

Phylogeny of Stephanidae (Hymenoptera: Apocrita) with a new genus from Upper Cretaceous Myanmar amber

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Abstract. A new wasp species, *Lagenostephanus lii* Li, Rasnitsyn, Shih and Ren **gen. et sp.n.**, is assigned to Stephaninae (Hymenoptera: Stephanidae). The specimen is described from the lowermost Upper Cretaceous Myanmar (Burmese) amber. This is the second stephanid wasp described from Myanmar amber after *Kronostephanus zigrasi* Engel & Grimaldi, representing the earliest fossil occurrences of Stephanidae. Based on morphological characters of all extant and extinct genera, a phylogenetic analysis of Stephanidae was conducted. The results indicate that Schlettereriinae and Stephaninae are monophyletic, whereas *Electrostephanus* Brues, as the only genus within Electrostephaninae, belongs to Stephaninae. Therefore, we propose a synonymy of Electrostephaninae with Stephaninae. Based on the fossil evidence, we infer that the family of Stephanidae was significantly diverse in the Late Cretaceous and that they originated during the Early Cretaceous or late Jurassic. A key to genera of Stephanidae is provided.

This published work has been registered in ZooBank, <http://zoobank.org/urn:lsid:zoobank.org:pub:C082C245-8FD3-42B0-880F-94333286500B>.

Introduction

Stephanidae are a relatively small family of parasitoid wasps that are considered in recent studies as the sister group to all other extant Apocrita (Sharkey *et al.*, 2012). This interesting lineage possesses peculiar characters that distinguish Stephanidae from all other families of extant and extinct Hymenoptera. Examples of the distinctiveness of Stephanidae include a distinct tuberculate crown that occurs on the head capsule, a hind femur usually swollen with two or three large, ventral tooth-like processes and several lesser denticles, a propodeum with dorsal profile that is continuously rectilinear throughout and the metasomal base nearly comes into contact with the metacoxa (Rasnitsyn & Zhang, 2010; Hong *et al.*, 2011).

Stephanid wasps are morphologically unusual and encountered rarely. The clade comprises ten extant genera with nearly 350 species (van Achterberg & Yang, 2004; Aguiar, 2004, 2006; Aguiar & Jennings, 2005; van Achterberg & Quicke, 2006; Aguiar *et al.*, 2010; Hong *et al.*, 2010; Hong & Xu, 2011). The geographical distribution of Stephanidae principally is in subtropical and tropical forests worldwide (van Achterberg,

2002; Aguiar, 2004; Hong *et al.*, 2011). Aguiar (2004) summarized the geographical distribution of extant species in Stephanidae as shown in Table 1. In contrast to their modern distribution, however, stephanids have a poor fossil record.

The fossil record of Stephanidae consists of six known genera. Three of these genera are of amber provenance, and consist of *Archaeostephanus* Engel & Grimaldi, from the Late Cretaceous of New Jersey, U.S.A.; *Kronostephanus* Engel & Grimaldi and *Lagenostephanus* **gen.n.** from the Late Cretaceous of Myanmar (Burma) (Engel & Grimaldi, 2004; Engel *et al.*, 2013, this study). The three genera of *Protostephanus* Cockerell, *Electrostephanus* Brues and *Denaestephanus* Engel & Grimaldi originate from Eocene deposits of Colorado, U.S.A., and the Baltic Region of northern Europe (Cockerell, 1906; Brues, 1933; Engel & Grimaldi, 2004; Engel, 2005; Engel & Ortega-Blanco, 2008). (See Table 2 for additional details.) The amber fossil species are widely distributed, whereas the only known compression fossil comes solely from Colorado, U.S.A. A combination of the phylogenetic relationships and data on the distribution of fossil and extant genera resulted in a preliminary assessment of the early palaeobiological history of Stephanidae, which elucidated the relationships among genera of this interesting group of insects.

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Table 1. Zoogeographical regions of extant genera within Stephanidae.

Genera	Zoogeographical regions
<i>Afromegischus</i> Achterberg, 2002 <i>Foenatopus</i> Smith, 1860	Nearctic; Palaearctic (including China and Japan); Afrotropical Neotropics (including the Caribbean and the whole of Mexico); Palaearctic (including China and Japan); Afrotropical; Oriental (including India and Myanmar); Australasian and Oceanian (inclusive of New Guinea and outlying islands to the east) Neotropics (including the Caribbean and the whole of Mexico)
<i>Hemistephanus</i> Enderlein, 1906 <i>Madagafoenus</i> Benoit, 1951 <i>Megischus</i> Brullé, 1846	Afrotropical Neotropics (including the Caribbean and the whole of Mexico); Palaearctic (including China and Japan); Afrotropical; Oriental (including India and Myanmar); Australasian and Oceanian (inclusive of New Guinea and outlying islands to the east)
<i>Parastephanellus</i> Enderlein, 1906	Palaearctic (including China and Japan); Oriental (including India and Myanmar); Australasian and Oceanian (inclusive of New Guinea and outlying islands to the east)
<i>Profoenatopus</i> Achterberg, 2002 <i>Pseudomegischus</i> Achterberg, 2002 <i>Schlettererius</i> Ashmead, 1900	Afrotropical Oriental (including India and Myanmar) Nearctic; Palaearctic (including China and Japan); Australasian and Oceanian (inclusive of New Guinea and outlying islands to the east)
<i>Stephanus</i> Jurine, 1800	Palaearctic (including China and Japan); Oriental (including India and Myanmar)

Although the biology of most stephanids still remains unknown, it appears that Stephanidae may have a natural history very similar to Orussidae based on observations of their biology, and this is apparently confirmed by their position in apocritan phylogeny (Sharkey *et al.*, 2012). As far as can be inferred from the published literature, stephanids are ectoparasitoids of wood-boring insect larvae. Stephanid hosts include larval Cerambycidae (Townes, 1949; Blüthgen, 1953; Taylor, 1967; Pagliano & Scaramozzino, 1990; Visitpanich, 1994; Völlger, 1994) and Buprestidae (Carlson, 1979; Braza, 1989) in Coleoptera, as well as larval Siricidae (Kirk, 1975) in Hymenoptera. One important anatomical trait of Stephanidae is the possible role of the subgenual organ in detecting vibrational sounds to locate suitable hosts within wood substrates (Vilhelmsen *et al.*, 2008). The subgenual organ is a chordotonal organ usually present on the legs of winged insects that employ echolocation or vibrational sounding to assist in detection of wood-boring larval hosts deeply concealed within hard substrates (Debaisieux, 1935, 1938; Yack, 2004).

