A new species of *Languidipes* Hubbard (Ephemeroptera, Polymitarcyidae) from Borneo (#93881)

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A new species of *Languidipes* Hubbard (Ephemeroptera, Polymitarcyidae) from Borneo

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The genus *Languidipes* is currently represented by three species distributed in southeastern Asian, India, and Sri Lanka. *Languidipes corporaali* is the most widely distributed species, and both, male and female imagos, as well as nymphs, are known. In contrast, the other species, *L. trapobanes* and *L. lithophagus*, are only known from nymphs. Here, we describe a new species, *Languidipes janae*, based on male imagos collected from Borneo, Indonesia. This new species is characterized by the presence of ommation on mesonotum, and penis almost completely divided, with sub-quadrate base and a small outer projection basal to the long and slender distal arms. This constitutes the first record of the genus for Borneo. A cladistic analysis of the subfamily Asthenopodinae corroborates its taxonomic status.

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Abstract

The genus *Languidipes* is currently represented by three species distributed in southeastern Asian, India, and Sri Lanka. *Languidipes corporaali* is the most widely distributed species, and both, male and female imagos, as well as nymphs, are known. In contrast, the other species, *L. trapobanes* and *L. lithophagus*, are only known from nymphs. Here, we describe a new species, *Languidipes janae*, based on male imagos collected from Borneo, Indonesia. This new species is characterized by the presence of ommation on mesonotum, and penis almost completely divided, with sub-quadrate base and a small outer projection basal to the long and slender distal arms. This constitutes the first record of the genus for Borneo. A cladistic analysis of the subfamily Asthenopodinae corroborates its taxonomic status.

Introduction

Polymitarcyidae (Ephemeroptera), with a worldwide distribution, includes large to medium-sized mayflies with burrowing nymphs (Kluge 2004, McCafferty 2004). Strong mandibular tusks of the immature forms are used to dig tunnels in varied kinds of underwater sediments, including mud, clay and even siliceous rocks (Molineri, Salles & Peters 2015, Bolotov et al. 2022). The additional particularity of producing silk in the malpighian ducts, allows them to coat their

burrowings with a thin mesh of this material (Sattler 1967), or even to construct silk cases where tunnels are impossible to dig (Molineri & Emmerich 2010, Pai et al. 2023). Furthermore, adults are so short-lived, that they do not present functional legs (except for the male forelegs, used to grab females in copula), spending their entire life in flight. This forces them to make their subimaginal molt in a unique manner, not shading their cuticle in the classic form (as an entire piece) but in flakes that come off the body and wings (Molineri 2010). Because of their unique biology, including nymphs hidden in the substrates and extremely short-lived adults, specimens of this group are infrequently collected.

The genus *Languidipes* was originally described for *Asthenopus corporaali* Lestage, 1922 from Java, Indonesia. *Languidipes corporaali* (Lestage) was subsequently recorded from other Indonesian localities (Sumatra and Simeulue), as well as from Malaysia and Thailand (Baumgardner et al. 2012). The genus *Languidipes* also includes the species *L. trapobanes* (Hubbard 1984, Rathinakumar et al. 2019, Pai et al. 2023), from India and Sri Lanka, and the recently described *L. lithophagus* (Bolotov et al. 2022) from Myanmar.

A phylogenetic framework has been proposed for the subfamily Asthenopodinae, where *Languidipes* is included together with partially sympatric *Povilla* and other three South American genera (Molineri, Salles & Peters 2015).

Here we describe a new species of *Languidipes* based on male imagos from Borneo, Indonesia, and test its phylogenetic relationships inside the subfamily.

Materials & methods

Specimens are fixed in alcohol 70º, wings of one of them were removed and mounted dry in microscope slides. Genitalia was dissected and temporarily mounted in gel alcohol for study and drawings with a camera lucida attached to a Olympus BX51 microscope. Photographs were taken with a Zeiss Axiocam ICc5 attached to a Zeiss Stemi 508 stereo microscope. Some images were processed with CombineZP software (Hadley, 2010) to improve focus.

Material is deposited in the following Institution: IBN (Instituto de Biodiversidad Neotropical, Tucumán), and FAMU (Florida A&M University, Tallahassee, FL).

