



The larval morphology of a new mid-Cretaceous stonefly and its systematic position in Plecoptera

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Abstract

The larval stage is an essential part of the life history of stoneflies and can provide substantial biological and evolutionary information. However, well-preserved fossils of larvae are sparse. Herein, the larval morphology of a new extinct stonefly, *Kachinoperla zwicki* **gen. et sp. nov.**, is described based on its exuvia in mid-Cretaceous Kachin amber from northern Myanmar (98.79±0.62 Ma). The new stonefly also represents a new family, Kachinoperlidae **fam. nov.** according to its unique characters. Kachinoperlidae can be distinguished from other stonefly families by the following larval characters: predaceous mouthparts, subequal glossae and paraglossae, subequal maxillary and labial palps, reduced apical maxillary palpomere, trapezoidal pronotum, banded and semicircular wing pads, invisible notal contour, absent thoracic gills, shortest first two tarsomeres, and strongly projected abdominal tergum 10. The morphological comparison and phylogenetic analysis suggested the new family as a basal lineage within Systellognatha. Evolutionary implications implied by the larval characters are also discussed.

Key words

Cenomanian, Kachin amber, *Kachinoperla*, Kachinoperlidae, phylogeny, Systellognatha

1. Introduction

Plecoptera (stoneflies) is a basal hemimetabolous order of the “lower” Neoptera and includes 17 extant families and over 3800 extant species of aquatic insects distributed on all continents and most major islands except Antarctica (Stewart 2009; DeWalt et al. 2022). Stoneflies are typically found in running water and waterside microhabitats. Their larvae are highly sensitive to water pollution and thus are important biological indicators of water quality. The latest classification system of Plecoptera is composed of 17 extant families in two suborders: the largely northern hemisphere’s Arctoperlaria and the ex-

clusively southern hemisphere’s Antarctoperlaria (Zwick 2000; South et al. 2021). Arctoperlaria is composed of two infraorders, Euholognatha (including Capniidae, Leuctridae, Nemouridae, Notonemouridae, Scopuridae, and Taeniopterygidae) and Systellognatha (including Chloroperlidae, Kathroperlidae, Peltoperlidae, Perlidae, Perlodidae, Pteronarcyidae, and Styloperlidae). Antarctoperlaria contains the remaining four families, Austroperlidae, Diamphipnoidea, Eustheniidae, and Gripopterygidae. The higher-level phylogeny and biogeographic history of stoneflies still remain unresolved despite the

most recent work using a large molecular dataset (Letsch et al. 2021).

Plecoptera is ancient, with its oldest representative extending into the Pennsylvanian (ca. 300 Ma) (Béthoux et al. 2011). Over 300 fossil species have been reported for the order, ranging in age from Carboniferous to Pliocene (DeWalt et al. 2022). However, some problems are encountered in the study of stonefly fossils: precise identifications are difficult to make for the poorly preserved fossils; the taxonomic system is not complete for the fossils; most original descriptions and illustrations are very simple and cause difficulties for later revisions; synonymy and homonymy often occur in the fossil studies (Sinitshenkova 1987). Amber inclusions are in a much better state of preservation and have more informative morphological characters than fossil impressions (Chen et al. 2018). The mid-Cretaceous Kachin amber from northern Myanmar has revealed many interesting stoneflies under four families: Perlidae, Peltoperlidae, extinct Petroperlidae, and extinct Perspicuusoperlidae (Sroka et al. 2018; Chen and Xu 2020; Chen 2022a).

Larvae of stoneflies consist of a series of morphological characters very informative in species delimitation, higher-level classification, phylogeny, and inferring the evolutionary history of the order (Zwick 1973, 1980, 2000; Nelson 1984; Whiting et al. 1997). However, among the extinct stoneflies in Kachin amber, only *Electroneuria* Sroka, Staniczek & Kondratieff, 2018 (Plecoptera: Perlidae) is described based on larval characters, although its holotype is likely to be an exuvia as stated by the authors (Sroka et al. 2018). Other immature stoneflies known from fossil impressions are poorly preserved and many important characters such as mouthparts remain unknown (Sinitshenkova 1987), which results in our poor understanding of the evolutionary history of the important larval stages of Plecoptera. In this study, the larval morphology of a new stonefly that represents a new extinct family is described based on a well-preserved exuvia from the mid-Cretaceous Kachin amber. Its systematic placement is also investigated based on morphological characters.

2. Material and methods

2.1. Materials

The Kachin amber studied herein was obtained from amber mines near Noije Bum (26°20'N, 96°36'E), Hukawng Valley, Kachin State of northern Myanmar. The geologic age was dated as earliest Cenomanian (98.79±0.62 Ma), early Late Cretaceous (Shi et al. 2012; Yu et al. 2019). The studied amber piece was grounded with different-sized emery papers and polished with polishing grinding paste. Both the amber piece (ICJUST, No. CZT-PLE-MA13) and examined extant specimens are deposited in the ICJUST – Insect Collection of Jiangsu University of Science and Technology, Jiangsu Province, China.

2.2. Photographing and drawing

Photographs were taken with a Canon EOS 6D digital camera equipped with a Canon MP-E 65 mm 5× macro lens. Line drawings were produced with the aid of an SDPTOP SZM45 stereomicroscope and ADOBE ILLUSTRATOR CS6. Photographs and drawings were adjusted and optimized with ADOBE PHOTOSHOP CS6.

2.3. Morphological phylogenetic analysis

Two methods of morphological phylogenetic analysis were used to evaluate the systematic placement of the new stonefly, including parsimony and Bayesian inference. The character list and data matrix were partially derived from a previously published dataset in Nelson (1984) (see Supplementary Files 1 and 2). To better accommodate the fossils, the invisible anatomical characters from Nelson (1984) were removed from the dataset. During the selection of taxonomic groups, the fossil impressions were excluded from the dataset because their outlines of bodies or fragmentary wings could not provide enough information for the phylogenetic analysis. Extinct taxa were restricted to the better-preserved Cretaceous stoneflies in Kachin amber, excluding the poorly described *Pinguisoperla* Chen, 2018 and the very likely synonym *Burmaperla* Jouault & Nel, 2022 of *Largusoperla* Chen, Wang & Du, 2018 (Jouault and Nel 2022). Another 24 extant genera of Arctoperlaria were also included in the analysis. The monophyly of Arctoperlaria is well supported by the inner anatomical characters (Zwick 2000) and thus the inclusion of antarctoperlarian taxa in the phylogenetic analysis based on external morphological characters would generate bizarre tree topology. Meanwhile, the comparative discussion has excluded the new fossil in this study from Arctoperlaria. Therefore, Arctoperlaria is removed from the dataset. A putative ancestor with all plesiomorphic character states was regarded as the outgroup. A final matrix of 41 taxa with 85 external morphological characters was constructed (see Supplementary File 2).

The data matrix was subjected to a parsimony analysis with the program TNT 1.5 (Goloboff et al. 2008; Goloboff and Catalano 2016) using new technology search. Other parameters were set as default. On the calculated strict consensus tree, character state transformations were visualized using WINCLADA (Version 1.00.08). The Bayesian inference was performed with the software MRBAYES 3.2.7 (Ronquist et al. 2012). The parameters used in MRBAYES were generations = 10,000,000, samplefreq = 50, rates = gamma, burn-in = first 25% of sampled trees. A majority consensus tree was built with the remaining generations, and the posterior probability of each node was calculated. Convergence was checked with the average standard deviation of split frequency, which remained below 0.01. All tree files were adjusted and visualized in FIGTREE v. 1.4.2 (available from <http://tree.bio.ed.ac.uk/software/figtree>).

