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ORIGINAL ARTICLE

The earliest Timematids in Burmese amber reveal diverse tarsal pads of stick insects in the mid-Cretaceous

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Abstract Many extant insects have developed pad structures, euplantulae or arolia on their tarsi to increase friction or enhance adhesion for better mobility. Many polyneopteran insects with euplantulae, for example, Grylloblattodea, Mantophasmatodea and Orthoptera, have been described from the Mesozoic. However, the origin and evolution of stick insects' euplantulae are poorly understood due to rare fossil records. Here, we report the earliest fossil records of Timematodea hitherto, *Tumefactipes prolongates* gen. et sp. nov. and *Granosicorpes lirates* gen. et sp. nov., based on three specimens from mid-Cretaceous Burmese amber. Specimens of *Tumefactipes prolongates* gen. et sp. nov. have extremely specialized and expanded euplantulae on their tarsomere II. These new findings are the first known and the earliest fossil records about euplantula structure within Phasmatodea, demonstrating the diversity of euplantulae in Polyneoptera during the Mesozoic. Such tarsal pads might have increased friction and helped these mid-Cretaceous stick insects to climb more firmly on various surfaces, such as broad leaves, wetted tree branches or ground. These specimens provide more morphological data for us to understand the relationships of Timematodea, Euphasmatodea, Orthoptera and Embioptera, suggesting that Timematodea might be monophyletic with Euphasmatodea rather than Embioptera and Phasmatodea should have a closer relationship with Orthoptera rather than Embioptera.

Key words euplantula; Myanmar; Polyneoptera; stick insects; survival strategy; Timematodea

Introduction

Many insects have developed specialized leg structures to adapt to their life styles and/or environment, for example, honeybee's pollen baskets in hind legs, male great diving beetle's predacious clasping front legs with suction cups, mole cricket's flattened fore legs for hole digging and the hind legs for pushing soil, adhesive pads on the tarsi of the housefly to stay upside down on smooth surfaces, and so on. Some insects have evolved with modified attachment structures on legs for adhering to surfaces (Grohmann *et al.*, 2015), a few having numerous setae directly on the ventral surface of the tarsi, for example, in Dermaptera (Haas & Gorb, 2004) and Coleoptera (Bullock & Federle, 2009; Gorb *et al.*, 2010), while others possessing attachment pads, such as euplantulae and arolia. The euplantulae may be smooth, as found in Orthoptera (Goodwyn *et al.*, 2006), Embioptera (Ross, 2000), Mantodea (Beutel & Gorb, 2001; Wieland, 2013) and Blattodea (Beutel & Gorb, 2001; Clemente & Federle, 2008), or nubby (ratio

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of length to width ≤4) in Phasmatodea (Bußhardt *et al.*, 2012) and Plecoptera (Nelson, 2009). Interestingly, both the smooth and nubby tarsal structures are found in *Acanthoproctus diadematus* (Orthoptera) (Grohmann *et al.*, 2015). In addition, some groups of Mantophasmatodea and Timematodea of Phasmatodea have long setae (ratio of length to width >4) on their euplantulae to increase friction (Beutel & Gorb, 2008).

Timematodea, only comprising 21 species within *Timema* of Timematidae, are considered to be the sister group of the remaining Phasmatodea (= Euphasmatodea) (Tilgner *et al.*, 1999; Wheeler *et al.*, 2001; Tilgner, 2002; Bradler *et al.*, 2003; Whiting *et al.*, 2003; Wheeler *et al.*, 2004). Up to date, there are no fossils that can be unambiguously assigned to Timematodea. Zompro placed a species, *Electrotimema carstengroehni*, from middle Eocene in Baltic amber to the Anareolatae in 2001 and mentioned it had five tarsomeres (Zompro, 2001). Later in 2005, he transferred this *E. carstengroehni* to Timematodea for its three tarsomeres, but did not declare whether the tarsi were trimerous or not (Zompro, 2005; Engel *et al.*, 2016). Therefore, the taxonomic position of *Electrotimema* Zompro, 2001 needs to be further studied.

Here, we described three stick insects from mid-Cretaceous Burmese amber and assigned them to Timematodea. Both specimens of *Tumefactipes prolongates* gen. et sp. nov. have very large and expanded euplantula structures on the tarsomere II. The first three tarsomeres of the originally five-segmented tarsus are fused, forming a trimerous tarsus, which is unique in stick insects, providing more evidence of ancient morphological characters of Timematodea. Although they are nymphs, they already have the basic characteristics of adults.

Materials and methods

Material and photography

All specimens described here are housed in the Key Lab of Insect Evolution and Environmental Changes, College of Life Sciences, Capital Normal University, Beijing, China (CNUB; Dong Ren, Curator). However, BU-001232 and BU-001959 will eventually be deposited in the Three Gorges Entomological Museum, Chongqing (specimens to be available for study by contacting DR or WWZ). A Leica M205C dissecting microscope and a Nikon ECLIPSE Ni microscope were used for observation of the amber specimens. All specimens were photographed under a Nikon SMZ 25 microscope with a Nikon DS-Ri 2 digital camera system and a Nikon ECLIPSE Ni microscope with a Nikon DS-Ri 2 digital camera system. Line drawings were prepared using Adobe Illustrator CC and Adobe Photoshop CC graphics software.

Micro-CT scanning and 3D reconstruction

The amber specimens BU-001232 and CNU-PHA-MA2017002 were scanned with a Micro-CT (nanoVoxel 3000D, Sanying Precision Instruments Co., Ltd., Tianjin, China) which was located at the School of Mathematical Sciences, Capital Normal University. The voltage of the scanning was 50 kV and the phase contrast enhancement technique was utilized to reconstruct the CT image with a higher contrast. The spatial resolution of specimens BU-001232 and CNU-PHA-MA2017002 were 0.499 μm and 0.438 μm, respectively. Based on the 3D CT image, tarsus structures of the two specimens were rendered with Amira 5.4 (Visage Imaging, San Diego, USA).

Systematic palaeontology

Order Phasmatodea Jacobson & Bianchi, 1902

Suborder Timematodea Kristensen, 1975

Family Timematidae Bradley & Galil, 1977

Genus *Tumefactipes* nov.

