Systematics, Morphology and Biogeography

New systematic position of *Itatingamyia* Albuquerque (Diptera, Muscidae) based on molecular evidence, and description of the female of *I. couriae*

Kirstern Lica F. Haseyama [a,*], Claudio J.B. de Carvalho [b], Ândrio Zafalon-Silva [b], Frederico D. Kirst [a]

[a] Universidade Federal de Minas Gerais, Laboratório de Sistemática de Insetos, Departamento de Zoologia, Belo Horizonte, MG, Brazil
[b] Universidade Federal do Paraná, Departamento de Zoologia, Curitiba, PR, Brazil

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ABSTRACT

Itatingamyia Albuquerque, 1979 is rare in collections, with only nine specimens preserved in museums. Two species are known, and their placement within Muscidae was never tested using molecular data. Here, we estimate the position of *Itatingamyia* within Muscidae with mitochondrial (COI) and nuclear (AATS, CAD, and EF1-α) markers using Maximum likelihood and Bayesian posterior probabilities as optimality criteria. According to our results, we propose to classify *Itatingamyia* as a Cyrtoneurininae. We also describe the previously unknown female and egg of *Itatingamyia couriae* Haseyama and de Carvalho, 2011 and expand the known distribution of this species to the state of Minas Gerais, Brazil, through the discovery of 13 new specimens there. We also argue that *Itatingamyia* distribution is restricted to Atlantic Forest areas.

Introduction

Itatingamyia Albuquerque, 1979 is a Neotropical genus of Muscidae with two described species, *I. bivittata* Albuquerque, 1979 and *I. couriae* Haseyama and de Carvalho, 2011. The genus was included in Mydaeinae by Albuquerque (1979), based on the shape of the ovipositor and female cercus. However, the author highlighted that *Itatingamyia* has a single post-sutural intra-alar seta, a characteristic found in many Cyrtoneurininae genera (sensu Snyder, 1954). Later, de Carvalho and Couri (1993) placed it in Reinwardtiini, a tribe of Azeliinae (sensu de Carvalho, 1989), based on the long female cercus projecting beyond the hypoproct, the developed sternite eight, and the anchor-shaped tergite six.

According to a phylogenetic analysis based on morphological data, *Itatingamyia* is the sister group of *Cariocamyia* Snyder, 1951 and *Charadrella* (Haseyama and de Carvalho, 2012). This clade, formed by three small Neotropical genera, was placed within an assembly of Paleotropical genera (*Alluaudinella* Giglio-Tos, 1895 and *Ochromusca* Malloch, 1927), and *Dichaetomyia* Malloch, 1921, a genus widespread from Africa to Australasia. Due to the close relationship with *Dichaetomyia*, *Charadrella* and allied genera were placed in Dichaetomyiinae (Haseyama and de Carvalho, 2012; see

also Couri and de Carvalho, 2003). On the other hand, some authors classified *Charadrella* and *Cariocamyia* within the Cyrtoneurininae (Hennig, 1965; de Carvalho et al., 2005). This was the first time a species of *Itatingamyia* was included in a phylogenetic study, but the results of that analysis cannot be considered conclusive because it employed a biased taxon sampling: rather than including representatives of the entire family, it mostly included genera that had previously been considered as closely-related to *Charadrella*.

Itatingamyia is rare in collections. *Itatingamyia bivittata* is known only from the type-series studied by Albuquerque (1979) (two males and six females) from Pedra Azul, state of Minas Gerais, Brazil. According to the author, the locality is 560 meters above sea level and is predominantly covered with Cerrado vegetation (Brazilian savanna; Albuquerque, 1979). *Itatingamyia couriae* is known only by its male holotype (Santa Teresa, state of Espírito Santo, Brazil; Haseyama and de Carvalho, 2011). It was collected using a Malaise trap in the Santa Lúcia Biological Station, at 867 meters above the sea level, in the Atlantic Forest biome.

Here, we use molecular data to estimate the phylogenetic position of *Itatingamyia*. We also describe the previously unknown female and egg of *I. couriae*. Finally, we describe morphological variations found among the males of this species, provide new geographic records for it, and discuss the geographic distribution of the genus.

* Corresponding author.
  E-mail: licahaseyama@gmail.com (K.L. Haseyama).

Material and methods

Sample design, DNA extraction, amplification, and sequencing

Taxon sampling included genera from the three subfamilies of Muscidae (Muscinae, Cyrtoneurininae, and Mydaeinae, according to Haseyama et al., 2015). We aimed for a denser sampling within the Cyrtoneurininae and Mydaeinae, subfamilies that included the groups previously hypothesized to contain *Itatingamyia* (see section 'Introduction'). We used previously published data available at GenBank, and new COI and nuclear protein-coding genes (AATS and EF1-α) sequences obtained for this study (Supplementary Table S1). Since most of the species with those genes also had CAD sequences, this nuclear protein-coding gene was also used in the analysis, even though we were not able to amplify it for *Itatingamyia*.

DNA was extracted using the Purelink Genomic DNA extraction kit (Invitrogen) following the protocol provided by the manufacturer, with the following modifications: (i) at the tissue lysis step, the specimen was not destroyed, allowing it to be pinned and deposited at the biological collection 'Universidade Federal de Minas Gerais, Centro de Coleções Taxonômicas' (CCT-UFMG; see also examined material in Taxonomy section). The whole fly was placed in tissue paper allowing for the evaporation of cryopreservation liquid (alcohol), and then incubated in proteinase K and lysis buffer; (ii) since the fly was not macerated, it was incubated for a longer period at 56 °C (approximately 90 h); (iii) aiming to obtain a concentrated genomic DNA, the final DNA elution used only 50 μL of buffer.