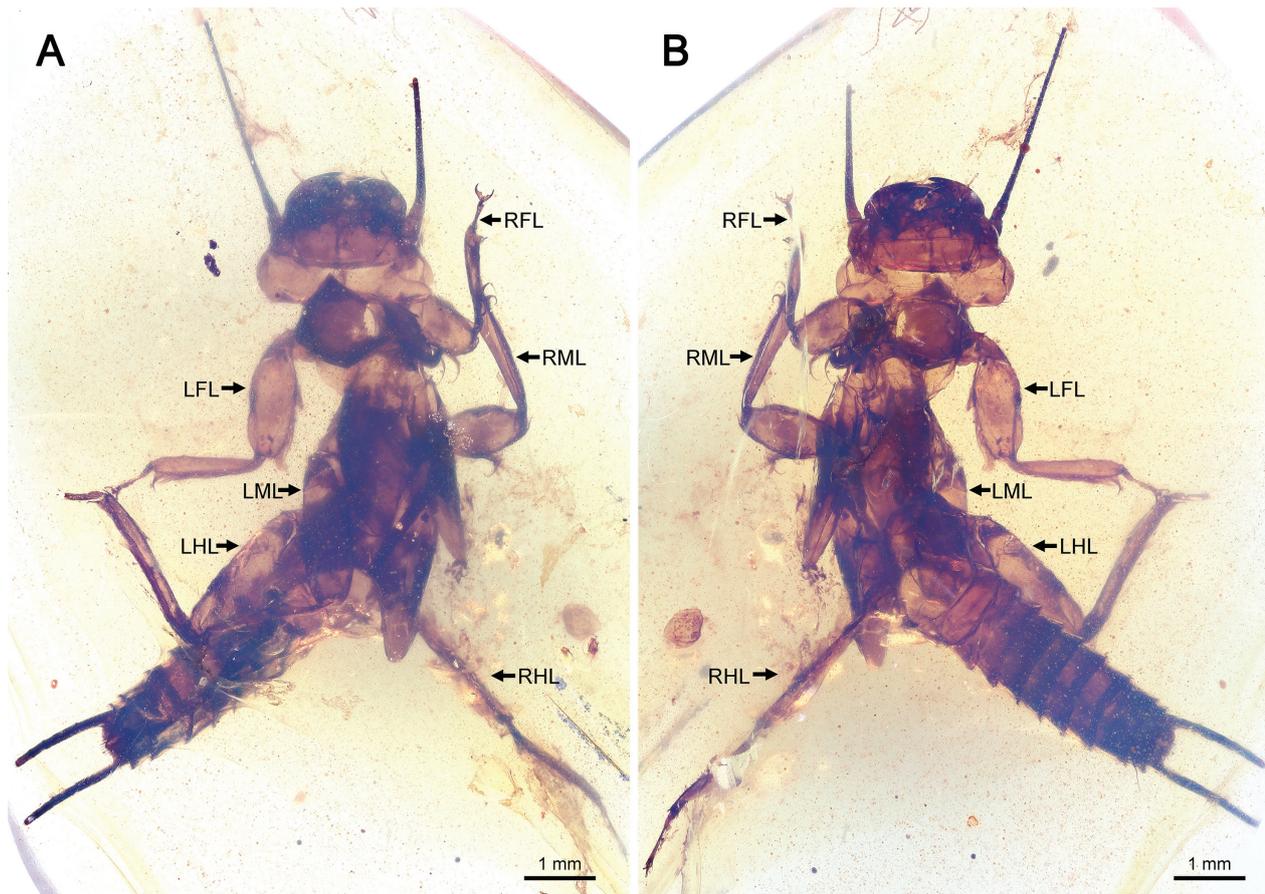


Figure 1. *Kachinoperla zwicki* gen. et sp. nov., holotype, CZT-PLE-MA13. **A** Habitus, dorsal view. **B** Habitus, ventral view. Abbreviations: LFL, left foreleg; LML, left midleg; LHL, left hind leg; RFL, right foreleg; RML, right midleg; RHL, right hind leg. Scale bars: 1 mm.

2.4. Abbreviations

UFP – is used for the undetermined family in Perloidea. The following institutional abbreviation is used: **ICJUST** – Insect Collection of Jiangsu University of Science and Technology, Jiangsu Province, China. The following abbreviations of morphological characters are used: **BL** – body length (excluding antennae and cerci); **HL** – head length; **HW** – head width; **ATL** – antenna length (partially preserved); **MPL** – maxillary palp length; **LPL** – labial palp length; **PL** – pronotal length; **PW** – pronotal width; **WL** – wing pad length; **FLL** – foreleg length; **MLL** – midleg length; **HLL** – hind leg length; **ABL** – abdomen length; **ABW** – abdomen width; **CL** – cercus length (partially preserved); **LFL** – left foreleg; **LML** – left midleg; **LHL** – left hind leg; **RFL** – right foreleg; **RML** – right midleg; **RHL** – right hind leg; **CE** – compound eye; **PR** – pronotum; **SM** – submentum; **GL** – glossa; **PG** – paraglossa; **CA** – cardo; **SS** – stipes; **LA** – lacinia; **LP** – labial palp; **MP** – maxillary palp; **AGR** – abdominal gill remnant; **CO** – coxa; **TR** – trochanter; **FE** – femur; **TI** – tibia; **TA** – tarsi; **CL** – claw; **T** – tergum; **ST** – sternum; **PP** – paraproct.

3. Systematic paleontology

Order Plecoptera Burmeister, 1839

Suborder Arctoperlaria Zwick, 1973

Family Kachinoperlidae fam. nov.

<http://zoobank.org/5C1011C7-9F17-4ACB-B6C1-E7B71A293320>

Type and only genus. *Kachinoperla* gen. nov.

Diagnosis. For larvae. Body robust and slightly oblate. Head wider than long; anterior ocellus present but tiny; postocular area with moderate setae; occipital row of short spinules irregularly scattered and sinuous; antennal segment near glabrous, apically with sparse short spines; predaceous mouthparts; glossae near as long as paraglossae; maxilla well-developed; cardo broad, laterally with dense short spines; stipes elongated, with rounded, gla-

brous outer margin; lacinia elongated, with two giant apical teeth and an inner comb of marginal spines; maxillary palpomeres elongated, apical palpomere much thinner and approximately half in length of penultimate segment; mandible with three pointed apical teeth, anterior half of inner margin with a comb of cylindrical spines; molar area unmodified; labial palp similar in length to maxillary palp, apical palpomere thin and slightly shortened, apex obliquely truncate. Pronotum near trapezoidal, with dark margins and two posteromedial pale spots, posterior margin with long spines. Wing pads banded, outline circular, dorsally and laterally covered with dense spinules; notal contour invisible; thoracic gills absent. Legs with dense long swimming hairs; tibia with two giant and several smaller apical spurs; tarsi with first two tarsomeres much shorter than third one. Abdominal segments covered and posteriorly fringed with long cylindrical spines; tergum 10 strongly projected backwards; paraprocts subtriangular, with straight inner margins and rounded posterior margins; cerci with sparse giant spines along inner and outer margins.

Genus *Kachinoperla* gen. nov.

<http://zoobank.org/24E713C3-D568-45A5-947B-52FA5E401ED3>

Type and only species. *Kachinoperla zwicki* sp. nov.

Etymology. The genus name is a combination of the type locality in the Kachin State of Myanmar and the stonefly genus *Perla* Geoffroy, 1762. The name is feminine in gender.

Diagnosis. By monotypy, as for the type species.

Kachinoperla zwicki sp. nov.

<http://zoobank.org/924CBD2C-1BAF-4410-AF89-4C81AD-28d94A>

Figs 1–5

Etymology. The species is named after Dr. Peter Zwick, who has made substantial contributions to the knowledge of Plecoptera.

Type material. Holotype: exuvia (No. CZT-PLE-MA13), deposited in the Insect Collection of Jiangsu University of Science and Technology (ICJUST).

Locality and horizon. Amber mine near Noije Bum Village (26°20'N, 96°36'E), Tanai Township, Myitkyina District, Kachin State, northern Myanmar. Horizon unnamed, mid-Cretaceous, Upper Albian to Lower Cenomanian (98.79±0.62 Ma).

Diagnosis. As for the genus.

Description. Body robust and slightly oblate (Fig. 1). Surface generally brown to dark brown, with hairs and setae of various size and shape (Fig. 1). — **Head:** Head wider than long (Fig. 2), anterior area of ecdysial suture dark brown. Anterior ocellus tiny; compound eyes large and rounded, length approximately 0.5× head length; postocular area fringed with moderately long setae. Occiput pale brown (Fig. 2A), with a transverse row of short spinules, the irregular spinules scattered and sinuous. Antennae partially preserved, dark brown and slender, each antennal segment wider than long, near glabrous, apically fringed with sparse short spines. Mouthparts of predaceous type (Fig. 2). Glossae of labium approximately as long as paraglossae. Maxilla well-developed; cardo broad, lateral surface covered with dense short spines; stipes elongated, with rounded, glabrous outer margin, boundary with lacinia invisible; lacinia long, outer margin circular, with two giant apical teeth and an inner comb of moderate marginal spines basal to the second apical tooth; the second shorter apical tooth of left lacinia truncate. Maxillary palp with four visible elongated palpomeres, distal palpomere much thinner and approximately half in length of previous segment. Mandible with three pointed apical teeth, anterior half of inner margin with a marginal comb of cylindrical, stout spines posterior to the base of apical teeth; molar area unmodified. Labial palp similar in length to maxillary palps, with three elongated palpomeres; distal palpomere thinner and slightly shorter than previous segments, apex obliquely truncate. All visible maxillary and labial palpomeres glabrous. — **Prothorax:** Pronotum wide and short with obtuse corners (Fig. 2A), mostly dark brown, margins darker, anterior margin longer than posterior margin; surface glabrous, posteromedial area near the median suture with two irregular pale spots, posterior margin fringed with long cylindrical spines (Fig. 2A, B). — **Meso- and metathorax:** Wing pads with anterior and posterior parts pale brown, medial part dark brown (Fig. 3); all wing pads with same size on meso- and metathorax; each wing pad with a transverse row of moderately long spines near anterior margin, dorsal surface and lateral margin mostly covered with dense patch of collapsed short spinules, apical one third of wing pad near glabrous; posterior notal contour not apparent. Thoracic gills not visible (Fig. 3D). — **Legs:** Legs covered by irregular patches of setae with various size and shape (Fig. 4). Coxae and trochanters dorsally with moderate spines along lateral margins. Femora dorsally and ventrally covered with long cylindrical spines along anterior margin and posterior areas, posterior margin with dense long swimming hairs. Tibiae with several moderate spines and dense long swimming hairs along posterior margin, dorsal and ventral surfaces without obvious spines. Two giant and several smaller tibial spurs present. Tarsi with first two tarsomeres very short, third one approximately 2× longer than first two combined and apically with several spines. Tarsal segments with sparse long swimming hairs along outer margin. Claws sharp and glabrous, inner margin with pronounced denticles. — **Abdomen:** Abdominal segments dark brown, each

