting the ventral rostral plate to the cephalon, the hypostomal suture connecting the hypostome to the cephalon, and/or a median suture running axially on the ventral side (Fig. 5.1). Opening of the facial sutures followed by release and/or inversion of the librigenae is the most common moulting characteristic for Trilobita. The facial sutures are a feature in trilobites and close relatives apparently existing only to facilitate moulting through creation of an anterior exuvial gape (Stubblefield, 1959; Du et al., 2018). In Salter's mode of moulting (Fig. 5.2) the joint connecting the cephalon to the thoracopygon is opened. In the Marginal Suture mode (Fig. 5.3), the marginal suture is opened (usually around the anterior of the cephalon; similar to moulting in xiphosurans; Sekiguchi et al., 1988; Loveland, 2002; Shuster and Sekiguchi, 2004), which is only found in certain derived trilobite groups. In all cases, at these openings an exuvial gape is produced through which the animal can anteriorly egress the old exoskeleton (Table 1). The trilobite individual would then be free to expand and harden the new exoskeleton.

A great deal of variation in trilobite moult configurations, the preserved assemblages of disarticulated sclerites, resulted from these three modes (see Budil and Bruthansová, 2005, fig. 3; Drage et al., 2018a; Chapter 5). Drage et al. (2018a, fig. 1) described six common trilobite moulting configurations resulting from the Sutural Gape

mode of moulting. These six are illustrated in Figure 5.1 A-F, and have varying configurations of the disarticulated librigenae, rostral plate and hypostome unit, and cranidium. Salter's mode (Henningsmoen, 1975) can produce two configurations; Salter's Configuration (with the entire disarticulated cephalon inverted, Richter, 1937; Fig. 5.2G), and the Zombie Configuration (with the cephalon displaced but not inverted; named here, Fig. 5.2H). Henningsmoen (1975) noted several additional configurations resulting from the Marginal Suture mode of moulting, which have also been discussed by other authors (e.g. Stubblefield, 1959). The *Harpes* Configuration represents moults where the individual has exuviated through an anterior gape at the marginal suture, but no exoskeletal parts have been disarticulated and the dorsal half subsequently returned to life position over the ventral part (Fig. 5.3I; Stubblefield, 1936). This is akin to that described above for some eurypterids (Tetlie et al., 2008) and horseshoe crabs (Sekiguchi et al., 1988). Maksimova's Configuration (named in Henningsmoen, 1975) is here considered as moults showing displacement of only the ventral doublure (usually as one unit) following opening of the marginal suture, with the dorsal exoskeleton left intact (Fig. 5.3J). Hupe's Configuration (named in Henningsmoen 1975; Fig. 5.3K) shows this ventral doublure inverted in relation to the dorsal exoskeleton. The preservation of these many moulting configurations not only show how variable trilobite moulting behaviour could be, but can also enable us to reconstruct the exuviating individual's movements. For example, figure 2 in Drage et al. (2018a) shows several specimens where the thoracopygon was initially shed, and the remaining cephalic sclerites were carried forward by the animal before being subsequently moulted. Non-cephalic parts of the exoskeleton could also become disarticulated during trilobite moulting, and these may have been important in certain rare moulting events if the cephalic gape sutures failed to open (e.g. see McNamara, 1986; Budil and Bruthansová, 2005; Paterson et al., 2007; Tortello and Clarkson, 2008). These

include separation between thoracic segments and between the thorax and pygidium, and these may be present in trilobites showing those configurations described above. Other research has suggested that the most parsimonious explanation if thoracic/pygidial disarticulations are present, but cephalic gapes are lacking, is that these other disarticulations result from decay and/or disruption to carcasses (Hunda et al., 2006).

Moult descriptions show that many trilobite taxa were not restricted to just one of these modes, and indeed could produce a number of the described configurations (e.g. Henningsmoen, 1975; Brandt, 2002; Budil and Bruthansová, 2005; Daley and Drage, 2016; Drage et al., 2018a and b). The moulting behaviours employed by the individual likely depended on a number of factors, including developmental stage and size (Crônier, 2013; Drage et al., 2018b), and ease of moulting in a particular environment. Additionally, there may have been morphological constraints, and some ecdysial characteristics may be constant for this reason. For example, Salter's mode of moulting was the norm for species with apparently inactive facial sutures, as in some phacopids (Henningsmoen, 1975; Drage et al., 2018b; Chapter 6). Other methods may have been key for species with more complex morphologies, such as the Marginal Suture mode (Fig. 5.3). We may also see trends in ecdysis through geological time, possibly in response to a phylogenetic or diversity signal (see Chapters 3 and 4). For example, Budil and Bruthansová (2005) found Middle Ordovician Czech taxa displayed more variation in moulting configurations (this period corresponding with morphological diversification of many trilobite groups; Harper, 2006).

Trilobites may have been as likely to moult dorsum-up as ventrum-up. This may be common across the class; a close to equal ratio between the two moult positions has been noted in trilobites such as olenids and paradoxidids (Clarkson et al., 2003; Ebbestad et al., 2013). However, other species show a bias towards one orientation (Karim and Westrop,

2002). For example, inverted *Phacops* Emmrich 1839 moults have been suggested to result from ventrum-up exuviation, this orientation being beneficial for opening of the ecdysial gape between cephalon and thorax (Speyer, 1985, 1987). Whereas exuviae of *Greenops* Delo 1935 are rarely found in this position, possibly indicating only dorsum-up exuviation (Speyer, 1985, 1987). Morphology may be key for determining moult orientation, although this is difficult to determine without preserved mass moult assemblages, and has seen very little research. Further, singular ventrum-up specimens could result from taphonomic effects, such as inversion via transport following ecdysis, rather than being informative for moulting behaviour. For orientation to inform on behaviours rapid burial and preservation would be required (Speyer, 1987).

Table 1: Definitions of general terms used regarding exoskeleton moulting.

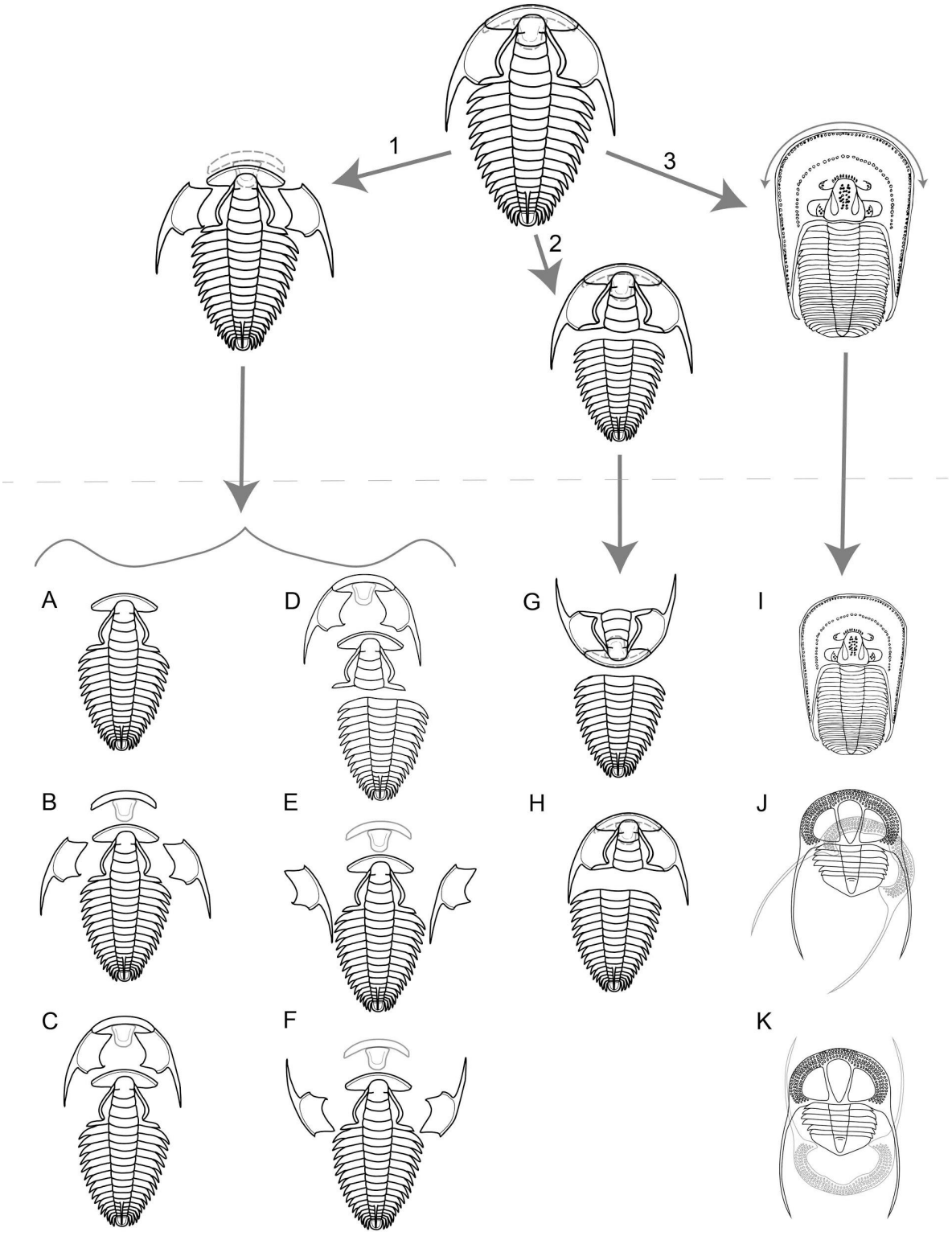
Moult configuration	The general term for any assemblage of disarticulated exoskeletal units (sclerites) produced through the behaviours of a moulting individual. This is left behind and becomes preserved in the fossil record (see Drage et al., 2018a; Chapter 5).	
Mode of moulting	The generalised type of exoskeleton moulting, involving a number of moulting characteristics, which produces a moulting configuration. Either Sutural Gape mode, Salter's mode, or Marginal Suture mode, defined below and in the main text.	
	Sutural Gape	Moulting through opening of the cephalic sutures, including any combination of the facial, rostral, hypostomal, and median sutures. The Sutural Gape mode therefore requires separation of one or more of the librigenae and ventral structures, and can include cranidium disarticulation.
	Salter's	Moulting involving opening of the cephalothoracic joint. Salter's mode therefore requires disarticulation of the cephalon or cranidium (if sutural gapes also used).

	Marginal Suture	An unusual mode for trilobites, involving anterior exuviation through a gape created by the opening of a suture running laterally along the anterior margin of the entire cephalon.
Exuviation	The act of an organism actually exiting the old exoskeleton.	
Exuvial gape	The opening produced in the old exoskeleton through which the organism exuviates.	

The general characteristics of trilobite moulting do not seem to specifically align them with any modern arthropod group. The facial sutures of trilobites represent a system of ecydsial suture that is unique in its complexity, with no known equivalent amongst modern arthropods or other ecdysozoans (Ax, 1987; Paterson and Edgecombe, 2006), although some of their closest relatives, for example the non-trilobite artiopodans (e.g. *Zhiwenia coronata* Du et al. 2018 and *Acanthomeridion serratum* Hou, Chen and Lu 1989), appear to show simple facial sutures that may have been involved in the moulting process for artiopodans (Hou et al., 2017; Du et al., 2018). Trilobites appear to show continuous, or indeterminate, growth, meaning that they continued to grow in size and moult after reaching their adult morphology (Hughes, 2007). Of extant arthropods, myriapods and crustaceans also continue to grow and moult as adults, whereas insects and arachnids show determinate growth, meaning they do not continue to grow and moult after reaching sexual maturity (Nijhout, 2013). Trilobites differ from extant arthropods in that there is no evidence for reabsorption of the old cuticle prior to moulting, as is seen in many groups (e.g. crustaceans; Miller and Clarkson, 1980; Mutvei, 1981; Ewer, 2005; Nijhout, 2013; Chapter 7). All preserved material also suggests trilobites exited their exuviae anteriorly, as is seen in modern xiphosurans, scorpions and myriapods, but which is unlike other arthropods, such as spiders and crabs, which exit their exuviae posteriorly

(Henningsmoen, 1975). Few ecdysial characteristics have been included in phylogenetic analyses of trilobite relationships beyond those associated with the morphology of facial sutures. Moulting behaviours as they have been published in the literature (except for subsequent published chapters included within this thesis) for each trilobite order are discussed below.

Figure 5 (next page): Summary of trilobite modes of moulting (top) and the most common trilobite moult configurations preserved in the fossil record (bottom). Generalised line drawings are based on *Redlichia takooensis* Lu 1950 from the Emu Bay Shale (1, 2, A-H), *Harpes* sp. Goldfuss 1839 (3, I), and *Marrolithus ornatus* Sternberg 1833 (J, K). Grey features represent ventral structures (rostral plate, hypostome, ventral doublure). 1: the Sutural Gape mode of moulting. 2: Salter's mode of moulting. 3: the Marginal Suture mode of moulting. A: Axial Shield. B: Nutcracker Configuration. C: Harrington's Configuration. D: Henningsmoen's Configuration. E: McNamara's Configuration. F: Somersault Configuration. G: Salter's Configuration. H: Zombie Configuration. I: *Harpes* Configuration. J: Maksimova's Configuration. K: Hupe's Configuration, as Maksimova's but with the ventral doublure (grey) flipped over.



Literature record of moulting in Redlichiida

Descriptions of ecdysial behaviour are most common in the literature for the speciose orders Phacopida and Redlichiida (Fig. 3). Most *Redlichia* individuals would

moult by opening of both the facial sutures (Öpik, 1958; McNamara, 1986; Fig. 5B, the Nutcracker Configuration) and the rostral sutures, the latter of which was necessary to release the "stitched rostral shield" (Öpik, 1958, pg. 28) that interlocks with the cranidium by a series of small nodes in some taxa. The cranidium would often detach (Henningsmoen's Confugration; Fig. 5D), leaving the thoracic segments and pygidium as a cohesive unit that is preserved (Öpik, 1958, 1970; McNamara, 1986). A moulted, but admittedly malformed, specimen of *R. hupehensis* Hsu 1948 shows only the detachment of the librigenae, i.e. a complete axial shield (cranidium, thorax and pygidium) (Han and Zhang, 1991). Specimens of *R. forresti* Etheridge 1890 individually show inversion of the pygidium (McNamara, 1986, figs. 1A, D, H, I), thoracic segments (Öpik, 1958, pl. 2, figs. 2, 3) and librigenae (McNamara, 1986, fig. 1B), whereas *R. idonea* Whitehouse 1939 (McNamara, 1986, fig. 1E) and *R. micrograpta* Öpik 1970 (McNamara, 1986, fig. 1C) each only show inversion of thoracic segments or librigenae respectively. *Redlichia takooensis* has so far shown only inversion of the librigenae (Drage et al., 2018a, fig. 4; Chapter 5).

McNamara (1986) highlights the unusual occurrence of inversion of part of the body (free cheeks, thoracic segments, and/or pygidium) during moulting. He focuses on three species of *Redlichia* with inverted elements and describes scenarios for each specimen that require partial enrolment (or 'dorsal flexure') of the specimen during ecdysis. This link between ecdysial moult configurations and enrolment behaviour was in contrast to previous suggestions that Cambrian micropygous trilobites were rigid and unable to enroll (Harrington, 1959; Bergström, 1973), although later studies have shown that at least flexing, if not total enrolment, was possible in these trilobites (Esteve et al., 2011, 2013; Ortega-Hernández et al., 2013). The dorsal spines pushing into the sediment and providing leverage may also have been key for this movement (as suggested for the

redlichiid *Paradoxides* by Whittington, 1990). McNamara (1986) stresses that inversions in *Redlichia* were "the exception, rather than the rule" (pg. 412), and should be considered in the context of the individual specimen (rather than generalised as behavioural differences between taxa). To account for the inverted body elements, McNamara (1986) invokes an ecdysial scenario that begins with a posterior movement of the trilobite in the early stages of exuviation, and suggests that this may have been used to separate the cephalon from the thorax in cases where the rostral suture failed to open. This implies a flexibility of ecdysial behaviour in *Redlichia* individuals and response to situations where the usual modes of moulting were unsuccessful.

Ebbestad et al. (2013) described redlichiid moults of two paradoxidid species, including one *Eccaparadoxides*, which also moulted using the cephalic sutures (Fig. 6). They particularly emphasise the open position of the facial sutures in all specimens (Ebbestad et al., 2013, figs. 3D-F), even where the librigenae remain somewhat in place, and a total absence of *in situ* hypostomes. Other paradoxidids, like *Paradoxides davidis* Salter 1836*,* also opened the cephalic sutures for moulting, displacing the librigenae and rostral-hypostomal plate as in the first step of ecdysis in *Redlichia* (Whittington, 1990).

Particular redlichiid trilobite taxa demonstrate the variability in ecdysial behaviour seen within species of other trilobite orders, employing several moulting behaviours. *Olenellus gilberti* Resser 1928 and *Nephrolenellus geniculatus* Palmer 1998 (Webster, 2007) seem to have moulted the hypostome and rostral plate before emerging ventrally through the resulting gape (Maksimova's Configuration; Fig. 5J), but occasionally specimens of the former also show dislocation and displacement of the thoracopygon beneath the cephalon (Webster, 2015, fig. 10). This differs from other redlichiids, such as *Eccaparadoxides* and *Redlichia*, because olenelloids lack facial sutures around the librigenae (Webster, 2015). *Balcoracania dailyi* Pocock 1970 appeared to have employed

the usual redlichiid style of ecdysis; strongly flexing the exoskeleton, pushing the spines into the sediment and allowing for opening of the cephalic sutures. The animal would emerge anteriorly, leading to the librigenae, rostral plate, hypostome, and/or cranidium becoming dislodged (Paterson et al., 2007, figs. 8A, B, E, H). However, at least one moult of *B. dailyi* displays separation between the first and second thoracic segments prior to opening of the facial sutures (Paterson et al., 2007, fig. 8C). As in *Redlichia*, this may well reflect flexibility in behaviour to overcome a particularly difficult moulting period (McNamara, 1986; Paterson et al., 2007), reinforcing the plasticity that some trilobite species can show during ecdysis.

Literature record of moulting in Phacopida

Budil and Bruthansová (2005) presented an initial exploration of the complicated nature of phacopid moulting, extolling the need for a more thorough analysis. In particular, there is great variability in ecdysial behaviours of dalmanitoid and acastoid phacopid taxa. The most common behaviour across all species described (and most other phacopid taxa) was complete disarticulation of the cephalon from the rest of the body (Salter's mode; Fig. 5.2), without the cephalic inversion involved in Salter's Configuration (i.e. the Zombie Configuration, Fig. 5I) (Budil and Bruthansová, 2005, fig. 4). However, particularly in *Ormathops* Delo 1935*,* as well as *Zeliszkella* Delo 1935*, Dalmanitina* Reed 1905 and *Kloucekia* Delo 1935*,* the break releasing the cephalon during ecdysis occurred several segments into the thorax. Thoraces broken into multiple segments in *Baniaspis (?) quadrata* Hawle and Corda 1847 (Budil and Bruthansová, 2005, figs. 6C, E, F) and *Zeliszkella cf. oriens* Barrande 1872 (Budil and Bruthansová, 2005, fig. 7E), potentially

representing multiple gape sutures, may indicate instances of problematic moulting as in some described redlichiids.

However, demonstrating the apparent versatility of these dalmanitoids, separation of the librigenae from the axial shield via opening of the facial sutures was common in young individuals of *Dalmanitina proaeva elfrida* Šnajdr 1982*, Dalmanitina proaeva proaeva* Emmrich 1839*,* and *Ormathops (Ormathops) atavus* Barrande 1872 (Budil and Bruthansová, 2005, figs. 7B, C; Drage et al., 2018b). Some specimens of *Ormathops (Ormathops) atavus* also show the librigenae disconnected from the separated cranidium (Budil and Bruthansová, 2005, figs. 4E, 5C; Henningsmoen's Configuration). However, Budil and Bruthansová (2005) state that moulting by opening of the facial sutures was replaced by more 'advanced modes' (Budil and Bruthansová, 2005, pg. 376), with a loss of facial suture functionality into adulthood of these phacopid trilobites causing moulting using cephalic or thoracic disarticulations (see Drage et al., 2018b; Chapter 6).

Similarly, cephalic sutures of *Trimerus stelmophorus* Busch and Schwartz 1985 (Homalonotidae) were non-functional during adulthood. Phacopids with this condition instead separated the cephalon and thorax (Busch and Swartz, 1985, fig. 1.8; Speyer, 1985). This often resulted in preservation of moults in Salter's Configuration, suggesting arching of the body with the dorsal cephalon towards the substratum (dorsal flexure, as in Drage et al., 2018a for *R. takooensis*). *Plagiolaria poothai* Kobayashi and Hamada 1968 (Crônier and Fortey 2006) and *Paciphacops* sp. Maksimova 1971 (Rustán et al., 2011, fig. 2) also moulted in this way, although the last is interpreted slightly differently. The *Paciphacops* figured by Rustán et al. (2011) may have utilised a gape suture between the cephalon and thorax for ecdysis while infaunal, but also displaced the cephalon dorsally over the thorax, leaving the cephalon pointing upwards at a 90^{o} angle to the thorax. *Phacops rana* Green 1832 and *Greenops boothi* Green 1837 presumably both moulted by

removing the cephalon, as determined by the prevalence of isolated thoracopygidia, although incidences of Salter's configuration were rare or absent for these species (Speyer, 1985).

From a single, complete inferred exuvia, McNamara and Tuura (2011) suggest a total moulting sequence for *Greenops widderensis* Lieberman and Kloc 1997. This begins with the familiar break of the join between cephalon and thorax, but also disarticulation of the pygidium (McNamara and Tuura, 2011, fig. 1). This is also the case for *Plagiolaria nandanensis* Zhang 1974 (Wang and Han, 1997). As with most other phacopids described, the facial sutures were not employed.

However, *Flexicalymene retrorsa* Foerste 1910 (Calymenidae) exuviae indicate the functionality of facial sutures, and the release of the librigenae, for ecdysis in some phacopid taxa (Hunda et al., 2006, fig. 9L). Subsequent rotation of the cranidium, they suggest, may reflect post-ecdysial disturbances (Hunda et al., 2006, fig. 9M). Some specimens of *F. retrorsa* showing an articulated thoracopygon without a cephalon have also been interpreted as moults, suggesting they may have occasionally utilised a gape suture behind the cephalon, as most phacopid trilobites did (Hunda et al., 2006, fig. 9N).

Literature record of moulting in Ptychopariida

Like redlichiid taxa, ptychopariid exuviae emphasise the importance of cephalic sutures for ecdysis across the order, often leading to the displacement of the hypostome and rostrum (Cederström et al., 2011, figs. 11C-F). This is certainly the case for moults of *Strenuaeva inflata* Ahlberg and Bergström 1978, although Cederström et al. (2011)

consider the opened facial sutures and displaced librigenae to result from taphonomic processes, with the ventral cephalic sutures being more important for exuviation.

Ctenopyge (Eoctenopyge) angusta Westergard 1922 utilised the facial sutures during moulting, as indicated by the preservation of separated yoked librigenae and hypostomes, with the cranidium also detached in some exuviae (Clarkson et al., 2003, pl. 8.1). Other olenids are described as having moulted similarly to this, releasing the librigenae with the axial shield remaining intact, including: *Acerocare ecorne* Angelin 1854 (Henningsmoen, 1957); *Parabolina frequens argentina* Kayser 1876 (Tortello and Clarkson, 2008, fig. 9.1); *Wujiajiania sutherlandi* Chatterton and Ludvigsen 1998 (Chatterton and Ludvigsen, 1998, fig. 10); and *Aciculolenus palmeri* Chatterton and Ludvigsen 1998 (Chatterton and Ludvigsen, 1998).

These olenid exuviae are often considered as preserved in 'Harrington's Configuration' (Henningsmoen, 1975; Drage et al., 2018a), with the shed librigenae (usually joined) displaced back and slightly below the axial shield (Tortello and Clarkson, 2008). Ptychopariids may also show within-taxon variation in moulting behaviour. For example, some moulted specimens of *Parabolina frequens argentina* have missing cranidia with the librigenae close to normal position (Tortello and Clarkson, 2008, figs. 8.7, 8.10, 9.3, 9.10), as do those of the olenid *Cloacaspis dejecta* Fortey 1974 (Fortey, 1974, pl. 12.1). *Parabolina frequens argentina* also displays signs of employing alternative behaviours such as disarticulated thoraces and displaced pygidia (Tortello and Clarkson, 2008, figs. 8-10) during difficult moulting periods, much like *Balcoracania dailyi* (Paterson et al., 2007) and *Redlichia* (McNamara, 1986). Unlike the other ptychopariids described, a single *Burnetiella leechi* Chatterton and Ludvigsen 1998 exuvia displays Salter's mode of moulting (Chatterton and Ludvigsen, 1998, fig. 22.6).

Literature record of moulting in Odontopleurida/Lichida

Descriptions of ecdysial behaviour are sparse for the remaining trilobite orders. *Odontopleura (Odontopleura) arctica* Adrain and Chatterton 1990 and *Odontopleura (Odontopleura) brevigena* Chatterton and Perry 1983 opened the facial and ventral sutures for ecdysis, releasing the librigenae and hypostome (Adrain and Chatterton, 1990, fig. 7.5). Edgecombe and Sherwin (2001) also note the librigenae, hypostome and rostral plate of *Odontopleura (Sinespinaspis) markhami* Edgecombe and Sherwin 2001 detached during ecdysis (Edgecombe and Sherwin, 2001, figs. 3D, 4A).

Literature record of moulting in Asaphida

Exoskeleton moulting in the Ordovician *Homotelus bromidensis* Esker 1964 took place through disarticulation of the cranidium, with the librigenae yoked anteriorly (Karim and Westrop, 2002). Conversely, preserved moulting configurations of *Labiostria westropi* Chatterton and Ludvigsen 1998*,* and occasionally *Pterocephalia norfordi* Chatterton and Ludvigsen 1998*,* usually consist of complete axial shields with the librigenae nearby, in a range of different positions (rotated, inverted, displaced), although some individuals may also have moulted the cranidium or cephalon (Chatterton and Ludvigsen, 1998, figs. 19, 29).

Literature record of moulting in Corynexochida and Proetida

Chen et al. (2008) describe a similar scenario of moulting to the redlichiid *Balcoracania dailyi* (Paterson et al., 2007) for the corynexochid trilobite *Oryctocephalus indicus* Reed 1910, including a downwards flexing to produce a break between the

cephalon and thorax, with the librigenae also becoming separated during this process. The cranidium then became inverted during exuviation of the animal from the old exoskeleton. The moulting of *Telephina (Telephus) spiniferus* Ulrich 1929 has been described as a very similar process to *O. indicus* (Fischer, 1946, fig. 1), involving displacement of the librigena and horizontal inversion of the cranidium (free cheeks "slightly shifted" with the glabella "overturned and carried forward", Fischer, 1946, p. 566). Glaessner (1948) notes that this moult configuration seems to span several trilobite families, but that preservation of all disarticulated moulted sclerites in position is extremely rare. A single moult of *Aulacopleura pogsoni* Edgecombe and Sherwin 2001 demonstrates that proetid trilobites could undergo ecdysis through disarticulation of the entire cephalon (Edgecombe and Sherwin, 2001).

Literature record of moulting in Agnostida

Descriptions of ecdysial modes in the literature are rare for Agnostida, considered by some to be the earliest diverging trilobite order (Cotton and Fortey, 2005), stem lineage mandibulates (Legg et al., 2013), or represent a paraphyletic group (Cederström et al., 2009). *Pagetia significans* Etheridge 1902 exuviae from the Cambrian Kaili Formation, China, suggest moulting occurred only through opening of the facial sutures, and disarticulation of the librigenae (Lin and Yuan, 2009). Complete cephala are common in the agnostoid fossil record, but these may result from taphonomic aspects. Whether Agnostida are trilobites, or close relatives of the Trilobita, improved knowledge of their moulting behaviours would allow better tracing of trends in arthropods with similar morphologies, and the origins of the patterns and variability in trilobite ecdysis.

Literature record of moulting in Harpetida

Modes of moulting are not readily discussed in the literature for Harpetida, or for other trilobites with a similar derived morphology (e.g. the Trinucleidae). This consists of a cephalon fused into a head shield with a dorsal and ventral component, and a marginal suture running between the two laterally around the cephalon (all other cephalic sutures are lost). Moulting therefore occurs via the opening of this marginal suture (in the 'Marginal Gape' mode of moulting, Fig. 5.3; Drage, accepted; Chapter 3), creating an anterior exuvial gape (Stubblefield, 1959; Henningsmoen, 1975; Drage, accepted; Chapter 3). This often leads to the dorsal and ventral halves returning to place after moulting, leaving a complete exoskeleton as in moulting of Xiphosurida (horseshoe crabs, Sekiguchi et al., 1988; the *Harpes* Configuration, Fig. 5I; Drage, accepted), or the ventral parts becoming displaced (Maksimova's and Hupe's Configurations, Fig. 5J and K respectively; see Henningsmoen, 1975; Drage, accepted). The *Harpes* Configuration was originally described from the harpetid genus *Harpes*, which had no cephalic sutures except the marginal suture (Henningsmoen, 1975). The Marginal Suture mode of moulting is therefore only seen in derived trilobites with this convergent morphology, although they may also display Salter's mode of moulting like other trilobites (Drage, accepted). The latter two configurations are more readily seen in other groups without cephalic sutures but with similar marginal sutures to harpetids (e.g. some early redlichiids; see Henningsmoen, 1975).

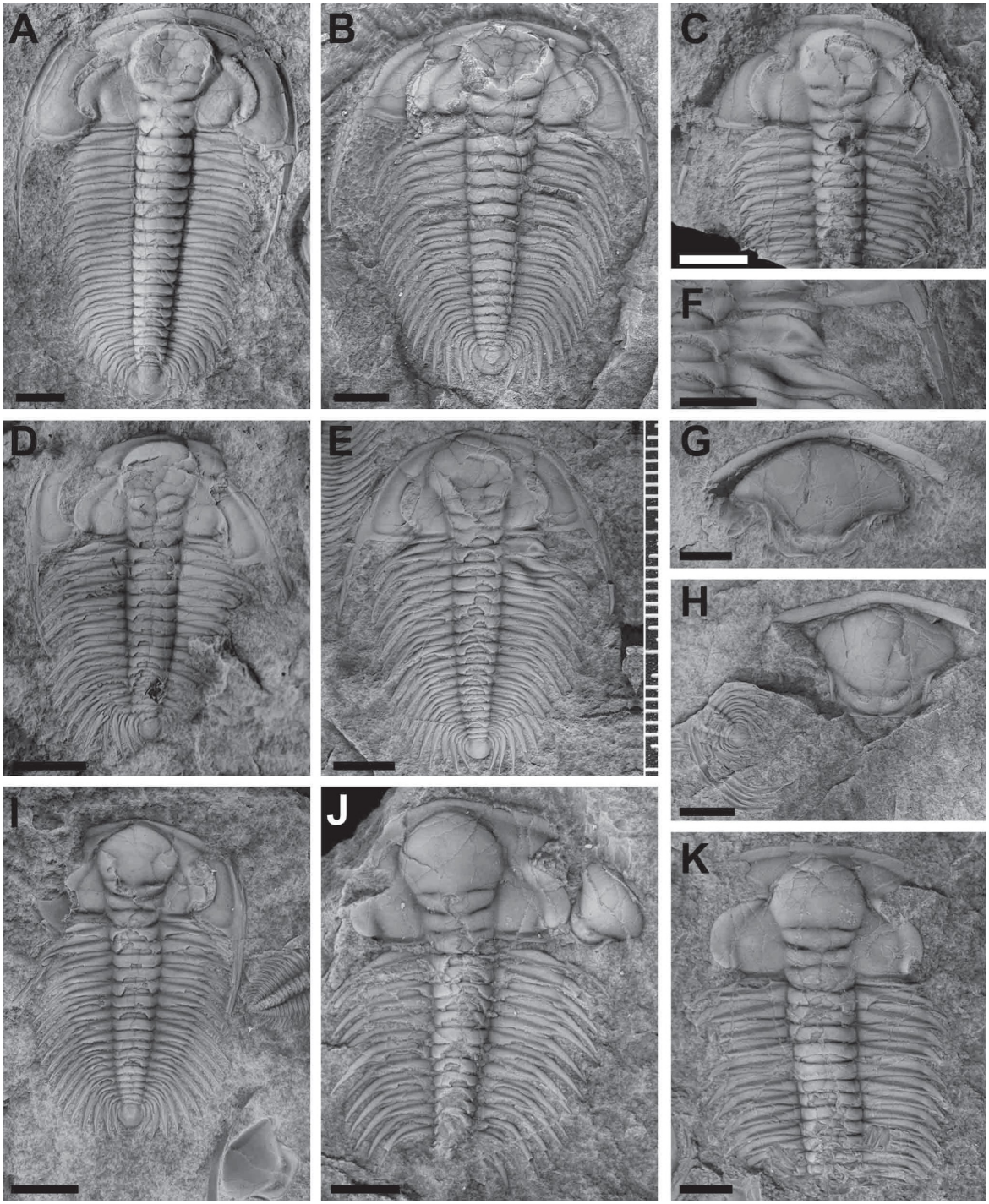


Figure 6: Moulted exuviae of paradoxidid trilobites from a mass moulting surface from the Cambrian of Jämtland, Sweden. A and B: Trilobites with open facial sutures (A: PMU 25711/29; B: PMU 25711/09b). C and D: Trilobites with open facial sutures and free cheeks slightly displaced (C: PMU 25711/59; D: PMU 25711/66). E and F: Specimen with facial sutures opened and damage to first thoracic segment on right side, probably caused by problems during moulting (PMU 25711/61; E whole specimen and F close-up of a damaged thoracic segment). G and H:

Moulted hypostome and rostral plate complexes (G: PMU 25711/45; H: PMU 25711/14). I: Facial sutures opened, left free cheek flipped and right free cheek displaced (PMU 25711/67). J: Free cheeks absent and hypostome displaced next to cephalon (PMU 25711/46). K: Free cheeks absent and break through thoracic segments (axial shield; PMU 25711/25). Scale bars 5 mm in A, B, D, E, and G-I; 3 mm in C, F, J, and K. All specimens are latex casts except for A, B, H, and K. Photo credit for all images: J. O. Ebbestad (specimens first described in Ebbestad et al., 2013).

Summary of trilobite moulting

Moulting utilising exuvial gapes created through opening of the facial (and often rostral) sutures is commonly observed across trilobite orders (the Sutural Gape mode, Fig. 5.1). This is to the exception of most phacopid trilobites, which had inactive facial sutures as adults; phacopid taxa typically exploit a gape suture created through the disarticulation of the cephalon from the thorax (Salter's mode, Fig. 5.2). As a generalisation, Redlichiida, Ptychopariida, Odontopleurida, Asaphida, and Agnostida all exhibit a high occurrence of facial suture moulting. Some Redlichiida, Asaphida, and Corynexochida tend to combine this with disarticulation of the cranidium. However, these are all very broad trends, and in reality those orders with ample moult descriptive literature (Redlichiida, Phacopida, Ptychopariida) show considerable flexibility in which moulting behaviours they employed. Whether the ecdysial methods discussed for Odontopleurida, Asaphida, Corynexochida, Proetida and Agnostida are representative of most taxa within their orders is unclear, as these are based on only a few species descriptions.

In a rare exploration of moulting and its broader evolutionary trends, Brandt (2002) analysed the interplay between trilobite moulting and geological survivorship. Brandt (2002) contrasted the extensive variation in trilobite ecdysial behaviour to the often-

stereotyped moult styles characterising other arthropod groups. In this way, trilobites were described as having an "inefficient moult habit" (Brandt, 2002, pg. 399), which, when coupled with the apparent need to form a new calcitic exoskeleton after each moult without the benefit of reabsorption of old cuticle material (Miller and Clarkson, 1980; Mutvei, 1981; Drage et al., accepted), may have been evolutionary disadvantageous and contributed to their ultimate decline. Further, the taxonomic survivorship analyses of Brandt (2002) support an association between morphology and moulting; taxa with simpler morphologies (in particular fewer thoracic segments) and therefore presumably easier ecdysis had longer chronological ranges. Öpik (1970) alternatively suggested that a decline in the efficiency of ecdysial hormones and moulting success factored into the extinction of the Cambrian *Redlichia* species. My quantitative research (Drage, accepted, and Drage, in review; Chapters 3 and 4) builds on the work of Brandt (2002) by investigating trends in ecdysial behaviours using a larger dataset, and considers the possible evolutionary links between moulting and morphology. Ultimately, we know very little about the broader evolutionary impacts of moulting behavioural variability in trilobites, but there is huge potential to explore these implications thanks to their extensive fossil record. Elucidating such trends may be a challenge given that higher-level trilobite phylogenetic relationships are currently in a state of flux, with essentially no resolution despite numerous analyses (Fortey, 2001; Adrain, 2011). However, further research along these lines could also help to resolve phylogenetic uncertainty within the group. Chapters 3 and 4 of this thesis focus on the process and results of broad-scale analyses of trilobite moulting behaviour, to quantify how variable they were in these behaviours, whether this differed with taxonomy or through time, and how this may have been linked to their morphology.

Other aspects of euarthropod moulting

Moulting may constitute a significant social behaviour in fossil euarthropods. Synchronised mass moulting events have been observed in many modern crustaceans, insects, and arachnids. These are variously triggered abiotically (for example circadian rhythms in aphids; Johnson et al., 1957) or through active communication (e.g. pheromones; Haug et al., 2013). These mass moultings were succinctly reviewed in Haug et al. (2013) for extinct euarthropod groups (Fig. 7A, B, F), and includes trilobites from the Cambrian (Fig. 7E; Paterson et al., 2007; Ebbestad et al., 2013), Ordovician (Karim and Westrop, 2002), and Devonian (Speyer and Brett, 1985); the soft-bodied arthropods *Canadaspis* and *Alalcomenaeus* from the Cambrian Burgess Shale (Fig. 7A, B, F; Haug et al., 2013); eurypterids from the Silurian (Braddy, 2001; Vrazo and Braddy, 2011); and shrimps from the Jurassic (Polz and Tischlinger, 2000), all of which consist of large numbers of empty exuviae in close proximity. These assemblages show no evidence for abiotic transport, reworking, or time-averaging, and so are generally accepted to represent actual biological mass moulting events. Another example of a supposed mass moult of mantis shrimps from the Carboniferous (Schöllmann, 2004) has been shown to co-occur with complete carcasses (Haug et al., 2013), and so may not represent a mass moult assemblage, but alternatively represents a mass moult preserved before completion (see Chapter 7 discussion).

Haug et al. (2013) also discuss the possible evolutionary functions of coordinated moulting. In extant arthropods, mating is often synchronised with moulting, particularly at the moult stage when the organism reaches sexual maturity (e.g. Kamio et al., 2014), as the associated gregarious behaviour of many individuals in close proximity would decrease the predation pressure on any one individual (Haug et al., 2013). Synchronised moulting, mating and spawning in adults would also lead to synchronised development in offspring,

which would then benefit from a similar reduction of predation pressure in crowded nursery habitats. This may explain trilobite (Speyer and Brett, 1985; Karim and Westrop, 2002; Paterson et al., 2007) and eurypterid (Braddy, 2001; Vrazo and Braddy, 2011) mass moult assemblages. However, Haug et al. (2013) and other researchers (e.g. Tetlie et al., 2008) reject this idea, although note that it may explain clusters of *Canadaspis perfecta* Briggs 1978 exuviae. The 'mass-mate-moult' hypothesis suggested for eurypterids (Speyer and Brett, 1985; Braddy, 2001), where females remain in shallow water post-mating and deposit their eggs prior to moulting, is dependent on the system of gender recognition. As pointed out by Haug et al. (2013), this hypothesis is influenced by comparisons to the extant horseshoe crab *Limulus*, which generally does not couple moulting with mating (Loveland, 2002; Shuster and Sekiguchi, 2004) even if large numbers of individuals aggregate during mating. Despite uncertainties regarding the motivation behind it, the repeated occurrence of mass moulting within trilobites and other Palaeozoic euarthropods suggests that this behaviour may have been common within early euarthropods, and even ancestral to Trilobita.

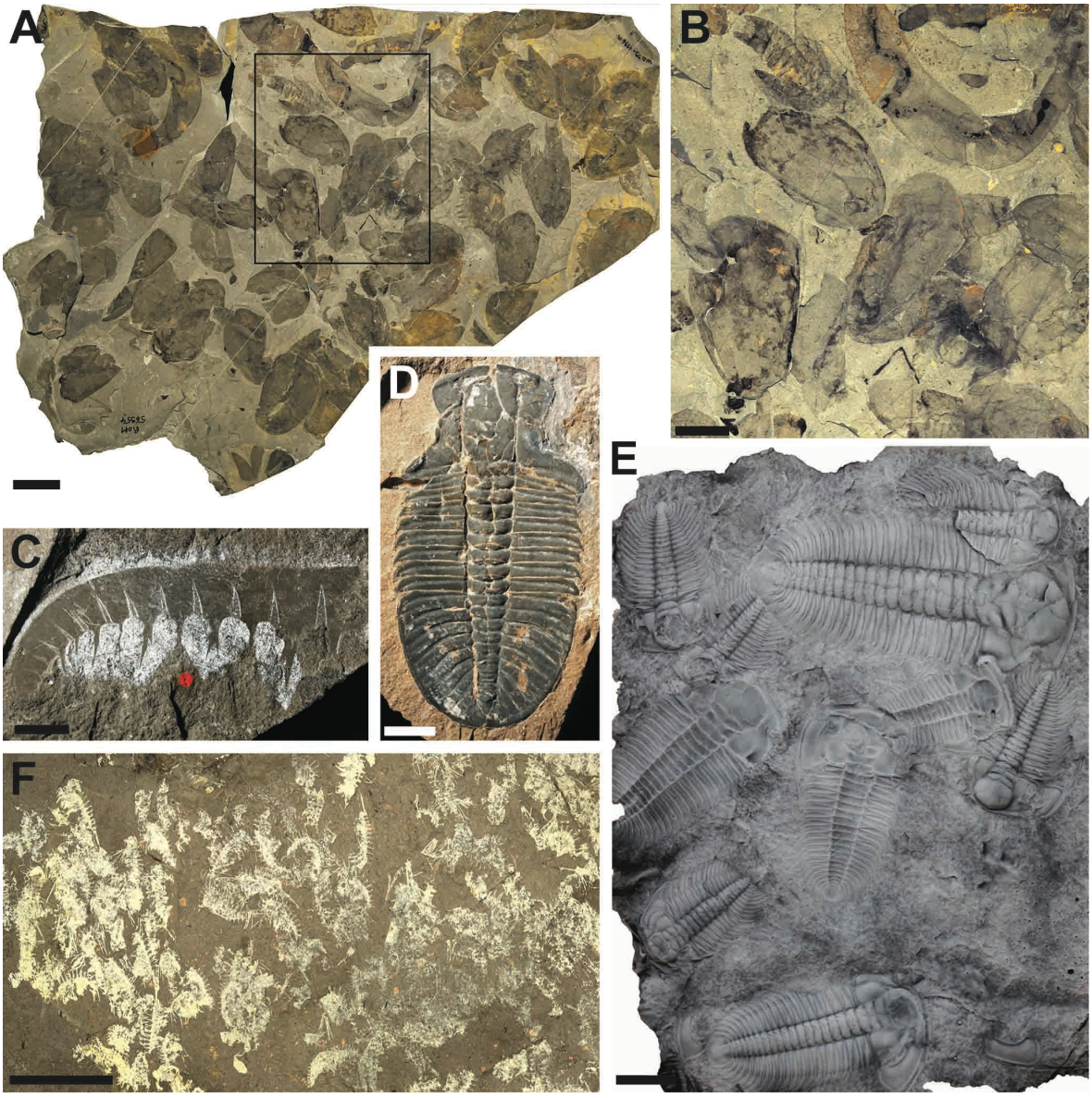


Figure 7: Mass moulting in the Cambrian fossil record. A: Complete slab showing synchronised moulting in *Canadaspis* from the Burgess Shale (ROM 62274; J. Haug, figured in Haug et al., 2013). Black box indicates close-up as B. C: Frontal appendage of *Anomalocaris canadensis* Whiteaves 1982 found with supposed trilobite mass moulting event in the Mount Stephen trilobite beds (GSC 3418a). D: Exuvia of *Ogygopsis klotzi* from mass moulting event at the Mount Stephen trilobite beds (OUMNH AT205). E: Cluster of paradoxidid trilobites preserved on a mass moulting surface from Jämtland, Sweden (PMU 25711/4-6, 74e79; J. O. Ebbestad, figured in Ebbestad et al., 2013). F: Synchronised moulting slab of megacheiran specimens from the Burgess Shale (ROM 62275; J. Haug, figured in Haug et al., 2013). Scale bars 10 mm in A, C, E; 5 mm in D, F.

Thesis outline

Following the above introduction and literature review (Daley and Drage, 2016), this thesis consists of a series of chapters, all of which are published or in press papers, that explore the evolution of trilobite exoskeleton moulting in the fossil record. Overall, I aim to significantly contribute to the limited body of work on moulting in the euarthropod fossil record, focusing on the trilobites in particular because their extremely abundant record provides an exceptional study group and they show uniquely high levels of moulting behaviour variability. Cuticle thickness and ultrastructure, while also potentially playing an important role in moulting behaviour and being useful in identifying and describing fossil moults, is not discussed within this thesis (with the exception of Chapter 7). This is because it is currently impossible to compare cuticle ultrastructure between trilobite species, as too little is known about their taxonomic differences and how different taphonomic regimes have affected their preservation. However, following future study, it could become apparent that cuticle thickness and ultrastructure also affected trilobite moulting behaviour. Regardless, I will demonstrate that trilobite moults can be identified from global fossil localities using rigorous criteria that can be broadly applied (not currently possible for cuticular morphology), and that these provide direct evidence of individual animal behaviours preserved in the fossil record. This enables us to explore aspects of trilobite life history and evolution related to moulting behaviour, in particular development, morphology, and ecology. Thus, I will emphasise the importance of understanding trilobite moulting in building a complete picture of the evolution of this euarthropod group.

Firstly, Chapter 2 (published as Drage and Daley, 2016) uses the context of a response to a published paper that inaccurately determined a recently-moulted trilobite in order to provide a much-needed update on how we identify moulted exoskeletons in the

fossil record, what we can learn from them, and why it is crucial that we do so correctly with rigorous criteria. The thesis then widens in scope to look at trilobite moulting behaviour as a whole, across the entire group and their Palaeozoic evolutionary history, forming Chapters 3 and 4 (as Drage, accepted, and Drage, in review). Chapter 3 uses a large purpose-built dataset to quantify the scope of inter- and intraspecific trilobite flexibility in moulting behaviour, and to quantitatively test for taxonomic and temporal trends across all of Trilobita and their evolutionary history. Chapter 4 specifically delves into whether it is possible to determine an association between the moulting behaviour used and trilobite morphology. The large datasets gathered for these studies will be made fully open access in the hope that future arthropod researchers will build upon them to test other broad-scale hypotheses surrounding trilobite moulting and evolution, such as their eventual decline and extinction.

Chapters 5 to 7 represent case studies that focus in detail on specific aspects of trilobite evolutionary history that were presumably related to moulting behaviour. These also indicate methodologies and direction for future studies of trilobite moulting behaviour. Chapter 5 (published as Drage et al., 2018a) looks at the trilobites of the Emu Bay Shale, South Australia, an important early Cambrian locality with exceptional fossil preservation, in order to demonstrate the huge wealth of behavioural and morphological information that can be gleaned from trilobite moults preserved under ideal conditions. Chapter 6 (published as Drage et al., 2018b) demonstrates the intrinsic link between moulting behaviour and development in trilobites, showing that this is key to many trilobite species' evolutionary history, phylogenetic relationships, and ontogenetic sequences. Chapter 7 (Drage et al., accepted) looks at the different moulting stages in trilobites, in particular the risky post-moult stage, which appear analogous to modern arthropods, and how these can be preserved in exceptional fossil localities. Finally,

Chapter 8 will present an overarching discussion and conclusion, summing up what value explorations of the fossil record of trilobite moulting can have, and emphasising the need for future work in this area.

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Chapter 2: Recognising moulting behaviour in trilobites by examining morphology, development, and preservation: Comment on Błażejowski et al. 2015

This chapter discusses the importance of the euarthropod fossil record of moulting, and how moult fossils are identified and distinguished from carcasses. It is written in the context of a response to Błażejowski et al. (2015), who described a putative just-moulted trilobite based on poorly-founded suppositions. This chapter therefore demonstrates why it is important that we use rigorous and replicable criteria to accurately distinguish moults and carcasses, and elaborates on how to do so.

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H.B.D. conceived the study and discussion points, wrote the majority of the paper, created the figures, and edited the paper. A.C.D. helped hone ideas, contributed to parts of the paper, and edited the paper.

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Recognising moulting behaviour in trilobites by examining morphology, development, and preservation: Comment on Błażejowski et al. 2015

Harriet B. Drage & Allison C. Daley

A 365 million year-old trilobite moult-carcass assemblage was described by Błażejowski et al. (2015) as the oldest direct evidence of moulting in the arthropod fossil record. Unfortunately, their suppositions are insufficiently supported by the data provided. Instead, the morphology, configuration, and preservational context of the highly fossiliferous locality (Kowala Quarry, Poland) suggest that the specimen consists of two overlapping, queued carcasses. The wider fossil record of moulting actually extends back 520 million years, providing an unparalleled opportunity to study behaviour, ecology, and development in early animals. Taking cues from modern analogues, it is possible to quantify precise details about moulting behaviour to determine broad-scale evolutionary trends, ontogenetic sequences, and morphological selection pressures. In this review, we argue that this rich source of data has been underused in evolutionary studies, though has great potential for investigating the life history and evolution of arthropods in deep time.

Key Words: Arthropod, Ecdysis, Ecdysozoa, Fossil Record, Trilobita, *Trimerocephalus*

Introduction

The extensive fossil record of Arthropoda provides a fascinating insight into the evolution of the most diverse and abundant animal phylum, upon its appearance amongst the earliest animals 541 million years ago [1-3]. Arthropod growth requires moulting of the exoskeleton (Fig. 1), a process known as ecdysis [4, 5], which is also observed in nematodes and other closely related phyla that make up the group Ecdysozoa. The

exoskeleton of arthropods provides protection and structural support, but has the disadvantage of needing to be moulted in order for the animal to grow. Each growth stage in arthropods involves a moulting event, which provides the opportunity not only for development towards an adult morphology (in juveniles) and an increase in body size, but also for the regeneration and repair of a damaged exoskeleton [5]. Owing to this multi-faceted function, the mode and method of moulting can greatly influence large-scale evolutionary trends within the arthropods; for example, the successful radiation of insects (Fig. 1A) was linked to the regulation of ecdysis-related hormones [6]. The evolutionary significance of moulting in extinct animals can also be examined because the empty moults, or exuviae, are discarded after ecdysis, providing a record of previous developmental stages, and have the potential to be fossilised. As one of the few behaviours with palaeontological evidence, moulting provides a rare opportunity to study ecology, growth and development in extinct arthropods, as recently reviewed by Daley and Drage [5] (Appendix 1).

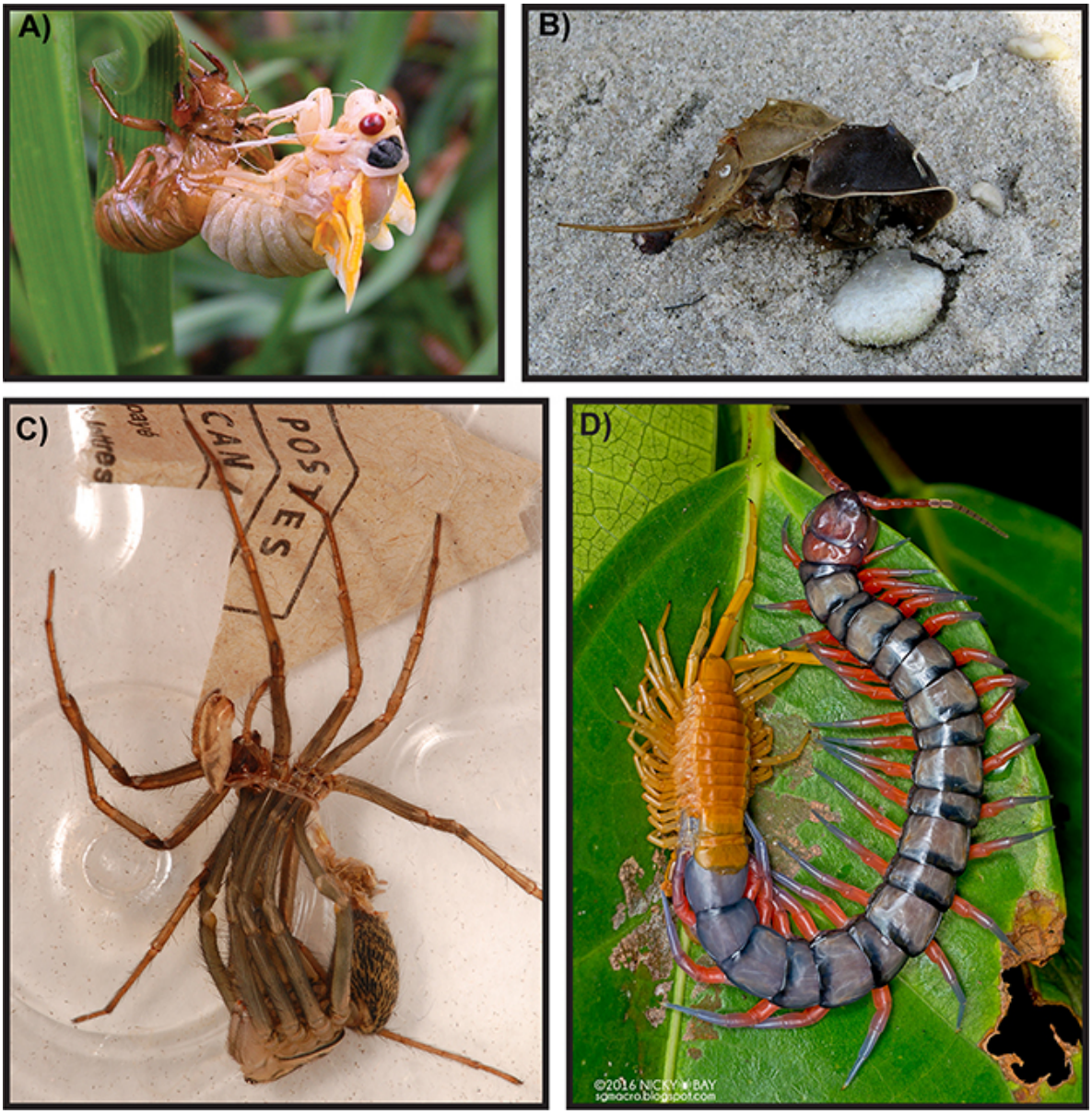


Figure 1: Exemplar photographs of modern arthropod moulting behaviours. A: cicada moulting through the dorsal part of the abdomen (image credit: Wikimedia Commons). B: horseshoe crab that died following exuviation through the anterior cephalic ecdysial gape (image credit: Wikimedia Commons). C: giant house spider (*Eratigena atrica* Koch 1843), extracting the legs while emerging mid-moult from the dorsal abdomen (image credit: Wikimedia Commons). D: mid-moult scolopendromorph centipede, emerging through an anterior cephalic gape suture (image credit: Nicky Bay, http://sgmacro.blogspot.com).

The fossil record of moulting is largely composed of exuviae, and fossils preserved during moulting are extremely rare because these events take place on a relatively rapid time scale [5]. The most prolific fossil record of moulting is observed in trilobites, an extinct group of marine arthropods that were very abundant and important members of animal ecosystems from 521 to 250 million years ago (Fig. 2). Their anatomy consists of a mineralised, segmented body with three longitudinal lobes (a central axial lobe, and left and right pleural lobes, hence the name "trilobite"), and an anterior to posterior regionalisation consisting of a head (cephalon), thorax, and tail (pygidium) (Fig. 2A). Building on this basic body plan, trilobites had a diverse morphology and ecology, occupying numerous niches in marine communities. Trilobites had distinct moulting behaviours that mostly utilised the facial sutures, thus allowing the lateral and anterodorsal parts of the cephalon to be displaced (the free cheeks, or librigenae), providing an opening through which the organism could shed the smaller exoskeleton (Fig. 2B). In some trilobites, the facial sutures were fused and the animal moulted by disarticulating the entire cephalon (Fig. 2C). With either scenario, the distinct configurations preserved as empty exuviae allow us to identify specimens as moults with relative ease. For other moulting arthropods with a known fossil record, such as decapods, scorpions, and eurypterids (sea scorpions), distinguishing between exuviae and carcasses is not as easy because ecdysial suture lines are less distinct and the moult assemblages are not as recognisable [5]. Trilobites, therefore, represent one of the best model taxa for studying the evolution of moulting, growth and development in extinct arthropods.

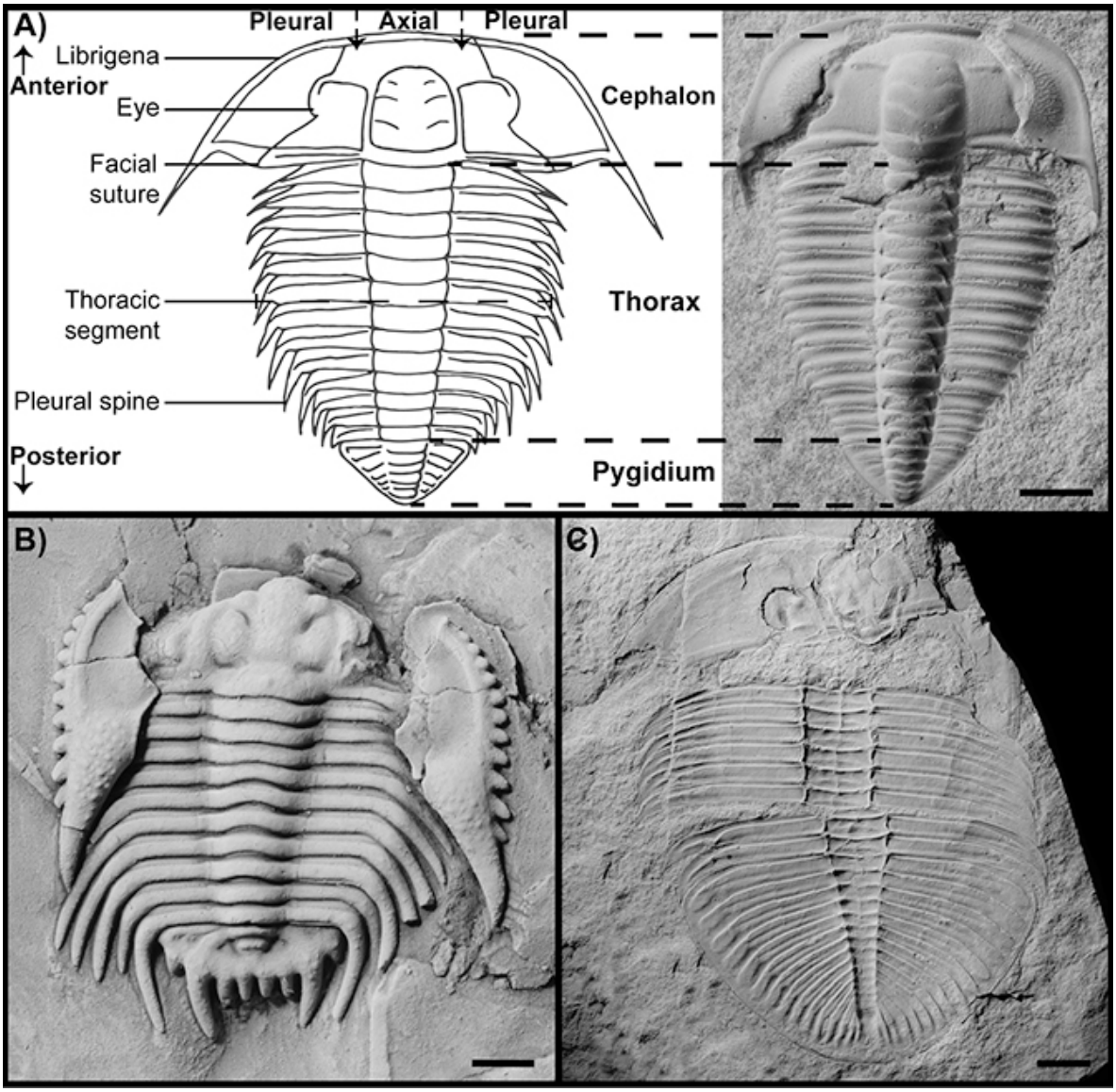


Figure 2: A: labelled line drawing of *Olenus* sp., Furongian, Alum Shale, Sweden, with the anterior and posterior directions indicated, and the cephalic, thoracic, and pygidial regions also indicated on an adjacent photograph of an *Olenus truncatus* Brünnich 1781 specimen (PMU, no number, 4 mm scale). B: *Acidaspis coronata* Salter 1853 moult, Upper Silurian, Wenlock Limestone, UK, showing disarticulation and backwards displacement of the free cheeks (librigenae) (OUMNH C.17494, 5 mm scale). C: *Ogygiocarella debuchii* Brongniart 1822 moult, Lower Ordovician, Llandeilo Series, UK, showing disarticulation of the cephalon and dislocation along the thorax (OUMNH B.263, 20 mm scale).

Trilobites have a rich fossil record of carcasses and exuviae, which has provided a wealth of information on developmental stages, behaviour, and interactions with the environment (for example, [5, 7, 8]). Trilobite specimens preserved in the short period immediately post-moulting are extremely rare. However, a recent paper by Błażejowski et al. [9] described an Upper Devonian fossil, approximately 365 million years old from the Kowala Quarry in the Holy Cross Mountains of central Poland, that consists of two stacked exoskeletons of the trilobite *Trimerocephalus chopini* Kin and Błażejowski 2013, which they interpret as *in situ* preservation of an individual having just emerged from its moulted exuvia, located directly above the carcass (Fig. 3C, F). The spectacular preservation of such a short-lived event would provide important information for studying the complex interplay between ontogeny, behaviour, and fossil preservation, and as such it is critical that such evidence is identified correctly in the fossil record. Unfortunately, the proposed trilobite carcass-moult assemblage hypothesised by Błażejowski et al. [9] is not well supported, and the lines of evidence on which their interpretation is based are discussed in order below. We then place our paper in the context of the excellent fossil record of moulting in general, and focus on its potential for studying the evolution of developmental processes in deep time.

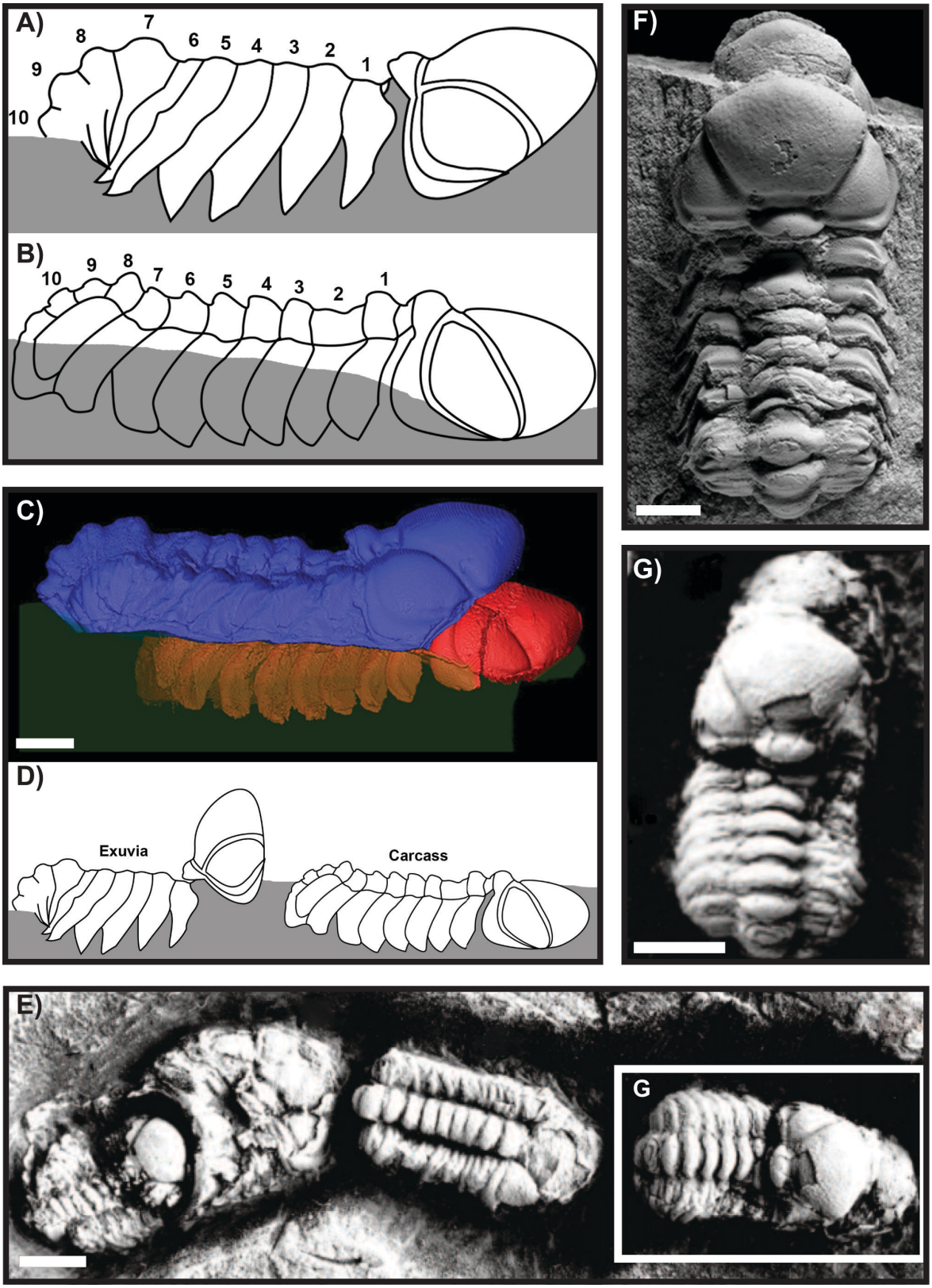


Figure 3: *Trimerocephalus* spp. McCoy 1849 specimens from the Lower Famennian (Upper Devonian), Holy Cross Mountains, central Poland. A: line drawing produced from the CT scan of the *Trimerocephalus chopini* "moult" figured by Błażejowski et al. [9]. B: line drawing from the

CT scan of the *T. chopini* "carcass". Numbering in A and B indicates 10 thoracic segments in each, particularly visible when using the appended CT scan video provided by Błażejowski et al. [9]. C: original lateral-view CT scan of the *T. chopini* specimen from Błażejowski et al. ([9] Fig. 2B, cc-by licensed), illustrating the relative positioning of their putative "carcass" and "moult". Measures of the lateral and dorsal lengths of the cephala, and of the individual thoracic segment lengths show no appreciable difference in size between the two individuals. Scale bar 2 cm, as given in Błażejowski et al. [9]. D: in comparison to C, the positioning of the labelled "carcass" and "moult" suggested for a true moult-carcass assemblage [17]. E: one of several *Trimerocephalus* spp. "queues" of overlapping trilobite carcasses from the Holy Cross Mountains, adapted from Plate 2 Fig. 4 of Radwański et al. [19]. The white box shows the contextual positioning of G. Scale bar 1 cm. F: dorsal-view photograph of the *T. chopini* suggested moult-carcass assemblage from Błażejowski et al. ([9] Fig. 2A, cc-by licensed). Scale bar 2cm, as given in Błażejowski et al. [9]. G: dorsal-view close-up of two overlapping *Trimerocephalus* spp. carcasses, adapted from Radwański et al. ([19] Plate 2 Fig. 4A, B) and shown *in situ* in E. Shown free of context in comparison to F, the two specimens appear extremely similar. Scale bar 1 cm.

The earliest fossil record of moulting extends back to the Cambrian

Błażejowski et al. [9] claimed that the three-dimensional specimen of *Trimerocephalus chopini* from Kowala Quarry in central Poland represents the oldest direct example of a fossilised moulting event at 365 million years old. However, in-the-act moulting is conclusively known from a number of older fossils from the Cambrian. An exquisite specimen of *Marrella splendens* Walcott 1912 from the middle Cambrian Burgess Shale (approximately 508 million years old) was illustrated emerging anteriorly from its exoskeleton, and therefore represents the earliest example of an arthropod preserved mid-moult [10]. Even older examples (from approximately 518 million years ago) are known for other ecdysozoans, including a loriciferan (microscopic marine sediment-affixed animals with a protective outer layer) from the lower Cambrian Sirius

Passet preserved exiting its exoskeleton [11], and a lobopodian (extinct worm-like ecdysozoan with paired limbs) with overlapping plates from the lower Cambrian of northern Greenland that suggests it was preserved during the process of moulting [12]. The early fossil record of moulting is also rich in examples of exuviae, particularly for Cambrian and Ordovician strongly biomineralised arthropods such as trilobites [5]. Several specimens from this time period show clusters of exuviae preserved in close proximity, indicating synchronous mass moulting events [13, 14]. This rich fossil record of moulting has allowed for quantitative analyses investigating moulting behaviour trends, and their impact on trilobite evolution [5, 15]. Therefore, the Devonian specimen of *T. chopini* described by Błażejowski et al. [9] cannot be considered "the oldest direct evidence for a moulting episode" ([9] p.1).

Specimen position, preservation and context suggest queueing trilobite carcasses

Many arthropods have distinct exoskeletal arrangements upon moulting, which are found preserved in the fossil record due to their reinforced cuticle. These moult assemblages can usually be recognised by a number of criteria, described by Henningsmoen [16] and expanded upon in Daley and Drage [5]. For example, opened gape sutures (created by the opening of suture lines or articulation points) in the exoskeleton and repeated configurations of disarticulated sclerites (individual units of the exoskeleton) are most commonly used to designate moults. Contextual information from the fossil locality (associated material, palaeoenvironment, preservational processes), and the distortion of sclerites in consideration with local preservational processes (flattening, superimposition of elements, telescoping), are important for differentiating between preserved arthropod moults and carcasses, and for distinguishing abiotic or microbial exoskeleton disarticulation from true moult assemblages. Using these criteria, moulted

trilobite exoskeletons are very often recognisable in the fossil record, and can provide a great deal of behavioural and ecological information. They display several distinct moult arrangements (such as those described in [16]) with clear exuvial gapes. Most frequently, trilobites created an exuvial gape by disarticulating the free cheeks (Fig. 2B), or entire cephalon (Fig. 2C), followed by the animal extracting itself from the remaining articulated sclerites by moving anteriorly [5]. Unfortunately, Błażejowski et al. [9] do not factor several crucial considerations into their description of a putative moult-carcass trilobite assemblage. In particular, the presumed exuvial gape, relative positioning of their specimen, and the context (other specimens from the locality, and preservational conditions) do not support their hypotheses.

Trimerocephalus chopini figured by Błażejowski et al. ([9] Fig. 2) shows two overlapping individuals, a situation that has been interpreted as a "moulted exoskeleton" positioned dorsal and slightly posterior to the "carcass". The putative moult is exposed, displaying a cephalon with slight dorsal angling, while the putative carcass was revealed in three-dimensions by CT-scanning. Błażejowski et al. [9] argued that this specimen shows the moult arrangement described for *Paciphacops* Maksimova 1972 preserved "immediately following moulting" ([9] p. 2). They (and we) reject the idea that this assemblage represents a specimen preserved during or immediately before moulting (with the "carcass" still enclosed in the soon-to-be moulted exoskeleton) because the two individuals are "separated by a layer of sediment" (Fig. 3F) and not "pancaked" ([9] p. 2), as would be expected if they were still articulated at the time of preservation. *Paciphacops* is thought to utilise infaunal moulting (following burrowing for protection from predators), which occurs through an anterior gape suture created by ventral disarticulation of the cephalon and thorax, causing the cephalon to hinge dorsally at an angle of nearly 90^{o} ([17] Fig. 2; our Fig. 3D). Other phacopid trilobite specimens also show possible infaunal

moulting with their cephala angled at more than 90° from the sediment [18]. However, the specimen of *T. chopini* ([9] Fig. 2) shows a much lower angle of cephalic hinging and displacement (Fig. 3C) than these definitive moult specimens, suggesting either very minor disruption during moulting or subsequent closure of the gape suture. Both scenarios would be unlikely if the animal had been moulting infaunally, because sediment would immediately infill the empty exoskeleton after moulting [18]. Even if the specimen were moulting aboveground, a larger displacement of the cephalon would still be expected, as is regularly seen in taxa that moult in this manner (Fig. 2C) [5, 16]. Regardless of whether *Trimerocephalus* was moulting infaunally or not, similar minor displacements of the cephala have frequently been observed in other trilobites from the Kowala Quarry, and interpreted as "resulting from a diagenetic event of compaction" ([19] p. 461), rather than moulting. This further suggests that the dorsal individual figured in Błażejowski et al. [9] is not a moult.

The position of the "carcass" relative to the "moult" raises further issues with the moult-carcass assemblage interpretation. The "carcass" component is positioned very much beneath, and only slightly forward, of the "moult" ([9] Fig. 2). As mentioned earlier, this cannot represent preservation before or during moulting, because the layer of sediment separating the dorsal and ventral individuals indicates that the supposed "carcass" was not enclosed in the soon-to-be moulted "exoskeleton". To result in this position in a specimen preserved immediately after moulting, the egressing individual would have had either to detach the dorsal exoskeletal shield as a single unit and move downwards vertically, or to have exited anteriorly through a ventral gape suture under the cephalon and burrowed backwards into the sediment. Trilobites do not have sutures in the thorax, meaning that the pleural and pygidial doublure (the parts of the thorax and pygidium that extend laterally onto the ventral side) would inhibit the first of these scenarios. Some trilobites did move

anteroventrally through the cephalon while exiting the old exoskeleton [16], but this would have required disarticulation of ventral sclerites (rostral plate, hypostome), and no convincing event involves backwards movements within the sediment post-moulting. The direction of exuvial movement in most trilobites is towards the anterior ([17] Fig. 3; [20] Figs. 2-6), and therefore a more convincing moult-carcass assemblage would have the carcass preserved entirely in front of the moult on a similar depositional plane (Fig. 3D). For example, Henningsmoen [16] identified a *Hanchungolithus primitivus* Born 1921 specimen ([21] Plate 5 Fig. 9) possibly preserved shortly after moulting in this specific configuration (Fig. 3D) "again [indicating] forwards movement of the exuviating trilobite, since the larger specimen lies in front of the smaller" ([16] p.188). The broader preservational context of the locality must also be considered. Kowala Quarry preserves aligned ("queued") carcasses of *Trimerocephalus* ([19] Plate 1 Fig. 5C-D, Plate 2 Figs. 3A-B, 4A-B, 6C-D) overlapping in the same way as the specimen illustrated by Błażejowski et al. [9]. Radwański et al. [19] described these very similar specimens as "live trilobites entombed while migrating" (p. 460), and these queues of up to nine trilobites do not show any clear evidence of moulting (e.g. disarticulated cephala) or the presence of disarticulated exuvial debris. The specimens described by Błażejowski et al. [9] more likely represent two individual aligned carcasses preserved on top of each other and separated by sediment deposition, in the queued arrangement typical of this locality [19]. While this does not necessarily exclude the specimen of Błażejowski et al. [9] from also being a moult assemblage, it demands a clearer explanation of how this particular specimen differs from the other completely preserved body clusters found there. In isolation, several queued carcasses from Radwański et al. ([19] Plate 2) are indistinguishable (see our Fig. 3E, G) from the specimen described by Błażejowski et al. [9].

Understanding moulting allows for the study of growth and development in trilobites

During development, juvenile arthropods grow in a stepwise pattern, with distinct instars separated by moulting events and accompanied by morphological change such as thoracic segment addition (anamorphic growth). Adult trilobites may have grown indeterminately, meaning they increased in size with each adult moult until death while maintaining the same morphology [22, 23]. Continued moulting during adulthood is suspected because of the presence of healed injuries in adult exoskeletons, a process that only takes place during moult growth events [24]. Examining moults therefore allows direct interpretation of growth and developmental patterns from the fossil record, and to date there have been numerous studies detailing variation in trilobite ontogeny (for example, [25-28]). Błażejowski et al. [9] interpreted their putative moult-carcass assemblage as a growing juvenile, based on a suggested increase in thoracic segment number. However, we find no clear evidence for the increased number of thoracic segments, and suggest that the lack of overall increase in body size argues against this being a moult assemblage.

Błażejowski et al. [9] argued that the "carcass" part of their specimen displays one additional thoracic segment as compared to the supposed empty moult, and use this to support the idea of a moult-carcass assemblage. This suggested increase in segment number may actually reflect complications in preservation of the dorsal specimen because posterior segments are obscured by pygidium enrolment. Błażejowski et al. [9] did not publish their segment number observations, but we can distinguish ten thoracic segments in the CT-scan reconstructions of both individuals (Fig. 3A, B). This argues against the juvenile moult assemblage interpretation. Furthermore, pygidium enrolment results from muscle contraction on death [19], and is a classic indicator of a preserved carcass rather than a moult, because complex musculature is required.

Our measurements also indicate that the overall body size and size of the individual sclerites of the ventral specimen are not significantly larger than the dorsal specimen. Trilobites grew at each moulting event [22], particularly when considered juvenile [29], as seen in the *Hanchungolithus primitivus* specimen figured in Dean ([21] Plate 5 Fig. 9) and suggested by Henningsmoen [16] to represent a larger carcass to the anterior of its moulted exoskeleton. While there may have been a very brief period of time immediately after moulting when inflation of body size had not yet occurred to an observable extent, the exoskeleton would necessarily be relatively soft during this freshly moulted stage, but there is little evidence of this in the Błażejowski et al. [9] specimen, as discussed in detail in the next section. A freshly moulted *Phacops* Emmrich 1839 trilobite specimen found immediately adjacent to its moulted exoskeleton ([30] Plate 1 Fig. 5) clearly has a larger body size and is in the softer, post-moult state, as evidenced by the flattened and wrinkled preservation. This specimen suggests that an increase in body size occurs almost immediately after exiting the exoskeleton, and should be observed if the specimen in Błażejowski et al. [9] really were a moult assemblage.

Preservation of freshly moulted trilobites is characterised by wrinkling and flattening

Several trilobite carcasses (Fig. 4) have been described as preserved very shortly after a moulting event in a soft-shelled state before the new exoskeleton mineralised and hardened [30-33]. In this duration of time arthropods are vulnerable to predation. Individuals of *Phacops rana* Green 1832 from the same beds as trilobite exuviae clusters at the Penn Dixie shale pit, New York (Fig. 4C), "exhibit a distinctly thinner, pale brown to grey cuticle, which may be flattened and slightly wrinkled" ([31] p.96) and are found directly associated with specimens showing "little or no compression" ([31] Fig. 3D p.91, reiterated in [32] Fig. 7E p.214). A single *Olenoides serratus* Röminger 1887 (Fig. 4A, B),

in contrast to other individuals of the species, has low relief, conspicuous wrinkling, poorly defined structures, and soft-part preservation of appendages and the entire alimentary canal (including midgut), the latter indicating that it is a carcass [33]. *Phacops rana milleri* Stewart 1927 trilobites from the Middle Devonian Silica Shale of Ohio show the stages of post-ecdysial cuticle hardening, freshly moulted individuals being "pale, thin and wrinkled" ([30] p.33). Cuticle thickness was quantitatively measured in thin section, and freshly moulted individuals had a thinner cuticle (25-135 μm) than intermoult specimens (300-500 μm) [30]. All of these specimens show a characteristic wrinkling, flattening, and thinness in the exoskeletons of freshly moulted trilobites. Wrinkles may have resulted from incomplete decompression of the new larger exoskeleton after exiting the old exoskeleton, and/or from deformation and compaction during burial in sediment, the latter of which also caused flattening of the soft exoskeleton [30]. Because other preservational processes unrelated to moulting (e.g. tectonic deformation) could have caused wrinkling and/or flattening in whole assemblages of trilobite exoskeletons [34], trilobites with flattened and wrinkled exoskeletons need also to be found in close proximity to trilobites with unwrinkled, high-relief, fully-mineralised exoskeletons before they can be soundly interpreted as freshly moulted individuals. Size frequency distributions of the assemblages should also be examined to determine if differences in relief could be the result of varying preservation mechanics of differently sized trilobites.

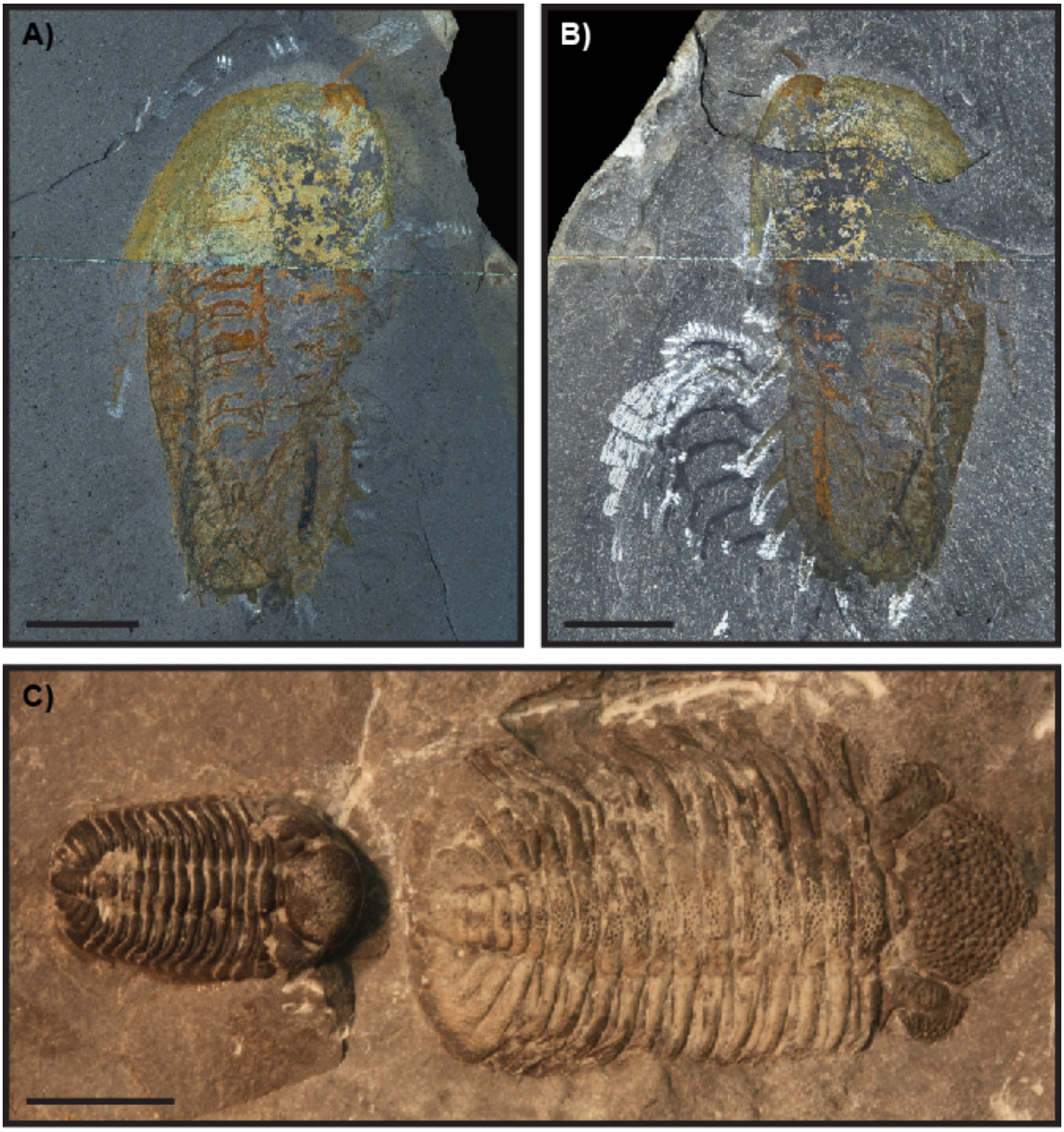


Figure 4: A and B: specimen of *Olenoides serratus* described as freshly moulted [33] (USNM 57685, part and counterpart). C: freshly-moulted specimen of *Phacops rana* (USNM 403875, described in [31, 32]). Both show wrinkling, flattening, and low relief of features; these are considered characteristic of freshly moulted trilobites and led to the original publications describing them as such. C is in contrast to an adjacent, smaller specimen considered a normal intermoult trilobite because it is darker in colour, and shows no flattening, wrinkling, or evidence of compression. Scale bars all 1cm.

Wrinkles in the anterior cephalon of the *T. chopini* "carcass" specimen are taken by Błażejowski et al. ([9] p.3) as evidence that the cuticle was soft as a result of recent moulting. However, these wrinkles are difficult to discern in specimen images ([9] Fig. 2E), and could alternatively be cracks in the rock, particularly given that numerous cracks are visible elsewhere in the "carcass" and "moult" ([9] Fig. 2C-F), and in other trilobites from this locality [19, 35], because trilobite exoskeletons are known to fracture during compression [30, 36]. Wrinkles alone would not be enough to identify a freshly moulted trilobite because all other documented examples (Fig. 4) show pronounced flattening in addition to wrinkles ([30] Plate 1 Fig. 5; [31] Fig. 3D; [33] Plate 17 Fig. 3, Plate 19), owing to the greater compressibility of the softer, newly moulted exoskeleton. In contrast, the Błażejowski et al. [9] specimen shows very little difference in the height of the "carcass" as compared to the "moult" (Fig. 3A, B), and this lack of flattening does not support the interpretation of a freshly moulted individual.

Modern analogues can be used to understand trilobite moulting

One of the better ways to understand the anatomy, behaviour and development of extinct animals is to compare them to modern animals with similar affinity, mode of life, or environmental preferences. The use of modern analogues has shed light on a diverse set of enigmatic palaeontological evidence. For example, a comparative anatomy approach allowed for the analysis of brain morphology in a 518 million year-old arthropod, *Fuxianhuia protensa* Hou 1987 from the Chengjiang Biota, by comparison with the neuroanatomy of modern hermit crabs [37]. Such approaches are particularly important when studying extinct animals with no direct modern relatives, as long as the modern analogue is appropriate in affinity and/or mode of life.

Błażejowski et al. [9] used horseshoe crabs as a comparison for understanding the preservation and behaviour of moulting trilobites. The horseshoe crab (Fig. 1B) is one of the most renowned "living fossils", generally considered to have a morphology that has changed little in over 400 million years. In reality, horseshoe crabs (xiphosurans) have undergone striking changes to their morphology during their long evolutionary history [38]. Horseshoe crabs may not be the most appropriate modern analogue for interpreting the trilobite fossil record of moulting. While trilobites and horseshoe crabs are both marine arthropods and some recent phylogenetic analyses ally them (and other Artiopoda, the extinct group containing trilobites) with the Chelicerata group to which horseshoe crabs belong [39], other analyses place trilobites with the Mandibulata, rather than the Chelicerata [1, 2, 40]. Further, xiphosurans possess an anterior exuvial gape that closes after egress, making it difficult to distinguish moults from carcasses in the fossil record [5]. Unlike most trilobites, horseshoe crabs have an exuvial suture that splits open along the anterolateral margin of the head, through which the animal exits, but during which no element of the head is removed [41]. The resulting uncertain moult fossil record makes it difficult to study evolutionary aspects of xiphosuran moulting behaviour. Horseshoe crabs also differ from trilobites in having determinate growth, meaning that they stop growing and moulting after reaching adulthood, whereas trilobites are suggested to have continued to moult and increase in size as adults [4, 22, 23, 42, 43].

Given that trilobites have been considered closely related to Mandibulata, modern analogues may be more appropriate if drawn from within this clade, which is comprised of the Myriapoda (centipedes and millipedes), Crustacea (crabs, lobsters, shrimp, etc.), and Hexapoda (insects). Many myriapods and crustaceans show continuous growth and moulting during adulthood [4, 44], as suggested for trilobites [22, 23, 42, 43]. In myriapods (Fig. 1D), moulting often proceeds by a transverse dorsal suture opening in the

head to create the exuvial gape, through which the animal egresses anteriorly [45, 46]. Other myriapods moult by disarticulating the entire head from the remainder of the body. The moulting behaviour and similar repeated nature of the trunk segments is therefore comparable to that of trilobites. The myriapod fossil record largely consists of millipedes, which have a robust cuticle reinforced with calcium carbonate, although most fossils are interpreted as carcasses rather than moults [47, 48]. This is because in centipedes the cuticle undergoes extensive reabsorption prior to moulting and the remaining moulted exuvia is almost always consumed by the individual, which greatly reduced the chance of exuviae being preserved in the fossil record [48]. Myriapods are appropriate modern analogues for studying general moulting modes, but because they are terrestrial rather than aquatic they make poor analogues for understanding trilobite exuvial behaviour and moult preservational processes. The density difference between air and water presumably leads to divergent exuvial procedures, requiring distinct movements for terrestrial compared to aquatic moulting. Marine isopod crustaceans may be a more useful comparative group. These are extant arthropods that have a unique biphasic moulting method, in which the posterior part of the body is moulted first, followed by the anterior sclerites a few days later [49-51]. Separate moulting of the body and head is reminiscent of moulting in trilobites, although in trilobites the cephalic sclerites are moulted first and the remainder of the exoskeleton is shed after. Biphasic moulting in marine isopods influences preservation of these arthropods in the palaeontological record, the majority of fossils being interpreted as moulted exuviae because they consist of posterior body regions only, without the head [50, 51]. Studying how marine isopod moulting affects their fossil preservation could therefore provide insight into the taphonomy and behaviour of moulting in the extinct trilobites.

Błażejowski et al. [9] compared their proposed trilobite moult assemblage to the fossil horseshoe crab *Crenatolimulus* Feldmann et al. 2011 from the Upper Jurassic Owadów-Brzezinki Quarry in Central Poland, to examine both preservation and behaviour. The proposed wrinkles on the exoskeletons of both the horseshoe crab and the trilobite are taken as evidence for these being freshly moulted individuals that have not yet undergone post-moulting "decompression" ([9] p. 3). This is in contrast to the well-established interpretation of wrinkles in otherwise complete horseshoe crab fossils as taphonomic artefacts resulting from sediment compaction and flattening of high relief structures [34, 41, 52]. Błażejowski et al. [9] imply that the horseshoe crab specimens, and by extension the *T. chopini* trilobite specimen, are too three-dimensional for the wrinkling seen on their exoskeletons to be from taphonomic processes. However, it has been shown that even small amounts of compression can cause taphonomic wrinkles in horseshoe crab specimens that retain high relief [34, 52]. The absence of pronounced flattening in these specimens suggests that these specimens had fully hardened exoskeletons at the time of preservation, and does not support the claim of Błażejowski et al. [9] that they were freshly moulted individuals.

Mass moulting occurs in modern arthropods and trilobites

One of the most significant social behaviours linked with moulting is the occurrence of synchronised moulting events, or "mass moulting", involving a large number of individuals [31, 53-57]. In modern arthropods, mass moulting events are sometimes triggered by external abiotic cues, as is seen in the lunar-rhythmic moulting of the crayfish *Astacus* Fabricius 1775 [53]. Direct communication between individuals can also trigger synchronised moulting, either through pheromones, as seen in some colonial spiders and Hexapoda such as beetles and springtails [54-56], or through visual

communication, as seen in krill [13, 57]. Mass moulting reduces predation pressure on individuals during this vulnerable stage of their development [58], but the benefit is balanced by higher disease transmission rates within the population [13, 59]. Synchronised moulting events are well established in modern arthropods, but identifying this behaviour in the fossil record is more difficult. Trilobites are one of the only fossil arthropod groups found in large enough numbers that we can access group behavioural information [19, 60-65] and examine synchronised moulting events in deep time.

Gregarious behaviour in trilobites has been documented in many assemblages [19, 60-63] for protection [14], moulting [31], burrowing [60], feeding [64], and reproduction [65]. Unlike in modern arthropods, where behaviour can be observed directly, trilobite fossil aggregations must be considered in context to try to assess whether they are the result of gregarious behaviour or abiotic accumulations. Currents, sediment reworking, gravity and other transportation mechanisms could act to accumulate numerous individuals in one area after death, leading to a time-averaging effect where individuals that were separated in space and time during life are combined and preserved together after death. Detailed analyses of the taphonomy, sedimentology, and palaeoenvironment of the locality and size-frequency distribution of the population have been successful in separating abiotic trilobite accumulations from actual examples of gregarious behaviour [31, 62, 65]. Błażejowski et al. [9] described their Devonian *Trimerocephalus chopini* trilobite assemblage as preserved in close association with many juvenile individuals, and suggest that they were "growing in a nursery ground" ([9] p. 4). Prior descriptions of synchronised moulting-mating behaviour in trilobites and migration of the offspring to nursery grounds were based on firmly documented evidence of moulting, in combination with quantitative analyses of the environmental context and population structure [31, 65]. These data were not described for the *Trimerocephalus* queues at the Kowala Quarry locality [9], but if

documented, could allow further inferences about trilobite gregarious behaviour to be made.

Why study moulting in trilobites?

The enormous wealth of trilobite moult fossils provides a unique opportunity to explore the development and behaviour of an ecologically important group during the early history of animal evolution. Each individual study on trilobite moulting feeds into a larger aggregation of occurrences and behavioural descriptions that can be analysed to interpret evolutionary trends for the group. This is why specimens such as that described by Błażejowski et al. [9] must be examined with a critical eye, in order to determine how appropriate they are for inclusion in future studies that examine broader trends in trilobite moulting. While most published occurrences of trilobite moulting are descriptive in nature, rare quantitative studies have given us a glimpse of the huge potential residing in this growing database of behavioural information. Brandt [15] contrasted trilobite moulting -- which is actually highly variable even within a single species [e.g. 66] and lacks reabsorption of the mineralised exoskeleton before moulting -- with the canalised habit and efficient reabsorption seen in modern arthropods. Preliminary survivorship analyses suggested that the ultimate extinction of trilobites may have been related to the cumulative effects of a variable and metabolically expensive moulting behaviour, revealing the evolutionary importance of ecdysial efficiency for taxonomic longevity [15], though the extent of this influence has been debated (see [67]). Our own recent analyses of published occurrences quantitatively showed that trilobites exhibit a high level of variability in moulting behaviour in all Orders and throughout their evolutionary history (Fig. 5, [5]). These proof-of-concept studies show that a huge breadth of information on early arthropod development remains to be extracted and interpreted from the moult fossil record.

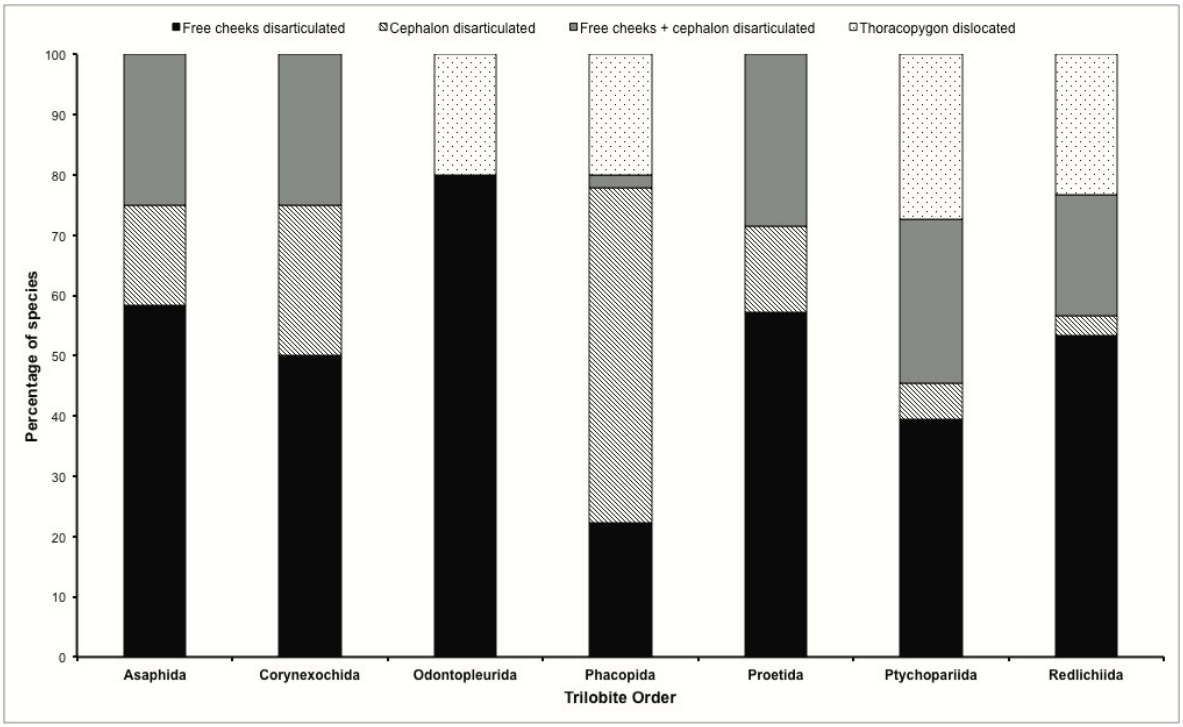


Figure 5: Percentage of sampled trilobite species from seven Orders that show each of four different moulting behaviours (disarticulation of: free cheeks; cephalon; free cheeks+remaining cephalon; thoracic segments). Data obtained from the descriptive literature (see [5] for methodology, data normalised to 100%).

Conclusions and outlook

Błażejowski et al. [9] described a specimen of *Trimerocephalus chopini,* from the Upper Devonian (365 million years ago) Kowala Quarry of central Poland, that they argued consisted of a freshly moulted individual preserved ventral to its recently moulted exoskeleton. This supposition is based on a suggested increase in the thoracic segment number and wrinkling of the "carcass". On consideration of the preservation, morphology, and arrangement of the specimen, and in comparison to other trilobite material from the locality, it is more likely that this specimen represents two separate trilobite individuals, which are queueing in a manner similar to that described for *Trimerocephalus* by Radwański et al. [19]. This type of critical analysis is important when investigating the

fossil record of moulting owing to the difficulty that exists in distinguishing moults from carcasses, which can be clarified by consideration of the contextual information of the fossil locality and comparison with modern analogues [5].

With every new discovery of moulting preserved in the fossil record, we are more able to discuss important aspects of the evolution of development and behaviour. The rare and exquisite preservation of caught-in-the-act moulting is informative for snapshot views of ecdysial behaviour [10-12], while larger scale quantitative analyses of abundant moult assemblages (i.e. readily identifiable trilobite exuviae) allow us to link trends in moulting modes with geological time and taxonomy [5, 15]. Quantitative studies on moulting in the fossil record should be further expanded, based upon their demonstrated potential to reveal information on the life histories and behaviour of extinct animals. Understanding what the fossil record can tell us about moulting and growth provides a unique deep-time insight into the evolution and development of Arthropoda and other members of Ecdysozoa.

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Chapter 3: Quantifying intra- and interspecific variability in trilobite moulting behaviour across the Palaeozoic

This chapter seeks to quantitatively answer the question "how variable were trilobites in their moulting behaviours?" I present a novel broad-scale database of trilobite moulting characteristics (locations of exoskeleton disarticulations, from moults identified based on discussions presented in Chapters 1 and 2), which is used to determine moulting variability in Trilobita. The data show that trilobites were ubiquitously intra- and interspecifically variable in moulting, and also reveal interesting taxonomic and temporal trends. Dataset sampling and trilobite diversity through the Palaeozoic are also explored.

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Quantifying intra- and interspecific variability in trilobite moulting behaviour across the Palaeozoic

Keywords: behaviour; morphology; Cambrian; exoskeleton; exuviation; moulting

ABSTRACT

Moulting of a protective exoskeleton is a defining characteristic of Euarthropoda, and its evolution can be explored through analysing moults preserved in the fossil record. Our most complete record comes from the Trilobita, which were uniquely flexible in moulting compared to other arthropod groups. This study presents the first broad-scale quantitative analysis of trilobite moulting. Trends in moulting variability with taxonomy and through the Palaeozoic are explored by looking at the occurrences of six moulting characteristics: opening of the facial and ventral sutures; and disarticulation of the cephalon, cranidium, thorax, and pygidium. Significant differences in moulting across taxonomic and temporal groups were identified using chi-squared analyses, and biases with sampling and diversity identified through correlation analyses. Occurrences of facial and ventral suture opening, and cephalic disarticulation, significantly varied between orders and Epochs. These likely result from the prevalence of ventral suture moulting in Redlichiida and cephalic disarticulation in Phacopida, and their relevant diversity patterns. The data show high levels of intraspecific variability in moulting; ~40% of species showed multiple moulting characteristics. Redlichiida and the early Cambrian are the most intraspecifically variable, with greater variability likely an adaptation to the initial radiation and establishment of trilobites into new niches. The longest-lived group, Proetida, showed the lowest levels of intraspecific variability, which may suggest greater specialism later in the Palaeozoic.

Ultimately, datasets such as this advocate the need to study behaviours in the fossil record on a broad-scale, because they help us build a comprehensive picture of extinct groups as living animals.

PLAIN LANGUAGE SUMMARY

Euarthropoda, the animal group including crustaceans, insects, and a wide diversity of extinct groups, all share a hard exoskeleton that functions as protection against predation and parasitism. However, this exoskeleton restricts growth of the individual, and therefore must be periodically shed (moulted) to facilitate development. Trilobites, one of the most abundant groups ever to have lived, had heavily reinforced exoskeletons and are therefore found preserved as fossils in great number. Empty moulted trilobite exoskeletons are common in the fossil record, and these can be used to interpret their behaviours relating to how they may have moulted. This study provides the first ever broad-scale dataset of trilobite moulting behaviours across the entire group and the whole of their geological history (~521-251 million years ago). The results show that trilobite moulting behaviour was particularly variable in comparison to other euarthropod groups, often differing both between individuals of the same species and of different species. The longest-lived trilobite group showed the lowest levels of variability, and the highest levels of variability were found in the earliest period of trilobite evolution. Ultimately, this study demonstrates that behaviours like moulting, which affect the broad evolutionary history of animal groups, can undergo quantitative study using the fossil record.

BACKGROUND

Arthropod moulting

Moulting is the process of shedding the old exoskeleton and replacing it with a new one. This characteristic unites all Ecdysozoa (Aguinaldo et al., 1997), while arthropods in particular often have exoskeletons reinforced by biomineralisation. This provides a tough covering (for example chitin or calcite) which protects the individual from predation and parasitism, prevents desiccation in terrestrial groups, and acts as a muscle attachment point (Ewer, 2005). However, arthropods must go through numerous successive moult cycles in their lifetime as the exoskeleton restricts growth. Moulting consequently allows the individual to inflate, develop their morphology, and repair damage. Arthropods are extremely vulnerable during and immediately after moulting (Henningsmoen, 1975), and moulting events, either due to a failure to separate from the old exoskeleton, or predation, are responsible for up to 80-90% of individual arthropod deaths (Clarkson, 1979; Brandt, 2002), making it one of the most crucial recurring events in arthropod life history. Moulting is intrinsically linked to the behaviour, biochemistry, reproduction, morphology, and ecology of arthropods, and consequently of primary importance in their evolutionary history (Vevea and Hall, 1984; Brandt, 2002; Ewer, 2005).

