

First Evidence of Indirect Oviposition in Phasiinae (Diptera: Tachinidae) with Insights on *Leucostoma* Meigen, its New Neotropical Distribution, and Host Association

Rodrigo de Vilhena Perez Dios^{1,*} , Talita Roell² , and Graciano Tejada Hurtado³ 

¹Laboratório de Diptera, Museu de Zoologia, Universidade de São Paulo, Av. Nazaré, 481, 04263-000, Ipiranga, São Paulo/SP, Brazil.

*Correspondence: E-mail: rodrigodios@gmail.com (Dios)

²Laboratório de Hemiptera, Museu de Zoologia, Universidade de São Paulo, Av. Nazaré, 481, 04263-000, Ipiranga, São Paulo/SP, Brazil.
E-mail: talitaroell@usp.br (Roell)

³Laboratorio de Entomología, Centro de Diagnóstico de Sanidad Vegetal, Servicio Nacional de Sanidad Agraria, SENASA, Av. La Molina 1981, La Molina. Lima, Perú. E-mail: gracionotejada@yahoo.com (Tejada)

Received 16 July 2024 / Accepted 19 October 2024 / Published 27 December 2024
Communicated by Daniel Stec

Flies of the family Tachinidae are known for being arthropod parasitoids. Phasiinae, the bug-killing flies, is one of its subfamilies, which is specialized in heteropteran hosts (Hemiptera: Heteroptera). Flies of this group are known to oviposit directly on their heteropteran hosts, and many parasitize important agricultural pests, making them relevant for biological control. Among phasiines, the tribe Leucostomatini is easily distinguished by their characteristic female terminalia, which has structures that resemble a pincer. *Leucostoma* Meigen is the largest genera with 28 species, and only 10 being recorded in the Neotropical Region. Herein, we present the first direct evidence of an indirect oviposition strategy in bug-killing flies. We observed females of *Leucostoma acirostre* Reinhard ovipositing directly on the host plant of their hosts. This possibility has already been considered in the literature, and a discussion of this new strategy is presented, as it is relevant for understanding Tachinidae evolution, as well as developing biological control strategies. This is the first record of *L. acirostre* for the Neotropical Region, as well as a new host record for the rhopalid *Harmostes parafraterculus* Göllner-Scheiding. Alongside, *Leucostoma aterrimum* (Villers) specimens were collected from *Liorhysus hyalinus* (Fabr.) of Peru, an important quinoa pest from the region, and the first record of the species and host association for the country. We update the distribution of both species, with new records for South America. Both species are redescribed, including photographs, new data for the puparium, and male and female terminalia (both illustrated). Lastly, we discuss the validity of the few *Leucostoma* non-heteropteran host records, as they are all probably mistakes, and comment on the widespread distribution of some of the genus' species and host use.

Key words: Biological control, Distribution, Leucostomatini, Oviposition strategy, Taxonomy

BACKGROUND

Among the highly diverse parasitoid flies (Diptera: Tachinidae), the subfamily Phasiinae are known as the bug-killing flies, due to their habit of using different heteropteran insects as hosts (Hemiptera: Heteroptera)

(Dupuis 1963; Blaschke et al. 2018). Some phasiines parasitize important crop-pests, including different *Trichopoda* Berthold species, with potential for biological control strategies; however, many of those still lack basic taxonomical knowledge (Guimarães 1977; Arnaud 1978; Dios and Nihei 2020).

Citation: de Vilhena Perez Dios R, Roell T, Tejada G. 2024. First evidence of indirect oviposition in Phasiinae (Diptera: Tachinidae) with insights on *Leucostoma* Meigen, its new neotropical distribution, and host association. Zool Stud 63:54. doi:10.6620/ZS.2024.63-54.

Leucostomatini is a peculiar group among the bug-killing flies that are easily distinguished by their remarkable female terminalia morphology. Many females of this tribe have modified parts of the terminalia which act as pincers and supposedly function to grasp the host (Dupuis 1963). Leucostomatini are usually small to medium-sized flies, with dark or grey coloration, contrary to many other phasiines that have striking coloration and/or hymenopteran mimicry (some exceptions in Leucostomatini are observed in species of *Brullaea* Robineau-Desvoidy and *Clairvillia* Robineau-Desvoidy) (Dupuis 1963; Blaschke et al. 2018; Dios and Nihei in prep). As the vast majority of Phasiinae, all Leucostomatini host records are from different families of Heteroptera (Hemiptera): Alydidae, Coreidae, Lygaeidae, Miridae, Nabidae, Pentatomidae, Rhopalidae, and Stenocephalidae (Arnaud 1978; Guimarães 1977; Tschorsnig 2017).

