The first twisted-wing parasitoids (Insecta: Strepsiptera) from the Early Eocene Green River Formation of Colorado

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Abstract. Strepsiptera is a clade of entomophagous parasitoid insects with fewer than 30 previously reported fossils. Two new species of *Caenocholax* (Strepsiptera: Myrmecolacidae) described here represent the first reported adult strepsipterans preserved as organic compression fossils. Their occurrence in the Early Eocene (*c.* 50 Ma) Green River Formation (Colorado, USA) is the northernmost New World record of Myrmecolacidae and the oldest record of *Caenocholax*. *Caenocholax barkleyi* **sp. nov.** and *Caenocholax palusaxus* **sp. nov.** are each known from one adult male. The aedeagus of *C. barkleyi* **sp. nov.** lacks a median projection and terminates in two hooks, an apomorphy of the species. *Caenocholax palusaxus* **sp. nov.** has distinctly intermediate wing-vein density and a larger ratio of antennomere 6 to antennomere 7 than any other species of *Caenocholax*. The fossils reported here expand the known insect biota of the formation in taxonomic richness as well as the fossil record of Strepsiptera in space and time. Moreover, the Eocene specimens hint at an unappreciated fossil diversity of endoparasitic insects.

Key words. Myrmecolacidae, organic compression fossils, lacustrine preservation, Ypresian, endoparasitoid insects, New World distribution.

Introduction

In pinned and fossil entomology collections Strepsiptera is one of the most rare cosmopolitan insect taxa, yet study of this small clade of tiny parasites has revolutionized our understanding of insect evolution. Sometimes called the twisted-wing parasites, Strepsiptera includes a few more than 600 recognized species, but fewer than 30 of these were described from fossils (Kathirithamby in press). Only adult males and first instar larvae (and in the basal clade Mengenillidia, last instar larvae) live outside a host; older instar larvae, pupae and adult females live as obligate endoparasites in other insects (Kathirithamby 1989). Adult females lack wings, legs and eyes, and they remain in their larval host while producing hundreds to hundreds of thousands of offspring through haemocoelous viviparity (Kathirithamby 1989; Maeta et al. 1998). Adult males emerge with membranous hindwings and live only long enough to track pheromones to females, no more than a few hours (Kathirithamby et al. 2015). Evolutionary biologists have studied Strepsiptera not only for the uniqueness of its life history traits, but also for the similarity of its morphology with certain other arthropods.

Morphological convergences with other endopterygote insect clades and with trilobites make Strepsiptera of paleontological and phylogenetic importance. The eyes of adult male strepsipterans represent the closest modern analogue in function and structure to those of schizochroal trilobites (Horváth et al. 1997; Buschbeck et al. 2003). Facets of these eyes are well separated and in Strepsiptera are called ‘eyelets’ (Buschbeck et al. 2003). Forewings of adult male Strepsiptera are reduced to halteres, which appear to aid in flight stabilization, as do the hindwing halteres of Diptera (Pix et al. 1993). Halter similarity with Diptera contributed to decades of fierce debate over the phylogenetic affinity of Strepsiptera, which recent morphological and genetic studies have resolved as a sister-clade relationship with Coleoptera (Niehuis et al. 2012; Boussau et al. 2014). The shared ancestry of Strepsiptera and Coleoptera implies that forewing halteres and elytra are homologous in these taxa. The ancestral state of the forewing remains speculative, as no Mesozoic transitional fossils have been found yet.

Biases in preservation mode, geography and taxonomy characterize the fossil record of most endoparasites. The small size of first instar larvae, the short lifespan of adult males, and the endoparasitic lifestyle of all other life stages limit the fossilization of Strepsiptera in particular. All but three previously described Strepsiptera fossils occur in amber. A Formicidae specimen preserved in Middle Eocene oilshale and hosting two pupae of male Myrmecolacidae (Lutz 1990) and a taxonomically unplaced first instar in Eocene Brown coal (Kinzelbach and Lutz 1985) are the only non-amber Strepsiptera fossils known besides those described here. All but four amber Strepsiptera fossils were collected in the Dominican Republic or the Baltic. Fossils collected outside these regions are the Cretaceous *Cretosylops engeli* Grimaldi and Kathirithamby, 2005 (Cretosylopidae) (in Grimaldi et al. 2005), *Kinzelbachilla ellenbergeri* Pohl and Beutel, 2016 (Kinzelbachillidae) and *Phthanoxenos nervosus* Engel et al., 2016 (Phthanoxenidae) from Burmese amber, and the Eocene *Kronomyrmecolax fushunicus* Wang et al., 2015 (Myrmecolacidae) from Chinese (Fushun) amber. About half of extinct Strepsiptera species belong to Myrmecolacidae, and about half of those (including the two described here) are assigned to *Caenocholax* (Kathirithamby in press). *Kronomyrmecolax fushunicus* is the oldest described myrmecolacid at 50—53 Ma (Wang et al. 2014).

