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Allan PM Santos, Daniela M Takiya, Jorge L Nessimian

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ABSTRACT

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INTRODUCTION

The microcaddisfly genus *Metrichia* Ross is included in the subfamily Ochrotrichiinae, which also includes *Ochrotrichia* Mosely, *Angrisanoia* Özdikmen, *Nothotrichia* Flint, *Rhyacopsyche* Müller, and the recently erected *Ragatrichia* Oláh & Johanson, all of them exclusively from New World. Based on adult morphology, Harris & Armitage (1997) and Oláh & Johanson (2011) also suggested three other genera to be included in this subfamily: *Dibusa* Ross, *Caledonotrichia* Sykora, and *Maydenoptila* Neboiss, respectively from U.S., New Caledonia, and Australia. However, because diversification of main lineages of hydroptilids has not been deeply studied, the placement of these genera remains dubious. As commented by Wells et al. (2013), relationships of these microcaddisflies need to be studied based on a rigorous analysis including molecular data.

Currently, *Metrichia* includes 107 species, found from the U.S. to South America, with highest known diversity in Central America (Flint, 1972; Marshall, 1979). *Metrichia* was considered as subgenus of *Ochrotrichia* due to similarities on adult morphology and almost indistinguishable larvae (Flint, 1968). This subgeneric status was followed by Marshall (1979), who also established the New World tribe that is now recognized as subfamily Ochrotrichiinae. Wiggins (1996) provided additional information on larval morphology of *Metrichia* and *Ochrotrichia*, reestablishing both as independent genera.

Diversity of Neotropical microcaddisflies is poorly known and usually several new species are found in collections or when collecting trips are made, even in localities previously studied by trichopterologists. This occurs because Hydroptilidae are very small and have complex male genitalia, making it difficult to observe in lower magnification microscopes and to understand homologies among some structures. Only one species of *Metrichia* has been described from Brazil so far, *M. pernambucana* Souza & Santos, but larvae are commonly identified from several localities (*e.g.*, Pes et al., 2005; Spies & Froehlich, 2006; Spies et al., 2009). It is not

surprising that material studied herein recently collected from different river basins in Brazil (Fig. 1, Supp. 1, Supp. 2) revealed so many new species.

Figure 1. Localities where *Metrichia* specimens studied herein where collected (●) distributed on seven of the large River Basins of Brazil.

Most *Metrichia* species exhibit a more restricted distribution, in other words, each species is recorded in relatively few close localities. Identification based mainly on features of male genitalia, revealed some interesting patterns, for example, one of the new species being found in three very distinctive biomes: Atlantic Forest (Southeastern Brazil), Caatinga (Northeastern

Brazil), and Cerrado (Midwestern Brazil). Although such wide distribution is not common for *Metrichia*, it is known for other Neotropical microcaddisflies, as *Oxyethira tica* Holzenthal & Harris, recorded from Mexico, Central, and South America (Flint et al., 1999).

Although molecular tools have become common in taxonomic studies to help in species delimitation, the use of these tools are still rare with Neotropical caddisflies. Using sequences of the mitochondrial cytochrome oxidase I gene (COI), the standard DNA barcode region for animals (Hebert et al., 2003), Pauls et al. (2010) were able to corroborate two new species of *Smicridea* (*Smicridea*) McLachlan from Chile, defined with morphological characters. In most studies with caddisflies, divergence in COI sequences show clear differences between intraspecific and interspecific variation, the so-called barcoding gap (Zhou et al., 2007; Pauls et al., 2010; Ruiter et al., 2013). Some species delimitation approaches rely exactly in distinguishing intra- and interspecific divergence, such as the Automatic Barcoding Gap Discovery (ABGD) (Puillandre et al., 2012). More sophisticated methods invoke coalescence and speciation models, such as, the General Mixed Yule Coalescent (GMYC) and are considered more robust to identify lineages when intra- and interspecific divergences overlap (Pons et al., 2006). Integrating independent data (*e.g.*, morphology and DNA sequences) and using different approaches are particularly interesting for the taxonomy of diverse and complex groups, such as microcaddisflies. In this work we applied both methodologies to evaluate our initial morphological identification.

Besides species delimitation, for caddisflies, DNA taxonomy has a valuable role in the associations between immature and adult stages (Graf et al., 2005; Waringer et al., 2007; Zhou et al., 2007; Ruiter et al., 2013). Traditional techniques to associate larvae and adults are more difficult because it involves rearing larvae in laboratory (not an easy task for many caddisfly groups) or luck in finding pharate adults in field. Indirect association, for example, by collecting

adults and larvae at the same locality are even more difficult and can result in misidentification, because different species of the same genus frequently co-occur.

Larvae of *Metrichia* have been associated for only two species: *M. nigritta* Ross, described by Edwards & Arnold (1961) and illustrated by Wiggins (1996), and *M. juana* (Flint) by Flint (1964) in the original description. In addition, Botosaneanu and Flint (1982) described a larva of *Metrichia* and its case from Venezuela and a pupal case from Ecuador; and Pes et al. (2005) illustrated larvae from Brazil and three different types of cases. In both works, specific names are not provided, since authors did not have the respective adults. *Metrichia* larvae build a typical oval purse-like case, made of silk and usually covered with algae filaments (Wiggins, 1996; Pes et al., 2005), sometimes also having mineral grains (Botosaneanu & Flint, 1982). Cases of some non-associated larvae show a pair of dorsal "chimneys", an uncommon feature also described and illustrated by Müller (1879, 1880) for *Dicaminus ladislavii* Müller, from Santa Catarina, Brazil. Based on larval cases from Central and South America, Botosaneanu & Flint (1982) pointed that *Dicaminus* is possibly synonymous with *Metrichia*, but this question remains open, since there are no male specimens from Müller work. Herein, DNA sequence data allowed the association of larvae from one locality in Mato Grosso do Sul State, where two distinct morphological adults have been collected. These larvae are atypical in the genus because were found in a river with calcareous sediments (calcareous tufa or travertine), making its cases with this same substance.

Almost nothing is known about the biology of *Metrichia* larvae. According to Wiggins (1996), larvae of *M. nigritta* were collected in association with filamentous algae on rock surface. In Brazil, *Metrichia* occurs in fast flowing streams, usually with associated algae. Herein, we report for the first time the occurrence of *Metrichia* in calcareous tufa. In fact, there are no hydroptilids reported from inhabiting calcareous tufa so far. Calcareous tufa or travertine is a terrestrial sedimentary rock, predominantly composed of carbonate minerals, calcite, and

aragonite (Drysdale, 1998). Travertines are formed by rapid precipitation of these minerals, producing large alterations on river morphology (Drysdale & Gale, 1997). Although it is recognized the importance of microbes on travertine formation, at least by creating surfaces to crystal nucleation, the knowledge of the importance of macroinvertebrates in this process is still poor (Drysdale, 1998; 1999; Paprocki et al., 2003). Studies of Drysdale (1998, 1999) pointed out that aquatic insects play important role in travertine biogenesis in Australian springs. According Drysdale (1998), Hydropsychidae (*Cheumatopsyche* Wallengreen) are the most "geomorphologic significant" in Australian travertines studied. Paprocki et al. (2003) also found a Hydropsychidae (*Smicridea* McLachlan) as an important organism in modifying travertine morphology in Venezuela.

MATERIAL AND METHODS

Morphological study

Specimens were collected manually (larvae or diurnal active adults) or using Malaise or light traps, and then fixed in 96% ethanol. Collecting permits in Brazil were issued by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (SISBIO 43047 and 14591). To observe genital structures, abdomen of males were removed and cleared in a heated solution of 10% KOH for 20 minutes. Then, abdomens were mounted in temporary slides, which were used to draw pencil sketches with compound microscope equipped with camera lucida. Vector graphics were traced in Adobe Illustrator CS6 (Adobe Systems Inc.) using pencil sketches as templates. Descriptions provided here were made with DELTA software (Description Language for Taxonomy) (Dallwitz et al., 1999). Terminology used throughout this paper follows that provided by Marshall (1979) and Bueno-Soria & Holzenthal (2003). Types for newly described species are deposited at Coleção Entomológica Prof. José Alfredo Pinheiro Dutra, Departamento de Zoologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro (**DZRJ**); Museu Nacional,

Universidade Federal do Rio de Janeiro, Rio de Janeiro (**MNRJ**); Instituto Nacional de Pesquisas da Amazônia, Manaus (**INPA**); Coleção Zoológica do Maranhão (**CZMA**); and Museu de Zoologia da Universidade Federal da Bahia, Salvador (**MZUFBA**).

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:D8D4049E-494B-4A30-92AC-F8F42D2B54B9. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central and CLOCKSS.

DNA extraction, PCR, and sequencing

Genomic DNA was extracted from head and thorax (or from the entire body) of fresh material using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany), without tissue maceration. After extraction, specimens were returned to ethanol and deposited in DZRJ collection as a DNA voucher. COI fragments were amplified using pair of primers: HCO-2198 (TAAACTTCAGGGTGACCAAAAAATCA) in combination with LCO-1490 (GGTCAACAAATCATAAAGATATTGG) (Folmer et al., 1994) or C1-J-1718 (GGAGGATTTGGAAATTGATTAGTTCC) (Simon et al., 1994). Polymerase chain reaction (PCR) conditions followed: initial denaturation at 94 ºC for 3 min; 35 cycles of denaturation at 94 ºC for 1 min, annealing at 50 ºC for 1 min, and extension at 72 ºC for 2 min; and final extension

166 at 72 ºC for 7 min. PCR products were sent to Macrogen Inc., Seoul, for purification and
167 sequencing reactions.

168 COI sequences of 64 specimens of 15 species of *Metrichia* were obtained. Additional
169 sequences were obtained for specimens of *Angrisanoia*, *Nothotrichia*, *Ochotrichia*, and
170 *Rhyacopsyche* (Table 1), included as outgroup in different analyses, as described below.

171 **Table 1.** Species of *Metrichia* and other hydroptilids with DNA barcode sequenced used in this
172 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	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 ♂	Costa Rica: Puntarenas	KU743404
Metrichia acuminata **sp. nov.**	ENT 2192 ♂ ENT 2282 ♂ ENT 2284-5 ♂ ENT 2779 ♂	Brazil: Alagoas: Quebrangulo	KU743406 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

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

Sequence editing, alignment, and analyses

Forward and reverse sequences of each sample were assembled and manually edited in Sequencher 4.1 (Gene Codes, Ann Arbor, Michigan, USA). Sequences were verified with the Blast tool in GenBank to check for contamination. Then, COI sequences were aligned with ClustalW implemented in MEGA 6 (Tamura et al., 2013) and translated into amino-acid sequences to check for stop codons. The final alignment resulted in a matrix with 577 bp (Supp. 3).

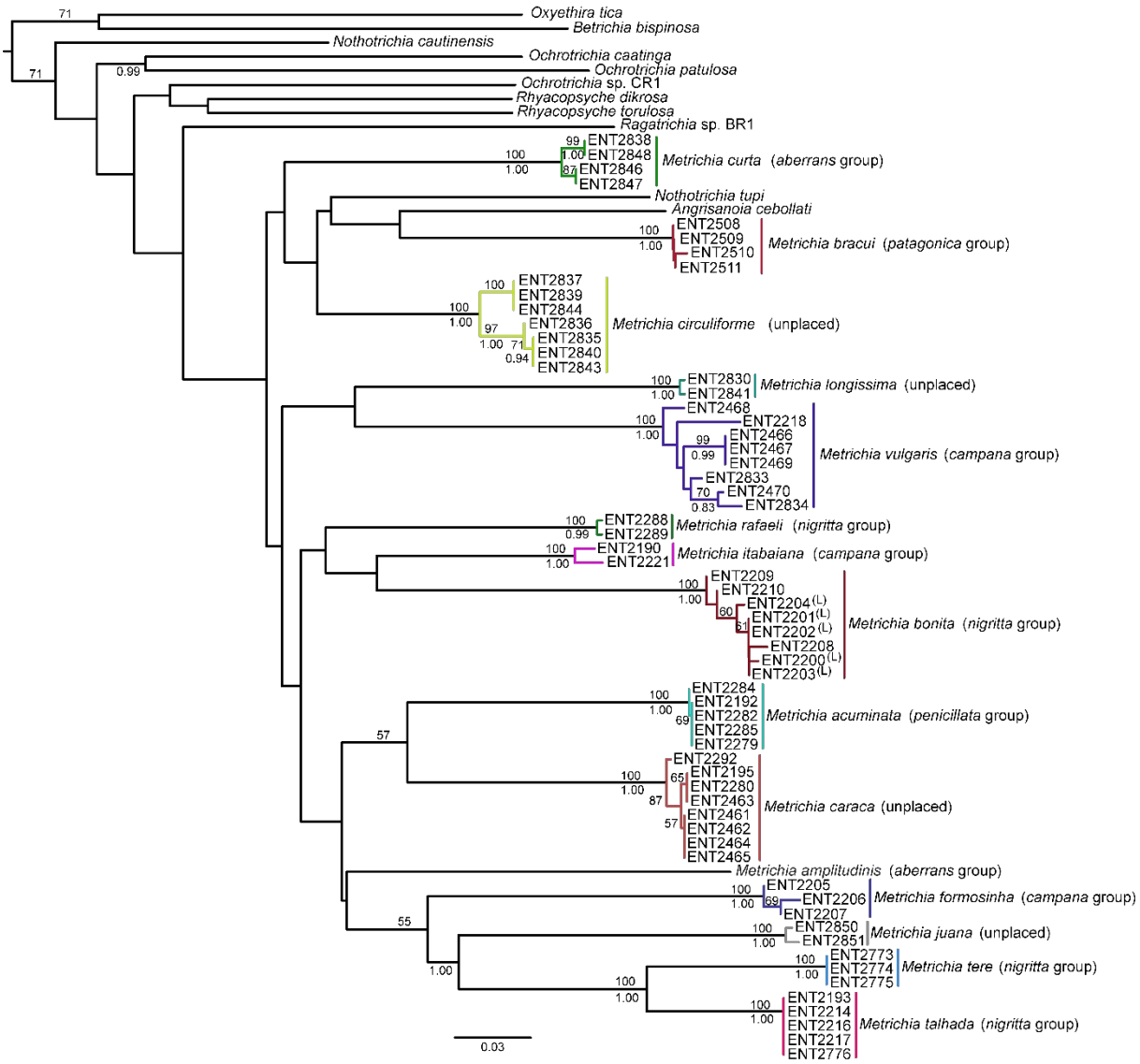
COI sequences were used to explore putative species limits with four different methodologies: (1) lineages recovered in neighbor-joining tree; (2) lineages recovered with Bayesian Inference; (3) ABGD; and (4) GMYC. The neighbor-joining tree was calculated in MEGA 6 using Kimura 2-Parameter (K2P) distances (Kimura, 1980), with partial deletion of missing information. Although the use of K2P distances in DNA barcoding is debated (Srivathsan & Meier, 2012), to allow comparison with previous works we also used this evolutionary model because it is frequently used in studies of species delimitation based on COI sequences. Branch

188 support of neighbor-joining tree was assessed with 1.000 pseudoreplicate of non-parametric
189 bootstrap (Felsenstein, 1985).
190 BI analysis was conducted with MrBayes v. 3.2.2 (Ronquist *et al*., 2012) with four
191 independent runs, each one with four MCMC chains running for 50,000,000 generations, with
192 sample frequency of 5,000. Convergence of sampled parameters was checked in Tracer v. 1.5
193 (Rambaut & Drummond, 2007) and the first 10% of sampled trees and parameters as burnin.
194 GTR+I+G was the best fit model selected by Akaike Information Criterion (AIC) with jModeltest
195 v. 0.1.1 (Posada, 2008) and it was applied in BI analysis in MrBayes. Branch support was
196 assessed by posterior probability (PP), presented on a 50% majority consensus tree.
197 ABGD analysis was run using on-line version available in
198 http://wwwabi.snv.jussieu.fr/public/abgd/, where COI alignment was uploaded. The analysis was
199 conducted with the following settings: Pmin=0.001; Pmax=0.1; steps=20; relative gap width=1.0,
200 also based on K2P model. This method statistically infers the DNA barcode gap in a single locus
201 alignment, partitioning the data based on this gap in putative species (Puillandre et al., 2012).
202 The GMYC analysis (Pons et al., 2006; Fujisawa & Barraclough, 2013) was performed in
203 R (R Development Core Team 2010) using the SPLITS package (Ezard et al., 2009) with single-
204 threshold method. Basically, the method estimates branching patterns on an ultrametric tree,
205 identifying the most likely transition point from coalescent to speciation branching. The
206 ultrametric tree used here was obtained with BEAST v. 1.8 (Drummond et al., 2012) under a
207 relaxed uncorrelated molecular clock (Drummond et al., 2006). The node including *Ochrotrichia*
208 species was calibrated based on fossil evidence with a lognormal distribution offset at 20 mya
209 and log(mean) = 2.8 to represent the possible range of 20-140 mya (Wells & Wichard, 1989); and
210 the divergence of Ochrotrichiinae was calibrated based on Malm et al. (2013) with a normal
211 distribution with mean 82.17 ± 12 mya. The BEAST analysis ran for 200,000,000 generations,

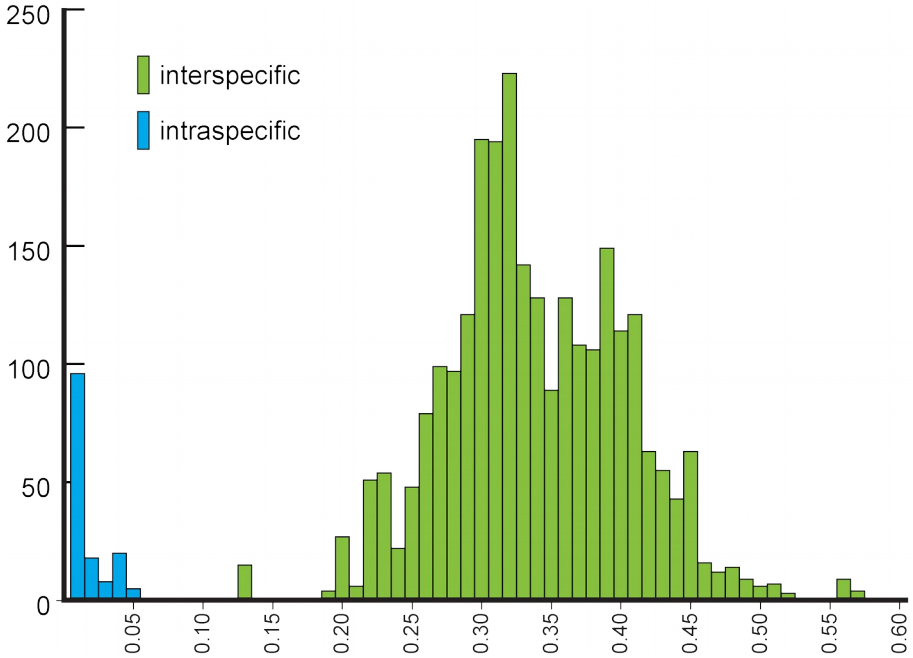
sampled every 10,000 generations. Convergence was verified with Tracer and a maximum credibility tree was written using TreeAnotator, discarding the first 10% as burnin.

RESULTS

NJ (Fig. 2) and BI (Supp. 4) trees corroborated morphological identification, with all 14 species of *Metrichia* with more than a single specimen recovered as monophyletic lineages with 100% bootstrap support. ABGD also returned the same species as they were previously delimited based on morphological features. A robust barcoding gap was found among *Metrichia* species (Fig. 3, Table 2). The maximum intraspecific divergence was observed within *Metrichia vulgaris* **sp. nov.** (0.048). The minimum interspecific divergence was found between specimens of *Metrichia talhada* **sp. nov.** and *Metrichia tere* **sp. nov.** (0.126).



