

A miniaturized beetle larva in Cretaceous Burmese amber: reinterpretation of a fossil “strepsipteran triungulin”

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Abstract

A wingless and eyeless tiny fossil embedded in Cretaceous amber from Myanmar is described and interpreted phylogenetically as beetle larva, very likely belonging to a cucujiform group of Coleoptera with parasitic habits, probably the family Ripiphoridae. Features supporting this are the lobe-like terminal elements of the legs and the pattern of setae on the abdomen. However the larva display specialized features differing from immatures of extant ripiphorid species, such as for instance the absence of stemmata and the presence of ventral transverse rows of spines. An earlier tentative assignment of a similar larva embedded in Cretaceous amber from Manitoba (Canada) to Strepsiptera is not followed here. We suggest that this larva is closely related to the beetle larva described here.

Keywords

amber; Burma; Coleoptera; fossils; larva; parasitic

Introduction

Burmese amber turned out as a rich source of information on Cretaceous insects and other groups of organisms, such as fungi, conifers, angiosperm plants, nematodes, ticks, velvet worms, spiders, and birds (Grimaldi et al. 2002). The age is estimated as 99 Ma (earliest Cenomanian) (Shi et al. 2012). A detailed survey on biotic diversity and other aspects is given in Grimaldi et al. (2002) and Ross et al. (2010).

Recently we discovered a strongly miniaturized wingless insect in a piece of amber from deposits in the Hukawng Valley (Fig. 1), currently considered to be of earliest Cenomanian age (ca. 99 mya) (Shi et al. 2012). Similarities with Cretaceous larvae (Canadian and Burmese amber) described earlier by Grimaldi et al. (2005) in a study with a main focus on an extinct ancestral species of Strepsiptera (†*Cretostylops engeli*



Fig. 1. Location of the amber mining area in the Hukawng Valley, Myitkina Province, Myanmar. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/1876312x>.

Grimaldi & Kathirithamby [adult male]) were apparent. The excellent preservation of the new material described here and some reservations concerning the interpretation of one of the larvae as a “triungulin” probably belonging to Strepsiptera (Grimaldi et al. 2005) induced us to investigate the specimens available to us. The main aim of the current study was to document the inclusions as detailed as possible using advanced photographic techniques and line drawings based on the images. The morphological findings are then interpreted with respect to the systematic position, structural affinities to the possible strepsipteran larva, and the biology of the species.

Material and Methods

The specimen was obtained from amber deposits in the Hukawng Valley of Myanmar (Fig. 1). The age is presently estimated as ca. 99 Ma (earliest Cenomanian) (Shi et al. 2012). The mining locality is at Noiye Bum, near Tanai Village (26°21'33.41"N, 96°43'11.88"E) (e.g., Grimaldi et al. 2002; Cruickshank & Ko 2003). The specimen (NO. BU-001009) is currently kept in the Institute of Zoology, Chinese Academy of Sciences (IZAS), and will eventually be deposited in the Three Gorges Entomological Museum, Chongqing, China (specimen available for study by contacting MB or WWZ).

The piece of amber containing the specimen was ground and polished to a 1.5 mm thick slice, mounted on a microscope slide using glycerine and examined with a compound microscope and reflected light at magnifications of 100 x. Photographs were taken with a Nikon D 90 digital SLR equipped with a Nikon M Plan 20 ELWD microscope lense, plus an adjustable extension bellows. The specimen was illuminated

by two flashlights. Helicon Focus Mac Pro X64 and Zerene Stacker 1.04 were used to combine a stack of several partially focused images.

Results

Morphology (Figs. 2 and 3)

Habitus. Extremely small, less than 0.45 mm excluding terminal bristles. Completely lacking wings or external wing pads. Moderately sclerotized on dorsal side, with largely smooth and shiny cuticle. Coloration light brown. Cuticle appears semitransparent. Vestiture of long setae present, but largely restricted to abdomen. Thorax distinctly longer than abdomen, with segments distinctly widening posteriorly, with rows of apically rounded spines on posteroventral segmental margins. Legs very well developed, with terminal pads. Abdomen widest in anterior third and very distinctly narrowing towards apex, with pair of long terminal bristles.

