

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

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

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# 13 ABSTRACT

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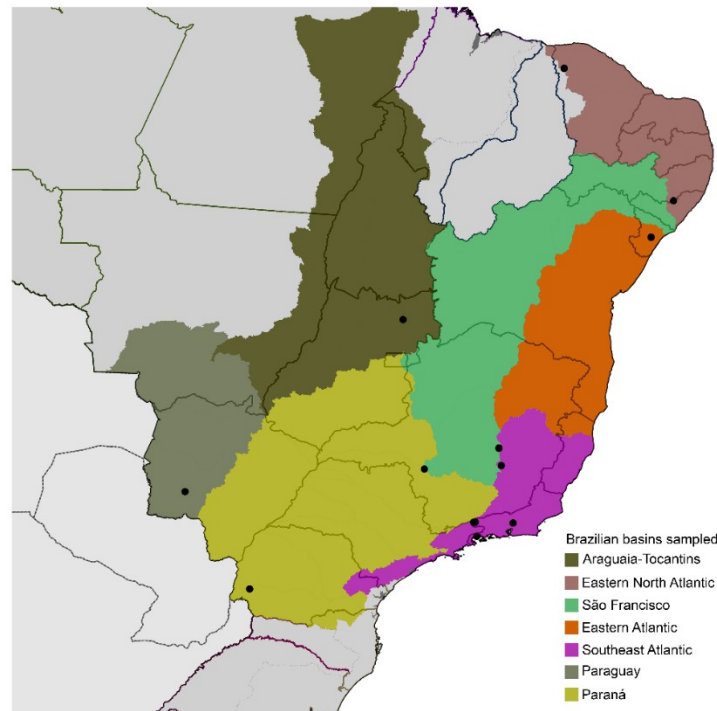
# INTRODUCTION

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. Oláh & Johanson (2011) assigned three other genera to 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 about 100 species, found from the U.S. to South America, with highest 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 larvae 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 tricopterologists. 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

61 surprising that material studied herein recently collected from different River Basins in Brazil  
 62 (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.

63 Most *Metrichia* species exhibit a more restricted distribution, in other words, each species  
 64 is recorded in relatively few localities, which are usually geographically close to each other.  
 65 Identification based mainly on features of male genitalia, revealed some interesting patterns, for  
 66 example, one of the new species being found in three very distinctive biomes: Atlantic Forest  
 67 (Southeastern Brazil), Caatinga (Northeastern Brazil), and Cerrado (Midwestern Brazil).  
 68 Although such wide distribution is not common for *Metrichia*, it is known for other Neotropical  
 69 microcaddisflies, as *Oxyethira tica* Holzenthal & Harris, recorded from Mexico, Central, and  
 70 South America (Flint et al., 1999).

71 Although molecular tools have become common in taxonomic studies to help in species  
 72 delimitation, in Neotropical caddisflies, these studies are still rare. Using sequences of the

mitochondrial cytochrome oxidase I gene (COI), the standard DNA barcode region for animals, Pauls et al. (2010) were able to corroborate two new species of *Smicridea* (*Smicridea*) McLachlan from Chile, previously 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 frequently co-occur.

Larvae of *Metrichia* have been associated only for two species: *M. nigrutta* 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. nigrutta* 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 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 Inc.) using pencil sketches as templates. Descriptions provided here were made with DELTA software (Description Language for Taxonomy) (Dallwitz et al., 1999). Most terminology used throughout this paper follows that provided by Marshall (1979). 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**).

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## 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 in combination with LCO-1490 (Folmer et al., 1994) or C1-J-1718 (Simon et al., 1994). Polymerase chain reaction (PCR) conditions followed: initial denaturation at 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 2 min; and final extension at 72 °C for 7 min. PCR products were sent to Macrogen Inc., Seoul, for purification and sequencing reactions.

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

**Table 1.** Species of *Metrichia* and other hydroptilids with DNA barcode sequenced used in this study, with respective information of specimen voucher and GenBank accession numbers.

Species	Voucher code and life stage	Collection site	GenBank accession number
<i>Angrisanoia cebolatti</i> (Angrisano, 1995)	ENT 2199 ♂	Brazil: Goiás: Alto Paraíso de Goiás	
<i>Betrichia bispinosa</i> Flint, 1974	ENT 2337 ♂	Brazil: Amapá	KU094961 <sup>b</sup>
<i>Nototrichia cautinensis</i> Flint, 1983	-	-	KC559534 <sup>a</sup>
<i>Nototrichia tupi</i> Holzenthal & Harris,	ENT 2460 ♂	Brazil: Minas Gerais: Catas	

1992			
<i>Ochrotrichia caatinga</i> Souza, Santos & Takiya	ENT 2472 ♂	Brazil: Ceará: Ubajara	
<i>Ochrotrichia patulosa</i> (Wasmund & Holzenthal, 2007)	ENT 2473 ♂	Brazil: Ceará: Ubajara	
<i>Ochrotrichia</i> sp. CR1	ENT 2279 ♂	Costa Rica: Puntarenas	KU094950 <sup>b</sup>
<i>Oxyethira tica</i> Holzenthal & Harris, 1992	ENT 0057 ♂	Brazil: Pará: Carajás	KU094940 <sup>b</sup>
<i>Ragatrichia</i> sp. BR1	ENT 2338 ♂	Brazil: Amapá	
<i>Rhyacopsyche dikrosa</i> Wasmund & Holzenthal, 2007	ENT 0122 ♂	Brazil: Rio de Janeiro: Teresópolis	KU094952 <sup>b</sup>
<i>Rhyacopsyche torulosa</i> Flint, 1971	ENT 2277 ♂	Costa Rica: Puntarenas	
	ENT 2192 ♂		
	ENT 2282 ♂		
<i>Metrichia acuminata</i> sp. nov.	ENT 2284-5 ♂	Brazil: Alagoas: Quebrangulo	
	ENT 2779 ♂		
<i>Metrichia amplitudinis</i> Bueno-Soria & Holzenthal, 2003	ENT 2278 ♂	Costa Rica: Puntarenas	
<i>Metrichia bonita</i> sp. nov.	ENT 2200-4 larvae	Brazil: Mato Grosso do Sul: Bonito	
<i>Metrichia bonita</i> sp. nov.	ENT 2208-10 ♂	Brazil: Mato Grosso do Sul: Bonito	
<i>Metrichia bracui</i> sp. nov.	ENT 2508-11 ♂	Brazil: Rio de Janeiro: Itatiaia	
	ENT 2195 ♂		
<i>Metrichia caraca</i> sp. nov.	ENT 2280 ♂	Brazil: Minas Gerais: Catas Altas	
	ENT 2461-5 ♂		
<i>Metrichia caraca</i> sp. nov.	ENT 2292 ♂	Brazil: Minas Gerais: São Roque de Minas	
<i>Metrichia circuliforme</i> sp. nov.	ENT 2835-40 ♂		
	ENT 2843-4 ♂	Brazil: Rio de Janeiro: Itatiaia	
<i>Metrichia curta</i> sp. nov.	ENT 2838 ♂		
	ENT 2846-8 ♂	Brazil: Rio de Janeiro: Itatiaia	
<i>Metrichia formosinha</i> sp. nov.	ENT 2205-7 ♂	Brazil: Mato Grosso do Sul: Bonito	
<i>Metrichia itabaiana</i> sp. nov.	ENT 2190 ♂	Brazil: Sergipe: Areia Branca	
<i>Metrichia itabaiana</i> sp. nov.	ENT 2220-1 ♂	Brazil: Goiás: Alto Paraíso de Goiás	
<i>Metrichia juana</i> (Flint, 1964)	ENT 2850-1 ♂	Puerto Rico	
<i>Metrichia longissima</i> sp. nov.	ENT 2330 ♂	Brazil: Rio de Janeiro: Teresópolis	
<i>Metrichia longissima</i> sp. nov.	ENT 2841 ♂	Brazil: Rio de Janeiro: Itatiaia	
<i>Metrichia rafaeli</i> sp. nov.	ENT 2288-9 ♂	Brazil: Ceará: Ubajara	
	ENT 2193 ♂		
	ENT 2214 ♂		
<i>Metrichia talhada</i> sp. nov.	ENT 2216-7 ♂	Brazil: Alagoas: Quebrangulo	
	ENT 2776 ♂		
<i>Metrichia tere</i> sp. nov.	ENT 2773-5 ♂	Brazil: Rio de Janeiro: Teresópolis	
<i>Metrichia vulgaris</i> sp. nov.	ENT 2218 ♂	Brazil: Goiás: Alto Paraíso de Goiás	
<i>Metrichia vulgaris</i> sp. nov.	ENT 2466-70 ♂	Brazil: Minas Gerais: Catas Altas	
<i>Metrichia vulgaris</i> sp. nov.	ENT 2833-4 ♂	Brazil: Rio de Janeiro: Itatiaia	

167 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 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, 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 support of neighbor-joining tree was assessed with 1.000 pseudoreplicate of non-parametric bootstrap (Felsenstein, 1985).

BI analysis was conducted with MrBayes v. 3.2.2 (Ronquist *et al.*, 2012) with four independent runs, each one with four MCMC chains running for 50,000,000 generations, with sample frequency of 5,000. Convergence of sampled parameters was checked in Tracer v. 1.5 (Rambaut & Drummond, 2007) and the first 10% of sampled trees and parameters as burnin. GTR+I+G was the best fit model selected by Akaike Information Criterion (AIC) with jModeltest v. 0.1.1 (Posada, 2008) and it was applied in BI analysis in MrBayes. Branch support was assessed by posterior probability (PP), presented on a 50% majority consensus tree.

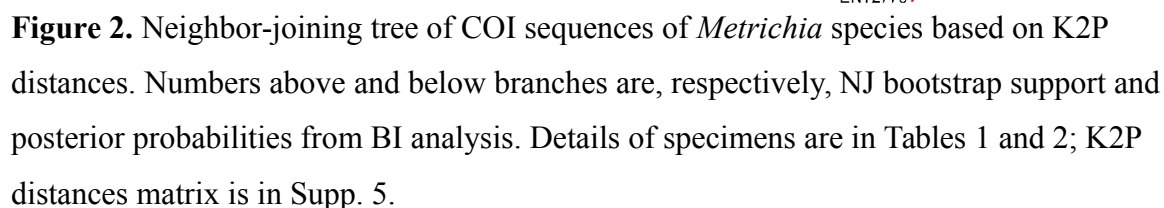
ABGD analysis was run using on-line version available in <http://www.wabi.snv.jussieu.fr/public/abgd/>, where COI alignment was uploaded. The analysis was conducted with the following settings: Pmin=0.001; Pmax=0.1; steps=20; relative gap width=1.0,

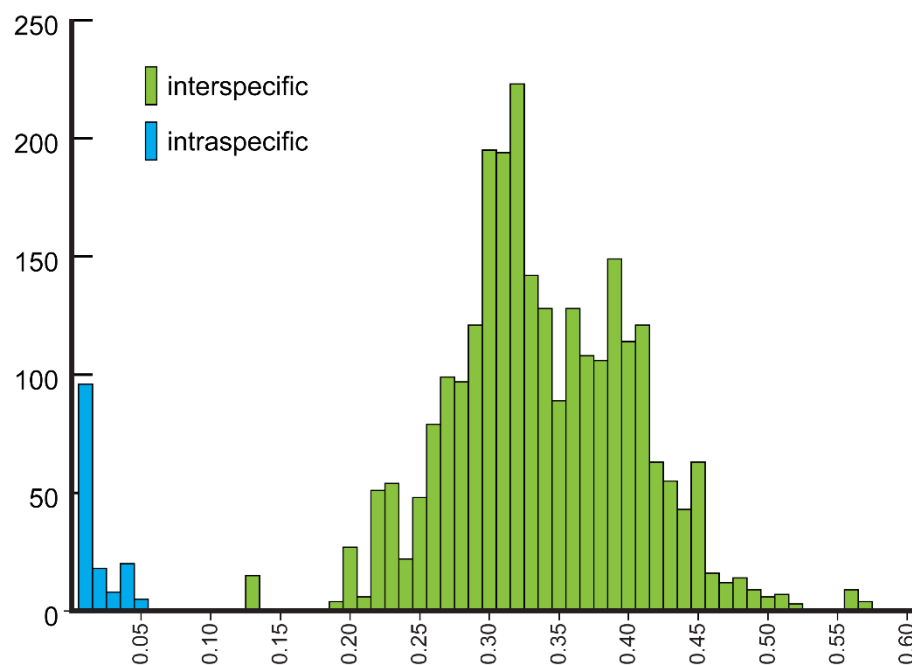
also based on K2P model. This method statistically infers the DNA barcode gap in a single locus alignment, partitioning the data based on this gap in putative species (Puillandre et al., 2012).

The GMYC analysis (Pons et al., 2006; Fujisawa & Barraclough, 2013) was performed in R (R Development Core Team 2010) using the SPLITS package (Ezard et al., 2009) with single-threshold method. Basically, the method estimates branching patterns on an ultrametric tree, identifying the most likely transition point from coalescent to speciation branching. The ultrametric tree used here was obtained with BEAST v. 1.8 (Drummond et al., 2012) under a relaxed uncorrelated molecular clock (Drummond et al., 2006). The node including *Ochrotrichia* species was calibrated based on fossil evidence with a lognormal distribution offset at 20 mya and  $\log(\text{mean}) = 2.8$  to represent the possible range of 20-140 mya (Wells & Wichard, 1989); and the divergence of Ochrotrichiinae was calibrated based on Malm et al. (2013) with a normal distribution with mean  $82.17 \pm 12$  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 TreeAnnotator, 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).

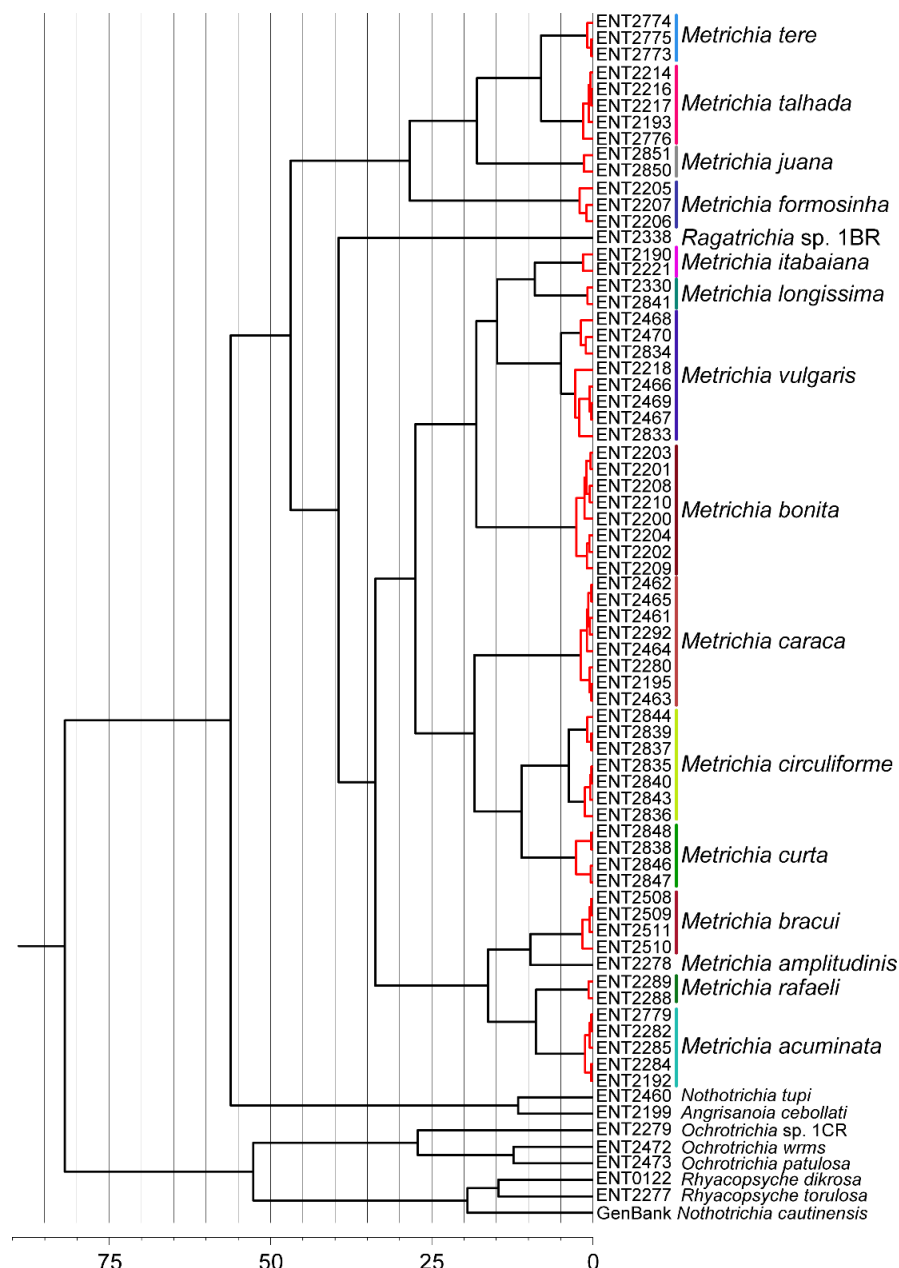




**Figure 3.** Histogram of number of pairwise comparisons of intra- (blue) and interspecific (green) K2P divergences among fifteen *Metrichia* species with COI sequences sampled.