The morphological matrix published in Molineri, Salles & Peters (2015) was revised, the new species amended, and some characters of *L. corporaali* were modified following the description of Baumgardner et al. (2012). All other taxa and characters in the matrix were not modified

(Appendix 1). TNT (Goloboff, Farris & Nixon 2008) was used to searching most parsimonious trees. Heuristic searches were conducted under implied weights (Goloboff, Mattoni & Quinteros 2006) with k = 3 and 100 replicates of tree bisection and reconnection. All characters were treated as non-additive except for continuous characters (chars. 0 to 26), for additional details see Molineri, Salles & Peters (2015). Group support was calculated with the method of frequency difference (Goloboff et al. 2003), using 1000 replications of symmetric jackknifing.

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Results

Description

Languidipes janae sp. nov. (Figures 1 – 3)

Type material. Holotype male imago from Indonesia (Borneo): Kalimantan, Timur Prov., Lake Semayang, nr. Kota Bangun, attracted to light on boat, 3.vii.1985, M. Christensen, specimen number IBN – E 6370. Paratypes: 4 male imagos, same data, all deposited in IBN (IBN – E – 6371, IBN – E – 6372, IBN – E – 6373 and IBN – E – 6374).

Additional material. We also examined 1 larvae of *L. trapobanes*, paratype, FAMU E2109, from Ceylon, Kollonawe, iv.1954 (no more data).

Diagnosis. The male imago of this species is characterized by the presence of ommation on mesonotum, and penis divided almost completely, with sub-quadrate base, small outer projection basally to the long and slender distal arms; distal arms with pointed apex.

Male imago. Length (mm): body, 10.0–14.0; forewing, 12.2–13.0; hind wing, 4.0–5.0; cercus, 26.0, terminal filament, 0.5-1.1. Head. Compound eyes large, black, covering most of head,

separated in the middle of head by a distance equal to 1/3 of the width of an eye (Figs. 1A, 1C); lateral ocelli large and pedunculated (Fig. 1C). Head brown dorsally, shaded with black mainly at the base of ocelli; ventrally much paler. Remnants of mouthparts whitish yellow. Antenna: scape and pedicel yellowish (flagellum broken-off and lost). Thorax. Pronotum reddish brown with black stippling on central area; anterior membranous portion blackish; sternum and pleura whitish. Mesonotum reddish brown slightly paler medially, shaded with black between PSP; ommation (oval whitish median area in basal ¼ of mesonotum) present (arrow in Fig. 1C); pleura and sternum light yellowish brown, furcasternal median impression translucent. Metanotum reddish brown shaded with black on median area and posterior margin, pleura yellowish, sternum whitish translucent. Forelegs relatively short (slightly shorter than ½ of body length), yellowish white (Fig. 1B). Middle and hind legs whitish, weak (Fig. 1D). Forewings (Fig. 2A) hyaline shaded with gray along costal margin and on membrane basal to vein A. Hindwings (Fig. 2A) hyaline, shaded with gray at costal and basal half of subcostal areas, and at base. Veins of both wings brownish, lighter toward apex, except cross veins on apical half of wing, translucent. Abdomen. Dorsum brownish shaded with black, ventrally whitish. Genitalia (Figs. 2B to 2E, 3A and 3B): forceps one-segmented, robust, distally with a patch of short and curved setae along the inner margin. Penis divided almost completely, penis base sub-quadrate with a small outer projection (arrow in Figs. 2E and 3B), distal arms long and slender with pointed apex. Cerci: whitish, shaded with light gray basally. Terminal filament as long as tergum X, whitish and thin.

Etymology. The specific name (noun in the genitive case) is a tribute to Janice Peters ("Jan"), who facilitated the material of the new species, and for her constant support.

Notes. In forewings, ICu veins presented variations among specimens. Frequently ICu1 is basally fused to CuA but may be basally free or joined to ICu2, additionally ICu2 may be basally free or fused to CuP.

Distribution. Data here presented constitute the first record of a *Languidipes* species in Borneo Island (Fig. 4).