Leucostoma Meigen is the most diverse Leucostomatini genera, with 28 species. Curiously, some species, such as *Leucostoma aterrimum* (Villers) and *L. simplex* (Fallén) (O'Hara et al. 2020) are widespread globally and probably follow some of their host distribution. Despite their diversity, ten species have been recorded for the Neotropical Region, and only three species have been recorded from Brazil (O'Hara et al. 2020; Nihei et al. 2024). The same scarcity is observed for Neotropical host records, with only three for South America: *Epicauta pilme* Molina (Meloidae: Coleoptera) for *Leucostoma aterrimum*, and *Oncopeltus fasciatus* (Dallas) and *O. unifasciatellus* Slater (Lygaeidae: Hemiptera) for *Leucostoma brasilianum* (Townsend) (Guimarães 1977).

Despite the characteristic pincer, Leucostomatini females also have the 8th sternite modified as a piercer, and their egg morphology points towards an injectable egg, with a membranous chorion and without a respiratory crypt (Pantel 1910; Dupuis 1963; Gaponov 2003). However, no observation on host oviposition has been made, along with no data regarding the oviposition process or how females use those peculiar pincers and short lance to infect hosts.

Here, we present new *Leucostoma* records for Brazil, *L. aciostre* Reinhard from São Paulo, SP, and Petrolina, PE, as well as a new host record for the species: *Harmostes parafraterculus* Göllner-Scheidig (Rhopalidae: Hemiptera). We also present new records of *L. aterrimum* for Brazil, as well as Peru, where it was found parasitizing *Liorhyssus hyalinus* (Fabr.) (Rhopalidae: Hemiptera), an important pest of quinoa crops (*Chenopodium quinoa* Willd.) in the region. Both species are redescribed, with new data on the puparium, along with descriptions and illustrations of the male and female terminalia, complementing previous works

(Reinhard 1956). A brief discussion about the validity of all non-Heteropteran Leucostomatini hosts is presented. We also provide new remarkable observations regarding the oviposition behavior of Leucostomatini species. This is the first record of an indirect oviposition strategy in Phasiinae, which could bring light to different uses for the characteristic female terminalia in Leucostomatini and understanding the evolution of oviposition strategies in Phasiinae.

MATERIALS AND METHODS

All examined material is deposited at the “Museu de Zoologia da Universidade de São Paulo,” São Paulo, SP, Brazil (MZSP) and “Coleção Entomológica do Instituto Oswaldo Cruz”, Rio de Janeiro, Brazil (Jane Costa). The holotype of *L. aciostre*, deposited at the Smithsonian National Museum of Natural History (NMNH), Washington, DC, USA, was examined by the first author. Morphological terminology follows Cumming and Wood (2017) and puparium follows Ziegler (1998).

Photographs of pinned specimens were taken using an AxioCam Mrc 5 camera attached to a Zeiss Discovery V20 stereomicroscope. The images were stacked with the software Helicon Focus 7.5.8, and edited in Adobe Photoshop CS6. Illustrations were made using a camera lucida attached to a Leica MZ16 stereomicroscope, and edited and arranged in Adobe Illustrator CS6. To digest tissues and clear structures, the last abdominal segments were placed in a glass tube containing a 10% KOH solution and heated in water at 70°C for 5 minutes, neutralized in a 5% acetic acid solution, and rinsed in distilled water. After examination, the dissected parts were placed in glycerin inside a plastic microvial pinned with the source specimen.

All the behavioral observations and photos were taken by RVPD in a community garden in Santo Amaro's neighborhood in São Paulo, SP, Brazil, using a smartphone Samsung M31 with an attached macro lens. Some additional distribution records were observed on iNaturalist (2024).

RESULTS

New distribution records for *Leucostoma aciostre* and *Leucostoma aterrimum*

Leucostoma aciostre: BRAZIL: Pernambuco, Petrolina, 5 males and 4 females (MZSP); São Paulo, São Paulo, 2 females and 1 male, (MZSP).

Leucostoma aterrimum: BRAZIL: Rio de Janeiro, Rio de Janeiro, 1 female (CEIOC), São Paulo, São Paulo, Cantareira, 1 female (MZSP), São Paulo, Bebedouro, Andes, 1 male (MZSP). PERU: Arequipa, Santa Rita, 4 males and 5 females (MZSP).