There has been a great deal of research on extant arthropod moulting behaviours, particularly in insects, and the biochemical pathways (involving ecdysones) that stimulate the moulting process (see e.g., Nijhout, 1994; Ewer, 2005; Song et al., 2017). However, there has been very little work using the fossil record to explore the evolution of this key characteristic. We can investigate moulting in the arthropod fossil record because moulted exoskeletons are preserved in much the same way as carcasses, and moult fossils are thought to be much more common than carcasses because each individual moults many

times during its lifetime but produces only one carcass (Daley and Drage, 2016). These moults are recognisable because the moulting process in extinct arthropods is thought to comprise the same stages as for modern groups, which is consistent despite differences in exoskeleton composition (Ewer, 2005). This comprises a pre-moult stage, where the animal prepares for moulting (secreting moulting fluids, detaching the old exoskeleton cuticle); exuviation (or ecdysis, the actual exiting of the old exoskeleton); and a post-moult stage, where the new exoskeleton decompresses and hardens; followed by a return to the intermoult stage (Krishnakumaran and Schneiderman, 1970; Henningsmoen, 1975). The most complete and abundant early fossil record of moulting belongs to Trilobita. This is in part because of their thickly biomineralised calcitic exoskeletons with a correspondingly high preservation potential, but also their incomparable diversity, abundance, and global distribution (Tarver et al., 2007). For the most part the fossil record of moulting is based on isolated or grouped empty moults, and these remain uncommon outside Trilobita. Arthropods caught 'in-the-act' of exuviation, such as an individual of *Marrella splendens* Walcott 1912 from the Burgess Shale (García-Bellido and Collins, 2004), are extremely rare, and no convincing example has been described for trilobites (Drage and Daley, 2016). Consequently, trilobites provide us with the only large dataset with which to explore the origins of moulting for total group Arthropoda, which may have been central to the development of their dominance throughout the Phanerozoic into the present (Vevea and Hall, 1984; Ewer, 2005). A complete review of trilobite and other arthropod moulting evidence from the fossil record published to-date is presented in Daley and Drage (2016).

Trilobite moulting behaviour, i.e., the lines the old exoskeleton splits along and the behaviours that an individual uses to accomplish exuviation, has been shown to be both inter- and intraspecifically variable despite the reasonably consistent body plan in this

group (Figure 1; Henningsmoen, 1975; Daley and Drage, 2016; Drage et al., 2018a). This is in contrast to the moulting behaviours of other arthropod groups, both extant and in the fossil record, which have morphologies and behaviours more specialised to one or two moulting methods (Daley and Drage, 2016). Trilobites, which appear earliest in the fossil record amongst crown-group arthropods (Daley et al., 2018), are therefore unique in their moulting. Previous studies of trilobite moulting, despite their clear importance, have been limited in scope or based on entirely qualitative observations (e.g., Henningsmoen, 1975; Whittington, 1980, 1990; McNamara and Rudkin, 1984; Busch and Swartz, 1985; Speyer, 1985; Speyer and Brett, 1985; McNamara, 1986; Brandt, 2002; Bruthansová, 2003; Clarkson et al., 2003; Budil and Bruthansová, 2005; Hunda et al., 2006; Paterson et al., 2007; Cederström et al., 2010; Rustán et al., 2011; Drage and Daley, 2016; Drage et al., 2018a), leaving many unanswered questions about the evolution of this behaviour. How flexible were trilobites in their moulting behaviour compared to other arthropods? What effect did this have on the evolution of their morphology, ecology, development, and geological longevity? Quantitative analysis of the history of trilobite moulting means that we can trace how this life history strategy affected the broad-scale evolution of a group.

This study presents the first broad-scale quantification of the variability of trilobite moulting behaviour. Using a large global dataset this will present the extent of the intra- and interspecific variability in moulting, and how this differed taxonomically and temporally. Results will first look at interspecific variability in trilobite moulting in the total group, within each order, and within families or superfamilies. The intraspecific moulting variability of the sampled species will then be presented, followed by the same analyses looking at variability across Palaeozoic time bins rather than taxonomic groups. This work significantly advances our ability to answer the key questions about the

evolutionary history of this ubiquitous characteristic central to the life histories of all arthropods.

Trilobite moulting

Investigating the fossil record of moulting relies on being able to accurately distinguish preserved moults and carcasses. Henningsmoen (1975) and Daley and Drage (2016) outlined a number of criteria with which to do this based on recurrent patterns of exoskeleton separation (i.e., the presence of specific moulting characteristics; Table 1), and contextual information (e.g., lack of abiotic/biotic disturbance etc.). However, this can be difficult for specimens for which separated moulted sclerites are not found in close association with the remainder of the exoskeleton, and so detailed explorations of moulting are limited to trilobite-bearing assemblages in which we can be certain of a moult identification (i.e., often Konservat-Lagerstätten; Drage et al., 2018a). However, well-preserved specimens with all moulted exoskeleton sclerites in close association are found in many locations worldwide, not just Konservat-Lagerstätten. For trilobite specimens that do fulfil these criteria, and are therefore confidently interpreted as moults, we can identify six key exoskeleton disarticulations, and which describe the moult; these are moulting characteristics (Table 1). These moulting characteristics are: open facial sutures (separating the librigenae from the cranidium); open ventral sutures (separating the rostral plate and/or hypostome from the cephalon); disarticulation of the cranidium; disarticulation of the cephalon; separation between thoracic segments; and disarticulation of the pygidium (Table 1). These represent the anatomical features of trilobite moults, and in combination are what most moult identifications are based upon (see Daley and Drage, 2016, and references therein). These six moulting characteristics are used throughout this

study to explore moulting trends, and can be identified from all trilobites if most of their exoskeletal sclerites are preserved in association.

TABLE 1. Definitions relating to trilobite moulting used within this work.

Moulting configuration	The general term for any assemblage of disarticulated exoskeletal units (sclerites) produced through the behaviours of a moulting individual. This is left behind and becomes preserved in the fossil record (see Drage et al., 2018a).
Style of moulting	The generalised type of exoskeleton moulting, involving a number of moulting characteristics, which produces a moulting configuration. There are two main styles, involving use of the cephalic sutures (facial, rostral, hypostomal, and median), or use of the cephalothoracic joint (joining the cephalon or cranidium to the thoracopygon), although a single moult specimen can show both of these together. A third unusual style involves the use of a marginal suture running laterally along the anterior margin of the entire cephalon.
Moulting characteristic	Any of the characteristic exoskeletal disarticulations produced during moulting, and found in a moulting configuration. Specifically, opening of the facial sutures and/or ventral sutures, and disarticulation of the cranidium, cephalon, thorax, and/or pygidium. The ***'normal' moulting characteristic*** is the one of these that is most commonly produced during moulting for a particular species.
Exuviation	The stage of moulting during which the organism actually sheds the old exoskeleton.
Exuvial gape	The opening produced in the old exoskeleton through which the organism exuviates.

These moulting characteristics identified from the fossil record of trilobites can be summarised into three general styles of moulting, indicating extensive flexibility in this behaviour (Figure 1). Firstly, usage of the cephalic sutures during moulting, involving the opening of the facial sutures connecting the librigenae to the cranidium (most commonly), the rostral suture connecting the ventral rostral plate to the cephalon, the hypostomal

suture connecting the hypostome to the cephalon, and/or a median suture running axially on the ventral side, depending on morphology. Some trilobites use the cephalothoracic joint to moult, meaning that the articulation connecting the cephalon to the thoracopygon is opened. Occasionally this may occur in association with use of the cephalic sutures in moulting. Finally, certain derived trilobite groups open the marginal suture during moulting, which is a suture usually found running around the anterior of the cephalon (similar to moulting in xiphosurans; Sekiguchi et al., 1988). In all cases, at these openings an exuvial gape is produced through which the animal can anteriorly egress the old exoskeleton. The trilobite individual would then be free to expand and harden the new exoskeleton.

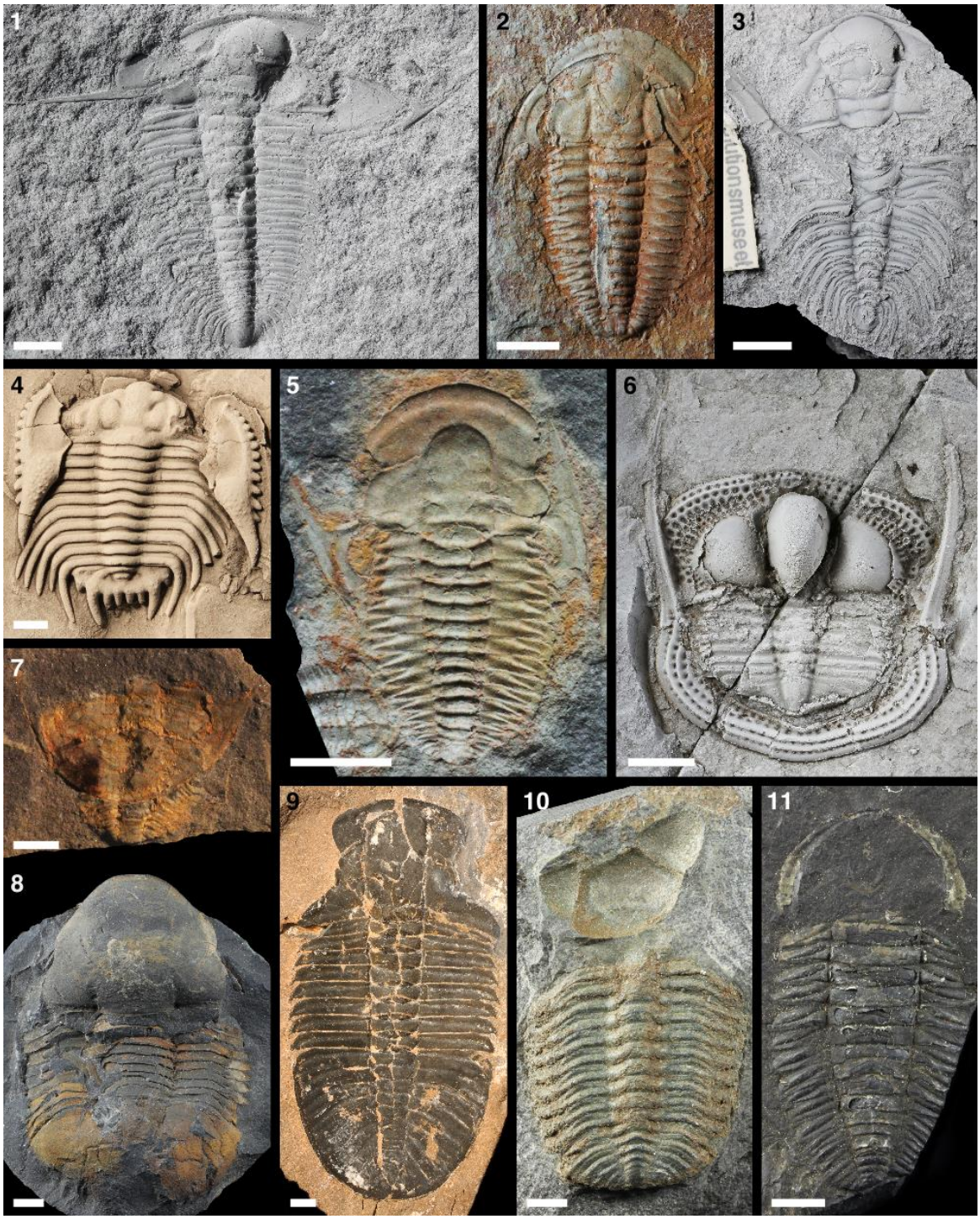


FIGURE 1. An example of the diversity of trilobite moult configurations preserved in the fossil record, see Drage et al. (2018a) for descriptions of the named configurations. 1: *Acadoparadoxides* sp. Šnajdr 1957 (PMU 25636, middle Cambrian, Czech Republic) in McNamara's or Somersault Configuration but with the librigenae also rotated; 2: *Estaingia bilobata* Pocock 1964 (SAM-P55734, early Cambrian, Australia) with the right librigena in McNamara's Configuration; 3: *Acadoparadoxides* sp. (PMU 25690, middle Cambrian, Czech Republic) showing disarticulation of

the librigenae, cranidium and thorax; 4: *Acidaspis coronata* Salter 1853 (OUMNH C.17494, Wenlock, UK) in the Nutcracker Configuration; 5: *E. bilobata* (SAM-P 54204, early Cambrian, Australia) in the Somersault Configuration; 6: *Marrolithus ornatus* Sternberg 1833 (NMP L15156, Ordovician, Czech Republic) in Hupe's Configuration; 7: *Redlichia takooensis* Lu 1950 (SAM-P55732, early Cambrian, Australia) in Salter's Configuration; 8: *Illaenus parabolinus* Novák 1918 (in Novák and Perner, 1918) (NHMUK I.15261, Lower Ordovician, Czech Republic), an axial shield missing the librigenae; 9: *Ogygopsis klotzi* Rominger 1887 (OUMNH AT.205, early Cambrian, Canada), a common axial shield; 10: *Trimerocephalus mastophthalmus* Richter 1856 (NHMUK In.22418, Upper Devonian, UK), in Salter's Configuration with the cephalon displaced forwards; 11: *Triarthrus beckii* Green 1832 (NHMUK In.19650, Upper Ordovician, USA), showing a Lower Cephalic Unit mostly *in situ* and a clearly displaced cranidium as a variant of Henningsmoen's Configuration.

METHODS

Data collection

This study aimed to provide the first broad-scale, quantitative exploration of trilobite moulting behaviour variability, both taxonomically and temporally throughout the Palaeozoic. Trilobite moults from museum collections were identified using the criteria discussed in the Background Section (Henningsmoen, 1975; Daley and Drage, 2016). Some localities and geological ages produce more disarticulated material than others, for example the early Cambrian and late in the Palaeozoic, however only reasonably complete specimens were used for data collection. Heavily disarticulated material (e.g., isolated sclerites; see Figure 2) cannot be confidently determined to represent a moult, and therefore less material from localities with poor preservation was included. From the identified moults, the six generalised moulting characteristics (locations of exoskeletal disarticulations associated with moulting, see Table 1) were recorded for 840 specimens

from 355 species. These were separation/disarticulation of the: rostral plate and/or hypostome (= opening of the ventral sutures); librigenae (= opening of the facial sutures); entire cephalon (= opening of the cephalothoracic joint); cranidium (the cephalon minus the librigenae); thorax (between any two thoracic segments); and pygidium (= opening of the pygidiothoracic joint). The species were also categorised into the two main styles of moulting, use of cephalic sutures (species showing separation of the librigenae, ventral structures, and/or cranidium), and use of the cephalothoracic joint (species showing disarticulation of the cephalon or cranidium; cranidium disarticulation therefore falls under both general styles), which produce the combinations of these six moulting characteristics (see Figure 2). The 'normal' moulting characteristic was also recorded, which counted the number of species which in the majority of their sampled specimens displayed each of the six moulting characteristics.

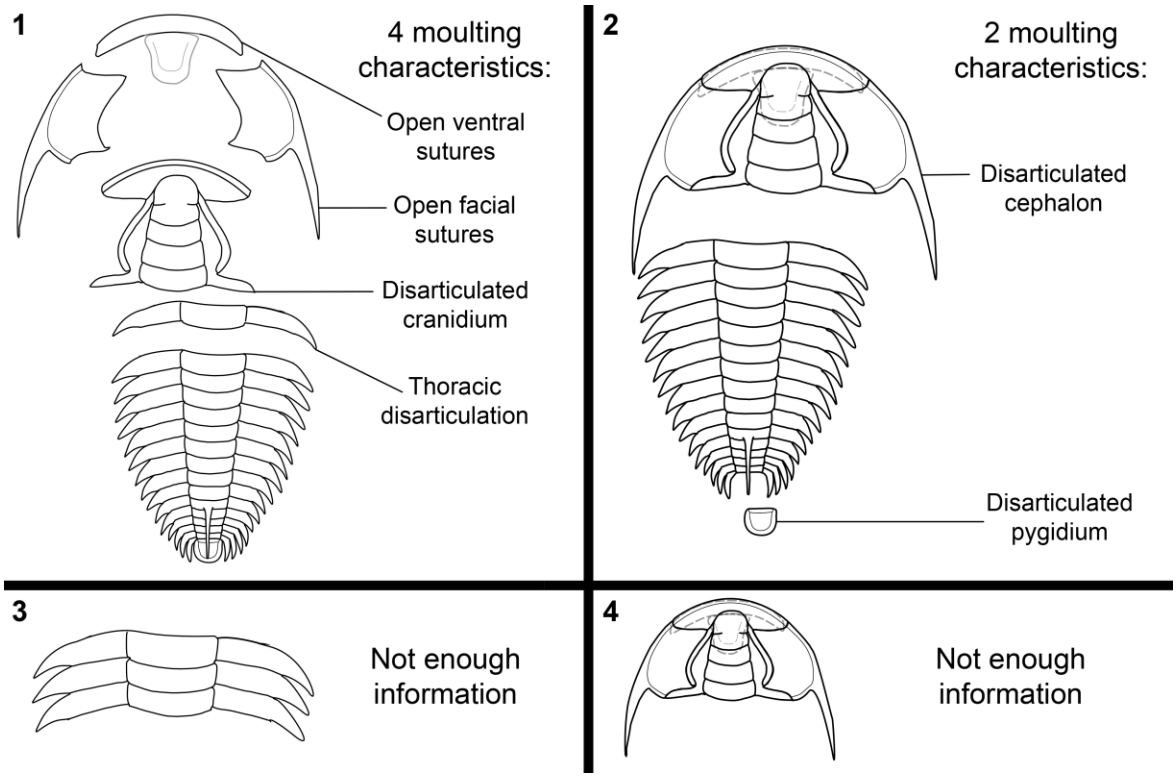


FIGURE 2. Hypothetical trilobite moult specimens, demonstrating the process of gathering data for the presented dataset. 1 and 2 represent specimens that can be confidently considered moult

configurations, whereas 3 and 4 (isolated thoracic segments and an isolated cephalon respectively) are too fragmented and lack sufficient information to identify as either moults or carcasses. 1: four moulting characteristics can be counted from this specimen, including open ventral sutures (and therefore separation of the rostral plate/hypostome), open facial sutures (separation of the librigenae), a disarticulated cranidium, and a disarticulated thorax. 2: two moulting characteristics can be counted from this specimen, including a disarticulated cephalon, and a disarticulated pygidium. Of these four specimens, only 1 and 2 would be recorded within the dataset. Dashed lines in 2 and 4 represent ventral structures under the dorsal exoskeleton.

The complete trilobite collections of the following museums were surveyed, and the identified moults sampled and incorporated in the dataset: Lapworth Museum of Geology, Birmingham (BIRUG); Natural History Museum, London (NHMUK); Oxford University Museum of Natural History (OUMNH); the Uppsala University Museum of Evolution (PMU). A number of specimens were also sampled from the South Australian Museum (SAM) and National Museum Prague (NMP). Finally, species were sampled from moult specimens figured or described in the trilobite literature, although specimen counts were often not reported for these.

Trilobites of the family Trinucleidae (Figure 3.1) and order Harpetida (Figure 3.2) were excluded from the moulting behaviour dataset. This is because they show an unusual derived morphology, with this necessitating a different moulting behaviour in comparison to those observed for the rest of Trilobita. Trinucleid and harpetid trilobites convergently evolved a fused cephalic shield, with a broader shape divided into dorsal and ventral halves (Stubblefield, 1959; Sekiguchi et al., 1988). This appears to have been a feeding adaptation, with the cephalic fringe pits in trinucleids apparently adaptive for a filter-feeding lifestyle (Fortey and Owens, 1999). Consequently, moulting in these groups

appears to have taken place through opening of the marginal suture (Figure 3.1, 3.2), with an anterior exuvial gape suture created between the dorsal and ventral parts of the cephalic shield. This would extend along the entire anterior margin on the cephalon, and the animal would then emerge forwards. Other trilobites also evolved similar cephalic structures, such as later brachymetopid species with a greatly expanded rostral plate and fused facial sutures (Fortey and Owens, 1975), and many olenid and phacopid species which secondarily lost the facial sutures and moulted through disarticulation of the entire cephalon (discussed in detail later; Crônier, 2013; Drage et al., 2018b). Modern xiphosurans moult in a comparable manner to harpetids and trinucleids, also having a fused cephalic shield (carapace) in which a marginal gape opens between the dorsal and ventral parts (Sekiguchi et al., 1988). For trinucleids, this could produce a moult assemblage with the dorsal cephalic fringe displaced away from the remainder of the exuvia (Figure 3.1). Moulted harpetids are difficult to recognise, like xiphosurans, as the anterior gape appeared to have closed post-moulting (Figure 3.2–3.4). These trilobites may also have moulted through disarticulating the entire cephalon, but complete configurations of these were rarely observed. Agnostida and uncategorised species were also removed dataset due to uncertain taxonomic assignment. Ordinal-level species assignments within the dataset therefore included the Asaphida, Corynexochida, Lichida (comprised of lichid and odontopleurid species), Phacopida, Proetida, Ptychopariida, and Redlichiida (see Whittington et al., 1997; Jell and Adrain, 2002).

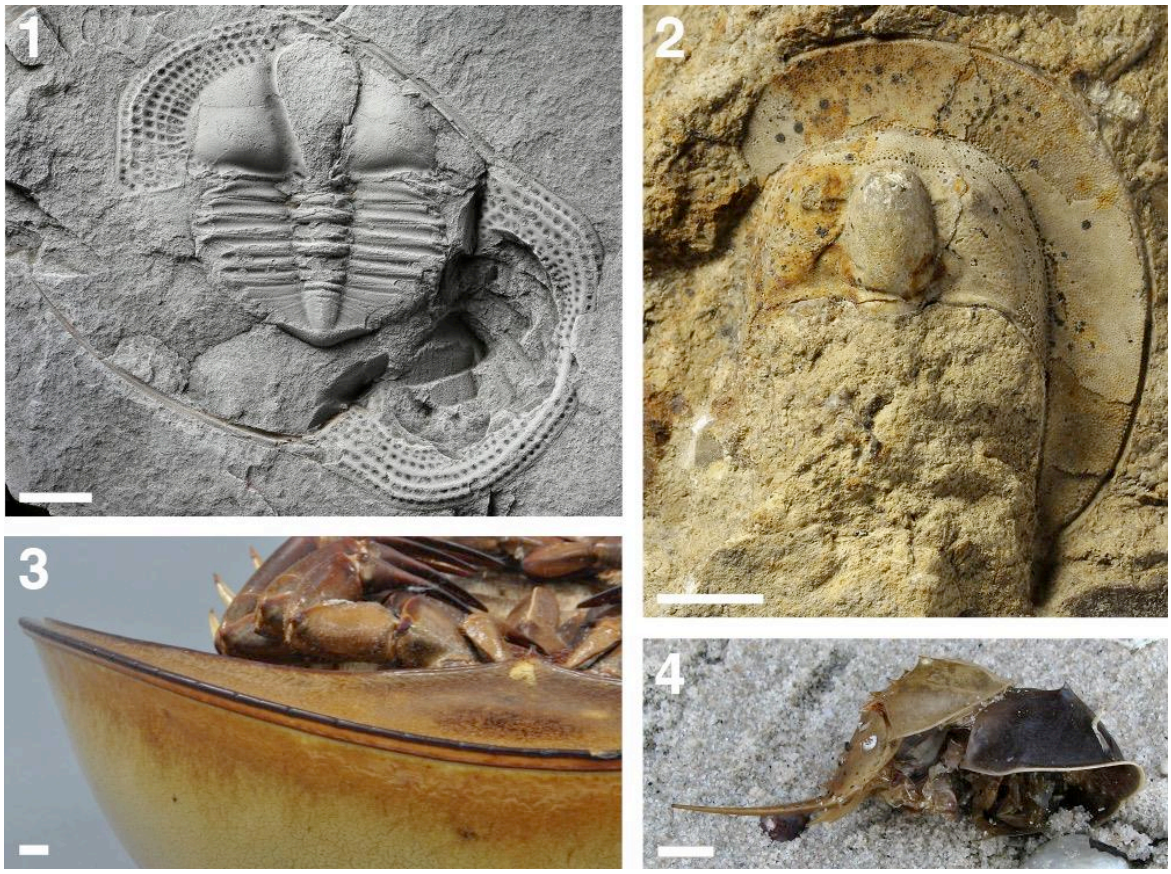


FIGURE 3. Potential moult assemblages and moulting morphology of specialised arthropods. 1: *Marrolithus ornatus senftenbergi* (Order Asaphida, Family Trinucleidae, L15157 from the NMP collections, Ordovician, Czech Republic) with the upper cephalic fringe inverted to rest posterior to the exuvia; 2: *Bohemoharpes ungula* Sternberg 1833 (Order Harpetida, L2959 from the NMP collections, Ordovician, Czech Republic), broken at some places at the marginal suture between the dorsal and ventral parts of the cephalic fringe; 3: *Limulus polyphemus* Linnaeus 1758 (Order Xiphosurida), carcass showing the comparable closed marginal suture (image by R.D.C. Bicknell, Va. 06 from the Natural History Museum of the University of New England, USA, recent); 4: *Limulus polyphemus* carcass mid-moult emerging through the marginal suture (Wikimedia Commons, recent, USA). Scale bars 5 mm for 1–3, 20 mm for 4.

All species' metadata were checked using comprehensive searches of the descriptive literature and the Paleobiology Database (Fossilworks, 2018; Kiessling et al., 2018). Species' age assignments were time-binned using the ICS International

Chronostratigraphic Chart 2017 (Cohen et al., 2013) into Palaeozoic Periods and Epochs. Taxa that occurred in two sequential time bins (i.e. across a boundary between two Periods or Epochs) were counted twice, once in each occupied time bin. Species taxonomic data (order, family) was obtained from Whittington et al. (1997) and Jell and Adrain (2002). Total-group trilobite diversity data was obtained from a FossilWorks and Palaeobiology Database search for all species entered and time-binned as Trilobita (Kiessling et al., 2018). These data were used to create raw and Shareholder Quorum Subsampled diversity curves using FossilWorks (from 26947 eligible occurrences, representing 8418 species throughout the Palaeozoic; generated 25/01/18; Fossilworks, 2018), which were then compared to sampling within the moulting dataset.

Country and GPS coordinates were obtained for all species for which collection metadata was available. For species recorded from the literature, either locality information from the descriptive article, or the type specimen locality was used, and the latitude and longitude coordinates obtained using Google Maps. Resulting coordinates were plotted for each sampled species on palaeogeographic maps representing the continental configurations at the mid-point age of each geological Period (Figure 4). These sampling maps were produced using GPlates and PaleoMap (Scotese, 2016). The Permian was excluded, as only one species was sampled.

Specimens were photographed using a Canon EOS 5D or 500D with a Macro Lens, under incident lighting, and using the Canon EOS Utility 2 remote camera access software. Graphs were produced in RStudio (RStudio Team, 2015) using custom code and the ggplot2 (Wickham et al., 2016) and Geoscale (Bell, 2015) packages. Figures were produced and/or modified using CS6 Adobe Illustrator and Photoshop.

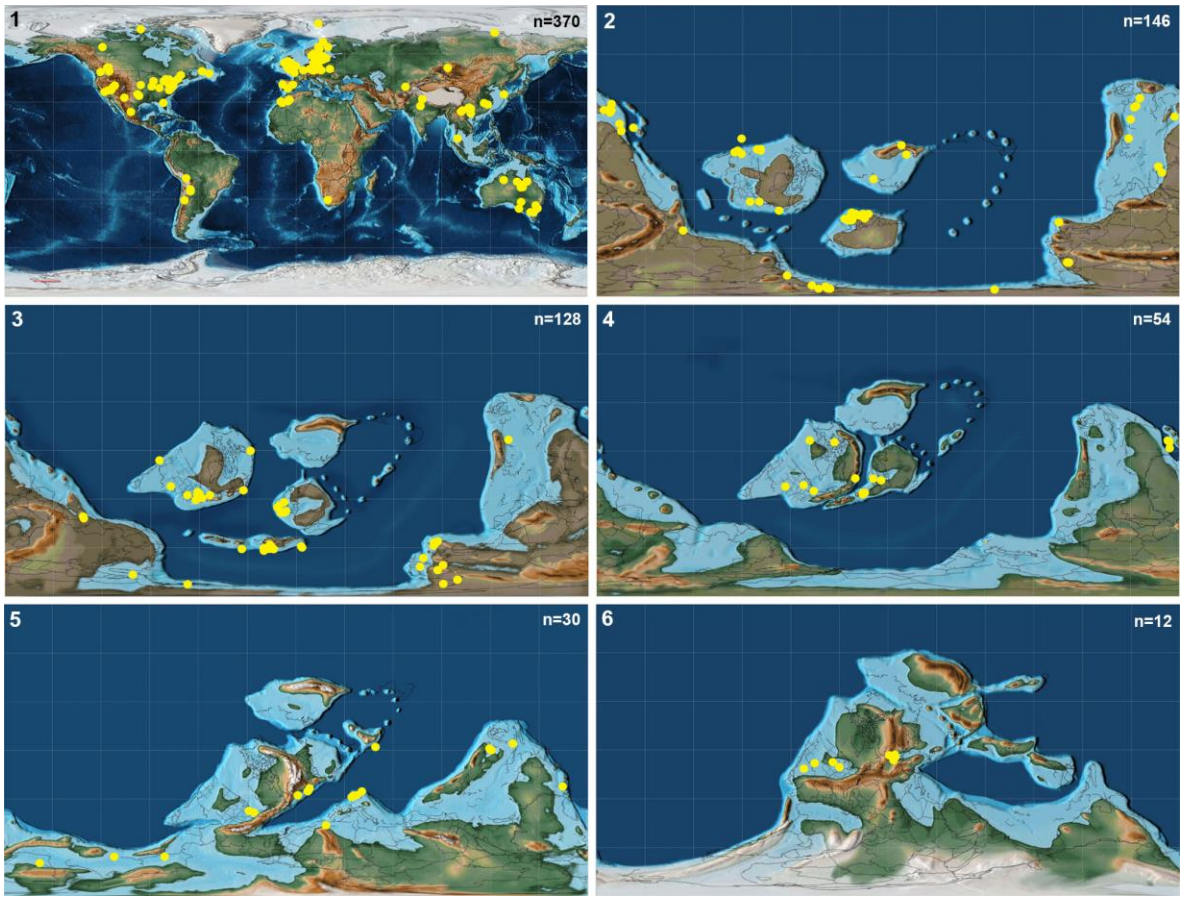


FIGURE 4. Palaeogeographic maps with yellow points representing the sampled species in each geological Period. 1: all sampled species on a modern map, n=370; 2: Cambrian, n=146; 3: Ordovician, n=128; 4: Silurian, n=54; 5: Devonian, n=30; 6: Carboniferous, n=12. Permian not displayed because n=1. Maps represent the mid-point age of each Period. Some specimens appear sampled from terrestrial areas owing to their lack of well-defined collection data. Total species count is higher than the number of unique species in the dataset because of the occurrence of some taxa across geological Period boundaries. Maps produced using GPlates and PaleoMap (Scotese, 2016).

Sampling

Overall, 29 species and 54 specimens were sampled from the BIRUG, 144 species and 336 specimens from the NHMUK, 70 species and 197 specimens from the OUMNH, 3 species and 31 specimens from the SAM, and 14 species and 142 specimens from the PMU,

although many more were observed. Finally, 148 species were sampled from the descriptive literature. A number of these provided data for the same species and not every species from the literature provided specimen measurement data, leaving a total of 355 species and 840 specimens. The number of species sampled for each order, suborder, superfamily, and family are displayed in Appendix 1, Table 1. The number of species sampled for each geological Epoch during the Palaeozoic are displayed in Appendix 1, Table 2, with a slightly higher total due to the presence of boundary taxa. Within the dataset, 58% of all trilobite species (196 species) were represented by moulting data from only one specimen, leaving 42% of species represented by two or more specimens (Figure 5). 19% of species (64) were represented by two specimens, 13% (45) by 3-5, and 10% (33) by 6+ specimens (Figure 5). Specimen sampling data was unavailable for 17 species due to being based on literature descriptions that did not provide specimen counts. All data used to produce the results presented in this study are available in Appendices 2–5.

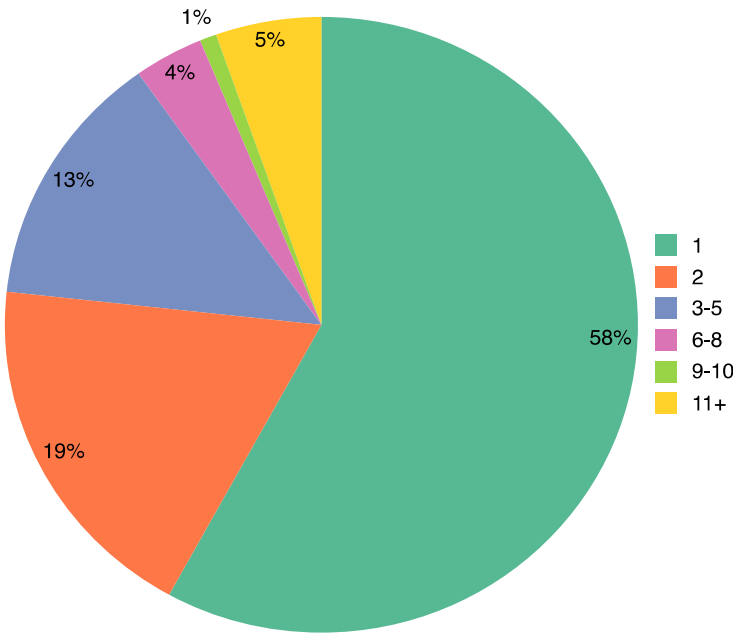


FIGURE 5. Percentage of all sampled trilobite species (n=338) that are represented by 1 specimen, 2, 3-5, 6-8, 9-10, and 11+ specimens. 17 species were not included in this count because the

number of representative specimens was unknown, as the data was derived from literature that did not provide a sampling estimate.

Analysis of interspecific moulting variability with taxonomy

Differences in the recorded moulting behaviours of trilobite taxonomic groups were explored over a number of levels. Firstly, at the superordinal-level, by comparing the proportion of all recorded trilobite species showing the two general styles of moulting (cephalic sutures, and cephalothoracic joint), and showing each of the six moulting characteristics. At the ordinal-level, comparisons were made of the proportions of species in each order showing both general styles of moulting (cephalic sutures, and cephalothoracic joint), and the moulting characteristics. For the latter, the 'normal' moulting characteristics were also plotted, i.e., the single moulting characteristic which each species most commonly displayed. Finally, the proportion of species for each family or superfamily (dependent on sampling) displaying the six moulting characteristics were compared within each order (Lichida and Proetida not included as sample sizes to low for robust statistical analysis, usually n=<5 for each family). Taxonomic sampling quality within the dataset was explored by plotting the ordinal sample diversity against that obtained from a full search of Trilobita occurrences recorded in Fossilworks and the Paleobiology Database (Kiessling et al., 2018; n=9753 for all trilobite orders; Appendix 3, Table 1).

Chi-squared analyses were performed based on the null hypothesis (H0) that the trilobite clades (orders, superfamilies, or families) did not show significant differences in their moulting characteristics. These tested the alternate hypothesis (H1) that the clades showed significant differences in the number of species displaying each moulting characteristic.

All analyses compared observed versus expected values. Expected numbers were calculated as the number of species that should display each moulting characteristic if all taxonomic groups moulted in the same way, when corrected for sampling. Analyses were performed in PAST3 (Hammer et al., 2001) with the "sample versus expected" setting. The total numbers (and proportions) of species summed to a greater total than that recorded in the dataset (or greater than 100%) because species could display more than one moulting characteristic. Full chi-squared tables for all analyses are available in Appendix 4. Correlation analyses (r^2, Spearman's Rank, and Kendall's tau) were calculated in PAST3 (Hammer et al., 2001) to test for significant differences in sample ordinal species diversity and total group diversity (the latter from Fossilworks and the Paleobiology Database; Fossilworks, 2018; Kiessling et al., 2018).

The chi-squared test was chosen for the moulting variability analyses because the results can directly inform on the question of interest; 'did trilobite moulting behaviour vary between taxonomic groupings?' The expected numbers used in analyses were corrected for uneven group sampling, to give an accurate comparison between observed and expected data (Hammer et al., 2001). Chi-squared is also particularly appropriate as it is a non-parametric test, and much of the data analysed here, because it is nominal, may violate assumptions of normality (McHugh, 2013). The test can have reduced accuracy when including expected values totalling <5, however this was inevitable given the behavioural nature of the variables tested (as some moulting characteristics occur rarely). Other statistical tests might be used to answer this question, for example ANOVAs or Mann-Whitney U tests to compare mean numbers of species displaying each moulting characteristic, however these are parametric, and would also require subsequent post-hoc tests to determine which groupings were causing the significant results. Tests such as

Kolmogorov-Smirnoff are not appropriate to apply to this dataset, because this compares distributions of two samples, not a number of variables with summed counts for each grouping. Bonferroni corrections were applied to all chi-squared test p-values, in order to confirm that significant results were not due to chance through multiple testing, thereby correcting for familywise error.

Analysis of intraspecific moulting variability with taxonomy

Intraspecific moulting variability between orders was explored by plotting the number of species in each order that displayed 1, 2, 3, 4, 5, or all 6 moulting characteristics. The impacts of dataset specimen sampling on this was determined by plotting the number of moulting characteristics shown by each species against the number of specimens sampled for it.

Analysis of interspecific moulting variability through time

Changes in the moulting behaviours of trilobites through time were explored by plotting the proportion of species in each geological Epoch displaying each of the six moulting characteristics. This was also plotted using the 'normal' moulting characteristics, i.e., the moulting characteristic most commonly observed for each species. Temporal sampling quality within the dataset was explored by plotting species diversity within each Epoch against total group diversity obtained from a full search of Trilobita occurrences recorded in Fossilworks and the Paleobiology Database (Fossilworks, 2018; Kiessling et al., 2018; n=8309; Appendix 3, Table 2), and against total diversity corrected for sampling using Shareholder Quorum Subsampling (SQS; carried out in Fossilworks, 2018). Geological time bins in the dataset were based on the International Chronostratigraphic Chart 2017 (Cohen et al., 2013). The time bins used by Fossilworks vary slightly, with the Cambrian

remaining delineated as the lower Cambrian-middle Cambrian-Furongian, whereas the sample dataset uses the global designations of Series 2-Miaolingian-Furongian.

Chi-squared analyses performed were based on the null hypothesis (H0) that the geological time bins (Epochs or Periods) did not show significant differences in the moulting characteristics of their constituent species. These tested the alternate hypothesis (H1) that the time bins showed significant differences in the number of species displaying each moulting characteristic. All analyses compared observed versus expected values. Expected numbers were calculated as the number of species that should display each moulting characteristic if all time bins showed the same moulting behaviour, when corrected for sampling. Analyses were performed in PAST3 (Hammer et al., 2001) with the "sample versus expected" setting. The total numbers (and proportions) of species summed to a greater total than that recorded in the dataset (or greater than 100%) because each could display more than one moulting characteristics. The Late Devonian to early Permian Epochs were removed due to having too small sample sizes for robust statistical analysis (n=<5). Full chi-squared tables are available in Appendix 4. Significant deviations in dataset and total/SQS diversity through time were tested for using correlation analyses (r^2, Spearman's Rank, and Kendall's tau) calculated in PAST3 (Hammer et al., 2001).

Analysis of intraspecific moulting variability through time

Intraspecific moulting variability between geological Epochs was explored by plotting the number of species in each Epoch that displayed 1, 2, 3, 4, 5, or all 6 moulting characteristics. The Pennsylvanian and early Permian Epochs were removed due to having too low sample sizes for robust statistical analysis (n=<5). The mean average and 95% Confidence Intervals for the number of moulting characteristics shown in each Epoch was calculated. ANOVA and Kruskal-Wallis tests were carried out in PAST3 (Hammer et al.,

2001) to compare the means, testing for a significant change in intraspecific moulting variability through time.

RESULTS

Superordinal-level interspecific moulting variability

The majority of trilobite species sampled (excepting those that display marginal suture moulting; Figure 3) show use of the cephalic sutures for moulting (80% of species), where cephalic sutures (facial, rostral, and/or hypostomal) are opened to create an anterior exuvial gape (Figure 6). Approximately 40% of species show use of the cephalothoracic joint where this is disarticulated to create an exuvial gape between the cephalon and thoracopygon (Figure 6). This means that for 20% of species moult configurations were found displaying both styles of moulting (i.e. 80+40=120%, therefore 20% of species must be included in counts for both styles of moulting), and these taxa had the flexibility to use both cephalic and cephalothoracic exuvial gapes.

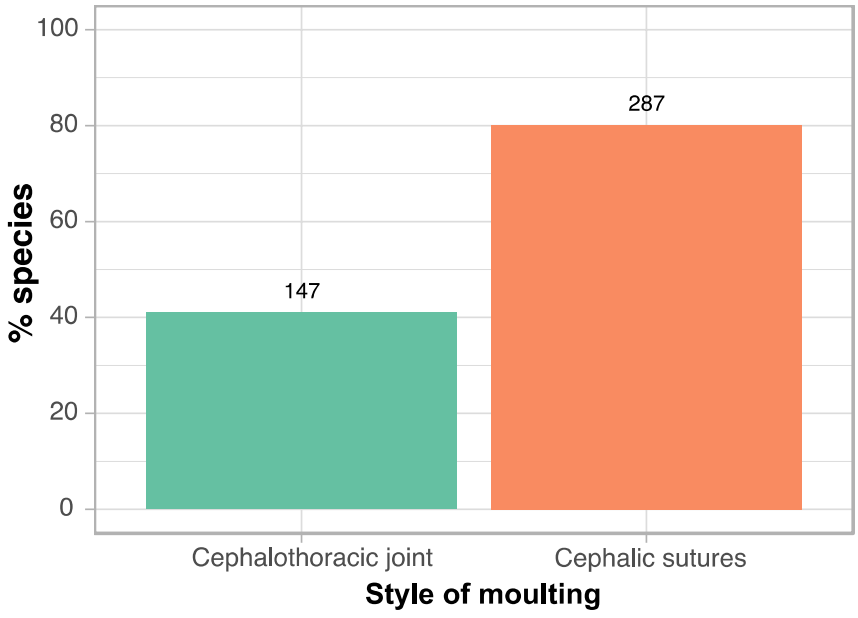


FIGURE 6. Percentage of all trilobite species displaying the two styles of moulting (opening of cephalic sutures, and opening of the cephalothoracic joint), with the total number of species

showing each above the bar. Species sum to more than the dataset total as each can show more than one style of moulting.

We can divide these general styles of moulting into the moulting characteristics they produced; opening of the ventral and/or facial sutures and cranidial disarticulation all showing use of cephalic sutures, and cephalic or cranidial disarticulation requiring opening of the cephalothoracic joint. Thoracic and pygidial disarticulations occur indiscriminately with the two styles, because they are not associated with the cephalon. 71% of all trilobite species display moult configurations with open facial sutures and disarticulated librigenae (Figure 7); the additional 9% utilising cephalic sutures (Figure 6) but not the facial sutures must have moulted via opening of the ventral sutures. The other moulting characteristics are all observed in fewer than 30% of species, with 18% showing disarticulated cranidia, 16% disarticulated pygidia, and 14% opening of the ventral sutures (Figure 7). An approximately equal proportion of species show disarticulation of the cephalon (25%) and the thorax (24%) in moult configurations.

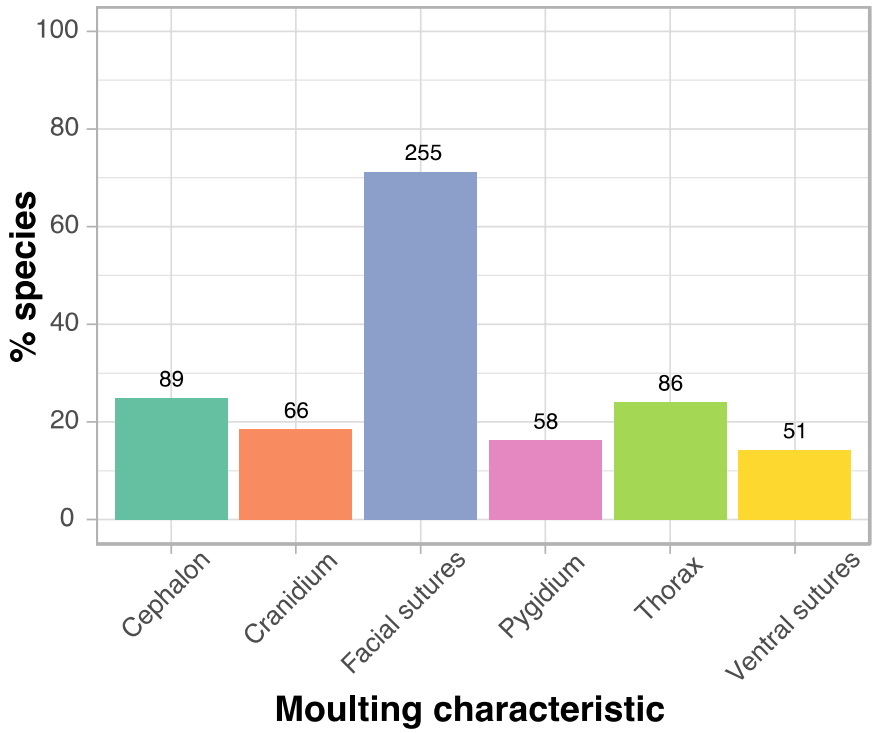


FIGURE 7. Percentage of all trilobite species displaying each of the six different moulting characteristics, with the total number of species showing each above the bar. Species sum to more than the dataset total as each can show more than one moulting characteristic.

Ordinal-level interspecific moulting variability

Total diversity (as sampled from Fossilworks; Kiessling et al., 2018) corresponds quite closely with database sampled diversity at an ordinal level (Figure 8; Appendix 3, Table 1). For the Asaphida, Corynexochida, Lichida, and Ptychopariida the sampled species diversity is reasonably similar to total diversity. However, the samples for Phacopida, Proetida, and Redlichiida are less representative of their total group diversities. Correlation analyses show that the sample diversity does not significantly correlate with total group diversity (r^2: 0.57, p=0.18; Spearman's Rank: 0.71, p=0.088; Kendall's tau: 0.52, p=0.099). Although for the non-parametric tests this is close to the significance threshold of α=0.05.

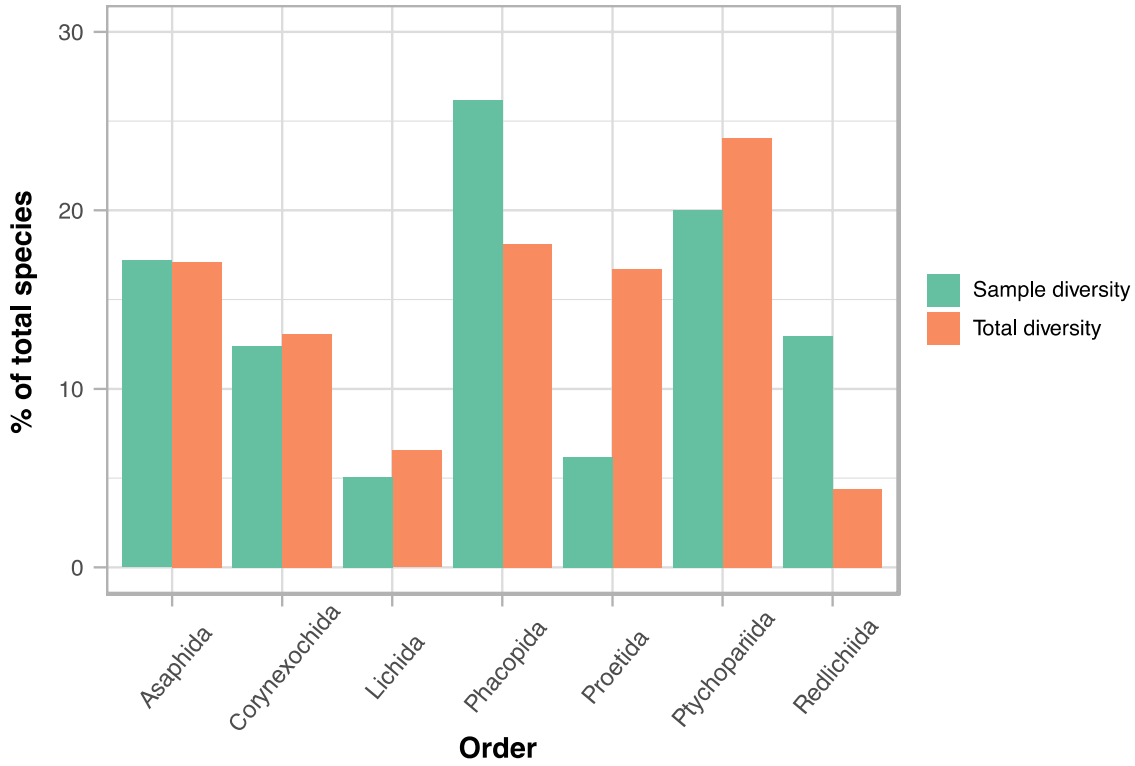


FIGURE 8. Trilobite taxonomic diversity in the sample dataset (green, n=355) plotted as a percentage of the total sample size, compared to total recorded diversity (orange, n=9753; downloaded from FossilWorks; Kiessling et al., 2018).

All seven trilobite orders display both styles of moulting in relatively high frequencies (Figure 9). Use of the cephalic sutures for moulting is the most common style for all orders except Phacopida, as expected by the almost ubiquitous presence of librigenae with facial sutures in trilobite morphology (Stubblefield, 1959; Whittington et al., 1997). For these six orders, the percentage of sampled species with at least one specimen displaying open cephalic sutures varies from 80% (Asaphida) to 100% (Redlichiida). However, 25% (Ptychopariida) to 41% (Asaphida) of species also show use of the cephalothoracic joint, previously considered to be very rare amongst non-phacopid trilobites (e.g., Henningsmoen, 1975). Phacopida is the only order to have a higher percentage of species displaying an open cephalothoracic joint (68%) than cephalic sutures (48%). Chi-squared

analyses show significant p-values and therefore reject the null hypothesis (p=0.027 and $1.8x10^{-4}$ respectively, at α=0.025; Appendix 4, Table 1), suggesting that the number of species displaying the two styles significantly differ between the orders. For use of the cephalic sutures this mainly results from corynexochid and ptychopariid species using these more than expected (41 observed to 34 expected species, and 68 observed to 56 expected, respectively), and less often than expected in phacopids (43 observed to 69 expected). Unsurprisingly the result is the opposite for use of the cephalothoracic joint in moulting, with phacopids displaying this more often than expected (63 observed to 37 expected), and corynexochids and ptychopariids less often (12 observed to 19 expected, and 18 observed to 30 expected). All other trilobite orders show counts close to those expected (Figure 9).

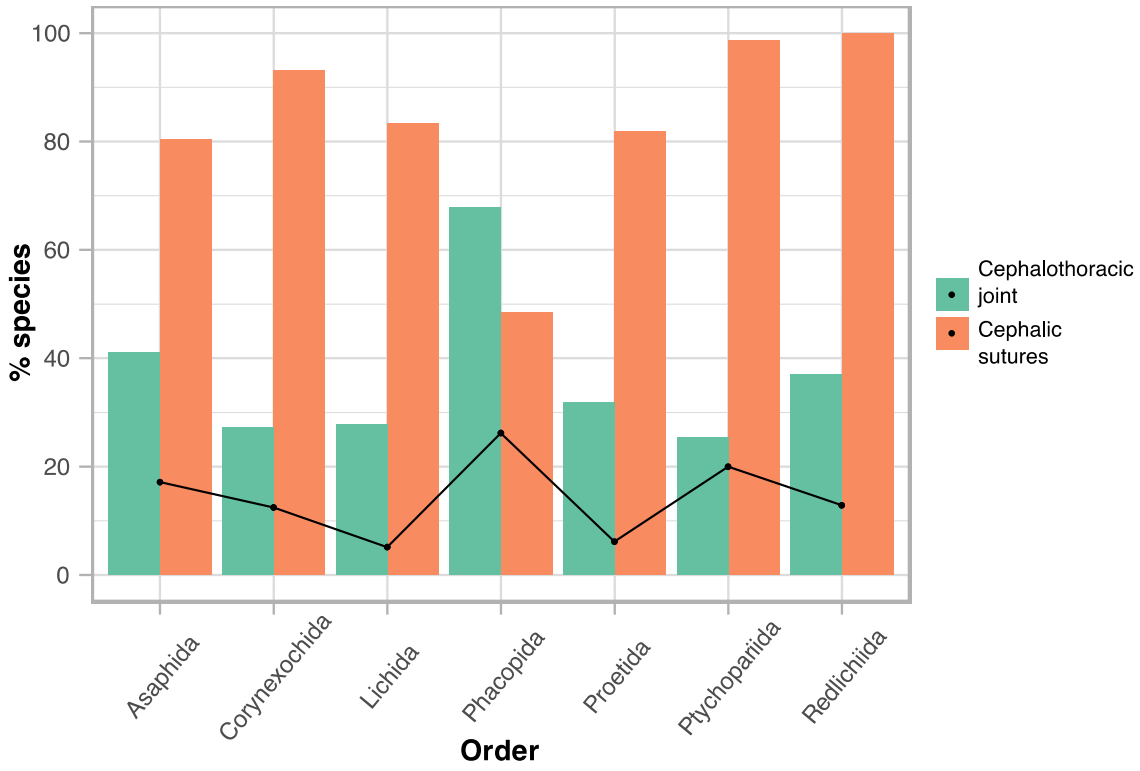


FIGURE 9. Percentage of species in each trilobite order (n=355) displaying the two styles of moulting (opening of cephalic sutures, and opening of the cephalothoracic joint), plotted against sampling (black line; the proportion of species sampled that belong to each order).

Variability in trilobite moulting characteristics (i.e., the specific disarticulations produced during moulting) is relatively consistent across all orders. All orders display each of the six moulting characteristics (Figure 10), even with the lowest sample size of 18 species (Lichida). Moulting behaviour, movements during exuviation, and moulting configurations are therefore extremely variable across all of Trilobita. Opening of the facial sutures is the most common moulting characteristic for all orders excepting Phacopida, meaning most of the species that used the cephalic sutures for moulting (Figures 6, 9) did so by disarticulating the librigenae (congruent with Figure 7). Opening of the ventral sutures and disarticulation of the rostral plate is also very common for Redlichiida (59% of species), meaning these sutures were important in redlichiids for moulting. Disarticulation of the cephalon is the most common moulting characteristic in phacopids (60%, Figure 10) because they more commonly opened the cephalothoracic joint (Figure 9). Proportions of the remaining moulting characteristics are reasonably consistent, and relatively uncommon, within all orders. Disarticulation of the pygidium in moult configurations varies between 26% of species at its highest incidence in Asaphida, to 5% in Proetida. Thoracic disarticulation is slightly more common, varying between 14% (Corynexochida) and 28% (Ptychopariida) of species. Opening of the cephalothoracic joint in tandem with the facial sutures, causing disarticulation of the cranidium, is relatively common in Redlichiida (35% of species) (Figure 10), but less so in other orders. Chi-squared analyses (Appendix 4, Table 2) indicate that the orders have significantly different numbers of species displaying opening of the ventral sutures ($p=1.3x10^{-9}$), facial sutures ($p=0.0038$), and cephalic disarticulation ($p=6.8x10^{-14}$). These are significant at the 0.05 threshold (with Bonferroni correction $\alpha=0.0083$). For opening of the ventral sutures in moulting this relates to its much greater prevalence in Redlichiida than expected (27 observed to 8 expected species), and low incidence in Phacopida (4 observed to 13 expected). For

opening of the facial sutures this results from the same trends seen for the broader styles of moulting (Appendix 4, Table 1), namely a greater count than expected in Corynexochida and Ptychopariida, and fewer than expected in Phacopida (Appendix 4, Table 2). The inverse is true for disarticulation of the cephalon, although Redlichiida also has fewer species showing this moulting characteristic than expected (2 observed to 15 expected). The remaining three moulting characteristics do not show significant results, indicating that the number of species displaying them do not vary between the orders.

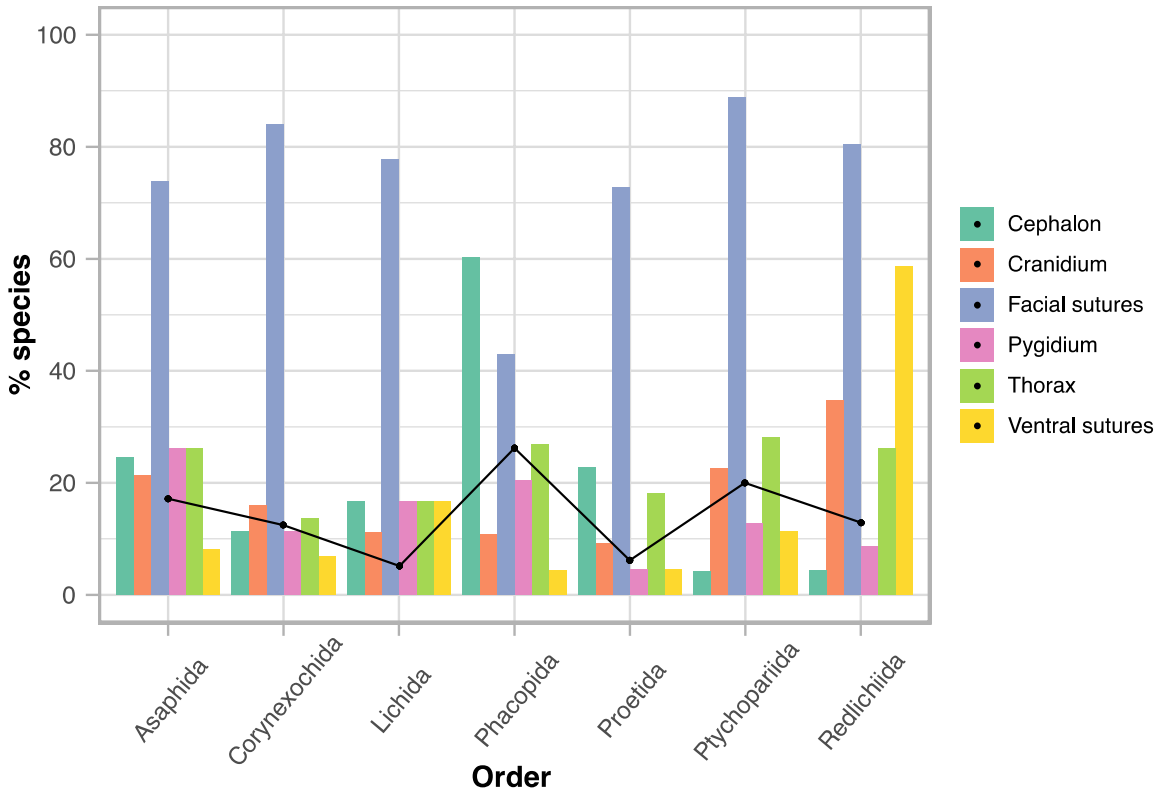


FIGURE 10. Percentage of species in each trilobite order (n=355) displaying each of the six different moulting characteristics (data legend), plotted against sampling (black line; the proportion of species sampled that belong to each order).

Differences in the moulting behaviours of the trilobite orders are more readily apparent when condensing the data to the 'normal' moulting characteristic of each species. This is the moulting characteristic most commonly observed for each species (Figure 11).

Moulting through opening of the facial sutures is again the most common characteristic for all orders (67-82% of species) except Phacopida (35%), which is dominated by a prevalence of cephalic disarticulation (57%). However, the other moulting characteristics display lower prevalences than when plotting all the data (Figure 10). Only 33% of redlichiids usually open the ventral sutures for moulting, compared to the 59% of species that show this characteristic in any of their sampled specimens (Figures 10, 11). Very few species show thoracic or pygidial disarticulation as their most commonly observed moulting characteristic, although these are slightly more common for Phacopida. Disarticulation of the cranidium for moulting, despite being reasonably prevalent in the sample as a whole (Figure 10), is not often a main moulting characteristic, with only Ptychopariida and Redlichiida containing 15% of species for which this is the case (Figure 11). The chi-squared analyses and corrected α-level show the same results (Appendix 4, Table 3) as for the analyses using all the data (Appendix 4, Table 2). The orders show significant differences in the number of species displaying rostral plate ($p=8.8x10^{-9}$) and facial suture opening ($p=0.0022$), and cephalic disarticulation ($p=9.9x10^{-14}$), with deviations in the same orders seemingly causing this result. Table 2 summarises the most commonly observed moulting characteristics and sampling for the seven trilobite orders.

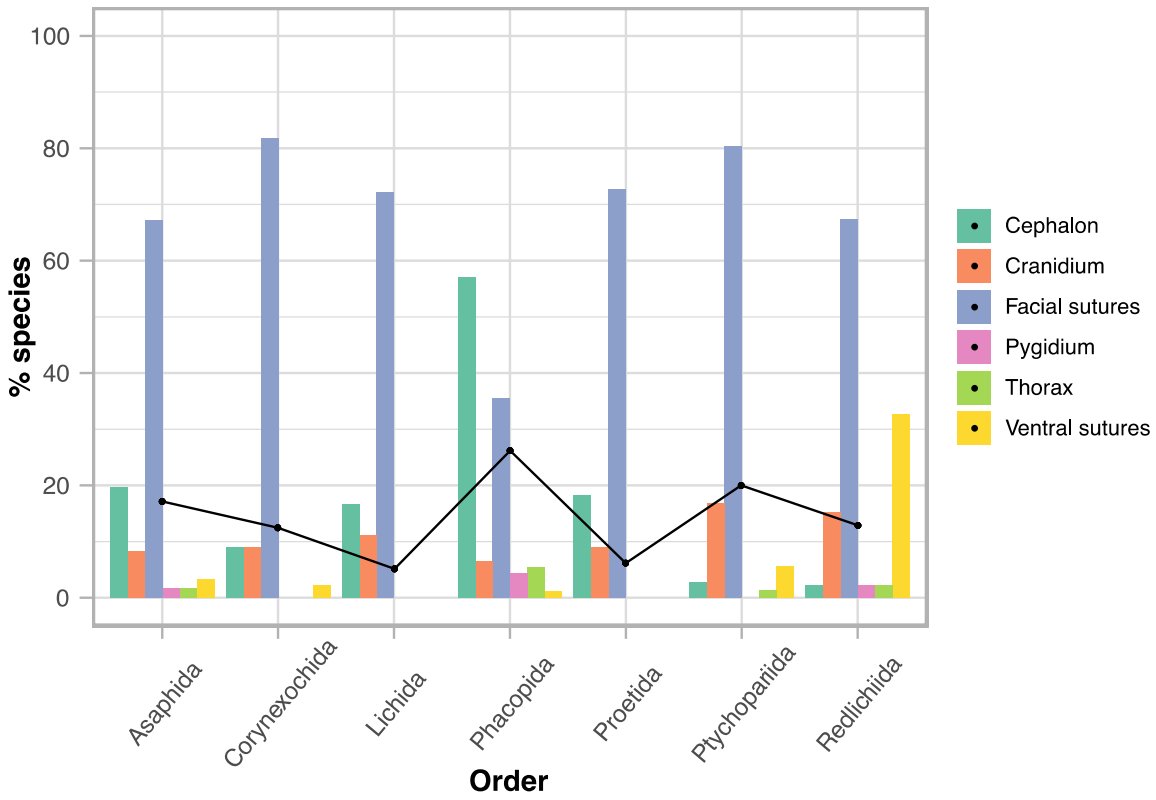


FIGURE 11. Percentage of species in each trilobite order (n=355) for which each moulting characteristic was the 'normal' (data legend); i.e., that characteristic most commonly observed in the moult configurations of each species, plotted against sampling (black line; the proportion of species sampled that belong to each order).

TABLE 2. Summary of moulting behaviour for the seven trilobite orders.

Order[1]	**Usual style of moulting**	**Moulting characteristics**[2]	**# species**	**# specimens**	**Geological range**
Asaphida	Cephalic sutures	1. Facial sutures =2. Thorax =2. Pygidium 3. Cephalon 4. Cranidium 5. Ventral sutures	61	150	Miaolingian – upper Silurian
Corynexochida	Cephalic sutures	1. Facial sutures 2. Cranidium 3. Thorax =4. Cephalon =4. Pygidium 5. Ventral sutures	44	112	Cambian Series 2 – Lower Devonian

Lichida	Cephalic sutures	1. Facial sutures =2. Cephalon =2. Ventral sutures =2. Thorax =2. Pygidium 3. Cranidium	18	33	Middle Ordovician – Middle Devonian
Phacopida	Cephalothoracic joint	1. Cephalon 2. Facial sutures 3. Thorax 4. Pygidium 5. Cranidium 6. Ventral sutures	93	140	Middle Ordovician – Upper Devonian
Proetida	Cephalic sutures	1. Facial sutures 2. Cephalon 3. Thorax 4. Cranidium =5. Pygidium =5. Ventral sutures	22	21	Upper Ordovician – lower Permian
Ptychopariida	Cephalic sutures	1. Facial sutures 2. Thorax 3. Cranidium 4. Pygidium 5. Ventral sutures 6. Cephalon	71	169	Cambrian Series 2 – Upper Ordovician
Redlichiida	Cephalic sutures	1. Facial sutures 2. Ventral sutures 3. Cranidium 4. Thorax 5. Pygidium 6. Cephalon	46	207	Cambrian Series 2 – Miaolingian

[1]For each order the number of species and specimens representing them in the dataset is given, and their geological age range. [2]The six moulting characteristics are in sequence from most to least common amongst species of that order.

Superfamilial/familial-level moulting variability

The well-sampled trilobite orders show some differences in the moulting characteristics observed within their constituent families or superfamilies (Figure 12).

Familial/superfamilial moulting variability seems relatively unaffected by sampling, as many of the clades with smaller sample sizes show almost all of the characteristics. Better sampled clades do not necessarily show more characteristics (Figure 12), and all orders show high variability in moulting behaviour. Phacopid families are notably disparate in their moulting characteristics. Seven phacopid families show a bias to cephalic

disarticulation over opening of the facial sutures for moulting (see the total-group phacopid data; Figures. 9-11), with only the Calymenidae, Cheiruridae and Encrinuridae showing the reverse, although all families contain species with moult configurations with disarticulated cephala (Figure 12.1). Thoracic disarticulation is relatively common across all phacopid families (>15% of species). The Acastidae and Dalmanitidae show high levels of pygidial disarticulation (33-50%), but they, along with the Pliomeridae, Calmoniidae and Phacopidae do not show cranidial disarticulation. Only two families (the Calymenidae and Pliomeridae) contain species that use the ventral sutures in moulting (Figure 12.1). The better sampled asaphid families show a tendency towards opening the facial sutures for moulting, except the Raphiophoridae which show high moulting variability, and the Cyclopygidae which only display disarticulation of the cephalon and pygidium (Figure 12.2). Within the Corynexochida (Figure 12.3) the Dorypygidae and Styginidae show greater variability in moulting characteristics, with a higher prevalence of thoracic disarticulation, as well as separation of the cranidium in the latter and the Oryctocephalidae. The Ptychopariida and Redlichiida (Figure 12.4, 12.5) displayed particularly high levels of variability, but this may be because their sample sizes meant testing at the superfamily-level. Although, it is probably a more true representation to consider the Ptychopariida and Redlichiida at the superfamily or family-level as these orders are widely considered to represent para- or polyphyletic groupings with unclear phylogenetic relationships (Fortey, 2001), and so order-level analyses may have lower accuracies for the Redlichiida and Ptychopariida. Ptychopariid superfamilies show relatively uniform variability in the six moulting characteristics with opening of the facial sutures being most common, although the Olenoidea and Ellipsocephaloidea contain higher proportions of species showing thoracic and cranidial disarticulation (Figure 12.4). All redlichiid superfamilies show disproportionately high incidences of ventral suture

opening for moulting. This is observable in at least 50% of species belonging to all superfamilies and is as common or more so than opening of the facial sutures for three out of five superfamilies (Figure 12.5). The Emuelloidea, Paradoxidoidea and Redlichioidea also show notably high proportions of cranidial and thoracic disarticulation.

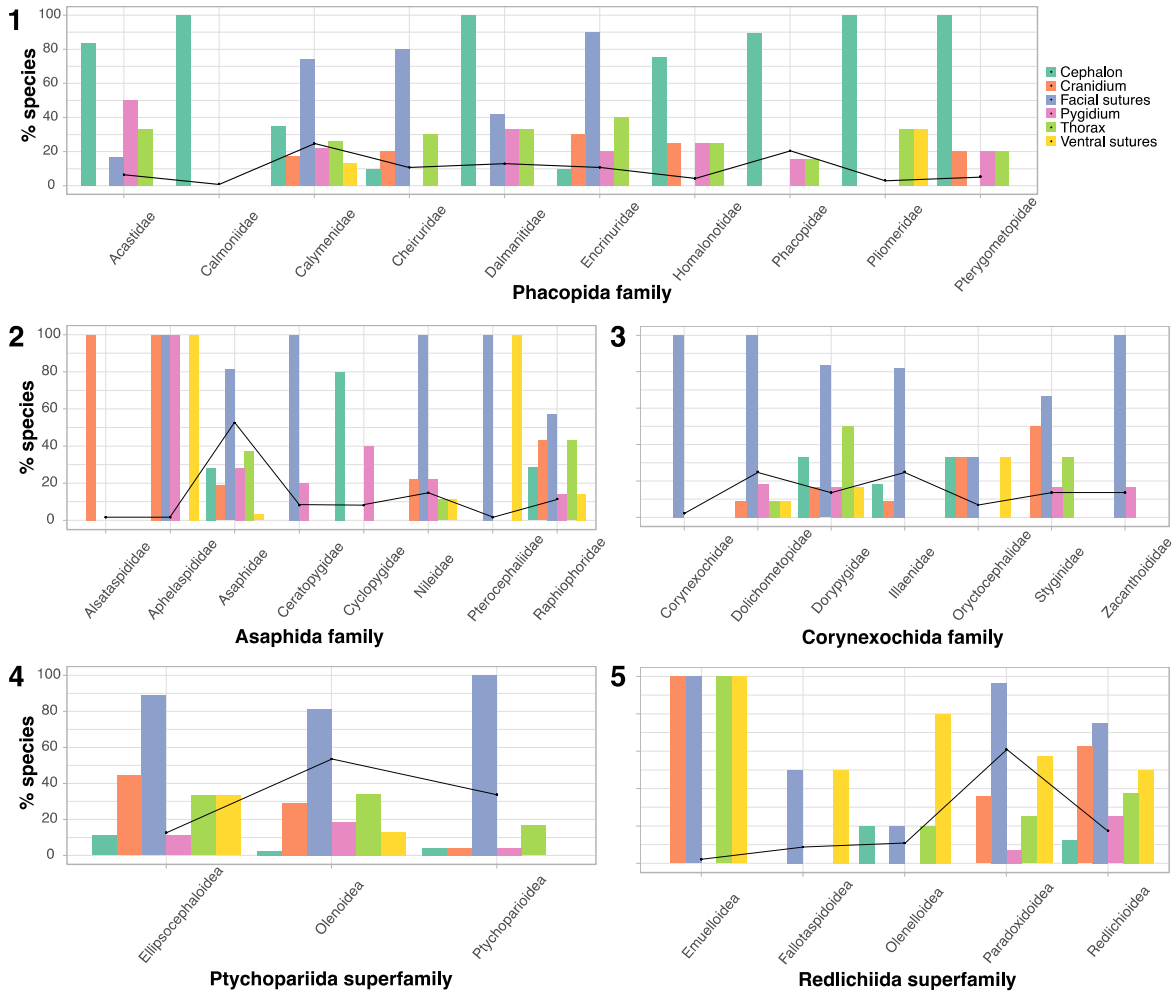


FIGURE 12. Percentage of species in each trilobite family or superfamily displaying the six different moulting characteristic disarticulations (data legend applies to all graphs), plotted against sampling (black line; the proportion of species sampled for the order that belong to each family or superfamily). The Lichida and Proetida are not included owing to their relatively low sample sizes. 1: Phacopida families, n=93; 2: Asaphida families, n=61; 3: Corynexochida families, n=44; 4: Ptychopariida superfamilies, n=71; 5: Redlichiida superfamilies, n=46. Lichiida and Proetida not included because their low sample sizes make them uninformative at the family-level.

The chi-squared analyses show little significant variation in these moulting characteristic occurrences within each order (Appendix 4, Table 4). Only the number of species opening the ventral sutures ($p=0.035$) and disarticulating the cephalon ($p=0.022$) during moulting vary significantly between asaphid families, and the facial sutures ($p=0.0036$) and cephalon ($p=0.002$) for Phacopida (Appendix 4, Table 4). For the cephalic disarticulation in Asaphida this is likely due to it occurring more often than expected in the Cyclopygidae and less than in the Nileidae, and in Phacopida this is more common than expected in the Pliomeridae, Acastidae, Dalmanitidae, Phacopidae and Pterygometopidae, but less than in the Calymenidae, Cheiruridae and Encrinuridae. Opening of the facial sutures shows the opposite pattern to this in Phacopida. For the Corynexochida, Ptychopariida, and Redlichiida the sampled families or superfamilies do not significantly vary in their displayed moulting characteristics.

Intraspecific moulting variability with taxonomy

Intraspecific moulting variability, or the proportion of species displaying multiple moulting characteristics, varies between the orders. Three of the orders (Asaphida, Phacopida, and Redlichiida) contain species that display all six of the moulting characteristics (Figure 13). These orders therefore appear to show greater intraspecific moulting variation than the others. Asaphida and Phacopida have large sample sizes, however, Ptychopariida has the second-largest sample size and contains only species displaying up to five of the moulting characteristics, while Lichida has the lowest sample size and contains a number of species showing four of the moulting characteristics (Figure 13). Overall, the patterns of intraspecific moulting variation are reasonably consistent across the trilobite orders. 43-77% of trilobite species display only a single moulting characteristic (see sampling discussion for Figure 5), and for all orders except Redlichiida

(with 80%), >90% of species displayed 3 or fewer moulting characteristics (Figure 13). Redlichiida is therefore the most intraspecifically variable order for moulting behaviour. It is uncommon for trilobite species to show more than two of the moulting characteristics, and so most species are either not at all or only moderately intraspecifically variable.

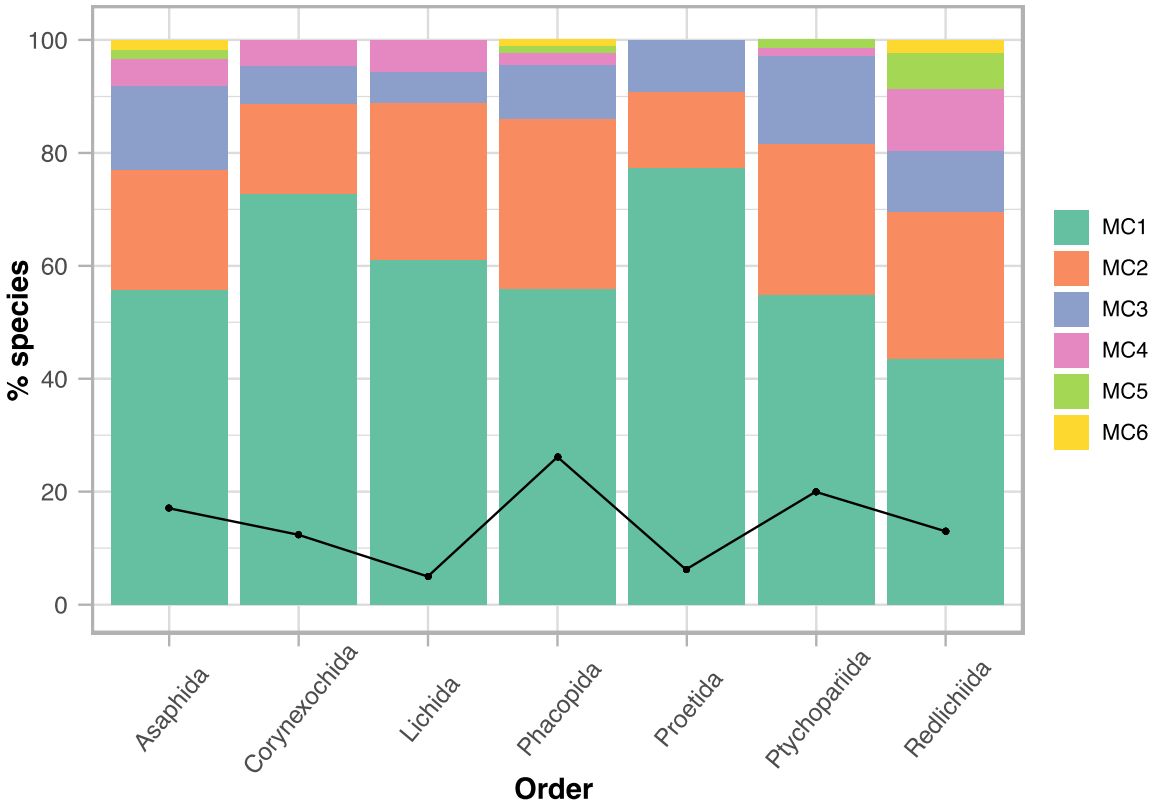


FIGURE 13. Percentage of species in each trilobite order (n=355) displaying 1 to 6 of the different moulting characteristics (MC1-6; data legend), plotted against sampling (black line; the proportion of species sampled that belong to each order). Number of moulting characteristics observed for a species functions as a proxy measure for its moulting variability.

However, the number of moulting characteristics shown per species does increase with the number of specimens sampled (Figure 14). This is supported by significant results from correlation tests (r^2: 0.48, $p=1.03 \times 10^{-19}$; Spearman's Rank: 0.49, $p=3.046 \times 10^{-21}$; Kendall's Tau: 0.44, $p=1.519 \times 10^{-32}$), however these values indicate only a moderate positive

correlation between specimen sampling and the number of moulting characteristics shown (i.e., below 0.5). This suggests that intraspecific moulting variability would increase with better specimen-level sampling, but likely only at the lower end of sampling (1-10 specimens), and not at higher levels of sampling, which do not fit the trend line as well (Figure 14).

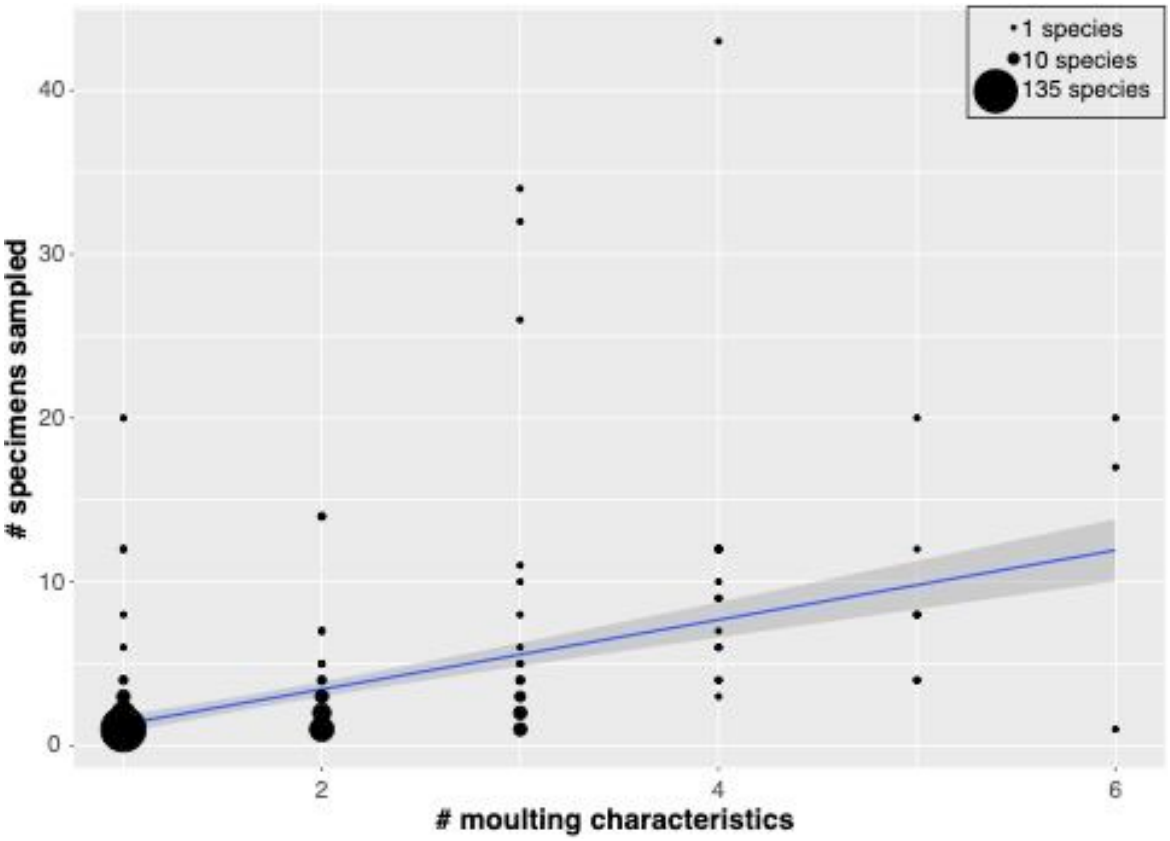


FIGURE 14. Number of specimens sampled for each trilobite species (y-axis), plotted against the number of the described moulting characteristics each species showed (x-axis). The size of each point represents the number of species sampled at that position.

Interspecific moulting variability through time

Raw total group trilobite species diversity across the Palaeozoic closely tracks the numbers of species sampled within the dataset (Figure 15; Appendix 3, Table 2). The lines are slightly offset from one another (i.e., the total diversity peaks occur slightly after the

sample diversity peaks), likely due to the difference in time bin dates between this study and Fossilworks (2018). An R^2 test shows that these are significantly correlated (0.68, p=0.0076), as do non-parametric tests for correlation (Spearman's rank: 0.77, p=0.0013; Kendall's tau: 0.58, p=0.0042). The raw total group diversity curve suggests trilobite diversity declined overall from the end-Cambrian, with these data not showing an early Ordovician radiation (unlike that observed for other groups during the Great Ordovician Biodiversification Event; see Servais et al., 2010), although there were clear diversity peaks in the Late Ordovician and Early Devonian (potentially reflecting the origination, and then diversification, of Phacopida; Crônier, 2013; and Proetida during the latter Epoch; Stubblefield, 1959). Figure 15 also supports the notion of trilobites having dramatically radiated early in the Cambrian (Webster, 2007). When corrected for sampling (using Shareholder Quorum Subsampling), the total group diversity shows similar trends (Figure 15), also closely tracing the sample diversity (again, with a significant positive correlation; r^2: 0.58, p=0.03; Spearman's rank: 0.7, p=0.0051; Kendall's tau: 0.55, p=0.0059), except for some slight divergences in the Ordovician.

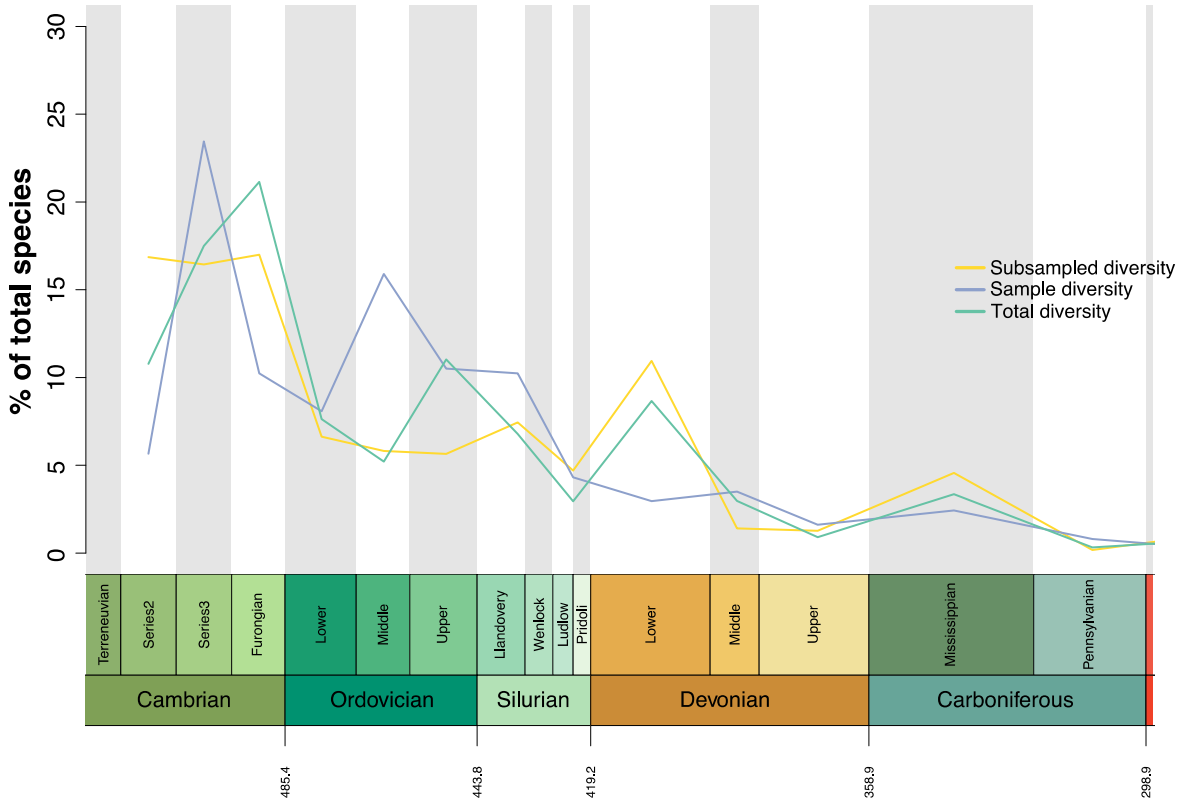


FIGURE 15. Trilobite species diversity through the Palaeozoic plotted as a percentage of the total diversity. Total group recorded diversity (green, n=8309; downloaded from Fossilworks; Kiessling et al., 2018) compared to dataset sample diversity (blue, n=370, sample size higher than dataset species total because taxa crossing multiple time bins were counted more than once). The yellow line represents the total group diversity corrected for sampling by using Shareholder Quorum Subsampling (using Fossilworks, 2018).

The proportion of all trilobite species displaying each of the six moulting characteristics varied through the Palaeozoic. Opening of the facial sutures and disarticulation of the cephalon during moulting display almost opposing trends (Figure 16). The former is extremely common from the early Cambrian to end Silurian (>50% of species), although in the Cambrian Epoch 2 moulting seems to be more variable, with all characteristics except pygidial disarticulation exhibiting a small peak (which do not correspond with a peak in sampling). When disarticulation of the cephalon is extremely prevalent during the

Devonian opening of the facial sutures is less common. Cephalic disarticulation also became conspicuously more common over the Ordovician Period. The chi-squared analyses over geological periods (Permian excluded due to sample size) indicate that the number of species displaying both of these characteristics during moulting is different to that expected (Appendix 4, Table 5; $p=0.0046$ and 6.1×10^{-18} respectively). This is also true of opening of the ventral sutures ($p=0.00053$), which is very common in the early Cambrian (48% of species in Epoch 2, decreasing to 24% in the Miaolingian), became less prevalent during the Ordovician and Silurian, and is absent by the start of the Devonian (Figure 16). The remaining three moulting characteristics follow similar patterns over a longer trajectory, becoming generally less prevalent from the early Silurian, but these do not have significant chi-squared results. None of the characteristics closely track the total number of species sampled (black lines, Figures 16, 17). The same chi-squared results were also produced when these data were further divided into geological Epoch bins. Opening of the facial sutures, ventral sutures, and cephalic disarticulation vary significantly (Appendix 4, Table 6; $p=0.018$, 0.0011, and 7.03×10^{-13} respectively), resulting from much the same deviations from expected values as described above.

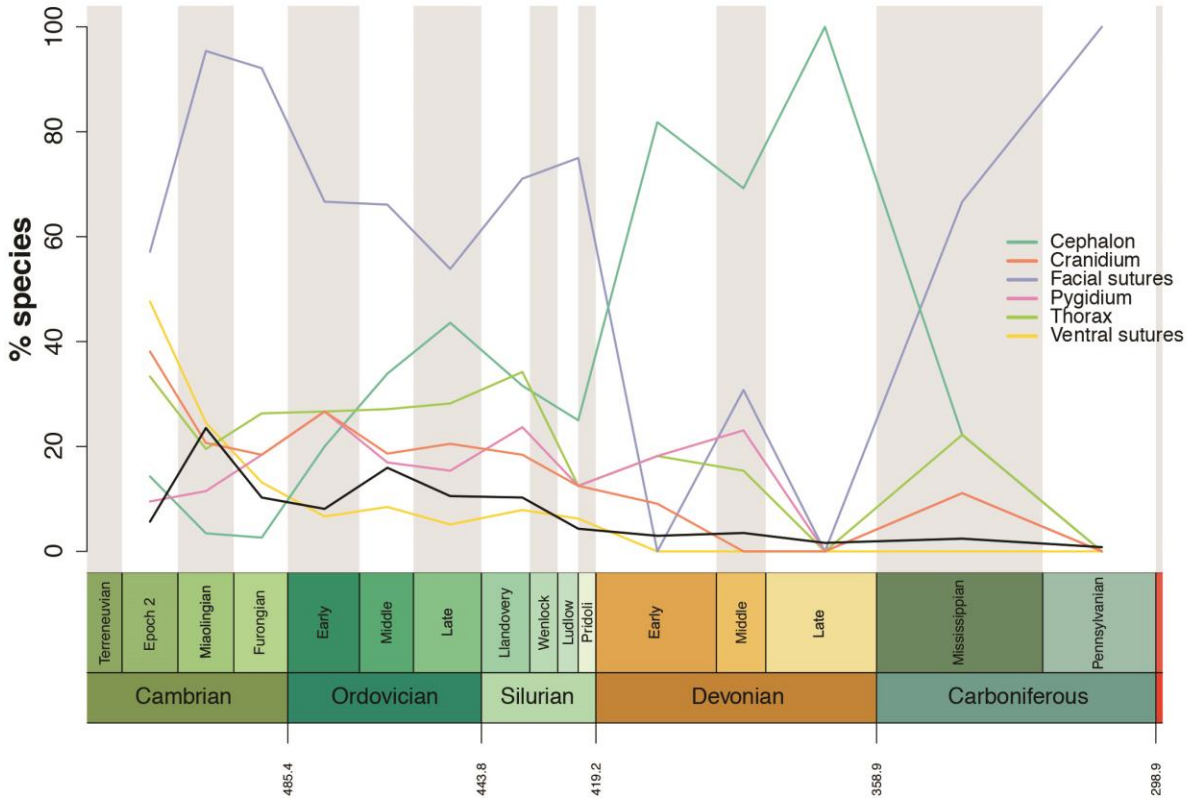


FIGURE 16. Percentage of trilobite species (n=370, sample size higher than dataset species total because taxa crossing multiple time bins were counted more than once) in each geological Epoch displaying each of the six different moulting characteristics (see data legend), plotted with total number of species sampled (black).

Plotting the 'normal' moulting characteristic for the species in each geological Epoch results in similar patterns (Figure 17 compared to Figure 16). Disarticulation of the cephalon again shows an opposing pattern to opening of the facial sutures, with the former becoming more prevalent during the Devonian (82-100% of species). The chi-squared results confirm that these two characteristics significantly vary through the Palaeozoic (Appendix 4, Table 6; $p=0.00098$ for the facial sutures and 1.03×10^{-15} for the cephalon). Presenting only the most common moulting characteristic for each species means that disarticulation of the thorax, pygidium, or cranidium decreases from around 10-40% species (Figure 16) to under 10% for the thorax and pygidium, and 18% of species at the

most for the cranidium (Figure 17). The chi-squared results suggest that any variation in these three characteristics through time is again not significant (Appendix 4, Table 7). The proportion of species most commonly displaying open ventral sutures in moults is also lower throughout the Palaeozoic, with no species using this as their main mode of moulting after the Late Ordovician (despite there being moult specimens with open ventral sutures until the Early Devonian; Figure 16). However, opening the ventral sutures is notably common during the Cambrian Epoch 2 at 43% of species (Figure 17), producing a significant chi-squared result (Appendix 4, Table 7; p=0.00096).

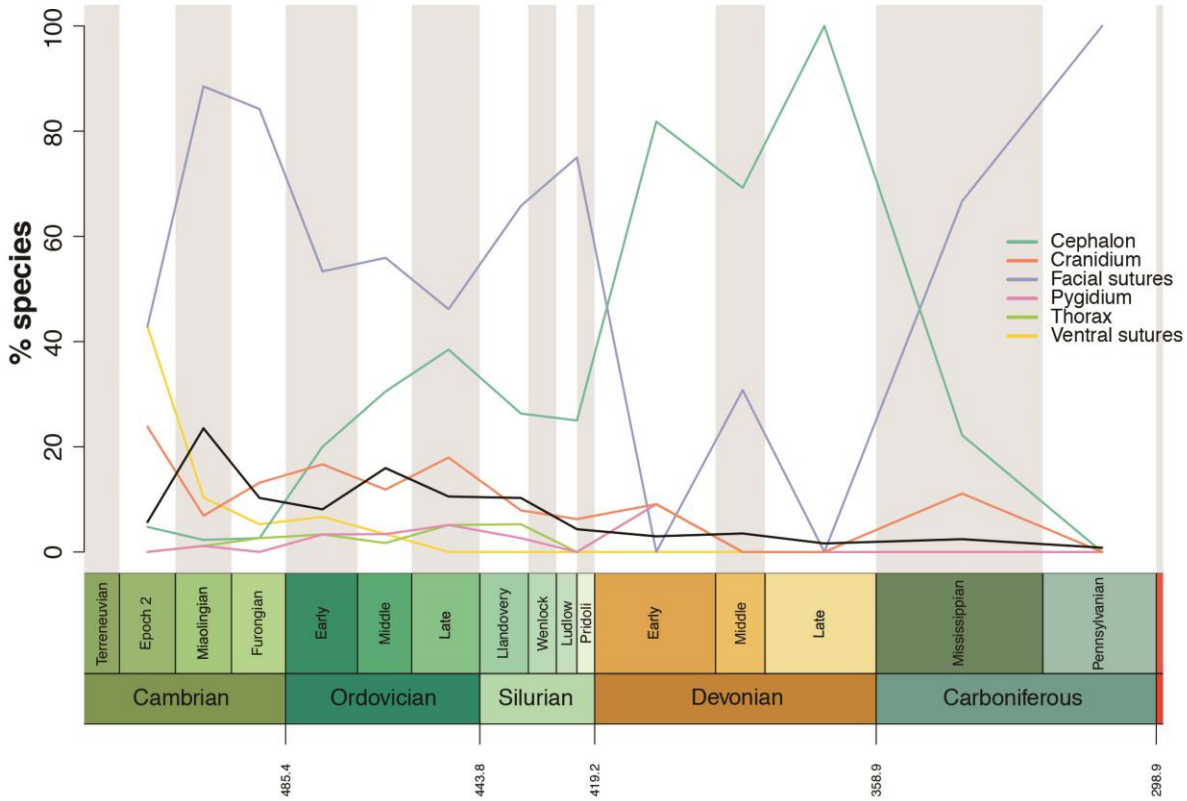


FIGURE 17. Percentage of trilobite species in each geological Epoch for which each moulting characteristic was the 'normal' (data legend); i.e., the characteristic most commonly observed in the moult configurations of each species. Plotted with total number of species sampled (black).

Intraspecific moulting variability through time

Intraspecific moulting variability also varies temporally within the dataset (Figure 18). In general, intraspecific moulting variability decreased over the Palaeozoic, going from 57% of species showing a single characteristic in the Cambrian Epoch 2 to 78% in the Mississippian (Figure 18). All Epochs from the early Cambrian to early Silurian contain species that displayed at least four moulting characteristics (between 29% in the Cambrian Epoch 2 and 3% in the Furongian), while the late Silurian and Mississippian both contain species showing 3 characteristics. The proportion of species showing 2 moulting characteristics varies stochastically through the Palaeozoic, but the overall proportion showing 3-6 moulting characteristics decreases through time (Figure 18). From the late Silurian this may be a product of smaller sample sizes, however this is not consistent with sampling bias prior to the Silurian. For example, the Cambrian Epoch 2 shows a high level of intraspecific moulting variation, with by far the highest proportion of species showing 3+ moulting characteristics (29%), but the lowest level of sampling (21 species) before the late Silurian. While the mean average number of moulting characteristics shown by the species is higher at this time (2.24) than in any other Epoch (1 to 1.89), the broad 95% confidence intervals overlap, thereby suggesting the means do not differ and that intraspecific moulting variability does not change through the Palaeozoic. However, these appear more different when comparing the means in the first half of the Palaeozoic to later Epochs. This is supported by ANOVA and Kruskal-Wallis tests comparing the means of the geological Periods, which are close to the significance threshold ($p=0.064$ for both). Decreasing moulting variability through the Palaeozoic (i.e., increasing proportion of 1 moulting characteristic, and decrease in 3-6) also trends with decreasing diversity through time (both in the sample, but also total group and subsampled diversity; see Figure 15).

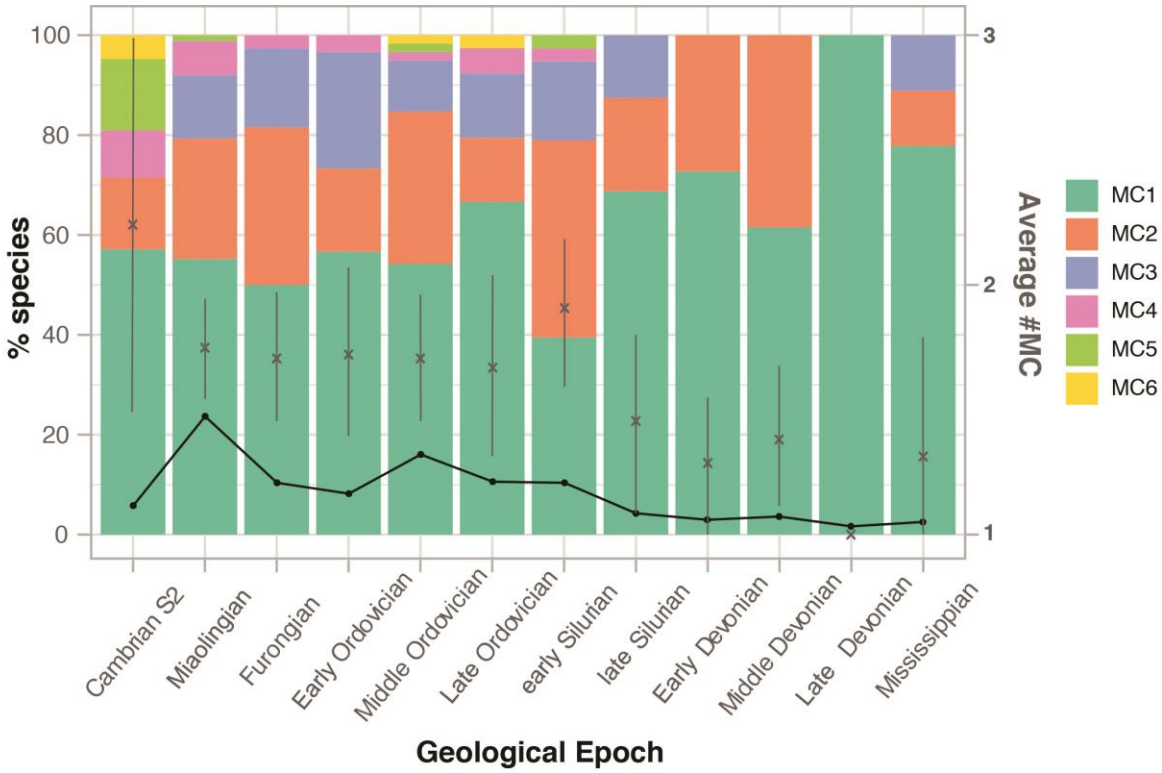


FIGURE 18. Percentage of species in each geological Epoch displaying 1 to 6 of the moulting characteristics (MC1-6; data legend), plotted against sampling (black line; the proportion of species sampled in each Epoch). Grey crosses and second y-axis represent the mean average number of moulting characteristics for species observed in each Epoch, with the 95% confidence intervals given. N=367; Pennsylvanian and early Permian removed due to sample sizes being too low for robust statistic analyses, species sample size higher than dataset total because taxa crossing Epoch boundaries were counted multiple times. Number of moulting characteristics observed functions as a proxy measure for intraspecific moulting variability.

Results summary

Trilobite moulting behaviour is interspecifically variable within all trilobite orders (Figures 6, 7, 9-11), and at lower taxonomic levels (Figure 12). Firstly, around 20% of all species show the use of both cephalic sutures and the cephalothoracic joint for moulting (Figure 6), despite these representing quite different methods of creating an anterior exuvial gape. Trilobite species commonly display moulting using the facial sutures (Figures 6, 7, 9). It is

thought that the facial sutures originated as a tool for moulting (Henningsmoen, 1975; Whittington, 1990), and are present in most species and in some closely-related, non-biomineralised artiopodans (Stubblefield, 1959; Hunda et al., 2006; Hou et al., 2017; Du et al., 2018), potentially even being the ancestral condition uniting these groups with trilobites (Du et al., 2018). All six moulting characteristics are seen in every order (Figure 10), and the family/superfamily-level data also shows high levels of interspecific variability with most groups showing at least 3 of the moulting characteristics (Figure 12). Moulting behaviour is similarly variable when plotting the data through geological time. All six moulting characteristics occur throughout the Palaeozoic, and usually during each Epoch, although ventral sutures were not used from the Devonian onwards, Figure 16). The sampled species are also generally intraspecifically variable in moulting behaviour, with moults of the same species often displaying more than one characteristic (Figure 18). This includes >30% of sampled species for most of the Palaeozoic, with the mean number of moulting characteristics per species in each Epoch ranging from 1.3-2.2. Trilobite orders are not consistent in their intraspecific moulting variability (Figure 13). All seven orders contain species showing at least four moulting characteristics, but differ in the proportion of species displaying 2 or more, with Redlichiida the most at 57% of species, to Proetida the least at 23% (Figure 13).

DISCUSSION

Trilobite moulting variability and diversity

This study presents the first broad-scale quantitative exploration of trilobite exoskeleton moulting behaviours. Previous qualitative studies named moult configurations and reconstructed behaviours, while noting that despite the overall similarity of bodyforms within Trilobita, their moulting behaviour is seemingly highly variable (see

Henningsmoen, 1975; Whittington, 1990; Brandt, 2002; Hughes, 2003; Daley and Drage, 2016; Drage and Daley, 2016; Drage et al., 2018a). This is in comparison to groups like chelicerates or decapod crustaceans which have several bodyforms and each has one specialised mode of moulting (see Daley and Drage, 2016, and references therein). This study demonstrates for the first time the unusually high behavioural variability in trilobite moulting, confirming the preliminary work of Daley and Drage (2016) that identified this from a smaller dataset over the Cambrian and Ordovician.

A number of differences in moulting variability between taxonomic groups and throughout the Palaeozoic have been identified and are summarised at the end of the Results section. These may be affected by sampling given the moderate positive correlation between specimen sampling and the number of moulting characteristics displayed (Figure 14). However, Redlichiida and Proetida are at the extremes of intraspecific moulting variability, but did not have the highest or lowest sample sizes (Figure 13). Intraspecific moulting variability also slightly decreases through the Palaeozoic (Figure 18), with the proportion of species showing only one moulting characteristic increasing through time. The Cambrian Epoch 2 shows the highest levels of intraspecific moulting variability. This corresponds with their elevated diversity (total group and sampled) in the Cambrian and Early Ordovician (Figure 15) and high levels of morphological variability (Fortey and Owens, 1999; Webster, 2007), presumably due to their ecological expansion after the Cambrian Explosion. Behavioural flexibility, particularly for a risky recurrent event like moulting, might be adaptive when expanding into new habitats, such as during the Cambrian. Both trilobite diversity and intraspecific moulting variability slowly decline after the Cambro-Ordovician, before the extinction of trilobites in the Permian (Song et al., 2013). This decrease in moulting variability is not simply a result of the reduced species

diversity because groups with the highest variability in moulting behaviour generally went extinct earlier, such as the Redlichiida (Stubblefield, 1959). Only the Proetida survived until the Permian (Fortey and Owens, 1975; Mikulic, 1981; Owens, 2003), but show low intraspecific variability (Figure 13) and comparably low morphological variability, particularly in the few surviving groups after the early Carboniferous (Fortey and Owens, 1975; Owens, 2003; Webster, 2007; Lerosey-Aubril and Feist, 2012). This suggests a curtailment in moulting behaviour variability and potentially specialisation in moulting behaviour over the Permian, which may have been an evolutionary advantage of the less intraspecifically flexible proetids. Brandt (2002) suggested that moulting flexibility may have been detrimental to trilobite evolutionary success, because later-evolving arthropod groups were more specialised and efficient in their moulting behaviours. As such, the less behaviourally diverse proetids may therefore have survived for such a prolonged period of time because they were more specialised. However, Lerosey-Aubril and Feist (2012) found that proetids underwent a generic-level diversification following Devonian and Carboniferous extinctions accompanied by a burst of adaptations to more ecological niches; as such, further investigation of Permo-Carboniferous proetid moulting behaviour might reveal an increase in flexibility comparable to the early Cambrian. Proetids sampled here mainly correspond to the Proetidae, although several aulacopleurid species were included which have an uncertain phylogenetic affinity (Adrain, 2011, removed them from Proetida, but phylogenetic analyses argue for their proetid assignment; Lamsdell and Selden, 2015) and this may have affected the results. In any case, future increased sampling of the Proetida, if suitable material were available, would be informative on late Palaeozoic changes in moulting behaviour.