Phylogenetic study

Only one shortest tree was recovered (Fig. 5), with a tree length of 270.8, a total fit of 5.8, and an adjusted homoplasy of 15.2. A high support was obtained for *Languidipes* (95%) and for the

sister group *Languidies* + *Povilla* (87%). The synapomorphies supporting the genus *Languidipes* (two species included) are: 1) ratio length second foretarsite / foretibia (char. 1 changes from 0.584-0.645 to 0.480), 2) ratio FW / foreleg length (char. 2, from 1.661-1.736 to 2.800), 3) ratio FW /cercus length (char. 3, from 0.339-0.347 to 0.375-0.464), 4) FW ratio length / width (char. 4, from 2.000-2.214 to 2.265), 5) ratio length FW / HW (char. 5, from 2.302-2.447 to 2.790), 6) penes, ratio basal width / subapical width (char. 17, from 1.300 to 2.000), 7) FW Cu sector, ICus joinning hind margin on different sides of tornus (char. 35): ICu1 close to tornus, ICu2 on basitornal margin, and 8) median plate of styliger (char 41) absent. The autapomorphies found for *Languidipes janae* are: 1) ratio subapical width of foretibia / subbasal width of tarsite 2 (char. 0, from 1.700 to 1.040), 2) ratio FW / cercus length (char. 3, from 0.375-0.464 to 0.500), 3) ratio marginal length between main longitudinal veins/imv length (mean of all values in a wing) (char. 9, from 1.653 to 1.745), 4) Rs stem length (FW male) / Rs from fork to margin (char. 10, from 0.235-0.241 to 0.220), 5) ratio total length of forceps / basal width (char. 13, from 4.545 to 4.300-4.500), 6) ratio length / basal width of penile lobe (char. 15, from 4.706-5.200 to 2.600), 7) penes, ratio basal width / subapical width (char. 17, from 2.000 to 3.125), and 8) male foretarsite 1 subrectangular (char. 29).

Discussion

The species of *Languidipes* seem restricted to southeastern Asia (Fig. 4). The range of *Languidipes corporaali* is the widest of the genus, being recorded in some Indonesian islands (Java, Sumatra, and Simeulue), Thailand, and Malaysia; with a doubtful record for Assam, India (Chopra 1927, cited in Hubbard 1984). Hubbard (1984) affirms that probably this last record will be a new species.

Most species of *Languidipes* are only known from nymphs. *Languidipes trapobanes* is known from Sri Lanka and the south of India, while *L. lithophagus* was recently described from Myanmar (Bolotov et al. 2022). It is possible that the males described here as *L. janae* represent the adult stage of one of them, but this seems unlikely. Nevertheless, we prefer to describe the new species because it constitutes the unique record from Borneo, and its size is relatively smaller than the other species (Hubbard 1984; Rathinakumar et al. 2019; Bolotov et al. 2022; Pai et al. 2023).

Styliger in *Languidipes* is reduced to pedestals, which appear to be the basal segment of forceps. Median plate of styliger is not present, contrary to *Povilla* and other Asthenopodinae, but similar to Campsurinae (Kluge 2004; Molineri, Salles & Peters 2015). Following this interpretation, forceps of *Languidipes* are one-segmented, and the diagnosis proposed by Baumgardner et al. (2012) including the statement “male genitalia without a remnant of styliger plate” should be amended to “male genitalia without a remnant of the median plate of styliger”.

Surprisingly, a weak small circular area in the center of the mesonotum (Fig. 1B) is present in the specimens here studied. This structure, much resembling the ommation of Caenidae and Neoephemeridae (Wang et al. 1997), is unique in the family Polymitarcyidae, and most probably is an independent acquisition.

Among the species of *Languidipes*, only *L. corporaali* is known from the male adult, and it presents a penis structure strongly different to *L. janae* sp. nov. The basal portion of the penis are wide and laterodistally rounded in *L. corporaali*, but is sub-quadrate and with an acute projection in outer margin in *L. janae*. Penis arms in *L. corporaali* ends more acutely than in the species described here. Finally, penis is divided from the base of the arms to the apex in *L. corporaali*, but *L. janae* presents a much deeper division including most of the basal portion of penis.

The previous phylogenetic hypothesis (Molineri, Salles & Peters 2015) is not modified by the inclusion of Languidipes janae. As expected, this species is grouped with L. corporaali in a well-defined group, sister to Povilla.

