Integrative taxonomy of *Metrichia* Ross (Trichoptera: Hydroptilidae: Ochrotrichiinae) microcaddisflies from Brazil: descriptions of twenty new species

Allan PM Santos, Daniela M Takiya, Jorge L Nessimian

Metrichia is assigned to the Ochrotrichiinae, a group of almost exclusively Neotropical microcaddisflies. *Metrichia* comprises over 100 described species and, despite its diversity, only one species has been described from Brazil so far. In this paper, we provide descriptions for 20 new species from 8 Brazilian states: *M. acuminata* **sp. nov.**, *M. azul* **sp. nov.**, *M. bonita* **sp. nov.**, *M. bracui* **sp. nov.**, *M. caraca* **sp. nov.**., *M. circuliforme* **sp. nov.**, *M. curta* **sp. nov.**, *M. farofa* **sp. nov.**, *M. forceps* **sp. nov.**, *M. formosinha* **sp. nov.**, *M. goiana* **sp. nov.**, *M. itabaiana* **sp. nov.**, *M. longissima* **sp. nov.**, *M. peluda* **sp. nov.**, *M. rafaeli* **sp. nov.**, *M. simples* **sp. nov.**, *M. talhada* **sp. nov.**, *M. tere* **sp. nov.**, *M. ubajara* **sp. nov.**, and *M. vulgaris* **sp. nov.** DNA barcode sequences (577 bp of the mitochondrial gene COI) were generated for 13 of the new species and two previously known species of *Metrichia* resulting in 64 sequences. In addition, COI sequences were obtained for other genera of Ochrotrichiinae (*Angrisanoia*, *Nothotrichia*, *Ochrotrichia*, *Ragatrichia*, and *Rhyacopsyche*). DNA sequences and morphological data were integrated to evaluate species delimitations. K2P pairwise distances were calculated to generate a neighbor-joining tree. COI sequences also were submitted to ABGD and GMYC methods to assess 'potential species' delimitation. Analyses showed a conspicuous barcoding gap among *Metrichia* sequences (highest intraspecific divergence: 4.8%; lowest interspecific divergence: 12.6%). Molecular analyses also allowed the association of larvae and adults of *Metrichia bonita* **sp. nov.** from Mato Grosso do Sul, representing the first record of microcaddisfly larvae occurring in calcareous tufa (or travertine). ABGD results agreed with the morphological delimitation of *Metrichia* species, while GMYC estimated a slightly higher number of species, suggesting the division of two morphological species, each one into two potential species. Because this could be due to unbalanced sampling and the lack of morphological diagnostic characters, we have maintained these two species as undivided.

1 **Integrative taxonomy of *Metrichia* Ross (Trichoptera: Hydroptilidae:**

2 **Ochrotrichiinae) microcaddisflies from Brazil: descriptions of twenty new**

3 **species**

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13   **ABSTRACT**

14   *Metrichia* is assigned to the Ochrotrichiinae, a group of almost exclusively Neotropical

15   microcaddisflies. *Metrichia* comprises over 100 described species and, despite its diversity, only

16   one species has been described from Brazil so far. In this paper, we provide descriptions for 20

17   new species from 8 Brazilian states: *M. acuminata* **sp. nov.**, *M. azul* **sp. nov.**, *M. bonita* **sp. nov.**,

18   *M. bracui* **sp. nov.**, *M. caraca* **sp. nov.**, *M. circuliforme* **sp. nov.**, *M. curta* **sp. nov.**, *M. farofa* **sp.**

19   **nov.**, *M. forceps* **sp. nov.**, *M. formosinha* **sp. nov.**, *M. goiana* **sp. nov.**, *M. itabaiana* **sp. nov.**, *M.*

20   *longissima* **sp. nov.**, *M. peluda* **sp. nov.**, *M. rafaeli* **sp. nov.**, *M. simples* **sp. nov.**, *M. talhada* **sp.**

21   **nov.**, *M. tere* **sp. nov.**, *M. ubajara* **sp. nov.**, and *M. vulgaris* **sp. nov.** DNA barcode sequences

22   (577 bp of the mitochondrial gene COI) were generated for 13 of the new species and two

23   previously known species of *Metrichia* resulting in 64 sequences. In addition, COI sequences

24   were obtained for other genera of Ochrotrichiinae (*Angrisanoia*, *Nothotrichia*, *Ochrotrichia*,

25   *Ragatrichia*, and *Rhyacopsyche*). DNA sequences and morphological data were integrated to

26   evaluate species delimitations. K2P pairwise distances were calculated to generate a neighbor-

27   joining tree. COI sequences also were submitted to ABGD and GMYC methods to assess

28   'potential species' delimitation. Analyses showed a conspicuous barcoding gap among *Metrichia*

29   sequences (highest intraspecific divergence: 4.8%; lowest interspecific divergence: 12.6%).

30   Molecular analyses also allowed the association of larvae and adults of *Metrichia bonita* **sp. nov.**

31   from Mato Grosso do Sul, representing the first record of microcaddisfly larvae occurring in

32   calcareous tufa (or travertine). ABGD results agreed with the morphological delimitation of

33   *Metrichia* species, while GMYC estimated a slightly higher number of species, suggesting the

34   division of two morphological species, each one into two potential species. Because this could be

35   due to unbalanced sampling and the lack of morphological diagnostic characters, we have

36   maintained these two species as undivided.

37   **INTRODUCTION**

38   The microcaddisfly genus *Metrichia* Ross, 1938 is included in the subfamily

39   Ochrotrichiinae, which also includes *Ochrotrichia* Mosely, 1934, *Angrisanoia* Özdikmen, 2008,

40   *Nothotrichia* Flint, 1967, *Rhyacopsyche* Müller, 1879, and the recently erected *Ragatrichia* Oláh

41   & Johanson, 2011, all of them exclusively from New World. Based on adult morphology, Harris

42   & Armitage (1997) and Oláh & Johanson (2011) also suggested three other genera to be included

43   in this subfamily: *Dibusa* Ross, 1939, *Caledonotrichia* Sykora, 1967, and *Maydenoptila* Neboiss,

44   1977, from the U.S.A., New Caledonia, and Australia, respectively. However, because

45   diversification of main lineages of hydroptilids has not been deeply studied, the placement of

46   these genera remains dubious. As noted by Wells et al. (2013), relationships of these

47   microcaddisflies need to be studied based on rigorous analyses including molecular data.

48   Currently, *Metrichia* includes 107 species, found from the U.S.A. to South America, with

49   highest known diversity in Central America (Flint, 1972; Marshall, 1979). *Metrichia* was

50   considered as a subgenus of *Ochrotrichia* due to similarities of adult morphology and almost

51   indistinguishable larvae (Flint, 1968). This subgeneric status was followed by Marshall (1979),

52   who also established the New World tribe that is now recognized as subfamily Ochrotrichiinae.

53   Wiggins (1996) provided additional information on larval morphology of *Metrichia* and

54   *Ochrotrichia*, reestablishing both as independent genera.

55   Diversity of Neotropical microcaddisflies is poorly known and usually several

56   undescribed species are found in collections or amongst recently collected material when

57   examined by experts, even in localities previously studied by trichopterologists. This likely

58   occurs because Hydroptilidae are very small and have complex male genitalia, making them

59   difficult to observe by lower magnification microscopes and to understand homologies among

60   some structures. Only one species of *Metrichia* has been described from Brazil so far, *M.*

61   *pernambucana* Souza & Santos, 2013, but larvae have been commonly identified from several

62   localities (*e.g.*, Pes et al., 2005; Spies & Froehlich, 2006; Spies et al., 2009). It is not surprising

63 that material studied herein recently collected from different river basins in Brazil (Fig. 1, Supp.

64 1, Supp. 2) revealed so many undescribed species.

65 **Figure 1.** Localities (●), distributed on seven of the large river basins of Brazil, where *Metrichia*

66 specimens studied herein were collected.

67   Most *Metrichia* species exhibit a more restricted distribution, in other words, each species

68 has been recorded only from type-localities or relatively few close localities. Herein we describe

69 one species with a wide distribution in Brazil, occurring in three very distinctive biomes: Atlantic

70 Forest (Southeastern Brazil), Caatinga (Northeastern Brazil), and Cerrado (Centralwestern

71   Brazil). Although such wide distributions are not common in *Metrichia*, similar examples are

72   known for other Neotropical microcaddisflies, as *Oxyethira tica* Holzenthal & Harris, 1992,

73   recorded from Mexico, Central, and South America (Flint et al., 1999).

74   Although molecular tools have become common in taxonomic studies to help in species

75   delimitation, their use is still rare with Neotropical caddisflies. Using sequences of the

76   mitochondrial cytochrome oxidase I gene (COI), the standard DNA barcode region for animals

77   (Hebert et al., 2003), Pauls et al. (2010) were able to corroborate two new species of *Smicridea*

78   (*Smicridea*) McLachlan, 1871 from Chile, defined with morphological characters. In most studies

79   with caddisflies, divergence in COI sequences has shown clear differences between intraspecific

80   and interspecific variation, the so-called 'barcoding gap' (Zhou et al., 2007; Pauls et al., 2010;

81   Ruiter et al., 2013). Some species delimitation approaches rely solely in distinguishing intra- and

82   interspecific divergence, such as the Automatic Barcoding Gap Discovery (ABGD) (Puillandre et

83   al., 2012). More sophisticated methods invoke coalescence and speciation models, such as the

84   General Mixed Yule Coalescent (GMYC), and are considered more robust for identifying

85   lineages when intra- and interspecific divergences overlap (Pons et al., 2006). Integrating

86   independent data (*e.g.*, morphology and DNA sequences) and using different approaches are

87   particularly interesting for the taxonomy of diverse and complex groups, such as

88   microcaddisflies. In this work, we applied both ABGD and GMYC methodologies to evaluate our

89   initial morphological identification.

90   Besides its use for species delimitation, DNA taxonomy has a valuable role in making

91   associations between immature and adult stages of caddisflies (Graf et al., 2005; Waringer et al.,

92   2007; Zhou et al., 2007; Ruiter et al., 2013). Traditional techniques to associate larvae and adults

93   are more difficult because they involve rearing larvae in the laboratory (not an easy task for many

94   caddisfly groups) or luck in finding pharate adults in field. Indirect association of stages, for

95   example, by collecting adults and larvae at the same locality, can result in misidentification,

96   because different species of the same genus frequently co-occur.

97   Larvae of *Metrichia* have been associated for only two species: *M. nigritta* (Banks, 1907)

98   described by Edwards & Arnold (1961) and illustrated by Wiggins (1996), and *M. juana* (Flint,

99   1964) by Flint (1964) in the original description. In addition, Botosaneanu and Flint (1982)

100   described a larva of *Metrichia* and its case from Venezuela and a pupal case from Ecuador; and

101   Pes et al. (2005) illustrated larvae from Brazil and three different types of cases. In both works,

102   specific names were not provided as authors did not have respective adults. *Metrichia* larvae

103   build a typical oval purse-like case, made of silk, usually covered with algae filaments (Wiggins,

104   1996; Pes et al., 2005), and sometimes also having sand grains (Botosaneanu & Flint, 1982).

105   Cases of some larvae that have not yet been associated with adults show a pair of dorsal

106   "chimneys", an uncommon feature also described and illustrated by Müller (1879, 1880) for

107   *Dicaminus ladislavii* Müller, 1879, from Santa Catarina, Brazil. Based on larval cases from

108   Central and South America, Botosaneanu & Flint (1982) pointed that *Dicaminus* is possibly

109   synonymous with *Metrichia*, but this question remains open, since there are no male specimens

110   from Müller's work.

111   Almost nothing is known about the biology of *Metrichia* larvae. According to Wiggins

112   (1996), larvae of *M. nigritta* were collected in association with filamentous algae on rock

113   surfaces. In Brazil, *Metrichia* occurs in fast flowing streams, usually with associated algae.

114   Herein, we report for the first time the occurrence of *Metrichia* in calcareous tufa. Calcareous

115   tufa or travertine is a terrestrial sedimentary rock, predominantly composed of carbonate

116   minerals, calcite, and aragonite (Drysdale, 1998). Travertines are formed by rapid precipitation of

117   these minerals, producing large alterations on river morphology (Drysdale & Gale, 1997).

118   Although the importance of microbes on travertine formation is recognized by creating surfaces

119   for crystal nucleation, our knowledge of the importance of macroinvertebrates in this process is

120   still poor (Drysdale, 1998; 1999; Paprocki et al., 2003). Studies by Drysdale (1998, 1999) pointed

121   out that aquatic insects play an important role in travertine biogenesis in Australian springs,

122   especially *Cheumatopsyche* Wallengreen, 1891 (Hydropsychidae) larvae. Paprocki et al. (2003)

123   also found another Hydropsychidae (*Smicridea*) to be an important organism in modifying

124   travertine morphology in Venezuela.

125   **MATERIAL AND METHODS**

126   **Morphological study**

127   Specimens were collected manually (larvae or diurnal active adults) or using Malaise or

128   light traps, and then fixed in 96% ethanol. Collecting permits in Brazil were issued by Instituto

129   Chico Mendes de Conservação da Biodiversidade (ICMBio) (SISBIO 43047 and 14591). To

130   observe genital structures, abdomen of males were removed and cleared in a heated solution of

131   10% KOH for 20 minutes. Then, abdomens were mounted in temporary slides, which were used

132   to draw pencil sketches with compound microscope equipped with camera lucida. Vector

133   graphics were traced in Adobe Illustrator CS6 (Adobe Systems Inc.) using pencil sketches as

134   templates. Descriptions provided here were made with DELTA software (Description Language

135   for Taxonomy) (Dallwitz et al., 1999). Terminology used throughout this paper follows that

136   provided by Marshall (1979) and Bueno-Soria & Holzenthal (2003). Types for newly described

137   species are deposited at Coleção Entomológica Prof. José Alfredo Pinheiro Dutra, Departamento

138   de Zoologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro (**DZRJ**); Museu Nacional,

139   Universidade Federal do Rio de Janeiro, Rio de Janeiro (**MNRJ**); Instituto Nacional de Pesquisas

140   da Amazônia, Manaus (**INPA**); Coleção Zoológica do Maranhão (**CZMA**); and Museu de

141   Zoologia da Universidade Federal da Bahia, Salvador (**MZUFBA**).

142   The electronic version of this article in Portable Document Format (PDF) will represent a

143   published work according to the International Commission on Zoological Nomenclature (ICZN),

144   and hence the new names contained in the electronic version are effectively published under that

145   Code from the electronic edition alone. This published work and the nomenclatural acts it

146   contains have been registered in ZooBank, the online registration system for the ICZN. The

147   ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed

148   through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The

149   LSID for this publication is: urn:lsid:zoobank.org:pub:D8D4049E-494B-4A30-92AC-

150   F8F42D2B54B9. The online version of this work is archived and available from the following

151   digital repositories: PeerJ, PubMed Central and CLOCKSS.

152   **DNA extraction, PCR, and sequencing**

153   Genomic DNA was extracted from head and thorax (or from the entire body) of fresh

154   material using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany), without tissue

155   maceration. After extraction, specimens were returned to ethanol and deposited in DZRJ

156   collection as a DNA voucher. COI fragments were amplified using pair of primers: HCO-2198

157   (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') in combination with LCO-1490 (5'-

158   GGTCAACAAATCATAAAGATATTGG-3') (Folmer et al., 1994) or C1-J-1718 (5'-

159   GGAGGATTTGGAAATTGATTAGTTCC-3') (Simon et al., 1994). Polymerase chain reaction

160   (PCR) conditions were as follows: initial denaturation at 94 ºC for 3 min; 35 cycles of

161   denaturation at 94 ºC for 1 min, annealing at 50 ºC for 1 min, and extension at 72 ºC for 2 min;

162   and final extension at 72 ºC for 7 min. PCR products were sent to Macrogen Inc., Seoul, for

163   purification and sequencing reactions.

164   COI sequences of 64 specimens of 15 species of *Metrichia* were obtained. Additional

165   sequences were obtained for specimens of *Angrisanoia*, *Nothotrichia*, *Ochrotrichia*, and

166   *Rhyacopsyche* (Table 1), included as outgroup in different analyses, as described below.

167 **Table 1.** Species of *Metrichia* and other hydroptilids with DNA barcodes sequenced and used in

168 this study, with respective information of specimen voucher and GenBank Accession Numbers.

Species	Voucher code and life stage	Collection site	GenBank Accession Number
Angrisanoia cebollati (Angrisano, 1995)	ENT 2199 ♂	Brazil: Goiás: Alto Paraíso de Goiás	-
Betrichia bispinosa Flint, 1974	ENT 2337 ♂	Brazil: Amapá	KU094961[b]
Nothotrichia cautinensis Flint, 1983	-	-	KC559534[a]
Nothotrichia tupi Holzenthal & Harris, 1992	ENT 2460 ♂	Brazil: Minas Gerais: Catas Altas	KU743400
Ochrotrichia caatinga Souza, Santos & Takiya, 2014	ENT 2472 ♂	Brazil: Ceará: Ubajara	KU743401
Ochrotrichia patulosa (Wasmund & Holzenthal, 2007)	ENT 2473 ♂	Brazil: Ceará: Ubajara	KU743402
Ochrotrichia sp. CR1	ENT 2279 ♂	Costa Rica: Puntarenas	KU094950[b]
Oxyethira tica Holzenthal & Harris, 1992	ENT 0057 ♂	Brazil: Pará: Carajás	KU094940[b]
Ragatrichia sp. BR1	ENT 2338 ♂	Brazil: Amapá	KU743403
Rhyacopsyche dikrosa Wasmund & Holzenthal, 2007	ENT 0122 ♂	Brazil: Rio de Janeiro: Teresópolis	KU094952[b]
Rhyacopsyche torulosa Flint, 1971	ENT 2277 ♂ ENT 2192 ♂	Costa Rica: Puntarenas	KU743404 KU743406
Metrichia acuminata **sp. nov.**	ENT 2282 ♂ ENT 2284-5 ♂ ENT 2779 ♂	Brazil: Alagoas: Quebrangulo	KU743427 KU743428-29 KU743452
Metrichia amplitudinis Bueno-Soria & Holzenthal, 2003	ENT 2278 ♂	Costa Rica: Puntarenas	KU743425
Metrichia bonita **sp. nov.**	ENT 2200-4 larvae	Brazil: Mato Grosso do Sul: Bonito	KU743409-13
Metrichia bonita **sp. nov.**	ENT 2208-10 ♂	Brazil: Mato Grosso do Sul: Bonito	KU743417-19
Metrichia bracui **sp. nov.**	ENT 2508-11 ♂	Brazil: Rio de Janeiro: Itatiaia	KU743444-47
Metrichia caraca **sp. nov.**	ENT 2195 ♂ ENT 2280 ♂ ENT 2461-5 ♂	Brazil: Minas Gerais: Catas Altas	KU743408 KU743426 KU743434-38
Metrichia caraca **sp. nov.**	ENT 2292 ♂	Brazil: Minas Gerais: São Roque de Minas	KU743432
Metrichia circuliforme **sp. nov.**	ENT 2835-7 ♂ ENT 2839-40 ♂ ENT 2843-4 ♂	Brazil: Rio de Janeiro: Itatiaia	KU743455-57 KU743459-60 KU743462-63
Metrichia curta **sp. nov.**	ENT 2838 ♂ ENT 2846-8 ♂	Brazil: Rio de Janeiro: Itatiaia	KU743458 KU743464-66
Metrichia formosinha **sp. nov.**	ENT 2205-7 ♂	Brazil: Mato Grosso do Sul: Bonito	KU743414-16
Metrichia itabaiana **sp. nov.**	ENT 2190 ♂	Brazil: Sergipe: Areia Branca	KU743405
Metrichia itabaiana **sp. nov.**	ENT 2220-1 ♂	Brazil: Goiás: Alto Paraíso de Goiás	KU743424
Metrichia juana (Flint, 1964)	ENT 2850-1 ♂	Puerto Rico	KU743467-68
Metrichia longissima **sp. nov.**	ENT 2330 ♂	Brazil: Rio de Janeiro: Teresópolis	KU743433
Metrichia longissima **sp. nov.**	ENT 2841 ♂	Brazil: Rio de Janeiro: Itatiaia	KU743461
Metrichia rafaeli **sp. nov.**	ENT 2288-9 ♂	Brazil: Ceará: Ubajara	KU743430-31
Metrichia talhada **sp. nov.**	ENT 2193 ♂ ENT 2214 ♂ ENT 2216-7 ♂ ENT 2776 ♂	Brazil: Alagoas: Quebrangulo	KU743407 KU743420-22 KU743451

Metrichia tere **sp. nov.**	ENT 2773-5 ♂	Brazil: Rio de Janeiro: Teresópolis	KU743448-50
Metrichia vulgaris **sp. nov.**	ENT 2218 ♂	Brazil: Goiás: Alto Paraíso de Goiás	KU743423
Metrichia vulgaris **sp. nov.**	ENT 2466-70 ♂	Brazil: Minas Gerais: Catas Altas	KU743439-43
Metrichia vulgaris **sp. nov.**	ENT 2833-4 ♂	Brazil: Rio de Janeiro: Itatiaia	KU743453-54

169   Sequences obtained from GenBank: (a) Malm *et al.* 2013; (b) Santos *et al.* 2016.

170   **Sequence editing, alignment, and analyses**

171   Forward and reverse sequences of each sample were assembled and manually edited in

172   Sequencher 4.1 (Gene Codes, Ann Arbor, Michigan, USA). Sequences were verified with the

173   Blast tool in GenBank to check for contamination. Subsequently, COI sequences were aligned

174   with ClustalW implemented in MEGA 6 (Tamura et al., 2013) and translated into amino-acid

175   sequences to check for stop codons. The final alignment resulted in a matrix with 577 bp (Supp.

176   3).

177   COI sequences were used to explore putative species limits with four different

178   methodologies: (1) lineages recovered in neighbor-joining tree; (2) lineages recovered with

179   Bayesian Inference; (3) ABGD; and (4) GMYC. The neighbor-joining tree was calculated in

180   MEGA 6 using Kimura 2-Parameter (K2P) distances (Kimura, 1980), with partial deletion of

181   missing information. Although the use of K2P distances in DNA barcoding is debated (Srivathsan

182   & Meier, 2012), to allow comparison with previous works we also used this evolutionary model

183   because it is frequently used in studies of species delimitation based on COI sequences. Branch

184   support of neighbor-joining tree was assessed with 1,000 pseudoreplicates of non-parametric

185   bootstrap (Felsenstein, 1985).

186   BI analysis was conducted with MrBayes v. 3.2.2 (Ronquist *et al.*, 2012) with four

187   independent runs, each one with four MCMC chains running for 50,000,000 generations, with

188   sample frequency of 5,000. Convergence of sampled parameters was checked in Tracer v. 1.5

189   (Rambaut & Drummond, 2007) and the first 10% of sampled trees and parameters discarded as

190   burnin. GTR+I+G was the best fit model selected by Akaike Information Criterion (AIC) with

191   jModeltest v. 0.1.1 (Posada, 2008) and it was applied in BI analysis in MrBayes. Branch support

192   was assessed by posterior probability (PP), presented on a 50% majority consensus tree.

193   ABGD analysis was run using the on-line version available in

194   http://wwwabi.snv.jussieu.fr/public/abgd/, where the COI alignment was uploaded. The analysis

195   was conducted with the following settings: Pmin=0.001; Pmax=0.1; steps=20; relative gap

196   width=1.0, also based on K2P model. This method statistically infers the DNA barcode gap in a

197   single locus alignment, partitioning the data based on this gap in putative species (Puillandre et

198   al., 2012).

