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Integrative taxonomy supports two new species of *Chimarra* Stephens, 1829 from Brazil (Trichoptera: Philopotamidae)

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Abstract

Chimarra Stephens, 1829 is the largest genus of the Philopotamidae with about 930 species and cosmopolitan distribution. Recent taxonomic revisions have subdivided the genus into four subgenera: *Chimarra*, *Curgia* Walker, 1860, *Chimarrita* Blahnik, 1997, and *Otarrha* Blahnik, 2002, the last three restricted to the New World. In this paper, we describe and illustrate two new species of *Chimarra* from Brazil, *C.* (*Otarrha*) *paraodonta* **sp. nov.** from Rio de Janeiro State and *C.* (*Chimarrita*) *truncata* **sp. nov.** from Amazonas and Pará states. Partial sequences of cytochrome oxidase subunit I (COI, DNA barcodes) were generated and integrated with morphological evidence to delimit the new species and evaluate their phylogenetic relationships within the genus. A maximum likelihood analysis of 48 COI sequences representing 19 species of *Chimarra* corroborated their subgeneric assignment based on morphology and highlighted their putative sister species. Both new species showed high K2P divergences when compared to their sister species: *Chimarra* (*O.*) *paraodonta* **sp. nov.** and *C.* (*O.*) *odonta* (17.4–21.3%) and *Chimarra* (*C.*) *truncata* **sp. nov.** and *C.* (*C.*) *simpliciforma* (20.0–21.3%). These distances are comparable to the range of interspecific distances calculated for the whole genus (13.6–22.7%), adding support to their description as new species. This analysis was especially important because of the high morphological similarity of *C. paraodonta* **sp. nov.** and *C. odonta*. Finally, analysis of the sequences of *Chimarra odonta* suggests that the nominal species may represent a complex of cryptic species with high intraspecific divergences (up to 18.1%), with at least two of those lineages co-occurring with *C. paraodonta* **sp. nov.** at Parque Nacional do Itatiaia.

Key words

Amazon, Atlantic Forest, caddisfly, DNA barcoding, finger-net caddisflies, Neotropics

1. Introduction

Philopotamidae comprise approximately 1,500 species in 26 extant genera distributed worldwide, but most of the diversity is found in the tropics (Holzenthal et al. 2018; Morse et al. 2019). Currently, the family is divided into three subfamilies: Rossodinae, with 16 species solely in one genus, endemic to Madagascar; the cosmopolitan Chimarrinae, with about 950 extant species distributed into three genera; and the Philopotaminae, with more than 400 species in 18 genera (Holzenthal et al. 2018; Morse 2021; Cartwright 2020). *Chimarra* Stephens, 1829 is one of the most diverse caddisfly genera with about 930 species (Kjer et al. 2014; Cartwright 2020); of these about 260 species occur in the Neotropical Region and 54 species in Brazil (Vilarino and Calor 2015; Holzenthal and Calor 2017; Dumas and Santos 2021). *Chimarra* adults range from 3−8 mm in size and are generally black or dark-brown colored, being commonly found near a wide variety of flowing waters, where larvae usually construct tubular, silken retreats attached beneath or between large substrates, like logs or rocks (Blahnik 1997; Holzenthal and Calor 2017). They can be characterized by having tibial spur formula 1–4–4 and presence of an anal loop on hind wing, in which the 2A vein is looped to join 1A vein (Blahnik 1998).

Currently, *Chimarra* is subdivided into four subgenera and the New World species have been the subject of relatively recent taxonomic revisions (Blahnik 1997, 1998, 2002; Flint 1998). The nominotypical subgenus *Chimarra* is the largest with about 550 species known from all zoogeographical regions, being more diverse in the Oriental Region with 321 species. In the New World approximately 100 species are recorded, being placed in 19 species groups, about half of them including only one or two species (Blahnik 1998; Kjer et al. 2014; Holzenthal and Calor 2017). The subgenera *Curgia* Walker, 1860, *Chimarrita* Blahnik, 1997, and *Otarrha* Blahnik, 2002 are confined to the New World, especially in the Neotropics (Blahnik and Holzenthal 2012). *Curgia* has 93 species distributed in 16 species groups ranging from the southwestern portion of the United States through Central and South America, extending into the Lesser and Greater Antilles (Flint 1998; Santos and Nessimian 2009). *Chimarrita* (21 spp.) and *Otarrha* (33 spp.) are endemic to the Neotropics, being widespread in Central and South America, including the Antilles (Blahnik 1997, 2002; Blahnik and Holzenthal 2012; Vilarino and Calor 2015; Desidério et al. 2018; Camargos 2016).

Recent phylogenetic analyses inferred from molecular data (Kjer et al. 2014; Wahlberg and Johanson 2014) support the monophyly of *Chimarra*, as well as that of its subgenera, but minor incongruences among some lineage relationships were found when compared with morphological data proposed in subgeneric revisions (Flint 1998; Blahnik 1997, 1998, 2002). Wahlberg and Johanson (2014) performed a biogeographical analysis pointing to an early Cretaceous origin, circa 138 million years ago, in the Neotropical region, followed by subsequent radiations into the Oriental, Palearctic, and Australasian regions, with several independent colonization events into the Afrotropical region. Both works are of great value as they provide unprecedented amounts of nuclear and mitochondrial sequence data for a large and worldwide distributed caddisfly genus.

In the past few decades, molecular techniques have been used widely for species separation and identification (Vogler and Monaghan 2006) and have become increasingly popular for taxonomic studies (Doyle 1992; Soltis et al. 2000; Moore et al. 2010). The DNA barcode, a short standard DNA region of the mitochondrial cytochrome oxidase I gene (COI), is the most commonly used marker for animal species identification, including several freshwater insect groups (e.g., Ephemeroptera - Ball et al. 2005; Trichoptera – Zhou et al. 2016; Simuliidae - Rivera and Currie 2009; Ephemeroptera, Plecoptera and Trichoptera – Morinière et al. 2017). DNA barcoding is not used only for species identification, but also for evolutionary, ecological, and conservation research (Hebert et al. 2003; Valentini et al. 2009; Leese et al. 2018). In addition, in recent years the use of DNA barcoding has increased the discovery of cryptic species in different taxa, habitats, and regions (e.g., Pfenninger and Schwenk 2007; Zakšek et al. 2009; Pauls et al. 2010; Jackson et al. 2014; Weiss et al. 2014).

In Trichoptera, studies using DNA barcodes have increased in recent decades. Initially, molecular data were used mainly to facilitate association of immatures with adults (e.g., Shan et al. 2004; Graf et al. 2005; Zhou 2009; Xu and Wang 2018; Stroil et al. 2018; Vitecek et al. 2020; Ruiz-Garcia et al. 2021) as an alternative to more traditional techniques, like rearing larvae/pupae in the laboratory or using the metamorphotype method (Milne 1938; Wiggins 1996). However, the use of DNA barcode has diversified for other types of applications, such as species delimitations (e.g., Balint et al. 2009; Zhou et al. 2009, 2010; Beermann et al. 2017; Hjalmarsson, 2019), cryptic diversity (e.g., Pauls et al. 2010; Zhou et al. 2011; Previšić et al. 2014; Wickson et al. 2014), biodiversity and conservation (e.g., Bozáňová et al. 2021), and integrative taxonomy (e.g., Salokannnel et al. 2010; Santos et al. 2016).

Although the use of COI sequences has become common in taxonomic studies at the species level, their use is still rare for Neotropical caddisflies. The first comprehensive work using this tool in the Neotropics was made by Pauls et al. (2010), where authors corroborated two new species and revealed the existence of cryptic diversity of *Smicridea* (*Smicridea*) McLachlan, 1871 in Chile. After that, only few works using DNA barcodes were carried out in order to associate different life-stages (e.g., Santos et al. 2016; Barcelos-Silva et al. 2018) and to evaluate species delimitation in integrative taxonomy efforts (e.g., Santos et al. 2016; Vilarino et al. 2019).

In this study two new species of *Chimarra* are described and illustrated in the subgenera *Chimarrita* and *Otarrha* through an integrative taxonomic approach based on adults from the Amazon and Atlantic Forest biomes of Brazil. In addition, DNA barcodes were gen-

erated and integrated in order to evaluate species delimitation and relationships of the new taxa within the genus *Chimarra*.