The initial flexibility in moulting behaviour may have been adaptive for trilobites owing to their generally thick, heavily-calcified exoskeleton, which would make moulting more problematic than for other arthropod groups (Miller and Clarkson, 1980; Fortey and Wilmot, 1991). This provided increased protection from predation and parasitism during the Cambrian Explosion and later in the Palaeozoic (Brandt, 2002), but was balanced by having exuvial gapes that were presumably harder to create. Trilobites may have therefore been prone to moulting failures, where the planes of weakness (facial and ventral sutures, and/or the cephalothoracic joint) did not open and the animal was trapped, necessitating the use of other 'back-up' moulting behaviours (McNamara, 1986; Brandt, 2002; Budil and Bruthansová, 2005; Paterson et al., 2007; Tortello and Clarkson, 2008). That this behavioural flexibility was adaptive and a response to dire circumstances is supported by looking at the 'normal' moulting behaviours of the sampled species. Most species known from multiple specimens (42% of the dataset, Figure 14) have one characteristic most commonly associated with their moult configurations, with other characteristics being generally rarer (Figure 11). Facial sutures (usually present in most species; Henningsmoen, 1975; Whittington, 1990; Brandt, 2002; Du et al., 2018) and the cephalothoracic joint are most commonly used for moulting, with a much smaller number of species usually showing cranidial disarticulation or opening of the ventral sutures (the latter being almost confined to Redlichiida; Figure 11). The lack of significant temporal or taxonomic variation (Appendix 4, Tables 1-7) in the percentage of species showing thoracic or pygidial disarticulation during moulting indicate that these characteristics were rarely involved in moulting and occurred incidentally, in association with another moulting characteristic. The comparable weakness at the articulation joints between thoracic segments, and the pygidiothoracic joint (see Whittington, 1980; McNamara and Rudkin, 1984; Drage et al., 2018a), meant these areas could disarticulate fairly readily when put

under pressure, such as during moulting. Some authors have considered most post-cephalic disarticulation to be the result of decay and/or post-mortem disturbance of trilobite carcasses, and not always attributed to moulting (e.g., Hunda et al., 2006). Although, decay and disturbance could equally happen to moults pre-preservation, which would also explain the occurrence of thoracic and pygidial disarticulation usually being associated with another moulting characteristic.

Moulting and morphology

Certain aspects of trilobite moulting variability can be explained by morphology and taxonomy. The significant chi-squared analyses for the temporal and taxonomic variation of ventral suture use in moulting (Figures 10, 11, 16, 17; Appendix 4, Tables 2, 3, 5-7) largely result from redlichiids opening the ventral sutures more than expected (27:8, Figure 10). This is because many taxa support a broad and easily recognisable rostral plate often with rostral sutures, a conterminant hypostome, and because the early Cambrian Olenelloidea do not have facial sutures like most trilobites (Stubblefield, 1959; Fortey, 1990). For many redlichiids usage of the functional ventral connective sutures (e.g., rostral, hypostomal) is therefore important for moulting (Fortey, 1990). Redlichiida are reasonably abundant in the early Cambrian (18/21 and 29/87 species in the Cambrian Epoch 2 and Miaolingian respectively) and went extinct soon after (in particular the olenellids; Stubblefield, 1959) explaining the significant Cambrian peak in ventral suture usage and its subsequent decrease in later Epochs (Figure 16). Stubblefield (1959) argued that groups with functioning facial sutures outcompeted these early-evolving redlichiids, and many of these later groups repeatedly lost the rostral sutures (Fortey, 1990), making moulting by ventral sutures alone less frequent. Although it is likely that Redlichiida

represents a para- or polyphyletic grouping (Fortey, 2001), and so further family-level study would be useful for further exploring this result.

Similarly, the significant variation in the proportion of species disarticulating the cephalon is likely linked to the diversity of the Phacopida. This is the only trilobite order with a higher percentage of species displaying disarticulation of the cephalothoracic joint for moulting (68%, Figure 9) than opening of the cephalic sutures (46%; Figures 10, 11). The suborder Phacopina has secondarily fused facial sutures during adulthood (Crônier, 2013; Drage et al., 2018b), preventing the librigenae from being disarticulated for moulting. Phacopines diversified later in the Palaeozoic (25/30 for the Devonian; Crônier, 2013), and repeated convergence of blindness and loss of facial sutures was common in Devonian trilobites (Feist, 1995), likely causing the peak in cephalic moulting at this point (Figures 16, 17). Those phacopid families that retained operable facial sutures, like other trilobite groups, separate the librigenae more often than disarticulating the cephalon in moulting (Figure 12.1). Use of the facial sutures and cephalic disarticulation are mutually exclusive moulting characteristics (together, they equate to cranidial disarticulation), and so the facial suture trends mirror the cephalic data. For the majority of orders and geological Epochs, separation of the librigenae is the most common moulting characteristic, owing to the almost ubiquitous existence of the facial sutures (potentially even as an ancestral condition in Artiopoda; Du et al., 2018), which probably originated as a tool for moulting (Stubblefield, 1959; Henningsmoen, 1975).

Disarticulation of the cranidium mostly follows the same trend as thoracic and pygidial disarticulation, and does not significantly vary or show much prevalence amongst the 'normal' moulting characteristics for the sampled species. This is in contrast to Drage et al.

(2018a), who found that disarticulation of the cranidium was extremely common for moult configurations of *Estaingia bilobata* Pocock 1964, and did not follow the stochastic occurrence of thoracic and pygidial disarticulations (again, presumably due to incidental occurrence during moulting, or because they would have readily occurred due to taphonomic effects pre-preservation). This may be because this characteristic is simply not morphologically or taxonomically linked (at least above species-level), and therefore does not show broader-scale trends.

Sampling a rarely preserved behaviour

Quantitative studies will always be enriched by sampling greater numbers of specimens. For example, this work shows a moderate positive correlation between specimen sampling and intraspecific moulting variability, probably owing to the 58% of species for which only one specimen was recorded (Figure 14). However, it would be almost impossible to sample moulting behaviour for the over 20,000 described species of trilobites (Lerosey-Aubril and Peel, 2018), especially as many are not known from fully-articulated specimens. Further, greater specimen sampling would likely reinforce the major findings of this work as more specimens would be expected to give even higher levels of intraspecific variability. Greater specimen sampling may even strengthen the trend of decreasing variability through time, since the longest surviving group, the proetids, appeared to be comparably specialised in moulting behaviour (Figure 13). In depth sampling of families from certain groups (in particular the ptychopariids and redlichiids) would also allow for analyses to be carried out predominantly at the family-level, which might circumnavigate issues of uncertain high-level trilobite phylogeny (Fortey, 2001).

Increased species-level sampling would likely have little effect on the broad-scale results. There is a significant correlation between sampled species diversity and total group (raw and subsampled) diversity (Figure 15), and these diversity curves are similar to those presented by other researchers (Brandt, 2002; Webster, 2007; Adrain, 2008). Further, with the exception of a couple of orders, taxonomic species sampling is also reasonably similar to total diversity (Figure 8; although data from Fossilworks and the Paleobiology Database are also subject to missing records; Fossilworks, 2018). These factors suggest that the dataset sampling is representative of the 'true' diversity of trilobites through the Palaeozoic, and that the interspecific moulting results are robust. Specimen sampling is also less of an issue than might be supposed. Firstly, specimen sampling is overall underestimated in the results reported here (Figure 14) because accurate counts were impossible to obtain from specimens described in the literature. Secondly, the total number of trilobite specimens actually surveyed and interpreted to produce the dataset was several times greater than the 840 specimens included (in the order of 5-10,000 accessioned specimens). Excepting for Konservat-Lagerstätten, the preservation of an unambiguous moult configuration is reasonably rare, and therefore these data are not available for the majority of Palaeozoic localities, or even many described species. This is because specimens must be mostly articulated (with most sclerites found in association) to be confidently identified as moults, helping to ensure carcasses that have simply been disarticulated prior to preservation (e.g., by water currents) are not falsely included within the dataset. For the majority of species in the dataset multiple specimens were therefore examined, but could not be included because the material was too disarticulated. These singleton species could be excluded from the dataset to improve overall specimen sampling, but, owing to their rarity, all of these are informative and important for broad-scale studies of behaviour. Given the requirement for reasonably articulated specimens, if

exploring trilobite behaviours at a finer-scale than that presented here, it would be logical to look at Konservat-Lagerstätten with their greater abundance of samples (e.g., Drage et al., 2018a).

Finally, the higher-level taxonomy used here is subject to change (Fortey, 1990) because of the problematic phylogenetic relationships of Trilobita. This is owing to widespread cryptogenesis within the group (Stubblefield, 1959) and a paucity of broad-scale phylogenetic studies, although the latter is greatly improving (see Lieberman and Karim, 2010). Hence, a more in-depth study on a family or superfamily-level (such as presented in Figure 12) would likely prove worthwhile for investigating phylogenetic differences in moulting. Further studies on aspects such as moulting and ontogeny (e.g., Drage et al., 2018b; Wolfe and Hegna, 2014) may even help to elucidate these problematic relationships.

CONCLUSIONS

This study presents the first quantification and detailed exploration of total-group trilobite moulting behaviour, and the largest dataset on trilobite behaviour ever assembled. It describes variation in trilobite moulting and tracks broad-scale trends in the occurrence of their characteristic moulting disarticulations. This has been explored both intra- and interspecifically on taxonomic and temporal scales, and compared to diversity estimates through the Palaeozoic. The sample presented here is representative of the total-group (Figure 15), and is currently the largest dataset feasible given the huge number of specimens examined to produce it and the reasonably high preservational requirements of valid specimens. The data capture a broad scale of morphological and taxonomic diversity, as well as rarer examples of moulting.

Despite their reasonably consistent body plan, trilobite moulting behaviour is extremely variable across all orders, superfamilies, and families (Figures 6, 7, 9-12), and throughout the Palaeozoic (Figures 16-18). This represents an inherent moulting flexibility within the group, and within a large proportion of trilobite species. Opening of the facial sutures and separation of the librigenae for moulting is generally ubiquitous across Trilobita because this represents a specialism for moulting found in most of the group (Du et al., 2018). Some groups and time bins show significant differences in the prevalence of moulting characteristics. In particular, Redlichiida from early Cambrian Epochs often moulted by opening the ventral sutures (Figures 11, 17). For many of the early trilobite species (e.g., olenellids) this is due to a morphological constraint, but this characteristic is also seen throughout most of the Palaeozoic and in all orders (Figure 10). Similarly, the Phacopida are biased towards disarticulating the entire cephalon for moulting (Figure 11), and are responsible for peaks in this behaviour during the Ordovician and Devonian when they were particularly diverse (Figure 17). Again, a subgroup of phacopids (the phacopines) were constrained to this behaviour (Drage et al., 2018b), but it is also frequently observed in all of the orders (Figures 7, 9) and throughout the Palaeozoic (Figure 16).

Moulting behaviour flexibility generally appeared to decrease through time in concert with decreasing trilobite diversity (Figures 15, 18). This may reflect high levels of behavioural flexibility (along with morphological variability) during their early radiation and establishment after the Cambrian Explosion, and subsequent specialism in the more conservative later-Palaeozoic groups (such as the long-lived Proetida; Figure 13).

This work conclusively demonstrates that quantitative behavioural hypothesis-testing using the fossil record is not only feasible, but can reveal broad-scale evolutionary trends.

Moulting is of singular importance in the life histories of arthropods, and has been central to their establishment as the most abundant and diverse animal group from the early Cambrian to the present. It has thereby dramatically shaped the evolution of groups like trilobites, and in doing so affected all of Earth's ecosystems, past and present. Quantitative analyses based on large datasets of moulting behaviour, beginning with that presented here, allow us to start to fully understand the evolution and importance of this behaviour in deep time.

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Chapter 4: Morphological controls on trilobite moulting variability

Following on from the previous chapter, this work focuses on the association between moulting and morphology to answer the question "can trilobite moulting behaviour variability be explained by differences in their body proportions, or their number of articulations?" The data indicate significant links between particular morphological aspects and moulting behaviour; providing one explanation for why trilobites were so variable in moulting (as seen in Chapter 3).

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Morphological controls on trilobite moulting variability

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Abstract

All euarthropods have an exoskeleton which protects them from predation and parasitism while acting as a muscle attachment point, but which must be moulted periodically in order to grow. Unlike for other euarthropod groups, trilobite moulting behaviour is uniquely variable, both between species and within individuals of the same species. Trilobites could seemingly disarticulate along any articulation point in the exoskeleton during moulting, and thereby produce a number of moulting configurations preserved in their fossil record. Euarthropod moulting behaviour is intrinsically linked to all aspects of their life histories (including development, survivability, and ecology), and therefore this variability in trilobites must have had evolutionary consequences.

This study uses a large dataset of trilobite moulting behaviour and body proportion measurements to explore the potential relationship between moulting and morphology.

Length/width ratios of the cephalon, cranidium, thorax, pygidium, and the entire body were measured, and thoracic segment number counted, for a sample of 756 moulted exoskeleton specimens representing 355 trilobite species. Each specimen was assigned a moulting characteristic, which represents the general exoskeleton disarticulations made during moulting; disarticulation of the cephalon or cranidium, and opening of the facial or ventral sutures. Analyses were performed to test for significant differences in the average of each morphological aspect between the four moulting characteristics.

Results show that overall shape of the body, including number of thoracic segments, is a significant influence on moulting behaviour. Trilobite moults with disarticulated cephala have a significantly wider body, wider cephalon, and/or fewer thoracic segments, whereas moults with open ventral sutures have a significantly longer thorax and body, narrower cranidium, and/or more thoracic segments. This quantitative exploration into trilobite moulting behaviour shows the potential for using information from fossil anatomy to predict behaviours, like moulting, in extinct groups.

Introduction

Exoskeleton moulting is a key recurrent event in the life histories of all euarthropods. The presence of an exoskeleton protects an individual from predation and parasitism, and is important for muscle attachment (Ewer, 2005). This characteristic morphologically unites all of Ecdysozoa (Aguinaldo et al., 1998), with the exoskeleton often being reinforced within Euarthropoda. The exoskeleton is naturally restrictive, and so must be periodically moulted in order for significant development and growth to occur. This is an energetically-expensive, difficult, and risky process. It requires resources to produce a new exoskeleton, and leaves the individual vulnerable to predation during

exuviation (exiting of the old exoskeleton) and immediately after, during hardening of the new exoskeleton (Vevea and Hall, 1984). Therefore, the behaviours and mechanisms involved in exoskeleton moulting are inherently linked to euarthropod morphology, development, ecology, and broad-scale evolution (Brandt, 2002; Daley and Drage, 2016). The fossil record of Euarthropoda in particular is extensive, largely owing to their tough exoskeletons, and consequently large moult sample sizes exist that can be used to explore many of these aspects.

Owing to the predation risk during moulting, and the risk of death if moulting fails, moulting behaviours within Euarthropoda are generally specialised. This means there is one usual 'mode' of moulting for each euarthropod group with a reasonably consistent bodyplan. For example, Chelicerata show two modes of moulting (see Daley and Drage, 2016), each producing a limited number of moulting configurations (the exact configuration of shed exoskeletal elements, which can be preserved in the fossil record; see Drage et al., 2018a). In one mode, an anterior suture in the head shield opens and the individual emerges forwards (e.g. in Eurypterida, Xiphosurida, and scorpions; Kjellesvig-Waering, 1986; Sekiguchi et al., 1988; Tetlie et al., 2008; McCoy and Brandt, 2009), and in the second the carapace disarticulates and the individual emerges backwards pulling the appendages out after it (e.g. spiders; Savory, 1928). Decapod crustaceans show the latter of these (in crabs; Daley and Drage, 2016), and a second mode in which the 'neck' joint opens and the individual emerges up and forwards (in lobsters and shrimp; Glaessner, 1969). Daley and Drage (2016) provided a full literature review of these modes of moulting throughout the euarthropod fossil record. Trilobites are unique amongst Euarthropoda because, despite having a quite constrained basic bodyplan, they show several modes of moulting (Daley and Drage, 2016), which produce a huge variety of moult configurations preserved in the fossil record (Drage et al., 2018a). Trilobites are also

one of the most successful groups of euarthropods to have lived based on their diversity of ecological niche, abundance, and global distribution (Fortey and Whittington, 1989; Fortey and Owens, 1999; Webster, 2007). Owing to their particularly thick and hard exoskeletons reinforced with calcite, both trilobite carcasses and moults are preserved in the fossil record in vast abundance. Groups of trilobites show the evolution and loss of a variety of sophisticated sutures involved in moulting. For example, the presence of cephalic facial sutures, which seem to exist purely to disarticulate the free cheeks (librigenae) to facilitate moulting (Stubblefield, 1959; Hou et al., 2017; Du et al., 2018); the secondary loss of these sutures in several groups (Drage et al., 2018b); a variety of ventral cephalic sutures used in moulting (which again are repeatedly lost); and the use of anterior marginal sutures in some derived groups (Stubblefield, 1959; Henningsmoen, 1975; Drage, accepted).

Consequently, trilobites display several modes of moulting, which can be generalised into: use of cephalic sutures (facial, ventral); use of the cephalothoracic joint (i.e. disarticulation of the cephalon, or cranidium if the librigenae are separated); and use of a marginal suture along the anterior margin of the cephalon (Henningsmoen, 1975; Drage, accepted). Coupled with the individual behaviours of trilobites, these produce an array of distinct configurations of exoskeletal sclerites (moulting configurations) that are preserved in the fossil record (Fig. 1; see Henningsmoen, 1975; Drage et al., 2018a; Drage, accepted). For most trilobite moult specimens, it can be difficult to distinguish some of the wide variety of moulting configurations (such as those illustrated in Drage et al., 2018a, from the Emu Bay Shale), particularly if preservation is not exceptional because of pre-preservational disturbance, such as by scavengers or water currents. However, for all specimens identified as moults (for identifying criteria see Henningsmoen, 1975; Daley and Drage, 2016; Drage and Daley, 2016), no matter the preservation, six disarticulations characteristic of moulting can be identified (‘moulting characteristics’), which together

make up the different moulting configurations (e.g. Drage et al., 2018a; Drage, accepted). These are the opening of the facial sutures and ventral sutures, and disarticulation of the cephalon, cranidium, thorax, and pygidium (see Fig. 2).

Despite the plethora of available trilobite moult material with which to work, and the knowledge of this extensive variability in moulting behaviour, little work has been done to quantify how variable these different behaviours are across Trilobita, and how this might relate to aspects of their evolutionary history. Brandt (2002) first attempted to address this, bringing to light a number of ways in which trilobite variability in moulting might have shaped long-term evolutionary trajectories in the Palaeozoic, their extinction, and how this might have been influenced by morphology. However, the hypothetical importance of moulting behaviours in larger-scale trilobite evolutionary studies has been controversial (e.g. Hughes, 2003a). Drage (accepted) gathered the first quantitative broad-scale dataset for trilobite moulting behaviour, building on the ideas put forth by Brandt (2002). This study recorded the proportion of specimens for 355 species that showed each of the six moulting characteristics identified, looking at their variability both inter- and intraspecifically. Moulting behaviour varied significantly throughout the Palaeozoic and with taxonomy, specifically that trilobite orders differed in how variable their moulting behaviours were, and that the early Cambrian showed the highest levels of variability (corresponding to when morphological diversity was high and trait polymorphism may have been adaptive; Foote, 1991; Webster, 2007). Use of the facial sutures to disarticulate the librigenae for moulting was found to be ubiquitous because these likely evolved to facilitate moulting (although there is debate surrounding whether these were ancestral, see Fortey and Whittington, 1989; Du et al., 2018). However there were particularly clear differences in which groups frequently used opening of the ventral sutures (usually disarticulation of the rostral plate; most often Redlichiida) and disarticulation of the entire

cephalon (most often Phacopida). Disarticulation of the thorax and pygidium were found to vary stochastically within the dataset (Drage, accepted), and may be in general less influenced by moulting and more so by decay (Hunda et al., 2006). Both studies exploring trilobite moulting variability (Brandt, 2002; Drage, accepted) suggest that it has evolutionary significance, with Brandt (2002) even indicating it may have been responsible for their ultimate demise.

By further analysing the reasons why trilobites were uniquely flexible in moulting behaviour we can quantitatively test the effects of this on aspects of their evolution, as suggested by Brandt (2002) and Drage (accepted). In particular, a link between morphology and moulting behaviour might indicate that moulting has imposed constraint on the evolution of this extremely diverse and significant animal group. This study explores the causes of trilobite moulting variability in the context of overall morphology and body proportions. Statistical analyses are carried out to compare occurrences of the four meaningful moulting characteristics in trilobites (disarticulation of the cephalon and cranidium, and opening of the facial and ventral sutures; see Drage, accepted) to proportions of exoskeleton tagma and number of thoracic segments. Analyses show that overall body shape and segment count are significantly linked to the occurrence of different moulting characteristics in total-group Trilobita.

Institutional abbreviations.—NHMUK, Natural History Museum, London, UK; SAM, South Australian Museum, Adelaide, Australia.

Other abbreviations.—CI, 95% confidence interval; ANOVA, Analysis of Variance.

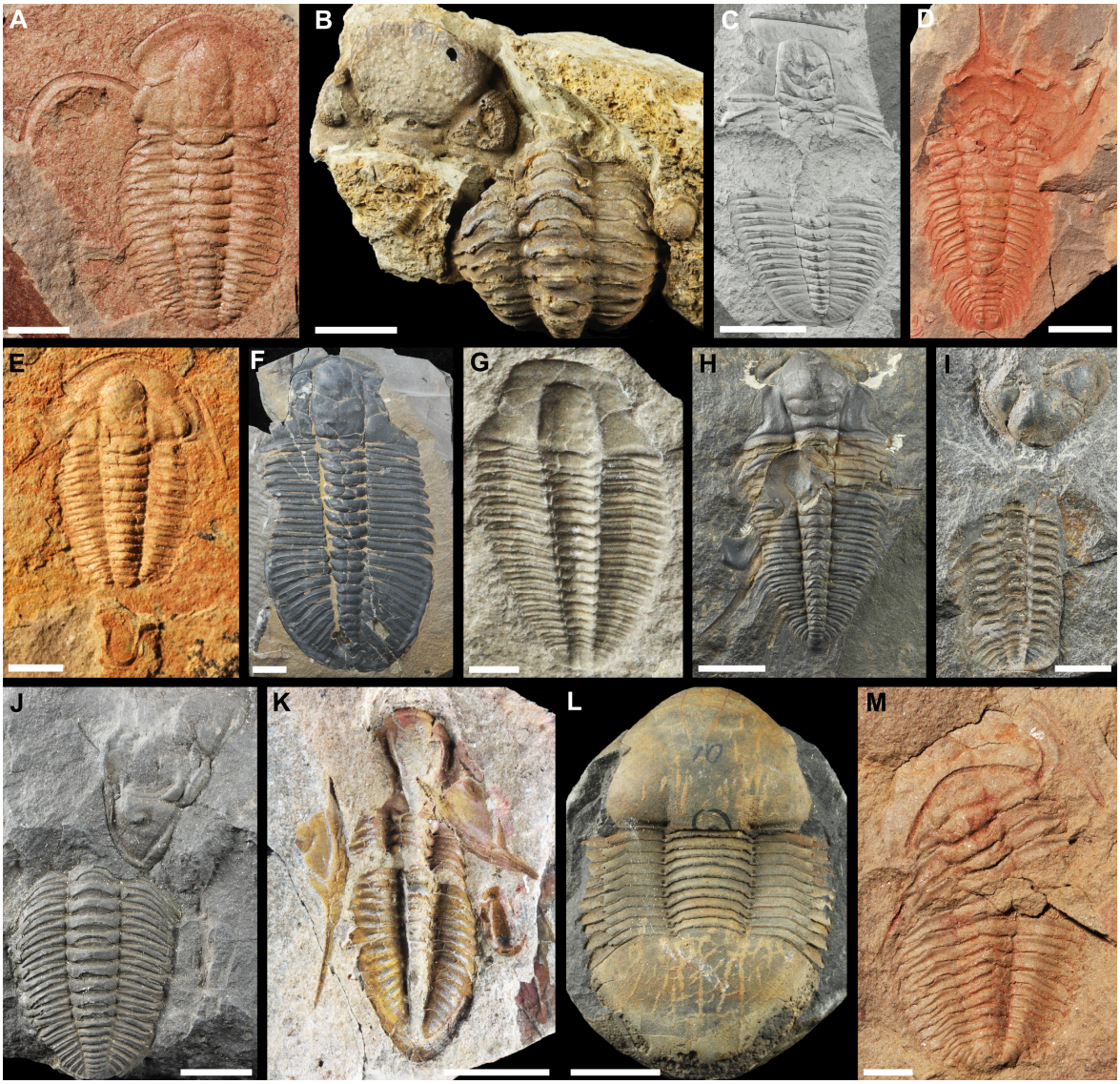


Figure 1: Examples of the wide diversity of trilobite moults preserved in the fossil record. A: *Estaingia bilobata* Pocock 1964 (SAM-P 43767, early Cambrian, Australia) with the librigenae missing and the rostral plate and hypostome displaced; B: *Phacops latifrons* Bronn 1825 (NHMUK It.5823, Middle Devonian, Belgium) in the Zombie Configuration with the entire cephalon displaced; C: *Parabolinites (Parabolinella) williamsoni* Belt 1868 (NHMUK 59291, Furongian, Wales) axial shield missing the librigenae with a thoracic break; D: *Redlichia takooensis* Lu 1950 (SAM-P45875, early Cambrian, Australia) in Salter's Configuration; E: *E. bilobata* (SAM-P 47947, early Cambrian, Australia) in McNamara's Configuration; F: *Ogygopsis klotzi* Rominger 1887 (NHMUK It.4442, Miaolingian, Canada) axial shield; G: *Elrathina brevifrons* Rasetti 1951 (NHMUK It.5956, Miaolingian, Canada) axial shield; H: *Paradoxides minor* Boeck 1827

(NHMUK 42443, Miaolingian, Sweden) axial shield with disarticulated and rotated librigenae; I: *Trimerocephalus mastophthalmus* Richter 1856 (NHMUK I.5100, Upper Devonian, UK) in the Zombie Configuration with the cephalon rotated; J: *Dalmanitina socialis proaeva* Emmrich 1839 (NHMUK 42341, Upper Ordovician, Czech Republic) in the Zombie Configuration; K: *Phillipsia* sp. Berkeley 1881 (NHMUK I.1092, early Carboniferous, UK) in McNamara's Configuration; L: *Illaenus katzeri* Barrande 1872 (NHMUK I.3746, Lower Ordovician, Sweden) axial shield; M: *E. bilobata* (SAM-P 44050, early Cambrian, Australia) in Harrington's Configuration with the rostral plate and left librigena displaced backwards, and a thoracic disarticulation. Scale bars: 2.5 mm for E, G, and M; 5 mm for A and K; and 10 mm for B-D, F, H-J, and L.

Material and methods

This study builds on the dataset presented in Drage (accepted; see also Drage et al. 2018). The dataset in Drage (accepted) gives the moulting behaviours for 355 trilobite species by recording the occurrences of six moulting characteristics in 840 specimens, from across the Palaeozoic and within all trilobite taxonomic orders (see Drage, accepted; Appendix 4). Only specimens which were reasonably complete with most disarticulated sclerites in association are able to be confidently considered moults, and entirely fragmentary material was not included (see Drage, accepted, fig. 2). The moulting characteristics are used because they represent the characteristic disarticulations involved in the main modes of moulting, and are reasonably simple to identify across the entire group regardless of preservation type and locality. However, the specific moulting configurations preserved (as in Drage et al., 2018a) are often hard to distinguish and represent a combination of these characteristics. Drage (accepted) therefore recorded the occurrences of the following characteristics in identified moulted exoskeletons: disarticulation of the cephalon; disarticulation of the cranidium; opening of the facial

sutures (and therefore separation of the librigenae); opening of the ventral sutures (often meaning disarticulation of the rostral plate and/or hypostome); thoracic disarticulation; and pygidial disarticulation.

In this study, the moulting characteristics used were limited to disarticulation of the cranidium, cephalon, librigenae, and ventral structures, with pygidium and thoracic disarticulation not included due to their stochastic variation (Drage, accepted). Cranidium disarticulation represents any displacement of the cranidium (which requires opening of the facial sutures and usually librigenal displacement). Opening of the facial sutures represents only disarticulation of the librigenae. Opening of the ventral sutures is any combination of rostral plate and/or hypostome displacement (which often occurs in association with the librigenae). Cephalon disarticulation is where the entire intact cephalon is displaced. Groups moulting through opening of a marginal suture (e.g. Trinucleidae and Harpetida) were not included as this requires a highly specialised morphology for trilobites (see Drage, accepted, fig. 3).

For the specimens with recorded moulting characteristics measurements were made of the length and width of the cephalon, cranidium, thorax, and pygidium (Fig. 2, right), and thoracic segment number was counted. Specimens from Drage (accepted) obviously thought to be tectonically deformed were excluded from the dataset. Specimens for which no morphological data was available were also removed, leaving 756 specimens total. All of these specimens were included in the analyses, although not all specimens provided adequate morphological data for all size measurements. Size measurements were made using digital calipers at millimetre-scale on moult specimens and digital images (the latter totalling <100 specimens). Body proportion ratios (i.e. length of the exoskeleton sclerite over its width) were used throughout because raw measurements would overemphasise size differences between trilobite species and individuals (which can vary greatly),

conflating the results. These consisted of (see Fig. 2): the cephalic ratio (axial length/width of cephalothoracic joint); cranidial ratio (axial length/width of the fixigenae at the anterior of the cranidium); thoracic ratio (axial length/greatest width); pygidial ratio (axial length/greatest width); and total body ratio (axial length from anterior of cephalon to posterior of pygidium/greatest width). Thoracic segment number was determined where possible by directly counting the number of articulated thoracic segments. If not all segments were present (e.g. in cases of thoracic disarticulation), the total segment number was derived from the literature if available for the species (Whittington et al., 1997). If a thoracic segment count was impossible to determine the specimen was not included in segment number analyses. Summary statistics (mean, median, mode, CIs) were calculated in PAST3 (Hammer et al., 2001). Graphs displaying body length/width ratios all plotted the values for each sampled specimen (x-axis) against their total body ratio (y-axis) in order to standardise for body size differences and allow for direct comparison of trends. All graphs and linear trend lines were constructed and plotted using ggplot2 (Wickham et al., 2016) in RStudio (RStudio Team, 2015). Figures were otherwise created and/or modified using Adobe Illustrator and Photoshop CS6.

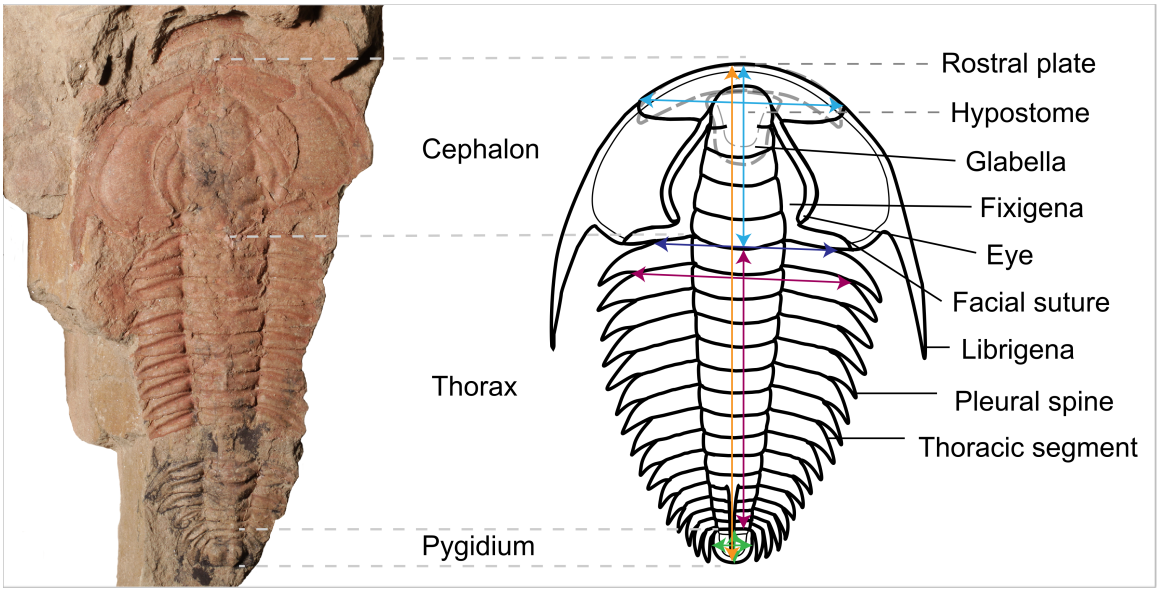


Figure 2: Photograph (left) of *Redlichia takooensis* from the early Cambrian Emu Bay Shale, South Australia (SAM-P 44544; Drage et al., 2018a, fig. 4D), and representative drawing (right) of a *Redlichia* (drawn based on specimens of *R. takooensis* from the Emu Bay Shale, e.g. Drage et al., 2018a, fig. 4). Important anatomical features are labelled, with dashed lines showing ventral structures. Coloured arrows represent measurements taken of trilobite specimens: light blue, greatest length and width of cranidium; orange, total body length; red, greatest width and length of thorax; dark blue, width of cephalothoracic joint; green, greatest width and length of pygidium.

Statistical analyses were performed to test for a difference in morphology between specimens showing each of the four moulting characteristics. The null hypothesis (H0) for all statistical analyses states that there is no difference in the average body proportions (or thoracic segment number) of trilobite specimens showing the different moulting characteristics. For each body ratio (cephalic, cranidial, thoracic, pygidial, and total body), and for thoracic segment number, the most appropriate test for comparison of averages was selected based on the data (see Fowler et al., 1998). This first required using Levene's test and the Shapiro-Wilks test to determine whether the data fitted the assumptions of a parametric test; that of equal sample variances and a normal distribution (respectively by the two tests). If neither test was significant the assumptions are not violated and a parametric Analysis of Variance (ANOVA), which compares the means, is appropriate. If Levene's test was significant the samples had unequal variances, and Welch's F test should be used to compare the sample averages. If the Shapiro-Wilks test is significant then the nonparametric Kruskal-Wallis H test, which does not assume normality and instead compares medians, is the most appropriate to test for differences in the averages of samples. In this study, all samples violated assumptions of normality, and therefore either the results for Welch's F test or the Kruskal-Wallis H test were the most appropriate. These tests compare multiple samples to identify significant differences amongst their

averages, but cannot pinpoint which pairings are responsible for a significant result. Therefore, nonparametric Mann-Whitney U tests, which do not assume normality and make pairwise comparisons of averages, were used to post-hoc identify the causes of significant test results. Full results for all multi-sample tests are given in Table 1, and results for all Mann-Whitney U tests in Table 2. All statistical analyses were carried out in PAST3 (Hammer et al., 2001).

Results

Moulting and thoracic segment number.—For all four moulting characteristics trilobite moult specimens show high variation in thoracic segment number. Specifically, there are moult specimens with 5 thoracic segments that show cranidial or cephalic disarticulation, or open facial sutures, and specimens with the maximum measured segment number of 20 showing cranidial disarticulation, or open facial or ventral sutures (Fig. 3). Specimens with cephalic disarticulation show 5-17 segments (Fig. 3A), both cranidial disarticulation and open facial sutures contain specimens with 5-20 segments (Fig. 3B and C), and moults with open ventral sutures show 6-20 segments (Fig. 3D; Appendix 5.1). The mode thoracic segment number is lowest for moults with open facial sutures at 8 thoracic segments (Fig. 3C), but the data are multimodal with further peaks at 10, 13, and 19 segments. The mode is 11 segments for moults with disarticulated cephala (Fig. 3A), but the data are left-skewed with almost all specimens having fewer than 14 thoracic segments. The modes are equal for moults with disarticulated cranidia (Fig. 3B) and open ventral sutures at 13 thoracic segments (Fig. 3D), but for the former the number of specimens showing all other segment numbers is roughly equivalent (between 1 and 4 specimens), and for open ventral sutures the data are bimodal with another distinct peak at 18-20 thoracic segments.

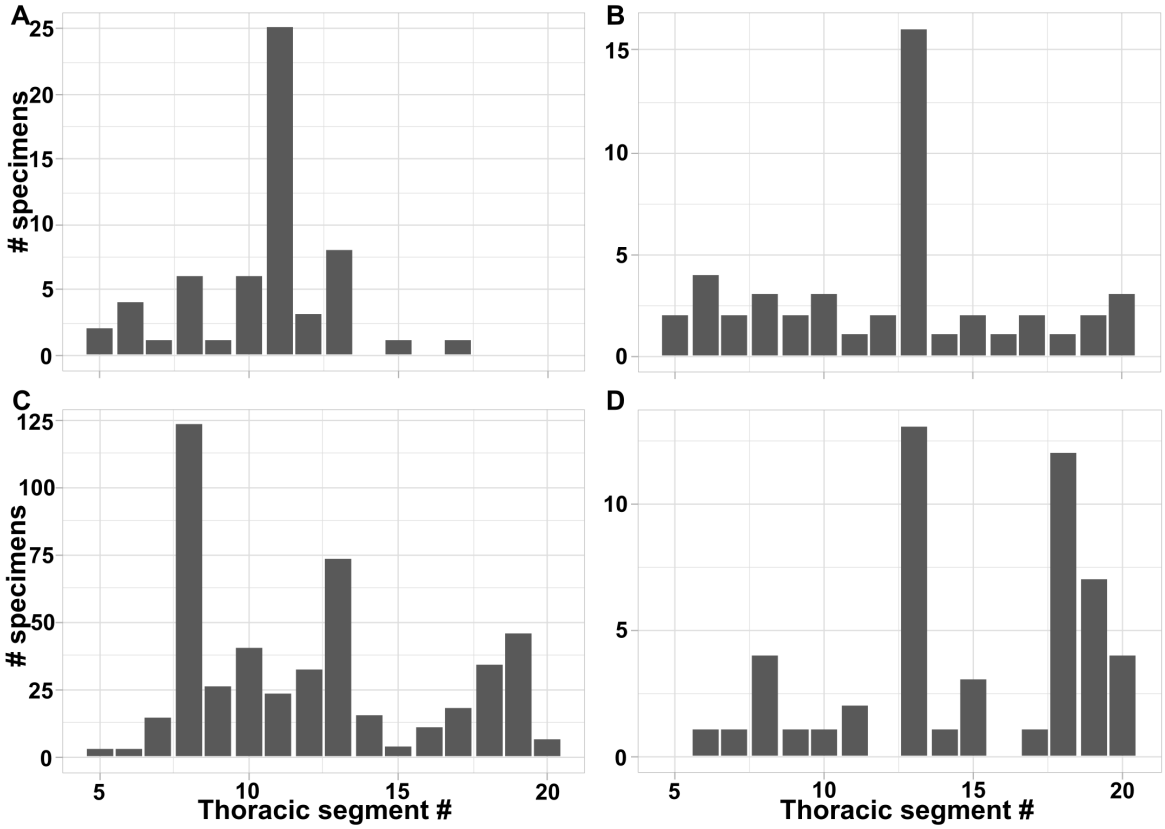


Figure 3: Thoracic segment number for trilobite moult specimens showing each of the four moulting characteristics. A: Thoracic segment number of moult specimens showing cephalic disarticulation (n=58). B: Thoracic segment number of moult specimens showing cranidial disarticulation (n=47). C: Thoracic segment number of moult specimens showing open facial sutures (n=471). D: Thoracic segment number of moult specimens showing open ventral sutures (n=51). Specimens were counted twice if showing both disarticulation of the cranidium and open ventral sutures.

Despite the reasonably consistent high variability in thoracic segment number, the summary statistics significantly differ between the moulting characteristics, and the mean averages differ from the modes. The mean thoracic segment number is much lower for moult specimens showing cephalic disarticulation (10 segments; Fig. 4) compared to specimens with cranidial disarticulation or open facial sutures (both 12 segments), and mean segment number is higher in specimens with open ventral sutures (15 segments).

Levene's and Shapiro-Wilks tests are both significant, showing that the data for thoracic segment counts violates parametric assumptions (Table 1). A Kruskal-Wallis H test is significant (as is an ANOVA; Table 1), indicating that average thoracic segment number does differ between specimens showing the four moulting characteristics. Post-hoc Mann-Whitney U tests (Table 2) indicate that this is caused by every pairing of moulting characteristics having significantly different average thoracic segment numbers, except for open facial sutures paired with cranidial disarticulation, which do not significantly differ in their mean thoracic segment number of 12.

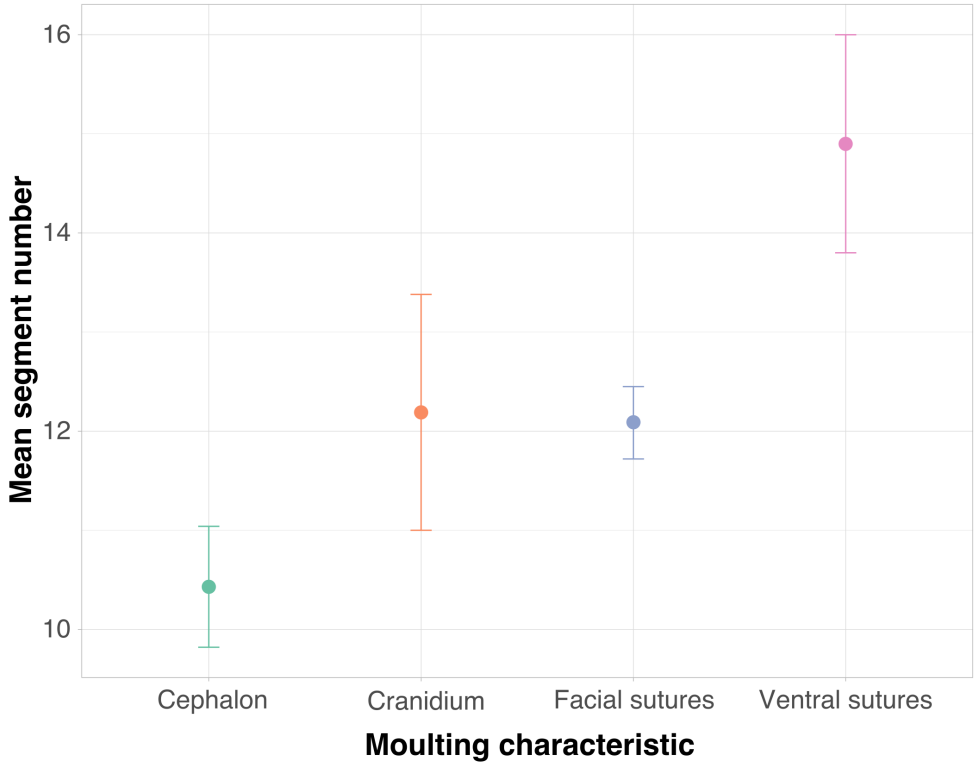


Figure 4: Average thoracic segment number for specimens showing each of the four studied moulting characteristics. Total n=627 specimens, each sample size same as for Figure 3. Points represent the means, and bars the 95% Confidence Interval for each mean. Test statistics and p-values for tests comparing the averages are in Table 1.

Moulting and body proportions.—Scatterplots of the cephalic, cranidial, thoracic, and pygidial ratios against total body ratio show different spreads in the body proportions of trilobite moult specimens, although for all body proportions the moulting characteristics show a great deal of overlap (Fig. 5). The cephalic ratios are mostly clustered around 0.4-0.8, equating to a cephalic width for most specimens of just under double the length (Fig. 5A), although a few specimens (mostly those with open facial sutures) have a ratio >1 with comparably longer or thinner cephala. The linear trend lines demonstrate that cephalic ratio is quite strongly positively correlated with specimen total body ratio (Fig. 5A). This means having a longer or thinner cephalon generally corresponds to a longer or thinner body, presumably because cephalon length contributes to total body length. The cranidial ratios show more spread, with most specimens having a cranidial ratio of 0.7-1.5 but with clustering round 0.7-1.3 (Fig. 5B). Moults with disarticulated cephala or open facial sutures generally maintain their overall body ratio (at about 1.75) as cranidial length increases relative to width, suggesting no real correlation with body ratio (Fig. 5B). However, for moult specimens with disarticulated cranidia and open ventral sutures cranidial ratio is negatively correlated with body ratio, with a longer or thinner cranidium occurring with a broader or shorter body. Thoracic ratio also shows a large degree of spread, with most specimens encompassed within 0.3-1.5 but with no obvious clustering (Fig. 5C), meaning specimens greatly differ in their thoracic proportions. However, all moulting characteristics show a similar positive correlation with body ratio (Fig. 5C); as thoracic ratio increases (a longer or thinner thorax), so does body ratio. As for the cephalic ratio, this is presumably because thoracic ratio directly affects total body ratio. Pygidial ratio has a spread from 0.3 to 1, clustering around 0.5 (a pygidial width approximately double the length; Fig. 5D). All moulting characteristics again show a positive correlation with body ratio, with a longer or thinner pygidium as the total body becomes longer or

thinner (Fig. 5D). Again, this can be explained by the fact that pygidial length is a constituent of total body ratio. The correlation is more strongly positive for specimens with open facial sutures or disarticulated cranidia, and less so for the other two moulting behaviours.

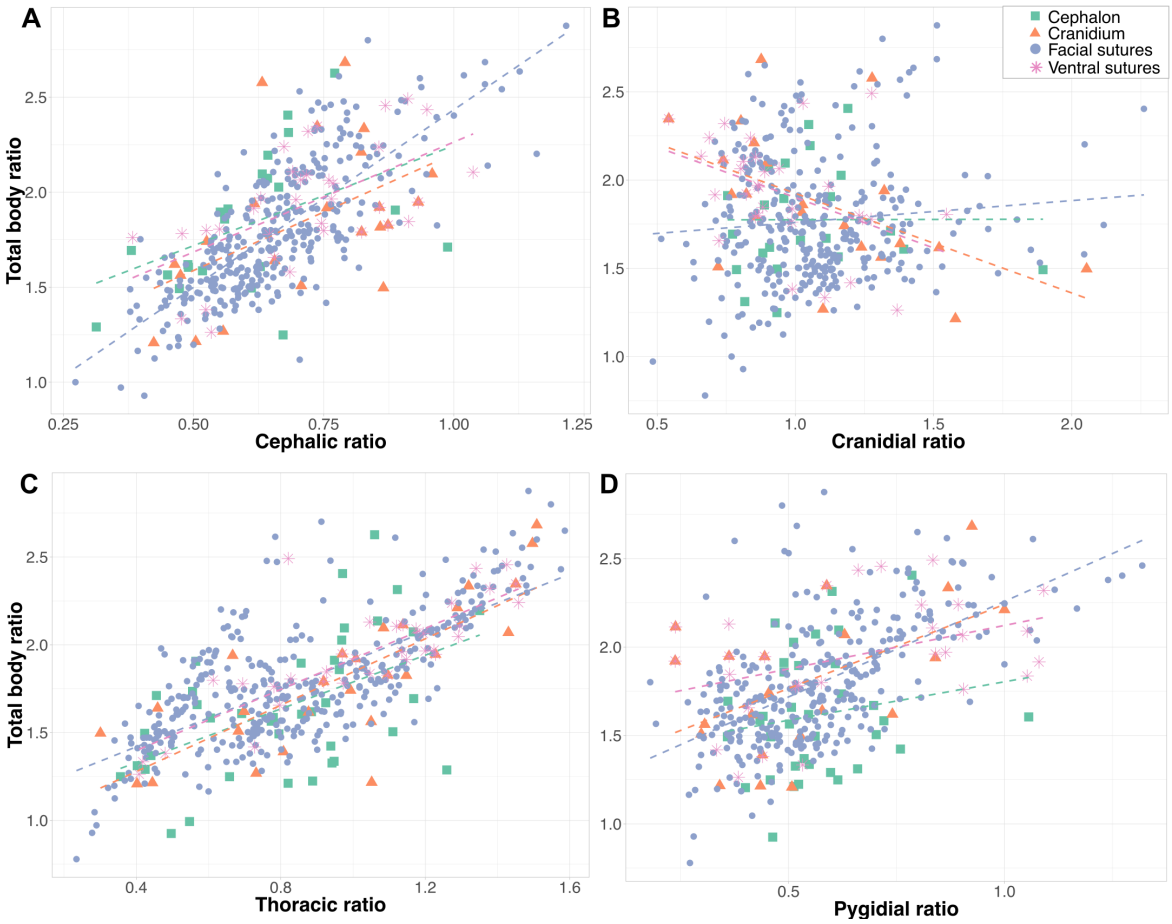


Figure 5: Body proportion ratios of trilobite specimens showing each of the four moulting characteristics, all plotted against total body ratio (body length/greatest width). A: cephalic ratio (cranidial length/cephalothoracic joint width), n=524 specimens. B: cranidial ratio (cranidial length/anterior width of cranidium), n=583 specimens. C: thoracic ratio (thoracic length/maximum thoracic width), n=500 specimens. D: pygidial ratio (pygidial length/maximum pygidial width), n=565 specimens. Five anomalous outlier specimens were excluded from the graphs in order to better display the cluster patterns. Dashed lines represent the linear trend lines for each moulting characteristic, identified by their different colours (see key).

It is difficult to distinguish any meaningful difference in the body proportion ratios for specimens showing the four moulting characteristics (Fig. 5), making statistical analyses extremely valuable (summarised in Tables 1 and 2). The mean cranidial ratio (Fig. 6A) of moult specimens showing open ventral sutures is 0.94 (a slightly wider cranidium than long), lower than for the other moulting characteristics (1.07 cephalon disarticulation, 1.04 cranidium disarticulation, and 1.07 for open facial sutures; all a slightly longer cranidium than wide) which overlap substantially in their Confidence Intervals (CIs). Levene's test is not significant (the variances are approximately equal), but the data are not normally distributed (Table 1), and so the Kruskal-Wallis H test is most appropriate to compare the samples. This is significant (an ANOVA is also close to the 0.05 threshold; Table 1). Post-hoc Mann Whitney U pairwise tests show a significantly lower cranidial ratio of specimens with open ventral sutures compared to those with open facial sutures or disarticulated cephala (Table 2).

The mean cephalic ratios of specimens showing cranidium disarticulation and open ventral sutures are similar to each other (0.74 and 0.71 respectively), and slightly higher than for those with open facial sutures (0.67), with all showing overlap in their CIs (Fig. 6B). The mean cephalic ratio is lower (0.61) for specimens with disarticulated cephala, and despite this having a large CI it only overlaps slightly with that of the mean for open facial sutures (Fig. 6B). All average ratios show that specimens generally have cephala that are wider than long, but slightly longer/thinner for moults with open ventral sutures and disarticulated cephala than for the other two moulting characteristics. Results of statistical analyses are similar to those for cranidial ratios (Table 1), with the Kruskal-Wallis H test being strongly significant, and an ANOVA on the significance threshold. Post-hoc Mann Whitney U pairwise tests (Table 2) confirm that mean cephalic ratio is significantly lower in specimens showing disarticulated cephala than the other moulting characteristics.

Further, they also suggest that the average cephalic ratios are significantly lower for specimens with open facial sutures than for those with open ventral sutures/disarticulated cranidia (Table 2).

Mean thoracic ratio is very similar for specimens showing cephalic disarticulation (0.83) and open facial sutures (0.84; Fig. 6C), both lower than for specimens with cranidium disarticulation (0.97) and open ventral sutures (1.04). Each pair show extensive overlap in their CIs. For specimens with disarticulated cephala or open facial sutures the average thorax is therefore wider than long, and for those with disarticulated cranidia or open ventral sutures the thorax is approximately equal in length or slightly longer than wide. Both a Kruskal-Wallis H test and ANOVA are strongly significant, indicating the average thoracic ratios differ between the moulting characteristics (Table 1). Post-hoc Mann-Whitney U tests confirm that specimens with disarticulated cranidia and open ventral sutures have significantly higher thoracic ratios in every pairwise combination with the other two moulting characteristics (Table 2).

Table 1: Statistical tests, with significant results shaded at a threshold of $\alpha=0.05$. Abbreviations: Ce, cephalic disarticulation; Cr, cranidial disarticulation; FS, open facial sutures; VS, open ventral sutures.

Test for difference in average:	Moulting characteristic	Mean	Median	Sample size	Levene's test[1]	Shapiro-Wilks[2]	ANOVA[3]	Welch's F test[4]	Kruskal-Wallis H test[5]
Thoracic segment #	Cephalic disarticulation	10 segments	11	58	$p=4.65\times10^{-7}$	0.94; $p=2.43\times10^{-15}$	12.13; $p=1\times10^{-7}$	17.07; $p=4.07\times10^{-9}$	29.75; $p=1.19\times10^{-6}$
	Cranidial disarticulation	12	13	47					
	Open facial sutures	12	12	471					
	Open ventral sutures	15	15	51					
Cranidial ratio	Ce	1.07	1.01	48	p=0.52	0.85; $p=4.89\times10^{-23}$	2.5; p=0.059	Not required	10.98; p=0.012
	Cr	1.04	0.92	51					
	FS	1.07	1.03	445					
	VS	0.94	0.87	39					
Cephalic ratio	Ce	0.61	0.6	26	p=0.95	0.7; $p=1.12\times10^{-29}$	2.61; p=0.051	Not required	18.08; $p=4.2\times10^{-4}$
	Cr	0.74	0.77	35					
	FS	0.67	0.65	424					
	VS	0.71	0.71	39					

Thoracic ratio	Ce	0.83	0.89	43	p=0.3	0.98; $p=2.43x10^{-5}$	7.12; $p=1.11x10^{-4}$	Not required	20.21; $p=1.54x10^{-4}$
	Cr	0.97	1.01	29					
	FS	0.84	0.79	389					
	VS	1.04	1.1	39					
Pygidial ratio	Ce	0.59	0.53	58	p=0.036	0.9; $p=2.72x10^{-19}$	1.85; p=0.14	2.14; p=1	5.24; p=0.16
	Cr	0.6	0.55	38					
	FS	0.64	0.58	433					
	VS	0.71	0.74	36					
Full body ratio	Ce	1.65	1.6	42	p=0.32	0.98; $p=2.3x10^{-5}$	4.91; $p=2.29x10^{-3}$	Not required	18.46; $p=3.5x10^{-4}$
	Cr	1.82	1.83	30					
	FS	1.78	1.73	377					
	VS	1.94	1.95	39					

[1]Test for equal variances between the samples. If significant should use Welch's F test to compare sample averages.

[2]Test for normal distributions of the samples. If significant the assumption of normality is violated and the Kruskal-Wallis H test should be used to compare sample averages.

[3]Parametric test to compare means of samples.

[4]Test which, unlike ANOVA, does not assume equal variances.

[5]Non-parametric test to compare medians of samples, which doesn't assume equal variances or a normal distribution (and is therefore more conservative).

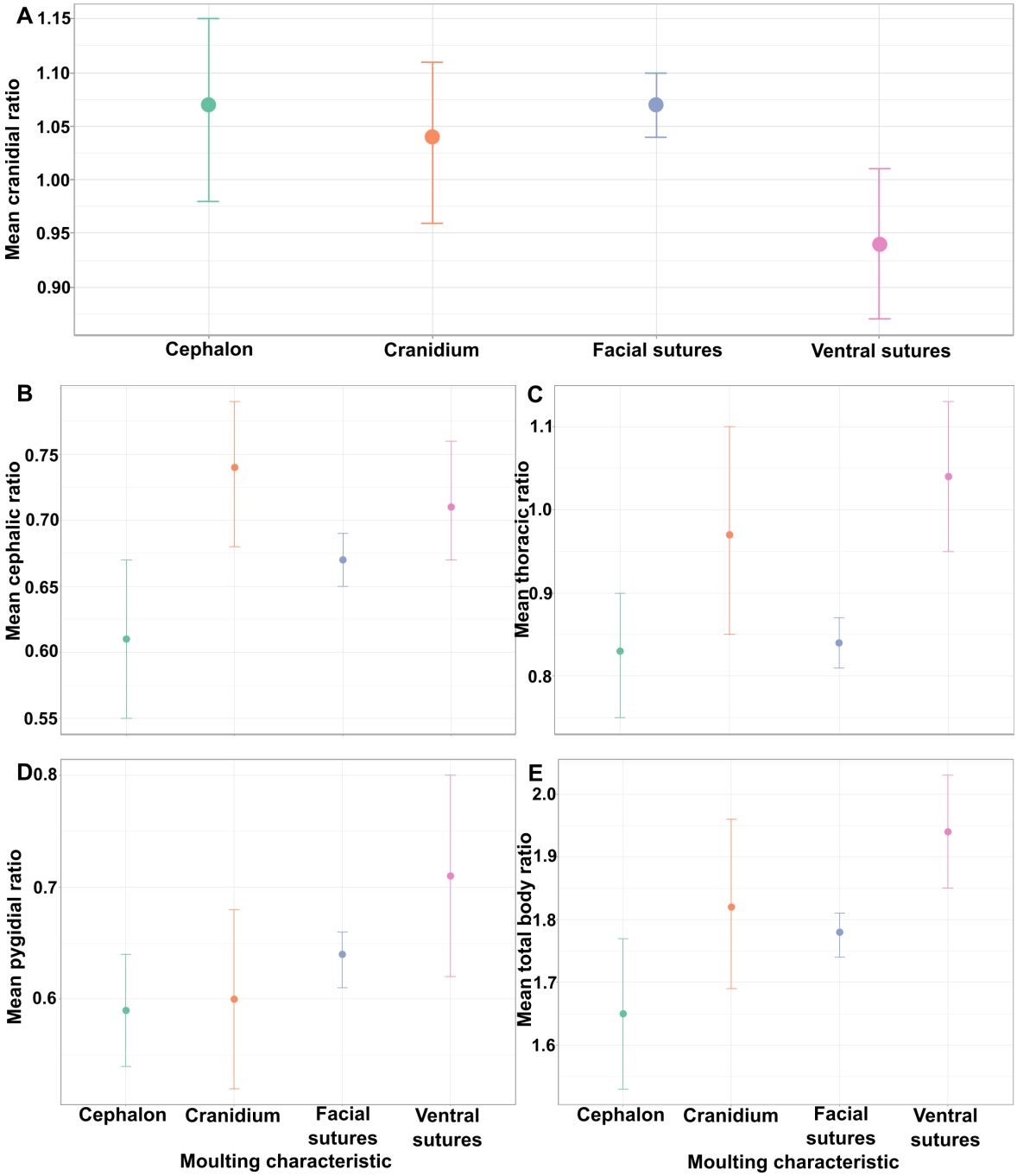


Figure 6: Mean body proportion ratio for specimens showing each of the four moulting characteristics. Points represent means and bars the 95% confidence intervals. A: mean cranidial ratio (cranidial length/anterior cranidial width), n=583. B: mean cephalic ratio (cranidial length/cephalothoracic joint width), n=524. C: mean thoracic ratio (thoracic length/maximum width), n=500. D: mean pygidial ratio (pygidial length/maximum width), n=565. E: mean total body ratio (total axial length/maximum width), n=488.

The mean pygidial ratios for all of the moulting characteristics overlap substantially in their CIs (Fig. 6D). The means are very similar for specimens with disarticulated cephala (0.59) and cranidia (0.6), slightly higher in those with opened facial sutures (0.64), and highest in moults with open ventral sutures (0.71; Fig. 6D). All statistical analyses fail to reject the null hypothesis, indicating that the average pygidial ratios do not significantly differ between specimens showing the moulting characteristics (Table 1).

Mean total body ratios (i.e. total length/greatest width) show substantial differences between the moulting characteristics. These are 1.65 for cephalon disarticulation, 1.78 for open facial sutures, 1.82 for cranidium disarticulation, and 1.94 for open ventral sutures (Fig. 6E). Both a Kruskal-Wallis H test and ANOVA strongly suggest these are significantly different (Table 1). Post-hoc Mann-Whitney U tests are significant for every pairing with cephalic disarticulation (Table 2), confirming these do have a lower total body ratio (a wider body compared to length) than specimens showing the other moulting characteristics. The pairing of specimens with open facial sutures compared to open ventral sutures is also significant, with the latter having a significantly longer or thinner body.

Table 2: Mann-Whitney U pairwise tests on the analyses that gave significant Kruskal-Wallis H tests (Table 1), with significant results shaded at a threshold of α=0.05. Top-right halves give p values, and bottom-left halves give the test statistic. Abbreviations: Ce, cephalic disarticulation; Cr, cranidial disarticulation; FS, open facial sutures; VS, open ventral sutures.

Difference in average:	**Mann-Whitney U pairwise test results**[1]				
Thoracic segment #		**Cephalic disarticulation**	**Cranidial disarticulation**	**Open facial sutures**	**Open ventral sutures**
	Cephalic disarticulation		$p=5.78\times10^{-3}$	p=0.024	3.6×10^{-9}
	Cranidial disarticulation	940.5		p=0.62	p=0.002
	Open facial sutures	11204	10586		$p=5.55\times10^{-6}$
	Open ventral sutures	519	770	7412.5	
Cranidial ratio		**Ce**	**Cr**	**FS**	**VS**
	Ce		p=0.44	p=0.8	p=0.017
	Cr	1112		p=0.22	p=0.15
	FS	10440	10147		$p=1.55\times10^{-3}$
	VS	656.5	815	6025.5	
Cephalic ratio		**Ce**	**Cr**	**FS**	**VS**
	Ce		$p=3.45\times10^{-3}$	p=0.058	$p=3.15\times10^{-3}$
	Cr	254		$p=2.3\times10^{-3}$	p=0.4
	FS	4293	5120		p=0.021
	VS	286	604.5	6428.5	
Thoracic ratio		**Ce**	**Cr**	**FS**	**VS**
	Ce		p=0.05	p=0.88	$p=5.34\times10^{-4}$
	Cr	452		p=0.032	p=0.44
	FS	8247	4292		$p=6.15\times10^{-5}$
	VS	465	503	4634	
Total body ratio		**Ce**	**Cr**	**FS**	**VS**
	Ce		p=0.05	p=0.018	$p=2.79\times10^{-4}$
	Cr	458		p=0.38	p=0.12
	FS	6155.5	5109		$p=6.48\times10^{-4}$
	VS	434	455.5	4913	

[1]Mann-Whitney U tests are used here as post-hoc non-parametric pairwise comparisons to determine which pairings caused the significant Kruskal-Wallis H test results reported in Table 1.

Discussion

How does morphology relate to moulting behaviour?.—The results show that trilobite moulting behaviour significantly varies in relation to changes in morphology. Specimens that disarticulated the entire cephalon during moulting display significantly fewer thoracic segments (Tables 1 and 2), and therefore also a wider thorax (compared to length) because the addition of more thoracic segments generally necessitates a longer trunk. This suggests that these trilobites needed a larger exuvial gape placed at the cephalothoracic joint, to accommodate exuviation of a broader thorax, rather than fitting it through the constrained gape at the anterior of the head shield (as created by the other moulting characteristics). Trilobite segmentation is notably interspecifically variable and increases through development, with many taxa also showing variable thoracic segment counts between individuals of the same species (e.g. see Hughes et al., 1999; Hughes, 2003b; Fusco et al., 2014). Given the significant link between them, the fact that thoracic segment number is highly variable within Trilobita may partially explain their variability in moulting behaviour. Both thoracic segment number and moulting behaviour appear to have become increasingly constrained later in trilobite evolution during the Palaeozoic (Hughes et al., 1999; Balint and Hughes, 2008; Drage, accepted). It has been posited that intraspecific adult segment count variability may relate to palaeoecological factors (Balint and Hughes, 2008), which might have a knock-on effect of changing the optimal moulting behaviour as thoracic segment number changes. Further, most trilobites grew by adding segments to the pygidium and releasing them periodically into the thorax (Hughes, 2003b; Fusco et al., 2014), which may therefore have brought about developmentally-linked changes in their moulting behaviour. For example, cephalic disarticulation may have been more common in early developmental stages, with a change to other moulting behaviours as thoracic

segment number increased during development, although for at least one group (Phacopina) morphological constraints caused the opposite to occur (Drage et al., 2018b), and this would require explicit testing through looking at intraspecific trilobite moulting variability over developmental stages.

The moulting behaviours employed are also significantly linked to the relative proportions of exoskeleton tagma. In particular, trilobite individuals that moulted by disarticulating the entire cephalon generally had a wider and/or shorter cephalon than those moulting using other modes (Fig. 6B; Tables 1 and 2). Although, the cranidium was not particularly shorter or wider than that of the other moulting characteristics (Fig. 6A), which suggests the width of the cephalothoracic joint is particularly important, being the measurement used to calculate cephalic ratio. The cephalothoracic joint represents the anatomical feature at which the exuvial gape is created for trilobites moulting through disarticulating the entire cephalon (or the cranidium plus librigenae). The cephalic ratio data thereby suggests trilobites with a broader cephalon needed a wider gape to moult than presumably given by opening of the cephalic sutures. Trilobites with narrower cephala were more likely to moult by opening some of the cephalic sutures (facial or ventral; Fig. 6B), suggesting that a broader exuvial gape at the cephalothoracic joint was unnecessary. The significantly lower cranidial ratio (Fig. 6A; Tables 1 and 2) in specimens that opened the ventral sutures for moulting indicates a comparably broader anterior of the head shield between the fixigenae (being the width measurement used to calculate cranidial ratio), which may translate to a broader ventral doublure area. Disarticulating the ventral sections, which almost always occurred in tandem with opening of the facial sutures and disarticulation of the librigenae (such as with the Lower Cephalic Unit often disarticulated by moulting *Estaingia bilobata*; Drage et al., 2018a), would in this case likely have provided a larger anterior exuvial gape for a broader cranidium to emerge through. A

number of specimens show both open ventral sutures and disarticulated cranidia, and therefore were counted in both groups; probably accounting for the lack of a significant Mann-Whitney U test in cranidial ratio for this pairing (Table 2).

The significantly higher thoracic ratio, and therefore longer thorax, in specimens with open ventral sutures (Fig. 6C; Tables 1 and 2) may be part of a larger trend discussed by Drage (accepted). In this study, redlichiids were found to disarticulate the rostral plate during moulting far more often than expected, probably in large part due to their generally broad rostral plate and the lack of facial sutures in earliest redlichiid taxa (Stubblefield, 1959; Fortey, 1990). Redlichiids also often have a high, more variable, number of thoracic segments (Hughes et al., 1999) and a long thorax, as well as a relatively broad anterior cranidium (Whittington et al., 1997; e.g. *Balcoracania dailyi* with the greatest number of segments known, Paterson et al., 2007), and therefore their abundance in the sample of moults with open ventral sutures may be the reason for the significant result obtained here. This is linked to the significant result for thoracic segment number, given a higher number of thoracic segments would in most cases produce a longer thorax and therefore a higher thoracic ratio (Figs 5 and 6; Tables 1 and 2). A longer thorax also likely creates greater complexity of form by way of more articulation points, as well as through an increased number of spines (both pleural and dorsal-facing) the segments usually support in trilobites, and therefore a larger opening at the anterior of the cephalon created by opening of both the facial and ventral sutures (and with a comparably broader cranidium) may have been important in extraction of these features. In keeping with these findings, the average total body ratio is significantly lower in moults with disarticulated cephala (Tables 1 and 2), showing that individuals with a broader overall body frequently disarticulated the entire cephalon for moulting. Conversely, total body ratio is significantly higher in moults with open ventral sutures, likely caused by their longer thorax.

Average pygidial ratio does not differ between the moulting characteristics. According to all current knowledge, trilobites exclusively moulted through emerging anteriorly from their old exoskeletons (Henningsmoen, 1975; Whittington, 1990; Whittington et al., 1997; Brandt, 2002; Daley and Drage, 2016; Drage and Daley, 2016; Drage et al., 2018a). Further, the posterior-end of trilobites is usually the smallest part of the form, excepting those with the isopygous or macropygous conditions (pygidium roughly the same size as or larger than the cephalon; Whittington et al., 1997), making it unsuitable for moulting events, although pygidial inversion is thought to occasionally result from redlichiid moulting (McNamara, 1986).

For most body proportion ratios the average values for individuals moulting through disarticulating the cranidium and opening the facial sutures are similar (Fig. 6; Table 1). This is because disarticulation of the cranidium inherently requires separation of the librigenae, in addition to opening of the cephalothoracic joint (without separation of the librigenae this would equal disarticulation of the cephalon). Cranidial disarticulation is therefore a hybrid between opening of the facial sutures and disarticulation of the cephalon, explaining the paucity of significant differences in the average body proportions obtained for this group of moults. Despite this, disarticulation of the cranidium seems to still be an important moulting characteristic in at least some early redlichiid and ptychopariid taxa (e.g. *Redlichia takooensis* and *Estaingia bilobata*; Drage et al., 2018a). Similarly, opening of the ventral sutures in trilobites is often associated with the facial sutures, however this does not appear to have much affected the results (Table 1), and a lack of significant Mann-Whitney U tests between open ventral sutures and disarticulated cranidia may be because specimens showing both were counted within both samples (Table 2; Appendix 5.2).

Sampling bias is not thought to be problematic in this study owing to the large sample sizes making up the dataset. All samples appeared to encompass a great deal of individual variability, showing a high spread in body proportions and thoracic segment numbers (Figs 3 and 5). The moulting characteristics generally show similar extremes in their spreads of data (i.e. total variability was similar between groups; Fig. 5), also indicating that sampling was sufficient. The confidence intervals for calculations of mean thoracic segment numbers and body proportions (Figs 4 and 6) are reasonably broad because, while the total sample sizes are very large (over 450 specimens) and the subset samples of statistically adequate sizes, they were always biased towards data from moults with open facial sutures as this is by far the most common moulting characteristic in trilobites (Stubblefield, 1959; Henningsmoen, 1975; Daley and Drage, 2016; Drage, accepted).

Future considerations.—The data presented here can be applied to explore a host of other outstanding questions about the evolutionary history of trilobites. For example, morphology is intrinsically linked to ecological aspects like habitat and mode of feeding (Fortey and Owens, 1999). Owing to the inherent moulting behaviour flexibility in most trilobite groups identified by Drage (accepted), it is therefore likely that moulting also differed depending on mode of life, with considerations to be made for things like risk of predation (Rustán et al., 2011). Additional morphological adaptations could also be considered, including spinosity (which was common and likely for defense against predation; Clarkson and Ahlberg, 2002; Pates and Bicknell, 2019) and overall complexity of form (e.g. different shapes, number of articulations). It is likely that specialisation in moulting behaviour was more important for these as spinosity and complexity presumably

further increased the time taken to moult (and therefore the risk of predation) and the chance of self-injury during moulting (Owen, 1985).

Some generalisations can be made about the link between trilobite morphology and moulting behaviour from these findings, which might allow for a tentative prediction of likely moulting behaviour based on the size proportions of articulated carcasses, or of isolated cephala in particular. This could be useful given many trilobite taxonomic assignments are based on isolated sclerites due to the absence or scarcity of articulated material from many fossiliferous Palaeozoic localities. A link between the 'normal' moulting behaviours usually shown by groups of trilobites (see Drage, accepted), moulting flexibility, and phylogeny could also be explored using broad-scale behavioural and morphological datasets. It is hoped that future studies will develop the large source of data presented herein and in Drage (accepted) to further examine these other key aspects of life history, and address the problem of how trilobite moulting variability affected their evolutionary history.

Conclusions

The data presented here show that trilobite moulting variation, which is uniquely and broadly variable across the whole group (Henningsmoen, 1975; Brandt, 2002; Daley and Drage, 2016; Drage, accepted), has significant associations with exoskeleton morphology. These highlight a number of potential morphological constraints on moulting behaviour, or conversely adaptations in morphological proportions that would facilitate moulting. Trilobite individuals that moulted through disarticulating the entire cephalon and emerged through an exuvial gape at the cephalothoracic joint generally have a significantly wider body, broader cephalon, and fewer thoracic segments than for the other moulting

characteristics. Individuals that used the ventral sutures during moulting (often in tandem with the facial sutures) have a significantly longer thorax, more thoracic segments, and a broad cranidium (but not necessarily cephalon). Moulting through opening the facial sutures and disarticulating the librigenae was likely the standard for all forms due to early trilobite morphology (Fortey and Whittington, 1989; Daley and Drage, 2016; Du et al., 2018).

The data presented here could also be built upon in order to test for a bias in moulting behaviour with other morphological factors, in particular factors of complexity like spinosity, which greatly vary between trilobite species. With further analysis of the aspects affecting moulting behaviour flexibility, i.e. these other morphological aspects or phylogeny, we can work towards building a complete picture of the impact of this flexibility on trilobite evolutionary history, and the causes of their eventual extinction. The creation of large behavioural and morphological databases, such as the one initially presented in Drage (accepted) and analysed here, is absolutely necessary for the quantitative testing of broad-scale evolutionary trends in extinct animals.

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Chapter 5: An exceptional record of Cambrian trilobite moulting behaviour preserved in the Emu Bay Shale, South Australia

This and subsequent chapters take a focused view on the trilobite fossil record of moulting, accompanying the broad-scale global view in the preceding chapters. In this chapter I demonstrate that an extremely high level of intraspecific behavioural variability in moulting can be described from trilobites preserved under ideal conditions. The chapter also includes detailed descriptions of a number of new and revised moulting configurations, which can be applied to trilobite moults found at other Konservat-Lagerstätte.

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H.B.D. conceived the study, gathered data, and wrote the majority of the paper. J.D.H. collected specimens, gathered EBS data with H.B.D., and contributed to writing of several sections. G.-B.D.C. and A.C.D. provided assistance, and all authors read and edited the manuscript.

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An exceptional record of Cambrian trilobite moulting behaviour preserved in the Emu Bay Shale, South Australia

Running header: Diverse trilobite moulting from the Emu Bay Shale

by Harriet B. Drage, James D. Holmes, Diego C. García-Bellido & Allison C. Daley

Trilobites dominate the Emu Bay Shale (EBS) assemblage (Cambrian Series 2, Stage 4, South Australia) in terms of numbers, with *Estaingia bilobata* Pocock 1964 being extremely abundant, and the larger *Redlichia takooensis* Lu 1950 being common. Many specimens within the EBS represent complete moulted exoskeletons, which is unusual for Cambrian fossil deposits. The abundance of complete moults provides an excellent record that has allowed the recognition of various recurrent moult configurations for both species, enabling the inference of movement sequences required to produce such arrangements. Moult configurations of *E. bilobata* are characterised by slight displacement of the joined rostral plate and librigenae, often accompanied by detachment of the cranidium, suggesting ecdysis was achieved by anterior withdrawal via opening of the cephalic sutures. Moulting in *R. takooensis* often followed the same method, but configurations show greater displacement of cephalic sclerites, suggesting more vigorous movement by the animal during moulting. Both species also show rare examples of Salter's configuration, with the entire cephalon anteriorly inverted, and several other unusual configurations. These results indicate that moulting in trilobites was a more variable process than originally thought. In contrast, other Cambrian Konservat-Lagerstätten with an abundance of trilobites (e.g., Wheeler Shale, USA, and Mount Stephen Trilobite Beds, Canada) show larger numbers of 'axial shields' and isolated sclerites, often interpreted as disarticulated exuviae. This points to a higher level of disturbance from factors such as

animal activity, depositional processes, or water movement, compared to that of the EBS, where quiescent conditions and intermittent seafloor anoxia contributed to an unparalleled trilobite moulting record.

Key words: Emu Bay Shale, Burgess Shale, trilobite, ecdysis, moult configuration, Cambrian, Lagerstätten

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Trilobite exoskeleton moulting behaviour is highly variable in comparison to what has been observed for other Cambrian arthropods, and for modern groups (Daley & Drage 2016). Trilobites moulted throughout their lifetime to develop, grow, and repair, and in doing so left various 'moult configurations' preserved in the fossil record. Data suggest that trilobite moulting behaviour and the resulting configurations were both inter- and intraspecifically variable (Daley & Drage 2016), and this variability appears to be consistent amongst trilobites with less derived morphologies (unlike more specialised groups such as the Phacopina, Daley & Drage 2016). Other Cambrian arthropods (although with less heavily calcified exoskeletons) moulted using more consistent standardised behaviours, producing a uniform location for the 'exuvial gape' (where the animal exits the old exoskeleton). For example, the anterior exuvial gape can be observed in an exceptional specimen of *Marrella splendens* Walcott 1912 from the Burgess Shale preserved midway through moulting (García-Bellido & Collins 2004). More recent arthropods also show consistent moulting behaviour, such as in many decapod crustaceans where moults can be identified by their characteristic Open Moult Position with dorsal carapace displaced from the abdomen (see Daley & Drage 2016). In comparison, studies of trilobite moulting have demonstrated the significant variability of the exuvial gape location (e.g. McNamara & Rudkin 1984; McNamara 1986; Brandt 2002; Budil & Bruthansova 2005; Paterson *et al.* 2007; Daley & Drage 2016). The evolutionary function of this variability is mostly untested, and the lack of moulting behaviour specialisation in trilobites has been suggested as responsible for their eventual progressive extinction (Brandt 2002), or contrastingly their geological longevity (Hughes 2003; Daley & Drage 2016).

In a comprehensive attempt to standardise and better enable research into trilobite moulting, Henningsmoen (1975) described and named a number of these behaviours and

their resulting configurations. However, these do not represent the full variability of trilobite moult configurations. Due to the susceptibility of arthropod exoskeletons to disarticulation from decay and physical processes (e.g. water currents), only under conditions of exceptional *in situ* preservation would the intraspecific variability of moult configurations be preserved. This means that a greater flexibility in moulting behaviour cannot be observed at most trilobite-bearing localities. However, the Cambrian Series 2 Emu Bay Shale (EBS) Konservat-Lagerstätte, with soft-body preserving beds containing abundant complete *in situ* trilobite moult configurations, presents a rare opportunity to document the full variability in these behaviours and the fossils they produce.

At Big Gully, on the north coast of Kangaroo Island, South Australia, the EBS houses a diverse Konservat-Lagerstätte assemblage containing over 50 taxa, including sponges, brachiopods, hyoliths, polychaetes, priapulids, lobopodians, radiodontans, other non-mineralised arthropods (e.g. nektaspids and other artiopodans, as well as megacheirans), trilobites, vetulicolians, and several problematic forms (Paterson *et al*. 2008, 2016; García-Bellido *et al*. 2009). Like the majority of Cambrian Lagerstätten, the assemblage is dominated by arthropods, however the EBS is unusual in that trilobites, and in particular *Estaingia bilobata* Pocock 1964, overwhelmingly dominate in numerical abundance. *E. bilobata* approaches densities of up to 300 individuals per square metre within the EBS (comprising up to 80% of individuals on each slab), and it has been suggested that this species was an exaerobic specialist, occupying a niche between oxic and anoxic areas of the seafloor (Paterson *et al*. 2016). Specimens of the larger trilobite *Redlichia takooensis* Lu 1950 are also very common. In contrast, the three other known species of trilobite from the EBS, *Balcoracania dailyi* Pocock 1970, *Megapharanaspis nedini* Paterson & Jago 2006, and *Holyoakia simpsoni* Paterson & Jago 2006, are all extremely rare (Paterson & Jago 2006; Paterson *et al*. 2007).

The majority of trilobite specimens (>90%; Gehling *et al*. 2011) from the EBS Lagerstätte probably represent carcasses showing a high degree of articulation, with the remainder being moulted exoskeletons. Many of these moults experienced little-to-no disturbance prior to burial, providing a unique opportunity to infer moulting behaviours (exuviation) by reconstructing sequences of movement and disarticulation. This is unlike the majority of Cambrian fossil deposits, where even minor amounts of disturbance have resulted in the movement and separation of disarticulated sclerites, making moult configurations difficult to interpret (Daley & Drage 2016). The EBS provides the perfect opportunity to study moulting behaviour in Cambrian trilobites, which we reconstruct here using detailed descriptions of moult configurations of *E. bilobata* and *R. takooensis*. These are compared with specimens from two other exceptionally preserved Cambrian assemblages, namely *Ogygopsis klotzi* Rominger 1887 from the Burgess Shale (British Columbia, Canada) and *Elrathia kingii* Meek 1870 from the Wheeler Shale (Utah, USA), covering three different Cambrian trilobite orders (Redlichiida, Ptychopariida, and Corynexochida). This study is intended to describe the variable configurations resulting from trilobite moulting, rather than as a quantitative case study, such that it can be used as a tool for future studies identifying preserved moulting behaviours.

Geological Setting

The EBS outcrops at the shoreline northwest of the Emu Bay jetty (Paterson *et al*. 2008; Jago & Cooper 2011) and at Big Gully, which is situated approximately 7 km east of the Emu Bay township, and 1.5 km west of White Point (see Paterson *et al*. 2016, fig. 1). Early collections from this second locality were sourced from the wave-cut platform and cliff exposures immediately to the East of the mouth of Big Gully, however, since 2007

excavations at ‘Buck Quarry’ approximately 400 m inland have yielded a richer assemblage and better quality preservation than previously encountered (García-Bellido *et al*. 2009). More recently, ‘Daily Quarry’ has been opened slightly closer to shore and is also yielding high-quality material.

The EBS forms part of the early Cambrian (Series 2) Kangaroo Island Group, a largely clastic shelf succession that outcrops on the central northern coast of the island. Structures found within the conglomerate facies of the EBS, as well as within the older White Point Conglomerate (that thin towards the south), suggest that the sequence was deposited adjacent to an active tectonic margin, with the source area immediately to the north of the present coastline (Daily *et al*. 1980; Gehling *et al*. 2011). Syndepositional faulting south of this margin may have resulted in the formation of isolated oxic-to-anoxic sections of the seafloor within which the Lagerstätte was rapidly deposited (Gehling *et al*. 2011). This near-shore depositional environment is very different from that observed for other Cambrian Konservat-Lagerstätten, which are generally deposited in outer shelf settings (Gaines 2014). This seems to be reflected in the unusual mode of preservation, which differs from that of other Burgess Shale-type (BST) localities. Preservation of non-mineralised material within Cambrian Konservat-Lagerstätten is usually represented by two-dimensional compression fossils composed of primary carbonaceous remains, a pathway termed ‘Burgess Shale-type preservation’ (Butterfield 2003). In contrast, preservation within the EBS seems to be somewhat different, with fossils exhibiting some three-dimensionality due to early-stage diagenetic mineralisation, including the phosphatisation of certain soft tissues (e.g. muscle and gut), as well as phosphatisation and pyritisation of cuticular structures such as eyes, and late diagenetic casting of fossils by fibrous calcite (see Paterson *et al*. 2016 and references therein).

Soft-bodied preservation occurs predominantly in the lowest 10–12 m of the EBS at Big Gully, within dark grey, micaceous, finely-laminated mudstone, interspersed with siltstone and fine sandstone horizons interpreted as gravity flow or storm deposits (Gehling *et al*. 2011). The majority of material collected from the EBS since 2007 (including the material discussed herein) originates from a c. 2 m thick section exposed in Buck Quarry, approximately 10 m above the base of the EBS. Material from Daily Quarry, approximately 50 m north of Buck Quarry along strike, was sourced from the same beds. A prominent sandstone bed c. 20 cm thick exposed in both quarries allows correlation between the two. The Konservat-Lagerstätten bearing mudstones are highly fossiliferous throughout this interval, with fossils preserved in millimetre-scale discontinuous laminate beds. Mudstone bedding planes do not appear to represent single events; rather, these are interpreted as background accumulations of fine sediment, with benthic taxa presumably tolerant of low oxygen conditions preserved *in situ,* and other elements being transported in or settling out of the water column (Paterson *et al*. 2016). Preservation of moult configurations appears to be consistent throughout this interval and between the two quarries.

Based on the presence of *E. bilobata*, *R. takooensis* and *B. dailyi* the EBS has been correlated with the *Pararaia janeae* trilobite zone of mainland South Australia (Jell in Bengtson *et al*. 1990; Jago *et al*. 2006; Paterson & Brock 2007), which is equivalent to the mid-late Botoman Stage of Siberia, the upper Nangaoan/lower Duyunian of South China, and the Dyeran of North America (Paterson & Brock 2007; Peng *et al*. 2012). Absolute age is estimated at *c*. 514 Ma based on correlation with the global Cambrian timescale presented in Figure 19.3 of Peng *et al*. (2012), corresponding to the Cambrian Series 2, early Stage 4. The comparative localities of the North American Burgess and Wheeler Shales are both middle Cambrian (Miaolingian) in age. The former is late Wuliuan

corresponding to *c.* 505 Ma, with the latter being early Drumian, *c.* 504 Ma (Collom *et al*. 2009; Robison & Babcock 2011; Peng *et al*. 2012).

Sedimentology and taphonomy of moult configurations

Context must be considered when examining trilobite moult assemblages, with a focus on understanding how taphonomic processes could have affected the relative arrangement of sclerites. Data on the sedimentology and taphonomy for the EBS are detailed (Gehling *et al.* 2011; McKirdy *et al.* 2011) and demonstrate its particular suitability for a study of *in situ* trilobite moult assemblages. All specimens of *E. bilobata* and *R. takooensis* used for this study originate from the same mudstone beds as the soft-bodied preservation at the EBS (e.g. Paterson *et al.* 2016).

Geochemical analyses conducted by McKirdy *et al*. (2011) suggest that the fossiliferous EBS mudstones were consistently deposited beneath a largely oxic water column, as is also suggested by the diverse free-swimming elements of the biota (Paterson *et al*. 2016). The original presence of pyrite within the sediment, impoverished representation of fixosessile taxa, and absence of trace fossils (which become abundant subsequent to the zone of exceptional preservation) suggest that the pore water below the sediment-water interface was anoxic, with low-oxygen conditions also occurring at least occasionally above the seafloor (Gehling *et al*. 2011; McKirdy *et al.* 2011). Geochemical content is relatively homogenous throughout the exceptional-preservation mudstones (McKirdy *et al.* 2011, fig. 4). The EBS study beds, and the trilobite moult configurations described herein, therefore show a consistent preservation style as part-counterpart internal-external moulds, which, although compressed, exhibit some three-dimensionality. This suggests no preservational bias is influencing the results from this study.

Further, the fossiliferous section of the EBS preserves soft tissue and *in situ* trilobite moult assemblages in part due to its almost complete lack of bioturbation, predation, or scavenging (Gehling *et al.* 2011; McKirdy *et al.* 2011), presumably as a consequence of the periodic low-oxygen conditions. This is consistent across all soft-bodied fossil-bearing mudstones, which retain their finely laminated texture, and in contrast to the silty, less fossiliferous intervening beds (McKirdy *et al.* 2011, fig. 2). There is rare evidence of predation or scavenging on *E. bilobata* by the chelicerate *Wisangocaris* Jago *et al.* 2016, as well as injuries inflicted upon specimens of *R. takooensis* (Conway Morris & Jenkins 1985) and the nektaspid *Emucaris* Paterson *et al.* 2010 (Nedin 1999), suggesting that at times the seafloor was at least partially oxygenated. However, most trilobite carcasses and moult configurations show a high degree of articulation. This may have resulted from depth fluctuations of the oxycline, causing mass asphyxiation of individuals, with the prevailing low-oxygen conditions helping to facilitate preservation by preventing scavenging and bioturbation prior to burial (Paterson *et al*. 2016). Mat-forming cyanobacteria may have helped maintain a sharp redox boundary between water column and sediment, and inhibited decay and disruption of soft tissues and articulated cuticle (McKirdy *et al.* 2011). The EBS also shows less evidence of weathering or thermal metamorphism than comparable early Cambrian Konservat-Lagerstätten (McKirdy *et al.* 2011).

In addition, Gehling *et al.* (2011) found no sedimentological evidence for water movement at the EBS. Large surfaces are not often recovered due to persistent cleavage and the lenticular nature of the bedding planes, however the trilobite moult configurations and intact carcasses show no consistent orientation. Different types of moult configurations for *E. bilobata* are also regularly found in close association, on the same slabs, which further argue against any sorting or bias to preservation across bed layers. In

addition, any possible bias could also be determined because of the rigorous method of sampling employed at the EBS, during which way up and bed level markers have been recorded on all specimens. These factors, combined with the high degree of fossil articulation, suggest that within the exceptional-preservation mudstones certain taxa are found not only *in situ*, but with essentially no disturbance prior to burial. Due to these combined sedimentological and taphonomic aspects producing the observed high fossil articulation and exceptional preservation, and the abundance of two trilobite species, the EBS is an ideal locality with which to explore trilobite moulting variability and behaviour.

Material and Methods

All figured material from the Emu Bay Shale was collected from Buck and Daily Quarries and is housed in the palaeontological collections of the South Australian Museum, Adelaide (prefix SAM). The Mount Stephen Trilobite Beds (Burgess Shale), British Columbia, and the Wheeler Shale, Utah, were chosen for comparison to the EBS due to their vast numbers of singular trilobite species (*Ogygopsis klotzi* and *Elrathia kingii,* respectively) and similar exceptional preservation conditions. Figured material from these localities is housed in the Natural History Museum, London (prefix NHMUK), Oxford University Museum of Natural History (prefix OUMNH), and Smithsonian National Museum of Natural History (prefix NMNH).

The problem of distinguishing between arthropod moults and carcasses has been discussed by a number of authors and a range of criteria for this proposed (e.g. Henningsmoen 1975; Whittington 1990; Daley & Drage 2016). In their review of the fossil record of moulting, Daley & Drage (2016) considered that the major requirements for the recognition of trilobite moults were, (1) evidence for the opening of exoskeletal sutures,

and (2) repeated configurations of displaced exoskeletal units, coupled with consideration of the preservational environment. These criteria were applied when identifying the moult configurations from the SAM, NHMUK, OUMNH, and NMNH. There are approximately 5000 specimens of *E. bilobata* and 300 of *R. takooensis* within the collections of the SAM, of which perhaps 10% represent moulted exoskeletons. Figured specimens were chosen as clear examples of the various configurations described herein due to their completeness and quality of preservation. 78 moult configurations of *O. klotzi* and 35 of *El. kingii* were compared to the selected EBS specimens, and appropriate representatives figured. In order to explore the relationship between trilobite orientation and moult configuration, a field sample of 100 randomly selected *E. bilobata* specimens taken from adjacent mudstone beds were examined.

Most specimens were photographed dry using Canon EOS 500D or 50D Digital SLR cameras, with a Canon EF-S 60 mm 1:2.8 Macro Lens under incident lighting from the standard NE direction. Particularly small specimens were photographed using a Canon MP-E 65 mm 1:2.8 1-5x Macro Lens. The camera was remotely controlled using the Canon EOS Utility 2.8.1.0 program. Additional specimens were photographed dry using a Canon SX60HS PowerShot digital camera with the above light settings. Photographs were adjusted and edited, and figures produced in Adobe Photoshop and Illustrator CS3 and CS6.

Results

Descriptions of recognised moult configurations

Minor variations on seven general types of moult configuration were recognised for the EBS specimens, three of which have been described in the literature for other trilobite species (Henningsmoen 1975). The existing configurations are presented here for clarity, and the four new configurations described and named. Individual moult configurations may vary slightly within this classification, such as in the specific placement of the separated sclerites. This will aid future studies of trilobite moulting in Cambrian Konservat-Lagerstätte, and be of value as generalised descriptions for describing trilobite moults throughout their evolutionary history (Fig. 1).

Trilobite moult configurations identified in the fossil record of the EBS (see Fig. 1):

1. *Axial shield* (Fig. 1B; named in Henningsmoen 1957). The cranidium, thorax, and pygidium are joined as a single unit (i.e. entire dorsal exoskeleton minus the librigenae). This is not a moulting configuration *per se*, but rather the product of displacement of sclerites (rostral plate, hypostome and librigenae) separated during moulting. As such, it can originate from Harrington's, Somersault, McNamara's or Nutcracker configurations described below.
2. *Harrington's configuration* (Fig. 1C; named in Henningsmoen 1975). Moult configuration comprising axial shield and displaced 'lower cephalic unit' (LCU; all parts of the cephalon except the cranidium, joined by integument or partially fused facial sutures, *sensu* Henningsmoen 1975). The joined LCU (comprising the librigenae and rostral plate, but usually lacking the hypostome) is often displaced posteriorly below the axial shield, or singular parts of the LCU may be disrupted presumably by decay of the integument or suture decalcification. The cranidium remains attached to the thorax.

3. *Henningsmoen's configuration* (Fig. 1D; named here). Similar to Harrington's configuration, although usually with minor displacement of the LCU, and showing displacement of the cranidium with respect to the trunk (joined thorax and pygidium). Named such because Henningsmoen (1975) notes the possible occurrence of this in addition to his Harrington's configuration (p. 194).
4. *Nutcracker configuration* (Fig. 1G; named here). Librigenae, and usually the rostral plate, separated and generally displaced outwards from the axial shield. The hypostome is usually displaced but may remain close to life position. Named for the postulated relatively simultaneous outwards displacement of most cephalic sclerites.
5. *Somersault configuration* (Fig. 1F; named here). Moult configuration with one or both librigenae disarticulated from the cranidium and anteriorly inverted, leaving the genal spine pointing forwards. The rostral plate may or may not be visibly displaced, and in some cases may be joined to the librigenae as an intact LCU. Named for the similar direction of movement in the acrobatic action.
6. *McNamara's configuration* (Fig. 1E; named here). Similar to the above configuration, but with one or both librigenae disarticulated and laterally inverted. Suggested by McNamara & Rudkin (1984, fig. 5) and McNamara (1986) to occur in *Batocara mitchelli* Foerste 1888*, Asaphiscus wheeleri* Meek 1873*, Redlichia forresti* Etheridge 1890*,* and *Redlichia micrograpta* Öpik 1970.
7. *Salter's configuration* (Fig. 1H; also called *Salterian configuration*, named in Richter 1937; Richter *et al.* 1959; Henningsmoen 1975). Cephalon is disarticulated from the trunk and anteriorly inverted, usually resulting in the anterior margin of the cephalon facing posteriorly and often underlapping the trunk.

All of these configurations (with the exception of Salter's) likely result from Henningsmoen's (1975) 'Sutural Gape' mode of moulting. This involved moulting through an anterior exuvial gape (opening in the old exoskeleton) produced by opening of the cephalic exoskeleton sutures (comprised of the facial, rostral, connective, and hypostomal sutures), with the cranidium and the LCU forming the dorsal and ventral exuvial elements, respectively. Different movements of the individual during this mode of moulting result in the above configurations preserved at the EBS. The exception, Salter's configuration, is produced through the 'Salterian' mode of moulting, which utilises an exuvial gape produced at the cephalothoracic joint. Other rarer modes of moulting, resulting in more unusual configurations, were also described by Henningsmoen (1975), such as the *Harpes* mode using the marginal-interlamellar cephalic suture (ibid. p. 189), or the *Ductina* mode using a marginal cephalic suture and sometimes resulting in 'Maksimova's configuration' (ibid. p. 190), or 'Hupe's configuration' with a displaced rostral plate (ibid. p. 192). However, these are linked to derived morphologies (e.g. fusion of the facial sutures), and are therefore not observed for *E. bilobata* or *R. takooensis*. The outlined configuration definitions are used below to describe the preserved moults of *E. bilobata* and *R. takooensis*, and thus to interpret their moulting behaviours and movements.

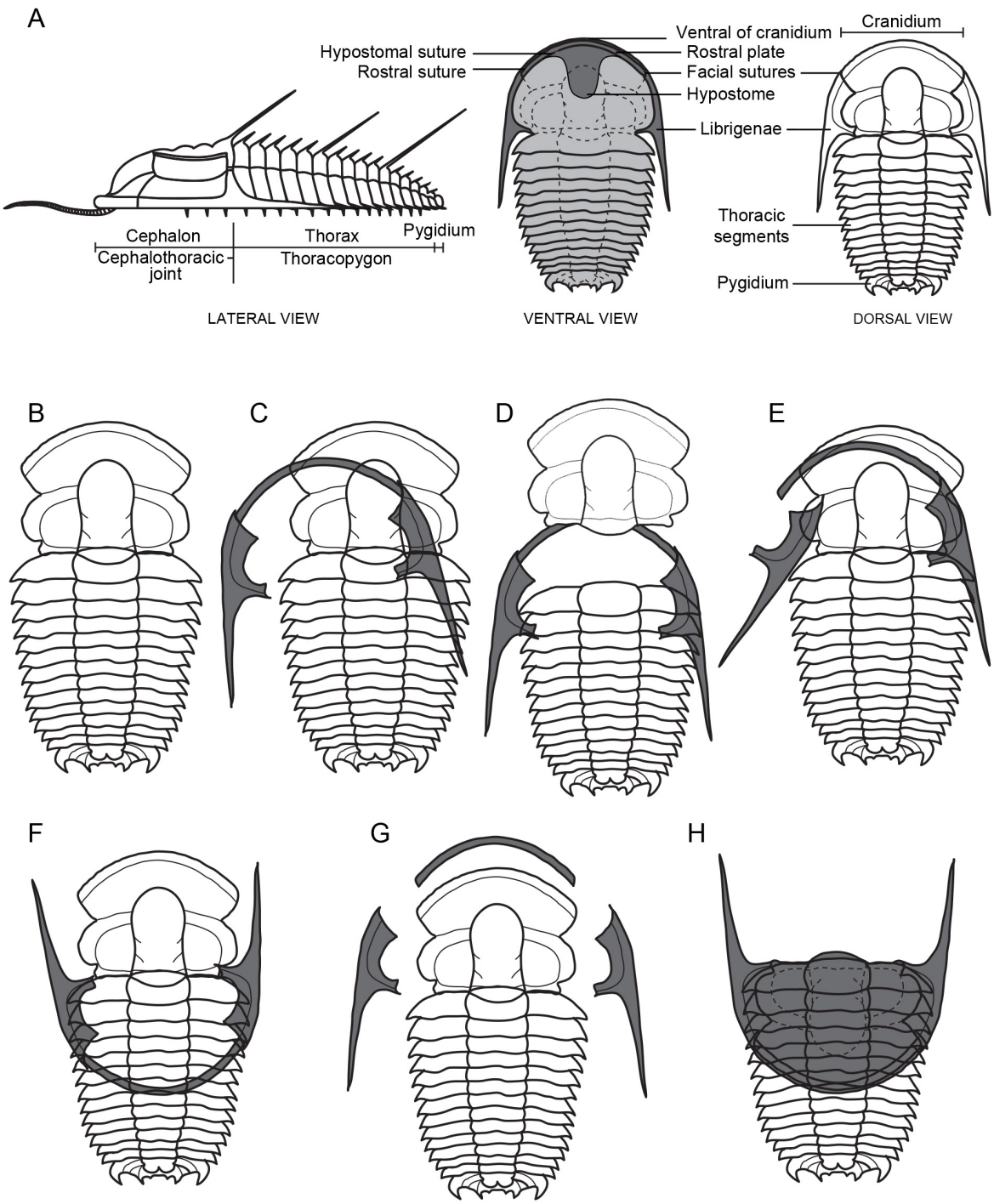


Figure 1: Trilobite anatomy (A) and moult configurations (B-H) described from the Emu Bay Shale and represented on the body of *Estaingia bilobata*. Ventral structures (rostral plate, hypostome), and structures that in some instances underlap the ventral exoskeleton due to moulting (librigenae, cephalon) are shaded grey. B, axial shield; C, Harrington's configuration (e.g. SAM-P 15339, see Fig. 3A); D, Henningsmoen's configuration (e.g. SAM-P 45519, see Fig. 2D); E, McNamara's configuration (e.g. SAM-P 48062 and 54207, see Figs. 4F and 3B respectively); F,

Somersault configuration (e.g. SAM-P 54204, see Fig. 3D); G, Nutcracker configuration (e.g. SAM-P 48085, see Fig. 4A); H, Salter's configuration (e.g. SAM-P 43593 and 15459, see Figs. 4I and 3E). Axial shields are incomplete moult configurations, Salter's configuration results from a 'Salterian' mode of moulting, and the remaining five complete configurations result from a 'Sutural Gape' (*sensu* Henningsmoen 1975) mode of moulting. Configurations C, D, and F are found at the EBS for only *Estaingia bilobata*; G for only *Redlichia takooensis*; and B, E, and H for both species.

Description of moulting in Estaingia bilobata *Pocock, 1964*

Estaingia bilobata is a small trilobite (up to *c.* 30 mm in length) with 13 thoracic segments, narrow librigenae bearing long genal spines, and a small pygidium. The opisthoparian facial and rostral sutures are operative, with the detached rostral plate slightly shorter than the anterior border of the cranidium (Pocock 1964). *Estaingia* is restricted to early Cambrian sediments of Australia, Antarctica and South China (Jell in Bengston *et al*. 1990; Palmer & Rowell 1995; Paterson 2005; Paterson & Brock 2007; Dai & Zhang 2012).

Pocock (1964, p. 461) noted that 'many specimens of *Estaingia* show that the cephalon has rolled forward over the detached rostral plate, and that the librigenae are free, but only slightly displaced; this appears to have been the characteristic mode of moulting in this trilobite'. Our observations support this interpretation, although moult configurations of *E. bilobata* within the EBS exhibit considerable variation. As discussed, the majority can be explained by the Sutural Gape mode of moulting, with the opening of the facial and rostral sutures, and common dislocation of the cephalothoracic joint, resulting in a range of configurations due to the movements of the moulting trilobite. The

vast majority of *E. bilobata* moults exhibit either Henningsmoen's (Figs 1–2) or Harrington's configurations (Figs 1, 3; around 35 and 55% of specimens respectively). Specimens in Harrington's configuration exhibit disarticulation and posterior displacement of the LCU (which may not always comprise both librigenae) relative to the cranidium. The positioning of the LCU relative to the axial shield varies, and occasionally displays slight disassociation or rotation of its components. Henningsmoen's configuration is similar, but with the cranidium displaced in addition to the LCU. Inversions of the librigenae (Somersault and McNamara's configurations) were very rare, as was Salter's configuration and instances of thoracic disarticulation.

SAM-P 43837 (Fig. 2A) shows an example of Henningsmoen's configuration in *E. bilobata*. The librigenae are slightly displaced, the rostral plate has moved 2–3 mm posteriorly relative to the cranidium, and the cranidium shows minor displacement from the thorax. SAM-P 43974 (Fig. 2B), 43402 (Fig. 2C), and 46933 (Fig. 2E) also display variations of Henningsmoen's configuration. Figure 2B displays anterior movement (*c*. 2 mm) and minor anti-clockwise rotation of the cranidium, lateral and anterior displacement of the left librigena, and the right librigena joined with the rostral plate. In Figure 2C the cranidium is only slightly anteriorly displaced, and the rostral plate and left librigena have been pushed backwards to underlie the occipital ring and anterior of the trunk. The right facial suture is open. In Figure 2E the rostral plate and left librigena appear connected and are slightly displaced outwards and rotated. The cranidium and poorly preserved right librigena are anteriorly displaced, with some anti-clockwise rotation. SAM-P 45519 and 46362 (Fig. 2D, F) show Henningsmoen's configuration, but differ from the regular pattern as their cranidia are anteriorly displaced (so that in Fig. 2D the middle of the glabella overlaps the rostral plate) but the joined LCU remains largely *in situ* with

negligible displacement. However, the rostral plate may have remained in place for Figure 2F based on the unusually deep anterior cephalic border furrow.