New record of *Leucostoma aciostre* in *Harmostes parafraterculus*, and evidence of an indirect oviposition strategy

One female of *L. aciostre* (Fig. 2 C, D) was reared from a female *Harmostes parafraterculus* (Fig. 1) in São Paulo. The larvae emerged on January 30th, 2024, and pupated immediately. The fly emerged on February 9th, 2024. A male (Fig. 2) and another female of *L. aciostre* were also collected at the same locality and similar date as mentioned above. This is the first host record and the first records (alongside with the specimens from Pernambuco) for the species in South America. Previously, *L. aciostre* was only known from the Nearctic Region (Reinhard 1956; O'Hara et al. 2020).

Observing the behavior of *L. aciostre* in *B. pilosa* (Fig. 3), the first author noticed that sometimes the female approached their abdominal rear end to the base of the capitulum in the process of fruiting. One female was photographed during that behavior in São Paulo, Brazil (Fig. 3A). This is a strong indication that these flies oviposit directly on the hemipteran host plant, and not in the host itself. Females also land and walk in buds with developing achenes as well as with fully developed achenes (Fig. 3). More on this behavior is discussed below.

Record of *Leucostoma aterrimum* in *Liorhyssus hyalinus* (Fabr., 1794)

The specimens from Santa Rita, Arequipa, Peru (five females and four males) were reared from *Liorhyssus hyalinus* collected in quinoa crops (*Chenopodium quinoa* Willd.) in 2015.

Taxonomy: *Leucostoma* Meigen, 1803: 279.

Type species: *Ocyptera simplex* Fallén, 1815, by subsequent monotypy of Meigen (1824: 234) [Sweden].

For a genus diagnosis and description, see Reinhard (1956), and for a full list of synonyms list, see O'Hara et al. (2020).

Leucostoma aciostre Reinhard, 1956 *Leucostoma aciostre* Reinhard 1956: 162

Diagnosis: The females are easily distinguished from other species of the genus as the tergite 6 pincers structures lack any denticles or strong setae on their inner margin. The males have some silver pruinosity on the abdomen, usually anteriorly on the last segments, as well as in the head. The male terminalia likely have some features that distinguish them from other *Leucostoma* species, but the single male was not dissected to compare.

Redescription: Male (Figs. 2A–B, 3D). Body length 4.5–5.2 mm.

Coloration: Head brown to black with strong silver and golden pruinosity; frontal vitta black. Antenna black; arista black. Palpus brown and prementum dark brown. Thorax black with silver pruinosity dorsally and laterally. Thoracic spiracles dark brown. Wing hyaline. Calypters white and partially hyaline. Halter dark brown to black. Legs dark brown to black; claws black; pulvilli pale brown. Abdomen black, tergites 3 to 5 anteriorly

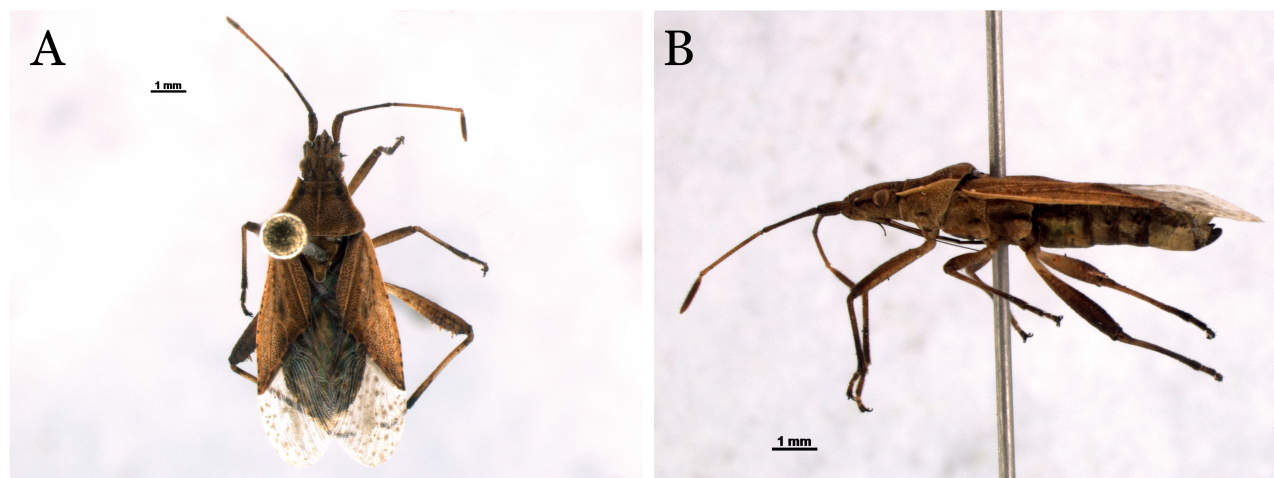


Fig. 1. *Harmostes parafraterculus*. A, dorsal view. B, Lateral view.