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

Species	Number of sequences	Max. intra. distance	Min. inter. Distance
<i>M. acuminata</i> <b>sp. nov.</b>	5	0.000	0.217
<i>M. amplitudinis</i> Bueno-Soria & Holzenthal, 2003	1	-	0.220
<i>M. bonita</i> <b>sp. nov.</b>	8	0.015	0.210
<i>M. bracui</i> <b>sp. nov.</b>	4	0.004	0.214
<i>M. caraca</i> <b>sp. nov.</b>	8	0.011	0.217
<i>M. circuliforme</i> <b>sp. nov.</b>	7	0.035	0.184
<i>M. curta</i> <b>sp. nov.</b>	4	0.015	0.184
<i>M. formosinha</i> <b>sp. nov.</b>	3	0.008	0.249
<i>M. itabaiana</i> <b>sp. nov.</b>	2	0.019	0.194
<i>M. juana</i> (Flint, 1964)	2	0.007	0.243
<i>M. longissima</i> <b>sp. nov.</b>	2	0.004	0.215
<i>M. rafaeli</i> <b>sp. nov.</b>	2	0.004	0.194
<i>M. talhada</i> <b>sp. nov.</b>	5	0.000	0.126
<i>M. tere</i> <b>sp. nov.</b>	3	0.000	0.126
<i>M. vulgaris</i> <b>sp. nov.</b>	8	0.048	0.246



**Figure 4.** Maximum credibility time tree from BEAST analysis based on COI sequences of *Metrichia* and other Ochrotrichiinae. Red branches represent species estimated by GMYC using single threshold in SPLITS. Timescale in million years.

217 GMYC analysis estimated a slightly higher number of putative species, with *Metrichia*  
 218 *circuliforme* **sp. nov.** and *Metrichia vulgaris* **sp. nov.** being each further divided as two species  
 219 (Fig. 4). Regarding all other species, GMYC results were congruent with other methods and with  
 220 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.**

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(Fig. 5, Fig. 26A)

**Adult male.** Length 2.1–2.5 mm (n=5). General color, in alcohol, brown. Head with no modifications. Three ocelli. 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; segment VII bearing a brush of very long setae dorsolaterally. 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 in ventral view, with anterior margin rounded; in lateral view narrower anteriorly than posteriorly. Inferior appendage subtrapezoidal in ventral view, covered by long setae; in lateral view, subtrapezoidal; apex with acute corners; dorsal hook short, almost half length of inferior appendage; in lateral view, downturned. Preanal appendage rounded in lateral view and bearing very long setae. Subgenital plate apparently absent. Tergum X membranous and truncate in dorsal view. 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.



**Holotype. BRAZIL: Ceará:** Ubajara, Parque Nacional de Ubajara, Cachoeira do Gameleira, 03°50'21"S 40°54'23"W, el. 880 m, 23.iv.2012, DM Takiya & JA Rafael, light trap, male (CZMA).

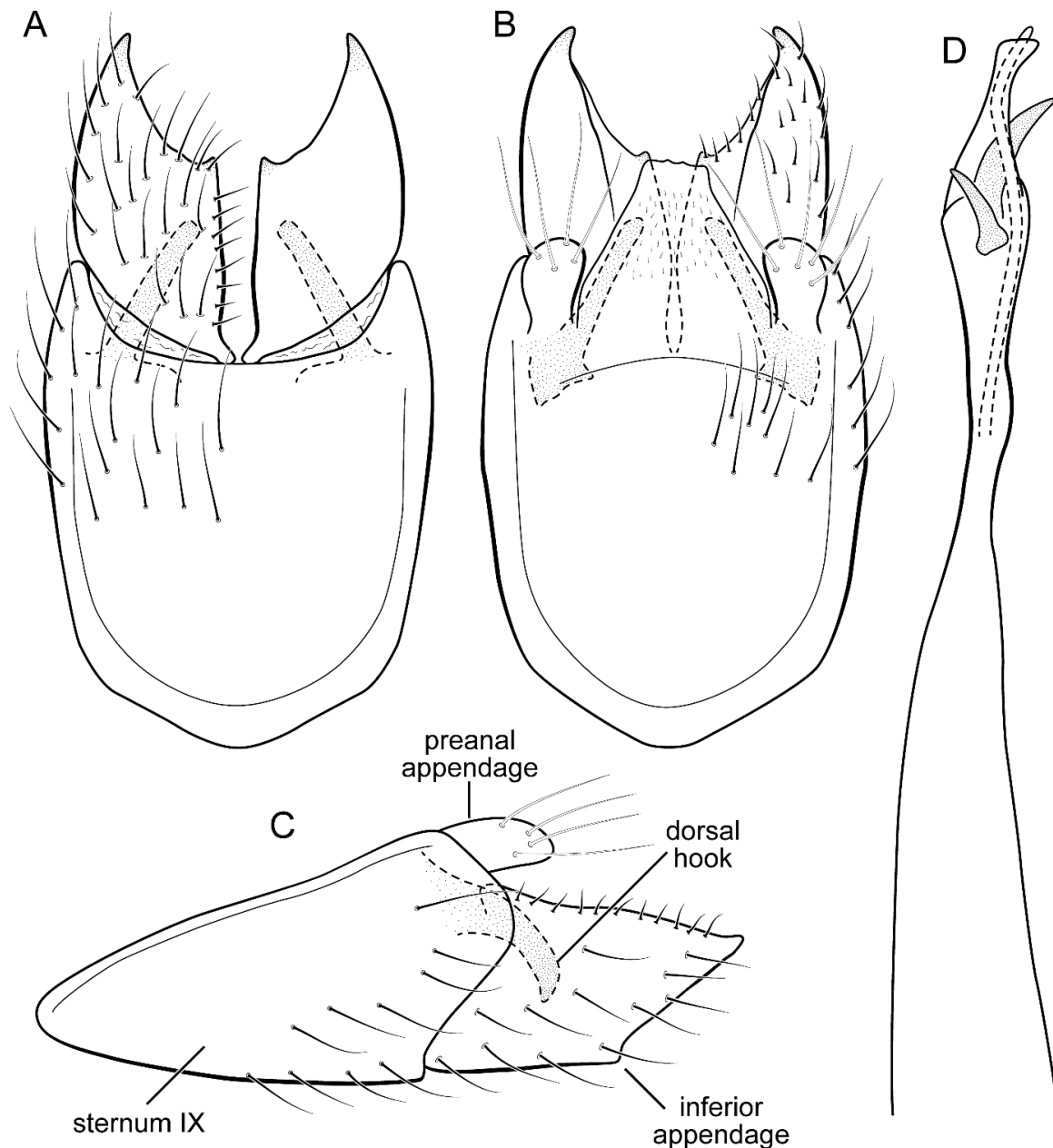
**Paratypes.** Same data as holotype, except, Rio das Minas, 03°50'03"S 40°54'18"W, el. 524 m, 13–17.ix.2012, JA Rafael et al., Malaise trap, 2 males (INPA); same data, except 14-16.ii.2013, DM Takiya, JA Rafael, RR Cavichioli & APM Santos, Malaise trap, 2 males (DZRJ). **Alagoas:** Quebrangulo, Reserva Biológica de Pedra Talhada, Rio Caranguejo, 09°15'26"S 36°25'08"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. nov.**, *M. acuminata* **sp. nov.** is readily recognized by the presence of setose lobes on abdominal segments V and VI.

We were not able to obtain COI sequences for individuals from Ceará State, so the five sequences analyzed belong to specimens from Alagoas State and shared the same haplotype. *M.*

269 *acuminata* **sp. nov.** was recovered as closely related to *M. caraca* **sp. nov.** (Fig. 2), but in both  
 270 Bayesian approaches these two species were not recovered as sister taxa (Fig. 4, Supp. 1).



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

271 *Metrichia azul* **sp. nov.**

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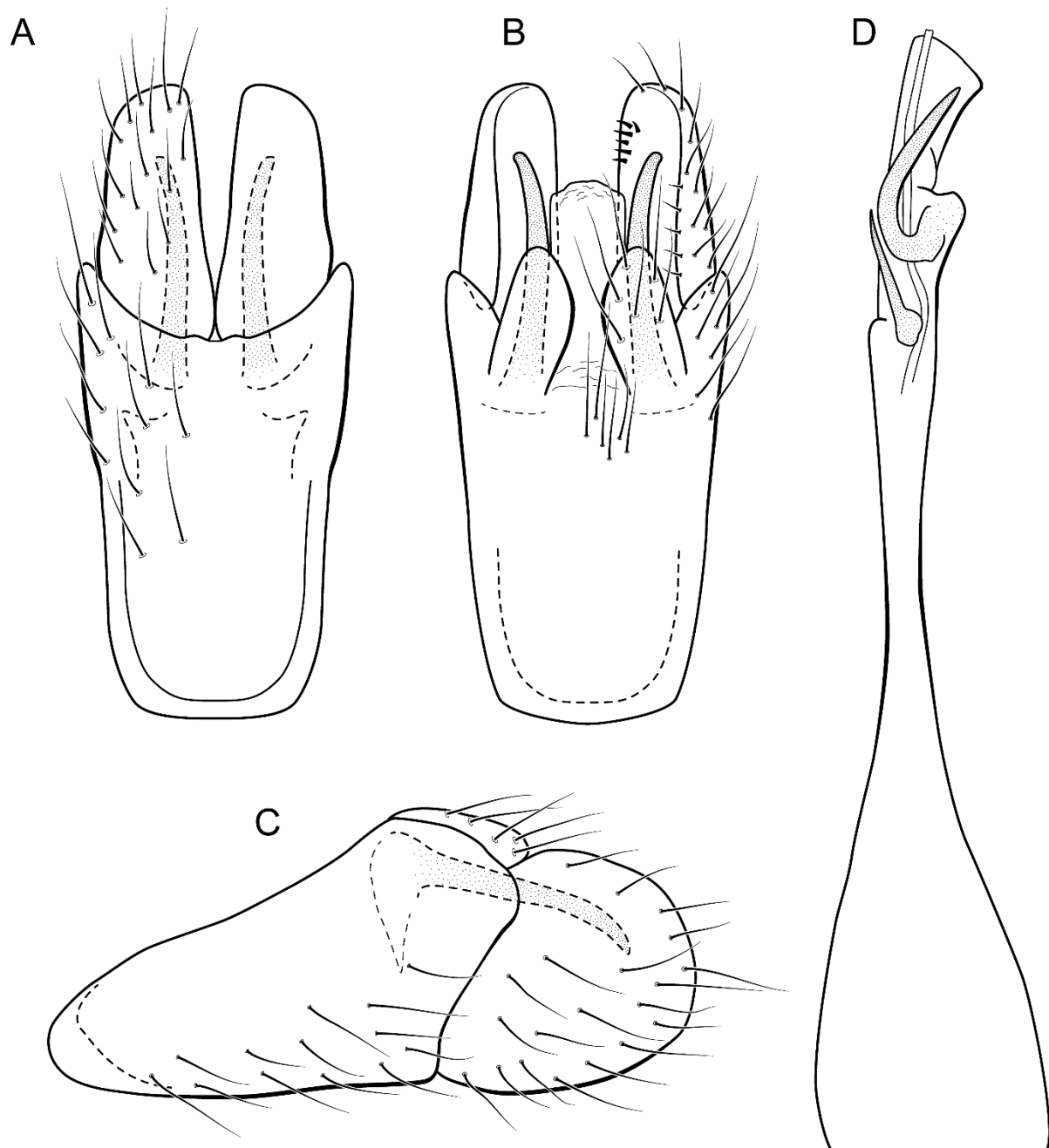
(Fig. 6)

**Adult male.** Length 2.0–2.1 mm (n=4). General color, in alcohol, brown. Head with no modifications. Three ocelli. 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 in ventral view; in lateral view narrower anteriorly than posteriorly. Inferior appendage subrectangular in ventral view, covered by long setae and with scale-like setae; in lateral view, rounded; apex rounded; dorsal hook long, more than half length of inferior appendage; in lateral view, slightly downturned. Preanal appendage elongate, but shorter than inferior appendage, and bearing very long setae. Subgenital plate apparently absent. Tergum X membranous and rounded in dorsal view. 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.

**Holotype. BRAZIL: Paraná:** Céu Azul, Parque Nacional do Iguaçu, Rio Azul, 25°09'21"S 53°47'44"W, el. 510 m, 6–8 ix.2012, APM Santos, DM Takiya, ALH Oliveira, GA Jardim & BHL Sampaio, 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.



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

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

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

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(Fig. 7, Fig. 8)

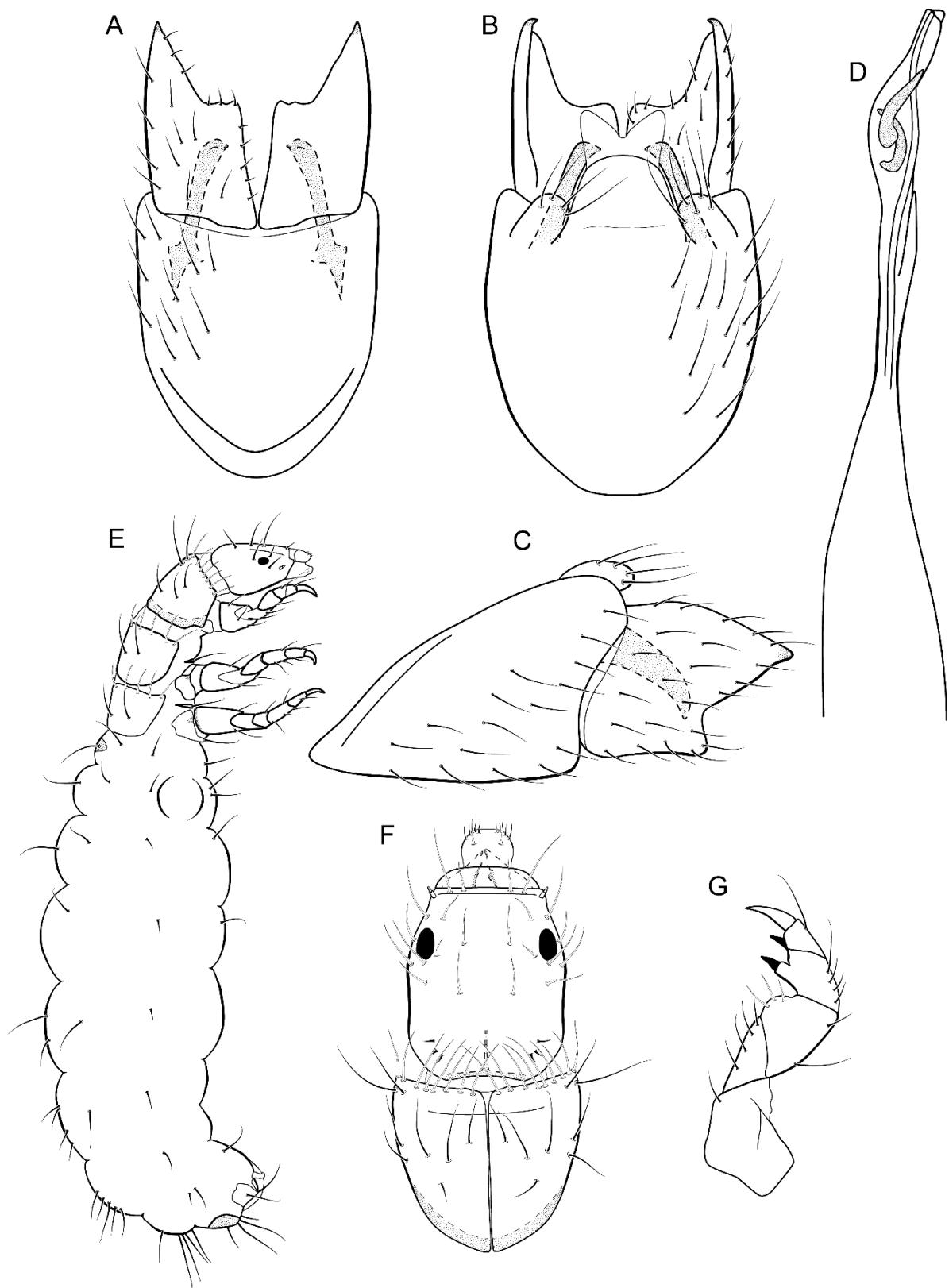
**Adult male.** Length 2.3–2.5 mm (n=4). General color, in alcohol, brown. Head with no modifications. Three ocelli. Antenna simple, 21-articulated. Maxillary palpus 5-articulated; labial palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular. Anterior femur without processes. 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 in ventral view, with anterior margin rounded; in lateral view narrower anteriorly than posteriorly. Inferior appendage subtrapezoidal in ventral view, covered by long setae; in lateral view, with an acute projection; apex with acute corners; dorsal hook short, almost half length of inferior appendage; in lateral view, slightly downturned. Preanal appendage short, rounded and bearing very long setae. Subgenital plate apparently absent. Tergum X membranous and with a shallow U-shaped incision. 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 protruding apically.

**Larva (5<sup>th</sup> instar).** Length 1.5-1.9 mm (n=10). Head dark brown, unpigmented around stemmata; slightly longer than broad; frontoclypeal and coronal sulci indistinct; with a few long setae. Antenna short, apparently 2-articulated and with no apical setae. Labrum with pair of stout setae. 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. 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; 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. 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. Constructed with calcareous particles (with no algal filaments added), forming two rigid and lateral valves, poorly closed dorsally and ventrally. External surface rugose.

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



**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, 21°10'16"S 56°26'47"W  
el. 275 m, 08-13.ix.2013, APM Santos & DM Takiya, Malaise trap, male (DZRJ).