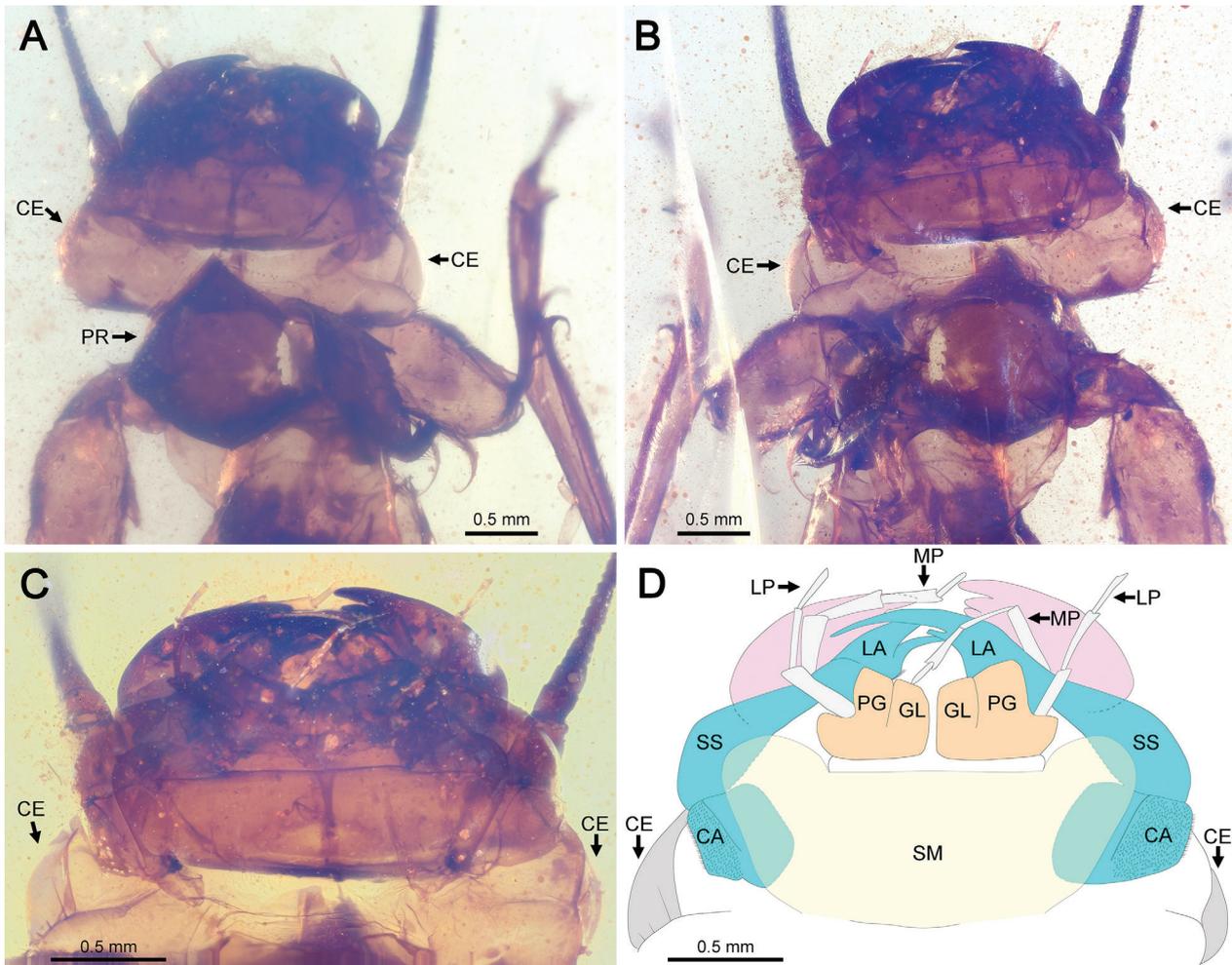


Figure 2. *Kachinoperla zwicki* gen. et sp. nov., holotype, CZT-PLE-MA13. **A** Head and prothorax, dorsal view. **B** Head and prothorax, ventral view. **C** Head, ventral view. **D** Drawing of head, ventral view. Abbreviations: CE, compound eye; PR, pronotum; SM, submentum; GL, glossa; PG, paraglossa; CA, cardo; SS, stipes; LA, lacinia; LP, labial palp; MP, maxillary palp. Scale bars: 0.5 mm.

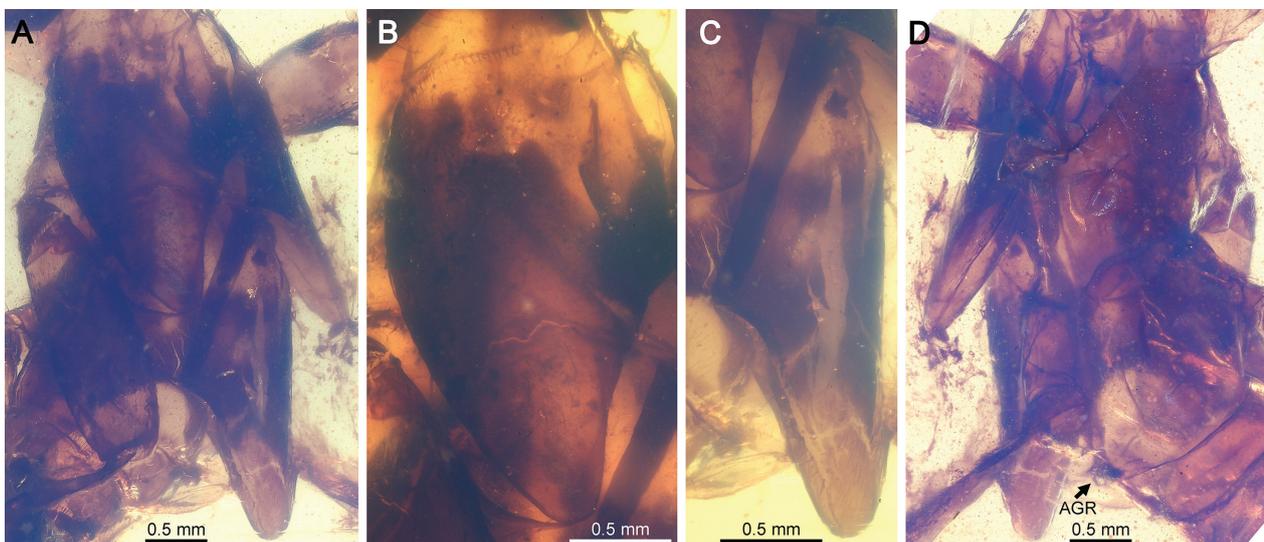


Figure 3. *Kachinoperla zwicki* gen. et sp. nov., holotype, CZT-PLE-MA13. **A** Mesothorax and metathorax, dorsal view. **B** Left forewing pad, dorsal view. **C** Right hind wing pad, dorsal view. **D** Mesothorax and metathorax, ventral view. Abbreviation: AGR, abdominal gill remnant. Scale bars: 0.5 mm.