Myrmecolacidae differs from other Strepsiptera taxa in host affinity and geographic distribution. Each sex in this basal clade of stylopid strepsipterans parasitizes a different insect clade (a condition termed heterotrophic heteronomy): males parasitize Formicidae (Hymenoptera), and females parasitize orthopteroid insects (Kathirithamby and Hamilton 1992; Kathirithamby 2009). All Strepsiptera clades traditionally ranked as families exhibit cosmopolitanism in extant species distributions except Bahiaxenidae, Bohartillidae and Myrmecolacidae. Each of these first two taxa is represented in the Recent by one species: *Bahiaxenos relictus* in Brazil (Bravo et al. 2009) and *Bohartilla megalognatha* in Central America (Kathirithamby and Grimaldi 1993; Cook 2015). Myrmecolacidae includes 80 extant species, all of which are restricted to tropical and sub-tropical latitudes, up to Texas and North Carolina in the New World (Kathirithamby in press). The clade's host taxa live worldwide.

Here we report two new species of *Caenocholax*. The specimens are preserved as compression fossils in thinly bedded limestone from the Early Eocene Green River Formation of Rio Blanco County, Colorado. The fossils described below are the first strepsipterans reported from the Green River Formation, the first adult strepsipterans preserved as compression fossils, and the oldest and northernmost occurrences of fossil or extant *Caenocholax* in the New World.

Materials and Methods

Jim Barkley collected the specimens in Rio Blanco County, Colorado, and donated them to the Division of Invertebrate Paleontology of the Peabody Museum of Natural History, Yale University, New Haven. The fossils are preserved as organic compressions in thinly bedded limestone. We immersed specimens in alcohol while photographing them with a Leica MZ-16 microscope and Leica Application Suite software, and we z-stacked images with Helicon Focus software to generate a single composite image of each specimen. We prepared line drawings from z-stacked images with Adobe Photoshop CS4. For the line drawing of YPM IP 320328, which has a part and counterpart slab, we horizontally reversed the image of one plate and superimposed it on the image of the other plate. We measured specimen anatomy with a Zeiss STEMI 2000-CS microscope and Q-Capture Pro software. We compared the slabs containing specimens against the Munsell Rock-Color Chart to describe shale color.

Terminology abbreviations. The wing venation abbreviations used in the text and figures follow standard entomology conventions: C, cubitus; Sc, subcubitus; R, radius; and CuA, anterior cubitus. Subscripts indicate wing vein branches, with the first branch defined as most proximal. Other anatomical terms are abbreviated in figures: e, eye; a, antennomere; fb, flabellum; h, haltere; fm, femur; tb, tibia; ts, tarsus; hd, head; le, leg; ab, abdominal segment; s, seta; ae, aedeagus. Antennal and abdominal segments are numbered from proximal to distal.

Institutional abbreviations. USNM, National Museum of Natural History, Washington D.C.; YPM IP, Peabody Museum of Natural History Division of Invertebrate Paleontology, New Haven, Connecticut.

The nomenclatural acts below have been registered with Zoobank: urn:lsid:zoobank.org:pub:340F120E-9B88-4B48-B6DD-3C94B1C4380E

Geologic Setting

The Green River Formation is a series of ponded basins, including lakes in what now forms northwest Colorado, northeast Utah and southwest Wyoming. The lakes formed as a result of drainage into areas of Late Cretaceous foreland basins deformed by the Laramide Orogeny (Dickinson et al. 1988). Diverse animal and plant fossils abound in the Green River Formation, a famous Eocene lacustrine Lagerstätte (Grande 1984; 2013). The fossils discussed here come from the former eastern shore of Lake Uinta, the largest of the Green River lakes, in the southeast part of the formation. Six members of Lake Uinta sediments have been identified; this material belongs to the Parachute Creek Member in the Piceance Creek Basin (Grande 2013). The Parachute Creek Member is thought to have formed in an evaporative event 49—50 Ma, during the Ypresian (Early Eocene) (Smith et al. 2008).

The fossils described here came from two adjacent properties in Rio Blanco County, Colorado (Fig. 1). YPM IP 320328 was collected at site B in Dayvault et al. (1995), in a quarry colloquially known as Gus’ Pit. The coordinates for this locality are 39° 43’ 38.10” latitude and 107° 58’ 34.14” longitude, *c.* 2 miles west of the Rio Blanco town store. YPM IP 389335 was collected at a ranch neighboring the quarry, at 39° 43' 38.13" latitude and -107° 58' 34.17" longitude. Both sites lie *c.* 45 m below the Mahogany Bed, at 2180 m elevation.

