A new species of Languidipes Hubbard (Ephemeroptera, Polymitarcyidae) from **Borneo** 2 3 Guillermo Eduardo Hankel^{1,2}, Carlos Molineri¹ 4 5 ¹ Instituto de Biodiversidad Neotropical (IBN), Consejo Nacional de Investigaciones Científicas 6 y Técnicas (CONICET), Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad 7 8 Nacional de Tucumán, Yerba Buena, Tucumán, Argentina. 9 ² Instituto de Invertebrados - Fundación Miguel Lillo. Miguel Lillo 251 - San Miguel de Formatted: Superscript 10 Tucumán - Tucumán - Argentina. CP: T4000JFE. 11 12 Corresponding author: Formatted: Spanish (Argentina) Guillermo E. Hankel 13 14 Tornquist s/n (alt. 1200), Colonia Juan Posse – Lastenia, Tucumán, CP 4111, Argentina. CP 15 guillehankel@gmail.com 16 17 18 **Abstract** 19 The genus Languidipes is currently represented by three species distributed in southeastern 20 Asian, India, and Sri Lanka. Languidipes corporaali is the most widely distributed species, and 21 both, male and female imagos, as well as nymphs, are known. In contrast, the other species, L. 22 trapobanest aprobanes and L. lithophagus, are only known from nymphs. Here, we describe a 23 new species, Languidipes janae sp nov, based on male imagos collected from Borneo, Indonesia. Formatted: Font: Not Italic This new species is characterized by the presence of ommation on mesonotum, and penis almost 24 25 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 26 of the subfamily Asthenopodinae corroborates supports its taxonomic status. 27 28 Introduction 29 30 Polymitarcyidae (Ephemeroptera), with a worldwide distribution, includes large to medium-sized 31 mayflies with burrowing nymphs (Kluge 2004, McCafferty 2004). The Strong mandibular tusks

- of the immature forms are used to dig tunnels in a varietyd kinds of underwater sediments,
- including mud, clay and even siliceous rocks (Molineri, Salles & Peters 2015, Bolotov et al.
- 34 2022). <u>In The</u> additional particularity of they produceing silk infrom the malpighian ducts,
- 35 allowings them to coat their tunnelsburrowings 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
- 37 et al. 2023). Furthermore, adults are so short-lived, that they do not present functional legs
- 38 (except for the male forelegs, used to graspb females during copula), spending their entire life in
- 39 flight. This forces them to make their subimaginal molt in a unique manner, not shading their
- 40 cuticle in the classic form (as an entire piece) but in flakes that come off the body and wings
- 41 (Molineri 2010). Because of their unique biology, including nymphs hidden in the substrates and
- 42 extremely short-lived adults, specimens of this group are infrequently collected.
- 43 The genus Languidipes was originally described for Asthenopus corporaali Lestage, 1922 from
- 44 Java, Indonesia. Languidipes corporaali (Lestage) was subsequently recorded from other
- 45 Indonesian localities (Sumatra and Simeulue), as well as from Malaysia and Thailand
- 46 (Baumgardner et al. 2012). The genus *Languidipes* also includes the species *L*.
- 47 trapobanes (Hubbard 1984) (Hubbard 1984, Rathinakumar et al. 2019, Pai et al.
- 48 2023), from India and Sri Lanka, and the recently described *L. lithophagus* (Bolotov et al. 2022)
- 49 from Myanmar.

55 56

- 50 A phylogenetic framework has been proposed for the subfamily Asthenopodinae, where
- 51 Languidipes is included together with partially sympatric Povilla and other three South American
- 52 genera (Molineri, Salles & Peters 2015).
- 53 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

- 57 Specimens awere fixed in alcohol 70°70 % (v/v) ethanol., wings of one of them were One wing
- 58 was removed and mounted dry oin microscope slides. Genitalia was dissected and temporarily
- 59 mounted in gel alcohol for study and drawings with a camera lucida attached to an Olympus
- 60 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)
- 62 to improve focus.