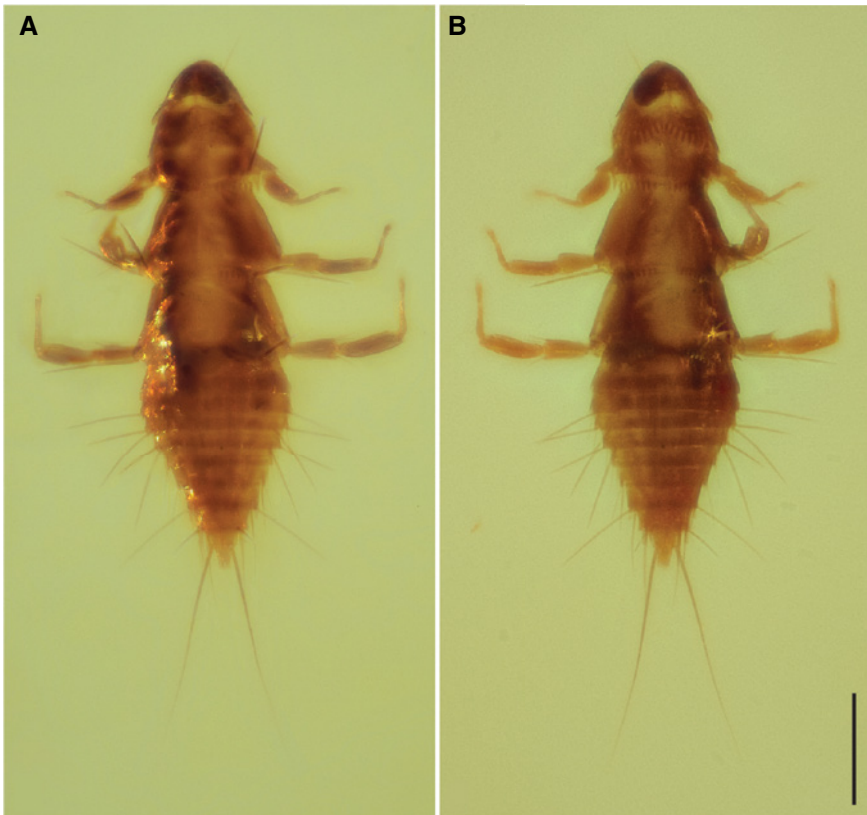


Fig. 2. Presumptive rhipiphorid larva in Cretaceous amber from Myanmar. A, dorsal view; B, ventral view. Photomicrograph. Scale bar: 100 μ m. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/journals/1876312x>.

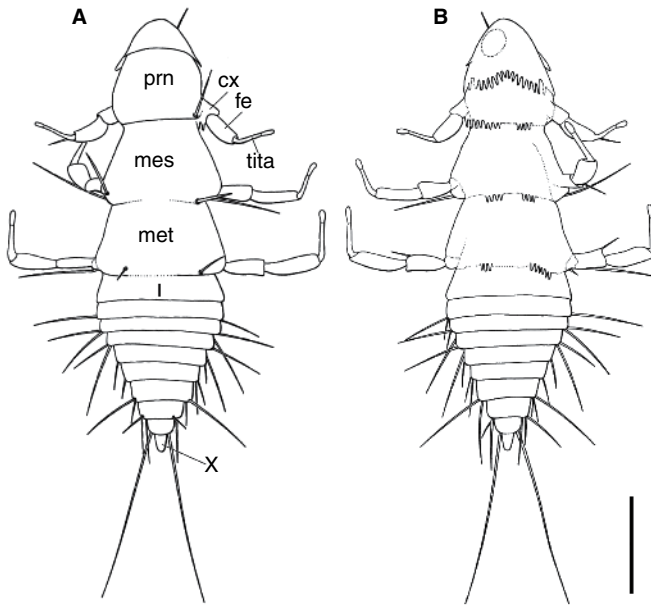


Fig. 3. Presumptive ripiphord larva in Cretaceous amber from Myanmar. A, dorsal view; B, ventral view. Drawing. cx – coxa, fe – femur, mes – mesonotum, met – metanotum, prn – pronotum, tita – tibiotalus, I – abdominal segment I, X – abdominal segment X. Scale bar: 100 μ m.

Head. Of parabolic shape, with evenly rounded anterior margin. Short, ca. 30 μ m from anterior to posterior margin in dorsal view. Ca. 80 μ m broad posteriorly. Bristle-like structure visible on right side, possibly representing a very slender antennae. Setae absent. Labrum and clypeus not recognisable as separate structures or regions. Epicranial sutures completely absent. Lateral eyes and ocelli lacking, irregular large spot visible on right side due to particle below head. Dorsal hind margin of head overlapping pronotum. Scale- or lobe-like extensions of head capsule recognizable posterolaterally, reaching anterior fourth of lateral pronotal margin. Mouthparts, sutures, ridges or gula not recognizable on ventral side. Tentorium or tentorial grooves not visible, presumably absent. Posteroventral edge with medially concave regular row of flattened spines resembling ctenidia (“pseudoctenidia”), but each less than 10 μ m long and with very narrow intervals.