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

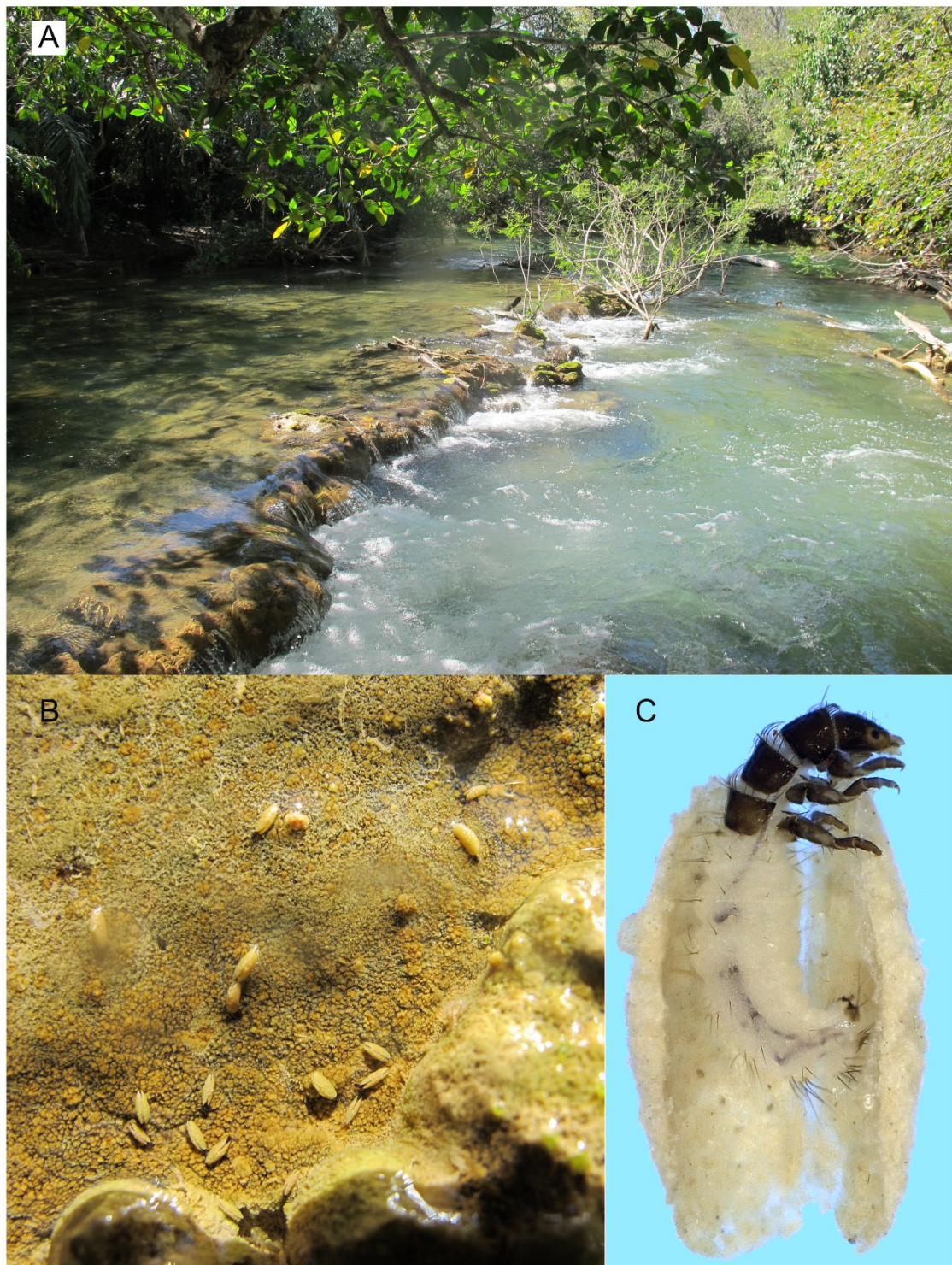
**Additional material.** Same data as holotype, except 13.ix.2013, manual, 10 larvae (DZRJ), 10  
larvae (MNRJ).

**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).





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

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

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

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(Fig. 9)

**Adult male.** Length 1.8–2.2 mm (n=3). General color, in alcohol, light brown. Head with no modifications. Three ocelli. 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 absent. Segment VIII shorter ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally; sternum subpentagonal in ventral view; in lateral view narrower anteriorly than posteriorly. Inferior appendage subrectangular in ventral view, covered by long setae and with scale-like setae; in lateral view, rounded; apex rounded; dorsal hook long, more than half length of inferior appendage; in lateral view, downturned. Preanal appendage elongate, as long as inferior appendage, and bearing very long setae. Subgenital plate apparently absent. Tergum X

membranous and with shallow U-shaped incision. 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 protruding apically.

**Holotype. BRAZIL: Rio de Janeiro:** Angra dos Reis, Rio Bracuí, 23°00'23"S 44°29'15"W, el. 75 m, 10–11.v.2002, JL Nessimian, light trap, male (DZRJ).

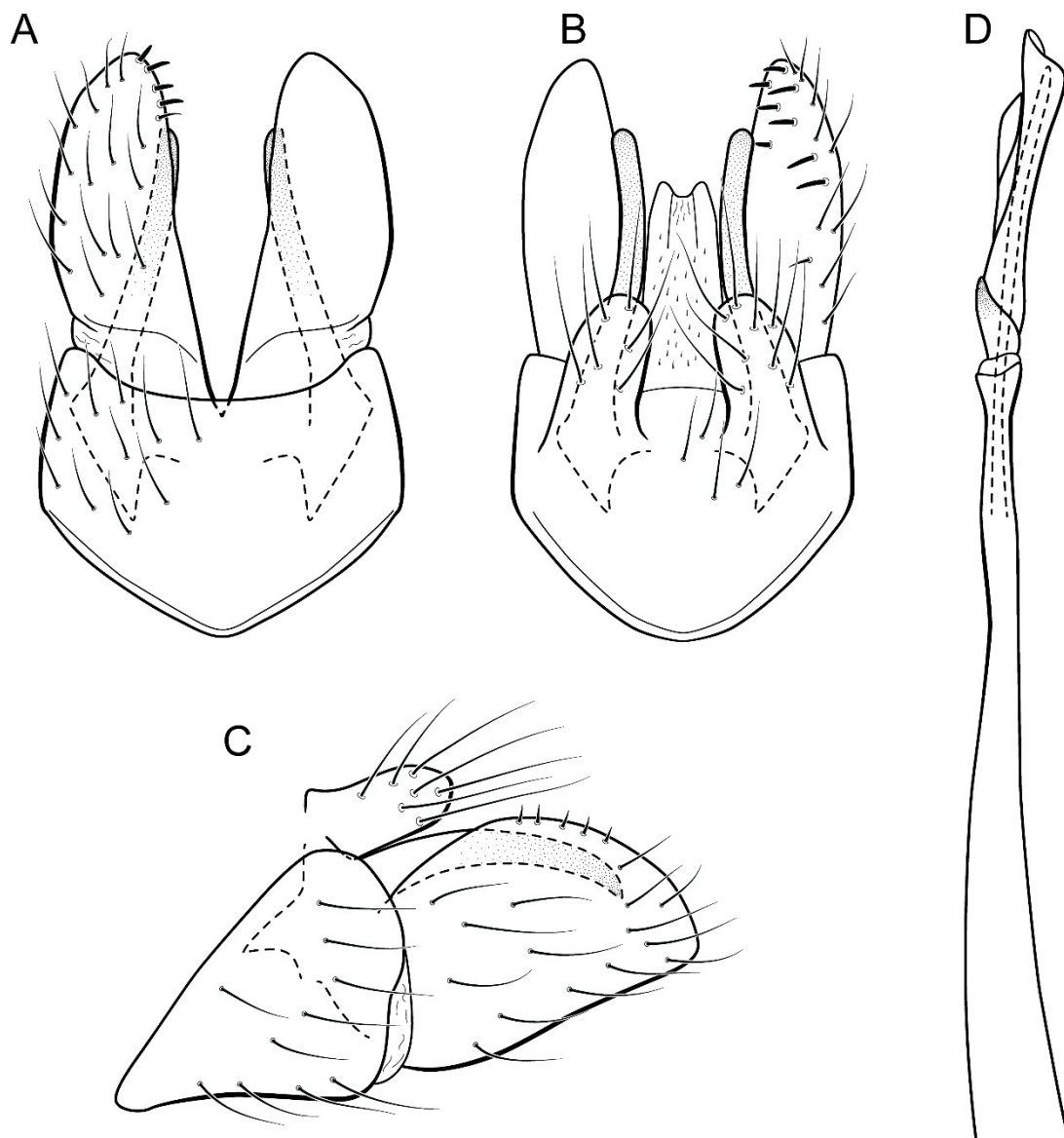
**Paratypes.** Same data as holotype, 2 males (MNRJ). **Rio de Janeiro:** Parque Nacional do Itatiaia, Córrego do Maromba, 22°25'32"S 44°37'03"W, el. 1250 m, 04.iv.15, APM Santos & DM Takiya, Malaise trap, 4 males (DZRJ).

**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 PN 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 cebolatti*. The position of *Angrisanoia* varied in the different phylogenetic analyses, suggesting that Ochotrichiinae genera still need a formal evaluation.





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

409 ***Metrichia caraca* sp. nov.**

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411 (Fig. 10)

412 **Adult male.** Length 2.5–3.0 mm (n=7). General color, in alcohol, brown. Head with no  
 413 modifications. Three ocelli. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial  
 414 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.  
 415 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 dorsolaterally; segment VII bearing brush of very long setae dorsolaterally. 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 in ventral view, with anterior margin rounded; in lateral view narrower anteriorly than posteriorly. Inferior appendage subtrapezoidal in ventral view, covered by long setae; apex oblique and projected dorsally into a large process bearing a stout spine-like setae; dorsal hook short and straight; in lateral view, truncate and broader apically. Preanal appendage short, rounded and bearing very long setae. Subgenital plate apparently absent. Tergum X membranous and truncate in dorsal view. Phallus tubular, elongate and slender, slightly constricted mesally; with two curved subapical spines, one short and another long; apex emarginate; ejaculatory duct sclerotized and protruding apically.

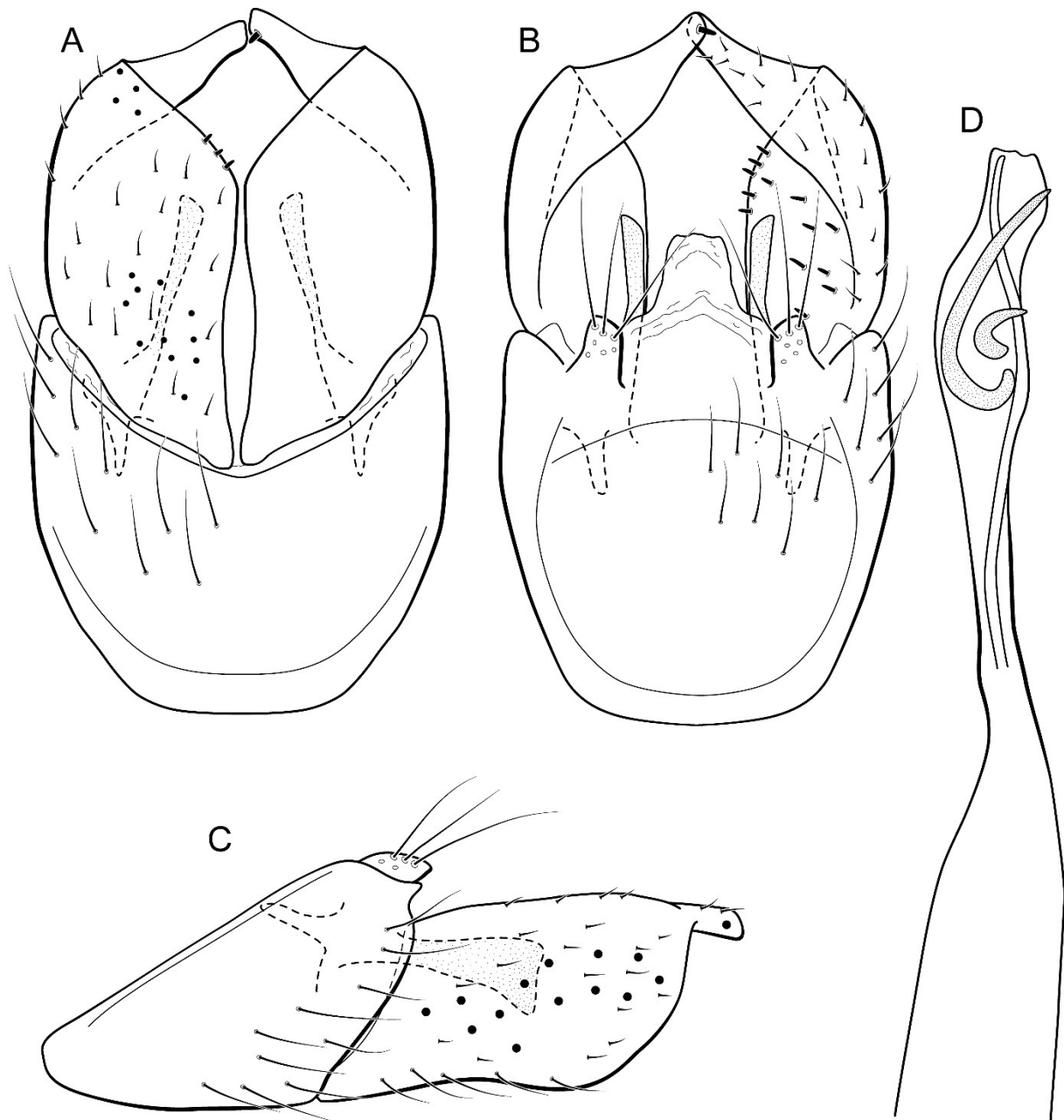
**Holotype. BRAZIL: Minas Gerais:** Catas Altas, RPPN Santuário do Caraça, Ribeirão Caraça, 11–13.vi.2013, ML Monné & JP Botero, Malaise trap, male (DZRJ).

**Paratypes.** Same data as holotype, 1 male (DZRJ). **Minas Gerais:** São Roque de Minas, Parque Nacional da Serra da Canastra, Fazenda Velha, Córrego dos Pombos, 20°14'57"S 46°38'05"W, el. 997 m, 02.iv.2014, JL Nessimian, ALH Oliveira, LL Dumas & SP Gomes, light trap, 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 mesally directed process of inferior appendages and dorsal hook, in lateral view, with apex broad and truncate.

439 COI distances within this species reached only 1.1% and the lowest interspecific distance  
 440 (21.7%) was found between specimens of *M. caraca* **sp. nov.** and *M. acuminata* **sp. nov.**, which  
 441 are very distinct based on morphological features.



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

442 *Metrichia circuliforme* sp. nov.

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444 (Fig. 11)

445 **Adult male.** Length 2.5–2.7 mm (n=4). General color, in alcohol, brown. Head with no  
446 modifications. Three ocelli. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial  
447 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.  
448 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both  
449 wings. Abdominal segment VI bearing brush of very long setae dorsolaterally; segment VII  
450 bearing a brush of very long setae dorsolaterally. Ventromesal process on segment VII present.  
451 Segment VIII shorter ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally;  
452 sternum subpentagonal in ventral view; in lateral view narrower anteriorly than posteriorly.  
453 Inferior appendage short, subrectangular in ventral view, covered by long setae; in lateral view,  
454 rounded; apex slightly truncate and bearing short spine-like setae; dorsal hook long, almost  
455 reaching the inferior appendage apex; in lateral view, downturned. Preanal appendage elongate,  
456 but shorter than inferior appendage, and bearing very long setae. Subgenital plate apparently  
457 absent. Tergum X membranous and truncate in dorsal view. Phallus tubular, elongate and slender,  
458 slightly constricted mesally; with two short subapical spines; apex rounded and sclerotized;  
459 ejaculatory duct sclerotized, straight and protruding apically.

460 **Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, Rio das Pedras, Cachoeira de Deus, 22°25'00"S  
461 44°32'50"W, el. 689 m, 06.iii.2008, JL Nessimian, LL Dumas & MR de Souza, light trap, male  
462 (DZRJ).

