#### A new species of Languidipes Hubbard (Ephemeroptera, Polymitarcyidae) from **Borneo** 2 3 Guillermo Eduardo Hankel<sup>1</sup>, Carlos Molineri<sup>1</sup> 4 5 <sup>1</sup> Instituto de Biodiversidad Neotropical (IBN), Consejo Nacional de Investigaciones Científicas 6 y Técnicas (CONICET), Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad 7 Nacional de Tucumán, Yerba Buena, Tucumán, Argentina. 8 9 10 Corresponding author: 11 Guillermo E. Hankel Tornquist s/n (alt. 1200), Colonia Juan Posse – Lastenia, Tucumán, CP 4111, Argentina. 12 guillehankel@gmail.com 13 14 15 **Abstract** The genus Languidipes is currently represented by three species distributed in southeastern 16 17 Asian, India, and Sri Lanka. Languidipes corporaali is the most widely distributed species, and 18 both, male and female imagos, as well as nymphs, are known. In contrast, the other species, L. 19 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 20 characterized by the presence of ommation on mesonotum, and penis almost completely divided, 21 22 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 23 Asthenopodinae corroborates supports its taxonomic status. 24 25 Introduction 26 27 Polymitarcyidae (Ephemeroptera), with a worldwide distribution, includes large to medium-sized 28 mayflies with burrowing nymphs (Kluge 2004, McCafferty 2004). The Strong-mandibular tusks of the immature forms are used to dig tunnels in a variety d kinds of underwater sediments, 29 including mud, clay and even siliceous rocks (Molineri, Salles & Peters 2015, Bolotov et al. 30 31 2022). The In addition, they all particularity of producing silk infrom the malpighian ducts,

- 32 allowings them to coat their burrowings tunnels 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
- 35 (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
- 37 the classic form (as an entire piece) but in flakes that come off the body and wings (Molineri
- 38 2010). Because of their unique biology, including nymphs hidden in the substrates and extremely
- 39 short-lived adults, specimens of this group are infrequently collected.
- 40 The genus Languidipes was originally described for Asthenopus corporaali Lestage, 1922 from
- 41 Java, Indonesia. Languidipes corporaali (Lestage) was subsequently recorded from other
- 42 Indonesian localities (Sumatra and Simeulue), as well as from Malaysia and Thailand
- 43 (Baumgardner et al. 2012). The genus Languidipes also includes the species L. trapobanes
- 44 (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.
- 46 A phylogenetic framework has been proposed for the subfamily Asthenopodinae, where
- 47 Languidipes is included together with partially sympatric Povilla and other three South American
- 48 genera (Molineri, Salles & Peters 2015).
- 49 Here we describe a new species of *Languidipes* based on male imagos from Borneo, Indonesia,
- 50 and test its phylogenetic relationships inside the subfamily.

## Materials & methods

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- 53 Specimens are fixed in alcohol 70°, wings of one of them were removed and mounted dry in
- 54 microscope slides. Genitalia was dissected and temporarily mounted in gel alcohol for study and
- 55 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
- 57 processed with CombineZP software (Hadley, 2010) to improve focus.
- 58 Material is deposited in the following Institution: IBN (Instituto de Biodiversidad Neotropical,
- 59 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
- 63 (Appendix 1).
- The TNT program (Goloboff, Farris & Nixon 2008) was used to searching most parsimonious
- 65 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
- 67 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
- 69 difference (Goloboff et al. 2003), using 1000 replications of symmetric jackknifing.
- 70 The electronic version of this article in Portable Document Format (PDF) will represent a
- 71 published work according to the International Commission on Zoological Nomenclature (ICZN),
- 72 and hence the new names contained in the electronic version are effectively published under that
- 73 Code from the electronic edition alone. This published work and the nomenclatural acts it
- 74 contains have been registered in ZooBank, the online registration system for the ICZN. The
- 75 ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed
- 76 through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The
- 77 LSID for this publication is: [LSIDurn:lsid:zoobank.org:act:048403BC-2E75-4C1B-AE70-
- 78 8DDF826FF9CA]. The online version of this work is archived and available from the following
- 79 digital repositories: PeerJ, PubMed Central SCIE and CLOCKSS.

## 81 Results

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- 82 <u>Description</u>
- 83 *Languidipes janae* sp. nov. (Figures 1 3)
- 84 Type material. Holotype male imago from Indonesia (Borneo): Kalimantan, Timur Prov., Lake
- 85 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 –
- 87 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
- 89 Ceylon, Kollonawe, iv.1954 (no more data).
- 90 Diagnosis. The male imago of this species is characterized by the presence of ommation on
- 91 mesonotum, and penis divided almost completely, with sub-quadrate base, small outer projection
- 92 basally to the long and slender distal arms; distal arms with pointed apex.