Acknowledgments

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Bibliography

Baumgardner, D. E., Peters, J. G., Ghani, I. A. & Hubbard, M. D. (2012). The adult stage of *Languidipes corporaali* (Lestage, 1922), new status and the validity of *Povilla* (Navas) (Ephemeroptera: Polymitarcyidae: Asthenopodinae). Aquatic Insects 34, 107–113. https://doi.org/10.1080/01650424.2012.713487

Bolotov, I. N., Kondakov, A. V., Potapov, G. S., Palatov, D. M., Chan, N., Lunn, Z., Bovykina G. V., Chapurina Y. E., Kolosova Y. S., Spitsyna E. A., Spitsyn V. M., Lyubas A. A., Gofarov M. Y., Vikhrev I. V., Yapaskurt V. O., Bychkov A. Y. & Pokrovsky, O. S. (2022). Bioerosion of siliceous rocks driven by rock-boring freshwater insects. npj Materials Degradation, 6(1), 3. https://doi.org/10.1038/s41529-022-00216-6

Chopra B. (1927). The Indian Ephemeroptera (mayflies). Part I. - The suborder Ephemeroidea: Families Palingeniidae and Polymitarcidae – Records of the Indian Museum 29, 91-138, pl. 8-10.

Goloboff P.A., Farris J.S., Kallersjo M., Oxelman B., Ramırez M.J., Szumik C.A. (2003). Improvements to resampling measures of group support. Cladistics 19, 324–332. https://doi.org/10.1111/j.1096-0031.2003.tb00376.x

Goloboff P.A., Farris J.S., Nixon K. (2008). TNT, a free program for phylogenetic analysis. Cladistics 24, 774–786. https://doi.org/10.1111/j.1096-0031.2008.00217.x

Goloboff P.A., Mattoni C., Quinteros S. (2006). Continuous characters analyzed as such. Cladistics, 22: 589–601. https://doi.org/10.1111/j.1096-0031.2006.00122.x

199 Hadley A. (2010). CombineZP software.
200 http://www.hadleyweb.pwp.blueyonder.co.uk/CZP/Installation.htm
201 Hubbard, M. D. (1984). A revision of the genus *Povilla* (Ephemeroptera: Polymitarcyidae).
202 Aquatic Insects 6, 17–35. https://doi.org/10.1080/01650428409361158
203 Kluge N.J. (2004) The phylogenetic system of Ephemeroptera. Kluwer, 442 pp.
204 Lestaje, J. A. (1922). Notes sur les genres *Asthenopus* - *Povilla* (Ephemeroptera) et description
205 d'une espèce javanaise nouvelle (*Asthenopus corporaali*). Annales de la Société
206 Entomologique de Belgique 62.
207 McCafferty W.P. (2004). Higher classification of the burrowing mayflies (Ephemeroptera:
208 Scapphodonta). Entomological News 115: 84–92.
209 Molineri C (2010) A cladistic revision of *Tortopus* Needham & Murphy with description of the
210 new genus *Tortopsis* (Ephemeroptera: Polymitarcyidae). Zootaxa 2481: 1–36.
211 Molineri C, Emmerich D (2010) New species and new stage descriptions of Campsurus major
212 species group (Polymitarcyidae: Campsurinae), with first report of silk-case construction
213 in mayfly nymphs. Aquatic Insects 32: 265–280. doi: 10.1080/01650424.2010.533131
214 Molineri, C., Salles, F. F., & Peters, J. G. (2015). Phylogeny and biogeography of
215 Asthenopodinae with a revision of Asthenopus, reinstatement of Asthenopodes, and the
216 description of the new genera *Hubbardipes* and *Priasthenopus* (Ephemeroptera,
217 Polymitarcyidae). ZooKeys, (478), 45. doi: 10.3897/zookeys.478.8057
218 Pai, S. G., Kalleshwaraswamy, C. M., Varanashi, K., Ranjith, M., & Rajkumar, M. (2023). First
219 record of Mayfly *Povilla* (*Languidipes*) *taprobanes* Hubbard from Karnataka. Indian
220 Journal of Entomology, 610-616. https://doi.org/10.55446/IJE.2021.392
221 Rathinakumar, T., Kubendran, T., & Balasubramanian, C. (2019). New record of the Genus
222 *Povilla* (Navas, 1912) (Ephemeroptera, Polymitarcyidae) from southern Western Ghats,
223 India. Journal of Entomological Research, 43(1), 89-92. DOI: 10.5958/0974-
224 4576.2019.00018.5
225 Sattler W. (1967). Über die Lebensweise, insbesondere das Bauverhalten, neotropischer
226 Eintagsfliegen-Larven (Ephemeroptera, Polymitarcidae). Beiträge zur Neotropischen
227 Fauna 5:89–110. doi: 10.1080/01650526709360399
228 Wang, T. Q., McCafferty, W. P., & Bae, Y. J. (1997). Sister relationship of the Neoephemeridae
229 and Caenidae (Ephemeroptera: Pannota). Entomological News, 108, 1: 52-56.

