SUPPLEMENTARY TEXT

The Tully Monster is a vertebrate

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INTERPRETATION OF MORPHOLOGY

We have re-examined the major morphological features of *Tullimonstrum gregarium* in 1,286 specimens, resulting in new interpretations which support the hypothesis of a chordate affinity (see main text). Here we provide (i) further discussion of major morphological features (presented in the order in which they are discussed in the main text: notochord, gut trace, brain, arcualia, myomeres and body shape, gill pouches, proboscis and buccal apparatus) and (ii) a discussion of additional morphological features (eyebar, tail and dorsal fin, naris, tectal cartilages and liver).

There are a number of other anatomical features that would help to determine the place of *Tullimonstrum* within the chordate clade[1]: a velum, a lateral line, the otic capsule (inner ear), and the presence or absence of a spiracle. However, none of these features are identifiable in the *Tullimonstrum* fossils. The velum, lateral line and the spiracle do not typically fossilize, being small, internal, or decay prone, and they are unknown in fossil lampreys. The velum persists through a few stages of chordate decay[2], but would likely be concealed in fossils because it is an internal structure. The otic capsule is evident in *Gilpichthys*[3], *Mayomyzon*[4], and possibly *Pipiscius*[3]; its apparent absence in *Tullimonstrum* may be a result of the predominance of dorsoventrally flattened specimens.

Notochord. A notochord was previously identified in only one Mazon Creek taxon, the stem hagfish *Gilpichthys greenei*, based on a number of morphological features: the notochordal sheath, the covering of the dorsal nerve cord, and a sheath enclosing the dorsal blood vessels. This tripartite arrangement is similar to that found in lampreys[3]. These morphological features allow the notochord to be distinguished from the gut. This provides a search image for the notochord in other taxa, where a 2D light-coloured linear structure matches the preservational style without necessarily displaying all the diagnostic morphology. The notochord is one of the most decay-resistant features in extant lampreys[2], and therefore likely to be preserved.

The notochord in *Tullimonstrum* (previously interpreted as a gut trace[5,6]) is a simple straight-sided linear structure lacking morphological features preserved in the notochord of *Gilpichthys*, but with the same general preservational style (two dimensional and light-coloured). The notochord is preserved in 204 of the 10658 specimens (about 19%) of *Tullimonstrum* that display at least part of the body. We examined 50 complete specimens of *Gilpichthys* and observed a notochord in 9 (18%), a similar frequency to that in *Tullimonstrum*. The notochord is more rarely preserved in the tail than in the body of both *Tullimonstrum* and *Gilpichthys* (e.g. Fig. 2b). In decay experiments on lampreys (and other chordates), the notochord was found to be more decay-prone at the posterior end[2].

Although a notochord has not been described in other Mazon Creek chordates, a structure with appropriate morphology and preservation is evident in the Mazon Creek stem lamprey *Mayomyzon*[7]. A dorsally preserved specimen shows a light-coloured linear, medial structure running the entire length of the body, bounded by darker material (similar to Fig. 2a,b); no gut trace is evident in this specimen.

Most specimens of *Tullimonstrum* (89%) are preserved dorsoventrally flattened (as indicated by a bilaterally symmetrical eyebar orthogonal to the trunk axis) so that all medial structures are superimposed, regardless of any dorsoventral offset in their original position. Although the few laterally preserved specimens (indicated by an asymmetrical eyebar) show structures offset dorsoventrally, previous authors have argued that it is impossible to distinguish dorsal from ventral[5,8]. With the identification of the notochord the position of other morphological features relative to it provides a means of distinguishing dorsal and ventral (Extended Data Fig.2 a-c). However other axial features, such as the dorsal fin and the arcualia, are rarely preserved with the notochord in laterally-preserved specimens; their position is determined in relation to the eyebar which is offset in the same direction as the notochord in laterally preserved specimens, and is therefore dorsal (Extended Data Fig. 2a).

Gut trace and oesophagus. The gut trace is evident in very few specimens of *Tullimonstrum,* in contrast to *Gilpichthys* which preserves it in 22 out of 50 specimens, or 44%. This may reflect the preservation of the great majority of *Tullimonstrum* specimens dorsoventrally in contrast to *Gilpichthys*, which is most commonly preserved laterally.