Polymerase chain reactions (PCR) were carried out using primers previously indicated by Haseyama et al. (2015) (Supplementary Table S2). Primers were used at a concentration of 10 pmol/μL for a total volume of 50 μL per reaction. GoTaq® G2 DNA Polymerase (Promega, Madison, USA) was used following the protocol provided by the manufacturer, except for the addition of 3 μL of $MgCl_2$ at 25 mM for all reactions. Moreover, 1-2 μL of each primer and 1-3 μL of genomic DNA were used for amplification. For nuclear genes, touchdown PCR was the chosen strategy. This strategy minimizes the amplification of spurious products through the successive decreasing of the annealing temperature (Don et al., 1991; Korbie and Mattick, 2008). PCR cycles are specified in Supplementary Tables S3 and S4.

PCR products were subjected to electrophoresis in agarose gel at 2.5% to discard possible unwanted amplifications. The products were stained with GelRed Stain (Biotium Inc., Hayward, USA) and the target bands were cut and purified using Wizard® SV Gel and PCR Clean-Up System kit (Promega, Madison, USA). The cleaned products were sent for sequencing in both directions at the Biochemistry Department at the Universidade Federal do Paraná, through WEMSeq Company (Curitiba, Brazil).

Chromatograms were analyzed using Chromas Lite 2.6.2 (Technelysium Pty, 2016) and sequences edited with BioEdit 7.2.5 (Hall, 1999, 2013). The quality index, which represents the base call probability, was analyzed for each nucleotide of the sequences and forward and reverse sequences were matched. A conservative approach was used for nucleotide positions with a quality index below 20 on each sequence, which were replaced with an 'N' on the consensus sequence. The consensus sequences were obtained through the Bayesian method implemented at SeqTrace 0.9 (Stucky, 2012).

Phylogeny and topology tests

Sequences were aligned using MAFFT online server (Katoh et al., 2017) by implementing an auto selection for aligning algorithm, while other options were used as default. As a first approach, each gene was analyzed separately using most available sequences on GenBank. Best models of nucleotide evolution were chosen by ModelFinder AUTO feature, simultaneously with tree reconstruction (Kalyaanamoorthy et al., 2017) with default options. Phylogenetic relationships were estimated using the Maximum likelihood (ML) software IQ-Tree (Trifinopoulos et al., 2016). Then, RogueNaRok (Aberer et al., 2013) web server (http://rnr.h-its.org/about) was used to identify rogue taxa in each analysis under default options. We chose this strategy to individually identify rogue sequences, instead of rogue taxa. Using this approach, if only a single gene sequence, or a subgroup of sequences, from a terminal is causing instability in the analysis, we can still preserve that terminal by using only the sequences with phylogenetic signal. After pruning rogues from individual matrices, alignments were concatenated using SequenceMatrix v. 1.7.8 (Vaidya et al., 2010).

ModelFinder (Kalyaanamoorthy et al., 2017) was used under default options with partition merging activated both for estimating the partitioning scheme and choosing the best nucleotide evolution models (Supplementary Table 5). Maximum likelihood (ML) was used as an optimality criterion through IQ-Tree (Trifinopoulos et al., 2016) with 20 independent searches using the standard options. ML branch support metrics were SH-aLRT, aBayes and Ultrafast Bootstrap (Anisimova and Gascuel, 2006; Guindon et al., 2010; Hoang et al., 2018), all calculated with IQ-Tree. Support was considered significant when over 80% for SH-aLRT, 0.95 for aBayes, and 95% for Ultrafast Bootstrap. Data was also analyzed under Bayesian Inference (BI) using MrBayes 3.2.2 (Ronquist et al., 2012) through the Cipres web server (Miller et al., 2010). The analysis used two simultaneous runs with four chains each for 2×10^8 generations with a sample every 1000 generations. The convergence of runs was checked using the standard deviation of split sequences. Bayesian posterior probabilities were used as branch support on the consensus tree, and consider significant when over 95%. Based on the current knowledge of the Muscidae phylogeny (Kutty et al., 2014; Haseyama et al., 2015) we rooted all trees between Muscinae and the clade we considered as the in-group, Cyrtoneurininae + Mydaeinae. Tree figures were generated using FigTree 1.4 (Rambaudt and Drummond, 2012).

The classification of *Itatingamyia* at subfamily level was also examined using topological tests. The tests were carried out using all metrics of statistical significance available on IQ-Tree (Kishino and Hasegawa, 1989; Shimodaira and Hasegawa, 1999; Strimmer and Rambaut, 2002; Shimodaira, 2002). Three constraints were created imposing alternative positions for *Itatingamyia*: (i) within Cyrtoneurininae; (ii) within Mydaeinae; (iii) within Muscinae. All tests were performed with 10,000 bootstrap resampling using the resampling estimated log-likelihood (RELL) method (Kishino et al., 1990).