with silver pruinosity forming bands, stronger laterally.

Head: Holoptic. Eye bare. Frontal vitta narrow, slightly narrower than fronto-orbital plate maximum width. Ocellar setae latero-clinate. Eight frontal setae, descending to pedicel level. Inner and outer vertical setae hair-like, similar to surrounding postocular setae. Postocellar setae hair-like. Antenna inserted at middle level of eye, short, not extending to vibrissa level. Lunule setulose. Scape short, about one-quarter length of pedicel. Pedicel setose dorsally, with 1 strong seta. First flagellomere slightly longer than pedicel, elliptical but broader distally. Arista micropubescent. Parafacial bare. Vibrissa strong, 4–5 subvibrissal setae. Eye about 0.85 to 0.9 head height. Gena about 0.1 to 0.05 eye height. Antennal axis slightly shorter than oral axis. Lower facial margin protruding. Palpus slightly clavate. Prementum long, slightly longer than head height. Labella well-developed, as long as first flagellomere.

Thorax: Prosternum bare. Proepisternum bare. One strong proepimeral seta, surrounded with weaker

setae. Anterior spiracle with both lappets well-developed, covering almost the entire opening. Posterior spiracle with posterior lappet slightly larger than anterior. Acrostichal setae 1+1, with another presutural long setula. Dorsocentral setae 2+3. Intra-alar setae 1+2. Supra-alar setae 1+2, the second postsutural short. Postpronotal lobe with 2 setae, and 3 anterior long setulae. One postalar seta. Scutellum with one basal, one lateral, one short subapical, and one decussate apical pairs of setae. Two katapisternal setae, with posterior seta longer. One anterior and five posterior anepisternal setae. One anepimeron seta. Katatergite and anatergite bare. Wing: subequal to thorax + abdomen length. Ratio of wing length/maximum wing width 2.4. Costal spine not differentiated. Costal vein without breaks, beyond intersection with vein Sc without ventral setulae. Cell r_{4+5} with long petiole, petiole more than three times r-m length, ending before wing apex. Base of vein R_{4+5} with 1 dorsal and ventral setae. Crossvein dm-cu slightly curved. Calypters rounded, large, almost