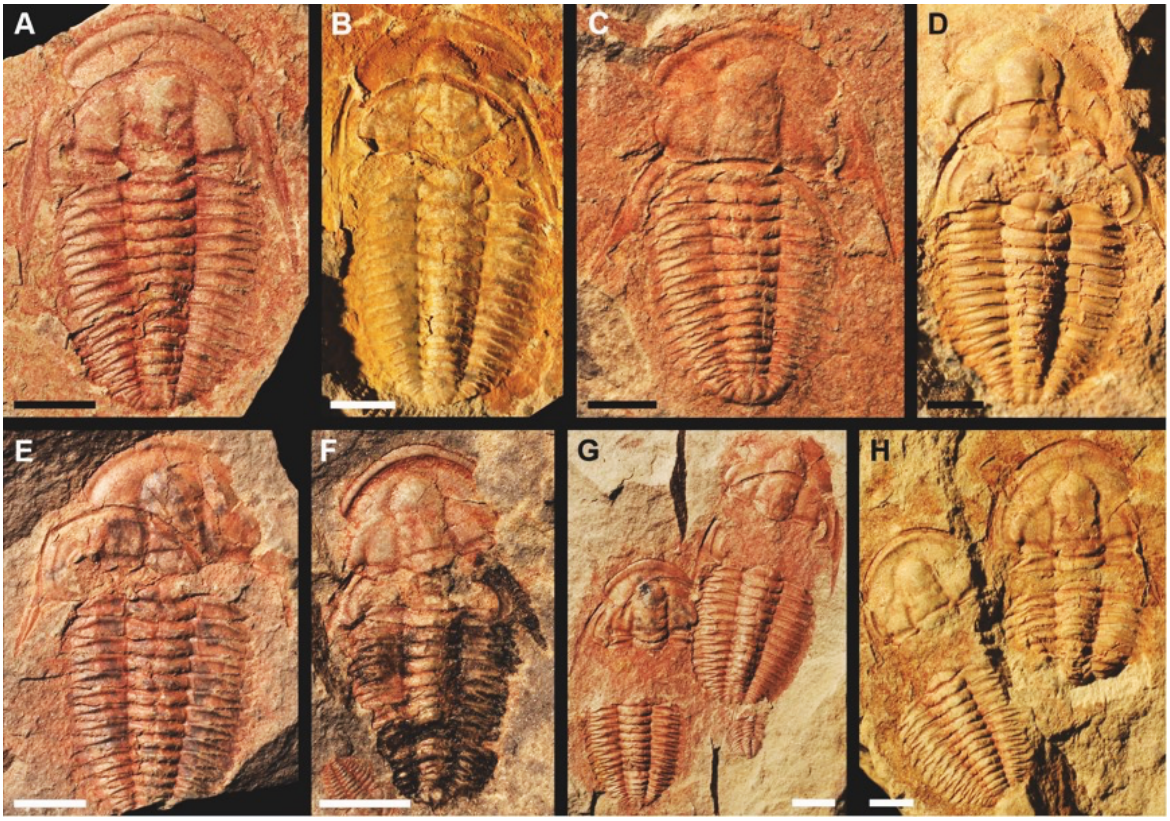


Figure 2: Example moult configurations of *Estaingia bilobata* from the Emu Bay Shale collections of the South Australian Museum, referred to in the text. A, SAM-P 43837; B, SAM-P 43974; C, SAM-P 43402; D, SAM-P 45519; E, SAM-P 46933; F, SAM-P 46362; G, SAM-P 46956; H, SAM-P 45697. A-H display Henningsmoen's configuration; G (left individual) and H (right individual) Harrington's configuration. Scale bars 5 mm, D and H scale bars 2.5 mm.

SAM-P 46956 (Fig. 2G) consists of a moult pair of *E. bilobata*. The right-hand specimen shows a variant of Henningsmoen's configuration, with the left librigena remaining joined to the trunk, the joined right librigena and rostral plate carried *c*. 3 mm forwards, and the cranidium deposited a further 2 mm in front. Unusually, the left specimen shows disarticulation between the first and second thoracic segments, rather than

at the cephalothoracic joint. The cephalic region with associated first thoracic segment is displaced forwards *c*. 6 mm and laterally offset. The rostral plate and joined right librigena are displaced to the right and rotated anti-clockwise. Other specimens, e.g. SAM-P 45697 (Fig. 2H), show similar moult configurations to this paired example.

SAM-P 15339 (Fig. 3A) exemplifies Harrington's configuration in *E. bilobata.* This specimen shows left-lateral displacement and minor anti-clockwise rotation of the LCU. The axial shield is otherwise preserved intact. Moult configurations similar to this are very common for *E. bilobata*. Moults showing inversions of the librigenae were generally much rarer than Henningsmoen's or Harrington's configuration (representing around 6% of specimens, combined), with two specimens clearly exhibiting the Somersault configuration (e.g. SAM-P 54204, Fig. 3D) and a further four showing variations of McNamara's configuration (e.g. SAM-P 54207, Fig. 3B).

SAM-P 54206 and 15459 (Fig. 3C, E) show *E. bilobata* moults in modified versions of Salter's configuration (representing around 4% of specimens), the former with the trunk resting on the inverted cephalon, and the latter with the cephalon inverted anteriorly but lying directly adjacent to the trunk. In the first specimen, both librigenae are displaced forwards relative to the cranidium (but towards the posterior of the specimen), and rotated outwards. Another, smaller specimen in the SAM collections shows an almost identical arrangement. In the second specimen, the left librigena remains in place relative to the cranidium and the right is detached and missing. Interestingly, one specimen (SAM-P 54205, Fig. 3F) has the final five thoracic segments and pygidium rotated 180^{o}, the rostral plate displaced backwards, and is possibly missing the left librigena. This appears to be a very unusual moult configuration for *E. bilobata*.

The moult configurations above were described from previously collected specimens in museum collections, and this was complemented by a field study that

examined the orientation of *E. bilobata* carcasses and moults. Of the 100 specimens of *E. bilobata* examined, 90 were oriented dorsum-down (overturned), six were upright, and four were small and/or partial specimens that were either enrolled, or potentially representing moulted exoskeletons in Salter's configuration. Of the six upright specimens, at least two likely represent moults and the remaining four were difficult to analyse owing to poor and/or partial preservation.

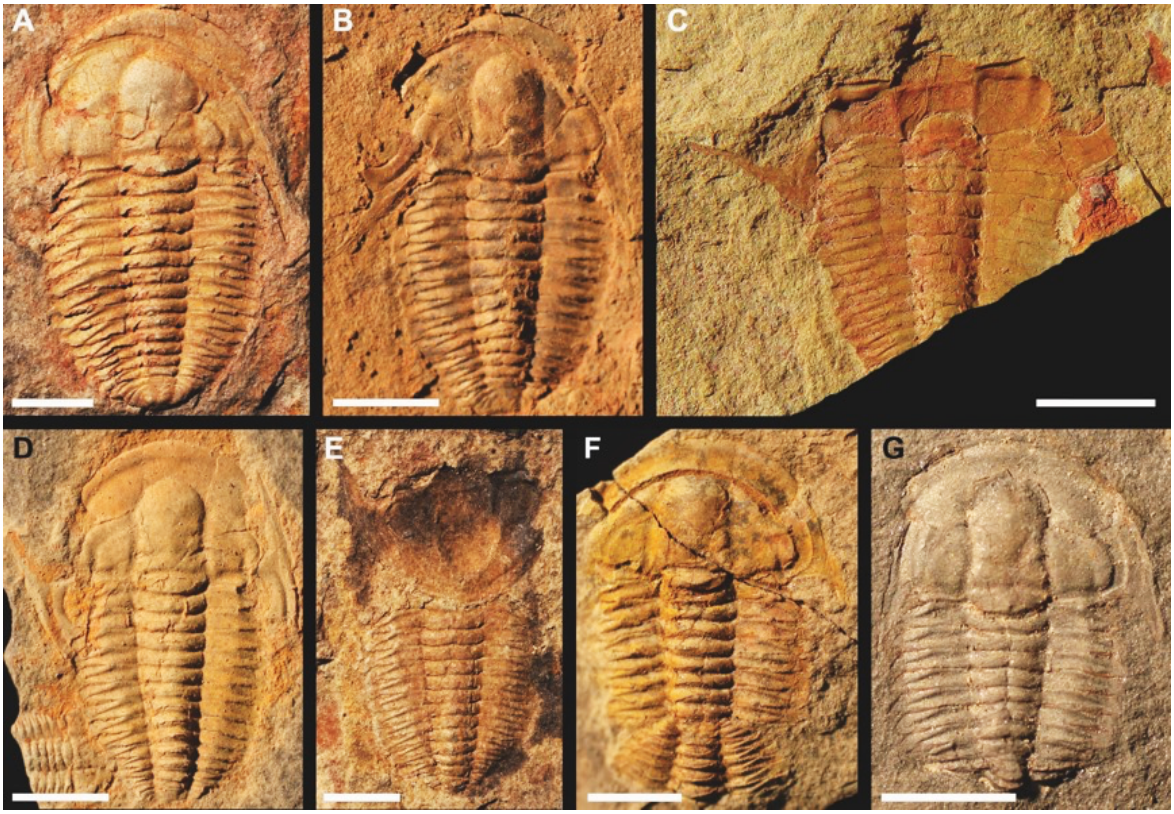


Figure 3: Example moult configurations of *Estaingia bilobata* from the Emu Bay Shale collections of the South Australian Museum, referred to in the text. A, SAM-P 15339; B, SAM-P 54207; C, SAM-P 54206; D, SAM-P 54204; E, SAM-P 15459; F, SAM-P 54205; G, SAM-P 54208. A and likely F display Harrington's configuration with F also showing rotation of the posterior thoracopygon; B McNamara's configuration; C and E Salter's configuration; D the Somersault configuration. G is a complete carcass. Scale bars 5 mm.

Description of moulting in Redlichia takooensis *Lu, 1950*

Redlichia takooensis is a large redlichiid trilobite known from lower Cambrian (late Botoman) sediments in Australia and South China (Zhang *et al*. 1980; Jell in Bengtson *et al*. 1990). The cephalic shield is semi-circular, with large librigenae bearing long genal spines that form a continuous curved border with the anterior of the cranidium, which in turn bears a long occipital spine. As in *E. bilobata*, the (opisthoparian) facial and rostral sutures are operative, with the rostral plate slightly narrower than the anterior of the cranidium (Jell in Bengtson *et al*. 1990). The thorax has 15 segments, each bearing a medial spine that is elongated on the 6th and particularly the 11th, and is relatively narrow compared to the width of the cephalon, especially in smaller specimens (Paterson & Jago 2006). The pygidium is small. Articulated specimens up to 25 cm long are known from Big Gully (Paterson *et al*. 2016), although most specimens from the Buck and Daily Quarries are 5–7 cm in length.

As with *E. bilobata*, specimens considered to be moults of *R. takooensis* exhibit considerable variation. *R. takooensis* also usually employed a Sutural Gape mode of moulting, but in general this produced different moult configurations to *E. bilobata*. Outwards displacement (away from the axial shield) of individual cephalic sclerites (e.g. the Nutcracker configuration, Fig. 1), rather than a joined LCU (this was not observed), is common, as is thoracic disarticulation. The latter is often associated with segments 6 and 11. The hypostome appears to have been shed during moulting, although displaced little and therefore often remained close to life position within the moult configuration.



Figure 4: Example moult configurations of *Redlichia takooensis* from the Emu Bay Shale collections of the South Australian Museum, referred to in the text. A, SAM-P 48085; B, SAM-P 44214; C, SAM-P 45440; D, SAM-P 44544; E, SAM-P 46207; F, SAM-P 48062; G, SAM-P 46047; H, SAM-P 45368; I, SAM-P 43593; J, SAM-P 45875; K, SAM-P 54209. A-E display the Nutcracker configuration; F and G McNamara's configuration; H-J Salter's configuration. K is a complete carcass. Scale bars 10 mm.

SAM-P 48085 (Fig. 4A), 44214 (Fig. 4B), and 44544 (Fig. 4D) illustrate the Nutcracker configuration for *R. takooensis* (representing around 90% of specimens). In

these moult configurations the librigenae are displaced outwards from the cranidium (by *c*. 1–10 mm), and show some rotation. Figure 4A and D have the rostral plate displaced forwards, although the anterior of the fossil is broken for Figure 4B. In Figure 4A the thorax is disarticulated between segments 11 and 12, with the posterior part of the trunk disrupted and rotated slightly clockwise. Figure 4D displays disarticulation between segments 3 and 4 (*c*. 2 mm), and 9 and 10 (*c*. 5 mm), and displacement of the hypostome to lie beneath the anterior cephalic border. SAM-P 45440 (Fig. 4C) is a small specimen of *R. takooensis* that also shows disarticulated librigenae, slight posterior displacement of the hypostome, and thoracic dislocation. The trunk posterior to segment 8 is missing, and segments 4–6 are heavily imbricated. The hypostome is much more visibly displaced and inverted in SAM-P 46207 (Fig. 4E), its anterior wings being adjacent to the right of segment 4. This specimen may also represent the Nutcracker configuration, as the librigenae are absent, although much of the specimen has been truncated by rock fracture.

Moult configuration SAM-P 48062 (Fig. 4F) shows the left librigena displaced outwards (*c*. 5 mm), however the right is laterally inverted so that the medial edge is facing away from the axial shield in the uncommon McNamara's configuration (<5% specimens). The cranidium is also slightly disrupted, and the rostral plate is not preserved due to truncation. SAM-P 46047 (Fig. 4G) also displays McNamara's configuration, with the right librigena laterally inverted, the cranidium and left librigena rotated anti-clockwise, and the rostral plate displaced.

SAM-P 45368, 43593, and 45875 (Fig. 4H–J) show Salter's configuration (representing around 5% of specimens). Figure 4H is incomplete posterior to the 11th segment, revealing the long medial thoracic spine. The occipital spine extending from the cranidial base is also preserved, confirming that the cephalon must have been inverted anteriorly during moulting, and that these are internal moulds (causing the underlapping

cephalon to be impressed upon the overlying trunk). This is also the case for the more complete specimens in Figure 4I and J. SAM-P 45875 (Fig. 4J) also shows dislocation between segments 4 and 5, and 11 and 12; there also appears to be extensional damage to the joint between the axial rings of segments 10 and 11, likely a result of pressure on the 11th segment's extended medial spine.

As seen from these examples, almost all associations at the EBS are relatively complete, although occasionally the posterior-most of the axial shield is missing. As such they are generally easily recognisable as moults or carcasses. Configurations consisting of entirely disarticulated sclerites, such that they are not behaviourally informative, are very unusual. In *R. takooensis* these usually consist of the rostral plate with attached hypostome and the individual librigenae, presumably from the same individual (they are size-compatible), but without a proximal axial shield. The corresponding axial shield may however have been preserved close by, but broken from the specimen during collecting. Isolated axial shields (e.g. Fig. 4E if it were not truncated) are also extremely rare for both *E. bilobata* and *R. takooensis*.

Description of trilobite moulting at other Cambrian Konservat-Lagerstätten

Mount Stephen Trilobite Beds, British Columbia, Canada.–The middle Cambrian Mount Stephen Trilobite Beds (Burgess Shale Formation, British Columbia) preserve trilobite fossils in great abundance (Fletcher & Collins 2003). In particular, specimens of *Ogygopsis klotzi* Rominger 1887 (Order Corynexochida), including moults, are extremely numerous, the locality often being referred to as the *'Ogygopsis* Shales' (Rudkin 2009). The majority of *O. klotzi* moults observed for this study (62 specimens at the NHMUK, OUMNH, and NMNH) comprise only axial shields (e.g. Fig. 5C), confirming the taxon

usually moulted by the Sutural Gape mode (Henningsmoen 1975). For these specimens no further details about moulting behaviour can be inferred as they lack associated disarticulated sclerites. Disarticulated associations seem more common than for the EBS (from more than 30 individual larger slabs with preserved trilobite material in background matrix housed at the NHMUK and NMNH), showing disarticulation and accumulation of individual sclerites such that they are indeterminable as moults or carcasses (e.g. Fig. 5F). Almost every observed larger slab displayed fragmentary *O. klotzi* material, usually alongside axial shields or complete trilobites.

In some cases where complete moult configurations are preserved, probable exuvial movements can be inferred in the same manner as for EBS trilobites. NHMUK I.4751(1) (Fig. 5A) is missing the librigenae, but also shows very slight displacement and rotation of the cranidium (akin to an incomplete Henningsmoen's configuration). Several configurations appear to represent more 'difficult' moulting events, as the full anterior exuvial gape failed to open (potentially damaging the individual moulting through this smaller gape). For example, NHMUK I.4741 (Fig. 5D) shows disruption to the left librigena and distortion of thoracic segments. NHMUK I.4751(2) (Fig. 5B) shows a relatively complete *O. klotzi* moult. The right librigena is missing, the left is displaced under the axial shield, and the middle thoracic segments have been displaced, leaving some remnants nearby. Two specimens from the NMNH represent similar moult configurations, with singular librigena rotated laterally (right) away from the axial shield, with one also showing thoracic disarticulation between segments 7 and 8. Pygidium disarticulation was also observed in two NMNH axial shield moult configurations.

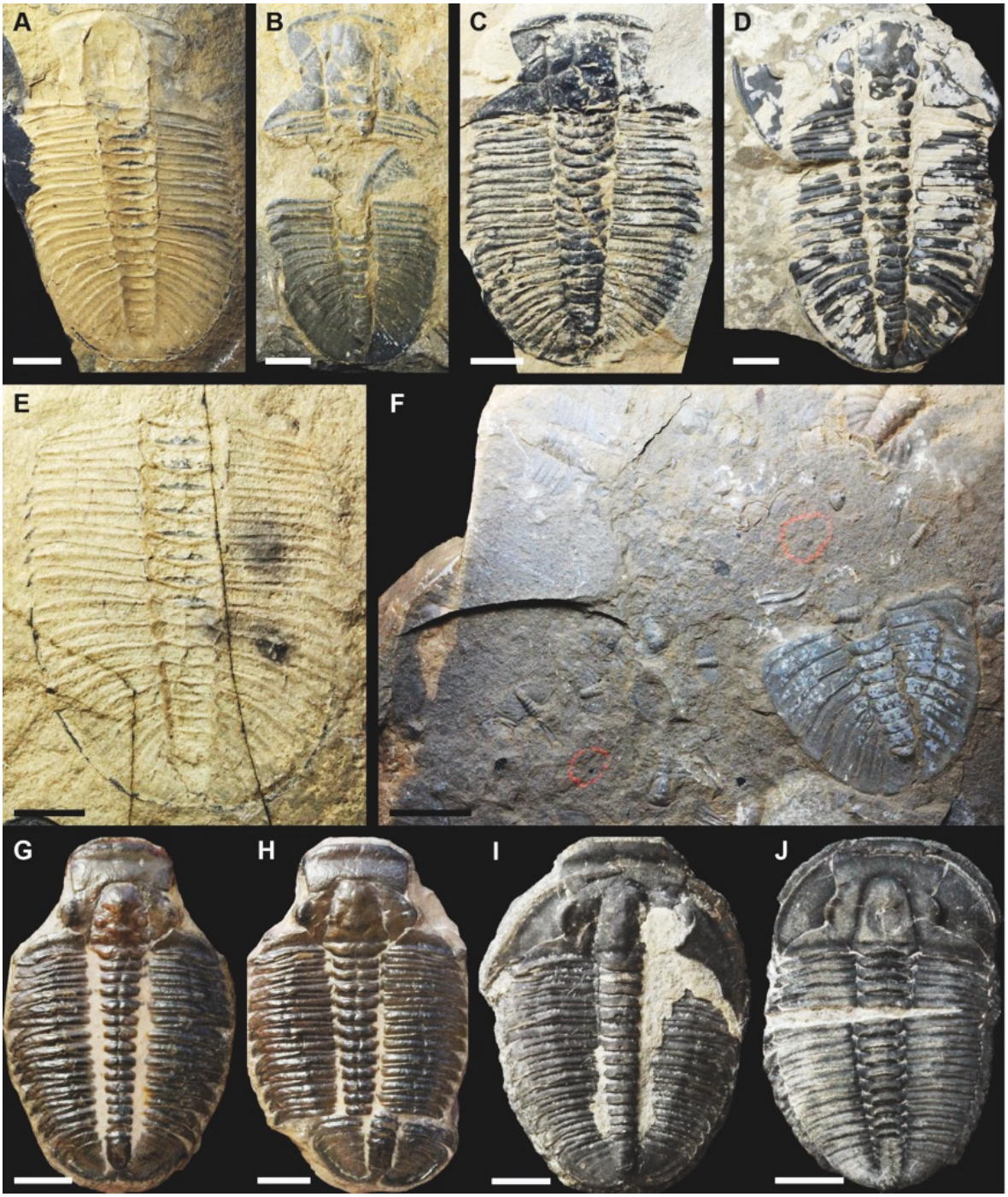


Figure 5: Example moult configurations of *Ogygopsis klotzi* from Mount Stephen Trilobite Beds, Burgess Shale, British Columbia (A–F), and *Elrathia kingii* from the Wheeler Shale, Utah (G–J), photographed from the Natural History Museum, London, collections. A, NHMUK I.4751(1); B, NHMUK I.4751(2); C, NHMUK I.4746; D, NHMUK I.4741; E, NHMUK In.19410; F, NHMUK I.4753; G, NHMUK It.21001; H, NHMUK It.20999; I, NHMUK It.21003; J, NHMUK It.5937. A, C, G and H are axial shields; B and D variants of the Nutcracker configuration; I Harrington's

configuration. E shows an isolated trunk, and J is a complete carcass. A–E scale bars 10 mm, F scale bar 20 mm, G, H, J scale bars 5 mm, I scale bar 2.5 mm.

Isolated thoracopyga were also observed (e.g. NHMUK In.19410, Fig. 5E), although not as complete moult assemblages as the disarticulated cephalic structures are not generally found in association. A single trunk (complete thorax and pygidium) from the NMNH was associated with the right librigena and potentially the fragmented hypostome, both displaced slightly to the left. Librigenal inversions (Somersault or McNamara's configurations) were not observed for *O. klotzi,* although one specimen showing anterior librigenal inversion was described by McNamara & Rudkin (1984, fig. 9), and one in McNamara's configuration figured by Rudkin (2009, fig. 1C). The cephalothoracic joint was not usually involved in moulting, nor was Salter's configuration observed. The rostral plate was rarely preserved, with the exception of one axial shield specimen with the fused rostral plate-hypostome complex found anterior to the cranidium.

Wheeler Shale, Utah, USA.–As at Mount Stephen, the exceptional Burgess Shale-type preservation at the middle Cambrian Wheeler Shale (House Range, Utah, USA) has produced well-preserved soft-bodied fossils. The Wheeler Shale seems to represent very similar exaerobic conditions to the EBS, with a single trilobite dominating, in this case *Elrathia kingii* (Order Ptychopariida) rather than *Estaingia bilobata* (Gaines & Droser 2003). This may also be the case within the Mount Stephen Trilobite Beds, where *O. klotzi* is found in great abundance (Rudkin 2009, 2011), although *Bathyuriscus rotundatus* Rominger 1887 may be similarly numerous. Specimens of *El. kingii* dominate at the Wheeler Shale, with up to 500 individuals per square metre, while other trilobite species are generally rare (Gaines & Droser 2003).

Unlike at the EBS, biomineralised *El. kingii* exoskeletons, owing to their nodular preservation from dolomite precipitation, are commonly found weathered out of their background matrix (Fig. 5G–J). This reduces identifiable moult assemblages in most museum collections to just axial shields (25 specimens at the OUMNH and NHMUK; e.g. NHMUK It.21001, Fig. 5G). This is also often the case for rarer Wheeler Shale trilobites (e.g. *Asaphiscus wheeleri*; McNamara & Rudkin 1984, fig. 6). A larger sample of specimens preserved within original slabs (i.e. through field rather than museum study) would likely provide more detailed information about *El. kingii* moulting, although figured slabs (e.g. Brett *et al.* 2009, figs 13–14) also appear to consist mostly of carcasses and isolated axial shields. These specimens indicate that, like for *O. klotzi, E. bilobata,* and *R. takooensis*, moulting through opening of the facial sutures (Sutural Gape; Henningsmoen 1975) was the normal mode. When identified *in situ*, moulted *El. kingii* axial shields are occasionally preserved in association with their disarticulated sclerites, although seemingly not with the frequency of those from the EBS. Several specimens show both librigenae displaced backwards similar to Harrington's configuration, but with the rostral plate not visible presumably because it is hidden beneath the dorsal cuticle (e.g. NHMUK It.21003, Fig. 5I). However, due to their minor displacement, this may also have resulted from decay and disturbance of the specimen. Some *El. kingii* moults show greater displacement of associated librigenae (e.g. Gaines & Droser 2003, fig. 1A), but their unusual positioning (both to the posterior-left of the axial shield) seem a product of transportation, rather than the movements of the exuviating trilobite.

Moult configurations from the Wheeler Shale, however, do regularly display thoracic disarticulation, or between the thorax and pygidium, and lateral displacement or rotation of this section (e.g. NHMUK It.20999, Fig. 5H), although individuals otherwise appearing as carcasses may also display thoracic disarticulations (such as NHMUK

It.5937, Fig. 5J). Very rarely, moults of *El. kingii* display cephalo-thoracic disarticulations, with the cranidium displaced. Librigenal or cephalic inversions (i.e. the Somersault, McNamara's, or Salter's configurations) were not observed. The rostral plate was also rarely observed.

Discussion

Inferred moulting behaviour of Estaingia bilobata

The prevalence of librigenal and rostral plate displacement in moult configurations of *E. bilobata* suggests that the operation of the facial and rostral sutures, producing an anterior exuvial gape in the manner described by Henningsmoen (1975), was the standard mode of ecdysis in this trilobite. This, coupled with the high proportion of specimens exhibiting anterior displacement of the cranidium, suggests that *E. bilobata* moulted similarly to a number of other Cambrian trilobite genera, e.g. *Ogygopsis*, *Paradoxides* (McNamara & Rudkin 1984; Whittington 1990), *Redlichia* (McNamara 1986), and *Balcoracania* (Paterson *et al.* 2007). This method involved an initial downward (towards the sediment) angling of the cephalon and upwards flexure of the dorsal thorax (producing a partial enrolment referred to as 'downwards flexure of the cephalon' in McNamara & Rudkin 1984, and termed 'dorsal flexure' here). This was followed by the opening of the cephalic sutures, allowing anterior egression of the trilobite from the old exoskeleton through the resulting exuvial gape. This may have been aided by relaxation and extension of the moulting trilobite from the partially enrolled exuviae (Whittington 1990). For *E. bilobata*, the LCU (forming the ventral section of the exuvial gape) was usually preserved intact, while the cranidium (the dorsal section) often became detached from the trunk.

Anterior inversions of the librigenae (Somersault configuration), and Salter's configuration where the entire cephalon is anteriorly inverted (Richter 1937), were rare (<10% of specimens, combined). Moult configurations showing these anterior inversions would require a relatively severe degree of dorsal flexure, with the cephalon at a high angle to the sediment. The forward movement of the moulting animal may then have resulted in the trunk being dragged over the librigenae or cephalon, causing them to become inverted (see McNamara & Rudkin 1984, fig. 11; Whittington 1990, fig. 6). However, the rarity of these configurations compared to the prevalence of Henningsmoen's and Harrington's configurations (around 90% of specimens) suggests that this was an unusual occurrence. This in turn indicates the degree of flexure in this species was perhaps less than for some other Cambrian trilobites, usually prohibiting anterior inversions.

The opening of the facial and rostral sutures in *E. bilobata* and the resulting exuvial gape was generally sufficient for moulting, with variations of Harrington's configuration (e.g. Fig. 3A) being very common (around 55% of specimens). Subsequently, the LCU would have been free of the cranidium, but seems to have generally remained articulated and connected to the thorax by integument. This explains why the LCU usually remained *in situ* or only slightly displaced relative to the trunk in Harrington's and Henningsmoen's configurations (e.g. Fig. 2A–B, D). Millimeter-scale displacements may have resulted from some degree of flexibility in the unmineralised cuticle connecting these sclerites, disturbance produced by the moulting animal, and/or subsequent decay of the connective integument. The LCU was often posteriorly displaced, presumably having been pushed backwards by the forwards movement of the individual during moulting (e.g. Fig 2D). The total absence of the Nutcracker configuration suggests that disarticulation of cephalic sclerites owing to the decay or breakage of unmineralised integument prior to burial was rare in *E. bilobata* compared to the larger *R. takooensis*. Despite this, it does not seem that

disarticulation of the LCU from the cranidium as a complete unit always occurred, or was necessary for successful moulting, with a number of specimens exhibiting rostral plates with one associated librigena, and the other remaining joined to the displaced cranidium (Fig. 2C, E, possibly B). Some moulting events may have involved multiple progressive breakages of the integument. For example, SAM-P 46956 (Fig. 2G) where the trunk and left librigena were initially shed. The trilobite then moved forward and discarded the rostral plate and right librigena, before leaving behind the cranidium.

Henningsmoen's configuration (Fig. 1) was also very common for *E. bilobata* (around 35% of specimens), suggesting that the cranidium easily became disarticulated during moulting, and in doing so presumably enlarging the exuvial gape. Whittington (1990), however, noted that the weakest part of a moult would have been the flexible membranes attaching the cranidium to the thorax, decay of which could easily result in disarticulation and transport. He therefore suggested caution in interpreting cranidium detachment as an aid in moulting. Nevertheless, the frequency of occurrence within the EBS and rarity of disarticulations in other joints suggest this is a legitimate biological pattern. Cranidial displacement was usually to the anterior, presumably having been carried forward by the post-ecdysial trilobite. The apparent weakness in the cephalothoracic joint may also explain the rare examples of Salter's configuration preserved for this species (Fig. 3C, E). This may have provided an alternative method of moulting following a failed opening of the facial and/or rostral sutures, with the trilobite extracting itself through the resulting exuvial gape. Given the displacement of cephalic elements in both figured specimens it is unlikely that complete failure of the sutures occurred in either of these cases.

Inferred moulting behaviour of Redlichia takooensis

In general, *Redlichia takooensis* also used the Sutural Gape mode of moulting via opening of the cephalic sutures. In contrast to *E. bilobata*, however, *R. takooensis* moults tend to show greater displacement of the cephalic sclerites in variations of the Nutcracker configuration (90% of moults), with the librigenae, rostral plate, and hypostome independent rather than joined in the LCU. This implies that breakage of the connective integument between sclerites prior to burial was common in the larger *R. takooensis* but rare in *E. bilobata*. This may indicate that exuvial movements in *R. takooensis* were more vigorous, resulting in more dispersed moult configurations. It is also possible that the connective integument was less robust in the larger species, and that this displacement may be a result of decay and subsequent disarticulation and movement (although we consider this less likely as there is no other evidence of decay). Assuming disarticulation occurred during moulting, the cephalic sclerites would then have fallen back down to the sediment and been displaced by any sideways and forward movements of the moulting animal, pushing them away from the axial shield (e.g. Fig 4A–B). The apparent breakage of integument and greater outwards displacement of the cephalic sclerites (producing the Nutcracker configuration, rather than Harrington's configuration) may indicate more extreme body movements during moulting for *R. takooensis* compared to *E. bilobata*, causing the sclerites to be pushed or carried further from the axial shield.

As has been suggested for other species of *Redlichia* (figs 2-5, McNamara 1986), it is probable that dorsal flexure played an important role in the standard mode of moulting in *R. takooensis*. The absence of anterior librigenal inversion (Somersault configuration, Fig. 1), however, suggests that enrolment may have been less acute prior to opening of the facial sutures, causing the librigenae to settle back down to the sediment following

moulting in their relatively normal orientations. This may also imply less pressure on the cephalothoracic joint, possibly explaining the relative rarity of cranidial displacement in moult configurations of *R. takooensis*. However, as Salter's configuration was slightly more common for *R. takooensis* than *E. bilobata* (although still relatively rare; around 5% of moults), the facial sutures may have failed to open more frequently. It also seems that in *R. takooensis* the facial sutures were likely to fail completely, rather than partially as in *E. bilobata*. In these scenarios, the individual may have employed a particularly high angle of dorsal flexure to enable the cephalothoracic joint to break. McNamara (1986, figs 1–2) observed pygidial inversion for *Redlichia forresti*, which he inferred to have resulted due to posterior movement during moulting to allow extraction from the cephalic region following failure of the sutures, increasing the angle of dorsal flexure at the pygidium. He also noted thoracic segment inversions for both *R. forresti* and *R. idonea* that he interpreted to have resulted from dorsal flexure, as well as a specimen of each with an inverted right librigena in variations of McNamara's configuration supposedly resulting from a zone of low pressure beneath the cephalon caused by rapid movement away from the sediment (McNamara 1986, figs 1B, C, E, 3). Whittington (1990) rejected the idea of posterior movement in this instance, and suggested such patterns of displacement and movement may simply be a result of transport and disturbance of partially decayed exuviae, or even carcasses (a possibility given the lack of evidence for the opening of facial sutures in certain specimens). We agree that specimens showing no evidence of opened cephalic sutures, other than full cephalon disarticulations (e.g. Salter's configuration), are unlikely to be moults. However, because multiple specimens of *R. takooensis* within the EBS collections also exhibit McNamara's configuration (around 5% of moult specimens), this arrangement likely represents a true biological signal. We consider that McNamara's configuration results simply from the librigenae occasionally

becoming laterally inverted due to the presumably spasmodic movement of the moulting trilobite, although a pressure gradient caused by this movement as suggested by McNamara & Rudkin (1984, fig. 5) is a possibility. Inversions, other than of cephalic structures, were not observed for *R. takooensis* or *E. bilobata*.

It is possible that the posterior-most pleural spines of *R. takooensis* were pushed into the sediment as an anchor for dorsal flexure, as suggested by Whittington (1990) for *Paradoxides*. Assuming the *Redlichia* specimens exhibiting pygidial inversion figured by McNamara (1986, figs 1–2) do represent moults, the pygidium may have been used in a similar way during moulting. The rotated pygidium and posterior-most thoracic segments observed in *E. bilobata* (Fig. 4F) can also be explained by its use as an anchor for dorsal flexure, particularly given the spinose nature of the pygidium in this species (Pocock 1964). Recurring presence of thoracic disarticulation in putative *R. takooensis* moult configurations immediately below segments 6 and 11, both of which supported extended dorsal spines (Paterson & Jago 2006), may have occurred during moulting. However, because of their orientation this is more likely the result of physical processes acting on the larger surface area of these segments prior to or during burial.

The normal sequence of moulting in *R. takooensis* therefore likely began with dorsal flexure away from the sediment, followed by opening of the cephalic sutures, with the animal exiting the exoskeleton through the resulting anterior exuvial gape. This likely involved more vigorous movements of the individual, causing the disarticulated librigenae to be displaced sideways and outwards from their usual position or, very occasionally, to become laterally inverted (Fig. 4F, G). Salter's configuration, although rare, is more common than for *E. bilobata*, suggesting that this was an important alternative strategy following the failure of the cephalic sutures to open.

Comparison with trilobite moulting at the Mount Stephen Trilobite Beds and Wheeler Shale

The favourable depositional conditions at the EBS have preserved essentially undisturbed moult configurations exhibiting high levels of variation in the positioning of moulted sclerites, allowing detailed reconstructions of exuvial movements (comparable to but more numerous than those illustrated in McNamara & Rudkin 1984 figs 5, 11; McNamara 1984, figs 2-5; Whittington 1990, figs 2-6). The Mount Stephen Trilobite Beds and the Wheeler Shale reveal that for *Ogygopsis klotzi* and *Elrathia kingii* respectively the standard mode of moulting is the same as for the EBS species, suggesting a common behaviour for Cambrian trilobites in the similar environmental and ecological settings of the Trilobite Beds and Wheeler Shale. However, even these exceptionally preserved deposits with very high trilobite abundances seemingly do not preserve such a complete and diverse record of moulting as the EBS, or are intensely impacted by historical sampling bias. The greater prevalence of isolated axial shields (e.g. Fig 5A, C, G), as well as aggregations of disarticulated specimens and fragmentary sclerites seen in the larger slabs (e.g. Fig 5F), may indicate slight transportation and disturbance of moult configurations prior to emplacement as even very minor currents may have been sufficient to transport disarticulated sclerites. It is also extremely difficult to determine orientations of specimens from the Trilobite Beds because they originate from non-*in situ* talus (Rudkin 2011). This makes it difficult to interpret aspects of movement such as dorsal flexure, because complete moult configurations are less frequently observed. However, the lack of associated sclerites may itself indicate a style of moulting involving greater displacement of sclerites than for *E. bilobata* and *R. takooensis*, for example if the librigenae of *O. klotzi* were carried further away by the post-ecdysial trilobite as suggested

by McNamara & Rudkin (1984). This may be supported by the moult configurations with librigenae displaced laterally. The presence of intact moult configurations within these deposits (Fig. 5, but see also McNamara & Rudkin 1984, figs 9, 10, 13A; Gaines & Droser 2003, fig. 1A; Rudkin 2009, fig. 1C), however, suggests a range of conditions. These deposits likely represent a combination of census and time-averaged assemblages as proposed by Caron and Jackson (2006) for the Greater Phyllopod Bed (Burgess Shale). While this is likely true for the EBS as well, conditions in general indicate extremely low levels of disturbance, with isolated axial shields and accumulations of disarticulated sclerites being extremely rare. It has been suggested that a fluctuating oxycline may have resulted in episodic mass kill events that excluded scavengers and inhibited decay at the EBS (Paterson *et al*. 2016), with carcasses (and moults) then smothered in a steady accumulation of fine sediment under extremely quiescent conditions. This could explain how such an exceptional *in situ* record of moulting was preserved at the EBS.

The intact moult configurations presented here for *O. klotzi* from the Mount Stephen Trilobite Beds show separation of the librigenae, and occasionally strong movements of the body to imbricate and displace the middle thoracic segments (Fig. 5B). Isolated axial shields seem generally more common than for *El. kingii* at the Wheeler Shale (and more common at both than at the EBS), but all of these Cambrian trilobite species still demonstrate a relatively high level of flexibility in their moulting behaviour. McNamara & Rudkin (1984) described two moults of *O. klotzi* (also from Mount Stephen), as well as a specimen of *Paradoxides davidis* Salter 1863, all in a modified version of the Somersault configuration, possibly with the entire LCU inverted to lie beneath the cranidium, and used this to argue for a sharp (greater than 90°) dorsal flexure to dislocate the cranidium from the thorax (McNamara & Rudkin 1984, fig. 11). Whittington (1990) considered this unlikely, based on the prevalence of complete axial

shields, as well as the fact that the facial sutures had operated in these specimens and therefore cranidium removal was not necessary. He argued that such a sharp angle would have dislocated all joints between the cephalon and the first three thoracic segments and instead advocated a more continuous enrolment for moulting, such that *O. klotzi* moulted in a manner similar to that described for *Paradoxides* (i.e. via the Sutural Gape mode with dorsal flexure facilitated by posterior thoracic pleural spines). Isolated thoracopyga of *O. klotzi* (e.g. Fig. 5E) may indicate instances of moulting through disarticulation at the cephalothoracic joint, as in the manner described for the EBS trilobites, although without the associated cephalon preserved this is difficult to confirm. It is also possible that these isolated fragments are the result of disarticulation resulting from decay and subsequent transport.

El. kingii moult specimens from the Wheeler Shale (Fig. 5G–J) indicate moulting was also accomplished via the Sutural Gape mode, but many provide no information on what additional behaviours may have been involved. This is because the location of disarticulated sclerites relative to the axial shield does not as often seem to reflect their moulted position (e.g. Gaines & Droser 2003, fig. 1A), suggesting higher levels of disturbance than at the EBS. The apparent scarcity of individual cephala or thoracopyga of *El. kingii* suggest that, as for *E. bilobata* and *R. takooensis*, moulting through other means (such as disarticulation of the entire cephalon) was very rare. The lack of inversions for *El. kingii* and *O. klotzi* potentially imply less dorsal flexure during moulting than for the EBS trilobites, although the *El. kingii* moult configurations may indicate greater angling of the pygidium due to their higher degree of posterior disarticulation and displacement.

Exploration of detailed moulting variation is made possible by access to the abundant trilobite collections at the SAM. This is not so readily accessible for *O. klotzi* and *El. kingii*, meaning results may also be more affected by collecting bias, and as such the

smaller sample sizes here represent a generalised rather than direct comparison of the three localities. This allows us to discern similarities like the shared preference for the Sutural Gape mode of moulting, and differences such as the greater prevalence of isolated axial shields within the two localities in comparison to the EBS. However, the limited sample size represented for *O. klotzi* and *El. kingii* likely has constrained the observed variation in their moulting configurations, and so the specimens presented here are not presumed to display their full moulting variation.

Recognising moult configurations

As for other arthropod groups, the sedimentological context and depositional environment must be considered when distinguishing moults and carcasses in the fossil record (Daley & Drage 2016). Determining the significance of thoracic segment disarticulation for exoskeleton moulting is particularly problematic, as thoracic breakages could be produced either during moulting or during decay of the integument articulating the segments in carcasses or moults. Specimens with systematic thoracic breaks may represent moult configurations, although as discussed above, the recurring dislocation below segments 6 and 11 in *R. takooensis* may have occurred after moulting due to physical processes acting on the enlarged spines of these segments. Specimens of *R. takooensis* with relatively minor cephalic displacement and thoracic breaks (e.g. Fig. 4D), or of *E. bilobata* with multiple thoracic dislocations are difficult to distinguish as moults or carcasses. SAM-P 54209 (Fig. 4K) shows a thoracic break in a specimen considered a carcass due to the closed cephalic sutures. Whittington (1990) explored this problem when reviewing some of the putative *Redlichia* moults figured by McNamara (1986), suggesting these could easily represent carcasses following decay and disturbance. We argue that the

presence of thoracic dislocation alone (i.e. without the disruption of the cephalic sutures to indicate an exuvial gape) is likely to represent decay rather than evidence of moulting, although some uncertainty remains. This is also true of specimens that show very minor disarticulation of cephalic sutures, and can make the identification of trilobite moults problematic.

Certain features, when more fully understood, may become useful tools in distinguishing trilobite carcasses and moults. In some cases, the presence/absence and positioning of ventral cephalic structures may be important for identifying moult configurations in trilobites with hypostomal and rostral sutures. The EBS trilobite moult configurations show that disarticulation of the rostral plate was integral to the opening of the anterior exuvial gape (separately for *R. takooensis,* and as part of the LCU for *E. bilobata*; e.g. Figs 2D, 3A, 4A), except perhaps for Salter's configuration. The hypostome was also frequently displaced for *R. takooensis* (e.g. Fig 4E), however several specimens otherwise considered moults due to their obvious anterior exuvial gapes seem to have the hypostome (Fig. 4B) and occasionally rostral plate (Fig. 4C) close to life position, possibly held in place via integument. It is therefore likely that specimens showing any evidence for opening of an anterior exuvial gape represent moults, regardless of the degree of hypostomal movement. Further, many global trilobite-bearing localities do not appear to preserve clear hypostomes, or they are obscured by the dorsal exoskeleton (HBD, pers. obs. 2016). At the EBS, ventral structures are easily observed due to the moldic preservation, without the calcitic exoskeleton. Delicate moulted parts, such as the antennae and legs, are rarely observed for this reason. Ventral structures can therefore aid in determining moulting if easily identifiable, but their absence or lack of movement does not conclusively define a carcass.

In complete carcasses of *E. bilobata*, the pygidium and final few thoracic segments are often curled under the thorax (e.g. Fig. 3G), presumably due to muscle contraction during death and/or decay. However, preserved moult configurations of *E. bilobata* do not seem to show pygidial enrolment, presumably due to the lack of required musculature. This may assist in moult identification for specimens of *E. bilobata* with only slight displacement of sclerites, and has the potential to be of broader use if identified in other trilobite species.

A large proportion of trilobite specimens within the EBS are preserved dorsum-down with previous sampling of a number of different surfaces indicating that greater than 75% of specimens are orientated in this manner (Gehling *et al*. 2011). In our field study, 90% of specimens were oriented dorsum-down, while at least two of the six specimens oriented upright were moults. Gehling *et al.* (2011) suggested that carcasses may be flipped dorsum-down due to the escape of gases from decay of internal soft tissues (discussed also by other authors, such as Speyer & Brett 1985; Speyer 1987; Ebbestad *et al.* 2013; etc.). It is therefore possible that the majority of upright specimens in this sample represent moults, as they would not have had much internal tissue to create enough decay gas to cause the exoskeleton to turn over. If this is indeed the case, it may provide additional evidence in identifying moults from carcasses at EBS, and potentially at other sites as well. However, Ebbestad *et al.* (2013) found an approximately equal number of dorsum-up and down moult specimens in their paradoxidid mass moult assemblage, and therefore suggested the represented species may have been equally able to moult in either orientation. Speyer (1987) also noted that moults preserved inverted may for some species represent a behavioural adaptation in moulting. This needs further quantitative examination on a larger scale at the EBS to determine whether there is a statistical bias towards moulting in one body orientation.

Collection bias

Several factors must be considered regarding the taphonomy, collection, and interpretation of preserved moult configurations. For example, the preservation of the unmineralised integument between exoskeleton sclerites may impact the moulting configurations seen, as is clear from the EBS material. The integument would likely have decayed rapidly post-moulting, resulting in disarticulation and displacement of configurations except under low hydrodynamic energy conditions (Whittington 1990), such as at the EBS. In localities with decay and disturbance prior to burial, configurations such as Henningsmoen's and Harrington's defined by the joined LCU would therefore not be observed. This would affect interpretations of the individual's movements during moulting.

Collecting bias for 'display-quality' specimens may also affect interpretations of moulting behaviour variability for trilobites, although it is difficult to predict whether complete moult configurations or articulated body fossils would be preferentially collected. For moult configurations, the more unusual behaviours may be chosen over those displaying subtler patterns (e.g. McNamara & Rudkin 1984). This may affect observations for species such as *E. bilobata*, as most *in situ* putative moults show only minor displacement of the LCU (Pocock 1964). Collection bias is likely also a problem for sampling of the Trilobite Beds and Wheeler Shale. It is likely that EBS trilobite moults are not significantly under or over-represented due to their frequent collection on samples containing other taxa of interest, and abundance in the SAM collections. Regardless, this study is a qualitative exploration to represent the range of observed moulting behaviours at the EBS, and we have only indicated relative proportions broadly estimated from the existing museum collections. A quantitative study of the numbers of these moulting

configurations (from a field sample), and how these differ between the two species, is beyond the scope of the present study, but would be extremely useful for furthering our understanding of trilobite exuvial variability.

Variation in trilobite moulting behaviour

The large, exceptionally preserved sample from the EBS revealed a broader range of intraspecific moulting behaviours and configurations than previously described for trilobites (e.g. in Henningsmoen 1975; Brandt 2002). Both discussed moulting methods (Sutural Gape and Salterian) have demonstrably occurred within a single species before (see Daley & Drage 2016), but without the number of configurations these have produced at the EBS (Fig. 1). The quiescent environmental conditions at the EBS were responsible for the preservation of this range in trilobite movement during moulting. Many localities with greater disturbance prior to burial therefore did not preserve the full variation in moulting behaviour and movement. Trilobite moulting was therefore a potentially more flexible behaviour than previously hypothesised, and much more variable than for other arthropods (for example decapod crustaceans, and chelicerates like eurypterids and scorpions; see Daley & Drage 2016). Brandt (2002), in one of few evolutionary studies of trilobite moulting, determined this flexibility was broadly detrimental to Trilobita, by their moult habit being unspecialised and therefore inefficient. However, the data from the EBS indicates this is a useful behavioural flexibility, in which rarer 'emergency behaviours' could increase individual survival chances during difficult moulting events to broaden the exuvial gape, or when the usual gape failed to open. This may have happened more frequently for trilobites than for other arthropods, due to their highly reinforced exoskeleton. On an individual and population level, this would have increased survival

rates, and therefore this variability may have contributed to their extremely long geological record (Hughes 2003).

Conclusions

Descriptions of the moulting behaviour for two abundant trilobite species from the early Cambrian Emu Bay Shale Konservat-Lagerstätte, *Estaingia bilobata* and *Redlichia takooensis*, have allowed for detailed interpretations of individual moulting movements (similar to those illustrated in McNamara & Rudkin 1984, fig. 11; Whittington 1990, figs 2-6). The moult configurations figured suggest that Cambrian trilobites, as observed from localities showing exceptional preservation, generally use a Sutural Gape mode of moulting (Henningsmoen 1975), involving opening of the cephalic sutures to create an anterior exuvial gape. However, the two species exhibit differing patterns of behavioural variation within this mode. For example, *E. bilobata* usually produces configurations in Harrington's or Henningsmoen's configuration with a joined Lower Cephalic Unit (*sensu* Henningsmoen 1975), whereas *R. takooensis* usually disarticulates the same sclerites but disconnected and displaced laterally outwards from the axial shield in the Nutcracker configuration. Both species preserve a range of moulting configurations including Salter's configuration through the alternative Salterian mode of moulting.

Minor enrolment, or 'dorsal flexure' of the exoskeleton away from the sediment, seems necessary to produce most of the configurations described here, which is consistent with the moulting methods determined in earlier literature (McNamara & Rudkin 1984; McNamara 1986; Whittington 1990; Paterson *et al.* 2007). More extreme movement, not just in an anterior direction, is inferred for many specimens, as rotation and lateral displacement of sclerites is common. There is an absence of inverted thoracic segments or

pygidia, possibly associated with the presence of elongated thoracic medial spines in *R. takooensis*.

The Wheeler Shale and Mount Stephen Trilobite Beds, both of which exhibit Burgess Shale-type preservation, contain a higher proportion of axial shields, which provide less information on trilobite movement during moulting than derived from the EBS. It is likely, therefore, that these sites reflect conditions of slightly higher hydrodynamic energy and/or slower burial, resulting in disarticulation of moults due to decay and transport (although it is possible this may also reflect greater movements of the exuviating trilobites). At the EBS, the lack of evidence for water movement, rapid burial, and detailed behavioural record, however, seem to confirm a difference in environment, as suggested by its unique depositional setting. It is this differing environment that appears responsible for highlighting the inherent biological flexibility in moulting for *E. bilobata* and *R. takooensis*. This variability appears widespread in trilobites not least due to its occurrence across the three Orders represented here (Corynexochida, Ptychopariida, Redlichiida), and may have been integral to their extremely long-lived and successful evolutionary history.

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Chapter 6: Post-embryonic development of *Dalmanitina*, and the evolution of facial suture fusion in Phacopina

This chapter looks at the link between moulting behaviour, ontogeny, and morphological development in trilobites. This is explored through describing a large sample of well preserved *Dalmanitina* (Phacopina) specimens that show a full ontogenetic sequence. The Phacopina are particularly interesting because dramatic morphological changes through their development led to important behavioural alterations, including to moulting, which affected their life history differently to other trilobite groups. Through looking at changes in moulting behaviours, we can thereby unpick the processes of developmental change in extinct euarthropod groups.

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Author Contributions

H.B.D. conceived the study, gathered data, and wrote the majority of the paper. L.L. carried out developmental analyses, and P.B. wrote geological settings. All authors edited the paper.

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Post-embryonic development of *Dalmanitina*, and the evolution of facial suture fusion in Phacopina

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Abstract.—A large sample of post-embryonic specimens of *Dalmanitina proaeva elfrida* Šnajdr 1982 and *D. socialis* Barrande 1846 from the Upper Ordovician (Sandbian to Katian) Prague Basin allows for the first reasonably complete ontogenetic sequence of Dalmanitoidea (Phacopina). The material provides an abundance of morphological information, including well-preserved marginal spines in protaspides and meraspides, and hypostome external surfaces throughout. The development of *D. proaeva elfrida* is unusual due to variability in timing of the first trunk articulation. While broadening our developmental understanding of Phacopina, a diverse group of phacopid trilobites, we are also able to study the evolution of their specializations in exoskeletal molting behavior. Adult phacopines, unlike most other trilobites, had fused facial sutures. This means that rather than molting through the Sutural Gape mode, characterized by opening of the facial sutures and separation of the librigenae, they disarticulated the entire cephalon in Salter's mode of molting. For other phacopine clades (Phacopoidea) the transition to Salter's mode occurs during the meraspid period or at the onset of holaspis, and its developmental timing is intraspecifically fixed. However, owing to the large sample size we can see that facial suture fusion likely occurred later in *Dalmanitina*, usually during the holaspid period, and was intraspecifically variable with holaspides of varying sizes showing unfused sutures.

Further, *D. proaeva elfrida* specimens showed an initial librigenal-rostral plate fusion event, where the librigenae began as separate entities but appear fused with the rostral plate as one structure (the 'Lower Cephalic Unit') from M1, and are discarded as such during molting. Dalmanitoidea is considered to represent the first phacopine divergence, occurring earliest in the fossil record. This therefore provides insight into how linked morphologies and behaviors evolved, potentially suggesting the timing of facial suture fusion in Phacopina moved earlier during development and became more intraspecifically fixed over geological time.

Introduction

The trilobite suborder Phacopina is partially defined based on the fusion of the facial sutures during development, which prevents the librigenae being separated from the cranidium (Jaanusson 1975). This means the Sutural Gape mode of molting, where cephalic sutures (facial, rostral, and hypostomal) are opened to create an anterior exuvial gape, is impossible (Drage et al. 2018). Instead the individual must disarticulate the entire cephalon from the trunk, called Salter's mode of molting (see Daley and Drage 2016; Drage et al. 2018). The developmental timing of facial suture fusion varies interspecifically within Phacopina and has so far been described from several species within the family Phacopidae (superfamily Phacopoidea) (Crônier et al. 2005; Crônier and Fortey 2006). Post-embryonic development is also comparatively well known for the Phacopidae (e.g. Osmólska 1963; Chatterton 1971, 1980; Chatterton and Speyer 1997; Crônier et al. 1999; Crônier 2007), but this group represents a more derived lineage within the Phacopina (Jaanusson 1975). On the contrary, superfamily Dalmanitoidea likely represents an earlier divergence of the phacopines, originating in the Lower Ordovician (Jaanusson 1975). Examining notable morphological and behavioral changes such as facial suture fusion in Dalmanitoidea is therefore crucial for understanding the evolution of

development within Phacopina. Considering the hypothesized decline in morphological variability and polymorphism in more derived members of various trilobite clades (e.g. Ramsköld 1991; Webster 2007), which has also been reported for order Phacopida (Webster 2007: Fig. 3E-F), we might expect that such changes in behaviour and morphology were less developmentally constrained in the earlier lineages of this group. To explore this notion, and to trace the origins of facial suture fusion in the Phacopina, we here examine the post-embryonic development of *Dalmanitina* (Dalmanitidae, Dalmanitoidea; following the taxonomy of Fortey 1997).

To-date, the development of Dalmanitoidea is known only from the family Dalmanitidae. In particular, several species of *Dalmanitina* and closely related taxa have seen descriptions of small numbers of juvenile specimens. These include the original description of *D. proaeva elfrida* from Bohemia (see Barrande 1852, also partially refigured by Whittington 1956 and Šnajdr 1990), *D. olini* from Sweden and Wales (Temple 1952, 1957), *D. nanchengensis* Lu 1957 from China (Lu and Wu 1983), *Dalmanites puticulifrons* Whittington and Campbell 1967 from North America (Whittington and Campbell 1967), and *Mucronaspis grandis* Barrande 1852 from Bohemia (Budil 1996). These works lack description of the full sequence of morphological change during ontogeny, as most studies consist of either too few specimens, or disarticulated material. For example, the material of *D. nanchengensis*, while numerous and permitting a description of the protaspid period, was inadequate for providing a full post-protaspid sequence because only a single articulated meraspid was identified.

This study presents the post-embryonic development of two species of *Dalmanitina* Reed (1905) from the Sandbian and Katian strata of the Prague Basin, and is the first comprehensive description of developmental patterns in Dalmanitoidea. While *Dalmanitina* is a more derived genus within the dalmanitoid lineage, which originated in

the Lower Ordovician (compared to the Middle Ordovician origins of *Dalmanitina*; Šnajdr 1982), *Dalmanitina* represents a comparatively large data set of early-diverging phacopine trilobites in the context of the greater body of work on ontogeny of the Phacopoidea. This data set was gathered by examining post-embryonic stages of *D. proaeva elfrida* and *D. socialis* from the National Museum Prague (NMP) and Czech Geological Survey (CGS), and has allowed for detailed biometric analysis of their development. In particular, this developmental sequence is significant due to its exemplification of the timing of facial suture fusion events, traceable by the noteworthy preservation of a large number of specimens.

Geological Setting

In the Prague Basin (Barrandian area, Czech Republic; see Havlíček 1981, 1982), all representatives of *Dalmanitina* occur exclusively in the strata representing the Berounian regional stage (corresponding approximately to the Sandbian and lower part of the Katian global stages; see Havlíček and Marek 1973; Havlíček and Fatka 1992; Havlíček et al. 1994; Fatka et al. 1995; Havlíček 1998; Fatka and Mergl 2009; Gutiérrez-Marco et al. 2017). The Berounian comprises in the Prague Basin the Libeň, Letná, Vinice, Zahořany, and Bohdalec Formations. Their constituent fauna are broadly comparable despite their different lithologies. Juveniles of *Dalmanitina* have historically been documented or collected at several localities for each of the Letná, Vinice, and Zahořany Formations in the Prague Basin. They are rare and poorly preserved, or absent, from all of the remaining stratigraphic levels of the Berounian. This study therefore focuses on the numerous and well-preserved specimens in the historical collections at the NMP and CGS from the Letná, Vinice, and Zahořany Formations (obtained from locations discussed

below; see Fig. 1), with an emphasis on the particularly rich material of *D. proaeva elfrida* occurring in the Vinice Formation.

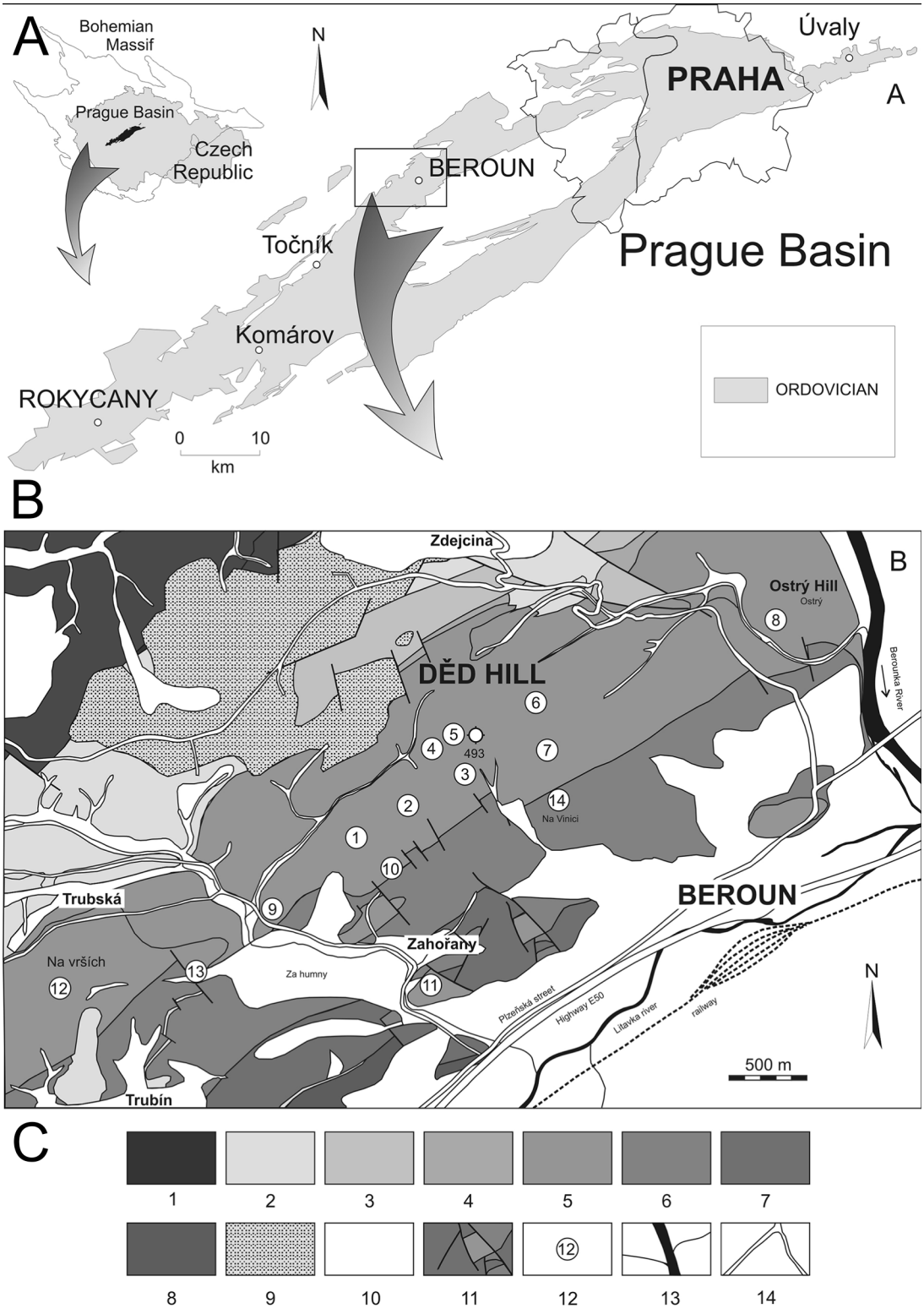


Figure 1: A, the Ordovician of the Prague Basin (after Manda 2008; Fatka et al. 2015). B, simplified geological map of the northwest neighborhood of Beroun (after Chlupáč 1965, 1993, 2002; Chlupáč et al. 1989; Havlíček et al. 1993). C, key to the geological map of the Prague Basin: 1, Neoproterozoic, Kralupy-Zbraslav Group; 2, Floian, volcanites of Komárov Volcanic Complex (Floian to Sandbian); 3, Libeň Formation, Řevnice Quartzite facies (Sandbian); 4, Libeň Formation, Libeň Shale facies (Sandbian); 5, Letná Formation (Sandbian); 6, Vinice Formation (Sandbian - Katian); 7, Zahořany Formation (Katian); 8, Bohdalec Formation (Katian); 9, Carboniferous; 10, Quaternary undivided; 11, faults; 12, position of localities; 13, rivers and brooks; 14, roads and highways. Key to the numbered localities in B (see Chlupáč 1965): 1-7, small pits and outcrops at the Děd Hill cumulatively named as "Drabov" in the 19th century (most museum samples have this name); 8, Ostrý Hill; 9, Čížovka; 10, Zahořany north of the village; 11, Zahořany, Háj; 12, fields between Trubín and Trubská villages; 13, Trubská rokle Gorge (cumulative name); 14, Vinice Farm, stratotype of Vinice Formation (cumulative name). 1-8 and 12 are Letná Formation localities, 10 and 11 Letná and Zahořany, 13-14 Letná and Vinice.

Juveniles of *D. socialis* were collected from multiple localities in the Letná Formation. Several small pits were created by fossil collectors at Děd Hill northwest of the town of Beroun in the 19th century (Fig. 1, localities 1-7), and cumulatively designated as from "Drabov". These pits correspond to several fossiliferous intervals, which can be difficult to distinguish (Chlupáč 1965, 1993, 2002). The exact geographic and stratigraphic source of NMP material of *D. socialis* from the locality Veselá is similarly uncertain (an old farm northeast of the town of Beroun, near the Vráž Village; see Chlupáč 2002; Vokáč et al. 2015: p. 150; also corresponds with localities 1-8).

Juveniles of *D. proaeva elfrida* are very common in the Vinice Formation. The rich material stored in the NMP collections (particularly the Barrande collection, representing many of the described specimens) comes mainly from Trubín. The exact geographic

position of this locality is poorly known. As for the Drabov localities in the Letná Formation, Trubín is likely represented by a series of recently overgrown natural outcrops and small collectors' pits exploited north of the village Trubín in the 19th century (Fig. 1, locality 12; see Chlupáč 2002). This cumulative locality name may also overlap with the better-known outcrop at Trubská rokle Gorge, north of Trubín (Fig. 1, locality 13), which provided material of *D. socialis* and *D. proaeva elfrida* from the Letná and Vinice Formations respectively. Some of the studied specimens of *D. proaeva elfrida* also come from Vinice, the type locality of the Vinice Formation. This was formerly exposed at the farm on the southeastern slope of Děd hill (now mostly overgrown collection pits, see Fig. 1, locality 14). Studied specimens accessioned at the CGS of *D. proaeva* had been collected from several localities in the Zahořany Formation (see localities 10 and 11 in Fig. 1 and Appendix 6.1).

The Letná Formation (Sandbian age) is characterized by alternation of coarser and finer sediments in beds several centimeters to decimeters thick (see Havlíček 1998), all with strong bioturbation and diverse ichnofossils (see Mikuláš 1998a, b). Fine grained sandstones to greywackes, sandy siltstones, clayey siltstones and clayey sandstones dominate, with rare clayey shales. The thickness of the formation varies between 650 m and 40 m (Havlíček 1998). *Dalmanitina socialis* and *Deanaspis goldfussi* Barrande 1846 dominate among trilobite species (with many other genera present); non-trilobite arthropods, bivalves, gastropods, conulariids, brachiopods, and echinoderms are also present. These are characteristic of the inshore quartzose sandstones, while a poor atheloptic trilobite association is typical for the offshore slope settings. Rare graptolites, trilobites of Cyclopygid Biofacies and brachiopods of the *Paterula* Community occur in poorly oxygenated black shales in central parts of the basin (for a summary see Fatka et al. 2013).

The Vinice Formation (uppermost Sandbian to Katian) is characterized by a marine transgression over the stable segments bounding the Prague Basin (see Havlíček 1998). Black shales are the dominant lithofacies. Due to anoxic or dysoxic near-bottom conditions, the benthic fauna of the Vinice Formation is reasonably monotonous and consists mostly of bivalves, gastropods, and hyolithids, accompanied by a few trilobite species (*D. proaeva elfrida* and *Deanaspis senftenbergi* Hawle and Corda 1847 in particular) and brachiopods (see Havlíček and Vaněk 1966). Ichnofossil diversity is much lower than in the underlying Letná Formation. The benthic fauna typical of the Vinice Formation supposedly inhabited a poorly aerated, soft-bottom environment. Rare graptolites, trilobites of the Cyclopygid Biofacies, and the *Paterula* brachiopod Community represent the pelagic and epibenthic forms. Diverse echinoderms, accompanied by trilobites, brachiopods, cephalopods, and conulariids are confined to the oolitic ironstones in the uppermost parts of the formation.

The Zahořany Formation (Katian) is comprised of siltstones and rare intercalations of shales and silty sandstones, with a thickness of between 70-400 m across the Prague Basin (Havlíček 1998). Storm sediments with hummocky cross-stratification are common within the formation at the thinner extremities, and bioturbation is frequent (Mikuláš 1998b). The fauna includes abundant brachiopods, gastropods, bivalves, hyolithids, trilobites (*D. proaeva* and *Marrolithus ornatus* Sternberg 1833 in particular), and echinoderms. In the deeper parts of the formation, brachiopods gradually decrease in number and the benthic fauna becomes poorly diversified (Havlíček 1998).

Materials and Methods

Material.—The material of *Dalmanitina proaeva elfrida* accessioned at the NMP that comes from the Vinice Formation is preserved as internal molds (usually covered by a

thin film of iron hydroxides) in dark grey (almost black) shales. Specimens of *Dalmanitina socialis* accessioned at the NMP from the Letná Formation are preserved in grey to brown siltstones to fine-grained sandstones. The specimens of *D. proaeva* from the Zahořany Formation accessioned at the CGS are preserved in grey siltstones. These have not been conclusively described to sub-species level (they are mostly represented by disarticulated cephala/cranidia), but are assigned to *D. proaeva* and are very closely-related (almost indistinguishable) to *D. proaeva elfrida* (see Šnajdr 1982: p.181). Generally, the material from the Vinice Formation is more dorso-ventrally flattened, but rarely affected tectonically and preserves more exoskeletal detail. By contrast, the material from the Letná Formation is less flattened, but in most cases poorly preserved due to the coarsely grained sediment. Sometimes, however, the specimens from the Letná Formation seem to preserve partially decalcified remains of exoskeleton with surface sculpture (see below). The material from the Zahořany Formation is more three-dimensional and generally well-preserved, but contains only holaspides.

Methods.—All measured specimens of *D. proaeva elfrida* (n=98), *D. socialis* (n=31), and *D. proaeva* (n=8) from the NMP and CGS (see Appendix 6.1) were coated with ammonium chloride sublimate and photographed using the digital microscope Keyence VHX-2000 and/or DSLR camera Canon EOS 6D. The material was subsequently measured using the optical image analyser TpsDig2 (Rohlf 2006). In all of these specimens two parameters were measured: 1) sagittal length of the cranidium/cephalon (crl); and 2) transverse width of the cranidium/cephalon across the occipital ring (crw, see Fig. 2). In the earliest (P1 to M2) complete individuals five parameters were measured in total: 1) sagittal length of the exoskeleton, excluding spines (exl); 2) transverse width of the cranidium across the occipital ring (crw); 3) sagittal length of the cranidium (crl); 4)

sagittal length of the trunk, excluding spines (trl); and width of the trunk (trw, see Fig. 3). Width and length measurements were displayed on scatter plots in PAST (ver. 2.13; Hammer et al. 2001) to examine developmental trajectory (using reduced major axis linear regression) and to distinguish individual developmental stages. Growth-related developmental traits (growth rate, GR, and index of conformity to Dyar's rule) were calculated according to Fusco et al. (2012) from arithmetic means of each cluster or stage.

Repositories and Institutional Abbreviations.—The studied material is housed in the collections of the National Museum, Prague, Czech Republic (prefix NMP), the Museum of Comparative Zoology, Cambridge, USA (prefix MCZ) and the Czech Geological Survey, Prague, Czech Republic (prefix CGS).

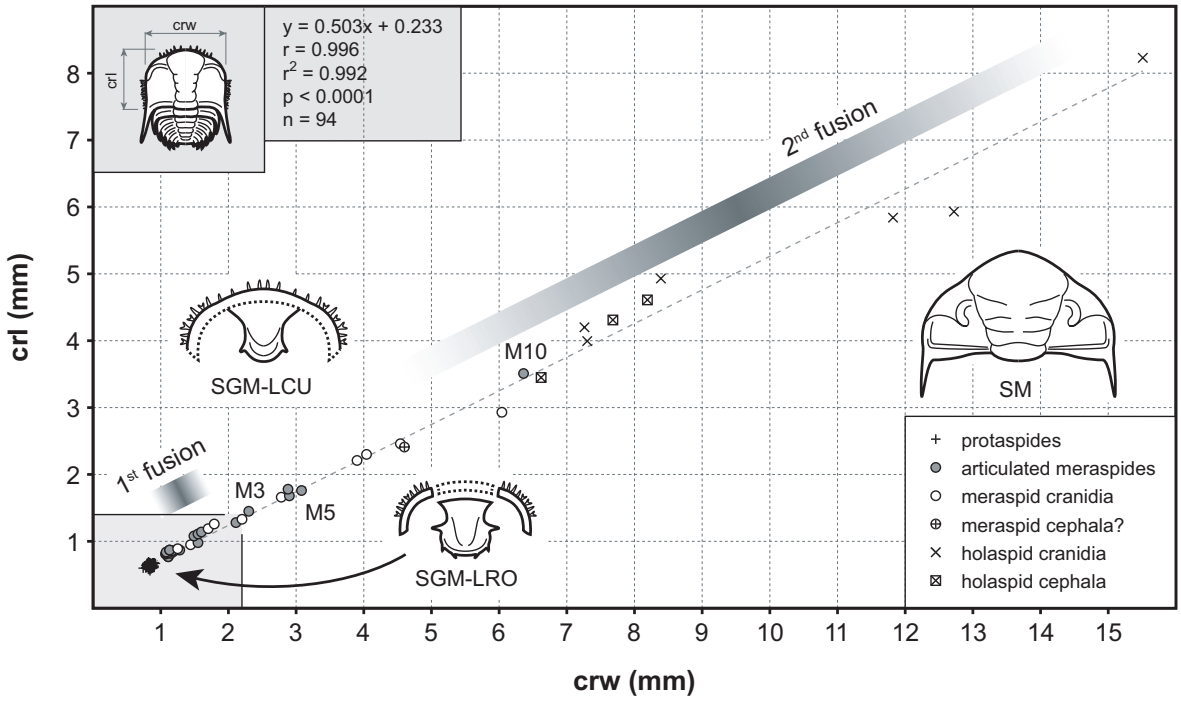


Figure 2: Scatter plot of cranidial or cephalic width (crw) vs. cranidial or cephalic length (crl) for *Dalmanitina proaeva elfrida* from the Vinice Formation (Sandbian to Katian) of the Prague Basin. Abbreviations: M3, meraspid degree 3; M5, meraspid degree 5; M10, meraspid degree 10; SGM-LRO, Sutural Gape Mode of molting separating librigenae (and doublure) and hypostome; SGM-

LCU, Sutural Gape Mode of molting with a joined Lower Cephalic Unit; SM, Salter's Mode of molting. Four holaspid specimens of *D. proaeva elfrida* and eight of *D. proaeva* (sizes detailed in Appendix 6.1), both with fused and unfused sutures, are greater than 8 mm in width. These were not included in the correlation calculations (all data in top-left box) because this analyzed data plotted within the graph and used to create the line of best fit.

Results

Post-Embryonic Developmental Sequence of *Dalmanitina proaeva elfrida*

The post-embryonic development of trilobites is traditionally divided into protaspid, meraspid and holaspid periods (cf. Chatterton and Speyer 1997, Hughes et al. 2006). The studied material of *D. proaeva elfrida* contains individuals belonging to all of these periods, ranging from 0.78 to 61.20 mm in sagittal exoskeletal length and 0.74 to 37.65 mm in maximum exoskeletal width. The size distribution of the individual cranidia and cephala during post-embryonic development can be described by reduced major axis linear regression (Fig. 2). Specimen measurements show size clustering, at least for the earliest developmental stages up to meraspid degree 1 (M1, Fig. 2). Protaspid stage 2 (P2) and meraspid degree 0 (M0) overlap considerably in size (forming the second cluster; Fig. 3) but have distinctly different morphologies (Figs 4 and 5). M0 specimens show an articulation structure and longer trunk with five axial rings (Fig. 5A and B), whereas P2 specimens show no articulation structure and have a shorter trunk with only four axial rings (Fig. 4H and I).

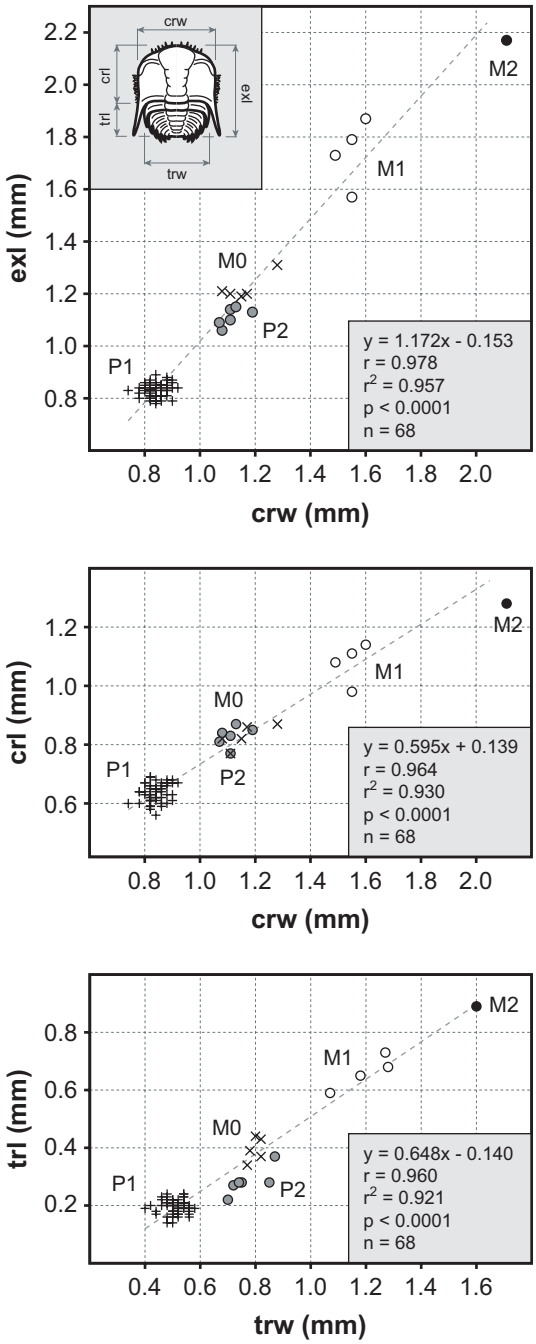


Figure 3 (right): Scatter plots of cranidial width (crw) vs. exoskeletal length (exl), cranidial width (crw) vs. cranidial length (crl), and width of the trunk (trw) vs. length of the trunk (trl) for *Dalmanitina proaeva elfrida* from the Vinice Formation (Sandbian to Katian) of the Prague Basin. Abbreviations: P1, first protaspid stage; P2, second protaspid stage; M0, meraspid degree zero; M1, meraspid degree one; M2, meraspid degree two.

There are some gaps in the ontogenetic sequence, particularly between meraspid degree 5 and 10, and within the holaspid period (Fig. 2). However, the calculated GR (Table 1) suggests that only one molting event occurred for each meraspid degree from M5 to M10 (assuming a more or less constant growth increment during development, which is typical for most trilobites; cf. Fusco et al. 2012). *Dalmanitina proaeva elfrida* displays protarthrous development as the full complement of 11 articulated thoracic segments is reached (Fig. 6A, with only 18 trunk segments) before the total number of trunk segments are present (20, counted from the largest specimens; Fig. 6B, C, E and F).

Protaspid Period.—The earliest post-embryonic stage (P1) of *D. proaeva elfrida* is roughly circular in outline, 0.78-0.89 mm long and 0.74-0.92 mm wide. The cranidium is semi-circular and 0.56-0.69 mm in length. The glabella expands anteriorly and has five lobes, including the occipital ring, and these are separated by deep glabellar furrows (e.g. Fig. 4D, E, and I). The anterior-most lobe is sub-rectangular and terminates just short of the anterior cephalic margin (Fig. 4D and E). A short (sag.) anterior border is developed. Postero-lateral edges of the cranidium protrude into long spines (Fig. 4C) that are directed immediately posteriorly and extend beyond the trunk (exsag.). Based on their topological position these seem to be homologous with the genal spines of later stages. The fixigenae are very convex, while the unfused librigenae (see Fig. 4B and F arrows, 4G) are narrow (tr.), and short (exsag.). The palpebral lobes are small and semi-circular, and placed far anterolaterally, with the anterior margin created by the librigenal sutures (e.g. Fig. 4E, with missing librigenae). Both the posterior marginal furrow and posterior border furrow are well developed, running from the occipital ring and curving backward abaxially. The margins of the cranidium and librigenae are fringed by small spines likely of sub-horizontal direction, which appear bifurcated on the lateral sides and singular towards the anterior (Fig. 4A and D, in particular the left margin of the latter). The trunk portion is broadly elliptical, about 60% of the maximum cranidial width, with a rounded semi-circular posterior margin, and two pairs of marginal spines. The axial part of the trunk is poorly differentiated but seems to contain two rings (Fig. 4A-G). The pleural parts are smooth without pleural or interpleural furrows. The hypostome, where visible (Fig. 4A inverted, in 4C potentially displaced to the bottom-left, impressions in 4F and G), seems in life to have been conterminant with a suture to the cephalic doublure (Fig 4F and G). It has a circular posterior margin, an internal marginal furrow, and likely laterally and posteriorly pointing marginal spines (Fig. 4C).

The second protaspid stage (P2) is morphologically comparable to P1, differing mainly by a slightly larger overall size and a larger trunk portion with four axial rings (Fig. 4H and I).



Figure 4: Protaspides of *Dalmanitina proaeva elfrida* from the Vinice Formation (Sandbian to Katian) of the Prague Basin. Red arrows in B and F point to locations of separated librigenae/doublure, and in C to the hypostome. A-C show these structures as independent (similar

to the Nutcracker molt configuration; Drage et al. 2018), while F and G are akin to Maksimova's molt configuration (Henningsmoen 1975). A-G, protaspid stage 1; H, I, protaspid stage 2. A, NMP L46704; B, NMP L15057; C, NMP L15046; D, NMP L46778; E, NMP L15056; F, NMP L46688; G, NMP L46713; H, NMP L46661; I, NMP L15050. All illustrated specimens are from Trubín (see Fig. 1, localities 12-14). Scale bars = 0.5 mm.

Meraspid Period.—The meraspid specimens range between 1.19-8.30 mm in sagittal exoskeletal length and 1.08-6.36 mm in maximum exoskeletal width (including the largest articulated meraspid specimen in M10). From M0 (Fig. 5A and B) the occipital ring is more defined, and distinct from the remaining four lobes of the glabella. The three pairs of glabellar furrows and occipital furrow remain well defined. The general shape of the glabella in the early meraspid stages is similar to that of the protaspides, but during subsequent development the glabella widens anteriorly and becomes more pentagonal shaped in outline with a diamond-shaped anterior lobe. The posterior border furrow becomes more prominent (e.g. Fig. 5B and C), leading to the genal spines and continuing around the lateral and anterior sides of the cephalon. The margin of the entire cephalon still bears numerous sub-horizontal spines (Fig. 5H magnified-view, also visible on left cephalic margin in Fig. 5F and right margin in Fig. 5G, on the anterior hypostome border in Fig. 5E and D, and on the right librigenal in 5B), which become less prominent during development and disappear in the late meraspid stages (present in M6, Fig. 5J arrow, but absent by M9, Fig. 5L). The facial sutures migrate towards the glabella throughout the meraspid period, with the librigenae consequently becoming broader and more triangular (e.g. arrows in Fig. 5B compared to 5J). This also caused the semi-circular eyes to move gradually away from the cephalic margin and more axially, while increasing in size and becoming more crescentic (Fig. 5L). In M0 the librigenae remain separate entities as in protaspides (Fig. 5A and B), but from M1 these structures appear to be joined together,

forming a Lower Cephalic Unit (LCU, Henningsmoen 1975) that separates from the cranidium as a single structure (seen disarticulated in Fig. 5C-E). The hypostome of the earliest meraspides is also similar to that in the protaspid period, but later appears to become parabolic to triangular in posterior outline rather than rounded and lacks spines (Fig. 5E and K). Throughout the meraspid period the trunk segments have small pleural spines, and five to seven axial rings can be recognized on the pygidium.

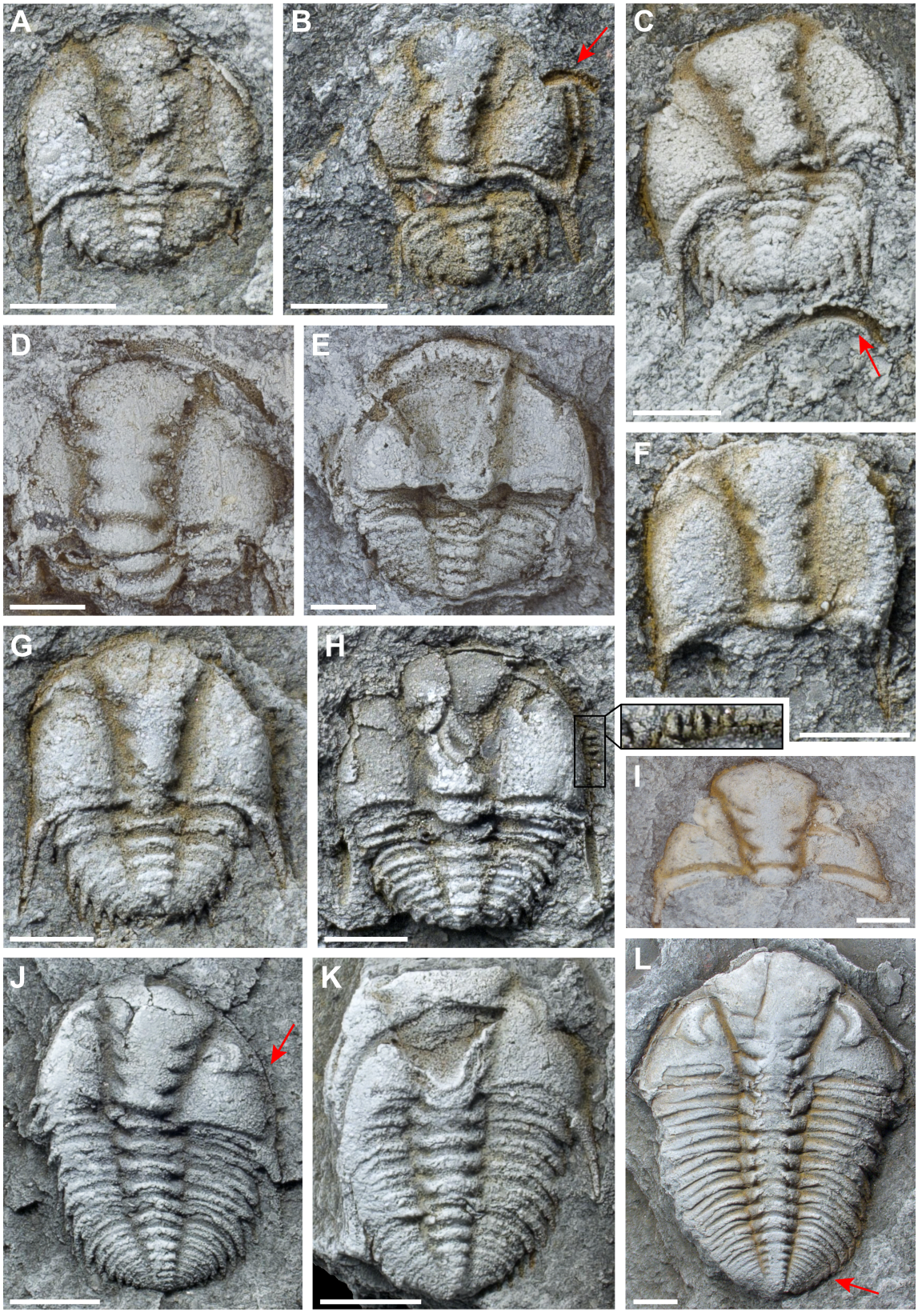


Figure 5: Meraspides of *Dalmanitina proaeva elfrida* from the Vinice Formation (Sandbian to Katian) of the Prague Basin. Red arrow in B points to a disarticulated librigena, in C to a disarticulated Lower Cephalic Unit, in J to the facial suture shape, and in L to location of the

thoracic-pygidial boundary. C-E and K show Harrington's molt configuration with the LCU detached. Magnified-view of H show numerous sub-horizontal spines round the cephalic margin. A, B, meraspid degree 0; C, G, H, meraspid degree 1; E, meraspid degree 2; K, meraspid degree 5; J, meraspid degree 6; L, meraspid degree 10. A, NMP L15049; B, NMP L15060a; C, NMP L15052; D, NMP L46798; E, NMP L46807; F, NMP L46694; G, NMP L15062; H, NMP L15051a; I, NMP L46796; J, NMP L15053; K, NMP L15061a; L, NMP L15064. All illustrated specimens are from Trubín (see Fig. 1, localities 12-14). Scale bars = 0.5 mm for A-H, and 1 mm for I-L.

Holaspid Period.—The exoskeleton of the smallest articulated holaspid specimen is 11.50 mm long and 7.26 mm wide. Holaspid individuals have eleven thoracic segments, but this condition occurs before the onset of epimorphic development and therefore some smaller holaspid specimens exhibit 7 or 8 axial rings on the pygidium (Fig. 6A) compared to 9 in larger individuals (Fig. 6B, C and F). The posterior of the pygidium forms a long terminal spine (Fig. 6E and F). During holaspis, the facial sutures become fused, meaning they can no longer be opened and the librigenae become fixed to the cranidium. This appears to be flexible in timing, as while the facial sutures are usually only fused in holaspid specimens based on their morphology (the full 11 thoracic segments), these are of greatly varying body sizes (as displayed in Figs 2 and 6; see Appendix 6.2 for more examples). For example, Figures 6B, E, G, H and J show a broad range of sizes and are all missing the librigenae, meaning that the facial sutures were still operable (also true for a number of additional specimens from the CGS, e.g. JK19125 with a cranidial width ~2 cm). Some specimens (e.g. Fig. 6J) are larger than or comparable to other specimens with fused sutures (e.g. Fig. 6F and I, although smaller than 6C and D), as are several other specimens of *D. proaeva* with missing librigenae from the CGS (e.g. PB130 with a cranidial width over 4 cm; Appendix 6.2).

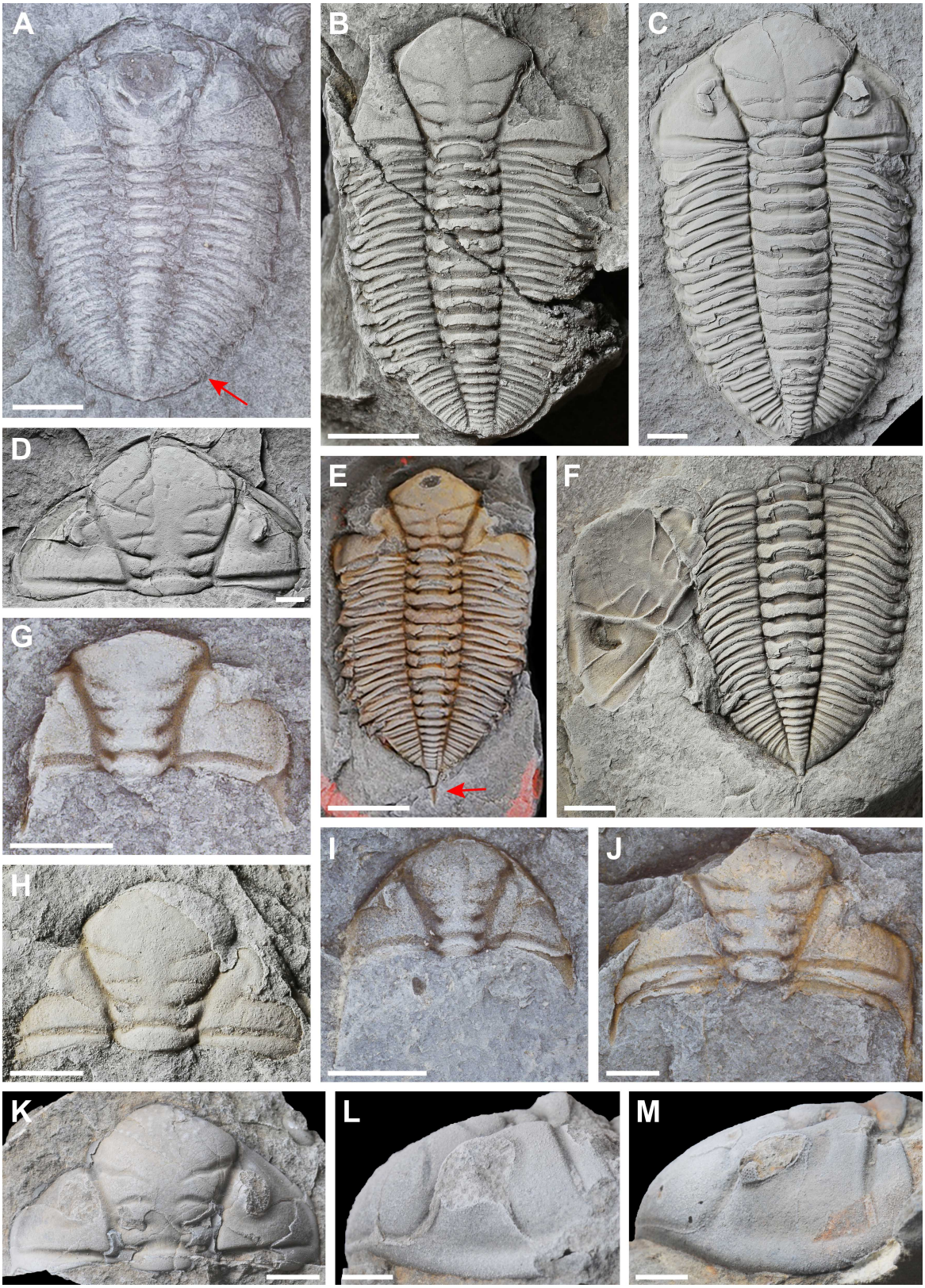


Figure 6: Holaspides of *Dalmanitina proaeva elfrida* and *D. proaeva* from the Vinice and Zahořany Formations respectively (Sandbian to Katian) of the Prague Basin. Red arrow in A points to the location of the thoracic-pygidial boundary, and in E to the terminal spine. Specimens B and

E are probable exuviae consisting only of the axial shields, and F is a molt in Salter's configuration (Drage et al. 2018). A, NMP L46804; B, NMP L46568; C, NMP L46821; D, NMP L46818; E, NMP L15055; F, NMP L46564; G, NMP L46793; H, NMP L46824; I, NMP L46708; J, NMP L46715; K, CGS MŠ2181a; L, CGS MŠ2671; M, CGS MŠ2673. All NMP specimens are from Trubín, and CGS specimens from Zahořany (localities 12-14, and 10 and 11 respectively, Fig. 1). Scale bars = 2.5 mm for A, G-J, L, M, and 5 mm for B-F, K.

Specimens with complete cephala, such as Figure 6C and M, are considered to have fused facial sutures because, while the lines of the sutures are still visible, they are by varying degrees much fainter and less defined (despite the excellent preservation) than sutures such as in Figure 6K, which are broader and clearly operable without the librigenae being separated from the cranidium (matrix is visible around the right librigena, and the left is displaced slightly inwards; probably also true for Fig. 6A with a broad right suture despite the poor preservation). Phacopine trilobites are defined, amongst other characteristics, by the proparian form of the suture lines that are ultimately fused by adulthood (Jaanusson 1975). When the librigenae are fused these sutures remain visible in varying degrees in holaspides, more so in dalmanitids than phacopids or pterygometopids (see Henry 1968; Eldredge 1972; Hammann 1976: Pl. 6; Holloway 1981 throughout; Šnajdr 1982, 1990; Budil and Bruthansová 2005; Sandford and Holloway 2006; Budil *et al.* 2009: Fig. 8L; Storey et al. 2016: Figs 5-8). For example, the suture lines are almost invisible (although still present) on less-well preserved holaspid cephala of *D. proaeva* (e.g. CGS MŠ2165; Appendix 6.2). There are many holaspid specimens from the CGS of *D. proaeva* of varying sizes which also have fused but faintly visible suture lines (e.g. MŠ2673, MŠ1245, MŠ2678, and MŠ2669 with cranidial widths of ~2-4 cm; see Appendix 6.2). Further, these specimens do not show even millimetre-scale librigenal displacement (even in otherwise disarticulated cephala, e.g. Fig. 6D, M), unlike holaspides with unfused

sutures (e.g. Fig. 6L and JV18212 from the CGS which both show librigenal displacement; see Appendix 6.2) or carcasses of other trilobite species with operable sutures that often show minor displacement through abiotic processes (e.g. Drage et al. 2018: Fig. 3G). The fused but faintly traced facial sutures are particularly clear in extremely three-dimensional specimens of *D. proaeva* (such as Fig. 6M). In the aulacopleurids, another trilobite group which underwent this fusion event later in development, Hughes et al. (2014) also specifically noted that, while the facial sutures were fused and Salter's mode of molting was used, "these sutures continued to be visible throughout the holaspid phase, […], indicating that they remained expressed as vestigial structures" (p. 231). This condition (inoperable, but still visible, facial sutures) is therefore not just restricted to dalmanitoids and has wider precedence within Trilobita.

The developmental timing of facial suture fusion in *D. proaeva elfrida* does not seem to be related to the onset of epimorphic growth or of the holaspid period, as specimens such as Figures 6B and E have unfused sutures and the full 11 thoracic segments. Further, Figure 6I, based on its size (Fig. 2), may indeed represent a late-stage meraspid with fused sutures and therefore suggest an even broader range of variability for the timing of this fusion event. Budil and Bruthansová (2005) similarly noted the occasional use of the facial sutures for molting in adult dalmanitoids and argued that this indicates facial suture fusion was not always genetically fixed in phacopines, agreeing with this interpretation of the *D. proaeva* specimens.

Post-Embryonic Developmental Sequence of *Dalmanitina socialis*

The specimens of *D. socialis* (Fig. 7) provide less detailed information on the species' ontogenetic sequence due to the coarser-grained preservation at the Letná Formation. Studied individuals of *D. socialis* have cranidia and cephala between 0.71-

11.56 mm in sagittal length and 0.99-30.40 mm in transverse width. Most of the specimens represent meraspid and holaspid stages, and the smallest individual (Fig. 7A) may be equivalent to P2 or M0 of *D. proaeva elfrida* due to its similar size and overall morphology. Morphological development in *D. socialis* was for the most part similar to that described for *D. proaeva elfrida*, including the changes in general morphology, and shape of the hypostome (Fig. 7C). When compared to specimens of *D. proaeva elfrida*, it appears that *D. socialis* enters the holaspid period at a comparably smaller size (Fig. 8), with holaspides being defined from the stable thoracic number of 11 segments. The smallest recorded holaspid of *D. socialis* with this complete number of thoracic segments has a cranidium 3.25 mm long and 5.20 mm wide (compared to 4.20 mm long and 7.26 mm wide in *D. proaeva elfrida*, while the M10 of *D. proaeva elfrida* is 3.51 mm long and 6.36 mm wide).

Some small individuals of *D. socialis* show a fairly densely pitted prosopon, in particular on the fixigenae. This is clear from the best-preserved early developmental stages (Fig. 7A-C), but not visible on the internal molds of *D. proaeva elfrida*. Where observable, the marginal cephalic spines in meraspid individuals actually appear longer than those seen in *D. proaeva elfrida*, and were similarly present until at least midway through the meraspid period (Fig. 7B and C visible around the left and anterior margin, and in 7D around the entire anterior). Facial suture fusion generally seemed to occur in *D. socialis* during early holaspid development, as far as this could be observed (e.g. Fig. 7G-I show larger adult cephala with fused sutures, but late meraspides such as 7E had unfused LCUs).

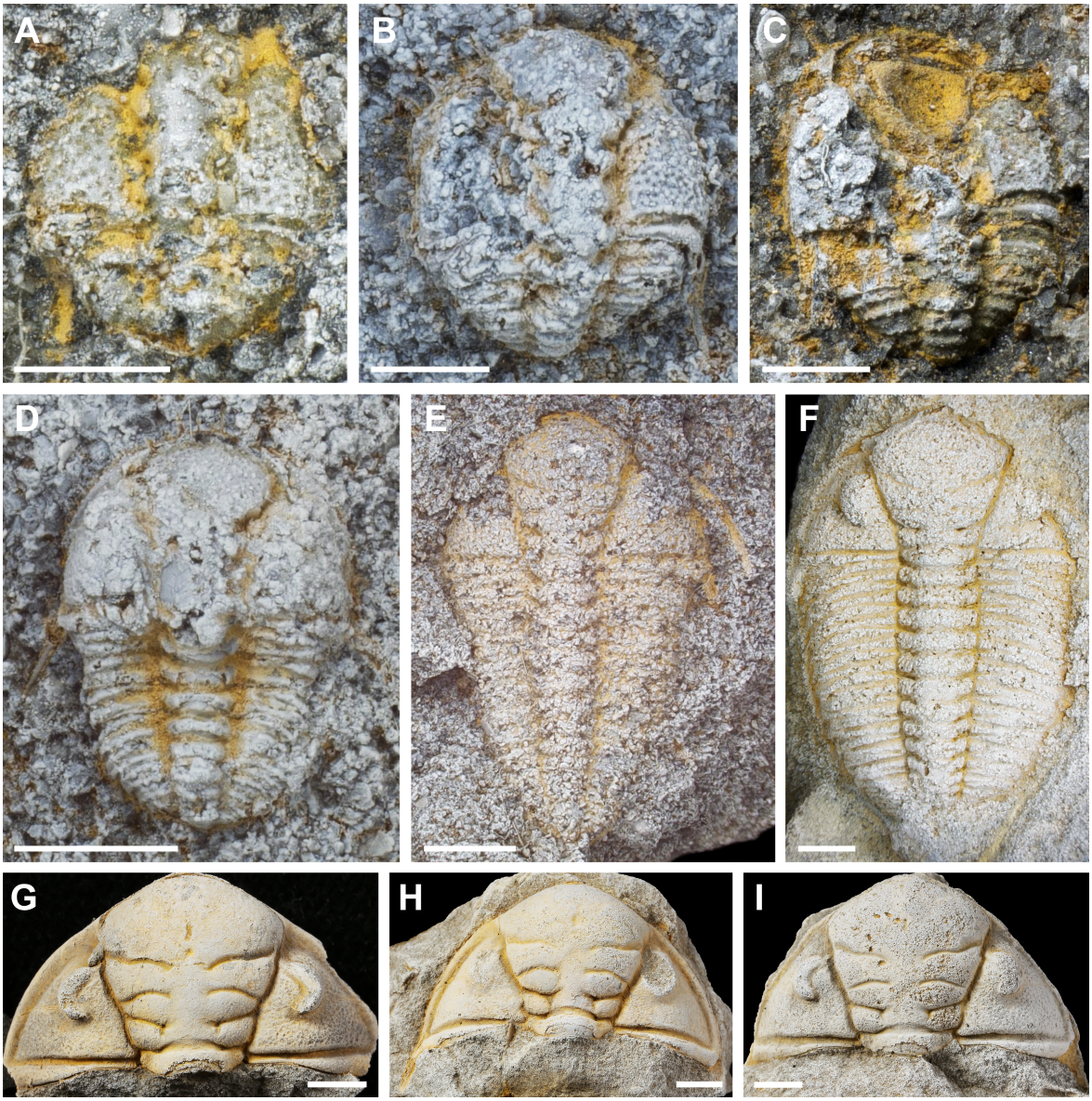


Figure 7: Specimens of *Dalmanitina socialis* from the Letná Formation (Sandbian) of the Prague Basin. A, NMP L46681; B, NMP L46725; C, NMP L46685; D, NMP L46739; E, NMP L46779; F, NMP L46785; G, NMP L46834; H, NMP L46566; I, NMP L46823. A-D are from Trubská, and E-I from Veselá (localities 12-14, and 1-7 respectively, Fig. 1). Scale bars = 0.5 mm for A-C, 1 mm for D and E, 2 mm for F, and 5 mm for G-I.

Exuviae of *Dalmanitina*

Many of the *D. proaeva elfrida* and *D. socialis* specimens from the NMP collections, and some of those displayed in Figures 4-7, are interpreted as molted exoskeletons

(exuviae). These show either the librigenae/LCU separated from the axial shield, or the cephalon disarticulated from the trunk (both to create an anterior exuvial gape to exit the old exoskeleton through, see Budil and Bruthansová 2005: Fig. 3A, B_1; Drage et al. 2018). For the protaspid period of *D. proaeva elfrida*, Figure 4B shows a singular separated left librigena displaced slightly outwards from the cranidium, which may also be the case for Figure 4C, and in both the right librigena appears missing as the eye ridge forms the outer margin. This is also true of Figure 4E, which is missing both librigenae. In Figure 4C the independent hypostome may also have been displaced to the left-posterior of the specimen. Figure 4F and G (and potentially 4H) show an impressed and posteriorly-displaced external mold of the two independent librigenae and doublure (i.e. the dorsal and ventral parts of the librigenae) with the hypostome (there is no visible connection between the left and right librigenae to the anterior of the hypostome). These molts are reminiscient of Maksimova's molt configuration (described by Henningsmoen 1975: pg. 190, Fig. 8) in which both sides of the doublure, often with the hypostome, are displaced as one unit below the axial shield. However, Maksimova's configuration was apparently not always produced, as Figures 4A-C and 5B show singular molted librigenae and/or hypostome (more comparable to the Nutcracker configuration from Drage et al. 2018). The P2 specimen in Figure 4I may also be lacking the librigenae, much like the M0 individual in Figure 5A, but this is difficult to ascertain due to the preservation. The latter specimen does appear to have an impression of the isolated hypostome displaced posteriorly, and Figure 5B (arrow) clearly shows the separated right librigena, and potentially the left librigena displaced outwards.

