First species of *Inbiomyia* from the Atlantic Forest of Brazil (Diptera: Inbiomyiidae)

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**ABSTRACT**

We describe and illustrate *Inbiomyia azevedoi*, sp. nov., the first species of Inbiomyiidae known from the Atlantic Forest in southeastern Brazil. SEM photos show that the bifurcated labella have on their ventral face rows of scalelike rasping teeth that may be used to graze on green algae, as the gut content of some specimens suggest. A reanalysis of the phylogeny of the genus using *Australimyza mcalpineorum* as designated outgroup and including *I. azevedoi* shows that all four groups of species previously proposed for the genus are monophyletic if *I. anodonta* is removed from the *scoliostylus* group. *Inbiomyia anodonta* and *I. azevedoi* are here transferred to the *anemosyris* group. In our results with implied weight, *I. exul* is sister to a clade including all other species of the genus, while the *scoliostylus* group appears as sister to the clade (*mcalpineorum* group + *anemosyris* group).

**INTRODUCTION**

The monogeneric Neotropical acalyptrate family Inbiomyiidae was first described by Buck (2006) based on *Inbiomyia mcalpineorum* Buck from Costa Rica. The family was placed in the superfamily Carnoidea as sister to Australimyzidae by Buck (2006) based on a phylogenetic study of the relationships between carnoid families. Australimyzidae occur in Australia, New Zealand, and their nearby islands (Brake and Mathis, 2007), while Inbiomyiidae is Neotropical.

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Inbiomyiidae is peculiar in having the posterior notopleural setae in a dorsal position, protruding eyes, head only with inner and outer vertical setae, one pair of inclinate fronto-orbitals, a typically very long arista, a strong subapical seta on the maxillary palpus, and both males and females without cerci (Buck, 2006).

Buck and Marshall (2006) later described 10 additional species, which extended the distribution of the family in the Neotropics with records for Colombia, Venezuela, Ecuador, Peru, Bolivia, and French Guiana. Buck and Marshall (2006) performed a cladistic analysis of the genus using morphological data. Their result show two major sister clades, in which they recognized four species groups—exul, scoliostylus, mcalpineorum, and anemosyris.

We here describe a new species of Inbiomymia from the Atlantic Forest based on males and females collected in southeastern Brazil. In our description, we include SEM images, illustrations, and a discussion of the phylogenetic relationships within the genus.

Material and Methods

Most of the material examined was collected along the Biota FAPERJ project. The holotype is deposited at the Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ), while paratypes are deposited at the MNRJ, the Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZUSP), the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil (INPA), and the Entomological Collection of the Universidade Federal do Espírito Santo (UFES). Part of the material initially studied was lost in the fire of the MNRJ that occurred on September 2, 2018 (Duarte, 2019). Fortunately, some of the specimens were in another lab and could be used as the formal type series.

Pictures were taken with LAS software and assembled in Helicon Focus 6. The terminalia were treated with KOH 10% at 40° C for 20 minutes, then neutralized in 1% acetic acid, and transferred to a microvial with glycerin. The wing and the antenna were mounted with Euparal between cover slips. Scanning electron micrographs (SEM) were obtained using a high vacuum JEOL JCM-6000. The morphological nomenclature follows Cumming and Wood (2017) and Buck and Marshall (2006).

In their phylogenetic study of the genus, Buck and Marshall (2006) used in the matrix a generalized “Australimyzidae” and an “ancestor” as terminals to root the analysis. The Australimyzidae had question marks for a number of characters of male and female terminalia. In this study, we used specimens of Australimyza mcalpinei Brake and Mathis to fill in some of the gaps on information about the generalized Australimyzidae, and we replaced it as a terminal in the matrix. We did not use an ancestor as a terminal and we rooted the analysis in Australimyza. We used Buck and Marshall’s (2006) original list of characters and data matrix to study the position of the Brazilian species within Inbiomyia and to reassess the relationships between the species of the genus, rooting the analysis in A. mcalpinei as the outgroup (table 1).

To understand the effects of the change of the outgroup and the addition of a new species, we ran the matrix including Inbiomyia azevedoi, sp. nov., using either Buck and Marshall’s (2006) Australimyzidae or A. mcalpinei as a terminal. We analyzed the data matrix using the
TNT 1.1 software under equal-weight scheme (Goloboff et al., 2008) and k = 3 under implied weight, so characters with a higher level of incongruence would have a smaller effect on the construction of the backbone of the phylogeny. The construction of the data matrix and the trees was made with the software Winclada (Nixon, 1999).