Figure 1

Languidipes janae sp. nov., male imago

1A, lateral habitus; 1B, foreleg, dorsal; 1C, dorsal habitus (wings removed); 1D, ventral habitus (wings removed).

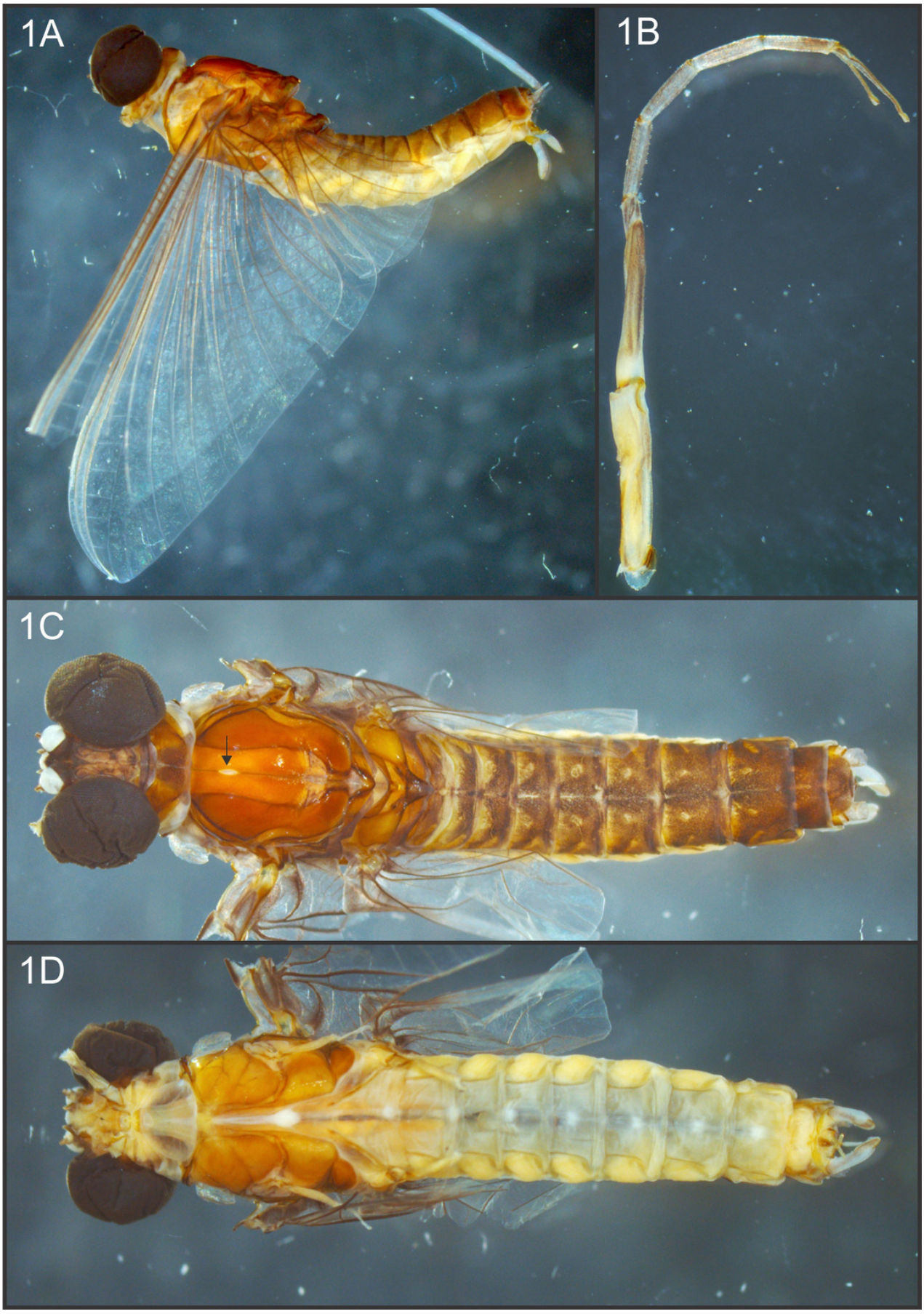
1A
1B
1C
1D

Figure 2

Languidipes janae sp. nov., male imago