2. Material and Methods

2.1. Material examined

Specimens of the new species were collected with Malaise traps (Gressit and Gressit 1962) and Pennsylvania light traps (Frost 1957) in two Brazilian biomes: Amazon Forest in Amazonas and Pará states and Atlantic Forest in Rio de Janeiro State (Fig. 1). Collected specimens were preserved in 96% ethanol. Specimens were identified based on male genitalic morphology using Blahnik (1997, 2002), Blahnik and Holzenthal (2012), Vilarino et al. (2015), Camargos (2016), and Desidério et al. (2018). In order to observe genital structures, the abdomen of each specimen was removed and cleared using heated 10% KOH or, alternatively, hot lactic acid for a few minutes (Blahnik et al. 2007), followed by a rinse in distilled water. After clearing, the abdomen was mounted on a temporary slide using glycerin or glycerin jelly, and it was examined under a Carl Zeiss Axiolab compound microscope equipped with a camera lucida. Finally, the abdomen was stored in a microvial with 96% ethanol together with the respective specimen. Pencil sketches were produced and then used as templates for vectorization in Adobe Illustrator CS6 (v. 16.0.0, Adobe Systems, Inc.) to create illustrations.

The distribution map was prepared using QGIS Las Palmas 2.18.10 software (QGIS Developed Team 2016). Morphological terminology for male genitalia follows that of Blahnik (1997, 2002). Type specimens are deposited in Coleção Entomológica Professor José Alfredo Pinheiro Dutra, Departamento de Zoologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (DZRJ), Coleção Entomológica do Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ), and Coleção de Invertebrados, Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA).

2.2. DNA extraction, amplification, sequencing, and alignments

Genomic DNA was extracted from legs of male adults through nondestructive methods using the DNeasy Blood & Tissue kit (Qiagen Inc., Hilden, Germany), optimizing the original protocol by incubating the tissue for lysis in Proteinase K for 48 hours and generating two separate 50µl elutions of DNA extract, instead of 100µl. Voucher specimens were deposited at DZRJ and INPA (Table 1).

Partial mitochondrial cytochrome oxidase c subunit 1 (COI) gene was amplified by using primers C1-J-1718 (5-GAGGATTTGGAAATTGATTAGTTCC-3) (Simon et al. 1994) and HCO-2198 (5-TAAACTTCAGGGTGACCAAAAAATCA-3) (Folmer et al. 1994). All PCR reactions had a total volume of 25µl and contained 5µl 5x Taq buffer (Promega), 3.5µl MgCl2 (25mM, Promega), 2µl BSA (10 mg/ml, Promega), 1µl dNTP mix (20mM, Promega), 0.5µl of each primer at 10mM (Invitrogen), 0.2µl Go®Taq DNA polymerase enzyme (Promega), and 3.0−8.0µl genomic DNA. The thermocycling profile consisted of 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 48°C, and 2 min at 72°C, with a final step of 7 min at 72°C.

PCR products were stained with GelRed™ (Biotium) and underwent agarose gel electrophoresis in 1.0% TBE and visualized under UV light. Amplicons were purified using ExoSAP-IT® (USB Affymetrix). DNA sequencing was performed in both forward and reverse directions using the same PCR primers by Macrogen (Seoul, South Korea). All sequences generated as part of this study were deposited in GenBank under accessions OM964809-OM964828.

Consensus sequences were constructed based on electropherogram assemblies in Geneious® v9.1.2 (Kearse et al. 2012) and used in a comparative similarity search at GenBank using the BLAST program (Altschul et al. 1990). Consensus sequences were aligned in Geneious using the ClustalW algorithm (Thompson et al. 1994) with a gap opening cost of 15 and a gap extension of 6.66. Amino acid translations were conducted to check for the absence of stop codons in the alignment.

2.3. Taxon sampling

In addition to sequences generated herein of the new species and two other *Chimarra* species, COI sequences publicly available from GenBank were included in the alignment to a total of 52 sequences (Table 1). As outgroups, four species of Philopotamidae were included: *Wormaldia planae* Ross & King (KX292642), *Philopotamus montanus* (Donovan, 1813) (MZ046700), *Sortosa chilensis* (Navás, 1918) (KM225345), and *Chimarrhodella peruviana* (Ross, 1956) (KX107274), the latter used for rooting according to Wahlberg and Johanson (2014). Sampling of *Chimarra* included the *incertae sedis C. usitatissima* Flint, 1971 and species representatives of the subgenera *Curgia* (2 spp.), *Chimarra* (2 spp.), *Chimarrita* (5 spp.), and *Otarrha* (6 spp.). In the latter, a more extensive sampling of individuals belonging to the *Chimarra odonta* complex (16 sequences) were also included. Although more species of *Chimarra* have COI sequences available in GenBank, we have selected species that were more closely related to the new species according to their morphology.

2.4. Phylogenetic analyses and K2P divergences

Phylogenetic relationships were estimated by maximum likelihood using RAxML v.8.2.11 (Stamatakis 2014) with 1,000 search replicates under GTR+I+G model. Model of

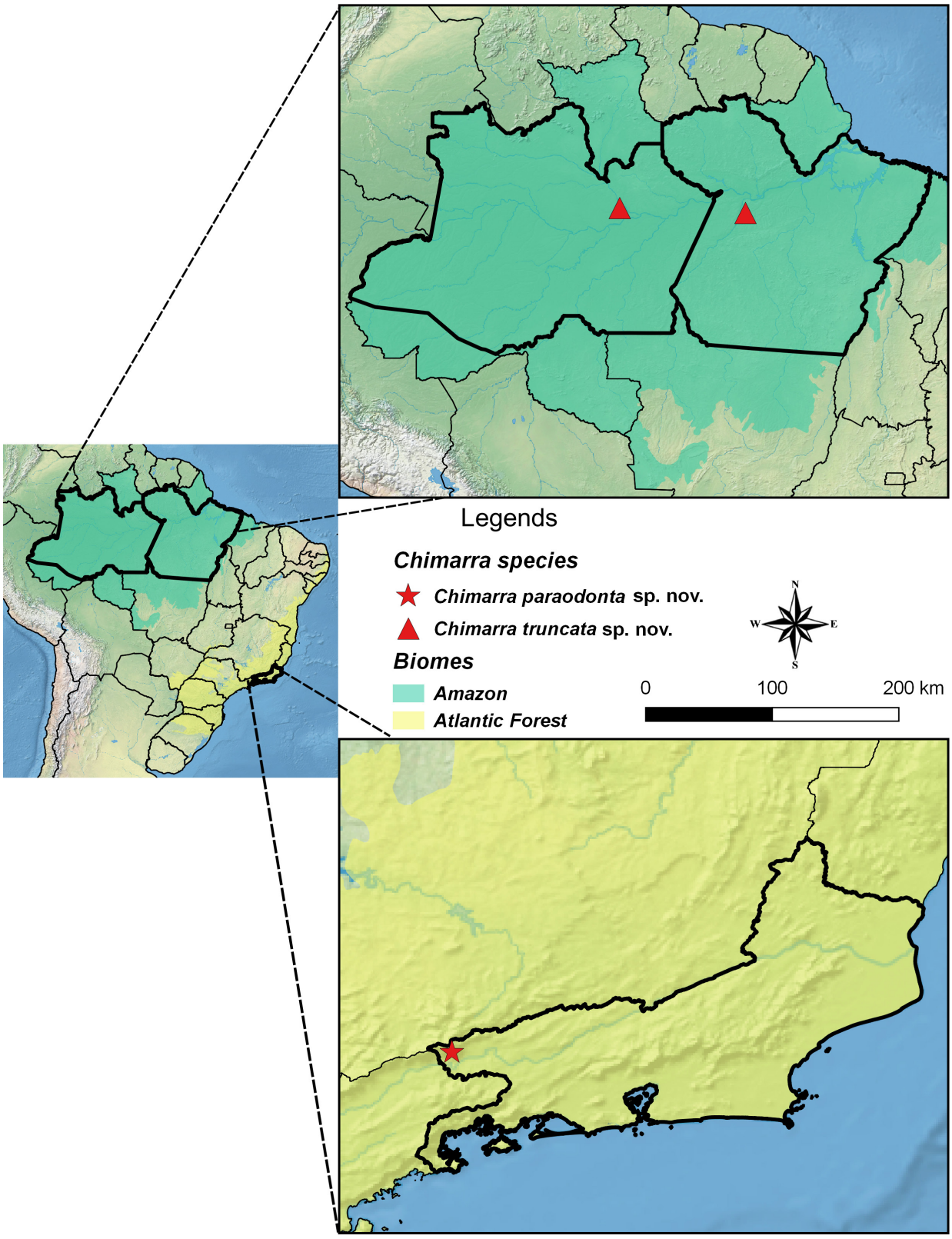

Figure 1. Distributional map of *Chimarra* (*Chimarrita*) *truncata* **sp. nov.** (Amazonas and Pará states) and *Chimarra* (*Otarrha*) *paraodonta* **sp. nov.** (Rio de Janeiro State) in Brazil.

nucleotide evolution was selected using the Akaike Information Criterion (AIC) (Akaike 1974) by jModelTest v.2.1.6 (Posada 2008). Bootstrap resampling was used to test support for tree nodes (Felsenstein 1985) and was calculated with 500 pseudoreplicate matrices. Newick tree files were viewed in FigTree v.1.4.4 (Rambaut 2014), exported as vector images, and later edited in Adobe Illustrator.