199   The GMYC analysis (Pons et al., 2006; Fujisawa & Barraclough, 2013) was performed in

200   R (R Development Core Team 2010) using the SPLITS package (Ezard et al., 2009) with single-

201   threshold method. Basically, the method estimates branching patterns on an ultrametric tree,

202   identifying the most likely transition point from coalescent to speciation branching. The

203   ultrametric tree used here was obtained with BEAST v. 1.8 (Drummond et al., 2012) under a

204   relaxed uncorrelated molecular clock (Drummond et al., 2006). The node including *Ochrotrichia*

205   species was calibrated based on fossil evidence with a lognormal distribution offset at 20 mya

206   and log(mean) = 2.8 to represent the possible range of 20-140 mya (Wells & Wichard, 1989); and

207   the divergence of Ochrotrichiinae was calibrated based on Malm et al. (2013) with a normal

208   distribution with mean 82.17 ± 12 mya. The BEAST analysis ran for 200,000,000 generations,

209   sampled every 10,000 generations. Convergence was verified with Tracer and a maximum

210   credibility tree was written using TreeAnotator, discarding the first 10% as burnin.

211   **RESULTS**

212   NJ (Fig. 2) and BI (Supp. 4) trees corroborated morphological identification, with all 14

213   species of *Metrichia* with more than a single specimen recovered as monophyletic lineages with

214   100% bootstrap support. ABGD also returned the same species as they were previously delimited

215   based on morphological features. A robust 'barcoding gap' was found among *Metrichia* species

216   (Fig. 3, Table 2). The maximum intraspecific divergence was observed within *Metrichia vulgaris*

217   **sp. nov.** (0.048). The minimum interspecific divergence was found between specimens of

218   *Metrichia talhada* **sp. nov.** and *Metrichia tere* **sp. nov.** (0.126).

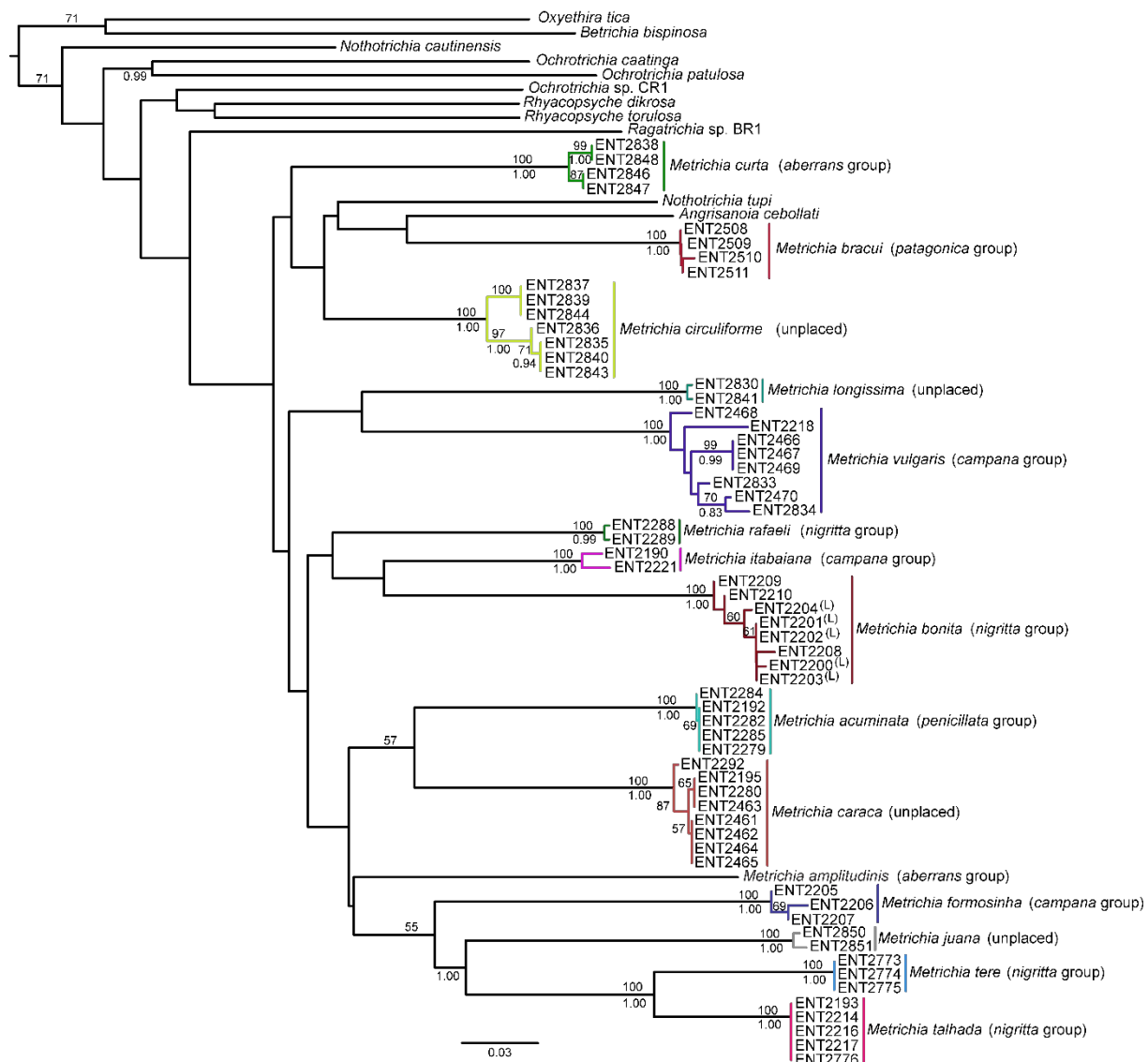
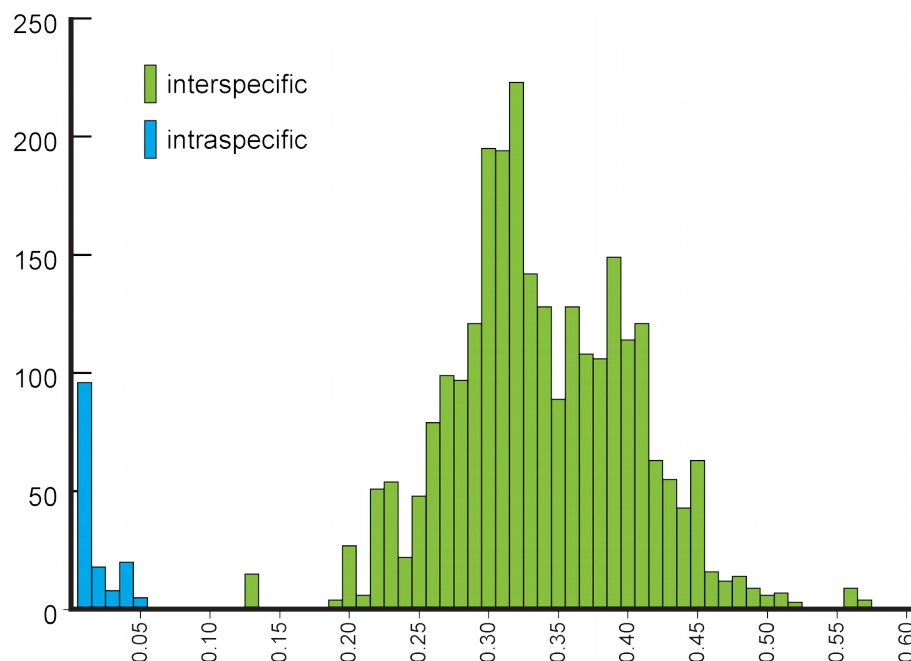



Figure 2. Neighbor-joining tree of COI sequences of *Metrichia* species based on K2P distances.

220   Numbers above and below branches are, respectively, NJ bootstrap support and posterior

221 probabilities from BI analysis. Details of specimens are in Tables 1 and 2; K2P distances matrix

222 is in Supp. 5.

223 **Figure 3.** Histogram of the number of pairwise comparisons of intra- (blue) and interspecific

224 (green) K2P divergences among 15 *Metrichia* species with COI sequences sampled.

225 **Table 2.** Maximum intra- and minimum interspecific K2P divergences of COI sequences among

226 and within *Metrichia* species.

Species	Number of sequences	Max. intra. distance	Min. inter. Distance
M. acuminata **sp. nov.**	5	0.000	0.217
M. amplitudinis Bueno-Soria & Holzenthal, 2003	1	-	0.220
M. bonita **sp. nov.**	8	0.015	0.210
M. bracui **sp. nov.**	4	0.004	0.214
M. caraca **sp. nov.**	8	0.011	0.217
M. circuliforme **sp. nov.**	7	0.035	0.184
M. curta **sp. nov.**	4	0.015	0.184
M. formosinha **sp. nov.**	3	0.008	0.249
M. itabaiana **sp. nov.**	2	0.019	0.194
M. juana (Flint, 1964)	2	0.007	0.243

M. longissima **sp. nov.**	2	0.004	0.215
M. rafaeli **sp. nov.**	2	0.004	0.194
M. talhada **sp. nov.**	5	0.000	0.126
M. tere **sp. nov.**	3	0.000	0.126
M. vulgaris **sp. nov.**	8	0.048	0.246

ENT2774
ENT2775 *Metrichia tere*
ENT2773
ENT2214
ENT2216
ENT2217 *Metrichia talhada*
ENT2193
ENT2776
ENT2851 *Metrichia juana*
ENT2850
ENT2205
ENT2207 *Metrichia formosinha*
ENT2206
ENT2338 *Ragatrichia* sp. 1BR
ENT2190 *Metrichia itabaiana*
ENT2221
ENT2330 *Metrichia longissima*
ENT2841
ENT2468
ENT2470
ENT2834
ENT2218 *Metrichia vulgaris*
ENT2466
ENT2469
ENT2467
ENT2833
ENT2203
ENT2201
ENT2208
ENT2210 *Metrichia bonita*
ENT2200
ENT2204
ENT2202
ENT2209
ENT2462
ENT2465
ENT2461
ENT2292 *Metrichia caraca*
ENT2464
ENT2280
ENT2195
ENT2463
ENT2844
ENT2839
ENT2837
ENT2835 *Metrichia circuliforme*
ENT2840
ENT2843
ENT2836
ENT2848
ENT2838 *Metrichia curta*
ENT2846
ENT2847
ENT2508
ENT2509 *Metrichia bracui*
ENT2511
ENT2510
ENT2278 *Metrichia amplitudinis*
ENT2289 *Metrichia rafaeli*
ENT2288
ENT2779
ENT2282
ENT2285 *Metrichia acuminata*
ENT2284
ENT2192
ENT2460 *Nothotrichia tupi*
ENT2199 *Angrisanoia cebollati*
ENT2279 *Ochrotrichia* sp. 1CR
ENT2472 *Ochrotrichia caatinga*
ENT2473 *Ochrotrichia patulosa*
ENT0122 *Rhyacopsyche dikrosa*
ENT2277 *Rhyacopsyche torulosa*
GenBank *Nothotrichia cautinensis*

75   50   25   0

227 **Figure 4.** Maximum credibility time tree from the BEAST analysis based on COI sequences of

228 *Metrichia* and other Ochrotrichiinae. Red branches represent species estimated by GMYC using

229 single threshold in SPLITS. Timescale in millions of years.

230   GMYC analysis estimated a slightly higher number of putative species, with *Metrichia*

231 *circuliforme* **sp. nov.** and *Metrichia vulgaris* **sp. nov.** being each further divided into two species

232 (Fig. 4). Regarding all other species, GMYC results were congruent with other methods and with

233 morphology.

234   In all analyses performed using DNA barcode, *Metrichia* larvae collected in calcareous

235 tufa were consistently associated with adult males of *Metrichia bonita* **sp. nov.** Therefore, in the

236 following section, we describe these larvae within that species.

237 **SPECIES DESCRIPTIONS**

238 *Metrichia acuminata* **sp. nov.**

239 urn:lsid:zoobank.org:act:01493211-CD39-4995-B2D7-8F3B44A0970E

240 (Fig. 5, Fig. 26A)

241 **Adult male.** Length 2.1–2.5 mm (n=5). General color, in alcohol, brown. Head with no

242 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

243 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

244 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

245 wings. Abdominal segment IV with pair of internal pouches in posterior area; segment V with

246 pair of internal pouches; segment VI with tergum as a sclerotized rounded plate, surrounded by

247 long setae (Fig. 26A); segment VII bearing a brush of very long setae dorsolateraly (Fig. 26A).

248 Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally and

249 bearing a brush of long setae dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum

250 subrectangular, with anterior margin rounded (Fig. 5A); in lateral view narrower anteriorly than

251 posteriorly (Fig. 5C). Inferior appendage covered by long setae; subtrapezoidal in ventral view

252 (Fig. 5A); in lateral view, subtrapezoidal, apex with acute corners (Fig. 5C). Dorsal hook short,

253 almost half length of inferior appendage; in lateral view, downturned (Fig. 5C). Preanal

254 appendage rounded in lateral view and bearing very long setae (Fig. 5C). Subgenital plate

255 apparently absent. Tergum X membranous and truncate (Fig. 5B). Phallus tubular, elongate and

256 slender, slightly constricted mesally, with two curved subapical spines, one short and another

257 long; apex truncate and sclerotized; ejaculatory duct sclerotized, sinuous, and protruding apically

258 (Fig. 5D).

259 **Holotype. BRAZIL: Ceará:** Ubajara, Parque Nacional de Ubajara, Cachoeira do Gameleira,

260 03º50'21"S 40º54'23"W, el. 880 m, 23.iv.2012, DM Takiya & JA Rafael cols., light trap, male

261 (CZMA).

262 **Paratypes.** Same data as holotype, except, Rio das Minas, 03º50'03"S 40º54'18"W, el. 524 m,

263 13–17.ix.2012, JA Rafael et al., Malaise trap, 2 males (INPA); same data, except 14–16.ii.2013,

264 DM Takiya, JA Rafael, RR Cavichioli & APM Santos, Malaise trap, 2 males (DZRJ). **Alagoas:**

265 Quebrangulo, Reserva Biológica de Pedra Talhada, Rio Caranguejo, 09º15'26"S 36º25'08"W, el.

266 550 m, 19–28.vi.2014, APM Santos, DM Takiya, WRM Souza, Malaise trap, 2 males (MNRJ), 3

267 males (MZUFBA), 13 males (DZRJ).

268 **Etymology.** The species is named in allusion to the pointed apices of inferior appendages (from

269 Latin, "acumin-" = "pointed").

270 **Remarks**. This new species belongs to the *penicillata* group based on: (1) internal pouches

271 between abdominal segments IV and V; (2) setal brushes on segments V, VI, and VII; and (3)

272 phallus with two subapical spines. The male genitalia and complex abdominal modifications

273 resemble *M. penicillata* (Flint, 1972) and *M. trigonella* (Flint, 1972). These three species have

274 inferior appendages with acute apices in lateral view; phallus with two subapical spines; and

275 abdominal terga with brushes of very long and stout setae. The new species can be distinguished

276   by inferior appendages more trapezoidal in lateral view, with acute corners posteriorly and dorsal

277   hook only slightly downturned in lateral view; and phallus with one larger and stouter subapical

278   spine. Although the male genitalia of this new species superficially resemble that of *M. bonita* **sp.**

279   **nov.**, *M. acuminata* **sp. nov.** is readily recognized by setose lobes on abdominal segments V and

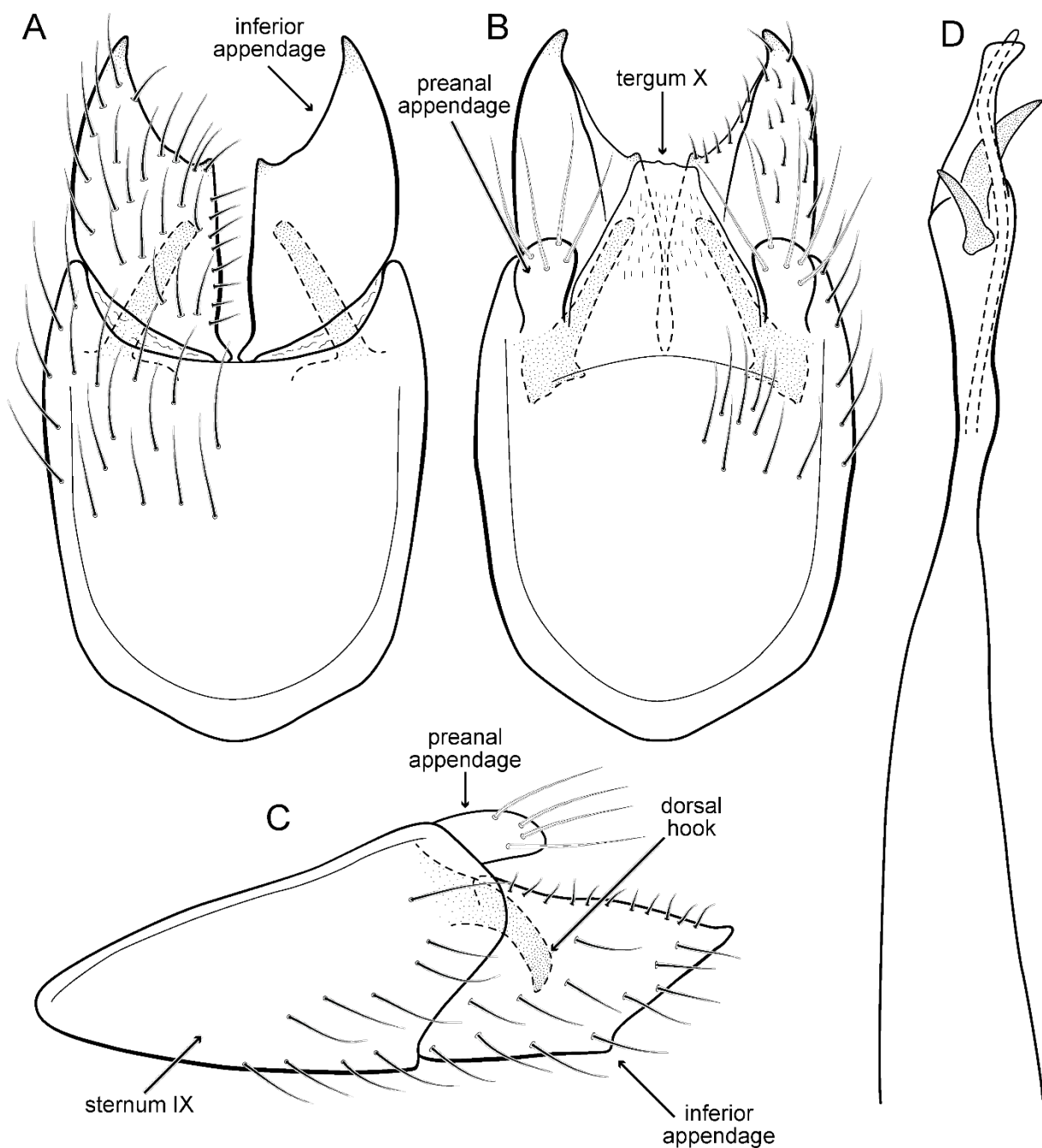
280   VI.

281   We were not able to obtain COI sequences for individuals from Ceará State, so the five

282   sequences analyzed belong to specimens from Alagoas State, which shared the same haplotype.

283   *Metrichia acuminata* **sp. nov.** was recovered as closely related to *M. caraca* **sp. nov.** (Fig. 2), but

284   in both Bayesian approaches these two species were not recovered as sister taxa (Fig. 4, Supp. 1).

Figure 5. *Metrichia acuminata* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C) lateral view; (D) phallus, dorsal view.

285 **Figure 5.** *Metrichia acuminata* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)

286 lateral view; (D) phallus, dorsal view.

287 *Metrichia azul* **sp. nov.**

288 urn:lsid:zoobank.org:act:E38ACAE9-61DC-4B61-A20D-1879520E1DD3

289 (Fig. 6)

290 **Adult male.** Length 2.0–2.1 mm (n=4). General color, in alcohol, brown. Head with no

291 modifications. Ocelli 3. Maxillary palpus 5-articulated, article IV broad and darkened; labial

292 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

293 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

294 wings. Abdominal segment V with pair of internal pouches; segment VI with pair of internal

295 pouches and pair of lateral external sacs with specialized setae. Ventromesal process on segment

296 VII present. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced

297 dorsally; sternum subrectangular (Fig. 6A); in lateral view narrower anteriorly than posteriorly

298 (Fig. 6C). Inferior appendage covered by long setae and with scale-like setae; subrectangular in

299 ventral view (Fig. 6A); in lateral view, rounded, apex rounded (Fig. 6C). Dorsal hook long, more

300 than half length of inferior appendage; in lateral view, slightly downturned (Fig. 6C). Preanal

301 appendage elongate, but shorter than inferior appendage, and bearing very long setae (Fig. 6B).

302 Subgenital plate apparently absent. Tergum X membranous and subrectangular (Fig. 6B). Phallus

303 tubular, elongate and slender, slightly constricted mesally, with two long, curved, subapical

304 spines; apex rounded and sclerotized; ejaculatory duct sclerotized, sinuous, and protruding

305 apically (Fig. 6D).

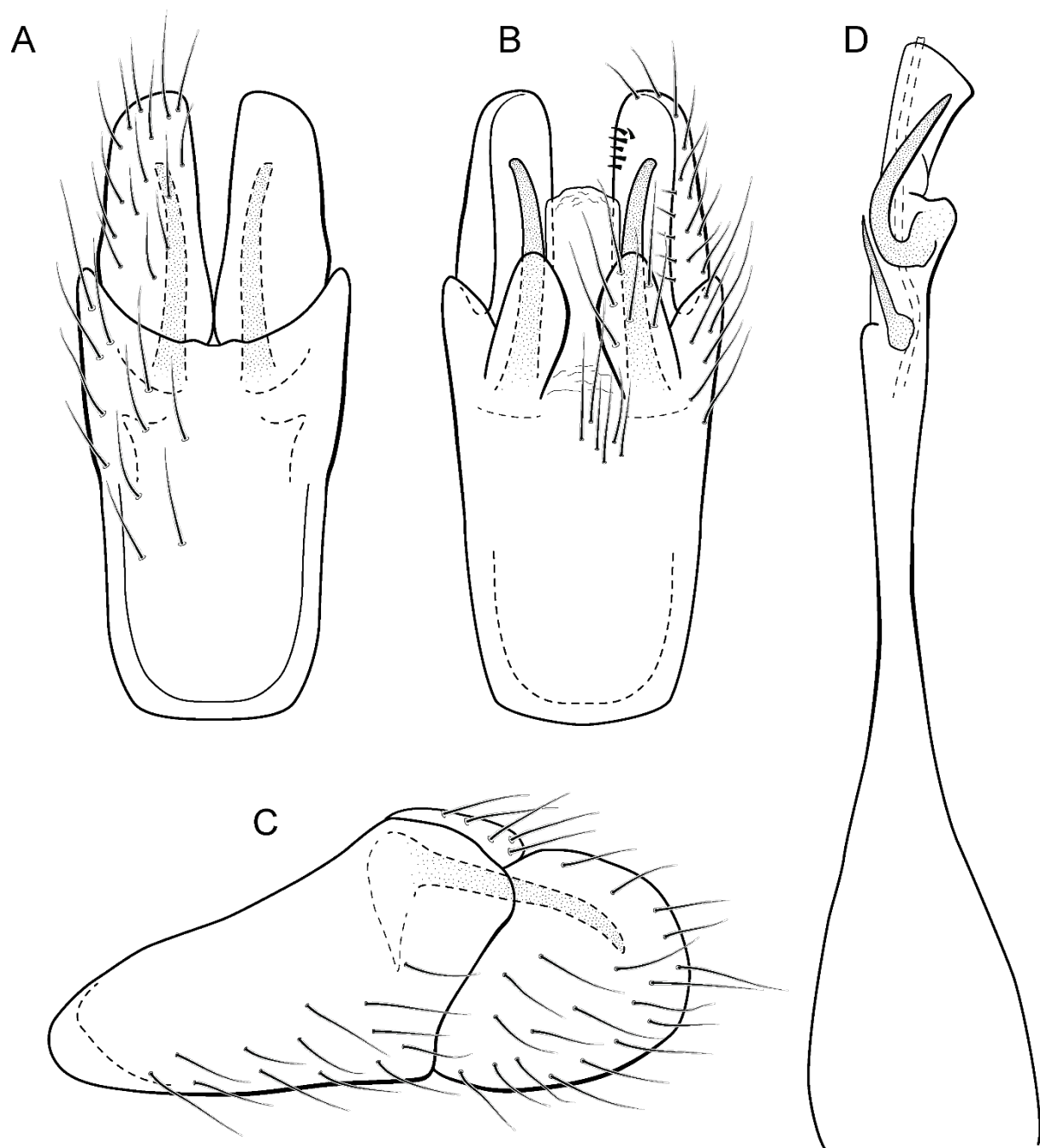
306 **Holotype. BRAZIL: Paraná:** Céu Azul, Parque Nacional do Iguaçu, Rio Azul, 25º09'21"S

307 53º47'44"W, el. 510 m, 6–8 ix.2012, APM Santos, DM Takiya, ALH Oliveira, GA Jardim &

308 BHL Sampaio cols., Malaise trap, male (DZRJ).

309 **Paratypes.** Same data as holotype, 2 males (DZRJ), 1 male (MNRJ).

