

Specialized ovipositor sensilla of Cretaceous wasps (Insecta: Hymenoptera) possibly reveal a unique way of host detection

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Abstract

Insects have evolved complex sensory systems that are important for feeding, defence and reproduction. Parasitoid wasps often spend much time and effort in searching for concealed hosts with the help of specialized sensilla. However, the early evolution of such behaviour and sensilla is poorly known. We describe two fossil female wasps, †*Tichostephanus kachinensis* sp. nov. and †*Tichostephanus longus* sp. nov., from mid-Cretaceous Kachin amber. Phylogenetic analyses based on morphological data retrieved †*Tichostephanus* as deeply nested within Evaniioidea and closely related to extant Gasteruptiidae and Evaniidae. Both of these Cretaceous wasps possess features, e.g. coronal tubercles and flexible ovipositor sheaths, that indicate that they might have laid eggs in wood where their larvae possibly parasitized insect larvae. They have a peculiar and unique ‘bottle brush’ of sensilla close to the apex of their ovipositor sheaths, which has not been observed in any extant parasitoid wasps. These sensilla comprise many regularly arranged plate-shaped setae, attached in relatively large sockets and with rows of longitudinal ridges. Such specialized sensilla perhaps served to enhance the ability to detect hosts inside wood.

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Introduction

Insects, the most abundant group of animals occurring in terrestrial habitats, have a substantial variety of sensilla for sensing their surroundings (Souto et al., 2021). The sensilla of insects are situated on various parts of the body, including the antennae, mouthparts, legs and ovipositor. The sensilla can detect a range of stimuli, e.g. touch, pressure, heat, vibration, stretching or chemical stimuli (Hartenstein, 2005; Chapman, 2007). These sensilla play crucial roles in almost all insect activities, such as detecting host

plants, feeding, avoiding predators, recognizing mates or locating oviposition sites (Souto et al., 2021).

Most extant parasitoid wasps have abundant sensory structures to process stimuli from hosts and environment, which are crucial for successful parasitization (Gauld and Bolton, 1988; Goulet and Huber, 1993). In addition to homing in on chemical cues from their hosts and symbiotic microorganisms in host galleries, or active or passive vibrational detection (Broad and Quicke, 2000; Vilhelmsen et al., 2001, 2008), successful host detection may also involve visual, tactile or even thermal clues (Quicke, 1997; Wang and Yang, 2008). Since the ovipositor plays a significant role in the search for concealed hosts in plant matter or other substrates (Papp, 1974; Vilhelmsen, 2003; Dweck et al., 2008), there are many

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varieties of sensilla consisting of short and soft setae on the ovipositor shaft and sheaths in parasitoid wasps (Shah, 2012; Ahmed et al., 2013).

We report two wasps from Kachin amber, one of the most diverse fossil insect faunas from the mid-Cretaceous (Ross, 2023). These two females display a remarkable ‘bottle brush’ assemblage of sensilla near the tip of the ovipositor sheaths, expanding the known diversity of insect sensilla and providing a possible example of host detection in 100 Ma old parasitoid wasps. Furthermore, we conduct parsimony analyses and Bayesian phylogenetic analyses based on a morphological dataset with a comprehensive sample across fossil Stephanoidea and Evanoidea, including the new fossil wasps. Based on the results of the analysis, we place the fossils in †*Tichostephanus* Engel, 2019 and revise the systematic placement of this genus, transferring it from Stephanoidea to Evanoidea.

Material and methods

Provenance

The Kachin amber specimen, CNU-HYM-MA-2014-050, was loaned for research by Mr Jun Li from the Huxuan Museum, Shandong, China. The Kachin amber specimen, CNU-HYM-MA-2014-051, reported in this study were purchased by Mr Fangyuan Xia on 1 September 2014 and donated to the Key Laboratory of Insect Evolution and Environmental Changes, College of Life Sciences, Capital Normal University, Beijing, China, in September, 2014. The fossils were all collected in full compliance with the laws of Myanmar and China. All specimens are permanently deposited in well-established, public museums (Huxuan Museum, Jinan, Shandong, China and Key Laboratory of Insect Evolution and Environmental Changes, College of Life Sciences, Capital Normal University, Beijing, China), in full compliance with the International Code of Zoological Nomenclature and the Statement of the International Palaeontological Society (International Commission on Zoological Nomenclature, 1999; Szwed et al., 2020).

Imaging

Amber specimens were examined under a Leica M205C dissecting microscope. Photographs of type specimens were taken with a Nikon SMZ 25 microscope with an attached Nikon DS-Ri2 digital camera system or a Nikon ECLIPSE Ni microscope with an attached Nikon DS-Ri2 digital camera system. Photography of extant wasps was done using a BK plus imaging system from Visionary Digital (Palmyra, PA, USA) equipped with a Canon 7D digital camera. Stacks of images from multiple focal planes were combined using Zerene Stacker (Zerene Systems LLC) and figure plates were composed in Photoshop CS6 (Adobe, San Jose, CA, USA). A Zeiss LSM 780 confocal laser scanning microscope (CLSM) equipped with 488 nm laser was employed to produce high-magnification images of the sensilla on the ovipositor sheaths.

Terminology

The wing venation terminology used herein follows that of Engel and Grimaldi (2004) and Engel (2019), with additional morphological terminology following that of Li et al. (2017, 2018).

Phylogenetic analyses

Our phylogenetic analyses included three species of Symphyta as outgroups (two fossil, one extant), 18 species of Stephanoidea (17 fossil, one extant) and 40 species of Evanoidea (35 fossil, five extant) as ingroups to clarify the taxonomic position of the two new fossil wasps. All taxa included are summarized in Appendix S1. Our taxon sample included representatives of all fossil and extant families of the Stephanoidea and Evanoidea. We scored 77 characters, of which some character definitions were taken from Li et al. (2018) or Jouault et al. (2022), and some new characters added. All characters are summarized in Appendices S2, S3 and S4.

Character coding was done in Nexus Data Editor version 0.5.0 (Roderic, 2001) and Mesquite version 2.75 (Maddison and Maddison, 2011). Maximum parsimony analyses were conducted with Tree Analysis Using New Technology (TNT) version 1.5 (Goloboff and Catalano, 2016). We used traditional search with tree bisection-reconnection and 10 000 replicates, saving 100 trees per replication. The analysis produced 12 most parsimonious trees. Bremer support values were obtained in TNT version 1.5 using 10 000 replicates. Bootstrap support was evaluated with 100 replicates. All characters were treated as unordered and of equal weight.