Taxonomy and distribution map

The studied material belongs to 'Universidade Federal de Minas Gerais, Centro de Coleções Taxonômicas' (CCT-UFMG), 'Universidade Federal do Paraná, Coleção Padre Jesus Santiago Moure' (DZUP) and 'Universidade de São Paulo, Museu de Zoologia da Universidade de São Paulo' (MZUSP). The new specimens, including the one used for DNA extraction, were captured using Malaise traps between 1999 and 2015. They were preserved in 70% ethanol at room temperature since they were collected. During this study, they were pinned and added to CCT-UFMG, and DZUP collections (see Examined material for details). The collecting sites are in Belo Horizonte municipality (Minas Gerais State, Brazil) or surrounding regions (within a 100 km ray). The collecting site at Belo Horizonte is a disturbed forest fragment in a highly urbanized area inside the campus of Universidade Federal de Minas Gerais. The traps placed at Nova Lima and Peti ecological station sites were in Atlantic forest fragments with nearby watercourses.

The morphological terminology of the adult follows Stuckenberg (1999), Nihei and de Carvalho (2007) and Cumming and Wood (2009). The following abbreviations are used in the description: $A_1 + CuA_2$ = anal 1 + anterior cubital 2 vein, A_2 = anal 2 vein, AD = anterodorsal, AV = anteroventral, D = dorsal, M = medial vein, P = posterior, PD = posterodorsal, PV = posteroventral, R = radial vein, and V = ventral. The female terminalia was prepared for observation using cold KOH and was drawn using a camera lucida.

The distribution map was built using data from the literature (Albuquerque, 1979) and museum specimens (see Examined material). The raster representing the continental surface was the 'Natural Earth I with shaded relief, water, and drainages' (Kelso and Patterson, 2010; https://www.naturalearthdata.com);
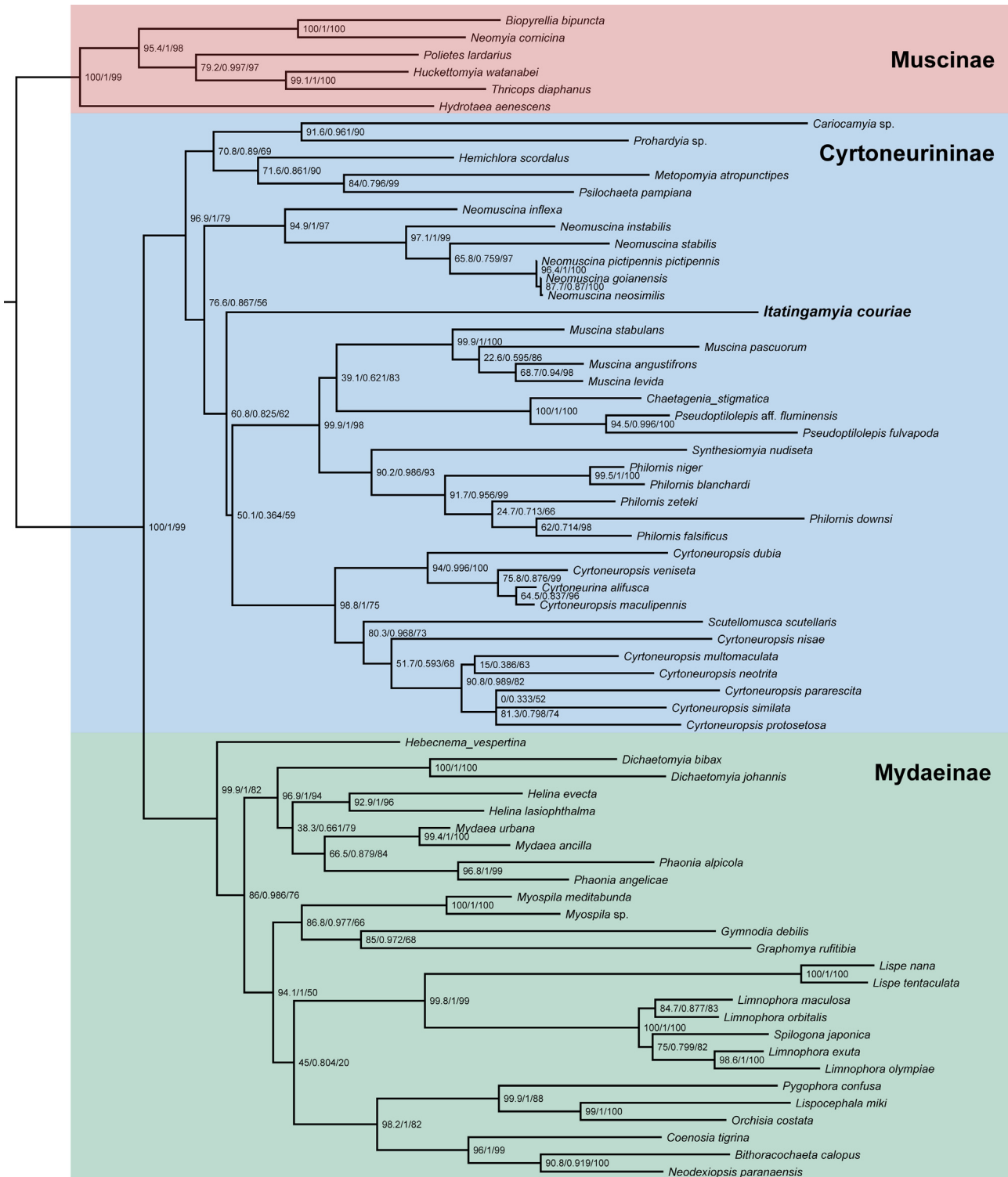

Fig. 1. Molecular phylogenetic hypothesis using Maximum Likelihood of the combined mitochondrial (COI) and nuclear (AATS, CAD, and EF1-a) protein-coding genes for 68 species of Muscidae highlighting the position of *Itatingamyia* within the Cyrtoneurininae. Subfamily-level classification follows Haseyama et al. (2015). Numbers on nodes are SH-aLRT, aBayes, and Ultrafast Bootstrap support values.

biomes boundaries were delimited using the shapefile from Ministério do Meio Ambiente (Brasil, 2018; http://mapas. mma.gov.br/i3geo/datadownload.htm).