Thorax. Large, more than 50% of total body length. Each segment distinctly widening posteriorly and width of segments increasing from prothorax to metathorax. Prothorax with well-developed pronotum without median ecdysial line and without well-defined lateral edge. Lateral margin rounded. Maximum width at posterior third. Posterior pronotal margin straight. One strongly developed and long seta (ca. 50 μ m) inserted on posterolateral corner. Ventral side without recognizable sternal plate. Posterior margin with almost straight row of apically rounded spines, similar to those of the posteroventral head margin. Forelegs with relatively short parallel-sided coxa distinctly protruding beyond lateral pronotal margin. Trochanter not recognizable like on middle

and hind legs. Femur moderately sized, ca. 30 μm long, fairly broad, with almost straight anterior margin and distinctly convex posterior margin. Distal segment probably composed of tibia and tarsus (tibiotarsus), very slender, with apical pad-like structure. Mesothorax slightly longer than prothorax, with distinctly widened and rounded posterolateral corners and long posterolateral seta. Mesonotum simple, without any differentiation or recognizable subdivision. Wings or wing buds completely absent. Posterior sternal margin with two recognizable groups of short, apically rounded spines, similar to those on prothorax. Very long laterally directed seta inserted posterolaterally on ventral side of segment. Middle legs with elongated, cylindrical, apically slightly widening coxa. Extending far beyond lateral mesonotal margin. Mesofemur longer and slightly narrower than profemur. Distal elements very similar to corresponding elements of proleg. Metathorax very similar in shape to mesothorax, but slightly shorter and slightly broader. Metanotum and mesonotum completely simple, with pair of setae close to hind margin, inserted relatively distant from lateral margin. Wings or wing buds completely missing. Very long seta inserted posterolaterally on ventral side of segment. Posterior sternal margin with one recognizable group of short, apically rounded spines visible on right side. Hind legs distinctly longer than forelegs and middle legs, very prominent. Metacoxae also elongate and cylindrical. Metafemur and distal elements slightly larger than those of anterior pairs of legs.

Abdomen. Abdomen ten-segmented, shorter than thorax (ca. 180 μm). All terga simple and sclerotized, terga I–VIII with straight posterior margin. All sterna with straight posterior margin without spines. Greatest width at segment II, strongly tapering towards apex. Segments II–VIII bearing long lateral setae. Segment I relatively long, widening posteriorly. Segments II–IV shorter, almost parallel-sided, III distinctly narrower than II, and IV narrower than III. Segments V–VIII slightly longer, each of them narrower than the preceding one. Segment IX about twice as wide as long, with rounded posterior margin and posterolaterally inserted caudal bristles as long as entire abdomen (180 μm). Segment X narrow, cone-shaped. Spiracles not recognizable.

Discussion

A series of features indicates clearly that the fossil specimen described here is a holometabolous larva: absence of compound eyes and ocelli, the strongly simplified or absent antennae, the complete absence of wings or wing buds, the lacking tarsal subdivision, the presence of only ten abdominal segments, and the complete absence of any recognizable genital structures. Affinities with wingless non-holometabolan groups can be easily excluded. The specimen differ fundamentally in its morphological features from entognathous insects including Protura (e.g., shape of head, morphology of forelegs, complete absence of abdominal appendages). Secondly flightless polyneopterans (e.g., Grylloblattodea) can be excluded by size and the complete lack of cerci and many other features (e.g., lack of well-developed eyes and antennae). Flightless Psocodea, i.e. Phthiraptera are also no serious option: lice are usually distinctly larger, even though species below 0.5 mm occur (e.g., Beutel et al. 2014). Phthiraptera differ greatly in their

cephalic organization and especially in the morphology of legs, which are usually characterized by clasping apparatuses and have always subdivided tarsi and distinct claws.

Within Holometabola all groups with eruciform larvae can be readily excluded, i.e., Hymenoptera, Mecoptera (excl. Nannochoristidae), and Lepidoptera, and also Trichoptera by size and the lack of terminal hooks in the specimen discussed here, and also Siphonaptera and Diptera, which are characterized by the complete absence of larval thoracic legs (e.g., Beutel et al. 2011). Larvae of Neuropterida are usually much larger and equipped with well-developed stemmata, antennae and mouthparts. The parasitic primary larvae (1st instar) of Mantispidae are small (ca. 1.2 mm) but differ strikingly in their cephalic morphology with an elongate head with prominent antennae and mouthparts (Aspöck & Aspöck 2007: fig. 35).