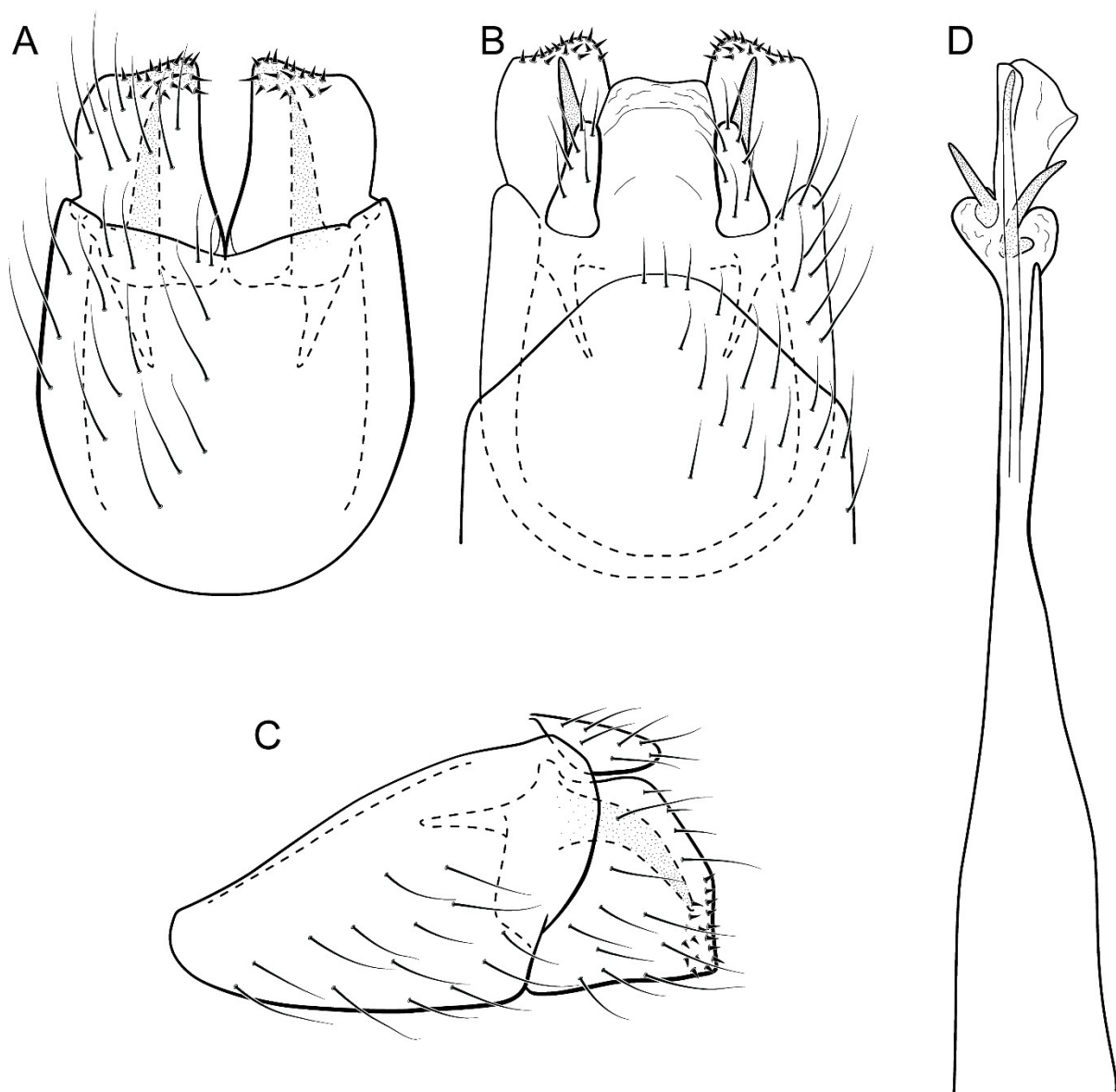
463 **Paratypes.** Same data as holotype, except Rio das Pedras, 22°24'33"S 44°33'08"W, el. 706 m,  
464 06.iii.2008, LL Dumas, JL Nessimian & MR de Souza, light trap, 1 male (DZRJ), 1 male  
465 (MNRJ); Parque Nacional do Itatiaia, Córrego Simon, 22°26'16"S 44°36'20"W, el. 1033 m,  
466 15.iv.07, LL Dumas, APM Santos, N Ferreira-Jr. & JL Nessimian, 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 rectangular 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 again, these two species are very distinct based on morphological features and apparently are not even closely related to each other (Fig. 2).





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

489 ***Metrichia curta* sp. nov.**

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491 (Fig. 12)

492 **Adult male.** Length 2.4–2.5 mm (n=9). General color, in alcohol, light brown. Head with no  
 493 modifications. Three ocelli. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial  
 494 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

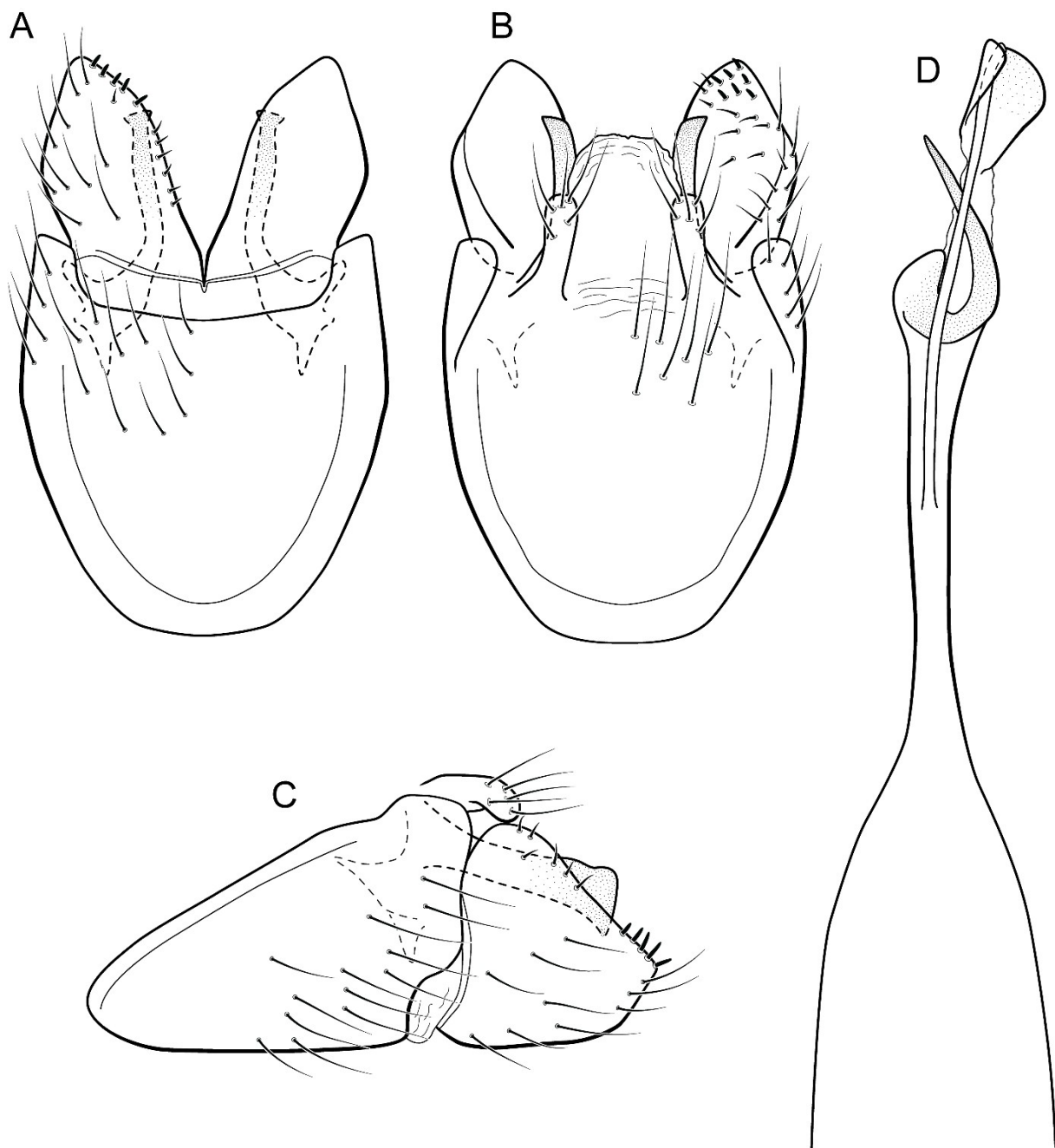
Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both wings. Abdomen without modifications. Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally. Inferior appendage subtrapezoidal in ventral view, covered by long setae; in lateral view, subtriangular; apex rounded; dorsal hook long, more than half length of inferior appendage; in lateral view, with apex slightly broader, downturned, and truncate. Preanal appendage elongate, but shorter than inferior appendage, and bearing very long setae. Subgenital plate apparently absent. Tergum X membranous and rounded in dorsal view. Phallus tubular, elongate and slender, slightly constricted mesally; with a stout subapical spine; apex rounded and folded; ejaculatory duct sclerotized, straight and protruding apically.

**Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, Rio das Pedras, 22°24'33"S 44°33'08"W, el. 706 m, 06.iii.2008, LL Dumas, JL Nessimian & MR de Souza, 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 assigned 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 subapically. *Metrichia amplitudinis* and *M. curta* **sp. nov.** share the widened dorsal hook, but in the new species this structure is only slightly wider and also truncate in lateral view. Maximum intraspecific divergence of COI sequences was 1.5% for *M. curta* **sp. nov.** and the minimum 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.

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

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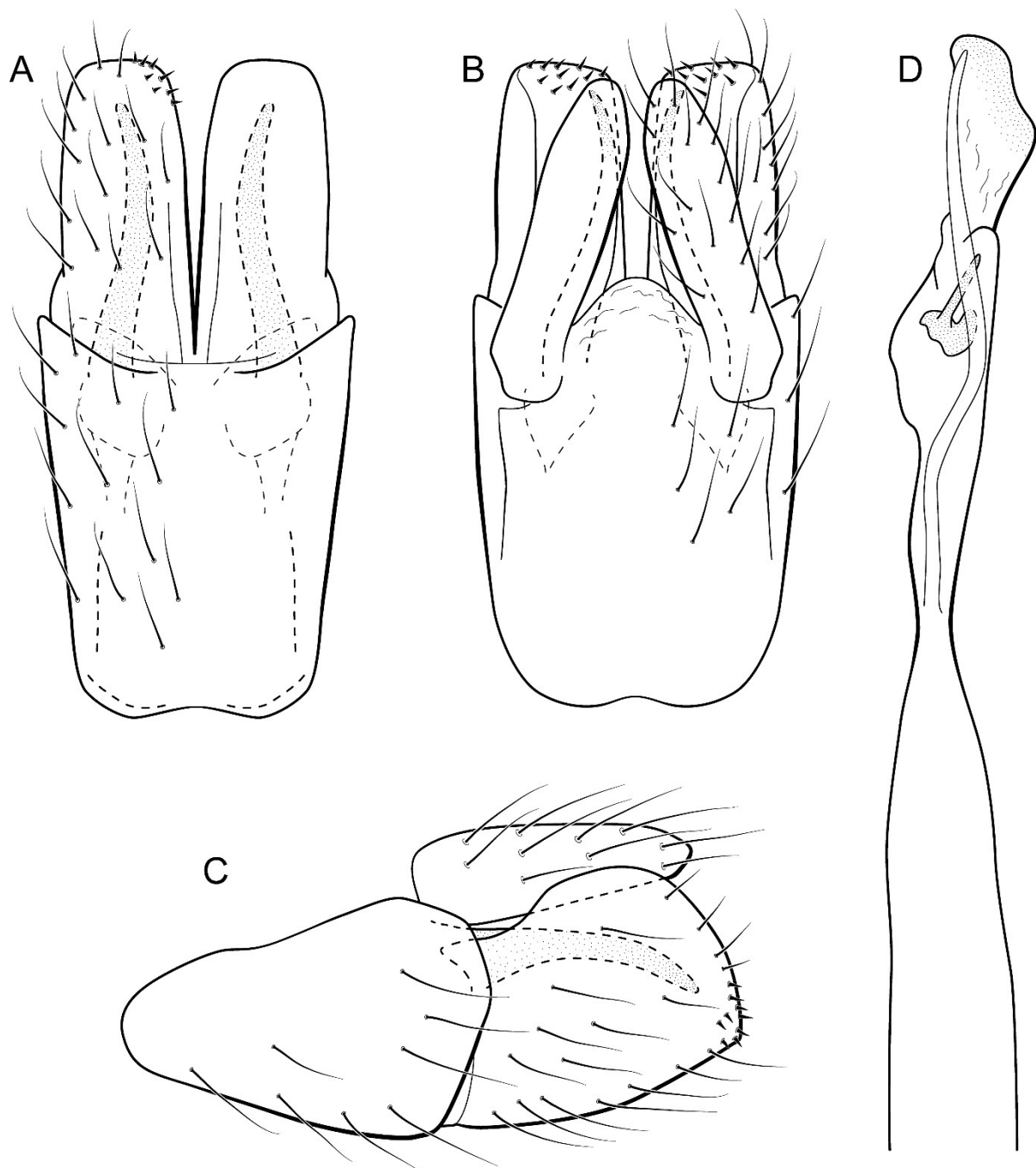
520 (Fig. 13)

**Adult male.** Length 1.8–2.1 mm (n=27). General color, in alcohol, light brown. Head with no modifications. Three ocelli. 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 in ventral view; in lateral view narrower anteriorly than posteriorly. Inferior appendage subrectangular in ventral view, covered by long setae; apex slightly truncate and bearing short spine-like setae; dorsal hook long, almost reaching the inferior appendage apex; in lateral view, downturned. Preanal appendage elongate, as long as inferior appendage, and bearing very long setae. Subgenital plate apparently absent. Tergum X membranous and rounded in dorsal view. Phallus tubular, elongate and slender, slightly constricted mesally; with a stout subapical spine; apex rounded and sclerotized; ejaculatory duct sclerotized, sinuous, and protruding apically.

**Holotype. BRAZIL: Minas Gerais:** Jaboticatubas, Parque Nacional da Serra do Cipó, Cachoeira da Farofa, 19°22'47"S 43°34'36"W, el. 811 m, 23.iv.2010, APM Santos & DM Takiya, manual, male (DZRJ).

**Paratypes.** Same data as holotype, 18 males (DZRJ), 8 males (MNRJ), 5 males (MZUFBA); same data, except Ribeirão Mascates, 19°24'02"S 43°34'35"W, el. 820 m, 09-11.xii.2011, APM Santos & DM Takiya, manual, 84 males (DZRJ).

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



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

543 **Remarks.** Due to absence of pouches in abdominal segments, *Metrichia farofa* **sp. nov.** can be  
 544 also included in the *aberrans* group. However, the new species has only a subapical spine in  
 545 phallus, like those species included *exclamationis* group. The new species can be easily

distinguished from any *Metrichia* species by the very long preanal appendages, reaching the apex of inferior appendages in dorsal and lateral views.

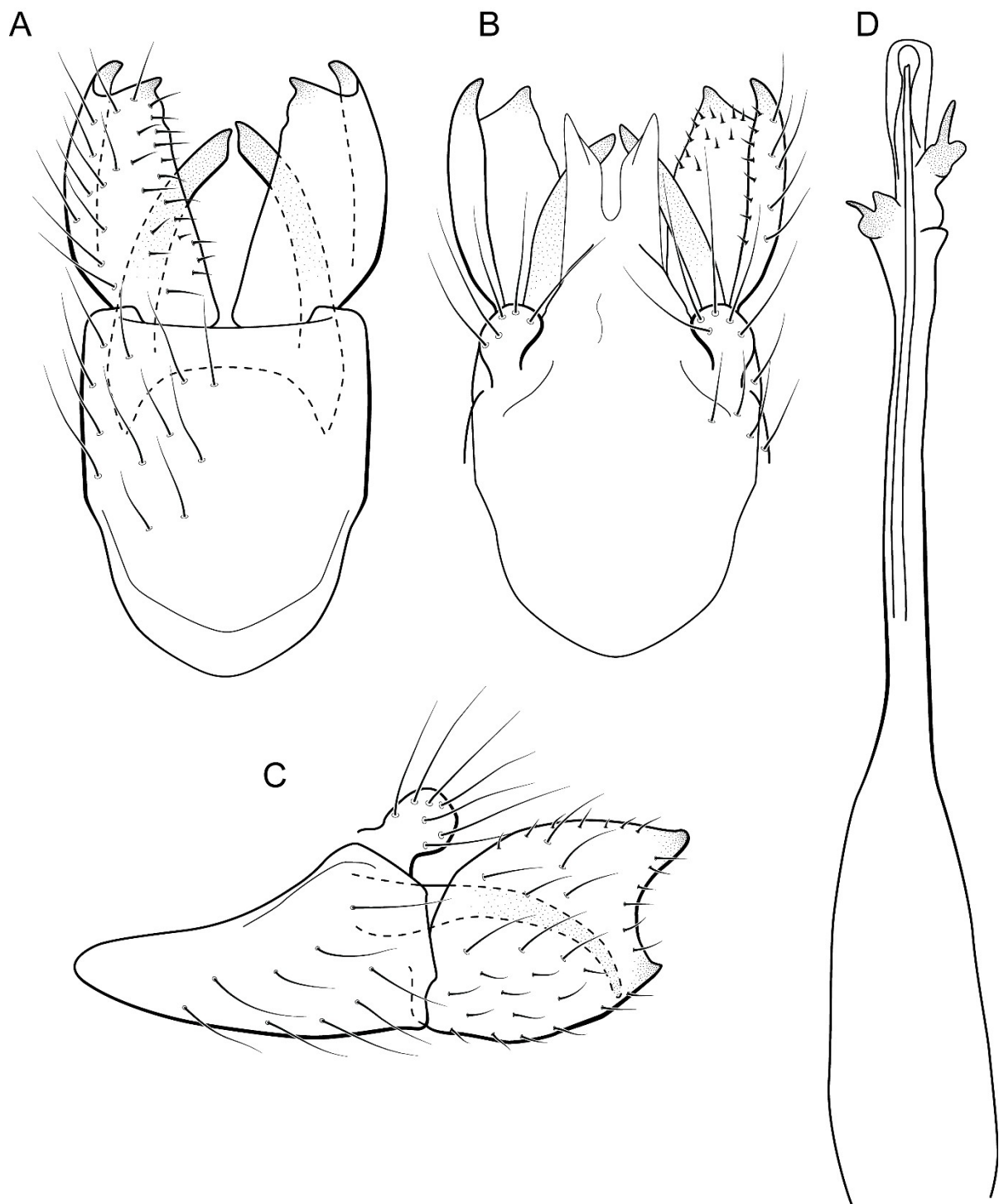
Although more than ten specimens of *M. farofa* **sp. nov.** were submitted to DNA extraction and many attempts of COI amplification via PCR were conducted, we were not able to obtain sequences of this species, even using recent material, collected after 2013.