Results

Phylogeny

A total of three new sequences were generated for *Itatingamyia couriae*. Only the forward strand of the AATS sequence was used, due to sequencing problems. Since the quality index was above the high probability threshold (>30), we felt confident enough to use the sequence based on a single strand. The final concatenated matrix had 4029 base pairs and 68 terminals.

The topology of the trees using both the ML and BI optimality criteria was similar, estimating the three subfamilies of Muscidae, Muscinae, Cyrtoneurininae and Mydaeinae (*sensu* Haseyama et al., 2015). *Itatingamyia* was nested in both cases within

Cyrtoneurininae, with significant support (Figs. 1 and 2). The internal relationships of this subfamily, however, differed according to the optimality criteria used. With ML, *I. couriae* is the sister-group of a clade comprising *Chaetagenia*, *Cyrtoneurina*, *Cyrtoneuropsis*, *Muscina*, *Philornis*, *Pseudoptilolepis*, *Scutellomusca* and *Synthesiomyia* (Fig. 1). In the BI analysis, the result is similar, but with lower resolution. In this case, *Itatingamyia* is placed in a polytomy, with *Chaetagenia*, *Cyrtoneurina*, some *Cyrtoneuropsis* species, *Muscina*, *Philornis*, *Pseudoptilolepis*, *Scutellomusca*, and *Synthesiomyia* (Fig. 2). All topological tests support *Itatingamyia* within the Cyrtoneurininae and reject its placement within alternative subfamilies (Table S6).

Taxonomy

Itatingamyia couriae Haseyama and de Carvalho, 2011
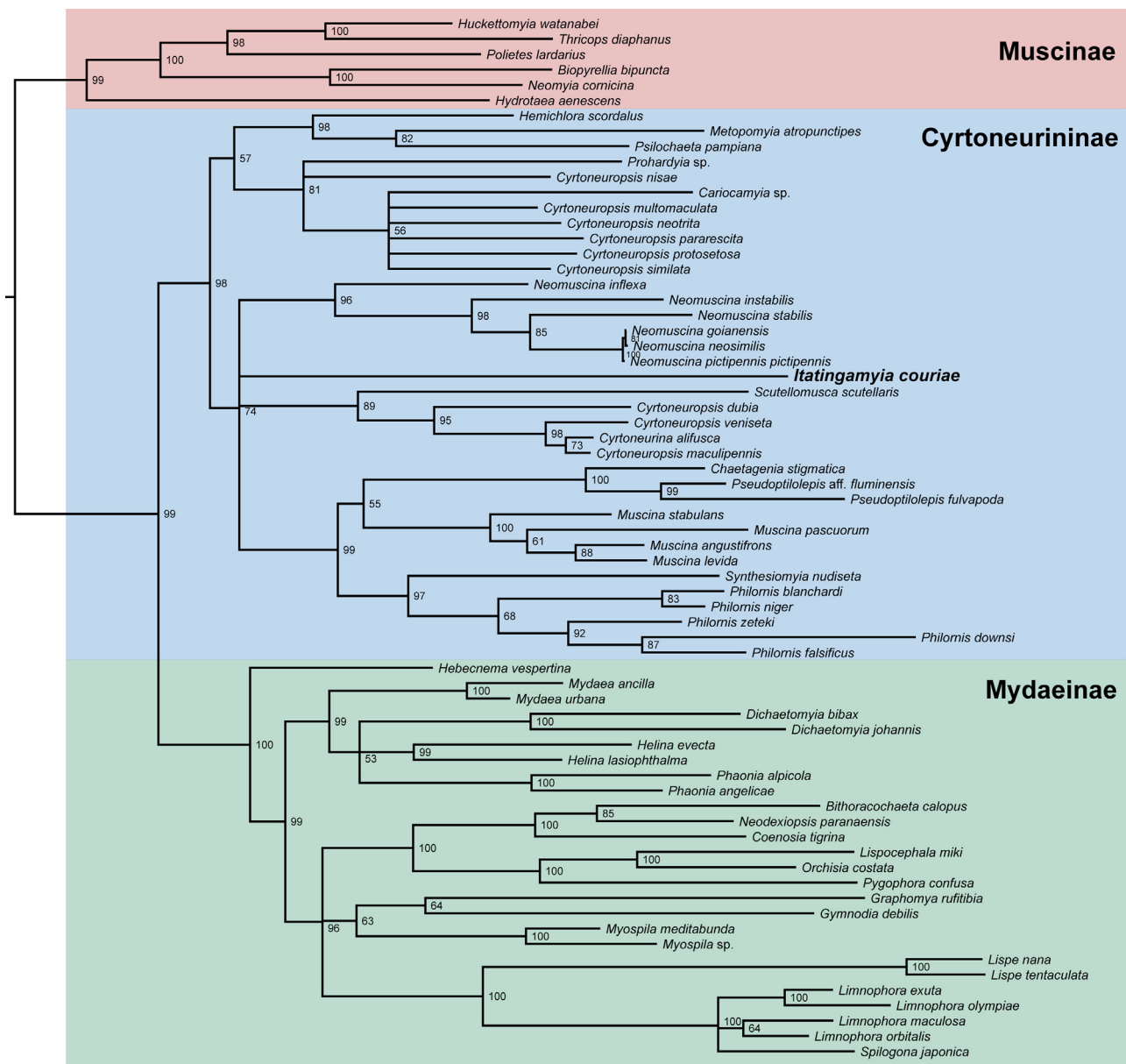(Figs. 3 and 4)