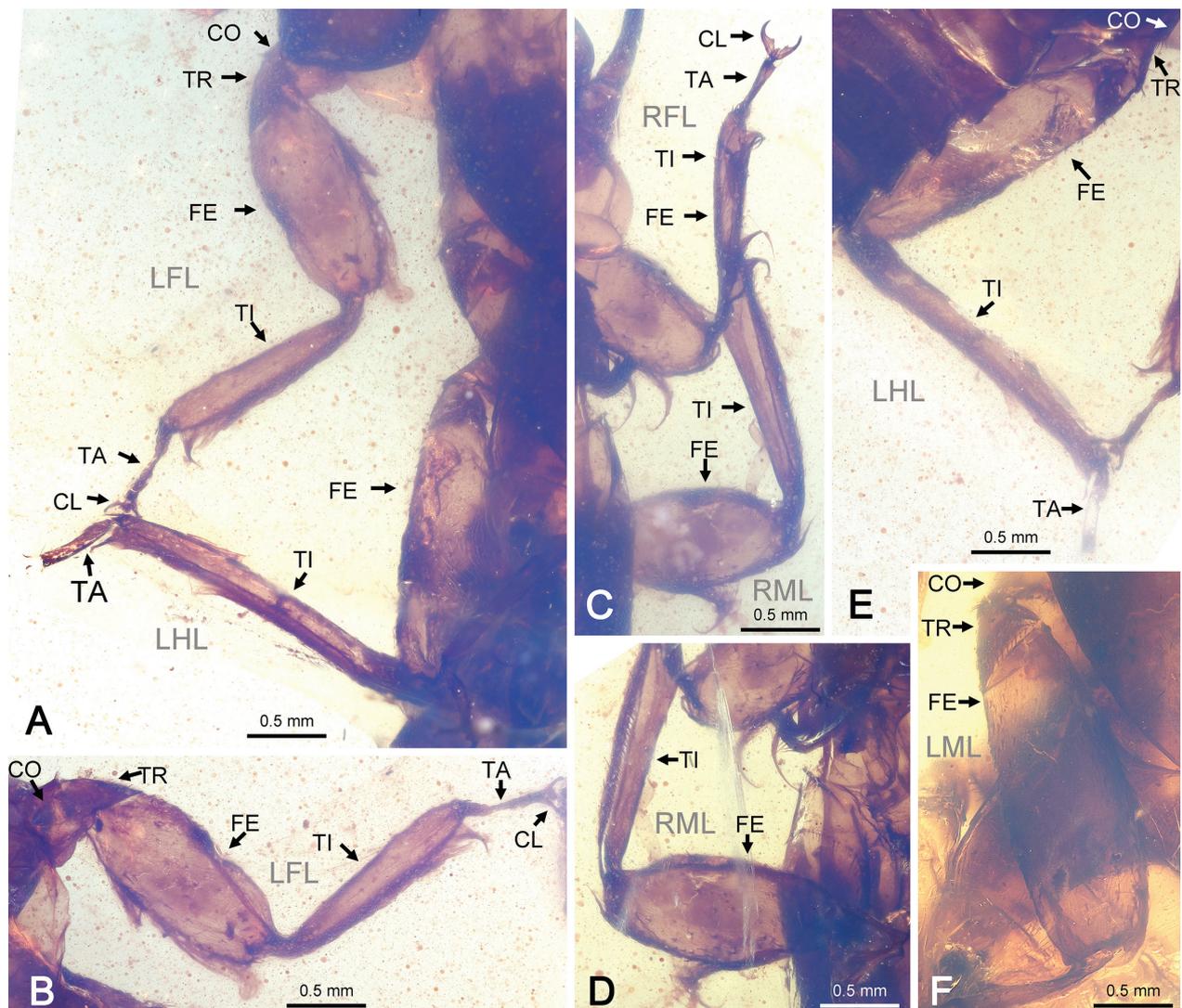


Figure 4. *Kachinoperla zwicki* gen. et sp. nov., holotype, CZT-PLE-MA13. **A** Left foreleg and left hind leg, dorsal view. **B** Left foreleg, ventral view. **C** Right foreleg and right midleg, dorsal view. **D** Right midleg, ventral view. **E** Left hind leg, ventral view. **F** Left midleg, dorsal view. Abbreviations: LFL, left foreleg; LML, left midleg; LHL, left hind leg; RFL, right foreleg; RML, right midleg; RHL, right hind leg; CO, coxa; TR, trochanter; FE, femur; TI, tibia; TA, tarsi; CL, claw. Scale bars: 0.5 mm.

segment covered and posteriorly fringed with long cylindrical spines (Fig. 5). Two distinct abdominal gill remnants present anterior to the first segment (Figs 3D; 5C). Tergum 10 strongly projected backwards, near triangular in shape, exceeding posterior margin of paraprocts (Fig. 5B). Paraprocts subtriangular, inner margins straight and with dense giant spines, posterior margins rounded and glabrous, posterior margins blunt (Fig. 5D). Cerci partially preserved, dark, inner and outer margins fringed with sparse rows of giant spines, each segment slightly longer than wide (Fig. 5B).

Measurements. BL 9.5 mm, HL 2.0 mm, HW 2.5 mm, ATL 4.0 mm (partially preserved), PL 1.2 mm, PW 2.0 mm, WL 3.0 mm, FLL 5.0 mm, MLL 6.0 mm, HLL 7.0 mm, ABL 5.0 mm, ABW 1.5 mm, CL 2.0 mm (partially preserved).

4. Discussion

4.1. Morphological phylogeny and systematic placement

The absence of well-developed abdominal gills as either lateral or anal appendages can easily exclude *Kachinoperla* gen. nov. from the suborder Antartoperlaria (Benedetto 1974; Heckman 2008; Pessacq et al. 2020). The toothed lacinia and sharp-cusped mandibles of *Kachinoperla* indicate its grasping and holding predaceous food habit, which excludes its placement in the phytophagous Euholognatha (Fig. 6A–I) of Arctoperlaria (Stewart 2009). The placement of *Kachinoperla* in the other arctoperlariid infraorder Systellognatha is well supported by its predaceous type of mouthparts and slender palps, which

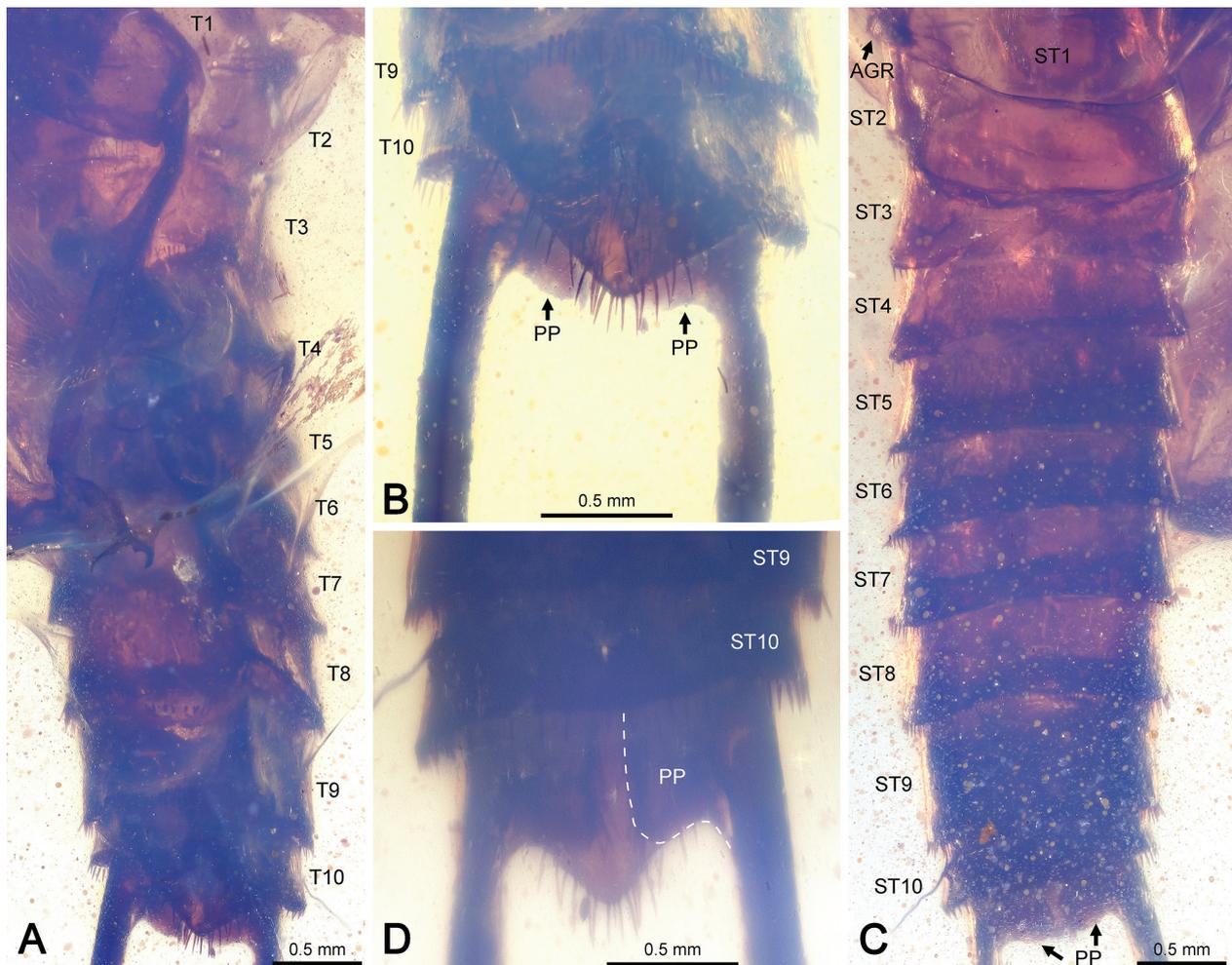


Figure 5. *Kachinoperla zwicki* gen. et sp. nov., holotype, CZT-PLE-MA13. **A** Abdomen, dorsal view. **B** Abdominal terminal segments, dorsal view. **C** Abdomen, ventral view. **D** Abdominal terminal segments, ventral view. Abbreviations: T, tergum; ST, sternum; PP, paraproct; AGR, abdominal gill remnant. Scale bars: 0.5 mm.