310 **Etymology.** The specific name refers to the type locality, Rio Azul in the municipality of Céu

311 Azul.

312   **Figure 6.** *Metrichia azul* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C) lateral

313   view; (D) phallus, dorsal view.

314   **Remarks**. This new species is another member of the *penicillata* group based on internal pouches

315   between segment V and VI and the long subapical spines of the phallus. The new species shares

316   similarities of the male genitalia with *M. biungulata* (Flint, 1972) and *M. decora* Bueno-Soria &

317   Holzenthal, 2003 particularly the rounded aspect of inferior appendages, but can be easily

318   distinguished from those species by the absence of tooth-like processes on inferior appendages;

319   more elongate preanal appendages; and dorsal hook only slightly downturned in lateral view.

320   *Metrichia bonita* sp. nov.

321   urn:lsid:zoobank.org:act:622BCD51-CC39-4D1F-BDCB-84A7DFF4F071

322   (Fig. 7, Fig. 8)

323   **Adult male.** Length 2.3–2.5 mm (n=4). General color, in alcohol, brown. Head with no

324   modifications. Ocelli 3. Antenna simple, 21-articulated. Maxillary palpus 5-articulated; labial

325   palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

326   Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

327   wings. Abdominal segment VI with dorsal pouches covered with setae directed inward.

328   Ventromesal process on segment VII absent. Segment VIII shorter ventrally than dorsally. **Male**

329   **genitalia**. Segment IX reduced dorsally; sternum subrectangular, with anterior margin rounded

330   (Fig. 7A); in lateral view narrower anteriorly than posteriorly (Fig. 7C). Inferior appendage

331   covered by long setae, subtrapezoidal in ventral view (Fig. 7A); in lateral view, with an acute

332   projection, apex with acute corners (Fig. 7C). Dorsal hook short, almost half length of inferior

333   appendage; in lateral view, slightly downturned (Fig. 7C). Preanal appendage short, rounded and

334   bearing very long setae (Fig. 7B). Subgenital plate apparently absent. Tergum X membranous and

335   with a shallow U-shaped incision (Fig. 7B). Phallus tubular, elongate and slender, slightly

336   constricted mesally, with a median process; with two curved subapical spines, one short and

337   another long; apex truncate and sclerotized; ejaculatory duct sclerotized and not protruding

338   apically (Fig. 7D).

339   **Larva (5th instar).** Length 1.5–1.9 mm (n=10). Head dark brown, unpigmented around stemmata

340   (Fig. 8C); slightly longer than broad; frontoclypeal and coronal sulci indistinct; with a few long

341   setae (Fig. 7F). Antenna short, apparently 2-articulated and with no apical setae (Fig. 7F).

342   Labrum with pair of stout setae (Fig. 7F). Mandibles with inner margin sinuous and darkened.

343   Thoracic nota sclerotized, dark brown, with a row of stout setae on anterior margin. Pro-, meso-,

344   and metanotum with middorsal ecdysial line. Thoracic segments with small pleurites (Fig. 7E).

345   Thoracic legs brown, short and stout, almost the same size of each other. Foreleg with stout setae;

346   tibia with a posteroventral lobe with a spine-like seta (Fig. 7G); femur bearing a spine-like setae;

347   tarsal claw simple. Mid- and hind legs with stout setae on posteroventral margin. Abdomen

348   almost white, with dark brown sclerites. Abdominal segment I with ellipsoid tergite; segments I-

349   V with pair of long, dorsal setae and pair of dorsolateral setae; segment VI with two pairs of long

350   dorsal setae and two pairs of dorsolateral setae; segments VII and VIII with three pairs of dorsal,

351   long setae and two pair of dorsolateral setae; segment IX with sclerotized tergite and several long

352   setae. Abdominal segments I, III, IV, and IX with pair of ventral, long setae; segment II with two

353   pairs of ventral, long setae and globose process on ventrolateral area (Fig. 7E). Anal proleg very

354   short not projecting prominently; with basal sclerite bearing long setae; anal claw simple.

355   **Larval case.** Length 1.5–2.0 mm (n=10). General color white (Figs. 8B, 8C). Constructed with

356   calcareous particles (with no algal filaments added), forming two rigid and lateral valves, poorly

357   closed dorsally and ventrally (Fig. 8C). External surface rugose.

358   **Biology.** Larvae were collected on calcareous tufa in a fast flowing river, approximately 10

359   meters wide (Fig. 8A). No pupae were found and adults were not seen active during the day.

A

B

D

median
process

E

globose
process

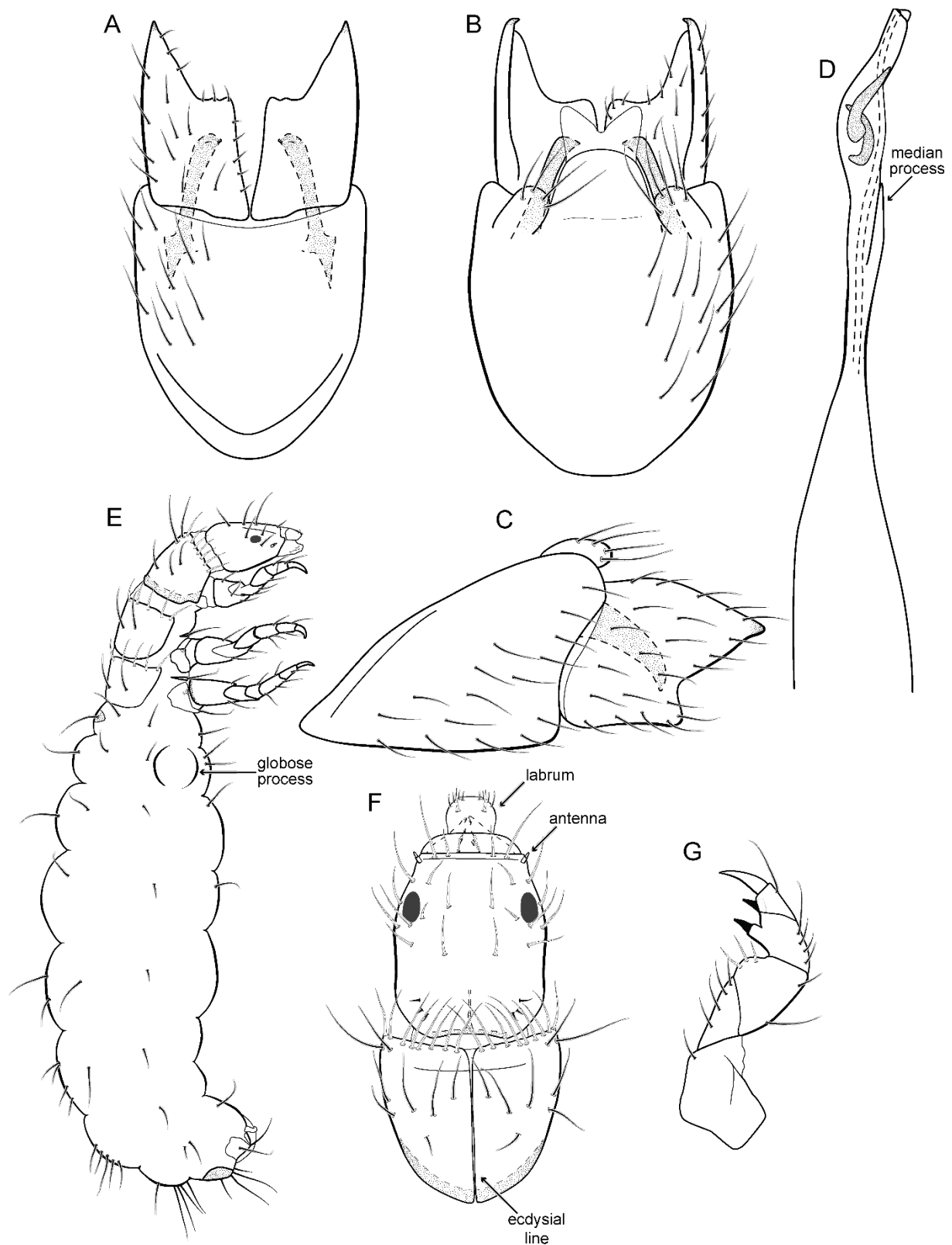
C

F

labrum

antenna

G

ecdysial
line

360 **Figure 7.** *Metrichia bonita* **sp. nov.**: (A) male genitalia, ventral view; (B) male genitalia, dorsal

361 view; (C) male genitalia, lateral view; (D) phallus, dorsal view; (E) larva, habitus, lateral view;

362 (F) larva, head and pronotum, dorsal view; (G) larva, foreleg, ventral view.

363 **Holotype. BRAZIL: Mato Grosso do Sul:** Bonito, Rio Formosinho, 21º10'16"S 56º26'47"W

364 el. 275 m, 08–13.ix.2013, APM Santos & DM Takiya cols., Malaise trap, male (DZRJ).

365 **Paratypes.** Same data as holotype, 3 males (DZRJ), 1 male (MNRJ).

366 **Additional material.** Same data as holotype, except 13.ix.2013, manual, 10 larvae (DZRJ), 10

367 larvae (MNRJ).

368 **Etymology.** This species is named in reference to the type locality (Fig. 8), the municipality of

369 Bonito in the state of Mato Grosso do Sul. In Portuguese, the word "bonita" (the feminine form)

370 means "beautiful".

371 **Remarks**. *Metrichia bonita* **sp. nov.** has features of the *nigritta* group: internal pouches between

372 segment V-VI and phallus with 2 spines and an acute process on distal portion. This new species

373 can be easily distinguished from other species in this group based on the shape of inferior

374 appendages, subtrapezoidal in ventral view, with dorsal corners acute and darkened. In addition,

375 inferior appendages have dorsal hooks, which are broad basally and slightly downturned in lateral

376 view. COI sequences showed maximum intraspecific distance of 1.5% and minimum interspecific

377 distance of 21.0% to its closest neighbor, *M. itabaiana* **sp. nov.** Although the male genitalia of

378 both species show some superficial resemblance, based on the abdominal modifications and

379 phallic aspect, *M. bonita* **sp. nov.** belongs to the *nigritta* group, whereas *M. itabaiana* fits better

380 in the *campana* group.

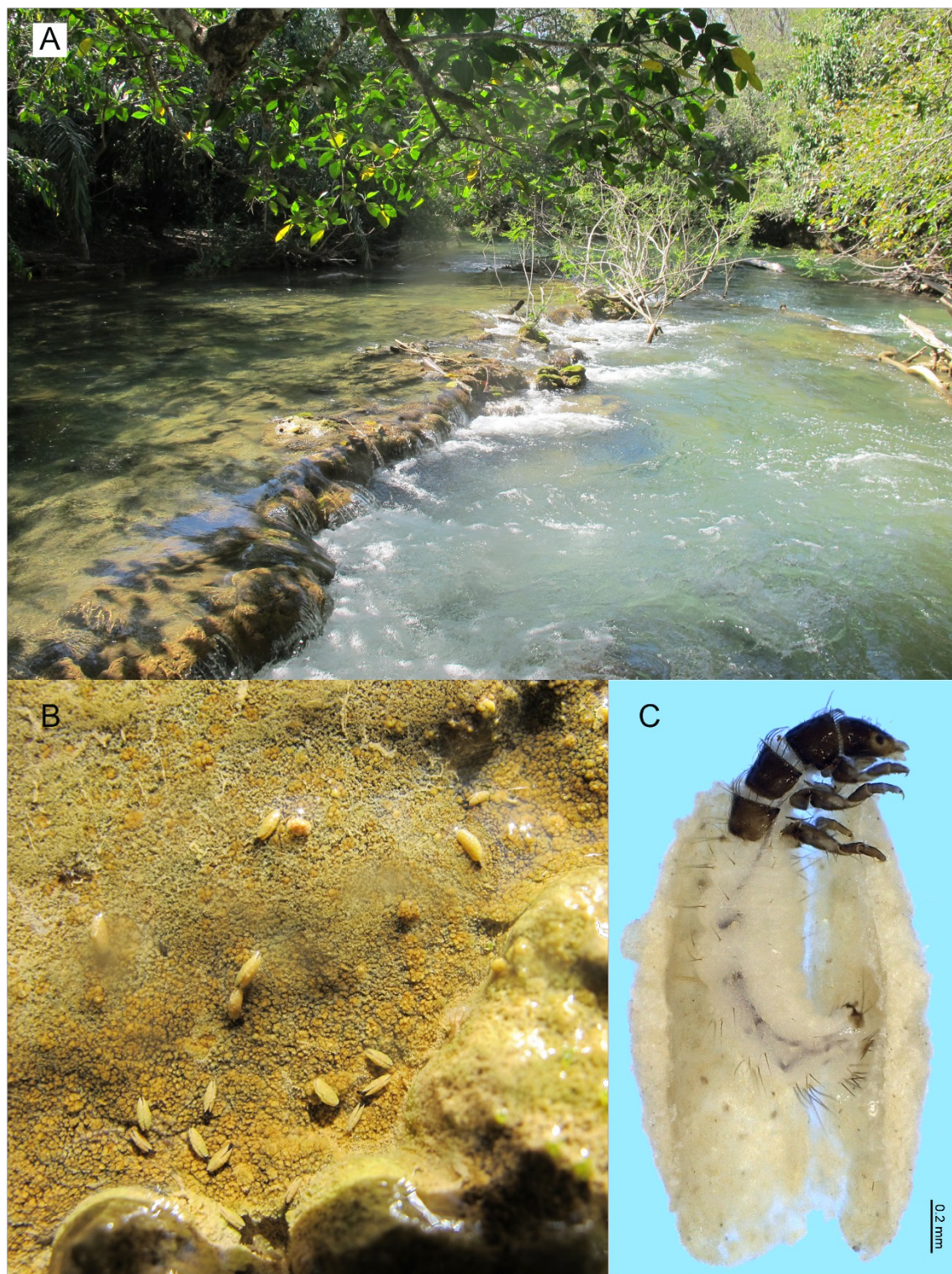
381 Larvae of *Metrichia bonita* **sp. nov.** are very similar to those previously described or

382 illustrated, including *M. nigritta*, *M. juana*, and unassociated larvae illustrated by Botosaneanu &

383 Flint (1982) from Venezuela and Ecuador; and by Pes et al. (2005) from Brazil. Actually, main

384   differences seem to be the shape and the material of larval cases. In this respect, larvae of

385   *Metrichia bonita* **sp. nov.** are unusual and easily recognized by having their case made entirely of

386   calcareous particles, without typical algal elements (Fig. 8).

387   **Figure 8.** *Metrichia bonita* **sp. nov.**, larva: (A) type locality, Rio Formosinho, Bonito

388   municipality, Mato Grosso do Sul, Brazil; (B) larvae on calcareous substrate; (C) larva and its

389   calcareous case.

390   Paprocki et al. (2003) discussed the role of *Smicridea travertinera* Paprocki, Holzenthal

391   & Cressa, 2003 in calcareous tufa formation (travertine). According to these authors, larvae of

392   that species interfere in the deposition and erosion of the calcareous substrate by their net-

393   building activities (Paprocki et al., 2003). Cyanobacteria and diatoms are known to participate in

394   travertine formation, but the role played by macroinvertebrates is poorly understood (Drysdale,

395   1998; 1999). It is possible that cases of *Metrichia bonita* **sp. nov.** are impregnated passively with

396   calcareous particles, but as commented by Drysdale (1999) for other aquatic insects, they could

397   be important in travertine biogenesis by producing new nucleation sites or eroding other ones.

398   *Metrichia bonita* **sp. nov.** is the only microcaddisfly known to inhabit (Fig. 8) and build cases

399   with calcareous tufa so far.

400   ***Metrichia bracui* sp. nov.**

401   urn:lsid:zoobank.org:act:07B44840-CAB0-4BBE-BD01-B62E885BE418

402   (Fig. 9)

403   **Adult male.** Length 1.8–2.2 mm (n=3). General color, in alcohol, light brown. Head with no

404   modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

405   palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

406   Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

407   wings. Abdomen without modifications. Ventromesal process on segment VII absent. Segment

408   VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum

409   subpentagonal in ventral view (Fig. 9A); in lateral view narrower anteriorly than posteriorly (Fig.

410   9C). Inferior appendage covered by long setae and with scale-like setae, subrectangular in ventral

411   view (Fig. 9A); in lateral view, rounded, apex rounded (Fig. 9C). Dorsal hook long, more than

412   half length of inferior appendage; in lateral view, downturned (Fig. 9C). Preanal appendage

413 elongate and bearing very long setae (Fig. 9B). Subgenital plate apparently absent. Tergum X

414 membranous and with shallow U-shaped incision (Fig. 9B). Phallus tubular, elongate and slender,

415 slightly constricted mesally; without spines, but with a sclerotized process arising from a

416 subapical constriction; apex rounded and folded; ejaculatory duct sclerotized, straight and not

417 protruding apically (Fig. 9D).

418 **Holotype. BRAZIL: Rio de Janeiro:** Angra dos Reis, Rio Bracuí, 23º00'23''S 44º29'15''W, el.

419 75 m, 10–11.v.2002, JL Nessimian col., light trap, male (DZRJ).

420 **Paratypes.** Same data as holotype, 2 males (MNRJ). **Rio de Janeiro:** Parque Nacional do

421 Itatiaia, Córrego do Maromba, 22º25'32''S 44º37'03''W, el. 1250 m, 04.iv.15, APM Santos & DM

422 Takiya cols., Malaise trap, 4 males (DZRJ).

423 **Etymology.** The species is named in allusion to the river where the holotype was collected.

424 **Remarks**. This new species can be assigned to the *patagonica* group because of the absence of

425 curved spines of the phallus. The general aspect of the male genitalia resembles *M. patagonica*

426 (Flint, 1983), *M. pernambucana*, and *M. pseudopatagonica* Bueno-Soria & Holzenthal, 2003.

427 *Metrichia bracui* **sp. nov.** differs from these species and others in the group specially by the

428 phallus bearing a sclerotized process on a constricted region.

429 The four COI sequences generated for *M. bracui* **sp. nov.** were from specimens collected

430 in a locality at Parque Nacional do Itatiaia, Rio de Janeiro, Brazil. The highest pairwise

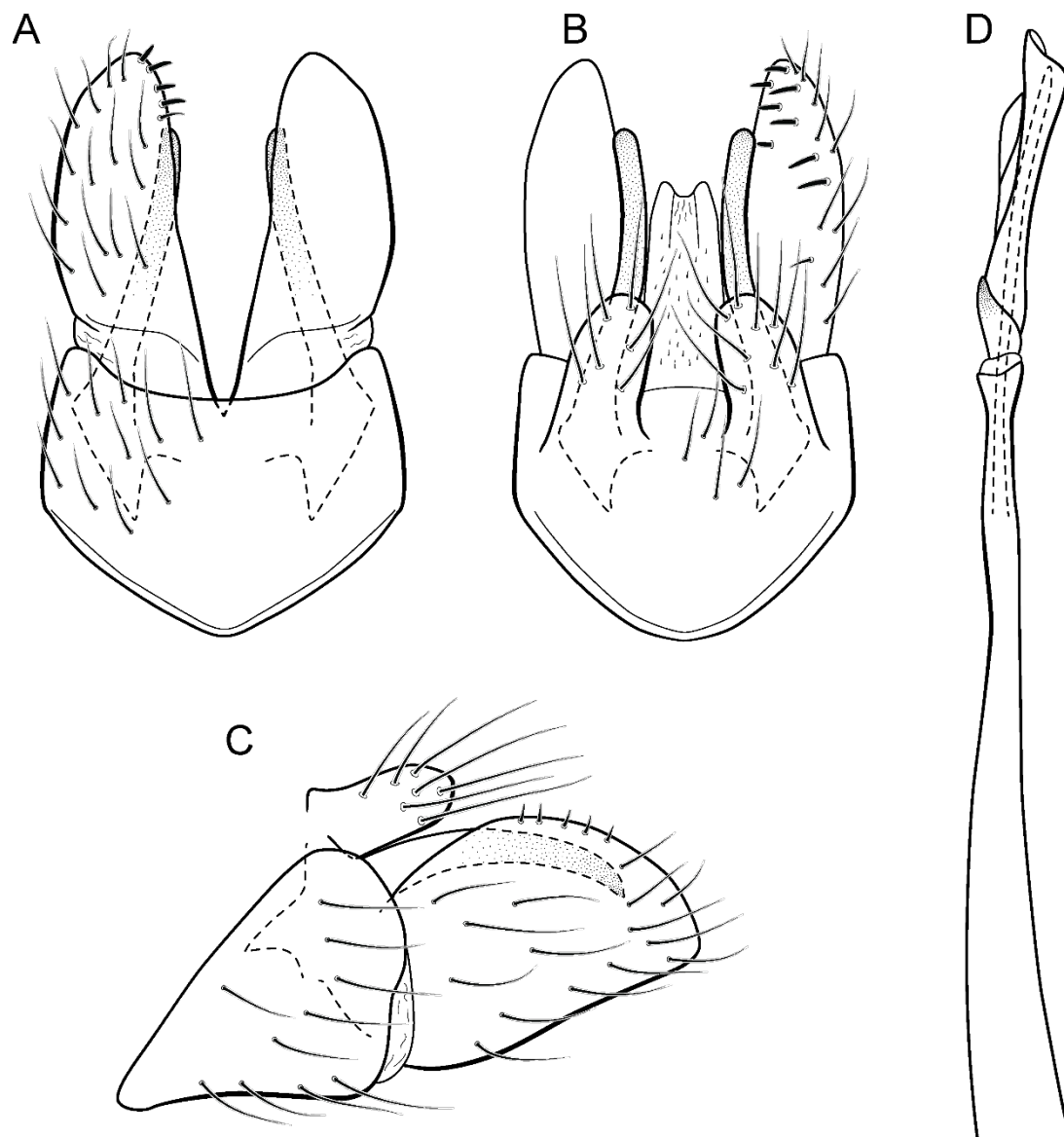
431 intraspecific divergence between sequences was 0.4%, and the lowest interspecific divergence

432 was 21.4% between *M. bracui* **sp. nov.** and *Angrisanoia cebollati* (Angrisano, 1995). Until now,

433 there have been no formal studies on the relationships among species or genera included in

434 Ochrotrichiinae. However, to infer phylogenetic hypothesis for the entire subfamily is beyond the

435 scope of this work, and also much more data and taxa sampling are necessary to generate robust

436 hypotheses.

437 **Figure 9.** *Metrichia bracui* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C) lateral

438 view; (D) phallus, dorsal view.