The maps (fig. 9) were constructed using data from the literature (Buck, 2006; Buck and Marshall, 2006) for the known species within Google Earth, then edited in Adobe Illustrator CS.

**TAXONOMY**

*Inbiomyia azevedoi*, sp. nov.

Figures 1–7

**Diagnosis:** Body entirely dark brown with brownish legs; proboscis with a pair of dark spots subapically in the prementum (fig. 1B); frontoclypeal membrane reaching dorsal part of lower facial ridge; second sector of the wing more than 2× longer than the third sector; male terminalia with ventral epandrial process articulated and apically rounded; surstylus wider distally, bearing a regular row of stout setae; female abdominal tergite 7 not divided medially and not fused laterally to sternite 7.

**Material examined:** Holotype male BRAZIL, Rio de Janeiro, Itatiaia, PNI Complexo do Maromba, PNI-M2B, S22°26′07″ W44°37′33.2″ 1234 m; vi-vii.2015, Malaise trap, Azevedo & equipe. col. [MNRJ]. Paratypes: 7 ♂ and 17 ♀, same data as holotype [2 ♀ MNRJ; 6 ♂ and 14 ♀ MZUSP; 1 ♂ and 1 ♀ INPA]; 1 ♀ BRAZIL, Espírito Santo, Divino de São Lourenço, Parque
Nacional do Caparaó, Trilha do Falcão, 1500 m, 20°24′30″S 41°47′06″W, 15-23.iii.2013, Malaise 21, C.O. Azevedo & F.B. Braga leg. [UFES].