Derivation of name *Tumefactipes* is a combination of the Latin word of tumefactus, meaning “expanded”, and pes, meaning, “leg”, referring to the euplantulae of tarsomere II expanded. Gender is feminine.

Type species *Tumefactipes prolongates* sp. nov.

Diagnosis Head and thorax smooth. Head ovoid, longer than width. Thoracic segments strikingly different in lengths. Pronotum significantly elongated, longer than width and about twice as long as mesonotum. Prosternum with a pair of conspicuous rough, wart-like structures (sensory areas), and profurcasternum lack of central sensory area (Fig. 2B). Abdominal tergum I longer than half of metanotum. Metafemora obviously broadened. The euplantulae of tarsomere II expanded. Arolia absent. Abdomen without longitudinal median carina. Tergum X broadly rounded apically and elongated forming a large, rectangular, transparent rostrum, edge serrated. Cerci cylindrical and not specialized.

Tumefactipes prolongates **sp. nov.** (Figs. 1–3)

Derivation of name The epithet, “*prolongatus*”, is a Latin word, referring to the elongated pronotum.

Holotype BU-001232; female (Figs. 1, 2).

Paratype CNU-PHA-MA2017001; female (Fig. 3).

Horizon and locality The amber specimens were collected from Kachin (Hukawng Valley) of northern

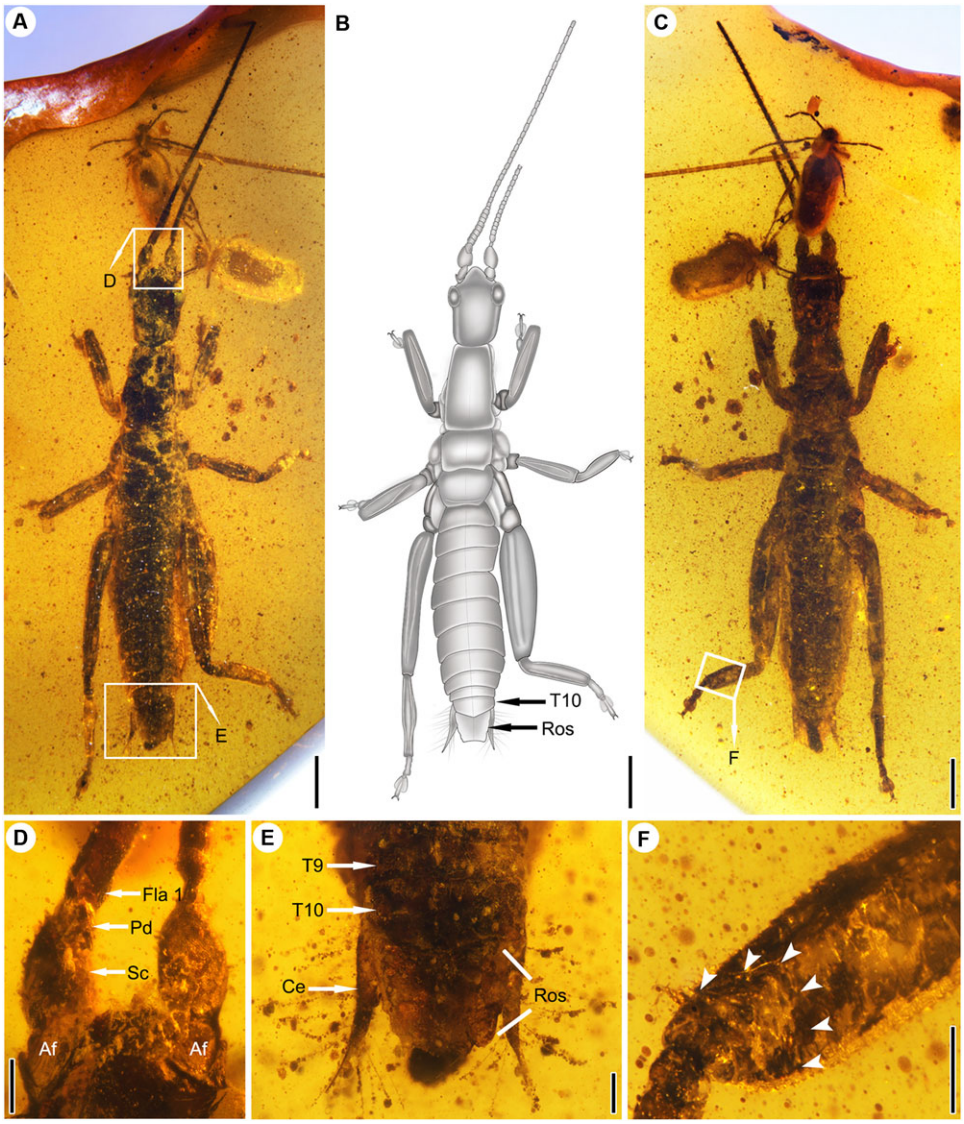

Fig. 1 Holotype of *Tumefactipes prolongates* gen. et sp. nov. from mid-Cretaceous Myanmar amber, No. BU-001232. Female. A and B, Photograph and line drawing in dorsal view. C, Photograph in ventral view. D, Basal part of antennae in dorsal view. E, Terminal in dorsal view. F, The area apicalis (arrows) of right metatibia in the ventral view. Abbreviations are as follows: Af, antennal field; Ce, cercus; Fla 1, flagellomere I; Pd, pedicellus; Ros, rostrum; Sc, scape; T9–10, abdominal terga 9–10. Scale bars represent 1 mm (A–C), 0.2 mm (D–F).

Myanmar, which was dated to be 98.79 ± 0.62 Ma (Cruickshank & Ko, 2003; Shi *et al.*, 2012; Ross, 2015).

Diagnosis As for genus.

Description Holotype No. BU-001232; nymph: body robust (Fig. 1A–C). Head unarmed, ovoid, roughly parallel-sided and about 1.6 times as long as width; ocelli absent; compound eyes ovoid, slightly exophthalmic; antenna filiform, longer than foreleg; left antenna with 38 antennomeres (as preserved); right antenna with 18 antennomeres (as preserved); scape broad, unarmed (Fig. 1D); pedicel cylindrical; antennomere III as long as IV; each flagellomere rounded in cross-section, basal eight flagellomeres short and thick, then gradually elongated and tapering toward apex; labrum and antennifer not clear due to the preservation.