In this report, we describe the second stephanid wasp from mid-Cretaceous Myanmar amber and present a phylogeny of Stephanidae based on the scoring of morphological features from all extinct and extant genera. This deposit previously has yielded well-preserved hymenopteran fossils (Li *et al.*, 2015a; Engel *et al.*, 2016). Although the age of Myanmar amber previously has been controversial (Zherikhin & Ross, 2000; Grimaldi *et al.*, 2002; Ross *et al.*, 2010), a recent radioisotopic date has been established at 98.79 ± 0.62 Ma (Shi *et al.*, 2012), equivalent to earliest Cenomanian and approximately 1 Ma within the boundary between the Early and Late Cretaceous epochs (Walker *et al.*, 2012).

Material and methods

Examined taxa and terminology

The amber deposits occur about 100 km south-west of Tanai Village, in the Hukawng Valley of Kachin Province, in northern Myanmar (Burma). The type material described in this paper is

deposited in the Key Lab of Insect Evolution and Environmental Changes, at the College of Life Sciences, Capital Normal University, in Beijing, China (CNUB; Ren Dong, Curator). All specimens were examined and photographed with a Leica DFC500 digital camera attached to a Leica MZ 16.5 dissecting microscope (Leica, Wetzlar, Germany). The figures were drawn using CorelDraw 12.0 and Adobe Photoshop Creative Suite, v5. The wing venation terminology used herein follows that of Engel & Grimaldi (2004) and Engel *et al.* (2013).

Phylogenetic analyses

The cladogram was rooted using *Praeratavites perspicuous* Rasnitsyn & Zhang (Karatavitidae), *Praeproapocritus flexus* Li, Shih & Ren (Ephialtitidae) and *Acephialtitia colossa* Li, Shih, Rasnitsyn & Ren (Ephialtitidae) as outgroups. The outgroup selection was based on a previously published phylogeny of Hymenoptera (Basibuyuk *et al.*, 2000; Sharkey *et al.*, 2012; Li *et al.*, 2015b). The ingroup comprises 16 stephanid genera described to date, including the new genus reported herein. Thirty-four characters from the adult morphology were identified and scored, listed in Table S1. A complete taxa and the character state matrix is presented in Table S2. The phylogenetic analysis was carried out in *NONA* (Goloboff, 1999) in conjunction with *WinClada* (Nixon, 2002). Tree searches were performed using an heuristic search method consisting of the following options: set to hold 10 000 trees, T1000 replications, 100 starting tree replications, and a multiple TBR + TBR search strategy. Character coding was established by Nexus Data Editor 0.5.0 (Roderic, 2001), with all characters unordered and of equal weight.

Results

Phylogeny analyses

Analyses of the morphological data matrix yielded four most-parsimonious trees (Figure S1). The resulting trees have the following characteristics: tree length (L) = 67, consistency index (CI) = 0.55 and retention index (RI) = 0.70. The consensus tree is shown in Fig. 1.

Table 2. Known extinct species of Stephanidae.

Species	Locality	Horizon	References
<i>Archaeostephanus corae</i> Engel & Grimaldi, 2004	New Jersey	Late Cretaceous	Engel & Grimaldi (2004)
<i>Kronostephanus zigrasi</i> Engel & Grimaldi, 2013	Myanmar	Late Cretaceous	Engel <i>et al.</i> (2013)
<i>Lagenstephanus lii</i> gen. et sp.n.	Myanmar	Late Cretaceous	This study
<i>Protostephanus ashmeadi</i> Cockerell, 1906	Florissant, Colorado	Late Eocene	Cockerell (1906)
<i>Denaeostephanus sculcatus</i> (Aguar & Janzen, 1999)	Baltic	Middle Eocene	Engel & Grimaldi (2004)
<i>Denaeostephanus petiolatus</i> (Brues, 1933)	Baltic	Middle Eocene	Engel & Grimaldi (2004)
<i>Denaeostephanus tridentatus</i> (Brues, 1933)	Baltic	Middle Eocene	Engel & Grimaldi (2004)
<i>Electrostephanus brevicornis</i> Brues, 1933	Baltic	Middle Eocene	Brues (1933)
<i>Electrostephanus neovenatus</i> Aguair & Janzen, 1999	Baltic	Middle Eocene	Aguair & Janzen (1999)
<i>Electrostephanus janzeni</i> Engel, 2005	Baltic	Middle Eocene	Engel (2005)

The family Stephanidae was confirmed as a monophyletic group, which includes 16 genera, and is supported by six apomorphic characters: (i) venation of the forewing that is slightly to strongly reduced (character 2:state 2); (ii) forewing with 1r-rs crossvein present with respect to the 2r-rs crossvein lost and traceless (4:1); (iii) forewing with the 3r-m crossvein absent (7:1); (iv) forewing with 2m-cu crossvein entirely lost (8:1); (v) hind femur with dentition present (25:1 or 2); (vi) a pygidial protuberance in the female (34:1). Within Stephanidae, Schlettereriinae (comprising three genera) was recovered as a monophyletic clade. The monophyly is supported by one unambiguous character: hind coxae having dorsal teeth (23:1). Stephaninae consists of nine extant genera and the three Eocene genera *Protostephanus*, *Denarostephanus* and *Electrostephanus*. Stephaninae is the second largest clade that includes one Mesozoic genus *Lagenostephanus* **gen.n.** in a relatively derived position and 12 other fossil and modern genera. This clade is supported by five characters: (i) crossvein cu-a of the hindwing absent (16:1); (ii) hind tibia distinctly narrowed at base (26:1); (iii) first metasomal segment distinctly longer than second one (30:1); (iv) depression along the mesial side of the hind tibia absent or weak (27:1); and (v) the first metasomal sternite is joined to the first tergite and retains some mobility (31:0). In addition, *Electrostephanus* is the only genus within Electrostephaninae, and based on these results belongs to Stephaninae. Notably, Electrostephaninae is not definitively separated from the other two subfamilies, and is erected only on the character of T1 and S1 having a not-fused condition. Hence, we propose that Electrostephaninae is synonymized with Stephaninae by abandoning Electrostephaninae as a distinct subfamily.

Taxonomy

Order Hymenoptera Linnaeus, 1785.

Superfamily Stephanoidea Leach, 1815.

Family Stephanidae Leach, 1815.

Subfamily Stephaninae Leach, 1815.

Genus *Lagenostephanus* **gen.n.**

<http://zoobank.org/urn:lsid:zoobank.org:act:E587967A-FD2D-4943-9845-70827B00887E>

Type species. Lagenostephanus lii **sp.n.**

Etymology. The new generic name is a combination of the Latin *lagena*, derived from the Greek *lagenos*, meaning a large jar with a narrow neck, similar to an amphora, referring to the narrowed tubular anterior and expanded posterior shapes of the mesosoma and metasoma of this genus. The *lagena* prefix is followed by the type genus of the family, *Stephanus*. The gender is masculine.