Fig. 2. Molecular phylogenetic hypothesis using Bayesian inference of the combined mitochondrial (COI) and nuclear (AATS, CAD, and EF1-a) protein-coding genes for 68 species of Muscidae highlighting the position of *Itatingamyia* within the Cyrtoneurininae. Subfamily-level classification follows Haseyama et al. (2015). Numbers are Bayesian posterior probabilities values.
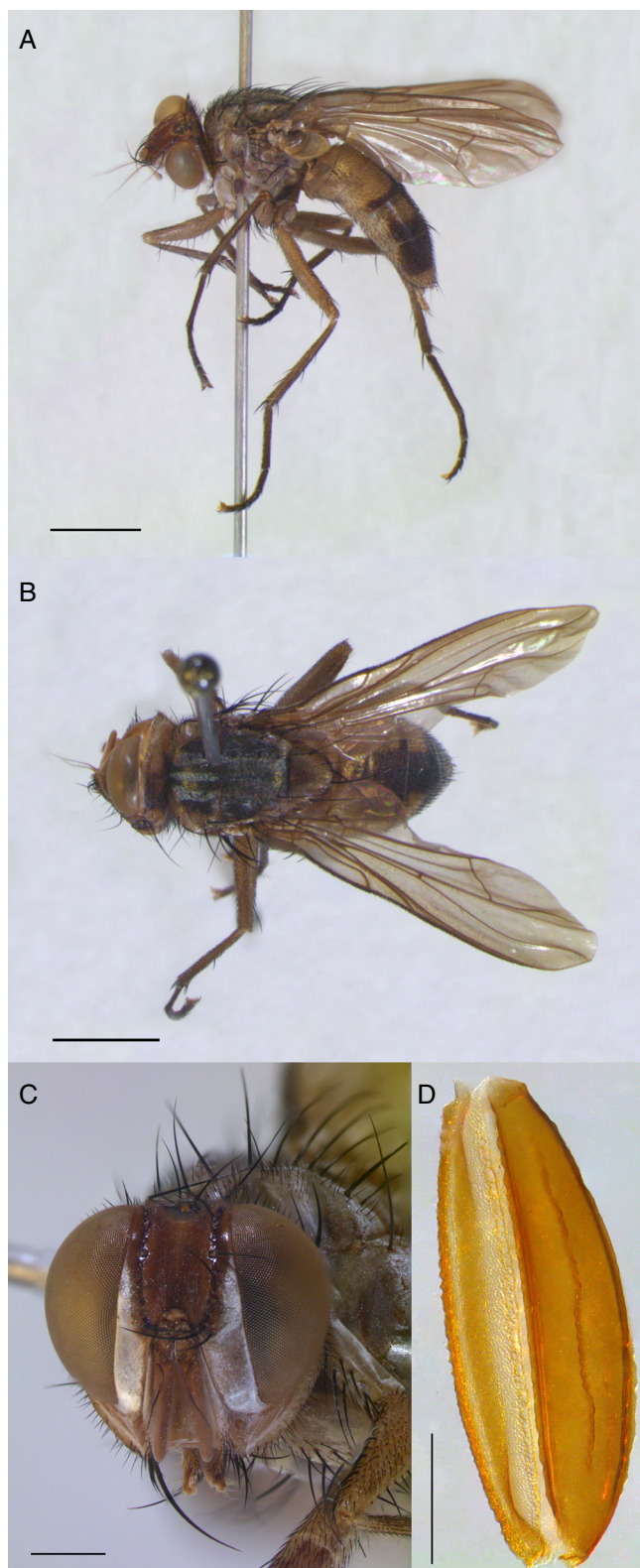
Fig. 3. *Itatingamyia couriae* Haseyama and de Carvalho, 2011, female UFMG-IDI-1600467. (A) lateral habitus view. Scale: 3 mm; (B) dorsal habitus view. Scale: 3 mm; (C) head, frontal view. Scale: 0.75 mm; (D) egg, dorsal view. Scale: 0.3 mm.
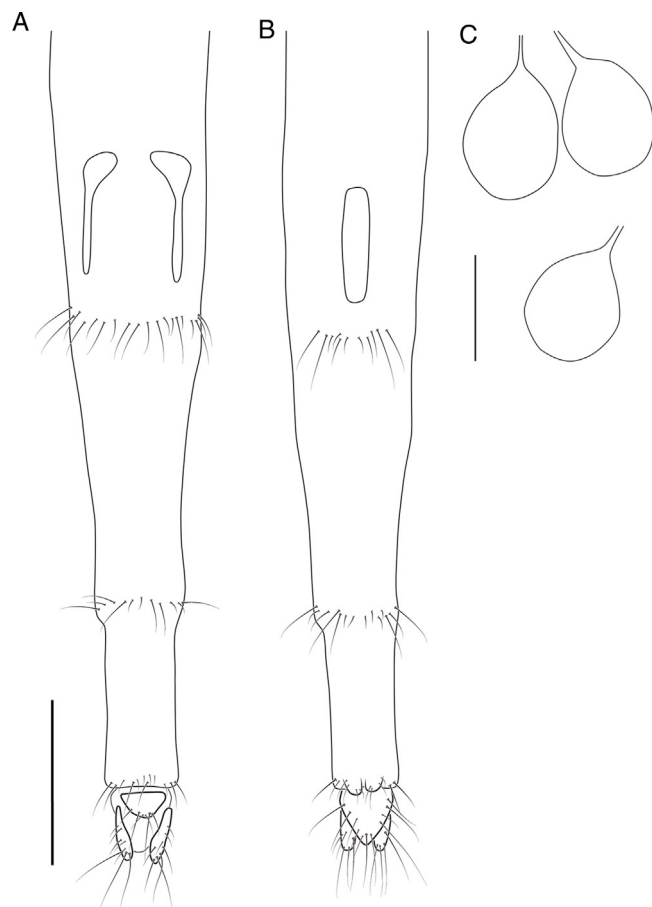