439 *Metrichia caraca* **sp. nov.**

440 urn:lsid:zoobank.org:act:34F912BC-1069-433E-AF16-A1B19D7FA622

441 (Fig. 10)

442 **Adult male.** Length 2.5–3.0 mm (n=7). General color, in alcohol, brown. Head with no

443 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

444   palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

445   Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

446   wings. Abdominal segment VI bearing brush of very long setae dorsolateraly; segment VII

447   bearing brush of very long setae dorsolateraly. Ventromesal process on segment VII absent.

448   Segment VIII shorter ventrally than dorsally and bearing brush of long setae dorsally. **Male**

449   **genitalia**. Segment IX reduced dorsally; sternum subrectangular, with anterior margin rounded

450   (Fig. 10A); in lateral view narrower anteriorly than posteriorly (Fig. 10C). Inferior appendage

451   covered by long setae, subtrapezoidal in ventral view (Fig. 10A), apex oblique and projected

452   mesad into a large process bearing a stout spine-like setae. Dorsal hook short and straight; in

453   lateral view, truncate and broader apically (Fig. 10C). Preanal appendage short, truncate and

454   bearing very long setae (Fig. 10B). Subgenital plate apparently absent. Tergum X membranous

455   and truncate (Fig. 10B). Phallus tubular, elongate and slender, slightly constricted mesally; with

456   two curved subapical spines, one short and another long; apex emarginate; ejaculatory duct

457   sclerotized and not protruding apically (Fig. 10D).

458   **Holotype. BRAZIL: Minas Gerais:** Catas Altas, RPPN Santuário do Caraça, Ribeirão Caraça,

459   11–13.vi.2013, ML Monné & JP Botero cols., Malaise trap, male (DZRJ).

460   **Paratypes.** Same data as holotype, 3 male (DZRJ). **Minas Gerais:** São Roque de Minas, Parque

461   Nacional da Serra da Canastra, Fazenda Velha, Córrego dos Pombos, 20º14'57"S 46º38'05"W, el.

462   997 m, 02.iv.2014, JL Nessimian, ALH Oliveira, LL Dumas & SP Gomes, light trap cols., 1 male

463   (MNRJ).

464   **Etymology.** This species is named in reference to the stream where type specimens were

465   collected.

466   **Remarks**. This new species has very distinctive male genitalia. Based on the dorsoapically

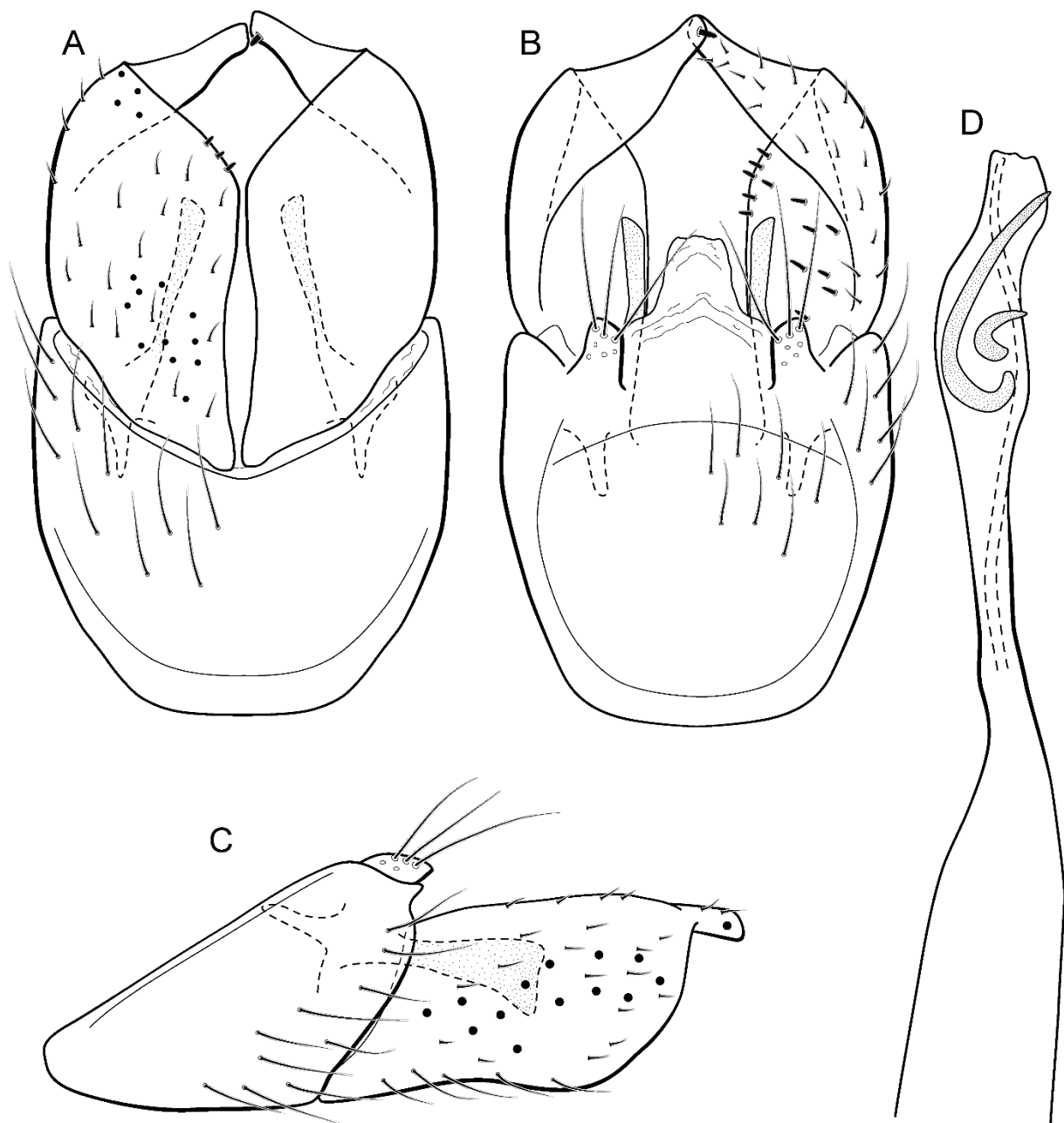
467   produced inferior appendages, it resembles *M. lenophora* (Flint, 1991). However, *M. caraca* **sp.**

468   **nov.** is easily recognized by the obliquely truncate and mesad directed process of inferior

469   appendages and dorsal hook, in lateral view, with apex broad and truncate.

470   COI distances within this species reached only 1.1% and the lowest interspecific distance

471   (21.7%) was found between specimens of *M. caraca* **sp. nov.** and *M. acuminata* **sp. nov.**, which

472   are very distinct based on morphological features.

473   **Figure 10.** *Metrichia caraca* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)

474   lateral view; (D) phallus, dorsal view.

475   *Metrichia circuliforme* **sp. nov.**

476   urn:lsid:zoobank.org:act:E539EF55-F963-433C-BB25-C9177339ED54

477   (Fig. 11)

478   **Adult male.** Length 2.5–2.7 mm (n=4). General color, in alcohol, brown. Head with no

479   modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

480   palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

481   Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

482   wings. Abdominal segment VI bearing brush of very long setae dorsolateraly; segment VII

483   bearing a brush of very long setae dorsolateraly. Ventromesal process on segment VII present.

484   Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally;

485   sternum subpentagonal (Fig. 11A); in lateral view narrower anteriorly than posteriorly (Fig. 11C).

486   Inferior appendage short, covered by long setae, subrectangular in ventral view (Fig. 11A); in

487   lateral view, rounded (Fig. 11C), apex slightly truncate and bearing short spine-like setae. Dorsal

488   hook long, almost reaching the inferior appendage apex; in lateral view, downturned (Fig. 11C).

489   Preanal appendage elongate, but shorter than inferior appendage, and bearing very long setae

490   (Fig. 11B). Subgenital plate apparently absent. Tergum X membranous and truncate (Fig. 11B).

491   Phallus tubular, elongate and slender, slightly constricted mesally and with a median process;

492   with two short subapical spines; apex rounded and sclerotized; ejaculatory duct sclerotized,

493   straight and not protruding apically (Fig. 11D).

494   **Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, Rio das Pedras, Cachoeira de Deus, 22º25'00"S

495   44º32'50"W, el. 689 m, 06.iii.2008, JL Nessimian, LL Dumas & MR de Souza cols., light trap,

496   male (DZRJ).

497 **Paratypes.** Same data as holotype, except Rio das Pedras, 22º24'33"S 44º33'08"W, el. 706 m,

498 06.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, 1 male (DZRJ), 1 male

499 (MNRJ); Parque Nacional do Itatiaia, Córrego Simon, 22º26'16"S 44º36'20"W, el. 1033 m,

500 15.iv.07, LL Dumas, APM Santos, N Ferreira-Jr. & JL Nessimian cols., light trap, 1 male (DZRJ).

501 **Etymology.** The new species name is an allusion to the rounded and simple inferior appendages,

502 derived from the Latin, "circuli-" and "form" meaning "rounded shape".

503 **Remarks**. This species has simple male genitalia and abdomen with only brushes of long setae

504 on segments VI and VII. General aspect of the male genitalia is similar to *M. riva* (Bueno-Soria,

505 1983) and *M. quadrata* (Flint, 1972), particularly their inferior appendages short and

506 subrectangular and phallus with two subapical spines. However, *M. circuliforme* **sp. nov.** can be

507 easily distinguished from *M. quadrata* by the absence of internal sacs in the abdomen. It can be

508 distinguished from *M. riva* by the elongate preanal appendages and phallus with subequal hook

509 spines subapically.

510 We obtained seven COI sequences for *M. circuliforme* **sp. nov.** and although all of them

511 came from specimens collected at the same locality, intraspecific divergences were relatively

512 high, reaching 3.5%. Besides that, GMYC estimated two species for these sequences instead of

513 one. Re-analysis of the morphology of these specimens did not reveal any conspicuous variation

514 that could justify splitting this species into two taxonomic groups. Compared to other studies

515 using DNA barcodes of caddisflies, this genetic distance is still low, for example Pauls et al.

516 (2010) found intraspecific divergences (K2P distance) up to 5.9% for Chilean *Smicridea*. Zhou et

517 al. (2011) found even higher intraspecific distances among caddisflies, reaching up to 14%.

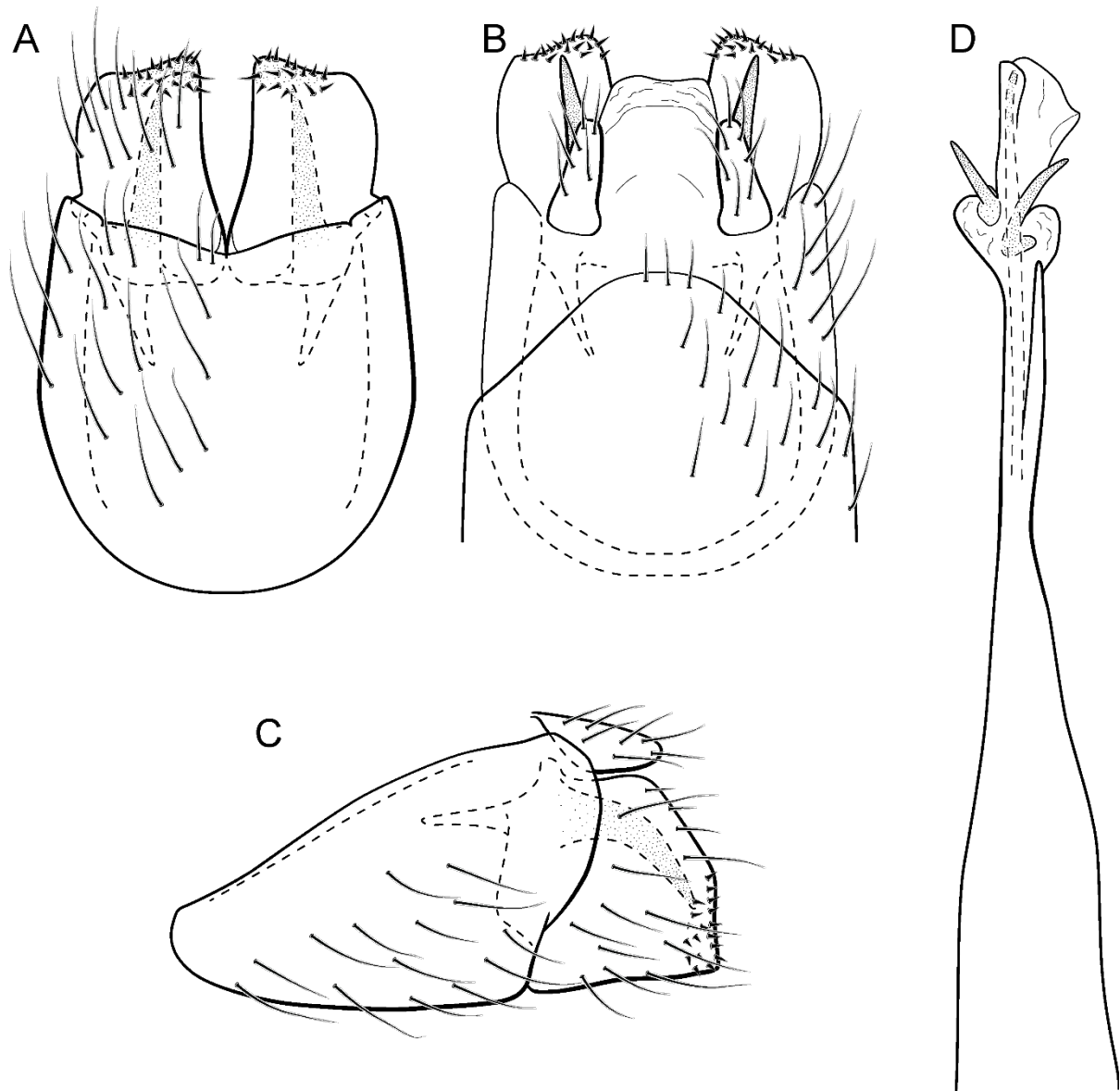
518 GMYC is known to be more sensitive to geographic range coverage and/or other sampling

519 schemes, resulting in oversplitting (Lohse, 2009; Talavera et al., 2013). Therefore, we consider

520 *M. circuliforme* **sp. nov.** as a robust species based on morphology as well as based on barcode

521 divergences. Minimum interspecific COI distances of *M. circuliforme* **sp. nov.** to *M. curta* **sp.**

522   **nov.** were 18.4%, and again, these two species are very distinct based on morphological features

523   and apparently are not even closely related to each other (Fig. 2).

524   **Figure 11.** *Metrichia circuliforme* **sp. nov.,** male genitalia: (A) ventral view; (B) dorsal view; (C)

525   lateral view; (D) phallus, dorsal view.

526   *Metrichia curta* **sp. nov.**

527   urn:lsid:zoobank.org:act:7EC0620B-D6F2-409C-8351-79A3D3FB77C5

528   (Fig. 12)

529   **Adult male.** Length 2.4–2.5 mm (n=9). General color, in alcohol, light brown. Head with no

530   modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

531   palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

532   Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

533   wings. Abdomen without modifications. Ventromesal process on segment VII present. Segment

534   VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum

535   subpentagonal (Fig. 12A); in lateral view, narrower anteriorly than posteriorly (Fig. 12C).

536   Inferior appendage covered by long setae, subtrapezoidal in ventral view (Fig. 12A); in lateral

537   view, subtriangular (Fig. 12C), apex rounded. Dorsal hook long, more than half length of inferior

538   appendage; in lateral view, with apex slightly broader, downturned, and truncate (Fig. 12C).

539   Preanal appendage elongate, but shorter than inferior appendage, and bearing very long setae

540   (Fig. 12B). Subgenital plate apparently absent. Tergum X membranous and rounded (Fig. 12B).

541   Phallus tubular, elongate and slender, slightly constricted mesally; with a stout subapical spine;

542   apex rounded and folded; ejaculatory duct sclerotized, straight and protruding apically (Fig.

543   12D).

544   **Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, Rio das Pedras, 22º24'33''S 44º33'08''W, el. 706

545   m, 06.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, male (DZRJ).

546   **Paratypes.** Same data as holotype, 3 males (DZRJ), 3 males (MNRJ).

547   **Etymology.** The specific name is a reference to the very short inferior appendage; in Portuguese

548   "curta" means "short".

549   **Remarks**. Based on the absence of modifications on abdominal segments, this new species can

550   be assigned to the *aberrans* group. Its phallus is similar to that of *M. amplitudinis* Bueno-Soria &

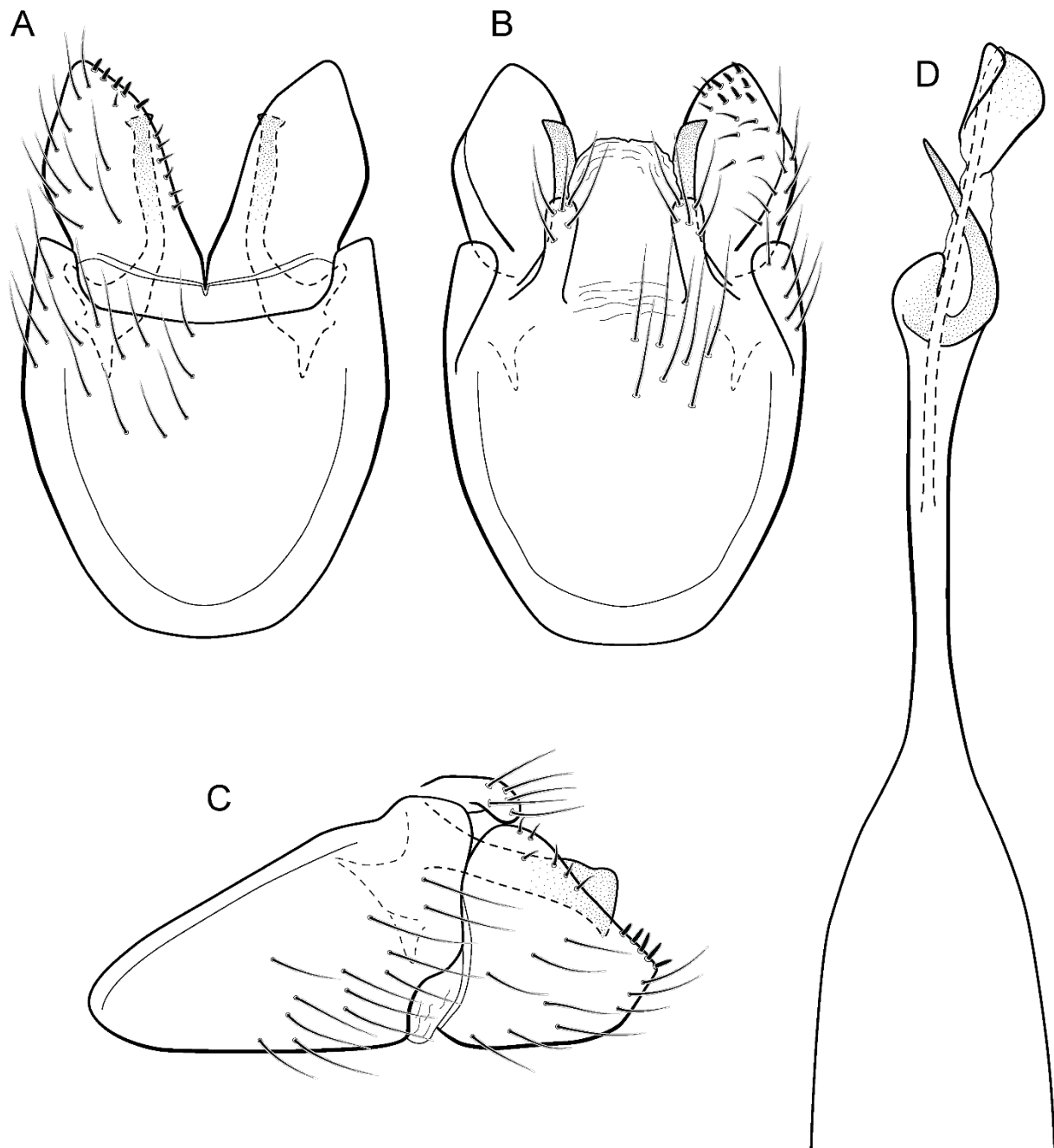
551   Hozenthal, 2003, with a long spine and an apical flap. The new species can be distinguished by

552   the triangular inferior appendages in lateral view and phallus with a strongly curved spine

553   subapically. *Metrichia amplitudinis* and *M. curta* **sp. nov.** share the widened dorsal hook, but in

554   the new species this structure is only slightly wider and also truncate in lateral view. Maximum

555   intraspecific divergence of COI sequences was 1.5% for *M. curta* **sp. nov.** and minimum

556   interspecific was to *M. circuliforme*, as mentioned above.

557   **Figure 12.** *Metrichia curta* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C) lateral

558   view; (D) phallus, dorsal view.

559   *Metrichia farofa* **sp. nov.**

560   urn:lsid:zoobank.org:act:BC4FF095-32BE-46A9-BEAB-28854E2F5BC7

561   (Fig. 13)

562   **Adult male**. Length 1.8–2.1 mm (n=27). General color, in alcohol, light brown. Head with no

563   modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

564   palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

565   Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

566   wings. Abdomen without modifications; segment VII bearing specialized setae dorsally.

567   Ventromesal process on segment VII absent. Segment VIII shorter ventrally than dorsally. **Male**

568   **genitalia**. Segment IX reduced dorsally; sternum subrectangular (Fig. 13A); in lateral view

569   narrower anteriorly than posteriorly (Fig. 13C). Inferior appendage covered by long setae,

570   subrectangular in ventral view (Fig. 13A); in lateral view, subrectangular (Fig. 13C), apex

571   slightly truncate and bearing short spine-like setae. Dorsal hook long, almost reaching the inferior

572   appendage apex; in lateral view, downturned (Fig. 13C). Preanal appendage elongate, as long as

573   inferior appendage, and bearing very long setae (Fig. 13B). Subgenital plate apparently absent.

574   Tergum X membranous and rounded (Fig. 13B). Phallus tubular, elongate and slender, slightly

575   constricted mesally; with a stout subapical spine; apex rounded and sclerotized; ejaculatory duct

576   sclerotized, sinuous, and protruding apically (Fig. 13D).