Bayesian analyses were performed in MrBayes version 3.2.7 (Ronquist et al., 2012). Only variable characters were coded, and non-applicable morphological characters were treated as missing data. Equal transition probabilities between the states and among-character rate variation were assumed, allowing the different characters to evolve at different rates. Seventeen multistate characters were treated as ordered. The Bayesian analyses were conducted with four independent runs. Convergence was assessed by the average standard deviation of split frequencies (ASDSF <0.01), and the potential scale reduction factor (PSRF <1.005). After 20 million generations, the topology converged with an ASDSF <0.005 and PSRF values <1.002. Half of the generations were then discarded as burn-in. The calculations were performed on the HPC cluster UBE-LIX (<http://www.id.unibe.ch/hpc>) at the University of Bern, Switzerland. For the data matrices and command scripts see Appendix S4.

To assess the placement of the new fossil species, we made use of the function `create.rogue.plots()` from the RoguePlot package (Klopfstein and Spasojevic, 2019) in R (version 4.0.2, R Core Team, 2020). In RoguePlots 1000 randomly selected trees from the posterior distribution are used together with the consensus tree, where the fossils of interest, in this case †*Tichostephanus hui* Engel, 2019 and the two new †*Tichostephanus* species, are excluded. This then summarizes the attachment probabilities of the specimens to the branches of the consensus tree.

Results

Phylogenetic analyses

Our parsimony analyses of the morphological dataset yielded 12 equally parsimonious trees, of which the strict consensus tree is displayed in Fig. 1. Support values are low in general. According to our phylogenetic results, Ephialtitidae are retrieved as a basal grade in Apocrita. †Praeaulacidae are a monophyletic group and sister to †Othniodelithidae + †Anomopterellidae + †Myanmarinidae + †Aptenoperissidae + Stephanidae + Aulacidae + †Baissidae + Gasteruptiidae + †Andreneliidae + Evaniidae. Stephanidae are a monophyletic group

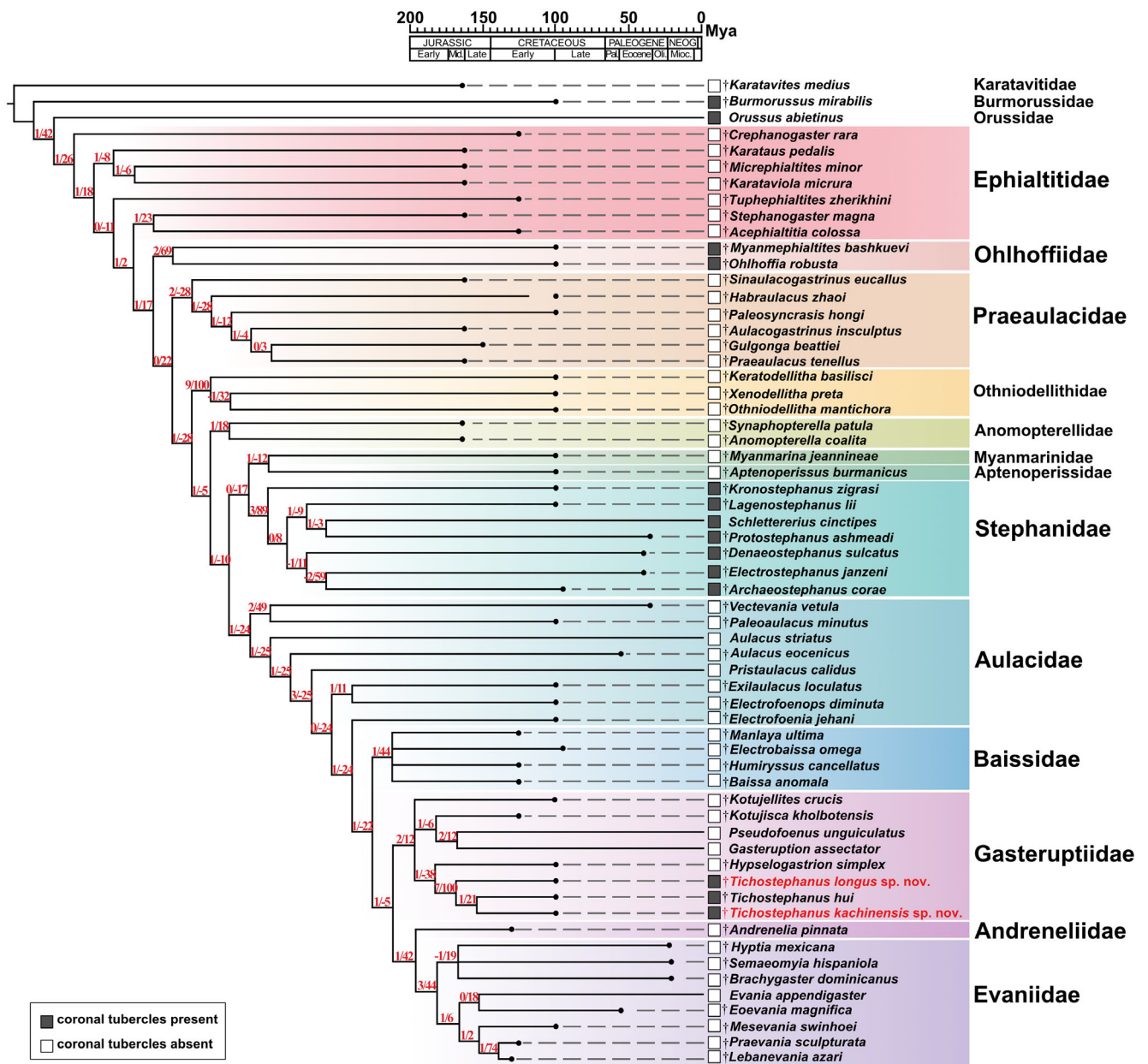


Fig. 1. Undated phylogeny of Stephanoidea and Evanioidea, based on morphological characters, and strict consensus tree; the time scale at the top indicates exclusively the age of the terminals, not the internal nodes in the cladogram. Tree length = 432, consistency index (CI) = 0.225, retention index (RI) = 0.673. The numbers at the branch points are Bremer support values (left) and bootstrap support values (right).

and are sister group to †Myanmarinidae + †Aptenoperissidae. Aulacidae are paraphyletic at the base of †Baissidae + Gasteruptiidae + †Andreneliidae + Evaniidae; the latter four families are all retrieved as monophyletic.