Fig. 4. *Itatingamyia couriae* Haseyama and de Carvalho, 2011, female genitalia UFMG-IDI-1600467. (A) Ovipositor, dorsal view. Scale: 1 mm; (B) Ovipositor, ventral view. Scale: 1 mm; (C) Spermathecae. Scale: 0.2 mm.

Description of female (similar to male). Measurements (2 females): 5.4–6.6 mm (length from the head to the posterior tip of the scutellum), 9.0–9.3 mm (wing length).

Color. Head. Parafacial yellow to light brown with silver pruinosity (Fig. 3C); gena, proboscis, and palpus yellow to light brown; postpedicel yellowish with silver pruinosity; arista yellow to light brown on basal fourth and reddish brown to the apex; frontal vitta reddish brown with minute silver setulae; ocellar triangle and fronto-orbital plate reddish brown, the latter with silver pruinosity anteriorly (Fig. 3C). Thorax laterally, postpronotal lobe, and notopleuron yellow to light brown (Fig. 3A); scutum dark brown with three silver pruinosity stripes; lower calypter yellowish; upper calypter hyaline with darkened borders (Fig. 3A). Wing hyaline yellowish, slightly darkened in the upper third. Legs yellow to light brown; claws brown with black apex; pulvilli yellowish.

Abdomen. Tergites 1–4 yellowish to light brown with a median brown stripe extending to the apical borders of the tergites (Fig. 3A,B); tergite 5 brown with yellowish apex (Fig. 3A).

Head. Dichoptic; eyes with short sparse setulae; frontal vitta bare; fronto-orbital plate with a few setulae; postpedicel ending before insertion of vibrissa; arista setulose, its longest setulae far exceeding width of base of arista; ventral setulae in arista present; secondary setulae on inner-dorsal surface of arista absent; parafacial setulose on lower third; facial ridge projected, partially hiding post-pedicel in lateral view; palpus cylindrical; proboscis retractile; vibrissa inserted under ventral level of the eye and fully developed, 2–3 setae longer than subvibrissal setae; inner vertical seta convergent; outer vertical seta divergent; postocular seta convergent, shorter than inner vertical seta; upward curved setae

in gena absent; ocellar triangle short, not reaching half the length of frontal vitta.

Thorax. Acrostichal setae: 0:1; dorsocentral setae: 2:3; postpronotal setae 2; presutural intra-alar seta present; presutural supra-alar seta present, twice longer than presutural intra-alar seta; notopleuron: 2 setae and with sparse coverage setulae around posterior seta; prealar seta absent; supra-alar postsutural setae: 2; intra-alar postsutural seta: 1; basal scutellar setae: 1 weak pair (about 80% of the lenght of scutellum); lateral scutellar setae: 1 pair 3–4 times longer than basal one; preapical scutellar setae: 1 pair, the same size as basal scutellar setae; apical scutellar setae: 1 pair, the same size as lateral scutellar setae; anepimeron (lower portion), anatergite, katatergite, katepimeron, meron, greater ampulla, post-alar wall, supra-squamal ridge and membrane between anepimeron and lower calypter and bare; proepisternum: 2 upward curved setae and with a few coverage setulae; proepimeron with a few coverage setulae; anepimeron (upper portion), katepisternum and basal lateral portion of scutellum setulose; katepisternals: 1:2; anepisternum with a row of 8 setae, with setulae posteriorly and at anterior dorsal corner; metathoracic spiracle larger than halter's knob and setulose at posterior portion; metakatepisternum with a few weak setulae; lateral basal membrane connecting upper and lower calypter absent; lower calypter glossiform.

Wing. Subcostal sinuous; $A_1 + CuA_2$ long, almost reaching the membrane border; A_2 curve smooth; subcostal sclerite bare; the apical portion of M straight, subparallel to R_{4+5}; microtrichia present in all the membrane; all veins bare except for costal vein.

Legs. Fore femur with a complete row of setae in PD, D, and PV surfaces; fore tibia with differentiated golden coverage setulae on AV surface, 2–3 median to submedian setae on the AD surface and a median seta on PD surface. Mid femur with 2 PD subapical setae, 1 strong median ventral and a series of distal slightly differentiated PV setae; mid tibia with 2–3 P median setae. Hind coxa bare on posterior face; hind femur on AV surface with a row of setae in apical half and a stronger submedian seta, an almost complete row of setae on AD surface, a subapical seta on PD surface, an irregular row of distal setae on PV surface and a stronger submedian seta; hind tibia with 1–2 submedian setae on AV surface, a submedian, a stronger median and a strong apical seta on AD surface; 1–2 median setae on PD surface; calcar absent.