577   **Holotype. BRAZIL: Minas Gerais:** Jaboticatubas, Parque Nacional da Serra do Cipó,

578   Cachoeira da Farofa, 19º22'47"S 43º34'36"W, el. 811 m, 23.iv.2010, APM Santos & DM Takiya

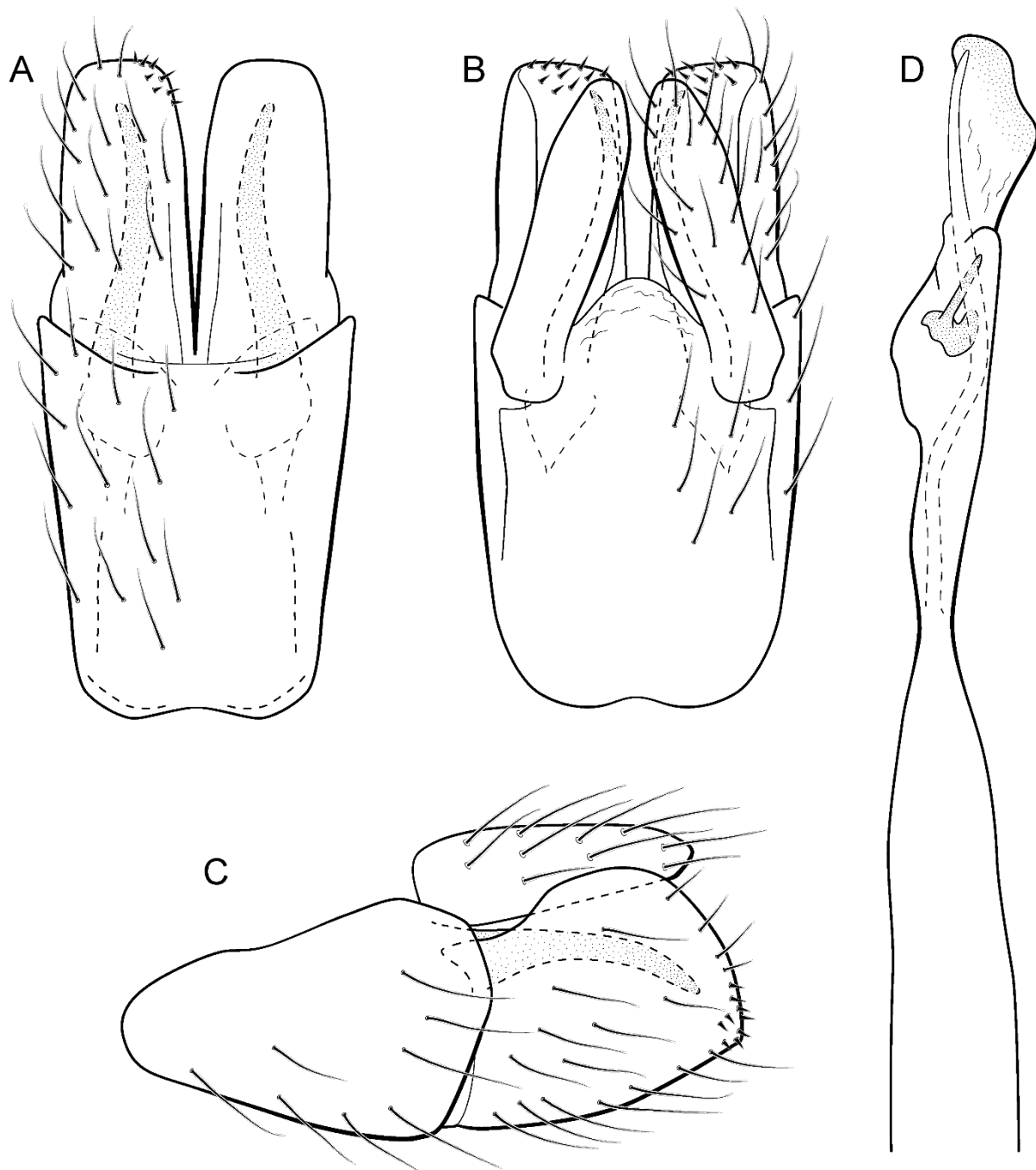
579   cols., manual, male (DZRJ).

580   **Paratypes.** Same data as holotype, 18 males (DZRJ), 8 males (MNRJ), 5 males (MZUFBA);

581   same data, except Ribeirão Mascates, 19º24'02"S 43º34'35"W, el. 820 m, 09–11.xii.2011, APM

582   Santos & DM Takiya cols., manual, 84 males (DZRJ).

583   **Etymology.** This new species is named in reference to the waterfall where specimens were

584   collected.

585   **Figure 13.** *Metrichia farofa* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)

586   lateral view; (D) phallus, dorsal view.

587   **Remarks**. Due to absence of pouches in abdominal segments, *Metrichia farofa* **sp. nov.** can also

588   be included in the *aberrans* group. However, the new species has a single subapical spine in

589   phallus, like those species included in the *exclamationis* group. The new species can be easily

590   distinguished from all other *Metrichia* species by the very long preanal appendages, reaching

591   apices of inferior appendages in dorsal and lateral views.

592   Although more than 10 specimens of *M. farofa* **sp. nov.** were submitted to DNA

593   extraction and many attempts of COI amplification via PCR were conducted, but we were not

594   able to obtain sequences of this species, even using recent material collected after 2013.

595   *Metrichia forceps* **sp. nov.**

596   urn:lsid:zoobank.org:act:8F25D006-D59B-4C83-8CDE-2398368917AD

597   (Fig. 14)

598   **Adult male**. Length 2.7–3.0 mm (n=2). General color, in alcohol, brown. Head with no

599   modifications. Ocelli 3. Antenna simple, 23-articulated. Maxillary palpus 5-articulated; labial

600   palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

601   Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation reduced

602   in both wings. Abdominal segment VII bearing internal pouches in anterior area. Ventromesal

603   process on segment VII absent. Segment VIII shorter ventrally than dorsally. **Male genitalia**.

604   Segment IX reduced dorsally; sternum subpentagonal (Fig. 14A); in lateral view, narrower

605   anteriorly than posteriorly (Fig. 14C). Inferior appendage covered by long setae, subrectangular

606   in ventral view (Fig. 14A); in lateral view, with posterior margin excavated and with two acute

607   and sclerotized process (Fig. 14C). Dorsal hook long, almost reaching the inferior appendage

608   apex; in lateral view, downturned (Fig. 14C). Preanal appendage short, rounded and bearing very

609   long setae (Fig. 14B). Subgenital plate apparently absent. Tergum X sclerotized, deeply notched

610   mesally, forming lateral curved processes (Fig. 14B). Phallus tubular, elongate and slender,

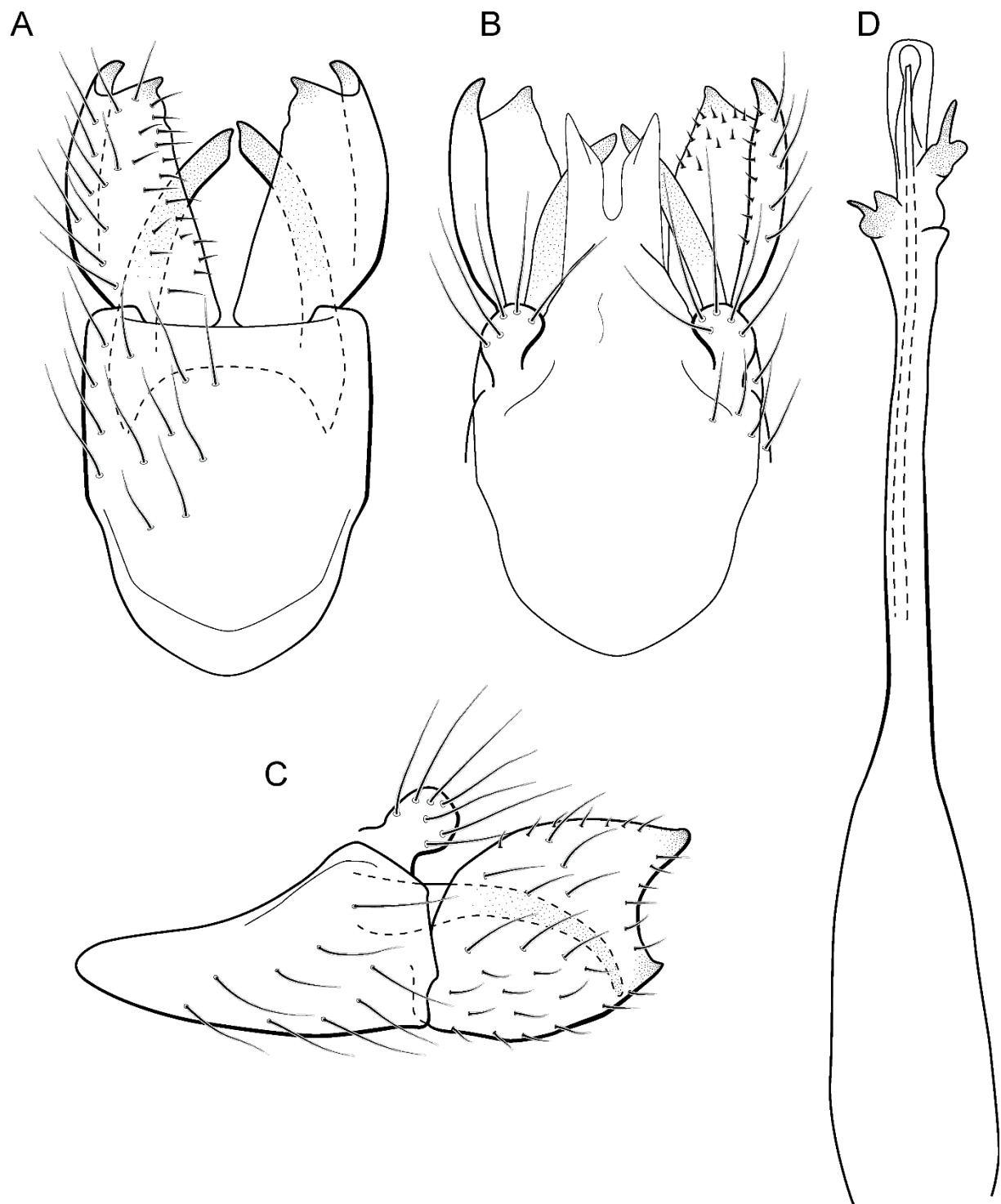
611   slightly constricted mesally; with two short subapical spines; apex rounded and folded;

612   ejaculatory duct sclerotized, straight and protruding apically (Fig. 14D).

A

B

D

C

613 **Figure 14.** *Metrichia forceps* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)

614 lateral view; (D) phallus, dorsal view.

615   **Holotype. BRAZIL: Paraná:** Céu Azul, Parque Nacional do Iguaçu, Rio Azul, 25º09'21"S

616   53º47'44"W, el. 510 m, 6–8 ix.2012, APM Santos, DM Takiya, ALH Oliveira, GA Jardim &

617   BHL Sampaio cols., Malaise trap, male (DZRJ).

618   **Paratypes.** Same data as holotype, 1 male (MNRJ).

619   **Etymology.** The name of this species is in reference to the dorsal hooks of inferior appendages,

620   which in ventral view resemble forceps.

621   **Remarks**. This new species belongs to the *campana* group, sharing the diagnostic internal

622   pouches between segments VI and VII, reduced spines on subapical region of phallus, and the

623   sclerotized and elongate tergum X. Within this group, *M. forceps* **sp. nov.** is most similar to *M.*

624   *campana* (Flint, 1968), *M. similis* (Flint, 1968), and *M. continentalis* (Flint, 1972), particularly by

625   their inferior appendages with excavate posterior margins, forming two pointed processes, one

626   ventral and another dorsal. The new species can be distinguished from the others by its deeply

627   notched tergum X; dorsal hook of inferior appendages elongate and downturned, and phallus

628   apex bearing two small spines and a sclerotized flap surrounding the protruding ejaculatory duct.

629   *Metrichia formosinha* **sp. nov.**

630   urn:lsid:zoobank.org:act:B8971D9B-7013-4213-970E-50A42CA0D1B7

631   (Fig. 15)

632   **Adult male**. Length 2.5–2.8 mm (n=2). General color, in alcohol, dark brown. Head with no

633   modifications. Ocelli 3. Antenna simple, 20-articulated. Maxillary palpus 5-articulated; labial

634   palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

635   Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation reduced

636   in both wings. Abdominal segment VI with pair of internal pouches in posterodorsal area.

637   Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally. **Male**

638   **genitalia**. Segment IX reduced dorsally; sternum subrectangular, with anterior margin rounded

639   (Fig. 15A); in lateral view narrower anteriorly than posteriorly (Fig. 15C). Inferior appendage

640   with peg-like setae, subrectangular in ventral (Fig. 15A) and lateral (Fig. 15C) views; apex with

641   acute corners. Dorsal hook long, almost reaching the inferior appendage apex; in lateral view,

642   with apex slightly broader, almost straight, and truncate (Fig. 15C). Preanal appendage short,

643   rounded and bearing very long setae (Fig. 15B). Subgenital plate apparently absent. Tergum X

644   membranous and rounded (Fig. 15B). Phallus tubular, elongate and slender, slightly constricted

645   mesally; with two curved subapical spines, one short and another one very long; apex rounded

646   and sclerotized; ejaculatory duct sclerotized, sinuous, and not protruding apically (Fig. 15D).

647   **Holotype. BRAZIL: Mato Grosso do Sul:** Bonito, Rio Formosinho, 21º10'16"S 56º26'47"W

648   el. 275 m, 08–13.ix.2013, APM Santos & DM Takiya cols., Malaise trap, male (DZRJ).

649   **Paratypes.** Same data as holotype, 3 males (DZRJ).

650   **Etymology.** This species is named in allusion to the river where type specimens were collected.

651   **Remarks**. This new species appears to be a member of the *campana* group because of internal

652   pouches between abdominal segments VI-VII and phallus with two subapical spines. General

653   aspect of the male genitalia of *Metrichia formosinha* **sp. nov.** is similar to *M. forceps* **sp. nov.,**

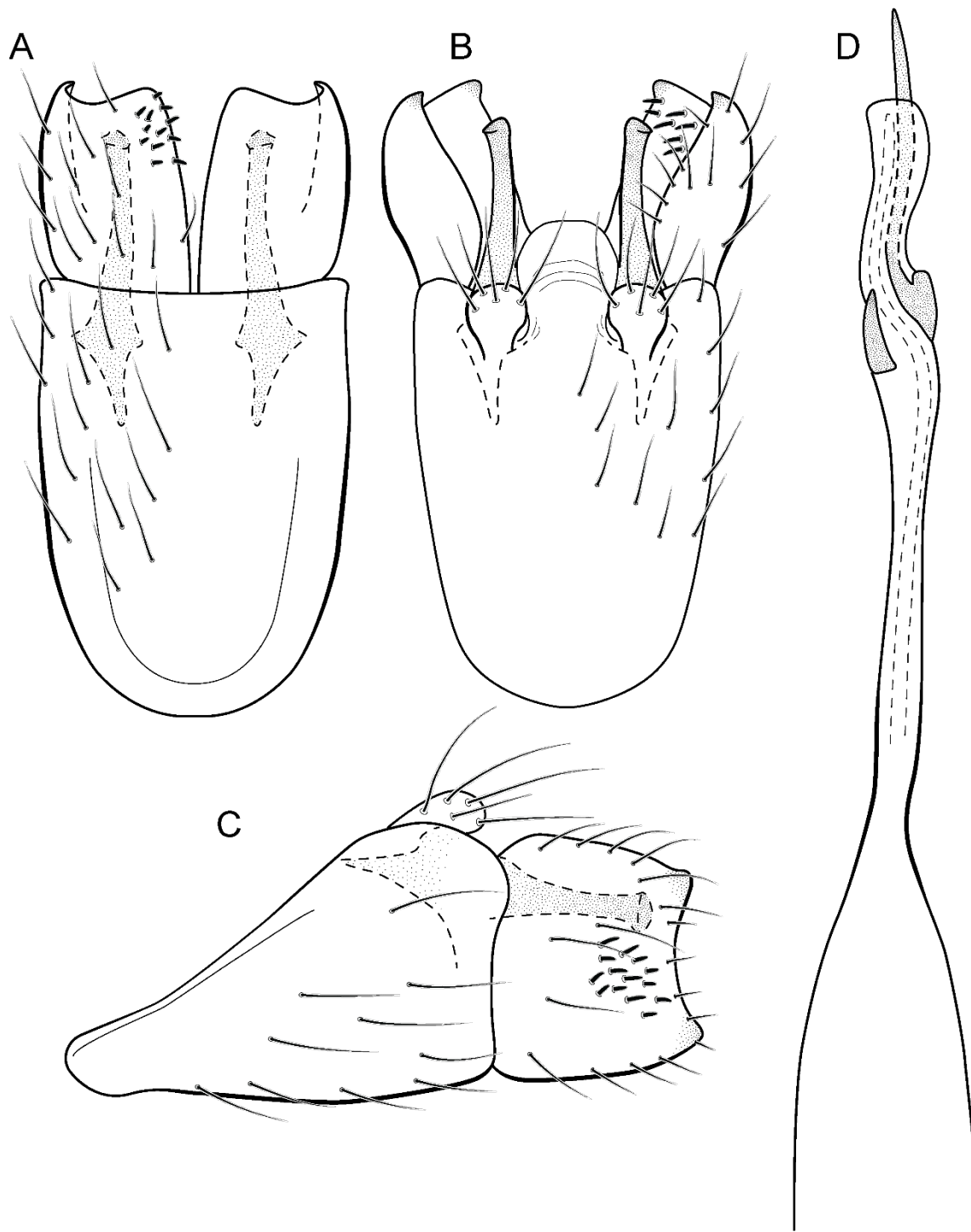
654   particularly in the inferior appendages with acute corners. However, *M. formosinha* **sp. nov.**

655   differs from the latter by dorsal hooks almost straight and capitate (strongly curved and acute in

656   *M. forceps* **sp. nov.** and in other species of the *campana* group) and phallus with a very long

657   subapical spine.

658   COI sequences of *M. formosinha* **sp. nov.** showed intraspecific divergences up to 0.8%

659   and minimum interspecific divergences of 24.9% compared to *M. talhada* **sp. nov.**

660 **Figure 15.** *Metrichia formosinha* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)

661 lateral view; (D) phallus, dorsal view.

662 *Metrichia goiana* **sp. nov.**

663 urn:lsid:zoobank.org:act:8726E665-1093-42EF-A1B6-E06178B2DAD1

664 (Fig. 16)

665 **Adult male**. Length 1.8–2.0 mm (n=6). General color, in alcohol, dark brown. Head with no

666 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated, article IV

667 broad and darkened; labial palpus 3-articulated. Mesoscutellum with transverse suture.

668 Metascutellum subtriangular. Anterior femur with small acute apical process. Tibial spur formula

669 1-3-4. Wing venation reduced in both wings. Abdominal segment V with pair of internal pouches

670 and pair of dorsolateral brushes; segment VI with a transverse sclerotized plate posteriorly on

671 dorsum; segment VII bearing specialized setae dorsally. Ventromesal process on segment VII

672 absent. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced

673 dorsally; sternum subrectangular, with anterior margin rounded (Fig. 16A); in lateral view

674 narrower anteriorly than posteriorly (Fig. 16C). Inferior appendage covered by long setae,

675 elongate and narrow in ventral view (Fig. 16A); in lateral view, rounded (Fig. 16A), apex

676 rounded and bearing a tooth-like projection (Fig. 16A). Dorsal hook short, less than half length of

677 inferior appendage; in lateral view, downturned (Fig. 16A). Preanal appendage short and bearing

678 very long setae (Fig. 16B). Subgenital plate apparently absent. Tergum X membranous and

679 truncate (Fig. 16B). Phallus tubular, elongate and slender, slightly constricted mesally; with two

680 long, curved, subapical spines; apex ending into two sclerotized and keel shaped processes;

681 ejaculatory duct sclerotized, straight and not protruding apically (Fig. 16D).

682 **Holotype. BRAZIL: Goiás:** Alto Paraíso de Goiás, Rio Bartolomeu tributary, 14º07'25"S

683 47º30'30"W, el. 1165 m, 22–25.iii.2013, APM Santos & DM Takiya cols., Malaise trap, male

684 (DZRJ).

685 **Paratypes.** Same data as holotype, 2 males (MNRJ), 1 male (DZRJ).

686   **Etymology.** The species is named in reference to Goiás State. "Goiana" is a Portuguese adjective

687   for people from Goiás.

688   **Remarks**. This is another member of *nigritta* group. Male genitalia of this species are similar to

689   *M. potosina* Bueno-Soria, 2002 and *M. ubajara* **sp. nov.**, due to rounded and elongate inferior

690   appendages in lateral view. This new species differs from *M. ubajara* **sp. nov.** by the presence of

691   an apical tooth on inferior appendages, also present in *M. potosina. Metrichia goiana* **sp. nov.** can

692   be distinguished from *M. potosina* by the two long subapical spines on phallus, whereas *M.*

693   *potosina* has three.

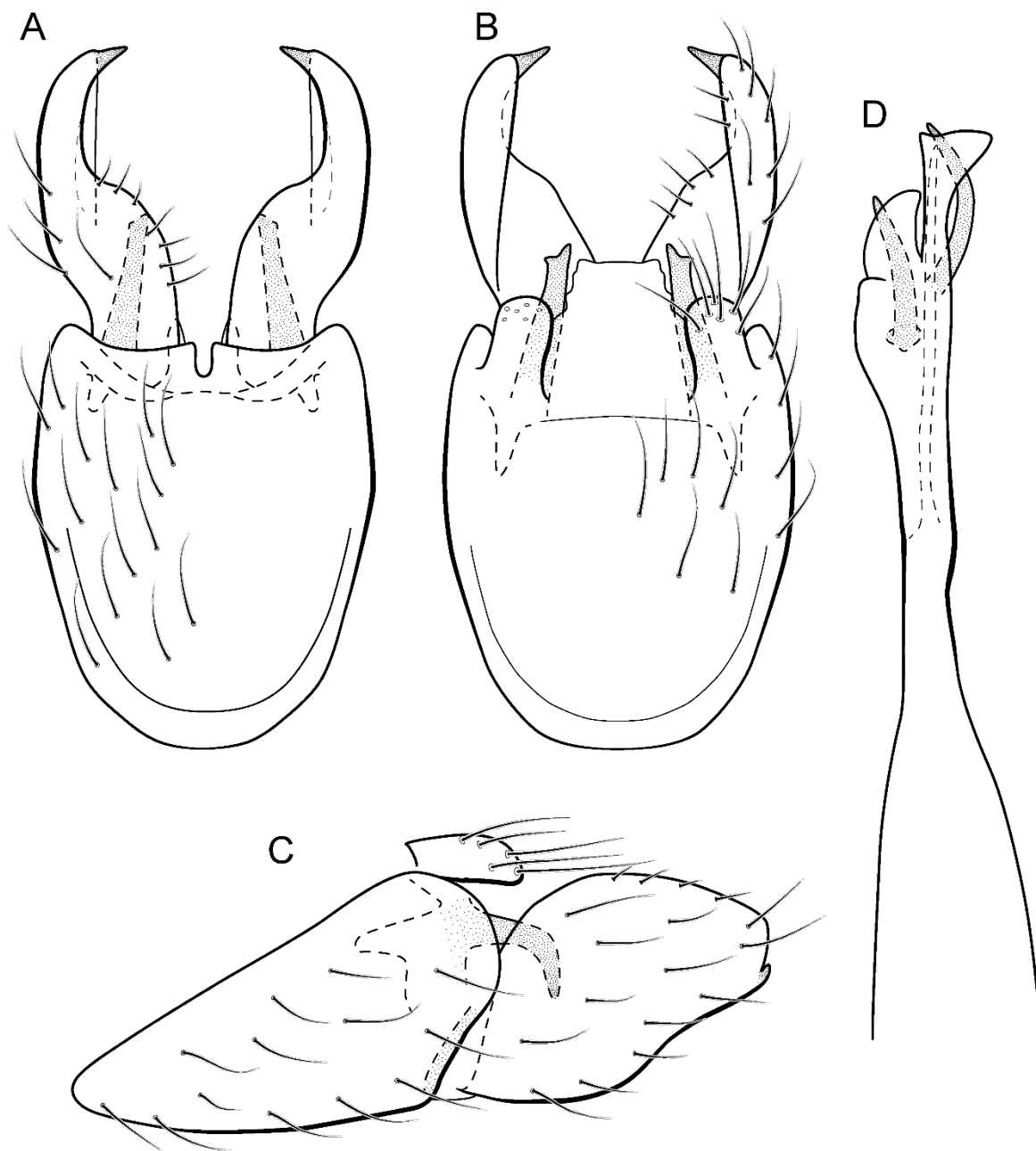



Figure 16. *Metrichia goiana* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C) lateral view; (D) phallus, dorsal view.

Metrichia itabaiana **sp. nov.**

urn:lsid:zoobank.org:act:1C902E75-7ECD-4875-A680-6440A3E5E9E9

(Fig. 17, Fig. 26B)

699 **Adult male**. Length 1.8–2.1 mm (n=3). General color, in alcohol, brown. Head with no

700 modifications. Ocelli 3. Antenna simple, 20-articulated. Maxillary palpus 5-articulated; labial

701 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

702 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

703 wings. Abdominal segment V ventrally with a mesal brush of long setae; segment VI with tergum

704 as a sclerotized triangular plate surrounded by specialized setae (Fig. 26B), internally with pair of

705 internal pouches; segment VII bearing specialized setae ventrally and dorsally (Fig. 26B).