Gasteruptiidae and †Andreneliidae + Evaniidae are retrieved as sister groups. Within Gasteruptiidae, †*Tichostephanus hui* and the two newly discovered female wasps form a clade with strong bootstrap and Bremer support. The sister group of this clade is

†*Hypsologastrion*; together they are the sister group of a clade comprising the extinct †*Kotujellitinae* and the two extant taxa *Gasteruption* and *Pseudofoenus*.

There is no significant difference between the results of the parsimony analyses and Bayesian inference analysis (Figs S2 and S3). However, in the latter †*Praeaulacidae* may be paraphyletic (Figs S2 and S3), but there is not enough support to confidently claim this. In the Bayesian inference analysis, many families are retrieved as monophyletic (e.g., Baissidae,

Stephanidae, Evaniidae, Gasteruptiidae). However, the relationships among most families, including †Praeaulacidae, are not resolved on the backbone of the phylogeny; this reflects the comparatively low numbers of morphological characters that can be scored from a dataset consisting primarily of fossil taxa. Nevertheless, the fossils of interest, †*Tichostephanus hui* and the two newly described species, group in a clade together with Gasteruptiidae + †Andreneliidae + Evaniidae, and more specifically within Gasteruptiidae (Fig. 1). In the RoguePlots all three †*Tichostephanus* species are attached to the same branches, resulting in the same plot (Fig. S1) with very similar probabilities (Appendix S5). †*Tichostephanus* is placed with almost equal probability within either Gasteruptiidae or Evaniidae. The summed up probabilities (not counting attachments below 1% probability) result in an attachment frequency of 47.4% within Gasteruptiidae and 43.7% within Evaniidae. Our conclusion is that †*Tichostephanus* should be assigned to Gasteruptiidae.

Systematic palaeontology

Order Hymenoptera Linnaeus, 1785.

Superfamily Evanioidea Latreille, 1802.

Family Gasteruptiidae Ashmead, 1900.

Genus †*Tichostephanus* Engel, 2019.

Type species. †*Tichostephanus hui* Engel, 2019.

Additional species. †*Tichostephanus kachinensis* Wang, Vilhelmsen, Rasnitsyn and Gao, sp. nov., †*Tichostephanus longus* Wang, Vilhelmsen, Rasnitsyn and Gao, sp. nov.

Diagnosis. Head with two unpaired anterior tubercles, and three prominent and one indistinct rows of coronal tubercles; antenna filiform with 13 antennomeres in female, 14 in male. Fore wing with pterostigma greatly elongate, narrow, more than six times as long as wide; r-rs distally positioned on pterostigma; R extending along wing margin to wing apex; 1Rs + M, Cu behind 2cu-a, 2 m-cu, 2r-m and 3r-m absent; 1 M confluent with or slightly predated 1cu-a; 2Rs + M + 1 m-cu forming elongate, continuous, straight vein between 2cu-a and 2Rs. Only Sc + R and R present in hind wing. Hind tibia strongly swollen distally, distal width four to eight times the basal width; tarsi five-segmented. Female with special ovipositor sheath. Ovipositor slightly longer than metasoma. ‘Bottle brush’ like sensilla of regularly arranged and plate-shaped setae on distal part of ovipositor sheath, extending from 1/3 to 1/9 of the distance to the apex of sheath. Male with genitalia exerted. Parameres tapering apically to broadly rounded apex, with fringe of setae along their inner margin; gonostipes broadly attached to paramere except apically distinct and

tapering to narrowly rounded apex; volsella with long, curved, apical lobe (possibly digitus) approaching apex of gonostipex; aedeagus narrow, apparently tubular, slightly narrower than parameres, not extending beyond paramere apices, apically broadly rounded.

Comments. In †*Tichostephanus*, the antennomere count is apparently sexually dimorphic, with 13 antennomeres in the females (†*T. kachinensis*, †*T. longus*) and 14 in the males (†*T. hui*); interestingly, this is reversed compared with other extant and fossil Gasteruptiidae (Rasnitsyn, 1988; Gauld and Hanson, 1995). †*Tichostephanus* has the fore wing without 2m-cu and 3r-m and the hind tibia swollen distally, unlike †Hypselogastrinae and †Kotujellitinae (Rasnitsyn, 1975; Engel and Wang, 2016). †*Tichostephanus* differs from Hyptiogastrinae in having a long ovipositor, almost as long as metasoma, and from Gasteruptiinae in not having an enclosed first discal cell in the fore wing (Rs + M lost) (Jennings and Austin, 2002).

†*Tichostephanus kachinensis* Wang, Vilhelmsen, Rasnitsyn and Gao, sp. nov. (Figs 2 and S4) [urn:lsid:zoo-bank.org:act:CF6C1E6E-67B6-44AD-AE0B-87BD36875C2C].

Etymology. The specific epithet refers to Kachin State in Myanmar, the region from which the amber originates.

Diagnosis. Female. Head with shorter eyes, wider temples, 13th antennomere about three times as long as 12th. Distinct propleuron with defined neck, 2Rs longer in fore wing. Metasomal second segment conical.

Type material. Holotype, CNU-HYM-MA-2014-050, female, well-preserved. Deposited in the Huxuan Museum, Jinan, Shandong, China.

Type locality. The amber specimen was collected from Kachin, northern Myanmar, lowermost Cenomanian, mid-Cretaceous, which was dated at 98.79 ± 0.62 Ma (Cruickshank and Ko, 2003; Shi et al., 2012).

Description. Male unknown. Female. Integument dark brown, nearly black in most body parts as preserved. Antenna with 13 antennomeres, scape about 1.6 times as long as wide; pedicel robust, about 2.2 times as long as wide; individual antennomeres 3–13 much longer than wide, antennomere 13 elongate, about three times as long as antennomere 12 (Fig. 2b). Frons with two single tubercles (tbZ and tbA) posterior to antennal bases; four rows of tubercles posterior to tbA (Figs 2c and S4b). Compound eye elliptical in shape, with scattered, minute setae, lateral ocelli close to compound eyes. Mouthparts hard to observe.