Diagnosis. Head elliptical in lateral view, with fine filiform antenna with at least 35 antennomeres. Mesosoma slender, pronotum prolonged as a thin neck. Forewing with 1-Rs and r-rs both straight and very short, 1-Rs as long as r-rs; 1-M weakly arched, slightly longer than 1 m-cu, c.5× as long as 1-Rs; 1cu-a postfurcal, nearly as long as 1 m-cu; free M far disconnected with Rs + M, free Rs, M and 2Cu₁ tubular. Fore- and mid-legs short and slender, hind legs long and swollen; hind coxa nearly as long as hind femur, hind femur greatly swollen, ventrally with two large tooth-like processes, each followed by a smaller one; hind tibia strongly petiolate, slender, gradually swollen from base to apex, hind tibia with two spurs. Metasomal T1 and S1 fused laterally, forming a petiole, the petiole length nearly as long as the second metasomal segment; from the second metasomal segment onward, the tergum and sternum not fused. Ovipositor length nearly as long as the metasoma.

Comments. The new genus can be attributed to Stephanidae by the following diagnostic characters: body long and slender, head globular with a few tooth-like spines around median ocellus; antenna with at least 35 flagellomeres; pronotum neck-like; metafemur enlarged bearing dentiform processes along ventral margin; ovipositor long (Goulet & Huber, 1993).

Lagenostephanus lii **sp.n.**

<http://zoobank.org/urn:lsid:zoobank.org:act:401419CA-FCD7-4B87-AC11-9A3408D73FB6>

Figs 2–4

Etymology. The specific name is dedicated to Mr. Jun Li for his generous donation of amber specimens for scientific research.

Diagnosis. See *Diagnosis* for the genus above.

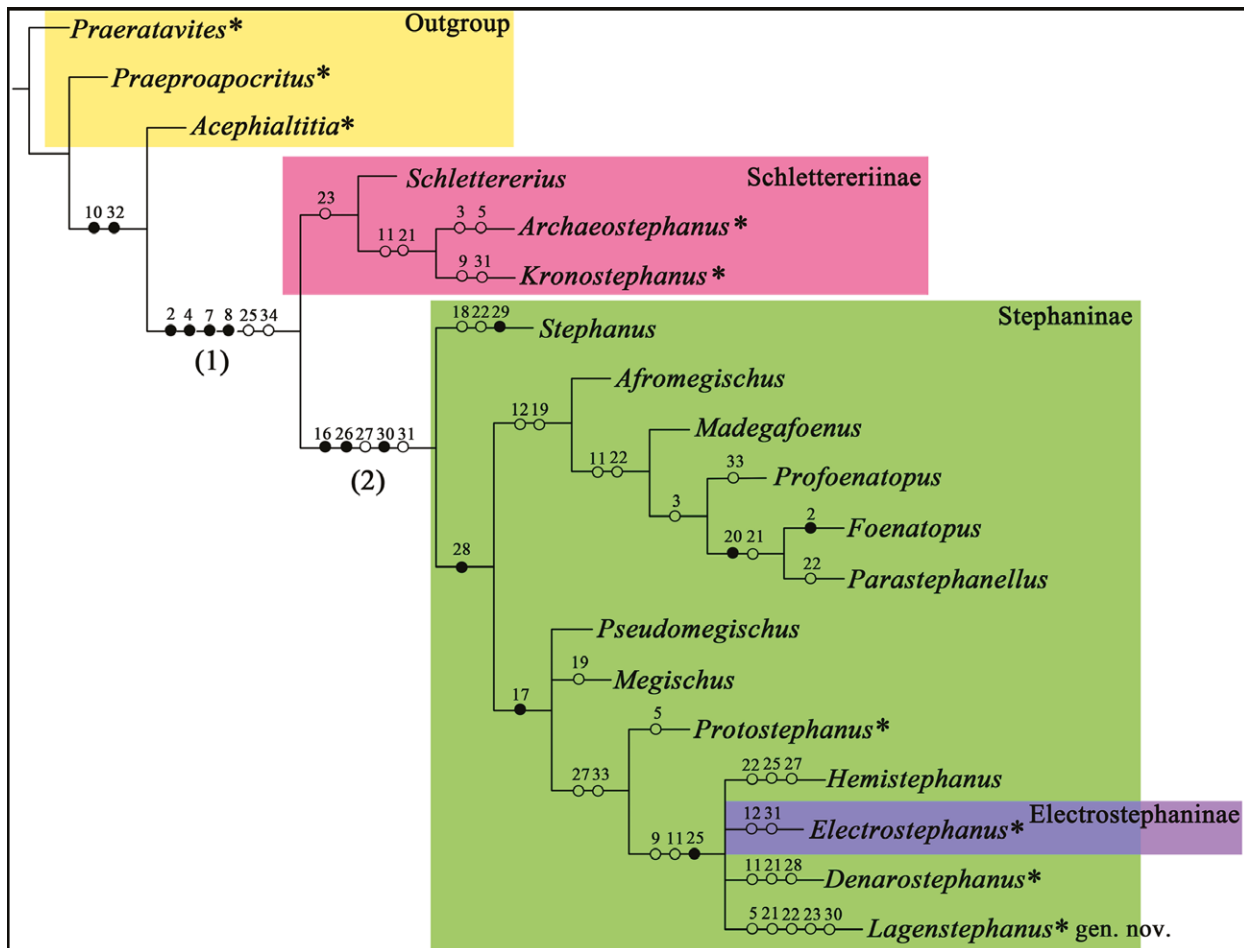


Fig. 1. The strict consensus tree of phylogenetic relationships for genera of Stephanidae, with tree length (L) = 70, consistency index (CI) = 0.52 and retention index (RI) = 0.67. Solid bullets (●) indicate nonhomoplastic characters; open bullets (○) indicate homoplastic characters; asterisks (*) denote extinct taxa. [Colour figure can be viewed at wileyonlinelibrary.com].