**Description:** Body length, 1.75–1.8 mm. Wing length, 2.1–2.2 mm. **Head** (figs. 1, 2, 3A): Broad, dark brown except for yellowish proboscis and labella. Antenna brown, almost concolor with frons, lighter toward apex. First flagellomere with a small sensory pore at outer face opening to wider cavity inside (fig. 3A). Postocellar and inner occipital setae absent. Inner vertical seta almost as long as the single strong fronto-orbital seta, with four additional frontal orbit hairs. Ocellar plate with sparse microtrichia, ocellar setulae minute, proclinate. Eyes reddish brown, protruding, densely covered with interommatidial setae, ventroposterior area of eyes strongly emarginated. Facial sclerotization extending ventrally to lower facial ridge; frontoclypeal membrane reaching dorsal part of lower facial ridge. Vibrissal angle rounded, vibrissa strong, slightly longer than fronto-orbital seta, no subvibrissal hairs. Gena with several setulae, in addition to some longer setae along ventral margin, postgena large, extending into eye emargination. Maxillary palpus concolor, with a strong, long typical seta. Proboscis elongate, with two pairs of setae and a dark apical spot on prementum, labella bifid, inner face of labellum at each side covered with rows of scalelike rasping teeth. **Thorax** (figs. 1A, 3B, 4A): Dark brown. Scutum pilose, with one pair of small setae on disc surface, anteriorly to margin (subequal in length to acrostichal hairs), and one pair of very long converging marginal scutellar setae, crossing apically. Postpronotum almost indistinct with one postpronotal seta as long as the presutural supraalar seta. Proepimeron and proepisternum bare, anepisternum entirely covered with setulae, no strong seta present. Katepisternum with anteriorly inclined posterodorsal seta, some setae on ventroanterior half, and setuale. Meron high, only with some setulae on dorsal third, ventrally to posterior spiracle. Halter dark brown. **Leg** (figs. 1A, 4A): Caramel brown. Forecoxa protruding anteriorly, with four small laterobasal spines (fig. 4A); mid and hind coxa with a longer central seta; antero- and posteroventral rows of setae of forefemur slightly stronger and darker than remaining setulae, posteroventral row slightly stronger than anteroventral row in both sexes. First tarsomere of foreleg shorter than mid and hind first tarsomeres; first tarsomeres with a ventral fringe of orangish short setae. Tarsal claws elongate, no pulvillus. **Wing** (figs. 1A, 4B): Membrane hyaline, iridescent, veins brownish. Wing sectors Cs1/Cs2/Cs3/Cs4: 3; 12; 4; 2. Well-defined subcostal and humeral breaks, C extending to tip of M1; setulae along C between apex of R1 and R2+3 well spaced, slightly longer and stouter than regular setae. Sc running close to R1, not sclerotized on distal half. R1 very short. R2+3 long, ending on distal fifth of wing. R4+5 ending almost at wing tip. M1 ending close to wing apex, slightly curved posteriorly at tip. M4 fading at tip, not reaching wing margin. CuA+CuP strong, not reaching wing margin, displaced anteriorly, close to wing margin; A1 entirely absent. Cell dm narrow, separated from cell bm by rather oblique dm-m. Cell cua closed, convex distally. Anal lobe not
FIGURE 1. *Inbiomyia azevedoi*, sp. nov., female paratype. **A**, habitus. **B**, head in frontal view (arrow indicates black spot on prementum)
FIGURE 2. Inbiomyia azevedoi. A, head, lateral view, female paratype. B, head, frontal view, male paratype. C, maxillary palpus, female paratype. D, inner face of labella, male paratype. Abbreviations: cly, clypeus; fc, face; lbl, labium; lbr, labrum; lsc, labial scale; mxp, maxillary palpus; mxs, maxillary palpus seta; pfc, parafacialia; prm, prementum; vb, vibrissa.
FIGURE 3. Inbiomyia azevedoi male paratype. A, antenna (arrow indicates the sensory pit). B, thorax, dorsal view. Abbreviations: acr, acrostichal setula; anpl, anterior notopleural seta; ar1, first aristomere; cxI, fore coxa; dc, dorsocentral seta; pnpl, posterior notopleural seta; psa, postsutural supralar seta; sct, apical scutellar seta.
FIGURE 4. *Inbiomyia azevedoi*. **A**, thorax, lateral view, female paratype (arrow indicating four small latero-basal spines). **B**, wing. Abbreviations: aep, anepimeron; ane, anepisternum; anpl, anterior notopleural seta; asp, anterior spiracle; bm, basal medial cell; bm-m, basal medial crossvein; CuA, anterior branch of cubital vein; CuA+CuP, anterior branch of cubital vein + posterior branch of cubital vein; dc, dorsocentral seta; dm-m, discal medial crossvein; h, humeral crossvein; ktp, katepisternum; M1, first branch of media; M4, fourth branch of media; mr, meron; pem, proepimeron; pep, proepisternum; pnpl, posterior notopleural seta; posi, postsutural supraalar seta; ppm, postpronotal seta; prsi, presutural supraalar seta; psa, postalar seta; psp, posterior spiracle; R1, anterior branch of radius; R2+3, second branch of radius; R4+5, third branch of radius; r-m, radial medial crossvein; Sc, subcostal vein; trs, transverse suture.
prominent. **Abdomen:** Tergites shinning brown, darker toward tip, terminalia brown. Tergites 1 and 2 fused, tergites much wider and well sclerotized than sternites 2–4. **Male terminalia** (figs. 5, 6): Synsternite 5+6+7 Y-shaped anteriorly, partially fused dorsally to sternite 8. Ring-shaped sclerite absent. Epandrium brown, elongated dorsoventrally, with posteroventral lobes paler, well developed; no anterodorsal apodemes developed; anteroventral process not fused to epandrium. Subanal plate continuous with epandrium. Perianal field elliptical dorsally, ventral margin clearly delimited from subanal plate. Surstylus brown, wider distally, with a regular row of stout setae along distal margin. Hypandrium connected to anteroventral margin of epandrium, with a long projection posteriorly. Postgonite short, brown, directed ventrally, with ca. 5 stout, peglike setae near apex and several small setulae. Epiphallus yellowish, slender, tip directed ventrodistally. Distiphallus short, microtrichose. **Female terminalia** (fig.7): Tergite 7 entire, laterally separate from sternite 7; tergite 8 without a dark brown apodeme, concolor with preceding tergites. Sternite 6 as wide as previous sternites, longer at lateral end, very short medially; sternite 7 evenly sclerotized. Ventral membrane internally at genital opening (possibly sternite 8) with rows of spines. Spermathecae weakly sclerotized, pale brown, slightly striate, central invagination at point of duct insertion.

**Etymology:** The specific epithet of this species is given to honor Leonardo Henrique Gil Azevedo, Brazilian dipterist working at the Museu Nacional do Rio de Janeiro. He has collected most of the specimens of the type series and has provided an important contribution to the systematics of the Neotropical Simuliidae and some other nematocerous families.

**Comments:** *Inbiomyia azevedoi* would run into couplet 8 of Buck and Marshall’s (2006) key to the species of *Inbiomyia*, along with *I. mcalpineorum* and *I. empheres* Buck, if we consider the C2/C3 proportions, but it would run to *I. anodonta* if we consider the distribution of setae on the surstylus. The postgonite in *I. mcalpineorum* is directed anteriorly, in such a way that the subanal plate and the hind margin of the epandrium almost form a right angle in lateral view, while in *I. empheres* and *I. azevedoi* the postgonite is directed ventrally and the subanal plate and hind margin of the epandrium form an obtuse angle. *I. azevedoi* differs from *I. empheres* by having the abdominal tergite 7 of females not divided medially and is not fused laterally to sternite 7, along with the ventral epandrial process apically rounded in the male terminalia (acute in *I. empheres*). *Inbiomyia azevedoi* resembles *I. anodonta* in the shape of the surstylus and of the epiphallus, with which the ventral process is articulated. *Inbiomyia anodonta* possesses the second sector of the wing much shorter than *I. azevedoi* and also has an anterior apodeme on the epandrium, which is absent in the new species. Green algae were found in abdomen of one specimen analyzed during the dissection of the male terminalia, probably belonging to the gut content.