Thorax robust, surface without spines or carinae (Fig. 2A); median line present; thoracic terga simple; pronotum rectangular, no lateral extension (Fig. 2C),

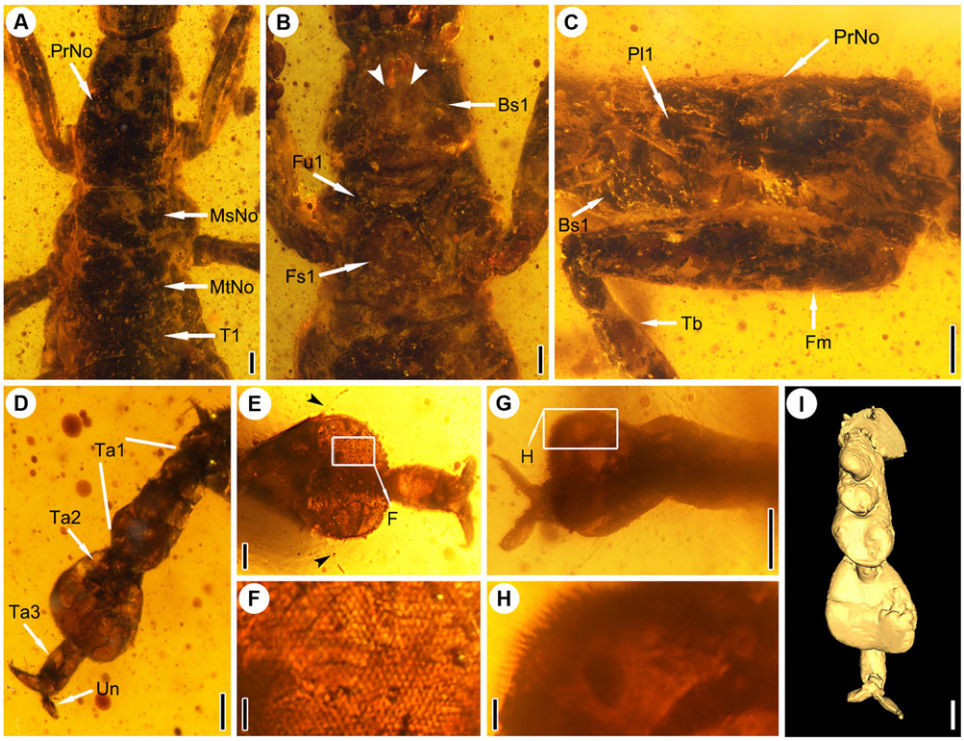

Fig. 2 Holotype of *Tumefactipes prolongates* gen. et sp. nov. from mid-Cretaceous Myanmar amber, No. BU-001232. Female. A, Thoracic nota and first abdominal tergum ('median segment'). B, Prosternum, profurca and profurcasternum, arrows indicating the sensory areas. C, The lateral view of prothorax. D, Right metatarsus in ventral view. E, The euplantula of tarsomere II of left mesotarsus, arrows indicating the long setae. F, Enlargement of the white rectangle in (E), showing the small square lines that may be setae. G, The euplantula of tarsomere II of right mesotarsus. H, Enlargement of the white rectangle in (G), showing the setae on the edge. I, 3D reconstructions based on micro-CT data, showing the ventral view of right metatarsus. Abbreviations are as follows: Bs1, prosternum; Fm, femur; Fs1, profurcasternum; Fu1, profurca; MsNo, mesonotum; MtNo, metanotum; Pl1, propleuron; PrNo, pronotum; T1, the first abdominal tergum (median segment); Ta1–Ta3, tarsomeres I–III; Tb, tibia; Un, claw. Scale bars represent 0.25 mm (A), 0.2 mm (B, C), 0.1 mm (D, G, I), 50 μm (E), 10 μm (F, H).

longer than head, and weakly widening toward posterior, prothoracic defensive glands not clear due to the preservation; mesonotum transversely rectangular, parallel-sided and much shorter than pronotum; metanotum slightly shorter than mesonotum, wider than length, widest at the middle point and with a transverse furrow at posterior; meso- and metasternum simple, no carinae; abdominal tergum I ('median segment') tectiform, shorter than metanotum and not associated with metanotum.

Legs slightly stout (Fig. 1A–C); foreleg emerging from the posterior of the pleura; trochanter fused with femur; profemora straight at the base; all femora and tibiae with obvious carinae; each femur and tibia almost rectangular in cross-section; tibiae with two longitudinal rows of minute spines in the ventral and two spines in the ventral apex; area apicalis present on the ventro-distal part of all tibiae (Fig. 1F); tarsus pseudotrimeric, euplantulae present on tarsomeres I and II and expanded on II (Figs. 2D–I, 5G, H); metafemora conspicuously broadened, reaching abdominal segment VII and metatibiae beyond apex of abdomen.

Abdomen broad; ten abdominal segments observed and median line present (Fig. 1A–C); abdominal sternum I associated with the metasternum; segments IV and V wider than others, the remaining segments gradually narrowing, wider than length; segment IX shortest (Fig. 1E); segment X posterior margin projecting and elongated forming a large, lamellar, rectangular, transparent rostrum, edge serrated and slightly rolled up; cerci unsegmented, long, cylindrical, gradually tapering toward apex, beyond the rostrum and bearing numerous elongate setae.

Measurements (in mm) Body 9.33 (excluding antennae), head 1.50, left antenna 5.26 (as preserved), right antenna 2.07 (as preserved), scape 0.40, pedicel 0.22, pronotum 1.70, mesonotum 0.73, metanotum 0.68,