Abdomen. First sternite setulose on the whole surface.

Terminalia. Ovipositor long; long setae present on the transition between ovipositor segments. Sixth sternite rectangular and poorly sclerotized, almost transparent and barely visible; seventh sternite not sclerotized (not visible); eighth sternite divided and clearly visible; hipoproct pentagonal; cerci digitiform; sixth tergite divided and anchor-shaped; seventh and eight sternites not sclerotized (not visible); epiproct triangular and poorly sclerotized, almost transparent (Fig. 4A,B); three spermathecae (Fig. 4C).

Egg (Fig. 3D). Color dark brown. The egg is Phaonia-type (Skidmore, 1985), elongated, rounded at both poles and with one longitudinally corrugated mark on ventral surface that extends almost from pole to pole. A similar mark is also found on each lateroventral and laterodorsal sides of the egg. Dorsal surface flat with a broad median area, and ventral surface convex. Hatching pleats foliaceous, parallel, and folding inwards. The outer margins are serrated, each peak with a delicate setula on the tip.

Male measurements (9 males): 4.6–6.1 mm (length from the head to the posterior tip of scutellum), 6.7–10 mm (wing length).

Morphological variations between new male specimens and the holotype. Scutellum varying from wholly yellow to yellow with a brown spot at the base. Parafacial setulose from the lower third to the lower half. Postocular setae convergent. Fore tibia with 1–2 A setae, the proximal only slightly differentiated from ground setulae; with or without a P median seta. Mid femur with a series of distal setae that are slightly differentiated from coverage setulae

on PV. Hind tibia with 1–2 submedian AV setae; 2–3 submedian AD setae; 1–2 submedian PD setae. The original description of the holotype reports a seta slightly differentiated from the coverage setulae as the parahumeral. As none of the newly observed material has this seta, we believe the parahumeral is actually not developed in this species. The same observation is valid for the intrapostalar seta.

Remarks. The original description of the holotype states that "parafacial setulose on ventral half (. . .) parafacial bare" (Haseyama and de Carvalho, 2011, p. 62). The parafacial is actually setulose in the lower half. Other characters not previously described are prosternum bare and notopleuron setulose only near the P seta. We had only two females available for this study, and both were dissected. We could not visualize the sixth tergite and sclerite and the epiproct in the first one. In the second female, these structures were poorly sclerotized and barely visible. The female is similar to male, regarding size, color, and chaetotaxy.

Examined material

Type-material. Holotype, male. Labels: "BIOTA-FAPESP/BRASIL: ES: Santa Teresa/Est. Biológica Santa Lúcia – 867 m/19 58 37 3 S 40 32 22 5 W/Arm. Malaise – Ponto 1 – Trilha/9-12 IV 2001/C.O. Azevedo & eq. Col." (MZUSP).

Additional material examined (n = 13). All specimens belong to CCT-UFMG, except where noted. Brazil, Minas Gerais. 1♂ Belo Horizonte, Campus UFMG, Estação Ecológica, 19°52'30"S, 43°58'20"W, 842 m, 20.i.1999, Malaise trap, A.F. Kumagai col. (UFMG-IDI-1700220); 1♀, same data, 17–23.xi.1991 (UFMG-IDI-1700221); 1♀ Belo Horizonte, Campus UFMG, Estação Ecológica, 10–16.xii.1991, A.F. Kumagai col. (UFMG-IDI-1600467); 1♂ Nova Lima, 19°58'7.4"S, 43°51'22.7"W, 27.x.2015, Malaise trap, A.R. Lima col. (UFMG-IDI-1701192; voucher of the sequences submitted to GenBank); 1♂ same data, 27.xii.2015 (UFMG-IDI-1700226); 1♂ São Gonçalo do Rio Abaixo, EPDA/Peti, PO-44, 19°52'49"S, 43°22'06"W, 14.ii.2003, Malaise trap, A.F. Kumagai col. (DZUP 245599); 1♂, same data, 14.xi.2013 (UFMG-IDI-1701194); 1♂, same data, 27.xii.2002, PO-37 (UFMG-IDI-1700225); 1♂, same data, 27.ii.2003, PO-47 (UFMG-IDI-1700224); 2♂, same data, 17.i.2003, PO-40 (UFMG-IDI-1700228, UFMG-IDI-1700229); 1♂, same data, 13.xii.2002, PO-35 (UFMG-IDI-1700223); 1♂, same data, 07.ii.2003, PO-43 (UFMG-IDI-1700222).

Geographical distribution (Fig. 5): Brazil: Espírito Santo, Minas Gerais (new record).

Discussion

Itatingamyia is highly supported as a Cyrtoneurininae by topological tests and phylogenetic estimation. Nevertheless, we were not able to consistently establish the sister-group of this genus, since Cyrtoneurininae internal relationships are not the same under ML and BI, and have low resolution on the latter criterion. More inclusive phylogenies, like those published by Kutty et al. (2014) and Haseyama et al. (2015) also have a low resolution for this clade, suggesting that a denser sampling of taxa or genes, or both, is needed to consistently solve the relationships within these genera.

