

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

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5 **ALLAN P M SANTOS^{1,2}, DANIELA M TAKIYA¹ & JORGE L NESSIMIAN¹**

6 ¹Departamento de Zoologia, Instituto de Biologia, Universidade Federal do Rio de Janeiro,
7 Rio de Janeiro, Rio de Janeiro, Brazil.

8 ²Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Estado do Rio
9 de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil.

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11 Corresponding Author:

12 Laboratório de Entomologia, Depto. de Zoologia, Inst. de Biologia, Universidade Federal do
13 Rio de Janeiro, Caixa Postal 68044, Rio de Janeiro, RJ, 21941-971, Brazil.

14 Email address: allanpms@gmail.com

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17 ABSTRACT

18 *Metrichia* is assigned to the Ochrotrichiinae, a group of almost exclusively Neotropical
 19 microcaddisflies. *Metrichia* comprises over 100 described species and, despite its diversity,
 20 only one species has been described from Brazil so far. In this paper, we provide descriptions
 21 for 20 new species from 8 Brazilian states: *M. acuminata* **sp. nov.**, *M. azul* **sp. nov.**, *M. bonita*
 22 **sp. nov.**, *M. bracui* **sp. nov.**, *M. caraca* **sp. nov.**, *M. circuliforme* **sp. nov.**, *M. curta* **sp. nov.**,
 23 *M. farofa* **sp. nov.**, *M. forceps* **sp. nov.**, *M. formosinha* **sp. nov.**, *M. goiana* **sp. nov.**, *M.*
 24 *itabaiana* **sp. nov.**, *M. longissima* **sp. nov.**, *M. peluda* **sp. nov.**, *M. rafaeli* **sp. nov.**, *M.*
 25 *simples* **sp. nov.**, *M. talhada* **sp. nov.**, *M. tere* **sp. nov.**, *M. ubajara* **sp. nov.**, and *M. vulgaris*
 26 **sp. nov.** DNA barcode sequences (577 bp of the mitochondrial gene COI) were generated for
 27 13 of the new species and two previously known species of *Metrichia* resulting in 64
 28 sequences. In addition, COI sequences were obtained for other genera of Ochrotrichiinae
 29 (*Angrisanoia*, *Nothotrichia*, *Ochrotrichia*, *Ragatrichia*, and *Rhyacopsyche*). DNA sequences
 30 and morphological data were integrated to evaluate species delimitations. K2P pairwise
 31 distances were calculated to generate a neighbor-joining tree. COI sequences also were
 32 submitted to ABGD and GMYC methods to assess ‘potential species’ delimitation. Analyses
 33 showed a conspicuous barcoding gap among *Metrichia* sequences (highest intraspecific
 34 divergence: 4.8%; lowest interspecific divergence: 12.6%). Molecular analyses also allowed
 35 the association of larvae and adults of *Metrichia bonita* **sp. nov.** from Mato Grosso do Sul,
 36 representing the first record of microcaddisfly larvae occurring in calcareous tufa (or
 37 travertine). ABGD results agreed with the morphological delimitation of *Metrichia* species,
 38 while GMYC estimated a slightly higher number of species, suggesting the division of two
 39 morphological species, each one into two potential species. Because this could be due to
 40 unbalanced sampling and the lack of morphological diagnostic characters, we have
 41 maintained these two species as undivided.

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51 INTRODUCTION

52 The microcaddisfly genus *Metrichia* Ross, 1938 is included in the subfamily
53 Ochrotrichiinae, which also includes *Ochrotrichia* Mosely, 1934, *Angrisanoia* Özdikmen,
54 2008, *Nothotrichia* Flint, 1967, *Rhyacopsyche* Müller, 1879, and the recently erected
55 *Ragatrichia* Oláh & Johanson, 2011, all of them exclusively from New World. Based on adult
56 morphology, Harris & Armitage (1997) and Oláh & Johanson (2011) also suggested three
57 other genera to be included in this subfamily: *Dibusa* Ross, 1939, *Caledonotrichia* Sykora,
58 1967, and *Maydenoptila* Neboiss, 1977, from the U.S.A., New Caledonia, and Australia,
59 respectively. However, because diversification of main lineages of hydroptilids has not been
60 deeply studied, the placement of these genera remains dubious. As noted by Wells et al.
61 (2013), relationships of these microcaddisflies need to be studied based on rigorous analyses
62 including molecular data.

63 Currently, *Metrichia* includes 107 species, found from the U.S.A. to South America,
64 with highest known diversity in Central America (Flint, 1972; Marshall, 1979). *Metrichia* was
65 considered as a subgenus of *Ochrotrichia* due to similarities of adult morphology and almost
66 indistinguishable larvae (Flint, 1968). This subgeneric status was followed by Marshall
67 (1979), who also established the New World tribe that is now recognized as subfamily
68 Ochrotrichiinae. Wiggins (1996) provided additional information on larval morphology of
69 *Metrichia* and *Ochrotrichia*, reestablishing both as independent genera.

70 Diversity of Neotropical microcaddisflies is poorly known and usually several
71 undescribed species are found in collections or amongst recently collected material when
72 examined by experts, even in localities previously studied by trichopterologists. This likely
73 occurs because Hydroptilidae are very small and have complex male genitalia, making them
74 difficult to observe by lower magnification microscopes and to understand homologies among

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85 some structures. Only one species of *Metrichia* has been described from Brazil so far, *M.*
86 *pernambucana* Souza & Santos, 2013, but larvae have been commonly identified from several
87 localities (e.g., Pes et al., 2005; Spies & Froehlich, 2006; Spies et al., 2009). It is not
88 surprising that material studied herein recently collected from different river basins in Brazil
89 (Fig. 1, Supp. 1, Supp. 2) revealed so many undescribed species.



90
91 **Figure 1.** Localities (●), distributed on seven of the large river basins of Brazil, where
92 *Metrichia* specimens studied herein were collected.

93
94 Most *Metrichia* species exhibit a more restricted distribution, in other words, each
95 species has been recorded only from type-localities or relatively few close localities. Herein

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削除: Identification based mainly on features of male genitalia, revealed some interesting patterns, for example,

106 | we describe one species with a wide distribution in Brazil, occurring in three very distinctive
 107 | biomes: Atlantic Forest (Southeastern Brazil), Caatinga (Northeastern Brazil), and Cerrado
 108 | (Centralwestern Brazil). Although such wide distributions are not common in *Metrichia*,
 109 | similar examples are known for other Neotropical microcaddisflies, such as *Oxyethira tica*
 110 | Holzenthal & Harris, 1992, recorded from Mexico, Central, and South America (Flint et al.,
 111 | 1999).

112 | Although molecular tools have become common in taxonomic studies to help in
 113 | species delimitation, their use is still rare with Neotropical caddisflies. Using sequences of the
 114 | mitochondrial cytochrome oxidase I gene (COI), the standard DNA barcode region for
 115 | animals (Hebert et al., 2003), Pauls et al. (2010) were able to corroborate two new species of
 116 | *Smicridea* (*Smicridea*) McLachlan, 1871 from Chile, defined with morphological characters.
 117 | In most studies with caddisflies, divergence in COI sequences has shown clear differences
 118 | between intraspecific and interspecific variation, the so-called 'barcoding gap' (Zhou et al.,
 119 | 2007; Pauls et al., 2010; Ruiter et al., 2013). Some species delimitation approaches rely solely
 120 | in distinguishing intra- and interspecific divergence, such as the Automatic Barcoding Gap
 121 | Discovery (ABGD) (Puillandre et al., 2012). More sophisticated methods invoke coalescence
 122 | and speciation models, such as the General Mixed Yule Coalescent (GMYC), and are
 123 | considered more robust for identifying lineages when intra- and interspecific divergences
 124 | overlap (Pons et al., 2006). Integrating independent data (*e.g.*, morphology and DNA
 125 | sequences) and using different approaches are particularly interesting for the taxonomy of
 126 | diverse and complex groups, such as microcaddisflies. In this work, we applied both ABGD
 127 | and GMYC methodologies to evaluate our initial morphological identification.

128 | Besides its use for species delimitation, DNA taxonomy has a valuable role in making
 129 | associations between immature and adult stages of caddisflies (Graf et al., 2005; Waringer et
 130 | al., 2007; Zhou et al., 2007; Ruiter et al., 2013). Traditional techniques to associate larvae and

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adults are more difficult because they involve rearing larvae in the laboratory (not an easy task for many caddisfly groups) or luck in finding pharate adults in field. Indirect association of stages, for example, by collecting adults and larvae at the same locality, can result in misidentification, because different species of the same genus frequently co-occur.

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Larvae of *Metrichia* have been associated for only two species: *M. nigrutta* (Banks, 1907) described by Edwards & Arnold (1961) and illustrated by Wiggins (1996), and *M. juana* (Flint, 1964) 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.

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In both works, specific names were not provided, as authors did not have respective adults. *Metrichia* larvae build a typical oval purse-like case, made of silk, usually covered with algae filaments (Wiggins, 1996; Pes et al., 2005), and sometimes also having sand grains (Botosaneanu & Flint, 1982). Cases of some larvae that have not yet been associated with adults show a pair of dorsal "chimneys", an uncommon feature also described and illustrated by Müller (1879, 1880) for *Dicaminus ladislavii* Müller, 1879, 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's work.

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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 surfaces. 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. 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 &

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删除: 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.

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删除: In fact, there are no hydroptilids reported from inhabiting calcareous tufa so far.

194 Gale, 1997). Although the importance of microbes on travertine formation is recognized by
195 creating surfaces for crystal nucleation, our knowledge of the importance of
196 macroinvertebrates in this process is still poor (Drysedale, 1998; 1999; Paprocki et al., 2003).
197 Studies by Drysdale (1998, 1999) pointed out that aquatic insects play an important role in
198 travertine biogenesis in Australian springs, especially *Cheumatopsyche* Wallengreen, 1891
199 (*Hydropsychidae*) larvae. Paprocki et al. (2003) also found another *Hydropsychidae*
200 (*Smicridea*) to be an important organism in modifying travertine morphology in Venezuela.

202 MATERIAL AND METHODS

203 Morphological study

204 Specimens were collected manually (larvae or diurnal active adults) or using Malaise
205 or light traps, and then fixed in 96% ethanol. Collecting permits in Brazil were issued by
206 Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) (SISBIO 43047 and
207 14591). To observe genital structures, abdomen of males were removed and cleared in a
208 heated solution of 10% KOH for 20 minutes. Then, abdomens were mounted in temporary
209 slides, which were used to draw pencil sketches with compound microscope equipped with
210 camera lucida. Vector graphics were traced in Adobe Illustrator CS6 (Adobe Systems Inc.)
211 using pencil sketches as templates. Descriptions provided here were made with DELTA
212 software (Description Language for Taxonomy) (Dallwitz et al., 1999). Terminology used
213 throughout this paper follows that provided by Marshall (1979) and Bueno-Soria &
214 Holzenthal (2003). Types for newly described species are deposited at Coleção Entomológica
215 Prof. José Alfredo Pinheiro Dutra, Departamento de Zoologia, Universidade Federal do Rio
216 de Janeiro, Rio de Janeiro (DZRJ); Museu Nacional, Universidade Federal do Rio de Janeiro,
217 Rio de Janeiro (MNRJ); Instituto Nacional de Pesquisas da Amazônia, Manaus (INPA);

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(

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in Australian travertines studied.

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229 Coleção Zoológica do Maranhão (CZMA); and Museu de Zoologia da Universidade Federal
230 da Bahia, Salvador (MZUFBA).

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

243 DNA extraction, PCR, and sequencing

244 Genomic DNA was extracted from head and thorax (or from the entire body) of fresh
245 material using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany), without tissue
246 maceration. After extraction, specimens were returned to ethanol and deposited in DZRJ
247 collection as a DNA voucher. COI fragments were amplified using pair of primers: HCO-
248 2198 ([5'-TAAACTTCAGGGTGACCAAAAAATCA-3'](#)) in combination with LCO-1490
249 ([5'-GGTCAACAAATCATAAAGATATTGG-3'](#)) (Folmer et al., 1994) or C1-J-1718 ([5'-](#)
250 [GGAGGATTTGGAAATTGATTAGTTCC-3'](#)) (Simon et al., 1994). Polymerase chain
251 reaction (PCR) conditions [were as follows](#): initial denaturation at 94 °C for 3 min; 35 cycles
252 of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 2

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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 barcodes sequenced and 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 cebollati</i> (Angrisano, 1995)	ENT 2199 ♂	Brazil: Goiás: Alto Paraíso de Goiás	-
<i>Betrichia bispinosa</i> Flint, 1974	ENT 2337 ♂	Brazil: Amapá	KU094961 ^b
<i>Nothotrichia cautinensis</i> Flint, 1983	-	-	KC559534 ^a
<i>Nothotrichia tupi</i> Holzenthal & Harris, 1992	ENT 2460 ♂	Brazil: Minas Gerais: Catas Altas	KU743400
<i>Ochrotrichia caatinga</i> Souza, Santos & Takiya, 2014	ENT 2472 ♂	Brazil: Ceará: Ubajara	KU743401
<i>Ochrotrichia patulosa</i> (Wasmund & Holzenthal, 2007)	ENT 2473 ♂	Brazil: Ceará: Ubajara	KU743402
<i>Ochrotrichia</i> sp. CR1	ENT 2279 ♂	Costa Rica: Puntarenas	KU094950 ^b
<i>Oxyethira tica</i> Holzenthal & Harris, 1992	ENT 0057 ♂	Brazil: Pará: Carajás	KU094940 ^b
<i>Ragatrichia</i> sp. BR1	ENT 2338 ♂	Brazil: Amapá	KU743403
<i>Rhyacopsyche dikrosa</i> Wasmund & Holzenthal, 2007	ENT 0122 ♂	Brazil: Rio de Janeiro: Teresópolis	KU094952 ^b
<i>Rhyacopsyche torulosa</i> Flint, 1971	ENT 2277 ♂	Costa Rica: Puntarenas	KU743404
<i>Metrichia acuminata</i> sp. nov.	ENT 2192 ♂	Brazil: Alagoas: Quebrangulo	KU743406
	ENT 2282 ♂		KU743427
	ENT 2284-5 ♂		KU743428-29
	ENT 2779 ♂		KU743452
<i>Metrichia amplitudinis</i> Bueno-Soria & Holzenthal, 2003	ENT 2278 ♂	Costa Rica: Puntarenas	KU743425
<i>Metrichia bonita</i> sp. nov.	ENT 2200-4 larvae	Brazil: Mato Grosso do Sul: Bonito	KU743409-13
<i>Metrichia bonita</i> sp. nov.	ENT 2208-10 ♂	Brazil: Mato Grosso do Sul: Bonito	KU743417-19
<i>Metrichia braconii</i> sp. nov.	ENT 2508-11 ♂	Brazil: Rio de Janeiro: Itatiaia	KU743444-47
<i>Metrichia caraca</i> sp. nov.	ENT 2195 ♂	Brazil: Minas Gerais: Catas Altas	KU743408
	ENT 2280 ♂		KU743426
	ENT 2461-5 ♂		KU743434-38
<i>Metrichia caraca</i> sp. nov.	ENT 2292 ♂	Brazil: Minas Gerais: São Roque de Minas	KU743432
<i>Metrichia circuliforme</i> sp. nov.	ENT 2835-7 ♂	Brazil: Rio de Janeiro: Itatiaia	KU743455-57
	ENT 2839-40 ♂		KU743459-60
	ENT 2843-4 ♂		KU743462-63
<i>Metrichia curta</i> sp. nov.	ENT 2838 ♂	Brazil: Rio de Janeiro: Itatiaia	KU743458

	ENT 2846-8 ♂		KU743464-66
<i>Metrichia formosinha</i> sp. nov.	ENT 2205-7 ♂	Brazil: Mato Grosso do Sul: Bonito	KU743414-16
<i>Metrichia itabaiana</i> sp. nov.	ENT 2190 ♂	Brazil: Sergipe: Areia Branca	KU743405
<i>Metrichia itabaiana</i> sp. nov.	ENT 2220-1 ♂	Brazil: Goiás: Alto Paraíso de Goiás	KU743424
<i>Metrichia juana</i> (Flint, 1964)	ENT 2850-1 ♂	Puerto Rico	KU743467-68
<i>Metrichia longissima</i> sp. nov.	ENT 2330 ♂	Brazil: Rio de Janeiro: Teresópolis	KU743433
<i>Metrichia longissima</i> sp. nov.	ENT 2841 ♂	Brazil: Rio de Janeiro: Itatiaia	KU743461
<i>Metrichia rafaelli</i> sp. nov.	ENT 2288-9 ♂	Brazil: Ceará: Ubajara	KU743430-31
	ENT 2193 ♂		KU743407
<i>Metrichia talhada</i> sp. nov.	ENT 2214 ♂	Brazil: Alagoas: Quebrangulo	KU743420-22
	ENT 2216-7 ♂		KU743451
	ENT 2776 ♂		
<i>Metrichia tere</i> sp. nov.	ENT 2773-5 ♂	Brazil: Rio de Janeiro: Teresópolis	KU743448-50
<i>Metrichia vulgaris</i> sp. nov.	ENT 2218 ♂	Brazil: Goiás: Alto Paraíso de Goiás	KU743423
<i>Metrichia vulgaris</i> sp. nov.	ENT 2466-70 ♂	Brazil: Minas Gerais: Catas Altas	KU743439-43
<i>Metrichia vulgaris</i> sp. nov.	ENT 2833-4 ♂	Brazil: Rio de Janeiro: Itatiaia	KU743453-54

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

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Sequence editing, alignment, and analyses

Forward and reverse sequences of each sample were assembled and manually edited in Sequencher 4.1 (Gene Codes, Ann Arbor, Michigan, USA). Sequences were verified with the Blast tool in GenBank to check for contamination. Subsequently, 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).

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

285 COI sequences. Branch support of neighbor-joining tree was assessed with 1,000
 286 [pseudoreplicates](#) of non-parametric bootstrap (Felsenstein, 1985).
 287 BI analysis was conducted with MrBayes v. 3.2.2 (Ronquist *et al.*, 2012) with four
 288 independent runs, each one with four MCMC chains running for 50,000,000 generations, with
 289 sample frequency of 5,000. Convergence of sampled parameters was checked in Tracer v. 1.5
 290 (Rambaut & Drummond, 2007) and the first 10% of sampled trees and parameters [discarded](#)
 291 as burnin. GTR+I+G was the best fit model selected by Akaike Information Criterion (AIC)
 292 with jModeltest v. 0.1.1 (Posada, 2008) and it was applied in BI analysis in MrBayes. Branch
 293 support was assessed by posterior probability (PP), presented on a 50% majority consensus
 294 tree.

295 ABGD analysis was run using [the](#) on-line version available in
 296 <http://www.wabi.snv.jussieu.fr/public/abgd/>, where [the](#) COI alignment was uploaded. The
 297 analysis was conducted with the following settings: Pmin=0.001; Pmax=0.1; steps=20;
 298 relative gap width=1.0, also based on K2P model. This method statistically infers the DNA
 299 barcode gap in a single locus alignment, partitioning the data based on this gap in putative
 300 species (Puillandre *et al.*, 2012).

301 The GMYC analysis (Pons *et al.*, 2006; Fujisawa & Barraclough, 2013) was
 302 performed in R (R Development Core Team 2010) using the SPLITS package (Ezard *et al.*,
 303 2009) with single-threshold method. Basically, the method estimates branching patterns on an
 304 ultrametric tree, identifying the most likely transition point from coalescent to speciation
 305 branching. The ultrametric tree used here was obtained with BEAST v. 1.8 (Drummond *et al.*,
 306 2012) under a relaxed uncorrelated molecular clock (Drummond *et al.*, 2006). The node
 307 including *Ochrotrichia* species was calibrated based on fossil evidence with a lognormal
 308 distribution offset at 20 mya and $\log(\text{mean}) = 2.8$ to represent the possible range of 20-140
 309 mya (Wells & Wichard, 1989); and the divergence of Ochrotrichiinae was calibrated based on

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312 Malm et al. (2013) with a normal distribution with mean 82.17 ± 12 mya. The BEAST
313 analysis ran for 200,000,000 generations, sampled every 10,000 generations. Convergence
314 was verified with Tracer and a maximum credibility tree was written using TreeAnotator,
315 discarding the first 10% as burnin.