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

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(Fig. 14)

**Adult male.** Length 2.7–3.0 mm (n=2). General color, in alcohol, brown. Head with no modifications. Three ocelli. Antenna simple, 23-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 VII bearing internal pouches in anterior area. Ventromesal process on segment VII absent. Segment VIII shorter ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally; sternum subpentagonal in ventral view; in lateral view narrower anteriorly than posteriorly. Inferior appendage subrectangular in ventral view, covered by long setae; apex excavated and with two acute and sclerotized process; dorsal hook long, almost reaching the inferior appendage apex; in lateral view, downturned. Preanal appendage short, rounded and bearing very long setae. Subgenital plate apparently absent. Tergum X sclerotized, deeply notched mesally, forming lateral curved processes. Phallus tubular, elongate and slender, slightly constricted mesally; with two short subapical spines; apex rounded and folded; ejaculatory duct sclerotized, straight and protruding apically.



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

568 **Holotype. BRAZIL: Paraná:** Céu Azul, Parque Nacional do Iguaçu, Rio Azul, 25°09'21"S  
 569 53°47'44"W, el. 510 m, 6–8 ix.2012, APM Santos, DM Takiya, ALH Oliveira, GA Jardim &  
 570 BHL Sampaio, Malaise trap, male (DZRJ).

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

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

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

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

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584 (Fig. 15)

585 **Adult male.** Length 2.5–2.8 mm (n=2). General color, in alcohol, dark brown. Head with no  
586 modifications. Three ocelli. Antenna simple, 20-articulated. Maxillary palpus 5-articulated; labial  
587 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.  
588 Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation reduced  
589 in both wings. Abdominal segment VI with pair of internal pouches in posterodorsal area.  
590 Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally. **Male**  
591 **genitalia.** Segment IX reduced dorsally; sternum subrectangular in ventral view, with anterior  
592 margin rounded; in lateral view narrower anteriorly than posteriorly. Inferior appendage  
593 subrectangular in ventral view, with peg-like setae; in lateral view, subrectangular; apex with  
594 acute corners; dorsal hook long, almost reaching the inferior appendage apex; in lateral view,



with apex slightly broader, almost straight, and truncate. Preanal appendage short, rounded and bearing very long setae. Subgenital plate apparently absent. Tergum X membranous and rounded in dorsal view. Phallus tubular, elongate and slender, slightly constricted mesally; with two curved subapical spines, one short and another long; apex rounded and sclerotized; ejaculatory duct sclerotized, sinuous, and protruding apically.

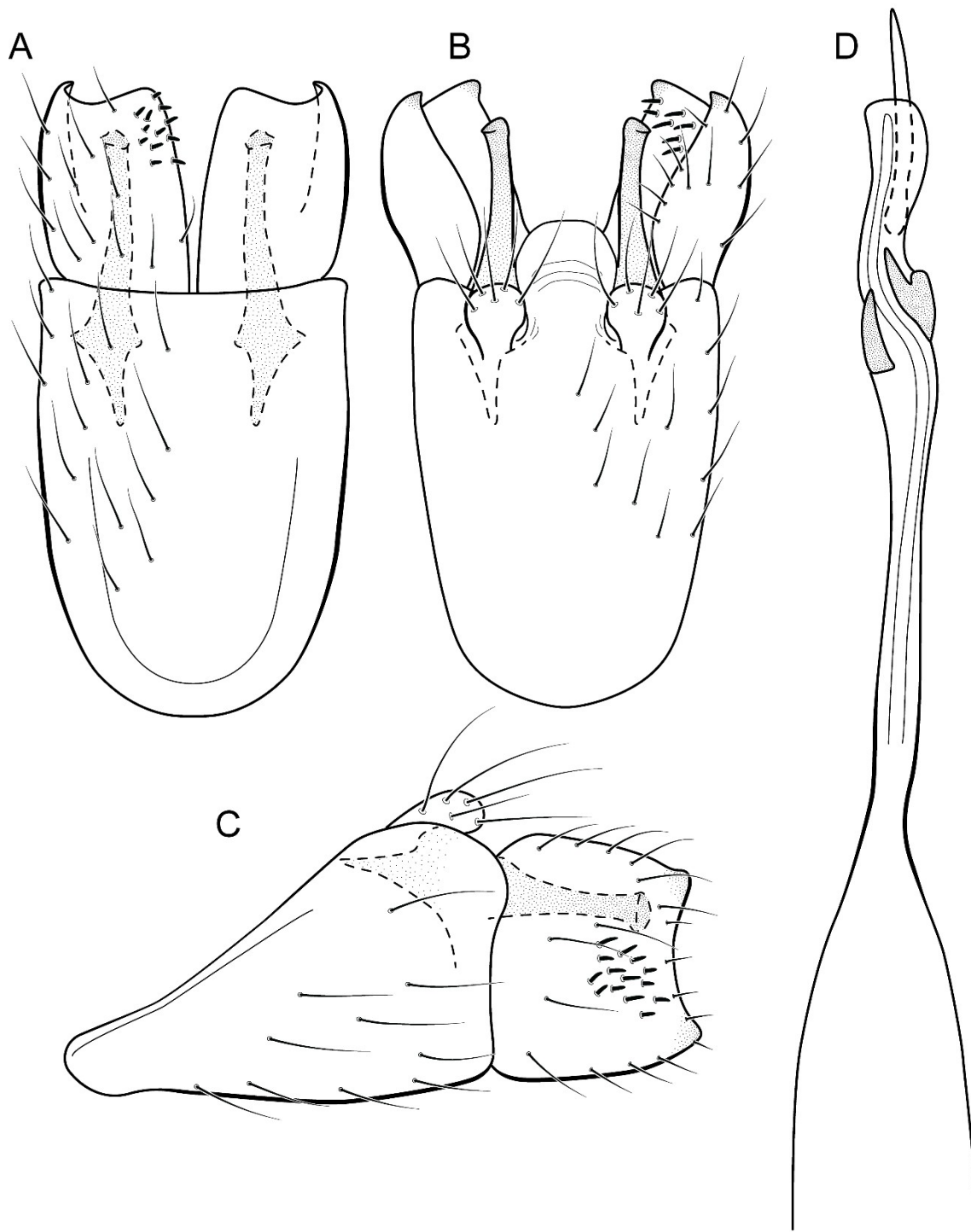
**Holotype.** BRAZIL: Mato Grosso do Sul: Bonito, Rio Formosinho, 21°10'16"S 56°26'47"W el. 275 m, 08–13.ix.2013, APM Santos & DM Takiya, Malaise trap, male (DZRJ).

**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 directed posteriorly and capitate (strongly curved and acute in *M. forceps* **sp. nov.** and in the other species of *campana* group); and by phallus with a longer spine on subapical area of phallus.

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.**



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

613 *Metrichia goiana* sp. nov.

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615 (Fig. 16)

616 **Adult male.** Length 1.8–2.0 mm (n=6). General color, in alcohol, dark brown. Head with no  
617 modifications. Three ocelli. Antenna simple, 18-articulated. Maxillary palpus 5-articulated, article  
618 IV broad and darkened; labial palpus 3-articulated. Mesoscutellum with transverse suture.  
619 Metascutellum subtriangular. Anterior femur with small acute apical process. Tibial spur formula  
620 1-3-4. Wing venation reduced in both wings. Abdominal segment V with pair of internal pouches  
621 and pair of dorsolateral brushes; segment VI with a transverse sclerotized plate posteriorly on  
622 dorsum; segment VII bearing specialized setae dorsally. Ventromesal process on segment VII  
623 absent. Segment VIII shorter ventrally than dorsally. **Male genitalia.** Segment IX reduced  
624 dorsally; sternum subrectangular in ventral view, with anterior margin rounded; in lateral view  
625 narrower anteriorly than posteriorly. Inferior appendage elongate and narrow in ventral view,  
626 covered by long setae; in lateral view, rounded; apex rounded and bearing a tooth-like projection;  
627 dorsal hook short, less than half length of inferior appendage; in lateral view, downturned.  
628 Preanal appendage short and bearing very long setae. Subgenital plate apparently absent. Tergum  
629 X membranous and truncate in dorsal view. Phallus tubular, elongate and slender, slightly  
630 constricted mesally; with two long, curved, subapical spines; apex ending into two sclerotized  
631 processes; ejaculatory duct sclerotized, straight and protruding apically.

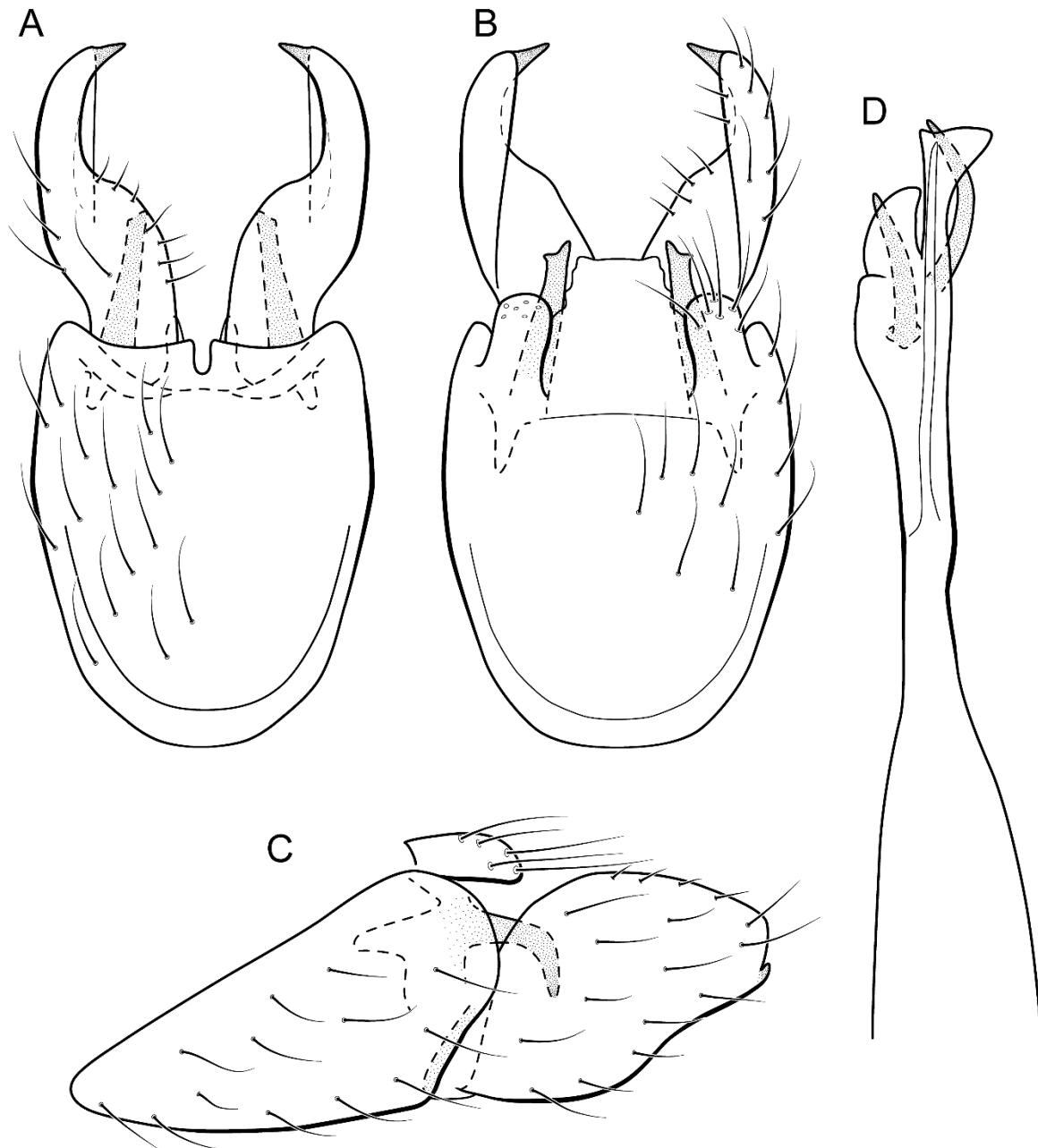
632 **Holotype. BRAZIL: Goiás:** Alto Paraíso de Goiás, afl. Rio Bartolomeu, 14°07'25"S  
633 47°30'30"W, el. 1165 m, 22–25.iii.2013, APM Santos & DM Takiya, Malaise trap, male (DZRJ).

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

635 **Etymology.** The species is named in reference to Goiás State.

636 **Remarks.** This is another member of *nigritta* group. Male genitalia of this species is similar to  
637 *M. potosina* Bueno-Soria and *M. ubajara* **sp. nov.**, due to rounded and elongate inferior  
638 appendage in lateral view. This new species differs from *M. ubajara* **sp. nov.** by the presence of  
639 an apical tooth on inferior appendages in lateral view, also present in *M. potosina*. *Metrichia*

640 *goiana* **sp. nov.** can be distinguished from *M. potosina* by the two long subapical spines on  
641 phallus, whereas *M. potosina* has three.



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

642 *Metrichia itabaiana* **sp. nov.**

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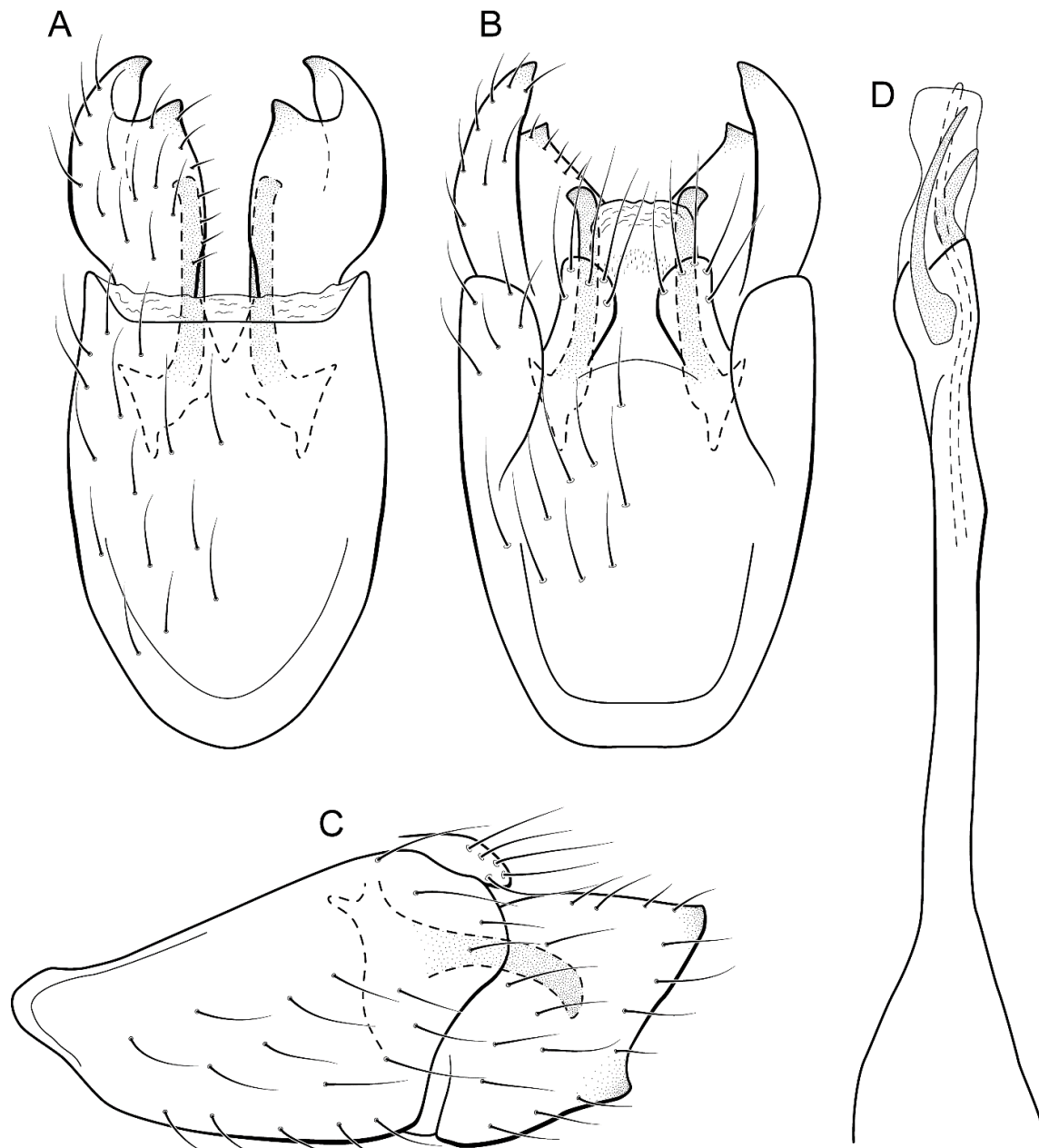
(Fig. 17, Fig. 26B)

**Adult male.** Length 1.8–2.1 mm (n=3). General color, in alcohol, brown. Head with no modifications. Three ocelli. 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, internally with pair of internal pouches; segment VII bearing specialized setae ventrally and dorsally. 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 in ventral view; in lateral view narrower anteriorly than posteriorly. Inferior appendage short, rounded, with apex slightly excavated in ventral view, covered by long setae; in lateral view, subtrapezoidal; apex excavated and with two acute and sclerotized process; dorsal hook short, almost half length of inferior appendage; in lateral view, slightly downturned. Preanal appendage elongate, but shorter than inferior appendage, and bearing very long setae. Subgenital plate apparently absent. Tergum X membranous and truncate in dorsal view. Phallus tubular, elongate and slender, slightly constricted mesally; with two long, curved, subapical spines; apex truncate and sclerotized; ejaculatory duct sclerotized, sinuous, and protruding apically.