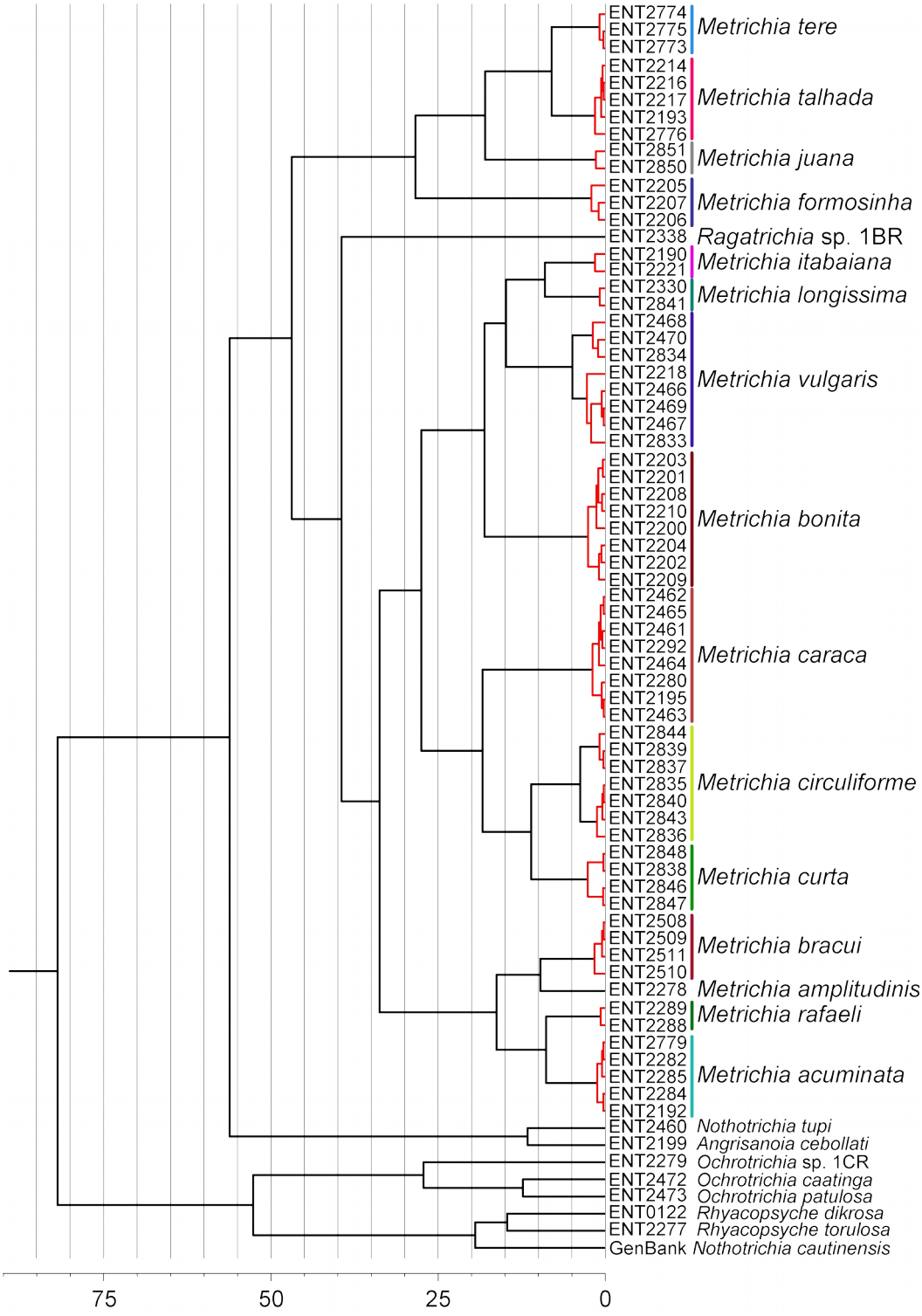
222 **Figure 2.** Neighbor-joining tree of COI sequences of *Metrichia* species based on K2P distances.
223 Numbers above and below branches are, respectively, NJ bootstrap support and posterior
224 probabilities from BI analysis. Details of specimens are in Tables 1 and 2; K2P distances matrix
225 is in Supp. 5.



226 **Figure 3.** Histogram of number of pairwise comparisons of intra- (blue) and interspecific (green)
227 K2P divergences among 15 *Metrichia* species with COI sequences sampled.

228 **Table 2.** Maximum intra- and minimum interspecific K2P divergences of COI sequences among
229 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



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

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

232 single threshold in SPLITS. Timescale in million years.

GMYC analysis estimated a slightly higher number of putative species, with *Metrichia circuliforme* **sp. nov.** and *Metrichia vulgaris* **sp. nov.** being each further divided as two species (Fig. 4). Regarding all other species, GMYC results were congruent with other methods and with morphology.

In all analyses performed using DNA barcode, *Metrichia* larvae collected in calcareous tufa were consistently associated with adult males of *Metrichia bonita* **sp. nov.** Therefore, in the following section, we describe this larva within that species.

SPECIES DESCRIPTIONS

Metrichia acuminata sp. nov.

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

(Fig. 5, Fig. 26A)

Adult male. Length 2.1–2.5 mm (n=5). General color, in alcohol, brown. Head with no modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdominal segment IV with pair of internal pouches in posterior area; segment V with pair of internal pouches; segment VI with tergum as a sclerotized rounded plate, surrounded by long setae (Fig. 26A); segment VII bearing a brush of very long setae dorsolateraly (Fig. 26A). Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally and bearing a brush of long setae dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subrectangular, with anterior margin rounded (Fig. 5A); in lateral view narrower anteriorly than posteriorly (Fig. 5C). Inferior appendage covered by long setae; subtrapezoidal in ventral view (Fig. 5A); in lateral view, subtrapezoidal, apex with acute corners (Fig. 5C). Dorsal hook short, almost half length of inferior appendage; in lateral view, downturned (Fig. 5C). Preanal

appendage rounded in lateral view and bearing very long setae (Fig. 5C). Subgenital plate apparently absent. Tergum X membranous and truncate (Fig. 5B). Phallus tubular, elongate and slender, slightly constricted mesally, with two curved subapical spines, one short and another long; apex truncate and sclerotized; ejaculatory duct sclerotized, sinuous, and protruding apically (Fig. 5D).

Holotype. BRAZIL: Ceará: Ubajara, Parque Nacional de Ubajara, Cachoeira do Gameleira, 03º50'21"S 40º54'23"W, el. 880 m, 23.iv.2012, DM Takiya & JA Rafael cols., light trap, male (CZMA).

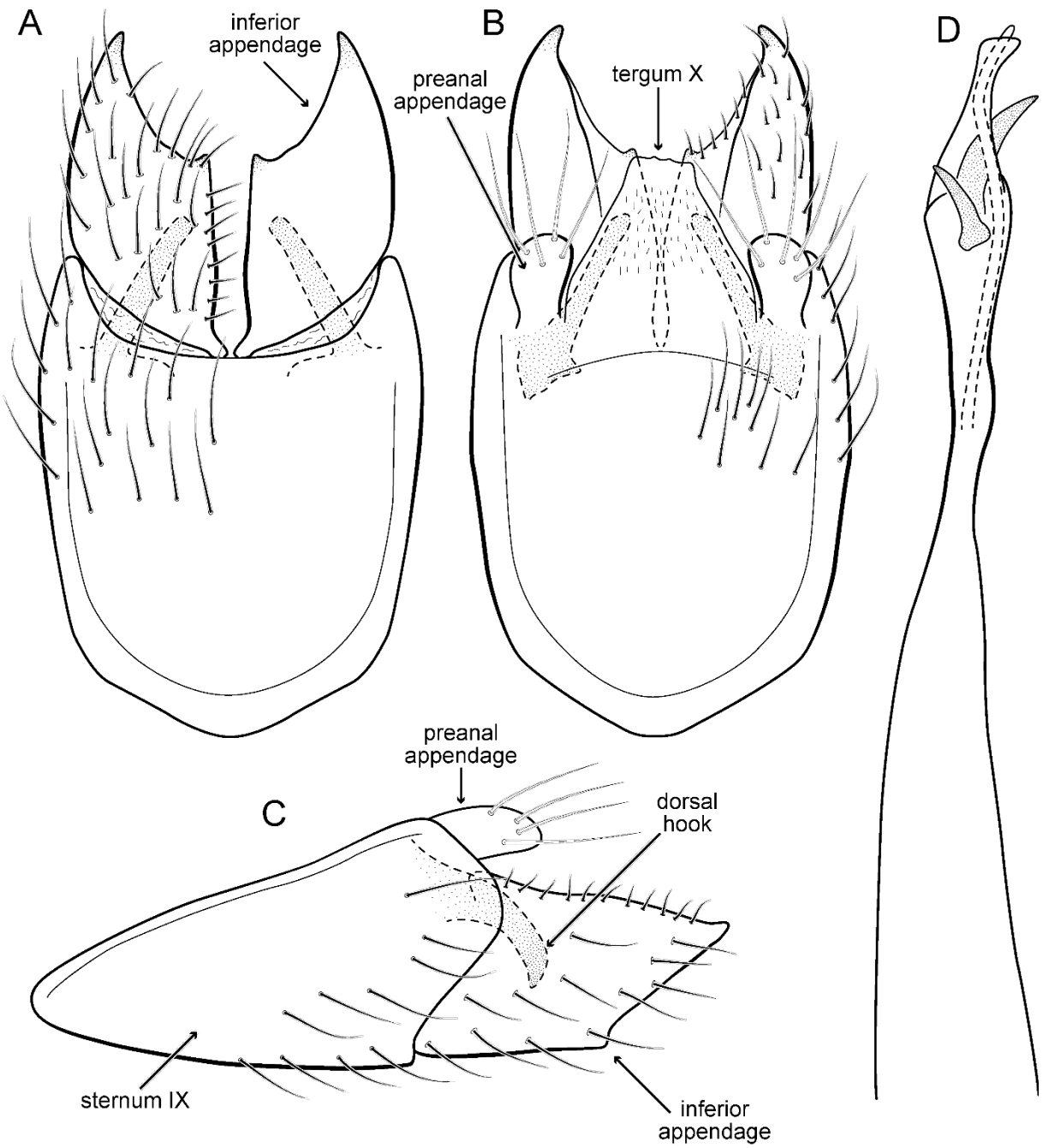
Paratypes. Same data as holotype, except, Rio das Minas, 03º50’03”S 40º54’18”W, el. 524 m, 13–17.ix.2012, JA Rafael et al., Malaise trap, 2 males (INPA); same data, except 14–16.ii.2013, DM Takiya, JA Rafael, RR Cavichioli & APM Santos, Malaise trap, 2 males (DZRJ). **Alagoas:** Quebrangulo, Reserva Biológica de Pedra Talhada, Rio Caranguejo, 09º15’26”S 36º25’08”W, el. 550 m, 19–28.vi.2014, APM Santos, DM Takiya, WRM Souza, Malaise trap, 2 males (MNRJ), 3 males (MZUFBA), 13 males (DZRJ).

Etymology. The species is named in allusion of the pointed apex of inferior appendages (from Latin, “acumin-”: “pointed”).

Remarks. This new species belongs to the *penicillata* group based on: (1) internal pouches between abdominal segments IV and V; (2) setal brushes on segments V, VI, and VII; and (3) phallus with two subapical spines. The male genitalia and complex abdominal modifications resemble *M. penicillata* Flint and *M. trigonella* Flint. These three species have inferior appendages with acute apex in lateral view; phallus with two subapical spines; and abdominal terga with brushes of very long and stout setae. The new species can be distinguished by inferior appendages more trapezoidal in lateral view, with acute corners posteriorly and dorsal hook only slightly downturned in lateral view; and phallus with one larger and stouter subapical spine. Although, the male genitalia of this new species superficially resembles that of *M. bonita* **sp.**

282 **nov.**, *M. acuminata* **sp. nov.** is readily recognized by the presence of setose lobes on abdominal
283 segments V and VI.

284 We were not able to obtain COI sequences for individuals from Ceará State, so the five
285 sequences analyzed belong to specimens from Alagoas State and shared the same haplotype. *M.*
286 *acuminata* **sp. nov.** was recovered as closely related to *M. caraca* **sp. nov.** (Fig. 2), but in both
287 Bayesian approaches these two species were not recovered as sister taxa (Fig. 4, Supp. 1).



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

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

290 ***Metrichia azul* sp. nov.**

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

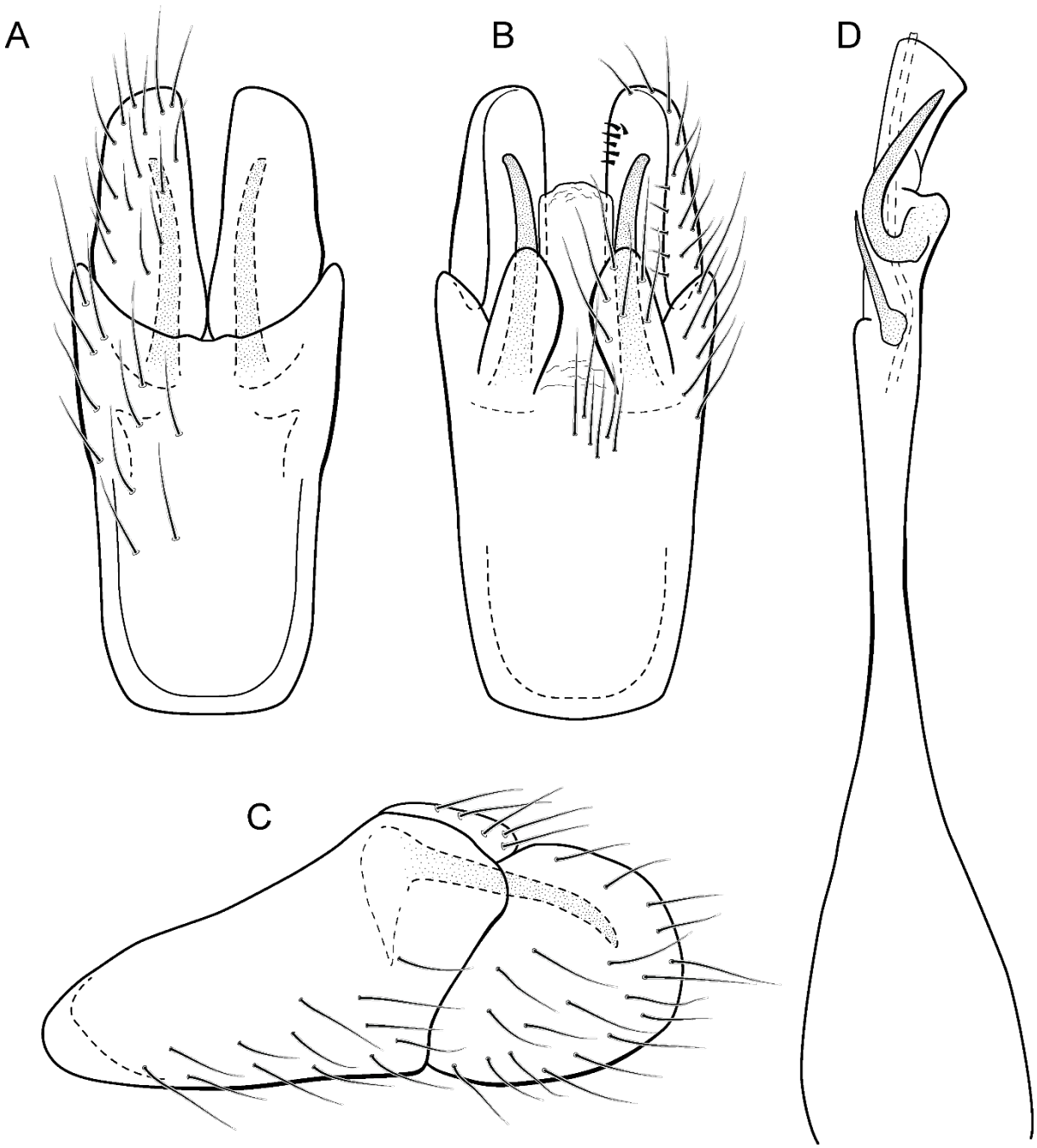
292 (Fig. 6)

Adult male. Length 2.0–2.1 mm (n=4). General color, in alcohol, brown. Head with no modifications. Ocelli 3. Maxillary palpus 5-articulated, article IV broad and darkened; labial palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdominal segment V with pair of internal pouches; segment VI with pair of internal pouches and pair of lateral external sacs with specialized setae. Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subrectangular (Fig. 6A); in lateral view narrower anteriorly than posteriorly (Fig. 6C). Inferior appendage covered by long setae and with scale-like setae; subrectangular in ventral view (Fig. 6A); in lateral view, rounded, apex rounded (Fig. 6C). Dorsal hook long, more than half length of inferior appendage; in lateral view, slightly downturned (Fig. 6C). Preanal appendage elongate, but shorter than inferior appendage, and bearing very long setae (Fig. 6B). Subgenital plate apparently absent. Tergum X membranous and subrectangular (Fig. 6B). Phallus tubular, elongate and slender, slightly constricted mesally, with two long, curved, subapical spines; apex rounded and sclerotized; ejaculatory duct sclerotized, sinuous, and protruding apically (Fig. 6D).

Holotype. BRAZIL: Paraná: Céu Azul, Parque Nacional do Iguaçu, Rio Azul, 25º09’21”S 53º47’44”W, el. 510 m, 6–8 ix.2012, APM Santos, DM Takiya, ALH Oliveira, GA Jardim & BHL Sampaio cols., Malaise trap, male (DZRJ).

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

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



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

317 **Remarks**. This new species is another member of *penicillata* group based on internal pouches
318 between segment V and VI and the long subapical spines of the phallus. The new species shares
319 similarities on male genitalia with *M. biungulata* Flint and *M. decora* Bueno-Soria & Holzenthal,

particularly the rounded aspect of inferior appendages, but can be easily distinguished from those species by the absence of tooth-like processes on inferior appendages; more elongate preanal appendages; and dorsal hook only slightly downturned in lateral view.

***Metrichia bonita* sp. nov.**

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

(Fig. 7, Fig. 8)

Adult male. Length 2.3–2.5 mm (n=4). General color, in alcohol, brown. Head with no modifications. Ocelli 3. Antenna simple, 21-articulated. Maxillary palpus 5-articulated; labial palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdominal segment VI with dorsal pouches covered with setae directed inward. Ventromesal process on segment VII absent. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subrectangular, with anterior margin rounded (Fig. 7A); in lateral view narrower anteriorly than posteriorly (Fig. 7C). Inferior appendage covered by long setae, subtrapezoidal in ventral view (Fig. 7A); in lateral view, with an acute projection, apex with acute corners (Fig. 7C). Dorsal hook short, almost half length of inferior appendage; in lateral view, slightly downturned (Fig. 7C). Preanal appendage short, rounded and bearing very long setae (Fig. 7B). Subgenital plate apparently absent. Tergum X membranous and with a shallow U-shaped incision (Fig. 7B). Phallus tubular, elongate and slender, slightly constricted mesally, with a median process; with two curved subapical spines, one short and another long; apex truncate and sclerotized; ejaculatory duct sclerotized and not protruding apically (Fig. 7D).

Larva (5th instar). Length 1.5–1.9 mm (n=10). Head dark brown, unpigmented around stemmata (Fig. 8C); slightly longer than broad; frontoclypeal and coronal sulci indistinct; with a few long

setae (Fig. 7F). Antenna short, apparently 2-articulated and with no apical setae (Fig. 7F). Labrum with pair of stout setae (Fig. 7F). Mandibles with inner margin sinuous and darkened. Thoracic nota sclerotized, dark brown, with a row of stout setae on anterior margin. Pro-, meso-, and metanotum with middorsal ecdysial line. Thoracic segments with small pleurites (Fig. 7E). Thoracic legs brown, short and stout, almost the same size of each other. Foreleg with stout setae; tibia with a posteroventral lobe with a spine-like seta (Fig. 7G); femur bearing a spine-like setae; tarsal claw simple. Mid- and hind legs with stout setae on posteroventral margin. Abdomen almost white, with dark brown sclerites. Abdominal segment I with ellipsoid tergite; segments I-V with pair of long, dorsal setae and pair of dorsolateral setae; segment VI with two pairs of long dorsal setae and two pairs of dorsolateral setae; segments VII and VIII with three pairs of dorsal, long setae and two pair of dorsolateral setae; segment IX with sclerotized tergite and several long setae. Abdominal segments I, III, IV, and IX with pair of ventral, long setae; segment II with two pairs of ventral, long setae and globose process on ventrolateral area (Fig. 7E). Anal proleg very short not projecting prominently; with basal sclerite bearing long setae; anal claw simple.

Larval case. Length 1.5–2.0 mm (n=10). General color white (Figs. 8B, 8C). Constructed with calcareous particles (with no algal filaments added), forming two rigid and lateral valves, poorly closed dorsally and ventrally (Fig. 8C). External surface rugose.