Mesosoma relatively slender, 3.1 times as long as wide, without sculpturing, with sparsely scattered short

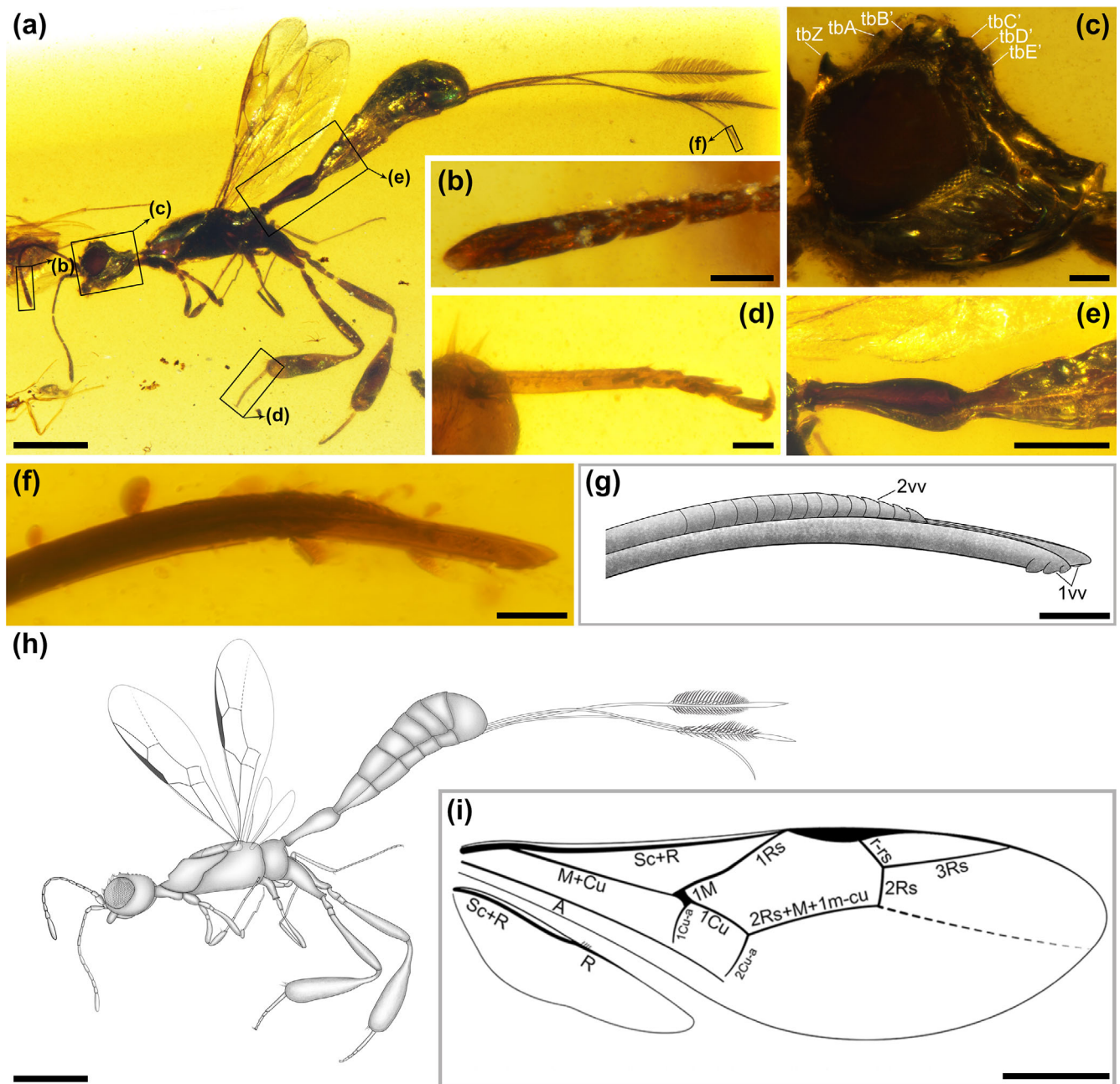


Fig. 2. Photographs and line drawings of †*Tichostephanus kachinensis* sp. nov. (CNU-HYM-MA-2014-050). (a) Habitus, lateral view; (b) the terminal parts of the right antenna; (c) head in lateral view, with tubercle positions indicated; (d) details of metatibial apex and metatarsus; (e) petiole in lateral view; (f) the first and second valvulae in lateral view; (g) line drawing of the first and second valvulae in lateral view; (h) line drawing of habitus in lateral view, wings are artificially extended from the body for ease of comparison; and (i) line drawing of wing venation. Scale bars for (a) and (h) = 1 mm; scale bars for (b)–(d) = 0.1 mm; scale bars for (e) and (i) = 0.5 mm; scale bars for (f) and (g) = 0.05 mm. Abbreviations: tbZ–tbE, tubercle Z–E; 1vv, the first valvulae of the ovipositor; and 2vv, the second valvulae of the ovipositor.

to minute setae. Propleuron with defined ‘neck’ anteriorly; pronotal fold lamellate and highly elevated above neck, with posterior area longer than neck and medial area combined. Mesopleuron elongate, integument without pronounced sculpturing; metanotum with pronounced sculpturing.

Fore wing with pterostigma greatly elongate, narrow, 12.5 times as long as wide; 1Rs straight, arising from Sc + R at base of pterostigma; 1cu-a postfurcal, nearly as long as length of vein 1Rs; 1Cu parallel to A; 1cu-a parallel to 2cu-a; 2Rs + M and 1m-cu forming composite straight line, demarcating large cell

combined by first submarginal and first discoidal; r-rs short, arising behind midlength of pterostigma, about as long as 2Rs; all veins tubular (Fig. S4c). Hind wing with only Sc + R and R present, thin and tubular (Fig. S4d).

Fore and mid legs very slender compared with hind legs. Hind coxa larger than fore and mid coxa, without dorsal tubercle; hind trochanter longer than wide; hind femur not swollen, without teeth ventrally; hind tibia distinctly narrowed at base and widened apically, distal width eight times the basal width; hind tarsi five-segmented; metabasitarsus longer than following tarsomeres combined (Fig. 2d). Tibial spur formula 1/2/2. Claws curved and simple.

Metasoma inserted on propodeum some distance from hind coxae, with eight visible segments, first tergum and sternum fused to form elongate petiole, petiole 3.4 times as long as wide. Second tergum and sternum not fused. Ovipositor comprising paired first valvulae with three sawteeth apically, second valvulae fused and with many small serrations apically, third valvulae forming ovipositor sheaths, with ‘bottle brush’ of regularly arranged plate-shaped setae extending from 1/3 to 1/9 of distance to apex of sheath (Figs 2f and S4e,f; see more details below).