316

317 RESULTS

318 NJ (Fig. 2) and BI (Supp. 4) trees corroborated morphological identification, with all
319 14 species of *Metrichia* with more than a single specimen recovered as monophyletic lineages
320 with 100% bootstrap support. ABGD also returned the same species as they were previously
321 delimited based on morphological features. A robust 'barcoding gap' was found among
322 *Metrichia* species (Fig. 3, Table 2). The maximum intraspecific divergence was observed
323 within *Metrichia vulgaris* **sp. nov.** (0.048). The minimum interspecific divergence was found
324 between specimens of *Metrichia talhada* **sp. nov.** and *Metrichia tere* **sp. nov.** (0.126).

325

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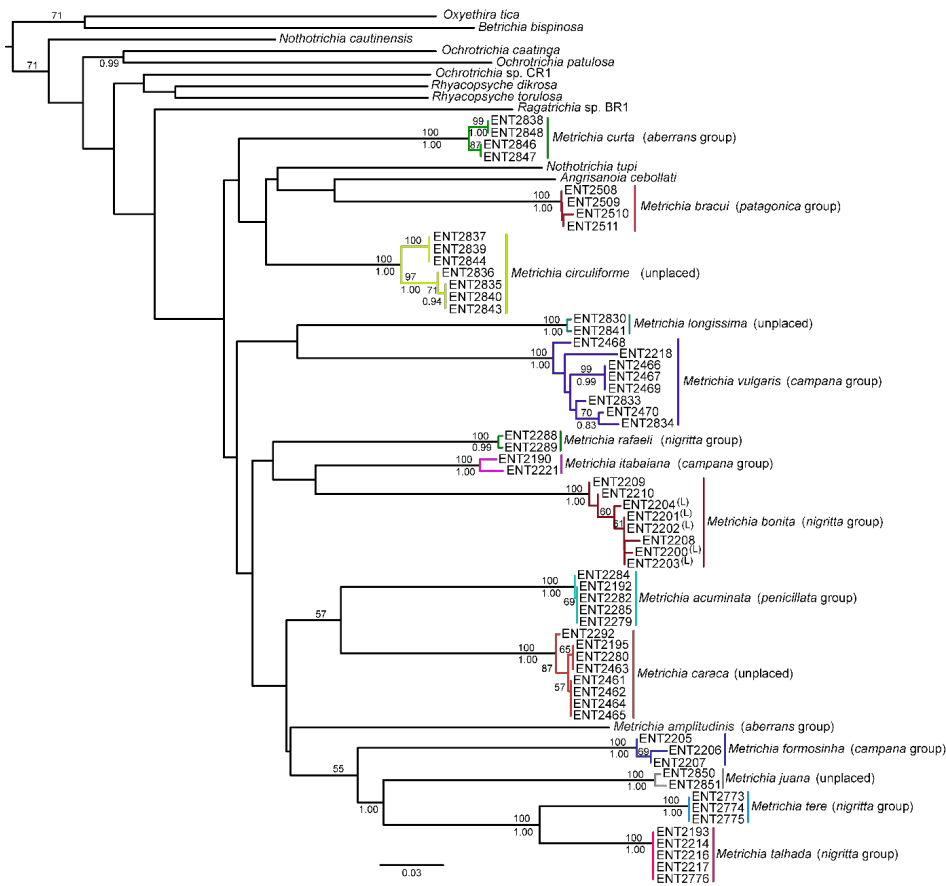


Figure 2. Neighbor-joining tree of COI sequences of *Metrichia* species based on K2P distances. Numbers above and below branches are, respectively, NJ bootstrap support and posterior probabilities from BI analysis. Details of specimens are in Tables 1 and 2; K2P distances matrix is in Supp. 5.

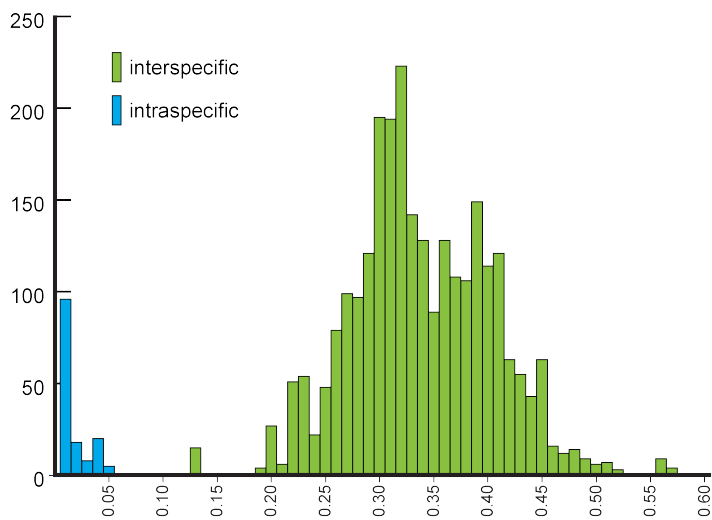


Figure 3. Histogram of the number of pairwise comparisons of intra- (blue) and interspecific (green) K2P divergences among 15 *Metrichia* species with COI sequences sampled.

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

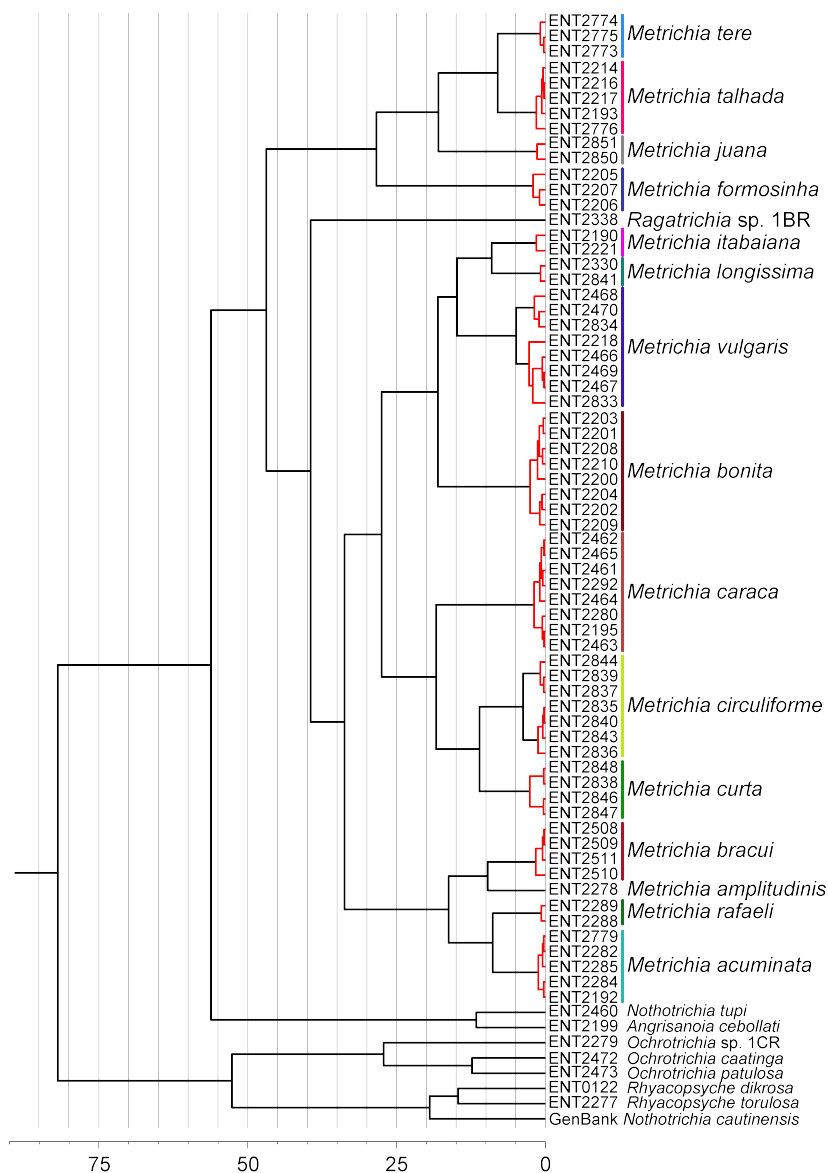


Figure 4. Maximum credibility time tree from the BEAST analysis based on COI sequences of *Metrichia* and other Ochrotrichiinae. Red branches represent species estimated by GMYC using single threshold in SPLITS. Timescale in millions of years.

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

348 In all analyses performed using DNA barcode, *Metrichia* larvae collected in
349 calcareous tufa were consistently associated with adult males of *Metrichia bonita* **sp. nov.**
350 Therefore, in the following section, we describe [these](#) larvae within that species.
351

352 SPECIES DESCRIPTIONS

353 *Metrichia acuminata* **sp. nov.**

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

355 (Fig. 5, Fig. 26A)

356 **Adult male.** Length 2.1–2.5 mm (n=5). General color, in alcohol, brown. Head with no
357 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial
358 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
359 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
360 wings. Abdominal segment IV with pair of internal pouches in posterior area; segment V with
361 pair of internal pouches; segment VI with tergum as a sclerotized rounded plate, surrounded
362 by long setae (Fig. 26A); segment VII bearing a brush of very long setae dorsolaterally (Fig.
363 26A). Ventromesal process on segment VII present. Segment VIII shorter ventrally than
364 dorsally and bearing a brush of long setae dorsally. **Male genitalia.** Segment IX reduced
365 dorsally; sternum subrectangular, with anterior margin rounded (Fig. 5A); in lateral view
366 narrower anteriorly than posteriorly (Fig. 5C). Inferior appendage covered by long setae;
367 subtrapezoidal in ventral view (Fig. 5A); in lateral view, subtrapezoidal, apex with acute
368 corners (Fig. 5C). Dorsal hook short, almost half length of inferior appendage; in lateral view,

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

376 **Holotype. BRAZIL: Ceará:** Ubajara, Parque Nacional de Ubajara, Cachoeira do Gameleira,
377 03°50'21"S 40°54'23"W, el. 880 m, 23.iv.2012, DM Takiya & JA Rafael cols., light trap, male
378 (CZMA).

379 **Paratypes.** Same data as holotype, except, Rio das Minas, 03°50'03"S 40°54'18"W, el. 524
380 m, 13–17.ix.2012, JA Rafael et al., Malaise trap, 2 males (INPA); same data, except 14–
381 16.ii.2013, DM Takiya, JA Rafael, RR Cavichioli & APM Santos, Malaise trap, 2 males
382 (DZRJ). **Alagoas:** Quebrangulo, Reserva Biológica de Pedra Talhada, Rio Caranguejo,
383 09°15'26"S 36°25'08"W, el. 550 m, 19–28.vi.2014, APM Santos, DM Takiya, WRM Souza,
384 Malaise trap, 2 males (MNRJ), 3 males (MZUFBA), 13 males (DZRJ).

385 **Etymology.** The species is named in allusion to the pointed apices of inferior appendages
386 (from Latin, “acumin-” = “pointed”).

387 **Remarks.** This new species belongs to the *penicillata* group based on: (1) internal pouches
388 between abdominal segments IV and V; (2) setal brushes on segments V, VI, and VII; and (3)
389 phallus with two subapical spines. The male genitalia and complex abdominal modifications
390 resemble *M. penicillata* (Flint, 1972) and *M. trigonella* (Flint, 1972). These three species have
391 inferior appendages with acute apices in lateral view; phallus with two subapical spines; and
392 abdominal terga with brushes of very long and stout setae. The new species can be
393 distinguished by inferior appendages more trapezoidal in lateral view, with acute corners
394 posteriorly and dorsal hook only slightly downturned in lateral view; and phallus with one
395 larger and stouter subapical spine. Although the male genitalia of this new species

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402 superficially resembles that of *M. bonita* **sp. nov.**, *M. acuminata* **sp. nov.** is readily
403 recognized by setose lobes on abdominal segments V and VI.
404 We were not able to obtain COI sequences for individuals from Ceará State, so the
405 five sequences analyzed belong to specimens from Alagoas State, which shared the same
406 haplotype. *Metrichia acuminata* **sp. nov.** was recovered as closely related to *M. caraca* **sp.**
407 **nov.** (Fig. 2), but in both Bayesian approaches these two species were not recovered as sister
408 taxa (Fig. 4, Supp. 1).

409

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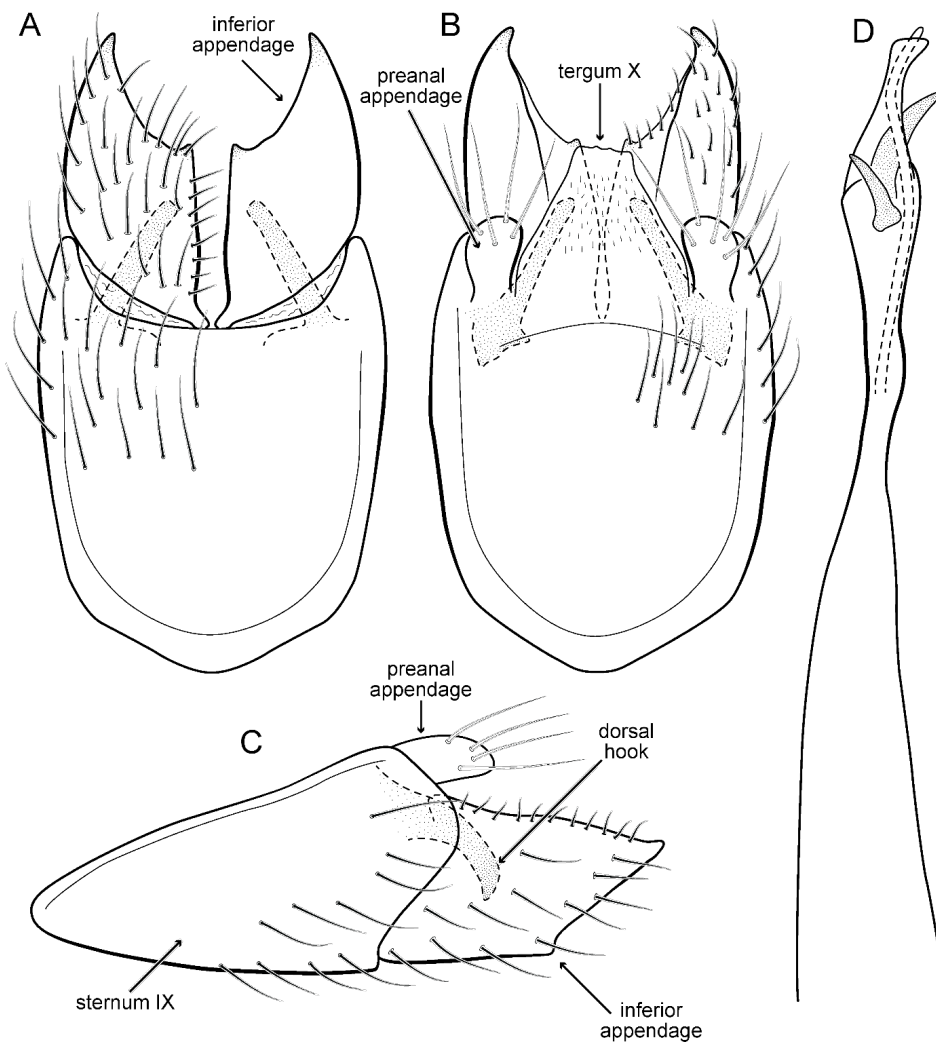


Figure 5. *Metrichia acuminata* sp. nov., male genitalia: (A) ventral view; (B) dorsal view; (C) lateral view; (D) phallus, dorsal view.

Metrichia azul sp. nov.

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(Fig. 6)

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

436 **Holotype. BRAZIL: Paraná:** Céu Azul, Parque Nacional do Iguaçu, Rio Azul, 25°09'21"S
437 53°47'44"W, el. 510 m, 6–8 ix.2012, APM Santos, DM Takiya, ALH Oliveira, GA Jardim &
438 BHL Sampaio cols., Malaise trap, male (DZRJ).

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

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

442

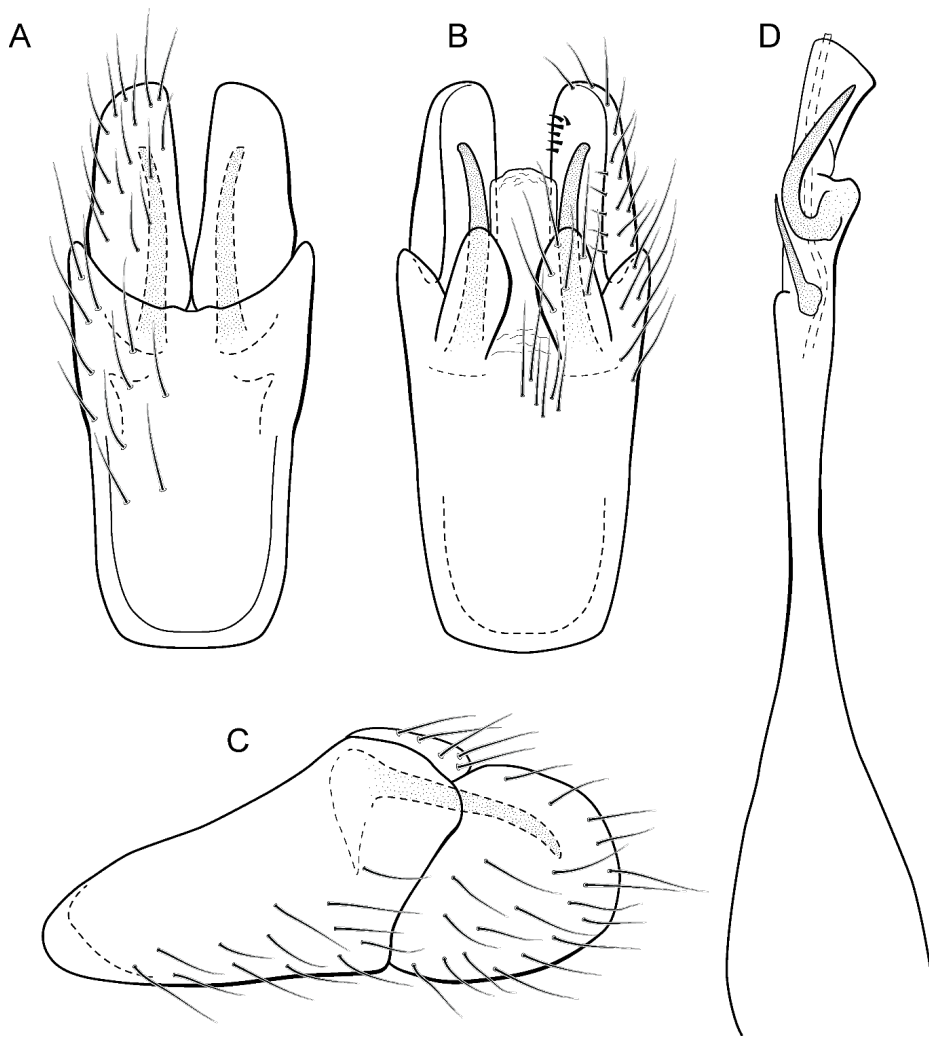


Figure 6. *Metrichia azul* sp. nov., male genitalia: (A) ventral view; (B) dorsal view; (C) lateral view; (D) phallus, dorsal view.

Remarks. This new species is another member of *the penicillata* group based on internal pouches between segment V and VI and the long subapical spines of the phallus. The new species shares similarities of the male genitalia with *M. biungulata* (Flint, 1972) and *M. decora* Bueno-Soria & Holzenthal, 2003, particularly the rounded aspect of inferior

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

455

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

457 [urn:lsid:zoobank.org:act:622BCD51-CC39-4D1F-BDCB-84A7DFF4F071](https://zoobank.org/urn:lsid:zoobank.org:act:622BCD51-CC39-4D1F-BDCB-84A7DFF4F071)

458 (Fig. 7, Fig. 8)

459 **Adult male.** Length 2.3–2.5 mm (n=4). General color, in alcohol, brown. Head with no
460 modifications. Ocelli 3. Antenna simple, 21-articulated. Maxillary palpus 5-articulated; labial
461 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
462 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
463 wings. Abdominal segment VI with dorsal pouches covered with setae directed inward.