Consistent with environmental reconstructions for other localities in the Green River Formation, the flora and fauna associated with the collection area indicate a low-energy wetland environment, possibly a lagoon. Equisetaceae, Typhaeceae and algae are present (Jim Barkley, pers. comm.), as well as insects in aquatic life stages. Some rock slabs exhibit ripple marks, but these slabs have not been found to contain fossils. The beds are rich in iron, with limonite and pyrite commonly occurring. Slabs with fossil insects also frequently contain pyrite, as well as aluminum oxide clays; dolomite and amber are present occasionally.

Systematics

Strepsiptera Kirby, 1813

Stylopidia Kinzelbach, 1969

Myrmecolacidae Saunders, 1872

Caenocholax Pierce, 1909

Type species. *Caenocholax fenyesi* Pierce, 1909; USNM 10081.

Included species. *Caenocholax barkleyi* **sp.nov.**; *C. brodzinskyi* Kathirithamby and Grimaldi, 1993; *C. dominicensis* Kathirithamby and Grimaldi, 1993; *C. fenyesi* Pierce, 1909; *C. groheni* Kathirithamby and Hendrickx, 2008; *C. palusaxus* **sp.nov.**

Diagnosis. Median and two lateral spines, or median spine only, on dorsally hooked aedeagus; abdominal segment X with enlarged lobate plate partially overlapping abdominal segment IX; hindwing R_3 absent (Kathirithamby and Johnston 1992)

Discussion. Synapomorphies of crown Strepsiptera present in *Caenocholax barkleyi* **sp.nov.** and *C. palusaxus* **sp.nov.** include a dorsally attached antenna, shortened coronal suture, and eye with well separated eyelets (Pohl and Beutel 2016). Flabella on the third of seven antennomeres and the absence of CuA_2 place the new species in Myrmecolacidae, the basal-most lineage of Stylopidia excluding Corioxenidae (McMahon et al. 2011). *Caenocholax barkleyi* **sp.nov.** and *C. palusaxus* **sp.nov.** are attributable to *Caenocholax*, sister clade to all other myrmecolacids, because each lacks R_3 and has a curved aedeagus with at least one hooked projection (McMahon et al. 2011).

Of the four previously described *Caenocholax* species, only *C. fenyesi* is extant. Genetic analysis indicates a substantial, unrecognized diversity within *C. fenyesi*, however. As many as 10 lineages within Recent *C. fenyesi* were found consistent with discrete species according to the evolutionary species concept Hayward et al. (2011). In addition to genetic differences among lineages, females host choice is diagnostic, and male morphology differs subtly in several but not all of these lineages (Hayward et al. 2011).

Caenocholax barkleyi ***sp.nov.***

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Figs 2—3

Fossil material. Holotype is YPM IP 389335, Green River Formation (Early Eocene; *c.* 50 Ma), Rio Blanco, Colorado. Adult male preserved in left lateral aspect on a single slab 25 x 29 mm. The right wing is extended, but the distal leg segments and mouthparts are obscured. The lithology is pale yellowish brown, with a greyish orange blush on the edge of the plate closest to the specimen.

Etymology. The species is named after Jim Barkley, who collected the holotypes described here.

Diagnosis. May be distinguished from other species in *Caenocholax* by the following combination of character states: (1) aedeagus with two dorsal spines [lacking central projection present in *C. fenyesi* (Kathirithamby and Johnston 1992)]; (2) presence of R_4 [absent in *C. dominicensis* (Kathirithamby and Grimaldi 1993)]; (3) small ratio of antennomere 5/flabellum [smaller than in *C. brodzinskyi* holotype (0.66) (Kathirithamby and Grimaldi 1993), *C. dominicensis* (0.75) (Kathirithamby and Grimaldi 1993) or *C. fenyesi* (0.60) (Kathirithamby and Johnston 1992)]; and (4) ratio of antennomeres 6/7 [smaller in *C. brodzinskyi* holotype (0.56) (Kathirithamby and Grimaldi 1993) or *C. groehni* (0.55) (Kathirithamby and Henderickx 2008);

larger in *C. dominicensis* (0.71) (Kathirithamby and Grimaldi 1993) and *C. fenyesi* (equal length) (Kathirithamby and Johnston 1992)].

Description.