Commented [DE1]: Revise wording

93	Male imago. Length (mm): body, 10.0–14.0; forewing, 12.2–13.0; hind wing, 4.0–5.0; cercus,
94	26.0, terminal filament, 0.5-1.1. Head. Compound eyes large, black, covering most of head,
95	separated in the middle of head by a distance equal to 1/3 of the width of an eye (Figs. 1A, 1C);
96	lateral ocelli large and pedunculated (Fig. 1C). Head brown dorsally, shaded with black mainly at
97	the base of ocelli; ventrally much paler. Remnants of mouthparts whitish yellow. Antenna: scape
98	and pedicel yellowish (flagellum broken-off and lost). Thorax. Pronotum reddish brown with
99	black stippling on central area; anterior membranous portion blackish; sternum and pleura
100	whitish. Mesonotum reddish brown slightly paler medially, shaded with black between PSP;
101	ommation (oval whitish median area in basal ¼ of mesonotum) present (arrow in Fig. 1C); pleura
102	and sternum light yellowish brown, furcasternal median impression translucent. Metanotum
103	reddish brown shaded with black on median area and posterior margin, pleura yellowish, sternum
104	whitish translucent. Forelegs relatively short (slightly shorter than ½ of body length), yellowish
105	white (Fig. 1B). Middle and hind legs whitish, weak (Fig. 1D). Forewings (Fig. 2A) hyaline
106	shaded with gray along costal margin and on membrane basal to vein A. Hindwings (Fig. 2A)
107	hyaline, shaded with gray at costal and basal half of subcostal areas, and at base. Veins of both
108	wings brownish, lighter toward apex, except cross veins on apical half of wing, translucent.
109	Abdomen. Dorsum brownish shaded with black, ventrally whitish. Genitalia (Figs. 2B to 2E, 3A
110	and 3B): forceps one-segmented, robust, distally with a patch of short and curved setae along the
111	inner margin. Penis divided almost completely, penis base sub-quadrate with a small outer
112	projection (arrow in Figs. 2E and 3B), distal arms long and slender with pointed apex. Cerci:
113	whitish, shaded with light gray basally. Terminal filament as long as tergum X, whitish and thin.
114	Etymology. The specific name (noun in the genitive case) is a tribute to Janice Peters ("Jan"),
115	who facilitated the material of the new species, and for her constant support.
116	Notes. In forewings, ICu veins presented variations among specimens. Frequently ICu1 is basally
117	fused to CuA but may be basally free or joined to ICu2, additionally ICu2 may be basally free or
118 119	fused to CuP.  Distribution. Data here presented constitute the first record of a <i>Languidipes</i> species in Borneo
TID	Distribution. Data here presented constitute the first record of a Languarpes species in Borneo

Phylogenetic study

Island (Fig. 4).

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- 123 Only one shortest tree was recovered (Fig. 5), with a tree length of 270.8, a total fit of 5.8, and an 124 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 125 (two species included) are: 1) ratio length second foretarsite / foretibia (char. 1 changes from 126 0.584-0.645 to 0.480), 2) ratio FW / foreleg length (char. 2, from 1.661-1.736 to 2.800), 3) ratio 127 FW /cercus length (char. 3, from 0.339-0.347 to 0.375-0.464), 4) FW ratio length / width (char. 4, 128 from 2.000-2.214 to 2.265), 5) ratio length FW / HW (char. 5, from 2.302-2.447 to 2.790), 6) 129 penes, ratio basal width / subapical width (char. 17, from 1.300 to 2.000), 7) FW Cu sector, ICus 130 joinning hind margin on different sides of tornus (char. 35): ICu1 close to tornus, ICu2 on 131 132 basitornal margin, and 8) median plate of styliger (char 41) absent. The autapomorphies found for
- 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

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- 143 The species of Languidipes seem restricted to southeastern Asia (Fig. 4). The range of
- 144 Languidipes corporaali is the widest of the genus, being recorded in some Indonesian islands
- 145 (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
- 150 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
- 154 et al. 2023).
- 155 Styliger in *Languidipes* is reduced to pedestals, which appear to be the basal segment of forceps.
- 156 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,
- 158 forceps of Languidipes are one-segmented, and the diagnosis proposed by Baumgardner et al.

- 159 (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".
- 161 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
- 163 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
- 167 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.
- 171 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-
- 173 defined group, sister to Povilla.

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