Gut contents are rarely identifiable in Mazon Creek fossils: the dark mineral infilling the gut most likely precipitated around the decaying contents. Previous authors have suggested that *Tullimonstrum* ate soft-bodied prey such as jellyfish[5] but this was based on the erroneous interpretation of the light coloured notochord as a gut trace with no contents. The true gut trace is preserved in a fashion typical of Mazon Creek fossils and provides no evidence of a particular diet. A less common pattern of gut preservation in Mazon Creek fossils comprises a light brown, irregularly shaped structure, crossed by multiple kaolinite-infilled cracks (Extended Data Fig. 8a). This is also the mode of preservation of coprolites[9], and was not observed in *Tullimonstrum.*

Some specimens of *Tullimonstrum* appear to show a trace of the digestive system running along the length of the proboscis: a linear, medial structure slightly darker than the surrounding tissue[5,6]. We interpret this structure as the oesophagus. It differs in preservation from the notochord and lacks the typical dark three-dimensional preservation of the post-cranial gut trace presumably because food did not remain in the oesophagus long enough to provide a locus for mineralization. Similarly, the dark three-dimensional gut trace in *Gilpichthys* does not continue all the way to the mouth (Fig. 2c).

Brain. Details of the tri-lobed brain are preserved clearly in three-dimensions in only a few of the studied *Tullimonstrum* specimens. In others the notochord appears to expand in the vicinity of the eye bar, or to extend anteriorly beyond it a small distance. These two styles of preservation – three-dimensional lobes similar to the arcualia (Extended Data Fig. 3a) or a light coloured stain similar to the notochord (Extended Data Fig. 3b) – suggest that the preserved structures represent some combination of elements of a cartilaginous neurocranium and nervous tissue. Decay experiments on modern lampreys have shown that elements of the braincase persist throughout decay, but are altered morphologically[2]. The preserved brain and braincase morphology in *Tullimonstrum* are likely to have degraded and therefore cannot be compared easily to that of modern lampreys. Molluscs also have a three-lobed brain, but, in contrast to chordates, the optic nerve connects to the anteriormost lobe rather than to the central lobe[10]. Thus the arrangement in *Tullimonstrum,* where the inferred optic nerves connect to the central lobe, is consistent with a chordate affinity.

Previous interpretations of the features interpreted here as the brain were based on the assumption that the notochord represented a gut trace. The expansion around the eyebar was assumed to be part of the digestive system, and was most commonly interpreted as a stomach[5,6].

Cartilaginous arcualia. About 34% of *Tullimonstrum* specimens preserve 1 to 8 arcualia, most commonly the anteriormost, in three dimensions. They are evident lying along the notochord in both dorsoventrally flattened and obliquely lateral specimens (Extended Data Fig. 1g, Extended Data Fig. 2c) but it is not possible to determine whether they were dorsal or ventral relative to the notochord. Arcualia are dorsal in position in lampreys and ventral in hagfishes[11]. There is one arcualium per myomere (previously reported as round impressions on the midline[6]), situated about its midlength or posteriorly (Extended Data Fig. 1d,g). The myomere separations rarely traverse the midline of the body, making it difficult to determine the position of the arcualia within the myomeres. In modern lampreys, each myomere encompasses two arcualia[11]. The different arrangement in *Tullimonstrum,* with one arcualium per segment, may reflect an evolutionary divergence, or each one may represent two arcualia positioned so closely that they cannot be distinguished. The arcualia are preserved as simple ovals or circles. This may represent an original simple morphology, or may be due to the loss of other morphological features.

As lampreys decay the fluid that fills the notochord clumps and condenses, which may give the appearance of repeated structures[2]. However, the fluid condenses irregularly and this does not result in a pattern resembling one arcualium per segment.

The arcualia were previously interpreted based on the assumption that the notochord represents a gut trace. Their serially repeated nature led to interpretations as nephridia, ganglia, gonads, or annulations of the gut[6].