From M1 several probable exuviae show a separated LCU, which is displaced backwards in Figure 5C, E, and K, with the hypostome attached in the latter two, and forwards in 5D. The *D. socialis* late meraspid in Figure 7E also appears to show a

displaced LCU to the right of the cranidium. These reflect Harrington's molt configuration (Henningsmoen 1975; Drage et al. 2018), although potentially with the LCU a single structure, rather than retaining the facial sutures and joined by integument (as for *Estaingia bilobata* Pocock 1964 in Drage et al. 2018). Axial shields (i.e. articulated but missing one/both the librigenae; Fig. 6B and E for *D. proaeva elfrida,* and Fig. 7F for *D. socialis* with the left librigena in place) are reasonably common for all but the largest *Dalminitina* holaspides (see Budil and Bruthansová 2005). Figure 6F presents a classic example of a molted exoskeleton in Salter's configuration (Henningsmoen 1975; Drage et al. 2018), albeit with the inverted cephalon facing in an unusual direction and slightly overlapping the trunk.

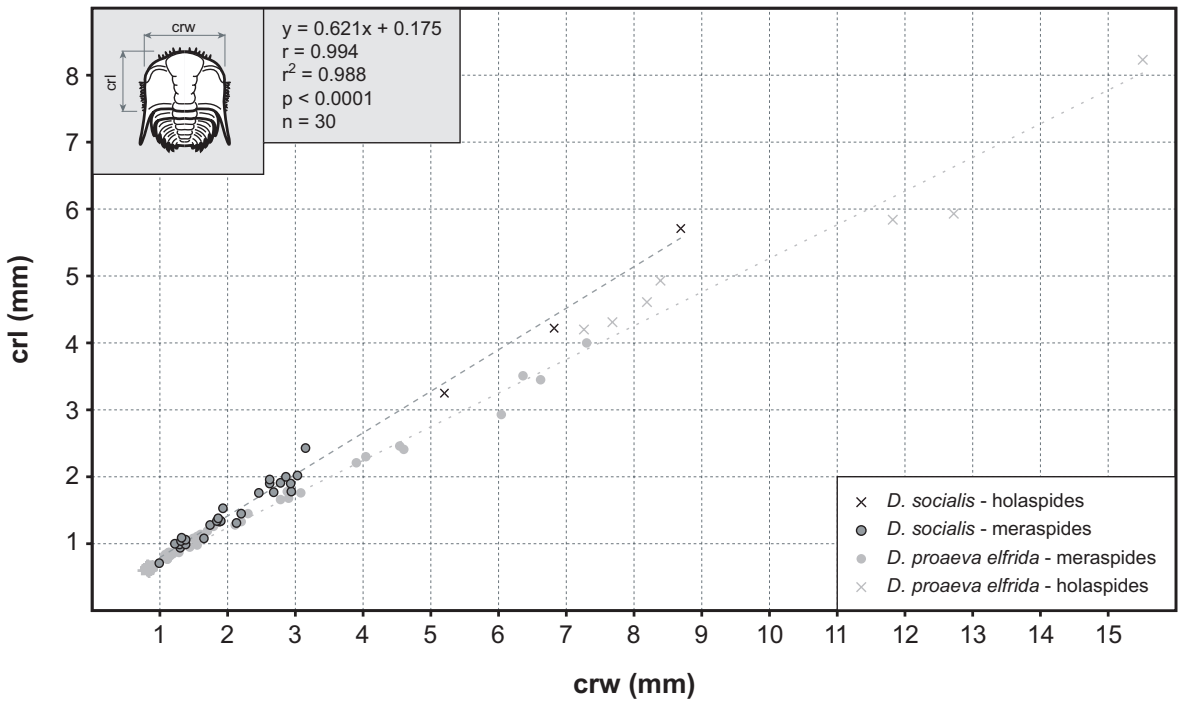


Figure 8: Scatter plot of cranidial or cephalic width (crw) vs. cranidial or cephalic length (crl) for *Dalmanitina socialis* from the Letná Formation (Sandbian) and *Dalmanitina proaeva elfrida* from the Vinice Formation (Sandbian to Katian) of the Prague Basin. Sample size and statistics in top left refer to the results for *D. socialis*.

Discussion

Comparative Development of Phacopina.—Early post-embryonic stages of *Dalmanitina* show most (but not all) of the distinctive characteristics of Phacopina, as summarized by Chatterton and Speyer (1997). In particular: 1) a proparian facial suture; 2) anteriorly located palpebral lobes; 3) forwardly expanding glabella; 4) four glabellar furrows; 5) pitted fixigenae (at least in small specimens of *D. socialis*); 6) short (sag.) anterior border; 7) marginal spines on the cranidium and librigenae; 8) distinct posterior marginal and border furrows; 9) sharp spines on the trunk beginning from the meraspid stage (see morphological reconstructions in Fig. 9); and 10) a terminal spine on the pygidium (Fig. 6E).

When compared with protaspides of the other phacopine families Phacopidae and Pterygometopidae, the dalmanitid protaspides lack the highly bulbous nonadult-like (pre-metamorphic) stage (see also Crônier 2007), instead showing only adult-like stages. All described post-metamorphic protaspides of Phacopina appear to have two dorsally-expressed segments in the trunk portion, as can be inferred from the axial section and the number of marginal trunk spines preserved (cf. Temple 1952; Whittington and Campbell 1967; Chatterton and Speyer 1997; Crônier 2007). Protaspides and earliest meraspides of the Pterygometopidae and Dalmanitidae also share unfused, independent librigenae (Chatterton and Speyer 1997: Figs 175.2, 175.10) and bifurcated marginal cranidial spines (Fig. 9; Chatterton and Speyer 1997: Fig. 175.8). In contrast, the earliest post-embryonic stages of the Phacopidae have fused librigenae, forming an LCU (Crônier 2007: Fig. 2.13), and the cranidial marginal spines do not seem to be bifurcated (Crônier et al. 1999: Fig. 5.7; Crônier 2007: Figs 2.13, 3.3). Further, the protaspid and early meraspid hypostomes of Dalmanitidae, with a rounded posterior lobe, resemble those of the Pterygometopidae rather than Phacopidae (cf. Chatterton and Speyer 1997; Crônier et al. 1999; Crônier

2007). Late meraspid stages of Dalmanitidae are distinct from the Phacopidae, as the latter have an extremely inflated anterior part of the glabella (Osmólska 1963; Crônier et al. 1999; Crônier 2007).

The trunk segmentation schedule described here for *D. proaeva elfrida* (Fig. 10) shows a protarthrous mode of segmentation because the onset of the holaspid period precedes the onset of the epimorphic phase of development (cf. Hughes et al. 2006). This is in contrast with the schedule so far described for Phacopidae, in which only synarthromeric and hypoprotomeric modes are known (Crônier 2010).

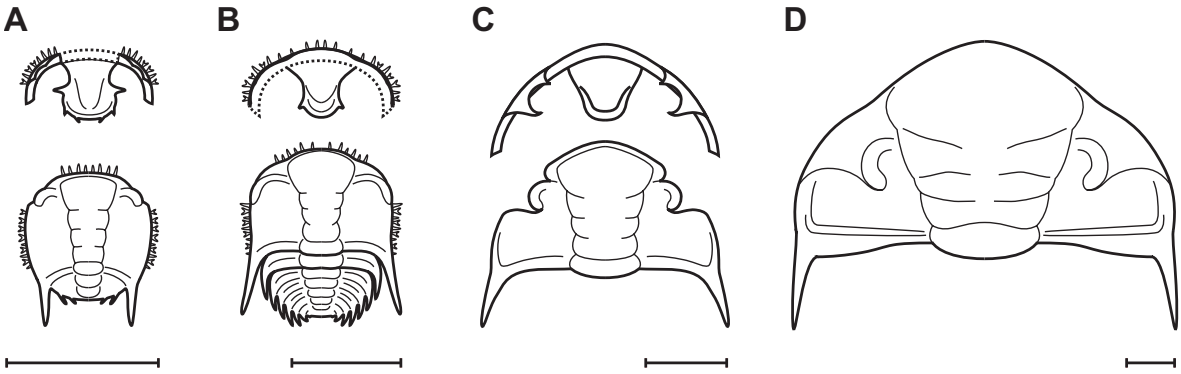


Figure 9: Reconstructions of selected developmental stages of *Dalmanitina proaeva elfrida* from the Vinice Formation (Sandbian to Katian) of the Prague Basin. A, first protaspid stage showing librigenae and hypostome; B, meraspid degree one showing Lower Cephalic Unit and hypostome; C, cranidium of meraspid degree five showing Lower Cephalic Unit and hypostome; D, cephalon of early holaspid stage. Dotted lines represent inferred structures. Scale bars = 1 mm.

It is particularly interesting that size-based clusters of *D. proaeva elfrida* stages can be recognized early in ontogeny because, while these individuals are from 'one locality' they most likely represent more than one population. The specimens are all from the Vinice Formation at Trubín and therefore of Sandbian-Katian age, but as historical specimens they may have been collected from different beds and collection pits, and

therefore subject to slight variations in age (see Geological Setting, Fig. 1). The size-clustering of the *D. proaeva elfrida* sample may therefore suggest the species was reasonably consistent in growth increment, and this was robust to differences in regional age and location. If found to be the case for other phacopine species, this could be useful for obtaining larger sample sizes to explore broad-scale ontogenetic trends.

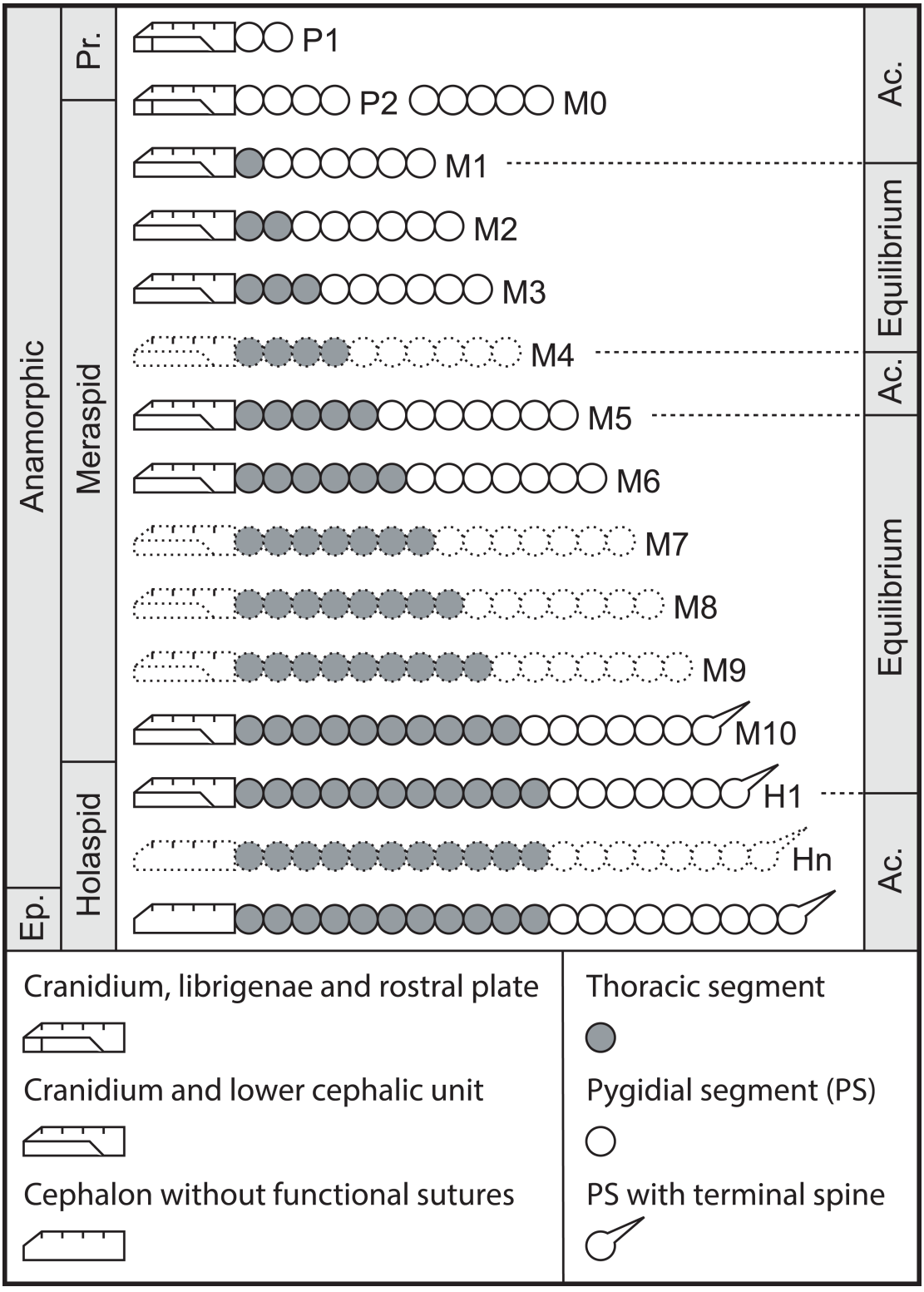


Figure 10: Trunk segmentation schedule of *Dalmanitina proaeva elfrida* from the Vinice Formation (Sandbian to Katian) of the Prague Basin. Dotted lines represent estimated, but not observed stages. Abbreviations: Ac., accumulation phase; Ep., epimorphic growth; H1-Hn, holaspid stages; M0-M10, meraspid degrees; P1-P2, protaspid stages; Pr., protaspid period.

Variability in the Timing of First Articulation.—Three clusters are apparent during the earliest post-embryonic development of *D. proaeva elfrida* (Fig. 3). The first and third of these clusters contain individuals that can be assigned to P1 and M1 respectively. By contrast, the second cluster (Fig. 3) contains two morphotypes that differ by the presence or absence of an articulation structure, the number of post-cephalic segments (four and five) and the size of the trunk (Figs 3-5). Therefore, the second cluster contains both individuals of P2 and M0. This unusual distribution can be explained in two ways. Firstly, the growth increment between P2 and M0 was smaller than during the rest of development, meaning the two stages greatly overlapped in their size ranges. Secondly, the timing of the first articulation and segment release was not developmentally constrained, producing a variability in the timing of the first articulation (and therefore variability of the protaspid-meraspid transition) and of segment number in individuals of similar sizes. Consequently, two models can be proposed to explore this distribution: the three stages model, and the four stages model. In the former, the developmental stages are based solely on size clustering, regardless of their morphology. In the four stages model, they are based on their morphology, irrespective of size clustering. We calculated several developmental traits under both models and compared the results with Fusco et al. (2012) to assess which scenario better fits the traits observed more generally in Trilobita.

The growth rate (GR) is a pre-molt/post-molt size ratio known for many trilobite taxa (e.g. Palmer 1957; Chatterton et al. 1990; Lerosey-Aubril and Feist 2005; Laibl et al. 2014; etc.). The GR in *D. proaeva elfrida* ranges from 1.351-1.364 for width and 1.397-1.497 for length under the three stages model; and from 1.039-1.337 for width and 1.099-1.424 for length under the four stages model (see Table 1). A constant rate of size increase between molts (Dyar's rule) is considered the null model for arthropod growth and is consistent with available evidence of the physiological mechanisms underlying growth and control of

molting (Klingenberg and Zimmermann 1992). Assuming Dyar's rule, variation in GR through ontogeny for a species should therefore be minimal. The three stages model better fits this assumption due to its low levels of GR variation (Table 1). This is also reflected by the values obtained for the index of conformity to Dyar's rule (IDC), which quantifies the level of adherence to a constant GR during development. The majority of trilobites (~80% with sufficient developmental data) apparently grew at a relatively constant rate, with IDCs over 0.8 (Fusco et al. 2012). In *D. proaeva elfrida*, the IDC is >0.9 for the three stages model, and for the four stages model it varies between 0.788-0.805 and 0.782-0.910 for width and length respectively (Table 1). For the former, this suggests a close fit with Dyar's rule, which may again support the three stages model. The assumption of variation in segment number in the second size cluster under the three stages model is not itself exceptional because segment number flexibility within trilobite stages is well known (e.g. Hughes et al. 2006; Hou et al. 2015; Dai et al. 2017).

Table 1: Results of developmental traits calculated for *Dalmanitina proaeva elfrida* from the Vinice Formation (Sandbian to Katian) of the Prague Basin. Abbreviations: AGI, average per-moult growth increment; Cl.1-Cl.3, cluster one to three; GR, growth rate; IDC, index of conformity to Dyar's rule; L, length; M0-M1, meraspid degrees; P1-P2, protaspid stages; W, width.

Three stages model						
Stage	W (mm)	L (mm)	GR (W)	GR (L)	IDC (W)	IDC (L)
Cl. 1	0.840	0.832	–	–	–	–
Cl. 2	1.135	1.162	1.351	1.397	0.985	0.906
Cl. 3	1.548	1.740	1.364	1.497	–	–
Four stages model						
Stage	W (mm)	L (mm)	GR (W)	GR (L)	IDC (W)	IDC (L)
P1	0.840	0.832	–	–	–	–
P2	1.115	1.112	1.327	1.337	0.805	0.910
M0	1.158	1.222	1.039	1.099	0.788	0.782
M1	1.548	1.740	1.337	1.424	–	–

However, the three stages model is problematic because it contradicts the general assumption that the timing of the first segment articulation is developmentally static within a species (and thereby indicates the onset of the meraspid period). Although, variability in the timing of first articulation within a single trilobite species has been suggested before. For example, Lee and Chatterton (2005) reported two late protaspid specimens with incomplete articulation in the Cambrian menomoniid *Bolaspidella housensis* Walcott 1886. Based on their size, these specimens clustered with P3, and were interpreted as potential inter-molt stages. They also described stage 2 protaspides of the lonchocephalid *Glaphyraspis parva* Walcott 1899 that clustered with two fully articulated (i.e. not inter-molt stages) specimens of meraspid degree 0 (Lee and Chatterton 2005: Fig. 6c), similar to for *D. proaeva elfrida* (Fig. 3). There is also some support for variability in the timing of the first articulation between two closely-related taxa (Park and Choi 2011). For example, *Asioptychaspis subglobosa* Sun 1924 had two protaspid stages, whereas its descendant species *Quadraticephalus elongatus* Kobayashi 1935 had only one protaspid stage, but its P1 and M0 corresponded with the size and morphology of P1 and P2 respectively in *A. subglobosa* (Kihm et al. 2013).

With the currently available data on trilobite development we cannot entirely rule out the four stages model. Hou et al. (2015) provided a detailed discussion related to overlapping clusters of various developmental stages. They suggested that a low growth increment (and therefore GR) might have been associated with size overlap among successive stages even during early post-embryonic ontogeny (as suggested under the four stages model). However, this is likely not the case for *D. proaeva elfrida*, which had a GR slightly higher than most other trilobites (cf. Fusco et al. 2012). The lower GR between P2 and M0 in the four stages model (1.039 width and 1.099 length) may be related to the

development of the articulation structure. Unfortunately, data concerning GR across the protaspid-meraspid transition that might inform on this are scarce (Fusco et al. 2012).

Based on the aforementioned evidence it seems more likely that there existed variability in the timing of the first articulation event in *D. proaeva elfrida* (three stages model). Some individuals apparently developed the first articulation (and thus entered the meraspid period) after one molt, whereas others molted without developing an articulation (and entered the P2 stage). During the subsequent molting event the M0 individuals developed another articulation, and the individuals of P2 may have developed two articulations (this has been described in *Sao hirsuta* Barrande 1846 and may have occurred in other trilobite taxa; Laibl et al. 2014). If the three stages model represents the true developmental sequence it suggests that the timing of the initial segment articulation event may have been variable even within one trilobite species. Further, it supports the view that the protaspid period is not biologically meaningful in terms of homology between developmental stages (see Park and Choi 2011), and that trilobite development reflects a complex combination of independent processes such as segmentation, articulation, and morphological changes (cf. Hughes et al. 2006).

Facial Suture Fusion in Phacopina.—Molted exoskeletons were reasonably common amongst the specimens of *D. proaeva elfrida* and *D. socialis* in the NMP collections, including, as described above, protaspides and earliest meraspides with individual separated librigenae (Figs 4B, C, F, G, and 5B), meraspides with a separated LCU (Figs 5C-E, K, and 7E), holaspid axial shields missing the librigenae (Figs 6B and E, 7F; Budil and Bruthansová 2005: Fig. 4F), and large holaspides with disarticulated entire cephala (e.g. Fig. 6F). Suborder Phacopina was partially defined based on facial suture fusion during ontogeny (Jaanusson 1975), where the sutures close and the librigenae can

no longer separate from the cranidium for molting. This fusion event therefore accounts for the occurrence of *Dalmanitina* meraspid molts with a separated LCU and holaspides with disarticulated cephala.

However, most ontogenetic descriptions for phacopine (Phacopidae) taxa have to-date noted only this single fusion event. In contrast to this, we also observed a primary fusion event that occurred between stages M0 and M1 in *D. proaeva elfrida,* when the librigenae became joined as a single unit (the LCU). The earliest developmental stages of phacopines for which a reasonably complete developmental sequence has been described display joined librigenae (Crônier et al. 2005; Crônier and Fortey 2006), rather than the small individual librigenae identified in the protaspides and M0 of *D. proaeva elfrida* (Figs 4B, C, F, G, and 5B). Further, Jaanusson (1975) in discussing the evolutionary trends of Phacopina asserted that their protaspides displayed an LCU. However, both separated LCUs and independent librigenae were recognized in juveniles of *Nephranops incisus incisus* Roemer 1866*,* belonging to the Phacopidae (Crônier 2007: Fig. 5), which also fused the facial sutures later in ontogeny, but this could not be associated with a specific fusion event during ontogeny as was possible for *D. proaeva elfrida*. In addition, figured protaspides of the pterygometopid *Calyptaulax annulata* Raymond 1905 appear to show independent librigenae retaining sutures on the doublure (Chatterton and Speyer 1997: Fig. 175.2 and .11), and the same with the encrinuroids *Physemataspis insularis* Shaw 1968 and *Cybeloides prima* Raymond 1905 (Chatterton and Speyer 1997: Fig. 176.2 and .6). This indicates that the primary fusion event, with its specific ontogenetic timing in *D. proaeva elfrida* (Dalmanitidae), was actually widespread within the Phacopina. Interestingly, some asaphids, for example *Dikelocephalus minnesotensis* Owen 1852, displayed a similar condition to this primary phacopine fusion event in which the median suture separating the two librigenae was fused, the timing of which varied even with single

bedding plans (Hughes 1994). Though several ptychaspidid species (also of the Asaphida) were described as showing an opposing pattern through ontogeny, with the fused librigenae eventually becoming separated into two individual structures later in development (Kihm et al. 2013).

Knowledge of the developmental timing of the secondary fusion event (that of the facial sutures) has been previously confined to the family Phacopidae, within which it is interspecifically variable. In *Weyerites ensae* Richter and Richter 1926)facial suture fusion takes place between stages M9-10 (Crônier et al. 2005; Crônier and Fortey 2006), and in *Trimerocephalus lelievrei* Crônier and Feist 1997 and *Plagiolaria poothaii* Kobayashi and Hamada 1968 it occurs at the onset of holaspis at M10-H1 (Crônier et al. 2005; Crônier and Fortey 2006). This secondary fusion is generally known to occur in dalmanitids, for example a number holaspides of various species have been described with fused facial sutures in detached cephala (see Henry 1968; Eldredge 1972; Holloway 1981; Šnajdr, 1982, 1990; Budil and Bruthansová 2005; Sandford and Holloway 2006; Budil et al. 2009: Fig. 8L; Story et al. 2016: Figs 5-8), however the data for *Dalmanitina* provide the first information on its developmental timing in Dalmanitidae. In comparison to these examples from the Phacopidae, the fusion of the facial sutures may occur later in development for *Dalmanitina.* In general, this is seemingly during the holaspid period and in individuals with a range of sizes (e.g. Fig. 6C, D, F, I, M; Appendix 6.2; Budil and Bruthansová 2005), rather than related to a specific developmental stage or to the meraspid-holaspid transition (Crônier et al. 2005; Crônier and Fortey 2006). This secondary fusion event is rare for trilobites outside of the Phacopina, but has been noted to occur during later development for some non-phacopines such as *Primaspis* [*Taemasaspis*] *campbelli* Chatterton 1971 and aulacopleurids (Hughes et al. 2014). These examples suggest that,

while the secondary fusion event appeared to follow a primary fusion event (producing the LCU) in Phacopina, this need not be the case in other trilobite groups.

These changes in morphology must represent significant events in the life history of phacopine trilobites because they necessitate alterations to exoskeletal molting behavior. *Dalmanitina proaeva elfrida* and *D. socialis*, like the Phacopidae, would have molted in their earlier developmental stages using the Sutural Gape mode (Henningsmoen 1975; Daley and Drage 2016; Drage et al. 2018), where the facial sutures opened to create an anterior gape through which the animal would have emerged. This would have involved separation of the smaller, independent librigenae in the P1-M0 stages, producing a molt similar to the Nutcracker configuration (Drage et al. 2018), and (following the primary fusion event) of the joined LCU, creating Harrington's configuration (Henningsmoen 1975; Drage et al. 2018) from the M1 stage until near the end of the meraspid period or during holaspis. Following the secondary fusion event, the complete closure of the facial sutures, the individual could no longer have molted using the Sutural Gape mode, instead by necessity employing Salter's mode where the entire cephalon was disarticulated from the trunk (Henningsmoen 1975; Daley & Drage 2016; Drage et al. 2018), presumably creating a wider exuvial gape. This produced Salter's molt configuration (e.g. Fig. 6F), and would have necessitated changes in behavior, such as different patterns of movement to cause the cephalothoracic articulation to break (see Drage et al. 2018, for descriptions of molting mechanisms).

The interspecific differences in the timing of the secondary fusion event in the Phacopidae may relate to differing body size proportions, and in particular that of the relative proportions of the cephalon to thorax. However, for *Dalmanitina*, it is likely unrelated to body size and proportions because of the observed variation in holaspid cranidium size with fused and unfused facial sutures. This indicates that facial suture

fusion was not a developmentally-constrained event, unlike for the Phacopidae or the primary fusion event in *D. proaeva elfrida* (as also suggested by Budil and Bruthansová 2005), and instead indicates the importance of an alternative trigger. Interestingly, the two *Dalmanitina* species may slightly differ in this regard, *D. socialis* having entered the holaspid period at a smaller size than *D. proaeva eflrida.* This may be related to a difference in habitat, being generally found in the Letná and Vinice Formations respectively, or a difference in the age of the two species (although these are very close, being late Sandbian and latest Sandbian/earliest Katian).

The Phacopoidea are thought to represent a more derived group within Phacopina (Jaanusson 1975; Budil & Bruthansová 2005). The earliest phacopoid representatives (family Pterygometopidae) emerged almost contemporaneously with the earliest Dalmanitoidea in the Floian - Darriwillian (latest Early – Middle Ordovician, see Adrain 2013; Bergström et al. 2013; Pärnaste and Bergström 2014), but the derived family Phacopidae appear later, in the latest Ordovician or earliest Silurian (Chlupáč 1977; Lespérance and Sheenan 1987; Eldredge 2001; Adrain 2013). Thus, it may be that the timing of the secondary fusion event became increasingly fixed during ontogeny, and therefore less intraspecifically variable, within the Phacopina throughout their evolution. This agrees with the Ordovician peak in species polymorphism and subsequent decline through the Palaeozoic described by Webster (2007) in the Phacopida (Webster, 2007: Fig. 3F). Alternatively, this intraspecific variability might also be a characteristic of the Dalmanitidae as the described specimens of *Dalmanitina* are not from the earliest origination of the Dalmanitoidea. If the timing of this secondary fusion event could be identified within additional early representatives of Phacopina, in particular the Pterygometopidae, these opposing hypotheses could be distinguished. Whether found more broadly within early phacopines, or unique to the Dalmanitidae, this developmental

variability might have been an evolutionary adaptation, potentially in response to an external trigger, in addition to the inherent interspecific developmental variability well-known within Trilobita (Hughes et al. 2006).

Conclusions

A large sample of protaspid to holaspid specimens of *D. proaeva elfrida* and *D. socialis* from the Letná, Vinice, and Zahořany Formations of the Prague Basin have allowed description of the first reasonably complete developmental sequence for the trilobite superfamily Dalmanitoidea. We have presented details of the morphological change in *Dalmanitina* from the protaspid to holaspid period, including the late disappearance of marginal cephalic spines and change in hypostome shape. Biometric data shows that the specimens cluster in distinct developmental stages, with probable variability in the timing of first articulation resulting in two different morphotypes (P2 and M0) with a significant overlap in size, and a broad range of body sizes in the holaspid period.

The developmental sequence for *D. proaeva elfrida* provides the first information on facial suture fusion for the Dalmanitoidea; a key characteristic of the suborder Phacopina. Between stages M0 and 1 the specimens display a fusion of the separate librigenae into a Lower Cephalic Unit, a morphological change that had not been observed as developmentally-fixed in phacopines. Further, during the holaspid period (or potentially from the latest meraspid), there occurs an intraspecifically variable fusion of the facial sutures. These are related to alterations in their exoskeleton molting behavior, the secondary fusion event requiring a change from the Sutural Gape mode of molting to Salter's mode (Drage et al. 2018). This would have produced the Nutcracker (or Maksimova's) molt configuration until M1, Harrington's configuration until around holaspis, and Salter's configuration when the facial sutures were inoperable (Drage et al.

2018). Facial suture fusion is a uniting characteristic of Phacopina, but generally occurs earlier in development and with no intraspecific variation in the family Phacopidae (Crônier et al. 2005; Crônier and Fortey 2006), which is closely related to Dalmanitoidea but occurs later in the fossil record. This may either reflect an increasing developmental constraint on the ontogenetic timings of these events within the Phacopina, as generally supported by decreasing intraspecific variability and polymorphism after the early Ordovician (Webster 2007), or a so-far unique condition amongst dalmanitids.

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Chapter 7: Sequence of post-moult exoskeleton hardening preserved in a trilobite mass moult assemblage from the Lower Ordovician Fezouata Konservat-Lagerstätte, Morocco

This chapter explores the mechanisms of the moulting process in trilobites, in particular looking at preservation of the post-moult soft-shell stage through description of a complete moulting sequence of *Symphysurus ebbestadi*. The post-moult stage is extremely short-lived, and this chapter describes the first incidence of a complete euarthropod moulting sequence; potentially reflecting an in-the-act mass moult. This work demonstrates that the biological process of moulting has been conserved through most of the evolutionary history of Euarthropoda, the stages of which have changed little from Lower Ordovician trilobites to modern groups.

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H.B.D. conceived the study, gathered data, and wrote the majority of the paper. T.R.A.V. and P.V.R. collected specimens, and wrote geological settings. All authors edited the paper.

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Sequence of post-moult exoskeleton hardening preserved in a trilobite mass moult assemblage from the Lower Ordovician Fezouata Konservat-Lagerstätte, Morocco

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Key words: Trilobita, mass moult, soft-shelled, post-moult, moulting, exoskeleton, Ordovician, Morocco.

Abstract

Euarthropods have a tough exoskeleton that provides crucial protection from predation and parasitism. However, this is restrictive to growth and must be periodically moulted. The moulting sequence is well-known from extant arthropods, consisting of: (i) the long inter-moult stage, in which no changes occur to the hardened exoskeleton; (ii) the pre-moult

stage where the old exoskeleton is detached and the new one secreted; (iii) exuviation, when the old exoskeleton is moulted; and (iv) the post-moult stage during which the new exoskeleton starts as soft, thin, and partially compressed and gradually hardens to the robust exoskeleton of the inter-moult stage. Trilobite fossils typically consist of inter-moult carcasses or moulted exuviae, but specimens preserving the post-moult stage are rare. Here we describe nine specimens assigned to *Symphysurus ebbestadi* Gutiérrez-Marco, Rábano and García-Bellido 2018 representing the first group of contemporaneous fossils collected that preserve all key stages of the moulting process in one taxon, including the post-moult stage. They were collected from a single lens in the Tremadocian part of the Fezouata Shale Formation, Morocco. Based on cephalic displacement and comparison to other trilobite moults, one specimen appears to represent a moulted exoskeleton. Four specimens are typical inter-moult carcasses. Four others are wrinkled and flattened, with thin exoskeletons compared to inter-moult specimens, and are considered post-moult individuals. These *S. ebbestadi* specimens illuminate the preservation and morphology of the post-moulting stage, characterised by strong anterior-posterior exoskeleton wrinkling, as well as overall body flattening and reduced visibility of thoracic articulations. Being found in the same lens, these specimens likely represent the first preserved in-the-act mass moulting event. The displayed sequence of moulting suggests the moulting process in trilobites was comparable to modern arthropods, and conserved within euarthropod evolutionary history.

Introduction

Trilobites had strongly reinforced exoskeletons that protected them from predation but, like other euarthropods, needed to be moulted periodically to allow growth and

development (see Daley and Drage 2016). These moults are readily preserved in the fossil record, and are particularly numerous for trilobites compared to other euarthropods because of their calcitic exoskeleton (Henningsmoen 1975; Daley and Drage 2016; Drage and Daley 2016; Drage et al. 2018a). The moulting sequence is inferred to be much the same as for other euarthropods (Henningsmoen 1975), including a pre-moult phase, followed by an exuviation event where the old exoskeleton is actually shed, then post-moulting expansion, biomineralisation, and hardening of the new exoskeleton (Ewer 2005). However, despite this assumption, little is known about the process of moulting in trilobites with the exception of the moment of exuviation and its associated behaviours, and the resultant moult configurations preserved in the fossil record (see Henningsmoen 1975; Whittington 1980, 1990; Daley and Drage 2016; Drage et al. 2018a, 2018b). Only two euarthropods have been confidently considered individuals preserved during the actual process of exuviation (Garcia-Bellido and Collins 2004; Yang et al. 2019), neither representing trilobites. A specimen of the trilobite *Trimerocephalus* McCoy 1849 from the Upper Devonian of the Holy Cross Mountains, Poland, was putatively preserved during the process of exuviation (Błażejowski et al. 2015). However, this was later shown to be two overlapping carcasses based on the morphology, configuration, and preservational context of these two individuals (Drage and Daley 2016), which are found at a locality where clusters and queues of trilobite carcasses are common (Radwański et al. 2009).

Aspects of the post-moult stage, also referred to as the soft-shell stage (Speyer and Brett 1985), have been described in respect to several trilobite species. Putative soft-shelled post-moult individuals have been described for *Phacops rana milleri* Stewart 1927 (Middle Devonian of Ohio, USA; Erdtmann 1978; Miller and Clarkson 1980), *Phacops rana rana* Green 1832 (Middle Devonian Hamilton Group, USA; Speyer 1983, 1987; Speyer and Brett 1985), and *Greenops boothi* Green 1837 (Hamilton Group; Speyer and

Brett 1985). The *P. rana* subspecies were described as having a much thinner exoskeleton of a lighter colour than other trilobites with normally preserved exoskeletons found in the same bed, and their cuticles were often flattened and wrinkled. Importantly, these soft-shelled individuals were always larger in size than the clusters of other individuals found in the same beds, suggesting preservation of the post-moult stage. Miller and Clarkson (1980) cross-sectioned cuticles of suspected post-moult *Phacops rana milleri* individuals, which showed wrinkling and flattening, and also revealed thinner exoskeletons than their concurrent conspecific counterparts. They therefore determined that these individuals were preserved during the post-moult phase prior to hardening and thickening of the new exoskeleton, which caused wrinkling; this allowed for a study of how microstructures in the cuticle and eyes changed as the exoskeleton hardened immediately after moulting. These post-moult stage specimens are extremely rare for trilobites, and have not been described for other extinct euarthropods, presumably because the individual is particularly at risk of predation owing to the softness of the new exoskeleton, and so there is evolutionary pressure to have the post-moult hardening phase as brief as possible (Henningsmoen 1975; Whittington et al. 1997). For example, lobsters occupy the post-moult stage for 5% or less of their total time because of this vulnerability (Phillips et al. 1980). In addition, post-moult individuals, displaying poor biomineralisation or sclerotisation, have a sharply reduced preservation potential, as they are essentially soft-bodied and hence require exceptional preservational circumstances to stand any chance of fossilisation. Publications on the structure of trilobite exoskeletons are limited but have important implications for understanding moulting processes. For example, trilobite taxa differ extensively in the structures and thicknesses of their exoskeletons (Teigler and Towe 1975; Fortey and Wilmot 1991), but it is unclear if this affected their moulting behaviour.

Nine specimens of *Symphysurus ebbestadi* (identified based on Gutiérrez-Marco et al. 2018), collected from a lens in the Lower Ordovician (upper Tremadocian) at the main level of exceptional preservation of the Fezouata Konservat-Lagerstätte, Tigzigzaouine area, Morocco (Van Roy 2006; Van Roy et al. 2010, 2015a, b; Martin et al. 2016a, b; Lefebvre et al. 2016, 2017), are described that display each of the moulting stages for trilobites (see Henningsmoen 1975). The morphology is described and compared for individuals representing a moulted exoskeleton, typical inter-moult specimens, and post-moult stage specimens. Thin sections demonstrate notable differences in exoskeleton thickness between specimens representing these stages. The material of *S. ebbestadi* represents a significant contribution to the trilobite fossil record of exoskeleton moulting, providing concrete indications of the preservation of post-moult stage arthropods.

Institutional abbreviations.—MGL, Museé Cantonal de Géologie Lausanne, Lausanne, Switzerland. UNIL, University of Lausanne, Lausanne, Switzerland. YPMIZ, Yale Peabody Museum, New Haven, USA.

Geological Setting

The Fezouata Shale Formation is an extensive, predominantly transgressive unit of fairly monotonous deposits of blue to green mudstone and siltstone with occasionally localised thin sandstone interbeds, ranging in age from the early Tremadocian to the late Floian. They were deposited at a high, southern sub-polar palaeolatitude (c. 65° S; Martin et al. 2016a). Historically, the Fezouata Shale was divided into the Lower (Tremadocian) and Upper (Floian) Fezouata formations, based on the occurrence of a glauconitic horizon separating the two (Destombes et al. 1985). This horizon, however, is not visible over a

large part of the outcrop area, including the Ternata Plains near Zagora where the units reach their greatest thickness, and generally, they can only be distinguished based on fossil content. For this reason, Martin et al. (2016a) rejected the largely artificial division in Lower and Upper Fezouata formations, uniting both as the Fezouata Shale Formation.

The Fezouata Shale Formation is a unique window on the Fezouata Biota, which represents the only diverse, open marine, exceptionally preserved fauna from the Ordovician (Van Roy 2006; Van Roy et al. 2010, 2015a, b). While exceptional fossil preservation was originally believed to occur throughout the Fezouata Shale Formation (Van Roy 2006; Van Roy et al. 2010), more recent work identified two specific intervals with exceptional preservation within the unit (Martin et al. 2016a). The lower interval, which is by far the most productive, is about 70 m thick and has a latest Tremadocian age (*Araneograptus murrayi* Biozone, *c.* 478-479 Ma, near the top of the Lower Fezouata Formation of Destombes et al. 1985; Gutiérrez-Marco and Martin 2016; Martin et al. 2016a). A second, *c.* 15 m thick interval with exceptional preservation occurs higher up, and has a mid Floian age (Gutiérrez-Marco and Martin 2016; Martin et al. 2016a). The occurrence of exceptional preservation within these intervals is not continuous, but spatially limited to lenticular bodies that may range in diameter from less than a metre to several tens of metres, and vary in thickness between less than 10 cm for the smallest lenses to over a meter for the largest bodies. They occur locally in high densities and often show marked differences in both diversity and faunal composition, even between adjacent collecting sites (Van Roy et al. 2015a). These lenses probably represent palaeo relief of the sea floor, being filled in by storm-generated sediments (Martin et al. 2016a; Vaucher et al., 2016). It was originally believed that the deposits preserving the Fezouata Biota formed in relatively deep water (Van Roy et al. 2010), but Martin et al. (2016a) and Vaucher et al. (2016) provided evidence for a relatively shallow open-marine setting around storm-wave

base, representing possibly even less than 30 m of water depth (Bernard Pittet, personal communication).

Non-biomineralised tissues in the Fezouata Biota are predominantly preserved within the mud- and siltstone as framboids of iron oxide pseudomorphs after pyrite, lending the fossils their vivid colours (Vinther et al. 2008). It seems, however, likely that organic carbon was also originally preserved, but was removed as the result of late deep weathering (Van Roy et al. 2015a). Although flattened, many specimens of non-biomineralised organisms preserved within the mudstone retain some three-dimensionality. Originally biomineralised parts have been replaced with clay minerals (Vinther et al. 2008; Van Roy et al. 2015a). In addition to the main mode of preservation in the shale, large organisms are also preserved within siliceous concretions, retaining their three-dimensionality (Van Roy and Briggs 2011; Gaines et al. 2012; Van Roy et al. 2015a, b).

Materials and Methods

The material for this paper (nine specimens total) was collected in early 2004 from a single excavation in the Tigzigzaouine area, c. 20 km north of Zagora, Morocco (exact GPS locality data available from the authors on request). This excavation falls within the lower c. 70 m thick level of exceptional preservation in the Fezouata Shale Formation (*Araneograptus murrayi* Biozone, 478-479 Ma; Martin et al. 2016a; Gutiérrez-Marco and Martin 2016). The material was briefly discussed and some of it figured by Van Roy (2006: 32-33), who considered the specimens to represent a moult sequence.

The specimens are accessioned in the MGL, numbers 102127-102135. They were photographed using a Leica M165C Z-Stack microscope controlled remotely by the Leica Application Suite program, and these were merged using Helicon Focus. Figures were

produced using Adobe Illustrator and Photoshop CS6. Several specimens were photographed in parts, and these merged in Adobe Photoshop CS6 using the automated photomerge tool. Measurements of specimens were taken using digital callipers and are provided in Supplementary 1. Some specimens (MGL 102127, 102130, 102133, and 102134) were sliced with a diamond saw through the cephalon, thorax, and pygidium. Slices were examined and photographed using an Olympus SZX10 microscope mounted with an Olympus SC50 digital camera. Exoskeletal material was distinguished from surrounding rock based on the colour of the material, and straight-line transverse measurements of exoskeletal thickness were obtained from digital photographs using ImageJ2 (Rueden et al. 2017), with measurements taken from the outer to inner layer of the exoskeleton in cross-section. For each specimen, multiple exoskeletal thickness measurements were made along the structure, producing a range of sizes.

Results

Description of specimens.—Nine complete or partial body fossils are here figured from the Fezouata Shale Formation. The specimens include five complete or mostly-complete trilobites (Fig. 1A-C, F, H), two with reasonably complete anterior halves (Fig. 1D, G), and two fragmentary specimens (Fig. 1E, I). All of the specimens share the same morphological characteristics. Four of the specimens, MGL 102128 and MGL 102132-4 (Fig. 1B, F-H respectively), show incidences of wrinkling of the exoskeleton. The extent of wrinkling varies between the specimens, with MGL 102132 (Fig. 1F) being severely wrinkled, and MGL 102128 (Fig. 1B) less so. In particular, MGL 102132 has extremely prevalent wrinkling, unconfined to the lateral margins and found across the entire dorsal exoskeleton (Fig. 2A). MGL 102134 (Fig. 2B) is slightly more convex, with less obvious

wrinkling in the axial portion of the pygidium, while MGL 102128 and 102133 (Fig. 2C, D respectively) show lesser degrees of wrinkling, mostly confined to the pleural parts of the thorax, and have reasonably convex cephala. However, in all of these specimens the wrinkling is largely confined to the lateral (i.e., pleural) parts of the exoskeletons, usually on all tagma but strongly visible on the thoracic segments and pygidium. Wrinkles are elongated parallel to the anterior-posterior axis of the body on the thoracic segments, and curved around parallel to the posterior margin of the pygidium. MGL 102127 and MGL 102129-31 (Fig. 1A, C-E) show no wrinkling of the exoskeleton, and are generally very effaced with only terrace ridges. The four wrinkled specimens also show flattening of the exoskeleton in comparison to the non-wrinkled specimens. They generally have reasonably flat cephala and pygidia, as well as the axial portion of the thorax, which in the other specimens are notably convex and well-rounded (Fig. 1). This flattening also means some morphological aspects are less visible; the thoracic segments in particular are often indistinct, such as on MGL 102132 (Fig. 1F), where the articulations are less obvious than in the convex specimens.

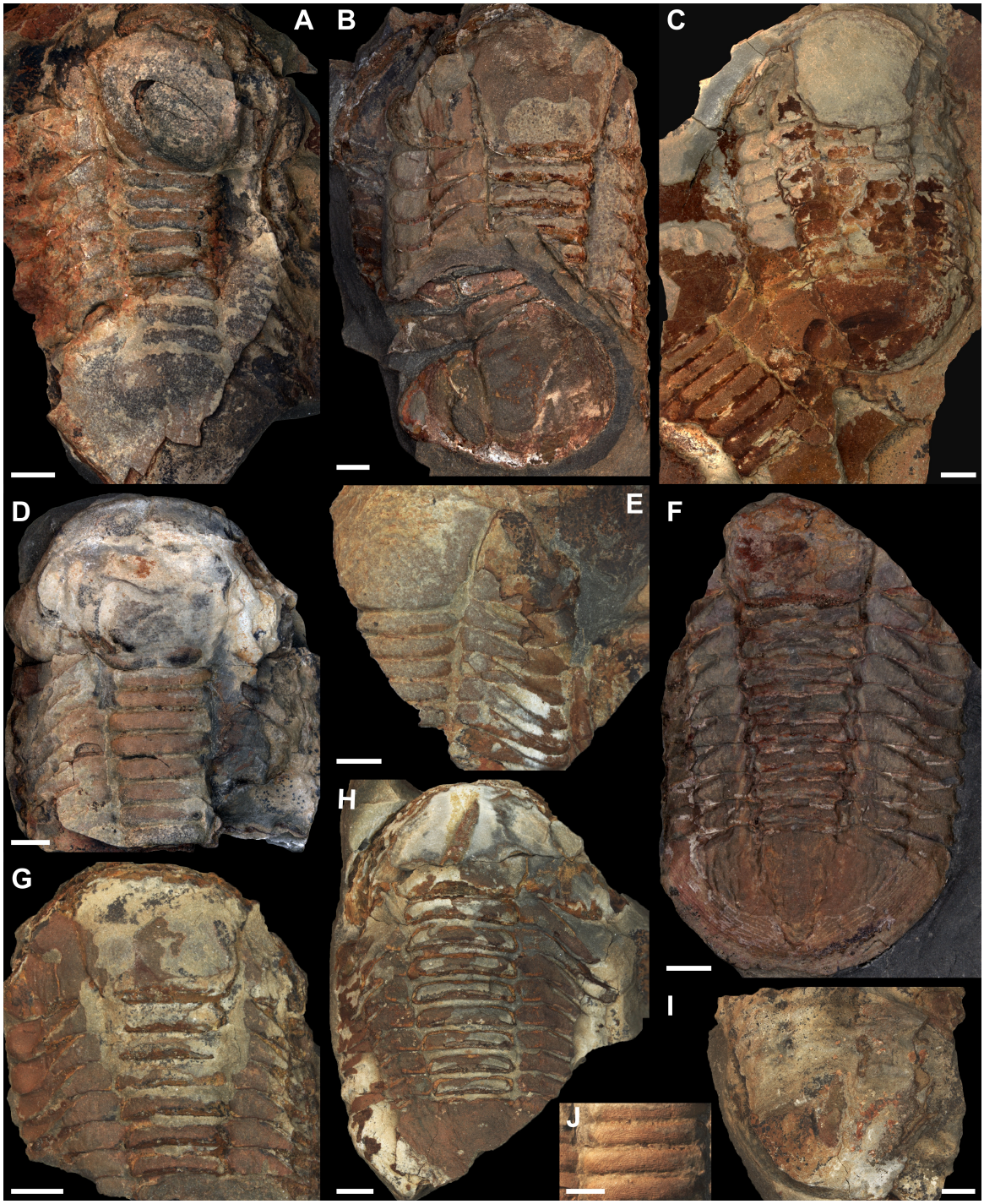


Fig. 1. The nine specimens of Moroccan trilobite referred to *Symphysurus ebbestadi*, from the early Ordovician of Morocco (Tigzigzaouine area), photographed dorsally under standard lighting. All scale bars = 5 mm. **A**. MGL 102127. **B**. MGL 102128. **C**. MGL 102129. **D**. MGL 102130. **E**. MGL 102131. **F**. MGL 102132. **G**. MGL 102133. **H**. MGL 102134. **I**. MGL 102135. **J**. close up of thorax axial rings in D, showing the clear terrace ridges.

Taxonomy.—All specimens are referred to *Symphysurus ebbestadi* following the full genus description in Goldfuss (1843) and Fortey (1986), further descriptions in (Ebbestad 1999), and species description in Gutiérrez-Marco et al. (2018). *Symphysurus* Goldfuss, 1843 has been previously described from complete or disarticulated fossils of the Lower Ordovician Moroccan Fezouata Shale Formation (*Symphysurus* sp. by Destombes 1967; *S. angustatus* Boeck 1838 by Martin et al. 2016b), and similar material has been assigned to the new and distinct species *S. ebbestadi* by Gutiérrez-Marco et al. (2018). The material here matches this description, with specimens being convex, with a large forwardly-expanding glabella, semi-circular eye ridges, and a high level of effacement of all features. The occipital furrow and median tubercle, the latter often used to diagnose the relevant family Nileidae and considered to function as a light-sensitive organ (Fortey and Clarkson 1976; Fortey 1986), are not obvious. The pygidium is subisopygous and semicircular. The thorax consists of eight fulcrate segments. The entire exoskeleton is covered with particularly clear terrace ridges, which vary in length and direction (Fig. 1J), in all but the least well-preserved specimens. These occur on all tagma (in contrast to the description of Fortey 1986), but are particularly prevalent running parallel to tergite margins and on axial rings. Librigenal morphology is difficult to interpret due to the preservation of the specimens. No ventral surfaces are exposed. The cephalic:thoracic:pygidial ratio is around 1:1.1:1.3. Additional *Symphysurus* material collected from the Fezouata Shale Formation was assigned to *S. sicardi* Bergeron 1895, but this is characterised by the near absence of terrace ridges (Gutiérrez-Marco et al. 2018), which are clearly present on the material figured here (Fig. 1J).

Interpretation of specimens.—Eight of the specimens appear to be carcasses. MGL 102128 displays two presumably complete specimens overlapping each other, facing opposing

directions (Fig. 1B). MGL 102127 (Fig. 1A) represents a putative moulted exoskeleton. This is based on comparison of the exoskeleton configuration to other trilobite moults generally (Drage et al. 2018a), but also close relatives. For example, the suggested moults of *Paciphacops* Maksimova 1971 figured in Rustán et al. (2011: fig. 2) consistently show an outstretched thoracopygon with the cephalon rotated backwards, with some sediment between the cephalon and thorax and a displaced doublure/hypostome. These were interpreted as moults with an anterior exuvial gape that did not quite disarticulate the entire cephalon at the dorsal part of the cephalothoracic joint. Other specimens of similar forms were also found in the Natural History Museum, London (e.g., NHMUK 58934). MGL 102127 displays the same moult configuration, with the cephalon displaced dorsally. Four of the eight carcass specimens of *S. ebbestadi* show ordinary exoskeleton morphology for the species, but four carcasses show the wrinkling, flattening, and indistinctiveness of features that is atypical for trilobites (MGL 102128, and 102132-102134). These four wrinkled carcasses are interpreted as ‘soft-shelled’ trilobites (Speyer and Brett 1985), preserved during the post-moult stage in which the new exoskeleton has not fully decompressed and hardened, and during which the thin and soft exoskeleton is vulnerable to deformation.

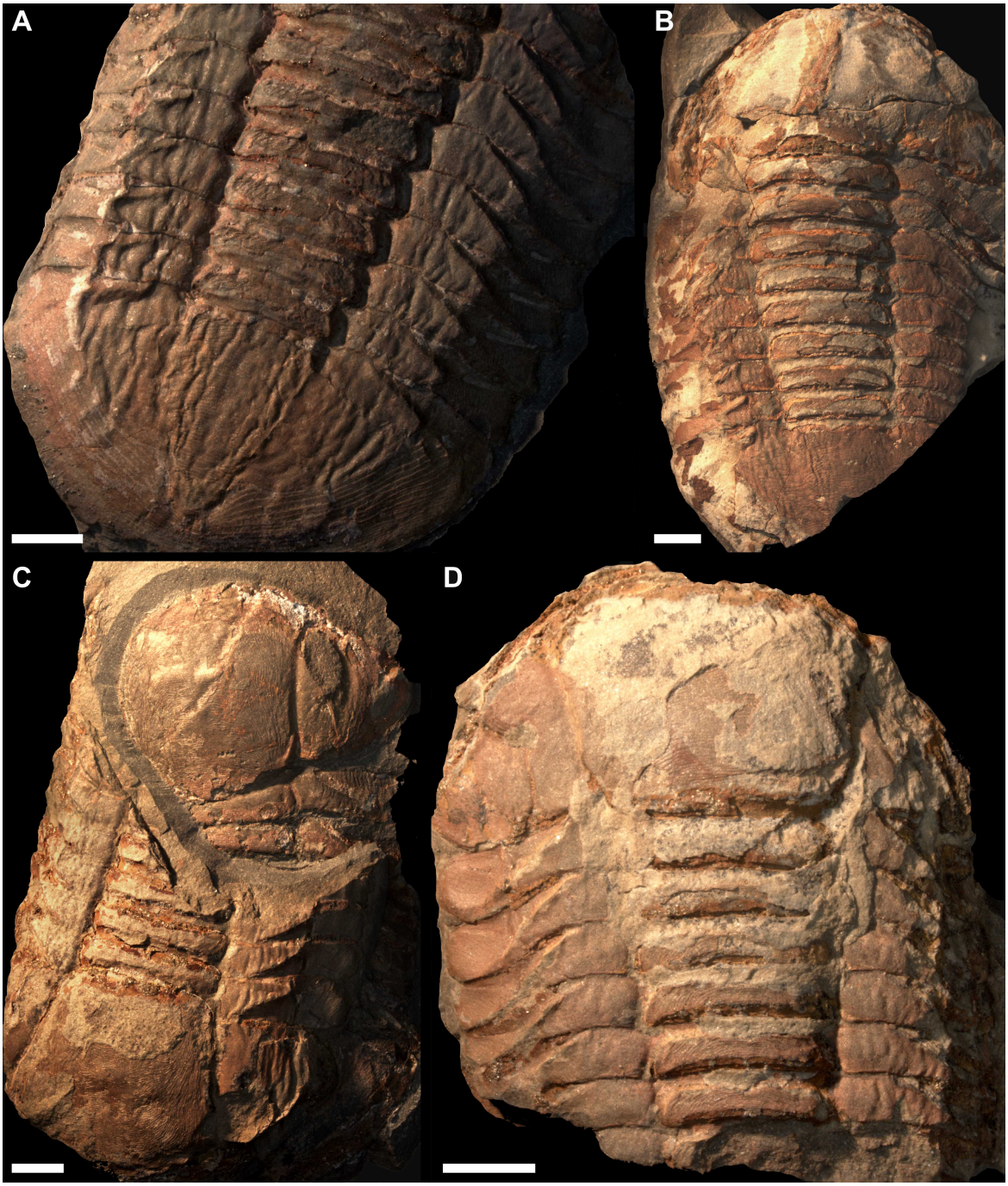


Fig. 2. Wrinkled specimens of *S. ebbestadi* Gutiérrez-Marco, Rábano and García-Bellido 2018, from the early Ordovician of Morocco, photographed under low-angle incident lighting, in order to emphasise the three-dimensional surface texture of their exoskeletons. Specimens are organised in relative order of exoskeleton hardening, from that with the most wrinkled and soft exoskeleton (A) to the least wrinkled (D) before being fully hardened. **A**. MGL 102132. **B**. MGL 102134. **C**. MGL 102128. **D**. MGL 102133. Scale bars all 5 mm.

Cuticular structure.—When viewed in transverse section and measured from outer to inner layer, cuticle thickness and structure in the cephalon (Fig. 3A-D) and the thorax (Fig. 3E-H) show differences between the moulted exuvia (Fig. 3A, E), the wrinkled post-moult individuals (Fig. 3C, D, G, H), and the inter-moult specimen with a fully-hardened exoskeleton (Fig. 3B, F). Although most fossils from the Fezouata Shale Formation have undergone some recrystallistion, replacement by clay minerals, and pyritisation (Vinther et al. 2008; Botting 2016; Gutiérrez-Marco and Martin 2016; Ortega-Hernández et al. 2016; Kouraiss et al. 2018), there is no obvious evidence for varying modes of exoskeleton recrystallisation, with the thin sections showing comparable structures between all of the *S. ebbestadi* specimens. Further, the specimens, having been found in the same lens, were very likely subjected to the same preservation mechanisms. Thus, their exoskeleton thicknesses should be similarly affected by taphonomic aspects like recrystallisation, allowing for an exploration of their relative differences in exoskeleton thickness.

For the sections through the cephala (Fig. 3A-D), parts of the doublure are visible for the inter-moult specimen and the post-moult individuals, (MGL 102130, 102133, and 102134), but not for the moulted exuvia (MGL 102127). The doublure may have been displaced from the cephalon by specimen disturbance after exuviation occurred, or during exuviation as suggested for *Paciphacops* by Rustán et al. (2011). The exoskeleton is much thicker for the inter-moult (830-220 μm) and moulted (760-200 μm) specimens (MGL 102127 and 102130) than for the wrinkled post-moult individuals (MGL 102133 and 102134; 170-50 μm and 240-70 μm, respectively), when measured from the anterior to the posterior of the cephalon (only measured from the dorsal exoskeleton layer and not the doublure; Fig. 4A). The thoracic exoskeletons of the post-moult specimens (MGL 102133 and 102134; 140-69 μm and 100-44 μm respectively) are also noticeably thinner than that of the moulted exuvia (MGL 102127; 340-210 μm) and the inter-moult individual (MGL

102130; 270-150 µm; Fig. 4B). The thorax generally seems to consist of thinner exoskeleton than the cephalon (Fig. 4).

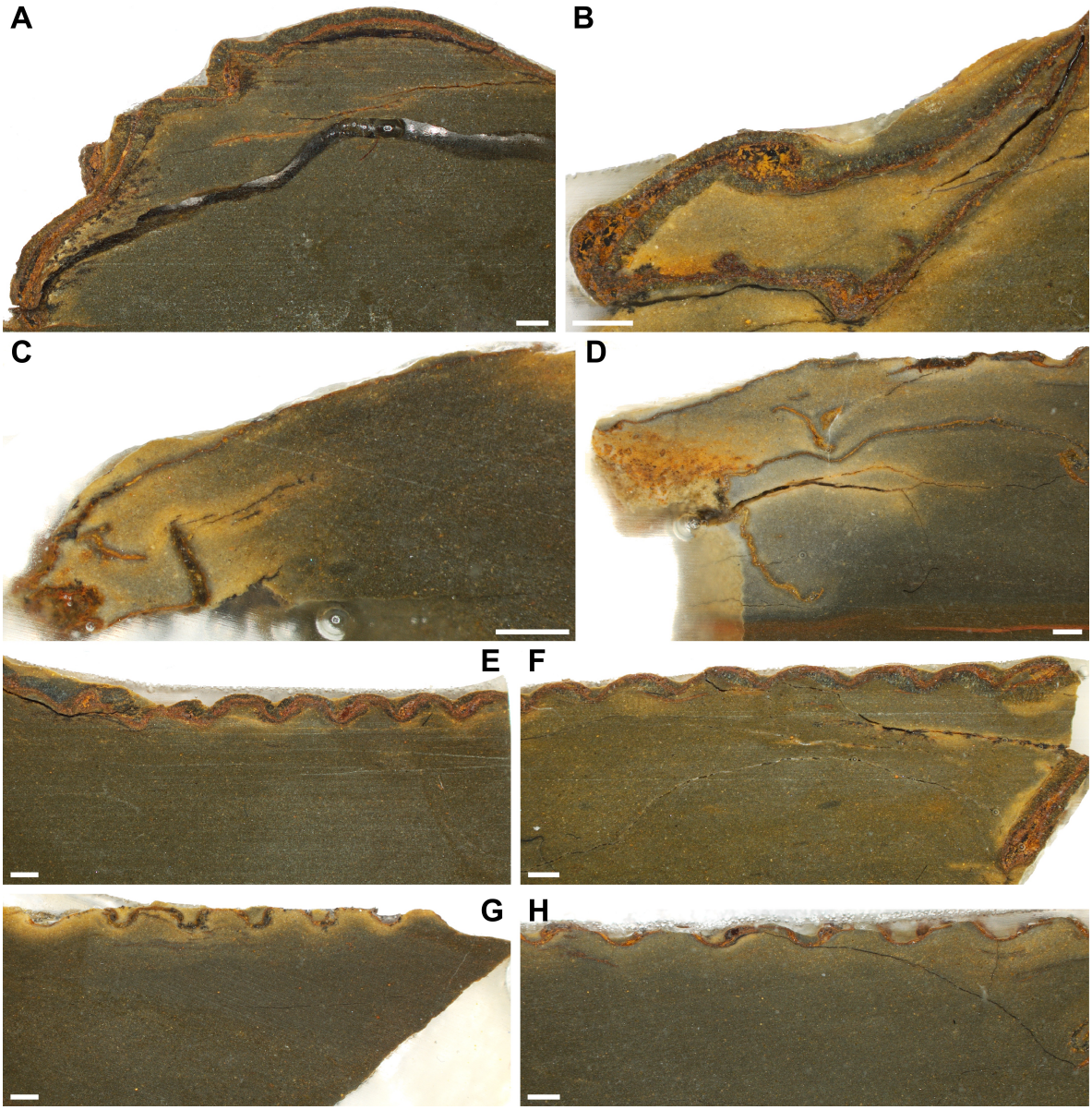


Fig. 3. Thin sections showing the cuticular structure of specimens referred to *Symphysurus ebbestadi* Gutiérrez-Marco, Rábano and García-Bellido 2018, from the early Ordovician of Morocco. **A-D**. show sections through the cephalon, all anterodorsal except for C, which represents a transverse section through the cephalon. **E-H**. show anterodorsal sections through the thorax. **A, E**. the putative moult, MGL 102127. **B, F**. sections through MGL 102130, a fully-hardened individual. **C, G**. MGL 102133, which shows medium levels of wrinkling. **D, H**. the most wrinkled

individual, MGL 102134. All scale bars 1 mm. B and C magnifications are 0.63X, all others 0.32X.

Composition of the exoskeleton also varies. All specimens had a reasonably clear orange-red layer, as well as at least one layer of grey crystalline material. The orange layer likely represents a layer of the exoskeleton that has been pyritised based on the colouration and the typical mode of preservation for Fezouata material (Van Roy et al. 2010, 2015; Gutiérrez-Marco and Martin 2016), and is apparently often seen in exoskeleton thin section (Wilmot 1990). This orange layer may represent the 'prismatic' outer layer of Dalingwater et al. (1999; considered the exocuticle by Mutvei 1981), although this layer is not ubiquitous amongst trilobite taxa (Teigler and Towe 1975). The larger grey crystalline layer is likely the 'principal layer' found in trilobite exoskeletons, which is always present, thickest, usually dark tan to almost black in colour (Wilmot 1990; considered the endocuticle by Mutvei 1981), and is the layer which tends to vary in thickness (Miller and Clarkson 1980). In the cephala of moulted exuvia and the inter-moult specimen (MGL 102127 and 102130), there was a grey crystalline layer either side of the orange layer (Fig. 3A, B), but the inside grey layer was usually lacking in the thinner exoskeletons of the post-moult specimens (MGL 102133 and 102134). The outer grey layer was also much thinner in the cephala of the post-moult specimens (MGL 102133 and 102134), although the orange layer is of reasonably comparable thickness to parts of the inter-moult individual and the moulted exuvia. It is primarily this outer grey layer that varies in thickness between the specimens. However, the level of preservation does not allow for much comparison of the crystalline structures of the two layers, but within the cephala the grey layer appears to consist of larger crystals orientated more dorso-ventrally than in the orange layer (Fig. 3A, B).

The thoracic exoskeleton has a similar structure to that of the cephalon, with a red-orange, presumably pyritised, layer and an outer grey layer (Fig. 3E-H). For the two specimens with thicker cuticles, particularly MGL 102130 (Fig. 3F), the placement of the red layer seems to interchange with the grey layer, likely because the slice is taken at a slightly oblique angle to the thoracic segments. The specimens with thinner cuticles, particularly MGL 102133 (Fig. 3G) seem to have a broader gap at the articulation point between the thoracic segments than the individuals with a thicker exoskeleton, which have segments with less of a gap (Fig. 3E, F). Slices through MGL 102133 and 102134 (Fig. 3C, D, G, H) all show flattening in cross-section of the morphological features, with both the cephala and the peaks of the thoracic segments appearing flatter than for the other specimens (Fig. 3A, B, E, F).

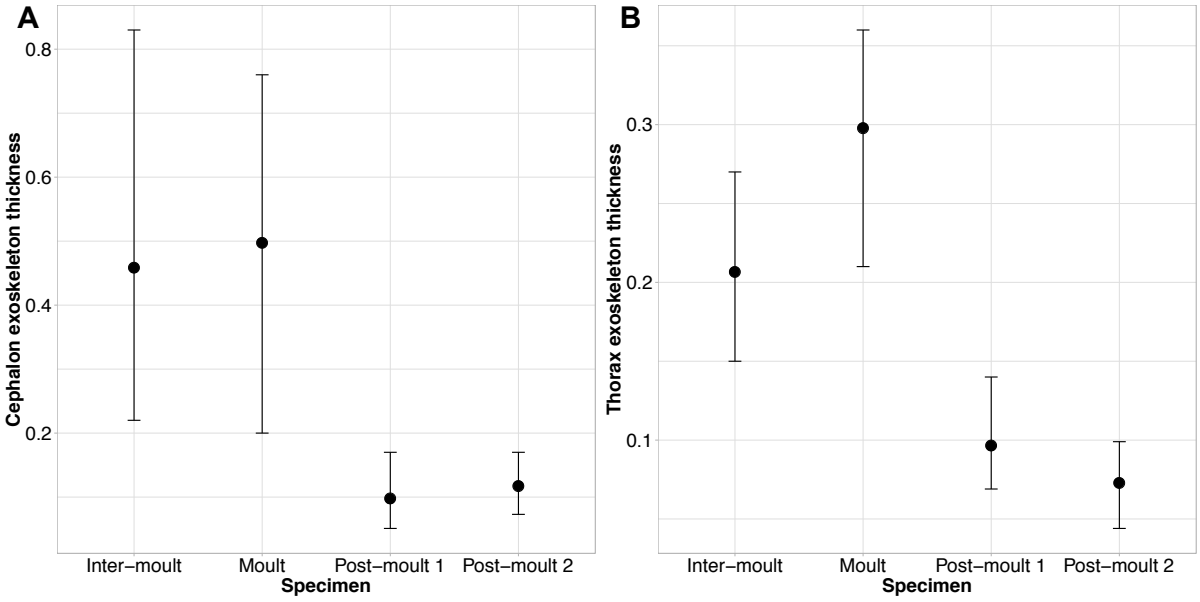


Fig. 4. Graphs showing means (points) and ranges (bars) of exoskeleton thickness for the cephala and thoraces of the four thin sectioned specimens (see x-axis, inter-moult: MGL 102130; post-moult 1: MGL 102133; post-moult 2: MGL 102134; moult: MGL 102127). **A**. exoskeleton thickness measurements (mm) for the cephala of the four specimens. **B**. exoskeleton thickness measurements (mm) for the thoraces of the four specimens.

Discussion

Recognising trilobites at stages of the moulting process.—Comprehensive studies of trilobite moults have been provided by works such as Henningsmoen (1975), Whittington (1980), Daley and Drage (2016), and Drage et al. (2018a). Henningsmoen (1975) and Daley and Drage (2016) in particular outlined a number of criteria with which to identify trilobite moults and accurately distinguish them from carcasses. Carcasses represent individuals preserved during the long inter-moult stage. There have been very few trilobites described as having been preserved in the pre-moult or post-moult phases, with most fossils representing exuviae or inter-moult carcasses. However, in exceptional circumstances, figured trilobite specimens have been described as 'soft-shelled' (Speyer and Brett 1985; or 'paper-shelled', Henningsmoen 1975), resulting from the individuals' preservation during the short-lived post-moult phase. Derived from the few published descriptions of post-moult trilobites, several distinct criteria have been determined that identify soft-shelled trilobites.

1. Flattening of the dorsal exoskeleton, so that it is less convex in lateral view than is usual for that taxon (Whittington 1975; Speyer and Brett 1985).
2. Obvious wrinkling of the cuticle (that is distinct from surface textures such as terrace ridges etc.). These are less visible in figures of the existing described 'soft-shelled' trilobites (e.g. Speyer and Brett 1985: fig. 3D), but are nonetheless implicated as being a key characteristic. Exoskeletal wrinkling is clear in moulting and immediately post-moult extant euarthropod individuals, for example, the horseshoe crab in Figure 5, which shows clear lateral wrinkling of the head shield (also see Fig. 2; Błażejowski et al. 2015: fig. 3).

3. Indistinct features of the dorsal exoskeleton. For example, structures such as the occipital ring, axial rings, or furrows of the rachis are less well defined than is usual for that species (e.g. Speyer and Brett 1985; Speyer 1987: fig. 7E).
4. Associated arthropod material within the same bed, ideally trilobites of the same species, which do not show criteria 1–3. I.e., putative soft-shelled trilobites are found in direct association with specimens that are normally convex, with no wrinkling, and distinct morphological features (Miller and Clarkson 1980). This rules out taphonomic causes of exoskeletal deformation.
5. In addition, thin sectioning may show these putative soft-shelled trilobites have thinner exoskeletons than the 'normal' trilobite carcasses found in association with them (Figs. 3, 4; Miller and Clarkson 1980; Mutvei 1981; Speyer and Brett 1985), possibly showing a reduced 'principal layer'.

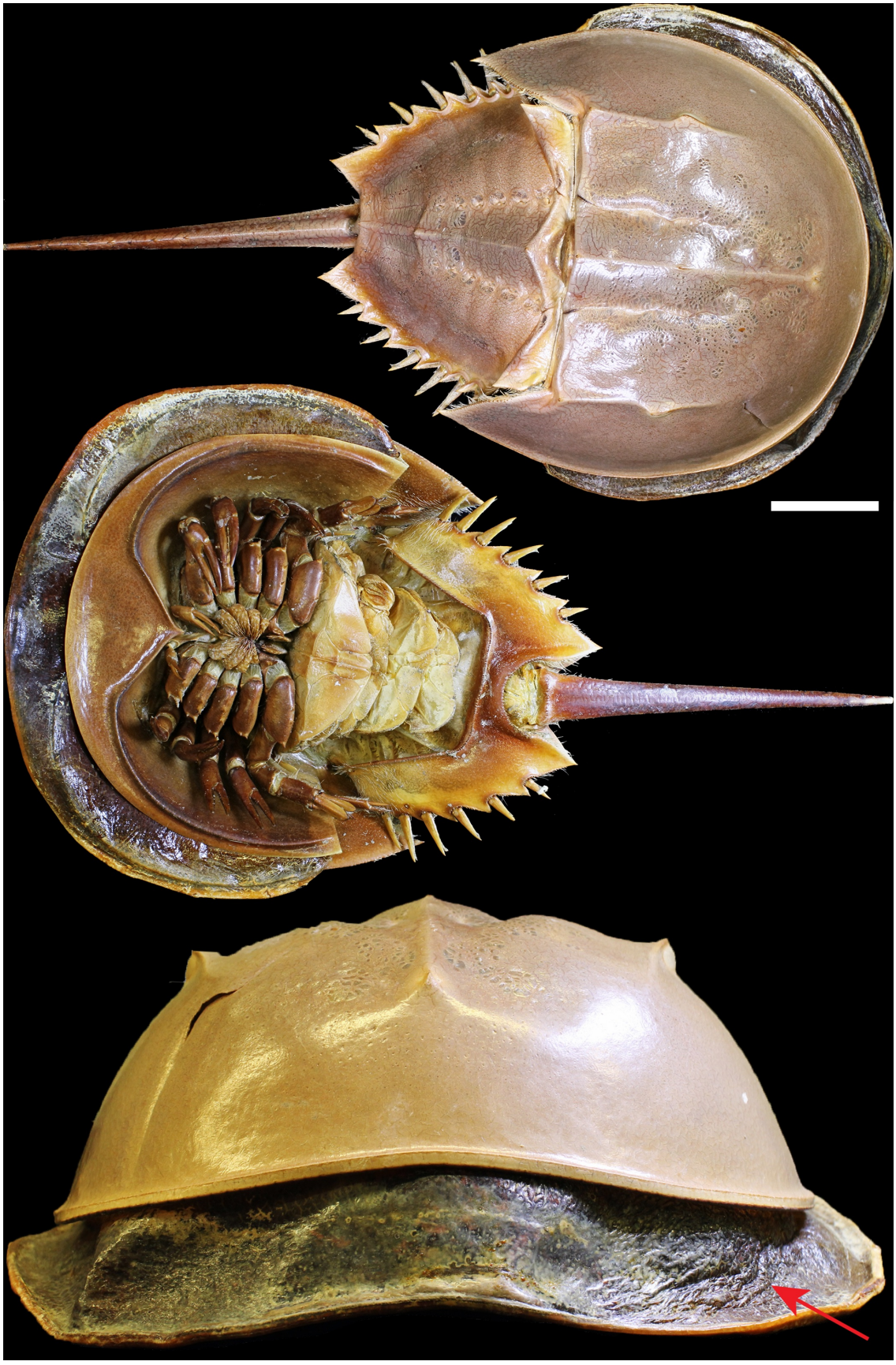


Fig. 5. Mid-moult specimen of *Limulus polyphemus* Linnaeus 1758, recent, USA, in dorsal, ventral, and anterior views (top to bottom; Yale Peabody Museum of Invertebrate Zoology

specimen 55597; photo Russell Bicknell). The moult is fully hardened and shows a robust convex exoskeleton, whereas the carcass partially emerged but trapped within the old exoskeleton shows extensive lateral wrinkling of its new exoskeleton (see arrow). Scale bar = 20 mm.

These features are interpreted as resulting from preservation prior to decompression and expansion of the new larger exoskeleton following moulting from the smaller restrictive one (producing wrinkling), and potentially deformation during burial prior to hardening (and biomineralisation) of the new soft exoskeleton (flattening and indistinct features). In comparison, the moulting horseshoe crab in Figure 5 shows that the larger exoskeleton is clearly soft and flexible in order to emerge from the restrictive old exoskeleton, with the wrinkles disappearing when the new exoskeleton decompresses and hardens. Owing to the short-lived nature of moulting events, very few trilobite individuals have been described as preserved in the immediate post-moult phase. Previously described putative soft-shelled post-moult individuals (Erdtmann 1978; Miller and Clarkson 1980; Speyer 1983, 1987; Speyer and Brett 1985) also generally match the five criteria listed above, with wrinkling and flattening resulting from recent moulting. In all existing descriptions of thin-shelled, wrinkled, and flattened trilobites the individuals co-occur contemporaneously with other individuals of the same species that lack these features (Speyer 1987). It is this contextual information that allows for a well-supported interpretation of a freshly-moulted soft-shell trilobite. Further examples of contemporaneous undeformed arthropods (i.e., with no wrinkling or flattening) allow us to confidently exclude a non-biological explanation for the occurrence of criteria 1–3. In addition to the biological causes discussed above, wrinkling of post-moult specimens may be further accentuated by burial and associated deformation of the particularly soft and thin new exoskeleton, and not from otherwise unrelated tectonic deformation (again, when also found in association with non-wrinkled contemporary specimens). Further,

compaction deformation more broadly in trilobites tends to occur as brittle deformation rather than plastic (Webster and Hughes 1999), and thus a soft-shell exoskeleton would usually be necessary for wrinkling to be related to burial.

The exoskeletons of the two non-wrinkled sectioned specimens (the putative moult, MGL 102127, Figs 1A, 3A, E; and one inter-moult specimen, MGL 102130, Figs 1D, 3B, F) are comparatively thicker than in most studies of trilobite exoskeleton structure. For example, the exoskeleton was between 220–300 μm in *Homalonotus rhinotropis* Angelin 1854 (Dalingwater et al. 1999), and up to 500 μm in *Phacops rana milleri* (Miller and Clarkson 1980). Fortey and Wilmot (1991) found a huge spectrum in trilobite exoskeleton thicknesses, with a 40-fold difference depending on palaeoenvironment as nearshore taxa had notably thick exoskeletons and those offshore being much thinner. This correlation between environment and exoskeletal thickness is also paralleled in extant arthropods, with the desert scorpion *Hadrurus arizonensis* Ewing 1928 having a cuticle that is about twice as thick as that of *Euscorpius italicus* Herbst 1800, which inhabits less arid regions (Pavan 1958; Filshie and Hadley 1979; Hadley and Filshie 1979; Hjelle 1990). The thinnest trilobite exoskeleton found by Fortey and Wilmot (1991) was of the cranidium of *Svalbardites* Fortey 1974 at 8 μm, although the thickest was 350 μm, with those <60 μm being considered “thin”, and those >130 μm “thick”. In contrast, the two thicker specimens studied here (Fig. 3A, B) gave measures upwards of 700–800 μm in the cephalon, therefore seeming comparably thicker. These are the putative moult and inter-moult specimens, which did not particularly differ in the ranges of their exoskeleton thicknesses (200–760 μm and 150–830 μm, respectively; Fig. 4). Mutvei (1981) also found that moults and carcasses did not differ and could not be distinguished at the exoskeletal level (see also Brandt 1993). This is supported by other studies finding no evidence for exoskeleton reabsorption pre-moulting, as is found in most crustaceans (Miller and Clarkson 1980;

Feldmann and Tshudy 1987; Speyer 1987). However, the two sectioned wrinkled specimens described here (MGL 102133 and 102134) not only have much thinner exoskeletons than MGL 102127 and 102130, but are comparably thinner than described for other trilobites. These were between 50–170 μm (Figs. 1G, 3C, G, 4) and 44–240 μm (Figs. 1H, 3D, H, 4), which puts their minima (and particularly their thoracic exoskeletal widths) within the "thin" category of Fortey and Wilmot (1991).

Studies of the relative effects of diagenesis on thin section ultrastructure of trilobite cuticles are scarce (Whittington et al. 1997), and this hinders comparisons between the specimens of *S. ebbestadi* figured here with other trilobite species invariably preserved under different taphonomic regimes (e.g., Fortey and Wilmot 1991; Dalingwater et al. 1999). For example, the middle orange-coloured layer of the exoskeleton (likely to be pyritic as for other Fezouata biota; Gutiérrez-Marco and Martin 2016) and the grey layers (potentially formed by replacement of calcite with clay minerals, as for the calcitic plates of *Plumulites bengtsoni*; Vinther et al. 2008), may have undergone associated thickness changes during pyritisation and clay-replacement which, while occurring to all specimens figured here and so not affecting comparisons between them, may affect comparisons with the other trilobite species discussed above (that have not undergone these preservational modes). Similarly, the outer cuticle layer of the *S. ebbestadi* specimens may be thinner than expected owing to the ubiquitous weathering found in the Fezouata Shale Formation (Gutiérrez-Marco and Martin 2016). Further analysis of the effects of different preservational regimes on the thickness and ultrastructure of trilobite exoskeletons would be invaluable to future studies of exoskeleton moulting and taphonomy.

A complete trilobite moulting sequence from the Fezouata Shale Formation.—The nine specimens of *S. ebbestadi* represent the first full exoskeleton moulting sequence for a

trilobite species. Arthropods spend most of their time in the inter-moult stage, between the relatively rapid moulting events, and thus individuals are preserved during this time in the vast majority of cases (with the exception of moulted exoskeletons, which are found in abundance for trilobites; Daley and Drage 2016). The *S. ebbestadi* specimens include one putative moult (Fig. 1A; similar to the configuration described by Rustán et al. 2011), four carcasses of standard inter-moult interval individuals (Fig. 1C-E; typical convex, effaced, and with no wrinkling), and four carcasses preserved during the post-moult soft-shell stage (Fig. 1B, F-H). The last of these are identifiable based on their previously described characteristics, in particular the strong lateral wrinkling of tagma (Fig. 2), and the notably thinner exoskeletons of two of these specimens (Figs 3, 4) compared to the putative moult and an inter-moult specimen. Thus, the nine contemporaneous specimens of *S. ebbestadi* collected from the same lens, contain all identifiable stages of the moulting process: a moulted exoskeleton, individuals in the long inter-moult stage, and individuals preserved immediately post-moulting. Pre-moult stage preserved carcasses will be almost impossible to distinguish, as this stage is also particularly short-lived, and characterised by the detachment of the old exoskeleton from the new cuticle, which would be extremely difficult to identify even in thin section of exceptionally preserved individuals.

Of the putative post-moult specimens, the degree of lateral wrinkling and flattening varies. MGL 102132 has extremely prevalent wrinkling (Fig. 2A), MGL 102134 (Fig. 2B) is slightly more convex with less obvious wrinkling, and MGL 102128 and 102133 (Fig. 2C, D respectively) show lesser degrees of wrinkling still. Further, the thoracic cuticle thickness of MGL 102134 (Fig. 2B) is slightly thinner than that of the less wrinkled MGL 102133 (Fig. 2D), with their ranges being 44–100 μm for MGL 102134 (Figs 2B, 3D, H) and 69–140 μm for MGL 102133 (Figs 2B, 3C, G). Their cephalic thicknesses, however, were similar (50–170 μm for MGL 102133 and 70–240 μm for MGL 102134), but both

were notably thinner than for the non-post-moult individuals (Fig. 3). The differences in the degree of the post-moult criteria outlined above suggest that these specimens varied in how susceptible their exoskeletons were to compression during preservation, and how much they had undergone post-moult inflation of the new exoskeleton. This therefore suggests that the four post-moult specimens were preserved at different points of exoskeleton hardening after different amounts of time had passed following exuviation (also considered in the case of specimens of *Phacops rana milleri* described by Miller and Clarkson 1980).

The collection of individuals from all stages of the moulting process from the same lens of the Fezouata Shale Formation suggests a mass moulting event that may have been preserved during-the-act. Specifically, the presence of variously hardened post-moult trilobites together suggests that the mass moulting event was ongoing. Mass moulting is a behaviour commonly seen in modern arthropods (e.g., Tarling and Cuzin-Roudy 2003; Haug et al. 2013), with its origins seemingly very early in the fossil record of Arthropoda (Daley and Drage 2016) and clear mass moult assemblages having been described for various trilobite groups (Speyer and Brett 1985; Karim and Westrop 2002; Paterson et al. 2007; Ebbestad et al. 2013). This appears to be a protective behaviour, and has been suggested to accompany mating events in some arthropods (Speyer and Brett 1985; Braddy 2001; Vrazo and Braddy 2011, although see Tetlie et al. 2008). However, all so-far described trilobite mass moults have been preserved following cessation of the event. This means that typically only exuviae are found, as the recently-moulted individuals have moved away from the preserved accumulation. An exception is the carcass congregations of *Phacops rana rana* described by Speyer and Brett (1985), which are interpreted as having been preserved after a mass-moulting event, except in this case the freshly moulted individuals are suggested to have remained in place following moulting for reproduction.

The Fezouata *S. ebbestadi* specimens, in contrast, originated from the same lens, with post-moult and pre-moult individuals co-occurring with at least one moulted exoskeleton. This represents the first occurrence of a probable trilobite mass moulting event preserved in-the-act, rather than post-event when soft-shelled individuals would not be present. Understanding the process and occurrence of mass moulting events in the fossil record is key to our knowledge of euarthropod behaviour. Mass moulting has distinct advantages in modern groups, and may therefore indicate similar ecological causes in the fossil record, for example an increased prevalence of predation within the locality.

Moulting sequence in extinct euarthropods is analogous to extant groups.—The description of the *S. ebbestadi* specimens as representing a post-moult sequence of hardening preserved during a mass moulting event adds to a growing body of evidence that the process of exoskeleton moulting has been strongly conserved throughout euarthropod evolutionary history, not just associated behaviours like mass moulting (e.g., Haug et al. 2013; Daley and Drage 2016). Although thin section work on trilobite exoskeletons has suggested a number of differences in structure to that of modern arthropods (which also vary themselves; see Krishnan 1953; Dalingwater 1973; Dalingwater et al. 1999), the results presented here support consistency in the biochemical and mechanical process of moulting throughout euarthropod evolution. Modern arthropods follow a well-known sequence of pre-moult preparation, ecdysis, and post-moult inflation and hardening before returning to the long inter-moult stage (Skinner 1962; Ewer 2005). The specimens of *S. ebbestadi* found contemporaneously represent each of these stages (although the pre-moult stage may be impossible to identify due to preservation). This strongly suggests, more than previous studies in which only singular post-moult trilobites were figured (Erdtmann 1978; Miller and Clarkson 1980; Mutvei 1981; Speyer and Brett 1985), that trilobites followed

the same sequence of moulting described in modern arthropods, as hypothesised by earlier workers (Henningsmoen 1975). The existence of a comparable moulting sequence between Early Ordovician trilobites and modern arthropods argues that the process of moulting is strongly conserved within euarthropods and has changed little over more than 400 million years of evolution. This is reasonable given the unparalleled importance, and riskiness, of exoskeleton moulting in the life histories of all euarthropods (Dalingwater et al. 1999; Daley and Drage 2016). Identification of specimens displaying the full moulting sequence in stem euarthropods would allow for further validation of the moulting process being conserved throughout Euarthropoda. The results do, however, support earlier evidence that unlike modern crustaceans, which also have thick calcitic exoskeletons, reabsorption of the exoskeletal material had not yet evolved in trilobites. To date, no evidence has supported reabsorption prior to moulting in trilobites, with trilobite moults and inter-moult carcasses having comparable exoskeleton thicknesses (Miller and Clarkson 1980; Mutvei 1981; Feldmann and Tshudy 1987; Speyer 1987; Brandt 1993), while this is not the case in extant arthropods that show exoskeleton reabsorption (Ewer 2005; Nijhout 2013). The reasons for this are unclear, as the recurrent production of calcitic exoskeletons would have been extremely energetically expensive, and thus reabsorption is critically important in modern crustaceans (Skinner 1962; Luquet and Marin 2004). However, evolutionary uncertainties such as this highlight the need for further analysis of both primary and diagenetic cuticular ultrastructures in thin sections of early euarthropod exoskeletons (Whittington et al. 1997).

Conclusions

Nine figured trilobite specimens collected from a lens of late Tremadocian age in the Fezouata Shale Formation, Morocco, are assigned to *Symphysurus ebbestadi* based on a recent description by Gutiérrez-Marco et al. (2018). These are considered to reflect all described stages of the exoskeleton moulting process. One specimen is a putative moulted exoskeleton with the cephalon displaced dorsally and no visible doublure. Several others show morphology typical of the species, with a notably convex and effaced dorsal exoskeleton, and are therefore considered to represent the inter-moult stage, as for most trilobite carcasses (Henningsmoen 1975; Daley and Drage 2016). Four specimens represent the short-lived post-moult stage, indicated by the presence of characteristics associated in the literature with deformation following burial and compression of the new soft exoskeletons (wrinkling, flattening, less distinct features). Thin sections of two post-moult specimens, the putative moult, and an inter-moult specimen show notable differences in exoskeleton thickness, with the former two being much thinner in both the cephalon and thorax than the latter two. The discovery of these specimens within the same lens suggests that they likely represent preservation of an in-progress mass moulting event. The specimens of *S. ebbestadi* also provide more detail on the morphological characteristics of post-moult trilobites, giving an important example for the future identification of post-moult extinct arthropods and the study of the fossil record of moulting, and suggest the sequence of moulting has been conserved in the evolutionary history of euarthropods.

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Chapter 8: Discussion, future work and conclusions

This final chapter comprises: a short summary drawing together the work within this thesis; discussion of some outstanding points relating to trilobite moulting; thoughts on a number of future directions into which research on trilobite (and more broadly, euarthropod) moulting behaviour might move; and some final conclusions.

I aim to avoid repeating the discussion sections within manuscript chapters of this thesis, which variously explore identification of euarthropod moults, descriptions of trilobite moults and associated difficulties, sampling, general moulting trends, etc. Instead, I focus on other considerations important to the study of trilobite, and euarthropod, moulting, such as preservation, interpretation, and data collection of fossil moults, trilobite phylogeny and taxonomy, and other relevant trilobite behaviours.

Discussion

Summary

This thesis provides a detailed analysis of moulting in trilobites, involving both quantitative studies looking at broad-scale evolutionary trends in moulting behaviour variability, and in-depth case studies to emphasise the importance of this characteristic for understanding species-level morphology, ecology, and development. Chapters 1 and 2 highlight ways to systematically distinguish moults and carcasses, and the importance of rigorously doing so. Chapters 3 and 4 document variability in trilobite moulting behaviour, and demonstrate that this is a broad-scale feature across Trilobita and throughout their Palaeozoic evolutionary history. These chapters also present statistical analyses that suggest taxonomic, temporal, and morphological signals reside in the fossil record of trilobite moulting. Moults are abundant in the trilobite fossil record, and understanding moulting behaviours is particularly useful in studies of Konservat-Lagerstätten where the presence of moult configurations can lead to extensive variation in trilobite preservation (Chapter 5). Dramatic changes in trilobite moulting behaviour can also be linked to significant morphological developments during ontogeny, which would have greatly affected their life histories (Chapter 6). But despite their extensive flexibility in moulting behaviours compared to other euarthropods, trilobites appear to show the same general processes and stages of moulting that we can examine in extant groups, suggesting that this has been a consistent and ubiquitous feature of euarthropod life histories since their earliest evolution (Chapters 1 and 7; Appendix 3). Trilobite moulting flexibility is in opposition to the "canalised ecdysial habit" of other euarthropods (Brandt, 2002, p. 399), where the moulting method is thought to be reasonably consistent for all members of a Genus, Family, or higher taxonomic classification (e.g. the "Open Moult Position" of decapod crustaceans, Glaessner, 1969; Bishop, 1972, 1986). In these extant arthropods, taxa with

similar bodyplans moult using the same method, whereas trilobite taxa sharing similar overall morphologies may have moulted using many different methods. This inconsistency in moulting behaviour within trilobites may have affected the evolutionary success and survivorship of the clade (Brandt 2002), although this is far from straightforward to determine. Results from Chapters 3 and 4 suggest that a quantitative approach to studying large-scale evolutionary trends of trilobite moulting is feasible and warrants further investigation.

Preservation and interpretation of moult fossils

Both the broad-scale analyses (Chapters 3 and 4) and detailed case studies (Chapters 5-7) in this thesis unequivocally demonstrate the intraspecific variability in trilobite moulting behaviour. Interspecific variability has been described previously in the literature (Henningsmoen, 1975; Busch and Schwartz, 1985; McNamara, 1986; Chatterton and Ludvigsen, 1998; Brandt, 2002; Budil and Bruthansová, 2005; Paterson et al., 2007; Tortello and Clarkson, 2008; Daley and Drage, 2016), although to a much lesser extent than revealed in Chapters 3 and 4, but intraspecific variability has received less attention. Konservat-Lagerstätten like the Emu Bay Shale provide rare opportunities to fully explore the limits of intraspecific variability in moulting behaviour by examining the different configurations preserved in the fossil record (Chapter 5; McNamara, 1986; Drage et al., 2018a). At localities without exceptional preservation (i.e., subjected to taphonomic effects like disarticulation and transportation by water currents), it is unlikely that the full range of moulting configurations produced by a single trilobite species would be preserved, because preservation of an identifiable moult is a much rarer occurrence. However, while we can extract data on the limits of intraspecific behavioural variability from Konservat-Lagerstätten, we are unable to look at long-term evolutionary trends without also gathering

data from global sites with 'normal' preservation. We must therefore balance the collection of data from both these types of locality because together they provide a more complete view of moulting evolutionary history.

It is also therefore of prime importance, when attempting to make inferences about behaviour, ecology, and morphology, that we interpret the moult fossil record correctly. This is often difficult in palaeontology, where authors frequently disagree on what fossils actually show, including for trilobite moults (see Brandt, 2002, and Hughes, 2003). One of the main aims of this thesis was to demonstrate that trilobite moults can be identified using a more rigorous framework than previously applied. This was presented in Chapter 1, and discussed in depth using the context of a poorly-supported putative 'post-moult trilobite' (Błażejowski et al., 2015) in Chapter 2. The remaining chapters, in particular those utilising the large database of trilobite moulting (Chapters 3 and 4), show that it is possible to record trilobite moulting behaviour from rigorous searches of museum and field collections across the world. However, it is crucial that taphonomic aspects are not ignored in this process, as has been repeatedly discussed throughout this thesis. For example, for trilobite specimens to be considered moults, they need to be incomplete (otherwise the most parsimonious explanation is that these are carcasses, although see the *Harpes* Configuration in Chapter 1) but with most of the disarticulated sclerites in close association. Fossils made up of a few disarticulated sclerites (e.g. the librigenae, ventral structures, or isolated thoracic segments; Drage, accepted; Chapter 3, fig. 2) cannot be confidently considered moults because it is impossible to determine whether their disarticulation resulted from behaviour of the individual or through taphonomic processes. Thus, criteria for rigorous identification of moults, which leave moulting as the most parsimonious explanation for the creation of specific exoskeletal configurations, are crucial to the study of the fossil record of moulting. Only specimens that conform to the

identification criteria presented and discussed within Chapters 1 and 2 were considered moults, and included within studies forming the rest of this thesis. Quantitative studies, with the exception of the small dataset analysed by Brandt (2002), had not previously been attempted, but it is clear that, with the application of thorough identification criteria, moults can be readily identified in pre-existing collections in high enough abundance for statistical analysis.

Data collection

The quantitative data presented in Chapters 3 and 4 represent the most extensive dataset ever generated for trilobite moulting, and had high enough sample sizes to allow for statistically sound quantitative analyses. Further, to produce all of the data presented in, and accompanying, this thesis I have looked at many more trilobite specimens than could be included. This is because, as discussed above, only specimens that were adequately preserved and possible to reasonably interpret as moults were included (see Chapter 3; Drage accepted, fig. 2). Thus, this thesis actually represents knowledge gained from observations and measurements of thousands of trilobites (*c.* 5000-10000 specimens) collected from many countries and at least three continents. It is impossible to gather data on moulting behaviour for some trilobite groups because they have only been described from fragmentary material, and moults and carcasses can only be confidently determined if most of the exoskeleton sclerites of an individual are found in reasonably close association. In addition, trilobites are extremely diverse, with the total number of described trilobite genera being well over 5000 (Jell and Adrain, 2002; Lerosey-Aubril and Feist, 2012), and it would take a vast amount of work to record moulting characteristics for all of these species. Despite this, exploration of the dataset I created largely from museum collections demonstrates that it is a representative sample of Trilobita, with good

geographical and taxonomic coverage (Chapter 3; Drage accepted, figs 3, 8, 15). Extensive museum collections made available to researchers are therefore an invaluable tool for the creation of broad-scale palaeontological databases.

Morphology and movement

The broad-scale trends in trilobite moulting behaviour presented in this thesis support the growing body of work that suggests the facial sutures, a feature unique to trilobites and their close relatives (together the Artiopoda; Hou and Bergström, 1997), evolved specifically as an adaptation to aid moulting, and that this was ancestral within Trilobita (Stubblefield, 1959; Daley and Drage, 2016; Hou et al., 2017; Du et al., 2018; Fig. 1). The use of the facial sutures in moulting was the most common characteristic by far observed in moult specimens for all trilobite orders (excepting Phacopida) and throughout most of the Palaeozoic (Drage, accepted; Chapter 3). It is not known why facial sutures are unique to Artiopoda (Fig. 1), or why these structures did not evolve in Euarthropoda more widely (Ax, 1987; Paterson and Edgecombe, 2006). However, certain groups do show convergent sutures analogous to the marginal suture described in some specialised trilobites that had lost the facial sutures (e.g. Harpetida, Trinucleida; Stubblefield, 1959; Fortey and Owens, 1999a). For example, a marginal suture is used for anterior exuviation in Xiphosurida (Sekiguchi et al., 1988) and Eurypterida (Tetlie et al., 2008), and a transverse suture in Chilopoda (Edgecombe, 2006). Some of the earliest trilobite groups completely lack the facial sutures (e.g. the Olenelloidea), which has led other researchers to argue that the facial sutures are not ancestral within Trilobita (Jell, 2003). However, the presence of facial sutures in non-trilobite artiopodans (see Hou et al., 2017; Du et al., 2018; Fig. 1), and the extensive variability in facial suture shape and its lack of phylogenetic signal within trilobites (see Stubblefield, 1936; Whittington et al.,

1997) suggest their use lies specifically for moulting (Stubblefield, 1936; Henningsmoen, 1975), and that the olenelloids (like later taxa) presumably secondarily lost their facial sutures. Alternatively, facial sutures as seen in later trilobite groups may not have evolved at this point in the early Cambrian, and olenelloids may display the ancestral condition of no facial sutures, with these developing (potentially several times) later in the Cambrian (Jell, 2003).



Figure 1: Facial sutures in Artiopoda. A: cephalon of *Dalmanitina proaeva* Emmrich 1839 from the Lower Ordovician Vinice Formation, Prague Basin (National Museum Prague), with facial suture on each side running around the visual surface; B and D: the artiopodan *Acanthomeridion serratum* Hou et al. 2017 from the early Cambrian Chengjiang Lagerstätte of China, showing a notch in the head shield with a suture (arrow, FS) and detached free cheek (arrow, FC; images taken from Hou et al., 2017, fig. 2); C and E: the artiopodan *Zhiwenia coronata* Du et al. 2018 from the early Cambrian Xiaoshiba Lagerstätte of China, also showing a notch (arrow, no) with suture (images taken from Du et al., 2018, fig. 2). Scale bars = 5 mm in A and C, 2 mm in B and D, 1 mm in E. Abbreviations: ant, antennae; exp, exopod; ey, eye; FC, free cheek; FS, facial suture; hs, head shield; hy, hypostome; no, notch; T*n*, trunk tergites.

Aspects of the research presented in this thesis also suggest that certain body movements, and their evolution through the Palaeozoic, may have been important to trilobite moulting behaviour. Dorsal-ventral movements, the same type of movement involved in full enrolment of the body, may have been key for moulting. Enrolment in trilobites is where the animal curls around itself to leave only the protective dorsal exoskeleton exposed (Esteve et al., 2017). In modern groups this is a mechanism to protect against predation (e.g. in extant isopods; Haug and Haug, 2014), which presumably was also the case for trilobites, with their thickly reinforced calcitic dorsal exoskeleton repelling attack (Bergström, 1973). The ability to at least partially enrol persisted in trilobites from the earliest groups present in the fossil record (e.g. the Olenelloidea, order Redlichiida; Bergström, 1973; Esteve et al., 2011, 2013; Ortega-Hernández et al., 2013; Fig. 2A) until the Permian (Lerosey-Aubril and Angiolini, 2009). Enrolment in early trilobites was originally considered impossible (Harrington, 1959; Bergström, 1973), but was merely imperfect (non-encapsulating), with the cephalon and pygidium not entirely locking together to create an impenetrable structure (in large part because they lack the

pleural fulcrum and cephala and pygidia that have coaptative structures to link together; Whittington et al., 1997; Ortega-Hernández et al., 2013; Gon, 2018). Perfect encapsulating enrolment, with the entire body enrolled and the vulnerable ventral side and cephalon enclosed (Fig. 2B), only evolved later in the history of trilobites (Ortega-Hernández et al., 2013).

Work on the moulting configurations produced by these earliest trilobites in the early-middle Cambrian suggested that the same movements involved in imperfect enrolment were important for creating exuvial gapes (McNamara and Rudkin, 1984; McNamara, 1986). This may be responsible for the reasonably high proportion of cranidial and cephalic disarticulation found by Drage et al. (2018a; Chapter 5) in *Estaingia bilobata* Pocock 1964 and *Redlichia takooensis* Lu 1950. Partial enrolment (or 'dorsal flexure' within Drage et al., 2018a) would presumably have put greater pressure on other articulations, in particular the cephalothoracic joint, while the anterior of the cephalon was pushing into the sediment (see Fig. 2C; McNamara and Rudkin, 1984; McNamara, 1986, figs 2-5; Whittington, 1990, figs 2-6). Enrolment behaviours, at least early in the evolution of trilobites, may therefore have performed a dual function as a defensive adaptation also facilitating moulting. This flexibility of movement in trilobites, resulting from a large thoracic region with moveable thoracic segments (owing to the articulating half-rings and fulcral processes and sockets between them that produce the structure of articulation; Whittington et al., 1997), would likely have contributed to their moult configuration variability.

Similarly, lateral movement may have been involved in producing some of the Cambrian moulting configurations figured and discussed within this thesis (see Chapters 1 and 5). For trilobite groups later in the Palaeozoic and ones occupying certain environments (e.g. deeper waters, like *Elrathia* Walcott 1924; Gaines and Droser, 2003),

lateral movement may have been impossible because the broader pleural fields of the thoracic segments, as compared to the axis, presumably mechanically prevented side to side movement of the exoskeleton. However, a number of fossils suggest that early groups (e.g. Redlichiida, Ptychopariida) had the capability for lateral movement (McNamara and Rudkin, 1984; Gon, 2018). This provides a putative explanation for how some of the moulting configurations of the early Cambrian *E. bilobata* and *R. takooensis* figured in Drage et al. (2018a; Chapter 5) may have formed, which have laterally displaced sclerites within a very low energy environment. Lateral movement may therefore have been involved in producing the higher levels of intraspecific moulting variability attributed to the Cambrian Redlichiida in Drage (accepted; Chapter 3). Greater ranges of movement, incorporating both enrolment and lateral movement in early trilobites, coupled with an exoskeleton composed of many articulated structures, would likely have caused a greater variety of disarticulations during moulting than in some other euarthropod groups. With on-going work into understanding mechanisms of trilobite enrolment (e.g. Esteve et al., 2017) it may be possible to further explore a potential link between these locomotive behaviours and moulting variability.