Description. Body length 4.6 mm (excluding ovipositor and antenna). Forewing length (estimated) 2.6 mm; hind wing length 1.5 mm. Petiole length 0.6 mm. Integument dark reddish brown, nearly black in most body parts (e.g. mesosoma and metasoma) as preserved; wing hyaline with sparse setae, veins black. Head oval in lateral view with compound eyes reniform; face with median-anterior tubercles high on head capsule, nearly at the peak of compound eyes. Antennae elongate, filiform, with at least 35 flagellomeres, scape $>2\times$ as long as radicle and pedicel axial length, flagellum distinctly narrower than pedicel. Chewing mouthparts visible, maxillary palp with three segments preserved. Mesosoma slender in lateral view; pronotum prolonged as a thin neck. Fore and mid legs very slim, hind leg longer and swollen, especially femur and distal part of tibia. Hind coxa 0.6 mm long, closer to and larger than fore and mid coxa; hind trochanter and trochantellus present; hind femur greatly expanded, with pronounced ventral dentition, two longer teeth slender and elongated, each followed by shorter denticles; hind tibia strongly petiolate with two, sharp metatibial spurs, narrowed basally and abruptly widened for apical 2/3 of length,

distinctly longer than femur. Hind tarsus with five tarsomeres, first one longer than other combined tarsomeres. Forewing with R very thin, but Sc thick, both veins extended to the wing base; pterostigma long and wide, distinctly wider than the length of vein r-rs; vein 1-Rs very short, about 1/5 length of vein 1-M; 1cu-a postfurcal vein, nearly as long as vein 1m-cu; vein r-rs very short, nearly as long as 1-Rs; vein r-rs issuing from pterostigma at its mid-length; free vein M tubular, not in contact with 2-Rs or Rs + M; $2Cu_a$ and $2Cu_b$ present and tubular; free vein M + Cu and A extended to the wing base (intersected as preserved). Hindwing with only outline discernible, without veins preserved. Metasoma with eight segments visible, attached to a relatively high position to the propodeum, in contact basally with foramina of hind coxae. First tergum and sternum fused laterally, forming a petiole nearly as long as second metasomal segment. Second tergum and sternum not fused; third metasomal segment and following metasomal segments distinctly shorter than petiole or second metasomal segment. Ovipositor sheath 2.2 mm long slightly shorter than metasoma, distinctly surpassing abdominal apex.

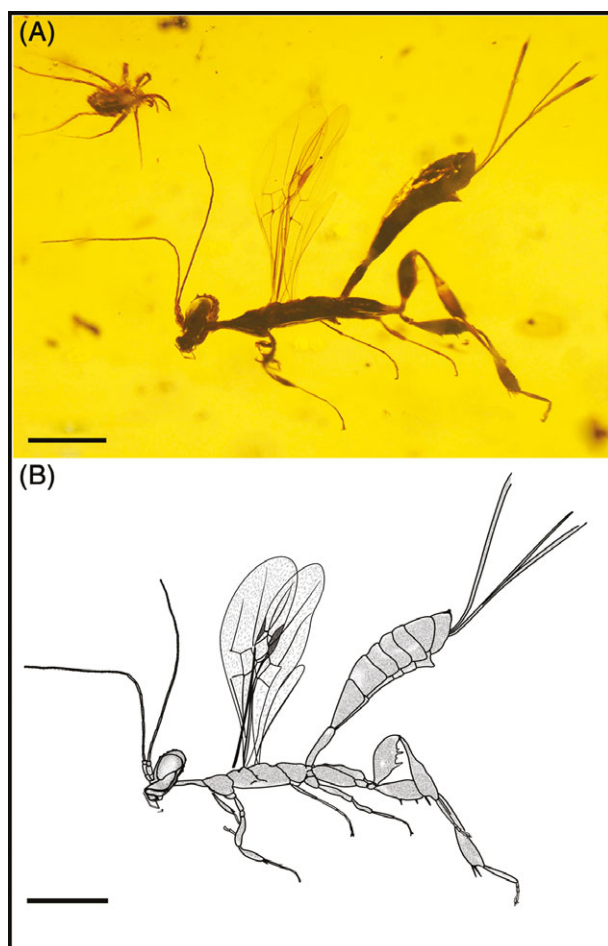


Fig. 2. *Lagenostephanus lii* gen. et sp.n. Holotype CNU-HYM-MA-2014010. (A) Photo of specimen. (B) Line drawing of habitus. Scale bars = 1 mm. [Colour figure can be viewed at wileyonlinelibrary.com].

Type material. Holotype, CNU-HYM-MA-2014-010, female, well preserved.

Locality and horizon. Hukawng Village, Kachin Province, Northern Myanmar; lowest Upper Cretaceous, equivalent to early Cenomanian.

1. Metacoxa with dorsal tooth-like process, hindwing vein with cu-a present.....2
 - Metacoxa without dorsal tooth, hindwing with cu-a absent.....4
2. Forewing with vein $2Cu_b$ absent or nebulous.....*Kronostephanus* Engel & Grimaldi
 - Forewing with vein $2Cu_b$ present and tubular.....3
3. Forewing with vein $Rs + M_b$ absent.....*Archaeostephanus* Engel & Grimaldi
 - Forewing with vein $Rs + M_b$ present.....*Schlettererius* Ashmead
4. First metasomal tergum and sternum distinctly separated laterally, female metatarsus

- 5-segmented.....*Electrostephanus* Brues
 - First metasomal tergum and sternum fused laterally, forming a completely tubular petiole, female metatarsus 3- or 5-segmented.....5
- 5. Hind tarsus of female 5-segmented.....6
 - Hind tarsus of female 3-segmented.....9
- 6. Forewing with vein $2Cu_b$ absent or nebulous.....*Denaeostephanus* Engel & Grimaldi
 - Forewing with vein $2Cu_b$ present and tubular.....7
- 7. Forewing with vein r-rs very long, about 2 times as long as 1Rs, A preserved beyond 1cu-a.....*Stephanus* Jurine
 - Forewing with vein r-rs short, nearly as long as 1Rs.....8
- 8. Forewing with vein $Rs + M_b$ absent, free M not in contact with $Rs + M$... *Lagenostephanus* gen.n.
 - Forewing with vein $Rs + M_b$ present, free M in contact with $Rs + M$*Protostephanus* Cockerell
- 9. First subdiscal cell of forewing not enlarged.....10
 - First subdiscal cell of forewing much wider than first discal cell.....12
- 10. Ovipositor sheath without ivory band.....*Pseudomegischus* Achterberg
 - Ovipositor sheath with ivory subapical band.....11
- 11. Hind tibia with triangular depression weakly developed (often absent), vein 2-1A reduced, setae on vein $M + Cu_1$ reduced.....*Hemistephanus* Enderlein
 - Hind tibia distinctly transversely depressed, vein 2-1A complete, setae on vein $M + Cu_1$ absent.....*Megischus* Brullé
- 12. Neck separated from middle pronotum, pronotum with medial concavity.....13
 - Neck connected with pronotum, pronotum without medial concavity.....14
- 13. Hind tibia not inflated, distinctly narrower than hind femur.....*Afromegischus* Achterberg
 - Hind tibia strongly inflated, nearly as wide as hind femur.....*Madegafoenus* Benoit
- 14. Neck simple and shortened, forewing with 2-1A reduced.....*Parastephanellus* Enderlein
 - Neck emarginated, with upcurved flanges anterolaterally.....15
- 15. Forewing venation strongly reduced, $Rs + M$ and 1 m-cu absent.....*Foenatopus* Smith
 - Forewing venation complete, $Rs + M$ and 1 m-cu present.....*Profoenatopus* Achterberg

