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Neoempheria Osten Sacken (Diptera: Mycetophilidae) from the Neotropical region: redescriptions of two species with complete life cycles

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Summary. Despite the rich biodiversity of the Neotropical Region, there is a dearth of recent studies on new Mycetophilidae species or their immature stages. To address this gap, we conducted a survey of *Neoempheria* species in the state of Paraná, Brazil, and provide the first descriptions of the immature stages of two species: *Neoempheria plaumanni* Edwards and *N. spinosa* Edwards. We also redescribed the adults of these two species, detailing their morphology and biology, including notes on their life cycles. Immatures were collected from a piece of decaying wood containing lichens and fungi, and were subsequently reared in the laboratory. Our findings represent the first records of both species in the State of Paraná, and we update the geographic distribution of these two species accordingly. Our study contributes to a better understanding of the diversity and ecology of Mycetophilidae in the Neotropical Region.

Résumé. *Neoempheria* Osten Sacken (Diptera : Mycetophilidae) de la région Néotropicale : redescriptions de deux espèces et premières mentions de leurs cycles de vie complets. Malgré la riche biodiversité de la région Néotropicale, les études récentes sur les nouvelles espèces de Mycetophilidae ou leurs stades immatures sont rares. Pour combler cette lacune, nous avons mené une étude sur les espèces de *Neoempheria* dans l'État du Paraná, au Brésil, et fourni les premières descriptions des stades immatures de deux espèces : *Neoempheria plaumanni* Edwards et *N. spinosa* Edwards. Nous avons également décrit les adultes de ces deux espèces, en détaillant leur morphologie et leur biologie, avec des observations sur leurs cycles de vie. Les immatures ont été prélevés sur un morceau de bois en décomposition contenant des lichens et des champignons, et ont ensuite été élevés en laboratoire. Nos découvertes représentent les premiers signalements des deux espèces dans l'État du Paraná, et nous mettons à jour la répartition géographique de ces deux espèces en conséquence. Notre étude contribue à une meilleure compréhension de la diversité et de l'écologie des Mycetophilidae dans la région néotropicale.

Keywords: Bibionomorpha; biodiversity; Mycomyinae; natural history; morphology; immature stages

Mycetophilidae, a family of fungus gnats in the suborder Bibionomorpha (Diptera), is a diverse group comprising more than 4500 species in over 230 genera (Pape et al. 2011; Oliveira & Amorim 2014), among which more than 1200 species in 55 genera are present in the Neotropical Region (Oliveira & Amorim 2014; Fitzgerald 2017; Amaral et al. 2022a; Kurina & Õunap 2023). These insects are generally found in damp habitats that favor wood-growing fungi, with larvae typically feeding on fungal fruiting bodies, while the diet of adults is poorly understood (Oliveira et al. 2015).

Mycetophilids comprise six subfamilies (Oliveira & Amorim 2021), including Mycomyinae, which encompasses the genus *Neoempheria* Osten Sacken, 1878. Of the 153 species of *Neoempheria* known worldwide (Fungus Gnats Online 2022), 69 occur in the Neotropical Region (41 in Brazil) (Oliveira & Amorim 2014). Edwards (1940) and Coher (1959) were the main researchers on the Neotropical fauna of *Neoempheria*, with the description of new species and proposition of species-groups. Amaral et al. (2022a) recorded the first occurrence of the genus from the Brazilian Amazon biome, *Neoempheria bilobata* Edwards, 1940. Amorim et al. (2022) and Riccardi et al. (2022) corroborated the presence of this genus in the Brazilian Amazon, with a record of 21 morphospecies from the state of Amazonas and four morphospecies from the state of Roraima, respectively, reinforcing the urgent need for taxonomic studies on this genus in the Neotropical Region.

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Based on wing features, length of ocellar bristle, and markings of thoracic pleura, Neotropical *Neoempheria* species were divided into eight groups by Edwards (1940: Groups A–H), who also described *Neoempheria spinosa* Edwards, 1940 (Group C) and *N. plaumanni* Edwards, 1940 (Group D). These groups were respectively named *spinosa*-group and *maculipennis*-group by Coher (1959) in a review on the Neotropical Mycomyinae. The species belonging to Group C or *spinosa*-group are characterized by the "Sc ending above base of Rs; Sc_2 oblique and practically at tip of Sc" and "pleurae with oblique dark stripe, extending from base wing across anepisternite to base of fore coxa" (Edwards 1940, p. 111). The original description of *N. spinosa* is based on the male holotype, as well as seven male and two female paratypes. Coher (1959) studied an additional male specimen from the type-locality.

The species belonging to Group D or *maculipennis*-group are characterized by the "trichiation of veins alike in the two sexes; Sc bare or almost so; Cu setose at base" and "two of the bristles immediately behind ocelli are long, extending forwards almost or quite as far as base of antennae" (Edwards 1940, p. 114). The original description of *N. plaumanni* is based on numerous specimens of both sexes including the holotype. Coher (1959) studied two male specimens from the type-locality and one male from the state of São Paulo. In the present study, these features are easily recognized in the species studied and highlighted in the Results section.

Both species were described from the district of Nova Teutônia, municipality of Seara, state of Santa Catarina. Although there are no additional records of *N. spinosa*, *N. plaumanni* is also known from São Paulo and Mato Grosso do Sul states (Brazil) and from Salta and Tucumán (in Argentina) (Oliveira & Amorim 2014). The types of both species are housed in the Natural History Museum, London, whereas photographs and type series information are available in Amorim & Oliveira (2013). Edwards (1940) provided male genitalia and wing illustrations and Coher (1959) an identification key for the species-groups of Neotropical *Neoempheria*. There is no information regarding the immatures or female genitalia illustrations, which are herein provided.

The larval stage of *Neoempheria puncticoxa* Edwards, 1940 was described for the first time by Oliveira et al. (2015) and was the first observation of immature fungus gnats in the Neotropical Region. Later descriptions of mycetophilids include *Mycomya chilensis* Freeman, 1951 (Passacq et al. 2017) and *Monoclona carambeiensis* Amaral, Oliveira & Falaschi, 2022. In this study, we present the complete life cycles of *Neoempheria plaumanni* and *N. spinosa*, the redescriptions of male and female adults, and a distribution map of these species. This is the first record of the genus for the state of Paraná, and these are the fourth and fifth Neotropical mycetophilid species with known life cycles.

Material and methods

The specimens were collected from two different municipalities of the state of Paraná, Brazil, from tree trunks with fruiting fungal bodies (Polyporaceae) in fragments of mixed ombrophilous forests (Figure 1). The immatures were found on the surface of the bark under a silk-like webbing. A portion of the trunk containing the fungi and immatures was transferred to a plastic container and covered with a fine mesh for observation in the laboratory. The material was kept moist during the development of the larvae and pupae. *Neoempheria plaumanni* was collected in the municipality of Ponta Grossa in the Itaiacoca district and *N. spinosa* was retrieved from the municipality of Carambeí. One larva of each species was fixed in 80% alcohol, the others kept alive until the adults emerged. All material is housed at the Museu de Zoologia da Universidade de São Paulo (MZUSP) entomological collection.

The fixed larvae, adult terminalia and wings were dissected for each species. The terminalia were dehydrated in 80% alcohol for 10 min and subsequently were placed in a solution of KOH on a hot plate at a temperature of 40°C for 20–40 min, followed by neutralization in 10% acetic acid and preservation in glycerin. The wings were dehydrated and slide-mounted with Canada Balsam. Photographs were taken with an MC170 HD camera (Leica, Wetzlar, Germany) coupled to an M205 C stereomicroscope (Leica) using the LAS 4.8.0 software (Leica Application Suite). The material prepared on permanent slides was photographed using an epifluorescence microscope (BX41, Olympus, Tokyo, Japan) with a digital camera (CCD DP71, Olympus) using DP controller software (Olympus). The vectorized illustrations were prepared using the Adobe Illustrator CC program. The adult specimens were identified by comparing the morphological characters with those described in the literature. The adult morphological terminology follows that of Søli (2017). The larvae terminology follows that of Oliveira et al. (2015). The map of the geographic distribution of the species was prepared using ArcGIS 10.1 (ESRI 2012).

Abbreviations

Adults. a spr anterior spiracle; **anepst** anepisternum; **C** costal vein; **cerc** cercus; **cerc 1** cercus one of female terminalia; **cerc 2** cercus two of female terminalia; **CuA** anterior branch of cubital vein; **CuP** posterior branch of cubital vein; **cx** coxa; **ddp** dorsal distal projection; **fal vn** false vein; **gen fk** genital fork; **goncx apod** gonocoxal apodeme; **goncx** gonocoxite; **gonst** gonostylus; **h** humeral crossvein; **hlt** halter; **hyp vlv** hypogynial valve; **kepst** katepisternum; **lbl** labellum; **ltg** laterotergite; $\mathbf{M_1}$ first branch of media; $\mathbf{M_{1+2}}$ medial vein; $\mathbf{M_2}$ second branch of media; $\mathbf{M_4}$ fourth branch of media; **mtepm** metepimeron; **mtg** mediotergite; **plp** palpal segment; **pm** paramere; **prn** pronotum; $\mathbf{R_1}$ anterior branch of radial vein; $\mathbf{R_{2+3}}$ second branch of radius; $\mathbf{R_{4+5}}$ third branch of radius; **r-m** radial-medial crossvein; **Rs** radial sector; **Sc** subcostal vein; **sc-r** subcostal-radial crossvein; **sct** scutum; **sctl** scutellum; **spmth** spermatheca; **st** sternite; **tg** tergite.

Larvae. eyesp eye spot; **lbr** labrum; **lp** lateral plate; **mx plp** maxillary palpus; **mpl** medium plate; **mx** maxilla; **mxp** maxillary plate; **of** occipital foramen; **premd** premandible.