Our results agree with de Carvalho and Couri (1993), who classified Itatingamyia within the Reinwardtiini, which in the sense used here includes the Cyrtoneurininae sensu Snyder (1954), the Reinwarditiini, the Eginiini, and partially the Phaoniini (Haseyama et al., 2015). Albuquerque (1979) classified the genus within the Mydaeinae, based on the ovipositor of I. bivittata. However, his illustration of this structure (Fig. 1, p. 325) is very different from the one by de Carvalho and Couri (1993) for the same species (Figs. 8 and 9, p. 593). In Albuquerque's interpretation and illustration, the
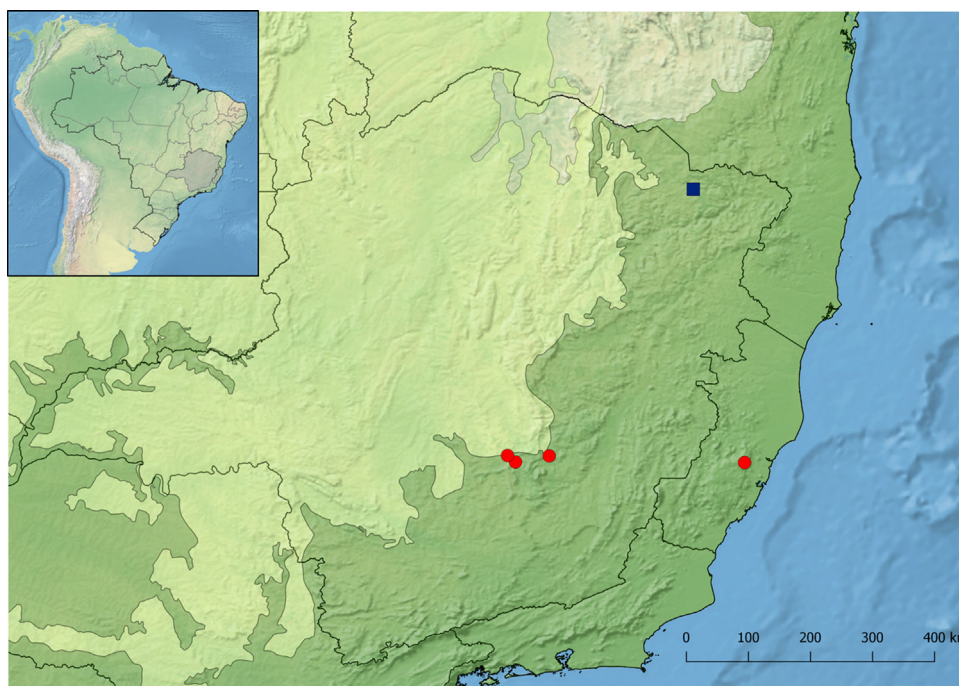
Fig. 5. Known collection sites for *Itatingamyia* species. Blue squares: *I. bivittata*. Red circles: *I. couriae*. Darker green area: Atlantic forest biome. Yellowish green area: Cerrado biome. Yellowish gray area: Caatinga biome.

ovipositor and cerci are short, like those found in many Mydaeinae, while in de Carvalho and Couri's illustration the ovipositor and cerci are long. This second interpretation is similar to our own (Fig. 4). The long ovipositor and the digitiform elongated cerci of the female are found in other Cyrtoneurininae genera (*e.g.*, *Chaetagenia*, *Neomuscina*, *Philornis*, among others) (Couri, 1983; Couri and Lamas, 1993; Pereira-Colavite and de Carvalho, 2012). Furthermore, short cerci are not exclusive of the Mydaeinae, and therefore, this character is not appropriate to determine the systematic position within Muscidae. Also, our analyses do not corroborate a close relationship between *Itatingamyia* and *Cariocamyia* (Haseyama and de Carvalho, 2012), which was estimated as the sister group of *Prohardyia* (ML, Fig. 1), or in a polytomy with *Cyrtoneuropsis* species (BI, Fig. 2).

The egg of *Itatingamyia couriae* is in general terms similar to those found in other Muscidae species with broadly foliaceous hatching plates (*i.e.*, *Phaonia*-type), such as *Mydaea lateritia* (Rondani, 1866) (Grzywacz and Pape, 2010), *Neodexiopsis rufipes* (Macquart, 1851) (Patitucci and Couri, 2018) and *Philornis torquans* (Nielsen, 1913) (Patitucci et al., 2017). Since these studies used electron microscopy and ours only light microscopy, we cannot compare the structures in detail. However, to our knowledge, it is the first time that setulae are observed in the hatching plate of Muscidae eggs.

Itatingamyia couriae is primarily distributed in Atlantic Forest areas (Fig. 5). On the other hand, according to Albuquerque (1979), *I. bivittata* was collected in a Cerrado area in the municipality of Pedra Azul, state of Minas Gerais. Interestingly, Pedra Azul is covered with dry forest, which is a kind of deciduous vegetation (Golfari, 1975). While some authors consider this kind of vegetation as a part of Cerrado biome (*e.g.*, Ribeiro and Walter, 1998), the Brazilian government places Pedra Azul within the boundaries of the Atlantic forest following law 11428/06 (Brasil, 2006). Therefore, the genus can be considered restricted to the Atlantic forest biome.

The discovery of the new individuals described here, especially the female, will facilitate future morphological and molecular systematic studies since the known specimens of *I. bivittata* are in poor condition (*e.g.*, Fig. 1 from Haseyama and de Carvalho, 2011). Even

though we were able to consistently propose a subfamily classification for the genus, further investigation is needed to understand the relationships within this clade and to uncover the sister-group of *Itatingamyia*.

Authors contribution

KLFH conceived the research project and analyzed the data. CJBC described the female. AZF produced the new DNA sequences and analyzed the data. FDK sorted the specimens and produced the images. All authors contributed to the analysis design, manuscript writing and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rbe.2018.10.003.

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