464 Ventromesal process on segment VII absent. Segment VIII shorter ventrally than dorsally.

465 **Male genitalia.** Segment IX reduced dorsally; sternum subrectangular, with anterior margin
466 rounded (Fig. 7A); in lateral view narrower anteriorly than posteriorly (Fig. 7C). Inferior
467 appendage covered by long setae, subtrapezoidal in ventral view (Fig. 7A); in lateral view,
468 with an acute projection, apex with acute corners (Fig. 7C). Dorsal hook short, almost half
469 length of inferior appendage; in lateral view, slightly downturned (Fig. 7C). Preanal
470 appendage short, rounded and bearing very long setae (Fig. 7B). Subgenital plate apparently
471 absent. Tergum X membranous and with a shallow U-shaped incision (Fig. 7B). Phallus
472 tubular, elongate and slender, slightly constricted mesally, with a median process; with two
473 curved subapical spines, one short and another long; apex truncate and sclerotized;
474 ejaculatory duct sclerotized and not protruding apically (Fig. 7D).

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

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477 with a few long setae (Fig. 7F). Antenna short, apparently 2-articulated and with no apical
478 setae (Fig. 7F). Labrum with pair of stout setae (Fig. 7F). Mandibles with inner margin
479 sinuous and darkened. Thoracic nota sclerotized, dark brown, with a row of stout setae on
480 anterior margin. Pro-, meso-, and metanotum with middorsal ecdysial line. Thoracic segments
481 with small pleurites (Fig. 7E). Thoracic legs brown, short and stout, almost the same size of
482 each other. Foreleg with stout setae; tibia with a posteroventral lobe with a spine-like seta (Fig.
483 7G); femur bearing a spine-like setae; tarsal claw simple. Mid- and hind legs with stout setae
484 on posteroventral margin. Abdomen almost white, with dark brown sclerites. Abdominal
485 segment I with ellipsoid tergite; segments I-V with pair of long, dorsal setae and pair of
486 dorsolateral setae; segment VI with two pairs of long dorsal setae and two pairs of
487 dorsolateral setae; segments VII and VIII with three pairs of dorsal, long setae and two pair of
488 dorsolateral setae; segment IX with sclerotized tergite and several long setae. Abdominal
489 segments I, III, IV, and IX with pair of ventral, long setae; segment II with two pairs of
490 ventral, long setae and globose process on ventrolateral area (Fig. 7E). Anal proleg very short
491 not projecting prominently; with basal sclerite bearing long setae; anal claw simple.

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

495 **Biology.** Larvae were collected on calcareous tufa in a fast flowing river, approximately 10
496 meters wide (Fig. 8A). No pupae were found and adults were not seen active during the day.

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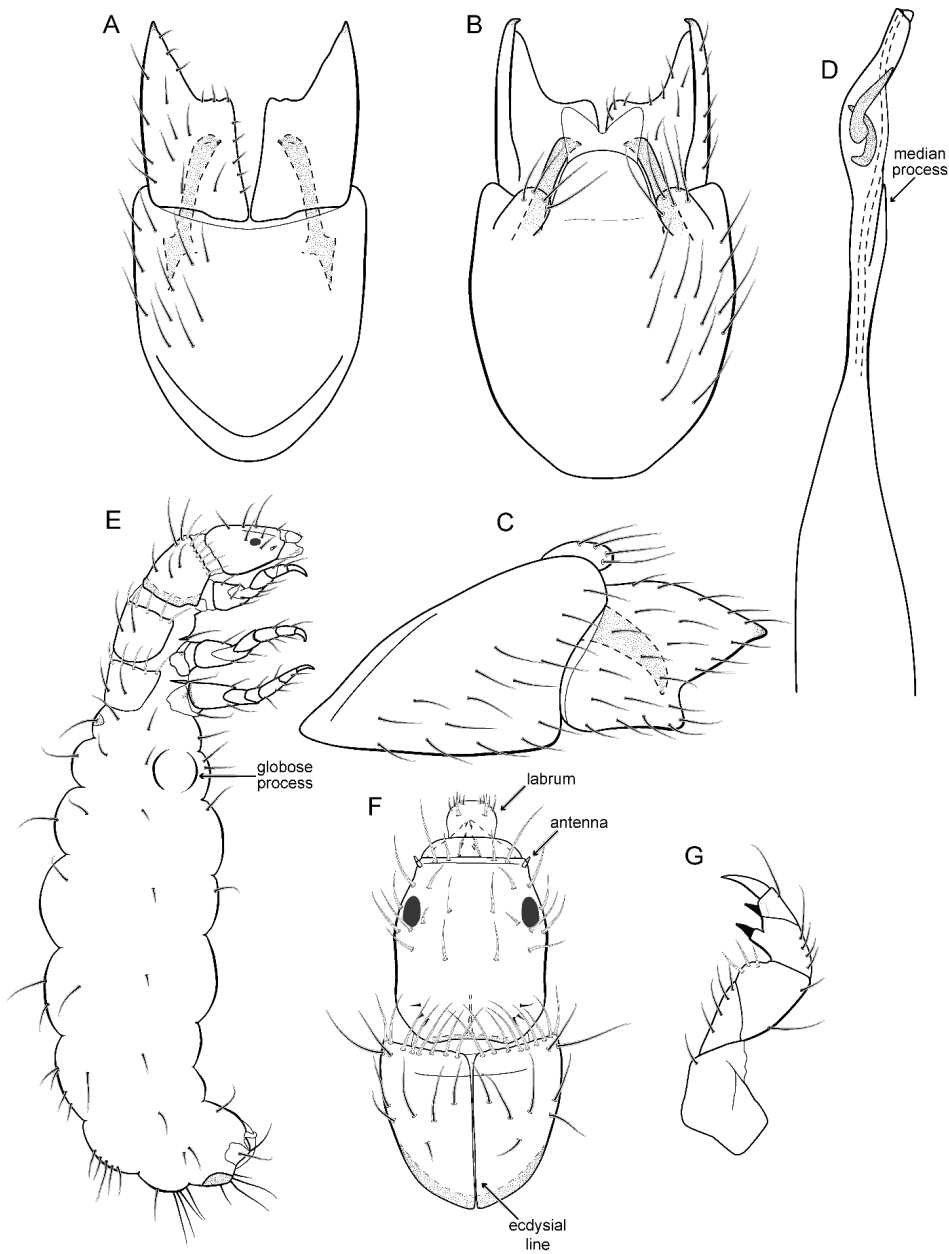
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502 **Figure 7.** *Metrichia bonita* **sp. nov.**: (A) male genitalia, ventral view; (B) male genitalia,
 503 dorsal view; (C) male genitalia, lateral view; (D) phallus, dorsal view; (E) larva, habitus,
 504 lateral view; (F) larva, head and pronotum,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

505

506 **Holotype. BRAZIL: Mato Grosso do Sul:** Bonito, Rio Formosinho, 21°10'16"S

507 56°26'47"W el. 275 m, 08–13.ix.2013, APM Santos & DM Takiya cols., Malaise trap, male

508 (DZRJ).

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

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

511 10 larvae (MNRJ).

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

513 of Bonito, [in the](#) state of Mato Grosso do Sul. In Portuguese, the word "bonita" (the feminine

514 form) means "beautiful".

515 **Remarks.** *Metrichia bonita* **sp. nov.** has features of [the](#) *nigritta* group: internal pouches

516 between segment V-VI [and](#) phallus with 2 spines and an acute process on distal portion. This

517 new species can be easily distinguished from other species in this group based on the shape of

518 inferior appendages, subtrapezoidal in ventral view, with dorsal [corners](#) acute and darkened.

519 [In addition](#), inferior appendages [have dorsal hooks](#), which are broad basally and slightly

520 downturned in lateral view. COI sequences showed maximum intraspecific distance of 1.5%

521 and minimum interspecific distance of 21.0% to its closest neighbor, *M. itabaiana* **sp. nov.**

522 Although the male genitalia of both species shows some superficial resemblance, based on the

523 abdominal modifications and phallic aspect, *M. bonita* **sp. nov.** belongs to [the](#) *nigritta* group,

524 [whereas](#) *M. itabaiana* fits better in the *campana* group.

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

526 illustrated, including *M. nigritta*, *M. juana*, and unassociated larvae illustrated by

527 Botosaneanu & Flint (1982) from Venezuela and Ecuador; and by Pes et al. (2005) from

528 Brazil. Actually, main differences seem to be the shape and the material of larval cases. In

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539 this respect, larvae of *Metrichia bonita* **sp. nov.** are unusual and easily recognized by having
540 their case made entirely of calcareous particles, without typical algal elements (Fig. 8).
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545 **Figure 8.** *Metrichia bonita* **sp. nov.**, larva: (A) type locality, Rio Formosinho, Bonito
546 municipality, Mato Grosso do Sul, Brazil; (B) larvae on calcareous substrate; (C) larva and its
547 calcareous case.

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548
549 Paprocki et al. (2003) discussed the role of *Smicridea travertinera* Paprocki,
550 Holzenthal & Cressa, 2003 in calcareous tufa formation (travertine). According to these
551 authors, larvae of that species interfere in the deposition and erosion of the calcareous
552 substrate by their net-building activities (Paprocki et al., 2003). Cyanobacteria and diatoms
553 are known to participate in travertine formation, but the role played by macroinvertebrates is
554 poorly understood (Drysedale, 1998; 1999). It is possible that cases of *Metrichia bonita* **sp.**
555 **nov.** are impregnated passively with calcareous particles, but as commented by Drysdale
556 (1999) for other aquatic insects, they could be important in travertine biogenesis by producing
557 new nucleation sites or eroding other ones. *Metrichia bonita* **sp. nov.** is the only
558 microcaddisfly known to inhabit (Fig. 8) and build cases with calcareous tufa so far.

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559
560 *Metrichia bracui* **sp. nov.**

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

562 (Fig. 9)

563 **Adult male.** Length 1.8–2.2 mm (n=3). General color, in alcohol, light brown. Head with no
564 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial
565 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
566 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
567 wings. Abdomen without modifications. Ventromesal process on segment VII absent.
568 Segment VIII shorter ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally;
569 sternum subpentagonal in ventral view (Fig. 9A); in lateral view narrower anteriorly than

572 posteriorly (Fig. 9C). Inferior appendage covered by long setae and with scale-like setae,
 573 subrectangular in ventral view (Fig. 9A); in lateral view, rounded, apex rounded (Fig. 9C).
 574 Dorsal hook long, more than half length of inferior appendage; in lateral view, downturned
 575 (Fig. 9C). Preanal appendage elongate and bearing very long setae (Fig. 9B). Subgenital plate
 576 apparently absent. Tergum X membranous and with shallow U-shaped incision (Fig. 9B).
 577 Phallus tubular, elongate and slender, slightly constricted mesally; without spines, but with a
 578 sclerotized process arising from a subapical constriction; apex rounded and folded;
 579 ejaculatory duct sclerotized, straight and not protruding apically (Fig. 9D).

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

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

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

586 **Remarks.** This new species can be assigned to the *patagonica* group because of the absence
 587 of curved spines of the phallus. The general aspect of the male genitalia resembles *M.*

588 *patagonica* (Flint, 1983), *M. pernambucana*, and *M. pseudopatagonica* Bueno-Soria &
 589 Holzenthal, 2003. *Metrichia bracui* **sp. nov.** differs from these species and others in the group
 590 specially by the phallus bearing a sclerotized process on a constricted region.

591 The four COI sequences generated for *M. bracui* **sp. nov.** were from specimens
 592 collected in a locality at Parque Nacional do Itatiaia, Rio de Janeiro, Brazil. The highest
 593 pairwise intraspecific divergence between sequences was 0.4%, and the lowest interspecific
 594 divergence was 21.4% between *M. bracui* **sp. nov.** and *Angrisanoia cebollati* (Angrisano,
 595 1995). Until now, there have been no formal studies on the relationships among species or
 596 genera included in Ochrotrichiinae. However, to infer phylogenetic hypothesis for the entire

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subfamily is beyond the scope of this work, and also much more data and taxa [sampling are](#)
[necessary](#) to generate robust hypotheses.

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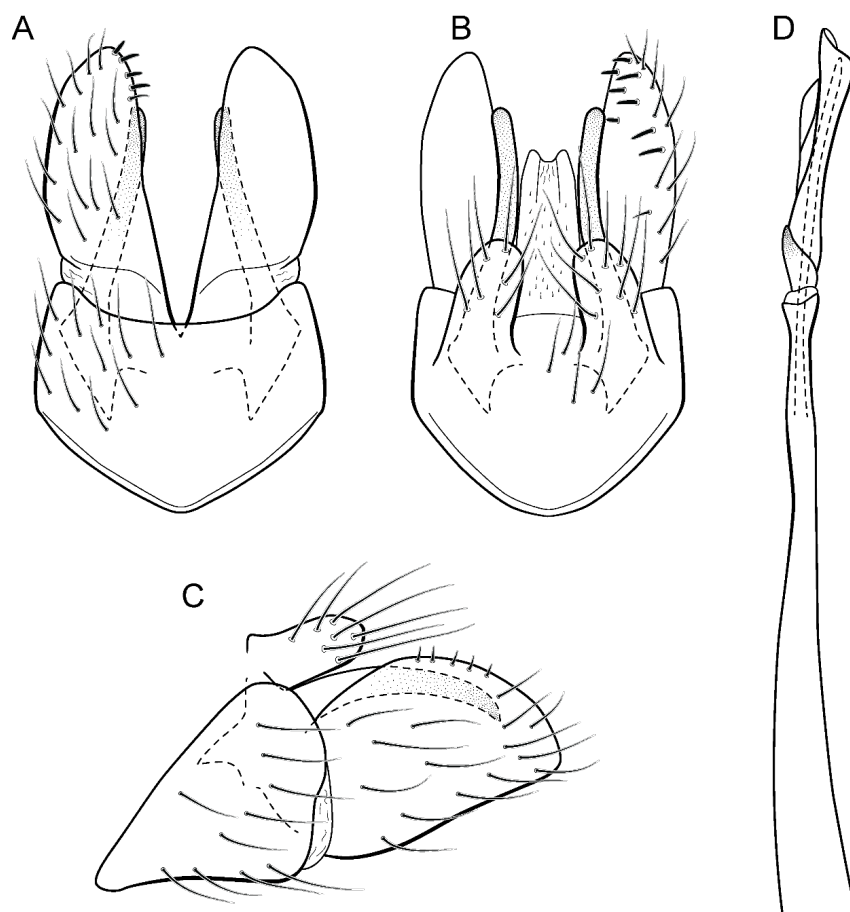


Figure 9. *Metrichia bracui* sp. nov., male genitalia: (A) ventral view; (B) dorsal view; (C) lateral view; (D) phallus, dorsal view.

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

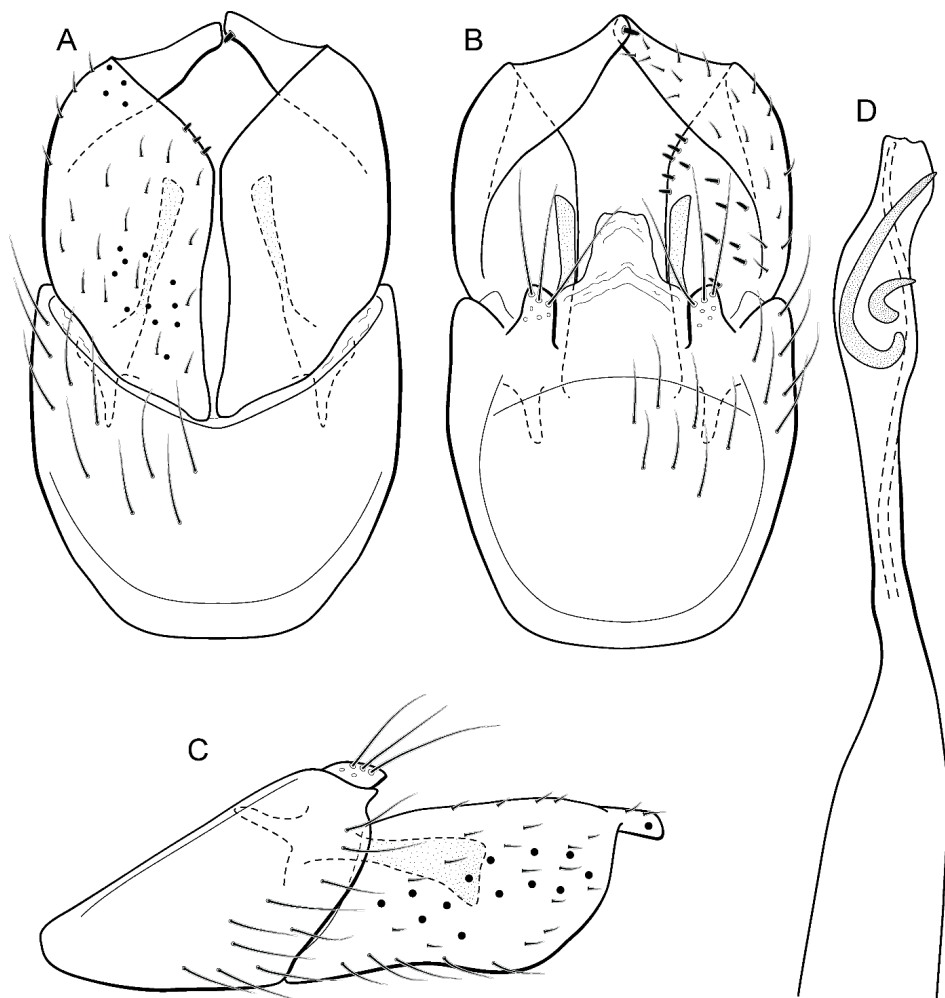
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(Fig. 10)

615 **Adult male.** Length 2.5–3.0 mm (n=7). General color, in alcohol, brown. Head with no
 616 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial
 617 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
 618 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
 619 wings. Abdominal segment VI bearing brush of very long setae dorsolaterally; segment VII
 620 bearing brush of very long setae dorsolaterally. Ventromesal process on segment VII absent.
 621 Segment VIII shorter ventrally than dorsally and bearing brush of long setae dorsally. **Male**
 622 **genitalia.** Segment IX reduced dorsally; sternum subrectangular, with anterior margin
 623 rounded (Fig. 10A); in lateral view narrower anteriorly than posteriorly (Fig. 10C). Inferior
 624 appendage covered by long setae, subtrapezoidal in ventral view (Fig. 10A), apex oblique and
 625 projected mesad into a large process bearing a stout spine-like setae. Dorsal hook short and
 626 straight; in lateral view, truncate and broader apically (Fig. 10C). Preanal appendage short,
 627 truncate and bearing very long setae (Fig. 10B). Subgenital plate apparently absent. Tergum X
 628 membranous and truncate (Fig. 10B). Phallus tubular, elongate and slender, slightly
 629 constricted mesally; with two curved subapical spines, one short and another long; apex
 630 emarginate; ejaculatory duct sclerotized and not protruding apically (Fig. 10D).
 631 **Holotype. BRAZIL: Minas Gerais:** Catas Altas, RPPN Santuário do Caraça, Ribeirão
 632 Caraça, 11–13.vi.2013, ML Monné & JP Botero cols., Malaise trap, male (DZRJ).
 633 **Paratypes.** Same data as holotype, 3 male (DZRJ). **Minas Gerais:** São Roque de Minas,
 634 Parque Nacional da Serra da Canastra, Fazenda Velha, Córrego dos Pombos, 20°14'57"S
 635 46°38'05"W, el. 997 m, 02.iv.2014, JL Nessimian, ALH Oliveira, LL Dumas & SP Gomes,
 636 light trap cols., 1 male (MNRJ).
 637 **Etymology.** This species is named in reference to the stream where type specimens were
 638 collected.

Remarks. This new species has very distinctive male genitalia. Based on the dorsoapically produced inferior appendages, it resembles *M. lenophora* (Flint, 1991). However, *M. caraca* sp. nov. is easily recognized by the obliquely truncate and mesad directed process of inferior appendages and dorsal hook, in lateral view, with apex broad and truncate.