2A, wings; 2B, genitalia, lateral; 2C, same, latero-dorsal; 2D, ventral; 2E, dorsal.

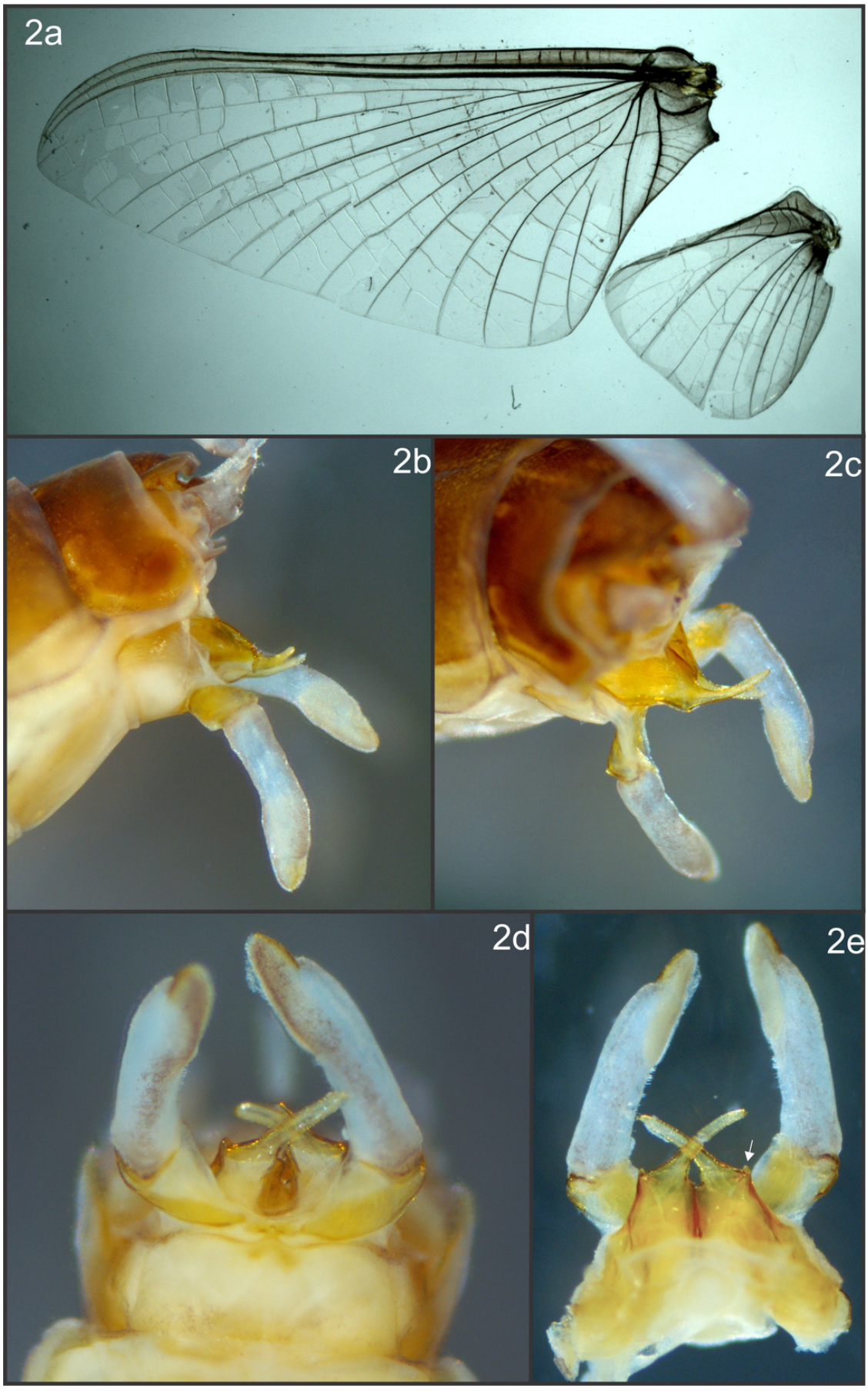
2a
2b
2c
2d
2e

Figure 3

Languidipes janae sp. nov., male imago

3A, genitalia, ventral; 3B, penis, dorsal.