Biology. Larvae were collected on calcareous tufa in a fast flowing river, approximately 10 meter wide (Fig. 8A). No pupa was found and no adult was seen active during the day.

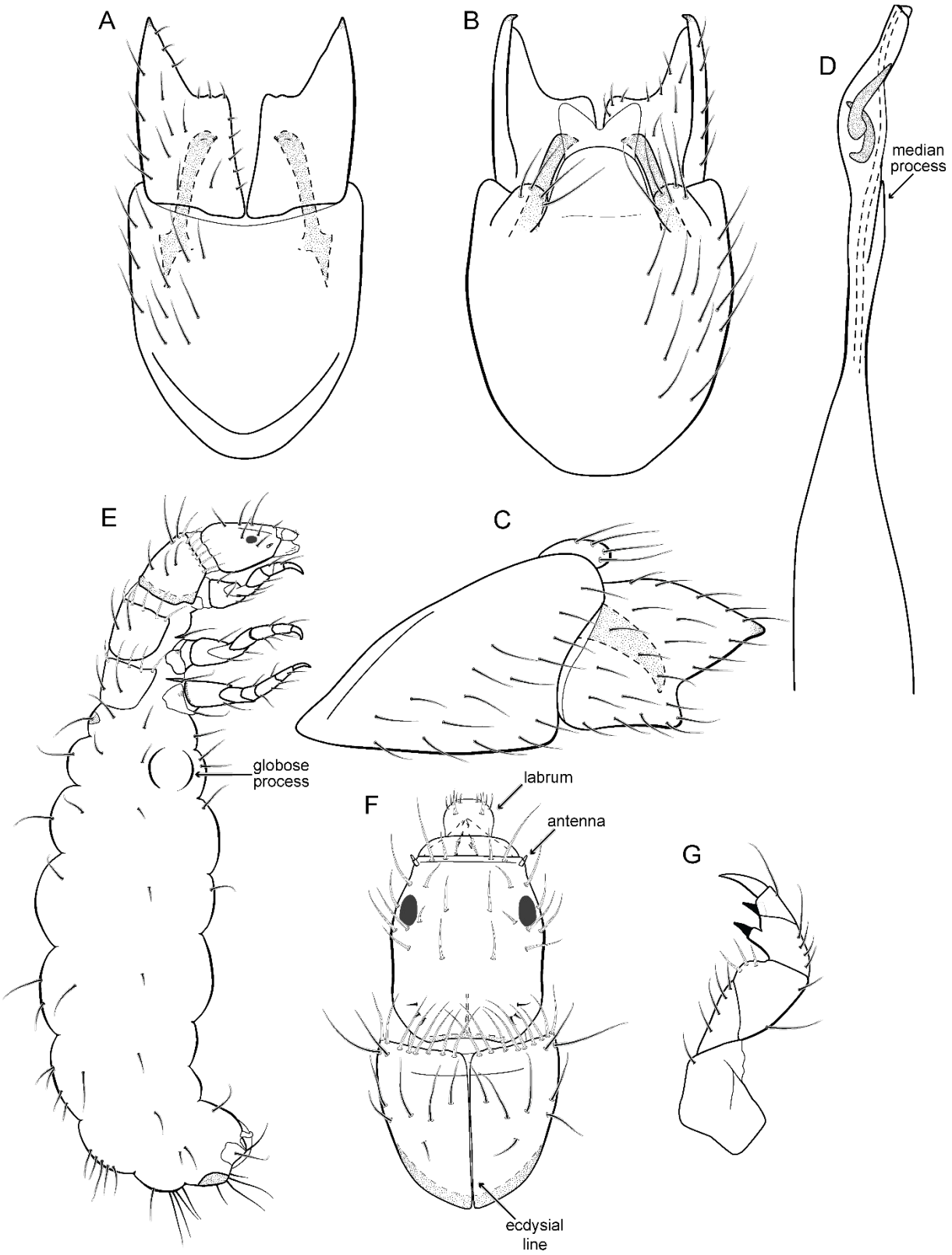

A
B
D
median process
E
C
globose process
labrum
F
antenna
G
ecdysial line

Figure 7. *Metrichia bonita* **sp. nov.**: (A) male genitalia, ventral view; (B) male genitalia, dorsal view; (C) male genitalia, lateral view; (D) phallus, dorsal view; (E) larva, habitus, lateral view; (F) larva, head and pronotum, dorsal view; (G) larva, foreleg, ventral view.

Holotype. BRAZIL: Mato Grosso do Sul: Bonito, Rio Formosinho, 21º10'16"S 56º26'47"W el. 275 m, 08–13.ix.2013, APM Santos & DM Takiya cols., Malaise trap, male (DZRJ).

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

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

Etymology. This species is named in reference to the type locality (Fig. 8), the municipality of Bonito, state of Mato Grosso do Sul. In Portuguese, the word "bonita" (the feminine form) means "beautiful".

Remarks. *Metrichia bonita* **sp. nov.** has features of *nigritta* group: internal pouches between segment V-VI, phallus with 2 spines and an acute process on distal portion. This new species can be easily distinguished from other species in this group based on the shape of inferior appendages, subtrapezoidal in ventral view, with dorsal corner acute and darkened. Besides, the dorsal hook of the inferior appendages, which are broad basally and slightly downturned in lateral view, also helps to differs this new species. COI sequences showed maximum intraspecific distance of 1.5% and minimum interspecific distance of 21.0% to its closest neighbor, *M. itabaiana* **sp. nov.** Although the male genitalia of both species shows some superficial resemblance, based on the abdominal modifications and phallic aspect, whereas *M. bonita* **sp. nov.** belongs to *nigritta* group, *M. itabaiana* fits better in the *campana* group.

Larvae of *Metrichia bonita* **sp. nov.** are very similar to those previously described or illustrated, including *M. nigritta* (Banks), *M. juana* (Flint), and unassociated larvae illustrated by Botosaneanu & Flint (1982), from Venezuela and Ecuador; and by Pes et al. (2005), from Brazil.

Actually, main differences seem to be the shape and the material of larval cases. In this respect, larvae of *Metrichia bonita* **sp. nov.** are unusual and easily recognized by having its case made entirely of calcareous particles, without the typical algal elements (Fig. 8).

390 **Figure 8.** *Metrichia bonita* **sp. nov.**, larva: (A) type locality, Rio Formosinho, Bonito
391 municipality, Mato Grosso do Sul, Brazil; (B) larvae on calcareous substrate; (C) larva and its
392 calcareous case.

393 Paprocki et al. (2003) discussed the role of *Smicridea travertinera* Paprocki, Holzenthal
394 & Cressa in calcareous tufa formation (travertine). According with these authors, larvae of that
395 species interfere in the deposition and erosion of calcareous substrate, by their net-building
396 activities (Paprocki et al., 2003). Cyanobacteria and diatoms are known to participate in
397 travertine formation, but the role played by macroinvertebrates is poorly understood (Drysdale,
398 1998; 1999). It is possible that cases of *Metrichia bonita* **sp. nov.** are impregnated passively with
399 calcareous particles, but as commented by Drysdale (1999) for other aquatic insects, they could
400 be important in travertine biogenesis by producing new nucleation sites or eroding other ones.
401 *Metrichia bonita* **sp. nov.** is the only microcaddisfly known to inhabit (Fig. 8) and build cases
402 with calcareous tufa so far.

403 ***Metrichia bracui* sp. nov.**
404 urn:lsid:zoobank.org:act:07B44840-CAB0-4BBE-BD01-B62E885BE418
405 (Fig. 9)
406 **Adult male.** Length 1.8–2.2 mm (n=3). General color, in alcohol, light brown. Head with no
407 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial
408 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
409 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
410 wings. Abdomen without modifications. Ventromesal process on segment VII absent. Segment
411 VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum
412 subpentagonal in ventral view (Fig. 9A); in lateral view narrower anteriorly than posteriorly (Fig.
413 9C). Inferior appendage covered by long setae and with scale-like setae, subrectangular in ventral
414 view (Fig. 9A); in lateral view, rounded, apex rounded (Fig. 9C). Dorsal hook long, more than
415 half length of inferior appendage; in lateral view, downturned (Fig. 9C). Preanal appendage

elongate and bearing very long setae (Fig. 9B). Subgenital plate apparently absent. Tergum X membranous and with shallow U-shaped incision (Fig. 9B). Phallus tubular, elongate and slender, slightly constricted mesally; without spines, but with a sclerotized process arising from a subapical constriction; apex rounded and folded; ejaculatory duct sclerotized, straight and not protruding apically (Fig. 9D).

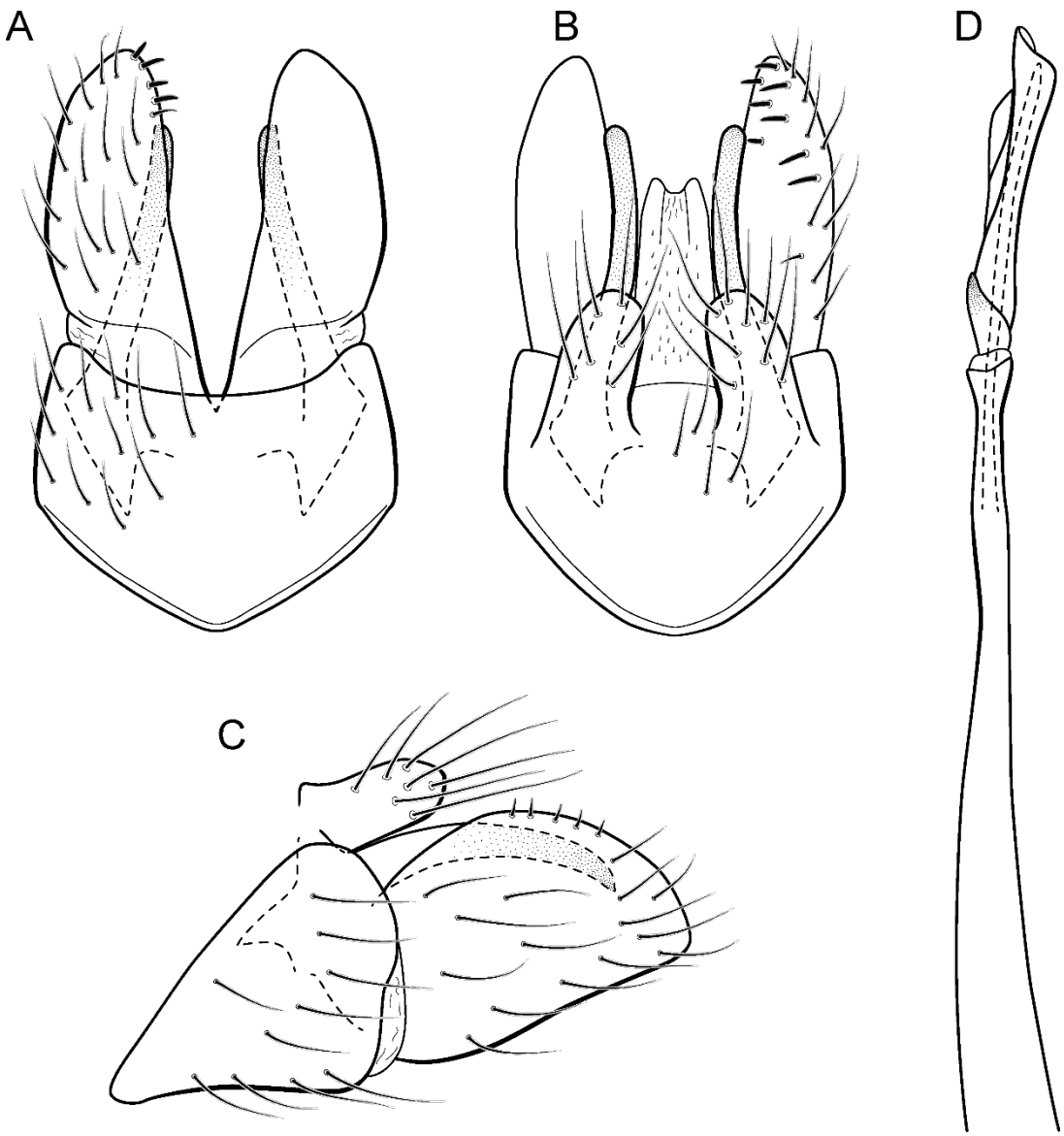
Holotype. BRAZIL: Rio de Janeiro: Angra dos Reis, Rio Bracuí, 23º00'23"S 44º29'15"W, el. 75 m, 10–11.v.2002, JL Nessimian col., light trap, male (DZRJ).

Paratypes. Same data as holotype, 2 males (MNRJ). **Rio de Janeiro:** Parque Nacional do Itatiaia, Córrego do Maromba, 22º25'32"S 44º37'03"W, el. 1250 m, 04.iv.15, APM Santos & DM Takiya cols., Malaise trap, 4 males (DZRJ).

Etymology. The species is named in allusion of the river where the holotype was collected.

Remarks. This new species can be assigned to the *patagonica* group because of the absence of curved spines of the phallus. The general aspect of the male genitalia resembles *M. patagonica* Flint, *M. pernambucana* Souza & Santos, and *M. pseudopatagonica* Bueno-Soria & Holzenthal. *Metrichia bracui* **sp. nov.** differs from these species and others in the group specially by the phallus bearing a sclerotized process on a constricted region.

The four COI sequences generated for *M. bracui* **sp. nov.** came from specimens collected in a locality at Parque Nacional do Itatiaia, Rio de Janeiro, Brazil. The highest pairwise intraspecific divergence between sequences was 0.4%, and the lowest interspecific divergence was 21.4% between *M. bracui* **sp. nov.** and *Angrisanoia cebollati*. Until now, there are no formal studies on relationships among species or genera included in Ochrotrichiinae. But to infer phylogenetic hypothesis for the entire subfamily is beyond the scope of this work, and also it is necessary much more data and taxa to generate robust hypotheses.



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

441 ***Metrichia caraca* sp. nov.**

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

443 (Fig. 10)

444 **Adult male.** Length 2.5–3.0 mm (n=7). General color, in alcohol, brown. Head with no
445 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdominal segment VI bearing brush of very long setae dorsolateraly; segment VII bearing brush of very long setae dorsolateraly. Ventromesal process on segment VII absent. Segment VIII shorter ventrally than dorsally and bearing brush of long setae dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subrectangular, with anterior margin rounded (Fig. 10A); in lateral view narrower anteriorly than posteriorly (Fig. 10C). Inferior appendage covered by long setae, subtrapezoidal in ventral view (Fig. 10A), apex oblique and projected mesad into a large process bearing a stout spine-like setae. Dorsal hook short and straight; in lateral view, truncate and broader apically (Fig. 10C). Preanal appendage short, truncate and bearing very long setae (Fig. 10B). Subgenital plate apparently absent. Tergum X membranous and truncate (Fig. 10B). Phallus tubular, elongate and slender, slightly constricted mesally; with two curved subapical spines, one short and another long; apex emarginate; ejaculatory duct sclerotized and not protruding apically (Fig. 10D).

Holotype. BRAZIL: Minas Gerais: Catas Altas, RPPN Santuário do Caraça, Ribeirão Caraça, 11–13.vi.2013, ML Monné & JP Botero cols., Malaise trap, male (DZRJ).

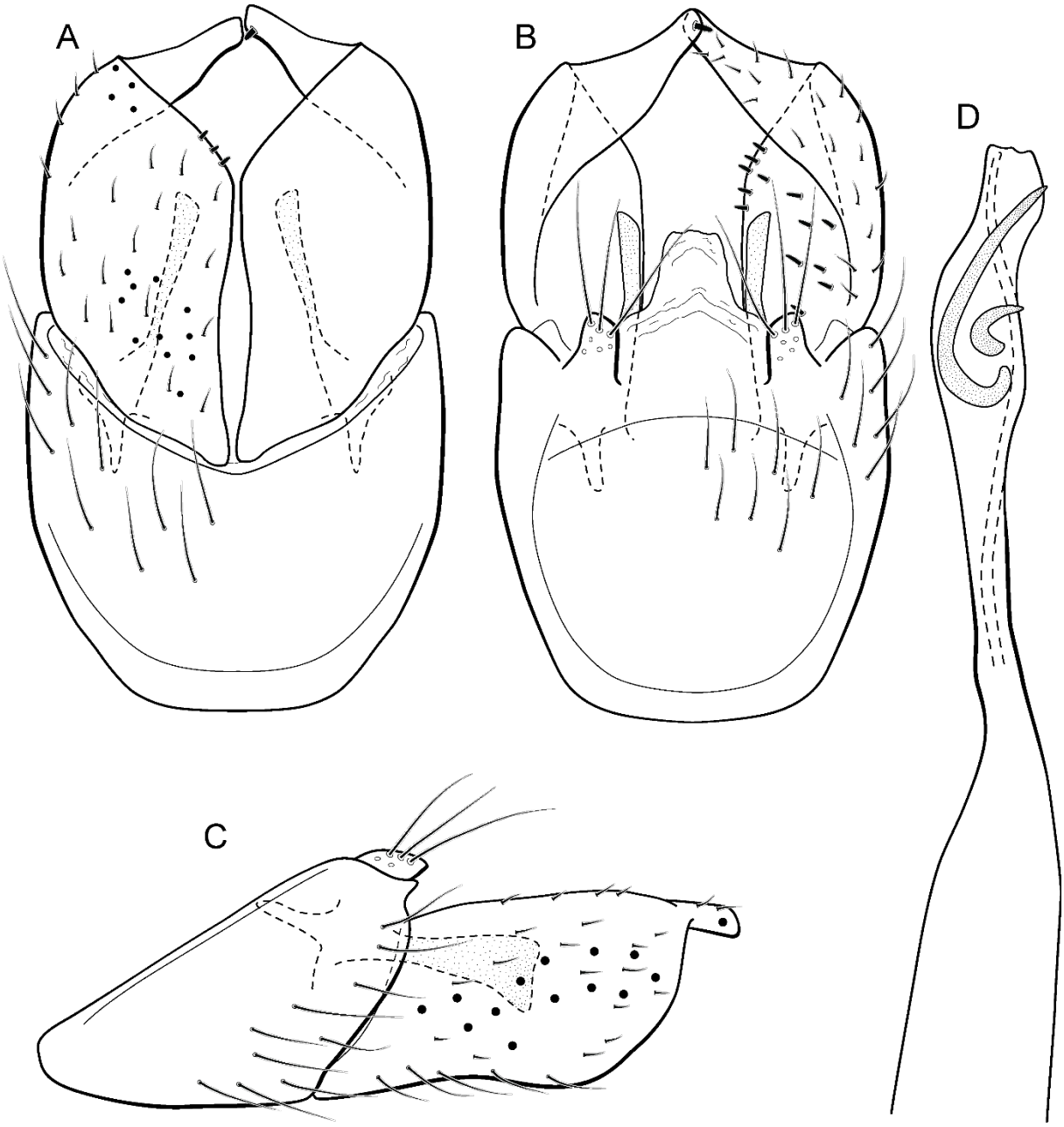
Paratypes. Same data as holotype, 3 male (DZRJ). **Minas Gerais:** São Roque de Minas, Parque Nacional da Serra da Canastra, Fazenda Velha, Córrego dos Pombos, 20º14’57”S 46º38’05”W, el. 997 m, 02.iv.2014, JL Nessimian, ALH Oliveira, LL Dumas & SP Gomes, light trap cols., 1 male (MNRJ).

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

Remarks. This new species has very distinctive male genitalia. Based on dorsoapically produced inferior appendages, this new species resembles *M. lenophora* (Flint). However, *M. caraca* **sp.**

nov. is easily recognized by the obliquely truncate and mesad directed process of inferior appendages and dorsal hook, in lateral view, with apex broad and truncate.

COI distances within this species reached only 1.1% and the lowest interspecific distance (21.7%) was found between specimens of *M. caraca* **sp. nov.** and *M. acuminata* **sp. nov.**, which are very distinct based on morphological features.



475 **Figure 10.** *Metrichia caraca* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)
476 lateral view; (D) phallus, dorsal view.