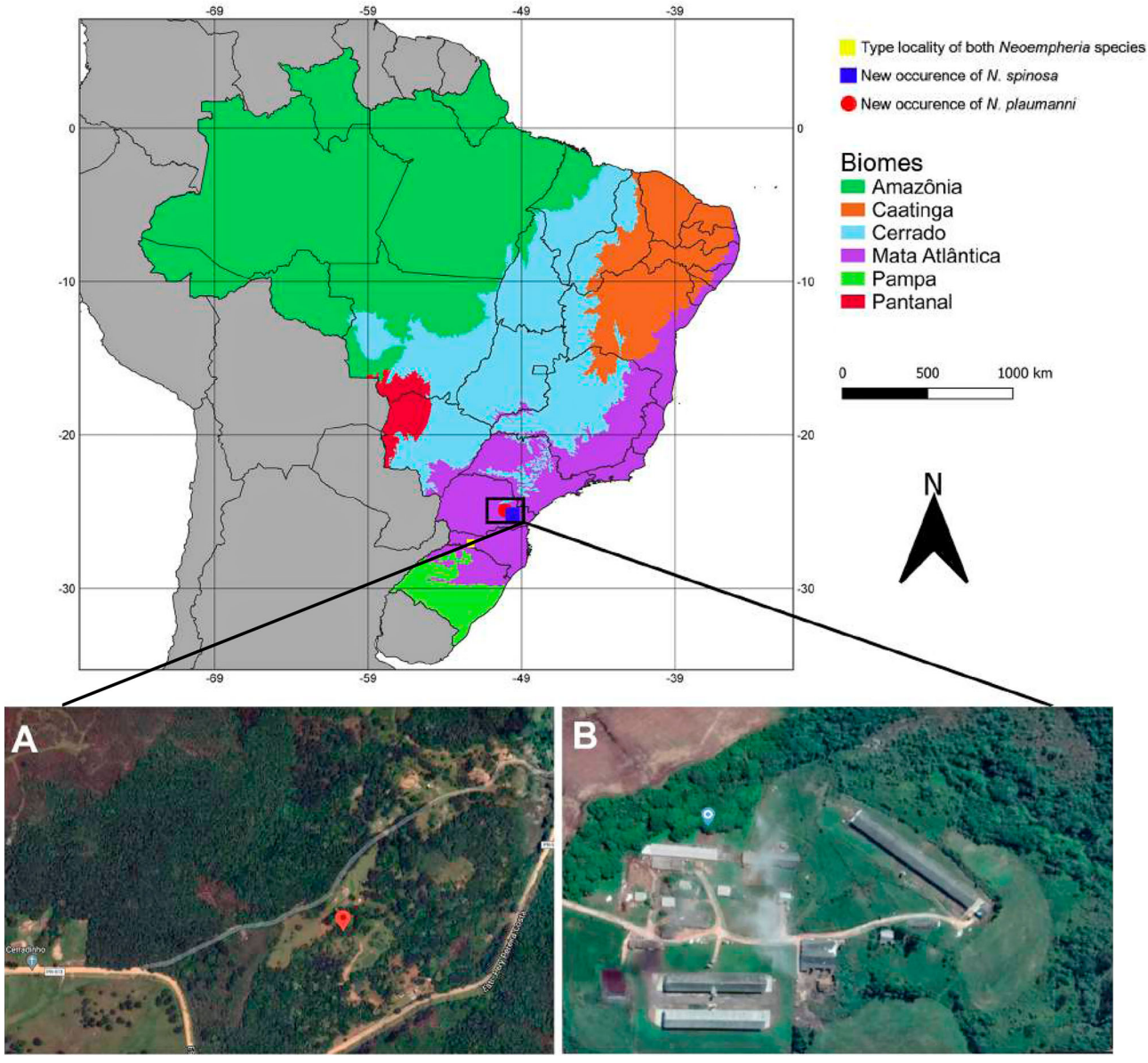


Figure 1. Map of the Brazilian biomes. **A,** Region of the municipality of Ponta Grossa, district of Itaiacoca, state of Paraná, where the immatures were found. **B,** Region of the municipality of Carambeí, state of Paraná, where the immatures were found.

Results

Taxonomy

Neoempheria Osten Sacken, 1878

Syn. *Empheria* Winnertz 1863, p. 783, non Hagen, 1856 (Psocodea). Type-species: *Sciophila striata* Meigen, 1818 (Coquillett 1910, p. 537).

Neoempheria Osten Sacken, 1878, p. 9 (replacement name for *Empheria* Winnertz). Type-species: *Sciophila striata* Meigen, 1818.

Syn. *Pleonazoneura* Enderlein 1910, p. 156. Type-species: *P. johannseni* Enderlein, 1910 (original designation).

Syn. *Neurocompsa* Enderlein 1910, p. 158. Type-species: *N. ornatipennis* Enderlein, 1910 (original designation).

Neoempheria plaumanni Edwards, 1940 (Figures 2–24)

Neoempheria plaumanni Edwards 1940, p. 114, fig. 4a (♂ terminalia), pl. 2, fig. 13 (wing). Type locality: Brazil, Santa Catarina, Seara, Nova Teutônia. Distr.: Brazil (Santa Catarina, São Paulo, Mato Grosso do Sul), Argentina (Salta, Tucumán). Reference: Amorim & Oliveira 2013, p. 68 (comments on types and label data), fig. 192 (habitus). Holotype: ♂, the Natural History Museum, London, UK.

Examined material

1♂, Brazil, Paraná, Ponta Grossa, Distrito de Itaiacoca, collected on 28.XI.2018, emerged in the laboratory on 10.XII.2018, manual collection in rotten wood with fungi,

Figures 2–5. *Neoempheria plaumanni* from Ponta Grossa, Itaiacoca District, state of Paraná. **2,** Male (dissected), habitus, dorsal view. **3,** Male (dissected), habitus, lateral view. **4,** Female (dissected), habitus, dorsal view. **5,** Female (dissected), habitus, lateral view. Scale bar: 2 mm.

25°08′55.34″S 49°53′23.96″W, Almeida M. C. Preserved in alcohol 70%, genitalia in glycerin and wing between two cover slides, in Canada Balsam [MZUSP-MZ053445]; 1♀, Brazil, Paraná, Ponta Grossa, Distrito de Itaiacoca, collected on 28.XI.2018, emerged in the laboratory on 13.XII.2018, manual collection in rotten wood with fungi, 25°08′55″S 49°53′23″W, Almeida M. C. Preserved in alcohol 70%, genitalia in glycerin and wing between two cover slides, in Canada Balsam [MZUSP-MZ053446]; Larvae, Brazil, Paraná, Ponta Grossa, Distrito de Itaiacoca, collected and fixed on 28.XI.2018, manual collection in rotten wood with fungi, 25°08′55″S 49°53′23″W, Almeida M. C. Preserved in slide preparation within Canada Balsam [MZUSP-MZ053447].

Redescription

Male (Figures 2, 3, 7, 9–14). Length: 8,10 mm.

Head (Figures 2, 3). Vertex brownish, with scattered setae, yellowish around eyes. Two ocelli medially on blackish occiput. Frons light brown. Face and clypeus yellowish, covered with setulae. Labellum yellowish, ventrally darker; maxillary palpi brownish, apical segment lighter, segments 3–5 of similar length, first two segments short. Scape and pedicel yellow, rounded, more setose anteriorly, longer setae posteriorly; flagellum yellowish, antennae shorter than thorax, flagellomeres slightly longer than wide.

Thorax (Figure 6). Prosternum yellowish. Pronotum yellow, with strong, long, black bristles. Propleuron yellowish, bare. Anepisternum yellowish with a clear brownish spot, bare. Katepisternum yellowish, with a clear brownish spot, bare. Anepimeron yellow, posterior margin brownish, bare. Laterotergite mostly yellow, anterodorsal margin brownish, bare. Mediotergite yellow ventrally, a brownish mark dorsally, bare. Metanepisternum, metepimeron and metakatepisternum yellow, entirely bare. Scutum yellow, with five brown stripes fusing posteriorly, covered with short and long setae, a pair of stronger dorsocentral, and a pair of stronger dorsolateral setae posteriorly. Scutellum yellow, with a pair of long scutellar bristles and a few scattered setulae. Legs yellow;

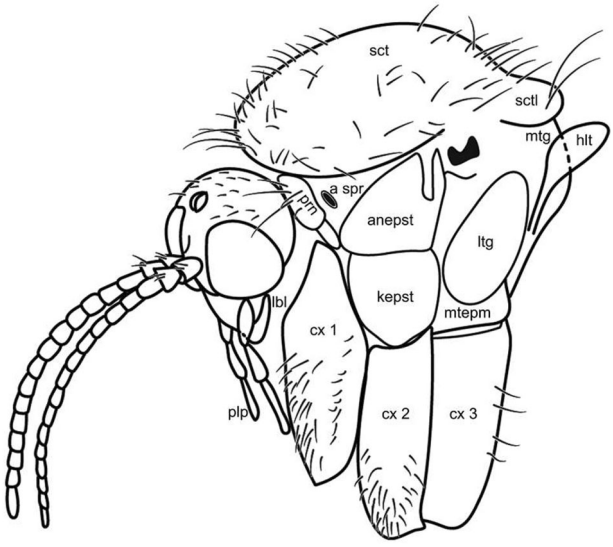


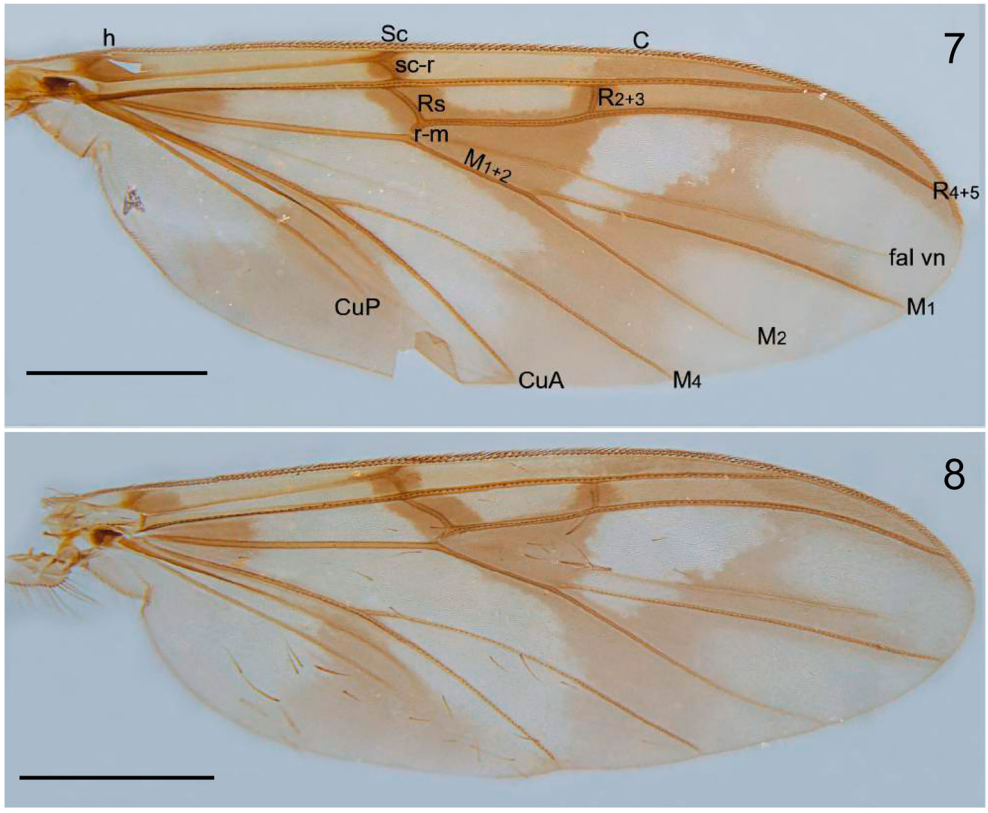
Figure 6. *Neoempheria plaumanni* from Ponta Grossa, district of Itaiacoca, state of Paraná, thorax illustration, lateral view. Abbreviations: **anepst**, anepisternum; **a spr**, anterior spiracle; **cx**, coxa; **hlt**, halter; **lbl**, labellum; **ltg**, laterotergite; **mtepm**, metepimeron; **mtg**, mediotergite; **plp**, palpal segment; **prn**, pronotum; **kepst**, katepisternum; **sct**, scutum; **sctl**, scutellum.