Basically only two options remain, the highly specialized Strepsiptera and the megadiverse Coleoptera. A similar larva embedded in Cretaceous amber from Manitoba (Canada) was tentatively assigned to Strepsiptera by Grimaldi et al. (2005). Several features are shared by this specimen, the larva described here and the extremely miniaturized, hypermetamorphic and endoparasitic larvae of Strepsiptera: very small size (ca. 340 μm in the Manitoban larva, ca. 200 μm on average in primary larvae of Strepsiptera; Grimaldi et al. 2005; Pohl 2000), the absence of antennae (possibly present in the short-legged larva described here), the unusual lobe-like distal element of the leg (pretarsal “bladder”) (Pohl & Beutel 2004; fig. 6), and the long terminal bristles (one pair, referred to as abdominal styli by Grimaldi et al. 2005). However, as already pointed out by Grimaldi et al. (2005) several features do not fit well with a placement in Strepsiptera: an apically pointed head is not found in any extant strepsipteran primary larvae (Pohl 2000), coarse ventral ctenidia are unknown in the group, and also 2-segmented tarsi, a condition entirely unknown in extant holometabolans. The presence of very long, strong setae also differs distinctly from known primary larvae of Strepsiptera. Additional arguments against a placement in this order are the indistinct or absent lateral eyes (well-developed and large individual stemmata are present in Strepsiptera; Pohl 2000, 2009), the strongly prominent, laterally protruding coxae (see Osswald et al. 2009), the absence of three strongly developed coxal spines (always present in Strepsiptera; Pohl 2000: figs 1, 2), the absence of spines and rows of setae from the abdominal sternites (present in Strepsiptera; Pohl 2000: figs 1, 2), and the insertion of a pair of terminal bristles on abdominal segment IX (on abdominal segment XI in Strepsiptera; Pohl 2000).

Like in the case of the Manitoban larvae it can be assumed that the similar larva described here is not closely related with Strepsiptera. A placement in the coleopteran suborders Archostemata and Adephaga can be easily excluded as the larvae known in these groups are not only distinctly larger (first instar of *Micromalthus debilis*: 2.7–3.3 mm; Beutel & Hörnschemeyer 2002a) but differ completely in their habitus and many individual characteristics (e.g., Beutel & Hörnschemeyer 2002a,b; Beutel & Leschen 2005). Affinities with Myxophaga are also very unlikely as the relatively small larvae (and adults) of this group live either in substrate in moist riparian zones or in hygrotropic habitats. Moreover, they possess well-developed antennae and stemmata and usually a unique type of spiracular gills (Beutel et al. 1998). A placement among the polyphagan Cucujiformia appears very likely, especially a close relationship with extant

species of the family Ripiphoridae, as suggested by Grimaldi et al. (2005) for their “Triungulin species B”. Fossil ripiphorid adults were described from Dominican amber (Miocene, 19–20 mya; e.g., Grimaldi 1995; Batelka et al. 2011), from Eocene Baltic amber (ca. 42 mya; Spahr 1981), from the Paleogene of Florissant (Colorado) (ca. 34 mya; Meyer 2003), from Lower Eocene French amber (ca. 53 mya; Batelka et al. 2006), from Albian-Cenomanian French amber (ca. 100 mya; Perrichot et al. 2007; Falin & Engel 2010), and from Burmese amber (99 mya; Grimaldi et al. 2002; Cruickshank & Ko 2003; Falin & Engel 2010; Shi et al. 2012). A feature strongly suggesting close affinities to this family are pad-like distal leg modifications which occur in ripiphorid larvae (absent in Pelecatominae; Švácha 1994; Lawrence et al. 2010) but not in any other group of Coleoptera. This unusual condition is apparently related with phoresy, i.e., the attachment of parasitic larvae on a hosts’s body surface (Švácha 1994). Another very unusual feature indicating ripiphorid affinities is the posterior head capsule distinctly overlapping the anterior pronotal margin. In its general shape the head is similar to the unusual condition found in ripidiine and ripiphorine larvae (Lawrence et al. 2010: figs. 11.8.4. F, G., 11.8.5. D), shield-like, widening posteriorly, with a truncate posterior margin. This suggests that the larva belong to a subgroup of the family that does not include Pelecatominae, whose slender, very elongate larvae differ strongly in their morphology (Švácha 1994). A considerable number of details (e.g., antennae, mouthparts) remain obscure and the larvae differ in a number of features from the few known primary larvae of Ripiphoridae. This includes the apparent absence of eyes, the distinctly trapezoid shape of the thoracic tergites, the absence of distinctly developed sternal plates (Lawrence et al. 2010: fig. 11.8.4. G), and especially the presence of the regular transverse rows of spines on the ventral side.

Considering the recognizable morphological features it appears likely that different highly miniaturized larvae embedded in Cretaceous ambers belong to a subgroup of the cucujiform Ripiphoridae. The pad-like attachment structures on the apex of the legs (and high numbers of similar larvae in some pieces of amber; Bo Wang, pers. commun., June 2015) support the interpretation as first instars of a parasitic species. The precise placement within Ripiphoridae remains obscure, partly due to features not recognizable in the fossils, but also due to the very limited knowledge of the primary larvae of this beetle family (Falin 2003).

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