Intra- and interspecific genetic divergences were calculated using the Kimura 2-parameter model – K2P (Kimura 1980) with pairwise deletion when missing data in MEGA7 (Kumar et al. 2016).

Table 1. Species sampled for the phylogenetic analysis of *Chimarra* with GenBank accession numbers for COI sequences and information on specimens sequenced herein (accession numbers in bold), such as voucher specimen code at DZRJ, collection locality, and adult gender.

Species	Voucher code	Gender	Collection locality	Accession number
OUTGROUP				
Chimarrhodella peruviana			Venezuela: Barinas	KX107274
Sortosa chilensis			Chile	KM225345
Philopotamus montanus			Switzerland: Grisons	MZ046700
Wormaldia planae			Costa Rica: Puntarenas	KX292642
INGROUP – *Chimarra* spp.				
C. usitatissima			Brazil: Minas Gerais	KX106978
C. (*Chimarra*) *forcipata*			French Guiana	KM225357
C. (*Chimarra*) *marginata*			Finland: Etela-Suomen Laani	KX295044
C. (*Chimarra*) *obscura*			Canada: Ontario	KM537514
C. (*Chimarrita*) *camella*			Brazil: Rio de Janeiro	KX102673
C. (*Chimarrita*) *camura*			Brazil: Rio de Janeiro	KX104186
C. (*Chimarrita*) *kontilos*			Brazil: São Paulo	KX103574
C. (*Chimarrita*) *simpliciforma*			Guyana: Upper Demerara-Berbice	HQ967557
C. (*Chimarrita*) *simpliciforma*	ENT4288	Male	Brazil: Pará, Tailândia, Rod. PA-150, Km 74, Agropalma	**OM964809**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT4292	Male	Brazil: Amazonas, Novo Airão, Rod. AM352, km-68, Igarapé Mato Grosso	**OM964817**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT4293	Male	Brazil: Amazonas, Novo Airão, Rod. AM352, km-68, Igarapé Mato Grosso	**OM964816**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT4294	Male	Brazil: Amazonas, Novo Airão, Rod. AM352, km-68, Igarapé Mato Grosso	**OM964815**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT4295	Male	Brazil: Amazonas, Novo Airão, Rod. AM352, km-68, Igarapé Mato Grosso	**OM964814**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT4296	Male	Brazil: Amazonas, Novo Airão, Rod. AM352, km-68, Igarapé Mato Grosso	**OM964813**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT4297	Male	Brazil: Amazonas, Novo Airão, Rod. AM352, km-68, Igarapé Mato Grosso	**OM964811**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT4298	Male	Brazil: Amazonas, Novo Airão, Rod. AM352, km-68, Igarapé Mato Grosso	**OM964812**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT5580	Male	Brazil: Pará, Belterra, BR 163, Km 85, Igarapé do Branco	**OM964819**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT5581	Male	Brazil: Pará, Belterra, BR 163, Km 85, Igarapé do Branco	**OM964810**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT5582	Male	Brazil: Pará, Belterra, BR 163, Km 85, Igarapé do Branco	**OM964823**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT5583	Male	Brazil: Pará, Belterra, BR 163, Km 85, Igarapé do Branco	**OM964820**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT5584	Male	Brazil: Pará, Belterra, BR 163, Km 85, Igarapé do Branco	**OM964821**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT5591	Female	Brazil: Pará, Belterra, BR 163, Km 85, Igarapé do Branco	**OM964818**
C. (*Chimarrita*) *truncata* **sp. nov.**	ENT5592	Female	Brazil: Pará, Belterra, BR 163, Km 85, Igarapé do Branco	**OM964822**
C. (*Curgia*) *braconoides*			Brazil: Al Ibateguara	KX144398
C. (*Curgia*) *peruviana*			Bolivia: Santa Cruz	KX104022
C. (*Otarrha*) *odonta*			Brazil: Rio de Janeiro, Parque Nacional da Serra dos Órgãos, Rio Beija-flor	KX104378
C. (*Otarrha*) *odonta*			Brazil: Rio de Janeiro, Parque Nacional do Itatiaia, Rio Campo Belo	KX105279
C. (*Otarrha*) *odonta*	ENT5640	Male	Brazil: Rio de Janeiro, Maricá, Caranguejo stream	**OM964825**
C. (*Otarrha*) *odonta*	ENT5642	Male	Brazil, Rio de Janeiro, Nova Iguaçu, Tinguá	**OM964827**
C. (*Otarrha*) *odonta*	ENT5641	Male	Brazil: Rio de Janeiro, Petrópolis, Araras, Araras stream	**OM964826**
C. (*Otarrha*) *odonta*	ENT5635	Male	Brazil: Rio de Janeiro, Itatiaia, Parque Nacional do Itatiaia, Córrego Maromba	**OM964828**
C. (*Otarrha*) *odonta* complex sp.			Brazil: Rio de Janeiro, Parque Nacional do Itatiaia	HQ582429
C. (*Otarrha*) *odonta* complex sp.			Brazil: Rio de Janeiro, Parque Nacional do Itatiaia	HQ582430
C. (*Otarrha*) *odonta* complex sp.			Brazil: São Paulo, Bananal	HQ582431
C. (*Otarrha*) *odonta* complex sp.			Brazil: Rio de Janeiro, Rio Macaé, Macaé de Cima	KX141696
C. (*Otarrha*) *odonta* complex sp.			Brazil: Santa Catarina, Parque Ecológica Spitzkopf, confl. Rio Ouro & Rio Caeté	KX142040
C. (*Otarrha*) *odonta* complex sp.			Brazil: Rio de Janeiro, Parque Nacional da Serra dos Órgãos, Rio Beija-flor	KX144047
C. (*Otarrha*) nr. *odonta*			Brazil: São Paulo	KX104578
C. (*Otarrha*) nr. *odonta*			Brazil: Rio de Janeiro, Parque Nacional do Itatiaia, Rio Campo Belo	KX105682

Species	Voucher code	Gender	Collection locality	Accession number
C. (*Otarrha*) nr. *odonta*			Brazil: São Paulo, 11 km SE Bananal, small stream on São Paulo Route 247	KX106200
C. (*Otarrha*) *parilis*			Peru: Madre de Dios	KX105173
C. (*Otarrha*) *patosa*			Bolivia: La Paz	KX106455
C. (*Otarrha*) *paraodonta* **sp. nov.**	ENT5577	Male	Brazil: Rio de Janeiro, Itatiaia, Parque Nacional do Itatiaia, Córrego Maromba	**OM964824**
C. (*Otarrha*) *peruana*			Peru	KM225365
C. (*Otarrha*) *phthanorossi*			Colombia: Choco	KX102748
C. (*Otarrha*) *rossi*			Costa Rica: Guanacaste	KX106996
C. (*Otarrha*) *tachuela*			Venezuela: Merida	KX103258