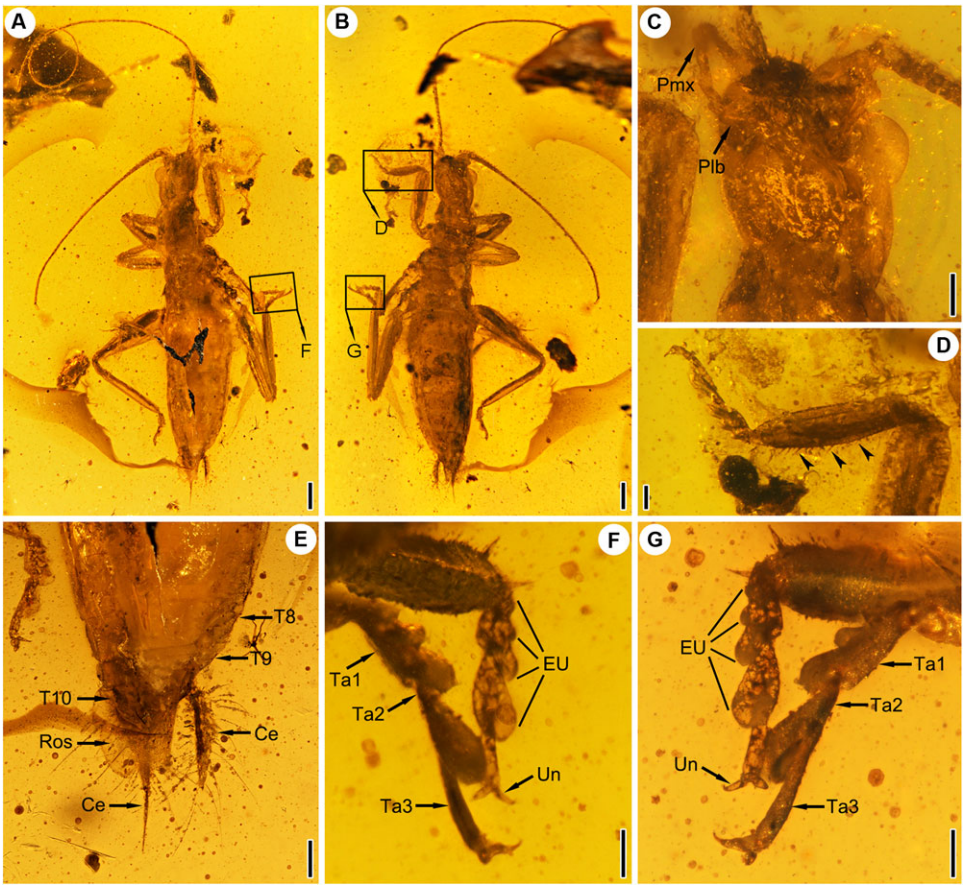

Fig. 3 Paratype of *Tumefactipes prolongates* gen. et sp. nov. from mid-Cretaceous Myanmar amber, No. CNU-PHA-MA2017001. Female. A and B, Photographs in dorsal and ventral view. C, Head in ventral view. D, Left hind leg in a lateral view, arrows showing the longitudinal row of spines. E, Terminal in dorsal view. F, The right mesotarsus and metatarsus in the dorsal view, showing the euplantulae. G, The left mesotarsus and metatarsus in the lateral view, showing the euplantulae. Abbreviations are as follows: Ce, cercus; EU, euplantula; Plb, labial palpus; Pmx, maxillary palpus; Ros, rostrum; T8–10, abdominal terga 8–10; Ta1–Ta3, tarsomeres I–III; Un, claw. Scale bars represent 0.5 mm (A, B), 0.2 mm (C, E), 0.1 mm (D, F, G).

abdominal tergum I ("median segment") 0.42, profemur 1.70, mesofemur 1.57, metafemur 2.68, metatibia 1.48, metatarsus 0.85 and abdomen 4.62.

Paratype No. CNU-PHA-MA2017001; nymph: body smaller than the holotype (Fig. 3A, B). Head prognathous and about 1.4 × as long as wide (Fig. 3C); left antenna preserved with only 36 antennomeres; right antenna, more than 80 antennomeres.

Thoracic structure similar to that in holotype, but the pronotum with similar width and metanotum without the transverse furrow at posterior.

Compared to holotype, the spines in the ventral of tibiae more obvious (Fig. 3D); euplantulae on tarsomeres I and II more prominent (Fig. 3F, G).

Ten abdominal segments preserved and abdominal tergum X also elongated forming a rostrum (Fig. 3E).

Measurements (in mm) Body 6.0 (excluding antennae), head 1.0, left antenna 4.0 (as preserved), right antenna 9.20 (as preserved), pronotum 1.0, mesonotum 0.48, metanotum 0.44, abdominal tergum I ("median segment") 0.27, profemur 1.07, protibia 0.72, protarsus 0.52, mesofemur 1.05, mesotibia 0.83, mesotarsus 0.47, metafemur 3.41, metatibia 3.20, metatarsus 1.21 and abdomen 3.0.

Genus *Granosicorpes* nov.

Derivation of name *Granosicorpes* is a combination of the Latin word granosus, meaning "particles", and corpus, meaning, "body", referring to the body with a large number of small protuberances. Gender is feminine.

Type species *Granosicorpes lirates* sp. nov.

Diagnosis Dorsal surfaces of head and thorax with a large number of small protuberances. Head ovoid, with

two parallel carinae, longer than width. Thoracic segments strikingly different in lengths. Pronotum significantly elongated, slightly longer than width and about three times as long as mesonotum. Prosternum without sensory areas, profurcasternum with the central sensory area. Mesonotum and metanotum with two fan-shaped depressions. Abdominal tergum I shorter than half of metanotum. Metafemora obviously broadened. The euplantulae of tarsomere II not expanded. Arolia absent. Abdomen with a longitudinal median carina, tergum X flat apically and the rostrum small, transversely rectangular, not transparent and edge smooth. Cerci cylindrical and not specialized.

Granosicorpes lirates gen. et sp. nov. (Fig. 4A–G)

Derivation of name The epithet, "*liratus*", is a Latin word, referring to the head and abdomen with carinae.

Holotype CNU-PHA-MA2017002; female (Fig. 4A–G).

Horizon and locality The amber specimen was collected from Kachin (Hukawng Valley) of northern Myanmar; mid-Cretaceous.

Diagnosis As for genus.

Description Nymph. Body robust (Fig. 4A, B, D). Head ovoid, longer than width, anterior margin with two rough warts; maxillary palps 5-segmented; labial palps 3-segmented; ocelli absent; compound eyes hemispherical; antenna filiform, longer than foreleg; left antenna with 40 antennomeres (as preserved); right antenna 33 antennomeres preserved; scape broad, unarmed; pedicel cylindrical, half as long as scape and thinner; antennomere III longer than IV; each flagellomere rounded in cross-section, basal six flagellomeres short and thick, then gradually elongated and tapering toward apex; labrum and antennifer not clear due to the preservation.