706 Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally and

707 bearing a brush of long setae dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum

708 subpentagonal (Fig. 17A); in lateral view narrower anteriorly than posteriorly (Fig. 17C). Inferior

709 appendage short, covered by long setae (Fig. 17A); in lateral view, subtrapezoidal (Fig. 17C),

710 apex excavated and with two acute and sclerotized process (Figs. 17A, 17B). Dorsal hook short,

711 almost half length of inferior appendage; in lateral view, slightly downturned (Fig. 17C). Preanal

712 appendage elongate, but shorter than inferior appendage, and bearing very long setae (Fig. 17B).

713 Subgenital plate apparently absent. Tergum X membranous and truncate (Fig. 17B). Phallus

714 tubular, elongate and slender, slightly constricted mesally; with two long, curved, subapical

715 spines; apex truncate and slightly sclerotized; ejaculatory duct sclerotized, sinuous, and

716 protruding apically (Fig. 17D).

717 **Holotype. Sergipe:** Areia Branca, Parque Nacional da Serra de Itabaiana, Rio dos Negros,

718 10º44'51"S 37º20'24"W, el. 208 m, 17.vi.2014, APM Santos, DM Takiya & WRM Souza cols.,

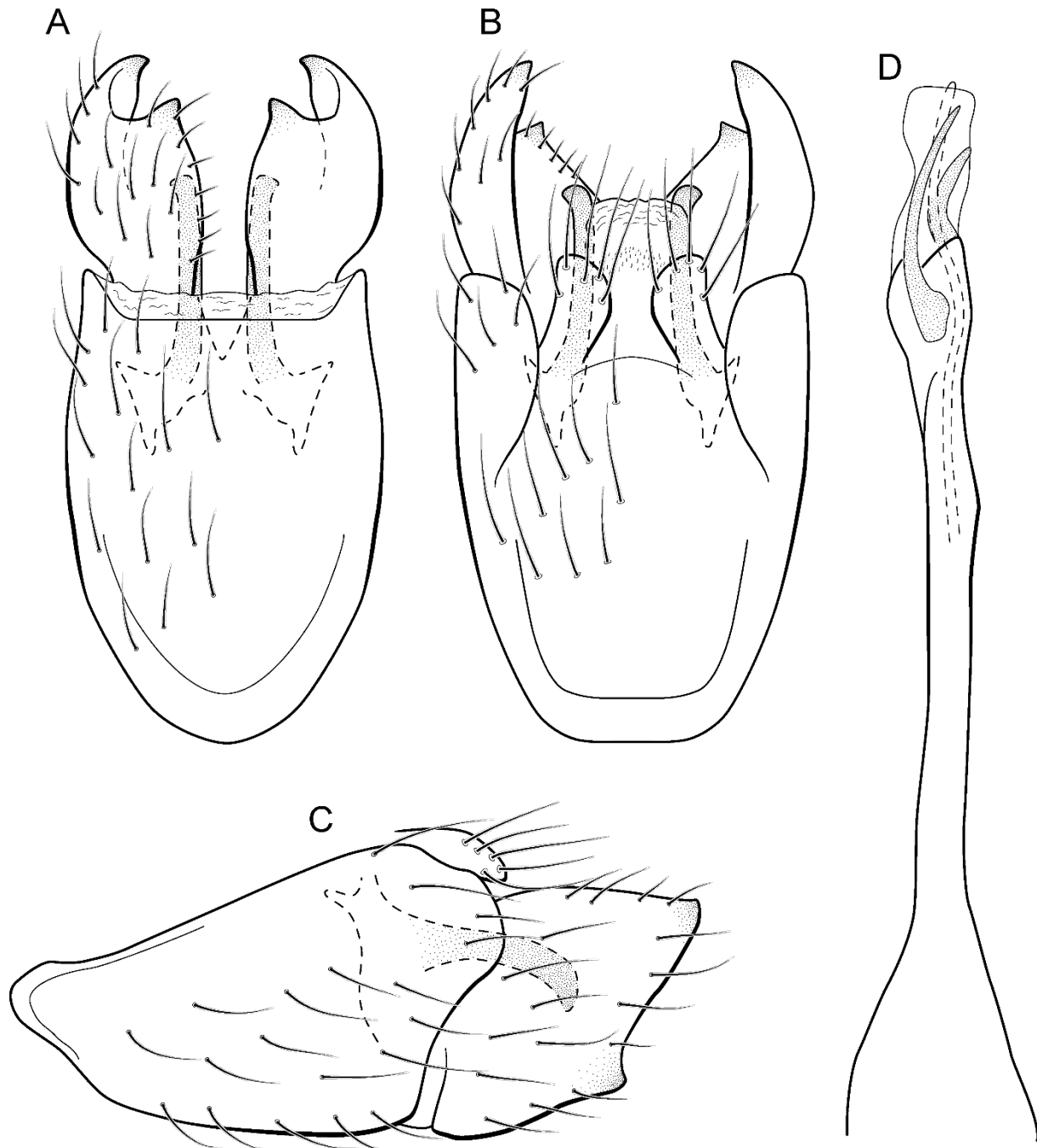
719 light trap, male (DZRJ).

720 **Paratypes.** Same data as holotype, 1 male (DZRJ), 1 male (MZUFBA); same data, except

721 Riacho Água Fria, 10º45'17"S 37º20'32"W, el. 196 m, 17–19.vi.2014, APM Santos, DM Takiya,

722 WRM Souza cols., Malaise trap, 2 males (MNRJ). **Goiás:** Alto Paraíso, Rio Bartolomeu tributary,

723   14º07'25"S 47º30'30"W, el. 1165 m, 22–25.iii.2013, APM Santos & DM Takiya cols., Malaise

724   trap, 1 male (DZRJ).

725   **Figure 17.** *Metrichia itabaiana* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)

726   lateral view; (D) phallus, dorsal view.

727 **Etymology.** This species is named in reference to Serra de Itabaiana, Sergipe, where the holotype

728 was collected.

729 **Remarks**. This new species appears to be a member of the *campana* group because of internal

730 pouches between abdominal segments VI and VII and pair of long subapical spines on phallus,

731 but it lacks the acute process on the mesal area of phallus. Male genitalia of *M. itabaiana* **sp. nov.**

732 resemble those of *M. campana* and *M. vulgaris* **sp. nov.**, particularly, in the excavated inferior

733 appendages, with acute and darkened corners. However, the new species can be recognized by

734 very long curved subapical spines on phallus and subtrapezoidal aspect of inferior appendages in

735 lateral view.

736   Only two COI sequences were obtained for *M. itabaiana* **sp. nov.**, one from Sergipe

737 (Northeastern Brazil) and another from Goiás (Centralwestern Brazil). The COI divergence

738 between these two samples was 1.9% and minimum interspecific distance was 19.4% in relation

739 to *M. rafaeli* **sp. nov.**, which belongs to a different species group based on morphological

740 features.

741 *Metrichia longissima* **sp. nov.**

742 urn:lsid:zoobank.org:act:F87C549F-6F84-4466-AFCE-940729F32F46

743 (Fig. 18, Fig. 26C)

744 **Adult male**. Length 2.5–2.7 mm (n=2). General color, in alcohol, brown. Head with no

745 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

746 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

747 Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation reduced

748 in both wings. Abdominal segment IV with dorsal area expanded posteriorly bearing stout setae;

749 segment VI with stout and striate setae (Fig. 26C); segment VII with stout and striate setae (Fig.

750 26C). Ventromesal process on segment VII absent. Segment VIII shorter ventrally than dorsally.

751 **Male genitalia**. Segment IX reduced dorsally; sternum subpentagonal (Fig. 18A); in lateral view

752 narrower anteriorly than posteriorly (Fig. 18C). Inferior appendage bearing scale-like setae, very

753 elongate; in ventral view, curved inward apically (Fig. 18A); in lateral view, tapering to a

754 rounded apex (Fig. 18C). Dorsal hook short and straight; in lateral view, slightly downturned

755 (Fig. 18C). Preanal appendage elongate, but shorter than half length of inferior appendage, and

756 bearing stout and striate setae (Fig. 18B). Subgenital plate apparently absent. Tergum X

757 membranous and truncate (Fig. 18B). Phallus tubular, elongate and slender, slightly constricted

758 mesally, with a median process; with two long, curved, subapical spines; apex rounded and

759 sclerotized; ejaculatory duct sclerotized and protruding apically (Fig. 18D).

760 **Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, Rio Palmital, 22º25'34"S 44º32'52"W, el. 637 m,

761 07.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, male (DZRJ).

762 **Paratype. Brazil: Rio de Janeiro:** Teresópolis, Parque Nacional da Serra dos Órgãos, Rio

763 Paquequer, 22º27'25"S 42º59'52"W, el. 1100 m, 15–18.ix.2011, APM Santos, DM Takiya, BM

764 Vasconcelos & RA Carvalho cols., Malaise trap, 1 male (MNRJ).

765 **Etymology.** The species name is an allusion to the elongate inferior appendages, unusual for

766 *Metrichia* species.

767 **Remarks**. The new species is most similar to *M. sesquipedalis* Bueno-Soria & Holzenthal, 2003,

768 sharing with it very long inferior appendages with very short dorsal hooks. The new species is

769 easily distinguished from the latter by their internal pouches in the male abdominal segment VI

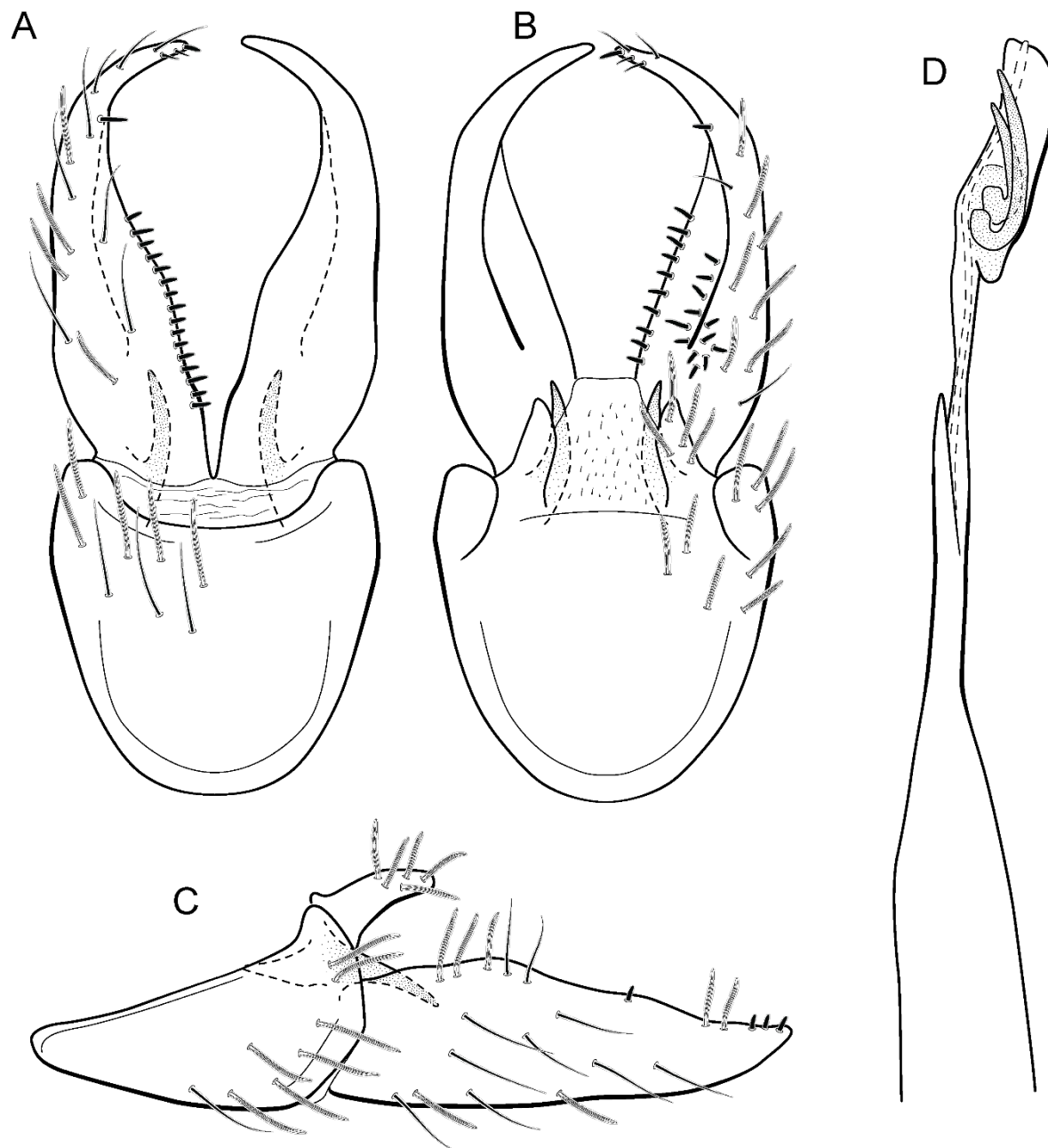
770 and phallus with only two subapical spines (three in *M. sesquipedalis*).

771   Two COI sequences were generated for *M. longissima* **sp. nov.**, one from a specimen from

772 Itatiaia and the other from Teresópolis in Rio de Janeiro State, localities in distinct mountain

773 ranges, Serra da Mantiqueira and Serra do Mar, respectively. The genetic distance between

774 sequences of *M. longissima* **sp. nov.** was 0.4%. The minimum interspecific distance was found to

775 *M. itabaiana* **sp. nov.** with 21.5% divergence.

776   **Figure 18.** *Metrichia longissima* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)

777   lateral view; (D) phallus, dorsal view.

778   *Metrichia peluda* **sp. nov.**

779   urn:lsid:zoobank.org:act:E1B7E1AE-5751-4D10-9B07-47A8CF849C7F

780   (Fig. 19, Fig. 26D)

781   **Adult male**. Length 2.7–3.0 mm (n=3). General color, in alcohol, dark brown. Head with no

782   modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

783   palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

784   Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

785   wings. Abdominal segment V with dorsolateral brushes of long setae; segment VI with

786   dorsolateral brushes of long setae (Fig. 26D). Ventromesal process on segment VII present.

787   Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally;

788   sternum subrectangular, with anterior margin rounded (Fig. 19A); in lateral view narrower

789   anteriorly than posteriorly (Fig. 19C). Inferior appendage covered by long setae, subtrapezoidal

790   in ventral view (Fig. 19A); apex excavated; in lateral view, rounded (Fig. 19C). Dorsal hook

791   short, almost half length of inferior appendage; in lateral view, slightly downturned (Fig. 19C).

792   Preanal appendage short and bearing very long setae (Fig. 19B). Subgenital plate apparently

793   absent. Tergum X membranous and truncate (Fig. 19B). Phallus tubular, elongate and slender,

794   slightly constricted mesally; with two curved subapical spines, one short and another long; apex

795   rounded and folded; ejaculatory duct sclerotized, straight and protruding apically (Fig. 19D).

796   **Holotype**. **BRAZIL: Rio de Janeiro:** Itatiaia, 1st order tributary of Rio Palmital, 22º25'40"S

797   44º32'46"W, el. 584 m, 07.iii.2008, JL Nessimian, LL Dumas & MR de Souza cols., light trap,

798   male (DZRJ).

799   **Paratypes.** Same data as holotype, 1 male (MNRJ); same data, except Rio Palmital, 22º25'34"S

800   44º32'52"W, el. 637 m, 07.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, 4

801   males (DZRJ).

802   **Etymology.** The name of this species refers to dense brushes of setae on the dorsal area of the

803   male abdomen. In Portuguese "peluda" means "hairy".

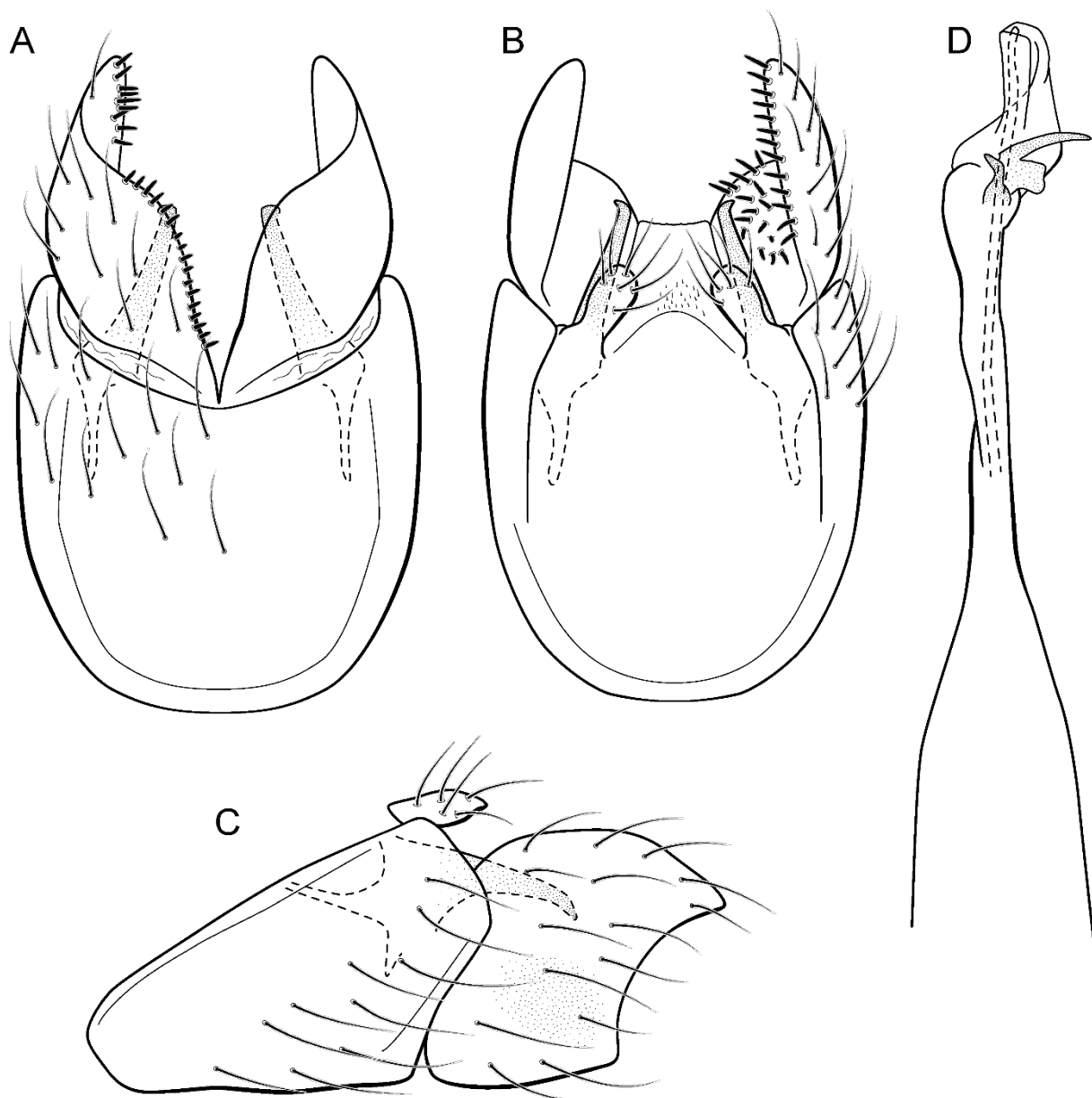



Figure 19. *Metrichia peluda* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C) lateral view; (D) phallus, dorsal view.

Remarks. Modifications on male abdominal segments V, VI, and VII suggest that this new species belongs to the *campana* group. The general aspect of inferior appendages is somewhat similar to *M. forceps* **sp. nov.** and *M. formosinha* **sp. nov.**, which are excavate posteriorly. However, *M. peluda* **sp. nov.** is readily identified by the dense brushes of setae on the dorsum of abdominal segments V, VI, and VII. Besides, the male genitalia of this new species differ from

811   those described for *M. forceps* **sp. nov.** and *M. formosinha* **sp. nov.** by the rounded corners of

812   inferior appendages instead of acute and by phallus with two subapical spines with unequal sizes.

813   ***Metrichia rafaeli* sp. nov.**

814   urn:lsid:zoobank.org:act:CBCADBB8-2C79-49AB-8345-CC1E6FA2AEE9

815   (Fig. 20)

816   **Adult male**. Length 2.0–2.5 mm (n=7). General color, in alcohol, dark brown. Head with no

817   modifications. Ocelli 3. Antenna simple, 20-articulated. Maxillary palpus 5-articulated; labial

818   palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

819   Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

820   wings. Abdominal segment V with pair of internal pouches; segment VI with pair of internal

821   pouches and pair of lateral external sacs with specialized setae; segment VII bearing specialized

822   setae dorsally. Ventromesal process on segment VII present. Segment VIII shorter ventrally than

823   dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subrectangular, with anterior

824   margin rounded (Fig. 20A); in lateral view narrower anteriorly than posteriorly (Fig. 20C).

825   Inferior appendage covered by long setae, short and rounded, with apex slightly excavated (Fig.

826   20A); in lateral view, rounded (Fig. 20C). Dorsal hook long, more than half length of inferior

827   appendage; in lateral view, downturned (Fig. 20C). Preanal appendage elongate, but shorter than

828   half length of inferior appendage, and bearing stout and striate setae (Fig. 20B). Subgenital plate

829   apparently absent. Tergum X membranous and truncate (Fig. 20B). Phallus tubular, elongate and

830   slender, slightly constricted mesally, with a median process; with two long, curved, subapical

831   spines, and a membranous lobe; apex rounded and sclerotized; ejaculatory duct sclerotized,

832   sinuous, and not protruding apically (Fig. 20D).

833   **Holotype male. BRAZIL: Ceará:** Ubajara, Parque Nacional de Ubajara, Rio das Minas,

834   03º50'03"S 40º54'18"W, el. 524, 17–18.ii.2013, DM Takiya, JA Rafael, RR Cavichioli & APM

835   Santos cols., Malaise trap (CZMA).

836   **Paratypes.** Same data as holotype, 1 male (MZUFBA); same data, except Rio das Minas,

837   03º49'58"S 40º53'53"W, el. 420 m, 20–23.iv.2012, F Limeira-de-Oliveira et al. cols., Malaise

838   trap, 1 male (CZMA); same data, except 14–16.ii.2013, DM Takiya, JA Rafael, RR Cavichioli &

839   APM Santos cols., 1 male (DZRJ).

840   **Etymology.** This species is named in honor of the Brazilian entomologist Dr. José Albertino

841   Rafael (INPA), who has collected many interesting caddisflies, including some species described

842   here.