477 ***Metrichia circuliforme* sp. nov.**

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

479 (Fig. 11)

480 **Adult male.** Length 2.5–2.7 mm (n=4). General color, in alcohol, brown. Head with no
481 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial
482 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
483 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
484 wings. Abdominal segment VI bearing brush of very long setae dorsolateraly; segment VII
485 bearing a brush of very long setae dorsolateraly. Ventromesal process on segment VII present.
486 Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally;
487 sternum subpentagonal (Fig. 11A); in lateral view narrower anteriorly than posteriorly (Fig. 11C).
488 Inferior appendage short, covered by long setae, subrectangular in ventral view (Fig. 11A); in
489 lateral view, rounded (Fig. 11C), apex slightly truncate and bearing short spine-like setae. Dorsal
490 hook long, almost reaching the inferior appendage apex; in lateral view, downturned (Fig. 11C).
491 Preanal appendage elongate, but shorter than inferior appendage, and bearing very long setae
492 (Fig. 11B). Subgenital plate apparently absent. Tergum X membranous and truncate (Fig. 11B).
493 Phallus tubular, elongate and slender, slightly constricted mesally and with a median process;
494 with two short subapical spines; apex rounded and sclerotized; ejaculatory duct sclerotized,
495 straight and not protruding apically (Fig. 11D).
496 **Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, Rio das Pedras, Cachoeira de Deus, 22º25'00"S
497 44º32'50"W, el. 689 m, 06.iii.2008, JL Nessimian, LL Dumas & MR de Souza cols., light trap,
498 male (DZRJ).

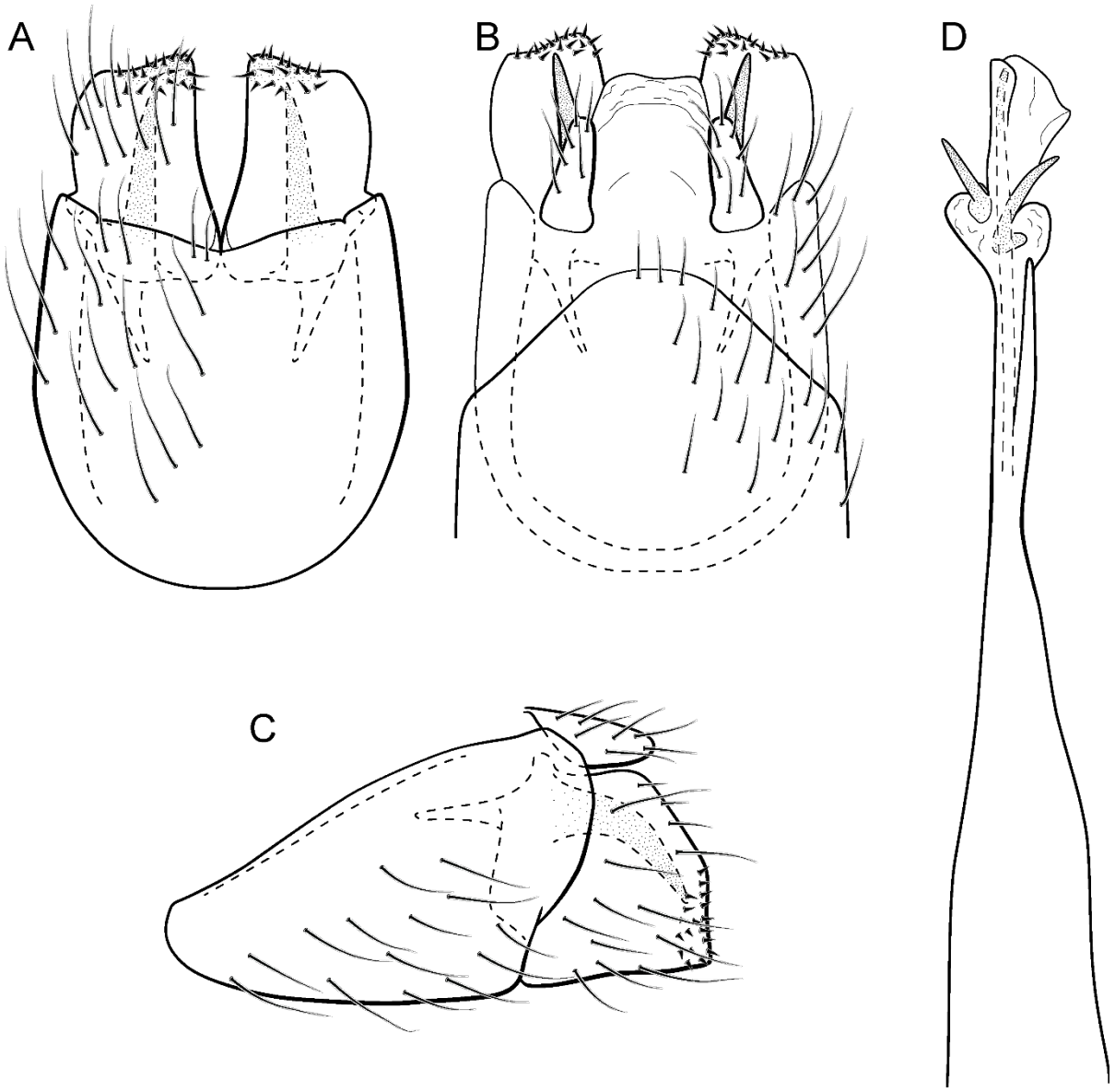
Paratypes. Same data as holotype, except Rio das Pedras, 22º24'33"S 44º33'08"W, el. 706 m, 06.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, 1 male (DZRJ), 1 male (MNRJ); Parque Nacional do Itatiaia, Córrego Simon, 22º26'16"S 44º36'20"W, el. 1033 m, 15.iv.07, LL Dumas, APM Santos, N Ferreira-Jr. & JL Nessimian cols., light trap, 1 male (DZRJ).

Etymology. From Latin, "circuli-" and "form" meaning "rounded shape"; in allusion to the rounded and simple inferior appendages.

Remarks. This species has a simple male genitalia and abdomen with only brushes of long setae on segments VI and VII. General aspect of the male genitalia is similar to *M. riva* (Bueno-Soria) and *M. quadrada* (Flint), particularly their inferior appendages short and subrectangular and phallus with two subapical spines. However, *M. circuliforme* **sp. nov.** can be easily distinguished from *M. quadrada* by the absence of internal sacs in the abdomen. It can be distinguished from *M. riva* by the elongate preanal appendages and phallus with subequal hook spines subapically.

We obtained seven COI sequences for *M. circuliforme* **sp. nov.** and, although all of them came from the same locality, intraspecific divergences were relatively higher, reaching 3.5%. Besides that, GMYC estimated two species for these sequences instead of one. Reanalysis of the morphology of these specimens did not reveal any conspicuous variation that could justify splitting in two taxonomic groups. Comparing to other studies using DNA barcodes of caddisflies, this genetic distance is still low, for example Pauls et al. (2010) found intraspecific divergences (K2P distance) up to 5.9% for Chilean *Smicridea*. Zhou et al. (2011) found even higher intraspecific distances among caddisflies, reaching up to 14%. GMYC is known to be more sensitive to geographic range coverage and/or other sampling schemes resulting in oversplitting (Lohse, 2009; Talavera et al., 2013). Therefore, we considered *M. circuliforme* **sp. nov.** as a robust species based on morphology as well as based on barcode divergences. Minimum interspecific COI distances of *M. circuliforme* **sp. nov.** to *M. curta* **sp. nov.** were 18.4%, and

523 again, these two species are very distinct based on morphological features and apparently are not
524 even closely related to each other (Fig. 2).



525 **Figure 11.** *Metrichia circuliforme* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)
526 lateral view; (D) phallus, dorsal view.

527 ***Metrichia curta* sp. nov.**

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

(Fig. 12)

Adult male. Length 2.4–2.5 mm (n=9). General color, in alcohol, light brown. Head with no modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdomen without modifications. Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subpentagonal (Fig. 12A); in lateral view, narrower anteriorly than posteriorly (Fig. 12C). Inferior appendage covered by long setae, subtrapezoidal in ventral view (Fig. 12A); in lateral view, subtriangular (Fig. 12C), apex rounded. Dorsal hook long, more than half length of inferior appendage; in lateral view, with apex slightly broader, downturned, and truncate (Fig. 12C). Preanal appendage elongate, but shorter than inferior appendage, and bearing very long setae (Fig. 12B). Subgenital plate apparently absent. Tergum X membranous and rounded (Fig. 12B). Phallus tubular, elongate and slender, slightly constricted mesally; with a stout subapical spine; apex rounded and folded; ejaculatory duct sclerotized, straight and protruding apically (Fig. 12D).

Holotype. BRAZIL: Rio de Janeiro: Itatiaia, Rio das Pedras, 22º24’33”S 44º33’08”W, el. 706 m, 06.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, male (DZRJ).

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

Etymology. The specific name is a reference to the very short inferior appendage, in Portuguese “curta” means “short”.

Remarks. Based on the absence of modifications on abdominal segments, this new species can be asigned to the *aberrans* group. This species has the phallus similar to *M. amplitudinis* Bueno-Soria & Hozenthal, with a long spine and an apical flap. The new species can be distinguished by the triangular inferior appendages in lateral view and phallus with a strongly curved spine

554 subapically. *Metrichia amplitudinis* and *M. curta* **sp. nov.** share the widened dorsal hook, but in
555 the new species this structure is only slightly wider and also truncate in lateral view. Maximum
556 intraspecific divergence of COI sequences was 1.5% for *M. curta* **sp. nov.** and the minimum
557 interspecific was to *M. circuliforme*, as mentioned above.

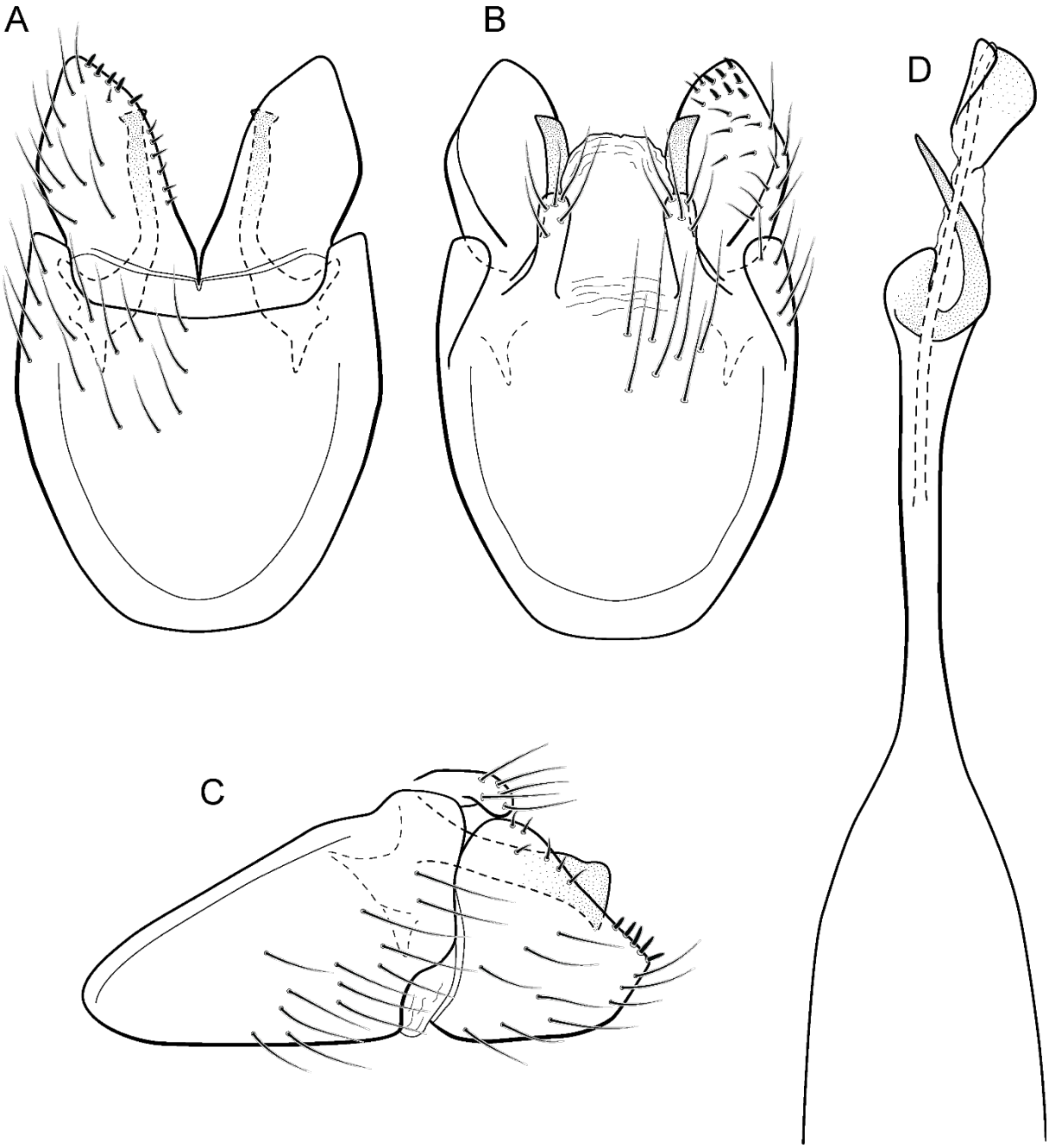


Figure 12. *Metrichia curta* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C) lateral view; (D) phallus, dorsal view.

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

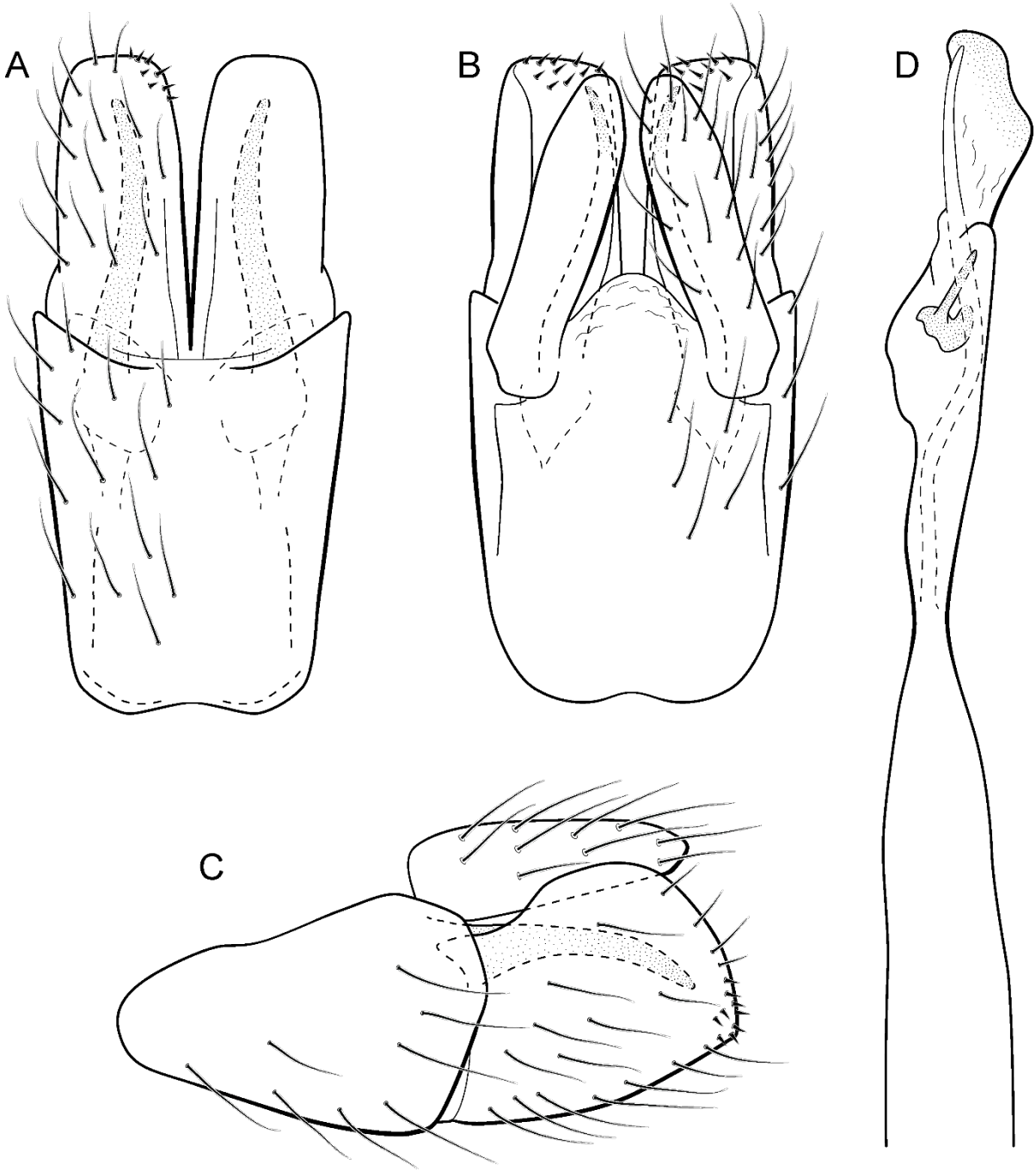
urn:lsid:zoobank.org:act:BC4FF095-32BE-46A9-BEAB-28854E2F5BC7

(Fig. 13)

Adult male. Length 1.8–2.1 mm (n=27). General color, in alcohol, light brown. Head with no modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdomen without modifications; segment VII bearing specialized setae dorsally. Ventromesal process on segment VII absent. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subrectangular (Fig. 13A); in lateral view narrower anteriorly than posteriorly (Fig. 13C). Inferior appendage covered by long setae, subrectangular in ventral view (Fig. 13A); in lateral view, subrectangular (Fig. 13C), apex slightly truncate and bearing short spine-like setae. Dorsal hook long, almost reaching the inferior appendage apex; in lateral view, downturned (Fig. 13C). Preanal appendage elongate, as long as inferior appendage, and bearing very long setae (Fig. 13B). Subgenital plate apparently absent. Tergum X membranous and rounded (Fig. 13B). Phallus tubular, elongate and slender, slightly constricted mesally; with a stout subapical spine; apex rounded and sclerotized; ejaculatory duct sclerotized, sinuous, and protruding apically (Fig. 13D).

Holotype. BRAZIL: Minas Gerais: Jaboticatubas, Parque Nacional da Serra do Cipó, Cachoeira da Farofa, 19º22’47”S 43º34’36”W, el. 811 m, 23.iv.2010, APM Santos & DM Takiya cols., manual, male (DZRJ).

581 **Paratypes.** Same data as holotype, 18 males (DZRJ), 8 males (MNRJ), 5 males (MZUFBA);
582 same data, except Ribeirão Mascates, 19º24’02”S 43º34’35”W, el. 820 m, 09–11.xii.2011, APM
583 Santos & DM Takiya cols., manual, 84 males (DZRJ).
584 **Etymology.** This new species is named in reference to the waterfall name, where specimens were
585 collected.



586 **Figure 13.** *Metrichia farofa* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)
587 lateral view; (D) phallus, dorsal view.

588 **Remarks**. Due to absence of pouches in abdominal segments, *Metrichia farofa* **sp. nov.** can be
589 also included in the *aberrans* group. However, the new species has only a subapical spine in
590 phallus, like those species included *exclamationis* group. The new species can be easily
591 distinguished from any *Metrichia* species by the very long preanal appendages, reaching the apex
592 of inferior appendages in dorsal and lateral views.

593 Although more than 10 specimens of *M. farofa* **sp. nov.** were submitted to DNA
594 extraction and many attempts of COI amplification via PCR were conducted, we were not able to
595 obtain sequences of this species, even using recent material, collected after 2013.