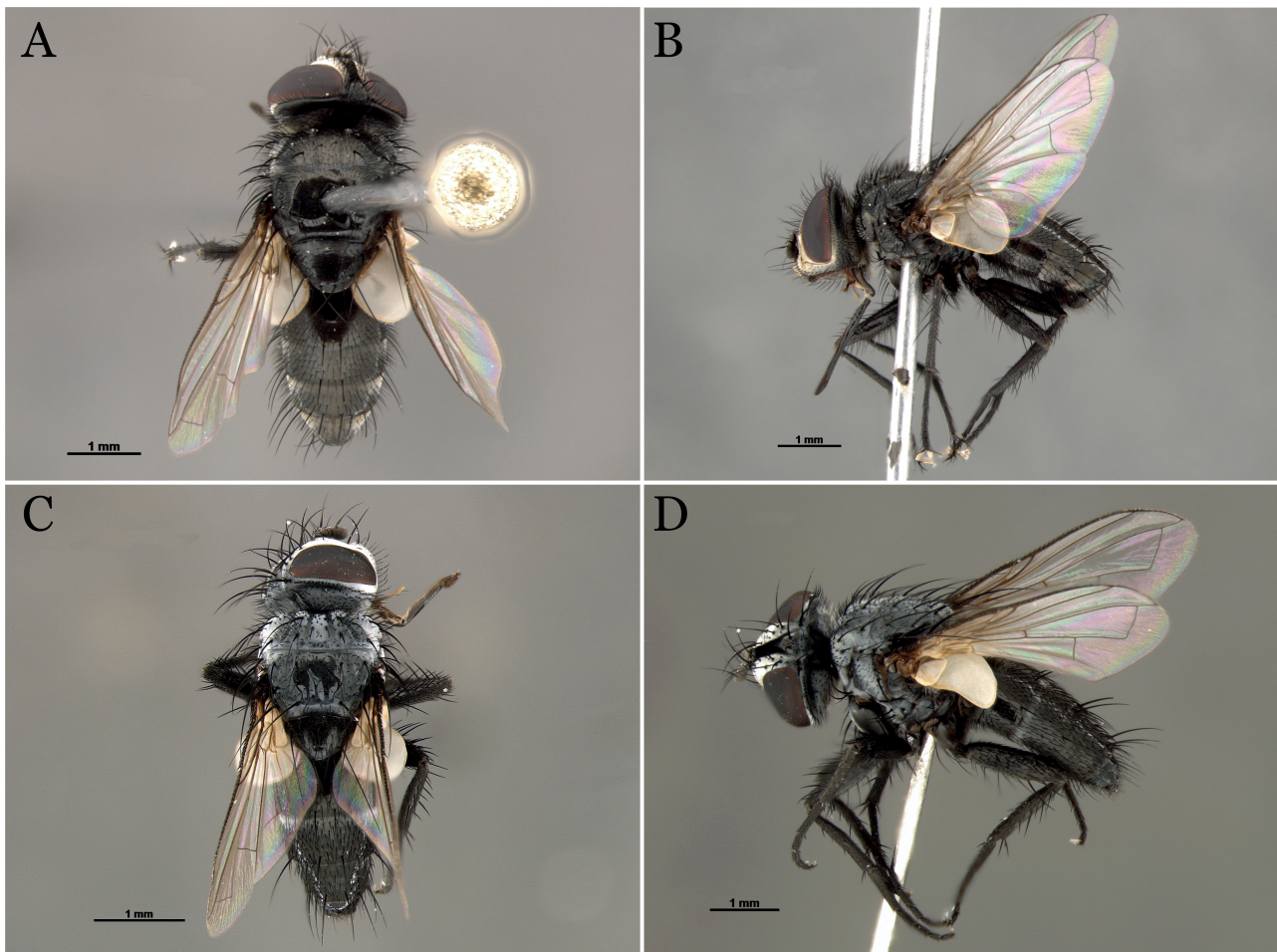


Fig. 2. *Leucostoma acirostre*. A, Male, dorsal view. B, Male, lateral view. C, Female, dorsal view. D, Female, lateral view. Specimens from São Paulo, SP, Brazil.

reaching end of syntergite 1+2. Legs: Fore coxa with many anterodorsal setae. Fore femur with row of posterodorsal and posteroventral seta. Fore tibia with one posteroventral seta. Mid coxa with row of setae on anterior surface. Mid femur with rows of anteroventral and posteroventral setae on basal half, two posterodorsal preapical and one posterior preapical setae. Mid tibia with three anterior, two posterodorsal and one ventral setae. Hind coxa with row of anterodorsal setae. Hind femur with rows of anterodorsal, anteroventral and posteroventral setae on basal half, two anteroventral preapical setae. Hind tibia with three anteroventral setae, row of anterodorsal short setae with two strong, three posterodorsal setae. Tarsal claws long, subequal to combined length of fourth and fifth tarsomere.

Abdomen: Elliptical, slightly pointed at apex. Median marginal setae and lateral marginal setae on all tergites, row of marginal setae on tergites 4 and 5. Abdomen 1.8–1.95 times as long as wide, 1.1–1.25 times as long as thorax in dorsal view. Sternites

completely overlapped by tergites. Sternite 5 plate-like, posterior margin slightly invaginated.

Terminalia: The single male was not dissected.

Female (Figs. 2C–D, 3A–C): Differs from male as follows. Head with only strong silver pruinosity and dichoptic. Ocellar setae stronger. Frontal setae stronger, with 6–7 setae, the posterior laterocline. Two proclinate fronto-orbital setae. Inner and outer verticals stronger, the latter directed laterally. Prementum slightly shorter. Thorax with stronger silver pruinosity; in presutural scutum sometimes delimiting two diverging median black stripes and two large lateral black spots. Three katepisternal setae. Wing slightly shorter, ratio of wing length/maximum wing width 2.6 to 2.7. Calypters slightly shorter. Fore tibia with three anterodorsal and one posteroventral setae. Mid tibia with four anterior setae. Tarsal claws shorter and more curved, pulvilli shorter. Abdomen with silver pruinosity band stronger on tergite 3, weak on tergite 4. Tergite 5 anterior part telescopically inserted in abdomen.