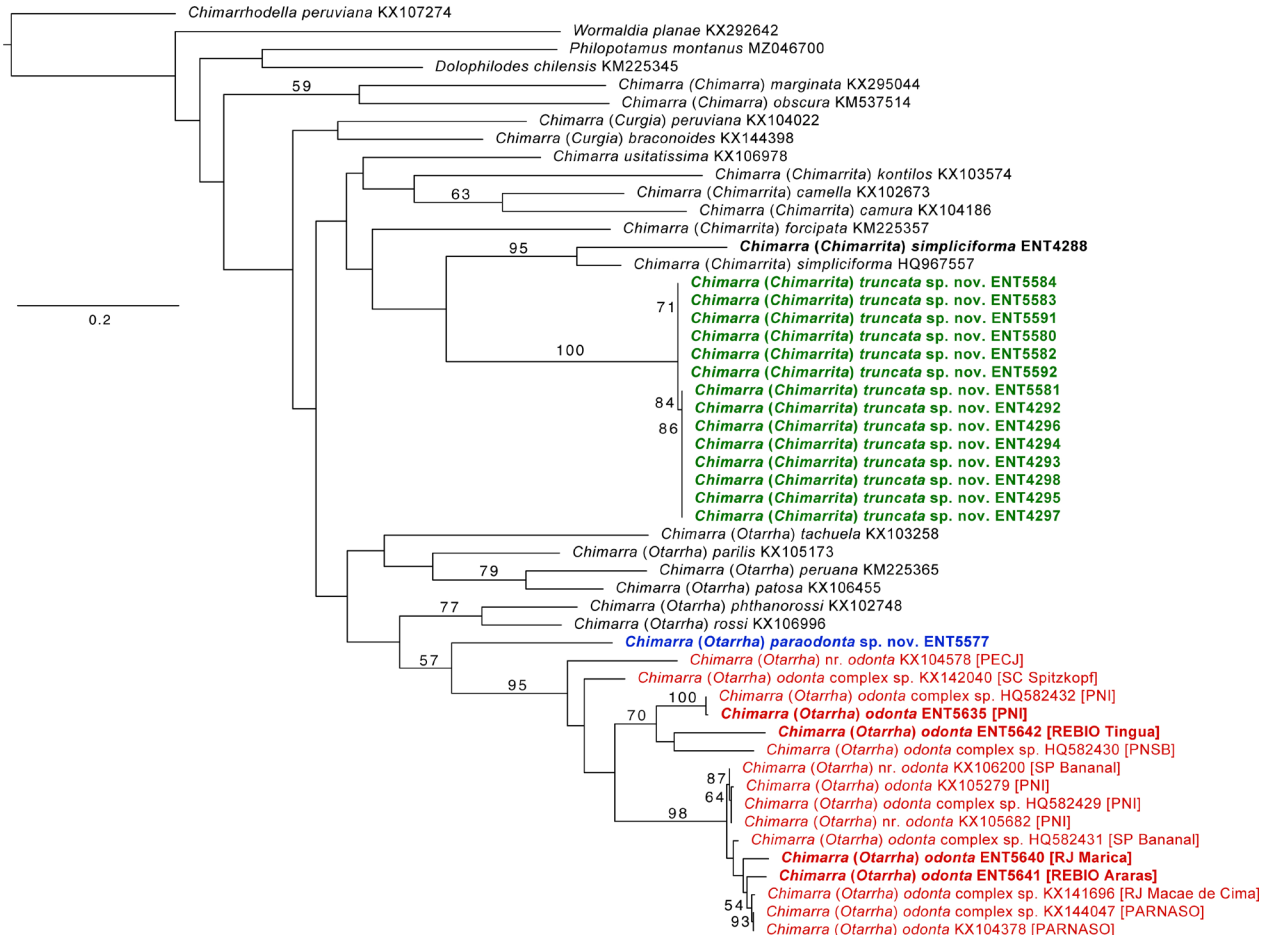

Figure 2. Maximum likelihood tree (–lnL = 5254.453966) of COI sequences of *Chimarra* species showing the phylogenetic placement of the two new species proposed, *C.* (*Chimarrita*) *truncata* **sp. nov.** (in green) and *C.* (*Otarrha*) *paraodonta* **sp. nov.** (in blue). *Chimarra* (*Otarrha*) *odonta* species complex in red. Terminals sequenced herein in bold. Values above branches are bootstrap percentages (>50%).

3. Results

3.1. Phylogenetic placement and COI divergences

The maximum likelihood tree (–lnL = 5254.453966, Fig. 2) of COI sequences corroborated the morphological identification of the two new species of *Chimarra* proposed herein within *Chimarrita* and *Otarrha* subgenera. The 14 individuals of *C.* (*Chimarrita*) *truncata* **sp. nov.** with COI sequences were recovered as a monophyletic lineage with maximum bootstrap support (Fig. 2, in green). Surprisingly, the maximum intraspecific K2P divergence for this species was low (0.5%), given that specimens were collected in two localities very distant geographically (approximately 660 km). Unfortunately, only a single individual of the other new species proposed, *C.*

(*Otarrha*) *paraodonta* **sp. nov.**, had the COI successfully sequenced (Fig. 2, in blue).

Both new species showed high interspecific K2P divergences when compared to its respective sister species (see Supplementary file 1: Table S1). *Chimarra* (*Chimarrita*) *truncata* **sp. nov.** and *C.* (*Chimarrita*) *simpliciforma* had distances ranging from 20.0−21.3% and *C.* (*Otarrha*) *paraodonta* **sp. nov.** and *C.* (*Otarrha*) *odonta* Blahnik ranging from 17.4−21.3%. These distances fall into the range of all *Chimarra* interspecific K2P distances calculated herein (13.6−22.7%), which provides support to description of the new taxa as distinct species.

It is important to note that, although we are treating *C.* (*Otarrha*) *odonta* (Fig. 2, in red) as a single nominal species (terminals labeled as *C. odonta*, *C.* nr. *odonta*, or *C. odonta* complex sp.), it is most likely a complex of cryptic species based on hitherto inconclusive morphological and molecular evidence, such as showing high intraspecific K2P COI divergences (up to 18.1%, see Discussion below).

3.2. Taxonomy

3.2.1. *Chimarra* (*Chimarrita*) *truncata* sp. nov.

http://zoobank.org/27CE7BA1-F2D6-46EF-9E29-302E548-44D84

Type locality. Igarapé Mato Grosso, Novo Airão, Amazonas State, Brazil.

Diagnosis. *Chimarra* (*Chimarrita*) *truncata* **sp. nov.** is most similar to *C.* (*Chimarrita*) *xingu* Blahnik, 1997 by the short, fully divided tergum X with dorsal ridges and foldlike areas on outer margin of each lobe and the simple, almost uniform in width inferior appendages. However, the new species has an apically subtruncated ventral process of segment IX, while in *C.* (*Chimarrita*) *xingu* this process is pointed. Furthermore, *C.* (*Chimarrita*) *truncata* **sp. nov.** can be recognized by inferior appendages with truncated apices both in lateral and ventral view (rounded in *C. xingu*).

Description. *Adult male*: forewing length 2.6–3.0 mm (n=6; holotype = 2.6 mm). General color (in alcohol) uniformly pale brown, except dorsum of the head dark brown, antennae and palps pale brown. Head with anterior, anteromesal, posterior, and posterolateral setal warts; posterior setal warts large, triangular, meeting broadly on median portion; postocular parietal sclerite triangular, slightly extending below eye. Maxillary palps relatively short, 2nd segment longer than 3rd segment, apicomesally with stout setae. Wing venation typical for the subgenus (Fig. 3); forewing with forks I, II, III, and V present, stem of Rs straight, crossveins *r*, *s*, *r-m*, and *m* nearly linearly arranged and unpigmented, crossvein *m-cu* and apex of Cu2 also hyaline; 2A apparently forked to 1A and 3A (Fig. 3A); hind wing venation not reduced, with forks I, II, III, and V present, Sc completely separated from R1; crossveins *s*, *r-m* and *m-cu* unpigmented, crossvein between 1A and 2A (Fig. 3B). Male protarsal claws nearly symmetrical; tibial spur formula 1–4–4.

Male genitalia (Fig. 4): Segment IX, dorsally with paired mesal and mesolateral ridges on each side of midline, mesal ones poorly developed (Fig. 4B); in lateral view, tall, with anterior margin concave, expanded anteroventrally and anterodorsally; posterolateral margin broadly convex (Fig. 4A); ventral process moderately elongate, approximately 1/3 as long as inferior appendages, wider at basal half, slender at distal half, with subtruncate apex (Fig. 4A, 4C). Tergum X short, fused to segment IX; in dorsal view, fully divided mesally, forming 2 lobes with distinct sensilla foldlike area on outer margin, folding itself dorsally; apex of each lobe distinctly sclerotized, acute and slightly turned inwardly (Fig. 4B); in lateral view, strongly humped dorsally, apex short, slender, curved apicoventrally (Fig. 4A). Preanal appendages short, rounded, fused dorsolaterally near base of tergum X (Fig. 4A, 4B). Inferior appendages of moderate length, simple in structure, almost uniform in width in lateral and ventral views, apex truncate, with slightly irregular margin (Fig. 4A, C). Phallic apparatus with phallotheca tubular, bearing basodorsal and apicoventral pointed extensions; phallic spine single, stout, with moderate length, emerging dorsally near base of phallotheca; endotheca elongate, inflated dorsoapically; phallotremal sclerite complex indistinct (Fig. 4D, 4E).

Etymology. The specific epithet is an allusion to the characteristic inferior appendages, which are apically truncated. Derived from the Latin, "*truncata*" = piece cut off, tip, end.