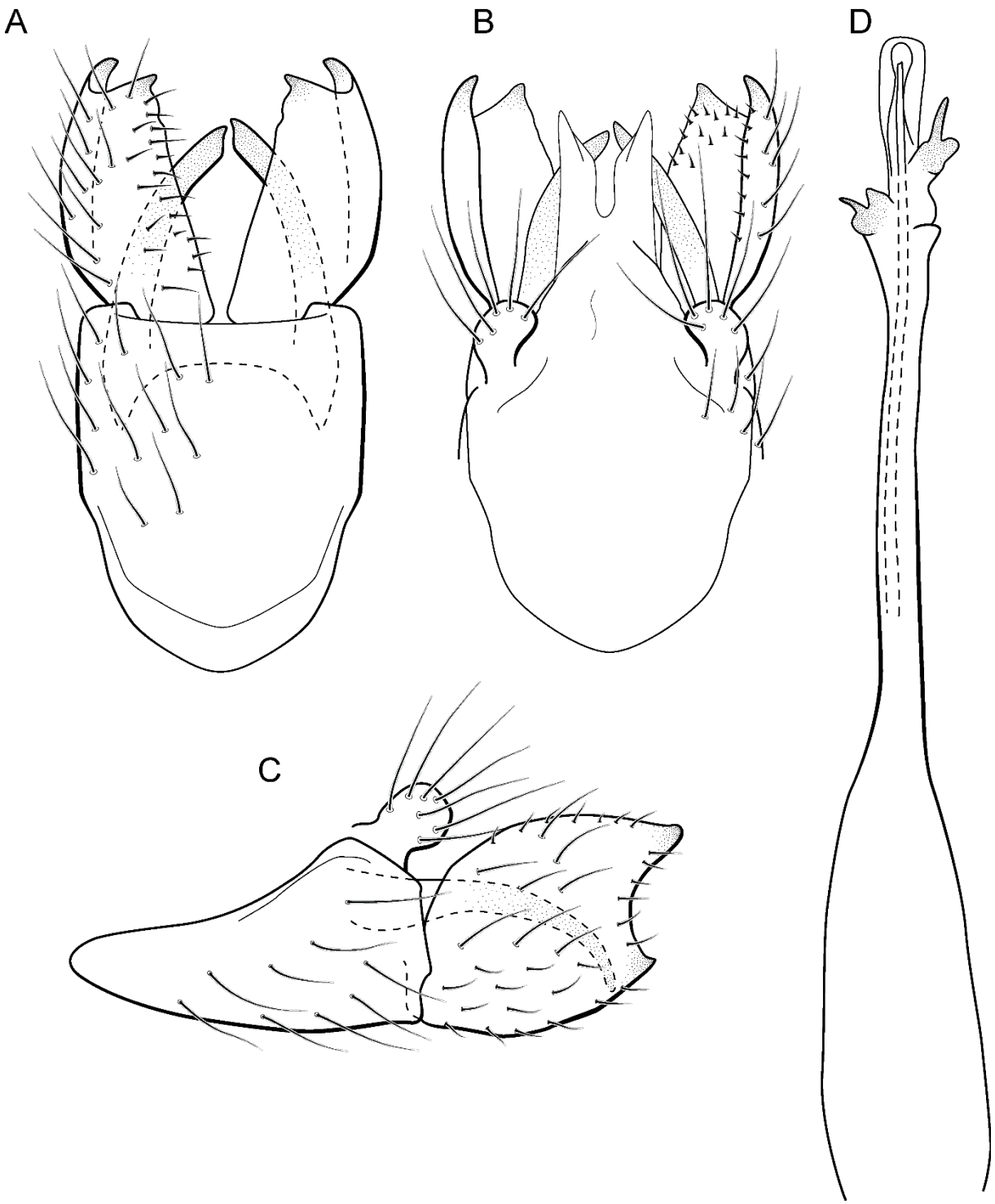
596 ***Metrichia forceps* sp. nov.**

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

598 (Fig. 14)

599 **Adult male**. Length 2.7–3.0 mm (n=2). General color, in alcohol, brown. Head with no
600 modifications. Ocelli 3. Antenna simple, 23-articulated. Maxillary palpus 5-articulated; labial
601 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
602 Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation reduced
603 in both wings. Abdominal segment VII bearing internal pouches in anterior area. Ventromesal
604 process on segment VII absent. Segment VIII shorter ventrally than dorsally. **Male genitalia**.
605 Segment IX reduced dorsally; sternum subpentagonal (Fig. 14A); in lateral view, narrower
606 anteriorly than posteriorly (Fig. 14C). Inferior appendage covered by long setae, subrectangular
607 in ventral view (Fig. 14A); in lateral view, with posterior margin excavated and with two acute
608 and sclerotized process (Fig. 14C). Dorsal hook long, almost reaching the inferior appendage

609 apex; in lateral view, downturned (Fig. 14C). Preanal appendage short, rounded and bearing very
610 long setae (Fig. 14B). Subgenital plate apparently absent. Tergum X sclerotized, deeply notched
611 mesally, forming lateral curved processes (Fig. 14B). Phallus tubular, elongate and slender,
612 slightly constricted mesally; with two short subapical spines; apex rounded and folded;
613 ejaculatory duct sclerotized, straight and protruding apically (Fig. 14D).



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

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

Holotype. BRAZIL: Paraná: Céu Azul, Parque Nacional do Iguaçu, Rio Azul, 25º09'21"S 53º47'44"W, el. 510 m, 6–8 ix.2012, APM Santos, DM Takiya, ALH Oliveira, GA Jardim & BHL Sampaio cols., Malaise trap, male (DZRJ).

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

Etymology. The name of this species is in reference to the dorsal hooks of the inferior appendages, which in ventral view resemble a forceps.

Remarks. This new species belongs to *campana* group, due to the internal pouches between segments VI and VII, reduced spines on subapical region of phallus, and the sclerotized and elongate tergum X. Within this group, *M. forceps* **sp. nov.** shares more similarities in male genitalia with *M. campana* Flint, *M. similis* Flint, and *M. continentalis* Flint, particularly by the inferior appendages with excavate posterior margin, forming two pointed processes, one ventral and another dorsal. This new species can be distinguished from the other by tergum X deeply notched; dorsal hook of inferior appendages elongate and downturned, and phallus apex bearing two small spines and a sclerotized flap surrounding the protruding ejaculatory duct.

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

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

(Fig. 15)

Adult male. Length 2.5–2.8 mm (n=2). General color, in alcohol, dark brown. Head with no modifications. Ocelli 3. Antenna simple, 20-articulated. Maxillary palpus 5-articulated; labial palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdominal segment VI with pair of internal pouches in posterodorsal area. Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subrectangular, with anterior margin rounded

(Fig. 15A); in lateral view narrower anteriorly than posteriorly (Fig. 15C). Inferior appendage with peg-like setae, subrectangular in ventral (Fig. 15A) and lateral (Fig. 15C) views; apex with acute corners. Dorsal hook long, almost reaching the inferior appendage apex; in lateral view, with apex slightly broader, almost straight, and truncate (Fig. 15C). Preanal appendage short, rounded and bearing very long setae (Fig. 15B). Subgenital plate apparently absent. Tergum X membranous and rounded (Fig. 15B). Phallus tubular, elongate and slender, slightly constricted mesally; with two curved subapical spines, one short and another one very long; apex rounded and sclerotized; ejaculatory duct sclerotized, sinuous, and not protruding apically (Fig. 15D).

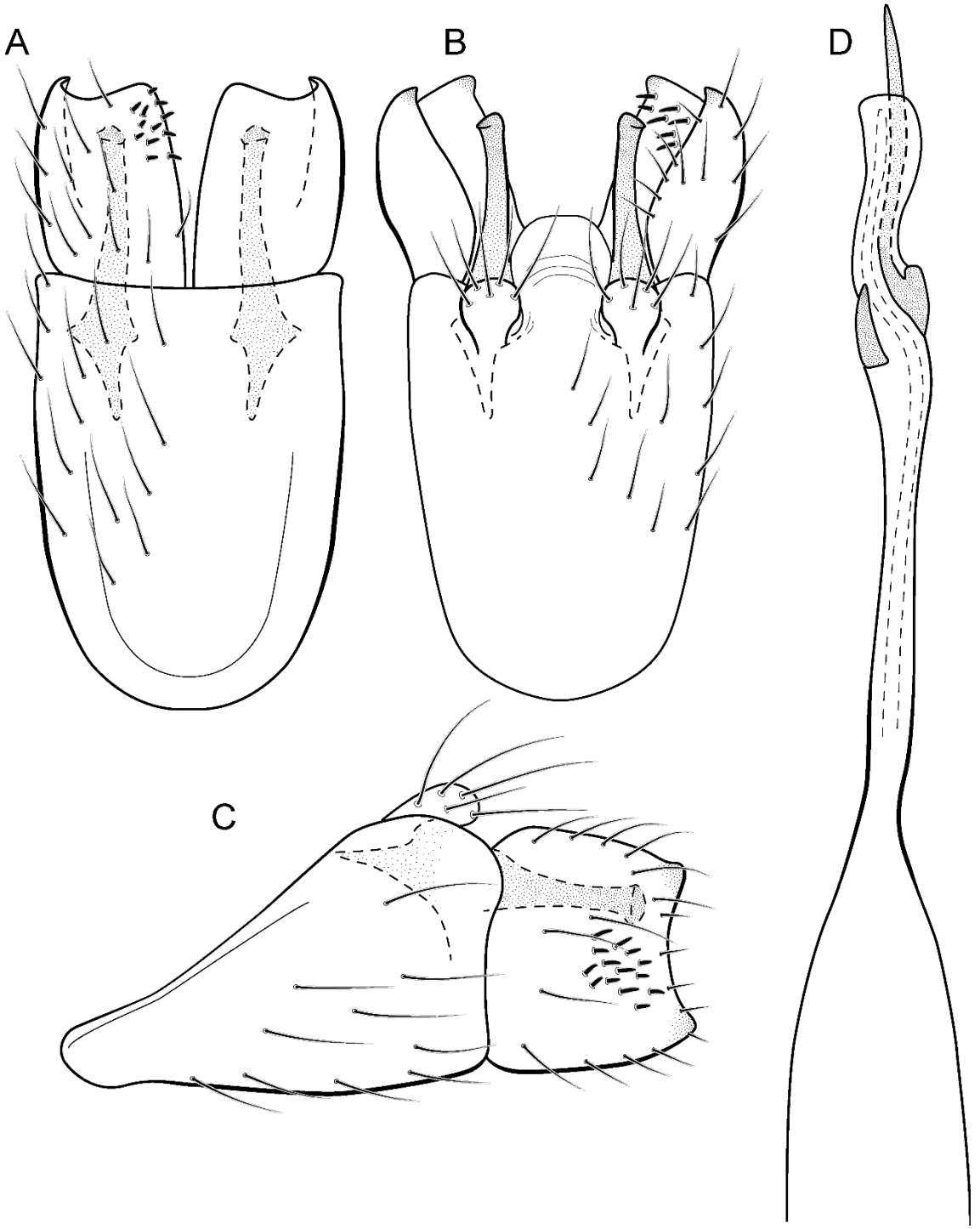
Holotype. BRAZIL: Mato Grosso do Sul: Bonito, Rio Formosinho, 21º10'16"S 56º26'47"W el. 275 m, 08–13.ix.2013, APM Santos & DM Takiya cols., Malaise trap, male (DZRJ).

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

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

Remarks. This new species appears to be a member of the *campana* group because of internal pouches between abdominal segments VI-VII and phallus with two subapical spines. General aspect of the male genitalia of *Metrichia formosinha* **sp. nov.** is similar to *M. forceps* **sp. nov.**, particularly the inferior appendages with acute corners. However, *M. formosinha* **sp. nov.** differs from it by the dorsal hook almost straight and capitate (strongly curved and acute in *M. forceps* **sp. nov.** and in the other species of *campana* group); and by phallus with a very long spine on the subapical area.

COI sequences of *M. formosinha* **sp. nov.** showed intraspecific divergences up to 0.8% and minimum interspecific divergences of 24.9% when compared with *M. talhada* **sp. nov.**



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

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

663 ***Metrichia goiana* sp. nov.**

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

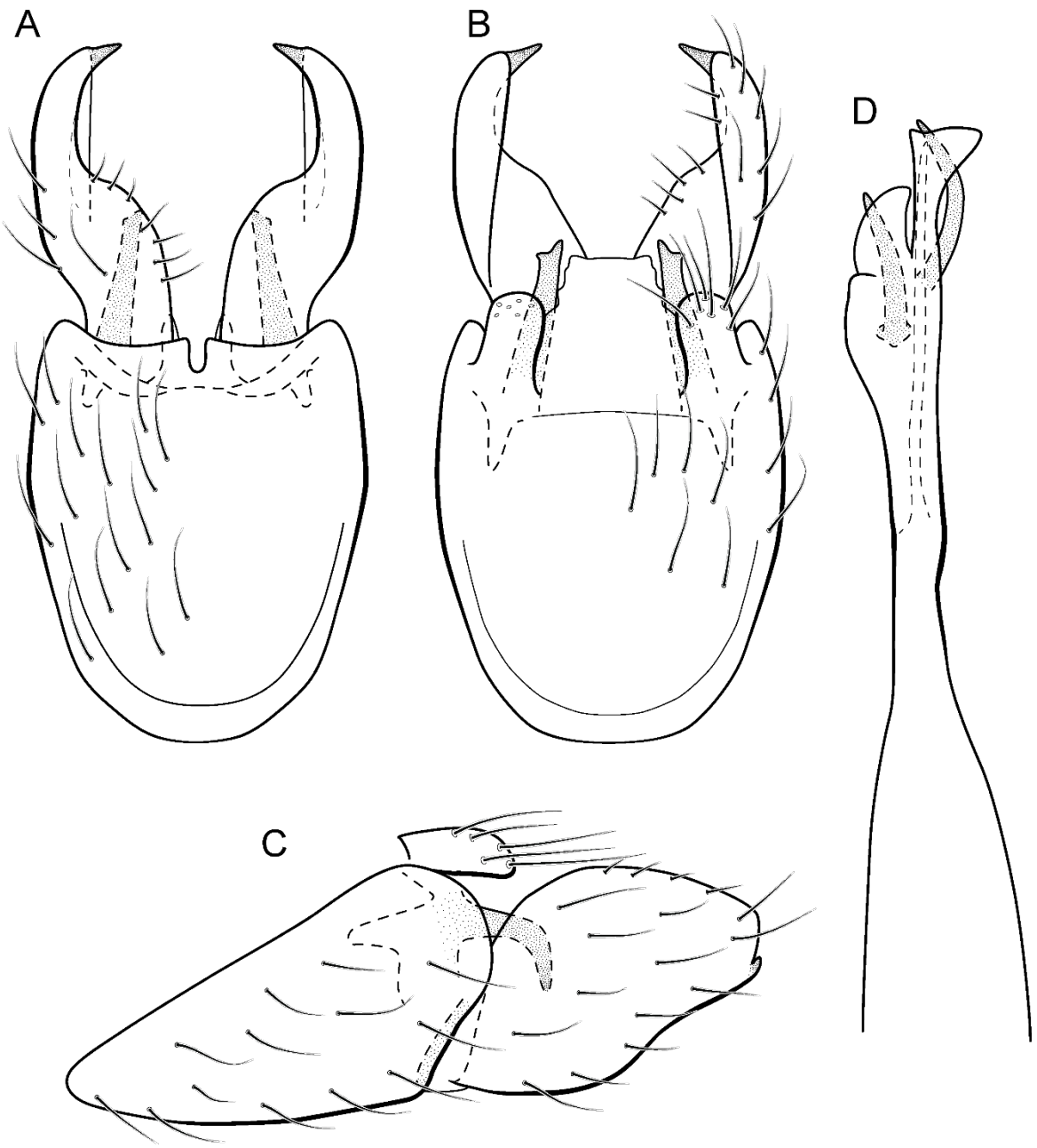
665 (Fig. 16)

666 **Adult male**. Length 1.8–2.0 mm (n=6). General color, in alcohol, dark brown. Head with no
667 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated, article IV
668 broad and darkened; labial palpus 3-articulated. Mesoscutellum with transverse suture.
669 Metascutellum subtriangular. Anterior femur with small acute apical process. Tibial spur formula
670 1-3-4. Wing venation reduced in both wings. Abdominal segment V with pair of internal pouches
671 and pair of dorsolateral brushes; segment VI with a transverse sclerotized plate posteriorly on
672 dorsum; segment VII bearing specialized setae dorsally. Ventromesal process on segment VII
673 absent. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced
674 dorsally; sternum subrectangular, with anterior margin rounded (Fig. 16A); in lateral view
675 narrower anteriorly than posteriorly (Fig. 16C). Inferior appendage covered by long setae,
676 elongate and narrow in ventral view (Fig. 16A); in lateral view, rounded (Fig. 16A), apex
677 rounded and bearing a tooth-like projection (Fig. 16A). Dorsal hook short, less than half length of
678 inferior appendage; in lateral view, downturned (Fig. 16A). Preanal appendage short and bearing
679 very long setae (Fig. 16B). Subgenital plate apparently absent. Tergum X membranous and
680 truncate (Fig. 16B). Phallus tubular, elongate and slender, slightly constricted mesally; with two
681 long, curved, subapical spines; apex ending into two sclerotized and keel shaped processes;
682 ejaculatory duct sclerotized, straight and not protruding apically (Fig. 16D).

683 **Holotype. BRAZIL: Goiás:** Alto Paraíso de Goiás, afl. Rio Bartolomeu, 14º07’25”S
684 47º30’30”W, el. 1165 m, 22–25.iii.2013, APM Santos & DM Takiya cols., Malaise trap, male
685 (DZRJ).

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

687 **Etymology.** The species is named in reference to Goiás State. “Goiana” is a gentile adjective in
688 Portuguese for people from Goiás.
689 **Remarks**. This is another member of *nigritta* group. Male genitalia of this species is similar to
690 *M*. *potosina* Bueno-Soria and *M*. *ubajara* **sp. nov.**, due to rounded and elongate inferior
691 appendage in lateral view. This new species differs from *M*. *ubajara* **sp. nov.** by the presence of
692 an apical tooth on inferior appendages, also present in *M. potosina*. *Metrichia goiana* **sp. nov.** can
693 be distinguished from *M*. *potosina* by the two long subapical spines on phallus, whereas *M*.
694 *potosina* has three.



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

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

697 ***Metrichia itabaiana* sp. nov.**

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

699 (Fig. 17, Fig. 26B)

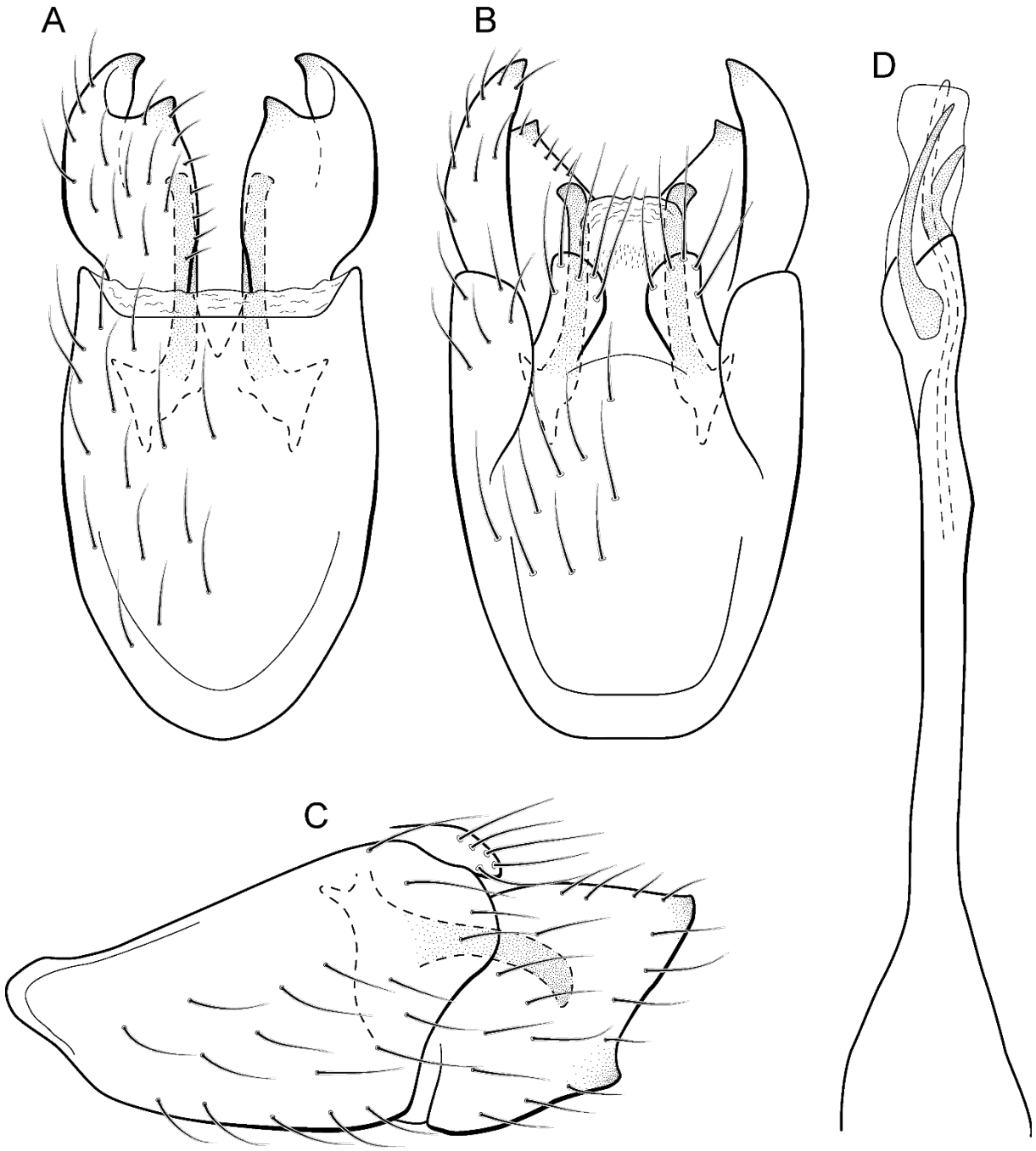
Adult male. Length 1.8–2.1 mm (n=3). General color, in alcohol, brown. Head with no modifications. Ocelli 3. Antenna simple, 20-articulated. Maxillary palpus 5-articulated; labial palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdominal segment V ventrally with a mesal brush of long setae; segment VI with tergum as a sclerotized triangular plate surrounded by specialized setae (Fig. 26B), internally with pair of internal pouches; segment VII bearing specialized setae ventrally and dorsally (Fig. 26B). Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally and bearing a brush of long setae dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subpentagonal (Fig. 17A); in lateral view narrower anteriorly than posteriorly (Fig. 17C). Inferior appendage short, covered by long setae (Fig. 17A); in lateral view, subtrapezoidal (Fig. 17C), apex excavated and with two acute and sclerotized process (Figs. 17A, 17B). Dorsal hook short, almost half length of inferior appendage; in lateral view, slightly downturned (Fig. 17C). Preanal appendage elongate, but shorter than inferior appendage, and bearing very long setae (Fig. 17B). Subgenital plate apparently absent. Tergum X membranous and truncate (Fig. 17B). Phallus tubular, elongate and slender, slightly constricted mesally; with two long, curved, subapical spines; apex truncate and slightly sclerotized; ejaculatory duct sclerotized, sinuous, and protruding apically (Fig. 17D).