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



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

651

652 ***Metrichia circuliiforme* sp. nov.**

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

654 (Fig. 11)

655 **Adult male.** Length 2.5–2.7 mm (n=4). General color, in alcohol, brown. Head with no
656 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial
657 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
658 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
659 wings. Abdominal segment VI bearing brush of very long setae dorsolaterally; segment VII
660 bearing a brush of very long setae dorsolaterally. Ventromesal process on segment VII present.
661 Segment VIII shorter ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally;
662 sternum subpentagonal (Fig. 11A); in lateral view narrower anteriorly than posteriorly (Fig.
663 11C). Inferior appendage short, covered by long setae, subrectangular in ventral view (Fig.
664 11A); in lateral view, rounded (Fig. 11C), apex slightly truncate and bearing short spine-like
665 setae. Dorsal hook long, almost reaching the inferior appendage apex; in lateral view,
666 downturned (Fig. 11C). Preanal appendage elongate, but shorter than inferior appendage, and
667 bearing very long setae (Fig. 11B). Subgenital plate apparently absent. Tergum X
668 membranous and truncate (Fig. 11B). Phallus tubular, elongate and slender, slightly
669 constricted mesally and with a median process; with two short subapical spines; apex rounded
670 and sclerotized; ejaculatory duct sclerotized, straight and not protruding apically (Fig. 11D).
671 **Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, Rio das Pedras, Cachoeira de Deus,
672 22°25'00"S 44°32'50"W, el. 689 m, 06.iii.2008, JL Nessimian, LL Dumas & MR de Souza
673 cols., light trap, male (DZRJ).

674 **Paratypes.** Same data as holotype, except Rio das Pedras, 22°24'33"S 44°33'08"W, el. 706 m,
 675 06.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, 1 male (DZRJ), 1 male
 676 (MNRJ); Parque Nacional do Itatiaia, Córrego Simon, 22°26'16"S 44°36'20"W, el. 1033 m,
 677 15.iv.07, LL Dumas, APM Santos, N Ferreira-Jr. & JL Nessimian cols., light trap, 1 male
 678 (DZRJ).

679 **Etymology.** The new species name is an allusion to the rounded and simple inferior
 680 appendages, derived from the Latin, "circuli-" and "form" meaning "rounded shape".
 681 **Remarks.** This species has simple male genitalia and abdomen with only brushes of long
 682 setae on segments VI and VII. General aspect of the male genitalia is similar to *M. riva*
 683 (Bueno-Soria, 1983) and *M. quadrata* (Flint, 1972), particularly their inferior appendages
 684 short and subrectangular and phallus with two subapical spines. However, *M. circuliforme* **sp.**
 685 **nov.** can be easily distinguished from *M. quadrata* by the absence of internal sacs in the
 686 abdomen. It can be distinguished from *M. riva* by the elongate preanal appendages and
 687 phallus with subequal hook spines subapically.

688 We obtained seven COI sequences for *M. circuliforme* **sp. nov.** and although all of
 689 them came from specimens collected at the same locality, intraspecific divergences were
 690 relatively high, reaching 3.5%. Besides that, GMYC estimated two species for these
 691 sequences instead of one. Re-analysis of the morphology of these specimens did not reveal
 692 any conspicuous variation that could justify splitting this species into two taxonomic groups.
 693 Compared to other studies using DNA barcodes of caddisflies, this genetic distance is still
 694 low, for example Pauls et al. (2010) found intraspecific divergences (K2P distance) up to
 695 5.9% for Chilean *Smicridea*. Zhou et al. (2011) found even higher intraspecific distances
 696 among caddisflies, reaching up to 14%. GMYC is known to be more sensitive to geographic
 697 range coverage and/or other sampling schemes, resulting in oversplitting (Lohse, 2009;
 698 Talavera et al., 2013). Therefore, we consider *M. circuliforme* **sp. nov.** as a robust species

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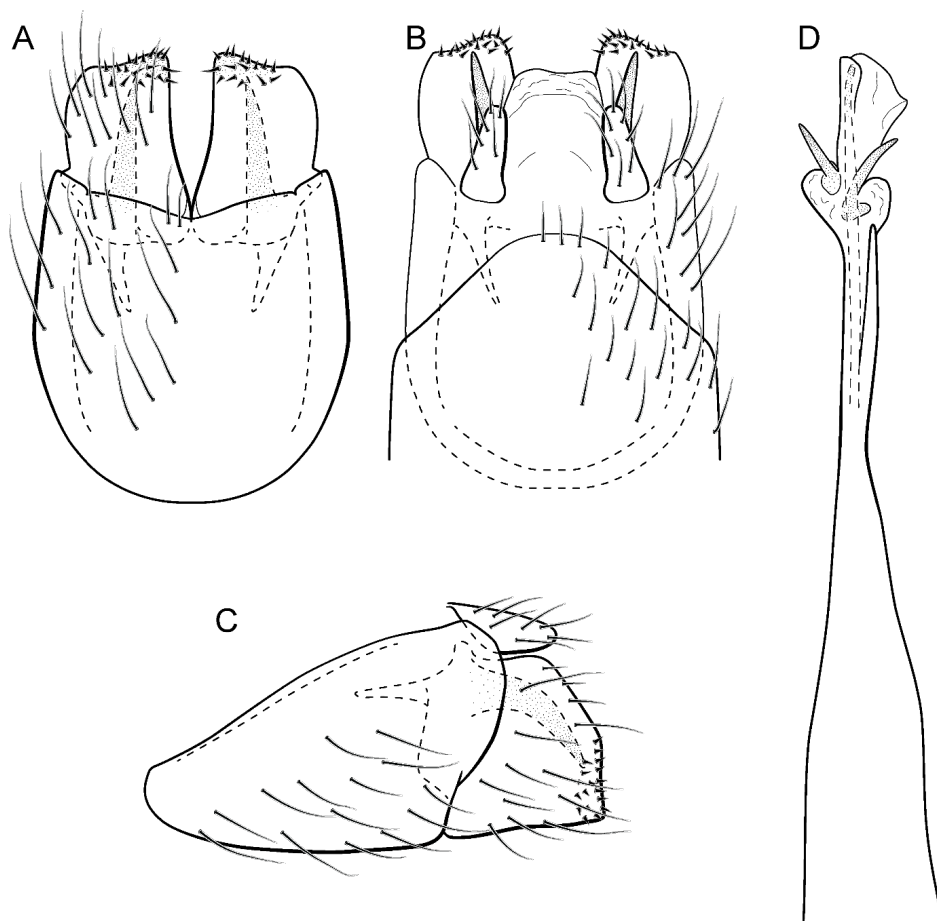
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709 based on morphology as well as based on barcode divergences. Minimum interspecific COI
710 distances of *M. circuliforme* **sp. nov.** to *M. curta* **sp. nov.** were 18.4%, and again, these two
711 species are very distinct based on morphological features and apparently are not even closely
712 related to each other (Fig. 2).

713



714

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

717

718 ***Metrichia curta* sp. nov.**

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

720 (Fig. 12)

721 **Adult male.** Length 2.4–2.5 mm (n=9). General color, in alcohol, light brown. Head with no
722 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial
723 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
724 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
725 wings. Abdomen without modifications. Ventromesal process on segment VII present.
726 Segment VIII shorter ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally;
727 sternum subpentagonal (Fig. 12A); in lateral view, narrower anteriorly than posteriorly (Fig.
728 12C). Inferior appendage covered by long setae, subtrapezoidal in ventral view (Fig. 12A); in
729 lateral view, subtriangular (Fig. 12C), apex rounded. Dorsal hook long, more than half length
730 of inferior appendage; in lateral view, with apex slightly broader, downturned, and truncate
731 (Fig. 12C). Preanal appendage elongate, but shorter than inferior appendage, and bearing very
732 long setae (Fig. 12B). Subgenital plate apparently absent. Tergum X membranous and
733 rounded (Fig. 12B). Phallus tubular, elongate and slender, slightly constricted mesally; with a
734 stout subapical spine; apex rounded and folded; ejaculatory duct sclerotized, straight and
735 protruding apically (Fig. 12D).

736 **Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, Rio das Pedras, 22°24'33"S 44°33'08"W, el.
737 706 m, 06.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, male (DZRJ).

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

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

741 **Remarks.** Based on the absence of modifications on abdominal segments, this new species
742 can be assigned to the *aberrans* group. Its phallus is similar to that of *M. amplitudinis* Bueno-

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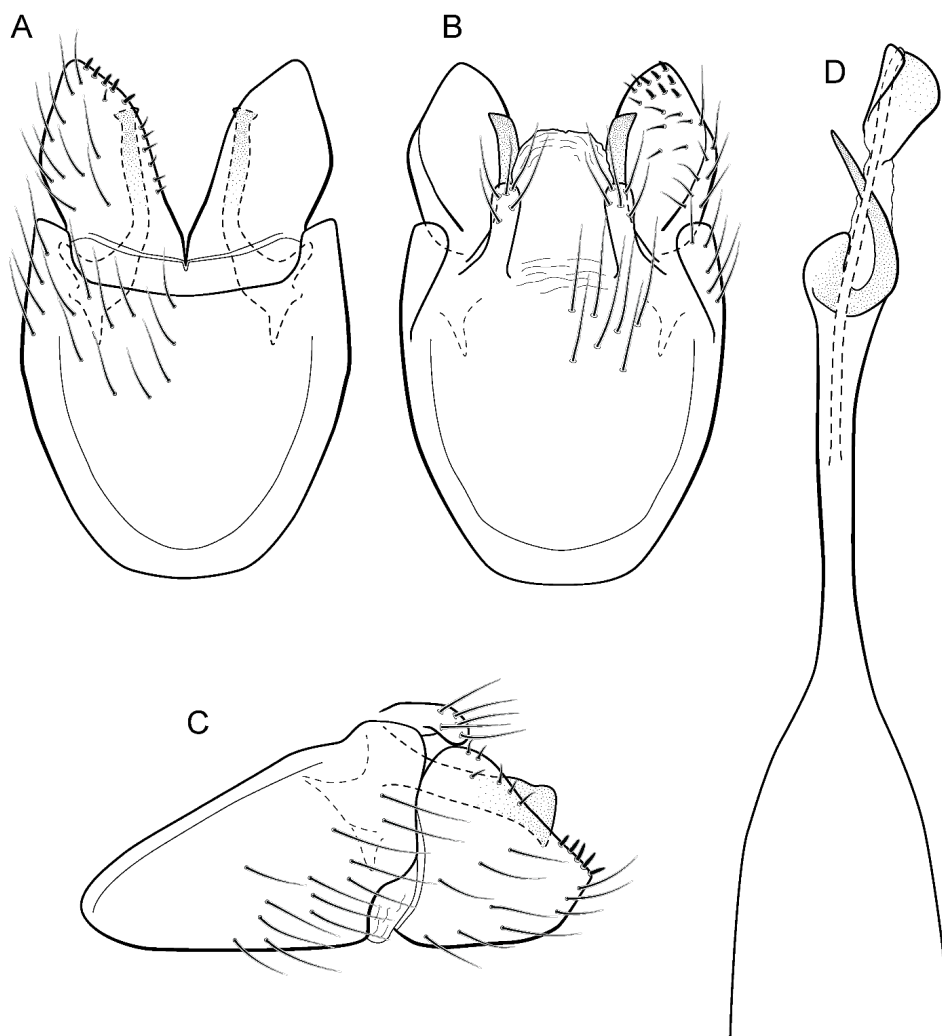
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745 | Soria & Hozenthal, 2003, with a long spine and an apical flap. The new species can be
 746 | distinguished by the triangular inferior appendages in lateral view and phallus with a strongly
 747 | curved spine subapically. *Metrichia amplitudinis* and *M. curta* **sp. nov.** share the widened
 748 | dorsal hook, but in the new species this structure is only slightly wider and also truncate in
 749 | lateral view. Maximum intraspecific divergence of COI sequences was 1.5% for *M. curta* **sp.**
 750 | **nov.** and minimum interspecific was to *M. circuliforme*, as mentioned above.

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752

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

756

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

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

759 (Fig. 13)

760 **Adult male.** Length 1.8–2.1 mm (n=27). General color, in alcohol, light brown. Head with no
761 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial
762 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
763 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
764 wings. Abdomen without modifications; segment VII bearing specialized setae dorsally.

765 Ventromesal process on segment VII absent. Segment VIII shorter ventrally than dorsally.

766 **Male genitalia.** Segment IX reduced dorsally; sternum subrectangular (Fig. 13A); in lateral
767 view narrower anteriorly than posteriorly (Fig. 13C). Inferior appendage covered by long
768 setae, subrectangular in ventral view (Fig. 13A); in lateral view, subrectangular (Fig. 13C),
769 apex slightly truncate and bearing short spine-like setae. Dorsal hook long, almost reaching
770 the inferior appendage apex; in lateral view, downturned (Fig. 13C). Preanal appendage
771 elongate, as long as inferior appendage, and bearing very long setae (Fig. 13B). Subgenital
772 plate apparently absent. Tergum X membranous and rounded (Fig. 13B). Phallus tubular,
773 elongate and slender, slightly constricted mesally; with a stout subapical spine; apex rounded
774 and sclerotized; ejaculatory duct sclerotized, sinuous, and protruding apically (Fig. 13D).

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

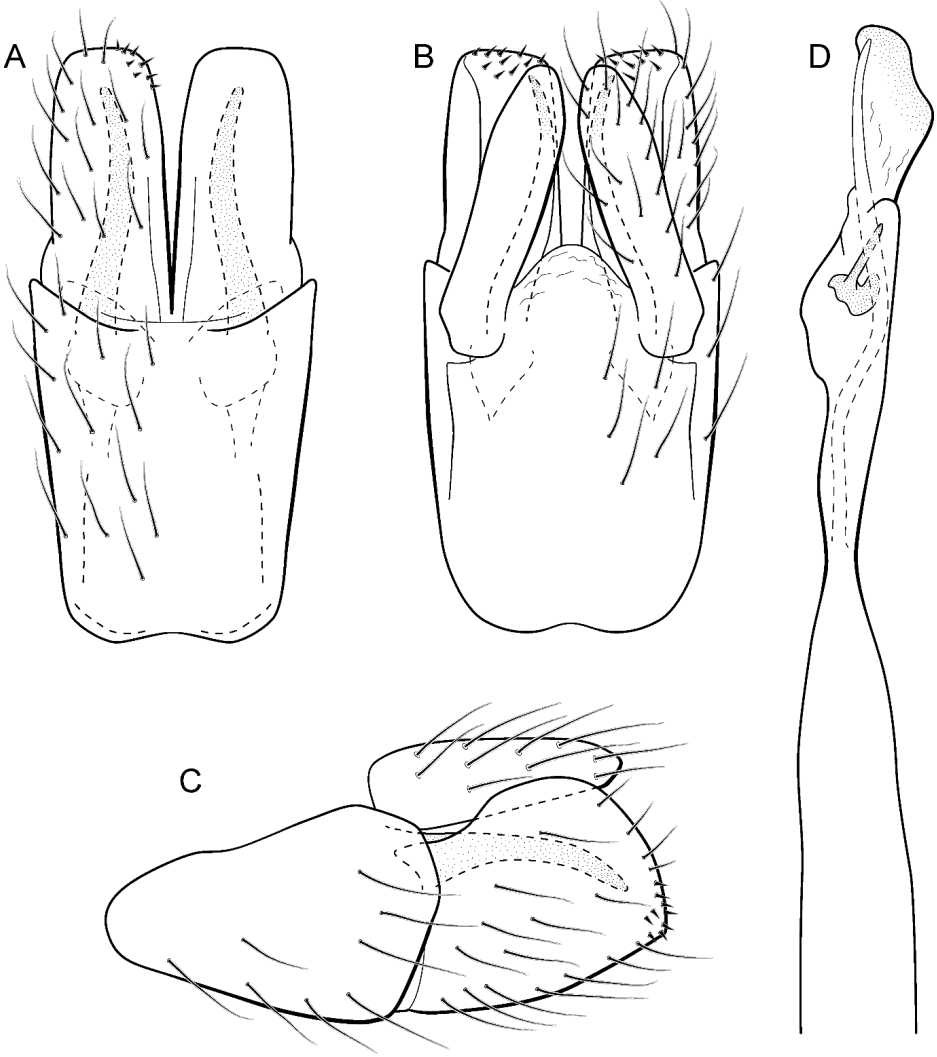
778 **Paratypes.** Same data as holotype, 18 males (DZRJ), 8 males (MNRJ), 5 males (MZUFBA);
779 same data, except Ribeirão Mascates, 19°24'02"S 43°34'35"W, el. 820 m, 09–11.xii.2011,
780 APM Santos & DM Takiya cols., manual, 84 males (DZRJ).

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

783

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784

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

788
789 **Remarks.** Due to absence of pouches in abdominal segments, *Metrichia farofa* **sp. nov.** can
790 also be included in the *aberrans* group. However, the new species has a single subapical spine
791 in phallus, like those species included in the *exclamationis* group. The new species can be
792 easily distinguished from all other *Metrichia* species by the very long preanal appendages,
793 reaching apices of inferior appendages in dorsal and lateral views.

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

797
798 ***Metrichia forceps* sp. nov.**

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

800 (Fig. 14)

801 **Adult male.** Length 2.7–3.0 mm (n=2). General color, in alcohol, brown. Head with no
802 modifications. Ocelli 3. Antenna simple, 23-articulated. Maxillary palpus 5-articulated; labial
803 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
804 Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation
805 reduced in both wings. Abdominal segment VII bearing internal pouches in anterior area.
806 Ventromesal process on segment VII absent. Segment VIII shorter ventrally than dorsally.

807 **Male genitalia.** Segment IX reduced dorsally; sternum subpentagonal (Fig. 14A); in lateral
808 view, narrower anteriorly than posteriorly (Fig. 14C). Inferior appendage covered by long
809 setae, subrectangular in ventral view (Fig. 14A); in lateral view, with posterior margin
810 excavated and with two acute and sclerotized process (Fig. 14C). Dorsal hook long, almost

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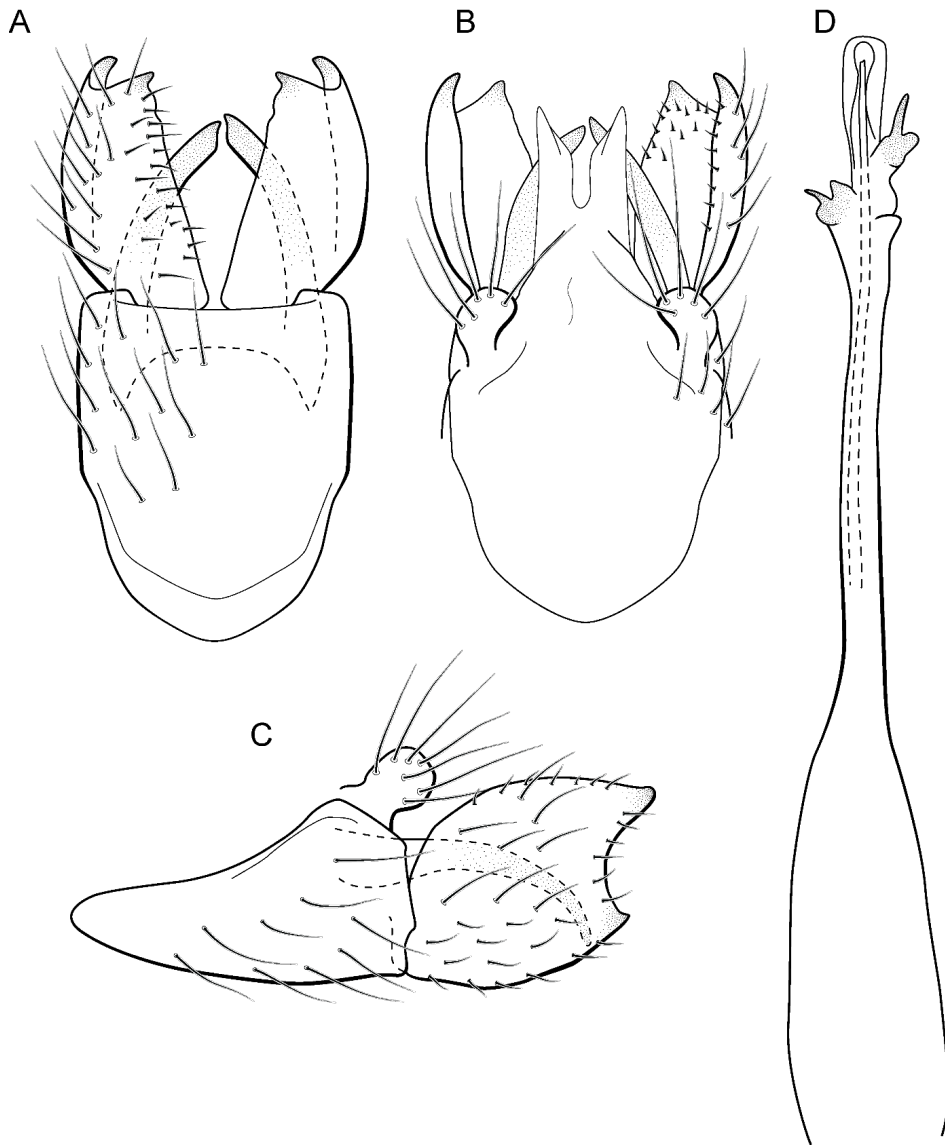
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816 reaching the inferior appendage apex; in lateral view, downturned (Fig. 14C). Preanal
817 appendage short, rounded and bearing very long setae (Fig. 14B). Subgenital plate apparently
818 absent. Tergum X sclerotized, deeply notched mesally, forming lateral curved processes (Fig.
819 14B). Phallus tubular, elongate and slender, slightly constricted mesally; with two short
820 subapical spines; apex rounded and folded; ejaculatory duct sclerotized, straight and
821 protruding apically (Fig. 14D).