Myomeres and general body shape. The myomeres were previously identified as segments[6], either as chordate myomeres[8] or as transverse muscle bands similar to those in some heteropod gastropods[5]. The patterns of myomere separation in *Tullimonstrum* closely match those observed in the decay of extant chordates[2] and differ from that in molluscs. In many specimens of *Tullimonstrum* the myomeres separate only at the edges of the body, not along the axis (Extended Data Fig. 1d-e). Similarly in experiments on lampreys the myomeres show most decay resistance along the axis in the vicinity of the notochord[2]. Myomere separation differs in shape and extent between the body and tail region of individual specimens of *Tullimonstrum* (extended Data Fig. 1e); in extant lampreys, the trunk and tail myomeres likewise differ in decay resistance[2].

Myomere separation in *Tullimonstrum* is evident in the body of 48% of the 1058 specimens that preserve at least part of the body, and in the tail (the part of the body surrounded by the tail fin) of 27% of the 510 specimens. Some specimens have myomere separation in both body and tail (Extended Data Fig. 1a-e). Myomeres are one of the most resistant structures during the decay of lampreys[2,12]. Where decay of *Tullimonstrum* was limited, the myomeres would not appear separated. Previous authors have noted that the 'segments' are not apparent in the specimens that are well preserved[5].

Myomere number is very weakly correlated with body length ($R^2 = 0.05$) (Extended Data Fig. 1j) whereas myomere length shows a stronger correlation ($R^2 = 0.57$) (Extended Data Fig. 1i). This suggests that *Tullimonstrum* grew by increasing the size of myomeres rather than their number.

The preserved soft tissues of *Tullimonstrum* are enriched in a variety of trace elements (Extended Data Fig. 5a-c). Zinc enrichment reveals the myomeres (Fig. 1e, Extended Data Fig. 5c) but a similar enrichment has not been observed in Mazon Creek taxa, including molluscs and chordates, which were analysed for comparison (Extended Data figure 6c,h,i,k).

The body of *Tullimonstrum* was tubular and short and stout compared to extant (and most fossil) lampreys. The length-width ratio of *Tullimonstrum* is similar whether it is preserved in dorsal (Fig. 2a) or lateral (Fig. 1c) view. In contrast, Mazon Creek fish such as *Esconichthys* appear wide when preserved laterally and narrow when preserved dorsally[7]. A short, stout body (i.e. one with a low length to width ratio) facilitates tail-propelled rather than undulatory swimming[13,14].

Gill pouches. Gill pouches, which have not been recorded previously in *Tullimonstrum*, were only identified in four specimens. The gill pouches are elliptical, similar in shape but wider than the gill pouches in the Mazon Creek stem lamprey *Mayomyzon*[5] and extant lampreys[2], which together phylogenetically bracket *Tullimonstrum* (Extended Data Fig. 7). In *Mayomyzon* the long axis of the gill pouches is oriented perpendicular to the notochord[4], as it is in *Tullimonstrum*, whereas the orientation is oblique in extant lampreys[2]. The gill pouches in *Tullimonstrum* (Fig. 1c, Extended Data Fig. 1h) and *Mayomyzon*[7,4] are both preserved as dark stains. *Mayomyzon* and modern lampreys both have seven gill pouches[4] but no more than five are evident in *Tullimonstrum*. This may represent a real difference, or additional gill pouches may not be preserved in *Tullimonstrum.* In extant lampreys the gill pouches can persist even after extensive decay[2], and gill pouches are commonly observed in fossil lampreys. The rare preservation of gill pouches in *Tullimonstrum* is most likely due to its typical dorsal orientation (in contrast to most fossil lampreys which are preserved laterally).

Proboscis and buccal apparatus. 729 specimens of *Tullimonstrum* preserve at least part of the proboscis. Of these, 153 are characterized by sharp bends (Fig. 3a) and the rest are either straight or too incomplete to detect evidence of flexion. The angle of the flexures shows a range from straight to almost 90°, indicating movement about the joints. Thus the evidence shows that the proboscis is articulated in three positions rather than flexible along its length.