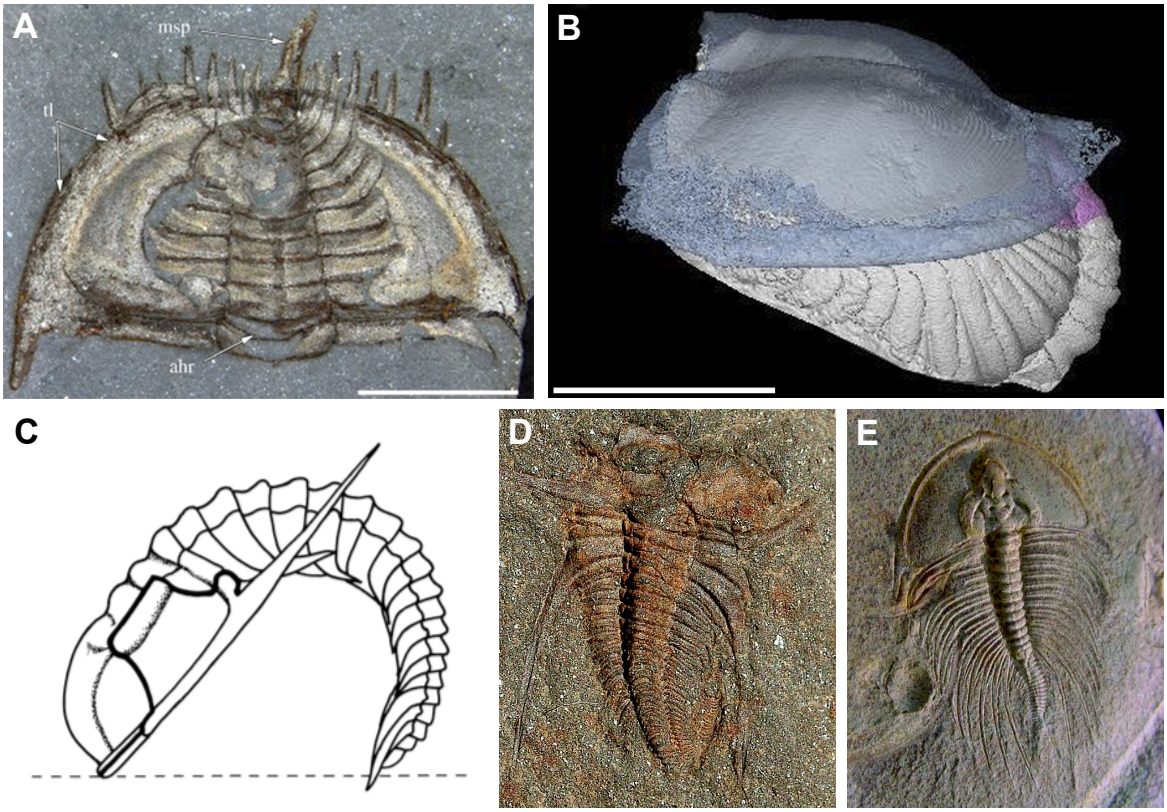


Figure 2: Enrolment and lateral movement behaviours in trilobites, which may have been involved in moulting. A: imperfect enrolment in *Mummaspis ?muralensis* Fritz 1992 from the early Cambrian Mural Formation, Canada, (image taken from Ortega-Hernández et al., 2013, fig. 1); B: CT scan of perfectly enrolled *Bailiaspis? glabrata* Angelin 1854 specimen from the Miaolingian Valtorres Formation, Spain (image taken from Esteve et al., 2017, fig. 1); C: partially enrolled drawing of *Paradoxides sp.* Brongniart 1822 with the tips of the cephalon and pygidium pushing into the sediment in order to facilitate moulting of the individual (drawing taken from Whittington, 1990, fig. 3); D and E: specimens of *Balcoracania dailyi* Pocock 1970 (early Cambrian of South Australia) and *Olenellus fowleri* Palmer 1998 (early Cambrian, Pioche Shale, USA) respectively displaying the capability for lateral movement in these early groups (photos taken from Gon, 2018, 'trilobite thorax'). Scale bars = 5 mm in A and B, unknown for D and E but estimated body lengths of adult representatives are usually ~1–2 cm for D and ~3–4 cm for E. Abbreviations: ahr, articulating half ring; tl, terrace lines; msp, macrospine.

Future work

The study of exoskeleton moulting is in its relative infancy for most of fossil Euarthropoda. Trilobites are the most abundant and varied sample group to look at how moulting behaviours are related to other evolutionary aspects, such as morphology, development, and ecology. However, the well-preserved fossil record of Euarthropoda (at least those that biomineralised, Conway Morris, 1986; Zhao et al., 2014; Holmes et al., 2018) provides extensive opportunity to study moulting. In addition, there should be more moults than carcasses in the euarthropod fossil record because each individual moults many times in its lifetime but only produces one carcass. Therefore there is great potential to further explore moulting in trilobites but also more broadly across the fossil record of Euarthropoda.

Trilobite taxonomy and phylogeny

Higher-level trilobite taxonomy, i.e. the definitions of the current 8-10 orders (depending on perspective; Whittington et al., 1997; Jell and Adrain, 2002), is variously poorly defined, ambiguous, and based on descriptive cladistic assessments. Phylogenetic work for Trilobita to date focuses on family-level or lower relationships, and so, while for some groups we have reasonably robust ideas of their relationships (e.g. Aulacopleuroidea, Encrinuridae, etc.), we know very little about how these groups relate to each other (Fortey, 1990). Many workers have noted the need for more broad-scope phylogenetic analyses (Fortey, 1990; see review by Liebermann and Karim, 2010), but a summary of current knowledge of putative broad-scale evolutionary relationships is displayed in Figure 3 (from Gon, 2018). We need a robust phylogenetic tree in order to confidently make interpretations about many aspects of trilobite evolutionary history, including morphology, development, behaviour, ecology, palaeobiogeography, impact on Palaeozoic ecosystems,

and extinction. However, this is complicated by the fact that a supermatrix phylogenetic analysis of such a massive and ecologically varied group would require collection of a vast amount of data, and cryptogenesis is rife within the group (i.e. the difficulty to align Cambrian species with post-Cambrian relatives; Stubblefield, 1959; Edgecombe, 1992; Lieberman and Karim, 2010).

Research is underway to produce a comprehensive phylogeny for Cambrian and Early Ordovician trilobites, which also looks at evolutionary rates, diversification, and morphological character change (pers. comm. J. Paterson and M. Lee). When such a broad-scale hypothesis of trilobite evolutionary relationships is published, it will provide significant opportunities for the exploration of moulting behaviour in the group. The data presented in Chapters 3 and 4 can be updated to reflect changes in phylogenetic relationships and taxonomy, and analysed on a smaller-scale to look at variability in moulting behaviour during particular geological ages or groups. Further, moulting behaviours (e.g. the mode of moulting, or the most common moulting characteristic used; Chapters 1 and 3; Drage accepted) may be coded as traditional phylogenetic characters and mapped across the tree, which might reveal interesting patterns of behavioural and morphological change with trilobite phylogenetic relationships and across the Palaeozoic.

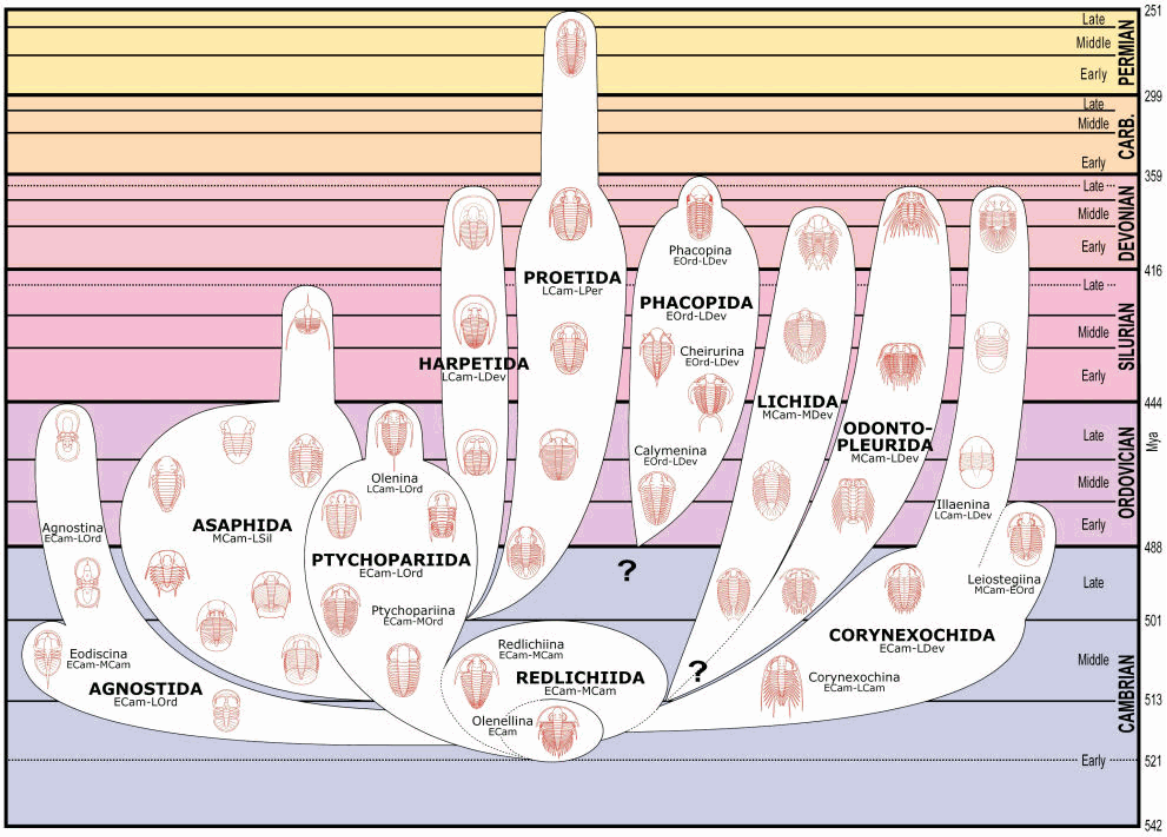


Figure 3: Representation of our current state of knowledge for broad-scale order-level trilobite phylogenetic relationships, and their evolutionary history, including general timings of origination and extinction. Created by Gon (2018).

Development and expansion of analyses

The datasets generated for this thesis, and the results presented, are intended to be available for further development and application. They provide a solid framework for future research to build on and analyse when quantitatively exploring trilobite evolutionary patterns. As it is, a number of other aspects could be tested, including more work on the diversity patterns presented in Chapter 3 (figs 8, 14), or detailed explorations of species/generic-level moulting flexibility in trilobites belonging to certain groups or from particular localities. For example, looking at moulting variability in the earliest trilobites (redlichiids) and their origins and diversification, or the Phacopida with their behavioural and morphological specialisations (fusion of the librigenae and facial sutures) that

occurred in one major group but not in others (Chapter 6). It also provides broad-scale quantitative morphological data for the first time (body proportions and thoracic segment counts for multiple specimens belonging to 355 species), which could be used to investigate other evolutionary trends such as body shape and size change across the Palaeozoic. The data could also be used to look at the links between behaviour and other morphological features in trilobites, such as spinosity (which varies in trilobites, presumably as a predation defence, and is lost entirely for a burrowing mode of life; Owen, 1985; Fortey, 1986; Pates and Bicknell, in press). As suggested above, in the future the data may also be used for looking at mapping behaviours onto phylogenetic trees, and looking for links with other broad-scale evolutionary trends like palaeobiogeography, data for which is also available in the presented dataset.

Ecology and general mode of life, like morphology, are likely inherently linked to moulting behaviour and moulting variability in trilobites. Trilobites were a hugely diverse group, which rapidly expanded to global dominance in the Cambrian, and partitioned niches in order to further diversify. They filled seemingly every generalised feeding mode possible, including predators of various sizes, scavengers, grazers, and filter-feeders, and every mode of locomotion, including benthic walkers, burrowers, pelagic drifters, and swimmers (Fortey, 1990; Whittington et al., 1997; Fortey and Owens, 1999b). It is likely that the morphologies and behaviours to allow for this also affected other aspects of life history, like moulting behaviour. For example, the morphology and behaviour necessitated for a burrowing lifestyle were presumably very different compared to a swimming lifestyle. Chapter 4 of this thesis demonstrates a statistically significant link between moulting and morphological variability, with environment presumably also important because it influences morphology. Using the dataset presented in Chapters 3 and 4 I have carried out a preliminary analysis that suggests there are some links between moulting

behaviour and feeding niche and habitat. Generalised ecology and feeding mode information was collected from Fossilworks (Fossilworks 2018; Kiessling et al., 2018) for all sampled species (355; see Chapter 4, Drage in review; Appendix 5), and these grouped according to whether they more commonly displayed cephalic or cranidial disarticulation, or facial suture or ventral suture opening during moulting. The proportion of species in each moulting category that occupied each of the ecological or feeding niches was calculated and compared using Chi^2 analyses. Analyses expected the proportion of species displaying each ecology/feeding mode to be comparable between the different moulting characteristics.

The majority of species in the dataset were carnivorous (50-80% for each moulting characteristic; Fig. 4). Only the species usually moulting through disarticulation of the cephalon showed all five feeding modes (Fig. 4). The trilobite species that were carnivores, detritivores, or suspension feeders did not significantly differ in their moulting characteristics. The remainder were mostly deposit feeders, with most of these moulting through opening the ventral sutures (45%) and the fewest through disarticulating the cephalon (11%; Fig. 4). Chi^2 analyses were significant for the number of species that were deposit feeders and microcarnivores (Chi^2 10.88, $p=0.013$ and 8.76, $p=0.033$ respectively). For the deposit feeders this is likely because fewer of these moulted via disarticulation of the cephalon than expected, and more utilised the facial and ventral sutures than expected. The sample size is unfortunately too low (n=4) to place any emphasis on the significant result for microcarnivore species.

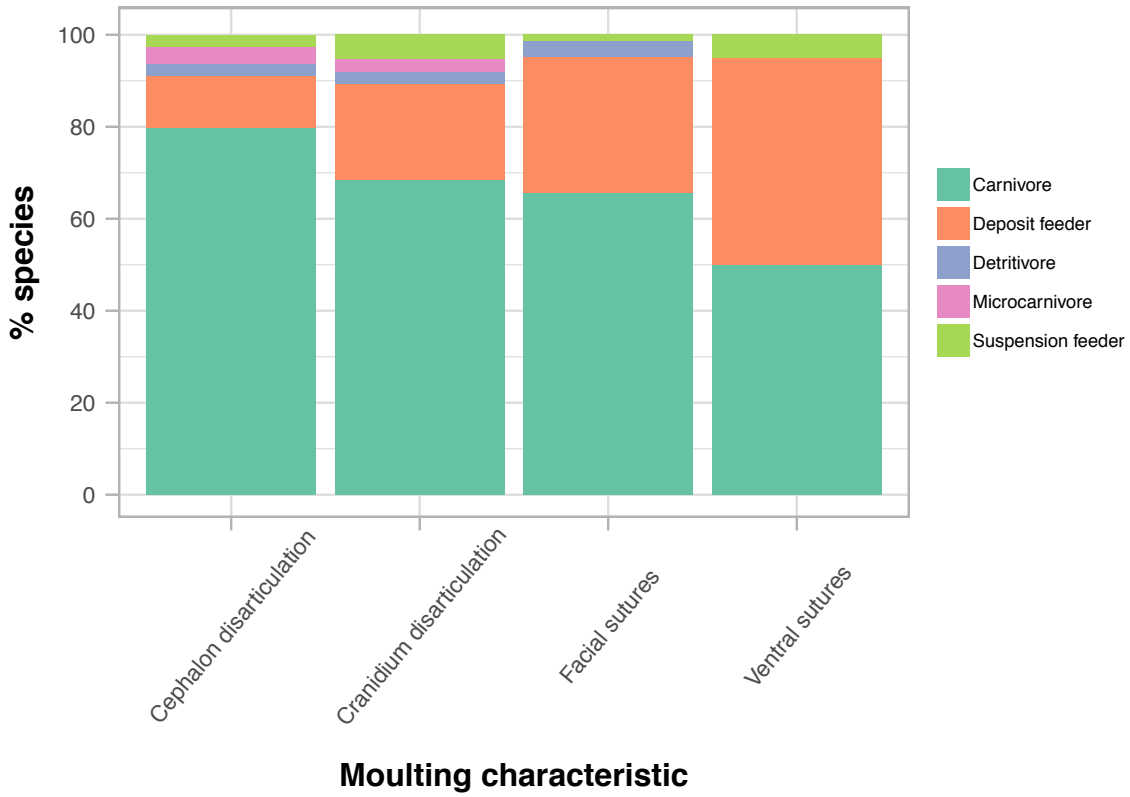


Figure 4: Proportion of species for each of the four moulting characteristics that displayed the five feeding modes (carnivore; deposit feeder; detritivore; microcarnivore; suspension feeder). Total n= 364 species. Ecological data from Kiessling et al. (2018).

The majority of species occupied epifaunal habitats (70-80% of species for each moulting characteristic), and 20-30% of species were nektobenthic, with only nektobenthic and epifaunal species showing cranidium disarticulation or opening of the facial or ventral sutures (Fig. 5). Only species showing cephalic disarticulation during moulting occupied all of the habitat types, although infaunal and nektonic species were rare (Fig. 5). Only infaunal trilobites showed significant variability in their moulting characteristics through Chi^2 analysis (10.94, p=0.012). This was because all recorded infaunal species disarticulated the entire cephalon for moulting, however the sample size was too low (n=3) for this to be a reliable result. Overall, therefore, trilobite species showing each of the four moulting characteristics did not much vary in their habitats, and varied little in their

feeding modes. However, this work is in need of expansion into a full-scale, robust study. In particular, too few non-carnivorous, and infaunal and nektonic taxa are included. This may be because the data at Fossilworks (Fossilworks, 2018; Kiessling et al., 2018) does not provide adequate detail and so most taxa are lumped into broad groups like 'carnivore', or because the original descriptive literature tends to be conservative in interpreting modes of life. More data could likely be gathered through looking at the associated lithologies within the descriptive literature for trilobite taxa to determine the paleoenvironment. This work needs to be expanded in both data collection and analysis in order to robustly test the hypothesis that mode of life was associated with trilobite moulting behaviour.

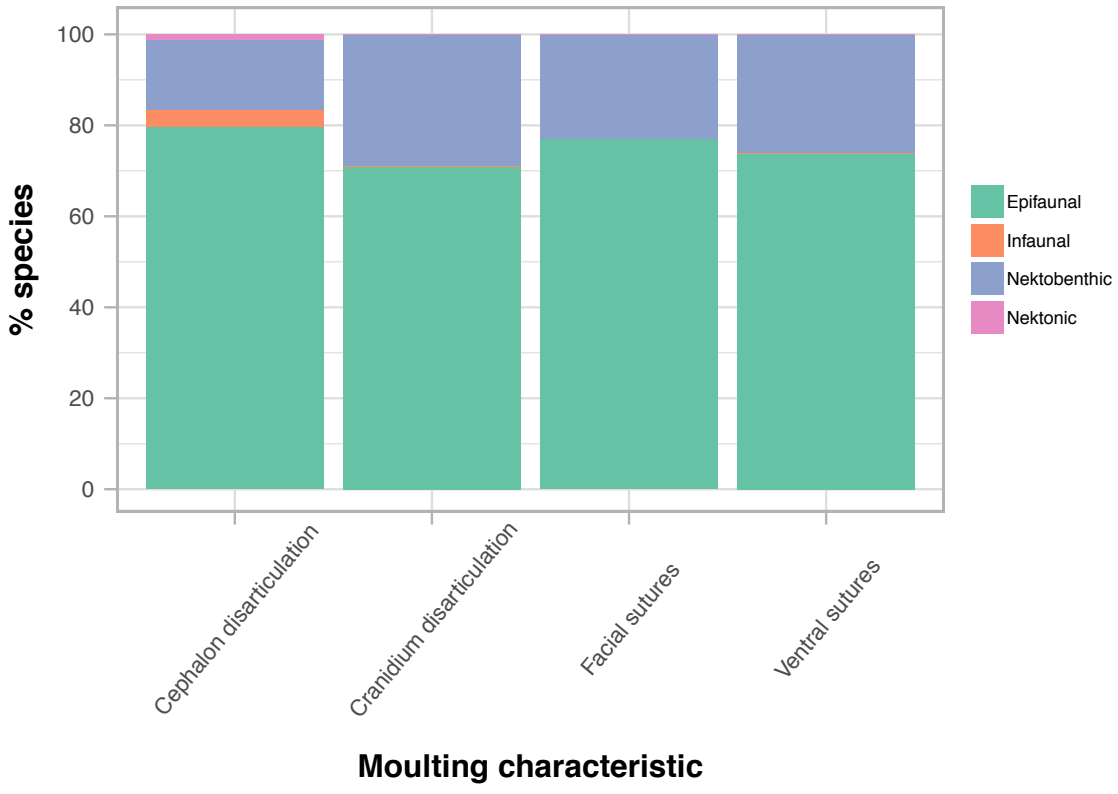


Figure 5: Proportion of species for each of the four moulting characteristics that displayed the four habitat modes (epifaunal; infaunal; nektobenthic; nektonic). Total n=367 species. Ecological data from Kiessling et al. (2018).

Further, geological longevity, i.e. the length of time a trilobite group is present in the fossil record, would also be worth studying in order to explore a link with moulting behavioural variability. Brandt (2002) suggested that trilobite moulting flexibility may have been inherently involved in their decline and ultimate demise in the late Palaeozoic, due to the steady diversification and domination of other euarthropod groups with more specialised moulting behaviours. She posited that this would mean these other groups, e.g. crustaceans, would moult faster and with a lower risk of failed moulting events, and might therefore have outcompeted the less well-adapted trilobites. However, the opposite is also plausible, in which behavioural flexibility facilitated the extreme diversity, abundance, and global dominance that trilobites maintained through much of the Palaeozoic (e.g. Hughes, 2003). For example, trilobites may have been able to use alternative methods of moulting if the main method failed (e.g. if the facial sutures did not open; see Paterson et al., 2007), or used different methods in certain environments (e.g. risky environments necessitating a faster method of moulting versus safer environments). Further, high levels of polymorphism and flexibility have been suggested to be important to the early establishment and radiation of Cambrian trilobites (Foote, 1991; Fortey and Owens, 1999; Webster, 2007). The data presented within Chapters 3 and 4 of this thesis could be adapted to test between these hypotheses. One method of doing so would be to gather data on geological longevity for the sampled taxa, and test for an association between longevity and the number of moulting characteristics each taxon or group shows. A higher average number of moulting characteristics for long-lived taxa would indicate that flexibility in moulting might have been adaptive, and a lower average for these would suggest it might have been maladaptive. The latter scenario may agree with some results presented in Drage (accepted, Chapter 3), which found trilobite taxa later in the Palaeozoic were less variable in moulting behaviour, although this might also result from a paucity of well-

preserved material from the Carboniferous and Permian. Further exploration might help to answer outstanding questions regarding the decline and extinction of this once extremely diverse and ecosystem-dominating group.

Finally, the likelihood of producing some of the trilobite moult configurations discussed within this thesis could be directly tested by the application of novel techniques. The production of certain configurations observed in early Cambrian taxa, e.g. Somersault and McNamara's Configurations (see Drage et al., 2018a, fig. 1; Chapter 5), were described by McNamara (1986) as relating to changes in water pressure currents, and by Drage et al. (2018a) as involving dorsal movements of the moulting individual. Given the marine environment of trilobites, these ideas could be directly tested by the application of Computational Fluid Dynamics (CFD). CFD reconstructs fluid flow variables (e.g. velocity and pressure) around modelled objects (see Rahman, 2017), and therefore can be used to test the likelihood of hypotheses such as those given above for the production of certain moult configurations. Using novel methods like CFD, we can quantitatively analyse the likelihood that extinct groups showed hypothesised behaviours. CFD is extremely versatile and can be used to test a vast array of hypotheses about the life histories, morphologies, development, and ecologies of extinct marine euarthropods, and to greatly further work on our understanding of trilobites as living animals.

Other euarthropods

This thesis provides the first detailed and quantitative exploration of moulting behaviour in an entirely extinct euarthropod group, owing to the abundance of well preserved and described trilobite moults in the fossil record. Isolated, qualitative studies of fossil Euarthropoda have described moulting configurations and behaviours of a number of non-trilobite species. For example, the decapod crustacean and eurypterid fossil records of

moulting are reasonably well known (see the full literature review in Daley and Drage, 2016; Appendix 1). However, expansion of work looking at moulting and exoskeleton structure in other extinct euarthropods would be useful. In particular, investigations of moulting in stem euarthropods, as most of our data is for crown groups (Daley and Drage, 2016), might demonstrate whether extensive intraspecific flexibility in moulting is unique to trilobites, or evolved earlier in Euarthropoda. Identification of consistent modes of moulting and relevant behaviours in earlier-evolving stem euarthropods may also suggest longer-term and across-group constraints that might have impacted the broad-scale evolution of Euarthropoda as a whole. For example, Yang et al. (2019; Appendix 3; Fig. 6) describes moulting behaviour from an early Cambrian stem-group euarthropod (*Alacaris mirabilis* Yang et al. 2018, Fuxianhuiida), which seems to utilise a similar mode of moulting to decapod crustaceans (specifically lobsters, in either the Open Moult Position or Overturned Carapace Position). This may indicate that modes like these and the morphology to facilitate them are ancestral within Euarthropoda, and were important behaviours early on in euarthropod evolution. Further, additional work on the moult fossil records of non-trilobite euarthropods may demonstrate comparable moulting behaviours to trilobites, and also comparable variability in the moulting configurations produced. For example, further analyses of decapod crustacean moults might indicate additional behaviours in fossil taxa that were used in moulting and produced exoskeletal disarticulations and configurations in addition to the two described to date (see Daley and Drage, 2016). Some groups, like the isopods, which have reasonably similar bodyplans to trilobites, have seen very little work on moulting in both their extant groups and fossil records (e.g. Wieder and Feldmann, 1989; Feldmann 2009; Hyžny et al., 2013). Moulting is a recurrent event intrinsically linked to the life histories of all euarthropods, and as such warrants further study in the fossil record and early evolution of non-trilobite groups.

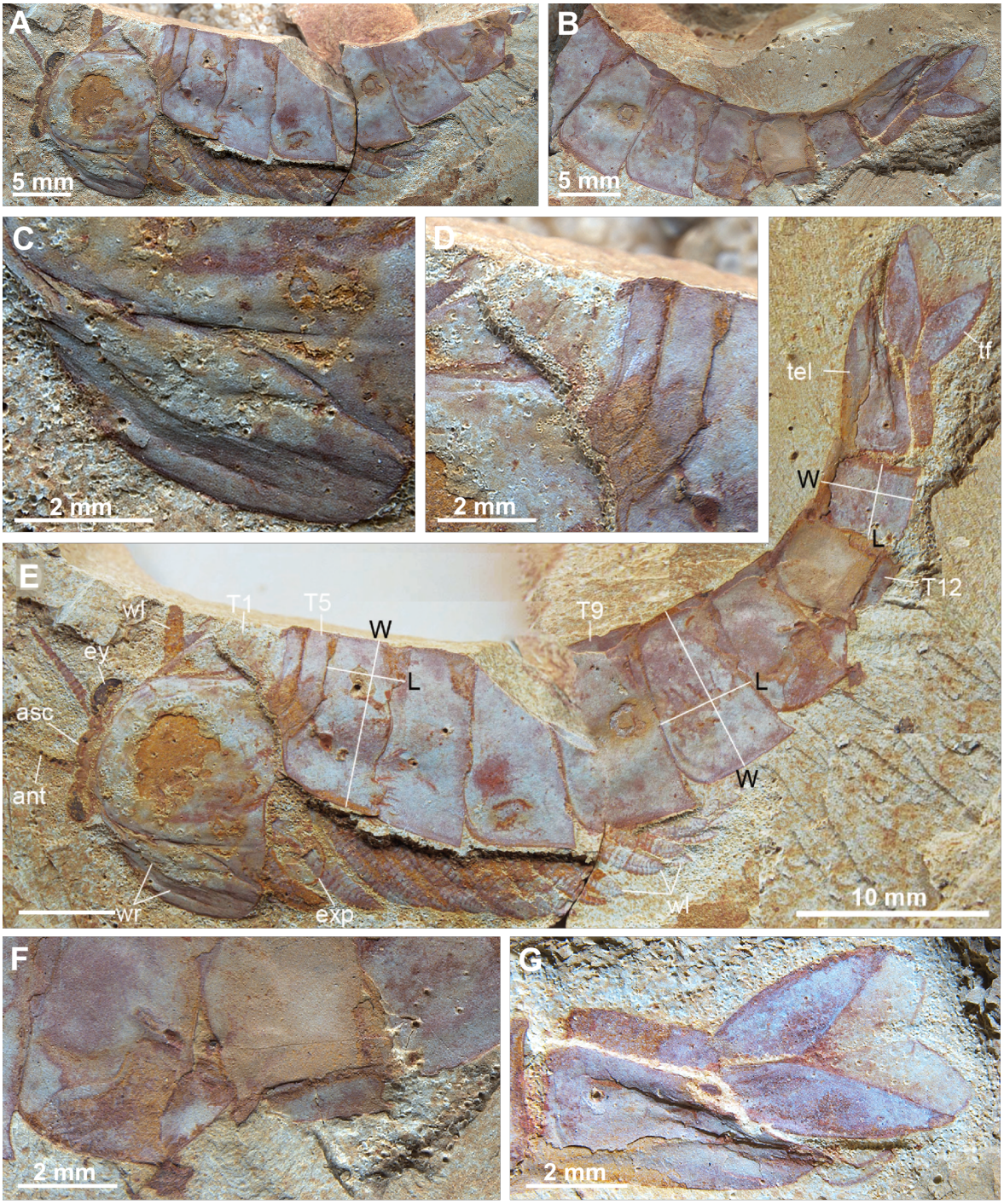


Figure 6: Preserved in the act of moulting specimen of the fuxianhuiid *Alacaris mirabilis*, from the Miaolingian Xiaoshiba Lagerstätte, South China. E represents a composite photograph of A and B. The specimen comprises a complete tergite series with telson on an upper sediment layer, and a wrinkled head shield attached to well-preserved appendages and a duplicated telson with tail flukes on a lower sediment level. Reproduced figure 1 of Yang et al. (2019; Appendix 3). Abbreviations:

ant, antennae; asc, anterior sclerite; exp, exopod; ey, eye; hs, head shield; tel, telson; tf, tail fluke; T*n*, trunk tergites; wl, walking legs; wr, wrinkles.

Conclusions

Based on the work presented in this thesis, trilobite moulting behaviours were notably variable, both inter- and intraspecifically, despite the reasonably conserved bodyplan of trilobites. We are able to reconstruct these behaviours through careful observation and analysis of the fossil record. Trilobites provide our best record of moulting in early euarthropods owing to their vast abundance and diversity. However, it is plausible that with further investigation, other fossil euarthropod groups might also show variability in the behaviours involved in moulting, and the exoskeletal configurations they produce. Trilobite moulting variability in large part presumably resulted from their extensive variability in morphology and life history across the group. They built on a similar bodyplan to diversify into a wide range of ecological niches, and also showed extensive flexibility in developmental patterning along with high levels of morphological polymorphism. This means that trilobites moulted using a number of different methods, including facial and/or ventral sutures, a marginal suture, or the cephalothoracic joint, as well as producing a range of configurations of the resulting disarticulated sclerites. Some other groups, e.g. eurypterids and decapod crustaceans, appear to show a certain amount of variation in the creation of exuvial gapes (Tetlie et al., 2008; Daley and Drage, 2016), although much less attention has been paid to the diversity of fossil moults these might produce and to the other behaviours involved in their moulting processes. The exceptional fossil record of trilobites provides excellent opportunity to gather an abundance of moult data, both globally and across the Palaeozoic (Chapters 3 and 4), and in high numbers from singular species present in localities with exceptional preservation (Chapters 5-7). This

record also allows the exploration of trilobite variability in other important areas, such as development flexibility and morphological polymorphism. Having these data available means we can begin to unpick the factors affecting trilobite modes of life and their evolutionary history.

Exceptional zones of preservation, such as the Cambrian Emu Bay Shale, Ordovician well-preserved strata of the Prague Basin, and Lower Ordovician Fezouata Shale featured in this thesis demonstrate the extremely high level of detail we can gather about trilobite moulting behaviours and related aspects from individual specimens (movement, development, and exoskeleton structure respectively for Chapters 5-7). However, this thesis also aims to show that the creation of large and robust datasets with which we can test broad-scale evolutionary hypotheses is feasible for extinct euarthropod groups. This is achievable by focusing more on methods and data collection, such as those presented within this thesis, and in particular making use of museum collections. The abundance of data presented in Chapters 3 and 4 was gathered solely from specimens in museum collections (and a small number from literature descriptions); these collections are extremely valuable in order to quickly build up large bodies of data to quantitatively test evolutionary hypotheses. Further, in-depth field studies at localities with exceptional preservation could provide vast stores of information, enabling us to test evolutionary and ecological hypotheses at the species-level. This also allows us to explore the coverage of museum collections and look at faunal changes through short periods of geological time.

Ultimately, reconstructing behaviours of extinct animal groups from the fossil record is a complex process, and is inherently linked to a vast number of variables. This means that these behaviours are difficult to understand, and to determine with confidence what variables they affect and what affects them. Despite this difficulty, looking at behaviours like moulting is highly worthwhile. This thesis emphasises the need to look at

extinct fossils as living animals, with a number of complex behaviours and variable modes of life, like in modern biology. To work towards building a more complete picture of the evolutionary history of trilobites and Euarthropoda, it is imperative that we not only look at morphology, taxonomy, and phylogeny, but also at reconstructing behaviours (moulting, feeding, etc.), development, and modes of life.

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Appendices

Appendix 1: Full published copy of Daley and Drage (2016), the edited version of which (trilobite-only sections) forms Chapter 1.
Daley, A.C. and Drage, H.B. 2016. The fossil record of ecdysis, and trends in the moulting behaviour of trilobites. *Arthropod Structure & Development* 45, 71–96.

Appendix 2: Additional published manuscript, in which we compile fossil record and molecular evidence for the timings of the origin of Euarthropoda, including a discussion of the earliest trilobite fossils.
Daley, A.C., Antcliffe, J.B., Drage, H.B. and Pates, S. 2018. Early fossil record of Euarthropoda and the Cambrian Explosion. *PNAS* 115, 5323–5331.

Appendix 3: Additional published manuscript, in which we describe the second ever fossil euarthropod preserved in the act of moulting (*Alacaris mirabilis*, from the Xiaoshiba Formation of China).
Yang, J., Ortega-Hernández, J., Drage, H.B., Du, K.-S. and Zhang, X.-G. Ecdysis in a stem-group euarthropod from the early Cambrian of China. *Scientific Reports* 5709, 1–9.

Appendix 4: Supplementary data files submitted with the manuscript constituting Chapter 3 (Drage, in press, *Palaeontologia Electronica*).

1. Dataset sampling summary
2. Raw counts for specimen sampling
3. Diversity and sampling data
4. Statistical analyses
5. Raw data for trilobite moulting behaviours – Excel file not included due to length; available if requested.

Appendix 5: Supplementary data files submitted with the manuscript constituting Chapter 4 (Drage, in review, *Acta Palaeontogica Polonica*).

1. Thoracic segment number and moulting behaviour statistical analyses
2. Body proportion and moulting behaviour statistical analyses

3. Raw data for trilobite moulting behaviours and body proportion measurements – Excel file not included due to length; available if requested.

Appendix 6: Supplementary data files submitted with the manuscript constituting Chapter 6 (Drage et al., 2018, *Paleobiology*)

1. Raw data for *Dalmanitina* specimen ontogenetic stages and body proportion measurements
2. Additional photos of specimens illustrating the primary and secondary fusion events

Appendix 7: Supplementary data files submitted with the manuscript constituting Chapter 7 (Drage et al., 2019, *Acta Palaeontologica Polonica*).

1. Body proportion measurements of described specimens
2. Measurements of exoskeleton thicknesses from thin sections of specimens

Appendix 1:

Daley, A.C. and Drage, H.B. 2016. The fossil record of ecdysis, and trends in the moulting behaviour of trilobites. *Arthropod Structure & Development* 45, 71–96.

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The fossil record of ecdysis, and trends in the moulting behaviour of trilobites

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ABSTRACT

Ecdysis, the process of moulting an exoskeleton, is one of the key characters uniting arthropods, nematodes and a number of smaller phyla into Ecdysozoa. The arthropod fossil record, particularly trilobites, eurypterids and decapod crustaceans, yields information on moulting, although the current focus is predominantly descriptive and lacks a broader evolutionary perspective. We here review literature on the fossil record of ecdysis, synthesising research on the behaviour, evolutionary trends, and phylogenetic significance of moulting throughout the Phanerozoic. Approaches vary widely between taxonomic groups, but an overall theme uniting these works suggests that identifying moults in the palaeontological record must take into account the morphology, taphonomy and depositional environment of fossils. We also quantitatively analyse trends in trilobite ecdysis based on a newly generated database of published incidences of moulting behaviour. This preliminary work reveals significant taxonomic and temporal signal in the trilobite moulting fossil record, with free cheek moulting being prevalent across all Orders and throughout the Phanerozoic, and peaks of cephalic moulting in Phacopida during the Ordovician and rostral plate moulting in Redlichiida during the Cambrian. This study and a review of the literature suggest that it is feasible to extract large-scale evolutionary information from the fossil record of moulting.

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1. Introduction

Ecdysis, the process of moulting an exoskeleton, is one of the key characters uniting arthropods, nematodes and a number of smaller phyla in the aptly named group Ecdysozoa (Fig. 1) (Telford et al., 2008). Ecdysozoans include both the most abundant and the most diverse animal groups on the planet today, representing over 80% of total biodiversity. They have been key components of ecosystems since the early Cambrian when trilobites and various soft-bodied Burgess Shale-type ecdysozoans including arthropods, lobopodians, priapulids and palaeoscolecids dominated marine animal communities (Budd and Jensen, 2000), and have remained important throughout the whole of the Phanerozoic. Understanding the processes and behaviour of ecdysis in the fossil record is important because this life history strategy would have imparted constraints on morphology throughout the evolution of modern animal groups. In addition, it allows us to access information on growth and development in deep time, which can help resolve the affinity of enigmatic taxa early in the evolution of animals. This is particularly important because many of these Cambrian fossils are early diverging stem lineage taxa that are critically important for breaking up long branches, helping us to resolve current ambiguous relationships in modern taxa (e.g. Legg et al., 2012). Additionally, studying ecdysis in the fossil record can provide insight into understanding ecology as one of the only examples of direct evidence of behaviour preserved in the fossil record.

For both extant and extinct taxa, moulting is best known for the arthropods. The arthropod external cuticle is a chitinous exoskeleton that provides protection from mechanical injury and dessication, while also giving strong structural support for muscle attachment and locomotion (Moussian, 2013). This rigid exoskeleton is moulted during normal growth and development, at which time regeneration and repair of damage may also occur. In extant taxa, moulting of the exoskeleton is a relatively rapid process, with actual exuviation and subsequent hardening of the new

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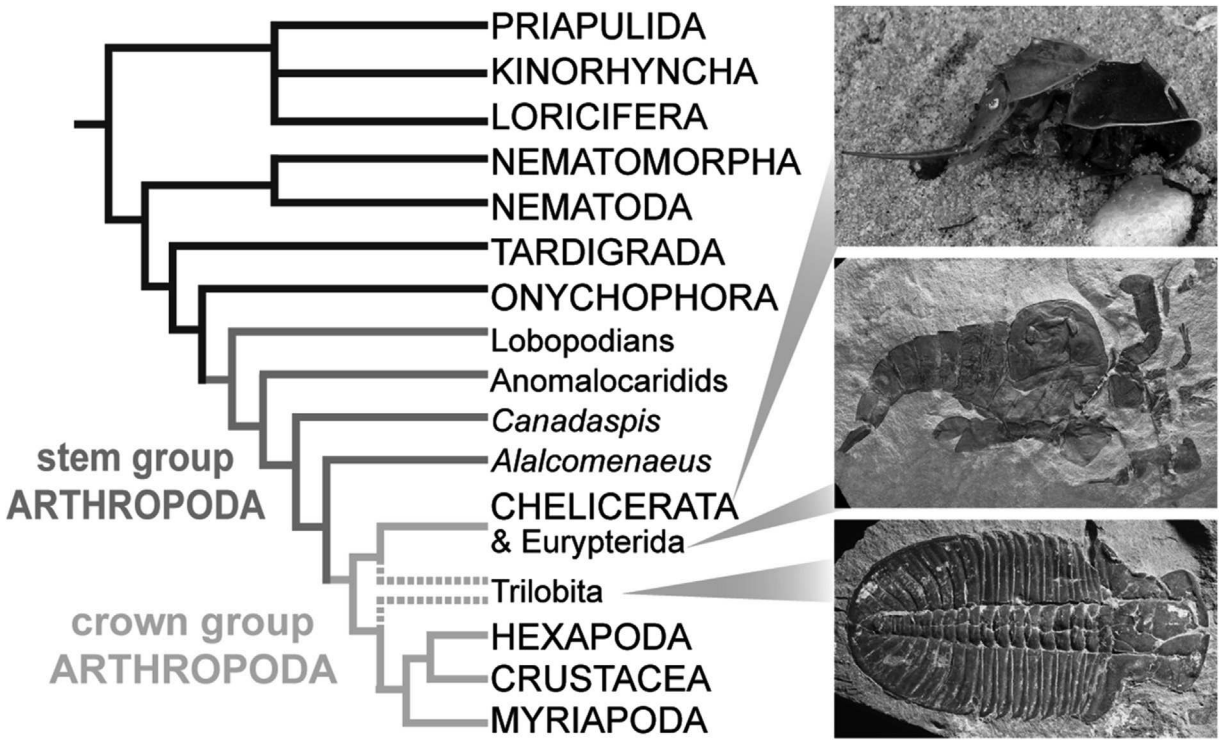


Fig. 1. Generalised phylogeny of ecdysozoans, with extant phyla in ALLCAPS and fossil groups/taxa in lowercase lettering. Phylogeny based on Dunn et al. (2014), Rota-Stabelli et al. (2013) and Legg et al. (2013). Images from top: Horseshoe crab in the process of moulting (Image credit: Wikimedia Commons); Eurypterid moult ensemble, YPM 208303 (Image credit: J. Utrup); Trilobite moulted exuvia, OUMNH AT.205 (Image credit: H. Drage).

exoskeleton taking only a brief period of time (minutes to hours generally, but highly variable between taxa). The brevity of this physical process is reflected in the fossil record by the rarity of specimens that are preserved mid-moult (e.g. García-Bellido and Collins, 2004; Peel et al., 2013) or during the immediate post-exuviation stage when the exoskeleton is not yet fully hardened (e.g. Whittington, 1990). During this process the arthropod is unable to move to escape threats and is vulnerable to desiccation, leading to an evolutionary constraint for exuviation to occur as rapidly as possible and behavioural adaptations that minimise risk (e.g. mass moulting behaviour and seeking shelter during moulting).

The full process of moulting begins with a complex series of biological interactions long before the actual act of exuviation takes place. The exoskeleton consists of cuticle that was secreted by underlying epidermal cells, to which it remains attached during its functional lifetime (Moussian, 2013). The process of moulting starts with the separation of the cuticle from the epidermis, referred to as apolysis, after which the epidermal cells undergo a round of cell division to increase surface area, followed by the secretion a new layer of cuticle, which remains soft until the old cuticle is shed (Nijhout, 2013). A moulting fluid is secreted between the old and new cuticles, and in some taxa is involved with digestion and reabsorption of the old exoskeleton. At some point, all structures associated with the old cuticle (e.g. muscle attachment, sensory neurons, circulatory system, etc.) must be transferred to the new cuticle (Ewer, 2005). The old cuticle is then split open along specific lines of weakness, usually by movement of the body or through an increase in internal pressure, so that the arthropod can emerge and the new cuticle can begin to harden. This process is controlled by the hormone ecdysone in all arthropods (Krishnakumaran and Schneiderman, 1970; Nijhout, 2013), although there is great variation in the number of moulting episodes within the phylum, with some taxa requiring many moults to reach adult size, while others can reach the same size in fewer moults by growing in larger increments at each moult (Nijhout, 2013).

Most palaeontological data that exist for arthropod moulting are derived from the trilobites, eurypterids and crustaceans. Research on moulting tends to be descriptive and focused on particular specimens or assemblages, with only a few studies examining the fossil record of ecdysis as a whole. Henningsmoen (1975) describes in detail the characteristic modes of moulting observed in different groups of trilobites, but does not examine the evolutionary significance of these trends. A broader look at such evolutionary trends is taken by Brandt (2002), who examines the link between trilobite moulting method and geological survivorship of taxa. A brief treatment of the fossil record of moulting is used by Valentine and Collins (2000) in conjunction with molecular phylogenetic information to make broad inferences on the process of evolution within Ecdysozoa, but this focuses mostly on developmental data in modern taxa and does not succinctly summarise or integrate the broad record known from fossils. A more fossil-based perspective is provided by Speyer (1990), who evaluates the reliability of the fossil record for understanding moulting behaviour in a wide variety of moulting groups, including trilobites (based heavily on Henningsmoen, 1975), decapod crustaceans, insects, and spiders. An informative and substantial body of research focused specifically on evidence of moulting in the fossil record, particularly for the arthropods, has been accumulating since these reviews. Here, we bring together this information into a large-scale literature review examining the prevalence and pattern of moulting in fossil ecdysozoans, with the aim of reviewing the current knowledge on this major morphological innovation. This literature review comprises the first half of our paper, with the second half focused on a quantitative evaluation of the patterns in ecdysial behaviour in trilobites in relation to taxonomic and temporal signal.

1.1. Criteria for distinguishing moults from carcasses in the fossil record

Describing patterns of ecdysis in the fossil record necessitates the ability to distinguish preserved exoskeleton moults from carcasses. Only in rare cases have specimens been preserved mid-moult (e.g. García-Bellido and Collins, 2004; Peel et al., 2013) with both the empty moult and the exiting animal in close association. In the vast majority of cases, individual specimens must be examined to determine if they are empty moults or full carcasses. Important aspects to consider when identifying moults include the locations of 'gape sutures', planes of weakness where the exoskeleton splits to produce an opening through which the individual exits; movements of the animal to facilitate opening of the exoskeleton 'gape sutures'; the direction of exuviation (anteriorly or posteriorly); the orientation during exuviation (dorsal- or ventral-up); and the final configuration of exuviae segments that may be recorded in the fossil record. Interpretations of moulting behaviour can only be made from definite exuviae, so as not to introduce misleading signals on, for example, the locations of 'gape sutures' identified from fragmented carcasses. Physical disturbance of carcasses (e.g. from scavenging, decay, currents, reworking, or compaction) can lead to preservation of disarticulated assemblages, and distinguishing these from true empty moults requires consideration of the morphology, taphonomic and depositional environment of the fossil. The procedure for identifying moults depends on the taxonomic group, owing to the broad morphological and behavioural variation within Ecdysozoa. This is more difficult for some groups, particularly those with suture lines that close following exuviation (e.g. modern *Limulus*; Vrazo and Braddy, 2011), but may be aided by consideration of the taphonomic aspects detailed below.

Numerous criteria have been suggested for identifying fossil moults, based on observations from fossil material in previous studies (Henningsmoen, 1975; Whittington, 1990; Braddy, 2001; Tetlie et al., 2008; Ebbestad et al., 2013). We suggest that at least Criteria 1 and/or 2 below are crucial for identifying a moulted exuvia. Criterion 3 may provide additional evidence for identifying moults, but only if Criteria 1 and/or 2 have been met and the depositional environment and preservational processes considered (Criterion 4). Criteria 4–6 provide further support and are dependent on the preservation type (soft-part; mineralised).

Criteria for identifying moults in the fossil record:

(1) Presence of suture lines showing evidence of having been opened. Criteria 1 and/or 2 are crucial for identification of a fossil moult specimen.
(2) Repeated configurations of specific exoskeletal parts (for examples see Figs. 2–6). Criteria 1 and/or 2 are crucial for identification of a fossil moult specimen.
(3) Disturbance of mineralised exoskeletal features in a systematic, repeated and characteristic way. This may include flattening or distortion of elements (Braddy, 2001); superimposition of dorsal and ventral surfaces; telescoping of segments (Braddy, 2001); and possibly crushing of the glabella (Figs. 2C and 4B) (Ebbestad et al., 2013). The exact nature of the disturbance may vary between different assemblages and types of fossils. Identification of these types of features may assist with recognising fossil moults in taxa where suture lines close after exuviation, for example with the scorpions where the relative positioning of anatomical features allows for identification of fossil moults even without suture lines (McCoy and Brandt, 2009). Generally, Criterion 3 would not be enough on its own to conclusively identify a moult assemblage, but may provide additional evidence if Criteria 1 and/or 2 are met and if Criterion 4 has been considered.
(4) Consideration of contextual information, including biostratinomic data, palaeoenvironmental conditions, and preservational processes. Evidence indicating a quiet depositional environment with low energy conditions and a low degree of transportation is preferred for moulting assemblages, as it would preclude post-depositional disarticulation that could cause carcasses to look like empty moults. Avoiding heavily reworked and/or time averaged deposits also reduces the chance of incorrectly identifying carcasses as moults. This type of information is not enough on its own to identify a moult, but would provide supporting evidence for moults identified using Criteria 1, 2 and/or 3.
(5) Lack of clear internal carcass features, including: the midgut and/or gut contents (as some arthropods moult part of the gut with the exuviae); musculature; and the nervous or circulatory system. This criterion is only applicable at localities known to have soft-part preservation, so is not diagnostic on its own for the arthropod taxa that comprise the vast majority of the fossil record of ecdysis, including trilobites, eurypterids and crustaceans. Only rarely are these taxa found with soft-part preservation (e.g. Whittington, 1990) and so a lack of internal organs in arthropod taxa does not necessarily indicate a moult unless minimally Criteria 1 and/or 2 are also met. Criterion 5 is more relevant when discussing the ecdysis fossil record of lobopodians, loriciferans, priapulids and stem-lineage arthropods without a hardened exoskeleton.
(6) Lack of evidence of behaviours associated with body fossils, such as predation and scavenging. For eurypterids and crustaceans at least, moult assemblages are thought to be better preserved than carcasses, because they would not be a target for scavenging and predatory activities (Clarke and Ruedemann, 1912; Feldmann and Tshudy, 1987; Braddy, 2001). Again, this criterion is not enough on its own to identify a moult assemblage, but provides supporting evidence for specimens that minimally meet Criteria 1 and/or 2.

Preservation potential must also be considered when investigating the moult fossil record. Moults in animals with thickened exoskeletons, such as Trilobita, may have increased preservation potential as compared to carcasses. This could result in a bias to moults in the fossil record of these groups, exacerbating inflation already present from the fact that one individual produces numerous moults during its lifetime (but only one carcass). This bias is also dependent on additional moulting behaviours. Specifically, some taxa consume their moults entirely, while others reabsorb exoskeletal material for energy conservation (Brandt, 2002). Consequently, moults of some clades may be more fragile (for example eurypterids; Vrazo et al., 2014), with decreased preservation potential compared to their respective carcasses. Further, body sections in segmented ecdysozoans are more likely to become disarticulated in the moult as connective tissue may be lacking. However, the opposite could also be true; preserved carcasses could appear to be moults (based on criterion 3 above) due to the presence of exoskeletal distortion following the rapid decay of soft connective tissue (Hegna, 2012). Factors such as these may decrease the likelihood of moult preservation, but the lack of published experimental taphonomic (decay) data comparing moults and carcasses compounds this knowledge gap (although see Hegna, 2012; for preliminary data on crustacean decay experiments). A number of criteria must therefore be considered when attempting to justify a moult identification, and decisions must be

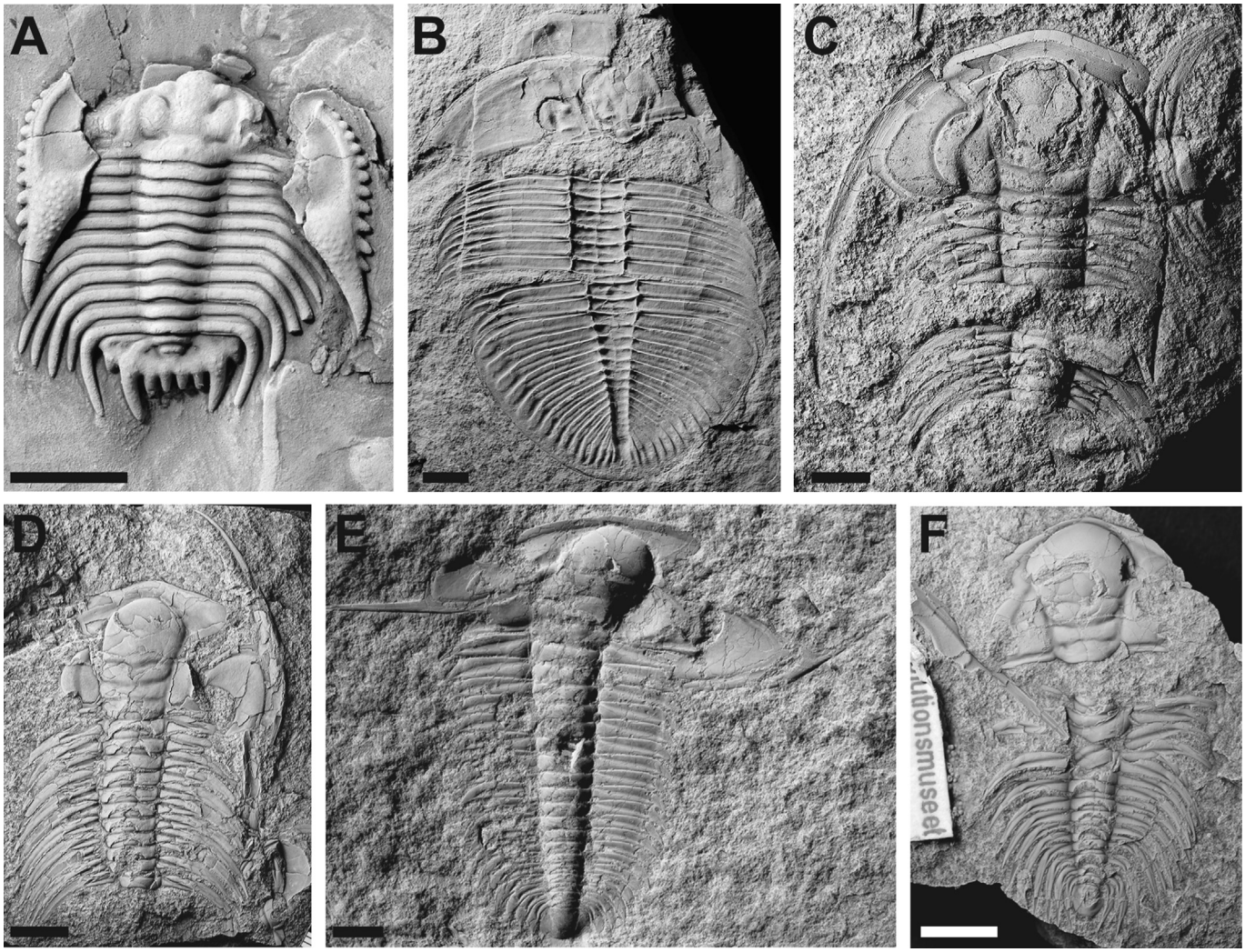


Fig. 2. Different moulting behaviours preserved as exuviae of trilobites. A: *Acidaspis coronata* from the Silurian Wenlock Shale in Malvern, UK, facial sutures opened and displaced slightly backwards. OUMNH C.17494. B: *Ogygiocarella debuchii* from the Ordovician Llandeilo Series in Meadowtown, Shropshire, with cephalon displaced and break through the thorax. OUMNH B.263. C–F: Cambrian trilobites from Jämtland, Sweden. C: *Acadoparadoxides* with left facial suture opened, crushed glabella, and middle thoracic segments displaced and rotated 90° over the right side of the cephalon. PMU 28736. D: *Acadoparadoxides* with left free cheek missing and right free cheek inverted. PMU 25995. E: *Eccaparadoxides* showing both free cheek inverted. PMU 25636. F: *Accadoparadoxides* with right free cheek missing, left free cheek flipped, cephalon removed, and disrupted thoracic segments. PMU 25690. Scale bars 5 mm in A, C, E–F and 10 mm in B, D.

rooted within specific contextual knowledge of the clade and depositional environment.

2. Literature review of the fossil record of moulting

The fossil record of moulting consists mainly of specimens belonging to the phylum Arthropoda, or falling on the stem lineage to this phylum (e.g. lobopodians), however the smaller ecdysozoan phyla Priapulida, Tardigrada and Loricifera, and the vermiform cycloneuralian group Palaeoscolecida, also have preserved evidence of moulting. The ecdysozoan phyla Nematoda, Nematomorpha, and Kinorhyncha have poor or absent fossil records and no fossil moulting assemblages have yet been found. In taxonomic studies, individual trilobites are often distinguished as an empty moult or an unmoulted carcass, providing information on the ecdysial mode for that group and on growth and development. The ecdysial fossil record also provides insight into unique behaviours, such as mass moulting and reabsorption/retention of previous moults.

2.1. Trilobite ecdysis

The majority of literature published on ecdysis in the fossil record concerns patterns of moulting in Class Trilobita. This group was extremely long-lived and diverse, living from the Early Cambrian until their disappearance in the fossil record at the end-Permian mass extinction, following a drawn-out decline (Brandt, 2002). In addition to being geologically successful and geographically diverse, trilobites filled numerous niches, including as predators, scavengers and filter-feeders. Trilobites have been variously interpreted as related to the mandibulates, chelicerates and as stem lineage arthropods (see Budd and Telford, 2009; Edgecombe, 2010 for a review), but regardless of their exact phylogenetic affinity, they are important as early arthropods owing to their abundance in Palaeozoic marine environments. Their thick cuticles had a high preservation potential; providing researchers with extensive collections of material with which to investigate biostratigraphy, biogeography, and their morphology, development and ecology. Trilobites were a key component of ancient marine ecosystems,

with a complex evolutionary history shaped by their ability to ecdyse. Understanding their ecdysial behaviours is central to the study of the early evolution of Arthropoda and its evolution for the next 540 million years (Henningsmoen, 1975).

Trilobite moult configurations (Figs. 2 and 3), the repeated patterns of fragmented exuviae preserved in the fossil record, demonstrate that the ecdysial behaviours employed by trilobites were highly varied. These behaviours have been reconstructed using the consistent patterns observed in empty moult configurations, because no specimens have yet been found that preserve a trilobite midway through the act of moulting (although see Błażejowski et al., 2015, for a suggested moult-carcass composite). The most common methods of ecdysis across Trilobita are the opening of the facial sutures (Figs. 2–4) (sometimes including the rostral suture, figs. 4G–J), usually followed by the release and/or inversion of the librigenae (free cheeks), and a disarticulation between the cephalon and the thoracopygon (the rest of the body, including thorax and pygidium). Other behaviours have also been described in the literature (Henningsmoen, 1975), including: breaks at other points along the thorax (Figs. 2B,C and 3K,L); displacement of the pygidium (Fig. 3J); removal of the rostral plate and ventral exuviation of the animal; and release of the librigenae followed by dislocation of the cranidium from the thorax (Fig. 2F). These modes have been dissected into the resulting 'characteristic' configurations that are preserved in the fossil record (Budil and Bruthansová, 2005, fig 3). For example, Salterian moulting (Henningsmoen, 1975) consists of a break at the joint between the cephalon and the thorax during ecdysis, and the subsequent inversion of the cephalon to rest with the anterior edge towards the thoracopygon (Fig. 3I), resulting in Salter's Configuration (Richter, 1937). The trilobite would arch its body during ecdysis, angling the dorsal cephalon towards the substrate. This mode of moulting may also have produced different exuviae configurations, if the cephalic angle was too small for inversion (for example, in *Trimerus stelmophorus*, Busch and Swartz, 1985).

Many adult trilobite taxa were not restricted to a single method of ecdysis. The mode employed by the individual likely depended on a number of factors, including developmental stage and size, and ease of moulting in a particular environment (and associated risk). Some taxa seem to have the ability to account for failure of the usual moulting mode by employing an unusual method if needed (McNamara, 1986; Budil and Bruthansová, 2005; Paterson et al., 2007; Tortello and Clarkson, 2008). Additionally, there may have been morphological constraints, and some ecdysial characteristics may be constant for this reason. For example, the Salterian mode of moulting likely occurred more frequently in species with apparently inactive facial sutures, as in some phacopids (Henningsmoen, 1975). Other methods may have been key for species with more complex morphologies. We may also see trends in ecdysis through geological time, possibly in response to a phylogenetic signal. For example, Budil and Bruthansová (2005) found Middle Ordovician taxa displayed more variation in modes of moulting.

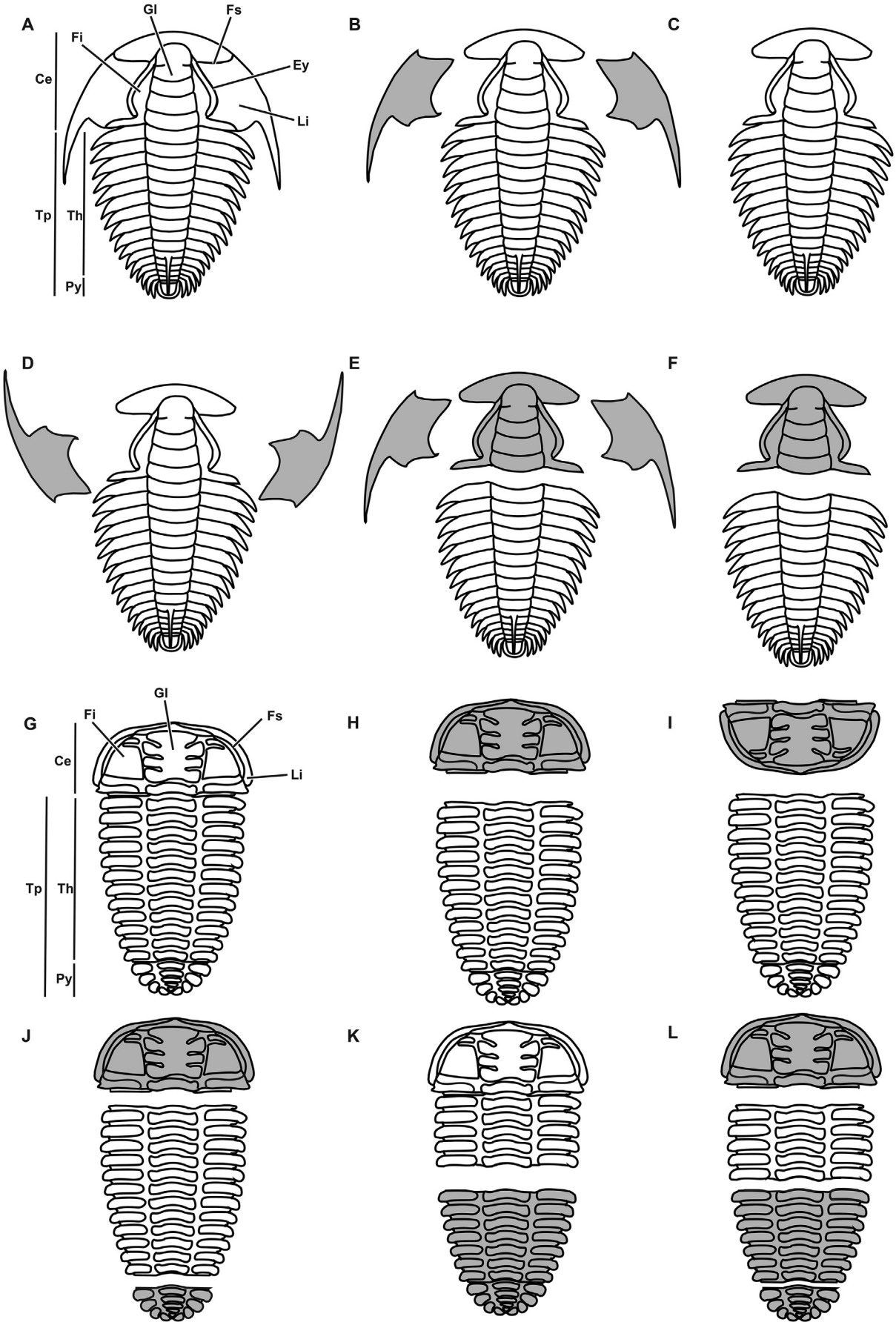
Trilobites may have been as likely to moult dorsal-up as ventral-up. This may be common across the Class; a close to equal ratio between the two moult positions has been noted in trilobites such as olenids and paradoxidids (Clarkson et al., 2003; Ebbestad et al., 2013). However, other species show a bias towards one orientation (Karim and Westrop, 2002). For example, inverted *Phacops* moults have been suggested to result from dorsum-down exuviation, this orientation being beneficial for opening of the ecdysial gape between cephalon and thorax (Speyer, 1985, 1987). Whereas exuvia of *Greenops* are rarely found in this position, possibly indicating only dorsum-up exuviation (Speyer, 1985, 1987). Morphology may be key for determining moult orientation, although this is difficult to determine without preserved mass moult assemblages. Further, singular ventral-up specimens could result from taphonomic effects, such as inversion via transport following ecdysis, rather than being informative for moulting behaviour. For orientation to inform exuvial behaviour, rapid burial and preservation would be required (Speyer, 1987).

The general characteristics of trilobite moulting do not seem to specifically align them to any modern arthropod group. The facial sutures of trilobites represent a system of ecydsial suture that is unique in its complexity, with no known equivalent amongst modern arthropods or other ecdysozoans (Ax, 1987; Paterson and Edgecombe, 2006). Trilobites show continuous, or indeterminate, growth, meaning that they continued to grow in size and moult after reaching their adult morphology (Hughes, 2007). Of extant arthropods, myriapods and crustaceans also continue to grow and moult as adults, whereas insects and arachnids show determinate growth, meaning they do not continue to grow and moult after reaching sexual maturity (Nijhout, 2013). Trilobites differ from extant arthropods in that there is no evidence for reabsorption of the old cuticle prior to moulting, as is seen in extant groups (Ewer, 2005; Nijhout, 2013). All preserved material also suggests trilobites exited their exuviae anteriorly, as is seen in modern xiphosurans and scorpions, but which is unlike other arthropods, such as spiders and crabs, which exit their exuviae posteriorly (Henningsmoen, 1975). Few ecdysial characteristics have been included in phylogenetic analyses of trilobite relationships beyond those associated with the morphology of facial sutures. Moulting behaviours for each trilobite Order are discussed below and summarised in Table 1.

2.1.1. Ecdysis in Redlichiida

Descriptions of ecdysial behaviour are more common in the literature for the speciose Orders Phacopida and Redlichiida (Fig. 3). Most *Redlichia* individuals would moult by opening of both the facial sutures (Fig. 3B) (Öpik, 1958; McNamara, 1986) and the rostral sutures, the latter of which was necessary to release the "stitched rostral shield" (Öpik, 1958; pg. 28) that interlocks with the cranidium by a series of small nodes. The cranidium also detaches (Fig. 3E), leaving the thoracic segments and pygidium as a cohesive unit that is often found preserved (Öpik, 1958, 1970; McNamara, 1986). A moulted, but admittedly malformed, specimen of *R. hupehensis* shows only the detachment of the librigenae, i.e. a complete axial shield (cranidium, thorax and pygidium) (Han and Zhang, 1991). Specimens of *R. forresti* individually show inversion of the pygidium (McNamara, 1986, figs. 1A, D, H, I), thoracic segments (Öpik, 1958; pl. 2, figs. 2, 3) and librigenae (McNamara, 1986, fig. 1B), whereas *R. idonea* (McNamara, 1986, fig. 1E) and *R. micrograpta* (McNamara, 1986, fig. 1C) each only show inversion of thoracic segments or librigenae respectively.

McNamara (1986) highlights the unusual occurrence of inversion of part of the body (free cheeks, thoracic segments, and/or pygidium) during moulting. He focuses on three species of *Redlichia* with inverted elements and describes scenarios for each specimen that requires partial enrolment of the specimen during ecdysis. This link between ecdysial moult configurations and enrolment behaviour was in contrast to previous suggestions that Cambrian micropygous trilobites were rigid and unable to enrol (Harrington, 1959; Bergström, 1973), although later studies have shown that at least flexing, if not entire enrolment, was possible in these trilobites (Esteve et al., 2011, 2013; Ortega-Hernández et al., 2013). McNamara (1986) stresses that inversions in *Redlichia* were "the exception, rather than the rule" (pg. 412), and should be considered in the context of the individual specimen (rather than generalised as behavioural differences between taxa). To account for the inverted body elements, McNamara (1986) invokes an ecdysial scenario that begins with a posterior movement of the trilobite in the early stages of exuviation, and suggests that this may have been


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used to separate the cephalon from the thorax in cases where the rostral suture failed to open. This implies a flexibility of ecdysial behaviour in *Redlichia* individuals and response to situations where the usual modes of moulting were unsuccessful.

Ebbestad et al. (2013) described redlichiid moults of two paradoxidid species, including one *Eccaparadoxides*, which also moulted using the cephalic sutures (Fig. 4). They particularly emphasise the open position of the facial sutures in all specimens (Ebbestad et al., 2013, figs. 3D–F), even where the librigenae are somewhat in place, and a total absence of *in-situ* hypostomes. Other paradoxidids, like *Paradoxides davidis*, also opened the cephalic sutures for moulting, displacing the librigenae and rostral-hypostomal plate as in the first step for ecdysis in *Redlichia* (Whittington, 1990).

Particular redlichiid trilobite taxa demonstrate the variability in ecdysial behaviour seen within species of other trilobite Orders, employing multiple methods for moulting. *Olenellus gilberti* and *Nephrolenellus geniculatus* (Webster, 2007) seem to have moulted the hypostome and rostral plate before emergence ventrally through the resulting gap, but occasionally specimens of the former also show a dislocation and displacement of the thoracopygon beneath the cephalon (Webster, 2015, fig. 10). This mode of ecdysis differs from other redlichiids, such as *Eccaparadoxides* and *Redlichia*, because olenelloids lack facial sutures around the librigenae (Webster, 2015). *Balcoracania dailyi* appeared to have employed the usual redlichiid style of ecdysis; strongly flexing the exoskeleton, pushing the spines into the sediment and allowing for opening of the cephalic sutures. The animal would emerge anteriorly, leading to the librigenae, rostral plate, hypostome and/or cranidium becoming dislodged (Paterson et al., 2007, figs. 8A, B, E, H). However, at least one moult of *B. dailyi* displays fragmentation between the first and second thoracic segments (Paterson et al., 2007, fig. 8C), prior to opening of the facial sutures. As in *Redlichia*, this may well reflect flexibility in behaviour to overcome a particularly difficult moulting period (McNamara, 1986), reinforcing the plasticity that some trilobite species can show during ecdysis.

2.1.2. Ecdysis in Phacopida

Budil and Bruthansová (2005) presented an initial exploration of the complicated nature of phacopid trilobite ecdysis, extoling the need for a more thorough analysis. In particular, there is great variability in ecdysial behaviours of dalmanitoid and acastoid phacopid trilobite taxa. The most common behaviour across all species described (and most other phacopid taxa) was complete disarticulation of the cephalon from the rest of the body (Fig. 3H), without the cephalon inversion involved in producing Salter's configuration (Fig. 3I) (Budil and Bruthansová, 2005, fig. 4). However, particularly in *Ormathops*, as well as *Zeliszkella*, *Dalmanitina* and *Kloucekia*, the break releasing the cephalon during ecdysis occurred several segments into the thorax (Fig. 3K). Thoraces broken into multiple segments in *Baniaspis* (*?*) *quadrata* (Budil and Bruthansová, 2005, figs. 6C, E, F) and *Zeliszkella cf. oriens* (Budil and Bruthansová, 2005, fig. 7E), representing multiple gape sutures, may indicate instances of problematic moulting as in some described redlichiids.

Demonstrating the apparent versatility of dalmanitoid trilobite moulting habit, separation of the librigenae from the remainder of the axial shield via opening of the facial sutures was common in young individuals of *Dalmanitina proaeva elfrida*, *Dalmanitina proaeva proaeva*, and *Ormathops* (*Ormathops*) *atavus* (Budil and Bruthansová, 2005, figs. 7B, C). Some specimens of *Ormathops* (*Ormathops*) *atavus* also show the librigenae disconnected from the separated cranidium (Budil and Bruthansová, 2005, figs. 4E, 5C). However, Budil and Bruthansová (2005) state that free-cheek moulting (by opening of the facial sutures) was replaced by more 'advanced modes' (Budil and Bruthansová, 2005; pg. 376), with a loss of facial suture functionality into adulthood of these phacopid trilobites and ecdysis using the cephalic or thoracic disarticulations.

Cephalic sutures of *Trimerus stelmophorus* (Homalonotidae), like most phacopids, were non-functional during ecdysis. Phacopids instead utilised the common behaviour of separating the cephalon and thorax (Busch and Swartz, 1985, fig. 1.8; Speyer, 1985). This often resulted in preservation of moults in Salter's Configuration, suggesting arching of the body with the dorsal cephalon towards the substratum. *Plagiolaria poothai* (Cronier and Fortey, 2006) and *Paciphacops* sp. (Rustán et al., 2011, fig. 2) also moulted in this way, although the last is interpreted differently. The species of *Paciphacops* figured by Rustán et al. (2011) may have utilised a gape suture between the cephalon and thorax for ecdysis while infaunal. A Devonian specimen of *Trimerocephalus chopini* from the Holy Cross Mountains, Central Poland, was also suggested by Błażejowski et al. (2015) to represent an infaunal moult-carcass composite, using the same exuvial method. *Phacops rana* and *Greenops boothi* both moulted by removing the cephalon, as determined by the prevalence of thoracopygidia, although Salterian configurations were rare or absent in these species (Speyer, 1985).

From a single, complete inferred exuvia, McNamara and Tuura (2011) suggest a total moulting sequence for *Greenops widderensis*. This begins with the familiar break of the join between cephalon and thorax, and leads to the disarticulation of the pygidium (McNamara and Tuura, 2011, fig. 1). This is also the case for *Plagiolaria nandanensis* (Wang and Han, 1997). As with most other phacopids described, the facial sutures were not employed, although *G. widderensis* ecdysis has only been interpreted from the one specimen.

However, *Flexicalymene retrorsa* (Calymenidae) exuviae indicate the functionality of facial sutures, and the release of the librigenae, for ecdysis in some phacopid taxa (Hunda et al., 2006, fig. 9L). The subsequent rotation of the cranidium, they suggest, may reflect post-ecdysial disturbances (Hunda et al., 2006, fig. 9M). Some specimens of *F. retrorsa* showing an articulated thoracopygon without a cephalon have been interpreted as moults, suggesting they may have occasionally utilised a gape suture behind the cephalon, as most phacopid trilobites did (Hunda et al., 2006, fig. 9N).

2.1.3. Ecdysis in Ptychopariida

Like redlichiid taxa, ptychopariid trilobite exuviae emphasise the importance of cephalic sutures for ecdysis across the Order, often leading to the displacement of the hypostome and rostrum (Cederström et al., 2011, figs. 11C–F). This is certainly the case for moults of *Strenuaeva inflata*, although Cederström et al. (2011) consider the opened facial sutures and displaced librigenae to

Fig. 3. Summary of the most common trilobite moult configurations preserved in the fossil record, illustrated by *Redlichia* (A–F, usually moulted via the facial sutures and librigenae) and *Placoparia* (G–L, usually moulted via disarticulation of the entire cephalon). Exoskeleton sections disarticulated during the moulting process shaded grey. A: Complete labelled dorsal exoskeleton of *Redlichia*. B: Facial sutures opened and librigenae displaced outwards. C: Facial sutures opened and librigenae missing. D: Facial sutures opened and librigenae horizontally flipped (possible if the individual arched during exuviation). E: Facial sutures and cephalothoracic joint opened, causing disarticulation of the librigenae and cranidium. F: As in E, but librigenae missing. G: Complete labelled dorsal exoskeleton of *Placoparia*. H: Cephalon disarticulated, leaving exuvial gape between cephalon and thorax. I: Cephalon disarticulated and flipped horizontally (Salter's configuration). J: Cephalon and pygidium disarticulated. K: Disarticulation along the thorax (unspecific location). L: Cephalon, thorax and pygidium disarticulated in the same exuvia. Other combinations of these moult configurations can be found preserved in the fossil record, for example disarticulation of the thorax with release of the librigenae. Abbreviations: Ce, cephalon; Ey, eye; Fi, fixigena (fixed cheek); Fs, facial suture; Gl, glabella; Li, librigena (free cheek); Py, pygidium; Th, thorax; Tp, thoracopygon. Cranidium is the cephalon minus the librigenae; axial shield is the cranidium plus the thoracopygon.

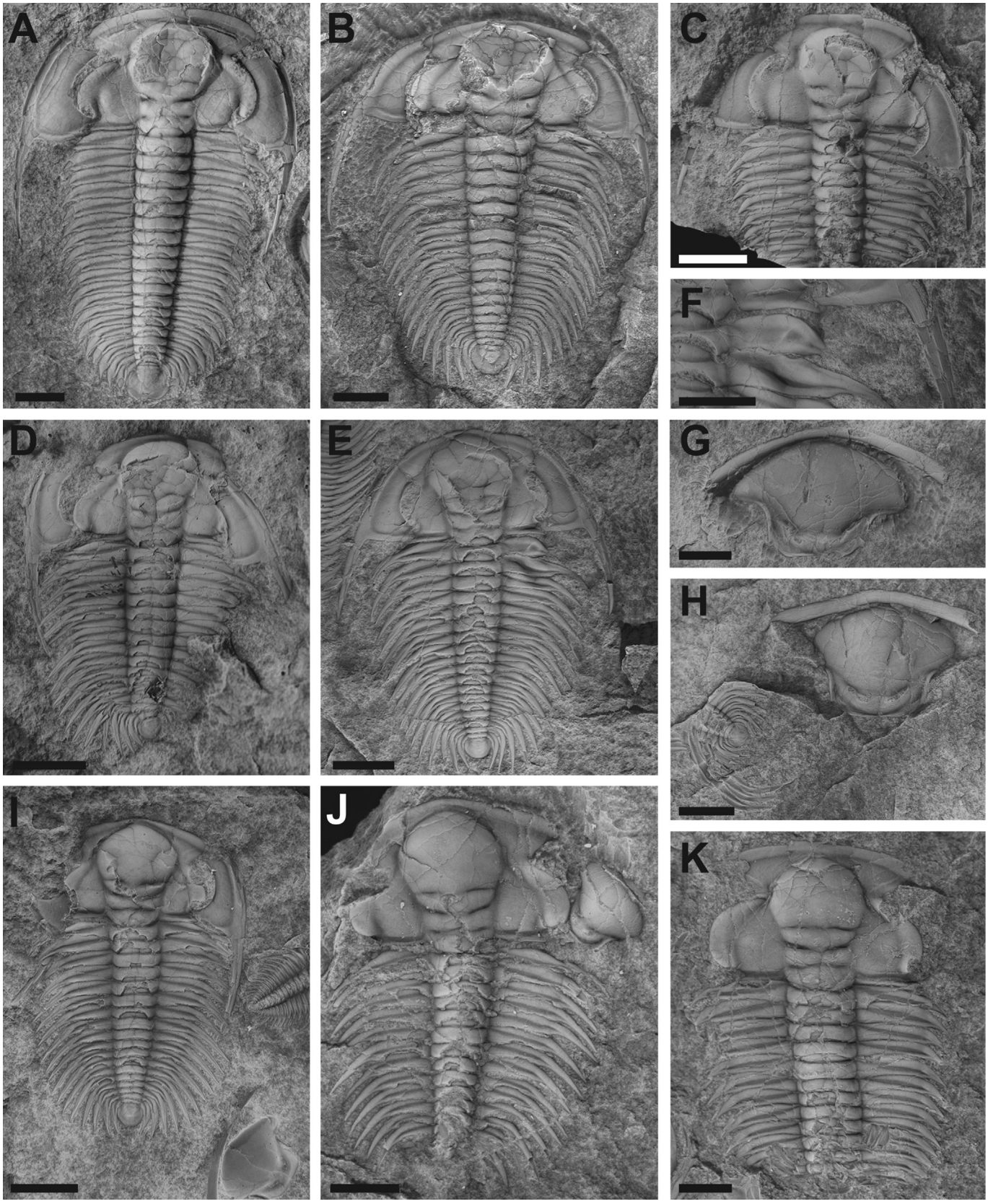


Fig. 4. Moulted exuviae of paradoxidid trilobites from a mass moulting surface from the Cambrian of Jämtland, Sweden. A–B: Trilobites with facial sutures opened. A: PMU 25711/29. B: PMU 25711/09b. C–D: Trilobites with facial sutures opened and free cheeks slightly displaced. C: PMU 25711/59. D: PMU 25711/66. E–F: Specimen with facial sutures opened and damage to first thoracic segment on right side, probably caused by problems during moulting. PMU 25711/61. E: Whole specimen. F: Closeup of damaged thoracic segment. G–H: Moulted hypostome and rostral plate complexes. G: PMU 25711/45. H: PMU 25711/14. I: Facial sutures opened, left free cheek flipped and right free cheek displaced. PMU 25711/67. J: Free cheeks absent and hypostome displaced next to cephalon. PMU 25711/46. K: Free cheeks absent and break through thoracic segments. PMU 25711/25. Scale bars 5 mm in A–B, D–E, G–I; 3 mm in C, F, J–K. All specimens are latex casts except for A–B, H and K, which are rock. Photo credit for all images: J. O. Ebbestad (Ebbestad et al., 2013).

result from taphonomic processes, with the other cephalic sutures being more important for emergence.

Ctenopyge (*Eoctenopyge*) *angusta* utilised the facial sutures during moulting, as indicated by the preservation of separated yoked librigenae and hypostomes, with the cranidium also detached in some exuviae (Clarkson et al., 2003; pl. 8.1). Other olenids may have moulted similarly, releasing the librigenae with the axial shield remaining intact, including: *Acerocare ecorne* (Henningsmoen, 1957); *Parabolina frequens argentina* (Tortello and Clarkson, 2008, fig. 9.1); *Wujiajiania sutherlandi* (Chatterton and Ludvigsen, 1998, fig. 10); and *Aciculolenus palmeri* (Chatterton and Ludvigsen, 1998).

These Olenidae trilobite exuviae are often considered as preserved in 'Harrington's Configuration' (Henningsmoen, 1975), with the shed librigenae found below the axial shield (Tortello and Clarkson, 2008). Ptychopariids may also show within-taxon variation in ecdysial pattern. For example, some moulted specimens of *Parabolina frequens argentina* have missing cranidia with the librigenae close to normal position (Tortello and Clarkson, 2008, figs. 8.7, 8.10, 9.3, 9.10), as do those of the olenid *Cloacaspis dejecta* (Fortey, 1974; pl. 12.1). *Parabolina frequens argentina* also displays signs of employing alternative ecdysial behaviours such as disarticulated thoraces and displaced pygidia (Tortello and Clarkson, 2008, figs. 8–10) during difficult moulting periods, much like *Balcoracania dailyi* (Paterson et al., 2007) and *Redlichia* (McNamara, 1986). Unlike all other ptychopariids described, a lone *Burnetiella leechi* exuvia displays a Salterian mode of moulting (Chatterton and Ludvigsen, 1998. fig. 22.6).

2.1.4. Ecdysis in Odontopleurida

Descriptions of ecdysial behaviour are sparse for the remaining trilobite Orders. *Odontopleura* (*Odontopleura*) *arctica* and *Odontopleura* (*Odontopleura*) *brevigena* opened the facial sutures for ecdysis, releasing the librigenae and hypostome (Adrain and Chatterton, 1990, fig. 7.5). Edgecombe and Sherwin (2001) also note the librigenae, hypostome and rostral plate of *Odontopleura* (*Sinespinaspis*) *markhami* detached during ecdysis (Edgecombe and Sherwin, 2001, figs. 3D, 4A).

2.1.5. Ecdysis in Asaphida

Exoskeleton moulting in the Ordovician *Homotelus bromidensis* took place through disarticulation of the cranidium, with the librigenae yoked anteriorly (Karim and Westrop, 2002). Conversely, preserved moulting configurations of *Labiostria westropi*, and occasionally *Pterocephalia norfordi*, usually consist of complete axial shields with the librigenae nearby, in a range of different positions (rotated, inverted, displaced), although some individuals may also have moulted the cranidium or cephalon (Chatterton and Ludvigsen, 1998, figs. 19, 29).

2.1.6. Ecdysis in Corynexochida and Proetida

Chen et al. (2008) describe a similar scenario of moulting to *Balcoracania dailyi* (Paterson et al., 2007) for the corynexochid trilobite *Oryctocephalus indicus*, including a downwards flexing to produce a break between the cephalon and thorax, with the librigenae also becoming disarticulated during this process. The remaining cranidium then became inverted during full exuviation of the animal from the old exoskeleton. The moulting of *Telephina* (*Telephus*) *spiniferus* has been described as a very similar process to *O. indicus* (Fischer, 1946, fig. 1), involving shifting of the librigena and horizontal inversion of the cranidium (free cheeks "slightly shifted" with the glabella "overturned and carried forward", Fischer, 1946, p. 566). Glaessner (1948) notes that this moult configuration seems to span several trilobite Families, but that preservation of all disarticulated moulted sclerites in position is extremely rare. A single moult of *Aulacopleura pogsoni* demonstrates that proetid trilobites could undergo ecdysis through disarticulation of the entire cephalon (Edgecombe and Sherwin, 2001).

2.1.7. Ecdysis in Agnostida

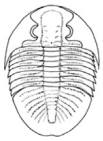
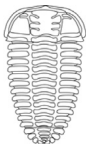
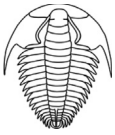
Descriptions of ecdysial modes in the literature are also rare for Agnostida, considered by some research to be the earliest diverging trilobite Order (Cotton and Fortey, 2005) or as stem lineage mandibulates (Legg et al., 2013). *Pagetia significans* exuviae from the Cambrian Kaili Formation, China, suggest ecdysis only through opening of the facial sutures, and disarticulation of the librigenae (Lin and Yuan, 2009), although entire cephala seem common in the agnostoid fossil record. Whether Agnostida are trilobites, or close relatives of the Trilobita, greater knowledge of their moulting methods would allow better tracing of trends in arthropods with similar morphologies, and the origins of the patterns and variability in trilobite ecdysis.

2.1.8. Summary of trilobite ecdysis

Ecdysis utilising gapes created through opening of the facial (and often anterior and rostral) sutures is commonly observed across trilobite Orders (Table 1) and through geological time (see Section 3.2). This is to the exception of most phacopid trilobites, which had inactive facial sutures; phacopid taxa typically exploit a gape suture created through the disarticulation of the cephalon from the thorax (Fig. 3G–L). As a generalisation, Redlichiida, Ptychopariida, Odontopleurida, Asaphida, and Agnostida all exhibit a high occurrence of facial suture moulting. Whereas some Redlichiida (*Redlichia*), Asaphida, and Corynexochida combine this with disarticulation of the cranidium. However, these are all very broad trends, and in reality those Orders with ample exuviae descriptive literature (Redlichiida, Phacopida, Ptychopariida) show considerable flexibility in which ecdysial method they employed. Whether the ecdysial methods discussed for Odontopleurida, Asaphida, Corynexochida, Proetida and Agnostida are representative of most taxa within their Orders is unclear, as these are based on few species descriptions. More detailed results from a preliminary quantitative analysis of these trends is presented in Section 3.2 of this work.

In a rare exploration of ecdysis and its broader evolutionary trends, Brandt (2002) analysed the interplay between trilobite moulting and geological survivorship. Brandt (2002) contrasted the extensive variation in trilobite ecdysial behaviour to the often-stereotyped moult styles characterising other arthropod groups. In this way, trilobites were described as having an "inefficient moult habit" (Brandt, 2002; pg. 399), which, when coupled with the apparent need to from a new calcitic exoskeleton after each moult without the benefit of reabsorption of old cuticle material, may have been evolutionary disadvantageous and contributed to their ultimate decline. Further, the taxonomic survivorship analyses of Brandt (2002) support an association between morphology and ecdysis; taxa with simpler morphologies (particularly fewer thoracic segments) and therefore easier ecdysis had longer chronological ranges. Our quantitative analysis (Section 3) builds on the works of Brandt (2002) by investigating more specific ecdysial behaviours and considering the possible evolutionary advantage of flexibility in ecdysis. Öpik (1970) alternatively suggested a decline in the efficiency of ecdysial hormones and moulting success factored into the extinction of *Redlichia* species. Ultimately, we know very little about the broader evolutionary impacts of moulting behaviour in trilobites, but there is huge potential to explore these implications thanks to their extensive fossil record. Elucidating such trends may be a challenge given that higher-level trilobite phylogenetic relationships are currently in a state of flux,

Table 1
Summary table of published descriptions of moulting behaviour in trilobite Orders.

Order	Morphology	Moulting behaviour[a]	#of sp.[b]	Age range	Key references[c]
Agnostida e.g. *Girvanagnostus*		1. Facial sutures opened	1	Cambrian–Ordovician	Lin and Yuan (2009)
Asaphida e.g. *Ogygiocaris*		1. Facial sutures opened 2. Cranidium removed 3. Cephalon removed	9	Cambrian–Silurian	Chatterton and Ludvigsen (1998), Karim and Westrop (2002)
Corynexochida e.g. *Olenoides*		1. Facial sutures opened 2. Rostral plate removed 2. Cephalon removed 2. Cranidium removed	3	Cambrian–Devonian	Chen et al. (2008)
Odontopleurida e.g. *Odontopleura*		1. Facial sutures opened 2. Rostral plate removed 2. Pygidium displaced	4	Cambrian–Devonian	Adrain and Chatterton (1990), Edgecombe and Sherwin (2001)
Phacopida e.g. *Placoparia*		1. Cephalon removed 2. Facial sutures opened 3. Pygidium displaced 4. Thoracic dislocation 5. Cranidium removed	32	Ordovician–Devonian	Budil and Bruthansová (2005), Hunda et al. (2006), Rustán et al. (2011)
Proetida e.g. *Cyphoproetus*		1. Facial sutures opened 2. Cranidium removed 3. Cephalon removed	7	Cambrian–Permian	Edgecombe and Sherwin (2001)
Ptychopariida e.g. *Olenus*		1. Facial sutures opened 2. Cranidium removed 3. Thoracic dislocation 4. Pygidium displaced 5. Rostral plate removed 5. Cephalon removed	21	Cambrian–Ordovician	Chatterton and Ludvigsen (1998), Clarkson et al. (2003), Tortello and Clarkson (2008)
Redlichiida e.g. *Redlichia*		1 Facial sutures opened 2. Rostral plate removed 3. Cranidium removed 4. Thoracic dislocation 5. Pygidium displaced 6. Cephalon removed	24	Cambrian	McNamara (1986), Öpik (1958), Paterson et al. (2007)

[a] In order of most common moulting behaviour (1) amongst individuals of species within that Order, to least common.
[b] Number of species from the Order in data set.
[c] A selection of references that specifically describe ecdysis for species of each Order.

with essentially no resolution despite numerous analyses (Adrain, 2011). However, further research along the lines of the preliminary analyses described in Section 3 could also help to resolve phylogenetic uncertainty within the group.

2.2. Eurypterids

The other main group of fossils from which ecdysis is relatively well known is the eurypterids (Figs. 1 and 5). There has been much debate on whether various eurypterid fossils represent exuviae or carcasses, much of which has focused on the abundant and well-preserved specimens of the Bertie Waterlime in New York State. For example, Andrews et al. (1974) consider the Bertie eurypterids to be carcasses, whereas Braddy (2001) and Tetlie et al. (2008) thought they were exuviae. Tetlie et al. (2008) considered the presence of musculature and the midgut as the only internal structures that would indicate a carcass (as opposed to an empty moult), owing to the tendency for other structures (i.e. fore- and hindgut; book lungs) to be shed with the exoskeleton. The only definitive carcasses that meet his criteria are specimens from the Devonian Gogo Formation (Tetlie et al., 2004) and the Ordovician Soom Shale (Braddy et al., 1995, 1999), but he also suggested that generally most specimens preserving any part of the gut are quite likely to be carcasses rather than exuviae. Preservation of these internal structures is still very rare, and so the majority of eurypterid(s) assemblages are considered to be exuviae rather than carcasses (e.g. Selden, 1981; Tetlie et al., 2008; Vrazo et al., 2014). In the Bertie Waterlime, the lack of evidence for scavenging is taken as further evidence for these specimens being exuviae (Clarke and Ruedemann, 1912), as is the presence of crumpling, flattening or

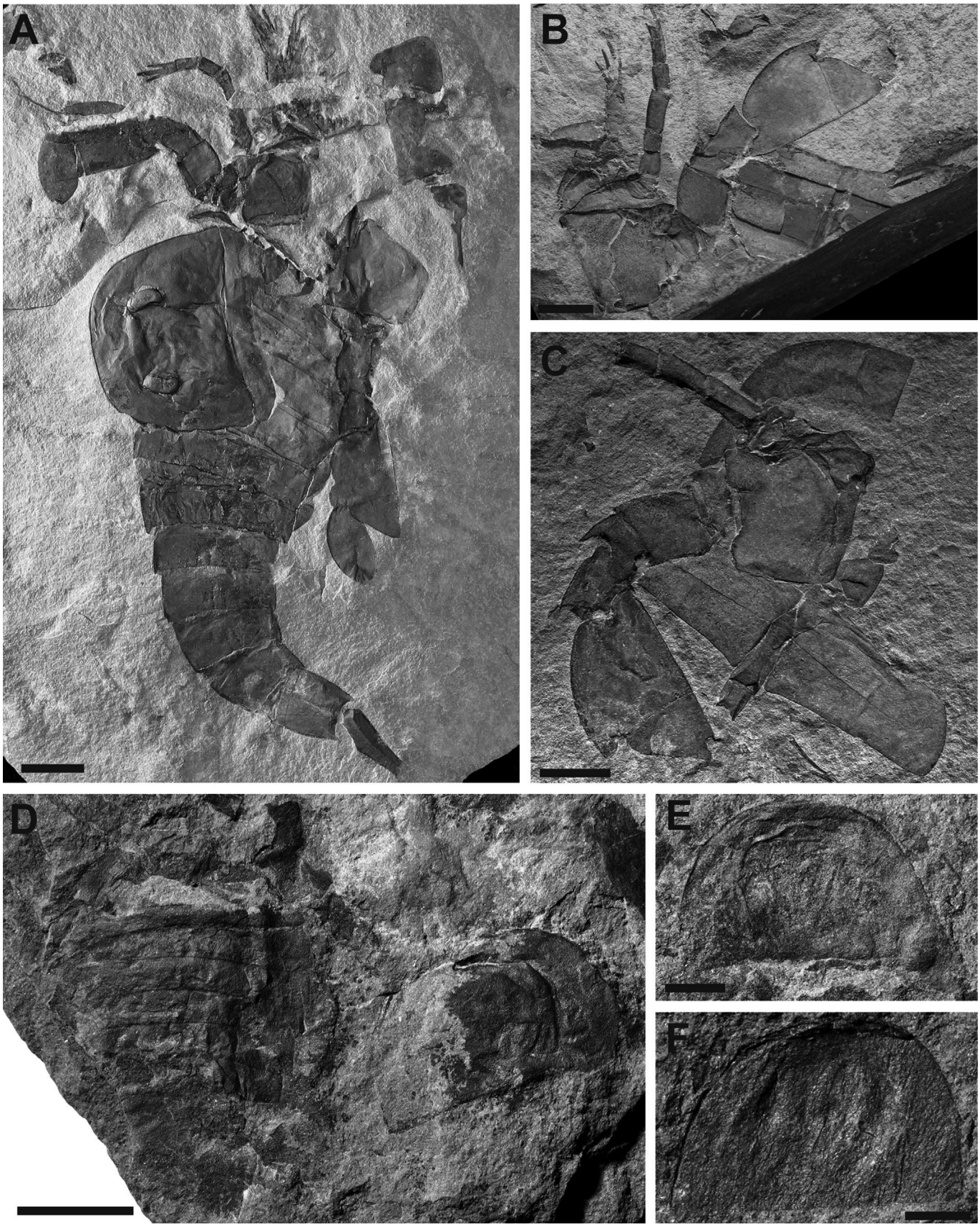


Fig. 5. Eurypterid moult assemblages. A–C: *Eurypterus lactustris* from the Williamsville Formation in Ridgemount Quarry, Ontario. Photo credit: J. Utrup. A: Mostly complete moult ensemble with cephalic carapace and one opisthosomal segment dislocated and superimposed on the abdomen. YPM 208303. Scale bar 10 mm. B: Dissociated ventral plates with prosomal appendage attached. YPM 208456. Scale bar 10 mm. C: Ventral plate and swimming leg in moult ensemble. YPM 209023. Scale bar 10 mm. D–F: Adelopthalmid eurypterid from the Devonian Lower Old Red Sandstone of the Dinas Estate in Brecon, Powys, Wales. D: Moult ensemble with cephalic carapace (right) dislocated from all opisthomal segments of the abdomen (left). OUMNH D.2184. Scale bar 10 mm. E–F: Dislocated cephalic carapaces preserved in isolation, without the first opisthomal segment. E: OUMNH D.2178. Scale bar 5 mm. F: OUMNH D.2173. Scale bar 3 mm.

distortions of the exoskeleton, superimposition of dorsal and ventral surfaces, and telescoping or other disturbance of disarticulated body elements (as in Selden, 1981).

As such, in eurypterid studies, the assumption seems to be that a specimen is a moulted exuviae unless it can be shown to be a carcass by the presence of very specific internal structures (i.e. midguts and musculature; Tetlie et al., 2008). As discussed in Section 1.1, these structures have a much lower preservation potential than the external exoskeleton, and so their absence alone may not be enough to discriminate moults from carcasses. This assumption is built on the idea that because every eurypterid individual produces multiple exuviae during its lifetime but only one carcass upon death, so there is a much higher probability of finding an exuvia rather than a carcass (Braddy, 2001). This is coupled with a higher preservation potential because of a lower probability of scavengers disrupting the exuviae after deposition (Clarke and Ruedemann, 1912; Braddy, 2001). This underlying assumption has important consequences for the understanding of eurypterid ecdysial processes. Tetlie et al. (2008), who take a quantitative approach to studying moulting patterns in a large sample of eurypterids from the Bertie Waterlime, began with the assumption that all specimens are empty exuviae and that the large number of complete and intact specimens must be indicating that eurypterids exuviae "behaved like horseshoe crab exuviae" (Tetlie et al., 2008; pg. 188) with ecdysial sutures closing after egress, leaving little evidence that moulting has taken place. An alternative, possibly simpler, hypothesis could be that the Bertie eury-pterid(s) represent a mix of carcasses and moulted exuviae.

The quantitative study of Eurypterid assemblages from the Bertie Waterlime was based on completeness estimates and counts of specimens showing characteristic combinations of body segments (Tetlie et al., 2008), making it possible to describe the moulting process in *Eurypterus*. This begins with the opening of suture joints between the ventral plates and the marginal carapace suture, followed by the separation of the head and first opisthomal segment as a single unit, after which the animal egressed anteriorly (Fig. 5) (Tetlie et al., 2008, fig. 10). This was based on an abundance of *Eurypterus remipes* assemblages that showed the cephalic carapace and first opisthomal segment together (228 specimens), however many specimens also showed the carapace in isolation (172 specimens), with the first 2 opisthomal segments (34 specimens), or rarely with breaks elsewhere along the opisthoma, indicating that there was variation in where this suture opening formed, even within one species. Variation must also exist in moulting behaviour between species, as indicated by comparison with adelopthalmid eurypterids from the Dinas estate in Brecon, Wales in the collections at the OUMNH (Fig. 5D–F). Moult ensembles of these eurypterids show a break between the cephalic carapace and the rest of the opisthomal segments during moulting (Fig. 5D), with many carapaces found in isolation without attached opisthomal segments (Fig. 5E–F).

The novel analytical approach employed by Tetlie et al. (2008) allowed for comparison with the moulting modes in aquatic chelicerates and scorpions, in line with a chelicerate affinity for the eurypterids. Further study is needed to identify if ecdysial mode provides insight into the phylogenetic uncertainty for the position of eurypterids within Chelicerata, namely whether they are most closely related to arachnids (Weygoldt and Paulus, 1979; Shultz, 1990, 2007; Kamenz et al., 2011; Lamsdell, 2013), and the scorpions in particular (Kjellesvig-Waering, 1986; Dunlop and Webster, 1999) or the more traditional view of being sister taxa with the xiphosurans (Woodward, 1867, 1872; Strømer, 1944, 1955) in Merostomata, which has also received recent support (Garwood and Dunlop, 2014). *Eurypterus* shares in common with modern *Limulus* the ventral anterior location of the prosomal suture and the tendency of the ecdysial suture to close after egress (Loveland, 2002; Shuster and Sekiguchi, 2004; Tetlie et al., 2008), but also shows similarity to scorpion ecdysis in that the more anterior opisthomal segments remain attached to the cephalic carapace during formation of the anterior ecdysial opening (Tetlie et al., 2008). Further documentation of ecdysial characters for eurypterids, and their inclusion in phylogenetic analyses may help resolve affinity disputes related to the position of these fossil chelicerates.

Tetlie et al. (2008) also attempted to determine if the eurypterid(s) were moulting prone or supine (as seen in modern scorpions). Although evidence for supine ecdysis in the prevalence of ventral-up specimens in other collections had been reported (Tollerton, 1997), Tetlie et al. (2008) did not identify a statistically significant difference in the number of ventral-up versus dorsal-up specimens, and interpreted this to mean that *Eurypterus* could moult in either position. It seems particularly difficult to identify eurypterid moulting orientation given the possibility that exuviae could have been reoriented by currents or other post-depositional processes (Tollerton, 1997; Tetlie et al., 2008). Given the conserved moulting behaviour within closely related modern scorpions, with different taxa consistently moulting either prone or supine (Rosin and Shulov, 1962; De Armas, 1986; Gaban and Farley, 2002), it seems unlikely that the eurypterids had an "absence of preferred dorsal or ventral moulting posture" (Tetlie et al., 2008; pg. 189). Rather, it could be that the data currently available do not allow us to determine this quantitatively. Some scorpion taxa begin moulting in the prone position, but flip over onto the dorsal surface once the legs are freed to continue moulting in the supine position (Gaban and Farley, 2002). Given that the majority of the ventral-up specimens are relatively incompletely preserved, and so were more likely to have been affected by post-depositional reorientation, the data of Tetlie et al. (2008) seem to suggest that moulting in the supine position may not have occurred in *Eurypterus*, or at most was occupied only very briefly during ecdysis.

Eurypterids form an exemplary case study for examining the evolutionary demands exerted on morphology by the ecdysial process. In eurypterids, mode of moulting may have imparted constraints on the size ratio of morphological features, owing to the necessity of large morphological features to pass through narrow spaces during egress (Tetlie et al., 2008). This is at odds with the ecological or functional morphological pressures to enlarge features such as the swimming paddles and telson (for greater lift, thrust and manoeuvrability during swimming) and the chelicerae (for increased predatory capability).

2.3. Other chelicerata

Non-eurypterid chelicerates generally have a poor fossil record of moulting, and this is particulary true for the marine groups. In pycnogonids, moulting changes with instars during development owing to substantial change in morphology, and they exhibit determinate growth such that they cease moulting after reaching adulthood (Nakamura, 1981; Tetlie et al., 2008). The thin cuticle and tendency to fragment means that all pycnogonid fossils have been interpreted as carcasses, not moults (Siveter et al., 2004; Tetlie et al., 2008). Horseshoe crabs (Fig. 1) also stop moulting at adulthood (Tetlie et al., 2008), so any moult of a horseshoe crab in the fossil record would have to represent a juvenile individual. Given that xiphosurans moult by exiting anteriorly through a wide suture along the prosoma that immediately closes after egress, the probability of distinguishing a xiphosuran exuvia is low (Babcock et al., 2000; Tetlie et al., 2008). It is also difficult to determine if fragmentary assemblages represent moults or carcasses that have disarticulated, particularly given the tendency for horseshoe crabs to moult in shallow, marginal marine environments with low

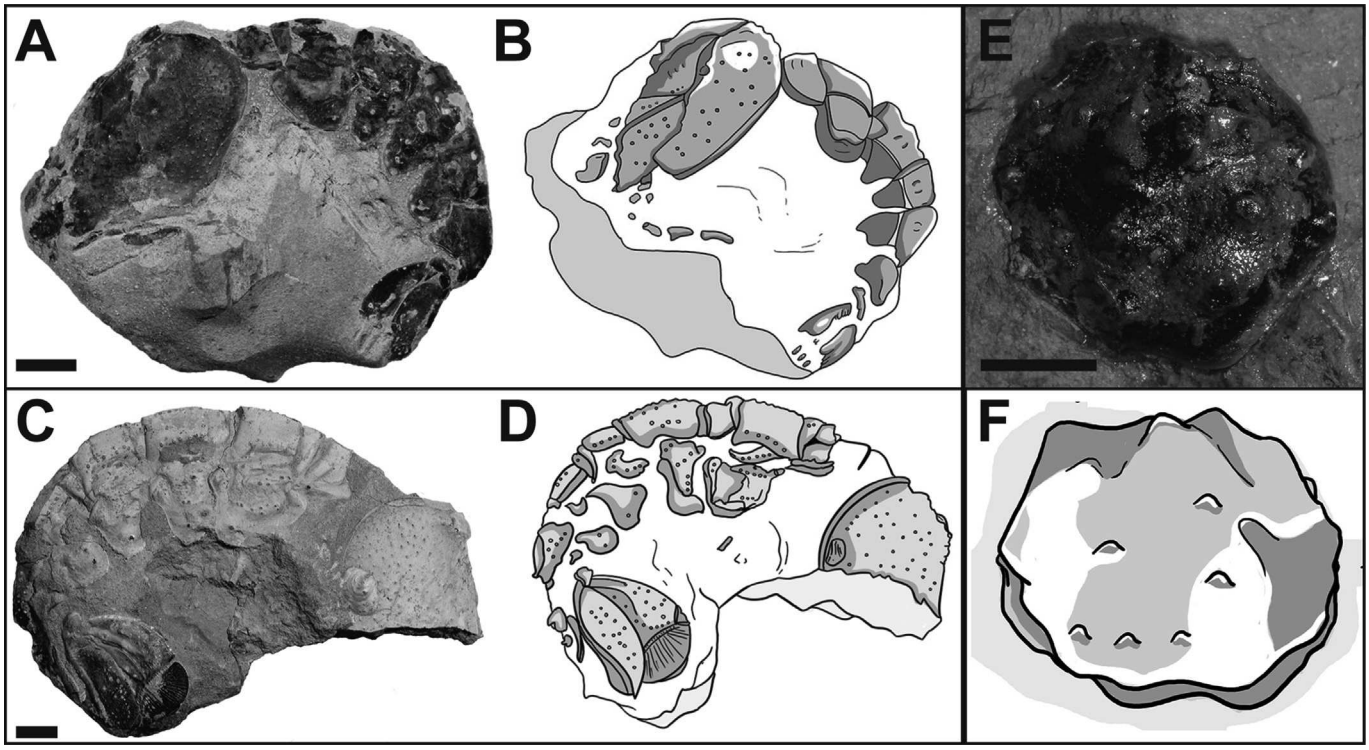


Fig. 6. Moulting in decapod crustaceans. A–D: Photographs and sketches of *Meyeria magna* from the Lower Greensand of Atherfield Clay, Isle of Wight, showing typical "Lobster Open Moult" position, with carapace displaced from the abdomen. A–B: OUMNH K.756. C–D: OUMNH K.755. E–F: Photograph and sketch of *Necrocarcinus labeschii* from the Cretaceous Gault Clay, Folkestone, UK, with disarticulated carapace broken along the pleural sutures. OUMNH K.64003. Scale bars 5 mm. Drawings by J. Bevis.

preservation potential and high energy (Babcock et al., 2000; Rudkin and Young, 2009).