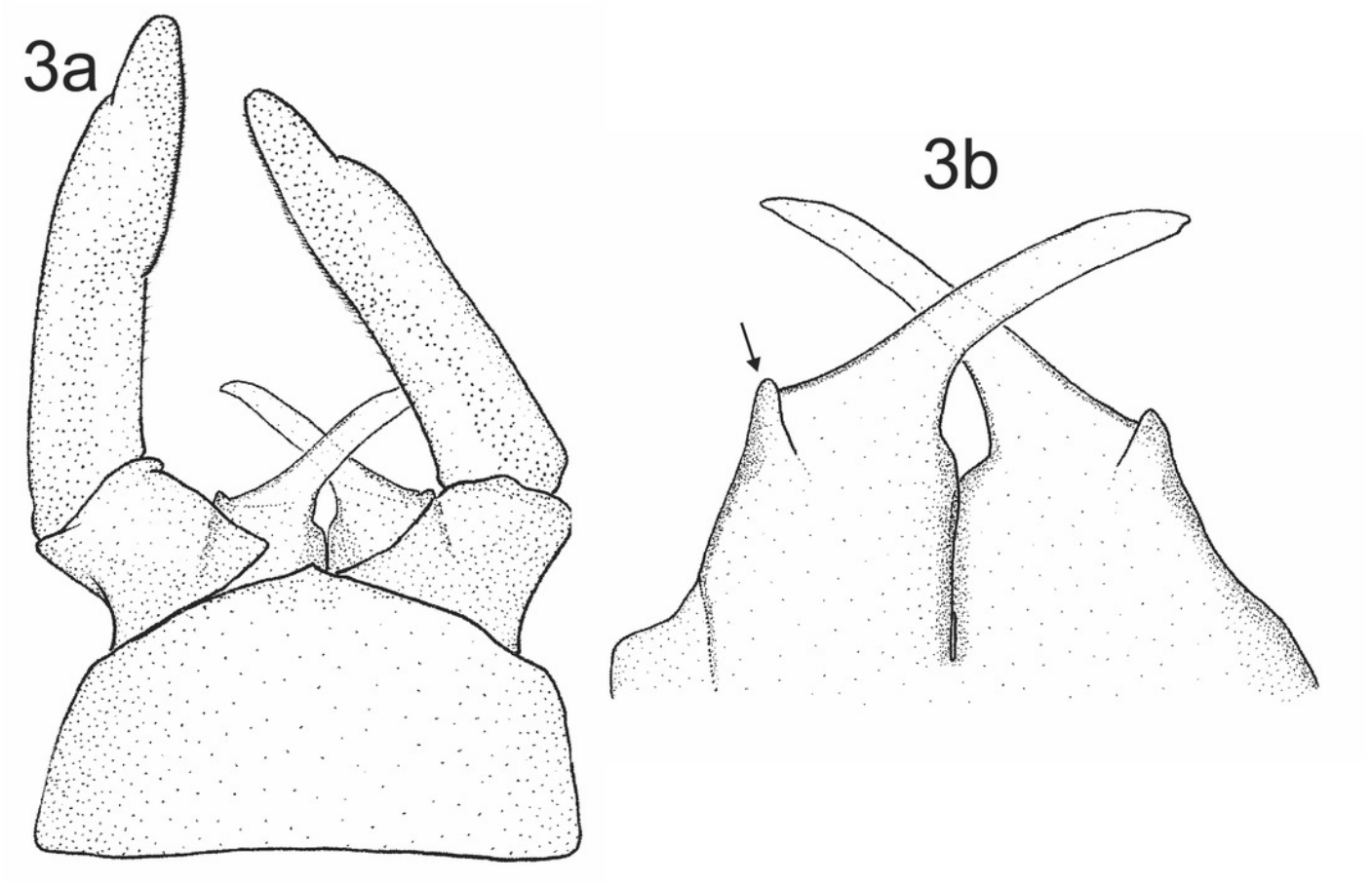

Figure 4

Map showing the distribution of all known *Languidipes* species.