The most proximal (posteriormost) joint is at the base of the proboscis. In laterally preserved specimens it is evident that the proboscis bends ventrally here (Fig. 1c). In dorsally preserved specimens, a fold is evident at this joint (Fig. 3a), as in the tail, reflecting vertical rather than lateral flexure. An angular flexure, which directs the oral hood ventrally, is also characteristic of extant lampreys and of *Pipiscius zangerli* (identified as a lamprey here: Extended Data Fig. 7) from Mazon Creek[7]. A second joint lies near the midlength of the proboscis, where it flexes in the opposite direction to the proximal one, directing the proboscis dorsally. The distal-most (anteriormost) joint lies immediately posterior to the base of the buccal apparatus where the buccal apparatus is flexed ventrally (Fig. 1b shows a reconstruction of the life position and relative orientations of the jointed proboscis and buccal apparatus). The thicker element of the bifurcate structure is dorsal in position. This reflects the arrangement of the oral hood in extant lampreys where the dorsal part of the oral hood is thicker than the ventral. Although flexure of the proboscis was predominantly vertical some horizontal movement may have been possible.

The only internal structure preserved in the buccal apparatus is at the base and we interpret it as a lingual organ. This structure shows a range of morphologies (Extended Data Fig. 4a-b), which we interpret as a result of decay and varying degrees of secondary mineralization. In one well-preserved example the outline of this feature resembles an inverted stylized comma (Extended Data Fig. 4a). The structure is similar in shape and position to that of the lingual organ of extant lampreys[15]. The lamprey lingual organ bears rasping teeth[15], but we have observed no evidence of such a structure in *Tullimonstrum*.

We have observed no direct morphological evidence of internal cartilaginous structures in *Tullimonstrum* that might represent supports for an oral hood. *Tullimonstrum* is phylogenetically bracketed (Fig. 1a) in our analysis by *Mayomyzon*, a Mazon Creek lamprey without an oral hood[4], and by a clade of modern and fossil lampreys with a hood[3,16](Extended Data Fig. 7). Therefore it is not clear whether or not the proboscis and buccal apparatus of *Tullimonstrum* are a highly derived form of the oral hood in extant lampreys. Without preserved internal structures (with the exception of the lingual apparatus), the *Tullimonstrum* proboscis and buccal apparatus cannot be compared to other anterior structures in lampreys or hagfish to determine how these features might have been derived.

However, given that recent analyses have identified homologies between the lamprey oral hood and the nasal-oral septum in hagfish[17], homologies for the *Tullimonstrum* proboscis and oral apparatus may be found among these structures. This would be consistent with our conclusion that the *Tullimonstrum* proboscis is jointed rather than fully flexible, because the components of the lamprey oral hood are likewise jointed[15].

There is evidence of teeth within the base of the buccal apparatus of *Tullimonstrum*, i.e. posterior to the bifurcate structure (Extended Data Fig. 4b), but this area is often obscured by pyrite and kaolinite which even conceal where the base splits into the bifurcate structure. This makes it difficult to determine the number of teeth within the base and how they are positioned. Nonetheless the presence of teeth in the base, their shape and posterior inclination, and the position of the oesophagus within the proboscis all indicate that the mouth lies within this buccal apparatus. This supports our interpretation of the anteriormost end of the buccal apparatus as a bifurcate structure, rather than representing a section of a hollow tube revealed by splitting the fossil as suggested by previous authors[5,8]. In any event it is unlikely that the anterior bifurcate part would be preserved in section when the posterior part is not.

Disparities in the spacing and number of teeth in different specimens of *Tullimonstrum* suggest that they are frequently lost[6], resulting in variations in the total number in individuals between 0 and 19. However, the maximum number observed in any one row is 8, and the buccal apparatus includes at least four rows. On this basis we estimate that *Tullimonstrum* possessed up to 32 teeth. Although the teeth in modern lampreys are decay resistant, they have been observed to disarticulate (fall out and are lost) in decay experiments[2].