843   **Remarks**. This new species belongs to the *nigritta* group due to internal pouches between

844   abdominal segments V and VI and long and acute process on phallus. The male genitalia of *M.*

845   *rafaeli* **sp. nov.** are more similar to *M. magna* Bueno-Soria & Holzenthal, 2003 with short and

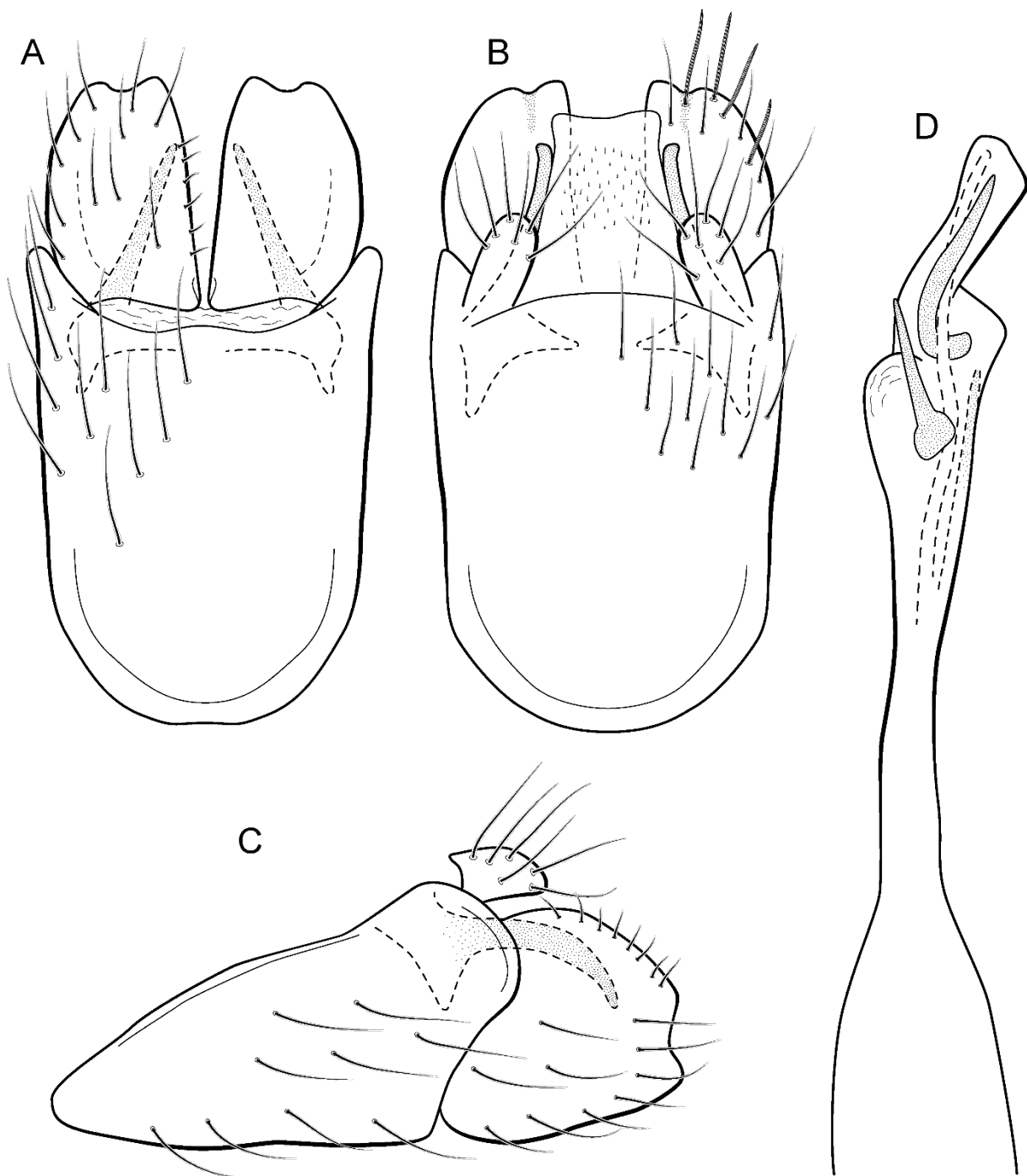
846   simple inferior appendages. However, the new species can be easily distinguished from the latter

847   and other *Metrichia* species in this group by the posterior margin of inferior appendages slightly

848   excavated and very long subapical spines of phallus.

849   Although specimens with barcode sequences of *M. rafaeli* **sp. nov.** were collected at the

850   same locality, haplotypes were not identical having intraspecific divergence of 0.4%. The lowest

851   interspecific distance was 19.4% when compared with *M. itabaiana* **sp. nov.**

852   **Figure 20.** *Metrichia rafaeli* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)

853   lateral view; (D) phallus, dorsal view.

854   ***Metrichia simples* sp. nov.**

855   urn:lsid:zoobank.org:act:05933E6E-A6FF-49DE-982E-54B263830D10

856   (Fig. 21, Fig. 26E)

857   **Adult male**. Length 2.1–2.2 mm (n=2). General color, in alcohol, brown. Head with no

858   modifications. Ocelli 3. Antenna simple, 19-articulated. Maxillary palpus 5-articulated; labial

859   palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

860   Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

861   wings. Abdominal segment IV with dorsal area expanded posteriorly bearing stout setae; segment

862   VI bearing very long setae laterally, with a brush of short setae covered dorsally by a triangular

863   plate (Fig. 26E); segment VII with a brush of short setae dorsally (Fig. 26E). Ventromesal process

864   on segment VII present. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment

865   IX reduced dorsally; sternum subpentagonal (Fig. 21A); in lateral view narrower anteriorly than

866   posteriorly (Fig. 21C). Inferior appendage covered by long setae, with apex obliquely truncate;

867   subtrapezoidal in ventral view (Fig. 21A); in lateral view, subtrapezoidal (Fig. 21C). Dorsal hook

868   short, almost half length of inferior appendage; in lateral view, slightly downturned (Fig. 21C).

869   Preanal appendage short, rounded and bearing very long setae (Fig. 21B). Subgenital plate

870   apparently absent. Tergum X membranous and rounded (Fig. 21B). Phallus tubular, elongate and

871   slender, slightly constricted mesally; with four subapical spines, three short and one long and

872   straight; apex rounded with a small sclerite; ejaculatory duct sclerotized and not protruding

873   apically (Fig. 21D).

874   **Holotype. BRAZIL: Paraná:** Céu Azul, Parque Nacional do Iguaçu, Rio Azul, 25º09'21''S

875   53º47'44''W, el. 510 m, 6–8 ix.2012, APM Santos, DM Takiya, ALH Oliveira, GA Jardim &

876   BHL Sampaio cols., Malaise trap, male (DZRJ).

877   **Paratypes.** Same data as holotype, 1 male (MNRJ).

878   **Etymology.** This species is named in reference to the simple aspect of the male genitalia and

879   abdomen, without modifications and processes seen in other *Metrichia* species.

880   **Remarks**. This species can be assigned to the *campana* group because of pouches in abdominal

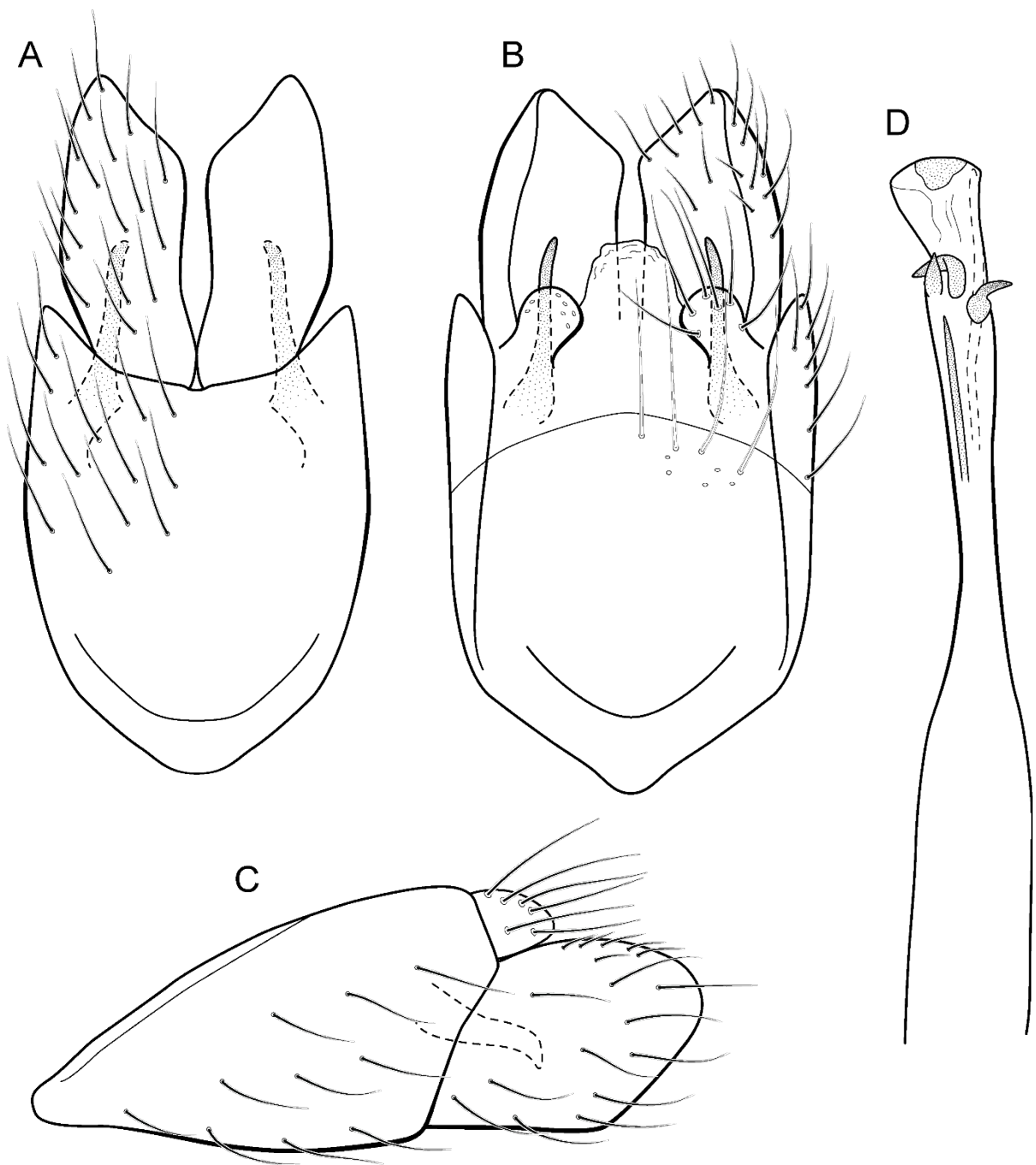
881   segments VI and VII. The new species shares the general aspect of the genitalia with *M.*

882   *quadrata*, particularly, in the quadrangular or rectangular shape of inferior appendages and the

883   short subapical spines on phallus. *Metrichia simples* **sp. nov.** can be recognized by the short

884   dorsal hook, reaching up to midlength of inferior appendages (subequal to inferior appendages in

885   *M. quadrata*), obliquely truncate apex of the inferior appendages, and strongly curved spines on

886   phallus.

887   **Figure 21.** *Metrichia simples* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)

888   lateral view; (D) phallus, dorsal view.

889   ***Metrichia talhada* sp. nov.**

890   urn:lsid:zoobank.org:act: 5456FEB8-5193-46DD-A10F-9FFADFCB59EC

891   (Fig. 22)

892 **Adult male**. Length 1.8–2.0 mm (n=10). General color, in alcohol, brown. Head with no

893 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

894 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

895 Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation reduced

896 in both wings. Abdominal segment V with pair of internal pouches and pair of dorsolateral

897 brushes; segment VI with dorsolateral brushes of long setae; segment VII bearing specialized

898 setae dorsally. Ventromesal process on segment VII absent. Segment VIII shorter ventrally than

899 dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subpentagonal (Fig. 22A); in

900 lateral view, narrower anteriorly than posteriorly (Fig. 22C). Inferior appendage elongate, apex

901 rounded and bearing a tooth-like projection; with a deep C-shaped notch in ventral view (Fig.

902 22A); in lateral view, with an acute projection (Fig. 22C). Dorsal hook short, less than half length

903 of inferior appendage (Fig. 22A); in lateral view, downturned. Preanal appendage short, rounded

904 and bearing very long setae (Fig. 22B). Subgenital plate apparently absent. Tergum X

905 membranous and truncate (Fig. 22B). Phallus tubular, elongate and slender, slightly constricted

906 mesally, with a median process; with two curved subapical spines, one short and another long;

907 apex rounded and sclerotized; ejaculatory duct sclerotized, straight and protruding apically (Fig.

908 22D).

909 **Holotype. BRAZIL: Alagoas:** Quebrangulo, Reserva Biológica de Pedra Talhada, Rio

910 Caranguejo, 09º15’26”S 36º25’08”W, el. 550 m, 19–28.vi.2014, APM Santos, DM Takiya, WRM

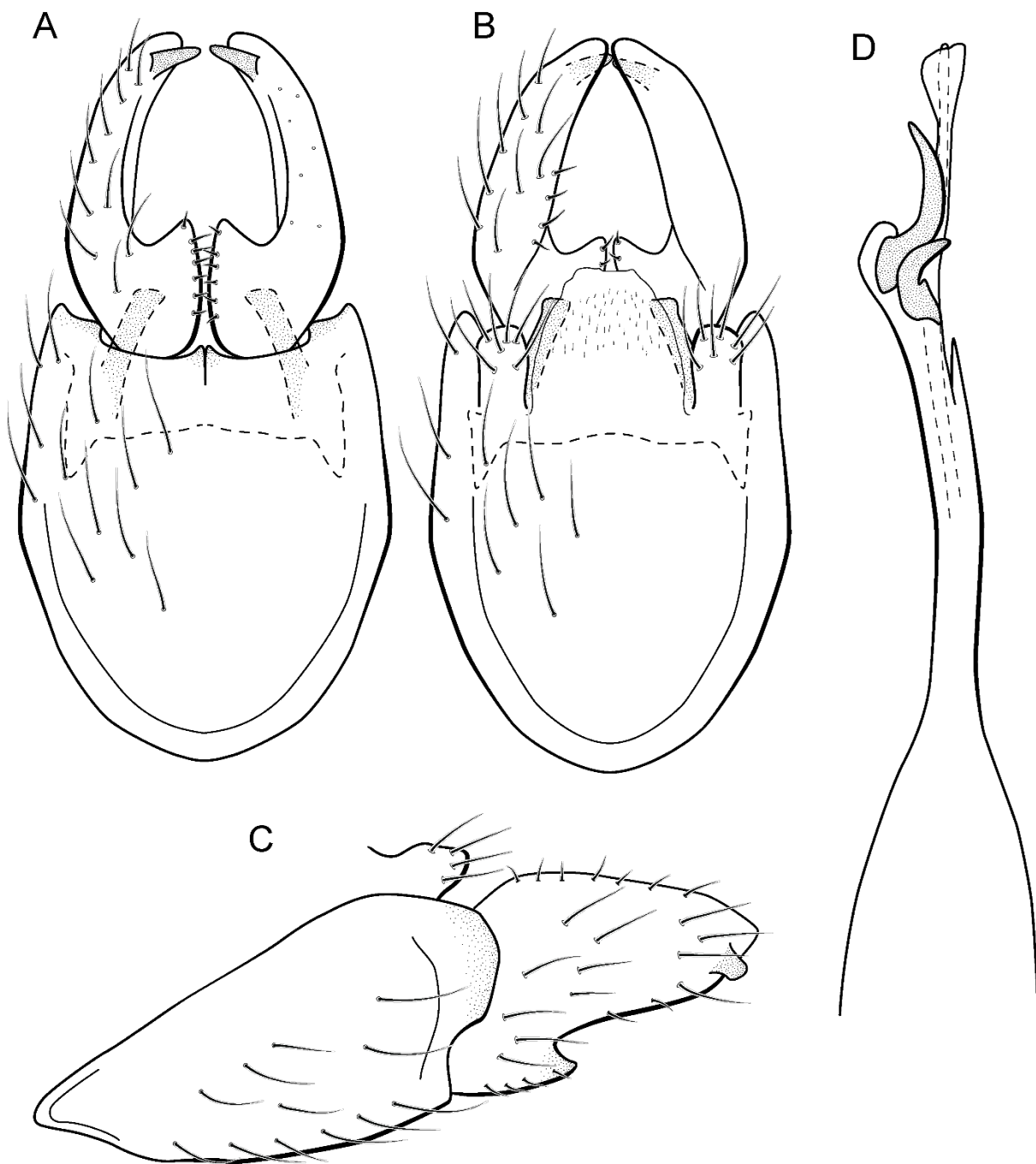
911 Souza cols., Malaise trap, male (DZRJ).

912 **Paratypes.** Same data as holotype, 8 males (DZRJ), 7 males (MZUFBA).

913 **Etymology.** This species is named in reference to its type locality, the Reserva Biológica de

914 Pedra Talhada.

A B D

C

915   **Figure 22.** *Metrichia talhada* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)

916   lateral view; (D) phallus, dorsal view.

917   **Remarks**. The new species belongs to the *nigritta* group because of their internal pouches

918   between abdominal segments V and VI and phallus with two subapical curved spines and an

919   acute process near mesal area. *Metrichia talhada* **sp. nov.** shares the apical tooth on inferior

920   appendages with *M. potosina*, *M. goiana* **sp. nov.**, and *Metrichia tere* **sp. nov.** From *M. potosina*

921   and *M. goiana* **sp. nov.**, it is easily distinguished by the shape of the inferior appendages, with a

922   deep C-shaped notch on ventral margin and subapical tooth stout and slightly truncate in lateral

923   view, and subapical spines on phallus, one long and another short. Although the male genitalia of

924   *M. talhada* **sp. nov.** are very similar to *M. tere* **sp. nov.**, these two species can be separated by the

925   shape of inferior appendages teeth, each slightly truncate and subapical in the former and acute

926   and apical in the later species.

927   Sequences of *M. talhada* **sp. nov.** showed 0.0% of divergence. Morphological similarity

928   between this species and its sister (*M. tere* **sp. nov.**) had a relatively low genetic divergence of

929   12.6%, the lowest interspecific distance found in our sampling. In addition to minor but stable

930   differences in genital structures, all molecular analyses with COI sequences (NJ, ABGD, GMYC)

931   corroborate the distinction between *M. talhada* **sp. nov.** and *M. tere* **sp. nov.**, which are formally

932   described here as different species.

933   ***Metrichia tere* sp. nov.**

934   urn:lsid:zoobank.org:act:21376F9C-6308-47BA-ADB8-A42848AD8FB5

935   (Fig. 23)

936   **Adult male**. Length 1.9–2.1 mm (n=10). General color, in alcohol, brown. Head with no

937   modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

938   palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

939   Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation reduced

940   in both wings. Abdominal segment V with pair of internal pouches and pair of dorsolateral

941   brushes; segment VI with dorsolateral brushes of long setae; segment VII bearing specialized

942   setae dorsally. Ventromesal process on segment VII absent. Segment VIII shorter ventrally than

943   dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subpentagonal (Fig. 23A); in

944   lateral view, narrower anteriorly than posteriorly (Fig. 23C). Inferior appendage elongate, apex

945   with an acute projection; with a deep C-shaped notch in ventral view (Fig. 23A); in lateral view,

946   with an acute projection (Fig. 23C). Dorsal hook short, less than half length of inferior

947   appendage; in lateral view, downturned (Fig. 23C). Preanal appendage short, rounded and bearing

948   very long setae (Fig. 23B). Subgenital plate apparently absent. Tergum X membranous and

949   truncate (Fig. 23B). Phallus tubular, elongate and slender, slightly constricted mesally, with a

950   median process; with two curved subapical spines, one short and another long; apex rounded and

951   sclerotized; ejaculatory duct sclerotized, straight and protruding apically (Fig. 23D).

952   **Holotype. BRAZIL: Rio de Janeiro:** Teresópolis, Parque Nacional da Serra dos Órgãos, Rio

953   Paquequer, 22º27'25"S 42º59'52"W, el. 1100 m, 15–18.ix.2011, APM Santos, DM Takiya, BM

954   Vasconcelos & RA Carvalho cols., Malaise trap, male (DZRJ).

955   **Paratypes.** Same data as holotype, 9 males (MNRJ), 19 males (DZRJ).

956   **Etymology.** This species is named in reference to the city of Teresópolis (= "City of Teresa"),

957   affectionately called as "Terê". The city was named in honor of Teresa Cristina, Brazilian

958   Empress from 1843 to 1889, wife of Dom Pedro II.

959   **Remarks**. This new species is very similar to the preceding one, also belonging to the *nigritta*

960   group. *Metrichia tere* **sp. nov.** can be distinguished from *M. talhada* **sp. nov.** by inferior

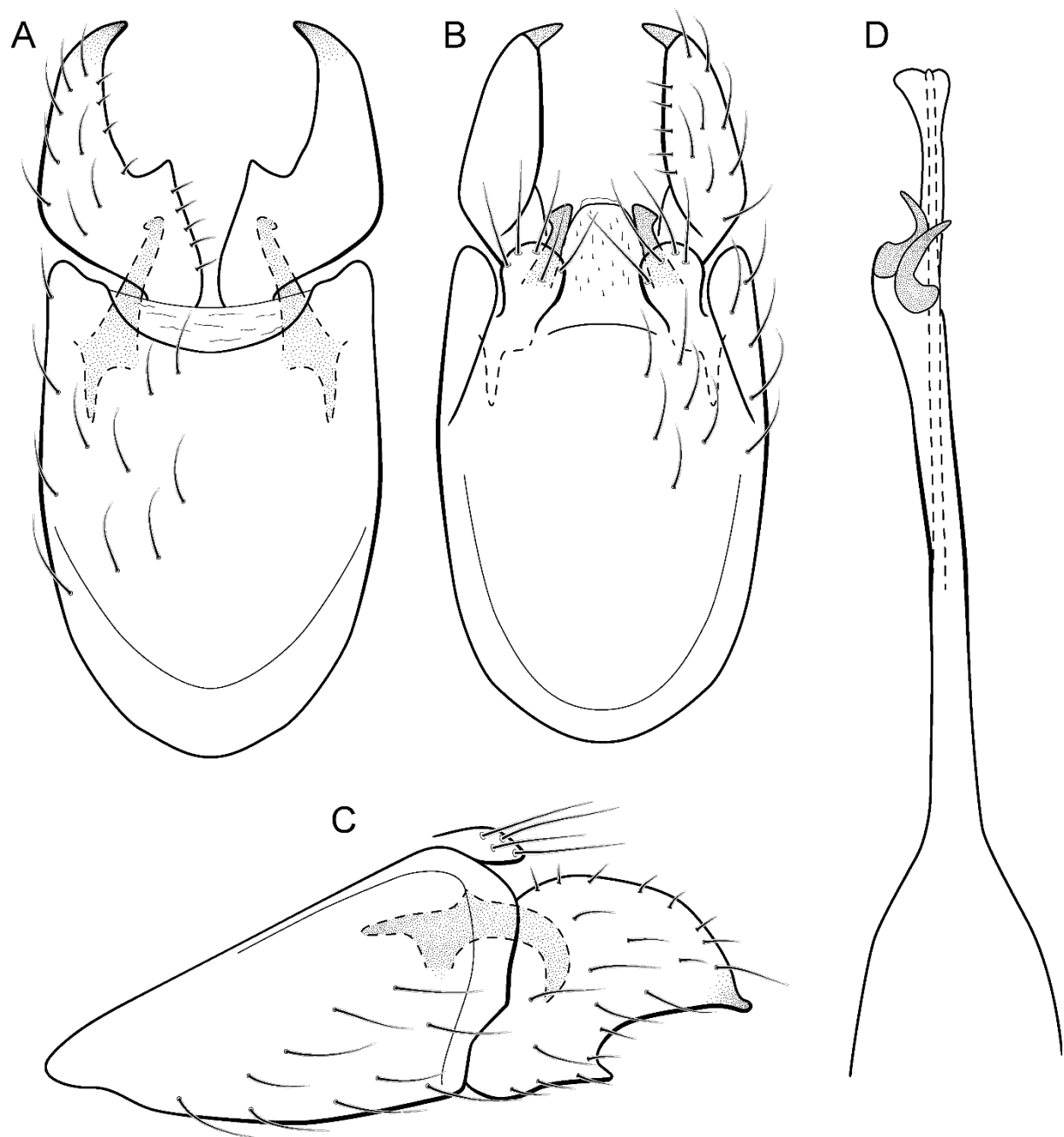
961   appendages with an apical acute projection, whereas in *M. talhada* **sp. nov.** inferior appendages

962   have a subapical tooth, which is more truncate than acute.