Measurements (mm): Total body length (excluding ovipositor and antenna) 6.13; head length 0.74, height 0.70; antenna length 1.53; mesosoma length 1.96, height across tegulae 0.63; fore wing length 3.13; hind wing length 1.08; forecoxa length 0.22, forecoxa width 0.15, foretrochanter length 0.20, foretrochanter width 0.06, foretrochantellus length 0.07, foretrochantellus width 0.04, forefemur length 0.47, forefemur width 0.07, foretibia length 0.31, foretibia width 0.09, lengths of foretarsal segments 0.22, 0.28, 0.20, 0.12 and 0.13, widths of foretarsal segments 0.03, 0.03, 0.03, 0.03 and 0.03; midcoxa length 0.32, midcoxa width 0.11, midtrochanter length 0.18, midtrochanter width 0.04, midtrochantellus length 0.12, midtrochantellus width 0.04, midfemur length 0.50, midfemur width 0.06, midtibia length 0.48, midtibia width 0.04, lengths of midtarsal segments 0.48, 0.16, 0.17, 0.06 and 0.06, widths of midtarsal segments 0.03, 0.03, 0.03, 0.03 and 0.03; hind coxa length 0.22, hind coxa width 0.11, hind trochanter length 0.24, hind trochanter width 0.04, hind trochantellus length 0.14, hind trochantellus width 0.04, hind femur length 0.90, hind femur width 0.06, hind tibia length 1.35, hind tibia width 0.04, lengths of hind tarsal segments 0.36, 0.08, 0.08, 0.06 and 0.07, widths of hind tarsal segments 0.03, 0.03, 0.03, 0.03 and 0.03; metasoma length 3.38; petiole length 0.88, petiole width 0.26; ovipositor sheaths length 4.33.

†*Tichostephanus longus* Wang, Vilhelmsen, Rasnitsyn and Gao, sp. nov. (Figs 3 and S5) [urn:lsid:

zoobank.org:act:02D5073A-F2E3-49A5-9EAD-B9ED78706533].

Etymology. The specific name is the Latin adjective ‘*longus*’ meaning long, referring to the fore wing with long 2Rs + M + 1 m-cu.

Diagnosis. Female. Head oval in lateral view with compound eyes reniform, 13th antennomere slightly longer than 12th. Mesosoma without neck. First and second metasomal segments together forming slender petiole. Second tergum and sternum not fused.

Type material. Holotype, CNU-HYM-MA-2014-051, female, well-preserved. Deposited in the Key Laboratory of Insect Evolution and Environmental Changes, College of Life Sciences, Capital Normal University, Beijing, China (CNUB).

Type locality and horizon. The amber specimen was collected from Kachin, northern Myanmar, lowermost Cenomanian, mid-Cretaceous, which was dated at 98.79 ± 0.62 Ma (Cruickshank and Ko, 2003; Shi et al., 2012).

Description. Male unknown. Female. Integument dark brown. Antenna with 13 antennomeres, scape robust, length same as width; pedicel robust, length same as width; individual antennomeres 3–13 longer than wide (Fig. S5b). Compound eye elliptical in shape, with scattered, minute setae, lateral ocelli close to compound eyes. Mouthparts mostly not preserved.

Mesosoma relatively slender, twice as long as high, without sculpturing, with sparsely scattered short to minute setae. Propleuron not distinctly elongate (no distinct ‘neck’). Mesopleuron elongate, length 0.6 times of mesosoma length, integument without pronounced sculpturing; metanotum with pronounced sculpturing.

Fore wing with pterostigma elongate, longer than wide, 6.5 times as long as wide; 1Rs straight, arising from Sc + R at base of pterostigma; 1Cu parallel to A; 1cu-a postfurcal, nearly as long as length of vein 1Rs, parallel to 2cu-a; 2Rs + M and 1 m-cu forming a composite straight line, thus demarcating massive cell combined by first submarginal and first discoidal; r-rs short, arising over than apical half of pterostigma, about as long as 2Rs; all veins tubular (Fig. S5c). Hind wing only with Sc + R and R present, thin and tubular (Fig. S5d).

Fore and mid legs shorter and very slim compared with hind legs. Hind coxa larger than fore and mid coxa; hind trochanter longer than wide; hind femur not swollen, without ventral teeth; hind tibia narrowed at base and widened apically, distal width four times basal width; hind tarsi five-segmented; metabasitarsus longer than following tarsomeres combined. Tibial spur formula 1/2/2. Claws long, curved, and simple.

Metasoma inserted on propodeum some distance from hind coxae, with eight segments. Petiole