The main mode of preservation of *Tullimonstrum* teeth is the same as that in jawless vertebrates (originally keratin[18]) and in molluscs (originally a chitin-protein complex[19]) at Mazon

Creek: a three-dimensional mould sometimes infilled with kaolinite[6] (Extended Data Fig. 4b-c, Extended Data Fig. 5k). However, *Tullimonstrum* teeth may also be preserved in pyrite[6] (Fig. 3b; Extended Data Fig. 4a, Extended Data Fig. 5j); about half of all *Tullimonstrum* teeth include some pyrite. Pyrite is rare and typically diffuse at Mazon Creek, and the sulphur is most commonly sourced from carcasses[9,20]. This suggests that the teeth of *Tullimonstrum* were originally sulphur-rich, a property shared with keratin, a connective tissue characterised by abundant disulphide bonds[18,21]. However, it is also possible that the specimens with more pyritized teeth were preserved in local microenvironments with higher sulphur levels. Chitinous tissues (e.g. arthropod cuticles), are often preserved in organic carbon at Mazon Creek (Extended Data Fig. 6l), a style of preservation that we have not observed in *Tullimonstrum* teeth. Keratinous teeth indicate a chordate affinity.

Eyebar. The *Tullimonstrum* eyebar is a rigid structure which projects beyond the margin of the body and bears an eye at each end[5,6] (Fig. 1c). Non-visual interpretations of this structure have also been offered, such as functioning in copulation, stabilization or locomotion[5,6,8]. However, morphological and taphonomic evidence supports its interpretation as bearing the eyes[5,6]. The distal ends of the bar are shaped as a cup enclosing a sphere which is frequently preserved as a dark stain composed of pyrite[6] enriched in zinc (Extended Data Fig. 5f,l). Both chordates and molluscs at Mazon Creek preserve eyes as a dark stain[5,22] and, at least in chordates, this stain is also composed of pyrite enriched in zinc (Extended Data Fig.6a-b). Experiments on modern lampreys have shown the eyes and eye pigments to be decay resistant[2].

The eyebar and body wall of one specimen of *Tullimonstrum* are completely pyritized, showing that the eyebar lies on the exterior of the body, at least for most of its length[6]. Its position relative to the notochord shows that the eyebar lies on the dorsal surface of the body (Extended Data Fig. 2a).

The eyebar is preserved three-dimensionally in many specimens, suggesting that it was formed of decay resistant tissue[6]. It may have comprised a sheath of resistant tissue around the optic nerves; given the interpretation of *Tullimonstrum* as a vertebrate, this tissue may be cartilage. The lack of preserved internal structures in the head of *Tullimonstrum*, with the exception of some elements of the braincase and tectal cartilages, prevents us froom determining the homology of the eyebar.

Among chordates, stalked eyes are known in hammerhead sharks and marine teleost larvae, including dragonfish. In hammerhead sharks, the separation of the eyes does not significantly change the visual field, but it alters the size of the anterior binocular zone. This zone is wider in hammerheads than in other sharks, and species with the widest heads have the widest anterior binocular zones[23]. In larval dragonfish moveable stalks and elliptical eyes expand the visual field[24]. The eyebar in *Tullimonstrum* must have impacted the size and/or shape of the visual field and may reflect the position of the jaw at the end of a proboscis.

Tail and dorsal fin. The tail of *Tullimonstrum* is a continuation of the body that bears a fin supported by fin rays[5,6,8] (Fig. 1c; Extended Data Fig. 1f). It was originally considered to be horizontal[3], but has been reinterpreted as vertical on the basis of fin asymmetry (Fig. 1c, Extended Data Fig. 1f), and evidence of a clear fold at the base of the tail when it is rotated into the same plane as the eye bar[5,8] (Extended Data Fig. 1b). A vertical tail with this general morphology is consistent with either a mollusc or chordate affinity[5,8]. We have observed a continuation of the dorsal lobe of the tail fin anteriorly, which we interpret as a narrow dorsal fin

(Extended Data Fig. 1b,c). Where the dorsal fin is preserved in specimens that also show the myomeres or arcualia, its anterior extremity consistently aligns with the fifth myomere or arcualium (Extended Data Fig. 1b,c). An asymmetric tail fin and long narrow dorsal fin are characteristic of basal jawless vertebrates such as lampreys[1] but this arrangement is also consistent with heteropod molluscs where the dorsal fin is highly modified for swimming[5]. In dorsoventral specimens where the tail fin is not preserved in outline (indicating that the tail is not twisted horizontally[8]), the dorsal fin is often preserved as a three-dimensional mould (Extended Data Fig. 1c). This suggests that it was resistant to decay as are the ray-supported dorsal and tail fins in extant chordates[2].