Pronotum rectangular, no lateral extension, slightly longer than width (Fig. 4E), with an irregular projection in the middle, prothoracic defensive glands not clear due to the preservation; mesonotum much shorter than pronotum, with two fan-shaped depressions; metanotum as long as mesonotum, slightly wider than mesonotum, with two fan-shaped depressions; meso- and metasternum simple, metasternum wider than mesosternum; abdominal tergum I ("median segment") transversely rectangular, about 1/3 the length of metanotum, not associated with metanotum.

Foreleg emerging from the posterior of the pleura (Fig. 4A, B, D); trochanter fused with femur; profemora straight basally; all femora and tibiae with obvious carinae, almost rectangular in cross-section; the ventral of tibiae with two longitudinal rows of minute spines and two spines in the ventral apex; area apicalis present on the ventro-distal part of all tibiae (Fig. 4F); tarsus pseudotrimeric, euplantulae present on tarsomeres I and II, but not expanded (Figs. 4G, 5E, F); metafemora conspicuously broadened.

Abdomen broad, with a longitudinal median carina and two lateral carinae (Fig. 4A, B, D); ten abdominal segments observed; abdominal sternum I associated with the metasternum; segments II to VIII of similar length and width; segment VI the longest; segments IX to X gradually narrowing, segment X longer than segment IX and elongated forming a small rostrum, transversely rectangular, not transparent and edge smooth (Fig. 4C); cerci unsegmented, cylindrical, beyond the rostrum, with a small amount of setae.

Measurements (in mm) Body 3.56 (excluding antennae), head 0.80, left antenna 4.70 (as preserved), right antenna 3.33 (as preserved), scape 0.16, pedicel 0.08, pronotum 0.72, mesonotum 0.25, metanotum 0.24, abdominal tergum I ('median segment') 0.08, profemur 0.65, protibia 0.62, protarsus 0.46, mesofemur 0.63, mesotibia 0.55, mesotarsus 0.49, metafemur 0.86, metatibia 0.74, metatarsus 0.63 and abdomen 1.55.

Remarks The new taxa share two synapomorphies of Phasmatodea: metasternum and abdominal sternum I fused (Tilgner *et al.*, 1999; Tilgner, 2002; Bradler, 2009; Bradler & Buckley, 2011) and cerci unsegmented (Tilgner *et al.*, 1999; Whiting *et al.*, 2003; Grimaldi & Engel, 2005; Wedmann *et al.*, 2007; Bradler, 2009). The prothoracic defensive glands and labrum are not clear due to the preservation. The new findings differ from the stem groups of Phasmatodea from the Mesozoic, such as *Hagiphasma paradoxa* Ren, 1997, *Aethephasma megista* Ren, 1997, *Orephasma eumorpha* Ren, 1997, *Renphasma sinica* Nel & Delfosse, 2011, *Adjacivena rasnitsyni* Shang, Béthoux, & Ren, 2011 and *Cretophasmomima melanogramma* Wang, Béthoux, & Ren, 2014 (Ren, 1997; Nel & Delfosse, 2011; Shang *et al.*, 2011; Wang *et al.*, 2014) and so on, mainly based on prothorax elongated, tarsus pseudotrimeric and arolia absent. The monophyly of Timematodea is supported by tarsus pseudotrimeric and the first article comprising three fused tarsomeres, the presence of the mesal lobe on the right cercus of the male (Kristensen, 1975; Tilgner *et al.*, 1999; Bradler *et al.*, 2003; Grimaldi & Engel, 2005) and the egg-coating behavior of the female (Tilgner *et al.*, 1999; Grimaldi & Engel, 2005). The latter two features are not visible in our specimens. However, based on the following features: large scape about twice as long as pedicel (Vickery, 1993); retention of prothoracic sternal apophyses (furca) (Kristensen, 1975; Grimaldi & Engel, 2005); abdominal tergum I not fused with metanotum; abdomen not showing any particular elongation (Kristensen, 1975); legs shorter compared with other