822



823

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

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

826

827 **Holotype. BRAZIL: Paraná:** Céu Azul, Parque Nacional do Iguaçu, Rio Azul, 25°09'21"S
828 53°47'44"W, el. 510 m, 6–8 ix.2012, APM Santos, DM Takiya, ALH Oliveira, GA Jardim &
829 BHL Sampaio cols., Malaise trap, male (DZRJ).

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

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

833 **Remarks.** This new species belongs to the *campana* group, sharing the diagnostic internal
834 pouches between segments VI and VII, reduced spines on subapical region of phallus, and the
835 sclerotized and elongate tergum X. Within this group, *M. forceps* sp. nov. is most similar to
836 *M. campana* (Flint, 1968), *M. similis* (Flint, 1968), and *M. continentalis* (Flint, 1972),
837 particularly by their inferior appendages with excavate posterior margins, forming two
838 pointed processes, one ventral and another dorsal. The new species can be distinguished from
839 the others by its deeply notched tergum X; dorsal hook of inferior appendages elongate and
840 downturned, and phallus apex bearing two small spines and a sclerotized flap surrounding the
841 protruding ejaculatory duct.

842

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

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

845 (Fig. 15)

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

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861 dorsally. **Male genitalia.** Segment IX reduced dorsally; sternum subrectangular, with anterior
862 margin rounded (Fig. 15A); in lateral view narrower anteriorly than posteriorly (Fig. 15C).
863 Inferior appendage with peg-like setae, subrectangular in ventral (Fig. 15A) and lateral (Fig.
864 15C) views; apex with acute corners. Dorsal hook long, almost reaching the inferior
865 appendage apex; in lateral view, with apex slightly broader, almost straight, and truncate (Fig.
866 15C). Preanal appendage short, rounded and bearing very long setae (Fig. 15B). Subgenital
867 plate apparently absent. Tergum X membranous and rounded (Fig. 15B). Phallus tubular,
868 elongate and slender, slightly constricted mesally; with two curved subapical spines, one short
869 and another one very long; apex rounded and sclerotized; ejaculatory duct sclerotized, sinuous,
870 and not protruding apically (Fig. 15D).

871 **Holotype. BRAZIL: Mato Grosso do Sul:** Bonito, Rio Formosinho, 21°10'16"S
872 56°26'47"W el. 275 m, 08–13.ix.2013, APM Santos & DM Takiya cols., Malaise trap, male
873 (DZRJ).

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

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

877 **Remarks.** This new species appears to be a member of the *campana* group because of
878 internal pouches between abdominal segments VI–VII and phallus with two subapical spines.

879 General aspect of the male genitalia of *Metrichia formosinha* **sp. nov.** is similar to *M. forceps*
880 **sp. nov.**, particularly in the inferior appendages with acute corners. However, *M. formosinha*
881 **sp. nov.** differs from the latter by dorsal hooks almost straight and capitate (strongly curved
882 and acute in *M. forceps* **sp. nov.** and in other species of the campana group), and phallus with
883 a very long subapical spine.

884 COI sequences of *M. formosinha* **sp. nov.** showed intraspecific divergences up to
885 0.8% and minimum interspecific divergences of 24.9% compared to *M. talhada* **sp. nov.**

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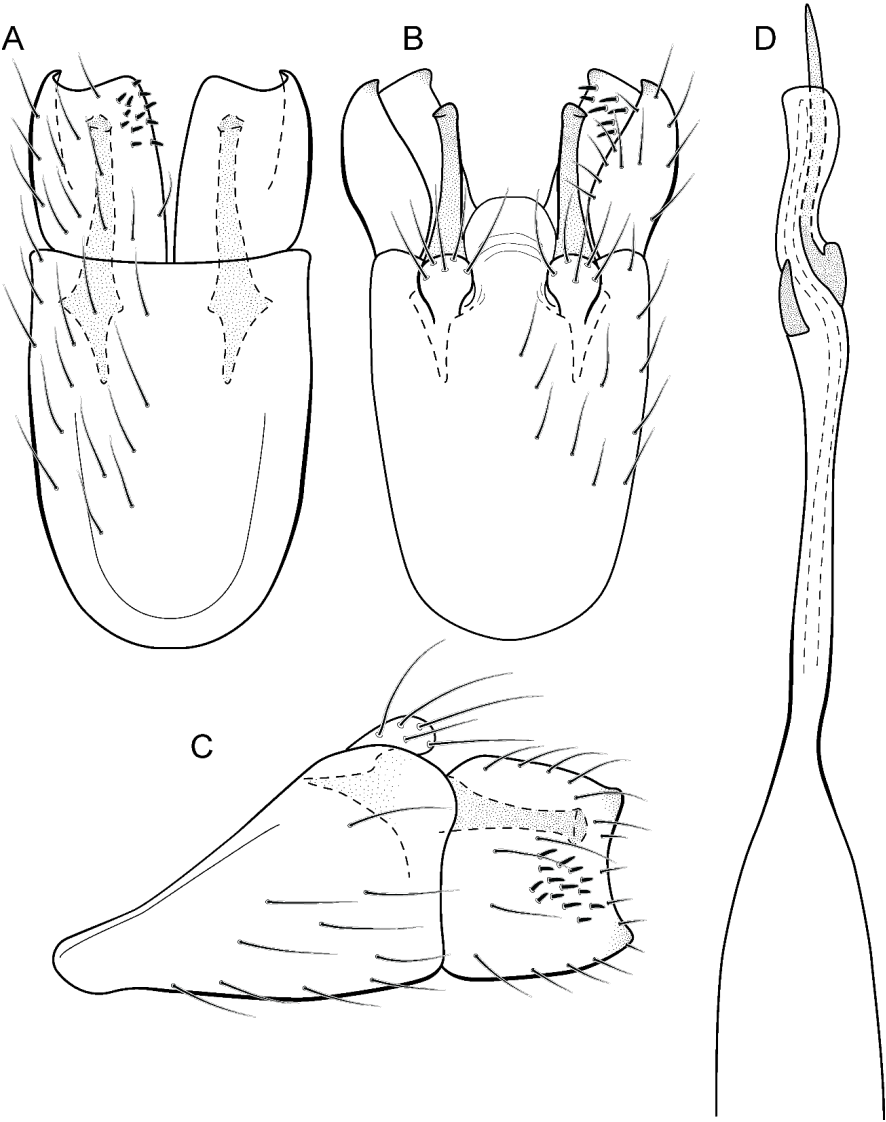
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896 **Figure 15.** *Metrichia formosinha* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view;
897 (C) lateral view; (D) phallus, dorsal view.

898

899 *Metrichia goiana* sp. nov.

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

901 (Fig. 16)

902 **Adult male.** Length 1.8–2.0 mm (n=6). General color, in alcohol, dark brown. Head with no
903 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated, article
904 IV broad and darkened; labial palpus 3-articulated. Mesoscutellum with transverse suture.
905 Metascutellum subtriangular. Anterior femur with small acute apical process. Tibial spur
906 formula 1-3-4. Wing venation reduced in both wings. Abdominal segment V with pair of
907 internal pouches and pair of dorsolateral brushes; segment VI with a transverse sclerotized
908 plate posteriorly on dorsum; segment VII bearing specialized setae dorsally. Ventromesal
909 process on segment VII absent. Segment VIII shorter ventrally than dorsally. **Male genitalia.**
910 Segment IX reduced dorsally; sternum subrectangular, with anterior margin rounded (Fig.
911 16A); in lateral view narrower anteriorly than posteriorly (Fig. 16C). Inferior appendage
912 covered by long setae, elongate and narrow in ventral view (Fig. 16A); in lateral view,
913 rounded (Fig. 16A), apex rounded and bearing a tooth-like projection (Fig. 16A). Dorsal hook
914 short, less than half length of inferior appendage; in lateral view, downturned (Fig. 16A).
915 Preanal appendage short and bearing very long setae (Fig. 16B). Subgenital plate apparently
916 absent. Tergum X membranous and truncate (Fig. 16B). Phallus tubular, elongate and slender,
917 slightly constricted mesally; with two long, curved, subapical spines; apex ending into two
918 sclerotized and keel shaped processes; ejaculatory duct sclerotized, straight and not protruding
919 apically (Fig. 16D).

920 **Holotype. BRAZIL: Goiás:** Alto Paraíso de Goiás, Rio Bartolomeu tributary, 14°07'25"S
921 47°30'30"W, el. 1165 m, 22–25.iii.2013, APM Santos & DM Takiya cols., Malaise trap, male
922 (DZRJ).

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

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925 **Etymology.** The species is named in reference to Goiás State. “Goiana” is a [Portuguese](#)
926 adjective [for people from Goiás](#).
927 **Remarks.** This is another member of *nigritta* group. Male genitalia of this species [are](#) similar
928 to *M. potosina* Bueno-Soria, [2002](#) and *M. ubajara* **sp. nov.**, due to rounded and elongate
929 inferior [appendages](#) in lateral view. This new species differs from *M. ubajara* **sp. nov.** by the
930 presence of an apical tooth on inferior appendages, also present in *M. potosina*. *Metrichia*
931 *goiana* **sp. nov.** can be distinguished from *M. potosina* by the two long subapical spines on
932 phallus, whereas *M. potosina* has three.
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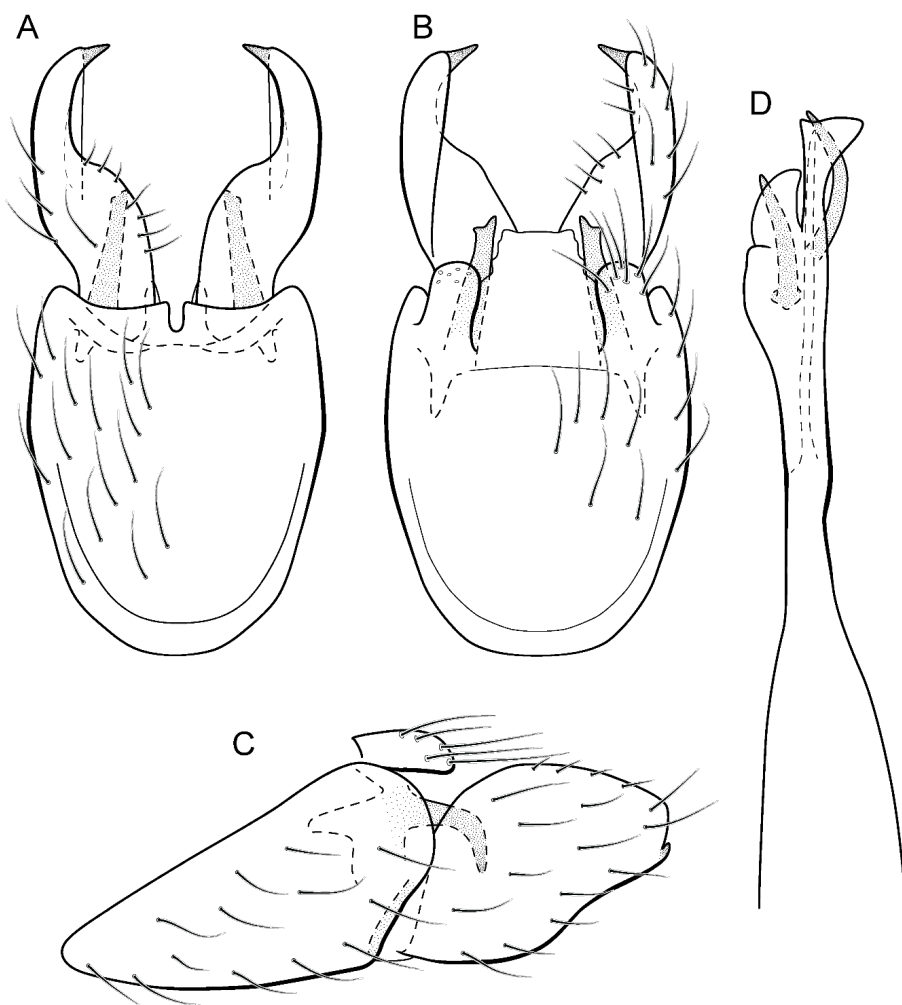
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939 **Figure 16.** *Metrichia goiana* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)
 940 lateral view; (D) phallus, dorsal view.

941

942 ***Metrichia itabaiana* sp. nov.**

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

944 (Fig. 17, Fig. 26B)

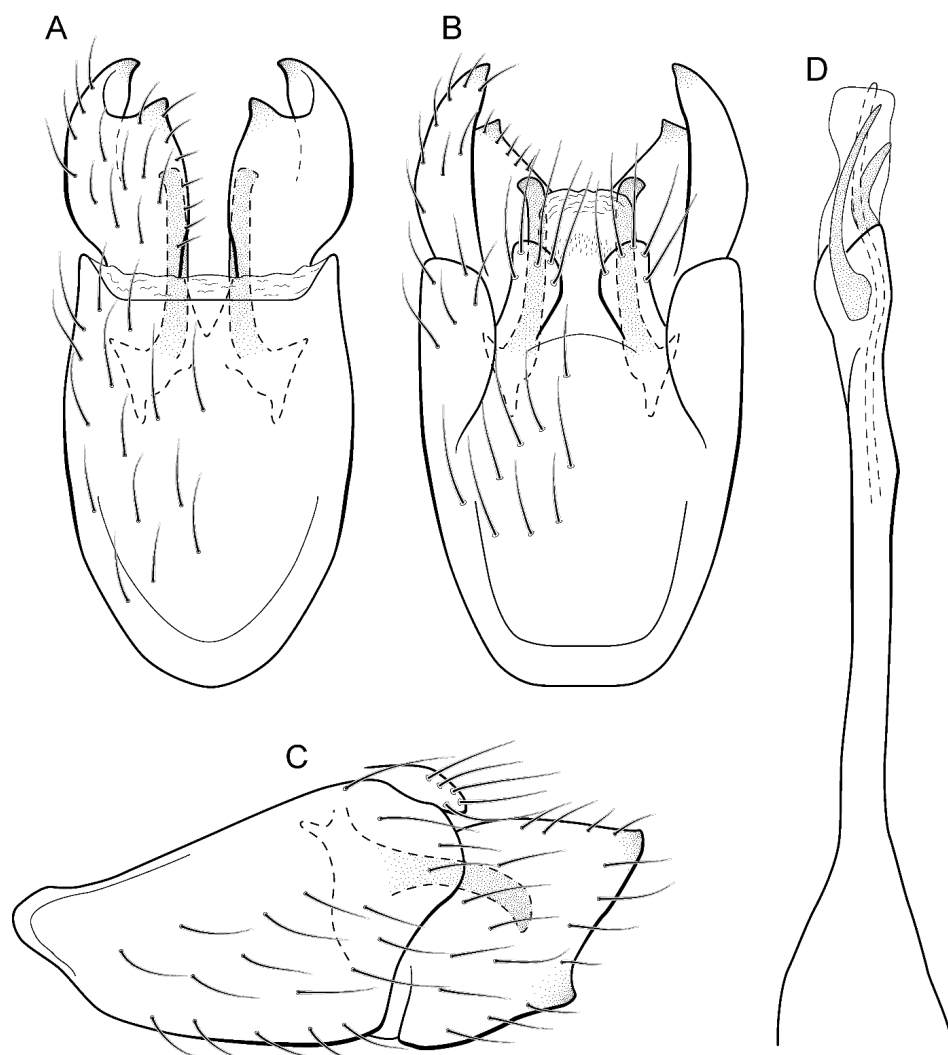
945 **Adult male.** Length 1.8–2.1 mm (n=3). General color, in alcohol, brown. Head with no
 946 modifications. Ocelli 3. Antenna simple, 20-articulated. Maxillary palpus 5-articulated; labial
 947 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
 948 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
 949 wings. Abdominal segment V ventrally with a mesal brush of long setae; segment VI with
 950 tergum as a sclerotized triangular plate surrounded by specialized setae (Fig. 26B), internally
 951 with pair of internal pouches; segment VII bearing specialized setae ventrally and dorsally
 952 (Fig. 26B). Ventromesal process on segment VII present. Segment VIII shorter ventrally than
 953 dorsally and bearing a brush of long setae dorsally. **Male genitalia.** Segment IX reduced
 954 dorsally; sternum subpentagonal (Fig. 17A); in lateral view narrower anteriorly than
 955 posteriorly (Fig. 17C). Inferior appendage short, covered by long setae (Fig. 17A); in lateral
 956 view, subtrapezoidal (Fig. 17C), apex excavated and with two acute and sclerotized process
 957 (Figs. 17A, 17B). Dorsal hook short, almost half length of inferior appendage; in lateral view,
 958 slightly downturned (Fig. 17C). Preanal appendage elongate, but shorter than inferior
 959 appendage, and bearing very long setae (Fig. 17B). Subgenital plate apparently absent.
 960 Tergum X membranous and truncate (Fig. 17B). Phallus tubular, elongate and slender,
 961 slightly constricted mesally; with two long, curved, subapical spines; apex truncate and
 962 slightly sclerotized; ejaculatory duct sclerotized, sinuous, and protruding apically (Fig. 17D).
 963 **Holotype. Sergipe:** Areia Branca, Parque Nacional da Serra de Itabaiana, Rio dos Negros,
 964 10°44'51"S 37°20'24"W, el. 208 m, 17.vi.2014, APM Santos, DM Takiya & WRM Souza
 965 cols., light trap, male (DZRJ).
 966 **Paratypes.** Same data as holotype, 1 male (DZRJ), 1 male (MZUFBA); same data, except
 967 Riacho Água Fria, 10°45'17"S 37°20'32"W, el. 196 m, 17–19.vi.2014, APM Santos, DM
 968 Takiya, WRM Souza cols., Malaise trap, 2 males (MNRJ). **Goiás:** Alto Paraíso, Rio

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970 | Bartolomeu [tributary](#), 14°07'25"S 47°30'30"W, el. 1165 m, 22–25.iii.2013, APM Santos &
971 DM Takiya cols., Malaise trap, 1 male (DZRJ).

972



973

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

975 (C) lateral view; (D) phallus, dorsal view.