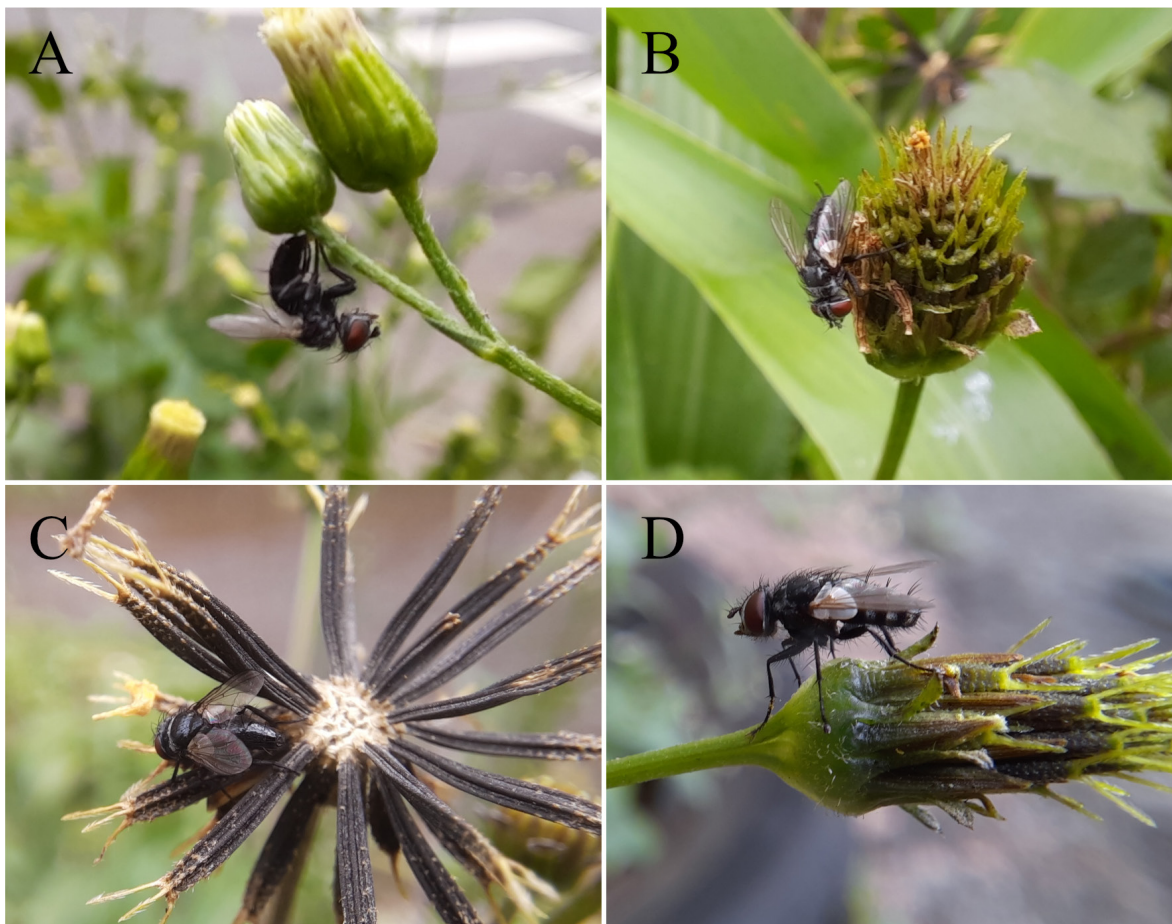


Fig. 3. *Leucostoma acirostre* in *Bidens pilosa* in São Paulo, SP, Brazil (photos taken between November and December 2021). A, Female ovipositing in the base of a capitulum in the process of fruiting. B, Female in a bud with developing achenes. C, Female in a bud with developed achenes. D, Male in a bud with developing achenes.