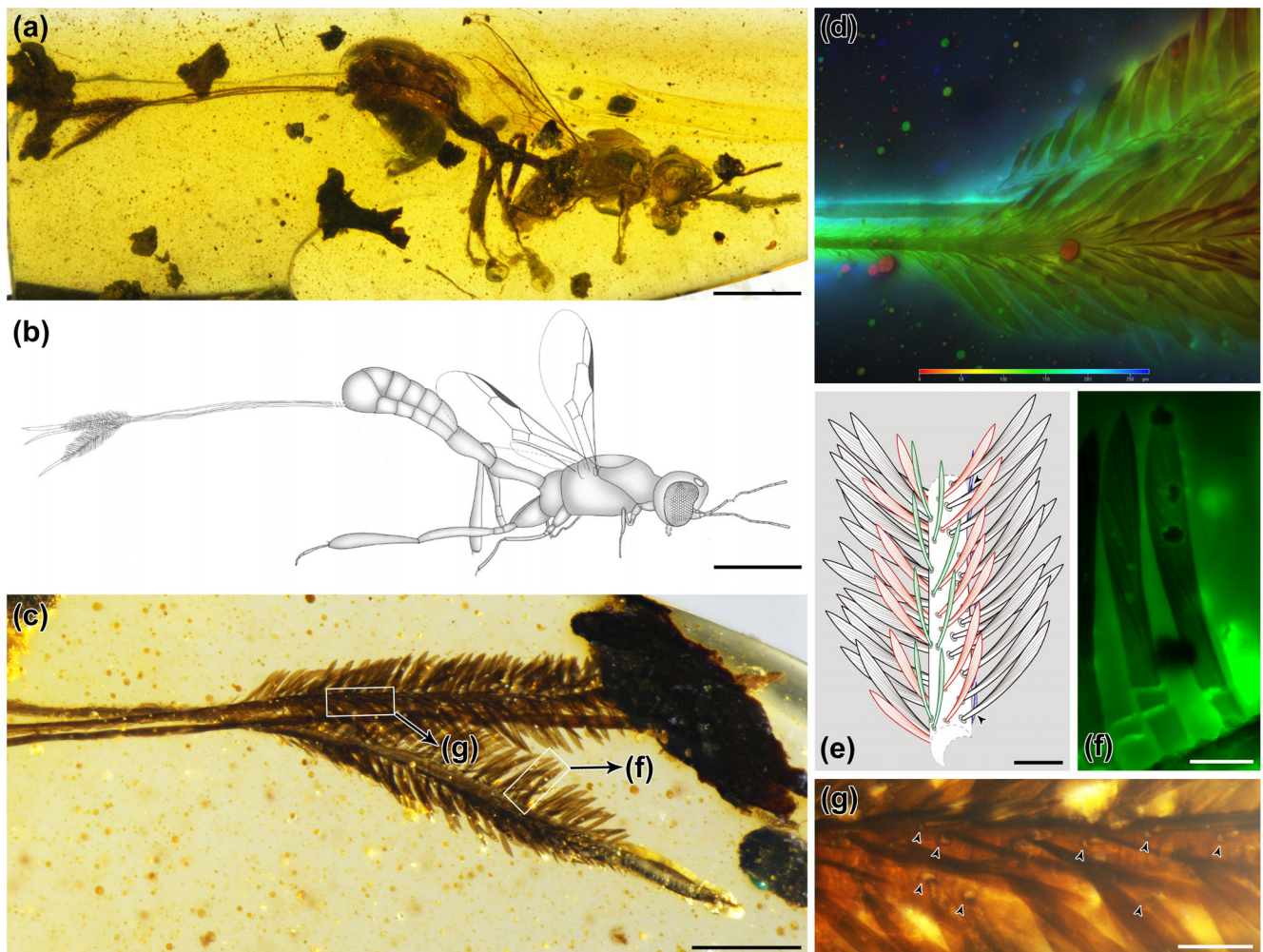


Fig. 3. Photographs and line drawings of †*Tichostephanus longus* sp. nov. (CNU-HYM-MA-2014-051). (a) Habitus, lateral view; (b) line drawing of lateral view, wings and legs are artificially extended from the body for ease of comparison; (c) the terminal parts of ovipositor in lateral view; (d) proximal plate-shaped setae on ovipositor sheath, under CLSM; (e) line drawing of plate-shaped setae on ovipositor sheath—the blue, green, red and black part represent four different sizes of plate-shaped setae, the black arrows pointing to blue plate-shaped setae; (f) the plate-shaped setae on ovipositor sheath, under CLSM; and (g) the black arrows pointing to the sockets in the base of plate-shaped setae. Scale bars for (a) and (b) = 2 mm; scale bar for (c) = 0.5 mm; scale bar for (e) = 0.1 mm; scale bars for (f) and (g) = 0.05 mm.

consisting of two segments, first tergum and sternum fused to form an elongate first petiolar segment. Second tergum and sternum fused, forming second petiolar segment (Fig. S5e). First tergum slightly longer than second one. Ovipositor comprising paired first valvulae with three sawteeth apically, fused second valvulae with many small serrations apically, paired third valvulae forming ovipositor sheaths with ‘bottle brush’ of regularly arranged plate-shaped setae extending from 1/3 to 1/9 of distance to apex of sheath (see below for more details).

Measurements (mm): total body length (excluding ovipositor and antenna) 9.17; head length 1.17, height 1.24; antenna length 2.49; mesosoma length 2.71, height across tegulae 1.38; fore wing length 3.93; hind

wing length 1.67; forecoxa length 0.44, forecoxa width 0.14, foretrochanter length 0.14, foretrochanter width 0.10, foretrochantellus length 0.07, foretrochantellus width 0.07, forefemur length 0.61, forefemur width 0.07, foretibia length 0.46, foretibia width 0.07, lengths of the first to fourth foretarsal segments 0.17, 0.10, 0.10 and 0.07, widths of the first to fourth foretarsal segments 0.03, 0.03, 0.03 and 0.03; midcoxa length 0.50, midcoxa width 0.28, midtrochanter length 0.20, midtrochanter width 0.14, midtrochantellus length 0.12, midtrochantellus width 0.07, midfemur length 0.69, midfemur width 0.07, midtibia length 1.06, midtibia width 0.07, lengths of midtarsal segments 0.22, 0.27, 0.15, 0.35 and 0.17, widths of midtarsal segments 0.03, 0.03, 0.03, 0.03

and 0.03; hind coxa length 1.40, hind coxa width 0.55, hind trochanter length 0.45, hind trochanter width 0.17, hind trochantellus length 0.25, hind trochantellus width 0.14, hind femur length 2.26, hind femur width 0.21, hind tibia length 1.77, hind tibia width 0.28, lengths of hind tarsal segments 0.17, 0.20, 0.34, 0.14 and 0.15, widths of hind tarsal segments 0.07, 0.07, 0.07, 0.07 and 0.07; metasoma length 5.03; first petiole length 1.20, first petiole width 0.41, second petiole length 1.17, second petiole width 0.48; ovipositor sheaths length 7.33.

Details of ‘bottle brush’ plate-shaped setae on ovipositor sheaths. The ‘bottle brush’ on the apical part of the ovipositor sheath comprises many regularly arranged plate-shaped setae, placed on the outside of the ovipositor sheath, and extending from 1/9 to 1/3 from the apex of the sheath (Fig. 3c,e). Each plate-shaped seta is flaky and very thin, with parallel longitudinal ridges that extend to the edge (Fig. 3f). These setae, with the same plate-shaped structure, appear in four different sizes on the ovipositor sheaths of †*T. longus* and †*T. kachinensis*: the smallest setae are found near the margin of the ovipositor sheath and are almost as long as the common setae (blue in Fig. 3e, and indicated by black arrows, 0.11 mm long in †*T. longus* and 0.08 mm long in †*T. kachinensis*); the second smallest setae are found in the median line of ovipositor sheath (green in Fig. 3e, 0.16 mm long in †*T. longus* and 0.12 mm long in †*T. kachinensis*); from the middle line to the edge of ovipositor sheath, two types of longer setae are found (red and black in Fig. 3e, shorter setae (red) is 0.23 mm long in †*T. longus* and 0.15 mm long in †*T. kachinensis*; longer setae (black) is 0.32 mm long in †*T. longus* and 0.23 mm long in †*T. kachinensis*). The distinct socket at the base of each seta is of approx. 10 µm diameter (Fig. 3g).