976

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

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

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

991

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

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

994 (Fig. 18, Fig. 26C)

995 **Adult male.** Length 2.5–2.7 mm (n=2). General color, in alcohol, brown. Head with no
996 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial
997 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
998 Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation
999 reduced in both wings. Abdominal segment IV with dorsal area expanded posteriorly bearing
1000 stout setae; segment VI with stout and striate setae (Fig. 26C); segment VII with stout and
1001 striate setae (Fig. 26C). Ventromesal process on segment VII absent. Segment VIII shorter

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1009 ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally; sternum subpentagonal
1010 (Fig. 18A); in lateral view narrower anteriorly than posteriorly (Fig. 18C). Inferior appendage
1011 bearing scale-like setae, very elongate; in ventral view, curved inward apically (Fig. 18A); in
1012 lateral view, tapering to a rounded apex (Fig. 18C). Dorsal hook short and straight; in lateral
1013 view, slightly downturned (Fig. 18C). Preanal appendage elongate, but shorter than half
1014 length of inferior appendage, and bearing stout and striate setae (Fig. 18B). Subgenital plate
1015 apparently absent. Tergum X membranous and truncate (Fig. 18B). Phallus tubular, elongate
1016 and slender, slightly constricted mesally, with a median process; with two long, curved,
1017 subapical spines; apex rounded and sclerotized; ejaculatory duct sclerotized and protruding
1018 apically (Fig. 18D).

1019 **Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, Rio Palmital, 22°25'34"S 44°32'52"W, el. 637
1020 m, 07.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, male (DZRJ).

1021 **Paratype. Brazil: Rio de Janeiro:** Teresópolis, Parque Nacional da Serra dos Órgãos, Rio
1022 Paquequer, 22°27'25"S 42°59'52"W, el. 1100 m, 15–18.ix.2011, APM Santos, DM Takiya,
1023 BM Vasconcelos & RA Carvalho cols., Malaise trap, 1 male (MNRJ).

1024 **Etymology.** The species name is an allusion to the elongate inferior appendages, unusual for
1025 *Metrichia* species.

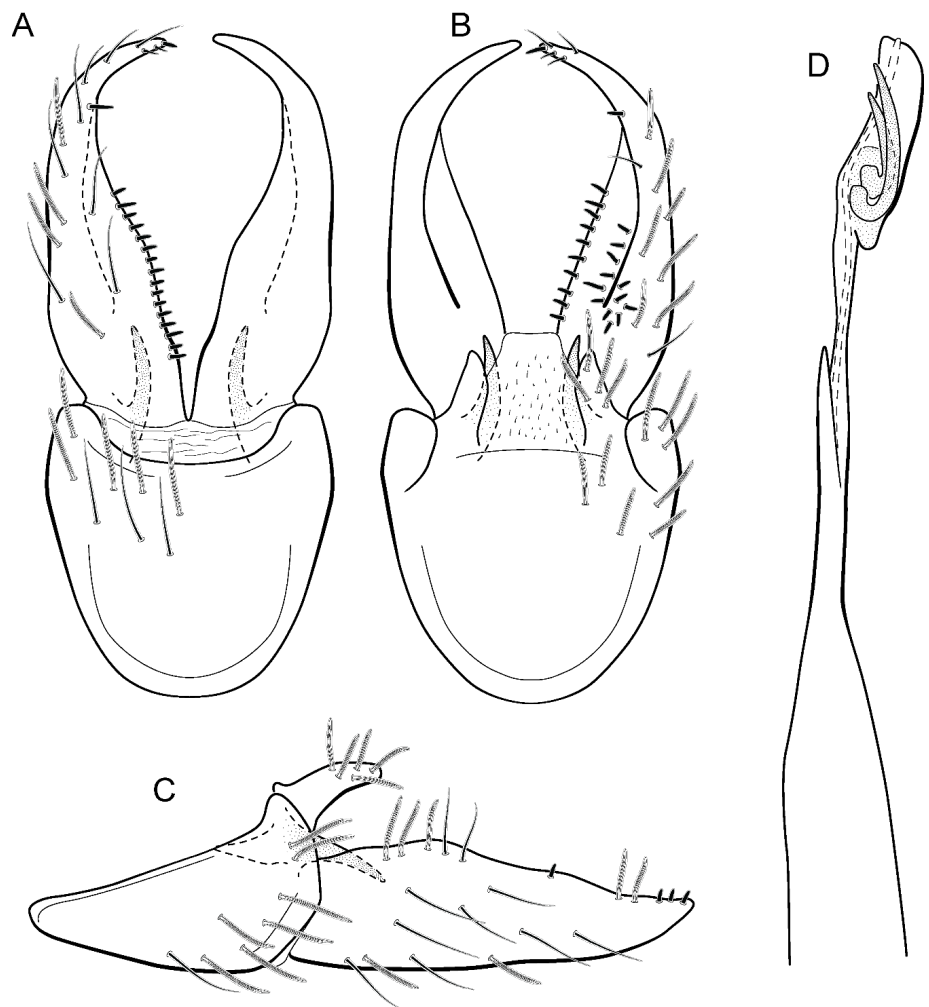
1026 **Remarks.** The new species is **most** similar to *M. sesquipedalis* Bueno-Soria & Holzenthal,
1027 2003, sharing with it very long inferior appendages with very short dorsal hooks. The new
1028 species is easily distinguished from the latter by their internal pouches in the male abdominal
1029 segment VI and phallus with only two subapical spines (three in *M. sesquipedalis*).

1030 Two COI sequences were generated for *M. longissima* **sp. nov.**, one from a specimen
1031 from Itatiaia and the other from Teresópolis, in Rio de Janeiro State, localities in distinct
1032 mountain ranges; Serra da Mantiqueira and Serra do Mar, respectively. The genetic distance

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1046 between sequences of *M. longissima* **sp. nov.** was 0.4%. The minimum interspecific distance
1047 was found to *M. itabaiana* **sp. nov.**, with 21.5% divergence.

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1049
1050 **Figure 18.** *Metrichia longissima* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view;
1051 (C) lateral view; (D) phallus, dorsal view.

1056 *Metrichia peluda* sp. nov.

1057 urn:lsid:zoobank.org:act:E1B7E1AE-5751-4D10-9B07-47A8CF849C7F

1058 (Fig. 19, Fig. 26D)

1059 **Adult male.** Length 2.7–3.0 mm (n=3). General color, in alcohol, dark brown. Head with no
1060 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial
1061 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
1062 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
1063 wings. Abdominal segment V with dorsolateral brushes of long setae; segment VI with
1064 dorsolateral brushes of long setae (Fig. 26D). Ventromesal process on segment VII present.
1065 Segment VIII shorter ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally;
1066 sternum subrectangular, with anterior margin rounded (Fig. 19A); in lateral view narrower
1067 anteriorly than posteriorly (Fig. 19C). Inferior appendage covered by long setae,
1068 subtrapezoidal in ventral view (Fig. 19A); apex excavated; in lateral view, rounded (Fig. 19C).
1069 Dorsal hook short, almost half length of inferior appendage; in lateral view, slightly
1070 downturned (Fig. 19C). Preanal appendage short and bearing very long setae (Fig. 19B).
1071 Subgenital plate apparently absent. Tergum X membranous and truncate (Fig. 19B). Phallus
1072 tubular, elongate and slender, slightly constricted mesally; with two curved subapical spines,
1073 one short and another long; apex rounded and folded; ejaculatory duct sclerotized, straight
1074 and protruding apically (Fig. 19D).

1075 **Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, 1st order tributary of Rio Palmital, 22°25'40"S
1076 44°32'46"W, el. 584 m, 07.iii.2008, JL Nessimian, LL Dumas & MR de Souza cols., light
1077 trap, male (DZRJ).

1078 **Paratypes.** Same data as holotype, 1 male (MNRJ); same data, except Rio Palmital,
1079 22°25'34"S 44°32'52"W, el. 637 m, 07.iii.2008, LL Dumas, JL Nessimian & MR de Souza
1080 cols., light trap, 4 males (DZRJ).

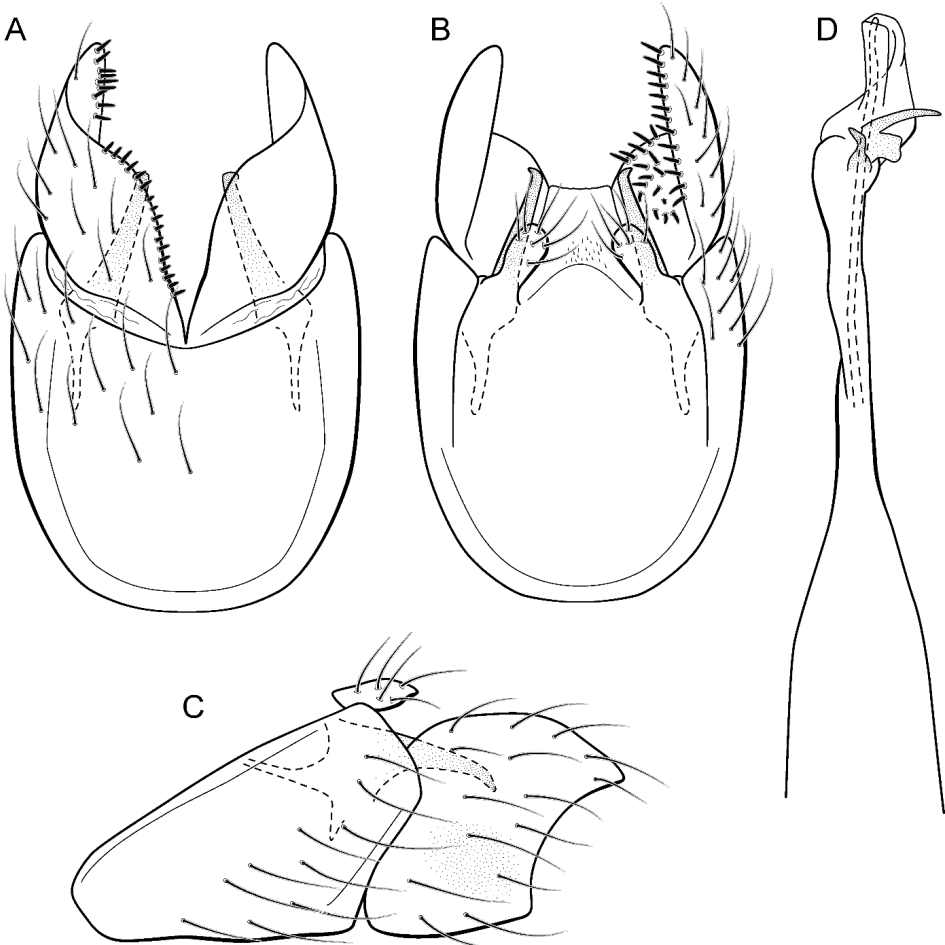
1081 | **Etymology.** The name of this species refers to dense brushes of setae on the dorsal area of the
1082 | male abdomen. In Portuguese, “peluda” means “hairy”.
1083 |

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1084 |
1085 | **Figure 19.** *Metrichia peluda* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)
1086 | lateral view; (D) phallus, dorsal view.
1087 |

1088 | **Remarks.** Modifications on male abdominal segments V, VI, and VII suggest that this new
1089 | species belongs to the *campana* group. The general aspect of inferior appendages is somewhat

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1093 similar to *M. forceps* **sp. nov.** and *M. formosinha* **sp. nov.**, which are excavate posteriorly.
 1094 However, *M. peluda* **sp. nov.** is readily identified by the dense brushes of setae on the dorsum
 1095 of abdominal segments V, VI, and VII. Besides, the male **genitalia** of this new species differ
 1096 from those described for *M. forceps* **sp. nov.** and *M. formosinha* **sp. nov.** by the rounded
 1097 corners of inferior appendages instead of acute and by phallus with two subapical spines with
 1098 unequal sizes.
 1099
 1100 ***Metrichia rafaeli* sp. nov.**
 1101 urn:lsid:zoobank.org:act:CBCADBB8-2C79-49AB-8345-CC1E6FA2AEE9
 1102 (Fig. 20)
 1103 **Adult male.** Length 2.0–2.5 mm (n=7). General color, in alcohol, dark brown. Head with no
 1104 modifications. Ocelli 3. Antenna simple, 20-articulated. Maxillary palpus 5-articulated; labial
 1105 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
 1106 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
 1107 wings. Abdominal segment V with pair of internal pouches; segment VI with pair of internal
 1108 pouches and pair of lateral external sacs with specialized setae; segment VII bearing
 1109 specialized setae dorsally. Ventromesal process on segment VII present. Segment VIII shorter
 1110 ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally; sternum subrectangular,
 1111 with anterior margin rounded (Fig. 20A); in lateral view narrower anteriorly than posteriorly
 1112 (Fig. 20C). Inferior appendage covered by long setae, short and rounded, with apex slightly
 1113 excavated (Fig. 20A); in lateral view, rounded (Fig. 20C). Dorsal hook long, more than half
 1114 length of inferior appendage; in lateral view, downturned (Fig. 20C). Preanal appendage
 1115 elongate, but shorter than half length of inferior appendage, and bearing stout and striate setae
 1116 (Fig. 20B). Subgenital plate apparently absent. Tergum X membranous and truncate (Fig.
 1117 20B). Phallus tubular, elongate and slender, slightly constricted mesally, with a median

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1123 process; with two long, curved, subapical spines, and a membranous lobe; apex rounded and
 1124 sclerotized; ejaculatory duct sclerotized, sinuous, and not protruding apically (Fig. 20D).

1125 **Holotype male. BRAZIL: Ceará:** Ubajara, Parque Nacional de Ubajara, Rio das Minas,
 1126 03°50'03"S 40°54'18"W, el. 524, 17–18.ii.2013, DM Takiya, JA Rafael, RR Cavichioli &
 1127 APM Santos cols., Malaise trap (CZMA).

1128 **Paratypes.** Same data as holotype, 1 male (MZUFBA); same data, except Rio das Minas,
 1129 03°49'58"S 40°53'53"W, el. 420 m, 20–23.iv.2012, F Limeira-de-Oliveira et al. cols., Malaise
 1130 trap, 1 male (CZMA); same data, except 14–16.ii.2013, DM Takiya, JA Rafael, RR
 1131 Cavichioli & APM Santos cols., 1 male (DZRJ).

1132 **Etymology.** This species is named in honor of the Brazilian entomologist Dr. José Albertino
 1133 Rafael (INPA), who has collected many interesting caddisflies, including some species
 1134 described here.

1135 **Remarks.** This new species belongs to the *nigritta* group due to internal pouches between
 1136 abdominal segments V and VI and long and acute process on phallus. The male genitalia of *M.*
 1137 *rafaeli* **sp. nov.** are more similar to *M. magna* Bueno-Soria & Holzenthal, 2003 with short and
 1138 simple inferior appendages. However, the new species can be easily distinguished from the
 1139 latter and other *Metrichia* species in this group by the posterior margin of inferior appendages
 1140 slightly excavated and very long subapical spines of phallus.

1141 Although specimens with barcode sequences of *M. rafaeli* **sp. nov.** were collected at
 1142 the same locality, haplotypes were not identical having intraspecific divergence of 0.4%. The
 1143 lowest interspecific distance was 19.4% when compared with *M. itabaiana* **sp. nov.**

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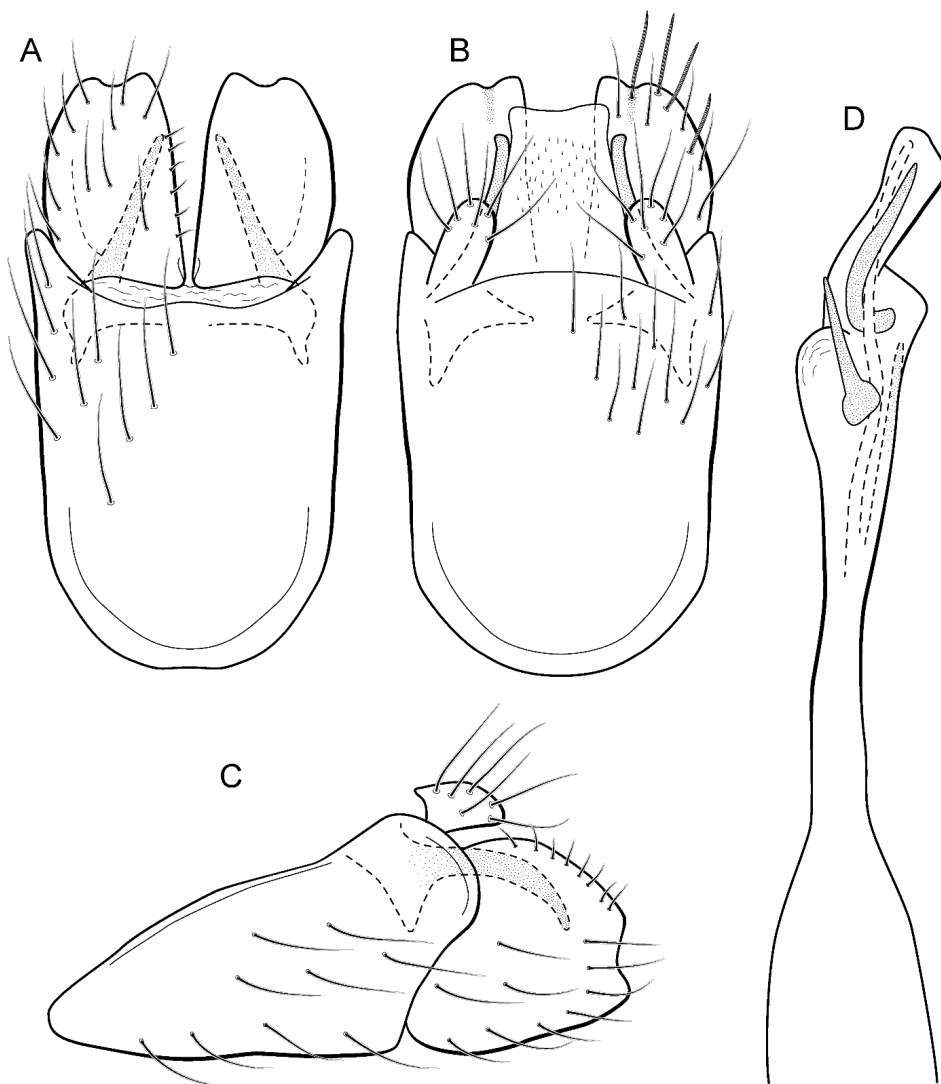


Figure 20. *Metrichia rafaelli* sp. nov., male genitalia: (A) ventral view; (B) dorsal view; (C) lateral view; (D) phallus, dorsal view.

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

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

(Fig. 21, Fig. 26E)

1169 **Adult male.** Length 2.1–2.2 mm (n=2). General color, in alcohol, brown. Head with no
1170 modifications. Ocelli 3. Antenna simple, 19-articulated. Maxillary palpus 5-articulated; labial
1171 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
1172 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
1173 wings. Abdominal segment IV with dorsal area expanded posteriorly bearing stout setae;
1174 segment VI bearing very long setae laterally, with a brush of short setae covered dorsally by a
1175 triangular plate (Fig. 26E); segment VII with a brush of short setae dorsally (Fig. 26E).
1176 Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally.
1177 **Male genitalia.** Segment IX reduced dorsally; sternum subpentagonal (Fig. 21A); in lateral
1178 view narrower anteriorly than posteriorly (Fig. 21C). Inferior appendage covered by long
1179 setae, with apex obliquely truncate; subtrapezoidal in ventral view (Fig. 21A); in lateral view,
1180 subtrapezoidal (Fig. 21C). Dorsal hook short, almost half length of inferior appendage; in
1181 lateral view, slightly downturned (Fig. 21C). Preanal appendage short, rounded and bearing
1182 very long setae (Fig. 21B). Subgenital plate apparently absent. Tergum X membranous and
1183 rounded (Fig. 21B). Phallus tubular, elongate and slender, slightly constricted mesally; with
1184 four subapical spines, three short and one long and straight; apex rounded with a small
1185 sclerite; ejaculatory duct sclerotized and not protruding apically (Fig. 21D).
1186 **Holotype. BRAZIL: Paraná:** Céu Azul, Parque Nacional do Iguaçu, Rio Azul, 25°09'21"S
1187 53°47'44"W, el. 510 m, 6–8 ix.2012, APM Santos, DM Takiya, ALH Oliveira, GA Jardim &
1188 BHL Sampaio cols., Malaise trap, male (DZRJ).
1189 **Paratypes.** Same data as holotype, 1 male (MNRJ).
1190 **Etymology.** This species is named in reference to the simple aspect of the male genitalia and
1191 abdomen, without modifications and processes seen in other *Metrichia* species.
1192 **Remarks.** This species can be assigned to the *campana* group because of pouches in
1193 abdominal segments VI and VII. The new species shares the general aspect of the genitalia

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1196 with *M. quadrata*, particularly, in the quadrangular or rectangular shape of inferior
1197 appendages and the short subapical spines on phallus. *Metrichia simples* **sp. nov.** can be
1198 recognized by the short dorsal hook, reaching up to midlength of inferior appendages
1199 (subequal to inferior appendages in *M. quadrata*), obliquely truncate apex of the inferior
1200 appendages, and strongly curved spines on phallus.