forecoxae with some brownish maculae anteriorly, with strong setae in a line on its posterior and ventral margins; tibial setae regularly aligned; tibial spur I almost twice length of tibial diameter at apex, tibial spurs II and III almost four times the length of tibial diameter at apex. Halter stem whitish, knob yellowish with brownish borders, setose.

Wings (Figure 7). Wing venation and color pattern as in Figure 7.

Abdomen (Figures 2, 3). Tergites and sternites mostly yellow; tg 1–tg 2 with one spot on dorsal view; tg 3, tg 5, tg 6 with spots on the sides in dorsal view extending towards the median region converging in a triangular shape; tg 4 with one spot in dorsal view and one on each side in lateral view; tg 7 with brown spots in dorsal view. st 2, st 4 and st 5 yellowish with lateral bands at the margins.

Terminalia (Figure 9–14). Yellow, except for long and brown gonostyle. tg 9 weakly developed and sclerotized, with a few setae distally. Gonocoxites with large dorsal projection as long as the apex of gonostylus, densely covered with setae on the external face, and basal region covered with microtrichia internally. Gonocoxite with a



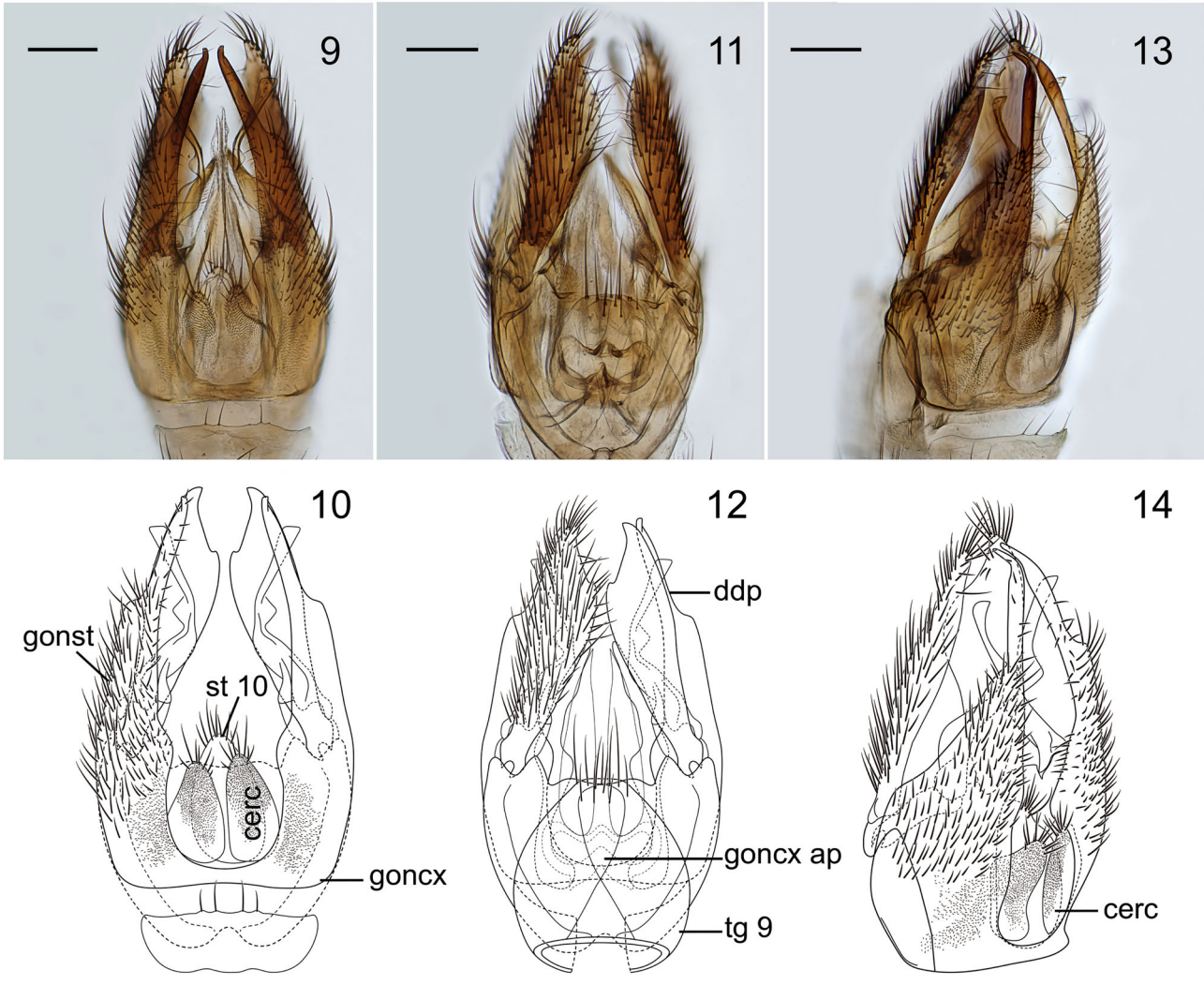
Figures 7, 8. *Neoempheria plaumanni* from Ponta Grossa, district of Itaiacoca, state of Paraná. **7,** Male wing. **8,** Female wing. Abbreviations: **C**, costal vein; **CuA**, anterior branch of cubital vein; **CuP**, posterior branch of cubital vein; **fal vn**, false vein; **h**, humeral crossvein; $\mathbf{M_1}$, first branch of media; $\mathbf{M_2}$, second branch of media; $\mathbf{M_4}$, fourth branch of media; $\mathbf{M_{1+2}}$, medial vein; $\mathbf{R_1}$, anterior branch of radial vein; $\mathbf{R_{2+3}}$, second branch of radius; $\mathbf{R_{4+5}}$, third branch of radius; **r-m**, radial-medial crossvein; **Rs**, radial sector; **Sc**, subcostal vein; **sc-r**, subcostal-radial crossvein. Scale bar: 1 mm.

dorsal distal projection, fingerlike, setose, and well sclerotized. Gonostylus well developed, deeply bifid, inner branch curved and bare, external branch strongly setose. Gonocoxal apodeme short, sclerotized; parameres strongly developed, with a dorsal, membranous, bare projection; cercus and st 10 rounded, with some small setae distally, covered with microsetae.

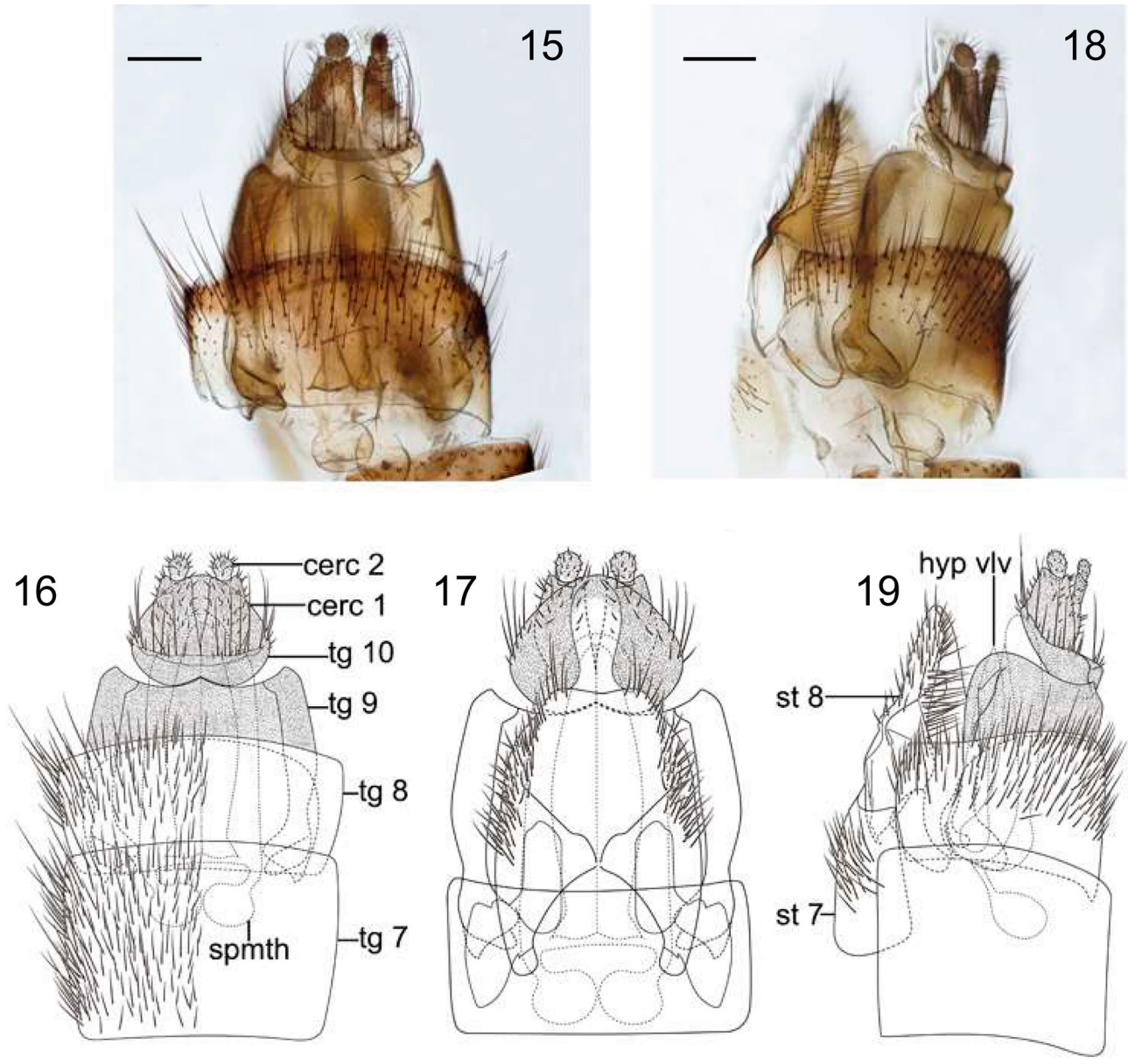
Female (Figures 4–6, 8, 15–19). Length: 8 mm. Similar to male.