Material Examined. ***Holotype.*** BRAZIL • ♂; Amazonas State, Novo Airão, Rod. AM352, km-68, Igarapé Mato Grosso; 02°48'58"S, 60°55'18"W; 21–31.vii.2016; J.A. Rafael & F.F. Xavier leg.; Malaise trap; INPA (DNA voucher ENT4293). — ***Paratypes.*** BRAZIL • 3 ♂♂; same data as for holotype; INPA (DNA vouchers ENT4292, ENT4294, ENT4295) • 3 ♂♂; same data as for holotype; except 08−20.v.2017; DZRJ 7825-DZRJ 7827 (DNA vouchers ENT4296-4298) • 4 ♂♂, 1 ♀; Pará State, Belterra, BR-163, Km 85, Igarapé do Branco, entrance LBA; 03°03'04"S, 54°55'29.3"W; 91 m a.s.l.; 11−12.xii.2014; J.O. Silva & S.M. Couceiro leg.; Pennsylvania trap INPA (DNA vouchers ENT5580-ENT5583, ENT5591) • 1 ♂, 1 ♀; same data as preceding, MNRJ (DNA vouchers ENT5584, ENT5592).

3.2.2. *Chimarra* (*Otarrha*) *paraodonta* sp. nov.

http://zoobank.org/FB039B0D-01E7-42E7-9938-ED5E9E243-0AD

Type locality. Cachoeira Véu da Noiva, Parque Nacional do Itatiaia, Itatiaia, Rio de Janeiro State, Brazil.

Diagnosis. The new species is closely similar to *C.* (*Otarrha*) *odonta* Blahnik, 2002 by some shared primitive characters of the subgenus, like hindwing venation

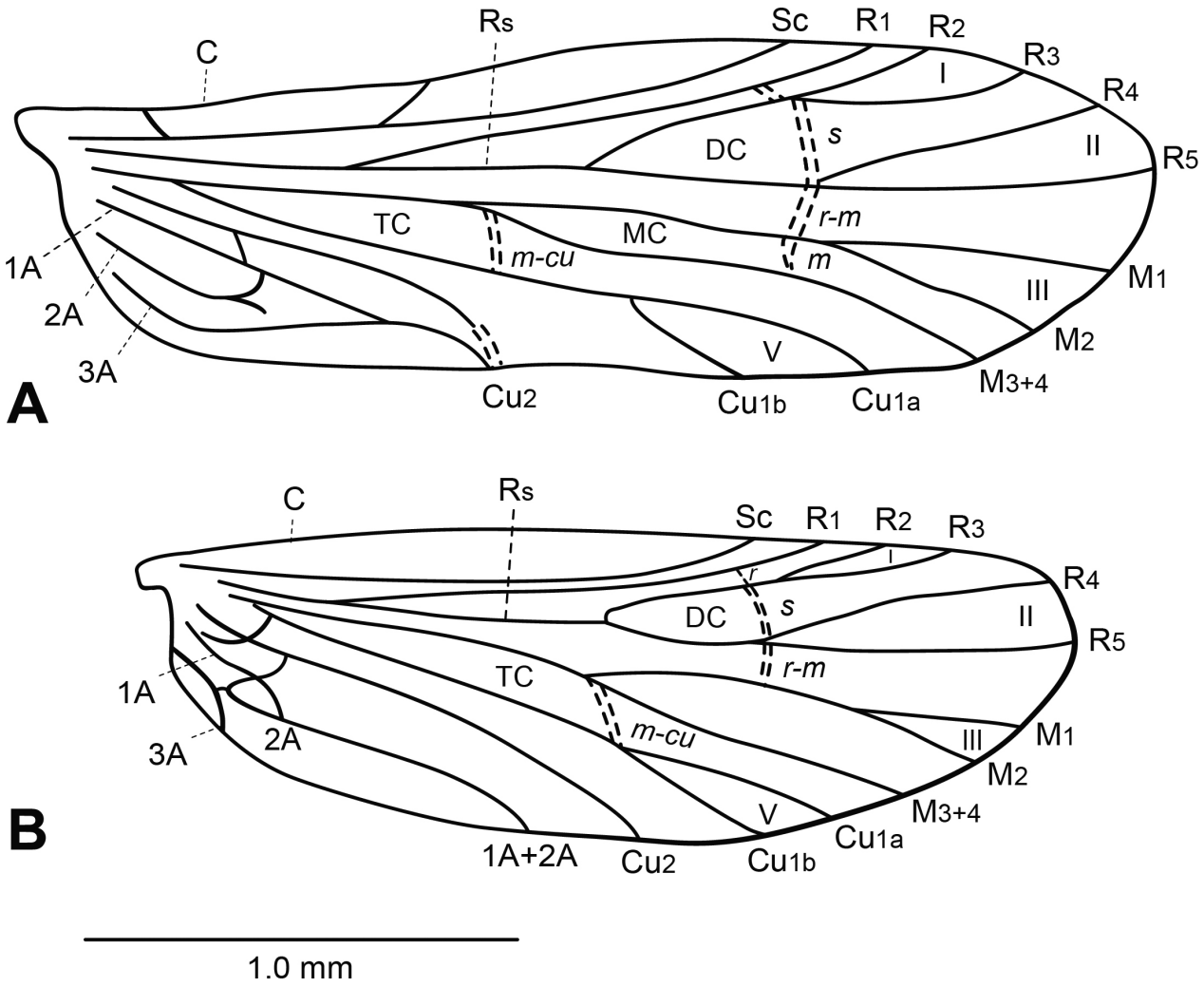

Figure 3. *Chimarra* (*Chimarrita*) *truncata* **sp. nov.**, holotype wing venation: **A** right forewing; **B** right hind wing. Abbreviations: DC, discoidal cell; MC, medial cell; TC, thyridial cell.

pattern with Rs 4-branched and the undivided anterior head setal warts. Both species also have a simple, subtriangular, and completely divided tergum X and an inner process on each inferior appendage. However, the new species has the *Otarrha* synapomorphic hindwing venation with Sc+R1 fused, narrower and more uniform lobes of tergum X, and inferior appendage rhomboidal (in lateral view) and more elongated and spatulated (in ventral view). Additionally, the dorsomesal process of the inferior appendage in *C.* (*Otarrha*) *paraodonta* **sp. nov.** is thorn-like, more robust, and positioned subapically; while in *C. odonta*, this process is tooth-like, blunt, and positioned more apically. Furthermore, *C.* (*Otarrha*) *paraodonta* **sp. nov.** can be recognized by its differently shaped tergum IX as viewed dorsally, the more robust ventral process, and simple phallotremal sclerite.

Description. *Adult male*: forewing length 5.2–5.8 mm (n=3; holotype = 5.8 mm). General color (in alcohol) uniformly golden brown, except dark brown dorsum of head. Dorsum of head with anterior, anteromesal, posterior, and posterolateral setal warts; posterolateral setal warts large; anterior setal warts each elongate and undivided; postocular parietal sclerite large, slightly extending below the eye. Maxillary palps relatively short, 2nd segment shorter than 3rd segment, apicomesally with stout setae. Wing venation typical for the subgenus (Fig. 5A−B), except Rs of hind wing 4-branched (Fig. 5B); forewing with forks I, II, III, and V present, stem of Rs almost straight, crossveins *s*, *r-m* and *m* linearly arranged and unpigmented, crossveins *m-cu* and *cu* and apex of Cu2 also hyaline; 2A not forked (Fig. 5A); hind wing with forks I, II and V present, R1 and Sc fused; crossveins *s* and *r-m* not aligned and unpigmented, crossvein *m-cu* apparently absent, *cu-a* present, anal loop very small (Fig. 5B). Tibial spur formula 1–4–4.

Male genitalia (Fig. 6): Segment IX, dorsally with anterior margin deeply concave, posterior margin almost straight (Fig. 6B); in lateral view, with anterior margin almost straight, somewhat projected in the anteroventral portion; posterior margin sinuous, with distinct dorsomedial and ventromedial invagination (Fig. 6A); ventral process elongate, about same length of inferior appendage as viewed laterally, enlarging apically with rounded apex (Fig. 6A, 6C). Tergum X, in dorsal view, completely divided mesally, forming elongate, paired narrow sclerotized lobes, slightly tapering apically; apex rounded (Fig. 6B); each lobe with numerous apical and basoventral