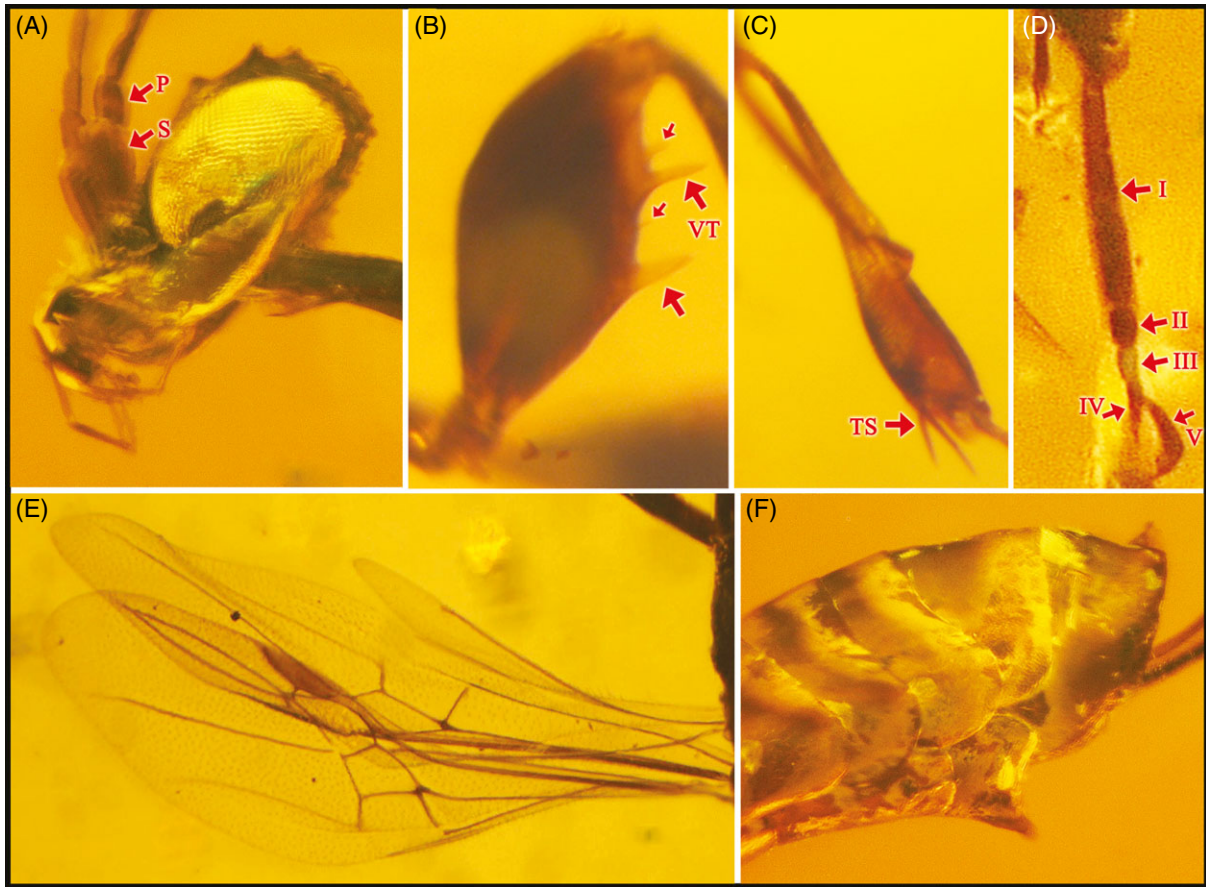


Fig. 3. *Lagenostephanus lii* gen. et sp.n. Holotype CNU-HYM-MA-2014010. Photographs of (A) Head in lateral view, (B) hind femur, (C) hind tibia, (D) hind tarsus, (E) wings and (F) part of metasoma in lateral view. S, scape; P, pedicel; VT, ventral tooth; TS, tibial spurs; I–V, five segments of tarsus. Scale bars = 0.1 mm. [Colour figure can be viewed at wileyonlinelibrary.com].

Discussion

Phylogenetic relationships within Stephanidae

Prior to this study, *Kronostephanus zigrasi* Engel & Grimaldi, from lowermost Upper Cretaceous Myanmar amber, was the earliest fossil record of Schlettereriinae within Stephanidae (Engel *et al.*, 2013). *Lagenostephanus lii* gen. et sp.n., the second discovered Myanmar amber species, provides new structural features which are distinctly different from *Kronostephanus zigrasi*. In particular, *Lagenostephanus lii* has T1 and S1 fused and the hind tibia is distinctly petiolate, both being important diagnostic characters for Stephaninae. However, these two species representing the earliest known fossil records of Stephaninae, have complete wing venation, with the veins and crossveins C, R, Rs, M, r-rs, Rs + M, M + Cu, A (at least basal of 1cu-a) and 1 m-cu present. The 1cu-a crossvein is postfurcal, and the M vein is free and not in contact with vein Rs + M. The hind tarsus of the female is five-segmented. Even though *K. zigrasi* and *L. lii* display characters of different subfamilies, they still demonstrate a combination of similar, plesiomorphic characters, such as the forewing with a postfurcal 1cu-a crossvein, the hind tarsus

of the female being five-segmented, and the presence of apomorphic characters that include fusion of T1 and S1, and hind tibia distinctly petiolate. The aforementioned character states suggest that the differentiation of these two stephanid subfamilies occurred significantly before the Early to Late Cretaceous boundary interval, before 100 Ma.

Based on the phylogenetic results presented in Fig. 1, Schlettereriinae is a monophyletic group supported by hind coxa with dorsal teeth (23:1). Engel (2005) stated that the separated first metasomal tergum and sternum certainly excludes *A. corae* from the Stephaninae. Only those taxa with weakly petiolate hind tibia are more similar to *Schlettererius* than to other stephanids, which suggests placement outside of Stephaninae. As *A. corae* does not have preserved hindwings or tarsi, it is prudent to retain it in Schlettereriinae, based on available morphological characters. Our present phylogeny results support Engel's (2005) attribution of *Archaeostephanus* to Schlettereriinae.