Holotype. Sergipe: Areia Branca, Parque Nacional da Serra de Itabaiana, Rio dos Negros, 10º44’51”S 37º20’24”W, el. 208 m, 17.vi.2014, APM Santos, DM Takiya & WRM Souza cols., light trap, male (DZRJ).

Paratypes. Same data as holotype, 1 male (DZRJ), 1 male (MZUFBA); same data, except Riacho Água Fria, 10º45’17”S 37º20’32”W, el. 196 m, 17–19.vi.2014, APM Santos, DM Takiya, WRM Souza cols., Malaise trap, 2 males (MNRJ). **Goiás:** Alto Paraíso, afl. Rio Bartolomeu,

724 14º07’25”S 47º30’30”W, el. 1165 m, 22–25.iii.2013, APM Santos & DM Takiya cols., Malaise

725 trap, 1 male (DZRJ).



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

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

728 **Etymology.** This species is named in reference to Serra de Itabaiana, Sergipe, where the holotype
729 was collected.
730 **Remarks**. This new species appears to be a member of the *campana* group because of internal
731 pouches between abdominal segments VI and VII and pair of long subapical spines on phallus,
732 but this species lacks the acute process on mesal area of phallus. Male genitalia of *M. itabaiana*
733 **sp. nov.** resembles those of *M. campana* Flint and *M. vulgaris* **sp. nov.**, particularly, the
734 excavated inferior appendages, with acute and darkened corners. However, this new species can
735 be recognized by the very long curved subapical spines on phallus and the subtrapezoidal aspect
736 of inferior appendages in lateral view.
737 Only two COI sequences were obtained for *M. itabaiana* **sp. nov.**, one from Sergipe
738 (Northeastern Brazil) and another from Goiás (Midwestern Brazil). The COI divergence between
739 these two samples was 1.9% and minimum interspecific distance was 19.4% in relation to *M.*
740 *rafaeli* **sp. nov.**, which belongs to a different species group based on morphological 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

narrower anteriorly than posteriorly (Fig. 18C). Inferior appendage bearing scale-like setae, very elongate; in ventral view, curved inward apically (Fig. 18A); in lateral view, tapering to a rounded apex (Fig. 18C). Dorsal hook short and straight; in lateral view, slightly downturned (Fig. 18C). Preanal appendage elongate, but shorter than half length of inferior appendage, and bearing stout and striate setae (Fig. 18B). Subgenital plate apparently absent. Tergum X membranous and truncate (Fig. 18B). Phallus tubular, elongate and slender, slightly constricted mesally, with a median process; with two long, curved, subapical spines; apex rounded and sclerotized; ejaculatory duct sclerotized and protruding apically (Fig. 18D).

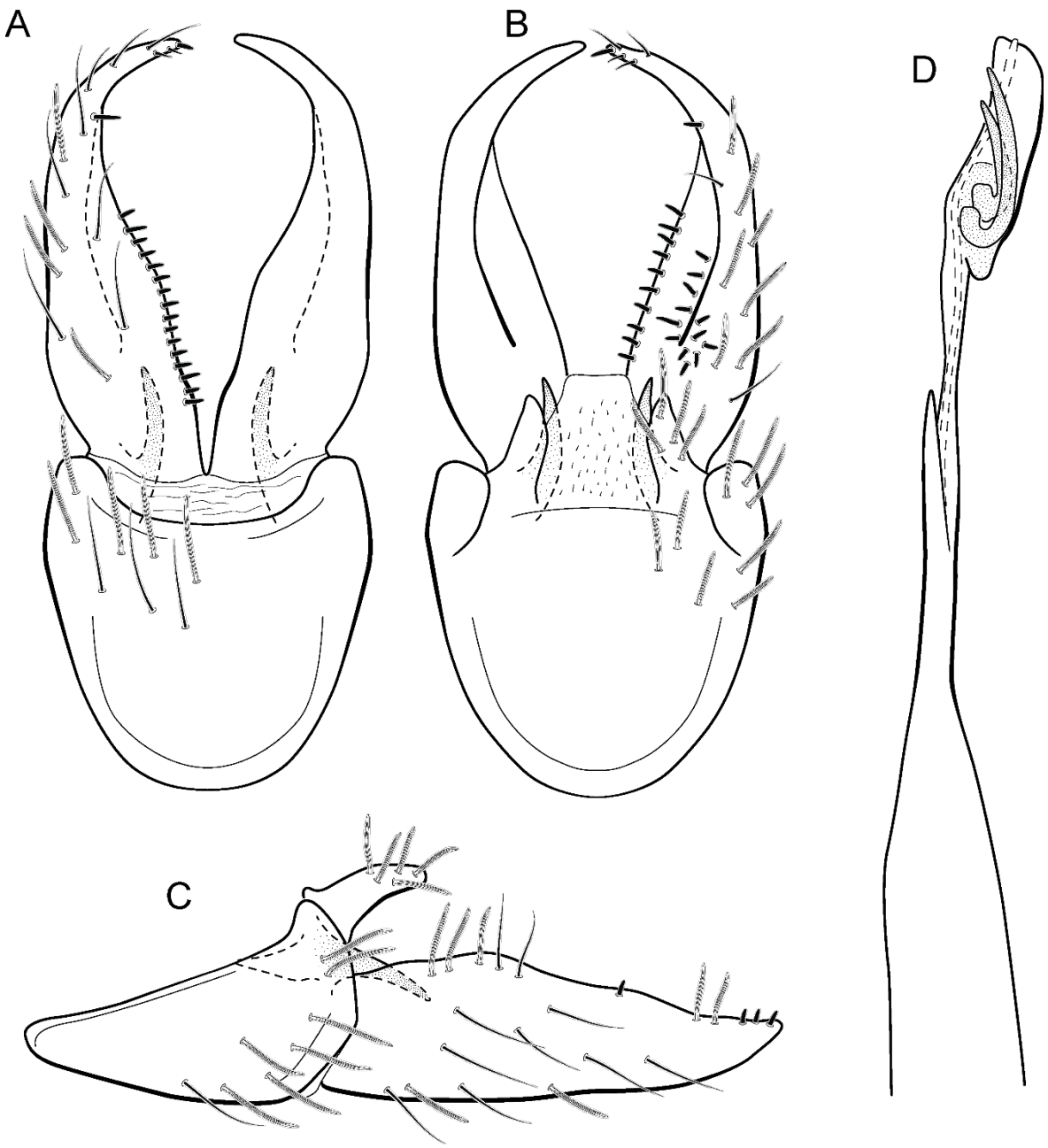
Holotype. BRAZIL: Rio de Janeiro: Itatiaia, Rio Palmital, 22º25'34"S 44º32'52"W, el. 637 m, 07.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, male (DZRJ).

Paratype. Brazil: Rio de Janeiro: Teresópolis, Parque Nacional da Serra dos Órgãos, Rio Paquequer, 22º27'25"S 42º59'52"W, el. 1100 m, 15–18.ix.2011, APM Santos, DM Takiya, BM Vasconcelos & RA Carvalho cols., Malaise trap, 1 male (MNRJ).

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

Remarks. Based on male genitalia, this species is more similar to *M. sesquipedalis* Bueno-Soria & Holzenthal. Both species have very long inferior appendages with very short dorsal hook. The new species is easily distinguished from *M. sesquipedalis* by their internal pouches in segment VI of the male abdomen and phallus with only two subapical spines (three in *M. sesquipedalis*).

Two COI sequences were generated for *M. longissima* **sp. nov.** and, in spite of exclusive occurrence in Rio de Janeiro State, one specimen from Itatiaia and the other from Teresópolis, these localities are in distinct mountain ranges, respectively, Serra da Mantiqueira and Serra do Mar. Distance between sequences of *M. longissima* **sp. nov.** was 0.4%. The minimum interspecific distance was related to *M. itabaiana* **sp. nov.**, with 21.5% of divergence.



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

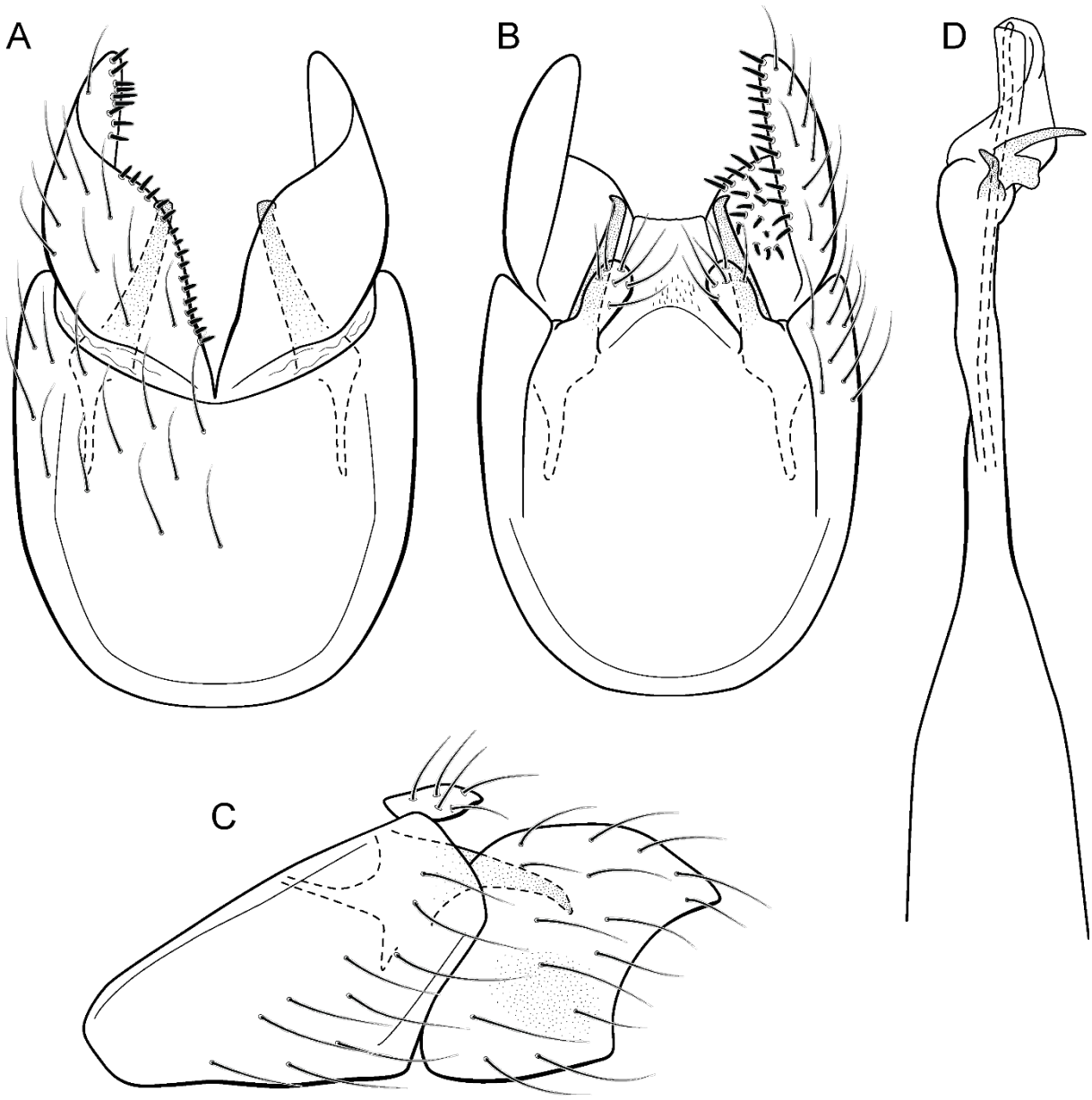
777 lateral view; (D) phallus, dorsal view.

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 dorsal area of male
803 abdomen. From Portuguese, “peluda” means “hairy”.



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

806 **Remarks**. Males with modifications on abdominal segments V, VI, and VII suggest that this new
807 species belongs to the *campana* group. The general aspect of inferior appendages is somewhat
808 similar to *M. forceps* **sp. nov.** or *M. formosinha* **sp. nov.**, which are excavate posteriorly. But *M.*
809 *peluda* is readily identified by the dense brushes of setae on the dorsum of abdominal segments
810 V, VI, and VII. Besides, the male genitalia of this new species differs from those described for *M.*

forceps **sp. nov.** and *M. formosinha* **sp. nov.** by the rounded corners of inferior appendages instead of acute and by phallus with two subapical spines with different sizes from each other.

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

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

(Fig. 20)

Adult male. Length 2.0–2.5 mm (n=7). General color, in alcohol, dark brown. Head with no modifications. Ocelli 3. Antenna simple, 20-articulated. Maxillary palpus 5-articulated; labial palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdominal segment V with pair of internal pouches; segment VI with pair of internal pouches and pair of lateral external sacs with specialized setae; segment VII bearing specialized setae dorsally. Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subrectangular, with anterior margin rounded (Fig. 20A); in lateral view narrower anteriorly than posteriorly (Fig. 20C). Inferior appendage covered by long setae, short and rounded, with apex slightly excavated (Fig. 20A); in lateral view, rounded (Fig. 20C). Dorsal hook long, more than half length of inferior appendage; in lateral view, downturned (Fig. 20C). Preanal appendage elongate, but shorter than half length of inferior appendage, and bearing stout and striate setae (Fig. 20B). Subgenital plate apparently absent. Tergum X membranous and truncate (Fig. 20B). Phallus tubular, elongate and slender, slightly constricted mesally, with a median process; with two long, curved, subapical spines, and a membranous lobe; apex rounded and sclerotized; ejaculatory duct sclerotized, sinuous, and not protruding apically (Fig. 20D).

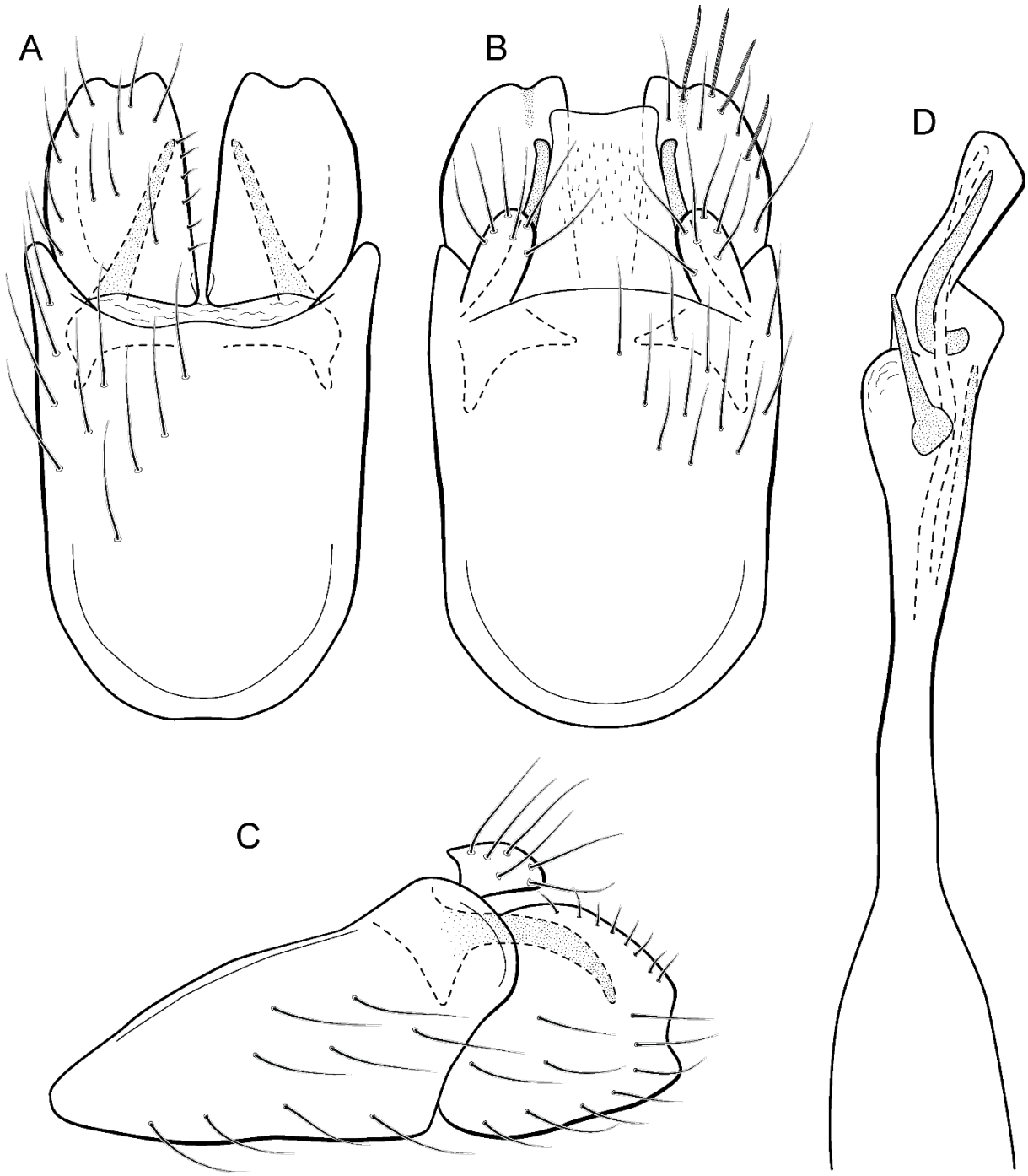
Holotype male. BRAZIL: Ceará: Ubajara, Parque Nacional de Ubajara, Rio das Minas, 03º50'03"S 40º54'18"W, el. 524, 17–18.ii.2013, DM Takiya, JA Rafael, RR Cavichioli & APM Santos cols., Malaise trap (CZMA).

Paratypes. Same data as holotype, 1 male (MZUFBA); same data, except Rio das Minas, 03º49'58"S 40º53'53"W, el. 420 m, 20–23.iv.2012, F Limeira-de-Oliveira et al. cols., Malaise trap, 1 male (CZMA); same data, except 14–16.ii.2013, DM Takiya, JA Rafael, RR Cavichioli & APM Santos cols., 1 male (DZRJ).

Etymology. This species is named in honor to the Brazilian entomologist Dr. José Albertino Rafael (INPA), who has collected a lot of interesting caddisflies, including some species described here.