The tail of *Tullimonstrum* has a higher aspect ratio than that in extant lampreys. Values for *Tullimonstrum* range exceed 1 whereas they are less than 1 in extant lampreys (Extended Data Table 1). A high tail aspect ratio facilitates tail-propelled rather than undulatory swimming.

Naris (nostril). A number of structures in the head of *Tullimonstrum* are preserved in three-dimensions or as a light coloured stain. One of the most notable is a crescent-shaped structure anterior to the eyebar, which is typically preserved three-dimensionally (Extended Data Fig. 3c). Its resistant nature is consistent with a cartilaginous composition, and its shape and position are reminiscent of a lamprey nasal organ. There is no evidence of a dividing septum, which indicates that it represents a single naris. In lampreys, the naris is a single crescent- to pouch-shaped structure, anterior to the eyes, supported by cartilage[25]. This structure in *Tullimonstrum* has previously been interpreted as a possible mouth[6], a hinge between the proboscis and the body[5], or a component of the pharynx[6].

Tectal cartilages. Two or three light coloured impressions or three-dimensional structures (Extended Data Fig. 8b) preserved anterior to the naris and immediately posterior to the proboscis in a few specimens of *Tullimonstrum* are interpreted as plate-like structures. They may represent tectal cartilages.

Liver. Some specimens of *Tullimonstrum* preserve a round, dark, off-centre structure in the trunk (Extended Data Fig. 8c) which we interpret as a liver. The style of preservation is similar to that of the liver in *Mayomyzon*[7] and *Gilpichthys*[3]. Experiments have shown that the liver is one of the most decay-resistant internal organs in chordates[2].

PHYLOGENETIC ANALYSIS

For the phylogenetic analysis, character codings for *Pipiscius*, *Gilpichthys* and *Tullimonstrum* were added to the data matrix of Conway Morris and Caron[26]. Although the total-group petromyzontiformes and total-group myxiniformes are internally resolved in our analysis, we recovered each of these lineages separately in a large polytomy with euconodonts, stem-gnathostomes, and other enigmatic vertebrates (Fig. 1a, Extended Data Fig. 7). This highlights the volatility of early vertebrate interrelationships and the need for continued investigation into the question of cyclostome monophyly. Future research may confirm the result of recent analyses supporting pan-cyclostome monophyly[17], but analysis of the morphological database used here indicates that *Tullimonstrum*, which falls crownward of *Mayomyzon*, lies on the stem of lampreys.

Codings of taxa included in the analysis of Conway Morris and Caron[26] were unchanged, except for Myxinoidea which was modified to reflect the morphological variation expressed by the range of genera within the group. This better represents the disparity in the clade, and reduces the possibility that poor sampling of polymorphisms will result in erroneous tree topologies[27] (a problem exacerbated by including higher taxa as operational taxonomic units). It reflects the observation that it is preferable to represent higher taxa in phylogenetic analyses with multiple exemplar taxa than by coding a single ‘gestalt’ entity[28].

One new character was added to the data matrix.

117. Tectal cartilages: absent = 0, present = 1.

Data set:

Hemichordata

0-00-0000-000-0---0---00--0000?0-0?000-000000---0----00000-000-0000-0000000000-0----0--
0---0--000-0---0-0-0--00?0-000

Myllokunmingia

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0--0---0-0???????????-?--?????21?

Pikaia

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-0---0--???????????-?--?????11?

Metaspriggina

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Haikouichthys

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Petromyzontida

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Gnathostomata

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Jamoytius

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Euphanerops

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Arandaspida

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Osteostraci

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Euconodonta

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Tunicata

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0-00-00000001011------0--1--0-010

Cephalochordata

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0-0-00-00000000011------0--1000-110

Mesomyzon

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Myxinoidea

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Myxinikela

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0-00-0-000??????0????-?--2????210

Priscomyzon

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Mayomyzon

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Heterostraci

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Astraspis

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Anaspida

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Galeaspida

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0-1001010100?????101???-?012?010210

Loganellia

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Turinia

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Pipiscius

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Gilpichthys

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Tullimonstrum

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SYSTEMATIC PALAEONTOLOGY

Phylum Chordata Haeckel, 1874

Subphylum Vertebrata Lamarck, 1801

Order Petromyzontiformes Berg, 1940

Tullimonstrum gregarium Richardson, 1966

Revised diagnosis. Mid-sized stem lamprey with stylophthalmine eyes set upon rigid bar; buccal apparatus extended into a cartilage-supported jointed proboscis with a bifurcate terminal structure bearing keratinous teeth; 20–25 myomeres; at least five gill pouches.