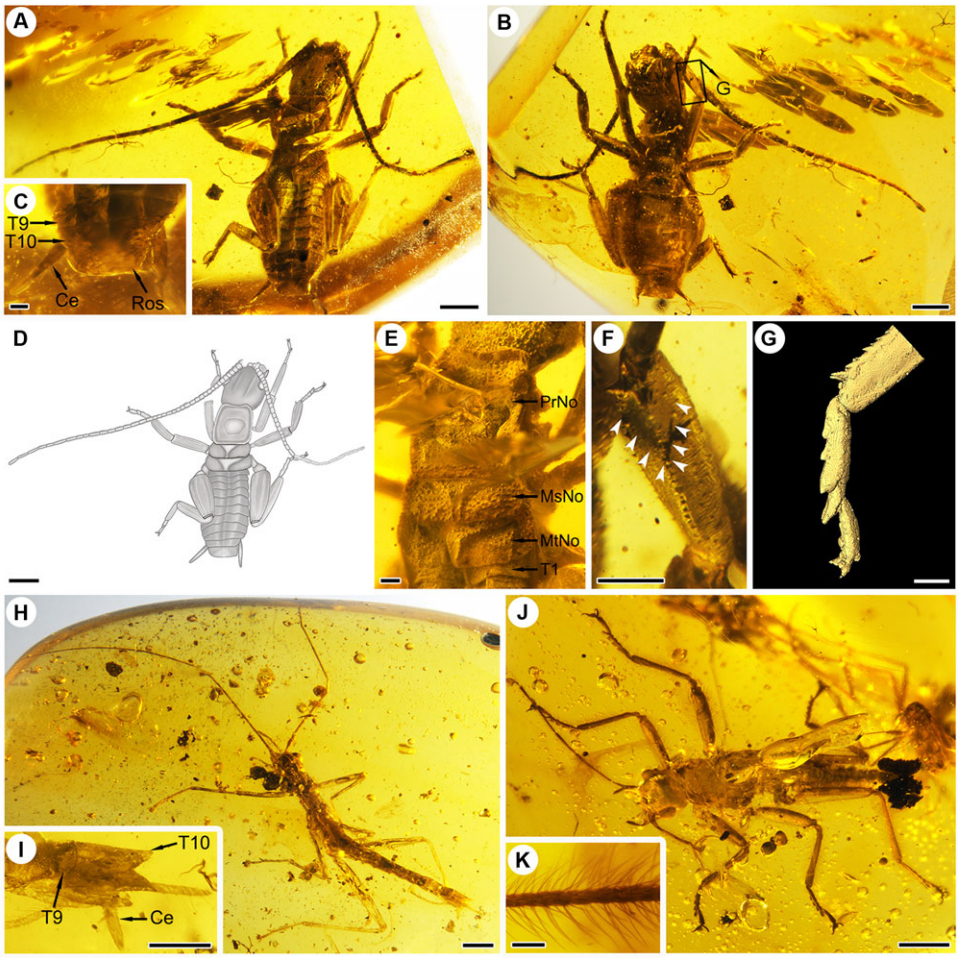

Fig. 4 A–G, Holotype of *Granosicorpes lirates* gen. et sp. nov. (No. CNU-PHA-MA2017002, female) from mid-Cretaceous Myanmar amber. A and B, Photographs in dorsal and ventral view. C, Terminal in dorsal view. D, Line drawing of (A). E, Thoracic nota and the first abdominal tergum ("median segment"). F, The area apicalis (arrows) of left protibia in the ventral view. G, 3D reconstructions based on micro-CT data, showing the lateral view of left protarsus. H, I, *Pseudoperla scapiforma* (No. CNU-PHA-MA2017003, male). H, Photograph in dorsal view. I, Terminal in dorsal view. J, K, *P. scapiforma* (No. BU-001959, female). J, Photograph in dorsal view. K, Part of antenna. Abbreviations are as follows: Af, antennal field; Ce, cercus; MsNo, mesonotum; MtNo, metanotum; Pmx, maxillary palpus; PrNo, pronotum; Ros, rostrum; T1, the first abdominal tergum (median segment); T9 and T10, abdominal terga 9 and 10. Scale bars represent 0.5 mm (A, B, D, I), 0.1 mm (C, E, G, K), 0.2 mm (F), 1 mm (H, J).

stick insects (Vickery, 1993) and area apicalis present; we temporarily place these two new genera within Timematodea. The prothoracic sternal apophyses are developed in Timematodea, Embioptera, Orthoptera and Plecoptera, but largely reduced in Euphasmatodea, which is considered to be an apomorphy of Euphasmatodea (Tilgner *et al.*, 1999; Bradler *et al.*, 2003; Bradler, 2009). The fusion of the metanotum with the first abdominal tergum is considered to be an apomorphy shared by Euphasmatodea (Tilgner *et al.*, 1999; Tilgner, 2002; Bradler, 2009; Bradler & Buckley, 2011) but they are separated in *Timema* and most other polyneopteran groups, so the discerption of metanotum and abdominal tergum should be a plesiomorphy of Phasmatodea (Tilgner *et al.*, 1999). Abdomen does not show any particular elongation, which is a primitive condition in *Timema* (Kristensen, 1975). These two new genera are differentiated from *Timema* based on the following characters: head longer than width; pronotum longer than width and much longer than mesonotum; thoracic segments strikingly different in lengths; metafemora conspicuously robust, no arolia; abdominal tergum X specialized as secondary ovipositor and cerci not specialized. The *Tumefactipes* gen. nov. is differentiated from *Granosicorpes* gen.

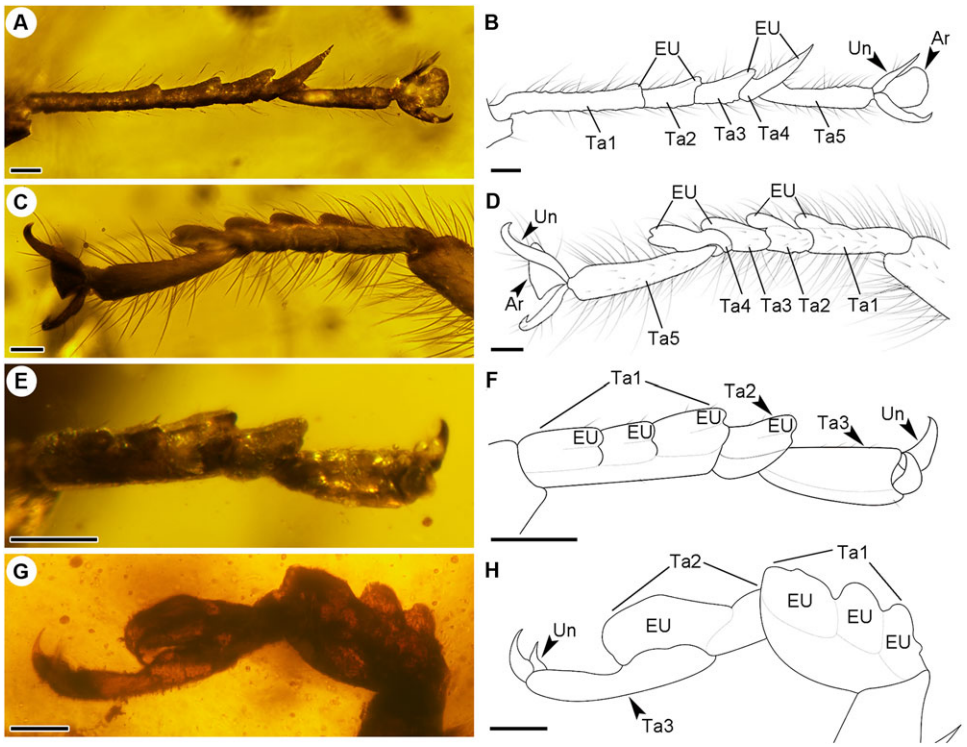

Fig. 5 The details of euplantulae. A, B, *Pseudoperla scapiforma* (No. CNU-PHA-MA2017003). A, Left mesotarsus in lateral view. B, Line drawing of (A). C, D, *P. scapiforma* (No. BU-001959). C, Left mesotarsus in lateral view. D, Line drawing of (C). E, F, *Granosicorpes lirates* gen. et sp. nov. (CNU-PHA-MA2017002). E, Left protarsus in lateral view. F, Line drawing of (E). G, H, *Tumefactipes prolongates* gen. et sp. nov. (No. BU-001232). G, Right metatarsus in lateral view. H, Line drawing of (G). Abbreviations are as follows: Ar, arolium; EU, euplantula; Ta1–Ta5, tarsomeres I–V; Un, claw. Scale bars represent 0.1 mm (A–H).