Remarks. This new species belongs to *nigritta* group due to the internal pouches between abdominal segments V and VI, and due to the long and acute process on phallus. Male genitalia of *M. rafaeli* **sp. nov.** is more similar to *M. magna* Bueno-Soria & Holzenthal, in respect to short and simple inferior appendages. This new species can be easily distinguished from that and other species in this group by the posterior margin of inferior appendages slightly excavated, and by very long spines in subapical region of phallus.

Although the specimens with barcode sequences of *M. rafaeli* **sp. nov.** came from the same locality, they were not identical, with intraspecific divergence of 0.4%. The lowest 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

(Fig. 21, Fig. 26E)

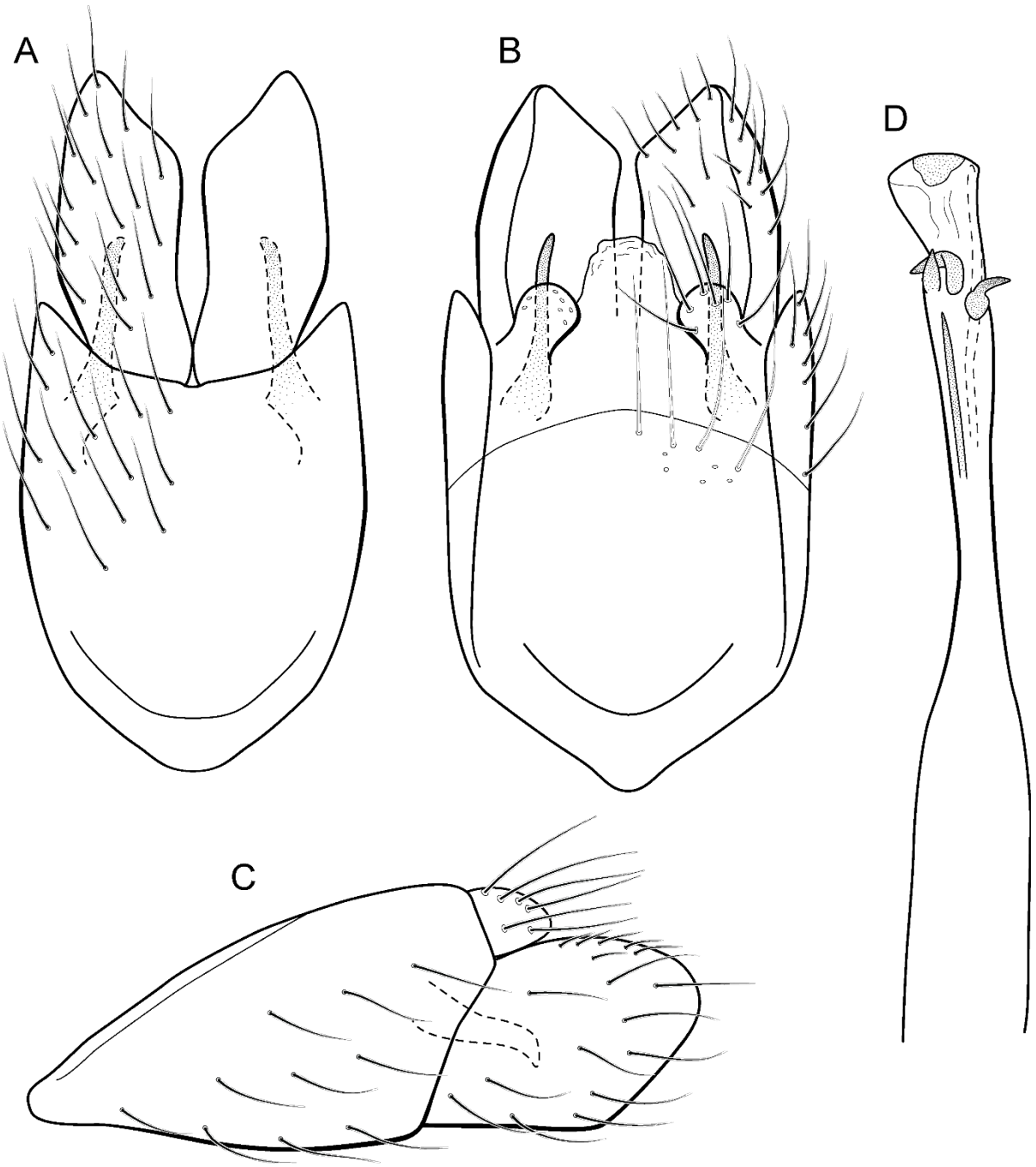
Adult male. Length 2.1–2.2 mm (n=2). General color, in alcohol, brown. Head with no modifications. Ocelli 3. Antenna simple, 19-articulated. Maxillary palpus 5-articulated; labial palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdominal segment IV with dorsal area expanded posteriorly bearing stout setae; segment VI bearing very long setae laterally, with a brush of short setae covered dorsaly by a triangular plate (Fig. 26E); segment VII with a brush of short setae dorsaly (Fig. 26E). Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subpentagonal (Fig. 21A); in lateral view narrower anteriorly than posteriorly (Fig. 21C). Inferior appendage covered by long setae, with apex obliquely truncate; subtrapezoidal in ventral view (Fig. 21A); in lateral view, subtrapezoidal (Fig. 21C). Dorsal hook short, almost half length of inferior appendage; in lateral view, slightly downturned (Fig. 21C). Preanal appendage short, rounded and bearing very long setae (Fig. 21B). Subgenital plate apparently absent. Tergum X membranous and rounded (Fig. 21B). Phallus tubular, elongate and slender, slightly constricted mesally; with four subapical spines, three short and one long and straight; apex rounded with a small sclerite; ejaculatory duct sclerotized and not protruding apically (Fig. 21D).

Holotype. BRAZIL: Paraná: Céu Azul, Parque Nacional do Iguaçu, Rio Azul, 25º09’21”S 53º47’44”W, el. 510 m, 6–8 ix.2012, APM Santos, DM Takiya, ALH Oliveira, GA Jardim & BHL Sampaio cols., Malaise trap, male (DZRJ).

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

Etymology. This species is named in reference to the simple aspect of male genitalia and abdomen, without the modifications and processes seen in other *Metrichia* species.

880 **Remarks**. This species can be assigned to the *campana* group due to the pouches in abdominal
881 segments VI and VII. The new species shares the general aspect of the genitalia with *M*.
882 *quadrata* Flint, particularly, a quadrangular or rectangular shape of inferior appendages and the
883 two short subapical spines on phallus. *Metrichia simples* **sp. nov.** can be recognized by the short
884 dorsal hook, reaching only the mesal area of inferior appendage (almost the same size of inferior
885 appendages in *M*. *quadrata*), obliquely truncate apex of the inferior appendages, and strongly
886 curved spines on phallus.



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

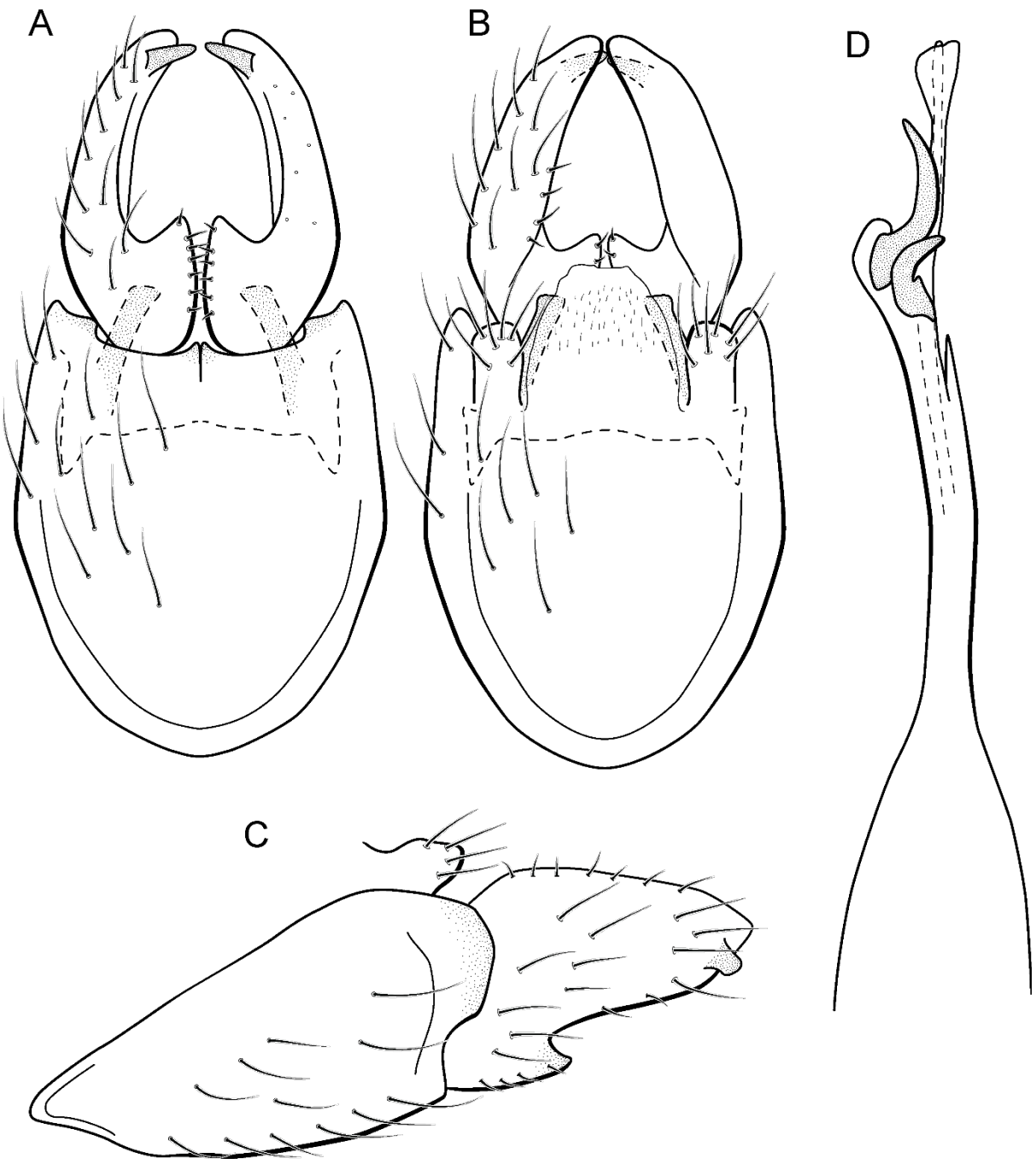
888 lateral view; (D) phallus, dorsal view.

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-shapped 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 the type locality, the Reserva Biológica de
914 Pedra Talhada.



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 due to internal pouches between
918 abdominal segments V and VI, phallus with two subapical curved spines and an acute process
919 near mesal area. *Metrichia talhada* **sp. nov.** shares the apical tooth on inferior appendages with

M. potosina Bueno-Soria, *M. goiana* **sp. nov.**, and *Metrichia tere* **sp. nov.** From *M. potosina* and *M. goiana* **sp. nov.**, it is easily distinguished by the shape of the inferior appendages, with a deep C-shaped notch on ventral margin and subapical tooth stout and slightly truncate in lateral view, and subapical spines on phallus, one long and another short. Although the male genitalia of *M. talhada* **sp. nov.** is very similar to *M. tere* **sp. nov.**, these two species can be separated by the tooth on inferior appendages, slightly truncate and subapical in the former, acute and apical in the later.

Sequences of *M. talhada* **sp. nov.** showed 0.0% of divergence. Morphological similarity between this species and its sister (*M. tere* **sp. nov.**) reflects the low genetic divergence of 12.6%, the lowest interspecific distance found in our sampling. Besides minor differences in genital structures and all molecular analyses with COI sequences (NJ, ABGD, GMYC) corroborate the distinction between *M. talhada* **sp. nov.** and *M. tere* **sp. nov.**, which are formally described here as different species.

***Metrichia tere* sp. nov.**

urn:lsid:zoobank.org:act:21376F9C-6308-47BA-ADB8-A42848AD8FB5

(Fig. 23)

Adult male. Length 1.9–2.1 mm (n=10). General color, in alcohol, brown. Head with no modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdominal segment V with pair of internal pouches and pair of dorsolateral brushes; segment VI with dorsolateral brushes of long setae; segment VII bearing specialized setae dorsally. Ventromesal process on segment VII absent. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subpentagonal (Fig. 23A); in

lateral view, narrower anteriorly than posteriorly (Fig. 23C). Inferior appendage elongate, apex with an acute projection; with a deep C-shaped notch in ventral view (Fig. 23A); in lateral view, with an acute projection (Fig. 23C). Dorsal hook short, less than half length of inferior appendage; in lateral view, downturned (Fig. 23C). Preanal appendage short, rounded and bearing very long setae (Fig. 23B). Subgenital plate apparently absent. Tergum X membranous and truncate (Fig. 23B). Phallus tubular, elongate and slender, slightly constricted mesally, with a median process; with two curved subapical spines, one short and another long; apex rounded and sclerotized; ejaculatory duct sclerotized, straight and protruding apically (Fig. 23D).

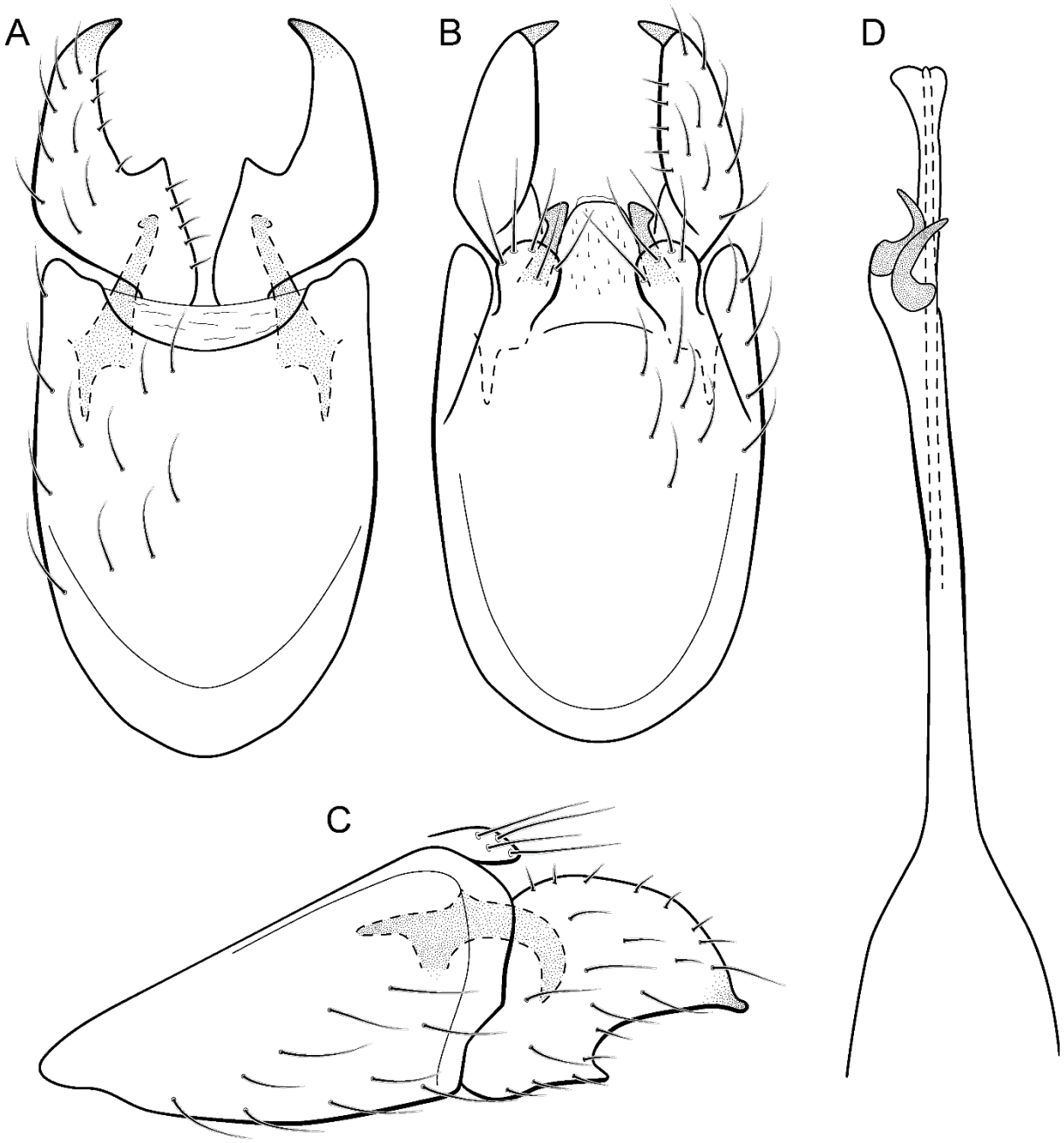
Holotype. BRAZIL: Rio de Janeiro: Teresópolis, Parque Nacional da Serra dos Órgãos, Rio Paquequer, 22º27'25"S 42º59'52"W, el. 1100 m, 15–18.ix.2011, APM Santos, DM Takiya, BM Vasconcelos & RA Carvalho cols., Malaise trap, male (DZRJ).

Paratypes. Same data as holotype, 9 males (MNRJ), 19 males (DZRJ).

Etymology. This species is named in reference to the city of Teresópolis, affectionately called as "Terê". The city was named in honor of Teresa Cristina (Teresópolis = "City of Teresa"), who was Empress, wife of Dom Pedro II, of Brazil (1831-1889).

Remarks. This new species is very similar to the preceding one, also belonging to the *nigritta* group. *Metrichia tere* **sp. nov.** can be distinguished from *M. talhada* **sp. nov.** by the inferior appendages with an apical acute projection, whereas in *M. talhada* **sp. nov.** the inferior appendages have a subapical tooth, which is more truncate than acute. Besides, the localities, where these two species occur, are very distant from each other, almost two thousands of kilometers.

Molecular data agrees with this morphological distinction of *M. tere* **sp. nov.** as commented above. Intraspecific variation among specimens sampled (n=3) of this species was 0.0%, and as mentioned in previous description, *M. talhada* **sp. nov.** and *M. tere* **sp. nov.** showed the lowest interspecific distance (12.6%).



969 **Figure 23.** *Metrichia tere* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C) lateral

970 view; (D) phallus, dorsal view.

971 ***Metrichia ubajara* sp. nov.**

972 urn:lsid:zoobank.org:act:68FD2A24-BC85-42A3-9007-96A42657DAE3

(Fig. 24)

Adult male. Length 2.0–2.7 mm (n=12). General color, in alcohol, brown. Head with no modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdominal segment V with pair of internal pouches and median internal plate in posterior region; with specialized setae on dorsum; segment VI with pair of internal pouches in posterodorsal area. Ventromesal process on segment VII absent. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subrectangular (Fig. 24A); in lateral view, narrower anteriorly than posteriorly (Fig. 24C). Inferior appendage covered by long setae, apex rounded; elongate and narrow in ventral view (Fig. 24A); in lateral view, rounded (Fig. 24C). Dorsal hook short, less than half length of inferior appendage; apex downturned; basally with a wide and sclerotized projection; in lateral view, C-shaped (Fig. 24C). Preanal appendage elongate, but shorter than half length of inferior appendage, and bearing stout and striate setae (Fig. 24B). Subgenital plate apparently absent. Tergum X membranous and truncate (Fig. 24B). Phallus tubular, elongate and slender, slightly constricted mesally; with two long, curved, subapical spines; apex rounded and folded; ejaculatory duct sclerotized, sinuous, and protruding apically (Fig. 24D).

Holotype. BRAZIL: Ceará: Ubajara, Parque Nacional de Ubajara, Rio das Minas, 03º49’58”S 40º53’53”W, el. 420 m, 20–23.iv.2012, DM Takiya, JA Rafael, F Limeira-de-Oliveira et al. cols., Malaise trap, male (CZMA).

Paratypes. Same data as holotype, 1 male (CZMA); same data, except 13–17.ix.2012, 25 males (CZMA), 12 males (DZRJ); same data, except 18–30.xi.2012, 9 males (INPA); same data, except 14–16.ii.2013, 5 males (MZUFBA); same data, except Rio das Minas, 03º50’03”S 40º54’18”W,

el. 524 m, 14–16.ii.2013, DM Takiya, JA Rafael, RR Cavichioli & APM Santos cols., Malaise trap, 5 males (MNRJ).