Comments. †*T. kachinensis* has the 13th (apical) antennomere about three times as long as the 12th; in †*T. hui* (Engel, 2019; Fig. S6 in Supplemental Material), the 14th (apical) antennomere is only twice as long as the 13th. In the fore wing of †*T. kachinensis* and †*T. longus*, veins 1Rs and 1M form clearly separate veins, whereas the two veins are continuous in a straight line in †*T. hui*. In †*T. kachinensis*, the petiole consists of only one segment, whereas †*T. hui* and †*T. longus* have two petiolar segments. Given the differences noted, we consider it prudent not to match †*T. hui* with any of the species represented by the females.

Key to †*Tichostephanus* spp. from Kachin amber:

1. Fore wing veins 1Rs and 1M form clearly separate veins. 2
- Fore wing veins 1Rs and 1M are continuous in a straight line (male). †*Tichostephanus hui*, Engel, 2019

2. Petiole consists of only one segment (male) †*T. kachinensis* sp. nov.
- Petiole consists of two segments (female). †*T. longus* sp. nov.

Discussion

Systematic placement of the new species

Owing to the leg structure, tubercles on frons, and fore wing venation, the two new species are considered to belong to the †*Tichostephanus*. †*Tichostephanus hui* was described from a single male specimen from Myanmar amber and placed in Stephanidae. This placement was based on the presence of a globular head with coronal tubercles anterodorsally, an elongate ‘neck’ (propleura), distinctive pronotal structure, a slender and elongate body and elongate legs. Characters diagnostic of †*T. hui* differ from extant Stephanidae, which have comparatively high antennomere count (30+), lateral displacement of the median metacoxal articulation and swollen hind femora with distinct spines (Gauld, 1995; Vilhelmsen et al., 2010); these are presumably ground plan characters for crown group Stephanidae and in the case of the hind leg characters apomorphic, but they are absent from †*T. hui*. Furthermore, the metasoma of †*T. hui* is inserted comparatively high on the mesosoma and the hind tibiae are distinctly swollen, indicating evaniid/gasteruptionid affinities.

The new fossils are retrieved in a well-supported clade (see Fig. 1) with †*Tichostephanus hui*, and this clade is nested deeply within Evanioidea. In the parsimony analyses, †*Tichostephanus* is grouped within a clade of both extant and extinct representatives of Gasteruptionidae; in the Bayesian inference analyses and the RoguePlots, †*Tichostephanus* is attached to branches within either Gasteruptionidae or Evaniidae. Despite the uncertainty highlighted by the RoguePlots (i.e. virtually equal probability for grouping with Evaniidae or Gasteruptionidae), we place †*Tichostephanus* in Gasteruptionidae. Two characters support this: the presence of an elongate propleuron and hind tibia swollen distally (Fig. S7). In contrast, there are no obvious character apomorphies shared with Evaniidae. A potential reason for the nearly equal probability of placement in these two families in the RoguePlots analysis could be inadequate selection of characters in the morphological matrix. Given the phylogenetic placement of the new fossils, we place them in †*Tichostephanus* Engel, 2019, and place the genus in Gasteruptionidae rather than Stephanidae or Evaniidae.

†*Tichostephanus* appears to be another example of an extinct insect taxon that displays a character combination unlike those observed in any extant taxa. Such taxa need to be integrated in a phylogenetic

framework to be correctly classified, rather than assigned to a given taxon based on a few characters purportedly diagnostic for some extant taxon, but that may have evolved independently in fossil representatives of other taxa. In the light of the phylogenetic analyses conducted here, the presence of coronal tubercles in †*Tichostephanus* has to be interpreted as a remarkable example of convergence (Fig. 1).

Adaptations in parasitoids of wood living insect larvae

The appearance of coronal tubercles on top of the head of †*Tichostephanus* is the first time that this character has been reported in Evanioidea. Coronal tubercles have previously been found in Stephanidae, Orussidae, †Burmorussidae and †Ohlhoftidae (Vilhelmsen and Turrissi, 2011; Li et al., 2017; Zhang et al., 2020; Jouault et al., 2021). Orussidae, †Burmorussidae and †Paroryssidae together form the Orussoidea, which is the sister group to Apocrita (e.g., Zhang et al., 2020); Stephanidae and †Ohlhoftidae are among the earliest diverging lineages of Apocrita. They are/were all presumably parasitoids on wood-boring insect larvae; the ocellar tubercles is probably correlated with this life style, as well as the presence of a long, flexible ovipositor sheath (Vilhelmsen and Turrissi, 2011). The structure of the coronal tubercles varies considerably among the taxa possessing them in Hymenoptera, and our cladogram shows that the presence of the coronal tubercles might have been acquired independently in Orussidae, †Burmorussidae (these two taxa were not retrieved in a single clade in our analyses), Stephanidae, †Ohlhoftidae and †*Tichostephanus* (Fig. 1). The tubercles could be used to brace the head against the walls of the pupal chamber inside the wood when the newly emerged adult has to chew an escape tunnel (Vilhelmsen and Turrissi, 2011; Zhang et al., 2020).

In the ovipositor, we found transverse subdivisions along most of the length of the third valvulae (Fig. 3g), indicating that they were flexible as in many extant parasitoid wasps with long ovipositors (Vilhelmsen, 2003). The tip of flexible ovipositor sheaths helps anchor the ovipositor during the initial stage of oviposition, after which the ovipositor obtains enough support on the surface of the wood to gain purchase. As drilling progresses, the flexible ovipositor sheaths can bend aside to allow the first and second valvulae (Fig. 2f,g) to penetrate deeper—support no longer needed when the latter are well anchored in the wood. (Vilhelmsen, 2003; Vilhelmsen and Turrissi, 2011).