**Holotype. Sergipe:** Areia Branca, Parque Nacional da Serra de Itabaiana, Rio dos Negros, 10°44'51"S 37°20'24"W, el. 208 m, 17.vi.2014, APM Santos, DM Takiya & WRM Souza, light trap, male (DZRJ).

**Paratypes.** Same data as holotype, 1 male (DZRJ), 1 male (MZUFBA); same data, except Riacho Água Fria, 10°45'17"S 37°20'32"W, el. 196 m, 17–19.vi.2014, APM Santos, DM Takiya, WRM Souza, Malaise trap, 2 males (MNRJ). **Goiás:** Alto Paraíso, afl. Rio Bartolomeu,

14°07'25"S 47°30'30"W, el. 1165 m, 22–25.iii.2013, APM Santos & DM Takiya, Malaise trap, 1  
male (DZRJ).



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

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

**Remarks.** This new species appears to be a member of the *campana* group because of internal pouches between abdominal segments VI and VII and pair of long subapical spines on phallus, but this species lacks the acute process on mesal area of phallus. Male genitalia of *M. itabaiana* **sp. nov.** resembles those of *M. campana* Flint and *M. vulgaris* **sp. nov.**, particularly, the excavated inferior appendages, with acute and darkened corners. However, this new species can be recognized by the very long curved subapical spines on phallus and the trapezoidal aspect of inferior appendages in lateral view.

Only two COI sequences were obtained for *M. itabaiana* **sp. nov.**, one from Sergipe (Northeastern Brazil) and another from Goiás (Midwestern Brazil). The COI divergence between these two samples was 1.9% and minimum interspecific distance was 19.4% in relation to *M. rafaelli* **sp. nov.**, which belongs to a different species group based on morphological features.

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

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(Fig. 18, Fig. 26C)

**Adult male.** Length 2.5–2.7 mm (n=2). General color, in alcohol, brown. Head with no modifications. Three ocelli. 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 IV with dorsal area expanded posteriorly bearing stout setae; segment VI with stout and striate setae; segment VII with stout and striate setae. Ventromesal process on segment VII absent. Segment VIII shorter ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally; sternum subpentagonal in ventral view; in lateral view narrower anteriorly than posteriorly. Inferior appendage very elongate bearing scale-like setae, in ventral view, curved inward apically; in lateral view, tapering to apex; apex rounded; dorsal hook short

and straight; in lateral view, slightly downturned. Preanal appendage elongate, but shorter than half length of inferior appendage, and bearing stout and striate setae. Subgenital plate apparently absent. Tergum X membranous and truncate in dorsal view. 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.

**Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, Rio Palmital, 22°25'34"S 44°32'52"W, el. 637 m, 07.iii.2008, LL Dumas, JL Nessimian & MR de Souza, light trap, male (DZRJ).

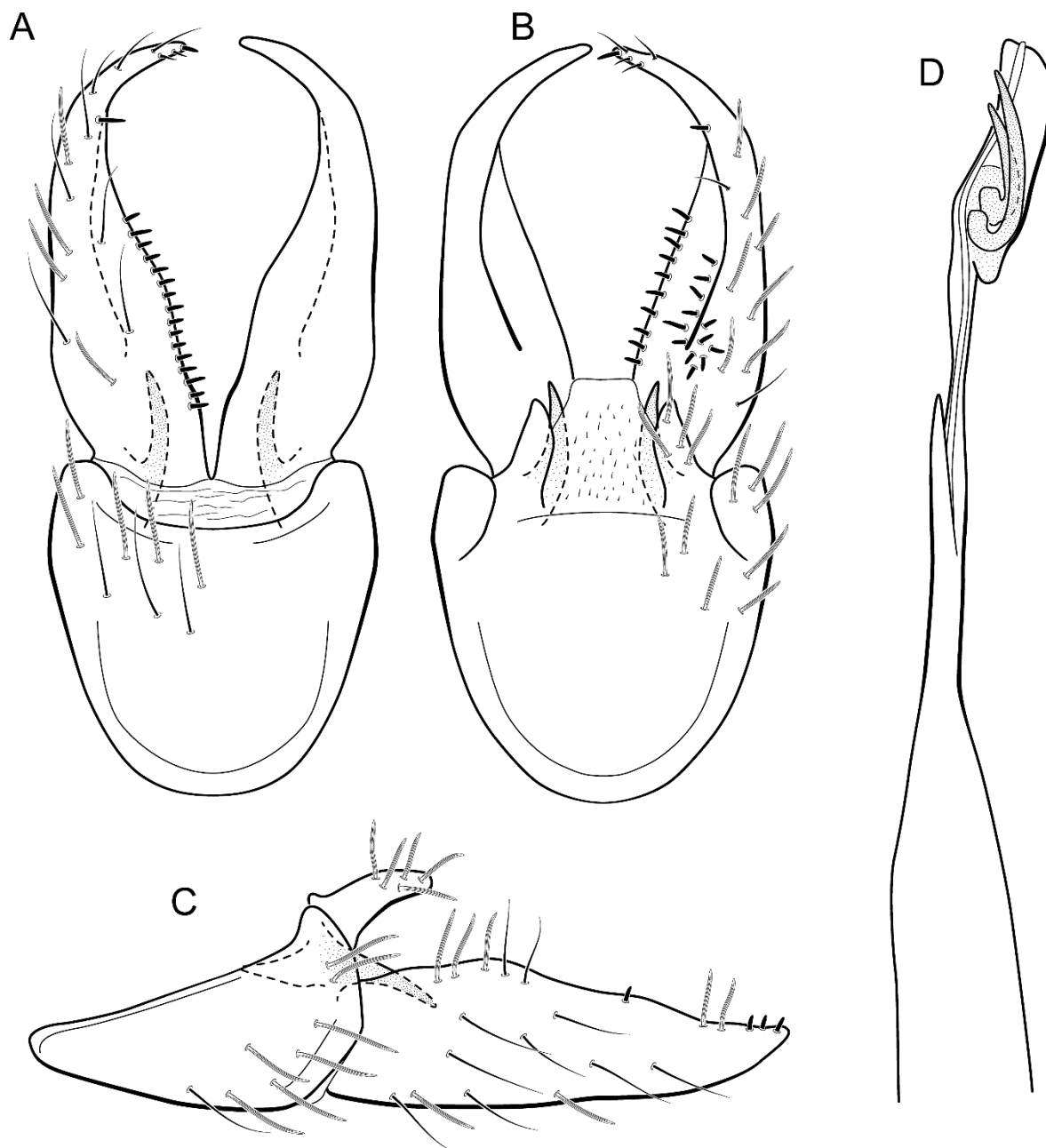
**Paratype. Brazil: Rio de Janeiro:** Teresópolis, Parque Nacional da Serra dos Órgãos, Rio Paqueta, 22°27'25"S 42°59'52"W, el. 1100 m, 15–18.ix.2011, APM Santos, DM Takiya, BM Vasconcelos, RA Carvalho, Malaise trap, 1 male (MNRJ).

**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.





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

717 ***Metrichia peluda* sp. nov.**

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719 (Fig. 19, Fig. 26D)

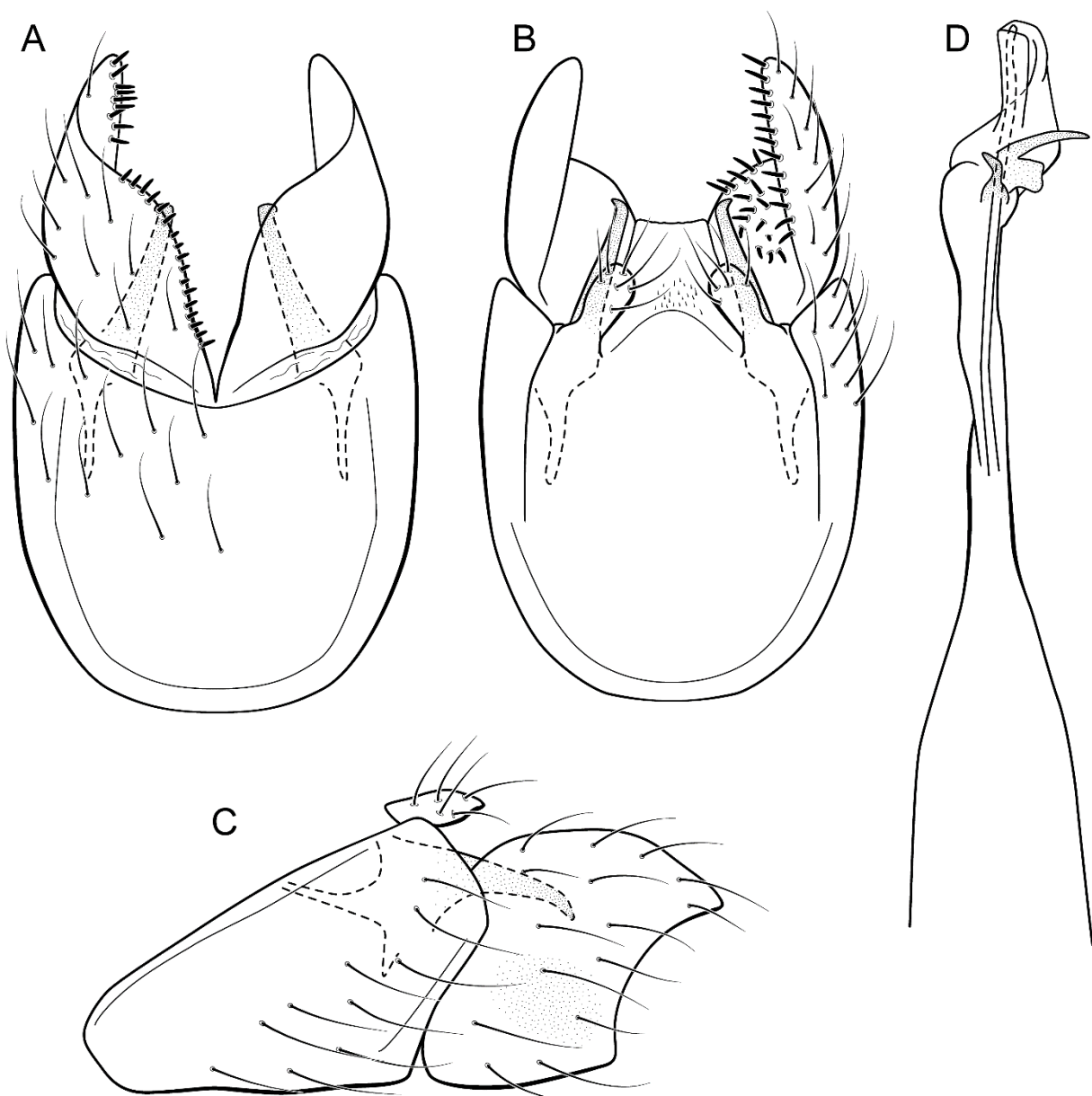
720 **Adult male.** Length 2.7–3.0 mm (n=3). General color, in alcohol, dark brown. Head with no  
721 modifications. Three ocelli. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

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 dorsolateral brushes of long setae; segment VI with dorsolateral brushes of long setae. Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally; sternum subrectangular in ventral view, with anterior margin rounded; in lateral view narrower anteriorly than posteriorly. Inferior appendage subtrapezoidal in ventral view, covered by long setae; in lateral view, rounded; apex excavated; dorsal hook short, almost half length of inferior appendage; in lateral view, slightly downturned. Preanal appendage short and bearing very long setae. Subgenital plate apparently absent. Tergum X membranous and truncate in dorsal view. Phallus tubular, elongate and slender, slightly constricted mesally; with two curved subapical spines, one short and another long; apex rounded and folded; ejaculatory duct sclerotized, straight and protruding apically.

**Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, 1st order tributary of Rio Palmital, 22°25'40"S 44°32'46"W, el. 584 m, 07.iii.2008, JL Nessimian, LL Dumas & MR de Souza, light trap, male (DZRJ).

**Paratypes.** Same data as holotype, 1 male (MNRJ); same data, except Rio Palmital, 22°25'34"S 44°32'52"W, el. 637 m, 07.iii.2008, LL Dumas, JL Nessimian & MR de Souza, light trap, 4 males (DZRJ).

**Etymology.** The name of this species refers to dense brushes of setae on dorsal area of male abdomen. From Portuguese, “peluda” means “hairy”.



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

743 **Remarks.** Males with modifications on abdominal segments V, VI, and VII suggest that this new  
 744 species belongs to the *campana* group. The general aspect of inferior appendages is somewhat  
 745 similar to *M. forceps* **sp. nov.** or *M. formosinha* **sp. nov.**, which are excavate posteriorly. But *M.*  
 746 *peluda* is readily identified by the dense brushes of setae on the dorsum of abdominal segments  
 747 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.**

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(Fig. 20)

**Adult male.** Length 2.0–2.5 mm (n=7). General color, in alcohol, dark brown. Head with no modifications. Three ocelli. 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 in ventral view, with anterior margin rounded; in lateral view narrower anteriorly than posteriorly. Inferior appendage short, rounded, with apex slightly excavated in ventral view, covered by long setae; in lateral view, rounded; apex slightly excavated; dorsal hook long, more than half length of inferior appendage; in lateral view, downturned. Preanal appendage elongate, but shorter than half length of inferior appendage, and bearing stout and striate setae. Subgenital plate apparently absent. Tergum X membranous and truncate in dorsal view. 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.

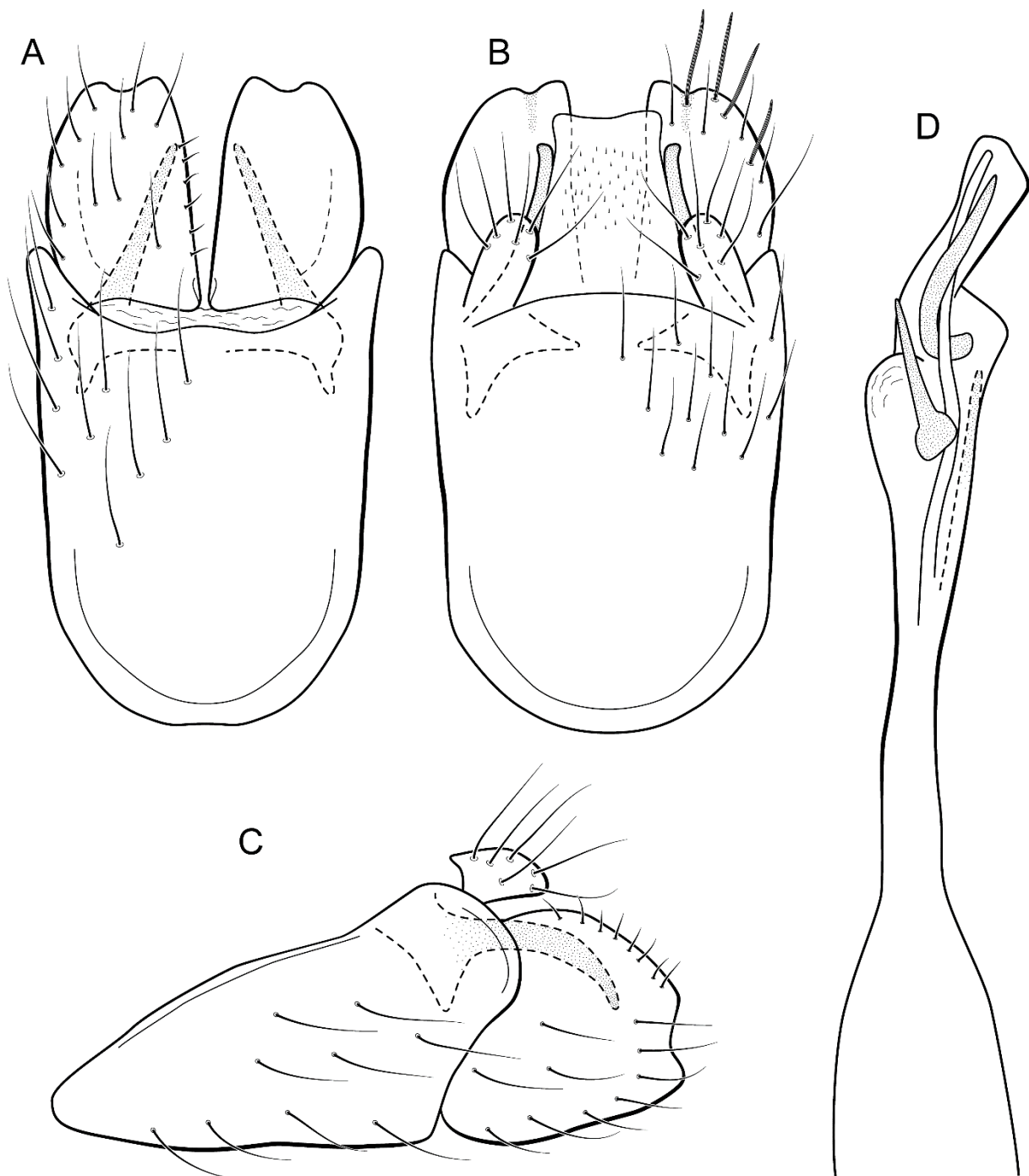
**Holotype male. BRAZIL: Ceará:** Ubajara, Parque Nacional de Ubajara, Rio das Minas, 03°50'03"S 40°54'18"W, el. 524, 17–18.ii.2013, DM Takiya, JA Rafael, RR Cavichioli & APM Santos, Malaise trap (CZMA).