- 63 Material is deposited in the following Institution: IBN (Instituto de Biodiversidad Neotropical,
- Tucumán), and FAMU (Florida A&M University, Tallahassee, FL).
- 65 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
- 68 (Appendix 1).
- 69 The TNT program (Goloboff, Farris & Nixon 2008) was used to searching most setup
- 70 parsimonious trees. Heuristic searches were conducted under implied weights (Goloboff, Mattoni
- 71 & Quinteros 2006) with k = 3 and 100 replicates of tree bisection and reconnection. All
- 72 characters were treated as non-additive except for continuous characters (chars. 0 to 26), for
- 73 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
- 75 jackknifing.
- 76 The electronic version of this article in Portable Document Format (PDF) will constitute a
- 77 published work as defined by the International Commission on Zoological Nomenclature (ICZN).
- 78 Consequently, the new names introduced in the electronic version are deemed effectively
- 79 <u>published under the Code solely from the electronic edition. This published work, along with the</u>
- 80 associated nomenclatural acts, has been registered in ZooBank, the online registration system for
- 81 the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be accessed and the relevant
- 82 <u>information viewed through any standard web browser by appending the LSID to the</u>
- 83 prefix http://zoobank.org/. The LSID for this publication is:
- 84 [LSIDurn:lsid:zoobank.org:act:048403BC-2E75-4C1B-AE70-8DDF826FF9CA]. The online
- 85 version of this work is archived and available from the following digital repositories: PeerJ,
- 86 <u>PubMed Central SCIE, and CLOCKSS. The electronic version of this article in Portable</u>
- 87 Document Format (PDF) will represent a published work according to the International
- 88 Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the
- 89 electronic version are effectively published under that Code from the electronic edition alone.
- 90 This published work and the nomenclatural acts it contains have been registered in ZooBank, the
- 91 online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be
- 92 resolved and the associated information viewed through any standard web browser by appending
- 93 the LSID to the prefix http://zoobank.org/. The LSID for this publication is:

94 [LSIDurn:lsid:zoobank.org:act:048403BC 2E75 4C1B-AE70-8DDF826FF9CA]. The online version of this work is archived and available from the following digital repositories: PeerJ, 95 PubMed Central SCIE and CLOCKSS. 96 97 Results 98 Description 99 *Languidipes janae* sp. nov. (Figures 1-3) 100 101 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 102 103 number IBN – E 6370. Paratypes: 4 male imagos, same data, all deposited in IBN (IBN – E – 104 6371, IBN -E - 6372, IBN -E - 6373 and IBN -E - 6374). 105 Additional material. We also examined 1 larvae of L. trapobanest aprobanes, paratype, FAMU E2109, from Ceylon, Kollonawe, iv.1954 (no more data). 106 107 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 108 basally to the long and slender distal arms; distal arms with pointed apex. 109 Male imago. Length (mm): body, 10.0–14.0; forewing, 12.2–13.0; hind wing, 4.0–5.0; cercus, 110 111 26.0, terminal filament parecercus, 0.5-1.1. Head. Compound eyes large, black, covering most of 112 head, separated in the middle of head by a distance equal to 1/3 of the width of an eye (Figs. 1Aa, 113 1€; lateral ocelli large and pedunculated (Fig. 1€). Head brown dorsally, shaded with black mainly at the base of ocelli; ventrally much paler. Remnants of mouthparts whitish yellow. 114 Antenna: scape and pedicel yellowish (flagellum broken-off and lost). Thorax. Pronotum reddish 115 116 brown with black stippling on central area; anterior membranous portion blackish, posterior 117 margin withish; sternum and pleura whitish. Mesonotum reddish brown slightly paler medially, 118 shaded with black between the posterior scutal protuberances PSP; ommation (oval whitish median area in basal-anterior 1/4 of mesonotum) present (arrow in Fig. 1cC); pleura and sternum 119 light yellowish brown, furcasternal median impression translucent. Metanotum reddish brown 120 shaded with black on median area and posterior margin, pleura yellowish, sternum whitish 121 122 translucent. Forelegs relatively short (slightly shorter than ½ of body length), yellowish white

(Fig. 1bB). Middle and hind legs whitish, weak (Fig. 1dD). Forewings (Fig. 2aA) hyaline shaded

with gray along costal margin and on membrane basal to vein A. Hindwings (Fig. 2aA) hyaline,

123 124

- 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. 2bb to 2eE, 3A and
- 128 3bb): 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. 2 et and 3 bb), distal arms long and slender with pointed apex. Cerci:
- whitish, shaded with light gray basally. Terminal filament Paracercus 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
- 137 fused to CuP.
- 138 Distribution. Data here presented constitute the first record of a Languidipes species in Borneo
- 139 Island (Fig. 4).