Of the terrestrial chelicerates, the scorpions have the best fossil record of moulting, partly because they possess an extra proteinaceous hyaline layer to their cuticle not present in other arthropods, which is thought to increase fossilisation potential (Selden and Jeram, 1989; Jeram, 2001; Gaban and Farley, 2002). Kjellesvig-Waering (1986) concluded that most scorpion fossils are moulted exuviae rather than carcasses, based on highly detailed examinations of numerous scorpion fossils. These exuvial fossils typically include booklung lamellae, pectines with sensory pegs, and bristles, hairs and sensilla on other parts. Taphonomic experiments conducted on modern scorpions moults and carcasses have established a set of criteria for distinguishing between them (McCoy and Brandt, 2009), and when applied to a collection of Late Silurian and Middle Pennsylvanian scorpions, approximately 89% met the criteria of being fossil moults. This supports the qualitative observation of Kjellesvig-Waering (1986) on the abundance of moults in the scorpion fossil record. The four main criteria for identifying fossil scorpion moults are (1) splayed walking leg posture; (2) distinctive position of the pedipalps with chelae angled inwards toward each other; (3) a curved body line; and (4) extended chelicerae (McCoy and Brandt, 2009). More fragmentary scorpion material was also thought to be derived from exuviae because they are delicate and easy to disperse (Wills, 1959; Jeram, 2001), however the taphonomic experiments of McCoy and Brandt (2009) showed that there is no significant difference in the time required for moults to disarticulate as compared to carcasses. For fossil scorpions, anatomical features related to the positioning of body elements during ecdysis are more useful for identifying moults than examination of the degree of exoskeleton disarticulation. Other examples of moulting in the scorpion fossil record include a specimen of *Compsoscorpius buthiformis* with distortion of the carapace thought to have occurred during ecdysis (Kjellesvig-Waering, 1986), and a tritonyph of a Cheliferoidea pseudoscorpion from the Lower Cretaceous amber of Archingeay, France, which was found partly enclosed in a layer of silk and is thought to represent the first fossil record of a moulting nest (Judson, 2009).

Other arachnid fossil evidence of moulting is scarce, perhaps owing to the relatively poor terrestrial record of arthropods in general. The preservation of spider exuviae in amber suggest that they may have moulted in a similar manner to modern spiders, whilst hanging from silk threads, leaving the exuvia hanging in the web, as was seen for the fossil taxon *Amaurobius succinic* (Petrunkevitch, 1942). Exuviae of this taxon were identified as such based on the presence of broken appendages and general disarticulation. The Devonian *Palaeocteniza crassipes* from the Rhynie Chert is thought to represent a moulted specimen, owing to its general condition of being crumpled and folded, the presence of a detached carapace in close proximity to the rest of the specimen, and the absence of the distal portions of the palps (Selden et al., 1991). The Permian spider *Permarachne novokshonovi* from the Ural Mountains, Russia, is also thought to represent a moulted exuvia owing to general displacement of the specimen and lateral orientation of the collapse chelicerae on top of one another (Eskov and Selden, 2005).

2.4. Crustaceans

The crustacean (Fig. 1) fossil record provides much insight for understanding ecdysis in this group of arthropods. The decapod crustacean moulting fossil record is best known and is described in detail in Section 2.4.2. However, even the earliest fossil record of the crustaceans contains information on moulting. Three-dimensionally preserved phosphatised fossils from the Upper Cambrian Orsten fauna of Sweden largely represent individual carcasses of larvae and adult arthropods <2 mm in size (e.g. Waloszek, 1993, 2003;

Waloszek and Maas, 2005; Hughes et al., 2008), and the occasional specimen found with a wrinkled and shrivelled appearance may represent an individual that died during ecdysis or shortly afterwards during the phase when the freshly moulting cuticle has not yet hardened (Müller, 1979; Müller and Waloszek, 1985). Additionally, the presence and position of moulting sutures in the Orsten fossils has proved useful for deciphering the affinity of enigmatic taxa, as in the identification of head shield sutures as one of the synapomorphic characteristics placing *Cambropycnogon kalusmuellerl* with Pycnogonida (Waloszek and Dunlop, 2002). The ontogenetic patterns and number of moulting stages in the Orsten bradoriid *Rehbachiella* were used to help identify its affinity within Branchiopoda (Waloszek, 1995).

The Bradoriids are another group of Early Palaeozoic taxa for which information is known about their moult stages. These small marine arthropods are characterised by a bivalve carapace, and were abundant in the fossil record in the Cambrian to early Ordovician (Shu et al., 1999; Duan et al., 2013). Their affinity has been debated, but they probably represent either stem or crown group crustaceans (e.g. Hou et al., 2010). The abundance of bradoriid bivalved carapaces in the fossil record, and the lack of obvious suture lines anywhere on the body, suggest that bradoriids may have moulted by directly shedding the old valves. The moulting behaviour and ontogeny of two supposed bradoriids from Australia were examined and found to be taxonomically informative (Zhang, 1987). A quantitative analysis of the size of *Phaseolella dimorpha* carapaces showed that these fossils do not segregate into well-defined size categories, as would be expected for a periodically moulting individual, suggesting that this taxon could actually belong to another group of bivalved arthropods, Phosphatocopida, which also produced moults continuously and at short intervals during their lives (Müller, 1979). *Phaseolella dimorpha* has since been synonymised with *Dabashanella hemicyclica* and placed withing the Order Phosphatocopida (Hou et al., 2001). On the other hand, *Houlongdongella xichuanensis* shows at least nine distinct moulting stages (Zhang, 1987), in a manner that seems typical for the bradoriids, with other fossil taxa such as *Kunmingella douvillei* reported to have at least five distinct moulting stages (Zhang, 2007; Duan et al., 2013).

Ostracods are the most abundant arthropod in the fossil record and thousands of species have been identified from their characteristic bivalved carapaces, the valves of which populate the fossil record from the Ordovician onwards (Siveter et al., 2003). Soft parts are extremely rare (Siveter et al., 2003, 2014) and it is difficult to distinguish fossil moults from decayed carcasses. Ostracods show determinate growth and moult up to nine times in total during their life (Turpen and Angell, 1971). Given the abundance of ostracod valves in the fossil record, it is possible to reconstruct the ontogeny and development of fossil taxa using their different moult stages (e.g. Shaver, 1953; Tinn and Meidla, 2003). Removal of the valves during ostacod moulting is usually complete (Turpen and Angell, 1971), however an enigmatic group probably belonging to Ostracoda, the Eridostracina, show an unusual moulting physiology and valve morphology (Olempska, 2012). These Middle Ordovician to Middle Carboniferous aged crustaceans produce a multi-layered calcified carapace by retaining unshed moults during growth and development. Successive newly deposited cuticles are larger than the previous carapace, such that they are visible on the external surface as growth bands. Retention of moults is thought to strengthen the carapace, while the characteristic number and arrangement of retained moults has useful taxonomic relevance (Olempska, 2012). Other arthropods exhibiting retention of moults include the spinicaudatan (conchostracan) branchiopod crustaceans, which are found in the fossil record from sites such as the Upper Carboniferous deposits of France and Ireland (e.g. Orr and Briggs, 1999; Vannier et al., 2003; Orr et al., 2008) and the Lower Devonian Willwerath Lagerstätte of Germany (Gross, 1934).

Early Devonian branchiopods such as *Lepidocaris rhyniensis* and *Castracollis wilsonae* from Rhynie Chert likely represent exuviae, whereas the oldest notostracan from the Upper Devonian Strud Locality, *Strudops goldenbergi*, likely represent whole body carcasses (Lagebro et al., 2015). This was determined based on the presence of a gut and its content, and owing to the intact nature of the body parts in most specimens. The preservation potential of notostracans have been studied using experiments where both carcasses and empty moults of modern notostracans were observed during decay under controlled conditions (Hegna, 2012). It was concluded that a carcass does come to resemble a moulted exoskeleton if decay is allowed to proceed to a certain point. These experiments confirmed that the presence of a gut can be used as evidence of a carcass, as can an undisturbed position for the thoracic appendage, abdominal segments and telson (Hegna, 2012).

2.4.1. Isopod crustaceans

The rest of the crustacean fossil record of moulting is focused on Mesozoic specimens, with little input from the fossil record of the later Palaeozoic. Decapods form the bulk of the crustacean ecdysis fossil record during the Mesozoic (Section 2.4.2), but it is also important to consider moulting when examining the fossil record of isopods. Living isopod crustaceans exhibit biphasic moulting, with the posterior part being moulted as an intact unit several days before the fragmentary moulting of the anterior carapace. Given that the majority of fossil isopods are of the posterior region of the body only, it is thought that they represent empty moults (Wieder and Feldmann, 1989; Feldmann, 2009). The near absence of anterior sclerites is likely owing to the fragmentation of these structures during moulting, such that the head, various limbs and the thoracic segments are disarticulated from each other. This fragmentation may be contributing to the poor fossil record of this group as a whole, leading to taxonomic confusion, as the posterior regions tend to be similar between separate species and it is likely that many species named only from posterior parts could be incorrectly included in the same fossil genus (Wieder and Feldmann, 1989). Only unusually are isopods found with anterior and posterior sections intact, such as in a single specimen of *Palaega goedertorum* from the late Oligocene Pysht Formation in Washington State (Wieder and Feldmann, 1989), some Early Cretaceous specimens including *Archaeoniscus aranguthyorum* from the Tlayúa Formation in Mexico (Feldmann et al., 1998), and a scavenging assemblage of complete *Brunnaega tomhurleyi* from the Toolebuc Formation of Queensland, Australia (Wilson et al., 2011). Another single specimen of the cirolanid isopod *Cirolana garassinoi* is thought to have been preserved in the pre-moult condition owing to the presence of three pairs of dermoliths (sites of calcium and phosphorous storage during the pre-moult condition of modern isopods) and a thin, apparently transparent cuticle (Feldmann, 2009). This shows a highly specific moulting behaviour in a fossil that had previously only been known from living taxa.

2.4.2. Decapod crustaceans

The decapod fossil record is particularly good owing to their heavily calcified skeletons, with lobsters and crabs having a greater abundance of fossilised moulted exuviae as compared to crayfish/shrimp, owing to the tendency of the latter to eat their own exuviae after moulting (Bishop, 1986). Moulted exuviae are so readily identifiable and abundant at many localities that it is generally thought that they may be biasing the fossil record of decapod crustaceans by inflating their overall numbers (Schäfer, 1972). Sure moults in the fossil record could outnumber carcasses by about five to one for decapod crustaceans (Mertin, 1941).

Many decapod exuvia fossils are preserved in a characteristic assemblage known as 'Open Moult Position' (or Salterian Position, or Salter's Position), which varies slightly for crabs and lobsters. In extant lobsters, moulting begins with the separation from the first abdominal segment, followed by a dorsal splitting of the carapace along the median line, through which the animal emerges, leaving a characteristic exuvial arrangement with the carapace displaced nearly 90° to the abdomen (Glaessner, 1969). The Lobster Open Moult Position refers to exuviae preserved on the side, with the carapace split medially and raised posteriorly relative to the abdomen (Fig. 6A–D), as is seen in moults of *Oncoparia*, *Linuparus* and *Hoploparia* from the Late Cretaceous Carlile fauna (Bishop and Williams, 1986) and *Oosterinkia neerlandica* from Middle Triassic Anisian sediments of the Winterswijk quarry in the Netherlands (Klompmaker and Fraaije, 2011). A specimen of the fossil lobster *Meyeria pueblaensis* from the Aptian deposits of the San Juan Raya Formation in Mexico is preserved in the Lobster Open Moult Position with its walking legs displaced; its identification as a moult is reinforced by its fully compressed preservation, and the fact that it exhibits less morphological detail on the carapace than carcass specimens from the same site (Feldmann et al., 1995). Another species of this genus, *Meyeria magna*, is also preserved in the characteristic Lobster Open Moult Position (González-León et al., 2014) (Fig. 6A–D). Other taxa preserved in this position include *Palaeohomarus* from the Upper Cretaceous, and *Glyphea* and *Protaxius* from the Jurassic (Glaessner, 1969).

When a crab moults, the carapace splits open along the posterior and sides (the pleural sutures), hinging near the mouth such that the carapace is pushed upwards and forward as the animal exits posteriorly. This Open Moult Position can be recognised in the fossil record by the carapace making an angle of approximately 90° to the sternum, and the splitting of the carapace along the pleural sutures such that parts of the carapace outside the pleural sutures remain attached to the sternal plastron (Fig. 6E,F). The crab *Carcineretes planetarius* is found abundantly as moults with articulated appendages in the Maastrichtian deposits of the Ocozocoautla Formation in Chiapas, southeastern Mexico (Vega et al., 2005). Other crab taxa showing Open Moult Position include *Ranina*, *Notopocorystes*, *Coeloma*, *Potamon* and *Macrophthalmus* (Glaessner, 1969; Bishop, 1972, 1986).

Given that the articulation between the cephalothorax and the abdomen tends to be a particularly weak joint, it had also been suggested that the arrangement of fossil lobsters in Open Moult Position alone was not enough to definitively identify the specimen as a moult (Bishop, 1986), particularly as it had long been thought that fossil crustacean cuticles did not preserve structures distinguishing between exuviae and unmoulted carapace (Glaessner, 1969; Schäfer, 1972). However, detailed examination of the cuticular ultrastructure of fossil specimens of *Hoploparia stokes* from the Maastrichtian of the Lopez de Bertodano Formation on Seymour Island, Antarctica, showed that moults can be distinguished from carcasses (Feldmann and Tshudy, 1987). Owing to reabsorption of the cuticle prior to moulting, exuviae are characterised by deterioration or complete loss of lamination in the inner endocuticle only (as compared to loss of laminations in other regions of the cuticle reflecting diagenetic alteration). These fossil moults tend to be extremely well preserved as compared to carcasses, probably owing to a higher rate of disturbance to the carcasses from post-mortem scavenging. Based on this ultrastructure, Feldmann and Tshudy (1987) were able to conclude that specimens found in the Salterian position were indeed moults, as were many of the more fragmented specimens. Other specimens from Antarctica have been identified as moults, such as *Metanephrops jenkinsi* from the Cretaceous and Paleocene of Seymour Island, which is preserved in the Salterian position (Feldmann, 1989), and a single specimen of *Antarctidromia infalta* from the early Miocene of Cape Melville, in which the abdominal structures are articulated but rotated out of their life-plane and remain attached to the cephalothorax only in a small region (Feldmann and Gaździcki, 1998).

Another characteristic moult assemblage for decapod crustaceans consists of the carapace preserved upside-down and rotated on the sternum, known as the Overturned-Carapace Moult Position (Bishop, 1972, 1986). Fossils found in this Position include specimens of the crab *Dakotiocancer overanus* from the Upper Cretaceous Pierre Shale in South Dakota (Bishop, 1972). Specimens of the fossil lobsters *Pseudoglyphea* cf. *P. spinosa* and *Clytiopsis argentoratensis* from Middle Triassic Anisian sediments of the Winterswijk quarry in the Netherlands preserve the cephalothorax upside down and separated from the abdomen, with occasional splitting of the cephalothorax along the median line (Klompmaker and Fraaije, 2011).

Decapod moults do not always take the form of one of the three Moult Positions described above. Specimens of *Carcineretes woolacotti* from the Maastrichtian Ocozocuautla Formation in Mexico were identified as moults based on the thickness of the carapace and displacement of the sternal plastron. This collection also includes six pairs of specimen found in close association, consisting of a smaller moult and a larger carcass, interpreted as a freshly moulted individual preserved with its recent exuvia (Vega et al., 2001). In all three pairs, the carcass was approximately 16% larger than the exuvia. Several specimens of the crab *Dakoticancer australia* from the Maastrichtian Difunta Group in Mexico are thought to represent moults owing to a lack of carapace material and preserved appendages, and displacement of the sternal plate (Vega and Feldmann, 1991). Similar sternal plate dislocation was used to determine that *D. australis* specimens found articulated in burrows at a nearby locality also represent moults, perhaps indicating a behaviour for these crabs of moulting in their burrows (Vega and Feldmann, 1991). The crab *Longusorbis cuniculosus* from the Upper Cretaceous Spray Formation of Vancouver Island, British Columbia, was found associated with filled burrows and encased in burrow-fill lithology, from which it was suggested that the specimens may have dug these burrows and moulted in enlarged terminal chambers (Richards, 1975). A specimen of carcineretid crab from the Late Cretaceous Point Loma Formation of San Diego, California has also been described in the terminal chamber of an burrow, but it is unknown if this specimen represents a moult or a carcass (Bishop, 1972). Additionally, specimens of the mud lobster *Thalassina* from Pleistocene deposits in northern Australia, New Guinea and Japan (Förster and Barthel, 1978; Murray and Hanley, 1986; Bishop and Williams, 2005) preserved in Lobster Open Moult Position have been found in the ends of tunnels, suggesting they may also moult in their burrows.

Polz (1995) reported the discovery of a fossil phyllosoma (lobster larval stage) being caught in the act of moulting, based on the presence of larval and "post-larval" features in the same specimen. Haug et al. (2009) alternatively suggest that this specimen, and another possible scyllarid larva, could actually be very late larval stages that already exhibit some "post-larval" characters. These specimens therefore need not represent crustaceans preserved mid-moult.

2.5. Hexapods

Moulted exuviae have been found in the hexapod fossil record. These empty moults may have a shrivelled appearance indicating partial desiccation, or deformations in the head or thorax. The exuvial fissure is usually located dorsally in the anterior region of the body (Rasnitsyn, 2002). These may also have a "composite nature" (Kukalova, 1968; pg. 311) owing to the dorsal and ventral surfaces pressing together during preservation such that impressions of structures from both surfaces are superimposed onto both

part and counterpart. Moulted cuticles identified in the literature include specimens of the mayfly nymph *Protereisma* from the Lower Permian of Oklahoma, USA, and Moravia, Czech Republic (Kukalova, 1968); a single specimen of the palaeodictyopteran *Lycocerus goldenbergi* from the Upper Carboniferous shales of Commentary, France (Kukalova, 1969; Kukalova-Peck, 1975); as well as numerous specimens of the mayfly *Ephemeropsis melanurus*, the beetle *Coptoclava longipoda* and the dragonfly *Hemeroscopus baissicus* in the Early Cretaceous of Baissa in Siberia (Rasnitsyn, 2002). A *Coptoclava* beetle specimen from the last locality has even been preserved mid-moult (Rasnitsyn, 2002, fig. 24). These published occurrences of fossil insect moults are preserved as compressions and impressions on fine-grained sediment, rather than preserved in amber.

The preservation of abundant moults in the insect fossil record could be effecting the ecological interpretation of assemblages by biasing the size and frequency distributions. In the lacustrine assemblage of the Early Cretaceous locality at Baissa, Siberia, where moults are abundant, dragonfly fossils are found two to ten times (and occasionally over 100 times) more frequently than beetles. Given that the assemblage is dominated by moulted exuviae, this discordance in abundance is likely reflecting the fact that dragonflies moult several more times during their lifetime than do beetles, rather than indicating that dragonflies were actually more abundant (Zherikhin, 1997; Rasnitsyn, 2002). Taphonomic experiments on extant decapod crustacean assemblages have suggested that a large abundance of empty moults in an assemblage can greatly affect the size-frequency distributions of samples in comparison with living populations, with moults outnumbering carcasses by 15:1 under some mortality regimes (Hartnoll and Bryant, 1990).

2.6. Other arthropods

The Cambrian record of soft-bodied taxa preserved in fossil lagerstätten such as the Burgess Shale have yielded abundant arthropod fossils, some of which help illuminate the early fossil record of ecdysis. The best example of a fossil preserved mid-moult is seen in a single specimen of the extremely abundant arthropod *Marrella splendens* (García-Bellido and Collins, 2004). An ecdysial suture is present at the anterior end of the head shield, through which the individual can be seen emerging (Fig. 7C). The wide lateral spines are folded inwards and backwards, indicating it was softer than usual during egress. Given the placement of this taxon in the stem lineage to mandibulata (Legg et al., 2013), its ecdysial behaviour can help identify the plesiomorphic condition for crustaceans and hexapods.

Other examples of arthropod moulting in the Cambrian fossil record are more circumstantial. For the trilobite-like arthropod *Naraoia* from the Chengjiang biota in China, the presence of isolated appendages, head shields, and pygidium elements are thought to indicate post-depositional disarticulation, possibly in connection with moulting (Hou et al., 1991). A single incomplete and partially degraded specimen of the stem lineage chelicerate *Sidneyia* from the Burgess Shale is preserved surrounded by numerous specimens of the scavenging priapulid-like worm *Ottoia* (Bruton, 2001). It was suggested that this assemblage could represent a dead, freshly moulted individual of *Sidneyia* that was providing a food source for the *Ottoia* worms (Bruton, 2001), although upon re-examination Vannier (2012) pointed out that the carcass appears quite decayed, making it difficult to ascertain if it really was a freshly moulted individual. *Sidneyia* specimens typically preserve guts, digestive glands and abdominal pockets in phosphate, indicating that specimens are carcasses rather than empty moults (Zacaï et al., 2016). A third example of possible moults from the Burgess Shale includes the highly disarticulated assemblages of the cephalic structures of the anomalocaridid *Hurdia*. This possible predator has a head bearing numerous partially sclerotized structures, including an oral cone with many plates, a pair of segmented appendages, a triangular central head shield element, and two lateral head shield elements (Daley et al., 2009, 2013). Disarticulated assemblages of these sclerotised head structures are found in consistent orientation in many specimens, suggesting that they may represent moult ensembles (Daley et al., 2013; pg. 749).

Large fossil surfaces covered in numerous empty moults of the stem-lineage arthropods *Canadaspis* and *Alalcomenaeus* from the Burgess Shale are thought to be indicative of mass moulting behaviour (Fig. 8A,B,F). This synchronized moulting is similar to that seen in a wide variety of modern crustaceans, insects and arachnids (Haug et al., 2013). The reasons for synchronized moulting are discussed in Section 2.8. A notable Cambrian mass moulting locality is the Mount Stephen Trilobite Beds of the Burgess Shale, where abundant trilobite exuviae (Fig. 8D) are found associated with numerous frontal appendages of the Cambrian apex predator *Anomalocaris canadensis* (Fig. 8C). It has been suggested that this predator was taking the opportunity of the mass moulting event to feast on the freshly moulted trilobites before their exoskeletons had the chance to completely harden after moulting (Rudkin, 1979, 2009).

2.7. Other ecdysozoans

The moulting fossil record of other ecdysozoans (Fig. 1) is mostly confined to the Cambrian, with specimens of lobopodians, Loricifera, palaeoscolecids and Priapulida providing insight into moulting behaviour in non-arthropod ecdysozoans. Sub-fossil Holocene moults from tardigrades have also been described (Gibson et al., 2007). The other ecdysozoan phyla Kinochyncha, Nematoda, and Nematomorpha have a very poor (or non-existent) fossil record generally, such that the odds of finding moults are low.

2.7.1. Loriciferans

As one of the most recently discovered metazoan phyla (Kristensen, 1983), it is not surprising that the fossil record of Loricifera is sparse and poorly known. These tiny marine animals live in the sediment inside a protective outer case called a lorica. The only plausible palaeontological record of loriciferans consists of the macrofossils *Sirilorica carlsbergi* and *Sirilorica pustulosa* from the early Cambrian Sirius Passet biota of Greenland (Peel, 2010). Although it is the less common of the two species, a single specimen of *S. pustulosa* has been preserved in the process of moulting (Peel et al., 2013). A nearly complete animal, including its anterior thorax with denticles, the loricate abdomen, and part of the anal field, is seen emerging from an opening between the anterior and posterior plate circlets of the lorica of the exuvia (Fig. 7A). This is exactly the same moulting behaviour observed in the modern loriciferan taxon *Rugiloricus* (Higgins and Kristensen, 1986; Peel et al., 2013). The fossil specimen is thought to represent a moult not only because of this orientation, but also because of the distorted and ruptured exuvial lorica, and the lack of an introvert in the exuvia.

2.7.2. Lobopodians

Lobopodians are soft-bodied Cambrian animals with an annulated body bearing paired limbs, distinguished by a variety of specialised appendages anteriorly and dermal sclerites along the body (Chen et al., 1995; Liu and Dunlop, 2014; Ma et al., 2014). As an abundant fossil of the Cambrian, studies of their phylogenetic affinities are revealing them to be early steam lineage members of both Arthropoda and Onychophora (Smith and Ortega-Hernández, 2014). Their fossil record consists of both full-body preservation in Cambrian lagerstätten such as the Chengijang Biota in China and

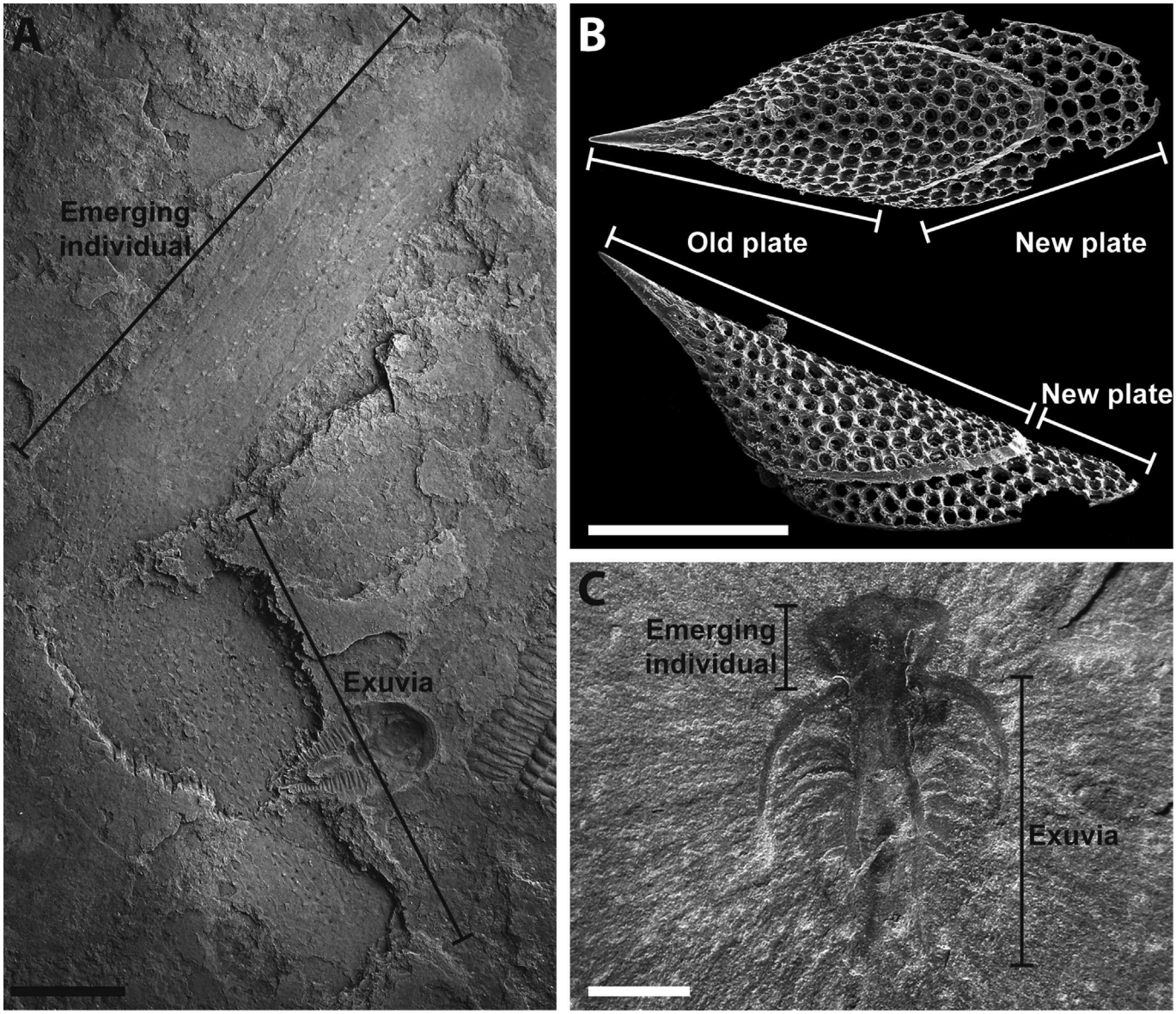


Fig. 7. Rare examples of Cambrian fossil preservation during ecdysis. A: The stem-lineage loriciferan *Sirilorica pustulosa* from the lower Cambrian Sirius Passet fauna of North Greenland, with a compressed specimen emerging from an exuvia. Scale bar 10 mm. Photo credit: J. Peel (Peel et al., 2013). B: Conjoined plates of the lobopodian *Onychodictyon* sp. from the lower Cambrian of North Greenland in plan (top image) and lateral (bottom image) view. The new, larger plate underlies the smaller, older plate. Scale bar 500 μm. Photo credit: T. Topper (Topper et al., 2013). C: The arthropod *Marrella splendens* from the middle Cambrian Burgess Shale. Scale bar 5 mm. Photo credit: D. García-Bellido (García-Bellido and Collins, 2004).

the Burgess Shale in Canada, and of sclerotized dermal sclerites that make up a large proportion of the mineralised microfossil record. A single body specimen of the xenusiid lobopodian *Hadranax* from the Sirius Passet biota of North Greenland was suggested to be a moulted exuvia because of its distorted preservation, and it has been suggested that xenuxian lobopodians in general shed their cuticle through a mid-ventral split in the body (Dzik and Krumbiegel, 1989; Bergström and Hou, 2001). However, Zhang and Aldridge (2007) considered these specimens as inconclusive evidence of ecdysis.

The dermal sclerites of lobopodians provide much more information on ecdysis in this group. These help illustrate lobopodian ontogeny, with the identification of discrete instars showing characteristic changes in morphology during growth, suggesting that lobopodians moulted as they grew, rather than having incremental growth (Zhang and Aldridge, 2007). The lobopodian sclerite fossil record also reveals specimens thought to have been preserved just prior to moulting, before the old sclerite was shed from the body. These conjoined specimens consist of a larger plate attached to the underside of a smaller plate, with the larger being in the range of 13–20% for *Microdictyon sinicum* from the Chengjiang Biota (Chen et al., 1995), and 24% in *Onychodictyon* sp. from North Greenland (Fig. 7B) (Topper et al., 2013). In *Microdictyon*, at least five full body specimens show multiple conjoined plates along the body of the lobopodian (Chen et al., 1995). Rare conjoined plates have also been found in large collections of isolated sclerites of *Microdictyon jinshaense*, *Microdictyon chinense* and *Quadratopora zhenbaensis*, showing that this type of ecdysis behaviour, with the new plate growing in underneath the old plate, is widespread in lobopodians. No indication of reabsorption was found in these conjoined sclerite specimens, with the old one showing no sign of deformation or wrinkling as compared to the new one (Zhang and Aldridge, 2007).

A different mode of moulting has been described for the Cambrian lobopodian *Hallucigenia* from the Burgess Shale, which has sclerites in the form of appendicular claws and dorsal spines. These sclerites have a unique "cone-in-cone construction" (Caron et al., 2013; pg. 5), which consists of a stack of three to five elements that are separated from each other along a logarithmic curve and grow from a basal accretional zone (Smith and Ortega-Hernández, 2014). These have been shown to have fundamentally the same construction as the claws in the extant onychophoran *Euperipatoides* (Caron et al., 2013; Smith and Ortega-Hernández, 2014). In both *Euperipatoides* and *Hallucigenia*, internal constituent elements are future replacements of the outermost elements, which are discarded during ecdysis. After the outermost element is discarded, a new innermost element is secreted and the bases of all existing elements are extended. This represents an ecdysial behaviour that is distinct from other ecdysozoans, being quite different from the direct replacement seen in some lobpodians (Zhang and Aldridge, 2007) and from the retained exuviae in Eridostracina ostracods (Olempska, 2012) where overlying moults are retained during ontogeny and accumulate as the organisms grows, without removal by ecdysis. Owing to this shared moulting behaviour, Smith and Ortega-Hernández (2014) suggest an onychophoran affinity for the enigmatic Cambrian taxon *Hallucigenia*.

2.7.3. Palaeoscolecids

Palaeoscolecids are a lower Palaeozoic group of vermiform animals that have a widely debated affinity, but are generally aligned with Cycloneuralia (ie. non-Panarthropoda ecdysozoans), and specifically either with Priapulida or Nematomorpha (García-Bellido et al., 2013). Their cuticle was annulated and covered in rows of sclerites, fragments or elements which are often found as 3-dimensionally preserved phosphatic small shelly fossils (SSF). Complete body fossils may also show soft-bodied preservation in fine-grained shales (e.g. Burgess Shale-type localities), and one such specimen of *Wronascolex antiguus* from the Emu Bay Shale in South Australia (Cambrian Series 2, Stage 4) is thought to represent an empty moult. It is distinctive in possessing a break through its middle and a pronounced wrinkle across its width, while also being strongly compressed with weakly expressed annulations and lacking surficial relief (García-Bellido et al., 2013, fig. 3D, E). The authors thought it unlikely that the tear through the trunk represented the position of the exuvial opening, based on comparison to the location of the rupture at the base of the introvert, and not in the middle of the trunk, in the extant *Priapulus caudatus*. Palaeoscoledid material from the middle Cambrian Georgina Basin of Queensland, Australia, is preserved as 3-dimensional Orsten-type fossils, two of which show a double-layer structure of the phosphatised cuticle with sclerites (Müller and Hinz-Schallreuter, 1993, text-figs. 12B, 14C). This has been interpreted to represent individuals preserved shortly before moulting (Müller and Hinz-Schallreuter, 1993; García-Bellido et al., 2013), in a manner similar to that described in Section 2.7.2 for lobopodian dermal sclerites.

2.7.4. Priapulida

Stem lineage priapulid body fossils are relatively abundant in some Cambrian Burgess Shale-type localities, while isolated microfossils of the spines lining the pharynx and introvert are widespread in Cambrian deposits. A detailed study of body fossils of *Ottoia prolifica* from the Burgess Shale revealed that the majority of published specimens were carcasses rather than moults, as indicated by the presence of gut structures and other internal organs (Conway Morris, 1977). Moulting capability in *Ottoia prolifica* is indicated by the frequent detachment of the body wall and cuticle, visible in the fossil as a contracted area of highly reflective body wall that has pulled away from the outer cuticle, which is left in place as an unreflective area that still maintains the original body outline (Conway Morris, 1977). One specimen in particular was described as "on the threshold of moulting" (Conway Morris, 1977; pg. 11) because its anterior end appears to have withdrawn, leaving behind a dark area. The cuticle seems to have been more resistant to decay than the internal structures, and some specimens consist of a collapsed and folded outer cuticle with an absence of nearly all internal organs, except for traces of the gut, which indicate that these fossils are decayed remnants of carcasses rather than moults (e.g. Conway Morris, 1977, text-figs. 49, 52, 64). Some specimens that are exceptionally well preserved seem not to have been affected by decay at all, and these have the body wall preserved as a highly reflective film that obscures details of the internal anatomy (Conway Morris, 1977; text-fig. 67). The lack of visible internal anatomy in these specimens could lead to them being interpreted as empty moults, however consideration of contextural information such as their completeness, lack of distortion and presence of some traces of internal anatomy should prevent them from being misidentified as moults. The only specimens suggested to actually represent moults of *Ottoia prolifica* are a few specimens consisting of the anterior proboscis only (e.g. Conway Morris, 1977, pg. 31, text-figs. 47, 48), based on comparisons to the moulted cuticle of the modern taxon *Priapulus*, where the anterior proboscis is everted and detached from the specimen during moulting (Lang, 1948).

The only other published description of moulting in the fossil record of Priapulida is a specimen of *Eximipriapulus globocaudatus* from the Chengjiang biota that has been interpreted as an empty moulted cuticle, based on the complete absence of internal anatomical features and its contracted and wrinkled appearance (Ma et al., 2014, figs. 1.5a, 1.5b, 2.7). This is inconsistent with the type of specimens described as moults by Conway Morris (1977), highlighting the need for more detailed study on moulting in Cambrian priapulid worms.

2.8. Mass moulting behaviour

Ecdysis may constitute a significant social behaviour in fossil ecdysozoans. Synchronised mass moulting events have been observed in many modern crustaceans, insects and arachnids. These are variously triggered abiotically (for example circadian rhythms in aphids; Johnson et al., 1957) or through active communication (pheromones) (Haug et al., 2013). These mass moultings were succinctly reviewed in Haug et al. (2013) for extinct arthropod groups. Mass moulting assemblages include trilobites from the Cambrian (Fig. 8E) (Paterson et al., 2007; Ebbestad et al., 2013), Ordovician (Karim and Westrop, 2002) and Devonian (Speyer and Brett, 1985); the soft-bodied arthropods *Canadaspis* and *Alalcomenaeus* from the Cambrian Burgess Shale (Fig. 8A,B,F) (Haug et al., 2013); eurypterids from the Silurian (Braddy, 2001; Vrazo and Braddy, 2011); and shrimps from the Jurassic (Polz and Tischlinger, 2000), all of which consist of large numbers of empty exuviae in close proximity. These assemblages show no evidence for abiotic transport, reworking or time-averaging, and so are generally accepted to represent actual biological mass moulting events. Another example of a supposed mass moult of mantis shrimps from the Carboniferous (Schöllmann, 2004) has been shown to co-occur with complete carcasses (Haug et al., 2013) and so may not represent a mass moult assemblage.

Haug et al. (2013) also discuss the possible evolutionary functions of coordinated moulting. In extant arthropods, mating is often synchronised with moulting, particularly at the moult stage when the organism reaches sexual maturity (e.g. Kamio et al., 2014), as the associated gregarious behaviour of many individuals in close

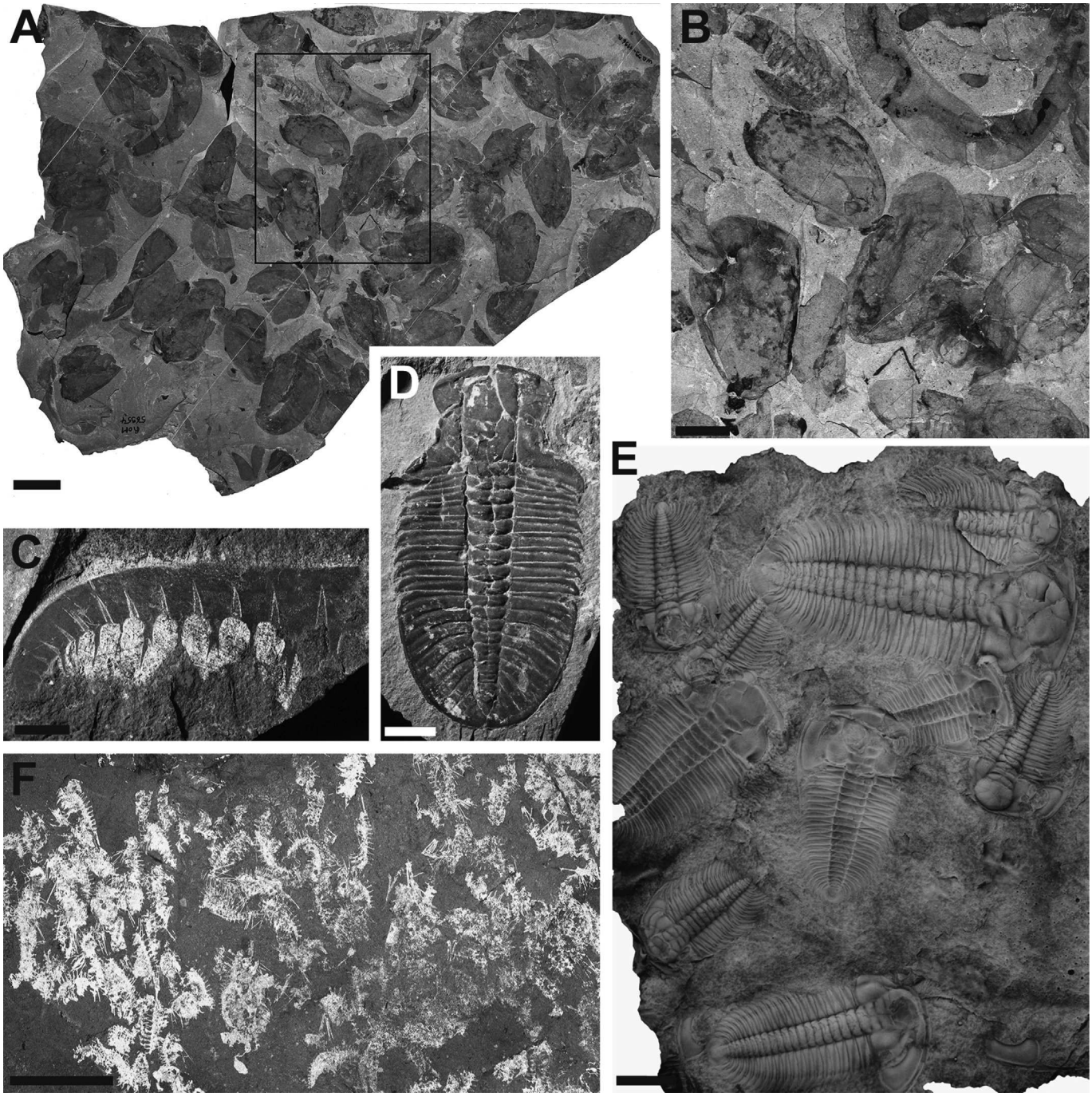


Fig. 8. Mass moulting in the Cambrian fossil record. A: Complete slab showing synchronized moulting in *Canadaspis* from the Burgess Shale. ROM 62274. Scale bar 10 mm. Photo credit: J. Haug (Haug et al., 2013). Black box indicates closeup in B. C: Frontal appendage of *Anomalocaris canadensis* found with supposed trilobite mass moulting event in the Mount Stephen trilobite beds. GSC 3418a. Scale bar 10 mm. D: Exuvia of *Ogygopsis klotzi* from mass moulting event at the Mount Stephen trilobite beds. OUMNH AT205. Scale bar 5 mm. E: Cluster of paradoxidid trilobites preserved on a mass moulting surface from Jämtland, Sweden. PMU 25711/4-6, 74–79. Scale bar 10 mm. Photo credit: J. O. Ebbestad (Ebbestad et al., 2013). F: Synchronized moulting slab of megacheiran specimens from the Burgess Shale. ROM 62275. Scale bar 5 mm. Photo credit: J. Haug (Haug et al., 2013).

proximity would decrease the predation pressure on any one individual (Haug et al., 2013). Synchronised moulting, mating and spawning in adults would also lead to synchronised development in offspring, which would then benefit from a similar reduction of predation pressure in crowded nursery habitats. This may explain trilobite (Speyer and Brett, 1985; Karim and Westrop, 2002; Paterson et al., 2007) and eurypterid (Braddy, 2001; Vrazo and Braddy, 2011) mass moult assemblages. However, Haug et al. (2013) and other researchers (for example Tetlie et al., 2008) reject this idea, although note that it may explain clusters of *Canadaspis perfecta* exuviae. The 'mass-mate-moult' hypothesis suggested for eurypterids (Speyer and Brett, 1985; Braddy, 2001), where females remain in shallow water post-mating and deposit their eggs prior to moulting, is dependent on the system of gender recognition. As pointed out by Haug et al. (2013), this hypothesis is influenced by comparisons to the extant horseshoe crab *Limulus*, which generally does not couple moulting with mating (Loveland, 2002; Shuster and Sekiguchi, 2004) even if large numbers of

individuals aggregate during mating. Despite uncertainties about the motivation behind it, the repeated occurrence of mass moulting within trilobites and other Palaeozoic arthropods suggests that this behaviour may have been common within early ecdysozoans, and even ancestral to Trilobita.

3. Quantitative analysis of the trilobite fossil record of moulting

Our review of the literature indicates that an abundance of information on moulting has been accumulating in publications on fossil ecdysozoans, with a particular focus on moulting methods within trilobites. Many of these publications are isolated reports on the taxonomy or systematics of particular groups, where identification of moult assemblages is included as part of the descriptive work, or they examine moulting behaviour within a particular trilobite group or geological time period (e.g. Whittington, 1990). Reviews examining patterns in moulting behaviour amongst all trilobites are rare (Henningsmoen, 1975; Speyer, 1990) but highly informative for examining large-scale trends in the evolution of trilobite ecdysis (Brandt, 2002). Enough published work has now accumulated that quantitative analyses are possible, and the prevalence of different moulting behaviours can be identified within trilobite Orders (Table 1) and traced throughout geological time. We amassed all published information on trilobite moulting behaviour into a database (Appendix 1) and subjected it to a series of statistical analyses designed to reveal the evolutionary patterns of this important behaviour and validate trends described qualitatively in previous publications (Henningsmoen, 1975; Speyer, 1990).

3.1. Methods

To facilitate visualisation and statistical analysis of the trends in trilobite moulting, a summary data set was produced from the literature (see Appendix 1). This scored the presence of six ecdysial behaviours preserved for a range of trilobite taxa across eight Orders from the Early Cambrian to Late Carboniferous. This included: opening of the facial sutures; removal of the rostral plate; removal of the cephalon; removal of the cranidium; displacement of the pygidium; and disarticulation along the thorax. We used Brandt (2002, Table 2) as a starting point, adding omitted descriptive literature and work published post-2002 with information about trilobite moulting. Only adult specimens specifically described as exuviae were included. A single taxon could display multiple ecdysial behaviours between or within specimens (e.g. opening of the facial sutures followed by disarticulation of the cranidium); this has been indicated when noted in the literature (Appendix 1). This produced a larger sample size of total occurrences (of ecdysial behaviours) than the total number of trilobite taxa sampled. For each entry in the database, the geological age of the specimen was determined from the publication where moulting was described, if this was easily translated to the most recent International Chronostratigraphic Chart (ICS) (Gradstein et al., 2012). For specimens where the given locality date did not correspond directly with the current Stages/Epochs of the ICS (e.g. Middle Cambrian), more recent literature or occurrence data (in the Palaeobiology Database) were consulted to place the localities in the current ICS framework and gather them into time bins corresponding to geological Periods (Fig. 10) and Series (Fig. 11).

Graphs created in R (R Development Core Team, 2008) display the variation in occurrence of the six different ecdysial behaviours between trilobite Orders and through geological time (Figs. 9–11). Chi^2 analyses were performed in PAST3 (Hammer et al., 2001) for each behaviour using the observed data paired with expected numbers of observations. Uneven sample sizes were corrected for in calculating the expected observations. Agnostida (n = 1) was removed for analyses testing the differences in mode of ecdysis between trilobite Orders. We tested H_0 = no significant difference in ecdysial behaviour across geological Periods or Series, or between the trilobite Orders.

Specimens were photographed by a Canon EOS 500D digital SLR under incident and polarised lighting. The camera was fitted with a Canon EF-S 60 mm Macro Lens, and remotely operated by the EOS Utility 2.8.1.0 program. Adobe Photoshop and Illustrator CS6 were used to make minor adjustments to the exposure of the images and removed unwanted background, and to make figures.

3.2. Results

Fig. 9 shows clear differences in ecdysial behaviour between and within the different trilobite Orders (summarised in Table 1), providing initial support for a link between phylogeny and moulting in Trilobita. Phacopid taxa show a significantly higher occurrence of cephalon removal during ecdysis ($p = 5.8 \times 10^{-7}$, Table 2) as compared to other moulting methods, although facial suture utilisation is also prevalent. Opening of the facial sutures during ecdysis, usually accompanied by removal of the librigenae, was the prevalent behaviour in all Orders excepting Phacopida (Fig. 9A).

Table 2
Chi^2 and p-values testing for variation in the six trilobite ecydsial behaviours, with significant results shaded in grey.

	Rostral plate removed	Facial sutures opening	Cephalon removed	Dislocation along thorax	Pygidium displaced	Cranidium removed
Geological Periods (Cambrian-Devonian)	$\chi^2 = 12.8$ p = 0.0084 n = 19	$\chi^2 = 7.77$ p = 0.051 n = 56	$\chi^2 = 29.1$ p = 2.18E-06 n = 33	$\chi^2 = 2.1$ p = 0.552 n = 13	$\chi^2 = 5.35$ p = 0.148 n = 13	$\chi^2 = 3.11$ p = 0.375 n = 22
Geological Series (Cambrian Series 2-Late Ordovician)	$\chi^2 = 23.4$ p = 0.00061 n = 21	$\chi^2 = 3.83$ p = 0.7 n = 49	$\chi^2 = 25.1$ p = 0.000328 n = 21	$\chi^2 = 5.4$ p = 0.494 n = 12	$\chi^2 = 1.07$ p = 0.983 n = 9	$\chi^2 = 2.89$ p = 0.823 n = 21
Orders (without Agnostida)	$\chi^2 = 25.9$ p = 0.000234 n = 19	$\chi^2 = 6.42$ p = 0.378 n = 57	$\chi^2 = 39.5$ p = 5.8E-07 n = 32	$\chi^2 = 4.02$ p = 0.674 n = 13	$\chi^2 = 3.47$ p = 0.748 n = 13	$\chi^2 = 10.9$ p = 0.0919 n = 22
Orders (without Agnostida, Corynexochida, Odontopleurida)[c]	$\chi^2 = 25.5$ p = 3.94E-05 n = 19	$\chi^2 = 4.91$ p = 0.296 n = 57	$\chi^2 = 38.2$ p = 1E-07 n = 32	$\chi^2 = 3.1$ p = 0.54 n = 13	$\chi^2 = 2.55$ p = 0.635 n = 13	$\chi^2 = 9.92$ p = 0.0418 n = 22

[a] Chi^2 value calculated in PAST3 (Hammer et al., 2001) for the observed versus expected observations of trilobite ecdysial behaviour. H_0 = no significant difference in ecdysial behaviour across geological Periods or Series, or between the trilobite Orders (see left-most column).
[b] Sample size (n) represents the number of occurrences of each ecdysial behaviour; this is greater than the number of taxa included because some specimens exhibit more than one behaviour.
[c] Chi^2 analyses run while excluding all trilobite Orders with a sample size of n = <5.

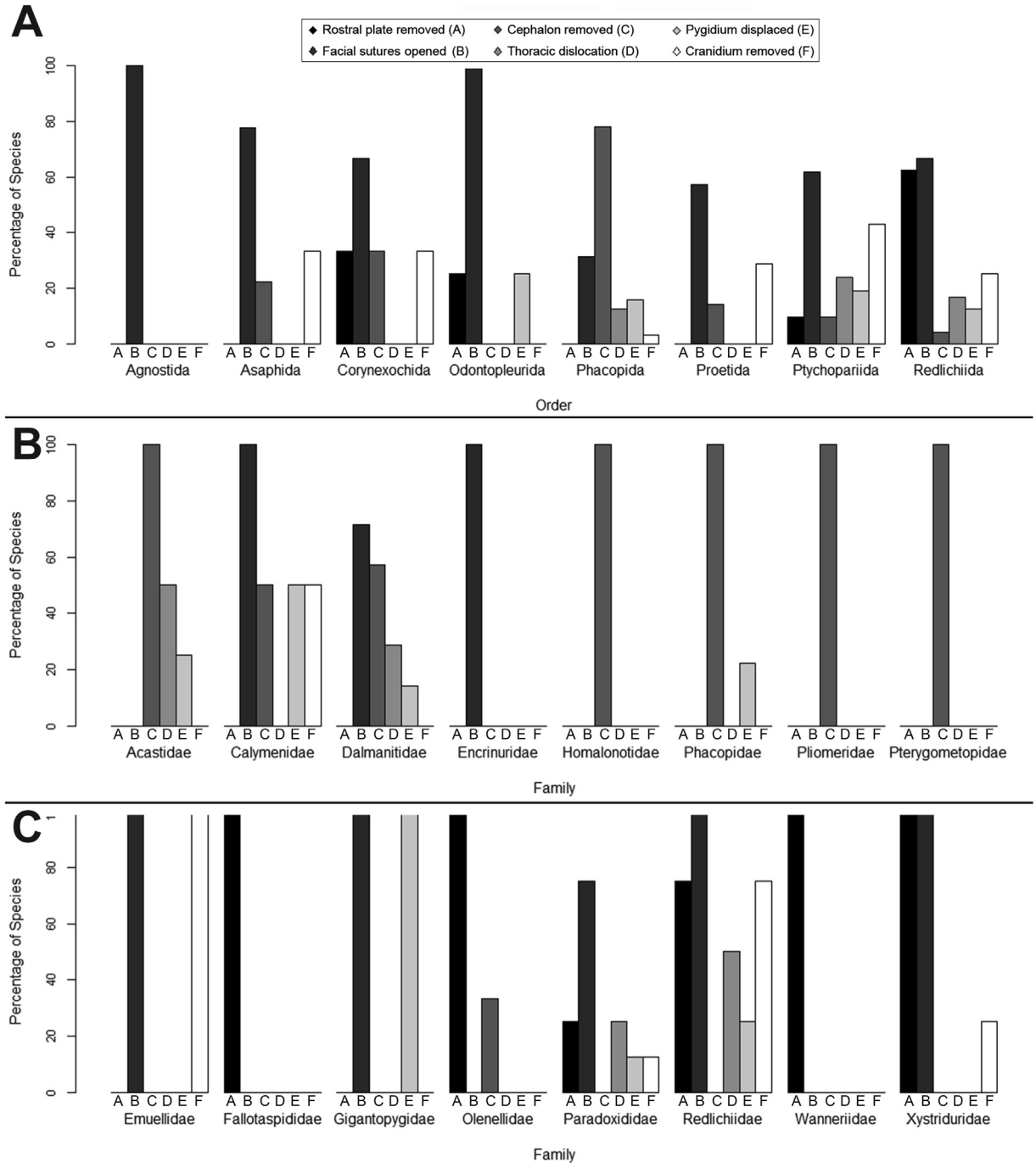


Fig. 9. Ecdysial behaviour displayed by trilobite Orders and Families. A: The percentage of trilobite species within different Orders displaying each of the six ecdysial behaviours. However, n = <5 for Agnostida, Corynexochida and Odontopleurida. B: The percentage of phacopid trilobite species within different Families displaying each of the six ecdysial behaviours. However, n = <5 for all Families but Dalmanitidae and Phacopidae. C: The percentage of redlichiid trilobite species within different Families displaying each of the six ecdysial behaviours. However, n = <5 for all Families but Paradoxididae. These behaviours are not mutually exclusive (i.e. one species may show two or three ecdysial behaviours), and so totals may add up to more than 100% for each Order.

This produced an insignificant result when applying the Chi^2 test ($p = 0.378$) because there is no clear difference between which Orders display the behaviour. Redlichiida also show a tendency towards removal of the rostral plate (possibly followed by ventral exuviation of the moult; $p = 0.00234$, Table 2) (Fig. 9A). The Chi^2 test applied to all Orders with sample sizes >5 also determined a significant difference in which Orders employed disarticulation of the cranidium (following detachment of the librigenae) during ecdysis ($p = 0.0418$, Table 2). Odontopleurida and Phacopida rarely, if ever, show this behaviour (Fig. 9A). Overall, Redlichiida and Ptychopariida display the greatest variation in ecdysial behaviour. This may be an artefact of their larger sample sizes, however it could also be reflecting the greater morphological plasticity of these trilobites during the Cambrian as compared to later trilobite groups (Webster, 2007).

Family-level variation in ecdysial behaviour for Phacopida indicates the secondary utilisation of facial sutures during moult exuviation is owing to the high prevalence of this moulting method

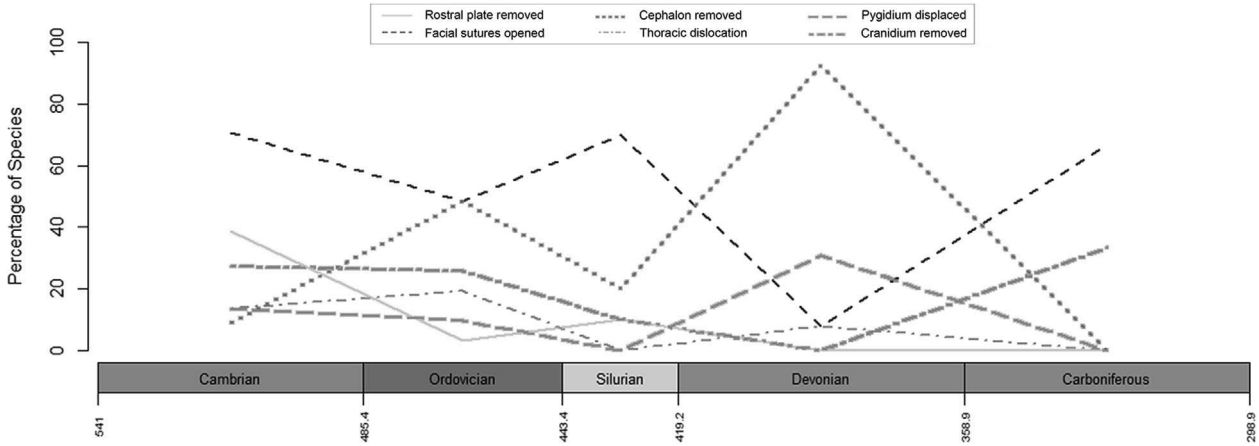


Fig. 10. The percentage of trilobite species across all eight Orders displaying each of the six ecdysial behaviours, through geological Periods from the Cambrian to Carboniferous. These behaviours are not mutually exclusive (i.e. one species may show two or three ecdysial behaviours), and so totals may add up to more than 100% for each time Period. Time scale after Gradstein et al. (2012).

in Calymenidae, Dalmanitidae and Encrinuridae (Fig. 9B). The other five Families show removal of the cephalon in 100% of the taxa sampled. Family-level data for Redlichiida reveals extensive variation in ecdysial habit within the Order (Fig. 9C). Four of the Families show removal of the rostral plate in 100% of taxa (Fallotaspididae, Olenellidae, Wanneriidae, Xystriduridae), and four employment of the facial sutures in 100% of taxa (Emuellidae, Gigantopygidae, Redlichiidae, and Xystriduridae) (Fig. 9C).

The results also suggest patterns in ecdysis varied through geological time. As expected, cephalic disarticulation and opening of the facial sutures were the most common methods of ecdysis throughout the majority of the Early Cambrian to Late Carboniferous (Figs. 10 and 11). These show an opposing pattern due to being mutually exclusive behaviours. The continued utilisation of the facial sutures resulted in an insignificant Chi^2 result ($p = 0.051$ and 0.7) (Figs. 10 and 11). Cephalon removal for ecdysis was at its peak during the Devonian (92% of taxa), resulting in only 8% of trilobite taxa displaying opened facial sutures (Fig. 10). This behaviour was also common during the Ordovician (48% of taxa, 71% in Late Ordovician) (Fig. 10). This temporal variation in a cephalon-thorax fracture during moulting is considered statistically significant ($p = 2.18 \times 10^{-6}$, Table 2).

Removal of the cranidium (necessitating release of the librigenae) for ecdysis is widespread (present in >25% of taxa) during the Cambrian, Ordovician and Carboniferous As expected this somewhat tracks trends in utilisation of the facial sutures (Figs. 10 and 11), although this was not significant. Loss of the rostral plate for ecdysis is common in the Cambrian (39% of taxa, >70% during the first half), but declines in frequency until its absence in the Devonian and Carboniferous (Figs. 10 and 11). This trend is statistically significant ($p = 0.0084$, Table 2). The other two ecdysial behaviours are less common. Pygidium displacement is present in 50% of taxa during the Middle Devonian (Figs. 10 and 11), but is otherwise rare or absent. Thoracic dislocation is very rare from the Middle Ordovician (present in 17% of taxa at this time).

3.3. Discussion

The preliminary results presenting variation in trilobite ecdysial behaviour suggest some chronological and taxonomic signal

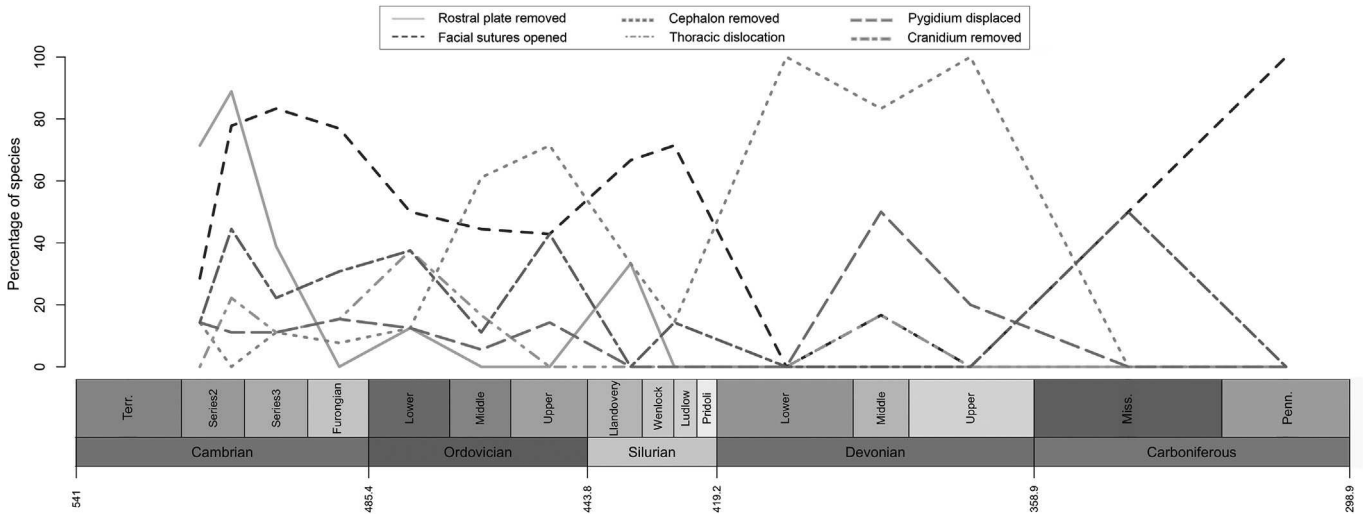


Fig. 11. The percentage of trilobite species across all eight Orders displaying each of the six ecdysial behaviours, through geological Series, from the Cambrian Series 2 (Early Cambrian) to Pennsylvanian (Late Carboniferous). These behaviours are not mutually exclusive (i.e. one species may show two or three ecdysial behaviours), and so totals may add up to more than 100% for each geological Series. Time scale after Gradstein et al. (2012). Abbreviations: Terr., Terreneuvian; Miss., Mississippian; Penn., Pennsylvanian.

resides in the moult fossil record. Generally, modes of ecdysis that vary significantly across trilobite Orders (disarticulation of the cephalon, and of the cranidium, Table 2) agreed with those qualitatively summarised in the literature review (Section 2.1). Further, the lack of significant taxonomic variation in facial suture moulting was expected from the prevalence of this behaviour across all Orders (Fig. 9A). The Family-level variation in phacopids and redlichiids described in the literature is also captured in Fig. 9B and C. Overall the results clearly support the well-known inter- and intraspecific variability in trilobite moulting. This high variability in the moulting method used by different trilobite individuals belonging to the same Family, Genus or even Species is in opposition to the "canalised ecdysial habit" of other arthropods (Brandt, 2002; pg. 399), where moulting method is robust and consistent for all members of a Genus, Family or higher taxonomic classification (e.g. the "Open Moult Position" of decapod crustaceans such as lobsters and crabs Glaessner, 1969; Bishop, 1972, 1986). In these extant arthropods, taxa with a similar overall morphology will moult using the same method, whereas trilobite taxa sharing similar overall morphology may moult using many different methods. This lack of consistency in moulting behaviour within trilobites may have affected the evolutionary success and survivorship of the clade (Brandt, 2002), although these links are far from straightforward. Our results suggest that a quantitative approach to studying large-scale evolutionary trends of trilobite ecdysis is feasible and warrants further investigation.

However, low sample sizes for several of the Orders (particularly Agnostida, Corynexochida, Odontopleurida, and Proetida) and during the Carboniferous indicate the need for more extensive sampling of trilobite moulting patterns before more rigorous statistical analysis. Perhaps fewer significant trends would be observed if all Order sample sizes were greater, and the full range of within-Order variation in ecdysis could be observed. Significant trends in variation of ecdysial behaviours through geological time may also be attributable to fluctuations in biodiversity and abundance of certain trilobite clades. For example, Phacopida trilobites moult by cephalon removal, and so a peak in this method of moulting is seen during the Ordovician coincidental with the origin and radiation of the Order.

The analysis presented exploring trends in trilobite moulting should be extended to encompass a much larger sample size, but also similar ventures made into detailing and quantifying the ecdysial patterns preserved in the fossil records of other arthropods. Following from the work of Brandt (2002), many other lines of inquiry may prove associations between ecdysial mode in different ecdysozoan groups to broader-scale macroevolutionary processes, such as development and body size, particular environmental cues, or phylogeny.

4. Conclusions

The focus on trilobite ecdysis in this paper reflects the bias to this group in the moult fossil record descriptive literature. However, we have detailed what information does exist for ecdysis in other marine arthropods (particularly Eurypterida and Crustacea), as well as for non-arthropod ecdysozoans. Work focussing on moulting in these groups is sparse, and in most cases limited to very few specimens of loriciferans, palaeoscolecids, priapulids, and lobopodians from the early Palaeozoic. Even rarer glimpses of exuviation preserved mid-moult (e.g. García-Bellido and Collins, 2004) have the potential to identify the taxonomic affinity of enigmatic fossils (Peel et al., 2013; Smith and Ortega-Hernández, 2014).

Methods of studying moulting in the fossil record are highly dependent on the group under examination, and no canonised set of rules or assumptions exists across Ecdysozoa generally. In some sclerotised taxa, such as eurypterids (Tetlie et al., 2008; Vrazo and Braddy, 2011; Vrazo et al., 2014) and decapods (Mertin, 1941; Schäfer, 1972), moults are thought to be more prevalent than carcasses in the fossil record, and specimens are assumed to be moults unless proven otherwise. Fossils of carcasses in these taxonomic groups are relatively rare. In Eurypterids, this is influenced by comparisons to modern taxa, such as *Limulus*, where the exuvial suture closes up after moulting, although this may not be the most appropriate analogue given the uncertain affinity of eurypterids (Tetlie et al., 2008) and the great expanse of geological time separating them. On the other hand, Mesozoic crustacean fossils benefit from having direct modern analogues, and so detailed information on their ultrastructure can provide more confidence in identifying moults in the fossil record.

A different tact is employed when studying the trilobite fossil record of moulting – more debate exists on deciding whether a specimen is a moult or not, with a tendency to assume complete trilobites are carcasses. Debate here focuses on whether disarticulated materials represent moult assemblages, or post-depositional disruption, meaning that more emphasis is placed on understanding the depositional environment (Henningsmoen, 1975). In contrast to eurypterids and even decapods, there is no published suggestion that trilobite ecdysial sutures could close after moulting, giving the false appearance of a complete carcass. For example, once the facial sutures opened in a trilobite, there is little chance they will close up again immediately afterwards. This is partly due to the mineralised nature of the trilobite exoskeleton, as compared to the sclerotized cuticles of other ecdysozoan taxa. As such, the trilobite fossil record of moulting contains important signal on temporal and taxonomic variation of this major morphological innovation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.asd.2015.09.004.

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Appendix 2:

Daley, A.C., Antcliffe, J.B., Drage, H.B. and Pates, S. 2018. Early fossil record of Euarthropoda and the Cambrian Explosion. *PNAS* 115, 5323–5331.

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PERSPECTIVE

Early fossil record of Euarthropoda and the Cambrian Explosion

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Euarthropoda is one of the best-preserved fossil animal groups and has been the most diverse animal phylum for over 500 million years. Fossil Konservat-Lagerstätten, such as Burgess Shale-type deposits (BSTs), show the evolution of the euarthropod stem lineage during the Cambrian from 518 million years ago (Ma). The stem lineage includes nonbiomineralized groups, such as Radiodonta (e.g., *Anomalocaris*) that provide insight into the step-by-step construction of euarthropod morphology, including the exoskeleton, biramous limbs, segmentation, and cephalic structures. Trilobites are crown group euarthropods that appear in the fossil record at 521 Ma, before the stem lineage fossils, implying a ghost lineage that needs to be constrained. These constraints come from the trace fossil record, which show the first evidence for total group Euarthropoda (e.g., *Cruziana*, *Rusophycus*) at around 537 Ma. A deep Precambrian root to the euarthropod evolutionary lineage is disproven by a comparison of Ediacaran and Cambrian lagerstätten. BSTs from the latest Ediacaran Period (e.g., Miaohe biota, 550 Ma) are abundantly fossiliferous with algae but completely lack animals, which are also missing from other Ediacaran windows, such as phosphate deposits (e.g., Doushantuo, 560 Ma). This constrains the appearance of the euarthropod stem lineage to no older than 550 Ma. While each of the major types of fossil evidence (BSTs, trace fossils, and biomineralized preservation) have their limitations and are incomplete in different ways, when taken together they allow a coherent picture to emerge of the origin and subsequent radiation of total group Euarthropoda during the Cambrian.

paleontology | Paleozoic | evolution | Arthropoda | Cambrian explosion

Euarthropoda (in the sense of ref. 1), consisting of the extant groups Chelicerata and Mandibulata, is the most abundant and diverse animal phylum, and is one of the best-studied in modern biology. For over 500 million years, euarthropods have been major components of animal ecosystems, as indicated by their rich fossil record (2, 3). The earliest fossil assemblages of euarthropods show a range of preservation types, and they provide the most complete metazoan example of phylum-level anatomical construction in the early evolutionary lineage (2, 4). Euarthropod fossils have been key for examining the dynamics of the rapid early radiation of animals during the Cambrian explosion (2, 4–7). This event is documented by the Cambrian fossil record, which depicts a coherent sequence of events, including the origins of bioturbation, biomineralization, animal reef systems, zooplankton, and the appearance of all major animal phyla in the 30 or so million years following the Ediacaran–Cambrian boundary (2, 7–10). However, Precambrian ancestors to the Metazoa have long been sought (11–13), with exploration efforts yielding numerous and diverse paleontological discoveries in this interval of time (8, 14).

We here examine the early fossil record of euarthropods (Fig. 1), and use it as a model to explore the quality of the fossil data as it relates to the Cambrian explosion. Numerous types of fossil preservation, including soft-bodied macrofossils from Burgess Shale-type (BST) localities, biomineralized exoskeletons, microfossils (phosphatic, siliceous, and carbonaceous), and trace fossils are compared and contrasted across the Ediacaran–Cambrian boundary to constrain when euarthropods first evolved. In congruence with the most recent analyses from molecular paleobiology

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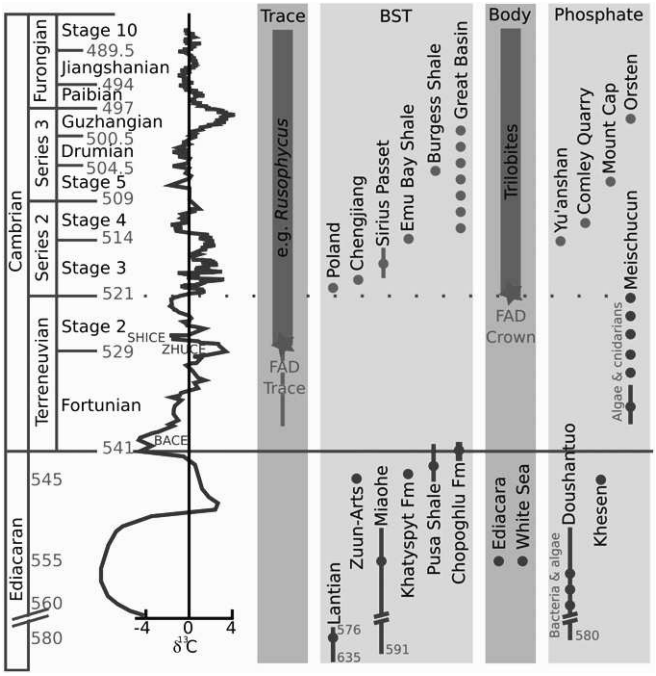


Fig. 1. Timescale of the Cambrian fossil evidence of euarthropods. Age of important localities shown in columns for trace fossils, BSTs, body fossils, and phosphatic microfossils. Localities in green show evidence of euarthropods, localities in blue do not. Orange stars indicate the FAD of total group Euarthropoda (FAD Trace) and crown group euarthropods (FAD Crown). Time in millions of years (red writing). Data from refs. 128 and 129.

(15–17), our comprehensive fossil dataset suggests an entirely Cambrian evolution for this phylum, as described below.

Cambrian BST Localities Reveal the Euarthropod Stem Lineage

The most renowned fossils of the Cambrian explosion are the diverse animal assemblages found at BST localities (Fig. 2), where soft-bodied fossils are preserved as compressions and carbonaceous films in fine-grained mudstones (18, 19). Euarthropods dominate Cambrian BSTs, and many possess morphologies that differ greatly from extant taxa. The stem and crown group concept (2) integrates Cambrian taxa into modern animal phylogeny, with the monophyletic crown group consisting of all extant members and their extant and extinct descendants of the last single-common ancestor uniting them. Stem lineage members are extinct taxa closely related to a crown group, but outside of it because they lack defining morphological characteristics of the crown (2). When stem taxa are arranged in a paraphyletic lineage leading to a crown group, they reveal the order of character acquisition and indicate homologies between living taxa.

Crown group euarthropods possess an exoskeleton, segmented body, jointed paired appendages, tagmosis, and specialized head appendages. A consensus view of how these characteristics were acquired along the euarthropod stem lineage is emerging (Fig. 3), although the interrelationships are often debated (1, 3, 4, 6). The base of the panarthropod tree is occupied by Onychophora, Tardigrada, and a grade of Cambrian lobopodians, including *Aysheaia* (Fig. 2*A*). The euarthropod lower stem lineage includes *Jianshanopodia* and *Megadictyon*, which have annulated bodies with unjointed lobopod walking limbs (3, 20). Next are the "gilled lobopodians" from Sirius Passet, *Kerygmachela*, and *Pambdelurion* (21), which possess lateral flaps and unsclerotized frontal appendages in addition to lobopod walking limbs. *Pambdelurion* also has sclerotized plates surrounding the ventral-facing mouth (21). The Burgess Shale taxon *Opabinia* (Fig. 2*B*) has a similar body morphology to the gilled lobopodians (22, 23), but a more developed head with compound eyes, a posterior-facing mouth, and a grasping appendage (24).

Radiodonta is a large clade (Fig. 2 *C*–*E*) that includes *Anomalocaris* and occupies the uppermost lower stem lineage position (4). Radiodonts lack lobopods and have a body with lateral flaps and setal blades, and a head with a pair of sclerotized appendages, circular mouthparts, and paired stalked compound eyes (4, 25–27). With over 20 taxa described, it is possible to examine ecological and evolutionary trends within this diverse and globally distributed clade. For example, radiodont frontal appendages (Fig. 2*D*) and mouthparts (Fig. 2*C*) were specialized either for active predation, scavenging, or filter feeding, so ecological dynamics can be examined (26, 27). Radiodonta also provide insight into euarthropod anatomical innovation. The evolutionary importance of the radiodont frontal appendage is seen in its homology to the labrum of modern euarthropods (6, 28). Important information is also revealed about the origin of the euarthropod biramous limb, a unique two-branched limb found in crustaceans and trilobites that is hypothesized to have formed from the fusion of two separate appendages (4, 22). The two pairs of swim flaps in radiodonts (27) indicate that the endopod (walking branch) and exopod (gill branch) of the biramous limb are homologous to a ventral set of walking limbs (in lobopodians, "gilled lobopodians," *Opabinia*) or ventral flaps (in radiodonts) and a dorsal set of flaps with setal structures, respectively. The paired flaps of radiodonts were used for swimming, as indicated by the prominent euarthropod-like musculature found at the bases of the flaps in *Anomalocaris* (white arrows in Fig. 2*E*) (29). Other euarthropod features of radiodonts include paired compound eyes of *Anomalocaris* from the Emu Bay Shale, each with 16,000 hexagonally packed ommatidial lenses (30), and complex cephalic carapaces, which reveal the segmental architecture of the euarthropod head region (4, 31). Radiodont anatomy also informs on the evolution of the euarthropod digestive system, with prominent gut glands (black arrows in Fig. 2*E*) indicating complexity in their feeding behavior (20). Radiodonts exemplify the importance of studying fossil stem lineage taxa to understand the anatomical innovation that led to the evolutionary success of the euarthropods.

Crownwards of Radiodonta is the Deuteropoda, a monophyletic clade that includes the upper stem and crown group Euarthropoda, and consists of a wide variety of taxa with contested interrelationships (1). Upper stem lineage euarthropods include the fuxianhuiids (32), *Leanchoilia* and other megacheirans ("great appendage" euarthropods) (33) (Fig. 2*F*), and bivalved taxa, such as *Canadaspis*, *Isoxys*, and *Perspicaris* (34) (Fig. 2*G*), all of which have a segmented body bearing biramous limbs and a multisegmented head with specialized appendages (1), although not all crown group head structures are seen. Some megacheirans have alternatively been placed within the crown as stem-lineage chelicerates (35). The euarthropod crown likely also includes the trilobites (33, 36, 37), trilobite-like taxa (Trilobitomorpha) such as *Helmetia* (Fig. 2*H*), and the vicissicaudates (38), including aglaspidids and taxa such as *Sidneyia* (Fig. 2*I*) and *Emeraldella* (Fig. 2*J*). Vicissicaudata and Trilobitomorpha are often united in the clade Artiopoda (Fig. 3), which has alternatively been aligned with the mandibulates (37) or the chelicerates (33).

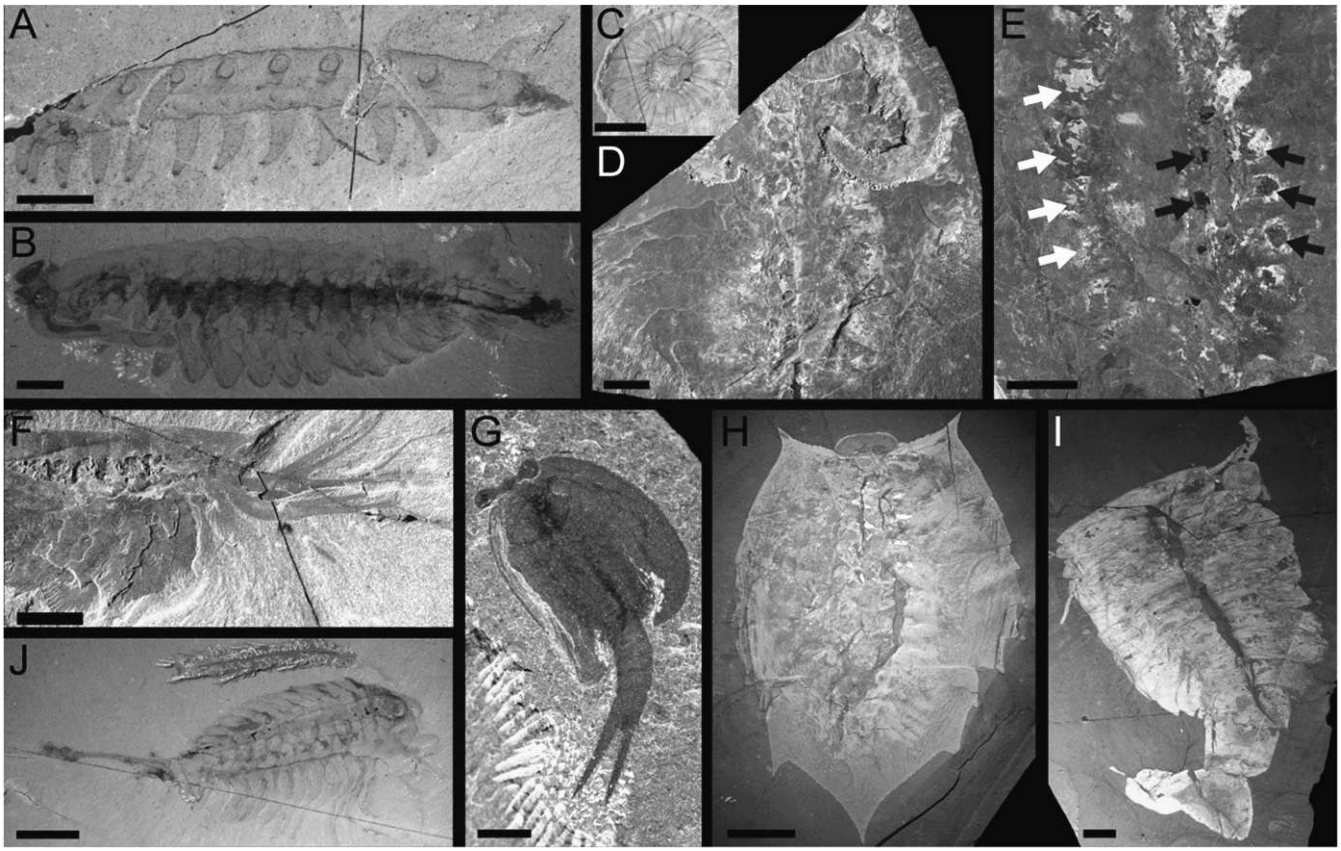


Fig. 2. Panarthropod fossils from the Burgess Shale. (*A*) Lobopodian *Aysheaia pedunculata*, USNM 83942. (*B–E*) Lower stem group euarthropods. (*B*) *Opabinia regalis*, USNM 155600. (*C–E*) Radiodont fossils. (*C*) *Hurdia* mouthpart, USNM 368583. (*D*) *Anomalocaris canadensis* body fossil, GSC 75535A. (*E*) *Anomalocaris canadensis* from *D* with musculature (white arrows) and gut glands (black arrows). (*F* and *G*) Upper stem group euarthropods. (*F*) Megacheiran *Leanchoilia superlata*, USNM 250299. (*G*) Bivalved arthropod *Perspicaris dictynna*, USNM 189245. (*H–J*) Crown euarthropods, artiopodans. (*H*) Trilobitomorph *Helmetia expansa*, USNM 83952. (*I* and *J*) Vicissicaudates. (*I*) *Sidneyia inexpectans*, USNM 250208. (*J*) *Emeraldella brocki*, USNM 57702. (Scale bars: 5 mm in *A*; 10 mm in *B–F* and *H–J*; 3 mm in *G*.)

The euarthropod stem lineage depicts a clear scenario of character acquisition, from the basal condition of an annulated body with lobopodous limbs through increasing levels of arthropodization. Stem lineage taxa originate from multiple BSTs, with the oldest being a single radiodont appendage from central Poland (39), which is only 1–2 million years older than the abundant and diverse arthropod assemblage of the Chengjiang biota at 518 million years old (40) (Fig. 1).

Microscopic Fossils of Extant Crown Group Euarthropods in the Cambrian

Microscopic fossils, such as small carbonaceous fossils (SCFs) and phosphatized "Orsten-type" fossils provide the earliest evidence of extant crown group euarthropods in the form of crustacean larvae and fragments. Three-dimensional phosphatic preservation is well known from the Upper Cambrian Orsten limestones of Sweden, and the Orsten-type preservational mode is widespread both temporally and geographically (41). The earliest crustaceans are *Yicaris dianensis* (Fig. 4*E*) and *Wujicaris muelleri* from the phosphatic Yu'anshan Formation, China (42, 43) and are at least 514 Ma (44). These taxa are comparable to larvae of crown group crustaceans such as cephalocarids, branchiopods, and maxillopods (43). The oldest nonlarval crown-group fossil crustacean is *Klausmuelleria salopensis* reported from the 511 Ma *Protolenus* Limestone, Comley, Shropshire, United Kingdom. *Klausmuelleria* is a phosphatocopid euarthropod, with paired appendages, a labrum, and a sternum preserved inside two carapaces (10).

The slightly younger Mount Cap and Deadwood Formations in western Canada preserve euarthropod appendage fragments as 3D microscopic SCFs (Fig. 4 *A–D*). These contain a variety of spines, setae, and setules for filter feeding (Fig. 4 *A–C*), and the molar surface of grinding mandibles (Fig. 4*D*) (45, 46). Comparison with modern phyla links the Mount Cap and Deadwood fossils to extant crustaceans, including branchiopods and malacostracans (45, 46). Similar fossils of copepod mandibles are reported from the Nolichucky Shale (Cambrian Series 3 to Furongian in age) of Tennessee (47).

Trilobites Appear Earlier than the BSTs and Microfossils

Crown group euarthropods appear even earlier in the Cambrian, at 521 Ma, in the form of trilobite body fossils (48) (Fig. 4*G*). The extinct trilobites are placed within crown group Euarthropoda (33, 36, 49) (Fig. 3) based on the presence of derived morphological features, such as biramous limbs, deutocerebral antennae (36), diverse feeding specializations, and a heavily biomineralized calcitic exoskeleton (36, 49). Their position within Euarthropoda is uncertain, with trilobites (and other Trilobitomorpha) being aligned with the Chelicerata, creating clade "Arachnomorpha" (33, 50, 51), or placed within the clade Mandibulata (15, 36, 37, 52).

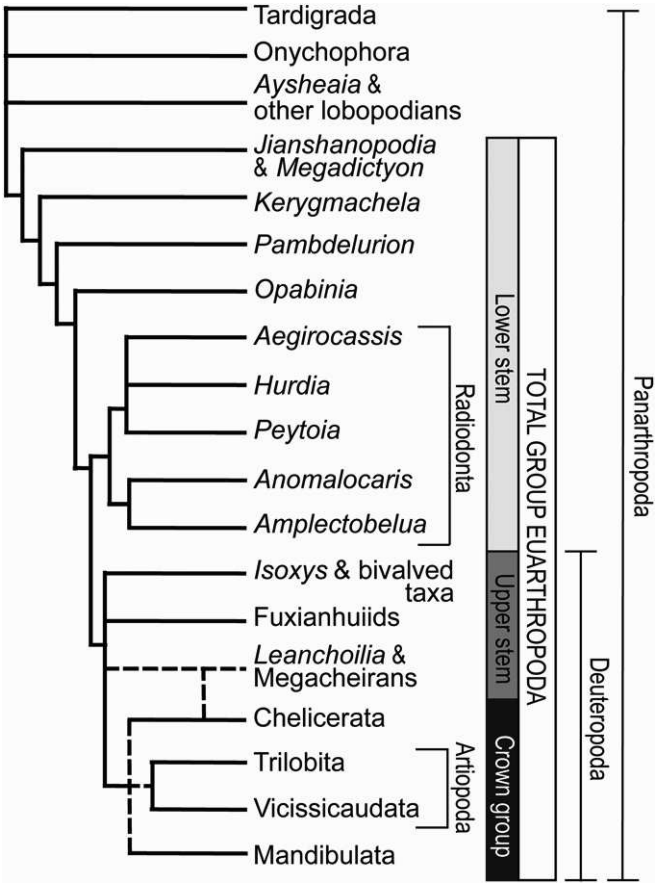


Fig. 3. A generalized phylogeny of panarthropod relationships, distinguishing the crown group Euarthropoda from the lower and upper stem lineage euarthropod taxa. Dashed lines indicates the uncertain phylogenetic placements of Megacheira and Artiopoda. Data from refs. 1, 4, 27, 31, 34, and 37.

The oldest trilobites currently define the beginning of Cambrian Series 2 Stage 3 at approximately 521 Ma (53) (Fig. 1), although formal designation has yet to be finalized for this boundary and the use of other sources (e.g., acritarch or molluscan biostratigraphy, isotopic curves) may mean the first appearance date (FAD) of trilobites could fall marginally into the latest Terreneuvian (54, 55). The oldest trilobites appear coevally at multiple localities, and include *Profallotaspis jakutensis* and *Profallotaspis tyusserica* from Siberia (48, 50, 56), *Hupetina antiqua* and fallotaspidids (Fig. 4*G*) from the lowest Igoudine Formation of Morocco (55), *Lunagraulos tamamensis* from the lowermost Ovetian of Spain (57), and *Fritzaspis generalis* from Laurentia (48), meaning that disparate clades appear simultaneously (54). These are rapidly followed by the earliest trilobites from Australia and China, *Abadiella* and *Parabadiella* (48, 53, 54, 58), and within a few million years, trilobite fossils can be found globally in vast numbers, showing high ecological and morphological diversity (54, 55). This rapid global distribution may be a result of a planktonic larval stage and adult stages (59), and was controlled by factors such as paleoclimatic variation and paleogeographical conditions, including carbonate productivity and periods of anoxia (60). Distribution was likely facilitated by patterns of oceanic circulation (faster equatorial flow and more open circulation) and newly developed environments resulting from Cambrian transgression (57, 60), such that if oceanic turnover time was similar to the present (20,000 to 30,000 years), trilobite diversification and dispersal would appear almost instantaneous given the resolution of the fossil record. Based on distribution patterns alone, there is no reason to suppose the existence of trilobites before their first appearance in the fossil record at 521 Ma (contrasting ref. 61).

It may seem counter-intuitive that crown group euarthropods appear at 521 Ma, while the first appearance of stem lineage euarthropods is not until 518 Ma. This makes sense in light of two points. First, trilobites have easily preserved, biomineralized skeletal elements, while stem lineage euarthropods lack biomineralized exoskeletons and require preservation of soft tissues in BSTs. Second, stem lineage euarthropod fossils do not represent the morphology at the divergence point to crown euarthropods, but are instead end members on a lineage that has undergone evolution since branching off from the lineage that led to crown Euarthropoda. However, stem lineage euarthropods would have evolved before trilobites, even if they are not preserved, so the real question is how much earlier

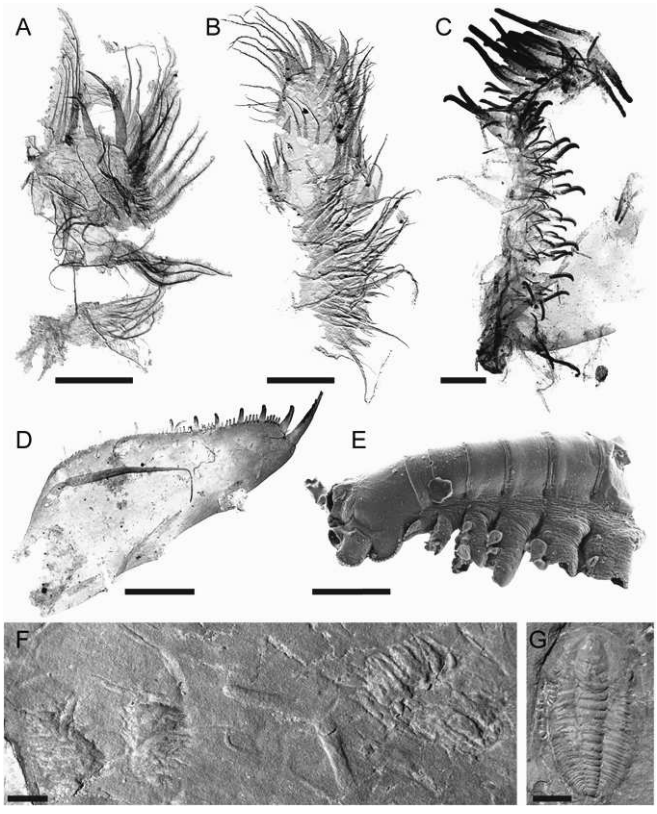


Fig. 4. Earliest fossil evidence of total group Euarthropoda, and extinct and extant crown group Euarthropoda. (*A–D*) SCFs of early crustaceans. Images courtesy of T. Harvey (University of Leicester, Leicester, United Kingdom) and N. Butterfield (University of Cambridge, Cambridge, United Kingdom). (*A* and *B*) Comparison of branchiopod filter plates from early Cambrian Mount Cap (*A*) and middle Cambrian Deadwood Formations (*B*, GSC 135392). (Scale bars, 50 μm.) (*C*) Appendage setae from Mount Cap, GSC 34928. (Scale bar, 70 μm.) (*D*) Branchiopod mandible from Mount Cap, GSC 34931. (Scale bar, 100 μm.) (*E*) Oldest crustacean *Yicaris dianensis*, YKLP 10840. (Scale bar, 150 μm.) (*F*) Earliest total group euarthropod evidence, *Rusophycus* trace fossil, GSC 85983. *E* and *F* reprinted with permission from ref. 44. (Scale bar, 20 mm.) (*G*) Fallotaspidid trilobite from Morocco, OUMNH AX.27. (Scale bar, 10 mm.)

than 521 Ma did they appear? The answer comes from the trace fossil record.

Oldest Euarthropod Evidence Comes from the Earliest Cambrian Trace Fossil Record

Trace fossils record the activities of animals as they interact with sediment, and can include tracks, burrows, feeding marks, and even death throws. Before the first appearance of trilobites at 521 Ma, there are 20 million years of the Terreneuvian for which trace fossils are abundant (9) (Fig. 1), including the euarthropod traces *Rusophycus*, *Diplichnites*, *Cruziana*, *Asaphoidichnus*, *Tasmanadia*, and *Cheiichnus*.

The general order for the first appearance of euarthropod traces has been described as *Monomorphichnus*, *Diplichnites*, *Rusophycus*, and *Cruziana* (62). Although *Monomorphichnus* appears coevally with *Treptichnus* at the base of the Cambrian, the latter's affinity as an euarthropod trace (62, 63) can be doubted because it consists of a single row of traces rather than being paired. In contrast, *Rusophycus* (Fig. 4*F*) provides definitive evidence of crown group Euarthropoda (44) as it records the activity of paired limbs across a bilaterally symmetrical body plan and are "undoubtedly resting excavations made by trilobites" (ref. 64, p. W101). Some specimens of *Rusophycus* have been found with the trilobite trace maker in situ (65). While *Rusophycus* appears later than *Diplichnites* in many sections worldwide, it appears before *Diplichnites* immediately above the basal Cambrian golden spike in the Chapel Island Formation of Newfoundland (Fig. 4*F*) (66) and immediately above *Treptichnus* in the Breidvika Formation of northern Norway (67). This makes *Rusophycus* the oldest euarthropod trace globally (see *SI Appendix* for details of other Terreneuvian euarthropod traces).

During the Ediacaran period, euarthropod trace fossils are "strikingly absent" (ref. 9, p. 3), but it is clear that euarthropod activity is abundant, diverse, and global before 528 Ma (based on correlations to the FAD of the marker *Watsonella crosbyi* at the base of Stage 2). *Rusophycus* likely appeared early in the Fortunian (53) based on biozone correlations (55). However, precise dates for these traces suffer from difficulties in correlating biostratigraphic information in the Fortunian and Cambrian Stage 2 and an absence of absolute dates in this interval (*SI Appendix*), but we can be confident that euarthropod traces appear substantially before the first appearance of euarthropod body fossils.

Modes of Fossil Preservation Are Comparable in the Cambrian and Precambrian

To search for euarthropod fossils earlier in the rock record requires comparable fossil preservation modes in the Precambrian, where exceptional preservation of soft-tissues abounds (14). Of the fossils described above, BST and phosphatic microfossil preservation are abundant in the Precambrian, and other modes of relevance include siliciclastic moldic and chert preservation (*SI Appendix*). These cover a wide variety of depositional environments, and preserve hard and soft organisms on macroscopic and microscopic scales.

BST preservation is characterized as organic preservation of soft-bodied macrofossils as carbonaceous films preserving primary tissues, rather than early authigenic mineral replacement (18, 19, 68). Later Phanerozoic carbonaceous fossils, such as algae, plants, graptolites, and eurypterid cuticle (68) preserve only selected refractory tissues and do not reach the same level of anatomical fidelity, abundance, or diversity as the Cambrian BSTs (69). Carbonaceous fossils are also abundant in Proterozoic mudrocks, and although detailed investigations of the depositional and diagenetic regimes of these localities are lacking, their preservation appears to be very similar to the Cambrian BSTs. Proterozoic carbonaceous compression fossils are seen, for example, in the Miaohe and Lantian biotas of South China (70, 71), Jinxian biota of North China (72), Pusa Shale of Spain (73, 74), Chopoghlu Shale of Iran (75), Khatyspyt Formation of Siberia (76), and Zuun-Arts biota of western Mongolia (77) (Fig. 1). The last four localities are latest Ediacaran in age (ca. 545 Ma), and BST preservation is then absent from the rock record until Cambrian Series 2, representing a gap of at least 20 million years (69). In Cambrian Series 2 and 3, as many as 50 BST localities are known (69), but BSTs are largely absent from the post-Cambrian rock record (68), a pattern that results from a combination of factors unique in Earth's history. Preservation is enabled by rapid accumulation of clays and silts beneath poorly oxygenated water masses, with stagnant anoxic conditions that reduce rates of microbial decay and bioturbation (18, 19, 78, 79), and the rapid early sealing of these entombing sediments by pervasive carbonate cements (80). Carbonate availability was likely amplified by enhanced weathering of continental basement rocks during the basal Cambrian marine transgression (79). Taken together with the increased use of carbonate as a biomineral during the Cambrian (14), these data show that carbonate became dramatically less available as a direct chemical cement by the end of the Cambrian, closing this taphonomic window.

Phosphatic microfossil preservation of soft tissues reached its greatest extent during the late Precambrian to early Cambrian (14, 81, 82). Phosphate precipitates on the seafloor within the photic zone under reducing conditions (82), when anoxic nutrient-rich water masses up-well into shallow water carbonate lagoons (14). It appears in the Ediacaran (14) as interbedded stratiform phosphate and chert layers in shallow water shale and limestone sequences (83) that formed along the northern margins of a vast ocean that now outcrops from south China through to Mongolia, west to the Arabian Peninsula, and into Oman (55). Numerous sections span the Ediacaran–Cambrian boundary and contain abundant bedded phosphates that are taphonomically, compositionally, and depositionally identical. High-quality post-Ordovician marine phosphate is unknown (14), indicating that the phosphorite preservation window is similar in length to that of BST deposits. This is because the phosphorus-rich redox boundary layer was shallow in Ediacaran oceans, allowing for early and rapid phosphatization within the photic zone, but during the Cambrian oxygen levels were increased in the water column by nekton and zooplankton (84, 85) and in the sediment by bioturbation (86), which forced the phosphogenic zone downward, through the water column and into the sediment profile.

Siliciclastic moldic preservation is associated with Ediacaran biota fossils, where fine to course sediments preserve external molds of soft organisms in open marine conditions. This preservation is abundant and widespread during the latest Ediacaran Period (87). At Ediacara in South Australia, rapid silicate lithification of overlying event sands preserve communities in situ by molding their upper surfaces (87), with the sands being stabilized through pervasive trapping and binding by microbial mats (87), which are preserved in detail in siltstones in Brazil (88) (*SI Appendix*). In sites across the Avalonian paleogeographic terrane, such as Mistaken Point, Newfoundland, Canada and Charnwood Forest, Leicestershire, United Kingdom, the lower surfaces of organisms are molded by smothering volcanic ash (87) and binding by microbial mats (89). Elsewhere, molds are formed from the rapid lithification of carbonates (White Sea, Russia) (90), or as

infaunal whole-body 3D preservation (Namibia) (91). Moldic preservation becomes less common at the start of the Cambrian (92–94) and rare later in the Phanerozoic, never again replicating the worldwide distribution seen in the Ediacaran (14), correlating with the decline and eventual disappearance of pervasive microbial mats during the Cambrian (14, 95).

These examples make it clear that the Cambrian is much more like the late Precambrian in terms of its taphonomy than it is like the rest of the Phanerozoic (14). A similar trend is also observed in chert deposits of cellular preservation (14) (*SI Appendix*), with high quality Precambrian and Cambrian preservation of soft tissues declining markedly in abundance and fidelity in post-Cambrian deposits. The differences between Ediacaran and Cambrian lagerstätten is mainly one of proportion rather than mode of preservation (14), for which numerous factors are responsible, as is also true for their decline after the Cambrian. The major factors are: alteration of ocean pH and Eh; dramatic reduction of phosphate, carbonate, and silica ion saturation in the seawater linked to abundant biomineralization in the Cambrian; oxygenation of upper sediment layers from increasing animal burrowing; lowering of the phosphate precipitation zone into deep sediment layers; and major erosional and facies differences related to global tectonics, which altered chemical availability for cement formation and changed ocean shelf areas (14, 78–80).

Ediacaran Biota Euarthropod Candidate Fossils Cannot Be Substantiated

Putative animals, including two euarthropod taxa, have been suggested from the siliciclastic moldic fossils of the Ediacaran biota, which consists of enigmatic discs and fronds (14). Animal affinities for the Ediacaran biota have been extensively debated (2, 11, 96). For example, although discoidal fossils were long regarded as cnidarian jellyfish (12), they are now known to instead consist of a mélange of microbialites, tool marks, gas escape structures, soft sediment deformation, and holdfasts (97). The suggested Pennatulacean affinity for Ediacaran rangeomorphs (11) has also been shown to be without any basis (98, 99). The numerous proposed affinities for *Dickinsonia* include Annelida (11), Placozoa (100), an extinct higher animal clade (Proarticulata) (101), Foraminifera (102), or an extinct eukaryotic kingdom (Vendobionta) (96). It is not our aim to review every enigmatic taxon, but only to emphasize the enigmatic nature of the Ediacaran biota and discuss putative euarthropod taxa.

Candidate euarthropod fossils include *Spriggina* and *Parvancorina* from the Ediacara Member in Australia and the White Sea locality in Russia (Fig. 1) (11, 12). No definitive characters have yet been identified that allow us to confidently place these fossils within Euarthropoda (or even within Metazoa). *Spriggina*, for example, does not possess bilateral symmetry, but instead has a marked offset along the midline (103), and this alone is sufficient to reject a euarthropod affinity. This mode of construction is common in the Ediacaran biota, and we emphasize that fossils should be analyzed in the context of their cooccurring biota to identify reliable characteristics for phylogenetic placement. *Spriggina* was also compared with *Metaspriggina* from the Cambrian Burgess Shale, with both considered euarthropods (104). *Metaspriggina* has since been shown to be a chordate (105, 106) and is unrelated to *Spriggina*, the affinity of which remains unknown.