Etymology. This species is named in reference to the Ubajara National Park, in the municipality with the same name, where the types where collected.

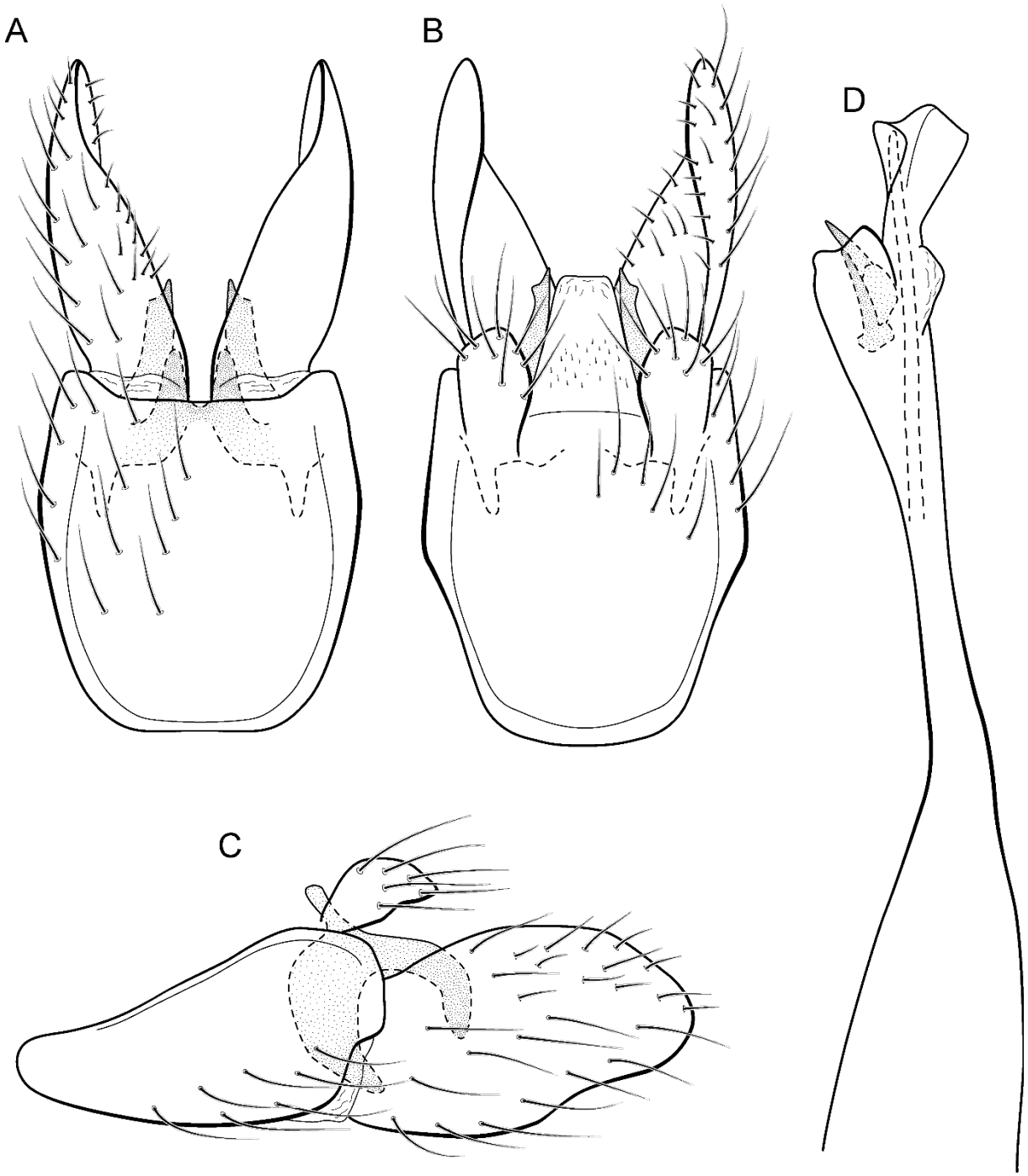


Figure 24. *Metrichia ubajara* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C) lateral view; (D) phallus, dorsal view.

Remarks. This new species appears to be a member of the *nigritta* group due to the internal pouches between abdominal segments V and VI and the presence of two long subapical spines on phallus. *Metrichia ubajara* **sp. nov.** resembles *M. potosina* Bueno-Soria and *M. goiana* **sp. nov.** because of the rounded and elongate inferior appendages in lateral view. However, in *M. ubajara* **sp. nov.** the inferior appendages lack the "tooth" mentioned for these two species. Besides, *M. ubajara* **sp. nov.** can be recognized by the phallic apex with a broad sclerotized plate wraping the ejaculatory duct.

***Metrichia vulgaris* sp. nov.**

urn:lsid:zoobank.org:act:BBC0387F-C111-4CA7-834A-F3711F7707F7

(Fig. 25)

Adult male. Length 2.7–3.1 mm (n=12). General color, in alcohol, brown. Head with no modifications. Ocelli 3. Antenna simple, 21-articulated. Maxillary palpus 5-articulated; labial palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdominal segment VI with pair of internal pouches in posterodorsal area. Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally. **Male genitalia**. Segment IX reduced dorsally; sternum subquadrangular (Fig. 25A); in lateral view, narrower anteriorly than posteriorly (Fig. 25C). Inferior appendage covered by long setae, apex excavated, posterodorsal margin acute and sclerotized; subtrapezoidal in ventral view (Fig. 25A); in lateral view, subretangular (Fig. 25C). Dorsal hook long and stout, almost reaching the inferior appendage apex; in lateral view, downturned (Fig. 25C). Preanal appendage elongate, rounded

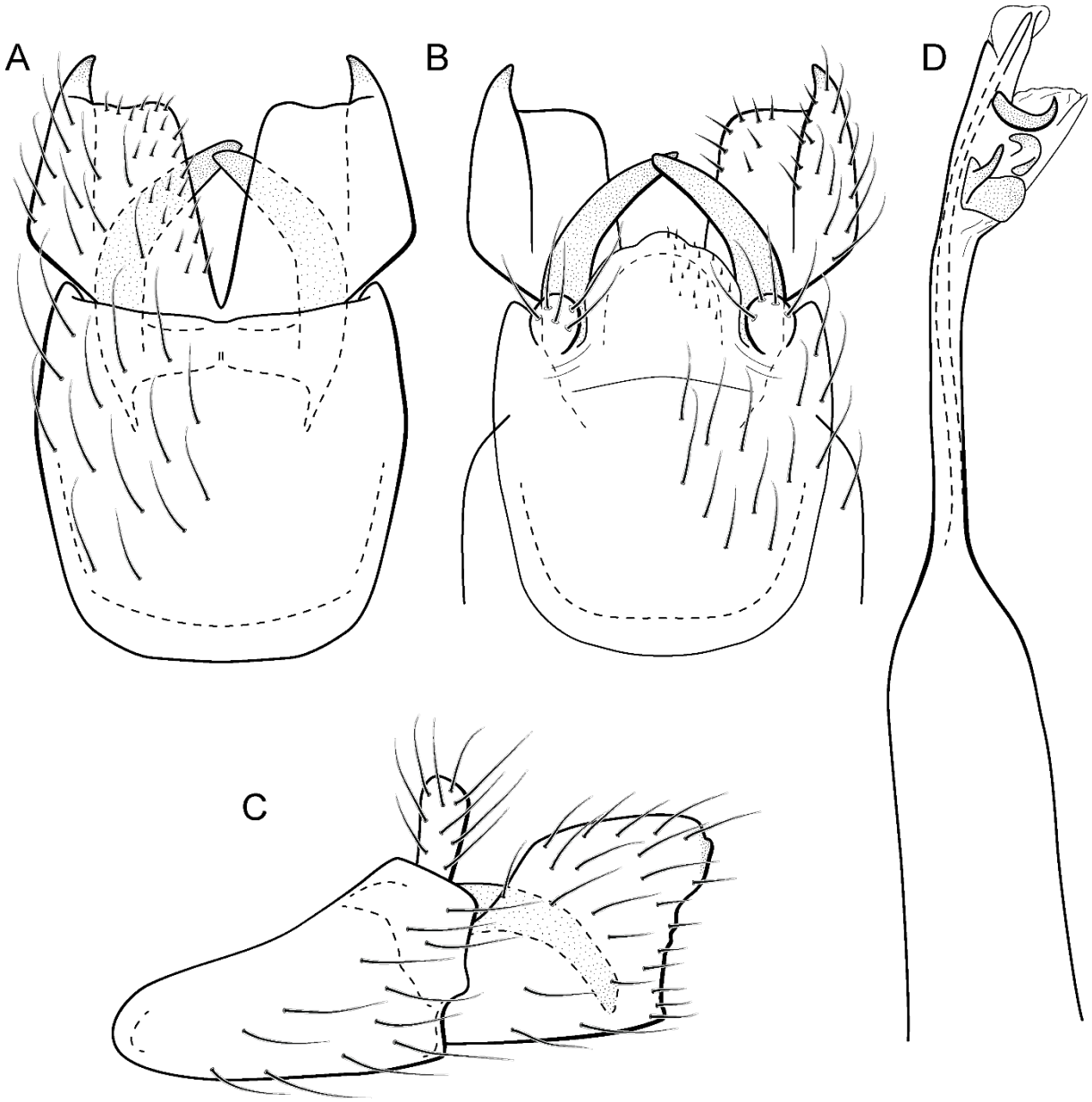
and bearing very long setae (Fig. 25B). Subgenital plate apparently absent. Tergum X membranous and rounded (Fig. 25B). Phallus tubular, elongate and slender, slightly constricted mesally; with two short subapical spines; apex rounded and folded; ejaculatory duct sclerotized and protruding apically (Fig. 25D).

Holotype. BRAZIL: Rio de Janeiro: Itatiaia, Rio Palmital, 22º25'34"S 44º32'52"W, el. 637 m, 07.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, male (DZRJ).

Paratypes. Same data as holotype, 1 male (DZRJ), 1 male (MNRJ); same data, except Rio das Pedras, 22º24'33"S 44º33'08"W, el. 706 m, 06.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, 4 males (MNRJ). **Ceará:** Ubajara, Parque Nacional de Ubajara, Rio Gameleira, 03º50'25"S 40º54'19"W, el. 874 m, 20–22.iv.2012, F Limeira-de-Oliveira et al. cols., Malaise trap, 1 male (CZMA). **Goiás:** Alto Paraíso, afl. Rio Bartolomeu, 14º07'25"S 47º30'30"W, el. 1270 m, 22–25.iii.2013, APM Santos & DM Takiya cols., Malaise trap, 3 males (DZRJ).

Etymology. This new species is named in allusion to its unusual wide distribution throughout Brazil. From the Latin "vulgaris" meaning "common".

Remarks. This new species belongs to the *campana* group due to the internal pouches between segments VI and VII, and the two small subapical spines in phallus. *Metrichia vulgaris* **sp. nov.** with *M. campana* Flint, *M. similis* Flint, and *M. itabaiana* **sp. nov.** share the general aspect of inferior appendages, with an excavated posterior margin. From these species, *M. vulgaris* **sp. nov.** can be distinguished by the inferior appendages, in lateral view, excavated but with ventral corner more rounded than acute; short and stout dorsal hook; and short and rounded tergum X.



1045 **Figure 25.** *Metrichia vulgaris* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)
1046 lateral view; (D) phallus, dorsal view.

1047 *Metrichia vulgaris* **sp. nov.** has an interesting distributional pattern, occurring in
1048 Southeastern, Midwestern, and Northeastern Brazil. Besides the geographic distance among
1049 localities where this species was found, they constitute very distinctive areas, for example, each
1050 one is in a different biome (Atlantic Forest, Cerrado, and Caatinga) and in four different large
1051 river basins (East North Atlantic, Southeast Atlantic, São Francisco, and Araguaia-Tocantins).

Barcode sequences corroborate these different populations being considered the same species, with K2P intraspecific divergences up to 4.8%. This is the highest intraspecific divergence found in our work, but still lower than in other caddisfly groups (Pauls et al., 2010, Zhou et al., 2011). GMYC analyses recovered sequences divided into two 'species', but the groups were not related to their geographic occurrence: one group included samples from Rio de Janeiro and Minas Gerais and the other group, samples from Rio de Janeiro, Minas Gerais, and Goiás. The broader sampling of this species (both in area and in specimens number), compared to others included in the analyses, associated with higher intraspecific divergences can justify the oversplitting with GMYC method, as discussed by Talavera et al. (2013).

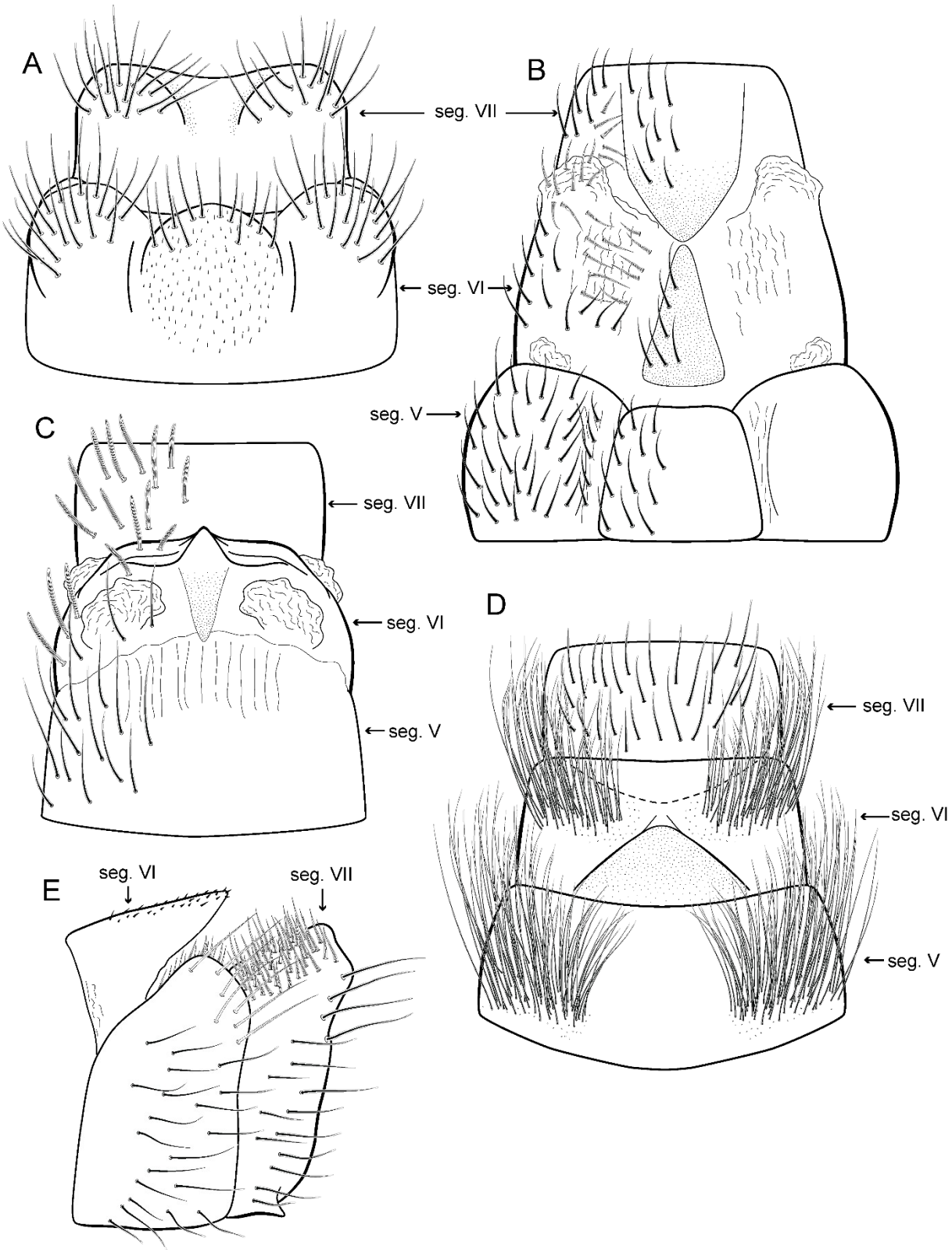
Although such wide distribution is not common for *Metrichia*, other microcaddisfly species can show continental distributions (*e.g.*, *Oxyethira tica* Harris & Holzenthal). Because the knowledge about Neotropical microcaddisflies is very poor, this pattern can be more common that we think.

DISCUSSION

Although, GMYC analysis overestimated the number of *Metrichia* species in our study (suggesting the split of *M. circuliforme* **sp. nov.** and *M. vulgaris* **sp. nov.** each one in two 'species'), COI sequences strongly corroborated the species limits previously defined based on morphological features. In general, caddisflies show a robust barcoding gap in reference to COI sequences (Pauls et al., 2010, Zhou et al., 2011), making it easy to use this molecular marker as a source of additional information to corroborate species delimitation or to associate different life stages.

Microcaddisflies are extremely diverse and poorly known, when associated with the morphology, the use of molecular information can result in a more robust taxonomy for this group. Although methods such ABGD and GMYC should not be used alone to determine

1076 'species', they are useful tools to identifying 'potential species' (Puillandre et al., 2012; Talavera
1077 et al., 2013), especially in very diverse groups and/or with dubious questions on morphology-
1078 based identification. The wide distribution of *Metrichia vulgaris* **sp. nov.** could indicate the
1079 existence of different species, but here morphology and barcodes agreed to define only one
1080 species. Although GMYC overestimated the number of *Metrichia* species in our analysis, this
1081 method is an important tool for preliminary distinction when taxonomic information is poor.
1082 Based on presence of abdominal modifications, such as internal pouches, external
1083 sclerotized plates, and brushes of long setae, and features of male genitalia, six species groups
1084 have been proposed for *Metrichia* (Flint, 1972; Bueno-Soria & Holzenthal, 2003). Although these
1085 modifications usually arise from segments V, VI, and VII, in general aspect, they are very
1086 distinctive from each other (Fig. 26), possibly representing non-homologous structures. Most of
1087 the species described herein fit in these groups and species with COI sequences represented five
1088 species groups. Except for *patagonica* group (represented by only one species, *M. bracui*), none
1089 of the species groups were recovered as monophyletic lineages in neighbor-joining (Fig. 2) or in
1090 BI (Supp. 4) analyses. Furthermore, the monophyly of *Metrichia* remains unclear, with COI
1091 sequences of other genera being more closely related to *Metrichia* sequences than sequences of
1092 other *Metrichia* species (Fig. 2). Nevertheless, relationships and monophyletic status of
1093 *Metrichia* and its species groups were not tested yet, due to the limited taxon sampling in our
1094 analyses.



1095 **Figure 26.** Abdominal modifications of *Metrichia* species: (A) *M. acuminata* **sp. nov.**, segments

1096 VI and VII, dorsal view; (B) *M. itabaiana* **sp. nov.**, segments V, VI, and VII, dorsal view; (C) *M.*

longissima **sp. nov.**, segments V, VI, and VII, dorsal view; (D) *M. peluda* **sp. nov.**, segments V, VI, and VII, dorsal view; (E) *M. simples* **sp. nov.**, segments VI and VII, lateral view.

We also used molecular data to associate larva and adult of *M. bonita* **sp. nov.** Larvae remain unknown for most Neotropical species of Trichoptera, and they are even less known for microcaddisflies. Rearing immatures is very difficult and association based in co-occurrence with adults is not possible when several species of the same genus co-occur. In this way, DNA barcodes are a powerful tool, allowing the association and description of immature stages (Shan et al., 2004; Pauls et al., 2010; Ruiter et al., 2013). Barcode reference libraries for caddisflies are available in other regions (Zhou et al., 2009; Zhou et al., 2011; Ruiter et al., 2013) and represent an important source of information for taxonomic work, as well as, for ecological or evolutionary studies. We expect that molecular data will become increasingly common for Neotropical caddisflies, as it does facilitate the understanding of their diversity in this region.

SUPPLEMENTAL INFORMATION

Collecting sites

Table with detailed list of collecting sites in Brazil where new species of *Metrichia* were found.

Georeferences of collecting sites

Google Earth (.kmz) file with collecting localities.

COI sequence alignment

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 (lnL= -5464.29). Values displayed near branches are posterior probability.

K2P pairwise divergences

Table matrix with pairwise divergences of COI of specimens included of *Metrichia* and related microcaddisflies.

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