140

141 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 *Languidipes* + *Povilla* (87%). The synapomorphies supporting the genus
- 145 Languidipes (two species included) are: 1) ratio length second foretarsite / foretibia (char. 1
- 146 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
- 148 / 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
- 150 sector, ICus joinning hind margin on different sides of tornus (char. 35): ICu1 close to tornus,
- 151 ICu2 on basitornal margin, and 8) median plate of styliger (char 41) absent. The autapomorphies
- 152 found for Languidipes janae are: 1) ratio subapical width of foretibia / subbasal width of tarsite 2
- 153 (char. 0, from 1.700 to 1.040), 2) ratio FW / cercus length (char. 3, from 0.375-0.464 to 0.500), 3)
- 154 ratio marginal length between main longitudinal veins/imv length (mean of all values in a wing)
- 155 (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. 1c²) 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-

158 159

160

161

162163

164

165166

167

168 169

170

171

172 173

174175

176

177

178

179

180

181 182

183

184

185

186

187 188

189 190

191

192

193

defined group, sister to Povilla.

Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic

Acknowledgments

195 We thank Luciana Cristobal for the map, and Janice Peters for providing the specimens here

196 described.

194

197

198

203

204

205 206

207

208

209

210

211

212

213

214 215

216 217

218

219

220 221

222 223

224 225

226 227

228

229

230

231

232

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

Hadley A. (2010). CombineZP software.

http://www.hadleyweb.pwp.blueyonder.co.uk/CZP/Installation.htm

- Hubbard, M. D. (1984). A revision of the genus *Povilla* (Ephemeroptera: Polymitarcyidae). Aquatic Insects 6, 17–35. https://doi.org/10.1080/01650428409361158
- Kluge N.J. (2004) The phylogenetic system of Ephemeroptera. Kluwer, 442 pp.
- Lestagje, J. A. (1922). Notes sur les genres *Asthenopus Povilla* (Ephemeroptera) et description d'une espèce javanaise nouvelle (*Asthenopus corporaali*). Annales de la Société Entomologique de Belgique 62<u>: 142-148</u>.
- McCafferty W.P. (2004). Higher classification of the burrowing mayflies (Ephemeroptera: Scapphodonta). Entomological News 115: 84–92.
- Molineri C (2010) A cladistic revision of *Tortopus* Needham & Murphy with description of the new genus *Tortopsis* (Ephemeroptera: Polymitarcyidae). Zootaxa 2481: 1–36.
- Molineri C, Emmerich D (2010) New species and new stage descriptions of *Campsurus* major species group (Polymitarcyidae: Campsurinae), with first report of silk-case construction in mayfly nymphs. Aquatic Insects 32: 265–280. doi: 10.1080/01650424.2010.533131
- Molineri, C., Salles, F. F., & Peters, J. G. (2015). Phylogeny and biogeography of
 Asthenopodinae with a revision of *Asthenopus*, reinstatement of *Asthenopodes*, and the
 description of the new genera *Hubbardipes* and *Priasthenopus* (Ephemeroptera,
 Polymitarcyidae). ZooKeys, (478), 45. doi: 10.3897/zookeys.478.8057

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

- Pai, S. G., Kalleshwaraswamy, C. M., Varanashi, K., Ranjith, M., & Rajkumar, M. (2023). First
 record of Mayfly *Povilla (Languidipes) taprobanes* Hubbard from Karnataka. Indian
 Journal of Entomology, 610-616. https://doi.org/10.55446/IJE.2021.392
- Rathinakumar, T., Kubendran, T., & Balasubramanian, C. (2019). New record of the Genus
 Povilla (Navas, 1912) (Ephemeroptera, Polymitarcyidae) from southern Western Ghats,
 India. Journal of Entomological Research, 43(1), 89-92. DOI: 10.5958/0974 4576.2019.00018.5

244

245

246

247

248

- Sattler W. (1967). Über die Lebensweise, insbesondere das Bauverhalten, neotropischer Eintagsfliegen-Larven (Ephemeroptera, Polymitarcidae). Beiträge zur Neotropischen Fauna 5:89–110. doi: 10.1080/01650526709360399
- Wang, T. Q., McCafferty, W. P., & Bae, Y. J. (1997). Sister relationship of the Neoephemeridae and Caenidae (Ephemeroptera: Pannota). Entomological News, 108, 1: 52-56.