Material. Holotype plus 1,287 specimens (Field Museum of Natural History) (Supplementary Table 1, Extended Data Table 2) and numerous other specimens in museums globally.

Locality and Geographic Horizon. The Pennsylvanian Mazon Creek fossil biota (Illinois, USA) was deposited in a deltaic environment[9], and the fossil assemblages reflect both the terrestrial/freshwater biota (the Braidwood assemblage) and the brackish/marine biota (the Essex assemblage)[29,30]; *Tullimonstrum* is known only from the Pit 11 locality of the Essex assemblage[6].

Description. The tubular body ranges in size from 2-15.4 cm[6] and extends anteriorly into the head (3.4-10.4 cm[6]) which bears a jointed proboscis ending in a bifurcate buccal apparatus (0.5-2.6 cm[6]) bearing multiple rows of small (0.1-0.2 cm long), sharp posteriorly-pointing keratinous teeth shaped as simple hollow cones. The proboscis is typically about one third the length of the body[6]. The buccal apparatus contains the mouth and a lingual organ. The eyes are borne on a long rigid bar (1.6-4.5 cm[6]) which lies on the dorsal surface of the body. The body consists of 22-25 myomeres. Serial cartilaginous arcualia are associated with the dorsal notochord. The gut is ventral. The tail (0.9-6.5 cm long[6]) bears an asymmetric fin; the dorsal lobe is longer but not as high as the ventral lobe. The tail is flexed ventrally. The dorsal lobe of the tail fin continues into a long narrow dorsal fin, which extends anteriorly into the fifth most posterior myomere. Specimens range from 8 to 35 cm in length[6] from the tip of the proboscis to the end of the tail.

SUPPLEMENTAL REFERENCES

1 Janvier, P. Early Vertebrates. *Oxford Monographs on Geology and Geophysics* **33** (1996).

2 Sansom, R. S., Gabbott, S. E. & Purnell, M. A. Atlas of vertebrate decay: a visual and taphonomic guide to fossil interpretation. *Palaeontology* **56**, 457-474 (2013).

3 Bardack, D. & Richardson Jr, E. S. New agnathous fishes from the Pennsylvanian of Illinois. *Fieldiana Geol.* **33**, 489-510 (1977).

4 Bardack, D. & Zangerl, R. First fossil lamprey: a record from the Pennsylvanian of Illinois. *Science* **162**, 1265-1267 (1968).

5 Foster, M. A reappraisal of *Tullimonstrum gregarium*. in *Mazon Creek fossils* (ed Nitecki, M. H.) 269-301 (Academic Press, 1979).

6 Johnson, R. G. & Richardson, E. S. Pennsylvanian invertebrates of the Mazon Creek Area, Illinois: the morphology and affinities of *Tullimonstrum*. *Fieldiana Geol.* **12**, 119-149 (1969).

7 Bardack, D. Fishes: 'Agnatha', Acanthodii, and Osteichthyes. in *Richardson's guide to the fossil fauna of Mazon creek* (eds Shabica, C. W. & Hay, A. A.), 226-243 (1997).

8 Beall, B. The Tully Monster and a new approach to analyzing problematica. in *The early evolution of metazoa and the significance of problematic taxa* (eds Simonetta, A. M. & Conway Morris, S.) 217-286 (Cambridge Univ. Press 1991).

9 Baird, G. C., Sroka, S. D., Shabica, C. W. & Kuecher, G. J. Taphonomy of Middle Pennsylvanian Mazon Creek area fossil localities, Northeast Illinois: significance of exceptional fossil preservation in syngenetic concretions. *Palaios* **1**, 271-285 (1986).