Terminalia (Figures 15–19). Yellow. st 8 covered with setae, inner margin slightly concave, with a pair of spine-like setae; tg 8 covered with microtrichia, bare of setae; genital fork (hypogynal valve) well developed, reaching segment 7 anteriorly; cercus short, apical cercomere rounded, ~1/4 the length of basal cercomere.

Mature larva [probably fourth instar] (Figures 20–24). Length: 12.3 mm. General body shape cylindrical, no projections, creamy white in color, whiter in prepupal stage, 12 apparent segments, segments 4–8 wider and longer than the others. Head capsule relatively well sclerotized, bare, triangular (anterior end slightly more slender than posterior end, as in the larvae of other mycetophilid genera, e.g. *Brachypeza* Winnertz, 1863; see Madwar 1937), and at least partially retractable into the first segment. The separation between dorsal plates of the head capsule is not evident, medial plate extending almost to the posterior capsule margin. Eyes posterolateral to the antennae. Occipital foramen ventrally triangular, at approximately distal fourth of head capsule. Mouthparts occupying the anterior third of the head capsule. Labrum wide, fleshy. Premandibles with a row of elongated,



Figures 9–14. *Neoempheria plaumanni* from Ponta Grossa, district of Itaiacoca, state of Paraná. **9,** Dorsal view of the male terminalia. **10,** Illustration of the dorsal view of the male terminalia. **11,** Ventral view of the male terminalia. **12,** Illustration of the ventral view of the male terminalia. **13,** Lateral view of the male terminalia. **14,** Illustration of the lateral view of the male terminalia. Scale bar: 0.2 mm. Abbreviations: **cerc**, cercus; **ddp**, dorsal distal projection; **goncx apod**, gonocoxal apodeme; **goncx**, gonocoxite; **gonst**, gonostylus; **pm**, paramere; **st**, sternite; **tg**, tergite.

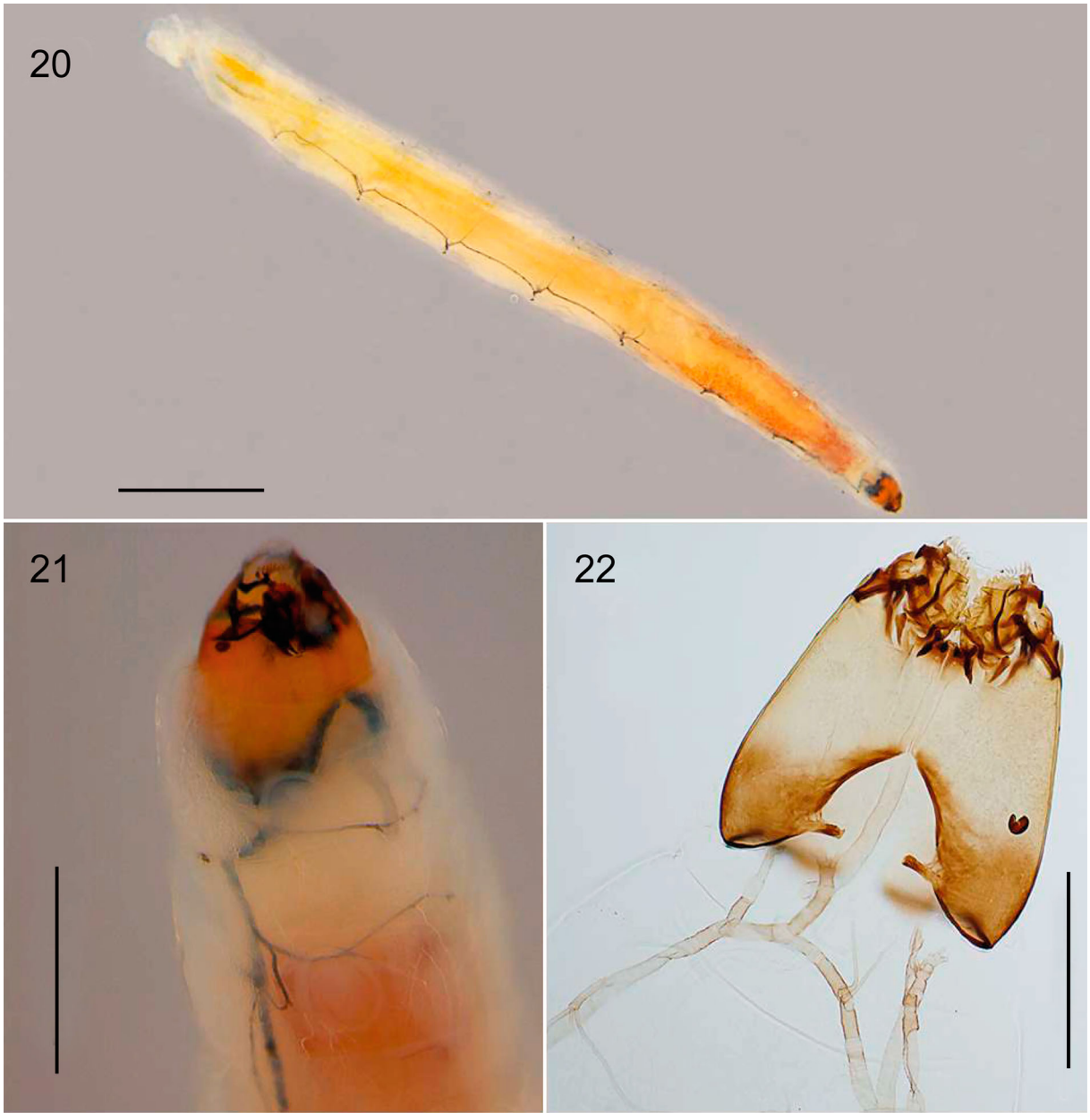


Figures 15–19. *Neoempheria plaumanni* from Ponta Grossa, district of Itaiacoca, state of Paraná. **15,** Dorsal view of the female terminalia. **16,** Illustration of the dorsal view of the female terminalia. **17,** Illustration of the ventral view of the female terminalia. **18,** Lateral view of the female terminalia. **19,** Illustration of the lateral view of the female terminalia. Scale bar: 0.2 mm. Abbreviations: **cerc 1**, cercus one of female terminalia; **cerc 2**, cercus two of female terminalia; **hyp vlv**, hypogynial valve; **spmth**, spermatheca; **st**, sternite; **tg**, tergite.

flexible teeth, supported by pair of lateral chitinous arms. Mandibles semicircular, bearing two rows of medially directed teeth as in other Mycomyinae species (Krivosheina & Zaitzev 2008). Maxillae rounded, medially directed, edge-bearing row of medially directed teeth. One pair of prothoracic, and seven pairs of abdominal, lateral spiracles; prothoracic spiracles only slightly larger than abdominal spiracles. Spiracles on short, scale-like sclerite with two small openings. Intersegmental areas with creeping welts (fleshy lobes slightly elongated across the body bearing rows of denticles). Each creeping welt includes part of an anterior and a posterior segment, anterior portion bears fewer, short rows of sparse denticles, posterior part bears more rows of dense denticles. Posterior end of abdomen with fleshy lobe folded ventrally.

Distribution

Brazil (States of Paraná, São Paulo, Santa Catarina). Oliveira et al. (2017) corrected a mistake made by Oliveira & Amorim (2014) regarding the distribution of this species and mentioned Papavero (1978) as the original reference for *N. plaumanni* distribution. However, Papavero did not consider Coher's work (1959), and São

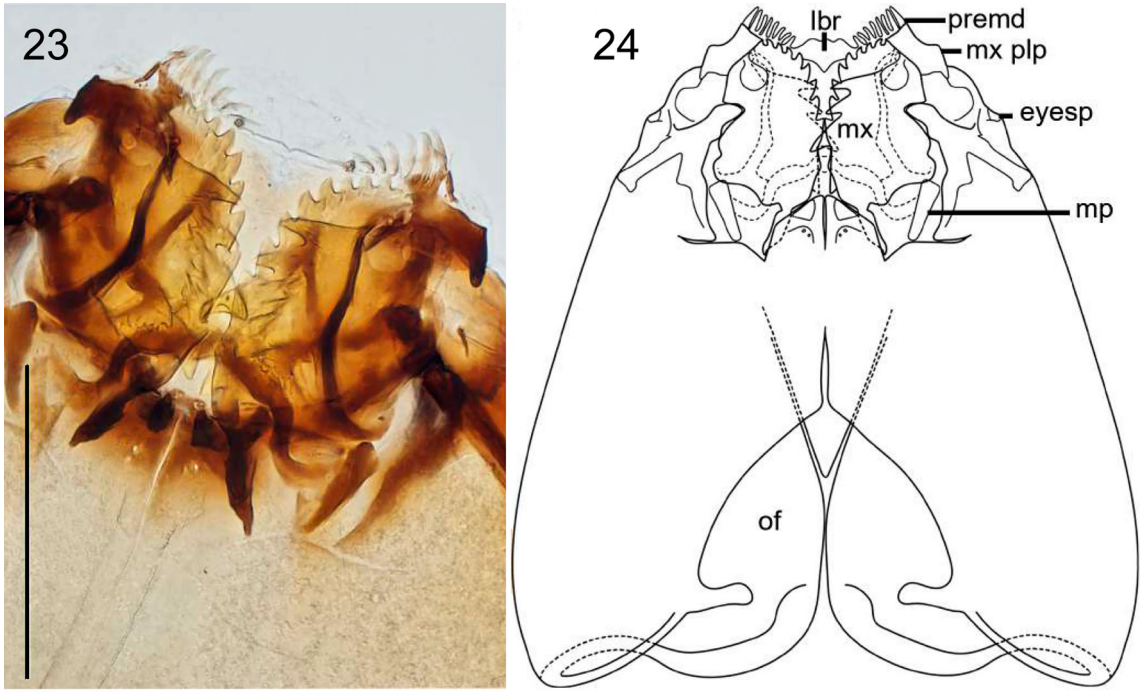


Figures 20–22. *Neoempheria plaumanni* from Ponta Grossa, district of Itaiacoca, state of Paraná. **20,** Dorsolateral view of the larva (scale bar: 2 mm). **21,** Lateroventral view of the head (scale bar: 0.5 mm). **22,** Dorsal view of the head capsule (scale bar: 0.2 mm).