Adult male. Eye very large; maximum width across eyes 0.72 mm; with at least 17 eyelets. Antenna with 7 segments and 1 flabellum (0.75 mm) on flagellomere 3. Antennomere lengths: 5 = 0.41 mm; 6 = 0.19 mm; 7 = 0.30 mm. Ratios: antennomere 5/flabellum = 0.55; antennomeres 6/7 = 0.63. Wing venation: R_2 short; R_3 and R_5 not apparent; R_4 1.11 mm. Wing expanse: maximum width 1.53 mm. Halter length 0.56 mm. Thorax humped: maximum width 0.64 mm at mesothorax. Maximum width of abdomen 0.31 mm. Aedeagus terminating in two symmetric, recurved spines. Aedeagus length 0.15 mm; width between apices of spines 0.13 mm. Total length 1.64 mm (measured from anteriormost visible part of vertex to end of abdomen, excluding aedeagus).

Remarks. Although the eyes of the holotype appear touching or nearly touching, this configuration is likely an artifact of two-dimensional preservation. While surrounding sediment compressed and dewatered, the specimen was angled on its right side, so the left eye appears to overlap the right eye. In life, the vertex may have separated the eyes as in all other *Caenocholax.* Similarly, although only 17 eyelets are visible in the holotype, several more eyelets may be present ventrally and obscured by rock matrix. We interpret the venation as having an absent R_5; in all other *Caenocholax* the R_5 is present. In the *C. barkleyi* holotype a short vein lies posterior to the R_4 in the distal portion of the wing, however, and it is possible that this fragment represents the R_5.

Caenocholax palusaxus **sp.nov.**

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Figs 4—5

Fossil Material. Holotype is YPM IP 320328, Green River Formation (Early Eocene; *c*. 50 Ma), Rio Blanco County, Colorado. Adult male preserved on two slabs 33 x 48 mm (part and counterpart). The rock is pale yellowish brown. The strepsipteran is exposed in dorsal aspect with the left wing extended and the right wing folded obscuring abdominal segment X and some of the aedeagus. Rock matrix conceals maxillae, mandibles, and tarsi on ventral side of specimen.

Etymology. The new species epithet is a combination of the noun *saxum* (Latin, meaning rock), referring to the novel preservation medium of the holotype, and the root *palu* (Latin, meaning swamp or marsh), indicating a habitat in which the species may have lived and the low-energy lacustrine environment that facilitated its preservation.

Diagnosis. May be distinguished from other species in *Caenocholax* by the following combination of character states: (1) ratio of antennomeres 6/7 [smaller in all other *Caenocholax* species]; (2) ratio of antennomere 5/flabellum [smaller in *C. barkleyi* **sp.nov.** (0.55); larger in *C. brodzinskyi* (0.66) (Kathirithamby and Grimaldi 1993) and *C. dominicensis* (0.75) (Kathirithamby and Grimaldi 1993)]; and (3) C, Sc and R_1 present and R_4 extending to wing margin [wing venation more reduced in *C. barkleyi* **sp.nov.**, *C. dominicensis* and *C. groehni*].

Description.

Adult male. Head length 0.38 mm. Eye large with minimum dorsal separation 0.18 mm; maximum distance across eyes 1.07 mm; with at least 18 eyelets. Antenna with 1 flabellum (0.93 mm) on flagellomere 3 and the following antennomere lengths: 5 = 0.56 mm; 6 = 0.35 mm; 7 = 0.43 mm. Ratios: antennomere 5/flabellum = 0.60; antennomeres 6/7 = 0.81 mm. Wing venation: C and Sc present, with Sc adjacent and parallel to C; R_1, R_2 and R_4 nearly touching or touching wing margin; R_3 not apparent. R_4 1.74 mm in length. Wing expanse (broadest width 1.93 mm). Halter length 0.56 mm (average of left and right halteres). Thorax symmetrically tapering anteriorly and posteriorly from maximum width of 0.93 mm across metanotum. Aedeagus broad, curved and with ≥1 hooked spine. Total length 2.42 mm (measured from anteriormost visible part of vertex to end of abdomen, excluding aedeagus).

Remarks. Although less than three millimeters long, the holotype of *Caenocholax palusaxus* **sp.nov.** is nearly 50 percent larger in body length than *C. barkleyi* **sp.nov.**, *C. dominicensis*, *C. fenyesi* or *C. groehni*. Preservation is poor in the wing region that contains the R_5 in other *Caenocholax.*

Superimposing a horizontally reversed image of one slab on an image of the other allows better resolution of some features in the holotype compression fossil than do photographs of either slab alone. The spine on the aedeagus of the holotype is one such structure that is split across two slabs and revealed in greater detail when slab images are superimposed.