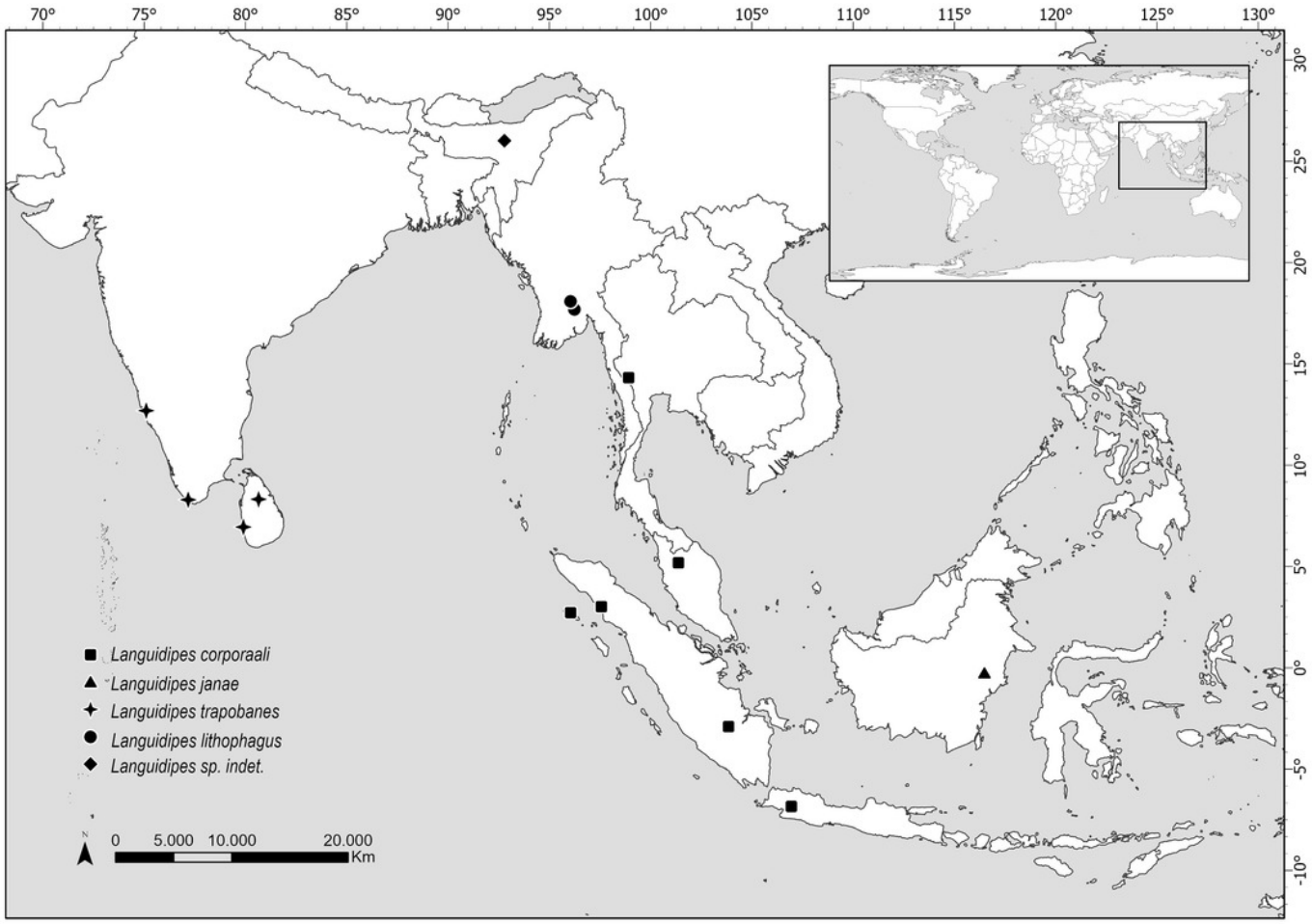

Figure 5

Phylogenetic tree of the Asthenopodinae subfamily, incorporating *Languidipes janae*.