**Paratypes.** Same data as holotype, 1 male (MZUFBA); same data, except Rio das Minas, 03°49'58"S 40°53'53"W, el. 420 m, 20–23.iv.2012, F Limeira-de-Oliveira et al., Malaise trap, 1 male (CZMA); same data, except 14–16.ii.2013, DM Takiya, JA Rafael, RR Cavichioli & APM Santos, 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.**



**Figure 20.** *Metrichia rafaelli* sp. nov., male genitalia: (A) ventral view; (B) dorsal view; (C) lateral view; (D) phallus, dorsal view.

788 *Metrichia simples* sp. nov.

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790 (Fig. 21, Fig. 26E)

**Adult male.** Length 2.1–2.2 mm (n=2). General color, in alcohol, brown. Head with no modifications. Three ocelli. 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 dorsally by a triangular plate; segment VII with a brush of short setae dorsally. Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally; sternum subpentagonal in ventral view; in lateral view narrower anteriorly than posteriorly. Inferior appendage subtrapezoidal in ventral view, covered by long setae; apex obliquely truncate; dorsal hook short, almost half length of inferior appendage; in lateral view, slightly downturned. Preanal appendage short, rounded and bearing very long setae. Subgenital plate apparently absent. Tergum X membranous and rounded in dorsal view. Phallus tubular, elongate and slender, slightly constricted mesally; with four subapical spines, three shorter and one longer and straight; apex rounded with a small sclerite; ejaculatory duct sclerotized and protruding apically.

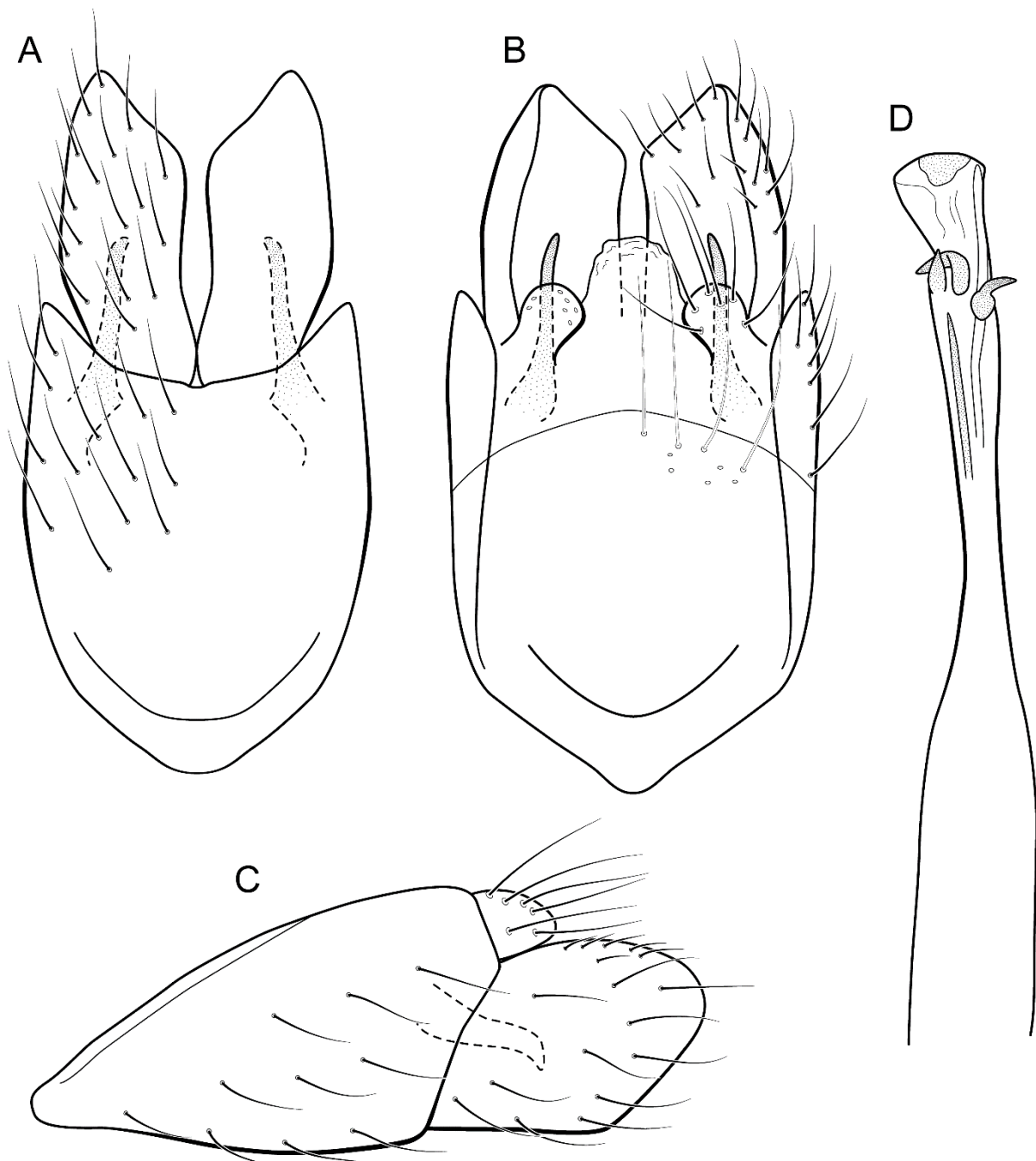
**Holotype. BRAZIL: Paraná:** Céu Azul, Parque Nacional do Iguaçu, Rio Azul, 25°09'21"S 53°47'44"W, el. 510 m, 6–8 ix.2012, APM Santos, DM Takiya, ALH Oliveira, GA Jardim & BHL Sampaio, Malaise trap, male (DZRJ).

**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.

**Remarks.** This species can be assigned to the *campana* group due to the pouches in abdominal segments VI and VII. The new species shares the general aspect of the genitalia with *M. quadrata* Flint, particularly, a quadrangular or rectangular shape of inferior appendages and the

816 two short subapical spines on phallus. *Metrichia simplex* **sp. nov.** can be recognized by the short  
 817 dorsal hook, reaching only the mesal area of inferior appendage (almost the same size of inferior  
 818 appendages in *M. quadrata*), obliquely truncate apex of the inferior appendages, and strongly  
 819 curved spines on phallus.



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



lateral view; (D) phallus, dorsal view.

820 ***Metrichia talhada* sp. nov.**

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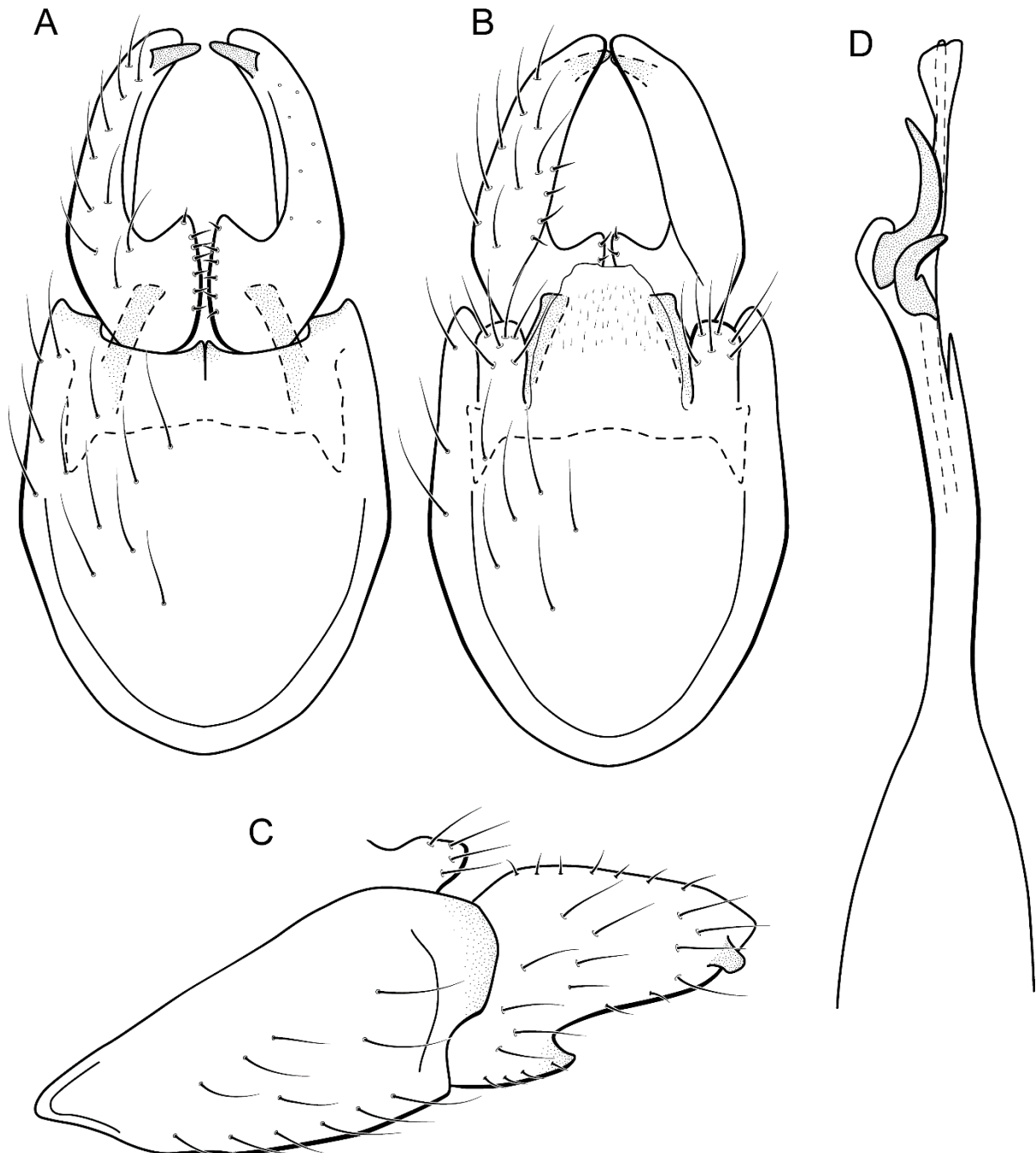
822 (Fig. 22)

823 **Adult male.** Length 1.8–2.0 mm (n=10). General color, in alcohol, brown. Head with no  
824 modifications. Three ocelli. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial  
825 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.  
826 Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation reduced  
827 in both wings. Abdominal segment V with pair of internal pouches and pair of dorsolateral  
828 brushes; segment VI with dorsolateral brushes of long setae; segment VII bearing specialized  
829 setae dorsally. Ventromesal process on segment VII absent. Segment VIII shorter ventrally than  
830 dorsally. **Male genitalia.** Segment IX reduced dorsally; sternum subpentagonal in ventral view;  
831 in lateral view narrower anteriorly than posteriorly. Inferior appendage elongate, with a deep C-  
832 shaped notch in ventral view; in lateral view, with an acute projection; apex rounded and  
833 bearing a tooth-like projection; dorsal hook short, less than half length of inferior appendage; in  
834 lateral view, downturned. Preanal appendage short, rounded and bearing very long setae.  
835 Subgenital plate apparently absent. Tergum X membranous and truncate in dorsal view. Phallus  
836 tubular, elongate and slender, slightly constricted mesally, with a median process; with two  
837 curved subapical spines, one short and another long; apex rounded and sclerotized; ejaculatory  
838 duct sclerotized, straight and protruding apically.

839 **Holotype. BRAZIL: Alagoas:** Quebrangulo, Reserva Biológica de Pedra Talhada, Rio  
840 Caranguejo, 09°15'26"S 36°25'08"W, el. 550 m, 19–28.vi.2014, APM Santos, DM Takiya, WRM  
841 Souza, Malaise trap, male (DZRJ).

842 **Paratypes.** Same data as holotype, 8 males (DZRJ), 7 males (MZUFBA).

843 **Etymology.** This species is named in reference to the type locality, the Reserva Biológica de  
844 Pedra Talhada.



**Figure 22.** *Metrichia talhada* sp. nov., male genitalia: (A) ventral view; (B) dorsal view; (C) lateral view; (D) phallus, dorsal view.

**Remarks.** The new species belongs to the *nigritta* group due to internal pouches between abdominal segments V and VI, phallus with two subapical curved spines and an acute process 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 stouter 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.**

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(Fig. 23)

**Adult male.** Length 1.9–2.1 mm (n=10). General color, in alcohol, brown. Head with no modifications. Three ocelli. 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 in ventral view; in lateral view narrower anteriorly than posteriorly. Inferior appendage elongate, with a deep C-shaped notch in ventral view; in lateral view, with an acute projection; apex with an acute projection; dorsal hook short, less than half length of inferior appendage; in lateral view, downturned. Preanal appendage short, rounded and bearing very long setae. Subgenital plate apparently absent. Tergum X membranous and truncate in dorsal view. 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.

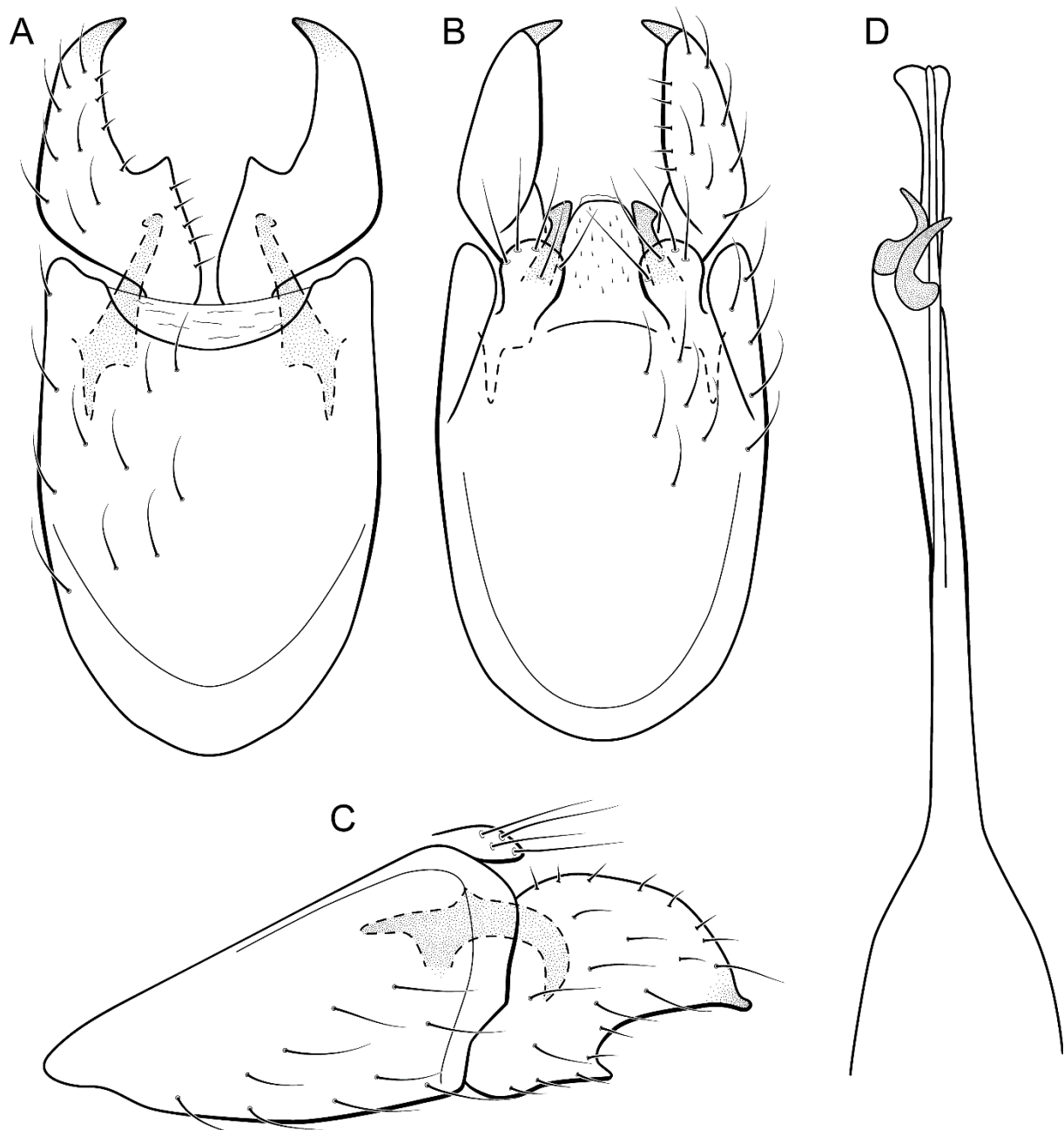
**Holotype. BRAZIL: Rio de Janeiro:** Teresópolis, Parque Nacional da Serra dos Órgãos, Rio Paqueta, 22°27'25"S 42°59'52"W, el. 1100 m, 15-18.ix.2011, APM Santos, DM Takiya, BM Vasconcelos, RA Carvalho, Malaise trap, male (DZRJ).

**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.

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



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

897 *Metrichia ubajara* sp. nov.