**DISCUSSION**

The species described here obviously fits in *Inbiomyia*. It shares with the remaining of the species of the genus distally bifid labella, a very conspicuous seta on the maxillary palpus, and an especially long arista. The fusion of sternites 5+6+7 forming an asymmetric, partly internal-
FIGURE 5. *Inbiomyia azevedoi*, male paratype. **A**, abdomen, lateral view. **B**, male terminalia, dorsal view. Abbreviations: ep, epandrium; eph, epiphallus; hy, hypandrium; pa, phallapodeme; S, sternite; ss, surstylus; sp, spiracle; T, tergite.
FIGURE 6. *Inbiomyia azevedoi*, male paratype, postabdomen. A, lateral view, dissected specimen. B, terminal view, dissected specimen. C, lateral view, illustration. D, dorsal view, illustration. Abbreviations: **bp**, basiphallus; **dp**, distiphallus; **ep**, epandrium; **hy**, hypandrium; **pa**, phallapodeme; **pog**, postgonite; **S**, sternite; **ss**, surstylus; **vp**, ventral process of epandrium.
ized structure in males and the absence of cerci in males and females are additional features of the terminalia in the Inbiomyiidae shared by *I. azevedoi*.

Our SEMs show that the labella have distally on the inner face six long rows of scalelike teeth, which are probably used to rasp the substrate. In some lauxaniids, the ventral surface of the labellum is adapted for fungal grazing (Broadhead, 1984), with teeth present along the pseudotracheal channels (Mello and Silva, 2006). The teeth of the labella found in *Inbiomyia azevedoi* (fig. 2D) differ in shape and position from those in lauxaniids and also from the pre-stomal teeth of calyptrates (Sukontason et al., 2005)—and represent obvious convergences. There are no field observation of adults feeding, nor an extensive study of the gut content of specimens in the family, but the observation of algae in the gut content of one of our specimens, as was also mentioned by Buck and Marshall (2006: 6), makes plausible an assumption that they graze wet surfaces to obtain algae for feeding.

In Buck’s (2006) phylogenetic study of the Carnoidea, both most-parsimonious trees have the Inbiomyiidae as sister to the Australimyzidae, this clade sister to Carnidae. The group (Carnidae + (Australimyzidae + Inbiomyiidae)) came out as sister of a large clade of Carnoidea of which Chloropidae, Milichiidae, Cryptochaetidae, and Canacidae are members. The main features gathering Inbiomyiidae and Australimyzidae in their study are the reduced ptilinium,
presence of a basal extension on the hypandrium (named hypandrial apodeme), hypandrium rigidly connected to the epandrium along its ventral margin, and a short ovipositor (Buck, 2006). Brake and Mathis (2007) also cited the absence of a subependrial sclerite as another feature shared by both families, but such a sclerite is extremely variable in carnoids, so it should not be regarded as a reliable character.

Wiegmann et al.’s (2011) phylogeny based on molecular data placed the Inbiomyiidae and the Australimyzidae in entirely different positions. Inbiomyiidae appeared as sister of the Neminidae, a clade that is sister to (Aulacigastridae + Nerioidea), while Australimyzidae came out as sister of Xenasteiidae, in a clade also including, e.g., Asteiidae, Ropalomeridae, Neurochaetidae, and Clusiidae—rather far from the Carnoidea. In addition, their position for Inbiomyiidae and Australimyzidae contradicts other sources of information (e.g., Buck, 2006). Finally, their phylogeny is based on a limited taxon sampling, which may have affected the position of some of the terminals. The rapid radiation of Schizophora during the Tertiary could be a factor prejudicing the comprehension of the suprafamilial relationships of this clade (Wiegmann et al., 2011). A more recent analysis made by Keith Bayless et al. (2018) using a wider dataset also dismantled Carnoidea and recovered Australimyzidae and Asteiidae as sister groups; Inbiomyiidae, however, was not included in the taxonomic sampling. It is evident that the relationships of the members of the Carnoidea still have to be reassessed, but this is outside the scope of this paper and a study of the position of the Inbiomyiidae in the context of the Schizophora major clades is to be made elsewhere.