Alternatively, *Electrostephanus*, as the only genus represented in Electrostephaninae, may belong to Stephaninae, an assignment inconsistent with the proposition of Engel (2005) that Electrostephaninae have an intermediary phylogenetic position between Schlettereriinae and Stephaninae. Our results

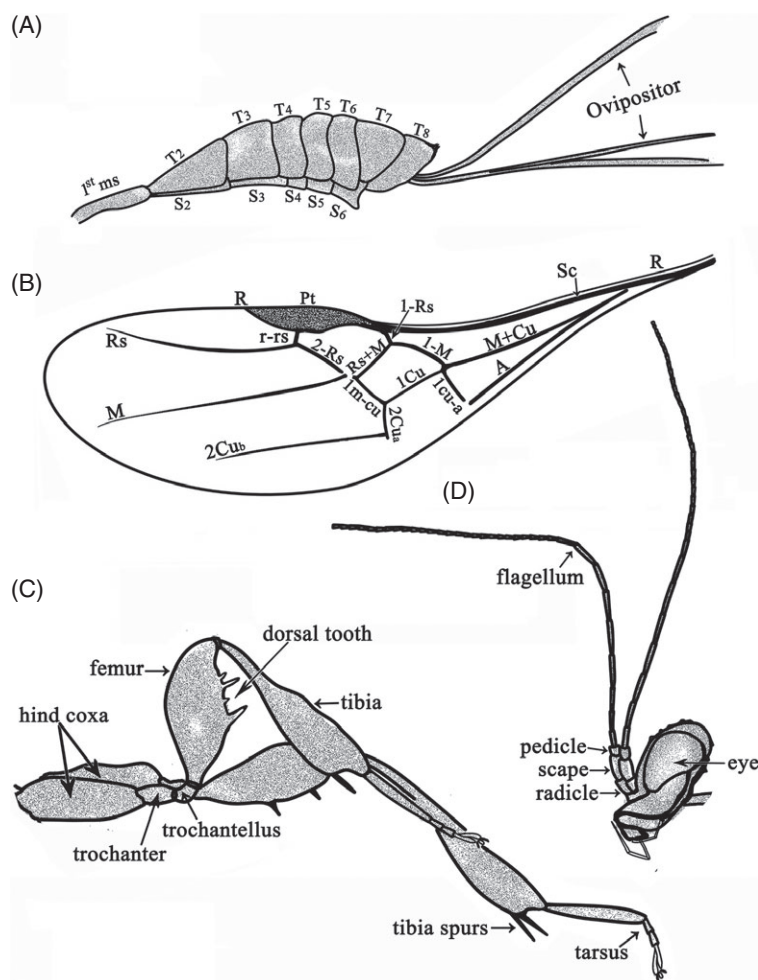


Fig. 4. *Lagenostephanus lii* gen. et sp.n. Holotype CNU-HYM-MA-2014010. Line drawings of (A) metasoma, (B) forewing, (C) hind legs and (D) head. Scale bars = 0.5 mm.

suggest that Electrostephaninae renders Stephaninae paraphyletic. Accordingly, we indicate that the cladogram (Fig. 1) depicts a reversal in the character of T1 and S1 as not fused in *Electrostephanus*, versus T1 and S1 fused in Stephaninae. Consequently, this reassignment does not contradict treating the genus as a member of Stephaninae. Because Electrostephaninae is deeply nested within Stephaninae, we propose the synonymy of Electrostephaninae with Stephaninae by abandoning Electrostephaninae as a distinct subfamily.

Clues on origin and migration of Stephanidae

The phylogenetic relationships of genera within Stephanidae are presented in a geo-chronological context consistent with their localities and palaeogeographical distribution plotted along a strict consensus tree (Fig. 5A). Because the two earliest amber stephanids are represented by the basal *Kronostephanus* in the subfamily Schlettereriinae and the more derived *Lagenostephanus* gen.n. in the subfamily Stephaninae, we suggest that Stephanidae were likely quite diverse during the Late Cretaceous. The earliest Late Cretaceous occurrences and diversification, imply that the origin of Stephanidae should be

placed earlier – perhaps significantly so – than the earliest Late Cretaceous.

It is notable that in the more basal subfamily Schlettereriinae, the amber genus *Kronostephanus* belongs to Eurasian-distributed clade, and the other amber genus *Archaeostephanus* from New Jersey, U.S.A., occurs in North America during the Late Cretaceous. The other derived amber genus, *Lagenostephanus* gen.n., also is from Myanmar. Two amber genera, *Electrostephanus* and *Denaestephanus*, from the Baltic Region occurs in the Eurasian Eocene, whereas a compression fossil genus of *Protostephanus* from Florissant, Colorado, U.S.A., has a North American Eocene provenance.

As shown in Fig. 5B, extant stephanid genera are widely distributed. An extant basal genus, *Schlettererius*, is distributed in the Palaearctic and Nearctic regions. In addition, *Stephanus* (Stephaninae) is distributed mainly in Eurasia, inhabiting the Oriental and Palaearctic regions. As northeastern Asia and northwestern North America became increasingly connected during the middle to Late Cretaceous from 80 to 100 Ma (Sanmartin *et al.*, 2001; Shih *et al.*, 2009, 2010), a pathway might have been present for some stephanid taxa to migrate from the Palaearctic to the Nearctic regions. Moreover, seven