The earliest parasitoid wasps most likely targeted woodboring insect larvae; the switch to a parasitoid lifestyle probably initiated the spectacular adaptive radiation of the apocritan lineages (Eggleton and Belshaw, 1992; Vilhelmsen and Turrissi, 2011). Many



Fig. 4. Ecological reconstruction of †*Tichostephanus kachinensis* sp. nov. painted by Xiaoran Zuo.

groups scattered across the Apocrita are also parasitoids of woodboring insects, e.g. Stephanidae, Aulacidae and Megalyridae, as well as some Ichneumonoidea, Chalcidoidea and Cynipoidea (Gauld and Bolton, 1988). In contrast, extant gasteruptionids have larvae that are predator-inquilines in the nests of cavity-nesting solitary bees, with some records for solitary wasps as hosts (e.g., Jennings and Austin, 2004; Parslow et al., 2020), whereas female evaniids oviposit into the oothecae of cockroaches and the larvae devour the cockroach eggs (Tee and Lee, 2017). For the two new species of †*Tichostephanus*, we found an ocellar corona (Figs 2c and S4b) and a long ovipositor with flexible sheaths (Fig. 3g; ovipositor sheath of extant Gasteruptionidae; see Fig. S7 in this paper and figs 28–30 in Johansson and Van Achterberg, 2016), which are characters typically associated with parasitoids of wood-living larvae. This suggests that †*Tichostephanus* spp. probably lived as parasitoids of wood-boring insects during the mid-Cretaceous (Fig. 4), similar to extant Aulacidae. The kleptoparasitoid lifestyle displayed by extant Gasteruptionidae and the predaceous habits of Evanioidea possibly arose comparatively late in the evolution of Evanioidea, and neither were probably the original lifestyle in the superfamily, this being more probably as a parasitoid of wood-boring insect larvae. The three extant evanioid lineages appear to be just the last remains of a clade that was much more diverse in the Cretaceous (e.g., Jouault et al., 2022), having radiated in the Jurassic together with, e.g. Stephanidae in what has been termed phase 1 of the mid-Mesozoic parasitoid revolution (Labandeira and Li, 2021).

Specialized sensilla for host searching

The plate-shaped setae near the tips of the ovipositor sheaths in †*Tichostephanus* are similar to scales in Trichoptera and Lepidoptera, which are modified setae

with a flattened blade and longitudinal ridges but lacking sockets (Frانيا and Wiggins, 1997; Dinwiddie et al., 2014). Lepidopteran scales are known to function in cryptic or warning coloration, in thermal regulation and in intraspecific signalling, and the scales fall off easily to protect the butterfly (Dinwiddie et al., 2014). In †*Tichostephanus*, the plate-shaped setae have no detached single setae but have sockets at the base, which are not like the scales of Lepidoptera. These plate-shaped setae with sockets are an unusual type of sensilla, the function of the socket being closely related to the reception of mechanical stimuli (Keil, 1997).

The sensilla on the ovipositor sheath of some extant parasitoid wasps sense vibrations from the hosts, thereby aiding the wasp in host detection, such as *Stephanus serrator* Fabricius, 1798 (Stephanidae) and *Bracon vulgaris* Ashmead, 1894 (Braconidae) (Alves et al., 2014; Hausl-Hofstätter and Bojar, 2016). The unusually enlarged plate-shaped setae found in †*Tichostephanus* have not been observed in any extant parasitoid wasps; *Torymus lasallei* Bubeníková et al., 2020 (Torymidae) and *Zaglyptogastra* Ashmead, 1900 (Braconidae) have long setae on the ovipositor sheaths, but they do not resemble those of †*Tichostephanus* (Quicke, 1991; Bubeníková et al., 2020; Chansri et al., 2022). Nevertheless, given their position, the plate-like sensilla of †*Tichostephanus* might have played a role in host detection. Given their unique nature, it is difficult to decide exactly how they might have worked, e.g. whether by mechano- or chemoreception, sensilla can detect vibrations or capture chemical molecules in the air. However, we cannot rule out the possibility that the ‘bottle brush’ setae on ovipositor sheath have other functions.

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Conflict of interest

The authors declare that there is no conflict of interest.

Data availability statement

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supporting Information.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Diversity and distribution of species in the phylogenetic analyses and references.

Appendix S2. Morphological characters coded in the phylogenetic analysis.

Appendix S3. Character matrix of TNT.

Appendix S4. Character matrix of MrBayes.

Appendix S5. The RoguePlot result.

Fig. S1. RoguePlot of the Bayesian phylogenetic analysis.

Fig. S2. Strict consensus tree from MrBayes, where all compatible clades are shown.

Fig. S3. Majority-rule consensus tree from MrBayes, where clades are shown when occurring at more than 50% in all generated trees.

Fig. S4. Photographs of †*Tichostephanus kachinensis* sp. nov. (a) Right lateral view; (b) head in dorsal view, with tubercle positions indicated; (c) fore wing; (d) hind wing; (e) the plate-shaped setae on the ovipositor sheath in lateral view; and (f) close-up of

plate-shaped setae on ovipositor sheath. Scale bar for (a) = 1 mm; scale bar for (b) = 0.25 mm; scale bar for (c) = 0.5 mm; scale bars for (d), (f) = 0.1 mm; scale bar for (e) = 0.2 mm. Abbreviations: tbZ-tbE, tubercle Z-tubercle E.

Fig. S5. Photographs of †*Tichostephanus longus* sp. nov. (a) Left lateral view; (b) antenna; (c) fore wing venation; (d) hind wing; (e) petiole in lateral view; and (f) the plate-shaped setae on the ovipositor sheath. Scale bar for (a) = 2 mm; scale bars for (b), (c) = 0.5 mm; scale bar for (d) = 0.25 mm; scale bar for (e) = 1 mm; scale bar for (f) = 0.1 mm.

Fig. S6. Photograph of †*Tichostephanus hui* Engle, 2019, adapted from Engel (2019). Scale bars: 1 mm.

Fig. S7. (a) Left lateral view of *Gasteruption cameroni* Hedicke, 1939; (b) ovipositor of *Gasteruption cameroni* Hedicke, 1939; (c) end of ovipositor sheath of *Gasteruption cameroni* Hedicke, 1939; (d) left lateral view of *Gasteruption erythrostomum* Dahlbom, 1831; (e) ovipositor of *Gasteruption erythrostomum* Dahlbom, 1831; and (f) end of ovipositor sheath of *Gasteruption erythrostomum* Dahlbom, 1831. Scale bars for (a), (b), (d), (e) = 2 mm; scale bars for (c), (f) = 0.5 mm.