are also known as diagnostic larval characters for the superfamily Perloidea (Fig. 7A–F) within Systellognatha (Zwick 1980). Conversely, in the other systellognathan superfamily Pteronarcyzoidea (Fig. 7G–L), mouthparts of the three families, Peltoperlidae, Pteronarcyzoidea, and Styloperlidae, are all phytophagous (Uchida and Isobe 1989; Stewart 2009). Apart from the mouthparts, *Kachinoperla* can further be distinguished from Peltoperlidae by lacking the cockroach-like larval habitus which has a much wider thorax than the head and abdomen (Uchida and Isobe 1989). *Kachinoperla* differs from Pteronarcyzoidea in the absence of pronotal projections and the invisibility of notal contours between wing pads (Nelson 1988; Myers and Kondratieff 2017). *Kachinoperla* is also easily separated from Styloperlidae by the presence of giant tibial spurs, which are replaced by numerous trifurcate setae in Styloperlidae (Uchida and Isobe 1989). Thus, *Kachinoperla* seems to be closely related to Perloidea. However, the subequal glossae and paraglossae of *Kachinoperla* firmly prevent attribution to Perloidea, since the glossae much shorter than paraglossae is an apomorphic character

defining the monophyly of Perloidea (Fig. 7A–F) (Zwick 2000). In addition to the proportion between glossae and paraglossae, the relative length between the maxillary and labial palps and the modification of their apical segments in both adult and immature stages are taxonomically informative, especially for higher taxa (families) and have been widely used in the classification of stoneflies (Stewart and Harper 1996; Stewart and Stark 2002; Jaihao and Phalaraksh 2011; Chen and Du 2018). Typical examples of apomorphically modified apical palpal segments are the circular and enlarged apical labial palpomere in Nemouridae (Fig. 6E) and the asymmetrically inserted, minute apical maxillary palpomere in Chloroperlidae (Fig. 7E–F). Characters of these palps are more useful in the fossil taxa because other characters of the fossils are not always well preserved and available for comparison. Apart from the subequal glossae and paraglossae, *Kachinoperla* is further excluded from the four extant families within Perloidea by the following diagnostic characters: from Perlidae by the absence of highly branched gills on sides and venter of all thoracic segments (Zwick 2000;

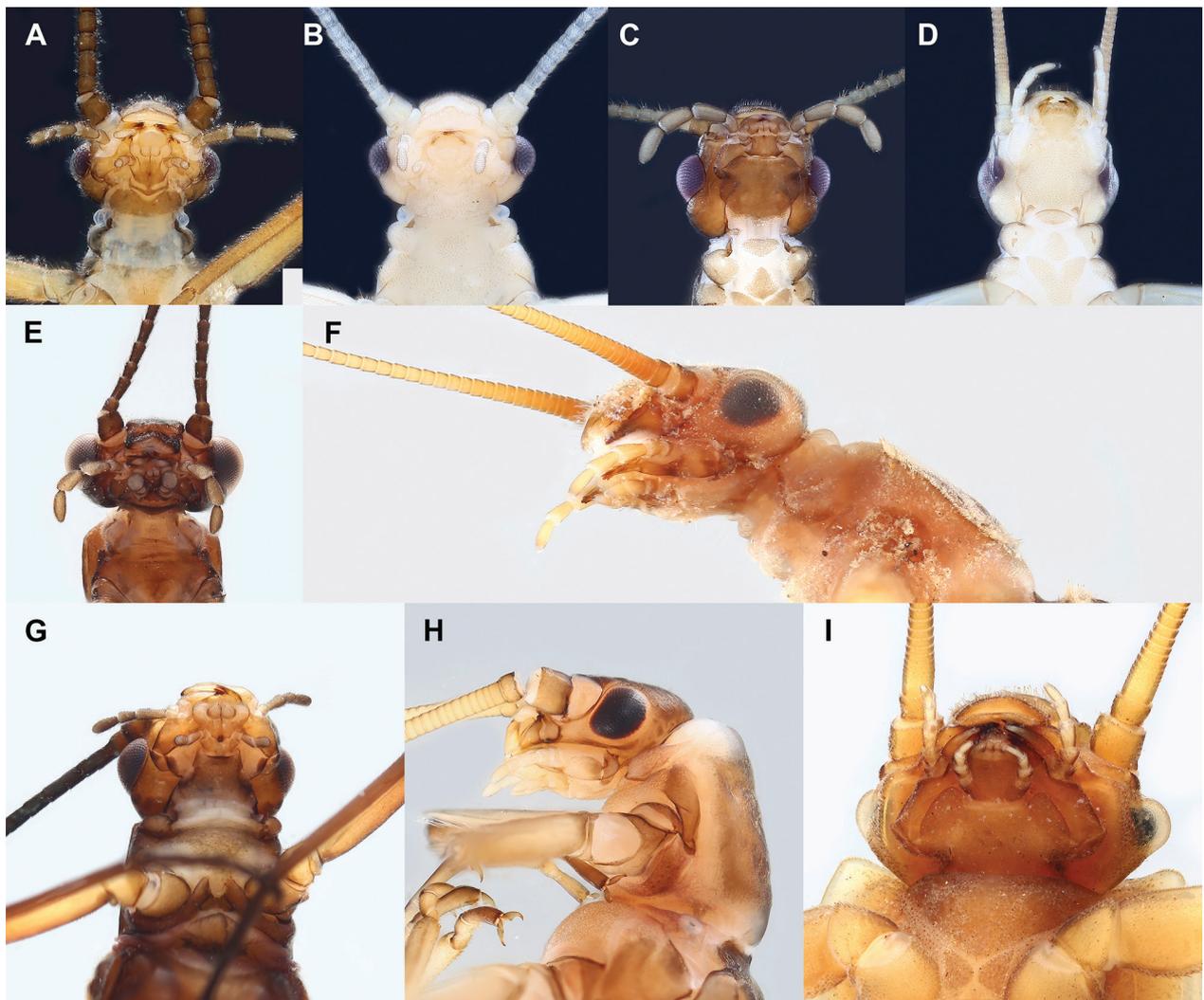


Figure 6. Some representatives of the extant Euholognatha, head in ventral view. **A** *Capnia zijingshana* Du & Chen, 2016 (Capniidae), adult. **B** *C. zijingshana*, larva. **C** *Rhopalopsole vespertilio* Chen & Du, 2017 (Leuctridae), adult. **D** *R. vespertilio*, larva. **E** *Nemoura nankinensis* Wu, 1926 (Nemouridae), adult. **F** *N. nankinensis*, larva. **G** *Taenionema* sp. (Taeniopterygidae), adult. **H** *Taenionema* sp., larva. **I** *Scopura longa* Uéno, 1929, larva.

Stewart and Stark 2002); from Chloroperlidae by the absence of asymmetrically inserted, minute apical maxillary palpomere, pronotum not oval, body not slender and metathoracic wing pads not parallel to the body axis (Zwick 2000; Stewart and Stark 2002); from Perlodidae by the absence of complicated pigmented pattern or chaetotaxy on the head and thoracic terga (Stewart and Harper 1996; Stewart and Stark 2002; Chen 2022b); and from Kathroperlidae by the absence of strongly elongated head and autapomorphic semiquadrate lacinia with no subterminal tooth (Zwick 2006; South et al. 2021). In conclusion, *Kachinoperla* undoubtedly belongs to the infraorder Systellognatha but cannot be attributed to any of the 17 extant stonefly families, including Perlidae and Peltoperlidae, which also comprise fossil records in Kachin amber (Chen et al. 2018; Chen and Xu 2020).