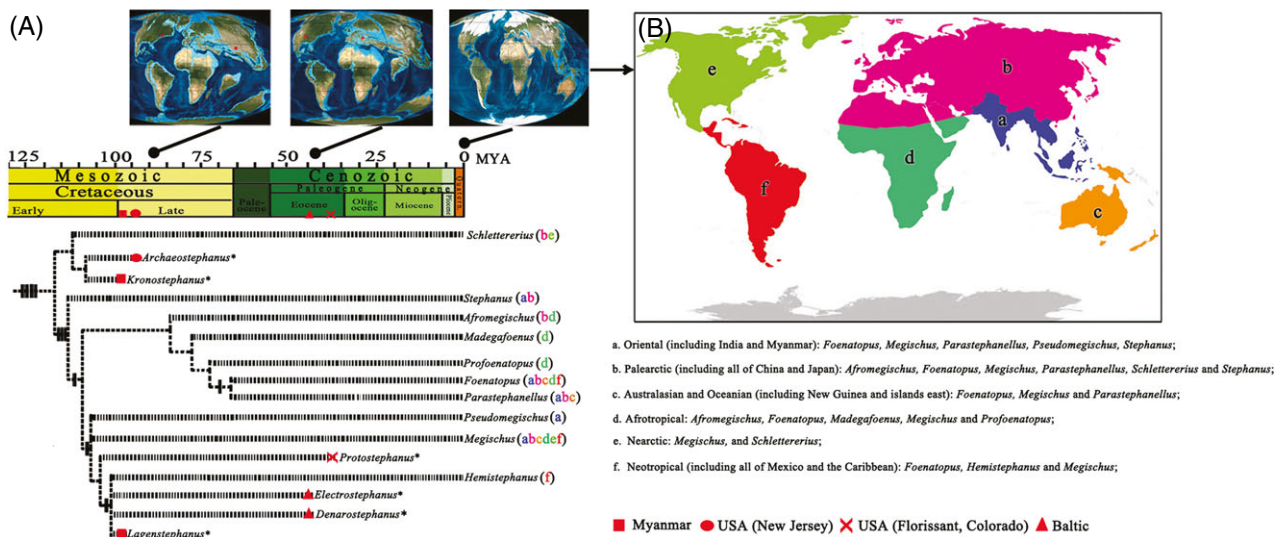


Fig. 5. (A) Time-calibrated phylogenetic relationships of Stephanidae genera, presented as a strict-consensus tree. Localities and distribution patterns are indicated. Asterisks (*) indicate extinct genera. (B) Distribution map of extant genera in Stephanidae. The geographical distribution of extant species is based on Aguiar (2004). [Colour figure can be viewed at wileyonlinelibrary.com].

genera: *Afromegischus*, *Foenatopus*, *Megischus*, *Pseudomegischus*, *Parastephanellus*, *Stephanus* and *Schlettererius* are distributed in Eurasia, which share three of the five genera of *Afromegischus*, *Foenatopus*, *Madagafoenus*, *Megischus* and *Profoenatopus* that are distributed in the Afrotropical Region. Four other modern biogeographical regions each harbour fewer than four extant genera. These deep-time and modern biogeographical data indicate clearly that historically Stephanidae were most diverse in Eurasia but have been biogeographically widely distributed during the past 100 Ma.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12202

Figure S1. The four most-parsimonious trees, with a tree length (L) = 67, a consistency index (CI) of 0.55 and a retention index (RI) of 0.70.

Table S1. Morphological characters coded in the phylogenetic analysis.

Table S2. Morphological data matrix including three out-group taxa and 16 ingroup taxa.

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References

- van Achterberg, C. (2002) A revision of the Old World species of *Megischus* Brullé, *Stephanus* Jurine and *Pseudomegischus* gen. nov., with a key to the genera of the family Stephanidae (Hymenoptera: Stephanioidea). *Zoologische Verhandelingen Leiden*, **339**, 1–206.
- van Achterberg, C. & Quicke, D.L.J. (2006) Taxonomic notes on Old World Stephanidae (Hymenoptera): description of *Parastephanellus matsumotoi* sp. n. from Japan, redescription of *Commatopus xanthocephalus* (Cameron) and keys to the genera *Profoenatopus* van Achterberg and *Megischus* Brullé. *Tijdschrift voor Entomologie*, **149**, 215–225.
- van Achterberg, C. & Yang, Z.Q. (2004) New species of the genera *Megischus* Brullé and *Stephanus* Jurine from China (Hymenoptera: Stephanioidea: Stephanidae), with a key to world species of the genus *Stephanus*. *Zoologische Mededelingen Leiden*, **78**, 101–117.
- Aguiar, A.P. (2004) World catalog of the Stephanidae (Hymenoptera: Stephanioidea). *Zootaxa*, **753**, 1–120.
- Aguiar, A.P. (2006) The Stephanidae (Hymenoptera) of Mexico, with description of six new species and key to western *Foenatopus* Smith. *Zootaxa*, **1186**, 1–56.
- Aguiar, A.P. & Janzen, J.W. (1999) An overview of fossil Stephanidae (Hymenoptera), with description of two new taxa from Baltic amber,