To determine the relationships among the species of Inbiomyia including the new species and to facilitate a comparison of our results with those of Buck and Marshall (2006), we removed the a priori “ancestor” as a terminal and instead used their “Australimyzidae” as a terminal and an Australimyza species as designated outgroup. Our reanalysis of Buck and Marshall’s (2006) data matrix under equal weight including I. azevedoi and keeping their data for “Australimyzidae” as a terminal disrupted their scoliostylus group (fig. 8A). Inbiomyia anodonta Buck and Marshall moved out of the scoliostylus group and appeared as sister to a clade including (I. mcalpineorum, I. empheres, I. scoliostylus, and I. zeugodonta). In this case, I. azevedoi is sister to a clade including I. anodonta and its sister clade; I. exul keeps its position as sister of the group anemosyris.

In our equal-weight analysis including I. azevedoi but changing the data of “Australimyzidae” for data of Australimyza mcalpinei, I. anodonta was again excluded from the scoliostylus group (fig. 8B). In this tree, the scoliostylus group, the mcalpineorum group, the anemosyris group, I. exul, I. azevedoi, and I. anodonta are at the same level in a polytomy at the base of the phylogeny of the genus. Finally, running this last matrix using implied weight with k = 3 (fig. 8C), E. exul is sister to the remainder of the genus. In this larger clade, the scoliostylus group without I. anodonta is sister to a clade including the mcalpineorum group and (I. anodonta + (I. azevedoi + (anemosyris group)))).

Indeed, the solution with k = 3 (fig. 8C) does not contradict the polytomy obtained with equal weight. We hence transfer I. anodonta to the anemosyris group, in which we also include I. azevedoi, based on the ventral process of the epandrium not being curved medially (char. 16:
FIGURE 8. Phylogenetic analyses of Inbiomyiidae. A, strict consensus of Buck and Marshall's (2006) analysis under equal weights including Inbiomyia azevedoi and excluding the hypothetical ancestor (two most parsimonious trees with 74 steps, consistency index 0.58 and retention index 0.7). B, strict consensus of the extended matrix (table 1) analysis under equal weights (13 most-parsimonious trees with 77 steps, consistency index 0.55 and retention index 0.68). C, most parsimonious tree of the extended matrix under implied weighting (k = 3, consistency index 0.55 and retention index 0.68). The green squares represent the scoliostylus group except I. anodontia; the orange squares represent the mcalpineorum group; and the purple squares represent the anemosyris group of Buck and Marshall (2006).
FIGURE 9. Distribution maps of the four species group of *Inbiomyia*. *I. anodonta* already transferred to the group *anemosyris* and *I. azevedoi* included in the same group. French Guyana occurrence of *I. zeugodonta* of Buck and Marshall (2006) was tentative and not included here. A, *Inbiomyia* group: *exul*. B, *Inbiomyia* group: *scoliostylus*. C, *Inbiomyia* group: *mcalpineorum*. D, *Inbiomyia* group: *anemosyris*. National boundaries in yellow; Brazilian states outlined in white.
state 1) and the apical portion of the surstylus greatly expanded (21: 2). This shows that the fusion of the ventral process to the epandrium (14: 0) and the absence of discal setae on the surstylus (22: 0) are plastic features that previously sustained the \textit{anemosyris} group. There is very good support of the monophyly of the \textit{anemosyris} group in its original delimitation and the connection of \textit{I. anodonta} and \textit{I. azevedoi} to the \textit{anemosyris} group results in a clade still restricted to South America (fig. 9), as noted by Buck and Marshall (2006).

Overall, our study results in limited changes to Buck and Marshall’s (2006) composition of the species groups. Basically, the species groups were confirmed to be monophyletic with the single exception of the position of \textit{I. anodonta}. Missing data in the original matrix and the inclusion of an additional species in our matrix are probably responsible for the differences found.

It is apparently early to more fully understand the evolution of the family in the Neotropical region. Additional undescribed species are known from the Amazon basin (J.A. Rafael, personal commun.) and a larger taxon sample in the family may be soon available. Nevertheless, it is interesting to observe that the relationship found among \textit{I. anodonta}, \textit{I. azevedoi}, and the clade with the remaining species of the \textit{anemosyris} group corresponds to an area cladogram that strictly correlates with the general pattern found for the Neotropical region (Amorim and Pires, 1996; Amorim, 2009) that has Central America–Mexico (with an extension west of the Andes in Colombia) sister to the Atlantic Forest, versus the Amazon Basin.

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