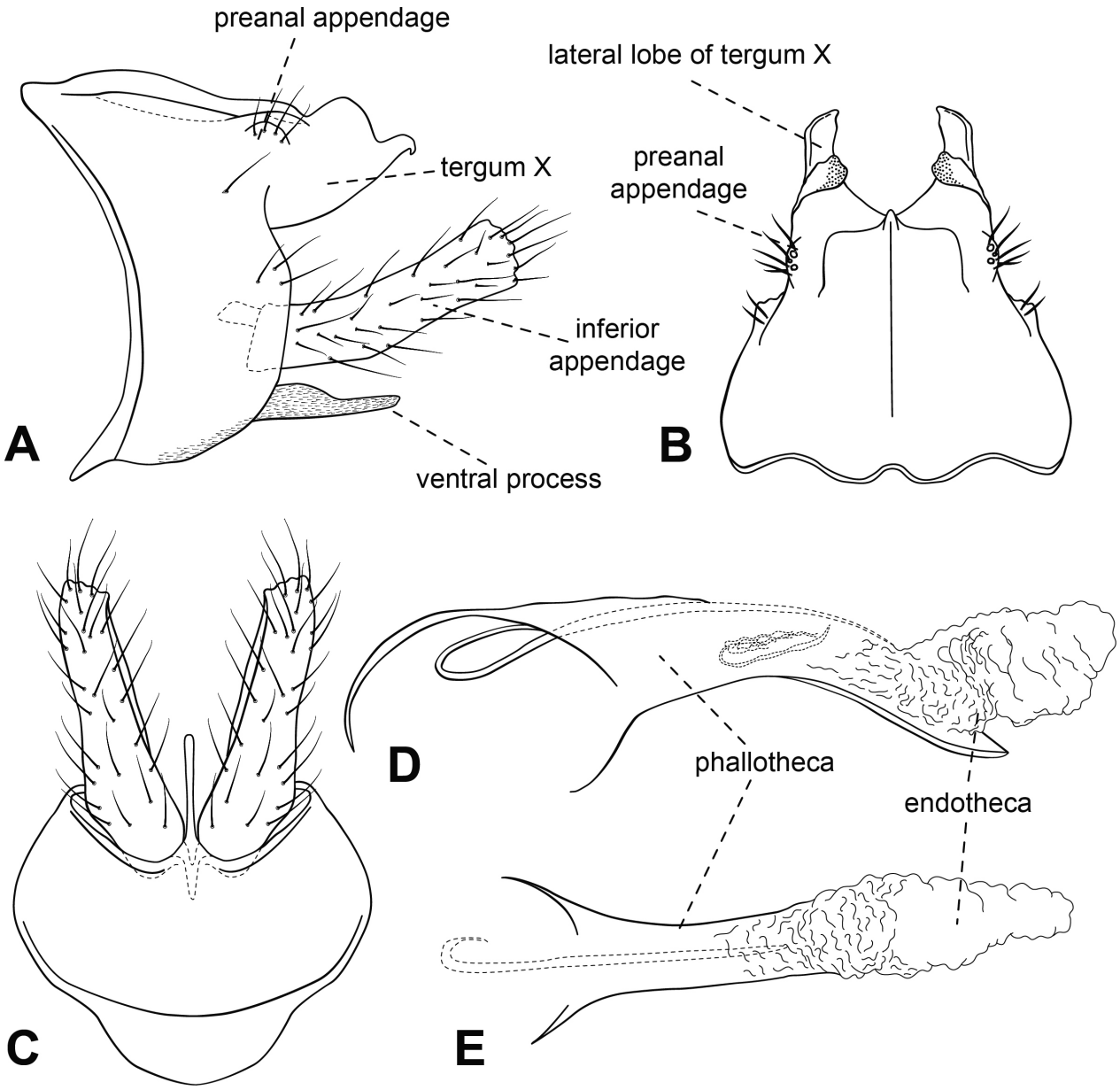

Figure 4. *Chimarra* (*Chimarrita*) *truncata* **sp. nov.**, holotype male genitalia: **A** left lateral view; **B** dorsal view; **C** ventral view; **D** phallus, left lateral view; **E** phallus, ventral view.

sensilla (Fig. 6A, 6B); in lateral view, subtriangular (Fig. 6A). Preanal appendages flattened, earlike, laterally directed (Fig. 6A, B). Inferior appendages, in lateral view, rhomboidal, relatively short (Fig. 6A); each appendage with subapical thorn-like process on the dorsomesal surface (Fig. 6A, C, D); in dorsal view, with outer lateral margin expanded distally (Fig. 6D); in ventral view, spatulate, with truncate distal margin (Fig. 6B). Phallic apparatus with phallotheca tubular, bearing basodorsal and basoventral pointed extensions; endotheca short, membranous, with 4 apical, robust, sclerotized spines; phallotremal sclerite simple, large, L-shaped (Fig. 6E, F).

Etymology. The specific epithet is a reference to the close similarity of the new species to *Chimarra* (*Otarrha*) *odonta*. Derived from the Greek, "*para*" = beside or near.

Material examined. ***Holotype*.** BRAZIL • ♂; Rio de Janeiro State, Itatiaia, Parque Nacional do Itatiaia, Complexo da Maromba, Cachoeira Véu da Noiva; 22°25'38.6"S, 44°37'9.7"W; el. 1140 m a.s.l.; 02−19.ii.2015; D.M. Takiya & A.P.M. Santos leg.; Malaise trap; DZRJ 7828 (DNA voucher ENT5579). — ***Paratypes*.** BRAZIL • 1 ♂; Rio de Janeiro State, Itatiaia, Parque Nacional do Itatiaia, Complexo da Maromba, Cachoeira Véu da Noiva [PNI-M2A]; 22°25'36.1"S, 44°37'05.80"W; el. 1153 m a.s.l; 02.x–02.xi.2015; M.L. Monné, J.P. Botero, Â.P. Pinto, L.H. Gil-Azevedo; Malaise trap, MNRJ (DNA voucher ENT5578) • 1 ♂; Rio de Janeiro State, Itatiaia, Parque Nacional do Itatiaia, abaixo da Cachoeira Véu da Noiva; 22°25'36.10"S, 44°37'05.80"W; el. 153 m a.s.l.; 02.x.2015; C.C.D. Corrêa & L.H. Gil-Azevedo leg.; INPA (DNA voucher ENT5577).

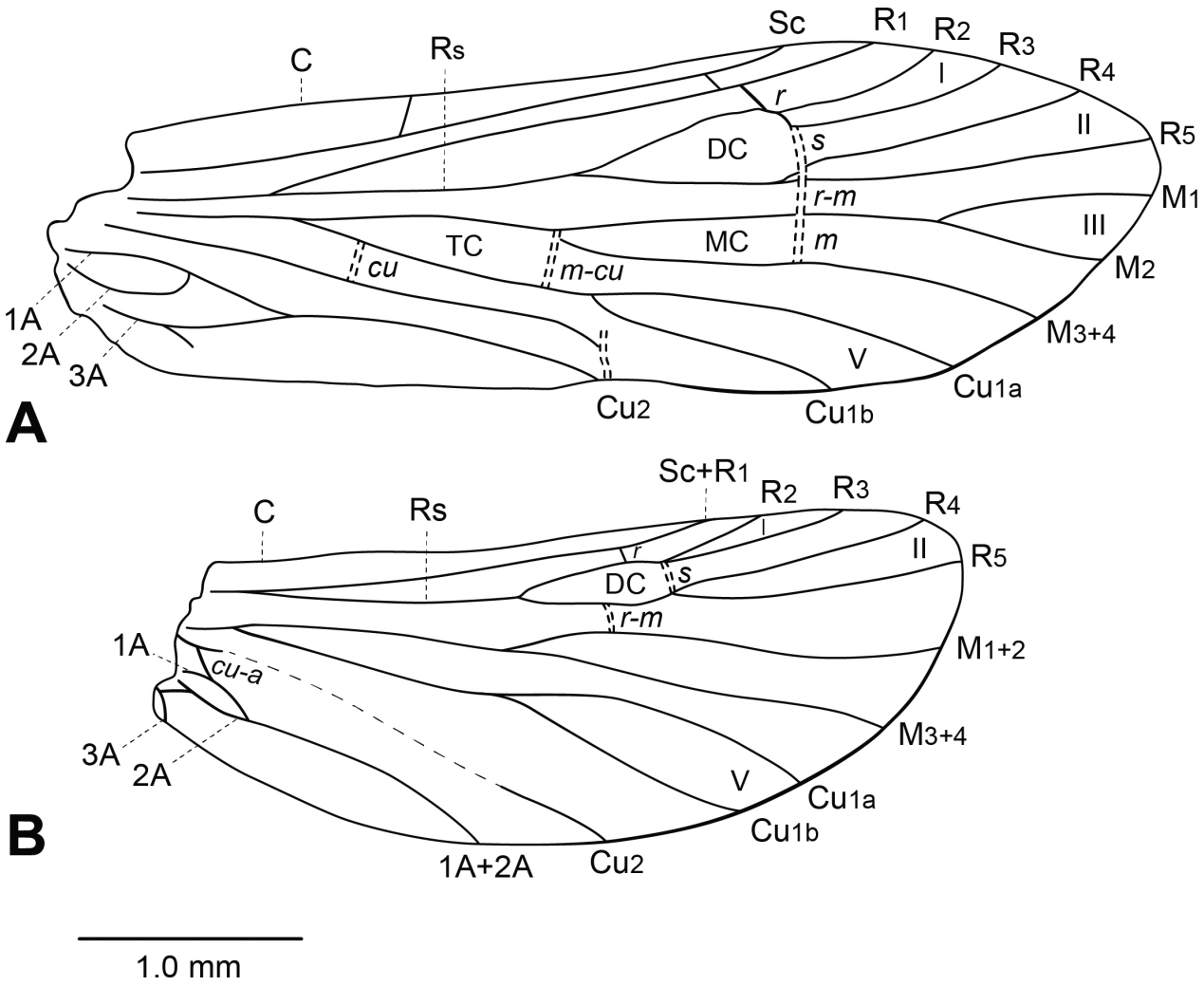

Figure 5. *Chimarra* (*Otarrha*) *paraodonta* **sp. nov.**, holotype wing venation: **A** right forewing; **B** right hind wing. Abbreviations: DC, discoidal cell; MC, medial cell; TC, thyridial cell.