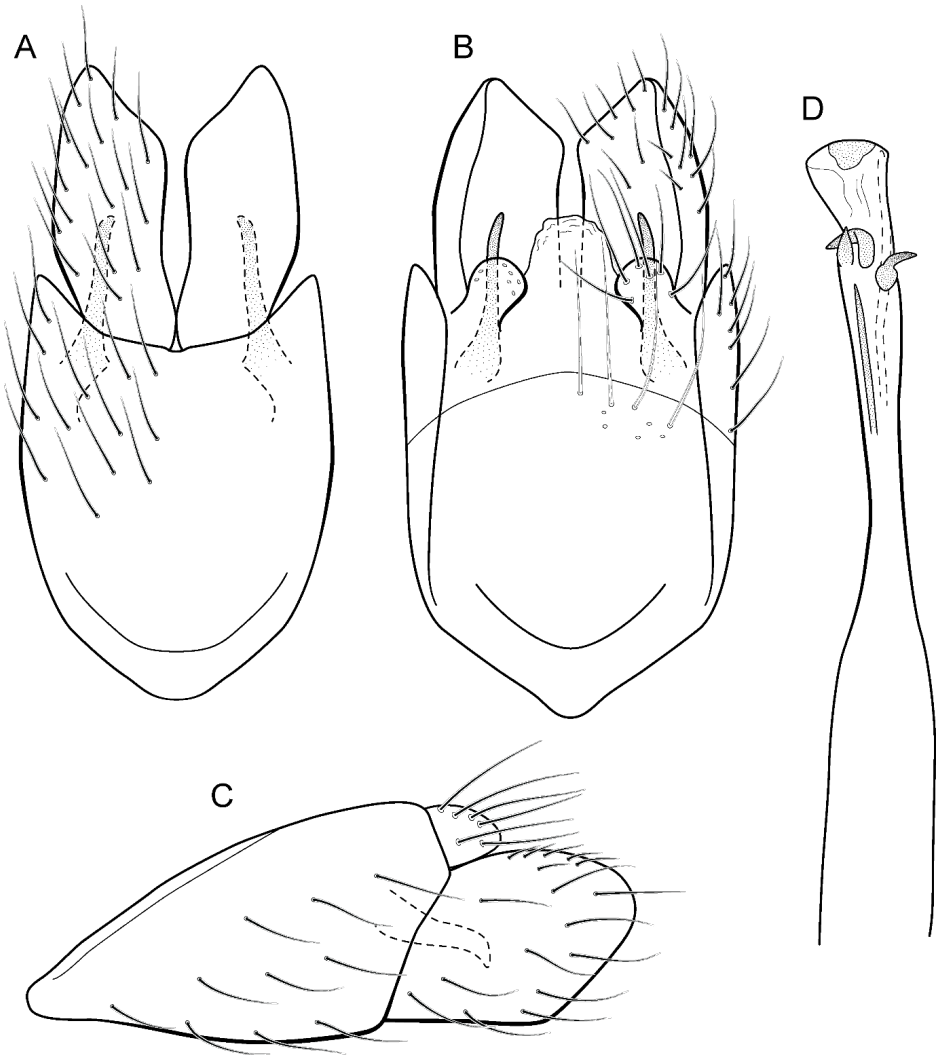
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(almost the same size)



1202

1208 **Figure 21.** *Metrichia simples* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)
1209 lateral view; (D) phallus, dorsal view.

1210

1211 *Metrichia talhada* **sp. nov.**

1212 urn:lsid:zoobank.org:act: 5456FEB8-5193-46DD-A10F-9FFADFCB59EC

1213 (Fig. 22)

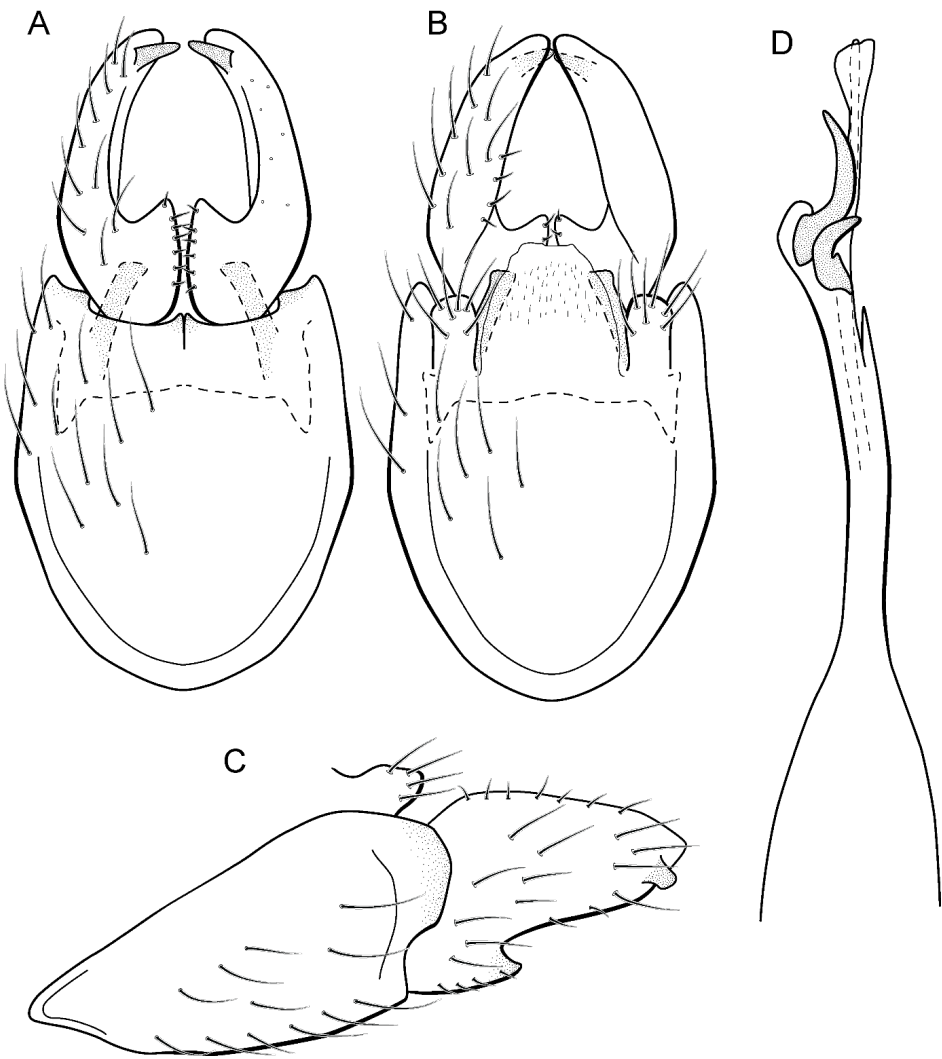
1214 **Adult male.** Length 1.8–2.0 mm (n=10). General color, in alcohol, brown. Head with no
1215 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial
1216 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
1217 Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation
1218 reduced in both wings. Abdominal segment V with pair of internal pouches and pair of
1219 dorsolateral brushes; segment VI with dorsolateral brushes of long setae; segment VII bearing
1220 specialized setae dorsally. Ventromesal process on segment VII absent. Segment VIII shorter
1221 ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally; sternum subpentagonal
1222 (Fig. 22A); in lateral view, narrower anteriorly than posteriorly (Fig. 22C). Inferior appendage
1223 elongate, apex rounded and bearing a tooth-like projection; with a deep C-shaped notch in
1224 ventral view (Fig. 22A); in lateral view, with an acute projection (Fig. 22C). Dorsal hook
1225 short, less than half length of inferior appendage (Fig. 22A); in lateral view, downturned.
1226 Preanal appendage short, rounded and bearing very long setae (Fig. 22B). Subgenital plate
1227 apparently absent. Tergum X membranous and truncate (Fig. 22B). Phallus tubular, elongate
1228 and slender, slightly constricted mesally, with a median process; with two curved subapical
1229 spines, one short and another long; apex rounded and sclerotized; ejaculatory duct sclerotized,
1230 straight and protruding apically (Fig. 22D).

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1232 **Holotype. BRAZIL: Alagoas:** Quebrangulo, Reserva Biológica de Pedra Talhada, Rio
1233 Caranguejo, 09°15'26"S 36°25'08"W, el. 550 m, 19–28.vi.2014, APM Santos, DM Takiya,
1234 WRM Souza cols., Malaise trap, male (DZRJ).
1235 **Paratypes.** Same data as holotype, 8 males (DZRJ), 7 males (MZUFBA).
1236 **Etymology.** This species is named in reference to [jts](#) type locality, the Reserva Biológica de
1237 Pedra Talhada.
1238

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1241 **Figure 22.** *Metrichia talhada* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)
1242 lateral view; (D) phallus, dorsal view.

1244 **Remarks.** The new species belongs to the *nigritta* group because of their internal pouches
1245 between abdominal segments V and VI and phallus with two subapical curved spines and an
1246 acute process near mesal area. *Metrichia talhada* **sp. nov.** shares the apical tooth on inferior
1247 appendages with *M. potosina*, *M. goiana* **sp. nov.**, and *Metrichia tere* **sp. nov.** From *M.*
1248 *potosina* and *M. goiana* **sp. nov.**, it is easily distinguished by the shape of the inferior
1249 appendages, with a deep C-shaped notch on ventral margin and subapical tooth stout and
1250 slightly truncate in lateral view, and subapical spines on phallus, one long and another short.
1251 Although the male genitalia of *M. talhada* **sp. nov.** are very similar to *M. tere* **sp. nov.**, these
1252 two species can be separated by the shape of inferior appendages teeth, each slightly truncate
1253 and subapical in the former and acute and apical in the later species.

1254 Sequences of *M. talhada* **sp. nov.** showed 0.0% divergence. Morphological similarity
1255 between this species and its sister (*M. tere* **sp. nov.**) had a relatively low genetic divergence of
1256 12.6%, the lowest interspecific distance found in our sampling. In addition to minor but stable,
1257 differences in genital structures, all molecular analyses with COI sequences (NJ, ABGD,
1258 GMYC) corroborate the distinction between *M. talhada* **sp. nov.** and *M. tere* **sp. nov.**, which
1259 are formally described here as different species.

1261 *Metrichia tere* **sp. nov.**

1262 urn:lsid:zoobank.org:act:21376F9C-6308-47BA-ADB8-A42848AD8FB5

1263 (Fig. 23)

1264 **Adult male.** Length 1.9–2.1 mm (n=10). General color, in alcohol, brown. Head with no
1265 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial

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1280 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.

1281 Anterior femur with small acute apical process. Tibial spur formula 1-3-4. Wing venation

1282 reduced in both wings. Abdominal segment V with pair of internal pouches and pair of

1283 dorsolateral brushes; segment VI with dorsolateral brushes of long setae; segment VII bearing

1284 specialized setae dorsally. Ventromesal process on segment VII absent. Segment VIII shorter

1285 ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally; sternum subpentagonal

1286 (Fig. 23A); in lateral view, narrower anteriorly than posteriorly (Fig. 23C). Inferior appendage

1287 elongate, apex with an acute projection; with a deep C-shaped notch in ventral view (Fig.

1288 23A); in lateral view, with an acute projection (Fig. 23C). Dorsal hook short, less than half

1289 length of inferior appendage; in lateral view, downturned (Fig. 23C). Preanal appendage short,

1290 rounded and bearing very long setae (Fig. 23B). Subgenital plate apparently absent. Tergum

1291 X membranous and truncate (Fig. 23B). Phallus tubular, elongate and slender, slightly

1292 constricted mesally, with a median process; with two curved subapical spines, one short and

1293 another long; apex rounded and sclerotized; ejaculatory duct sclerotized, straight and

1294 protruding apically (Fig. 23D).

1295 **Holotype. BRAZIL: Rio de Janeiro:** Teresópolis, Parque Nacional da Serra dos Órgãos, Rio

1296 Paquequer, 22°27'25"S 42°59'52"W, el. 1100 m, 15–18.ix.2011, APM Santos, DM Takiya,

1297 BM Vasconcelos & RA Carvalho cols., Malaise trap, male (DZRJ).

1298 **Paratypes.** Same data as holotype, 9 males (MNRJ), 19 males (DZRJ).

1299 **Etymology.** This species is named in reference to the city of Teresópolis (= “City of Teresa”),

1300 affectionately called as “Terê”. The city was named in honor of Teresa Cristina, [Brazilian](#)

1301 Empress [from 1843 to 1889](#), wife of Dom Pedro II.

1302 **Remarks.** This new species is very similar to the preceding one, also belonging to the *nigritta*

1303 group. *Metrichia tere* **sp. nov.** can be distinguished from *M. talhada* **sp. nov.** by inferior

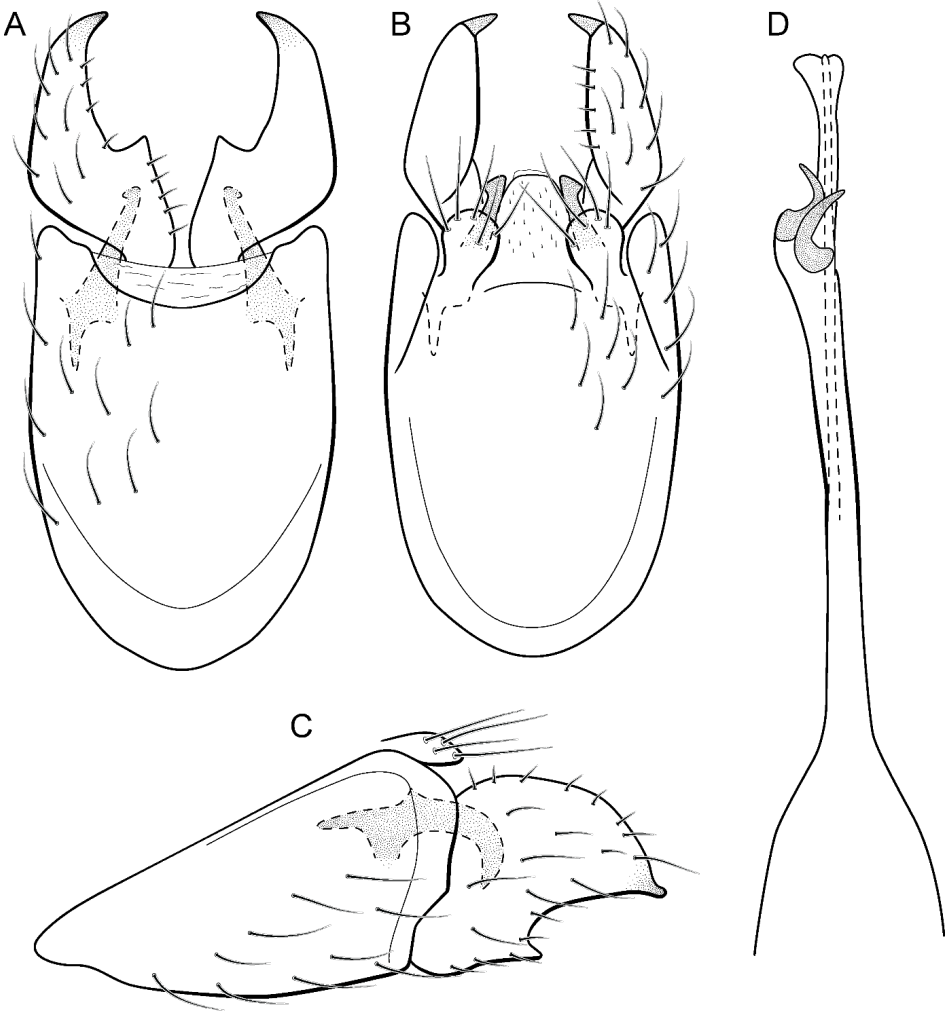
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1307 appendages with an apical acute projection, whereas in *M. talhada* **sp. nov.** inferior
1308 appendages have a subapical tooth, which is more truncate than acute.
1309 Molecular data agree with the morphological distinction of *M. tere* **sp. nov.** as
1310 commented on above. Intraspecific **K2P divergence** among specimens sampled (n=3) of this
1311 species was 0.0%, and as mentioned in the previous description, *M. talhada* **sp. nov.** and *M.*
1312 *tere* **sp. nov.** showed the lowest **observed** interspecific distance (12.6%).
1313

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1324 **Figure 23.** *Metrichia tere* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C)
1325 lateral view; (D) phallus, dorsal view.

1326

1327 *Metrichia ubajara* **sp. nov.**

1328 urn:lsid:zoobank.org:act:68FD2A24-BC85-42A3-9007-96A42657DAE3

1329 (Fig. 24)

1330 **Adult male.** Length 2.0–2.7 mm (n=12). General color, in alcohol, brown. Head with no
1331 modifications. Ocelli 3. Antenna simple, 18-articulated. Maxillary palpus 5-articulated; labial
1332 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
1333 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
1334 wings. Abdominal segment V with pair of internal pouches and median internal plate in
1335 posterior region; with specialized setae on dorsum; segment VI with pair of internal pouches
1336 in posterodorsal area. Ventromesal process on segment VII absent. Segment VIII shorter
1337 ventrally than dorsally. **Male genitalia.** Segment IX reduced dorsally; sternum subrectangular
1338 (Fig. 24A); in lateral view, narrower anteriorly than posteriorly (Fig. 24C). Inferior appendage
1339 covered by long setae, apex rounded; elongate and narrow in ventral view (Fig. 24A); in
1340 lateral view, rounded (Fig. 24C). Dorsal hook short, less than half length of inferior
1341 appendage; apex downturned; basally with a wide and sclerotized projection; in lateral view,
1342 C-shaped (Fig. 24C). Preanal appendage elongate, but shorter than half length of inferior
1343 appendage, and bearing stout and striate setae (Fig. 24B). Subgenital plate apparently absent.
1344 Tergum X membranous and truncate (Fig. 24B). Phallus tubular, elongate and slender,
1345 slightly constricted mesally; with two long, curved, subapical spines; apex rounded and
1346 folded; ejaculatory duct sclerotized, sinuous, and protruding apically (Fig. 24D).