Discussion

The two Early Eocene (*c.* 50 Ma) adult strepsipterans described here are the oldest Myrmecolacidae reported thus far except for *Kronomyrmecolax fushunicus* from Fushun Coalfield amber in northeastern China, which is coeval or up to 4 m.y. older (Wang et al. 2015).

The age of the Early Eocene *Caenocholax* species described here lies within the range of divergence dates of stem *Caenocholax* estimated from a fossil-calibrated molecular dating of 44.0—55.0 Ma (95% highest posterior density) (Hayward et al. 2011; McMahon et al. 2011). The Green River fossils restrict the origin of *Caenocholax* to at least 49 Ma, however. Relationships of *C. barkleyi* **sp.nov.** and *C. palusaxus* **sp.nov.** to other *Caenocholax* species remain unresolved because phylogenies of *Caenocholax* to date are based exclusively on genetic data unavailable in fossils (Hayward et al. 2011).

The preservation of extinct *Caenocholax barkleyi* **sp.nov.** and *C. palusaxus* **sp.nov.** in limestone differs from that of all previously described fossil strepsipterans, which, except for two male pupae parasitic in an ant in oilshale (Lutz 1990) and a first instar larva in brown coal (Kinzelbach and Lutz 1985), occur exclusively in amber. The lithification of these Green River specimens gives further evidence for the exceptional quality of preservation in the formation and for the high preservation potential of insects in lacustrine environments in general (Smith 2012). Famous as both a Concentration and Conservation Lagerstätte, the Green River Formation has previously yielded 20 major clades of insects traditionally ranked as orders: Blattodea, Coleoptera, Dermaptera, Diptera, Ephemeroptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Mantodea, Mecoptera, Neuroptera, Odonata, Orthoptera, Plecoptera, Pscocoptera, Raphidioptera, Siphonaptera, Thysanoptera and Trichoptera (Susan Butts, pers. comm.; Talia Karim, pers. comm.; Grande 2013). As the first examples of Strepsiptera reported from the formation, the fossils discussed here are a significant addition to the known Green River paleobiota.

The Colorado occurrence of *Caenocholax barkleyi* **sp.nov.** and *C. palusaxus* **sp.nov.** illuminates the biogeographic history of Myrmecolacidae. Rio Blanco County is the northernmost New World record of fossil or Recent Myrmecolacidae. Extant members of the clade occupy a circumtropical distribution, with the northernmost range extent in North Carolina (Fig. 1) (Kathirithamby in press). The Eocene presence of *Caenocholax* in Colorado indicates the colonization and subsequent extinction of Myrmecolacidae in the Green River region (*c.* 40° N). From the Late Paleocene to Early Eocene, the Earth experienced a rapid rise in global temperatures and CO_2 concentrations termed the Paleocene-Eocene Thermal Maximum (PETM), in which megathermal climates extended to high latitudes (Kennett and Stott 1991; Greenwood and Wing 1995; Zachos et al. 2003). Like members of several other animal clades adapted to warm climates, including stem-relatives of tse-tse flies (Insecta: Diptera: *Glossina*) (Grimaldi 1992), diverse primates (Mammalia: Placentalia) (Ni et al. 2013), frigatebirds (Aves: Suliformes) (Olson 1977), and several clades of crocodilians (Reptilia: Archosauria: Crocodylia) (Selden and Nudds 2008), myrmecolacids lived in the Green River region during the warmer and wetter conditions of the PETM but became restricted to lower latitudes by the Recent.

Conclusion

As for many entomophagous endoparasites, the fossil record of Strepsiptera is scarce and is biased in taxonomy, geography, and type of preservation. We describe two new species of myrmecolacid strepsipterans from the Eocene Green River Formation, extending the known New World geographic distribution of the clade. The specimens described here are also the only adult strepsipterans preserved in rock and the oldest fossils of *Caenocholax* reported thus far. The detailed preservation of tiny males, which live for no more than a few hours as adults, highlights the exceptional preservation potential of lacustrine environments.

We encourage careful examination of insect compression fossils to discover small but significant specimens, particularly parasites, which might otherwise remain unnoticed. Particularly promising sites for future fossil parasite prospecting include those that, like the Green River, harbor abundant insect compression fossils from lacustrine environments. Examples of such sites include Florissant, Grube Messel and the Ruby Paper Shale (Monroe 1981; Wilson 1988; Schaal and Ziegler 1992). Further examination of insect fossils from these and other Conservation Lagerstätten may yield rarely preserved taxa that could expand our understanding of Paleogene ecological community composition and the evolution of species interactions, including host-parasite interactions.

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