Larval descriptions are available for many extinct stoneflies of Systellognatha: Permian Palaeoperlidae,

Tshekardoperlidae, and *Tungussonympha* (undetermined family in Perloidea, abbreviated as UFP below); Triassic *Berekia* Sinitshenkova, 1987 (UFP) and *Triassoperla* Lin, 1977 (UFP); Jurassic *Derancheperla* Sinitshenkova, 1990 (Perlodidae), *Isoperlodes* Sinitshenkova, 1992 (Perlodidae), Platyperlidae, *Bestioperlisca* Sinitshenkova, 1990 (UFP), *Chloroperloides* Sinitshenkova, 1985 (UFP), *Perlisca* Sinitshenkova, 1985 (UFP), *Perlomimus* Sinitshenkova, 1985 (UFP), *Trianguliperla* Sinitshenkova, 1985 (UFP), and *Perlitodes* Sinitshenkova, 1987 (UFP); and Cretaceous *Dipsoperla* Sinitshenkova, 1987 (Chloroperlidae), *Ecdyoperla* Sinitshenkova, 1998 (UFP), *Savina* Sinitshenkova, 1987 (UFP), and the *Electroneuria* (Perlidae) known from Kachin amber.

Almost all known larval fossils of Plecoptera are poorly preserved as fossil impressions and their descriptions and comparisons with other larvae or adults are restricted to the shapes and ratios of general body parts, whereas

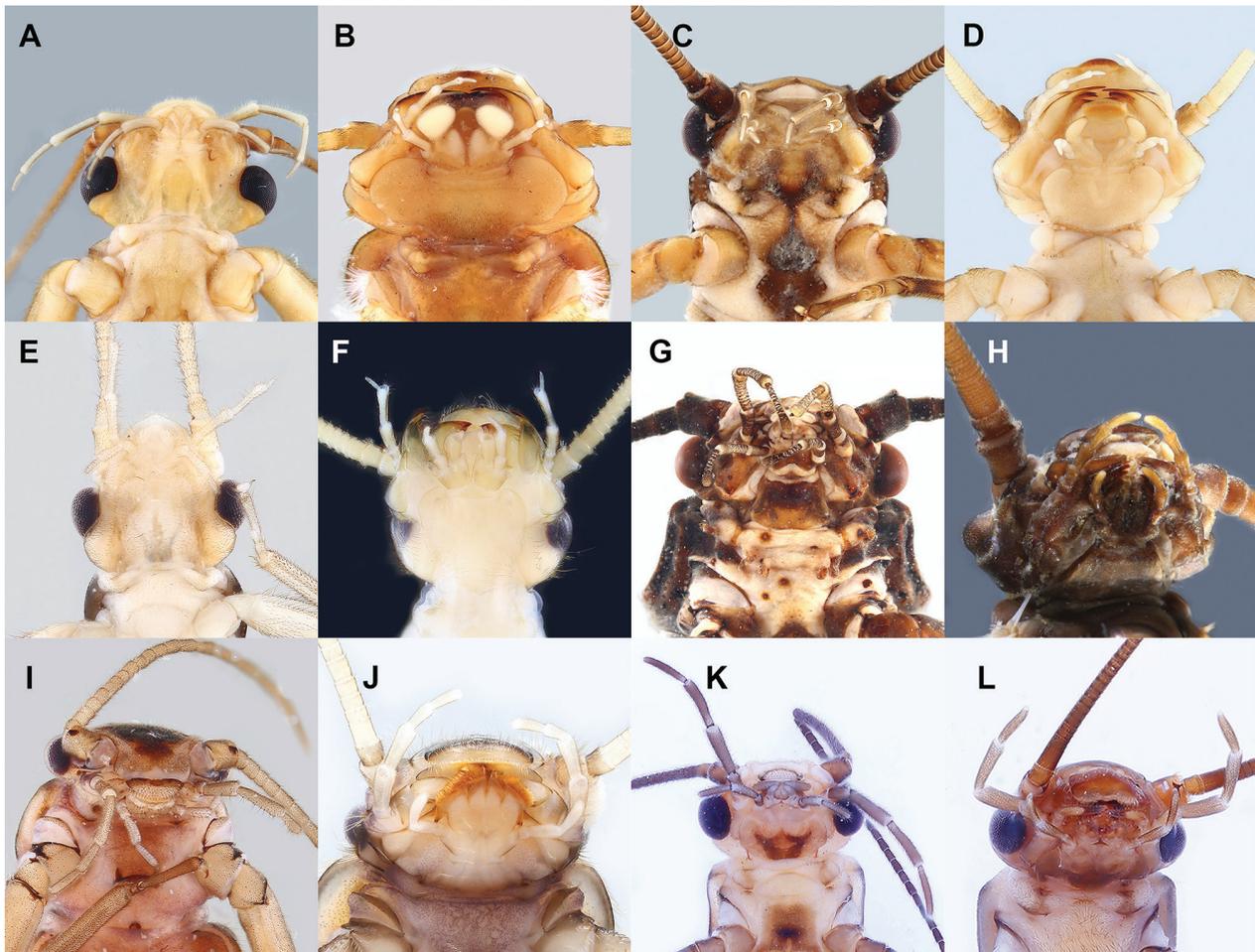


Figure 7. Some representatives of the extant Systellognatha, head in ventral view. **A** *Kamimuria petasus* Chen, 2019c (Perlidae), adult. **B** *K. petasus*, larva. **C** *Neofilchneria wanglana* Chen, 2019d (Perlodidae), adult. **D** *N. wanglana*, larva. **E** *Suwallia wolongshana* Du & Chen, 2015 (Chloroperlidae), adult. **F** *S. wolongshana*, larva. **G** *Pteronarcys sachalina* Klapálek, 1908 (Pteronarcyidae), adult. **H** *P. sachalina*, larva. **I** *Microperla retroloba* (Wu, 1937) (Peltoperlidae), adult. **J** *M. retroloba*, larva. **K** *Styloperla inae* Chao, 1947 (Styloperlidae), adult. **L** *S. inae*, larva.

the more informative structures such as the mouthparts remain unclear (Sinitshenkova 1987). Apart from the apparent difference in age, *Kachinoperla* notably differs from the Permian Palaeoperlidae, Tshekardoperlidae, and *Tungussonympha* by the thoracic segments without clearly visible notal contours between wing pads, and furthermore from Tshekardoperlidae by each antennal segment not elongated (Sinitshenkova 1987; Sroka et al. 2018). *Kachinoperla* apparently differs from the Triassic *Berekia* and *Triassoperla* by the head much larger than the pronotum, pronotum trapezoidal, and wing pads broad and semicircular; in the two latter genera, the head is rounded and much smaller than the pronotum, the pronotum is respectively horseshoe-shaped and rectangular, and the wing pads are slender and near cylindrical (Lin 1977; Sinitshenkova 1987).

When compared with the Jurassic taxa, *Kachinoperla* differs from *Derancheperla* by the invisibility of notal contour between wing pads (Sinitshenkova 1990); from *Isoperlodes* by the pronotum near twice as long as the

width, subequal in width to meso- and metanota (vs. pronotum length subequal to the width, much narrower than meso- and metanota), wing pads on mesonotum with circular lateral margins (vs. wing pads with angled anterolateral margins and near straight lateral margins), and by the invisibility of notal contour between wing pads (Sinitshenkova 1992); from Platyperlidae by the head broad and near trapezoidal (vs. head small and circular), and the invisibility of notal contour between wing pads (Sinitshenkova 1985, 1987); from *Bestioperlisca* by the wing pads not parallel to the body axis (vs. parallel), lateral pronotal margins not straight (vs. straight), and the femora at least 2.5 times wider than tibiae (vs. femora slightly wider than tibiae) (Sinitshenkova 1990); from *Chloroperloides* by the head broad and wider than pronotum (vs. head small and circular, much smaller than pronotum), and the wing pads large (vs. wing pads small) (Sinitshenkova 1985); from *Perlisca* by the head broad and wider than pronotum (vs. head small and circular, much smaller than pronotum), and the invisibility of notal contour

between wing pads (Sinitshenkova 1985); from *Perlo-mimus* by the head broad and wider than pronotum (vs. head small and circular, much smaller than pronotum), thorax normal, meso- and metanota near as wide as pronotum (vs. thorax strikingly widened, meso- and metanota at least 1.5 times wider than pronotum) (Sinitshenkova 1985); from *Trianguliperla* by the head broad and much larger than the trapezoidal pronotum (vs. head triangular, much smaller than the elliptical pronotum), and the presence of large wing pads (vs. complete absence of wing pads) (Sinitshenkova 1985); and from *Perlitodes* by all wing pads with the same size (vs. posterior wing pads distinctly wider than anterior ones) and the invisibility of notal contour between wing pads (Sinitshenkova 1987).