Paulo state was not included as a distributional range for the species. Hence, the distributional pattern herein presented, following Edwards (1940) and Coher (1959), is updated and should be taken as correct for this species.

Comments

Edwards (1940, p. 115) mentions a female specimen named "*N. plaumanni* var. ?" which "resembles *N. plaumanni* in most aspects, including the presence of a dark streak on the outer side of hind coxa, but differs from that species and the other two described below in that the wings (Pl. II, fig. 14) have no dark areas in base of cell R_5 and immediately below Cu.". Amorim & Oliveira (2013, p. 68) did not find the specimen mentioned by Edwards in the Natural History Museum collection; only the slide containing the wing is preserved. As the specimen is lost, this is not a taxonomic problem, as noted by Amorim & Oliveira (2013). The specimens herein analyzed fit the description of *N. plaumanni* according to Edwards (1940) and were formally redescribed and illustrated, and we have no doubts regarding its taxonomic position. The general morphology of the genitalia of this species, highlighting the bifid gonostylus, resembles



Figures 23, 24. *Neoempheria plaumanni* from Ponta Grossa, district of Itaiacoca, state of Paraná, larval stage. **23,** Ventral view of the head capsule. **24,** Illustration of the ventral view of the head. Scale bar: 0.1 mm. Abbreviations: **eyesp**, eye spot; **lbr**, labrum; **lp**, lateral plate; **mx plp**, maxillary palpus; **mpl**, medium plate; **mx**, maxilla; **mxp**, maxillary plate; **of**, occipital foramen; **premd**, premandible.

N. puncticoxa, which also belongs to the species group D or *maculipennis*-group. *N. puncticoxa* was recently redescribed and is the first Neotropical species with life cycle known (see images in Oliveira et al. 2015).

***Neoempheria spinosa* Edwards, 1940 (Figures 25–44)**

Neoempheria spinosa Edwards 1940, p. 111, fig. 2 (♂ terminalia), pl. 1, fig. 7 (wing). Type-locality: Brazil, Santa Catarina, Seara, Nova Teutônia. Distr.: Brazil (Santa Catarina). Reference: Amorim & Oliveira 2013, p. 70 (comments on types and label data), fig. 198 (habitus). Holotype: ♂, the Natural History Museum, London, UK.

Examined material

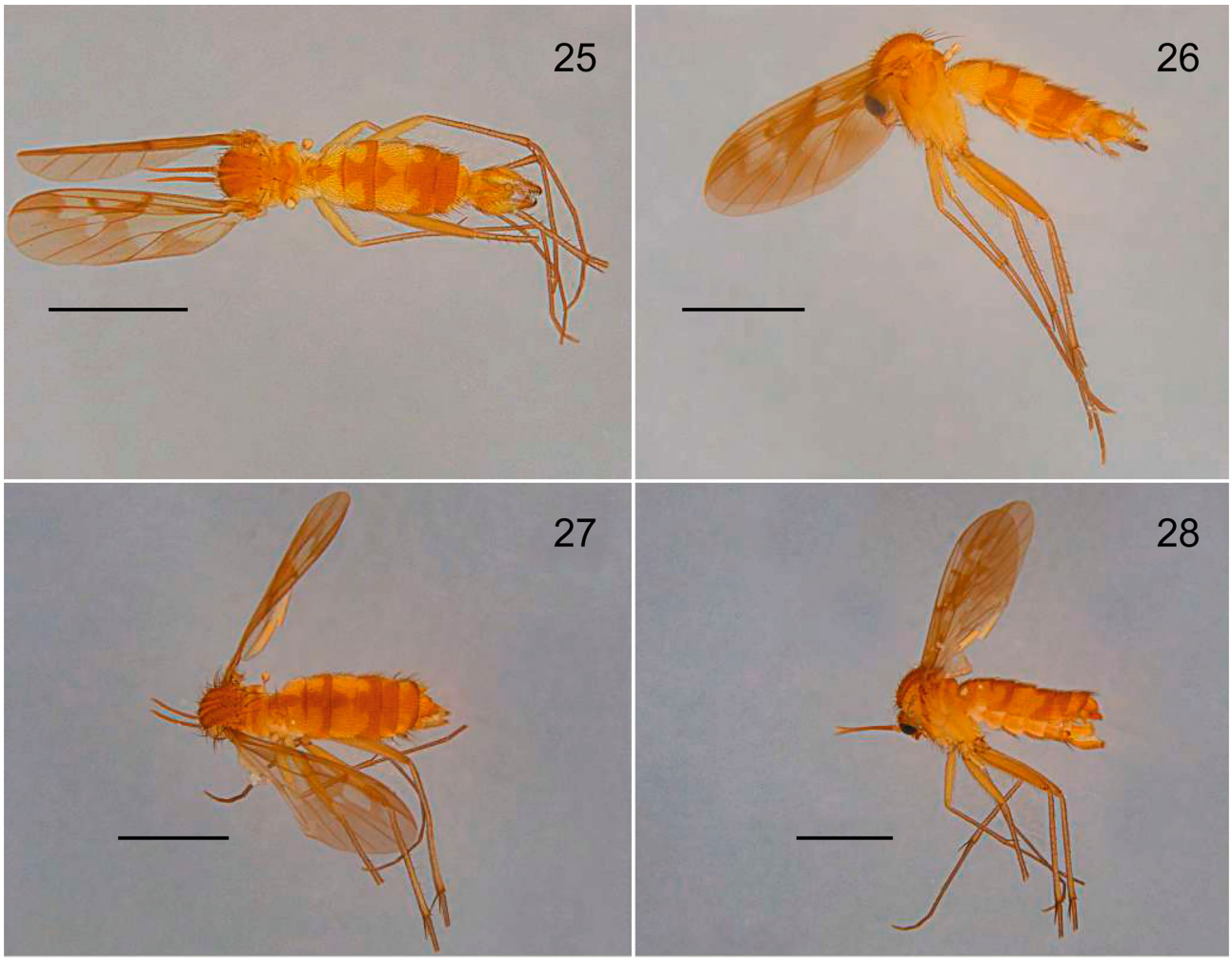
1♂, Brazil, Paraná, Carambeí, collected on 12.I.2019, emerged in the laboratory on 19.I.2019, manual collection in wood with fungi, 24°54′57,6″S 50°05′24″W, Amaral E. M. Preserved in alcohol 70%, genitalia in glycerin and wing between two cover slides, in Canada Balsam [MZUSP-MZ053448]; 2♂, same data as previous male, except: emerged in the laboratory on 23.I.2019. Preserved in slide preparation within Canada Balsam [MZUSP-MZ053449]; 2♂, same data as previous male, except: emerged in the laboratory on 26.I.2019. Preserved in alcohol 70% [MZUSP-MZ053450]; 1♀, same data as male, except: emerged in the laboratory on 27.I.2019. Preserved in alcohol 70%, genitalia in glycerin and wing between two cover slides, in Canada Balsam [MZUSP-MZ053451]; 1♀, same data as male, except: emerged in the laboratory on 22.I.2019. Preserved in slide preparation within Canada Balsam, genitalia in glycerin [MZUSP-MZ053452]; 1♂, same data as previous female. Preserved in slide preparation within Canada Balsam [MZUSP-MZ053453]; 2 larvae, Brazil, Paraná, Carambeí, collected and fixed on 16.I.2019, manual collection in wood with fungi, 24°54′57,6″S 50°05′24″W, Amaral E. M. Preserved in glycerin [MZUSP-MZ053454]; 1 larva, Brazil, Paraná, Carambeí, collected and fixed on 20.I.2019, manual collection in wood with fungi, 24° 54′57,6″S 50°05′24″W, Amaral E. M. Preserved in slide preparation with Canada Balsam [MZUSP-MZ053455].

Redescription

Male (Figures 25, 26, 29, 31–34). Length: 8,10 mm.

Head (Figures 25, 26). Vertex brownish, with scattered setae, yellowish around eyes. Two ocelli medially on blackish occiput. Frons light brown. Face and clypeus yellowish, covered with setulae. Labellum yellowish, ventrally darker; maxillary palpi brownish, apical segment lighter, segments 3–5 of similar length, first two segments short. Scape and pedicel yellow, rounded, more setose anteriorly, setae longer posteriorly; flagellum brownish, antennae shorter than thorax, flagellomeres slightly longer than wide.