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899 (Fig. 24)

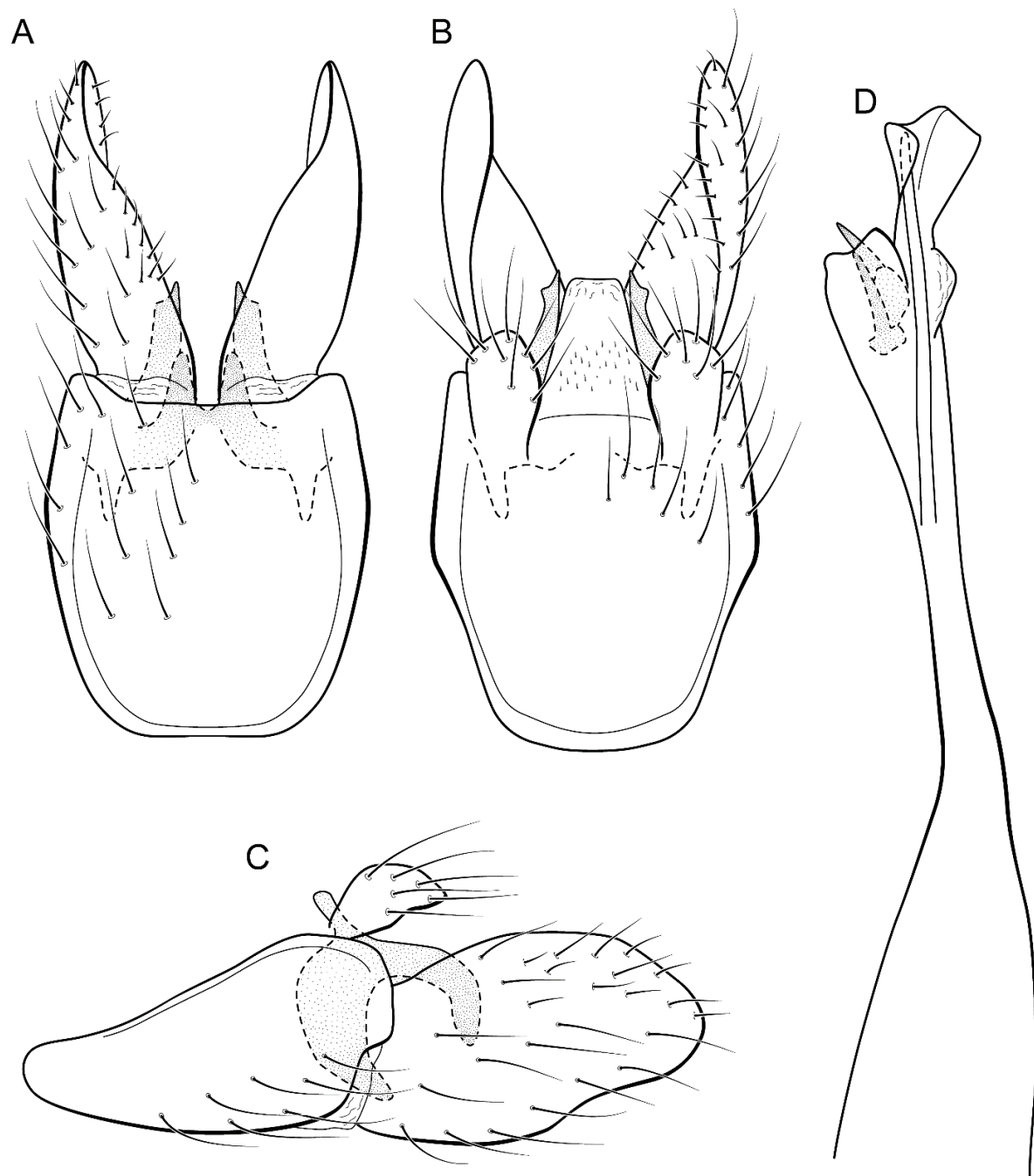
900 **Adult male.** Length 2.0–2.7 mm (n=12). General color, in alcohol, brown. Head with no  
 901 modifications. Three ocelli. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial  
 902 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.  
 903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

915 **Holotype. BRAZIL: Ceará:** Ubajara, Parque Nacional de Ubajara, Rio das Minas, 03°49'58"S  
 916 40°53'53"W, el. 420 m, 20–23.iv.2012, DM Takiya, JA Rafael, F Limeira-de-Oliveira et al.,  
 917 Malaise trap, male (CZMA).

918 **Paratypes.** Same data as holotype, 1 male (CZMA); same data, except 13-17.ix.2012, 25 males  
 919 (CZMA), 12 males (DZRJ); same data, except 18-30.xi.2012, 9 males (INPA); same data, except  
 920 14-16.ii.2013, 5 males (MZUFBA); same data, except Rio das Minas, 03°50'03"S 40°54'18"W,

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

923 **Etymology.** This species is named in reference to the Ubajara National Park, in the municipality  
924 with the same name, where the types were 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 wrapping the ejaculatory duct.

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

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(Fig. 25)

**Adult male.** Length 2.7–3.1 mm (n=12). General color, in alcohol, brown. Head with no modifications. Three ocelli. 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 in ventral view; in lateral view narrower anteriorly than posteriorly. Inferior appendage subtrapezoidal in ventral view, covered by long setae; in lateral view, subretangular; apex excavated, posterodorsal margin acute and sclerotized; dorsal hook long and stout, almost reaching the inferior appendage apex; in lateral view, downturned. Preanal appendage elongate, rounded and bearing very long setae. Subgenital plate apparently absent. Tergum X membranous and rounded in dorsal view. Phallus tubular, elongate



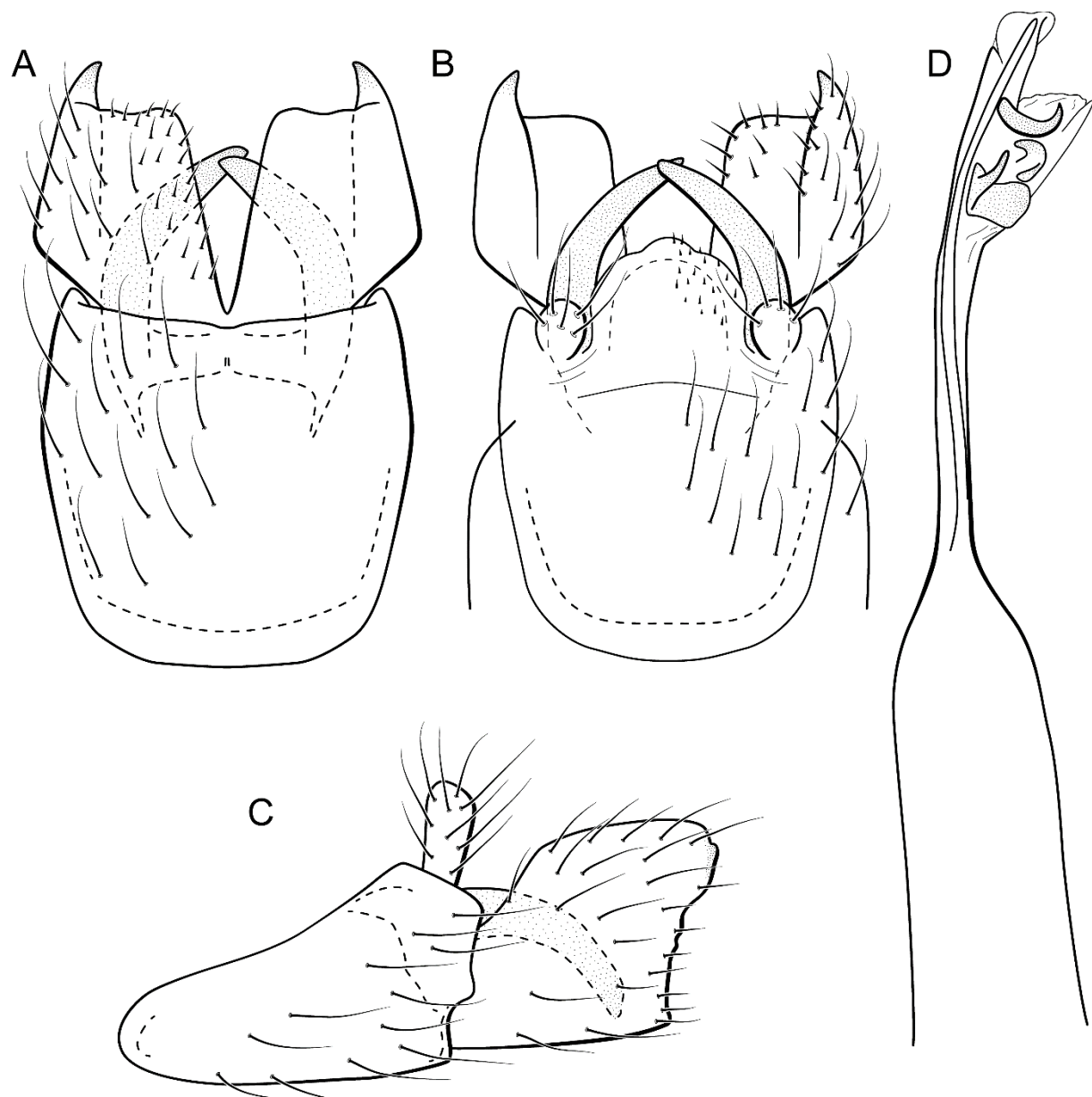
947 and slender, slightly constricted mesally; with two short subapical spines; apex rounded and  
948 folded; ejaculatory duct sclerotized and protruding apically.

949 **Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, Rio Palmital, 22°25'34"S 44°32'52"W, el. 637 m,  
950 07.iii.2008, LL Dumas, JL Nessimian & MR de Souza, light trap, male (DZRJ).

951 **Paratypes.** Same data as holotype, 1 male (DZRJ), 1 male (MNRJ); same data, except Rio das  
952 Pedras, 22°24'33"S 44°33'08"W, el. 706 m, 06.iii.2008, LL Dumas, JL Nessimian & MR de  
953 Souza, light trap, 4 males (MNRJ). **Ceará:** Ubajara, Parque Nacional de Ubajara, Rio Gameleira,  
954 03°50'25"S 40°54'19"W, el. 874 m, 20–22.iv.2012, F Limeira-de-Oliveira et al., Malaise trap, 1  
955 male (CZMA). **Goiás:** Alto Paraíso, afl. Rio Bartolomeu, 14°07'25"S 47°30'30"W, el. 1270 m,  
956 22–25.iii.2013, APM Santos & DM Takiya, Malaise trap, 3 males (DZRJ).

957 **Etymology.** This new species is named in allusion to its unusual wide distribution throughout  
958 Brazil. From the Latin “vulgaris” meaning “common”.

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



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

965 *Metrichia vulgaris* sp. nov. has an interesting distributional pattern, occurring in  
 966 Southeastern, Midwestern, and Northeastern Brazil. Besides the geographic distance among  
 967 localities where this species was found, they constitute very distinctive areas, for example, each  
 968 one is in a different biome (Atlantic Forest, Cerrado, and Caatinga) and in four different large  
 969 river basins (East North Atlantic, Southeast Atlantic, São Francisco, and Araguaia-Tocantins).  
 970 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.

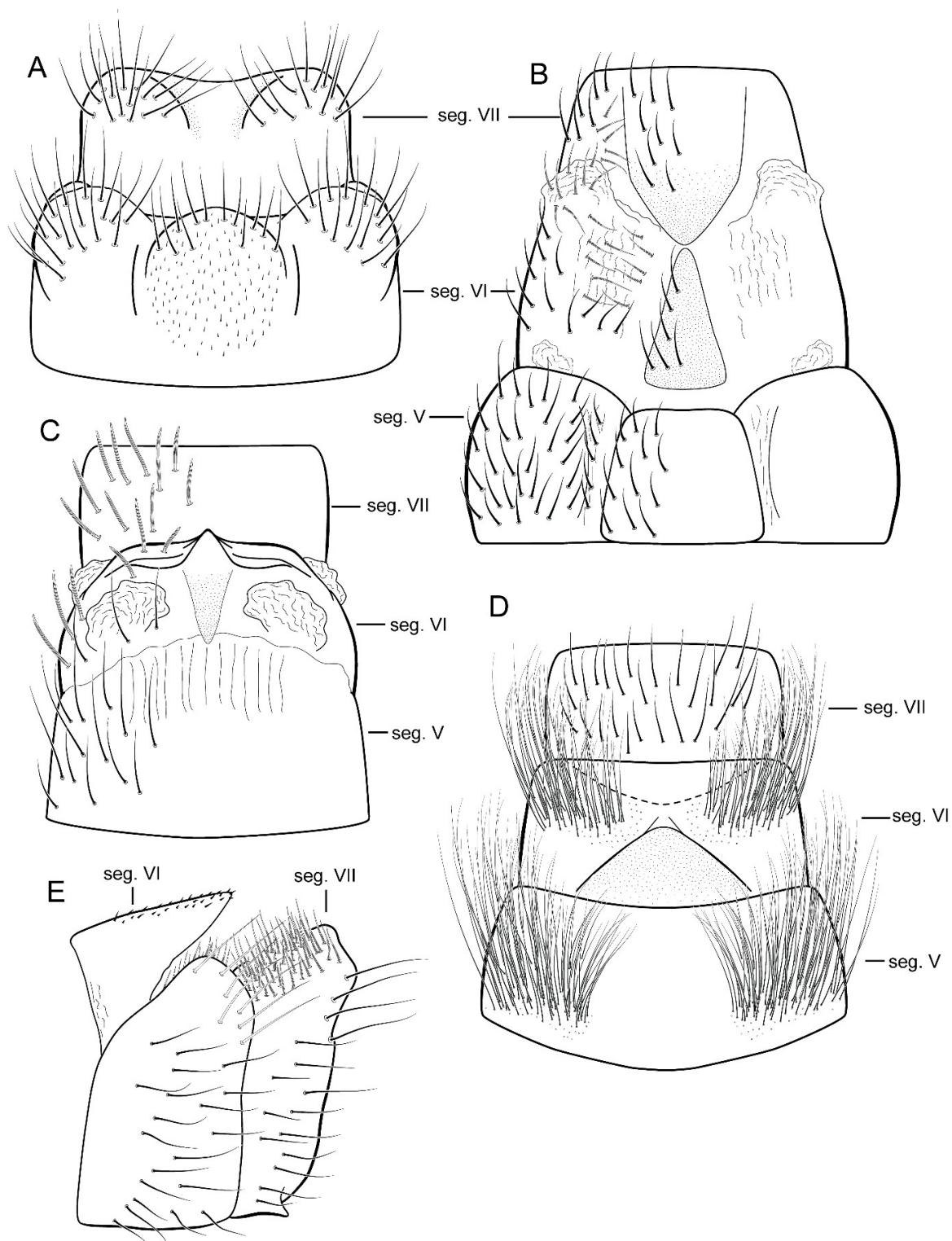
## DISCUSSION

Species limits previously defined based on morphological features were strongly corroborated by COI sequences, independent of the analyses implemented. Barcode studies with Neotropical caddisflies are still rare despite being a very common method nowadays. COI sequences seem to be very useful in the taxonomy of caddisflies, helping particularly in species delimitation and adult-immature associations (Zhou et al., 2007; Pauls et al., 2010, Zhou et al., 2011; Ruiter et al., 2013). 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.

In this work, we used DNA barcodes to corroborate previous species delimitation based on adult morphology. 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 cannot be used alone to determine ‘species’, they are useful tools to identifying ‘potential species’ (Puillandre et al., 2012; Talavera et al., 2013), especially in very diverse groups and/or with dubious questions on morphology-based identification. The wide distribution of *Metrichia vulgaris* **sp. nov.** could indicate the existence of different species, but here morphology and barcodes agreed to define only one species. Although GMYC overestimated the number of *Metrichia* species in our analysis, this method is an important tool for preliminary distinction when taxonomic information is poor.

Based on presence of abdominal modifications, such as internal pouches, external sclerotized plates, and brushes of long setae, and features of male genitalia, six species groups have been proposed for *Metrichia* (Flint, 1972; Bueno-Soria & Holzenthal, 2003). Although these modifications usually arise from segments V, VI, and VII, in general aspect, they are very distinctive from each other (Fig. 26), possibly representing non-homologous structures. Most of the species described herein fit in these groups and species with COI sequences represented five species groups. Except for *patagonica* group (represented by only one species, *M. bracui*), none of the species groups were recovered as monophyletic lineages in neighbor-joining (Fig. 2) or in BI (Supp. 4) analyses. Furthermore, the monophyly of *Metrichia* remains unclear, with COI sequences of other genera being more closely related to *Metrichia* sequences than sequences of other *Metrichia* species (Fig. 2). Nevertheless, relationships and monophyletic status of *Metrichia* and its species groups were not tested yet, due to the limited taxon sampling in our analyses.



**Figure 26.** Abdominal modifications of *Metrichia* species: (A) *M. acuminata* **sp. nov.**, segments VI and VII, dorsal view; (B) *M. itabaiana* **sp. nov.**, segments V, VI, and VII, dorsal view; (C) *M. 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**

1033 Consensus phylogram (50% majority-rule) from BI analyses of COI sequences (lnL= -5464.29).  
1034 Values displayed near branches are posterior probability.

# 1035 **K2P pairwise divergences**

1036 Table matrix with pairwise divergences of COI of specimens included of *Metrichia* and related  
1037 microcaddisflies.

# 1038 **ACKNOWLEDGEMENTS**

1039 We thank the Laboratório de Entomologia, UFRJ team for helping in field trips. Additional  
1040 specimens used in this study were provided by J. A. Rafael (INPA), M. L. Monné (Museu  
1041 Nacional, UFRJ), and C. H. Dietrich (University of Illinois at Urbana-Champaign).

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