1347 **Holotype. BRAZIL: Ceará:** Ubajara, Parque Nacional de Ubajara, Rio das Minas,
1348 03°49'58"S 40°53'53"W, el. 420 m, 20–23.iv.2012, DM Takiya, JA Rafael, F Limeira-de-
1349 Oliveira et al. cols., Malaise trap, male (CZMA).
1350 **Paratypes.** Same data as holotype, 1 male (CZMA); same data, except 13–17.ix.2012, 25
1351 males (CZMA), 12 males (DZRJ); same data, except 18–30.xi.2012, 9 males (INPA); same
1352 data, except 14–16.ii.2013, 5 males (MZUFBA); same data, except Rio das Minas,
1353 03°50'03"S 40°54'18"W, el. 524 m, 14–16.ii.2013, DM Takiya, JA Rafael, RR Cavichioli &
1354 APM Santos cols., Malaise trap, 5 males (MNRJ).
1355 **Etymology.** This species is named in reference to [Ubajara National Park](#), in the municipality
1356 with the same name, where the types were collected [from](#).
1357

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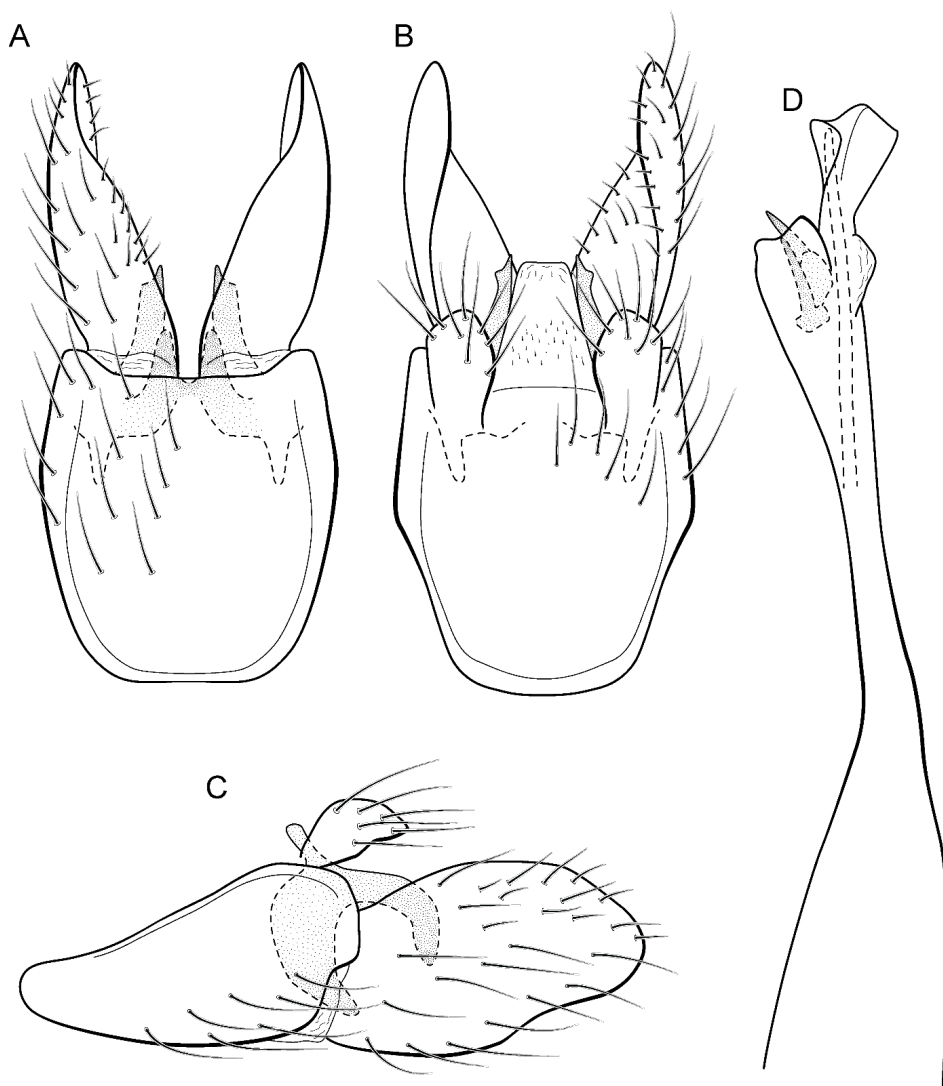


Figure 24. *Metrichia ubajara* **sp. nov.**, male genitalia: (A) ventral view; (B) dorsal view; (C) lateral view; (D) phallus, dorsal view.

Remarks. This new species appears to be a member of the *nigritta* group because of their 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* and *M. goiana*

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1369 **sp. nov.** because of the rounded and elongate inferior appendages in lateral view. However, in
1370 *M. ubajara* **sp. nov.** the inferior appendages lack the "tooth" mentioned for these two species.
1371 As well, *M. ubajara* **sp. nov.** can be recognized by the phallic apex with a broad sclerotized
1372 plate **wrapping** the ejaculatory duct.
1373

1374 ***Metrichia vulgaris* sp. nov.**
1375 urn:lsid:zoobank.org:act:BBC0387F-C111-4CA7-834A-F3711F7707F7
1376 (Fig. 25)
1377 **Adult male.** Length 2.7–3.1 mm (n=12). General color, in alcohol, brown. Head with no
1378 modifications. Ocelli 3. Antenna simple, 21-articulated. Maxillary palpus 5-articulated; labial
1379 palpus 3-articulated. Mesoscutellum with transverse suture. Metascutellum subtriangular.
1380 Anterior femur without processes. Tibial spur formula 1-3-4. Wing venation reduced in both
1381 wings. Abdominal segment VI with pair of internal pouches in posterodorsal area.
1382 Ventromesal process on segment VII present. Segment VIII shorter ventrally than dorsally.
1383 **Male genitalia.** Segment IX reduced dorsally; sternum subquadrangular (Fig. 25A); in lateral
1384 view, narrower anteriorly than posteriorly (Fig. 25C). Inferior appendage covered by long
1385 setae, apex excavated, posterodorsal margin acute and sclerotized; subtrapezoidal in ventral
1386 view (Fig. 25A); in lateral view, subretangular (Fig. 25C). Dorsal hook long and stout, almost
1387 reaching the inferior appendage apex; in lateral view, downturned (Fig. 25C). Preanal
1388 appendage elongate, rounded and bearing very long setae (Fig. 25B). Subgenital plate
1389 apparently absent. Tergum X membranous and rounded (Fig. 25B). Phallus tubular, elongate
1390 and slender, slightly constricted mesally; with two short subapical spines; apex rounded and
1391 folded; ejaculatory duct sclerotized and protruding apically (Fig. 25D).
1392 **Holotype. BRAZIL: Rio de Janeiro:** Itatiaia, Rio Palmital, 22°25'34"S 44°32'52"W, el. 637
1393 m, 07.iii.2008, LL Dumas, JL Nessimian & MR de Souza cols., light trap, male (DZRJ).

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1396 **Paratypes.** Same data as holotype, 1 male (DZRJ), 1 male (MNRJ); same data, except Rio
1397 das Pedras, 22°24'33"S 44°33'08"W, el. 706 m, 06.iii.2008, LL Dumas, JL Nessimian & MR
1398 de Souza cols., light trap, 4 males (MNRJ). **Ceará:** Ubajara, Parque Nacional de Ubajara, Rio
1399 Gameleira, 03°50'25"S 40°54'19"W, el. 874 m, 20–22.iv.2012, F Limeira-de-Oliveira et al.
1400 cols., Malaise trap, 1 male (CZMA). **Goiás:** Alto Paraíso, Rio Bartolomeu tributary,
1401 14°07'25"S 47°30'30"W, el. 1270 m, 22–25.iii.2013, APM Santos & DM Takiya cols.,
1402 Malaise trap, 3 males (DZRJ).

1403 **Etymology.** This new species is named in allusion to its unusually wide distribution
1404 throughout Brazil. From the Latin “vulgaris” meaning “common”.

1405 **Remarks.** This new species belongs to the *campana* group due to their internal pouches
1406 between segments VI and VII, and the two small subapical spines in phallus. *Metrichia*
1407 *vulgaris* **sp. nov.** shares with *M. campana*, *M. similis*, and *M. itabaiana* **sp. nov.** the general
1408 aspect of inferior appendages, with an excavated posterior margin. From these species, *M.*
1409 *vulgaris* **sp. nov.** can be distinguished by their inferior appendages, in lateral view, excavated
1410 but with ventral corner more rounded than acute, and short and stout dorsal hook, and tergum
1411 X short and rounded,

1412

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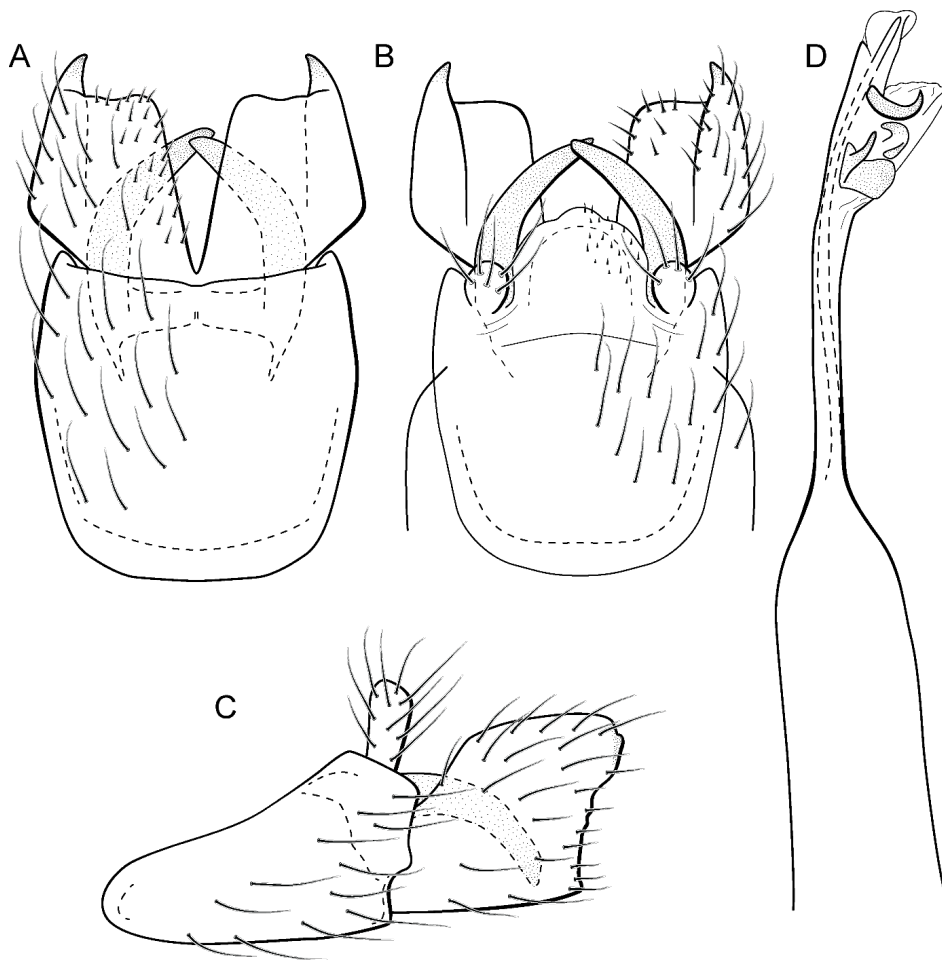


Figure 25. *Metrichia vulgaris* sp. nov., male genitalia: (A) ventral view; (B) dorsal view; (C) lateral view; (D) phallus, dorsal view.

Metrichia vulgaris sp. nov. has an interesting distributional pattern, occurring in very distant localities in Southeastern, Centralwestern, and Northeastern Brazil. In addition to their geographic distance, these localities are included in very distinctive areas, encompassing three biomes (Atlantic Forest, Cerrado, and Caatinga) and four large river basins (East North Atlantic, Southeast Atlantic, São Francisco, and Araguaia-Tocantins). Barcode sequences

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1439 corroborate these different populations as the same species, with K2P intraspecific
 1440 divergences up to 4.8%. This is the highest intraspecific divergence found in our work, but
 1441 this value is still lower than observed within other caddisfly groups' species (Pauls et al.,
 1442 2010, Zhou et al., 2011). GMYC analyses recovered sequences divided into two 'species', but
 1443 these groups were not related to their geographic occurrence: one group included samples
 1444 from Rio de Janeiro and Minas Gerais and the other group, samples from Rio de Janeiro,
 1445 Minas Gerais, and Goiás. The broader sampling of this species (both in area and in specimen
 1446 numbers), compared to others included in the analyses, associated with higher intraspecific
 1447 divergences, can justify the oversplitting with the GMYC method, as discussed by Talavera et
 1448 al. (2013).

1449 Although such wide distribution is not common for *Metrichia* species, other
 1450 microcaddisflies can show continental distributions (e.g., *Oxyethira tica*). Because the
 1451 knowledge about Neotropical microcaddisflies is very poor, this pattern may be more
 1452 common than currently thought.

1454 DISCUSSION

1455 Although GMYC analysis overestimated the number of *Metrichia* species in our study
 1456 (suggesting the split of *M. circuliforme* **sp. nov.** and *M. vulgaris* **sp. nov.** each into two
 1457 'species'), COI sequences strongly corroborated species limits previously defined based on
 1458 morphological features. In general, COI sequences of caddisflies show a robust 'barcoding
 1459 gap' (Pauls et al., 2010, Zhou et al., 2011), making this molecular marker appropriate as a
 1460 source of additional information to corroborate species delimitation or associations of
 1461 different life stages.

1462 Microcaddisflies are extremely diverse and poorly known, and when associated with
 1463 morphologic data, the use of molecular information can result in a more robust taxonomy for
 1464 this group. Although methods such ABGD and GMYC should not be used alone to determine

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1484 'species', they are useful tools to identifying 'potential species' (Puillandre et al., 2012;
1485 Talavera et al., 2013), especially in very diverse groups and/or with dubious morphology-
1486 based identification. The wide distribution of *Metrichia vulgaris* **sp. nov.** could indicate the
1487 existence of different **cryptic** species, **however** morphology and barcode **data** agreed to define
1488 **this group as a single** species. Although GMYC overestimated the number of *Metrichia*
1489 species in our analysis, **we consider** this method **an** important tool for preliminary distinction
1490 when taxonomic information is poor.

1491 Based on **the** presence of abdominal modifications, such as internal pouches, external
1492 sclerotized plates, and brushes of long setae, and features of male genitalia, six species groups
1493 have been proposed for *Metrichia* (Flint, 1972; Bueno-Soria & Holzenthal, 2003). **These**
1494 **abdominal** modifications usually arise from segments V, VI, and VII, **and** in general aspect,
1495 **are** very distinctive from each other (Fig. 26), possibly representing non-homologous
1496 structures. **Nevertheless, most** of the species described herein fit in these **previously defined**
1497 groups. **Although, the present analyses of COI sequences (Fig. 2 and Supp. 4) did not**
1498 **corroborate the monophyly of all four** species groups, **with more than one species included**
1499 **(neither of *Metrichia*), these relationships need to be analyzed further with better taxon** and
1500 **molecular marker** sampling.

1501

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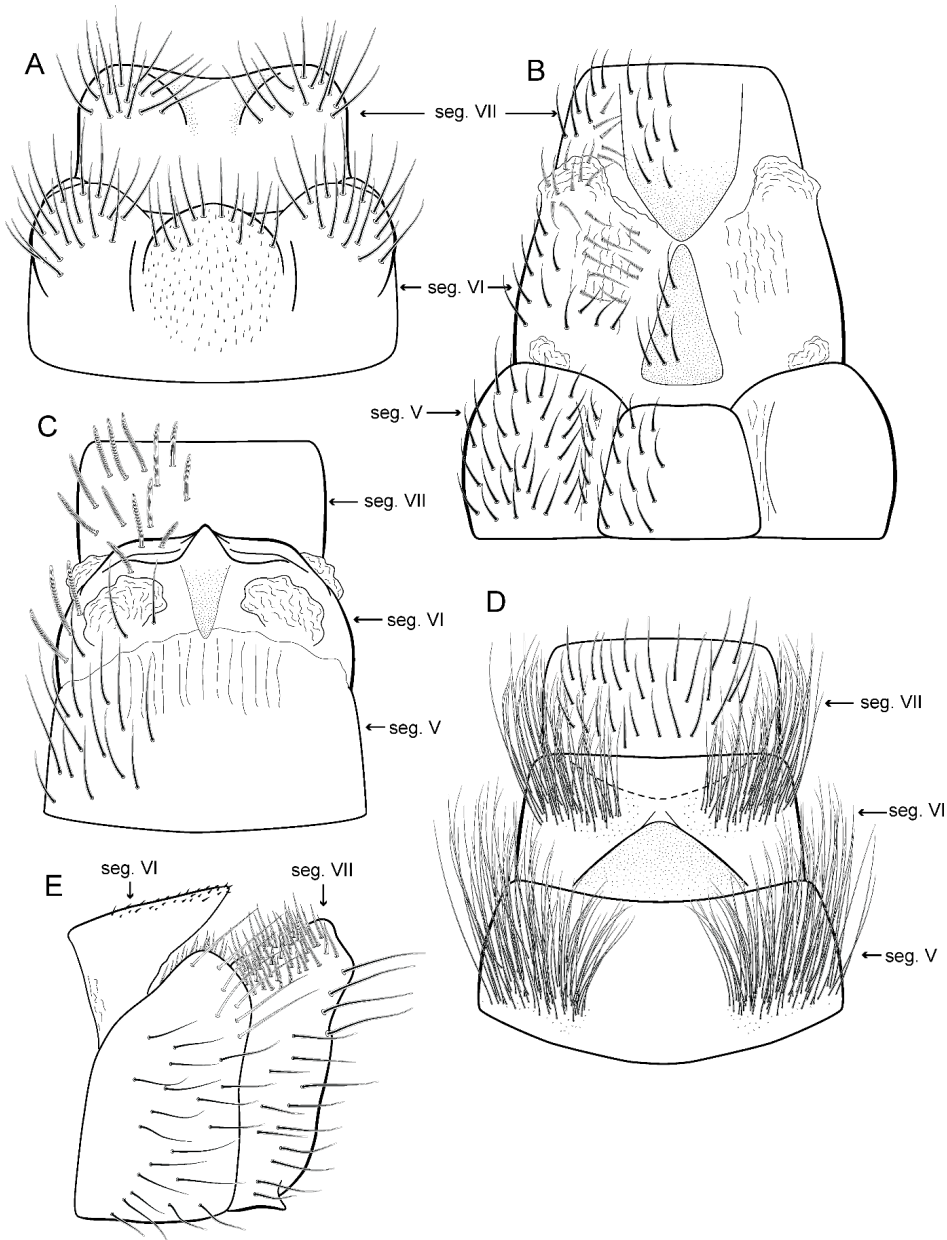
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1531

1532 **Figure 26.** Abdominal modifications of *Metrichia* species: (A) *M. acuminata* **sp. nov.**,
 1533 segments VI and VII, dorsal view; (B) *M. itabaiana* **sp. nov.**, segments V, VI, and VII, dorsal
 1534 view; (C) *M. longissima* **sp. nov.**, segments V, VI, and VII, dorsal view; (D) *M. peluda* **sp.**

1535 **nov.**, segments V, VI, and VII, dorsal view; (E) *M. simples* **sp. nov.**, segments VI and VII,
1536 lateral view.

1537

1538 [Herein, we have](#) also used molecular data to associate [larvae](#) and [adults](#) of *M. bonita*
1539 **sp. nov.** Larvae remain unknown for most Neotropical species of Trichoptera, and they are
1540 even less known for microcaddisflies. Rearing immatures is very difficult and association
1541 based in co-occurrence with adults is not possible when several species of the same genus co-
1542 occur. In this way, DNA barcodes are a powerful tool, allowing the association and
1543 description of immature stages (Shan et al., 2004; Pauls et al., 2010; Ruiter et al., 2013).
1544 Barcode reference libraries for caddisflies are available [for specimens from](#) other regions
1545 (Zhou et al., 2009; Zhou et al., 2011; Ruiter et al., 2013) and represent an important source of
1546 information for taxonomic work [as well as](#) [for ecological](#) [and](#) evolutionary studies. We expect
1547 that molecular data will become increasingly common for Neotropical caddisflies [as it](#)
1548 [facilitates](#) the understanding of their diversity in this region.

1549

1550 **SUPPLEMENTAL INFORMATION**

1551

1552 **Collecting sites**

1553 [Detailed](#) list of collecting sites in Brazil where new species of *Metrichia* were found.

1554

1555 **Georeferences of collecting sites**

1556 Google Earth (.kmz) file with collecting localities [of new *Metrichia* species](#).

1557

1558 **COI sequence alignment**

1559 FASTA format alignment of COI sequence data of *Metrichia* and related microcaddisflies.

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1571 **Bayesian inference estimated tree**

1572 Consensus phylogram (50% majority-rule) from BI analyses of COI sequences ([mean](#) lnL= -
1573 5464.29) of *Metrichia* and related microcaddisflies. Values displayed near branches are
1574 posterior [probabilities](#).

1575

1576 **K2P pairwise divergences**

1577 [Pairwise K2P](#) divergences of COI [sequences](#) of *Metrichia* and related microcaddisflies.

1578

1579 **ACKNOWLEDGEMENTS**

1580 We thank the Laboratório de Entomologia, UFRJ team for helping in field trips. Additional
1581 specimens used in this study were provided by J. A. Rafael (INPA), M. L. Monné (Museu
1582 Nacional, UFRJ), and C. H. Dietrich (University of Illinois at Urbana-Champaign). We also
1583 thank [the academic editor J.D. Reimer and two anonymous reviewers](#) for valuable comments
1584 [and A. M. Ray \(Xavier University\) for conducting an English review.](#)

1585

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