Thorax. Prosternum yellowish. Pronotum yellow, with strong, long, black bristles. Propleuron yellow and



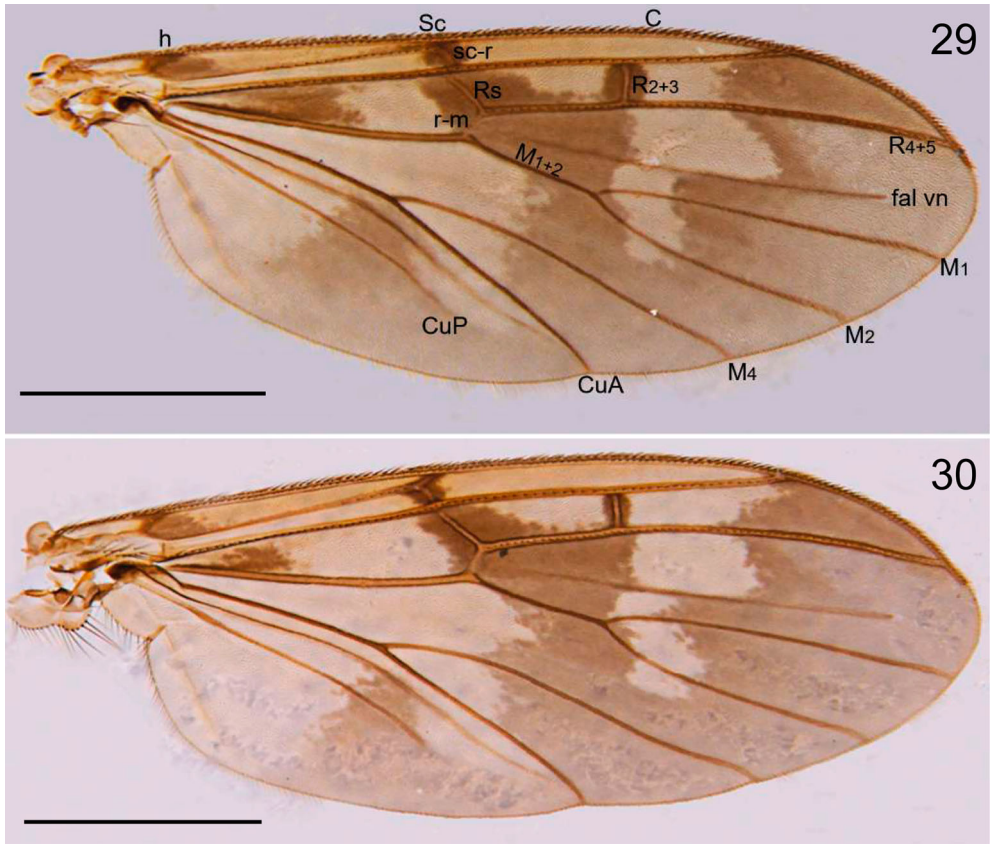
Figures 25–28. *Neoempheria spinosa* from Carambeí, state of Paraná. **25,** Male, habitus, dorsal view. **26,** Male, habitus, lateral view. **27,** Female, habitus, dorsal view. **28,** Female, habitus, lateral view. Scale bar: 2 mm.

bare. Anepisternum and katepisternum both yellow with a light brownish spot, bare. Anepimeron yellow, posterior margin brownish, bare. Laterotergite mostly yellow, anterodorsal margin brownish, bare. Mediotergite yellow ventrally, a brownish mark dorsally, bare. Metanepisternum, metepimeron, and metakatepisternum yellow, entirely bare. Scutum yellow, with five brown stripes fusing posteriorly, covered with short and long setae, a pair of stronger dorsocentral and a pair of stronger dorsolateral setae posteriorly. Scutellum yellow, with a pair of long scutellar bristles and a few scattered setulae. Legs yellow; forecoxae with some brownish maculae anteriorly, with strong setae in a line on posterior and ventral margins; tibial setae regularly aligned; tibial spur I almost twice the length of tibial diameter at apex, tibial spurs II and III almost four times the length of tibial diameter at apex. Halter stem whitish, knob yellowish with brownish borders, setose.

Wings (Figure 29). Wing venation and color pattern as in Figure 29.

Abdomen (Figures 25, 26). Tergites and sternites mostly yellow; tg 1 with two spots in dorsal view converging in a triangle; tg 2 and tg 4 yellowish with a mesal triangular spot on the basal portion of the tergite in dorsal view; tg 3 and tg 5 with triangular spots on the sides in dorsal view extending towards the median region; tg 6 entirely brownish; tg 7 entirely yellowish. Sternites yellowish with lateral bands at the margins.

Terminalia (Figures 31–34). Yellow; tg 9 weakly developed and sclerotized, with a few setae distally. Gonocoxites with a bifid distal dorsal projection, the more external large and well sclerotized, extending beyond apex of gonostylus, densely covered with setae on the external face and 11–12 inner spines, beside an inner projection, finger-like, half the length of the external projection, covered with setulae. Gonostylus well developed and sclerotized, with two main elongated projections, both curved medially; external part of one more densely setose, and an inner branch wider and bare, with a spun-like apex. Gonocoxal apodeme short, sclerotized;



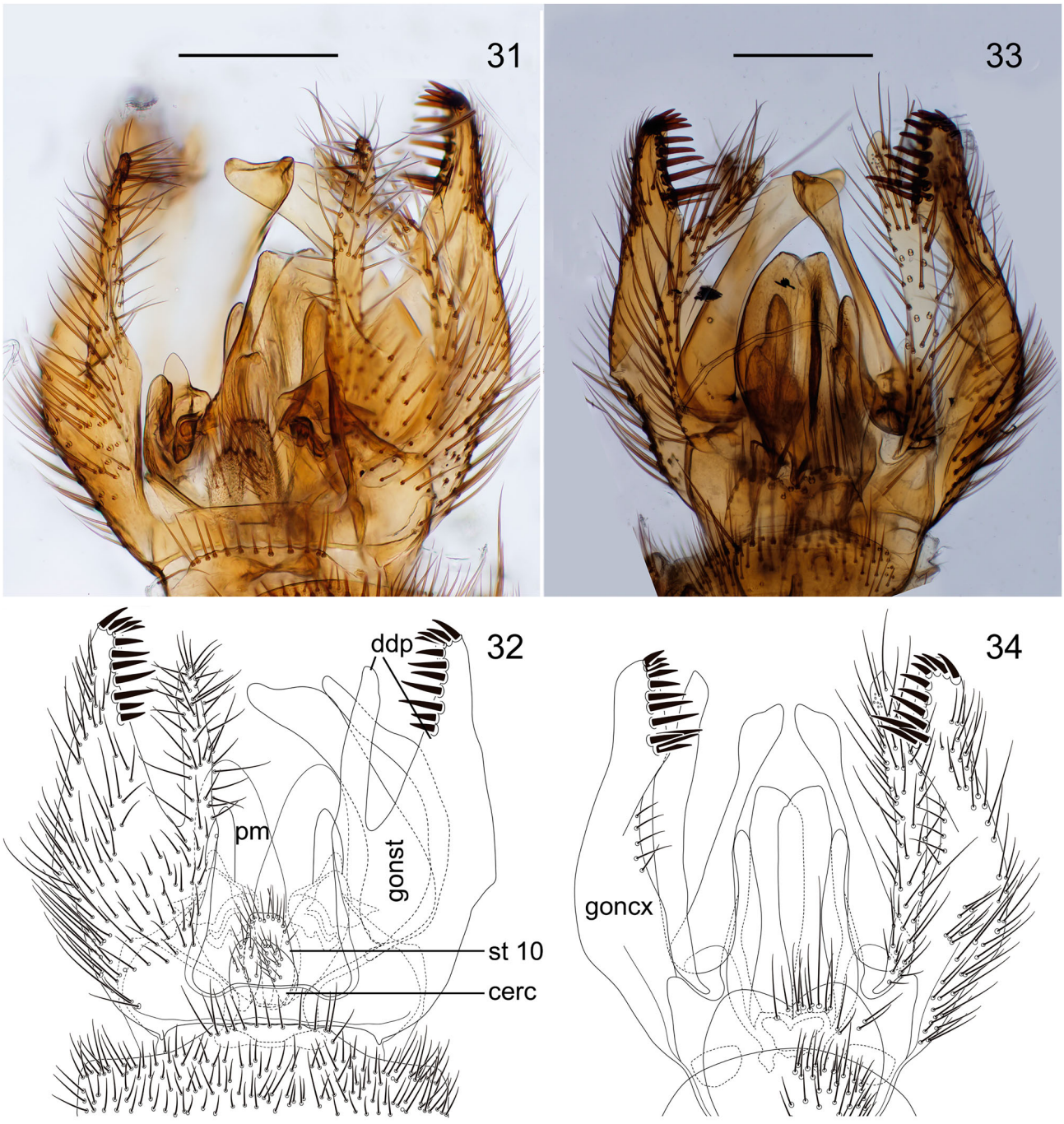
Figures 29, 30. *Neoempheria spinosa* from Carambeí, state of Paraná. **29,** Male wing. **30,** Female wing. Scale bar: 1 mm. Abbreviations: **C**, costal vein; **CuA**, anterior branch of cubital vein; **CuP**, posterior branch of cubital vein; **fal vn**, false vein; **h**, humeral crossvein; $\mathbf{M_1}$, first branch of media; $\mathbf{M_2}$, second branch of media; $\mathbf{M_4}$, fourth branch of media; $\mathbf{M_{1+2}}$, medial vein; $\mathbf{R_1}$, anterior branch of radial vein; $\mathbf{R_{2+3}}$, second branch of radius; $\mathbf{R_{4+5}}$, third branch of radius; **r-m**, radial-medial crossvein; **Rs**, radial sector; **Sc**, subcostal vein; **sc-r**, subcostal-radial crossvein.

parameres strongly developed, rounded, membranous, bare; cercus and st 10 well developed, rounded, with some small setae distally, covered by microsetae.

Female (Figures 27, 28, 30, 35–40). Length: 8 mm. Similar to male.

Terminalia (Figures 35–40). Yellow. st 8 covered with setae, inner margin slightly concave; tg 8 covered with microtrichia, bare of setae; st 7 with small lateral expansion rounded, with brownish spot, scattered setae; cercus short, apical cercomere rounded, ~1/4 the length of basal cercomere.

Mature larva [Probably fourth instar] (Figures 41–44). Length: 12.3 mm. General body shape cylindrical, no projections, creamy white in color, whiter in prepupal stage, 12 apparent segments, segments 4–8 wider and longer than the others. Head capsule relatively well sclerotized, bare, subrectangular (anterior end slightly more slender than posterior end, as in the larvae of other mycetophilid genera, e.g. *Brachypeza* Winnertz, 1863; see Madwar 1937), and at least partially retractable into the first segment. The separation between dorsal plates of head capsule not clearly evident, medial plate extending almost to the posterior capsule margin. Eyes posterolateral to the antennae. Occipital foramen ventrally triangular, at approximately the distal fourth of the head capsule. Mouthparts occupying the anterior third of the head capsule. Labrum well developed and sclerotized. Premandibles present but not visualized in detail. Mandibles with a plaque resembling a comb with many small teeth as seen in other Mycomyinae species (Krivosheina & Zaitzev 2008). Maxillae medially directed, edge bearing a row of medially directed cuspidate teeth. One pair of prothoracic, and seven pairs of abdominal, lateral spiracles; prothoracic spiracles only slightly larger than abdominal ones. Spiracles on short, scale-like sclerite with a couple of small openings. Intersegmental areas with creeping welts (fleshy lobes slightly elongated across the body bearing rows of denticles). Each creeping welt includes part of an anterior and a posterior segment, the anterior portion bears fewer, short rows of sparse denticles, the posterior part bears



Figures 31–34. *Neoempheria spinosa* from Carambeí, state of Paraná. **31,** Dorsal view of the male terminalia. **32,** Illustration of the dorsal view of the male terminalia. **33,** Ventral view of the male terminalia. **34,** Illustration of the ventral view of the male terminalia. Scale bar: 0.02 mm. Abbreviations: **cerc**, cercus; **ddp**, dorsal distal projection; **goncx**, gonocoxite; **gonst**, gonostylus; **pm**, paramere; **st**, sternite.