When compared with the three Cretaceous genera known by fossil impressions, *Kachinoperla* can be distinguished from *Dipsoperla* by the head two times longer than pronotum (vs. head almost as long as pronotum), abdominal sterna complete (vs. abdominal sterna divided by a longitudinal median membrane) (Sinitshenkova 1987); from *Ecdyoperla* by the pronotum without posterior projections (vs. pronotum with projections on hind angles), and the wing pads long and semicircular (vs. wing pads short and rounded) (Sinitshenkova 1998); from *Savina* by the large head longer and wider than pronotum (vs. head small rounded, subequal in size and shape to pronotum), the wing pads broad (vs. wing pads slender), and the invisibility of notal contour between wing pads (Sinitshenkova 1987). The only well-preserved fossil stonefly exuvia is that of *Electroneuria* in Kachin amber, which provided the first chance to study the larval mouthparts and chaetotaxy of extinct stoneflies in a modern way. *Electroneuria* was attributed to the family Perlidae based on the predaceous mouthparts, long and slender palps, the presence of an occipital row of short spinules (although this character is also found in Perlodidae, see recent examples in Chen 2022b), the presence of thoracic gills, the invisibility of notal contour between wing pads, robust body, and long cerci (Sroka et al. 2018). The absence of thoracic gills (diagnostic of Perlidae) and the presence of projected abdominal tergum 10, which is an indication of a giant supra-anal process or epiproct in the male adult (reduced in Perlidae), are the most crucial characters separating *Kachinoperla* from *Electroneuria* (Zwick 2000; Sroka et al. 2018).

Apart from *Electroneuria*, all other stoneflies in Kachin amber were described based on adult morphology. Among these, all the male specimens of Perlidae were exclusively and certainly attributed to the subfamily Acroneuriinae Klapálek, 1914 by the presence of hammer on abdominal sternum 9, absence of projected hemiterga, and absence of hair brush on mesal areas of abdominal sterna (Sivec et al. 1988; Zwick 2000; Chen et al. 2018; Sroka et al. 2018; Chen 2019a, 2019b, 2020a, 2022c; Chen and Xu 2021; Jouault et al. 2022). *Starkoperla* Chen & Wang, 2020 is a perlid genus erected based on female morphology, which exhibits much longer and anteriorly located paraglossae than glossae, and the apomorphic elongation of the neck and cercal segments that can easily distinguish it from *Kachinoperla* (Chen and Wang 2020). Another genus of Perlidae with a female holotype is *Burperla* Chen, 2020,

which is mainly characterized by the strikingly elongated maxillary and labial palps (longer than the head), RP vein reaching wing apex, and the broad and rounded subgenital plate with projected posterior margin (Chen 2020b). The non-elongated palps much shorter than the head exclude the close relationship between *Kachinoperla* and *Burperla*.

The two genera of Peltoperlidae, *Zwickoperla* Chen & Wang, 2020 and *Borisoperla* Chen & Xu, 2020, are characterized by the shortened head strongly inserted into the angulate prothorax (autapomorphy of Peltoperlidae) and share other similar characters with the extant peltoperlids (Chen and Xu 2020). The undescribed larvae of the two peltoperlids are expected to be cockroach-like as typical for the family (Zwick 2000) and which is apparently divergent from the regular larval habitus of *Kachinoperla*.

The subequal glossae and paraglossae of *Kachinoperla* are reminiscent of another extinct family Petroperlidae known from Kachin amber (Sroka et al. 2018; Sroka and Staniczek 2020). Petroperlidae is currently represented by four species in four genera, *Petroperla mickjaggeri* Sroka, Staniczek & Kondratieff, 2018, *Lapisperla keithrichardsi* Sroka, Staniczek & Kondratieff, 2018, *Branchioperla ianstewarti* Sroka & Staniczek, 2019, and *Ovaloperla staniczeki* Chen & Xu, 2022. The four species share the common characters distinguishable from *Kachinoperla*: the apical maxillary palpomere is subequal in length to previous segments but distinctly shortened in *Kachinoperla*; the labial palps are rather short, near half in the length of maxillary palps, whereas they are very long and about as long as maxillary palps in *Kachinoperla*; the apical labial palpomere is longer than previous segments (evident in the three former species but hardly visible in *O. staniczeki*) whereas it is shorter than previous segments in *Kachinoperla*; the pronotum has parallel lateral margins but it is trapezoidal in *Kachinoperla* (Sroka et al. 2018; Sroka and Staniczek 2020). Furthermore, the striking retention of highly-branched cervical and abdominal gills in the adult of *B. ianstewarti* suggests the existence of such developed gills in the larval stages of Petroperlidae, which is distinctly divergent from the state (absence of such highly-branched gills) in *Kachinoperla*.

Perspicuosoperlidae is another recently established extinct family from Kachin amber, which is regarded as the stem group of Euholognatha + Systellognatha (Chen 2022a). Although the maxillae and mandibles are vestigial in the female adult of Perspicuosoperlidae and are not available for comparison, the family was excluded from Systellognatha based on the unique egg structure and wing venation (Chen 2022a). On the contrary, *Kachinoperla* is well supported in Systellognatha based on its predaceous larval mouthparts (Zwick 1980). Moreover, the apical maxillary palpomere is distinctly shortened in *Kachinoperla* but remains unmodified in Perspicuosoperlidae; the labial palp is very long (about as long as maxillary palp) in *Kachinoperla* but it is short (length near $\frac{2}{3}$ of maxillary palp) in Perspicuosoperlidae (Chen 2022a).

Maximum parsimony searches and Bayesian inference were conducted to investigate the systematic position of *Kachinoperla* (Fig. 8). The monophyly of the following