- and key to species of *Electrostephanus* Brues. *Entomologica Scandinavica*, **30**, 443–452.
- Aguiar, A.P. & Jennings, J.T. (2005) First record of Stephanidae (Hymenoptera) from New Caledonia, with descriptions of four new species of *Parastephanellus* Enderlein. *Zootaxa*, **1001**, 1–16.
- Aguiar, A.P., Jennings, J.T. & Turrisi, G.F. (2010) Three new Middle-Eastern species of *Foenatopus* Smith (Hymenoptera: Stephanidae) with a new host record and key to species with two spots on the metasoma. *Zootaxa*, **2714**, 40–58.
- Basibuyuk, H.H., Rasnitsyn, A.P., Fitton, M.G. & Quicke, D.L.J. (2000) An archaic new genus of Evaniidae (Insecta: Hymenoptera) and implications for the biology of ancestral evanioids. *Bulletin of the Natural History Museum London (Geology)*, **56**, 53–58.
- Blüthgen, P. (1953) Zur biologie von *Stephanus serrator* F. (Hym. Stephanidae). *Zoologischer Anzeiger*, **150**, 229–234.
- Braza, R.D. (1989) Parasitoids of immature stages of *Agrilus sexsignatus* (Fisher) (Coleoptera: Buprestidae) attacking *Eucalyptus deglupta* Blume in Surigao del Sur. *Philippine Entomologist*, **7**, 479–483.
- Brues, C.T. (1933) The parasitic Hymenoptera of the Baltic amber. Part 1. *Bernstein Forschungen*, **3**, 4–178.
- Carlson, R.W. (1979) Stephanidae. *Catalog of Hymenoptera in America North of Mexico* (ed. by K.V. Krombein, P.D. Hurd Jr., D.R. Smith and B.D. Burks), pp. 143–144, 740–741. Smithsonian Institution Press, Washington, DC.
- Cockerell, T.D.A. (1906) Fossil Hymenoptera from Florissant, Colorado. *Bulletin of the Museum of Comparative Zoology*, **50**, 33–58.
- Debaisieux, P. (1935) Organes scolopidiaux des pattes d'insectes I. Lepidoptères et Trichoptères. *Cellule*, **44**, 271–314.
- Debaisieux, P. (1938) Organes scolopidiaux des pattes d'insectes II. *Cellule*, **47**, 77–202.
- Engel, M.S. (2005) The crown wasp genus *Electrostephanus* (Hymenoptera: Stephanidae): discovery of the female and a new species. *Polskie Pismo Entomologiczne*, **74**, 317–332.
- Engel, M.S. & Grimaldi, D.A. (2004) The first Mesozoic stephanid wasp (Hymenoptera: Stephanidae). *Journal of Paleontology*, **78**, 1192–1197.
- Engel, M.S. & Ortega-Blanco, J. (2008) The fossil crown wasp *Electrostephanus petiolatus* Brues in Baltic Amber (Hymenoptera, Stephanidae) designation of a neotype, revised classification, and a key to amber Stephanidae. *Zookeys*, **4**, 55–64.
- Engel, M.S., Grimaldi, D.A. & Ortega-Blanco, J. (2013) A stephanid wasp in mid-Cretaceous Burmese amber (Hymenoptera: Stephanidae), with comments on the antiquity of the hymenopteran radiation. *Journal of the Kansas Entomological Society*, **86**, 244–252.
- Engel, M.S., Huang, D.Y., Alqarni, A.S. & Cai, C.Y. (2016) A remarkable evanioid wasp in mid-Cretaceous amber from northern Myanmar (Hymenoptera: Evanioidea). *Cretaceous Research*, **60**, 121–127.
- Goloboff, P. (1999) *NONA (No Name) Version 2*. Published by the Author, San Miguel de Tucumán. [WWW document]. URL <http://www.cladistics.org> [accessed on 11 April 2009].
- Goulet, H. & Huber, J.T. (1993) *Hymenoptera of the World: An Identification Guide to Families*. Centre for Land and Biological Resource Research, Ottawa.
- Grimaldi, D., Engel, M.S. & Nascimbene, P. (2002) Fossiliferous Cretaceous amber from Myanmar (Burma): its rediscovery, biotic diversity, and paleontological significance. *American Museum Novitates*, **3361**, 1–72.
- Hong, C.D. & Xu, Z.F. (2011) A newly recorded genus and species of Family Stephanidae (Hymenoptera, Stephanoidea) from China. *Entomotaxonomia*, **33**, 71–73.
- Hong, C.D., van Achterberg, C. & Xu, Z.F. (2010) A new species of *Megischus* Brullé (Hymenoptera, Stephanidae) from China, with a key to the Chinese species. *ZooKeys*, **69**, 59–64.
- Hong, C.D., van Achterberg, C. & Xu, Z.F. (2011) A revision of the Chinese Stephanidae (Hymenoptera, Stephanoidea). *Zookeys*, **110**, 1–108.
- Kirk, A.A. (1975) Siricid woodwasps and their associated parasitoids in the south-western United States (Hymenoptera: Siricidae). *The Pan-Pacific Entomologist*, **51**, 57–61.
- Li, L.F., Rasnitsyn, A.P., Shih, C.K. & Ren, D. (2015a) A new genus and species of Praeaulacidae (Hymenoptera: Evanioidea) from Upper Cretaceous Myanmar amber. *Cretaceous Research*, **55**, 19–24.
- Li, L.F., Shih, C.K., Rasnitsyn, A.P. & Ren, D. (2015b) New fossil ephialtids elucidating the origin and transformation of the propodeal-metasomal articulation in Apocrita (Hymenoptera). *BMC Evolutionary Biology*, **15**, 1–17.
- Nixon, K.C. (2002) *WinClada Version 1.00.08*. Published by the Author, Ithaca, New York., Renner and Weerasooriya, USA [WWW document]. URL <http://www.cladistics.com> [accessed on 11 April 2009].
- Pagliano, G. & Scaramozzino, P. (1990) Elenco dei generi di Hymenoptera del mondo. *Memorie della Società Entomologica Italiana*, **68**, 3–210.
- Rasnitsyn, A.P. & Zhang, H.C. (2010) Early evolution of Apocrita (Insecta, Hymenoptera) as indicated by new findings in the Middle Jurassic of Daohugou, Northeast China. *Acta Geologica Sinica (English Edition)*, **84**, 834–873.
- Roderic, D.M. (2001) *NEXUS Data, Version 0.5.0*. University of Glasgow, Glasgow.
- Ross, A., Mellish, C., York, P. & Crighton, B. (2010) Burmese amber. *Biodiversity of Fossils in Amber from the Major World Deposits* (ed. by D. Penney), pp. 208–235. Siri Scientific Press, Manchester.
- Sanmartin, I., Enghoff, H. & Ronquist, F. (2001) Patterns of animal dispersal, vicariance and diversification in the Holarctic. *Biological Journal of the Linnean Society*, **73**, 345–390.
- Sharkey, M.J., Carpenter, J.M., Vilhelmsen, L. et al. (2012) Phylogenetic relationships among superfamilies of Hymenoptera. *Cladistics*, **28**, 80–112.
- Shi, G.H., Grimaldi, D.A., Harlow, G.E. et al. (2012) Age constraint on Burmese amber based on U-Pb dating of zircons. *Cretaceous Research*, **37**, 155–163.
- Shih, C.K., Liu, C.X. & Ren, D. (2009) The earliest fossil record of peleciniid wasps (Insecta: Hymenoptera: Proctotrupoidea: Peleciniidae) from Inner Mongolia, China. *Annals of the Entomological Society of America*, **102**, 20–38.
- Shih, C.K., Feng, H., Liu, C.X., Zhao, Y.Y. & Ren, D. (2010) Morphology, phylogeny, evolution, and dispersal of peleciniid wasps (Hymenoptera: Peleciniidae) over 165 million years. *Annals of the Entomological Society of America*, **103**, 875–885.
- Taylor, K.L. (1967) Parasitism of *Sirex noctilio* F. by *Schlettererius cinctipes* (Cresson) (Hymenoptera: Stephanidae). *Journal of the Australian Entomological Society*, **6**, 13–19.
- Townes, H. (1949) The Nearctic species of the family Stephanidae (Hymenoptera). *Proceedings of the United States Natural Museum*, **99**, 361–370.
- Vilhelmsen, L., Turrisi, G.F. & Beutel, R.G. (2008) Distal leg morphology, subgenal organs and host detection in Stephanidae (Insecta, Hymenoptera). *Journal of Natural History*, **42**, 1649–1663.
- Visitanich, J. (1994) The parasitoid wasps of the Coffee Stem Borer, *Xylotrechus quadripes* Chevrolat (Coleoptera, Cerambycidae) in Northern Thailand. *Japanese Journal of Entomology*, **62**, 597–606.
- Völger, E. (1994) *Stephanus serrator* (Fabricius, 1798) in Sachsen-Anhalt (Hym., Stephanidae). *Entomologische Nachrichten und Berichte*, **38**, 276.

- Walker, J.D., Geissman, J.W., Bowring, S.A. & Babcock, L.E. (2012) The Geological Society of America time scale. *Geological Society of America Bulletin*, **125**, 259–272.
- Yack, J.E. (2004) The structure and function of auditory chordotonal organs in insects. *Microscopical Research and Technique*, **63**, 315–337.
- Zherikhin, V.V. & Ross, A.J. (2000) A review of the history, geology and age of Burmese amber (Burmite). *Bulletin of the Natural History Museum London (Geology)*, **56**, 3–10.

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