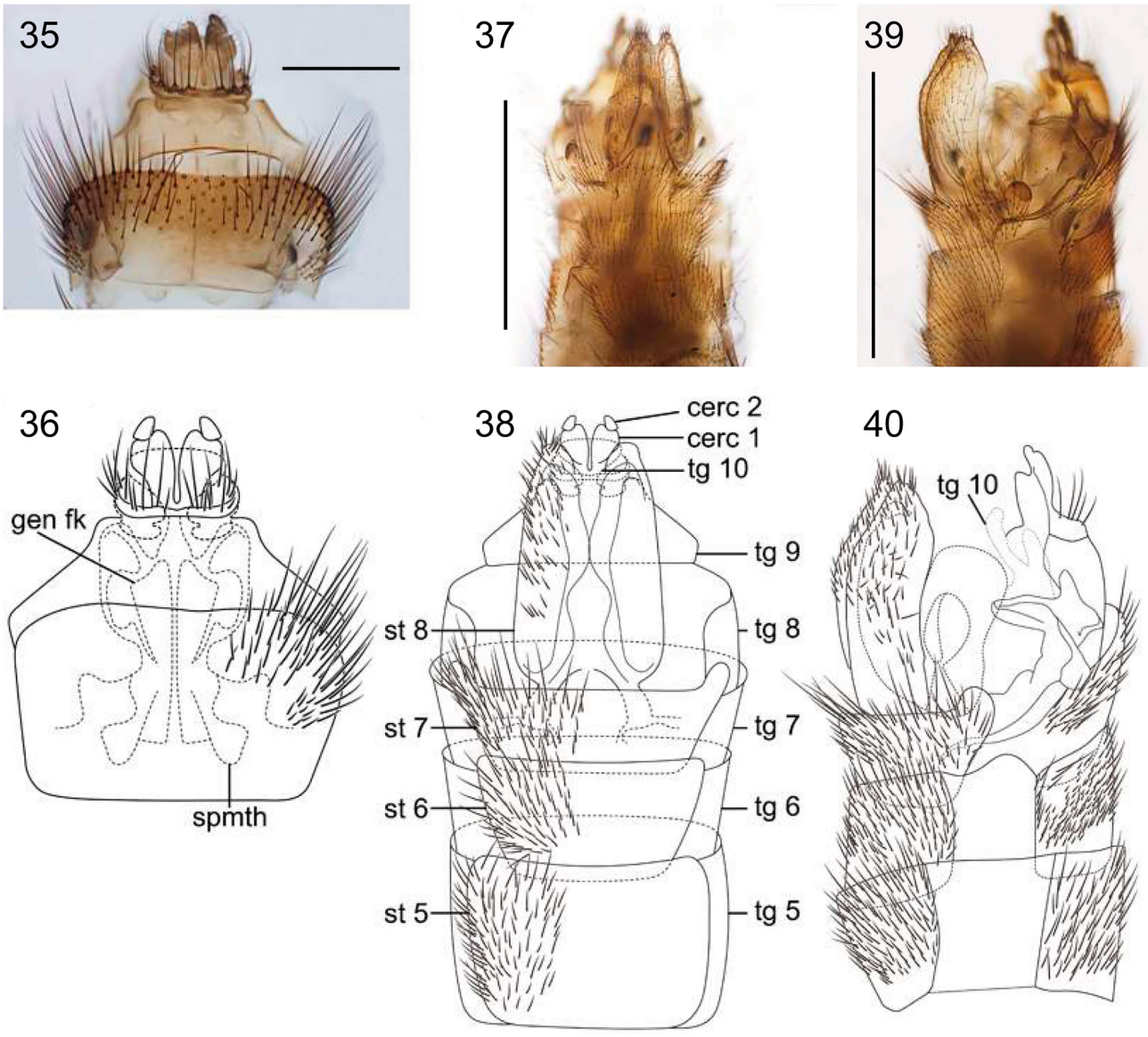
more rows of dense denticles. Posterior end of abdomen with fleshy lobe folded ventrally.

Distribution

Brazil (states of Paraná, Santa Catarina).

Comments

The general morphology of the genitalia of this species, highlighting the bifid gonostylus, resembles *N. bilobata*, which also belongs to the group C or *spinosa*-group. *Neoempheria bilobata* was recently redescribed by Amaral et al. (2022a).



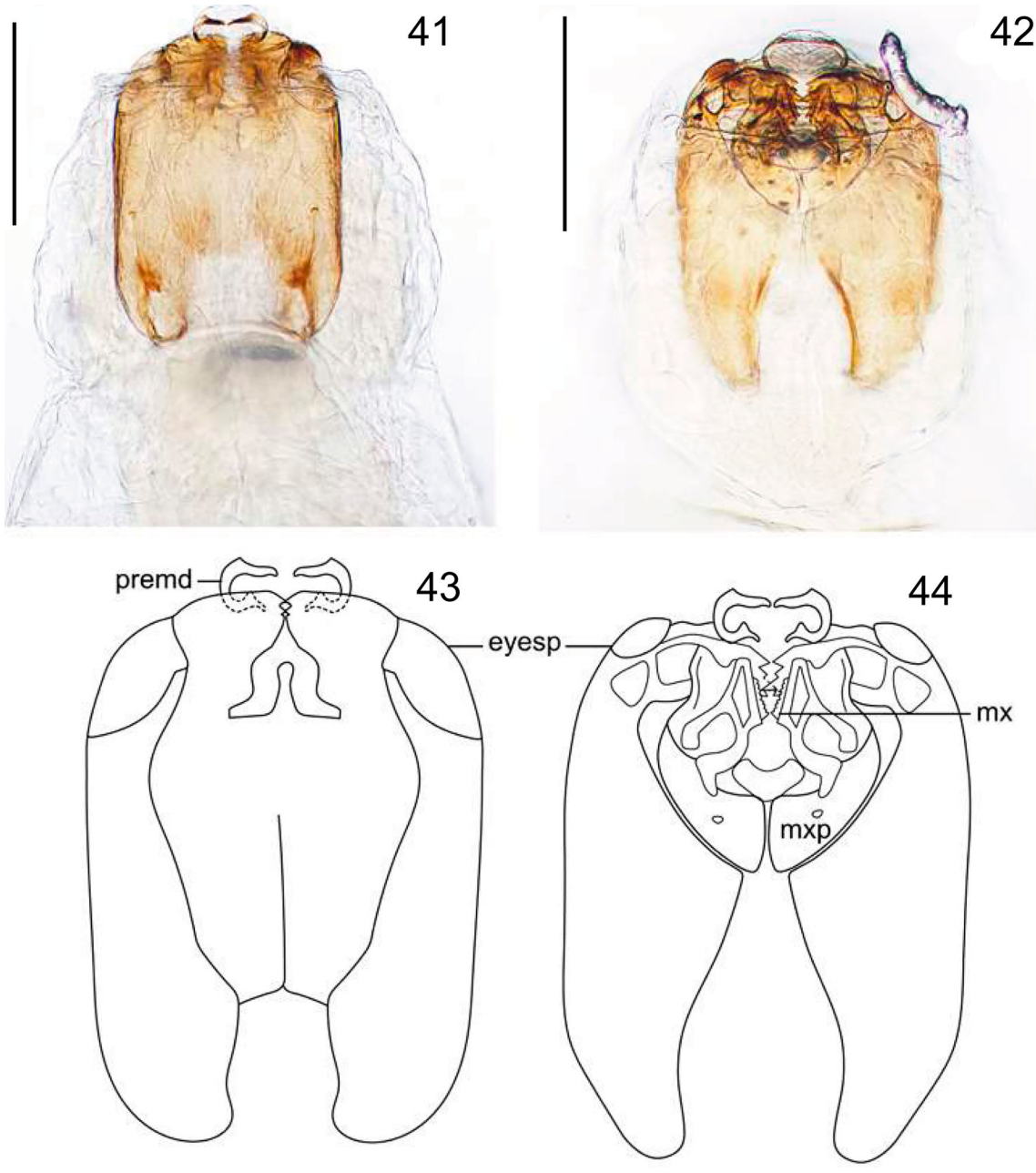
Figures 35–40. *Neoempheria spinosa* from Carambeí, state of Paraná. **35,** Dorsal view of the female terminalia (scale bar: 0.05 mm). **36,** Illustration of the dorsal view of the female terminalia. **37,** Ventral view of the female terminalia (scale bar: 1 mm). **38,** Illustration of the ventral view of the female terminalia. **39,** Lateral view of the female terminalia (**s**cale bar: 1 mm). **40,** Illustration of the lateral view of the female terminalia. Abbreviations: **cerc 1**, cercus one of female terminalia; **cerc 2**, cercus two of female terminalia; **gen fk**, genital fork; **spmth**, spermatheca; **st**, sternite; **tg**, tergite.

Biology observations

Both species exhibit the same development pattern described here. The larvae were found in a fallen tree trunk in an open area of a small rural property. This area had some livestock and a small plantation field surrounded by a fragment of mixed ombrophilous forest. The trunk was covered with a species of Basidiomycota fungus, and the immatures were possibly feeding on the spores. The larvae weave slimy-looking webs, which they use to move and hold the pupae. In the pre-pupal stage, the larvae seek a sheltered place in the substrate and weave an irregular web, approaching a loosely entwined cocoon and pupate in the center of this cocoon while hovering over the substrate with the ventral part of the head facing down. The adults emerge after approximately five days.