Terminalia (Fig. 4A): *Terminalia*. Sternite 5 plate-like. Tergite 6 strongly modified as two pincer structures; each posterior projection conical, without strong setae modified as spines, ending in 3 to 4 small projections; not fused dorsally; not fused to sternite 6. Sternite 6 subquadrate, not posteriorly invaginated. Tergite 7 divided in two separated pieces, with anterior elongation, and with a setulose lobe posteriorly. Sternite 7 as an elongated plate, with two short anterior projections; posterior margin deeply invaginated medially, forming two elongated lobes with setulae. Segment 8 fused; with a narrow-fused arch dorsally (probably part of tergite 8); ventral part elongated anteriorly, with a posterior bent piercer, projecting dorsally, then curving downwards (sternite 8). Epiproct and hypoproct not recognizable. Cerci elongate and thin, setulose posteriorly. Three spherical spermathecae.

Puparium (Fig. 5A): Elliptical, without horns or conspicuous spines. Posterior spiracle at the end of conical tubercle, with two very sinuous spiracle openings.

Distribution: U.S.A. (O’Hara et al. 2020); Brazil new record.

Examined material: USA. Holotype, ♂, Texas, Brownsville, iv.1929, Townsend col (NMNH). BRASIL: 5 ♂, 4 ♀, Pernambuco, Petrolina, UNIVASF (CCA), 22.xi.2020, Martins, H.O.F. col (MZSP); 1 ♀, Mato Grosso do Sul, Cassilândia, Vinicius Souza, 3.ii.2022, (<https://www.inaturalist.org/observations/106111066>, photographic record, accessed on 12th July, 2024); São Paulo, São Paulo, Santo Amaro, R. Dios col. 1 ♀ (MZSP), exit host: 30.i.2024, emerged from puparium: 9.ii.2024, host: *Harmostes parafraterculus*; 1 ♂ (MZSP), 10.ii.2024; 1 ♀ (MZSP), 30.xii.2023.