4. Discussion

Currently, four subgenera are recognized in *Chimarra*: *Chimarra*, *Chimarrita*, *Curgia*, and *Otarrha*, all of them recently reviewed (Blahnik 1997, 1998, 2002; Flint 1998). *Curgia* was originally established for *C. braconoides* by Walker (1860) but was treated by Ulmer (1905) under *Chimarra*, and the genus was officially placed as a subgenus within *Chimarra* by Milne (1936). Until the end of the 1990s, many species were described and generally assigned to the subgenera *Curgia* and *Chimarra*, but without clearly defining them. Even after revisional works for *Chimarra* (Blahnik 1998) and *Curgia* (Flint 1998) proper diagnostic characters for them remain ambiguous, although subgeneric limits were better established. The nominotypical *Chimarra* is defined by the stem of forewing Rs vein with conspicuous curvature before discoidal cell, but it is less conspicuous in members of *aterrima* and *obscurum* Groups from East North America and is not true for several Afrotropical, Australasian, and Oriental species (Blahnik 1998; Wahlberg and Johanson 2014). Besides that, the male tergum X completely divided and widely separated is also pointed out as diagnostic for the subgenus, although it is not exclusive (Blahnik 1998). *Curgia* can be diagnosed by an anal vein of forewings with 2A looped to 1A, without forks, and by a combination of more or less inconclusive male genital characters, such as tergum X often entire or knoblike, or when it is apically separated, lobes are not totally divided mesally, and the short and linear inferior appendages (Blahnik 1998; Flint 1998).

Blahnik (1997, 2002) erected the subgenera *Chimarrita* and *Otarrha*. *Chimarrita* was created in 1997 to include several species from South America and one from the Greater Antilles besides three described species formerly placed in subgenus *Chimarra*: *C. simpliciforma* Flint, 1971, *C. rosalesi* Flint, 1981, and *C. maldonadoi* Flint, 1964. Phylogenetic analysis based on morphological data (Blahnik 1997) support the monophyly of the subgenus mainly by male abdominal and genital characters: ventral process of segment IX elongate, narrow, acute, and projecting; anteroventral margin of segment IX distinctly projecting and narrowed, acute mesally; preanal appendages very short and fused basally; and phallic spines with at least a slight helical twist. *Chimarra*

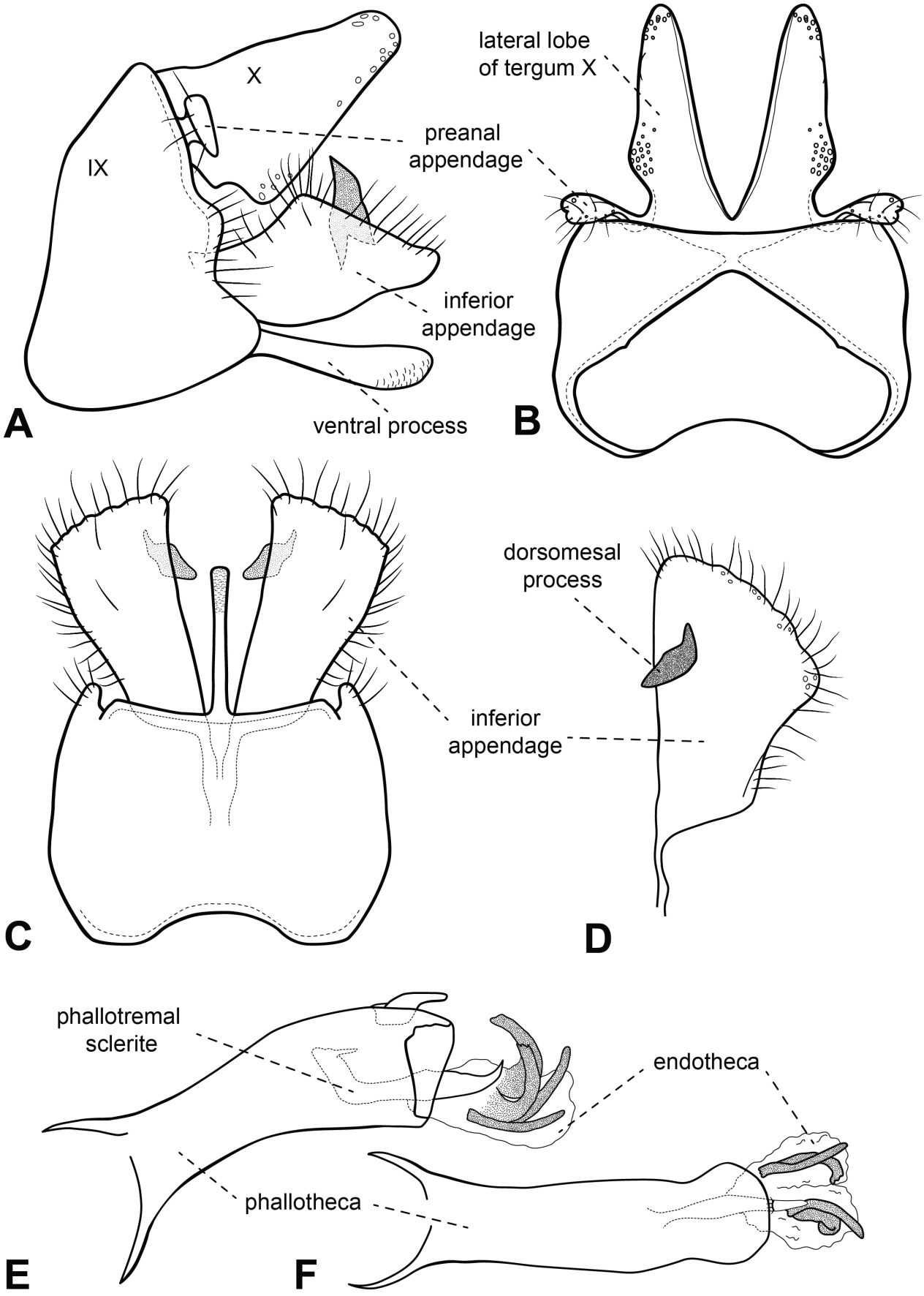

Figure 6. *Chimarra* (*Otarrha*) *paraodonta* **sp. nov.**, holotype male genitalia: **A** left lateral view; **B** dorsal view; **C** ventral view; **D** left inferior appendage, dorsal view; **E** phallus, left lateral view; **F** phallus, ventral view.

(*Chimarrita*) *truncata* **sp. nov.** has all of these characters, being readily placed within the subgenus.

Chimarrita was originally divided into three species groups by Blahnik (1997): *maldonadoi*, *rosalesi*, and *simpliciforma* Groups. However, Kjer et al. (2014) did not recover a monophyletic *Chimarrita* because of the position of *C. maldonadoi* as sister to *Otarrha*. It corroborates the speculative position of the *maldonadoi* Group in the original subgenus description based primarily on the structure of the female genitalia, which is distinctly elongate, although less so than in the other members of *Chimarrita* (Blahnik 1997). Therefore, based on this phylogenetic placement, this group, comprising two Antillean species, was considered *incertae sedis* within the genus, leaving *Chimarrita* with only two species groups (Kjer et al. 2014). The *simpliciforma* Group is easily recognized by the single, elongate spine emerging basodorsally from the phallotheca of the phallic apparatus. Interestingly, four out of the five *Chimarrita* species described recently belong to the *simpliciforma* Group (Blahnik and Holzenthal 2012; Vilarino and Calor 2015; Desidério et al. 2018), suggesting that, although this species group is the most diverse for the Neotropical Region, there are most likely many more species to describe. *Chimarra* (*Chimarrita*) *truncata* **sp. nov.** is clearly a member of the *simpliciforma* Group based on the characteristic of the group mentioned above. In the Kjer et al. (2014) phylogenetic analysis based on molecular data, they did recover the *simpliciforma* Group as monophyletic, but only sampled two species. In our analysis, *C.* (*Chimarrita*) *truncata* **sp. nov.** was recovered as sister to *Chimarra simpliciforma*, corroborating its position in the *simpliciforma* Group, although morphological analysis suggests that the new species is most similar (and likely more related to) *C. xingu*, which unfortunately was not sampled herein. However, the *simpliciforma* Group represented by five species was not recovered as monophyletic, but this result should be taken lightly as taxon sampling was low and internal branches had no significant support in the present analysis.