Male genitalia highlights

In 1940, Edwards conducted a study on the Neotropical fauna of *Neoempheria*, where he noted that in dry specimens, the male terminalia could be rotated, and the presence of the paired cerci and S10 could aid in understanding the dorsal and ventral surface of the



Figures 41–44. *Neoempheria spinosa* from Carambeí, state of Paraná, larva. **41, 42,** Head capsule (scale bar: 0.02 mm): **41,** dorsal view; **42,** ventral view. **43,** Illustration of the dorsal view of the head. **44,** Illustration of the ventral view of the head. Abbreviations: **eyesp**, eye spot; **mx**, maxilla; **mxp**, maxillary plate; **premd**, premandible.

genitalia. In our study of fresh material, we have observed the same phenomenon. Edwards also mentioned a large tergite 9 that was divided into two lobes with long dorsal projections. However, this was a misinterpretation of the male genitalia by Edwards. In our dissection of fresh material, we found that the tg 9 is developed but not significant, and the gonocoxites, on the other hand, are widely projected through the dorsal region of the male genitalia (like a bridge) and bear both dorsal and distal projections, which Edwards mistakenly interpreted as belonging to the tergite 9. The gonocoxites also feature a bifid and articulate gonostyle, and the distal and dorsal projection of the gonocoxite and the gonostyle are in the same plate, as depicted in Figures 9, 11, 13, 31, and 33. This is consistent with the Mycetophilidae male terminalia *bauplan* described by Søli (2017), and is also observed in *N. puncticoxa*, as redescribed by Oliveira et al. (2015), and *N. bilobata*, as redescribed by Amaral et al. (2022a). The presence of an

articulated gonostylus remains the easiest way to recognize the gonocoxite.

Discussion

Herein, we expanded the distribution of *N. spinosa* and *N. plaumanni* to other temperate areas in southern Brazil. In addition to the type locality, Edwards (1940) also described other species of *Neoempheria*, including *N. biflagellata*, *N. enderleini*, *N. lutzi*, *N. muelleri*, *N. neivai*, and *N. simplex*, which have since been documented in areas outside the original locality, and are also distributed throughout São Paulo state; see Oliveira & Amorim (2014) for detailed information.

Oliveira et al. (2015) and Amaral et al. (2022a) recorded species of *Neoempheria* described by Edwards in 1940 in other Brazilian regions. Oliveira et al. (2015) updated the taxonomical, morphological, and distributional information on *N. puncticoxa*, highlighting its presence in the states of Santa Catarina, São Paulo, Mato Grosso, Mato Grosso do Sul, and Goiás, Brazil, and in the provinces of Salta and Tucumán, Argentina. This species is related to *N. plaumanni* and also belongs to the *maculipennis*-group. Amaral et al. (2022a) updated the taxonomical, morphological, and distributional information on *N. bilobata*, highlighting its presence in the states of Santa Catarina and Pará, Brazil. This species is related to *N. spinosa* and also belongs to the *spinosa*-group.

According to Oliveira & Amorim (2014), the species *N. brevicauda*, *N. costalimai*, *N. enderleini*, *N. flavicoxa*, *N. lindneri*, *N. pilosa*, *N. rostrata*, *N. shannoni*, *N. unispinosa*, and *N. subclavata*, all described by Edwards in 1940, from Nova Teutônia, Seara, Brazil, are known only from the type locality. The literature on Neotropical mycetophilids has slowly improved in recent years in terms of the description of new species (e.g. Kurina & Oliveira 2015; Amorim et al. 2018; Kurina et al. 2018; Amaral et al. 2022b; Kurina & Õunap 2023), survey inventories (Amorim et al. 2022; Riccardi et al. 2022), and description of immature stages (Oliveira et al. 2015; Passacq et al. 2017; Amaral et al. 2022a, 2022b). Additional records of these species not related to the type locality and information on life cycles and morphology will probably be available in the future. The morphological groups established by Edwards (1940) and Coher (1959) are becoming better known and illustrated, but a phylogenetic framework is still remaining in the literature as a knowledge gap.

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References

Amaral EA, Oliveira SS, Falaschi RL. 2022a. A hidden Amazonian fauna: first record of *Neoempheria bilobata* Edwards, 1940 (Diptera: Mycetophilidae) in Pará State, Brazil. EntomoBrasilis. 15:e988. doi:10.12741/ebrasilis.v15.e988.

Amaral EA, Oliveira SS, Falaschi RL. 2022b. An unknown world in the Neotropical region: a complete life cycle of a new species of *Monoclona* Mik, 1886 (Diptera: Mycetophilidae: Sciophilinae). Zootaxa. 5091:107–130. doi:10.11646/zootaxa.5091.1.4.

Amorim DS, Brown BV, Boscolo D, Ale-Rocha R, Alvarez-Garcia DM, Balbi MIPA, Barbosa AM, Capellari RS, Carvalho CJB, Couri MS, et al. 2022. Vertical stratification of insect abundance and species richness in an Amazonian tropical forest. Scientific Reports. 12:1734. doi:10.1038/s41598-022-05677-y.

Amorim DS, Oliveira SS. 2013. Types of Neotropical Mycetophilidae. (Diptera) at the Natural History Museum collection, London. Zootaxa. 3726:1–119. doi:10.11646/zootaxa.3726.1.1.

Amorim DS, Oliveira SS, Henao-Sepúlveda AC. 2018. A new species of *Eumanota* Edwards (Diptera: Mycetophilidae: Manotine) from Colombia: evidence for a pseudogondwanan pattern. American Museum Novitates. 3915:1–19.

Coher EI. 1959. A synopsis of American Mycomyiini with description of new species (Diptera: Mycetophilidae). Entomologica Americana, n.s. 37:1–155.

Coquillett DW. 1910. The type-species of the north American genera of Diptera. Proceedings of the United States National Museum. 37:499–647. doi:10.5479/si.00963801.37-1719.499

Edwards FW. 1940. Neotropical *Neoempheria* (Diptera, Mycetophilidae). Novitates Zoologicae. 42:107–129.

Enderlein G. 1910. Neue Gattungen und Arten aussereuropäischen Fliegen. Stettiner entomologische Zeitung. 72:135–209.

Environmental Systems Research Institute (ESRI). 2012. ArcGIS Release 10.1. Redlands, CA. Available from URL: https://www.esri.com/news/arcnews/spring12articles/introducing-arcgis-101.html.

Fitzgerald SJ. 2017. *Phthinia* Winnertz (Diptera: Mycetophilidae): new species and records from the Neotropical and Oriental regions. Zootaxa. 4231:107–113. doi:10.11646/zootaxa.4231.1.7.

Fungus Gnats Online. 2022. Classification of *Neoempheria* [updated 02 Oct 2022; cited 05 Dec 2022]. Available from URL: https://sciaroidea.myspecies.info/taxonomy/45437.

Hagen HA. 1856. Die Odonaten-Fauna des russischen Reichs. Stettiner Entomologische Zeitung. 17:363–381.

Krivosheina NP, Zaitzev AI. 2008. Trophic relationships and main trends in morphological adaptations of larval mouthparts in sciaroid dipterans (Diptera, Sciaroidea). Bulletin of the Russian Academy of Sciences. 35:606–614. doi:10.1134/S1062359008060071.

Kurina O, Hippa H, Amorim DS. 2018. A contribution to the systematics of the genus *Manota* Williston (Diptera: Mycetophilidae) in Brazil. Zootaxa. 4472:1–59. doi:10.11646/zootaxa.4472.1.1.

Kurina O, Oliveira SS. 2015. A new case of an Holarctic element in the Colombian Andes: first record of *Cordyla* Meigen (Diptera, Mycetophilidae) from the Neotropical Region. ZooKeys. 520:87–108. doi:10.3897/zookeys.520.6142.

Kurina O, Õunap E. 2023. Morphological and molecular data reveal fifteen new sympatric *Zygomyia* Winnertz, 1864 (Diptera: Mycetophilidae) species from Ecuador. Zootaxa. 5227:1–49. doi:10.11646/zootaxa.5227.1.1.

Madwar S. 1937. I-Biology and morphology of the immature stages of Mycetophilidae (Diptera, Nematocera). Philosophical Transactions of the Royal Society of London Series B, Biological Sciences. 227:1–110.

Oliveira SS, Albertoni FF, Borkent CJ, Amorim DS. 2015. First record of neoempheria osten sacken (diptera, mycetophilidae) biology in the neotropical region, with associations between its larvae and fungi. Biodiversity Data Journal. 3: e5073. doi:10.3897/BDJ.3.e5073.

Oliveira SS, Amorim DS. 2014. Catalogue of Neotropical Diptera. Mycetophilidae. Neotropical Diptera. 25:1–87.

Oliveira SS, Amorim DS. 2021. Phylogeny, classification, Mesozoic fossils, and biogeography of the Leiinae (Diptera: Mycetophilidae). Bulletin of the American Museum of Natural History. 446:1–108. doi:10.1206/0003-0090.446.1.1

Oliveira SS, Falaschi RL, Urso-Guimarães MV, Amorim DS. 2017. Lista das espécies de Bibionomorpha (Diptera) do Estado do Mato Grosso do Sul, Brasil. Iheringia, Série Zoologia. 107(supl.):e2017127. doi:10.1590/1678-4766e2017127.

Osten Sacken CR. 1878. Catalogue of the described Diptera of North America. (2nd ed.). Smithsonian Miscellaneous Collections. 16:1–276.

Papavero N. 1978. 19E. Family Mycetophilidae. In: Papavero N, editor. A catalogue of the Diptera of the Americas south of the United States. São Paulo: Museu de Zoologia, Universidade de São Paulo; p. 1–78.

Pape T, Blagoderov V, Mostovski MB. 2011. Order DIPTERA Linnaeus, 1758. In: Zhang Z-Q, editor. Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. Zootaxa. 3148, p. 222–229. doi:10.11646/zootaxa.3148.1.42

Passacq P, Omad G, Kerr PH, Pardo C. 2017. First studies on Patagonian immature Mycetophilidae: description of the larva and pupa: redescription and comments on the biology of *Mycomya chilensis*. Revista Mexicana de Biodiversidad. 88:815–819. doi:10.1016/j.rmb.2017.10.022.

Riccardi PR, Fachin DA, Ale-Rocha R, Amaral EM, Amorim DS, Gil-Azevedo LH, Capellari RS, Carmo DDD, de Carvalho CJB, Ferro GB, et al. 2022. Checklist of the dipterofauna (Insecta) from Roraima, Brazil, with special reference to the Brazilian Ecological Station of Maracá. Papéis Avulsos de Zoologia. 62:e202262014. doi:10.11606/1807-0205/2022.62.014.

Søli GEE. 2017. 20. Mycetophilidae (fungus gnats). In: Kirk-Spriggs AH, Sinclair BJ, editor. Manual of Afrotropical Diptera. Vol. 2 Nematocera & Lower Brachycera. Pretoria: SANBI Publishing; p. 533–555.

Winnertz J. 1863. Beitrag zu einer Monographie der Pilzmücken. Verhandlung der Zoologisch-botanischen Gesellschaft in Wien. 13:637–964.