963   Molecular data agree with the morphological distinction of *M. tere* **sp. nov.**, as

964   commented on above. Intraspecific K2P divergence among specimens sampled (n=3) of this

965   species was 0.0%, and as mentioned in the previous description, *M. talhada* **sp. nov.** and *M. tere*

966   **sp. nov.** showed the lowest observed interspecific distance (12.6%).

967 **Figure 23.** *Metrichia tere* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C) lateral

968 view; (D) phallus, dorsal view.

969 *Metrichia ubajara* **sp. nov.**

970 urn:lsid:zoobank.org:act:68FD2A24-BC85-42A3-9007-96A42657DAE3

971 (Fig. 24)

972 **Adult male**. Length 2.0–2.7 mm (n=12). General color, in alcohol, brown. Head with no

973 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

974 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

975 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

976 wings. Abdominal segment V with pair of internal pouches and median internal plate in posterior

977 region; with specialized setae on dorsum; segment VI with pair of internal pouches in

978 posterodorsal area. Ventromesal process on segment VII absent. Segment VIII shorter ventrally

979 than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subrectangular (Fig. 24A);

980 in lateral view, narrower anteriorly than posteriorly (Fig. 24C). Inferior appendage covered by

981 long setae, apex rounded; elongate and narrow in ventral view (Fig. 24A); in lateral view,

982 rounded (Fig. 24C). Dorsal hook short, less than half length of inferior appendage; apex

983 downturned; basally with a wide and sclerotized projection; in lateral view, C-shaped (Fig. 24C).

984 Preanal appendage elongate, but shorter than half length of inferior appendage, and bearing stout

985 and striate setae (Fig. 24B). Subgenital plate apparently absent. Tergum X membranous and

986 truncate (Fig. 24B). Phallus tubular, elongate and slender, slightly constricted mesally; with two

987 long, curved, subapical spines; apex rounded and folded; ejaculatory duct sclerotized, sinuous,

988 and protruding apically (Fig. 24D).

989 **Holotype. BRAZIL: Ceará:** Ubajara, Parque Nacional de Ubajara, Rio das Minas, 03º49'58"S

990 40º53'53"W, el. 420 m, 20–23.iv.2012, DM Takiya, JA Rafael, F Limeira-de-Oliveira et al. cols.,

991 Malaise trap, male (CZMA).

992 **Paratypes.** Same data as holotype, 1 male (CZMA); same data, except 13–17.ix.2012, 25 males

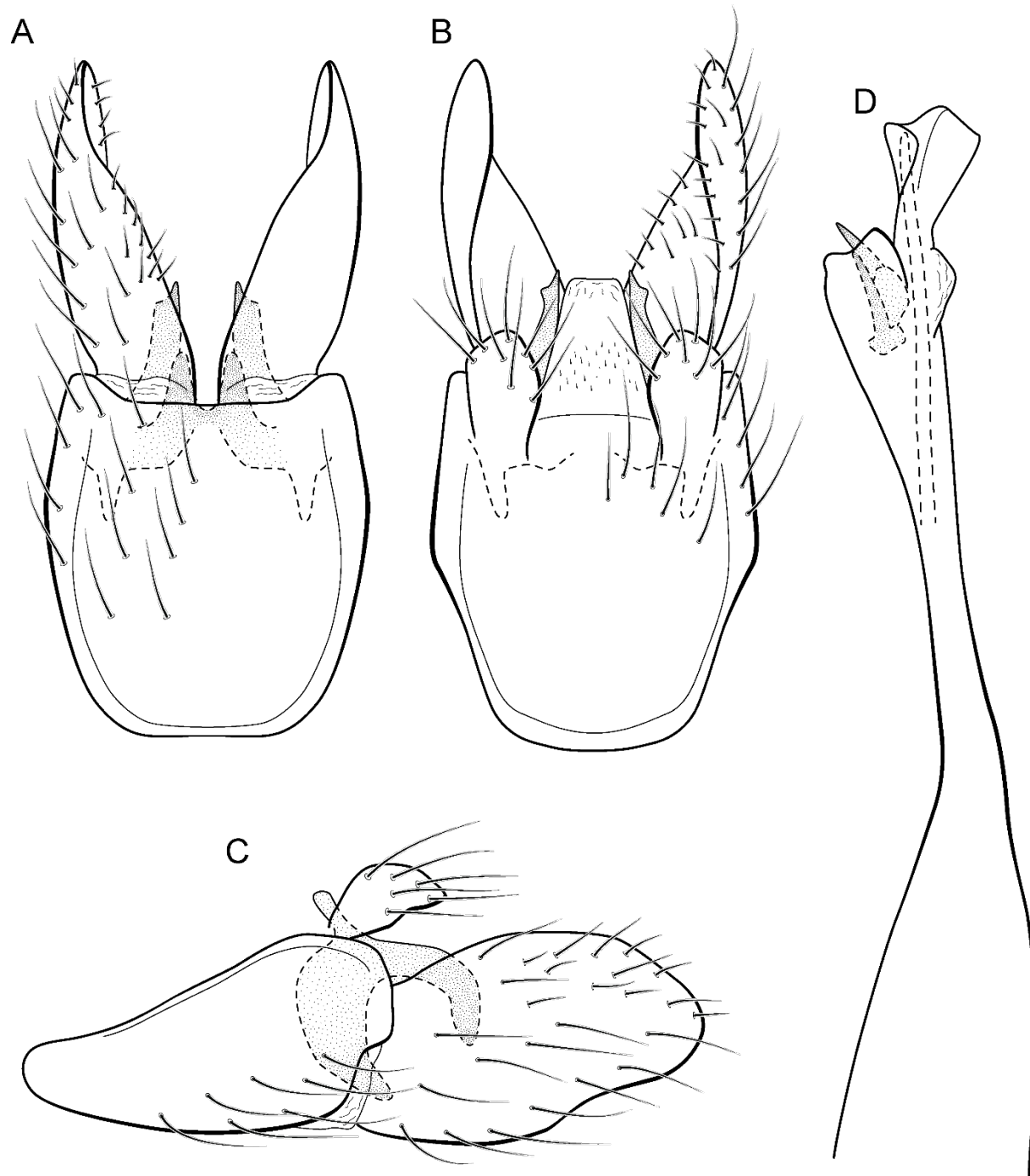
993 (CZMA), 12 males (DZRJ); same data, except 18–30.xi.2012, 9 males (INPA); same data, except

994 14–16.ii.2013, 5 males (MZUFBA); same data, except Rio das Minas, 03º50'03"S 40º54'18"W,

995 el. 524 m, 14–16.ii.2013, DM Takiya, JA Rafael, RR Cavichioli & APM Santos cols., Malaise

996 trap, 5 males (MNRJ).

997   **Etymology.** This species is named in reference to Ubajara National Park, in the municipality with

998   the same name, where the types were collected from.

999   **Figure 24.** *Metrichia ubajara* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)

1000   lateral view; (D) phallus, dorsal view.

1001 **Remarks**. This new species appears to be a member of the *nigritta* group because of their

1002 internal pouches between abdominal segments V and VI and the presence of two long subapical

1003 spines on phallus. *Metrichia ubajara* **sp. nov.** resembles *M. potosina* and *M. goiana* **sp. nov.**

1004 because of the rounded and elongate inferior appendages in lateral view. However, in *M. ubajara*

1005 **sp. nov.** the inferior appendages lack the "tooth" mentioned for these two species. As well, *M.*

1006 *ubajara* **sp. nov.** can be recognized by the phallic apex with a broad sclerotized plate wrapping

1007 the ejaculatory duct.

1008 *Metrichia vulgaris* **sp. nov.**

1009 urn:lsid:zoobank.org:act:BBC0387F-C111-4CA7-834A-F3711F7707F7

1010 (Fig. 25)

1011 **Adult male**. Length 2.7–3.1 mm (n=12). General color, in alcohol, brown. Head with no

1012 modifications. Ocelli 3. Antenna simple, 21-articulated. Maxillary palpus 5-articulated; labial

1013 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

1014 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both

1015 wings. Abdominal segment VI with pair of internal pouches in posterodorsal area. Ventromesal

1016 process on segment VII present. Segment VIII shorter ventrally than dorsally. **Male genitalia**.

1017 Segment IX reduced dorsally; sternum subquadrangular (Fig. 25A); in lateral view, narrower

1018 anteriorly than posteriorly (Fig. 25C). Inferior appendage covered by long setae, apex excavated,

1019 posterodorsal margin acute and sclerotized; subtrapezoidal in ventral view (Fig. 25A); in lateral

1020 view, subretangular (Fig. 25C). Dorsal hook long and stout, almost reaching the inferior

1021 appendage apex; in lateral view, downturned (Fig. 25C). Preanal appendage elongate, rounded

1022 and bearing very long setae (Fig. 25B). Subgenital plate apparently absent. Tergum X

1023 membranous and rounded (Fig. 25B). Phallus tubular, elongate and slender, slightly constricted

1024 mesally; with two short subapical spines; apex rounded and folded; ejaculatory duct sclerotized

1025 and protruding apically (Fig. 25D).

1026 **Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, Rio Palmital, 22º25'34"S 44º32'52"W, el. 637 m,

1027 07.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, male (DZRJ).

1028 **Paratypes.** Same data as holotype, 1 male (DZRJ), 1 male (MNRJ); same data, except Rio das

1029 Pedras, 22º24'33"S 44º33'08"W, el. 706 m, 06.iii.2008, LL Dumas, JL Nessimian & MR de

1030 Souza cols., light trap, 4 males (MNRJ). **Ceará:** Ubajara, Parque Nacional de Ubajara, Rio

1031 Gameleira, 03º50'25"S 40º54'19"W, el. 874 m, 20–22.iv.2012, F Limeira-de-Oliveira et al. cols.,

1032 Malaise trap, 1 male (CZMA). **Goiás:** Alto Paraíso, Rio Bartolomeu tributary, 14º07'25"S

1033 47º30'30"W, el. 1270 m, 22–25.iii.2013, APM Santos & DM Takiya cols., Malaise trap, 3 males

1034 (DZRJ).

1035 **Etymology.** This new species is named in allusion to its unusually wide distribution throughout

1036 Brazil. From the Latin "vulgaris" meaning "common".

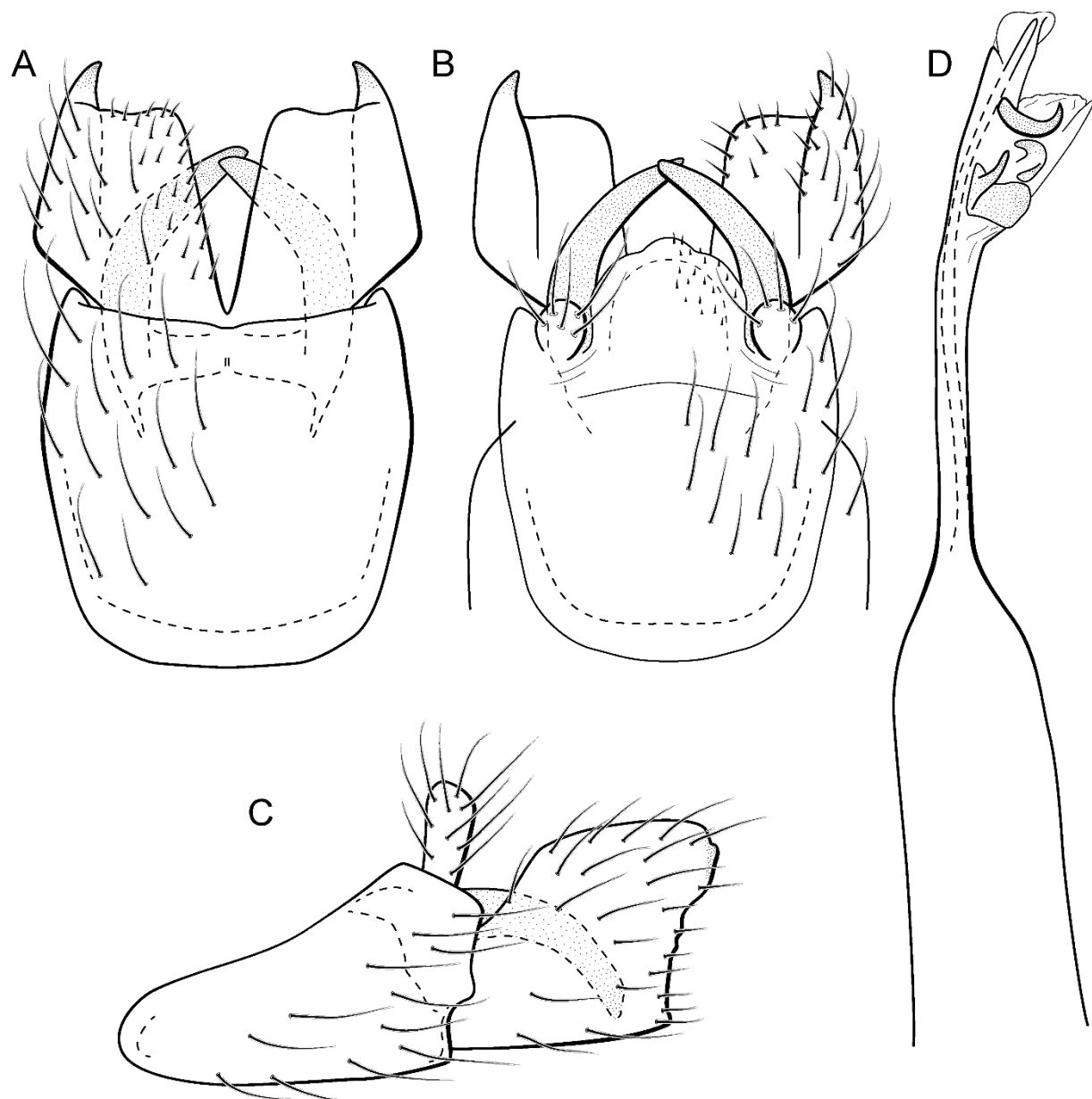
1037 **Remarks**. This new species belongs to the *campana* group due to their internal pouches between

1038 segments VI and VII and the two small subapical spines in phallus. *Metrichia vulgaris* **sp. nov.**

1039 shares with *M. campana*, *M. similis*, and *M. itabaiana* **sp. nov.** the general aspect of inferior

1040 appendages, with an excavated posterior margin. From these species, *M. vulgaris* **sp. nov.** can be

1041 distinguished by their inferior appendages, in lateral view, excavated but with ventral corner more

1042 rounded than acute and short and stout dorsal hook and tergum X short and rounded.

1043   **Figure 25.** *Metrichia vulgaris* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)

1044   lateral view; (D) phallus, dorsal view.

1045   *Metrichia vulgaris* **sp. nov.** has an interesting distributional pattern, occurring in very

1046   distant localities in Southeastern, Centralwestern, and Northeastern Brazil. In addition to their

1047   geographic distance, these localities are included in very distinctive areas: encompassing three

1048   biomes (Atlantic Forest, Cerrado, and Caatinga) and four large river basins (East North Atlantic,

1049   Southeast Atlantic, São Francisco, and Araguaia-Tocantins). Barcode sequences corroborate these

1050   different populations as the same species, with K2P intraspecific divergences up to 4.8%. This is

1051   the highest intraspecific divergence found in our work, but this value is still lower than that

1052   observed among species in other caddisfly groups (Pauls et al., 2010, Zhou et al., 2011). GMYC

1053   analyses grouped sequences into two 'species', but these groups were not related to their

1054   geographic distribution: one group included samples from Rio de Janeiro and Minas Gerais and

1055   the other group, samples from Rio de Janeiro, Minas Gerais, and Goiás. The broader

1056   geographical and specimen sampling of this species, which is directly associated with its higher

1057   intraspecific divergences, may explain the oversplitting by the GMYC method, as discussed by

1058   Talavera et al. (2013).

1059   Although such wide distribution is not common for *Metrichia* species, other

1060   microcaddisflies can show continental distributions (e.g., *Oxyethira tica*). Because the knowledge

1061   about Neotropical microcaddisflies is very poor, this pattern may be more common than currently

1062   thought.

1063   **DISCUSSION**

1064   Although GMYC analysis overestimated the number of *Metrichia* species in our study

1065   (suggesting the split of *M. circuliforme* **sp. nov.** and *M. vulgaris* **sp. nov.** each into two 'species'),

1066   COI sequences strongly corroborated species limits previously defined based on morphological

1067   features. In general, COI sequences of caddisflies show a robust 'barcoding gap' (Pauls et al.,

1068   2010, Zhou et al., 2011), making this molecular marker appropriate as a source of additional

1069   information to corroborate species delimitation or associations of different life stages.

1070   Microcaddisflies are extremely diverse and poorly known, and when associated with the

1071   morphological data, the use of molecular information can result in a more robust taxonomy for

1072   this group. Although methods such ABGD and GMYC should not be used alone to determine

1073   'species', they are useful tools to identifying 'potential species' (Puillandre et al., 2012; Talavera

1074   et al., 2013), especially in very diverse groups and/or with dubious morphology-based

1075   identification. The wide distribution of *Metrichia vulgaris* **sp. nov.** could indicate the existence of

1076   different cryptic species, however morphology and barcode data agreed to define this group as a

1077   single species. Although GMYC overestimated the number of *Metrichia* species in our analysis,

1078   we consider this method an important tool for preliminary distinction when taxonomic

1079   information is poor.

1080   Based on the presence of abdominal modifications, such as internal pouches, external

1081   sclerotized plates, and brushes of long setae, and features of male genitalia, six species groups

1082   have been proposed for *Metrichia* (Flint, 1972; Bueno-Soria & Holzenthal, 2003). These

1083   abdominal modifications usually arise from segments V, VI, and VII, and, in general aspect, are

1084   very distinctive from each other (Fig. 26), possibly representing non-homologous structures.

1085   Nevertheless, most of the species described herein fit in these previously defined groups. Present

1086   analyses of COI sequences (Fig. 2 and Supp. 4) recovered neither the monophyly of *Metrichia*

1087   nor any of the species groups tested (*aberrans*, *campana*, and *nigritta* groups). However, these

1088   relationships need to be analyzed further with better taxon and molecular marker sampling.

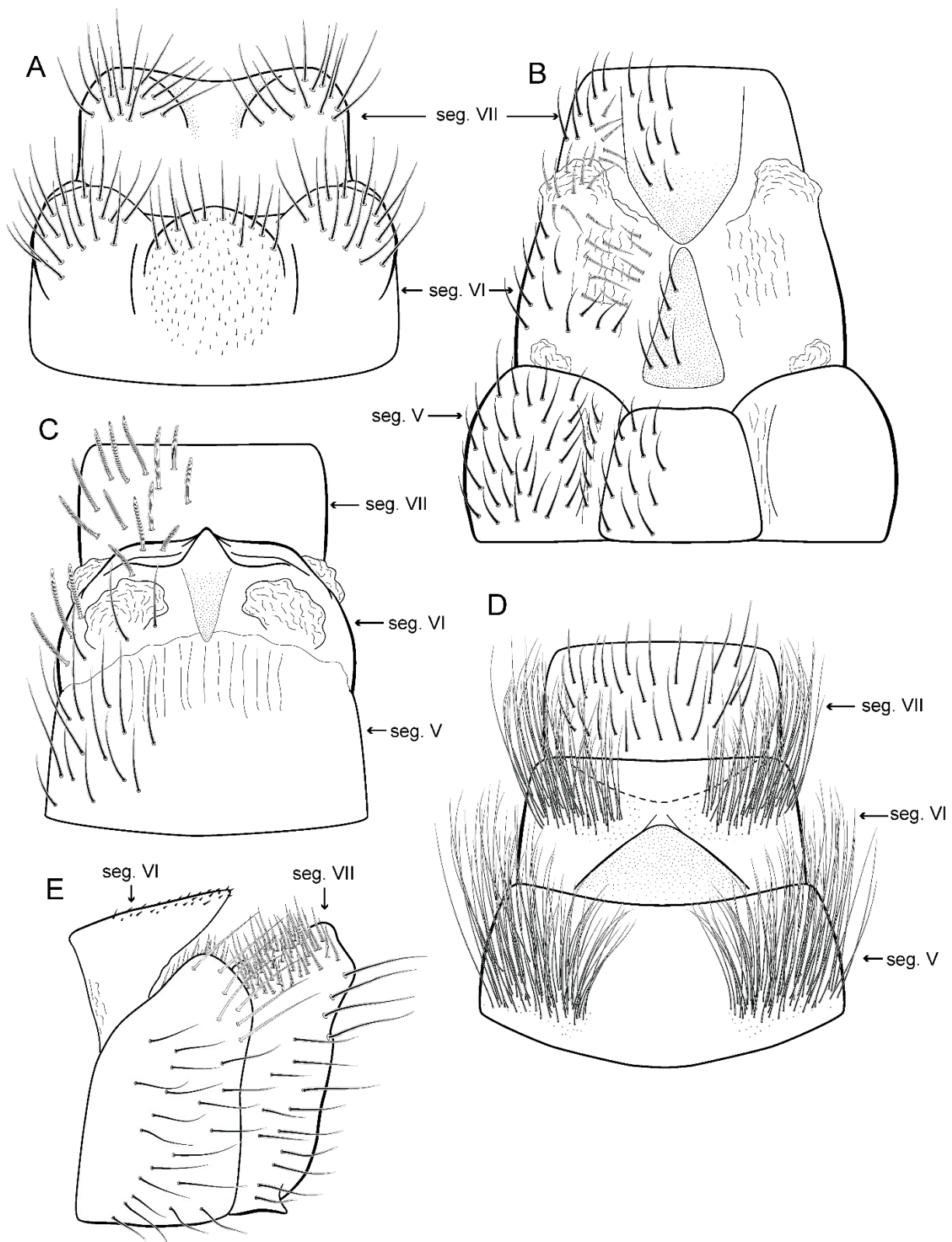



Figure 26. Abdominal modifications of *Metrichia* species: (A) *M. acuminata* **sp. nov.**, segments VI and VII, dorsal view; (B) *M. itabaiana* **sp. nov.**, segments V, VI, and VII, dorsal view; (C) *M.*

1091   *longissima* **sp. nov.**, segments V, VI, and VII, dorsal view; (D) *M. peluda* **sp. nov.**, segments V,

1092   VI, and VII, dorsal view; (E) *M. simples* **sp. nov.**, segments VI and VII, lateral view.

1093   Herein, we have also used molecular data to associate larvae and adults of *M. bonita* **sp.**

1094   **nov.** Larvae remain unknown for most Neotropical species of Trichoptera, and they are even less

1095   known for microcaddisflies. Rearing immatures is very difficult and association based in co-

1096   occurrence with adults is not possible when several species of the same genus co-occur. In this

1097   way, DNA barcodes are a powerful tool, allowing the association and description of immature

1098   stages (Shan et al., 2004; Pauls et al., 2010; Ruiter et al., 2013). Barcode reference libraries for

1099   caddisflies are available for specimens from other regions (Zhou et al., 2009; Zhou et al., 2011;

1100   Ruiter et al., 2013) and represent an important source of information for taxonomic work as well

1101   as for ecological and evolutionary studies. We expect that molecular data will become

1102   increasingly common for Neotropical caddisflies as it facilitates the understanding of their

1103   diversity in this region.

1104   **SUPPLEMENTAL INFORMATION**

1105   **Collecting sites**

1106   Detailed list of collecting sites in Brazil where new species of *Metrichia* were found.

1107   **Georeferences of collecting sites**

1108   Google Earth (.kmz) file with collecting localities of new *Metrichia* species.

1109   **COI sequence alignment**

1110   FASTA format alignment of COI sequence data of *Metrichia* and related microcaddisflies.

Bayesian inference estimated tree

Consensus phylogram (50% majority-rule) from BI analyses of COI sequences (mean lnL= -5464.29) of *Metrichia* and related microcaddisflies. Values displayed near branches are posterior probabilities.

K2P pairwise divergences

Pairwise K2P divergences of COI sequences of *Metrichia* and related microcaddisflies.

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