Otarrha was established in 2002 to accommodate eighteen species formerly placed either in the subgenus *Chimarra* or unplaced to subgenus (informally referred to as *patosa* Group) and thirteen new species. The subgenus is particularly well represented in the Lesser and Greater Antilles, and northern South America, with a few species recorded from Central or southern South America (Blahnik 2002). As in *Chimarrita*, morphological phylogenetic analyses were performed (Blahnik 2002), revealing a monophyletic group. However, universal characters applicable to all species inserted into the subgenus are hard to define and some combinations of head warts, wing venation, and male genital structure are necessary. Most *Otarrha* species can be diagnosed by the anterior setal warts divided and reduced venation of hindwing, with R1 fused to Sc, Rs three-branched, and M two-branched. In addition, male genital apparatus of most species have ear-like preanal appendages and ventral process relatively elongate and wider subapically. However, *C.* (*Otarrha*) *paraodonta* **sp. nov.** and the very closely related *C.* (*O.*) *odonta* are the only species of the subgenus that share the absence of some subgeneric diagnostic characters in head anterior setal warts, which are not divided, and in the reduced venation of hindwings, which bear fork I (Rs four-branched). Nevertheless, both species fit all male genital characters typical for the subgenus and are found herein nested within *Otarrha* in the phylogenetic analysis.

Although there is weak morphological support for most subgenera, recent species-level molecular data phylogenies, using a large subset of taxa and multiple molecular markers (Kjer et al. 2014; Wahlberg and Johanson 2014), corroborate the monophyly of *Chimarra* and all of its subgenera, except *Chimarrita*, which was recovered as monophyletic only with the exclusion of *C. maldonadoi* (Kjer et al. 2014), which appears closer to *Otarrha*. Furthermore, in Kjer et al. (2014), *Chimarra usitatissima* was unplaced to subgenus, corroborating the *incertae sedis* position established by Blahnik (2002) for it by its unusual morphology. In our present analysis, *Chimarrita* was recovered as monophyletic only with the inclusion of *C. usitatissima*, although internal branches did not have significant support (probably an artefact of low gene and taxon sampling), thus our results are inconclusive. Phylogenetic relationships among *Chimarra* subgenera are mostly consensual in that the subgenus *Chimarra* is sister to a clade containing all other *Chimarra* species, but relationships within this other clade varies among published analyses. In the maximum likelihood tree based on COI and 28S, Kjer et al. (2014) recovered *Curgia* and *Otarrha* in a clade together with the unplaced *maldonadoi* Group and *C. usitatissima*, this clade being sister to *Chimarrita*. Wahlberg and Johanson (2014) presented two different analyses based on COI, CAD, and POL-II: in the parsimony analysis of the data, *Chimarrita* was recovered as sister to all *Chimarra*, which was divided in two clades: the subgenus *Chimarra* and a clade with *Otarrha*+*Curgia*; while in the Bayesian analyses *Otarrha* was recovered as sister to *Chimarrita*+*Curgia*. However, it is important to consider that some relationships of the mentioned analyses between subgenera had low branch support and *Chimarrita* was represented by a single species in Wahlberg and Johanson (2014).

Of a total of 33 species, only three species of *Otarrha* are recorded from Brazil: *C. diakis* Flint, 1971 (Amazon), *C. odonta* (Atlantic Forest), and *C. machadoi* Camargos, 2016 (Cerrado) (Blahnik 2002; Blahnik and Holzenthal 2012; Camargos 2016). The finding of a new species of *Otarrha* from Parque Nacional do Itatiaia is actually surprising, considering that it is one of the most well sampled national parks in Brazil for caddisflies (Dumas and Nessimian 2012), exposing that taxonomic gaps may still exist in Southeastern Brazil. Although no formal quantitative study was performed, the new species does appear to be rare when compared to the sympatric and morphologically similar *C. odonta*. While sorting Malaise traps from where only the three type specimens of *C. paraodonta* **sp. nov.** were found, over 200 specimens of *C. odonta* were identified.

Considering the high morphological resemblance of *C. paraodonta* **sp. nov.** to *C. odonta*, we decided to include

in the present study all *C. odonta* barcode sequences available (from Zhou et al. 2016) and generated a few more. Based on the present molecular phylogenetic analysis and K2P divergences, it seems evident that *C. odonta* is likely a species complex, comprised of at least four distinct genetic species distributed from Rio de Janeiro to Santa Catarina states in the southeastern and southern Brazil, respectively. Two of these species apparently co-occur in Parque Nacional do Itatiaia, together with *C. paraodonta* **sp. nov.** These genetic species may be supported by fine morphological features of the phallus, as variation seems to be found in this complex (R Blahnik, in litt. 2021). However, in order to robustly delimit this species complex, an integrative analysis is needed comprising a detailed morphological study, especially embracing its full distributional range (Vilarino and Calor 2015), and sequencing of additional molecular markers.

Our study shows that the combination of detailed morphological observation and molecular sequence data is constructive in discovering and describing new species and provides another example of the effectiveness of DNA barcodes as a tool for species delimitation. Due to efforts of the global initiative Trichoptera Barcode of Life (TBOL) that started in 2007, a comprehensive COI barcode reference library is available for about one-third of the described caddisfly species (Zhou et al. 2016). So, this work helps to expand this database, and thus facilitate the knowledge of caddisfly diversity and provide tools for fast and reliable identification. Most barcode studies on Trichoptera show clearly distinguishable intraspecific and interspecific variability, clearly seen in this study, which is known as a barcoding gap (e.g., Graf et al. 2005; Waringer et al. 2008; Zhou et al. 2010; Pauls et al. 2010; Previšić et al. 2014; Santos et al. 2016), but in some taxa levels of these variations are not conclusive (e.g., Waringer et al. 2007; Pauls et al. 2010). Potential limitations of using mtDNA to infer species boundaries include retention of ancestral polymorphism, male-biased gene flow, selection on any mtDNA nucleotide (as the whole mitogenome is one linkage group), introgression following hybridization, and paralogy resulting from transfer of mtDNA gene copies to the nucleus (Moritz and Cicero 2004). As a result, solely using mtDNA divergence or phylogenetic signal may lead us to biased species delimitations. Therefore, using multilocus sequence data can increase delimitation success (Dupuis et al. 2012) and provide support for species delimitations under different theoretical models that combine species phylogenies and gene genealogies via ancestral coalescent processes (Yang and Rannala 2010). Thus, integrative taxonomy approaches should ideally combine morphology, ecology and/or behavior with multi-locus nu/mtDNA data.

Caddisflies are excellent freshwater biological indicators due to their ecological diversity and intolerance of most species to pollution and disturbances (Resh 1993; Houghton 2004). However, the practical use of caddisfly larvae in bioassessment monitoring programs are limited in many areas by the lack of lower-level taxonomic resolution. Higher levels of identification, such as family or even genus, can often mask the variability of environment and species interaction, causing ecological information loss or redundancy (Resh and Unzinckker 1975; Ruiter et al. 2013). In the Neotropical Region only about 9% of caddisfly species have their immature stages described (Pes et al. 2018). COI sequences have proven successful in associating larval and adult stages in Neotropical Trichoptera species (e.g., Santos et al. 2016; Barcelos-Silva et al. 2018), and its use can considerably reduce this gap of knowledge. Although life stage association was not the purpose of this work, the barcode data generated here will provide a valuable tool for future *Chimarra* larvae association, also contributing to biodiversity and conservation assessments.

5. Authors' contributions

All authors contributed to the study conceptualization and design. PDM, LLD, and GRD identified specimens and produced illustrations and maps. PDM and MPR carried out the molecular laboratory work under supervision of DMT. All authors contributed to the draft of the manuscript. All authors read and approved the final manuscript.

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Supplementary material 1

Table S1

Authors: Moreira P, Dumas LL, Rozo MP, Desidério G, Takiya D (2022)
Data type: .xlsx
Explanation note: Pairwise K2P divergences of 48 COI sequences of *Chimarra* species.

Link: https://doi.org/10.3897/asp.80.e76559.suppl1