nov. mainly by the characters head and thorax are smooth; pronotum obviously longer than width and about twice as long as mesonotum; prosternum with a pair of sensory areas, but the central sensory area on profurcasternum absent; abdominal tergum I longer than half of metanotum; the euplantulae of tarsomere II expanded; abdomen without longitudinal median carina; tergum X broadly rounded apically and the rostrum larger, transparent and edge serrated. The rostrum is a part of the secondary ovipositor for depositing eggs in soil and other substrates due to abdominal tergum X elongated (Lelong, 1995; Buckley *et al.*, 2009); all the new findings have clear rostrum structures (Figs. 1E, 3E, 4C), which suggest that the three specimens are female. In addition, *Tumefactipes* gen. nov. and *Granosicorpes* gen. nov. do not have traces of gonapophyses or the developing subgenital plate on the eighth sternum (Figs. 1C, 3B, 4B), which indicate that these specimens are nymphs.

Family Archipseudophasmatidae Zompro, 2001

Genus *Pseudoperla* Berendt & Pictet, 1854

Pseudoperla scapiforma Chen, Shih & Ren, 2017 (Chen *et al.*, 2017) (Fig. 4H–K)

Description of new material Male. Specimen No. CNU-PHA-MA2017003 (Fig. 4H, I). Female. Specimen No. BU–001959 (Fig. 4J, K). (Details of Description and Remarks, see on-line Supplementary Information).

Discussion

The morphology of euplantulae

The tarsus of *Tumefactipes prolongates* gen. et sp. nov. consists of three tarsomeres in dorsal view, and the basitarsomere clearly has three attachment pads (euplantulae), which are separated by moderately deep transverse notches on the ventral side and fused laterally (Figs. 3F, G, 5G, H). The second tarsomere is much shorter than the basitarsomere, but its attached euplantula is disc-like, strongly expanded and considerably much longer and broader (Figs. 3F, G, 5G, H). The euplantula on tarsomere II seems to be composed of two areas. The apical part shows many small tessellations formed by very short setae (Fig. 2E, F). Several long setae and a lot of short

setae about 4 to 5 μm, are distributed on the edge (Fig. 2E, G, H). The third and distal tarsomere, lacking an euplantula, is thinner than the other two tarsomeres, and equal to basitarsomere in length.

The tarsus of *Granosicorpes lirata* gen. et sp. nov. is similar to that of *T. prolongates* gen. et sp. nov., but the euplantulae on basitarsomere and tarsomere II are very small, smooth and bilobed (Figs. 4G, 5E, F).

The new material of *Pseudoperla scapiforma* Chen, Shih & Ren, 2017 (No. CNU-PHA-MA2017003 and No. BU-001959) have five-segmented tarsi (Figs. 4H–K, 5A–D), the same as that of all extant stick insects of Euphasmatodea. The bilobed euplantulae of No. BU-001959 is obviously larger than those of No. CNU-PHA-MA2017003. The euplantulae of tarsi are very different in shape and structure between *Pseudoperla scapiforma* and *T. prolongates* sp. nov. In addition, arolia are present in the *Pseudoperla scapiforma*, but absent in all the legs of *T. prolongates* sp. nov. and *G. lirata* sp. nov. (Fig. 5)

Functions of the expanded euplantulae in T. prolongates gen. et sp. nov

There are two types of attachment pads on tarsi of stick insects: a "heel" pad (euplantula) that provides strong friction and a "toe" pad (arolium) that enhances the power of adhesion (Labonte & Federle, 2013). In *T. prolongates* sp. nov., the euplantulae of tarsomere II on all legs are significantly expanded, and these widened and soft pads would have increased the contact area and produced strong friction between the pads and surface of their habitat. As a consequence, this structure helped these stick insects climbing and moving more firmly on various surfaces, such as broad leaves, wetted surfaces or ground. No traces of arolia are found in *T. prolongates* gen. et sp. nov., although most groups of extant *Timema* possess strongly enlarged and pan-shaped arolia. However, *G. lirata* sp. nov. has neither expanded euplantulae on tarsomere II, nor arolia. Therefore, it is difficult for us to conclude whether there is a causal-effect relationship between absence of arolia and the expanded euplantulae.

The first fossil records of euplantulae in Phasmatodea

Attachment pads are vital in the evolution of Hexapoda (Beutel & Gorb, 2001, 2006). The arolia, which are likely a derived ground plan feature of Neoptera, are strongly enlarged and pan-shaped in many groups of *Timema* and Mantophasmatodea (Beutel & Gorb, 2006, 2008). Euplantulae are considered to be a potential synapomorphy within the Phasmatodea, Mantophasmatodea, Orthoptera, Grylloblattodea and Dictyoptera (Beutel & Gorb, 2006, 2008). These new specimens, *T. prolongates* sp. nov. and *G. lirata* sp. nov., from the mid-Cretaceous Burmese amber, have no arolia structures on any legs, but *T. prolongates* possesses strongly expanded euplantulae, a feature that is not present in living groups of *Timema* or Euphasmatodea to our knowledge. It is interesting to note that some extant taxa in Phaneropterinae of Orthoptera (nymph, Fig. S1A–D) and Acromantinae of Mantodea (Fig. S1E–I) have strongly expanded euplantulae, similar as those of *T. prolongates*, on their tarsomeres III and IV. Several fossil examples of euplantula structures have been described for fossil specimens of Blattaria (Garwood & Sutton, 2010), Grylloblattodea (Huang *et al.*, 2008a), Mantophasmatodea (Huang *et al.*, 2008b) and Orthoptera (Gu *et al.*, 2010), but they have not been hitherto documented in any known fossil stick insects. Therefore, this is the first and the earliest fossil record with euplantula structures in Phasmatodea so far, providing unique and important morphological information about the early features of attachment pads in Phasmatodea and demonstrating the diversity of euplantula structures in Polyneoptera in the Mesozoic.