Parvancorina has been compared with the Burgess Shale euarthropod *Skania* (104, 107) and the related marrellomorphs (13) based on similarity of the anchor-shaped anterior region, and coding these characters as homologous in a phylogenetic analysis resolves *Parvancorina* as a stem group euarthropod (108). However, the anchor of *Parvancorina* lacks the anterior doublure and medial keel of the cephalic structure of *Skania*, meaning these structures are not homologous (109). The growth trajectory of *Parvancorina* does not match that of *Skania* (109, 110), and neither do the segmentation arrangement and attachment location of the supposed appendages (13, 109, 111). The Ediacaran taxa *Vendia* and *Praecambridium* were also suggested to have euarthropod-like guts, gonads, and intestinal caeca (112), but these are better understood as postdeath wrinkling and osmotic contractions, in concert with xenomorphism of the thin bodied organism over the irregular sediment surface, comparable to the taphonomy of other taxa from the White Sea and global specimens of *Dickinsonia* (113). No euarthropod claim from the Ediacaran biota can therefore be substantiated.

There Are No Euarthropods Preserved in Ediacaran BSTs, Phosphorites, or Cherts

The lack of euarthropod body fossils in the Ediacaran biota is mirrored in all other preservational regimes in the Precambrian, including BSTs, phosphatised microfossils, and chert deposits (Fig. 1 and *SI Appendix*). Numerous Precambrian BSTs (70–77) contain disk-shaped fossils of prokaryote colonies (71), such as *Beltanelloides* (73), or various carbonaceous ribbons and filaments interpreted as algae (14, 71). Over 15 algal taxa are known from the Miaohe assemblage of the Doushantuo Formation of China (71) and the Lantian Formation, a basinal equivalent of the Doushantuo Formation (70). These are comparable to compressions of multicellular benthic algae found from the Ediacaran–Cambrian transition in Avalonia and Baltica (114). As discussed above, these algal-bearing Ediacaran sites exhibit the same preservation as the Cambrian BSTs, which also preserve metazoans (18, 19). *Eoandromeda* is the only known Ediacaran fossil found in both BST preservation and siliclastic moldic preservation (115), but this crossing of taphonomic windows does not illuminate its affinity, which is thought by some (115) but not all (116) to be a putative ctenophore.

While BSTs preserve macroscopic soft-bodied fossils, phosphorites replicate microscopic organisms, preserving a different part of the biosphere. The biological content of abundant late Precambrian phosphorites (14, 81, 82) has been the subject of much debate. The Doushantuo Formation of China (~580 Ma) (117) was thought to contain animal embryos; however, these structures have now been shown to be nonmetazoan, containing algal thalli, acritarch vesicles, and nonmetazoan cell clusters (118). Some may even be nonbiological, as comparable structures can be generated during experimental precipitation of apatite (119). Other late Precambrian phosphate deposits contain a similar suite of single-celled/colonial eukarya and bacteria (81–83, 117), and even in the Khesen Formation of Mongolia, which is immediately below the base of the Cambrian, no metazoan remains are found (120, 121). The Doushantuo is continuous with the early Cambrian Meischucun phosphorites of South China, which preserve embryos and hatchlings of cnidarians (122). Rare examples of phosphatized animal embryos are also found throughout the middle and late Cambrian and early Ordovician (122). While Ediacaran phosphorites preserve only single-celled/colonial eukarya and bacteria, Cambrian localities with identical preservation contain metazoans. Phosphatic preservation of clustered coccoid benthic algae and photoautotrophs is rare from the base of the Cambrian onwards (14), with filaments distorted in a way that suggests

metazoan fecal processing (14). As outlined earlier, euarthropods are preserved in several Cambrian phosphorites, including the 514 Ma Yu'anshan Formation, China (42–44), the 511 Ma Comley Quarry limestone, United Kingdom (10), and the Upper Cambrian Orsten limestone, Sweden (41), but none has been reported from earlier in the Cambrian (i.e., Euarthropods are absent from Fortunian and Stage 2 Small Shelly Fossils) (*SI Appendix*) or the Precambrian.

When Did Euarthropods First Evolve?

The exact timing of the origination of animals has long been the subject of debate, with many claims being made for Ediacaran age animals (12, 13), including euarthropods (11, 103, 112). The development of molecular clocks during the 1990s (123) provided a stimulus for investigating the late Precambrian record for the earliest evolution of animals, as did the biomolecular preservation of sterols from approximately 640 Ma or younger (124, 125) (*SI Appendix*). This inspired field exploration efforts that led to the discovery of at least 30 new Ediacaran age lagerstätten in the last 20 years across the full range of taphonomic possibilities (siliciclastics, chert, phosphate, BST). These allow us to test the hypothesis of deep time euarthropods, as suggested by the earliest molecular clocks, which originally suggested the euarthropod divergence occurred at 1,200 Ma (123) to 610–700 Ma (8, 126). More recent molecular clocks place the euarthropod split in the late Ediacaran (15, 17), or constrained between 561–530 Ma and thereby spanning into the Cambrian (16), showing remarkable congruence with the fossil record. Divergence time analyses using morphological data also recover a Cambrian origin for Euarthropoda (127). These analyses benefit from refined analytical techniques (15–17) and the inclusion of well-defined and dated fossil calibrations (44), leading to increasingly precise results. Such divergence estimates require greatly enhanced rates of evolution in comparison with the Phanerozoic norm because it is not until ∼940 Ma that rate parity would be achieved (7), but confining euarthropod evolution to the late Precambrian or early Cambrian only requires a fractionally more elevated evolutionary rate than having the origination at 680 Ma (7).

The early fossil record of euarthropods presents a robust and coherent picture of evolutionary processes at this time. The first arthropod traces (*Rusophycus*) appear at approximately 537 Ma, shortly after the start of the Cambrian at approximately 540 Ma. Crown group euarthropods (trilobites) appear at 521 Ma and crustacean microfossils at 514 Ma, with soft-bodied stem lineage fossils illustrating the sequence of character acquisition in BSTs starting from 518 Ma. In contrast, BSTs of latest Ediacaran age (e.g., Miaohe, 555 Ma) are abundantly fossiliferous with single-celled/colonial eukarya but completely lack any evidence of euarthropods. Other windows of preservation, such as phosphatic microfossils, also lack any evidence of euarthropods in the Ediacaran yet contain abundant euarthropods in the Cambrian. Claims of Ediacaran age euarthropods, such as *Spriggina* and *Parvancorina*, all lack compelling euarthropod, or even animal, characters. Taken together, these data provide a convincing argument to reject the hypothesis of euarthropods originating before the Cambrian. The absence of animals from Ediacaran age rocks has been explained by either poor fossilization at this time (8) or by being too small to fossilize (e.g., the meiofauna of ref. 5) (88). Hypotheses that regard Precambrian preservation as insufficient to preserve euarthropods can no longer be sustained, given the abundant lagerstätten from the Ediacaran Period. Similarly, claims that euarthropods evolved as a tiny and soft-bodied meiofauna that escaped preservation cannot be substantiated because of how commonly the phosphate window is found in the Ediacaran and lower Cambrian, with microscopic euarthropods not appearing until 514 Ma.

The abundant sources of geological data spanning the Ediacaran and lower Cambrian can be used to constrain the origin of euarthropods. If it is accepted that as active motile organisms euarthropods are marked by their first appearance in the trace fossil record of sediment disturbance, then this constrains the first appearance date of total group Euarthropoda to near the base of the Cambrian at ∼541 Ma. The taphonomic evidence of the lack of euarthropods in Ediacaran lagerstätten conservatively constrain the appearance of total group Euathropoda to younger than ∼550 Ma. Even the conservative estimate of 541 Ma would then allow around 20 million years for the evolution of the first crown group euarthropods at the base of Cambrian Stage 3.

Each of the major types of fossil evidence (BSTs, trace fossils, and biomineralized hard parts) have their limitations and are incomplete in different ways, but when they are taken together they are mutually illuminating and allow a coherent picture to emerge of the origin and radiation of total group Euarthropoda during the lower to middle Cambrian. The fossil record of euarthropods provides our most complete view of the origin and radiation of a major phylum during the Cambrian explosion. Rather than being a sudden event, this diversification unfolded gradually over the ∼40 million years of the lower to middle Cambrian, with no evidence of a deep Precambrian history.

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Appendix 3:

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Ecdysis in a stem-group euarthropod from the early Cambrian of China

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Moulting is a fundamental component of the ecdysozoan life cycle, but the fossil record of this strategy is susceptible to preservation biases, making evidence of ecdysis in soft-bodied organisms extremely rare. Here, we report an exceptional specimen of the fuxianhuiid *Alacaris mirabilis* preserved in the act of moulting from the Cambrian (Stage 3) Xiaoshiba Lagerstätte, South China. The specimen displays a flattened and wrinkled head shield, inverted overlap of the trunk tergites over the head shield, and duplication of exoskeletal elements including the posterior body margins and telson. We interpret this fossil as a discarded exoskeleton overlying the carcass of an emerging individual. The moulting behaviour of *A. mirabilis* evokes that of decapods, in which the carapace is separated posteriorly and rotated forward from the body, forming a wide gape for the emerging individual. *A. mirabilis* illuminates the moult strategy of stem-group Euarthropoda, offers the stratigraphically and phylogenetically earliest direct evidence of ecdysis within total-group Euarthropoda, and represents one of the oldest examples of this growth strategy in the evolution of Ecdysozoa.

The process of moulting consists of the periodical shedding (i.e. ecdysis) of the cuticular exoskeleton during growth that defines members of Ecdysozoa[1], a megadiverse animal group that includes worm-like organisms with radial mouthparts (Priapulida, Loricifera, Nematoida, Kinorhyncha), as well as more familiar forms with clawed paired appendages (Euarthropoda, Tardigrada, Onychophora). Ecdysozoans are among the most ubiquitous group of animals throughout the Phanerozoic, and by virtue of their ecological success they have become a fundamental component of the biosphere since their appearance during the Cambrian Explosion more than 500 million years ago[2,3]. Given their diversity of forms, the phylogenetic relationships between extant Ecdysozoa are usually recognized through similarities at the molecular level[1–4]. Despite the scarcity of morphological characters uniting Ecdysozoa, the group is named for the archetypal growth strategy that is shared among all its members; that is the periodical shedding of the cuticular exoskeleton in order to accommodate changes in body size and/or shape[5]. Ecdysis is intricately linked with ontogenetic development, and therefore most ecdysozoans will typically undergo several moulting cycles over their lifespan. This carries important implications for understanding the evolution of this process in deep time, as the fossil record provides insights on diverse moulting adaptations in extinct ecdysozoan groups[6]. All ecdysozoans have the potential to produce several exuviae (i.e. shed exoskeletons) throughout their lifetime, increasing the likelihood of becoming preserved in the rock record. However, most fossil evidence of moulting is biased in favour of organisms with heavily biomineralised exoskeletons (e.g. trilobites, decapod crustaceans)[7–10], or those whose behaviour may lead to the burial of numerous exuviae simultaneously (e.g. eurypterid mass moulting events)[6,11]. By contrast, information on the moulting strategies of soft-bodied forms is comparatively rare, and only found under exceptional circumstances such as those conferred by Konservat-Lagerstätten[6,12–14]. Prominent instances of moulting in Cambrian soft-bodied forms include euarthropods (*Marrella*[12], *Canadaspis*[15], *Alacomenaeus*[13], *Houlongdongella*[16]), lobopodians with stacked sclerites (*Onychodictyon*[17], *Hallucigenia*[18,19], *Collinsium*[20], *Microdictyon*[21]), and scalidophoran worms (*Sirilorica*[14],

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Wronascolex[22]). From these examples, however, only the crown-group euarthropod *Marrella splendens*[12] and the stem-group loriciferan *Sirilorica pustulosa*[14] capture the moment at which the old exoskeleton is discarded, and thus provide a narrow perspective on the diversity of ecdysis for early Ecdysozoa. Here, we describe the moulting behaviour of the fuxianhuiid *Alacaris mirabilis* from the early Cambrian (Stage 3) Xiaoshiba biota in South China[23]. Our findings offer the first characterisation of ecdysis in an upper stem-group euarthropod[24], and illuminate the ancestral moulting behaviour within this diverse animal group.

Results

The dorsal exoskeletal configuration of *Alacaris mirabilis* (Chengjiangocarididae, Fuxianhuiida)[23] consists of a semicircular anterior sclerite with stalked lateral eyes[25] that is articulated dorsally with a heart-shaped head shield covering the anterior body region. As with other fuxianhuiids[26–28], the ventral surface of the head of *A. mirabilis* features a pair of pre-oral, multisegmented antennae, followed by well-developed specialised post-antennal appendages (SPAs) in a para-oral position. The mouth is covered by a broad sclerotised hypostome that overlies the basal portions of the SPAs. The trunk of *A. mirabilis* consists of 13 trunk tergites; the five anterior-most tergites are greatly reduced relative to the maximum body width and are covered by the head shield in life position. The remaining tergites gradually taper in width towards the posterior. Ventrally the trunk bears more than a dozen pairs of biramous limbs, including a differentiated gnathobasic protopodite (expressed only on the first three sets of post-oral appendages), a multipodomerous endopod, and a flap-like oval exopod with short marginal setae. The conical telson has paired paddle-like tail flukes fringed with posterior-facing elongate setae.

Specimen YKLP12270 is an articulated dorso-ventrally flattened individual with well-preserved soft tissues, including the stalked eyes connected to the anterior sclerite, multisegmented antennae, and 19 sets of biramous trunk appendages (Figs 1 and 2). YKLP12270a preserves the anterior half of the individual (Fig. 1a). The counterpart YKLP12270b contains the posterior body termination (Fig. 1b); a digital composition of the part and counterpart photographs provides a clear view of the whole individual (Fig. 1e). The orientation of the specimen reveals most of the head shield and the entire left side of the trunk, including its corresponding limbs. The anterior right half of the trunk, comprising part of the first to eighth tergites, is missing. YKLP12270 is identified as *A. mirabilis* based on the possession of 13 trunk tergites, of which the five anterior-most are greatly reduced relative to the maximum body width (Fig. 1a,d). However, the configuration of the dorsal exoskeleton preserved in YKLP12270 differs substantially from that of the type material[23], as well as other fuxianhuiids more generally[26,27,29,30]. The five reduced tergites of chengjiangocaridids are concealed beneath the head shield in life position[23,27,30] (Fig. 3); by contrast, the entire trunk tergite sequence of YKLP 12270 is preserved on a level above that of the head shield and its associated structures (Figs 1a,d,e and 2), with the five reduced tergites overlapping the head shield but without indication of the trunk being displaced otherwise. The presence of a layer of sediment separating the head shield and biramous appendages from the overlying dorsal exoskeleton (Fig. 1c–e) demonstrates that these anatomical components were physically disconnected prior to burial.

A closer examination of individual structures reveals differences compared to previously described carcasses of *A. mirabilis*[23] (Fig. 3b,c). For instance, the head shield of YKLP 12270 is connected with the eye-bearing anterior sclerite and the antennae, suggesting *in situ* burial with minimal taphonomic alteration due to transport or decay. However, the dorsal cuticle of the head shield also shows substantial lateral wrinkling and flattening (Figs 1a,e and 2). The texture of the head shield in YKLP 12270 (Fig. 3a) contrasts with the smooth surface typical of well-preserved chengjiangocaridids (Fig. 3b). Xiaoshiba fuxianhuiids frequently show the so-called "taphonomic dissection" of the head shield[27], in which this structure is displaced forwards (and commonly overturned) but remains attached to the body through its connection close to the anterior sclerite (Fig. 3c–e). Fuxianhuiids with taphonomically-displaced head shields frequently have lateral wrinkles associated with softening of the cuticle, likely due to decay and post-burial compaction (Fig. 3c–e). The head shield of YKLP 12270 also shows wrinkling relative to the cuticle of the trunk tergites preserved in the same specimen, which have a smooth surface (Fig. 1a,b,e). In addition, the trunk tergites of YKLP 12270 show some irregularities. The distal pleural tips of the eleventh and twelfth tergites reveal aduplicated tergite underneath (Fig. 1b,f), clearly identifiable for the eleventh tergite because its lateral border is complete adjacent to this additional fragment. The presence of extra exoskeletal elements is also found in the posterior-most body region, as exemplified by the peculiar duplication of the telson (Figs 1b,e,g and 2). The upper telson articulates with the trunk tergites, possesses a slight three-dimensionality, has the typical conical morphology of *A. mirabilis*, and possesses two small, slightly displaced, flukes that likely correspond to the bases of the tail flukes (Figs 1g and 2). These two flukes are impressed with the left side, one on top of the other, which is consistent with the articulated trunk being preserved in a somewhat lateral orientation with only the left side fully displayed. The underlying complete telson is flattened and supports well-preserved tail flukes bearing delicate posterior-facing setae (Fig. 1g). Similar to the head shield and anterior trunk tergites, the duplicated telsons are separated by a layer of sediment. Lastly, the underlying telson and tail flukes have a dorsal orientation that parallels that of the head shield and appendages relative to the articulated trunk (Fig. 1b,e–g), and the overlying telson follows the curvature of the trunk.

The preservation of YKLP 12270 differs from other fully articulated individuals of *A. mirabilis* from the Xiaoshiba biota, as well as other members of Chengjiangocarididae[22,26,29] (Fig. 3). These taphonomic irregularities include: the overlap pattern of the dorsal trunk tergite series relative to the well-preserved head shield and associated *in situ* structures (Figs 1a,d and 2); wrinkling restricted to the head shield (Figs 1a,e and 3a); different orientations of the trunk series and head shield (Fig. 1a,b,e);and the duplication of tergite margins and the entire telson in the posterior body region (Fig. 1g). These taphonomic features suggest that YKLP 12270 represents an individual at the exuviation stage of moulting, buried during the process of shedding the old exoskeleton[31]. The well-sclerotised trunk tergite series with conical telson corresponds to the discarded exoskeleton, and lies above the actual carcass of the emerging individual (partially encased within the exuvia), represented by the head shield with articulated eyes and antennae, the sets of ventral biramous appendages, and the pristine telson

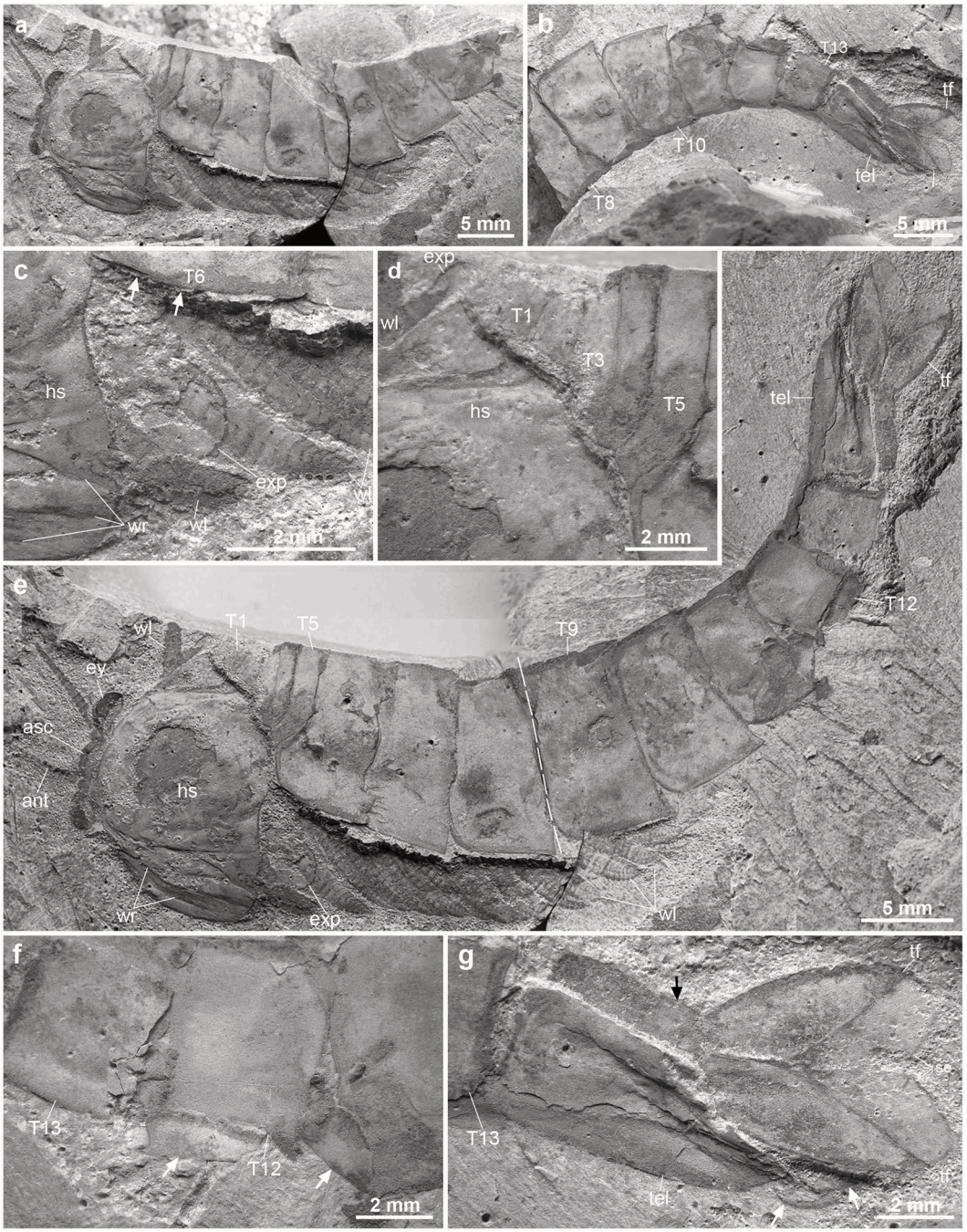


Figure 1. *Alacaris mirabilis* from the Cambrian (Stage 3) Xiaoshiba Lagerstätte in Kunming, southern China. (**a**) YKLP 12270a, dorsal view of the head shield and anterior portion of trunk. (**b**) YKLP 12270b (counterpart), showing the posterior portion of trunk and telson. (**c**) Close-up showing the layer of sediment (arrowed) separating the well sclerotized trunk tergites from the wrinkled head shield. (**d**) Close-up of the five reduced anterior tergites articulated to the remainder of the trunk, and overlapping the carcass head shield. (**e**) Restored specimen by digitally combining YKLP 12270a and 12270b by computer amalgamation (using photomerge software), the dashed line indicates the contact of YKLP 12270a by 12270b. (**f**) Close-up view of 11^{th} to 13^{th}tergites with fragments of duplicated tergites (arrows). (**g**) Close-up of the moulted telson articulated with the complete trunk tergite series and displaying paired tail flukes (white arrows), overlapping the pristine underlying telson (black arrow) with complete flukes and posteriorly-pointing setae. Abbreviations: ant, antenna; asc, anterior sclerite; exp, exopod; ey, eye; hs, head shield; tel, telson; tf, tail fluke, T*n*, trunk tergites; wl, walking leg; wr, wrinkles.

with tail flukes (Fig. 2). This configuration implies that the old head shield corresponding to the exuvia is no longer present, having been separated from the body following disarticulation for moulting (Fig. 4b), and only the attached head shield of the carcass is visible on the specimen. Most of the dorsal trunk tergite series of the

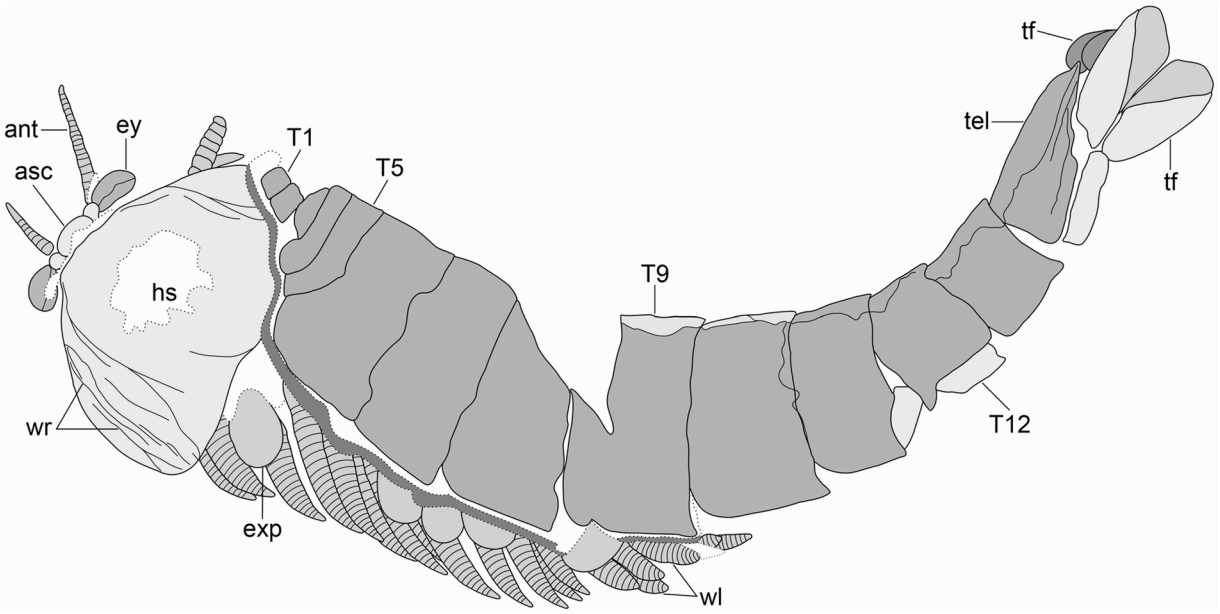


Figure 2. Interpretative diagram of YKLP 12270. Diagram based on the digitally reconstructed complete specimen depicted in Fig. 1e. The exuvia includes the entire trunk tergite series and telson (*orange*), and is preserved above the soft emerging individual represented by the head shield with articulated anterior sclerite, stalked eyes and antennae, as well as ventral limbs and duplicated telson with attached tail flukes (*yellow*). Abbreviations as in Fig. 1.

emerging individual – except for the posterior body termination – cannot be observed on the specimen. The missing trunk tergites are likely concealed within the layer of sediment separating the carcass from the discarded exoskeleton, and also obscured by the overlaying old tergite series (Fig. 1c–e). Based on the orientation of YKLP 12270, the moulting process of *A. mirabilis* most likely involved the posterior separation of the head shield from the body, comparable to the configuration observed in taphonomically-dissected fuxianhuiid specimens from the Xiaoshiba Lagerstätte[23,27]. This disarticulation pattern would facilitate the egress of the emerging individual through the resulting ample gape at the anterior of the body (Fig. 4d). The preservation of duplicated exoskeletal elements at the posterior of YKLP 12270b would have resulted from the lateral rupturing of the old exoskeleton during exuviation, revealing the new telson with articulated flukes and some tergite margins (Fig. 1b,e–g).

Discussion

Comparison with the fossil record of euarthropod ecdysis[6,10] further supports the interpretation of YKLP 12270 as an individual preserved while moulting. The lateral wrinkling of the flattened head shield closely evokes the appearance of rare trilobites that were buried shortly after moulting, and prior to the expansion and hardening of the new exoskeleton (i.e. the 'soft shell' stage)[6,9,31,32]. By comparison, the articulated trunk tergite series of YKLP 12270 completely lacks wrinkles (Fig. 1a,b,d,e), which is consistent with the typical well-sclerotised exoskeleton observed between moulting events (Fig. 3b). In soft-shelled trilobites the wrinkling is often confined to the lateral sections (Harriet Drage personal observation), similar to the situation observed in YKLP 12270 (Fig. 3a). The only other specimens of *A. mirabilis* that display a comparable degree of head shield wrinkling are those with taphonomically displaced head shields[23] (Fig. 3c), which can be attributed to the effect of post-mortem decay and burial.

The overall configuration of the exoskeleton in YKLP 12270 broadly resembles that of some decapod crustacean fossils, particularly those of lobsters and shrimps. Decapod moults are readily recognisable in both extant and extinct representatives owing to their heavy exoskeleton calcification and correspondingly high preservation potential[6]. Decapod exuviae are often found in the so-called 'Open Moult Position', in which the posterior margin of the dorsal carapace (comparable to the head shield) becomes separated from the succeeding tergites and rests at a right angle relative to the trunk; the carapace often becomes displaced when the individual escapes through the resultant opening[33–35] (Fig. 3). Fossilised decapod moults usually consist of the articulated trunk tergite series and a disarticulated carapace that often remains partly attached to the rest of the body[6]. Fossilised lobster moults may also show the similar 'Overturned-Carapace Moult Position', in which the carapace has entirely separated from the body and become inverted[34], closely resembling the head shield configuration in taphonomically-dissected Xiaoshiba fuxianhuiids[23,27] (Figs 3c,d and 4c,d). Despite the considerable phylogenetic distance between decapods and fuxianhuiids, the common moulting strategy of these organisms appears to be defined by the presence of a broad head shield or carapace covering the dorsal anterior body that needs to become disarticulated in order to enable exuviation. Due to the absence of the discarded head shield in YKLP 12270, it is not possible to unequivocally conclude that the head shield disarticulated at the posterior joint with the trunk as occurs in decapods. An alternative possibility could be an anterior disarticulation of the head shield and forwards exuviation as observed in some trilobites (e.g. harpetids and trinucleids)[31] and aquatic chelicerates (e.g. eurypterids)[6]. However, we

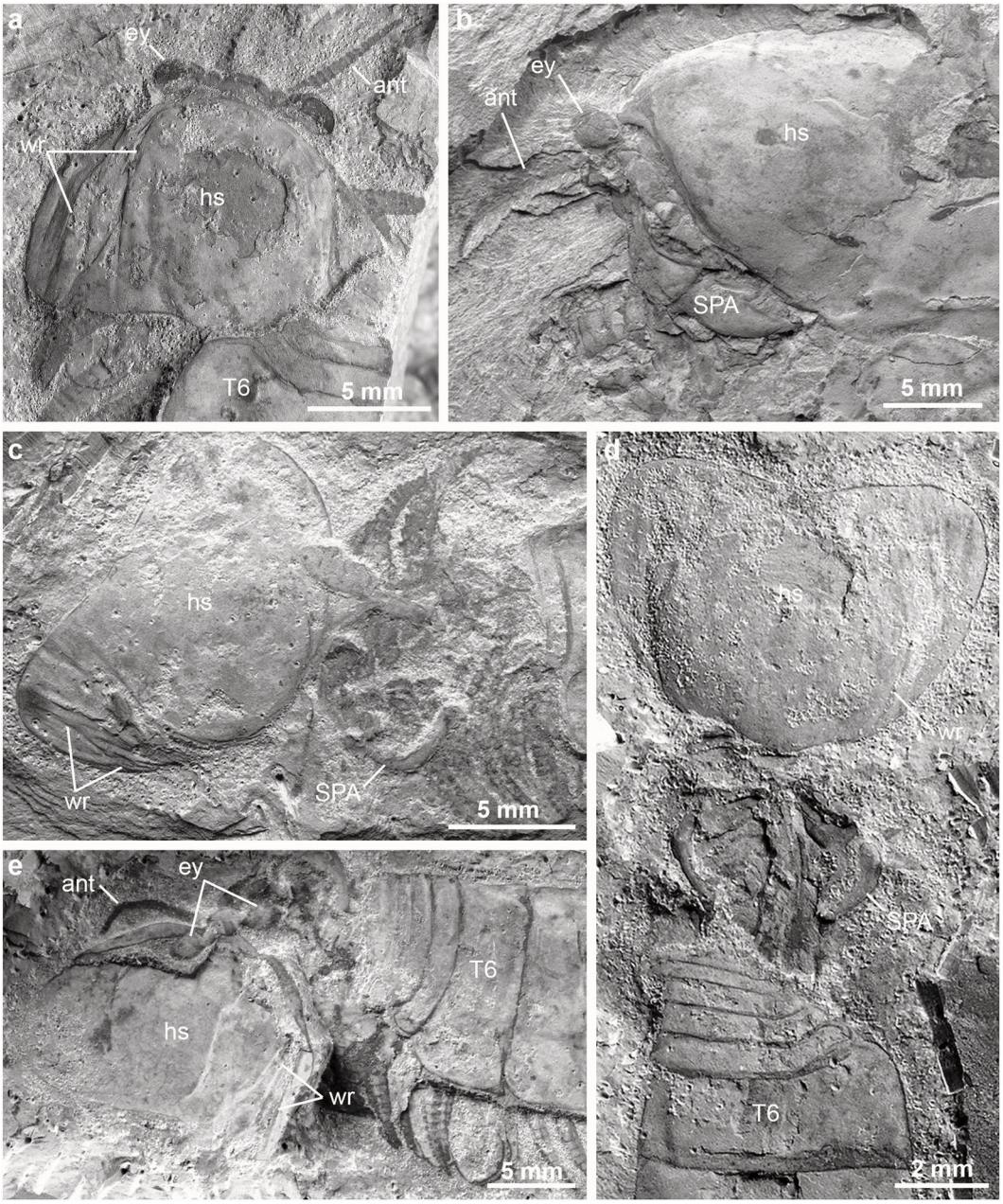


Figure 3. Taphonomic variability in specimens of *Alacaris mirabilis* and *Chengjiangocaris kunmingensis* from the early Cambrian Xiaoshiba biota. (**a**) *Alacaris mirabilis*, YKLP 12270, individual preserved in the act of moulting with wrinkled head shield, eyes and antennae in life position. (**b**) *A. mirabilis*, YKLP 12272, specimen preserved in life position. The presence of articulated eyes and antennae with the head shield, and absence of cuticular wrinkles, indicate that this specimen is a carcass from an inter-moult individual. (**c**) *A. mirabilis*, YKLP 12276, specimen preserved with 'taphonomic dissection', defined by the disarticulation of the head shield whilst still partially attached to the body anteriorly; note the presence of wrinkles on the head shield resulting from post-mortem compaction and decay. (**d**) *Chengjiangocaris kunmingensis*, YKLP 12020. (**e**) *C. kunmingensis*, YKLP 12024, specimen preserved with 'taphonomic dissection'. Abbreviations: SPA, specialised post-antennal appendages; others as in Fig. 1.

regard this option as less likely given the consistent articulation of the head shield to the anterior sclerite observed in taphonomically dissected fuxianhuiids[23,27] (Fig. 3c–e), which suggests a strong attachment site that remained stable even after death, and not a point of weakness that would disarticulate with ease.

We consider that the moulting behaviour reconstructed for *A. mirabilis* was likely common among fuxianhuiids given their similar exoskeletal construction[23,27–30], and potentially ancestral for members of Deuteropoda[24] more generally. A decapod-like moulting style and production of the Open Moult Position is supported by instances of Cambrian bivalved euarthropods in which the carapace is preserved at a right angle relative to the

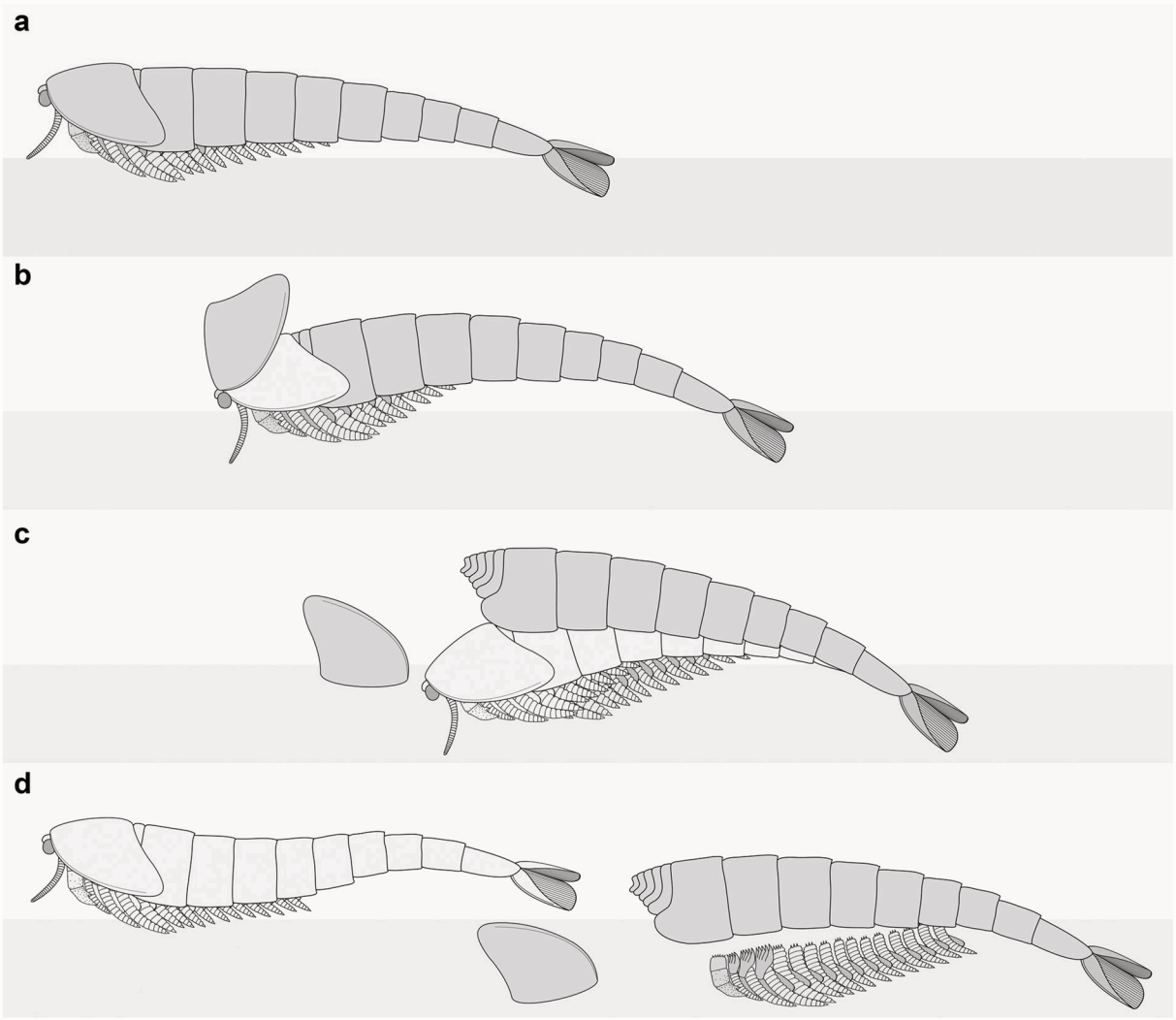


Figure 4. Reconstruction of ecdysis in *Alacaris mirabilis* based on the Open Moult Position strategy. (**a**) Intermoult stage; note that the anterior reduced tergites are covered by the head shield in life position. (**b**) Ecdysis begins with the posterior detachment of the head shield, partially revealing the underlying anterior reduced tergites. (**c**) As the emerging individual pushes against the exuvia, the old head shield is inverted and separated from the body, and the appendages become separated from the trunk; the preservation of YKLP 12270 suggests it was buried during this stage. (**d**) The soft individual emerges completely through the resulting gape, leaving the partially articulated exuvia behind.

trunk (see Fig. 8f in ref.[36]), and mass moulting assemblages of the megacheiran *Alalcomenaeus* sp. in which the head shield is consistently missing from the exuviae[13]. The Open Moult Position would appear to represent the common mechanism for performing ecdysis without the aid of specialized exoskeletal adaptations (e.g. suture lines), which are common in more phylogenetically-derived forms such as trilobites[7–9], and appear to may have evolved multiple times amongst various groups of Cambrian euarthropods[37,38]. The occurrence of the Open Moult Position seems constrained by the overall construction of the exoskeleton, particularly in taxa characterized by the presence of an extensive head shield or carapace covering the anterior region. By contrast, benthic euarthropods that have a dorsoventrally flattened profile, or that possess a proportionally smaller head shield, usually perform ecdysis facilitated through the integration of weakness lines in the exoskeleton, coupled with different patterns of breakage of the exuviae[6–9,31]. Although the exoskeletal configuration of the fuxianhuiid head shield most closely resembles that of decapod crustaceans among extant representatives in this context, it does not represent a perfect comparison given differences in terms of the site of attachment of this structure. Whereas the carapace in decapods and other crustaceans originates from the dorsal side of the trunk[39,40], the fuxianhuiid head shield is attached to the anterior margin of the head, and thus the similarities in mechanics of exuviation are most likely a result of convergent evolution resulting from a superficially similar exoskeletal organization.

Specimen YKLP 12270 makes *A. mirabilis* the second Cambrian euarthropod known to date to have been preserved during ecdysis, and also the phylogenetically earliest diverging taxon that illustrates the moulting behaviour of total-group Euarthropoda (Fig. 5). The direct record of ecdysis in Cambrian euarthropods is

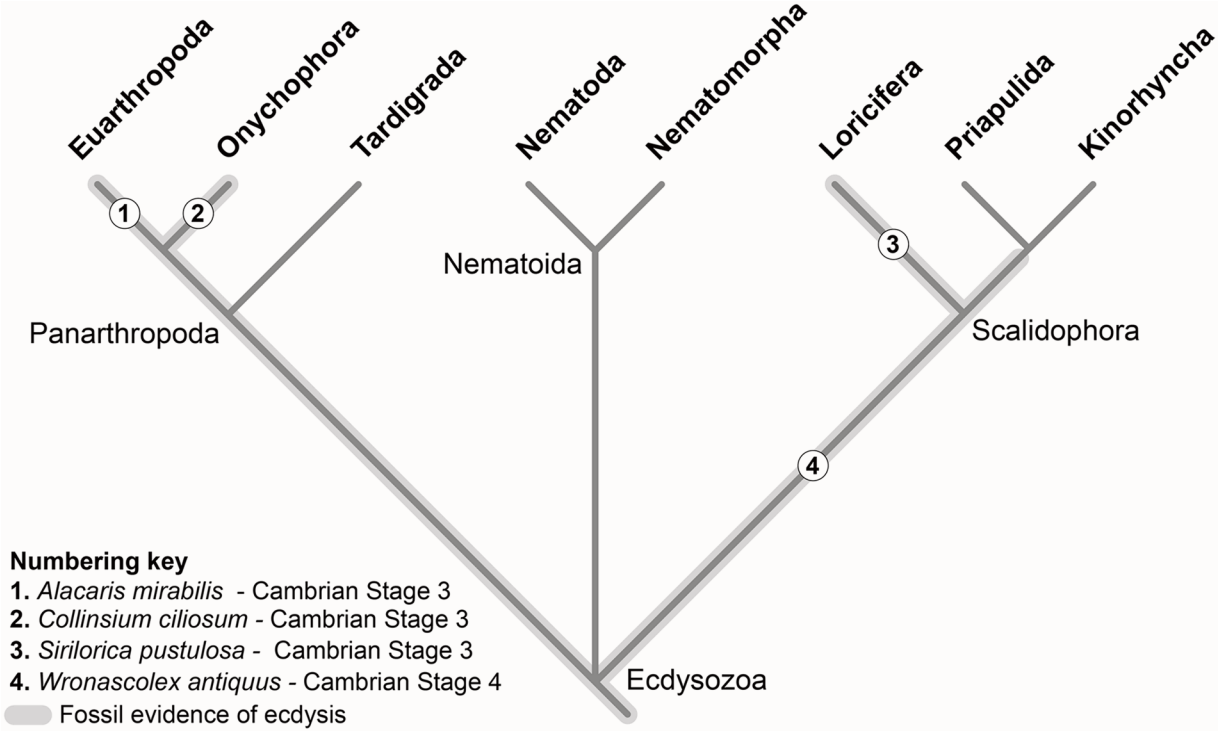


Figure 5. Simplified phylogeny of Ecdysozoa. Tree depicts the stratigraphically oldest and phylogenetically most ancestral fossil evidence of ecdysis for the major groups. Coloured lines indicate phylogenetic coverage of *Alacaris mirabilis*[23], *Collinsium ciliosum*[20], *Sirilorica pustulosa*[14], and *Wronascolex antiquus*[22]. Topology based on refs[3,4].

extremely limited as freshly-moulted individuals[9,31,32] are particularly vulnerable, and therefore cuticular hardening occurs as quickly as possible in order to avoid predation[6,41]. The other known case of a Cambrian euarthropod preserved whilst shedding the old exoskeleton is a specimen of *Marrella splendens* from the middle Cambrian (Wuliuan) Burgess Shale, whose soft emerging lateral spines are still partially trapped within the exuvia[12]. A stratigraphically-younger report of an individual of the Late Devonian trilobite *Trimerocephalus chopini* was described as preserved immediately post-exuviation[42], but the original interpretation of this specimen has been questioned and is instead regarded as two overlapping carcasses[7]. *A. mirabilis* represents one of the stratigraphically-oldest ecdysozoan fossils that capture the process of moulting[6] (Fig. 5). The only other specimen of comparable age that demonstrates ecdysis corresponds to the stem-group loriciferan *Sirilorica pustuolosa* from the Cambrian (Stage 3) Sirius Passet in North Greenland, where a complete emerging individual is associated with the shed lorica (Fig. 7 in ref.[14]). A specimen of the palaeoscolecid *Wronascolex antiquus* from the Cambrian (Stage 4) Emu Bay Shale in Australia has also been tentatively regarded as a putative exuvia (Fig. 3d in ref.[22]), although the authors did not rule out the possibility that it could also represent a decayed carcass. Despite the availability of additional Cambrian fossils that exemplify different modes of moulting[6], none of them capture the moment of ecdysis. For example, armoured lobopodians possess dorsal hardened sclerites for protection located above most of the limb pairs throughout the trunk[43]. Data from complete body fossils and/or isolated sclerites indicate that these structures were replaced regularly, as shown by the presence of multiple stacked sclerites in the plates of *Onychodictyon*[17], as well as the spines of *Hallucigenia*[18], *Microdictyon*[21], and the luolishaniid *Collinsium*[20]. Likewise, the terminal claws of *Hallucigenia* also possess multiple stacked elements, similar to the condition observed in extant onychophorans[19]. The rarity of direct fossil evidence demonstrating ecdysis in extinct organisms highlights the significance of the *A. mirabilis* specimen caught in the act of moulting. Our findings contribute towards a better understanding of the diversity and evolution of this critical growth strategy, which typifies the body plan of one of the most diverse and ecologically dominant group of animals that originated during the Cambrian Explosion.

Methods

Fossil repository and imaging. The study is based on a single specimen (YKLP 12270a,b) of the fuxianhuiid *Alacaris mirabilis*, Yang *et al.*[20], from the Cambrian (Stage 3; local lower Canglangpuan Stage) Xiaoshiba section in Kunming, South China (see locality details in refs[20,27,37]). The specimen is deposited at the Key Laboratory for Palaeobiology, Yunnan University, Kunming, China. Fossils were photographed with a Nikon D3X fitted with a Nikon AF-S Micro Nikkor 105 mm lens, with directional illumination provided by a LEICA LED5000 MCITM.

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Author Contributions

X.G.Z. and J.Y. conceived the study. J.Y. and K.S.D. collected the material. J.Y. prepared specimens for photography. J.O.-H., H.B.D., and X.G.Z. performed research and wrote the manuscript with input from the other authors. X.G.Z., and J.O.-H. prepared the figures. All authors discussed and approved the manuscript.

Additional Information

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Appendix 4.1: data set sampling summary

Table 1: Number of species sampled for seven trilobite Orders (Asaphida, Corynexochida, Lichida, Phacopida, Proetida, Ptychopariida, and Redlichiida), the taxonomically problematic Agnostida, and one incerti ordinis group. Orders are then divided into Suborders, Subfamilies, and Families, showing the taxon sampling for each. Trinucleidae, Harpetida, Agnostida, and a burlingiid species have been removed from the data set. Total species sampled = 355.

Order	#	Suborder	#	Superfamily	#	Family	#
Asaphida	61	Asaphina		Anomocaroidea	2	Aphelaspididae	1
						Pterocephaliidae	1
				Asaphoidea	37	Asaphidae	32
						Ceratopygidae	5
				Cyclopygoidea	14	Cyclopygidae	5
						Nileidae	9
				Trinucleioidea	8	Alsataspididae	1
						Raphiophoridae	7
Corynexochida	44	Corynexochina	27	Corynexochoidea	27	Corynexochidae	1
						Dolichometopidae	11
						Dorypygidae	6
						Oryctocephalidae	3
						Zacanthoididae	6
		Illaenina	17	Illaenoidea	17	Illaenidae	11
						Styginidae	6
Lichida/ Odontopleurida	18	Lichina	4	Lichoidea	4	Lichidae	4
		Odontopleurina	14	Odontopleuroidea	14	Odontopleuridae	14
Phacopida	93	Calymenina	27	Calymenoidea	27	Calymenidae	23
						Homalonotidae	4
		Cheirurina	23	Cheiruroidea	23	Cheiruridae	10
						Encrinuridae	10
						Pliomeridae	3
		Phacopina	43	Acastoidea	7	Acastidae	6
						Calmoniidae	1

				Dalmanitoidea	12	Dalmanitidae	12
				Phacopoidea	24	Phacopidae	19
						Pterygometopidae	5
Proetida	22	Proetina	22	Aulacopleuroidea	5	Aulacopleuridae	5
				Bathyuroidea	1	Telephinidae	1
				Proetoidea	16	Proetidae	15
						Tropidocoryphidae	1
Ptychopariida	71	Olenina	38	Olenoidea	38	Olenidae	38
		Ptychopariina	33	Ellipsocephaloidea	9	Agraulidae	3
						Ellipsocephalidae	5
						Estaingiidae	1
				Ptychoparioidea	24	Alokistocaridae	2
						Conocoryphidae	1
						Conokephalinidae	1
						Dokimocephalidae	1
						Elviniidae	2
						Kingstoniidae	1
						Marjumiidae	2
						Papyriaspididae	1
						Proasaphiscidae	1
						Ptychopariidae	6
						Solenopleuridae	6
Redlichiida	46	Olenellina	9	Fallotaspidoidea	4	Fallotaspididae	4
				Olenelloidea	5	Holmiidae	1
						Olenellidae	4
		Redlichiina	37	Emuelloidea	1	Emuellidae	1
				Paradoxidoidea	28	Centropleuridae	2
						Paradoxididae	22
						Xystriduridae	4

				Redlichioidea	8	Gigantopygidae	1
						Menneraspidae	1
						Redlichiidae	6

Table 2: Number of species sampled for each Epoch of the Palaeozoic Era, with their upper and lower boundary dates, and resulting bin lengths (in millions of years). Epoch dates taken from the ICS (2017). Total species sampled = 371, as species that crossed multiple Epochs have been counted within each Epoch they occurred in.

Geological Epoch	**Lower boundary (Ma)**	**Upper boundary (Ma)**	**Bin size (Ma)**	**Designation**	**# Species**
Cambrian S2	521	509	12	1	21
Cambrian S3	509	497	12	2	87
Furongian	497	485.4	11.6	3	38
Lower Ordovician	485.4	470	15.4	4	30
Middle Ordovician	470	458.4	11.6	5	59
Upper Ordovician	458.4	443.8	14.6	6	39
Lower Silurian (Llandovery & Wenlock)	443.8	427.4	16.4	7	38
Upper Silurian (Ludlow & Pridoli)	427.4	419.2	8.2	8	16
Lower Devonian	419.2	393.3	25.9	9	11
Middle Devonian	393.3	382.7	10.6	10	13
Upper Devonian	382.7	358.9	23.8	11	6
Mississippian	358.9	323.2	35.7	12	9
Pennsylvanian	323.2	298.9	24.3	13	3

Appendix 4.2: Raw counts for specimen sampling

Tables 1 and 2: The number of trilobite specimens sampled for each species (in each order). '?' = unknown sampling counts due to the species only being known from the descriptive literature.

Order	1 specimen	2	3	4	5	6	7	8	9	10	11+	?	Total
Asaphida	37	9	3	2	0	2	1	3	0	1	2	1	**61**
Corynexochida	25	14	2	1	0	0	1	0	0	0	1	0	**44**
Lichida/Odontopleurida	11	2	3	1	0	0	0	0	0	0	1	0	**18**
Phacopida	47	15	9	6	3	0	0	0	1	0	5	7	**93**
Proetida	17	4	1	0	0	0	0	0	0	0	0	0	**22**
Ptychopariida	37	13	5	2	1	1	1	1	0	0	5	5	**71**
Redlichiida	22	7	3	3	0	1	0	1	0	1	4	4	**46**
TOTAL	**196**	**64**	**26**	**15**	**4**	**4**	**3**	**5**	**1**	**2**	**18**	**17**	

Order	1	2	3-5	6-8	9-10	11+	?
Agnostida							
Asaphida	37	9	5	6	1	2	1
Corynexochida	25	14	3	1	0	1	0
Lichida/Odontopleurida	11	2	4	0	0	1	0
Phacopida	47	15	18	0	1	5	7
Proetida	17	4	1	0	0	0	0
Ptychopariida	37	13	8	3	0	5	5
Redlichiida	22	7	6	2	1	4	4
TOTAL	**196**	**64**	**45**	**12**	**3**	**18**	
% TOTAL	**57.988 16568**	**18.934 91124**	**13.313 60947**	**3.5502 95858**	**0.8875 73964**	**5.3254 43787**	

Table 3: The number of trilobite specimens sampled for each species that displayed 1-6 moulting characteristics.

# moulting characteristics shown	# specimens sampled	Count
1	**1**	135
	2	33
	3	10
	4	4
	6	1
	8	1
	12	2
	20	1
2	**1**	37
	2	20
	3	10
	4	4
	5	2
	7	2

	14	3
3	**1**	10
	2	10
	3	6
	4	4
	5	2
	6	1
	8	1
	10	1
	11	1
	26	1
	32	1
	34	1
4	**3**	1
	4	2
	6	2
	7	1
	9	2
	10	1
	12	3
	43	1
5	**4**	2
	8	2
	12	1
	20	1
6	**1**	1
	17	1
	20	1

Appendix 4.3: Diversity and sampling data

These data represent the total diversity of the dataset created for this study (i.e. using the species counts also included in Additional file 1), and the dataset generated from the online database of fossil occurrences at Fossilworks/The Paleobiology Database. Accessed 25/01/2018, and publically available at http://fossilworks.org.

Table 1: Trilobite taxonomic diversity data. Total diversity represents all occurrences of trilobite species for each order recorded in the Paleobiology Database and Fossilworks (Kiessling *et al.,* 2018), downloaded 25/01/18. Sample diversity represents all species recorded within this data set.

Order	# species - total diversity	# species - sample diversity
Asaphida	1668	61
Corynexochida	1276	44
Lichida	639	18
Phacopida	1766	93
Proetida	1629	22
Ptychopariida	2346	71
Redlichiida	429	46
TOTAL	9753	355

Table 2: Trilobite diversity data through the Palaeozoic. Total diversity represents all occurrences of trilobite species for each Epoch recorded in the Paleobiology Database and Fossilworks (Kiessling *et al.,* 2018), downloaded 25/01/18. SQS diversity is the same data corrected for sampling using Shareholder Quorum Subsampling through Fossilworks. Sample diversity represents all species recorded within this data set (boundary species recorded twice). Numbers for the Cambrian are slightly offset because the epochs are delineated differently within Fossilworks (lower Cambrian, middle Cambrian, Furongian) compared to this data set (following the ICS, 2017; Cambrian Series 2, Miaolingian, Furongian).

Geological Epoch	# species - total diversity	# species - SQS diversity	# species - sample diversity
lower Cambrian	896	563	21
middle Cambrian	1454	549	87
Furongian	1757	567	38
Lower Ordovician	634	221	30
Middle Ordovician	433	194	59
Upper Ordovician	917	189	39
lower Silurian	564	248	38

upper Silurian	245	157	16
Lower Devonian	720	366	11
Middle Devonian	247	47	13
Upper Devonian	75	43	6
Mississippian	279	153	9
Pennsylvanian	27	6	3
lower Permian	61	36	1
TOTAL	8309	3339	371

Appendix 4.4: Statistical analyses

Table 1: Chi^2 values with their associated p-values (at 6 degrees of freedom). Test of ordinal-level variability in numbers of species (observed versus expected) for each mode of moulting (Sutural Gape and Salter's). Statistical significant analyses shaded.

Order	Total # species	Sutural Gape mode (observed)	Sutural Gape mode (expected)	Salter's mode (observed)	Salter's mode (expected)
Asaphida	61	49	48.1	25	25.9
Corynexochida	44	41	34.45	12	18.55
Lichida	18	15	13	5	7
Phacopida	93	43	68.9	63	37.1
Proetida	22	18	16.25	7	8.75
Ptychopariida	71	68	55.9	18	30.1
Redlichiida	46	39	36.4	17	19.6
TOTAL	355	273	273	147	147
Chi^2 analyses α=0.05 (0.025 with Bonferroni correction)		**14.299 p=0.027**		**26.556 p=0.00018**	

Table 2: Chi^2 values with their associated p-values (at 6 degrees of freedom). Test of ordinal-level variability in numbers of species (observed versus expected) for each of the six moulting characteristics. Statistically significant analyses shaded. VS (O) and (E), observed and expected number of species with open ventral sutures respectfully; FS (O) and (E), observed and expected number of species with open facial sutures respectfully; Ce (O) and (E), observed and expected number of species with disarticulated cephala respectfully; Th (O) and (E), observed and expected number of species with thoracic disarticulation respectfully; Py (O) and (E), observed and expected number of species with disarticulated pygidia respectfully; Cr (O) and (E), observed and expected number of species with disarticulated cranidia respectfully.

Order	Total # species	VS (O)	VS (E)	FS (O)	FS (E)	Ce (O)	Ce (E)	Th (O)	Th (E)	Py (O)	Py (E)	Cr (O)	Cr (E)
Asaphida	61	5	9.33	45	46.12	15	16.29	16	15.74	16	10.43	13	12.08
Corynexochida	44	3	5.35	37	26.42	5	9.33	6	9.01	5	5.98	7	6.92
Lichida	18	3	2.38	14	11.74	3	4.15	3	4.01	3	2.66	2	3.07
Phacopida	93	4	13.07	40	64.57	56	22.81	25	22.04	19	14.61	10	16.91
Proetida	22	1	2.46	16	12.1	5	4.29	4	4.1	1	2.7	2	3.18

					6				5		5		
Ptychopariida	71	8	10.1	63	49.9	3	17.62	20	17.03	9	11.29	16	13.07
Redlichiida	46	27	8.32	37	41.09	2	14.51	12	14.02	4	9.29	16	10.76
TOTAL	355	51	51	252	252	89	89	86	86	57	57	66	66
Chi^2 analyses α=0.05 (0.0083 with Bonferroni correction)		**52.779 p=0.0000000013**		**19.115 p=0.004**		**73.782 p=0.000000000000068**		**2.48 p=0.87**		**9.09 p=0.17**		**6.9194 p=0.33**	

Table 3: Chi^2 values with their associated p-values (at 6 degrees of freedom). Test of ordinal-level variability in numbers of species (observed versus expected) that 'normally' display (i.e. data condensed to that which they use most often) each of the six moulting characteristics. Statistically significant analyses shaded. VS (O) and (E), observed and expected number of species with open ventral sutures respectfully; FS (O) and (E), observed and expected number of species with open facial sutures respectfully; Ce (O) and (E), observed and expected number of species with disarticulated cephala respectfully; Th (O) and (E), observed and expected number of species with thoracic disarticulation respectfully; Py (O) and (E), observed and expected number of species with disarticulated pygidia respectfully; Cr (O) and (E), observed and expected number of species with disarticulated cranidia respectfully.

Order	**Total # species**	**VS (O)**	**VS (E)**	**FS (O)**	**FS (E)**	**Ce (O)**	**Ce (E)**	**Th (O)**	**Th (E)**	**Py (O)**	**Py (E)**	**Cr (O)**	**Cr (E)**
Asaphida	61	2	3.74	41	36.94	12	12.86	1	1.3	1	0.98	5	6.18
Corynexochida	44	1	2.72	36	26.81	4	9.33	0	0.94	0	0.71	4	4.89
Lichida	18	0	1.09	13	10.72	3	3.73	0	0.38	0	0.28	2	1.8
Phacopida	93	1	6.16	33	60.77	53	21.15	5	2.14	4	1.61	6	10.17
Proetida	22	0	1.33	16	13.11	4	4.56	0	0.46	0	0.35	2	2.19
Ptychopariida	71	4	4.59	57	45.28	2	15.76	1	1.6	0	1.2	12	7.58
Redlichiida	46	15	3.38	31	33.36	1	11.61	1	1.18	1	0.88	7	5.59
TOTAL	355	23	23	227	227	79	79	8	8	6	6	38	38
Chi^2 analyses α=0.05 (0.0083 with Bonferroni correction)		**48.643 p=0.0000000088**		**20.609 p=0.0022**		**72.991 p=0.00000000000099**		**5.918 p=0.43**		**6.1189 p=0.41**		**4.97 p=0.55**	

Table 4: Chi^2 values (left-hand column in each pair) with their associated p-values (n-1 degrees of freedom, right-hand column in each pair, α=0.05, 0.0083 with Bonferroni correction). Test of familial/superfamilial-variability in numbers of species (observed versus expected) that display each of the six moulting characteristics. Statistically significant analyses shaded. Only tests indicated with * had close to sufficient numbers of expected samples for a reliable test.

Order	Ventral sutures		Facial sutures		Cephalon		Thorax		Pygidium		Cranidium	
Asaphida	15.068	p=0.035	*7.5447	*p=0.37	*16.362	*p=0.022	4.7795	p=0.69	2.7808	p=0.9	10.318	p=0.17
Corynexochida	5.1779	p=0.52	*4.2538	*p=0.64	6.2269	p=0.4	6.1255	p=0.41	2.1792	p=0.9	5.3548	p=0.5
Phacopida	11.86	p=0.22	*24.505	*p=0.0036	*26.026	*p=0.002	1.0944	p=1	4.9277	p=0.84	9.0536	p=0.43
Ptychopariida	4.1625	p=0.12	*4.8357	*p=0.089	0.84624	p=0.66	*0.53597	*p=0.76	1.6154	p=0.45	*3.4243	*p=0.18
Redlichiida	*3.5856	*p=0.46	*2.3727	*p=0.67	7.3333	p=0.12	1.1626	p=0.88	2.2473	p=0.69	*2.7019	*p=0.61

Table 5: Chi^2 values with their associated p-values (at 4 degrees of freedom). Test of temporal (Geological Periods) variability in numbers of species (observed versus expected) for each of the six moulting characteristics. Statistically significant analyses shaded. VS (O) and (E), observed and expected number of species with open ventral sutures respectfully; FS (O) and (E), observed and expected number of species with open facial sutures respectfully; Ce (O) and (E), observed and expected number of species with disarticulated cephala respectfully; Th (O) and (E), observed and expected number of species with thoracic disarticulation respectfully; Py (O) and (E), observed and expected number of species with disarticulated pygidia respectfully; Cr (O) and (E), observed and expected number of species with disarticulated cranidia respectfully.

Geological Period	Total # species	VS (O)	VS (E)	FS (O)	FS (E)	Ce (O)	Ce (E)	Th (O)	Th (E)	Py (O)	Py (E)	Cr (O)	Cr (E)
Cambrian	145	35	20.16	129	108.39	7	36.13	33	36.97	19	24.37	32	28.99
Ordovician	125	9	16.69	79	89.68	39	29.89	34	30.59	23	20.16	27	23.99
Silurian	51	4	7.04	37	37.83	15	12.61	15	12.9	10	8.5	8	10.12
Devonian	29	0	2.93	4	15.73	23	5.24	4	5.36	5	3.54	1	4.21
Carboniferous	12	0	1.19	9	6.38	2	2.13	2	2.17	1	1.43	1	1.71
TOTAL	362	48	48	258	258	86	86	88	88	58	58	69	69

Chi^2 analyses α=0.05 (0.0083 with Bonferroni correction)	**19.878 p=0.00053**	**15.036 p=0.0046**	**86.873 p=$6.1x10^{-18}$**	**1.508 p=0.83**	**2.5821 p=0.63**	**3.8704 p=0.42**

Table 6: Chi^2 values with their associated p-values (at 9 degrees of freedom). Test of temporal (Geological Epochs) variability in numbers of species (observed versus expected) for each of the six moulting characteristics. Statistically significant analyses shaded. VS (O) and (E), observed and expected number of species with open ventral sutures respectfully; FS (O) and (E), observed and expected number of species with open facial sutures respectfully; Ce (O) and (E), observed and expected number of species with disarticulated cephala respectfully; Th (O) and (E), observed and expected number of species with thoracic disarticulation respectfully; Py (O) and (E), observed and expected number of species with disarticulated pygidia respectfully; Cr (O) and (E), observed and expected number of species with disarticulated cranidia respectfully.

Geological Epoch	Total # species	VS (O)	VS (E)	FS (O)	FS (E)	Ce (O)	Ce (E)	Th (O)	Th (E)	Py (O)	Py (E)	Cr (O)	Cr (E)
Cambrian Series 2	**21**	10	3.41	12	17.62	3	5.85	7	6.13	2	4.11	8	4.88
Miaolingian	**87**	21	12.35	83	63.77	3	21.17	17	22.18	10	14.87	18	17.65
Furongian	**38**	5	5.28	35	27.27	1	9.05	10	9.49	7	6.36	7	7.55
Lower Ordovician	**30**	2	4.23	20	21.82	6	7.24	8	7.59	8	5.09	8	6.04
Middle Ordovician	**59**	5	8.21	39	42.38	20	14.07	16	14.74	10	9.88	11	11.72
Upper Ordovician	**39**	2	5.28	21	27.27	17	9.05	11	9.49	6	6.36	8	7.55
early Silurian	**38**	3	5.77	27	29.79	12	9.89	13	10.36	9	6.95	7	8.24
late Silurian	**16**	1	1.87	12	9.65	4	3.2	2	3.36	2	2.25	2	2.67
Lower Devonian	**11**	0	1.14	0	5.87	9	1.95	2	2.04	2	1.37	1	1.63
Middle Devonian	**13**	0	1.46	4	7.55	9	2.51	2	2.63	3	1.76	0	2.09
TOTAL	352	49	49	253	253	84	84	88	88	59	59	70	70
Chi^2 analyses α=0.05 (0.0083 with Bonferroni correction)		**27.582 p=0.0011**		**20.02 p=0.018**		**76.78 p=$7.03x10^{-13}$**		**3.1046 p=0.96**		**6.2279 p=0.72**		**5.445 p=0.79**	

Table 7: Chi^2 values with their associated p-values (at 4 degrees of freedom). Test of temporal (Geological Periods) variability in numbers of species (observed versus expected) that 'normally' display (i.e. data condensed to that which they use most often) each of the six moulting characteristics. Statistically significant analyses shaded. VS (O) and (E), observed and expected number of species with open ventral sutures respectfully; FS (O) and (E), observed and expected

number of species with open facial sutures respectfully; Ce (O) and (E), observed and expected number of species with disarticulated cephala respectfully; Th (O) and (E), observed and expected number of species with thoracic disarticulation respectfully; Py (O) and (E), observed and expected number of species with disarticulated pygidia respectfully; Cr (O) and (E), observed and expected number of species with disarticulated cranidia respectfully.

Geological Period	Total # species	VS (O)	VS (E)	FS (O)	FS (E)	Ce (O)	Ce (E)	Th (O)	Th (E)	Py (O)	Py (E)	Cr (O)	Cr (E)
Cambrian	145	20	9.85	117	95.18	4	32	2	3.69	1	2.87	16	16.41
Ordovician	125	4	8.25	67	79.71	36	26.8	4	3.09	4	2.41	19	13.74
Silurian	51	0	3.32	35	32.12	13	10.8	2	1.25	1	0.97	3	5.54
Devonian	29	0	1.85	4	17.85	23	6	1	0.69	1	0.54	1	3.08
Carboniferous	12	0	0.74	9	7.14	2	2.4	0	0.28	0	0.22	1	1.23
TOTAL	362	24	24	232	232	78	78	9	9	7	7	40	40
Chi^2 analyses α=0.05 (0.0083 with Bonferroni correction)		**18.565 p=0.00096**		**18.516 p=0.00098**		**76.34 p=1.03x10^{-15}**		**1.9118 p=0.75**		**2.8896 p=0.58**		**4.6293 p=0.33**	

Appendix 5:

Appendix 5.1: Statistical analyses comparing thoracic segment number and moulting behaviour

Table 1: Number of specimens displaying each moulting characteristic (CE, cephalic disarticulation; CR, cranidial disarticulation; FS, open facial sutures; VS, open ventral sutures) showing 5-20 thoracic segments. Sample size = 619 specimens total; CE=58; CR= 47; FS= 471; VS=51 (CR+VS counted twice).

Segment #	CE	CR	FS	VS
5	2	2	3	0
6	4	4	3	1
7	1	2	14	1
8	6	3	123	4
9	1	2	26	1
10	6	3	40	1
11	25	1	23	2
12	3	2	32	0
13	8	16	73	13
14	0	1	15	1
15	1	2	4	3
16	0	1	11	0
17	1	2	18	1
18	0	1	34	12
19	0	2	46	7
20	0	3	6	4

Table 2: Statistical analyses comparing the average thoracic segment number for specimens showing the four moulting characteristics. Statistically significant tests showing differences in average segment number between moulting behaviours are shaded.

	Cephalon disarticulation	Cranidium disarticulation	Facial suture opening	Ventral suture opening
MEAN	10.43103448	12.19148936	12.08704883	14.90196078
MEDIAN	11	13	12	15
Standard deviation	2.370225	4.168402	4.032694	4.026189
Standard error	0.3112258	0.608024	0.1858167	0.5637792
CI	0.609991339	1.191704989	0.364194101	1.10498698
-95% CI	9.821043144	10.99978437	11.72285473	13.7969738
+95% CI	11.04102582	13.38319435	12.45124293	16.00694776
n	58	47	471	51
ANOVA	12.13	p=1.00E-07	< sig diff between mean segment numbers	
Variances not equal (Levene's test for means p=4.65E-07) so should use Welch F test				
Welch F test	17.07	p=4.074E-09	< sig diff between mean segment numbers	
Residuals and Shapiro-Wilks show not normally distributed: 0.9391, p=2.433E-15, so should use non-parametric K-W				
Kruskal-	29.75	p=1.194E-06	< sig diff between median segment	

Wallis			numbers
Tukey post-hoc tests	CR-CE; VS-ALL	p=0.04761; 7.721E-06; 0.000478; 0.0002106	< presumably more segments CR than CE; more VS than the rest (CE fewer segments than rest)

Mann-Whitney posthoc pairwise comparisons to determine which pairings had significantly different average segment numbers

	CE	**CR**	**FS**	**VS**
CE	X	940.5	11204	519
CR	p=0.005783	X	10586	770
FS	p=0.023896	p=0.61844	X	7412.5
VS	p=3.5998x10E-9	p=0.0019984	p=5.5504x10E-06	X

Appendix 5.2: Statistical analyses comparing body proportion ratios and moulting behaviour

Table 1: Comparison of average cephalic ratio (length/width of thoracic joint of cephalon) for specimens showing the four different moulting characteristics (CE, cephalic disarticulation; CR, cranidial disarticulation; FS, open facial sutures; VS, open ventral sutures). Statistically significant tests showing differences in body proportion and moulting are shaded.

Cephalic ratio (L/width thorax attachment)				
	CE	**CR**	**FS**	**VS**
n	26	35	424	39
MEAN	0.6085614	0.7352612	0.6709263	0.7138774
MEDIAN	0.6049186	0.7716763	0.65236	0.7117117
Standard deviation	0.1492411	0.154979	0.20795	0.145484
Standard error	0.0292686	0.02619624	0.01009895	0.02329609
CI	0.057365378	0.051343672	0.019793568	0.045659486
-95% CI	0.551196022	0.683917528	0.651132732	0.668217914
+95% CI	0.665926778	0.786604872	0.690719868	0.759536886
ANOVA	2.606	p=0.05105		

Variances equal (Levene's test means 0.9521)
Residuals and Shapiro-Wilks show not normally distributed due to some extreme values: 0.6955 p=1.119E-29, so should use non-parametric K-W?

Kruskal-Wallis	18.08	p=0.0004233	< sig diff between median cephalic ratio
Tukey post-hoc tests	CR-CE	p=0.01786	< longer in CR than CE

Mann-Whitney posthoc pairwise comparisons to determine which pairings had significantly different average cephalic ratios

	CE	**CR**	**FS**	**VS**
CE	X	254	4293	286
CR	p=0.0034544	X	5120	604.5
FS	p=0.058353	p=0.0022976	X	6428.5
VS	p=0.0031508	p=0.4014	p=0.021457	X

Table 2: Comparison of average cranidial ratio (length/width of anterior between fixed cheeks) for specimens showing the four different moulting characteristics (CE, cephalic disarticulation; CR, cranidial disarticulation; FS, open facial sutures; VS, open ventral sutures). Statistically significant tests showing differences in body proportion and moulting are shaded.

Cranidial ratio (L/W anterior)				
	CE	**CR**	**FS**	**VS**
n	48	51	445	39
MEAN	1.064919	1.034591	1.068505	0.9409506
MEDIAN	1.007937	0.9207921	1.03	0.871875
Standard deviation	0.29257	0.2886611	0.2899767	0.2157347
Standard error	0.04222884	0.04042064	0.01374621	0.0345452
CI	0.08276701	0.079222997	0.026942084	0.067707346
-95% CI	0.98215199	0.955368003	1.041562916	0.873243254
+95% CI	1.14768601	1.113813997	1.095447084	1.008657946
ANOVA	2.5	p=0.05861		
Variances equal (Levene's test means 0.5234) Residuals and Shapiro-Wilks show not normally distributed: 0.8507 p=4.887E-23, so should use non-parametric K-W?				
Kruskal-Wallis	10.98	p=0.01184	< sig diff in median cranidial ratio	
Tukey post-hoc tests	None sig			
Mann-Whitney posthoc pairwise comparisons to determine which pairings had significantly different average cranidial ratios				
	CE	**CR**	**FS**	**VS**
CE	X	1112	10440	656.5
CR	p=0.435	X	10147	815
FS	p=0.798	p=0.21562	X	6025.5
VS	p=0.017255	p=0.14497	p=0.0015459	X

Table 3: Comparison of average thoracic ratio (length/greatest width) for specimens showing the four different moulting characteristics (CE, cephalic disarticulation; CR, cranidial disarticulation; FS, open facial sutures; VS, open ventral sutures). Statistically significant tests showing differences in body proportion and moulting are shaded.

Thoracic ratio (L/widest)				
	CE	**CR**	**FS**	**VS**
n	43	29	389	39
MEAN	0.824554447	0.973043874	0.836143994	1.040902015
MEDIAN	0.884892086	1.007936508	0.787564767	1.096153846
Standard deviation	0.2555852	0.351003	0.3021314	0.2855486
Standard error	0.03897637	0.06517963	0.01531867	0.04572437
CI	0.076392296	0.127749709	0.030024042	0.089618119
-95% CI	0.74816215	0.845294165	0.806119953	0.951283896
+95% CI	0.900946743	1.100793583	0.866168036	1.130520133
ANOVA	7.107	p=0.0001106	< sig diff between mean thoracic ratio	
Variances equal (Levene's test means 0.2973) Residuals and Shapiro-Wilks show not normally distributed: 0.9839, p=2.431E-05, so should use non-parametric K-W?				

Kruskal-Wallis	20.21	p=0.0001535		
Tukey post-hoc tests	VS-CE; VS-FS	p=0.002873; 0.005542	< presumably because longer in VS (bigger value)	

Mann-Whitney posthoc pairwise comparisons to determine which pairings had significantly different average thoracic ratios				
	CE	**CR**	**FS**	**VS**
CE	X	452	8247	465
CR	p=0.049608	X	4292	503
FS	p=0.88131	p=0.031727	X	4634
VS	p=5.3343x10E-04	p=0.44196	p=6.1483x10E-05	X

Table 4: Comparison of average pygidial ratio (length/greatest width) for specimens showing the four different moulting characteristics (CE, cephalic disarticulation; CR, cranidial disarticulation; FS, open facial sutures; VS, open ventral sutures). Statistically significant tests showing differences in body proportion and moulting are shaded.

Pygidial ratio (L/W)				
	CE	**CR**	**FS**	**VS**
n	58	38	433	36
MEAN	0.5872817	0.5973726	0.6372814	0.7067197
MEDIAN	0.5272268	0.5527726	0.5822785	0.7364532
Standard deviation	0.1914454	0.2484993	0.2655857	0.2815662
Standard error	0.02513801	0.04031191	0.01276323	0.0469277
CI	0.049269599	0.079009891	0.025015479	0.091976602
-95% CI	0.538012101	0.518362709	0.612265921	0.614743098
+95% CI	0.636551299	0.676382491	0.662296879	0.798696302
ANOVA	1.852	p=0.1366	< no sig diff between mean pygidial ratio	
Variances not equal (Levene's test for means p=0.03642) so should use Welch F test				
Welch F test	2.143	p=1.011		
Residuals and Shapiro-Wilks show not normally distributed: 0.8941 p=2.72E-19, so should use non-parametric K-W				
Kruskal-Wallis	5.24	p=0.155		

Table 5: Comparison of average body ratio (total length/greatest width) for specimens showing the four different moulting characteristics (CE, cephalic disarticulation; CR, cranidial disarticulation; FS, open facial sutures; VS, open ventral sutures). Statistically significant tests showing differences in body proportion and moulting are shaded.

Total body ratio (L/W)				
	CE	**CR**	**FS**	**VS**
n	42	30	377	39
MEAN	1.646502	1.823521	1.776205	1.940052
MEDIAN	1.595477	1.826213	1.727273	1.947761
Standard deviation	0.393622	0.3846388	0.347959	0.2968021
Standard error	0.06073719	0.07022512	0.0179208	0.04752637
CI	0.119042711	0.137638698	0.035124123	0.093149978
-95% CI	1.527459289	1.685882302	1.741080877	1.846902022

+95% CI	1.765544711	1.961159698	1.811329123	2.033201978
ANOVA	4.905	p=0.002288		
Variances equal (Levene's test means 0.3238) Residuals almost normally distributed, but not Shapiro-Wilks: 0.9834 p=2.302E-05, so should use non-parametric K-W?				
Kruskal-Wallis	18.46	p=0.0003535	< sig diff in mean body ratio	
Tukey post-hoc tests	VS-CE; almost CR-CE	p=0.0003028; 0.06934	< probably CE basically shorter than the others (VS and CR)	

Mann-Whitney posthoc pairwise comparisons to determine which pairings had significantly different average total body ratios

	CE	**CR**	**FS**	**VS**
CE	X	458	6155.5	434
CR	p=0.050127	X	5109	455.5
FS	p=0.018004	p=0.37903	X	4913
VS	p=2.7872x10E-04	p=0.11839	p=6.4778x10E-04	X

Appendix 6

Appendix 6.1: Raw data for specimens

Dalmanitina proaeva elfrida

Repository	No.	Stage	exl (mm)	crl (mm)	trl (mm)	crw (mm)	trw (mm)	Molting	Locality
NMP	L46703	P1	0.83	0.6	0.23	0.74	0.46	-	Trubín
NMP	L46653	P1	0.82	0.64	0.18	0.78	0.5	-	Trubín
NMP	L46678	P1	0.8	0.6	0.2	0.78	0.42	-	Trubín
NMP	L46663	P1	0.84	0.64	0.2	0.78	0.46	SGM-LRO	Trubín
NMP	L46707	P1	0.84	0.64	0.2	0.8	0.5	-	Trubín
NMP	L46651	P1	0.85	0.67	0.18	0.8	0.5	-	Trubín
NMP	L46744	P1	0.86	0.63	0.23	0.8	0.48	-	Trubín
NMP	L46698	P1	0.83	0.67	0.16	0.8	0.48	-	Trubín
NMP	L46792	P1	0.83	0.61	0.22	0.82	0.5	SGM-LRO	Trubín
NMP	L46772	P1	0.83	0.59	0.24	0.82	0.54	-	Trubín
NMP	L46670	P1	0.8	0.61	0.19	0.82	0.52	-	Trubín
NMP	L46686	P1	0.81	0.59	0.22	0.82	0.46	SGM-LRO	Trubín
NMP	L46650	P1	0.79	0.58	0.21	0.82	0.48	-	Trubín
NMP	L46664	P1	0.87	0.66	0.21	0.82	0.5	-	Trubín
NMP	L46659	P1	0.8	0.62	0.18	0.82	0.52	-	Trubín
NMP	L46757	P1	0.81	0.61	0.2	0.82	0.52	-	Trubín
NMP	L46755	P1	0.86	0.69	0.17	0.82	0.52	-	Trubín
NMP	L46750	P1	0.85	0.69	0.16	0.82	0.5	-	Trubín
NMP	L46660	P1	0.82	0.63	0.19	0.82	0.52	-	Trubín
NMP	L46731	P1	0.83	0.63	0.2	0.82	0.5	-	Trubín
NMP	L15046	P1	0.83	0.59	0.24	0.82	0.48	-	Trubín
NMP	L15057	P1	0.84	0.65	0.19	0.82	0.4	SGM-LRO	Trubín
MCZ	182149	P1	0.83	0.66	0.17	0.82	0.44	-	Trubín
NMP	L46774	P1	0.84	0.65	0.19	0.84	0.56	-	Trubín
NMP	L46769	P1	0.78	0.56	0.22	0.84	0.5	-	Trubín
NMP	L46778	P1	0.86	0.65	0.21	0.84	0.54	-	Trubín
NMP	L46688	P1	0.81	0.62	0.19	0.84	0.54	SGM-LRO	Trubín
NMP	L46680	P1	0.79	0.65	0.14	0.84	0.48	-	Trubín
NMP	L46665	P1	0.85	0.62	0.23	0.84	0.54	SGM-LRO	Trubín
NMP	L46657	P1	0.81	0.64	0.17	0.84	0.5	-	Trubín

NMP	L46748	P1	0.81	0.61	0.2	0.84	0.52	-	Trubín
NMP	L46682	P1	0.89	0.68	0.21	0.84	0.54	SGM-LRO	Trubín
NMP	L46706	P1	0.79	0.62	0.17	0.86	0.52	-	Trubín
NMP	L46671	P1	0.84	0.64	0.2	0.86	0.48	-	Trubín
NMP	L46656	P1	0.85	0.67	0.18	0.86	0.5	-	Trubín
NMP	L46649	P1	0.85	0.66	0.19	0.86	0.52	-	Trubín
NMP	L46736	P1	0.85	0.65	0.2	0.86	0.56	-	Trubín
NMP	L46713	P1	0.81	0.6	0.21	0.86	0.5	SGM-LRO	Trubín
NMP	L15045	P1	0.81	0.59	0.22	0.86	0.52	-	Trubín
NMP	L15056	P1	0.83	0.65	0.18	0.86	0.56	-	Trubín
NMP	L46655	P1	0.83	0.67	0.16	0.88	0.56	-	Trubín
NMP	L46662	P1	0.87	0.63	0.24	0.88	0.54	-	Trubín
NMP	L46705	P1	0.83	0.6	0.23	0.88	0.54	-	Trubín
NMP	L46697	P1	0.85	0.66	0.19	0.88	0.58	-	Trubín
NMP	L46730	P1	0.84	0.68	0.16	0.88	0.52	-	Trubín
NMP	L46719	P1	0.88	0.67	0.21	0.88	0.54	-	Trubín
NMP	L46709	P1	0.81	0.67	0.14	0.88	0.5	-	Trubín
NMP	L46771	P1	0.79	0.61	0.18	0.9	0.44	-	Trubín
NMP	L46673	P1	0.86	0.67	0.19	0.9	0.56	-	Trubín
NMP	L15058	P1	0.84	0.63	0.21	0.9	0.48	-	Trubín
MCZ	182163	P1	0.87	0.68	0.19	0.9	0.54	-	Trubín
NMP	L46777	P1	0.84	0.67	0.17	0.92	0.56	-	Trubín
NMP	L15059	P2	1.09	0.81	0.28	1.07	0.75	SGM-LRO	Trubín
NMP	L46720	P2	1.06	0.84	0.22	1.08	0.7	SGM-LRO	Trubín
NMP	L15047b	M0	1.21	0.82	0.39	1.08	0.78	-	Trubín
NMP	L46652	M0	1.2	0.77	0.43	1.11	0.82	SGM-LRO	Trubín
NMP	L46661	P2	1.1	0.83	0.27	1.11	0.72	-	Trubín
NMP	L15050	P2	1.14	0.77	0.37	1.11	0.87	SGM-LRO	Trubín
NMP	L46694	M	-	0.87	-	1.13	-	-	Trubín
NMP	L15047a	P2	1.15	0.87	0.28	1.13	0.74	-	Trubín
NMP	L15049	M0	1.19	0.82	0.37	1.15	0.82	SGM-LRO	Trubín
NMP	L46797	M0	1.2	0.86	0.34	1.17	0.77	SGM-LRO	Trubín

NMP	L46805	P2	1.13	0.85	0.28	1.19	0.85	-	Trubín
NMP	L46718	M	-	0.89	-	1.25	-	-	Trubín
NMP	L15060	M0	1.31	0.87	0.44	1.28	0.8	SGM-LRO	Trubín
NMP	L46747	M	-	0.95	-	1.44	-	-	Trubín
NMP	L15062	M1	1.73	1.08	0.65	1.49	1.18	-	Trubín
NMP	L15051	M1	1.79	1.11	0.68	1.55	1.28	-	Trubín
NMP	L15052	M1	1.57	0.98	0.59	1.55	1.07	SGM-LCU	Trubín
NMP	L46790	M1	1.87	1.14	0.73	1.6	1.27	-	Trubín
NMP	L46690	M	-	1.19	-	1.7	-	-	Trubín
NMP	L46791	M	-	1.26	-	1.79	-	-	Trubín
NMP	L46807	M2	2.17	1.28	0.89	2.11	1.6	SGM-LCU	Trubín
NMP	L46798	M	-	1.33	-	2.2	-	SGM-LCU	Trubín
NMP	L15063	M3	-	1.45	-	2.3	-	-	Trubín
NMP	L46749	M	-	1.66	-	2.78	-	-	Trubín
MCZ	182116	M5	-	1.78	-	2.88	-	-	Trubín
NMP	L15061	M5	-	1.68	-	2.9	-	SGM-LCU	Trubín
NMP	L15053	M5	-	1.76	-	3.08	-	-	Trubín
NMP	L46723	M	-	2.21	-	3.9	-	-	Trubín
NMP	L46783	M	-	2.3	-	4.04	-	-	Trubín
NMP	L46796	M	-	2.46	-	4.54	-	-	Trubín
NMP	L46708	M	-	2.41	-	4.6	-	SM?	Trubín
NMP	L46793	M	-	2.93	-	6.04	-	-	Trubín
NMP	L15064	M10	-	3.51	-	6.36	-	SGM-LCU	Trubín
NMP	L46717	H?	-	3.45	-	6.62	-	SM	Trubín
CGS	MŠ1247	H	-	4.2	-	7.26	-	SGM-LCU	Beroun - Na Vinici
NMP	L46788	H?	-	4	-	7.3	-	-	Trubín
NMP	L46804	H	-	4.31	-	7.68	-	SM	Trubín
NMP	L46562	H	-	4.61	-	8.19	-	SM	Trubín
NMP	L46824	H	-	4.93	-	8.39	-	SGM-LCU	Trubín
NMP	L46715	H	-	5.84	-	11.82	-	SGM-LCU	Trubín
NMP	L15055	H	-	5.93	-	12.72	-	SGM-LCU	Trubín
NMP	L46568	H	-	8.23	-	15.51	-	SGM-LCU	Trubín

NMP	L46564	H	-	13.05	-	24.31	-	SM	Trubín
CGS	MŠ1245	H	-	14.84	-	27.23	-	SM	Vráž near Beroun
NMP	L46821	H	-	17.78	-	32.37	-	SM	Trubín
NMP	L46818	H	-	27.99	-	52.1	-	SM	Trubín

Dalmanitina proaeva

Repository	No.	Stage	crw (mm)	crl (mm)	Molting	Locality
CGS	MŠ2165	H	45.04	23.91	SM	Praha - Libeň
CGS	MŠ2181a	H	26.98	15	SGM-LCU	Praha - Spořilov
CGS	MŠ2669	H	24.84	13.93	SM	Praha - Libeň
CGS	MŠ2671	H	19.78	12.42	SGM-LCU	oholy - Nová Dubeč
CGS	MŠ2673	H	43.98	26.97	SM	oholy - Nová Dubeč
CGS	MŠ2678	H	29.42	22.21	SM	Radotín
CGS	JV18212	H	20.82	13.03	SGM-LCU	Praha - Dubeč
CGS	PB130	H	45.92	24.79	SGM-LCU	Praha - Dubeč

Dalmanitina socialis

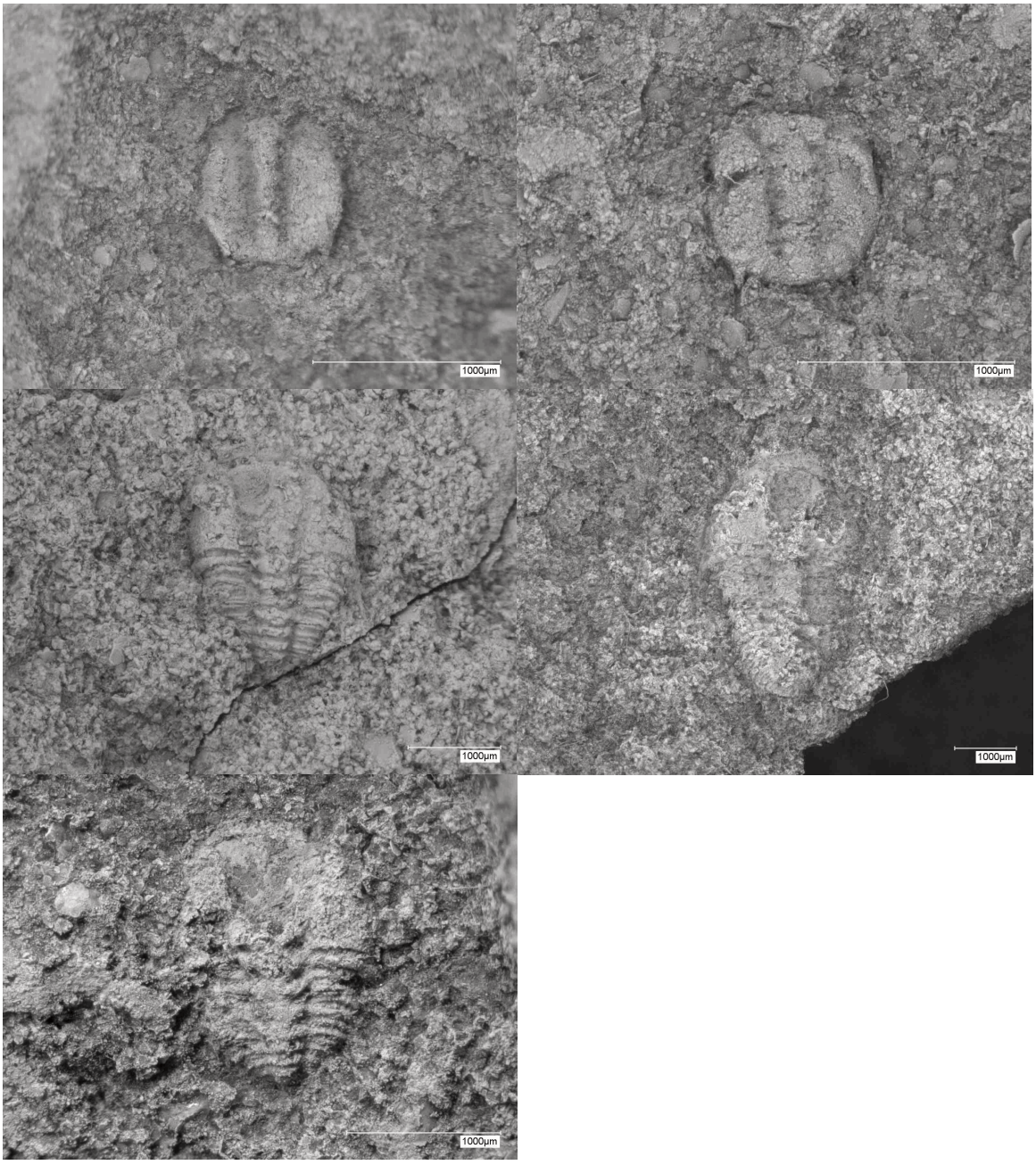
Repository	No.	Stage	crw (mm)	crl (mm)	Locality
NMP	L15054	H	5.2	3.25	Drabov
NMP	L46672	M	2.86	2	Trubsko
NMP	L46674	M	2.68	1.77	Trubsko
NMP	L46677	M	1.84	1.34	Trubsko
NMP	L46679	M	2.46	1.76	Trubsko
NMP	L46681	P/M?	0.99	0.71	Trubsko
NMP	L46693	M	1.65	1.08	Trubsko
NMP	L46696	M	1.32	1.09	Trubsko
NMP	L46700	M	1.74	1.28	Trubsko
NMP	L46702	M	1.38	1.06	Trubsko
NMP	L46711	M	1.38	0.99	Trubsko
NMP	L46714	M	2.13	1.31	Trubsko

NMP	L46716	M	2.62	1.96	Trubsko
NMP	L46721	M	1.93	1.53	Trubsko
NMP	L46725	M	1.29	0.99	Trubsko
NMP	L46727	M	1.22	1	Trubsko
NMP	L46732	M	2.78	1.91	Trubsko
NMP	L46737	M	2.94	1.78	Trubsko
NMP	L46739	M	1.86	1.38	Trubsko
NMP	L46762	M	2.62	1.9	Trubsko
NMP	L46764	M	1.9	1.34	Trubsko
NMP	L46765	M	2.2	1.45	Trubsko
NMP	L46767	M	3.03	2.02	Trubsko
NMP	L46768	M	1.31	1.04	Trubsko
NMP	L46768	M	1.88	1.33	Trubsko
NMP	L46779	M	2.93	1.9	Veselá
NMP	L46784	H	6.82	4.22	Veselá
NMP	L46786	M	1.3	0.94	Trubsko
NMP	L46789	H	8.69	5.71	Veselá
NMP	L46802	M	3.15	2.43	Trubsko

Table abbreviations: NMP repository, National Museum Prague, Czech Republic; CGS, Czech Geological Survey, Czech Republic; crw, cranidial width; crl, cranidial length; MCZ, Museum of Comparative Zoology, USA; exl, exoskeletal length; trl, trunk length; trw, trunk width; SM, Salter's mode of moulting; SGM-LCU, Sutural Gape mode of moulting with Lower Cephalic Unit; SGM-LRO, Sutural Gape mode of moulting with separate librigenae; H, holaspid stage; M(n), meraspid stage; P(n), protaspid stage.

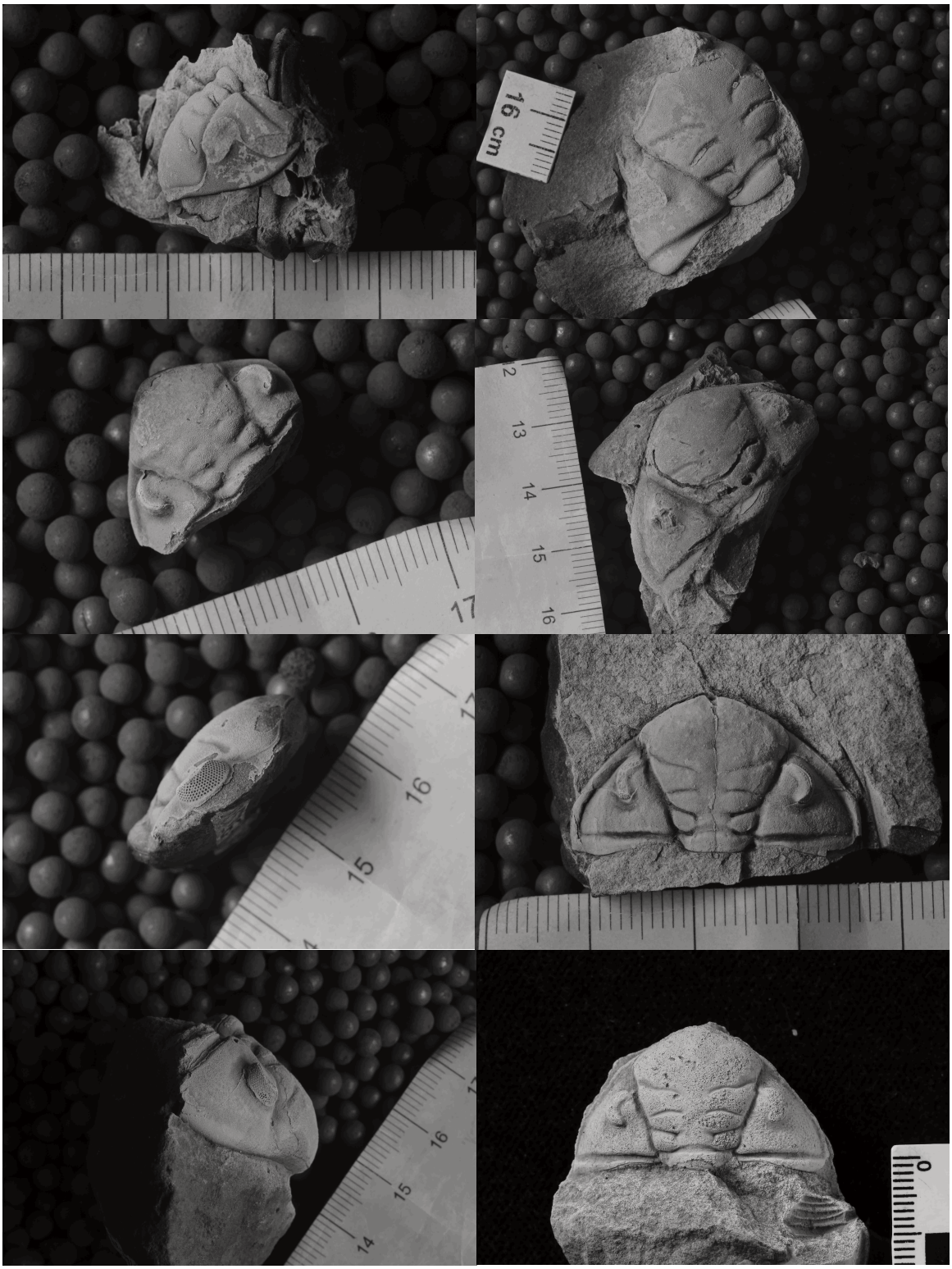
Appendix 6.2: Additional photos supporting the primary and secondary fusion events in *Dalmanitina* (from National Museum Prague and Czech Geological Society)

Primary fusion:



All specimens accessioned in the National Museum Prague, but without single identifier numbers. First two show still separate librigenae, the remainder a single joined bar (the LCU).

Secondary fusion:



Specimen numbers in order: JV18212; PB130; MŠ2669; MŠ2165; MŠ2678; MŠ1245; MŠ2674; L46923, from the National Museum Prague and Czech Geological Survey. First two show clearly operable sutures, others appear fused.

Appendix 7:

Appendix 7.1: Measurements (in mm) of *Symphysurus ebbestadi* specimen body proportions using digital callipers

Measurement abbreviations: incomp, incomplete; pyg, pygidium; ce, cephalon; th, thorax; ax ring, axial ring; th segment, thoracic segment; ant, anterior; post, posterior; occ ring, occipital ring.

1: Length: 47.7mm full - 14.8 (incomp) pyg - 17.6 ce - 17 th
Width: 25.1 (incomp) pyg - 26 ce - 26.6 (incomp) th - 10.2 1st ax ring - 8.3 8th ax ring

2: Length lower specimen: 50.2mm (incomp?) full - 16.4 pyg - 17.5 (incomp?) ce - 20.8 th
Width lower specimen: all incomp
Length upper specimen: 38.6 (incomp) full - 17.6 ce - pyg missing
Width upper specimen: 28.1 (incomp?) ce - 29.3 th - 11.8 1st ax ring - 5.8 left adaxial 1st th segment - 5.5 left abaxial 1st th segment - 18.2 glabella ant - 12.3 glabella post

3: Length right specimen: 43.4mm full - 12.8 pyg - 14.8 ce - 17.1 th
Width right specimen: 24.5 pyg - 29 ce - 27 th - 11.2 1st ax ring - 7.9 8th ax ring - 6 left adaxial 1st th segment - 5.1 left abaxial 1st th segment - 16 glabella ant - 11.9 glabella post (occ ring location)
Length left specimen: 19.9 th
Width left specimen: 11 2nd ax ring - 9.1 8th ax ring

4: Length: 18.4mm ce - 19.2 th
Width: 31.8 ce - 34.2 th - 12.4 1st ax ring - 8.9 8th ax ring

5: Too poorly preserved

6: Too poorly preserved

7: Length: 11.4 ce
Width: 25.4 ce - 25.3 th - 15.3 glabella ant - 11.3 glabella post - 10.2 1st ax ring - 6.1 left adaxial 1st th segment - 3.7 left abaxial 1st th segment

8: Length: 47 (incomp) full -14.4 (incomp?) ce - 23.2 th

Width: 23.5 (incomp) pyg - 32.9 ce - 32.9 th - 13.1 1st ax ring - 9.5 8th ax ring - 6.1 left adaxial 1st th segment - 5.3 left abaxial 1st th segment

9: Length: 14.4mm pyg - 20.1 th
Width: 28.5 th - 26.9 pyg - 11 1st ax ring - 8 8th ax ring - 5.9 right adaxial 2nd th segment - 6.1 right abaxial 2nd th segment - 11.8 glabella post

Table 1: summary of body proportion measurements for each specimen of *Symphysurus ebbestadi*. Abbreviations: length P:T:C, length of pygidium:thorax:cephalon; C W, cephalic width; C L, cephalic length; C W/L, cephalic width/length ratio; T W, thoracic width; T L, thoracic length; T W/L, thoracic width/length ratio; P W, pygidial width; P L, pygidial length; P W/L, pygidial width/length ratio; MGL, Museé Cantonal de Géologie Lausanne.

Specimen	Length P:T:C	C W (mm)	C L (mm)	C W/L	T W (mm)	T L (mm)	T W/L	P W (mm)	P L (mm)	P W/L
1 (MGL 102127)	X	26	17.6	1.48	X	17	X	X	X	X
2 (MGL 102128)	1:1.07:1.27 X	28.1 X	17.6 17.5	1.6 X	29.3 X	X 20.8	X X	X X	X 16.4	X X
3 (MGL 102129)	1:1.16:1.34	29	14.8	1.96	27	17.1	1.58	24.5	12.8	1.91
4 (MGL 102130)	X	31.8	18.4	1.73	34.2	19.2	1.78	X	X	X
5 (MGL 102131)	X	X	X	X	X	X	X	X	X	X
6 (MGL 102132)	X	X	X	X	X	X	X	X	X	X
7 (MGL 102133)	X	25.4	11.4	2.23	25.3	X	X	X	X	X
8 (MGL 102134)	X	32.9	14.4	2.28	32.9	23.2	1.42	X	X	X
9 (MGL 102135)	X	X	X	X	28.5	20.1	1.42	26.9	14.4	1.87

Appendix 7.2: Measurements (in mm) of cuticle thickness from thin sections of *Symphysurus ebbstadi* specimens

Cephalon

Specimen									Mean	Median
1 (MGL 102127), Slice 7.1	0.76	0.59	0.68	0.5	0.49	0.46	0.3	0.2	0.4975	0.495
4 (MGL 102130), Slice 3.4	0.83	0.7	0.61	0.42	0.29	0.3	0.22	0.3	0.45875	0.36
7 (MGL 102133), Slice 6.4	0.11	0.17	0.12	0.11	0.063	0.051	0.063		0.098142857	0.11
8 (MGL 102134), Slice 12.1	0.14	0.13	0.17	0.13	0.073	0.081	0.097		0.117285714	0.13

Thorax

Specimen										Mean	Median
1 (MGL 102127), Slice 10.2	0.33	0.34	0.33	0.25	0.3	0.31	0.25	0.21	0.36	0.297777778	0.305
4 (MGL 102130), Slice 1.3	0.18	0.24	0.19	0.15	0.22	0.19	0.27	0.18	0.24	0.206666667	0.198333333
7 (MGL 102133), Slice 5.2	0.093	0.072	0.075	0.14	0.11	0.11	0.13	0.069	0.071	0.096666667	0.094833333
8 (MGL 102134), Slice 12.3	0.076	0.044	0.083	0.07	0.097	0.099	0.058	0.061	0.068	0.072888889	0.071444444