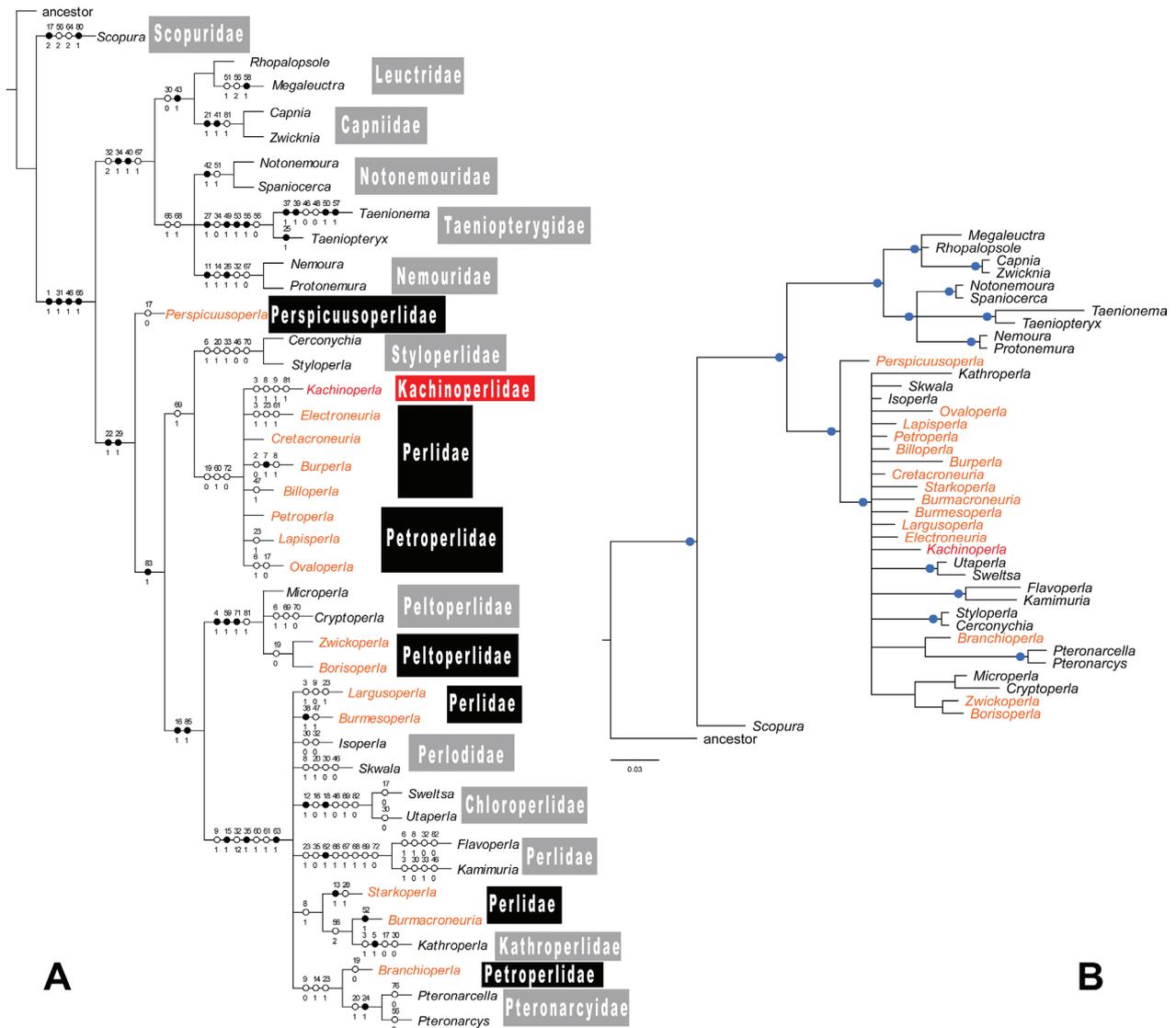


Figure 8. Phylogenetic trees based on morphological characters. **A** The strict consensus tree of the most parsimonious trees generated by TNT with morphological characters visualized on the cladogram using WINCLADA. The white and black circles represent homoplastic and non-homoplastic characters, respectively. Names of the taxa from Kachin amber are indicated with orange and red. The family names are in gray boxes for the extant taxa and in black boxes for the extinct taxa. The numbers above the circles are the character numbers; the numbers below are character states. **B** Bayesian tree. Blue circles at nodes represent posterior probabilities equal to or higher than 0.8.

extant families is supported in both analyses: Leuctridae, Capniidae, Notonemouridae, Taeniopterygidae, Nemouridae, Styloperlidae, Chloroperlidae, and Pteronarcyidae. A basal position of Scopuridae in Arctoperlaria was recovered by the external morphology, but the family has been widely supported as the sister-group of the superfamily Nemouroidea comprising Leuctridae, Capniidae, Notonemouridae, Taeniopterygidae, and Nemouridae (Zwick 2000). The monophyly of Nemouroidea is supported herein. The two extinct genera of Peltoperlidae were grouped with their extant relatives by several autapomorphies. As a highly diverse family both at present and during the Cretaceous, Perlidae was not supported as monophyletic. The Cretaceous perlids are scattered

in the big clade of Systellognatha. The extinct Perspicuusoperlidae was recovered as a sister group of Systellognatha, which is in agreement with the speculation of Chen (2022a) that Perspicuusoperlidae is a stem group of Euholognatha + Systellognatha. The monophyly of another extinct family, Petroperlidae, was not supported in the analyses, but its close relationship with several extinct genera of Perlidae and the newly described *Kachinoperla* is suggested. *Kachinoperla* is recovered in a relatively basal position within Systellognatha but does not form any further terminal clades with other stoneflies known from Cretaceous Kachin amber. The main cause for several unclear relationships might be the lack of sufficient morphological characters available in these fossils.

Although larval characters of stoneflies can vary between different instars, the studied exuvia corresponds to the mature (last instar) larva, which has more stable and distinct characters than younger instars and is the research object of almost all larva-related taxonomic and phylogenetic studies in Plecoptera. During the comparison of stoneflies in Kachin amber, the main diagnostic characters include the size of mouthparts, the length of cercal segments, the shape of pronotum, and the body color. Abundant extant evidence (Figs 6–7; Stewart and Harper 1996; Stewart and Stark 2002; Jaihao and Phalaraksh 2011; Chen and Du 2018; Chen 2020c, 2022b; Chen et al. 2021) supports the tendency to retain the above-mentioned diagnostic characters from mature larva to adult stages. The body color in the character list (see Supplementary File 1) has two extreme character states (colorless or patterned) to distinguish the single entirely colorless *Burperla* from other taxa, which does not affect the comparison between *Kachinoperla* and other stoneflies. Therefore, the selected morphological characters in this study are reliable and comparable between larval and adult stages.

Based on the comparative discussions and phylogenetic analysis, *Kachinoperla* differs from any related extant and extinct taxa and is considered a basal group in Systelognatha according to its larval structures and other general external characters.

4.2. Evolutionary and paleoecological implications

The hitherto five stonefly families known from Kachin amber of northern Myanmar, i.e., Perlidae, Petroperlidae, Peltoperlidae, Perspicuosoperlidae, and Kachinoperlidae, have exhibited both predaceous and phytophagous types of mouthparts, which suggests that the divergent evolution of feeding habits in stoneflies must have started earlier than the mid-Cretaceous. The various types of mouthparts and diverse feeding habits persist in extant Plecoptera, which allow different species to fill every conceivable major food niche in streams (Stewart 2009).

The coloration of *Kachinoperla* is composed of a bicolor head, a dark brown pronotum with pale spots, banded wing pads, banded femora of pale brown legs, dark brown abdominal segments, dark brown antennae, and cerci, which contrasts with the unpigmented larva (more likely to be an exuvia) of *Electroneuria* known also from Kachin amber. Such contrasting larval coloration might be non-functional, but it has apparently diverged from the common situation of aquatic insect larvae usually exhibiting subdued, cryptic coloration due to constraints imposed by the light environment of freshwater habitats (Hutchinson 1981). Coloration might be a product of sexual selection or Batesian mimicry, or even visual signals of aposematism to warn the potential predators (Foster et al. 2021). Even if *Kachinoperla* had aposematic larvae, it is not possible to examine the chemical compositions of defensive secretions or testing its unpalatability to predators. Such methodological difficulty in analyzing chemical defenses also exists in extant

semi-aquatic insect orders including stoneflies (Newman 1991).

The dorsal ecdysial suture of *Kachinoperla* consists of a Y-shaped epicranial suture, and a longitudinal thoracic suture extended from the anterior of pronotum to the posterior of metanotum, which is identical to all extant stoneflies (Stewart and Stark 2002; Heckman 2008). This suggests that the emergence pathway of Plecoptera is highly conserved, i.e., the adult gradually emerges from the mature larva through the split dorsal ecdysial suture (Snodgrass 1947).

The giant larval wing pads of *Kachinoperla* are signals of macropterous adults in the family. All other adults reported from Kachin amber are also macropterous. The reduction or complete loss of wings is quite common in extant stoneflies but it is rarely reported from fossils. Except for the genetic factor (Nebeker and Gaufin 1967), numerous environmental factors are related to the brachypterous condition of stonefly adults, including higher altitudes (Hynes 1941, 1974; Brinck 1949), poor nutrition and low temperatures (Lillehammer 1976), long photoperiod that reduces larval growth (Khoo 1968), isolated habitats (Brinck 1949), more lentic water habitats such as lakes (Donald and Patriquin 1983), glaciation in mountains (Donald and Patriquin 1983), and other stimuli delaying the small larvae to emerge late in the season (Khoo 1964, 1968). The absence of wing reduction or loss in stoneflies from Kachin amber indicates that the situation in the ancient Burmese forest was, in principle, contrary to the above-mentioned environmental factors.

The well-developed swimming hairs on all larval legs of *Kachinoperla* as well as in *Electroneuria* are correlated in extant taxa with strong swimming ability to move upstream in order to avoid passive transportation by fast-flowing streams; such ability is variable among different taxonomic groups in Plecoptera (Hynes 1976; Otto and Sjöström 1986).

The strong and sharp larval claws of *Kachinoperla* are similar to those in *Electroneuria* (Sroka et al. 2018). Such developed larval claws could increase the area and reach for anchoring on the substratum and holding the prey during feeding (Jindal and Singh 2020), facilitating movement and clinging on rough surfaces, especially before emergence (Nelson 1991). This ability would have helped these larvae to explore different niches in the stream such as riffles, pools and swift velocity regions (Jindal and Singh 2020). The discovery of larval characters of *Kachinoperla* provides important evolutionary and paleoecological data for Plecoptera. Future discoveries of plecopteran immature stages will keep shedding light on the evolutionary history of stoneflies.

5. Availability of data and material

All data generated or analyzed during this study are included in this published article. The new material studied is preserved in the Insect Collection of Jiangsu University of Science and Technology (ICJUST, No. CZT-PLA-MA13), Jiangsu Province, China

6. Competing interest

The author declares no competing interests.

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Supplementary material 1

File 1

Authors: Chen Z-T (2022)

Data type: .rtf

Explanation note: List of 85 morphological characters used in the phylogenetic analyses (partially derived from Nelson 1984).

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Link: <https://doi.org/asp.80.e82549.suppl1>

Supplementary material 2

File 2

Authors: Chen Z-T (2022)

Data type: .rtf

Explanation note: Character matrix used in the phylogenetic analyses (partially adapted from Nelson 1984). Not applicable character indicated by “?”.

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