The earliest fossil records of Timematodea

Timematodea as sister to other extant Phasmatodea has been confirmed by many phylogenetic studies based on morphological characters or DNA sequence data (Tilgner *et al.*, 1999; Wheeler *et al.*, 2001; Tilgner, 2002; Bradler *et al.*, 2003; Whiting *et al.*, 2003; Wheeler *et al.*, 2004). However, there are no definite fossil groups assigned to Timematodea up to date, especially in the Mesozoic strata, although many winged groups of Phasmatodea have been reported from the Jurassic and the Cretaceous (Ren, 1997; Nel & Delfosse, 2011; Shang *et al.*, 2011; Wang *et al.*, 2014). To date, *T. prolongates* gen. et sp. nov. and *G. lirata* gen. et sp. nov. are the earliest fossil records of Timematodea, dating the suborder back to the mid-Cretaceous and providing transitional morphological characters (area apicalis, the elongated prothorax and the absence of arolia) to enhance our understanding of the early evolution of the Timematodea.

The phylogenetic position of Timematodea based on the new findings

Tumefactipes gen. nov. and *Granosicorpes* gen. nov. differ from Orthoptera mainly based on the following features: head rectangular, prognathous; antennae projecting

forward; pronotum without lateral extension; metasternum fused with the abdominal sternum I; metatibia without paired, longitudinal rows of teeth or spines on the dorsal surface; stridulum and auditory tympanum absent, but area apicalis present on the tibiae. The area apicalis is considered to be a plesiomorphic character within Phasmatodea (Bradler, 1999, 2009) and present in *Timema* and some groups of Embioptera (Bradler, 2009; Bradler & Buckley, 2011), but does not exist in Orthoptera (Bradler, 2009). However, in some Euphasmatodea, for example, Pseudophasmatinae and *Bacillus*, the area apicalis is also present and might be a secondary condition (Bradler, 2009). In all stick insects and some stoneflies, the abdominal sternum I is fused with the metasternum, but in Orthoptera, they are separated (Tilgner, 2002; Bradler, 2009). The present fossils have similar prognathous heads with three groups of Prognathogryllus (*Leptogryllus*, *Prognathogryllus* and *Thaumatogryllus*) within Orthoptera. However, these new findings clearly differ from the latter based on these characters: stridulum and auditory tympanum absent, pronotum without lateral lobes, base of profemur square, metatibia without denticulations or serrations along dorsal margins and tarsal claws lack teeth (Gurney & Rentz, 1978).

Zompro (2004, 2005) supposed that Timematodea should be a sister group of webspinners rather than remaining stick insets based on the structure of egg, reduced ocelli and the male right cercus of two parts in Timematodea (Zompro, 2004, 2005), which is not recognized yet (Bradler, 2009). The new taxa share some characters with Embioptera, for example, ocelli absent and enlarged metafemur, but these features are also present in some groups of Euphasmatodea. The robust metafemora of the new findings are similar to jumping legs available within several groups of living stick insects, for example, the male of *Dryococelus*, *Eurycantha* and *Thaumatobactro* (Hennemann & Conle, 1997; Buckley *et al.*, 2009), and such legs are very common within Orthoptera. In addition, although some groups of Embioptera also have three-segmented tarsi, the trimerous condition of Embioptera is not homologous with that in Timematodea (Grimaldi & Engel, 2005). It is supposed that Timematodea might be monophyletic with Euphasmatodea rather than Embioptera.

Bradler *et al.* (2015) dated the splitting time between *Timema* and Euphasmatodea back to about 100 million years ago (Bradler *et al.*, 2015); the new finding suggests that Timematodea have been much more diverse in mid-Cretaceous than today. The elongated head and thoracic segments strikingly different in lengths occur in the new specimens, which are different from *Timema*, but similar to some groups of Euphasmatodea. Sensory areas on the prosternum or profurcasternum are considered to be an autapomorphy in Heteropterygidae (Bradler, 2009; Hennemann *et al.*, 2016), but also present in some Pseudophasmatidae (Conle *et al.*, 2014) and these two species of Timematodea described here. In addition, *Tumefactipes* gen. nov. and *Granosicorpes* gen. nov. have elongated prothorax and lack the structure of arolia, and both characters are unusual in Phasmatodea. However, these features are very common in many groups of Orthoptera and Mantodea. Pseudotrimeric tarsus is also similar to some taxa of Orthoptera. So we think Phasmatodea should have a closer relationship with Orthoptera rather than Embioptera. However, all these new specimens are female and we know little about the male from same stratum. Therefore, new and well-preserved fossils of timematods found in the future might help to address and clarify the natural phylogenetic relationships of Timematodea, Euphasmatodea, Orthoptera and Embioptera.

Conclusion

We documented the earliest fossil records of Timematodea, hitherto *Tumefactipes prolongates* gen. et sp. nov. and *Granosicorpes lirates* gen. et sp. nov., enhancing our understanding of the origin and early evolution of Timematodea and providing important morphological information about the relationships of Timematodea, Euphasmatodea, Orthoptera and Embioptera. These fossil stick insects with tarsal pad structures reveal diverse tarsal pads of Polyneoptera in the Mesozoic. The expanded euplantulae of *T. prolongates* gen. et sp. nov. were probably used to increase friction for the stick insects to climb more firmly on various surfaces.

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Disclosure

The authors declare that they have no competing interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Photographs A–D. The representative of extant Phaneropterinae of Orthoptera from Nanling, Guangdong, China. A, The habitus of entire specimen. B, Right mesotarsus in dorsal view. C, The lateral view of right metatarsus, showing the euplantulae. D, The ventral view of left mesotarsus, showing the euplantulae. Photographs E–I. The representative of extant Acromantinae of Mantodea from South Kunshan, Guangdong, China. E, The habitus of entire specimen. F, The dorsal view of left mesotarsus, showing the euplantulae. G, The enlargement of the tarsomere IV in (F). H, The ventral view of right protarsus, showing the euplantulae. I, The enlargement of the tarsomere IV in (H). Abbreviations are as follows: CP, claw pad; E1p and E1d, proximal and distal euplantulae of tarsomere 1; E1-E4, euplantulae of tarsomeres I–IV; Ta1–Ta5, tarsomeres I–V; Un, claw. Scale bars represent 4 mm (A, E), 0.6 mm (B, C), 0.4 mm (D), 0.5 mm (F), 0.1 mm (G, I), 1 mm (H).