10 Brusca, R. C. & Brusca, G. J. *Invertebrates*. Second Edition, (Sinauer Associates, Inc., 2003).

11 Ota, K. G., Fujimoto, S., Oisi, Y. & Kuratani, S. Identification of vertebra-like elements and their possible differentiation from sclerotomes in the hagfish. *Nat. Comms.* **2**, 373 (2011).

12 Sansom, R. S., Gabbott, S. E. & Purnell, M. A. Decay of vertebrate characters in hagfish and lamprey (Cyclostomata) and the implications for the vertebrate fossil record. *Proc. Roy. Soc. Lond. B: Biological Sciences* **278**, 1150-1157 (2011).

13 McDowall, R. Variation in vertebral number in galaxiid fishes, how fishes swim and a possible reason for pleomerism. *Rev. Fish Biol. Fisheries* **13**, 247-263 (2003).

14 McDowall, R. Jordan's and other ecogeographical rules, and the vertebral number in fishes. *J. Biogeog.* **35**, 501-508 (2008).

15 Yalden, D. Feeding mechanisms as evidence for cyclostome monophyly. *Zool. J. Linn. Soc.* **84**, 291-300 (1985).

16 Conway Morris, S. & Caron, J.-B. A primitive fish from the Cambrian of North America. *Nature* **512**, 419-422 (2014).

17 Oisi, Y., Ota, K. G., Kuraku, S., Fujimoto, S., & Kuratani, S. Craniofacial development of hagfishes and the evolution of vertebrates. *Nature* **493**, 175-180 (2013).

18 Alibardi, L. & Segalla, A. The process of cornification in the horny teeth of the lamprey involves proteins in the keratin range and other keratin–associated proteins. *Zool. Stud.* **50**, 416-425 (2011).

19 Scheltema, A. H., Kerth, K. & Kuzirian, A. M. Original molluscan radula: comparisons among Aplacophora, Polyplacophora, Gastropoda, and the Cambrian fossil *Wiwaxia corrugata*. *J. Morph.* **257**, 219-245 (2003).

20 Woodland, B. G. & Stenstrom, R. C. The occurrence and origin of siderite concretions in the Francis Creek Shale (Pennsylvanian) of northeastern Illinois. in *Mazon Creek fossils* (ed. Nitecki, M. H.) 69-103 (Academic Press, 1979).

21 McKittrick, J. *et al.* The structure, functions, and mechanical properties of keratin. *JOM* **64**, 449-468 (2012).

22 Kluessendorf, J. & Doyle, P. *Pohlsepia mazonensis*, an early 'octopus' from the Carboniferous of Illinois, USA. *Palaeontology* **43**, 919-926 (2000).

23 McComb, D., Tricas, T. & Kajiura, S. Enhanced visual fields in hammerhead sharks. *J. Exp. Biol.* **212**, 4010-4018 (2009).

24 Weihs, D. & Moser, H. G. Stalked eyes as an adaptation towards more efficient foraging in marine fish larvae. *Bull. Mar. Sci.* **31**, 31–36 (1981).

25 Forey, P. & Janvier, P. Agnathans and the origin of jawed vertebrates. *Nature* **361**, 129-134 (1993).

26 Conway Morris, S. & Caron, J.-B. A primitive fish from the Cambrian of North America. *Nature* **512**, 419–422 (2014).

27 Watanabe, A. The impact of poor sampling of polymorphism on cladistic analysis. *Cladistics*, doi:10.1111/cla.12130 (2015).

28 Brusatte, S., Montanari, S., Yi, H-Y. & Norell, M. A. Phylogenetic corrections for morphological disparity analysis: a new methodology and case studies. *Paleobiology* **37**, 1–22 (2011).

29 Johnson, R., G. & Richardson, E. S. A remarkable Pennsylvanian fauna from the Mazon Creek Area, Illinois. *J. Geol.* **74**, 626-631 (1966).

30 Baird, G. C., Shabica, C. W., Anderson, J. L. & Richardson, E. S., Jr. Biota of a Pennsylvanian muddy coast: habitats within the Mazonian delta complex, Northeast Illinois. *J. Paleont.* **59**, 253-281 (1985).