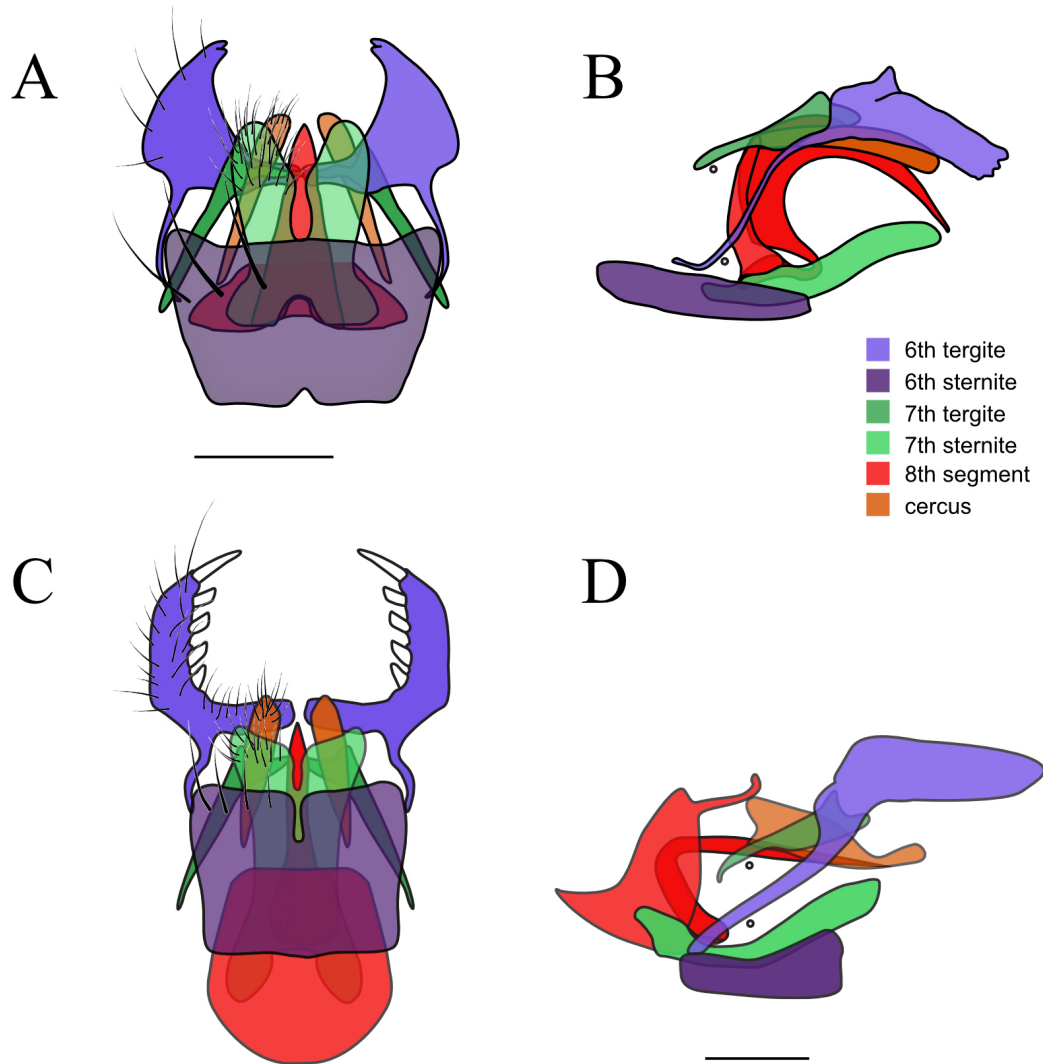


Fig. 4. Female terminalia, scale bar = 0.25mm, color legends on illustration. A, *Leucostoma acirostre*, ventral view. B, *L. acirostre*, lateral view. C, *L. aterrimum*, ventral view. D, *L. aterrimum*, lateral view.

Host list

Hemiptera (Heteroptera), Coreidae

Harmostes reflexulus (Say) (Arnaud, 1978)

Hemiptera (Heteroptera), Rhopalidae

Harmostes parafraterculus Göllner-Scheiding
new record

Leucostoma aterrimum (Villers, 1789)

Musca aterrima Villers, 1789: 548.

Leucostoma neomexicana Townsend, 1892: 169.

Cyclodionaea acuminata Townsend, 1915: 234.

Diagnosis: Females are easily distinguished from other *Leucostoma* by their long and slender abdomen, tapering distally (Fig. 6). All abdominal segments are similar in length/width ratio, however, the fourth tergite is telescopically retracted and seems wider (the retracted anterior portion lacks strong setulae). The males resemble *L. simplex*, according to Reinhard (1956), but the abdomen has pruinosities on the last two segments and is narrowed distally, not as much in females. Other characteristics are used in Reinhard's identification key. The male terminalia (Fig. 4) likely has some features that will distinguish them from other *Leucostoma* species, but we lack material and illustrations to compare.

Redescription: Male (Fig. 6A–B). Body length 4.6–5.5 mm.

Coloration: Head brown to black with strong silver pruinosity; frontal vitta black. Scape and pedicel brown, first flagellomere black, brown basally; arista brown. Palpus pale tawny and prementum brown. Thorax black to brown with weak silver pruinosity dorsally and laterally. Thoracic spiracles brown. Wing hyaline, veins at base pale yellow. Calypters white.

Halter pale brown. Legs dark brown to black; claws black; pulvilli pale tawny. Abdomen dark brown to black, tergites 4 to 5 fully covered with silver pruinosity.

Head: Holoptic. Eye bare. Frontal vitta narrow, slightly narrower than fronto-orbital plate maximum width. Ocellar setae hairlike, laterocline. Eight to 11 frontal setae, hairlike, descending to pedicel level. Fronto orbital plate and dorsal part of parafacialia covered with setulae. Inner and outer vertical setae not differentiated from surrounding postocular setae. Postocellar setae hair-like. Antenna inserted at middle level of eye, short, not extending to vibrissa level. Lunule setulose. Scape short, about one-quarter length of pedicel. Pedicel setose dorsally, with 1 strong seta. First flagellomere slightly longer than pedicel, elliptical but broader distally. Arista micropubescent. Vibrissa strong, 4–5 subvibrissal setae. Eye about 0.8 to 0.9 head height. Gena about 0.1 eye height. Antennal axis slightly subequal to oral axis. Lower facial margin slightly protruding. Palpus slightly clavate. Prementum short, about half eye height. Labella well-developed, as long as first flagellomere.

Thorax: Prosternum bare. Proepisternum bare. One strong proepimeral seta, surrounded with weaker setae. Anterior spiracle with both lappets well-developed, covering almost entire opening. Posterior spiracle with posterior lappet slightly larger than anterior. Acrostichal setae 1+1, with another presutural long setula. Dorsocentral setae 2+3. Intra-alar setae 1+2. Supra-alar setae 1+3, the first and third postsutural short. Postpronotal lobe with 3 setae, and 2 anterior long setulae. One postalar seta. Scutellum with one basal, one lateral, and one decussate apical pairs of setae. Two katapisternal setae, with posterior seta longer. One anterior and five posterior anepisternal setae. One anepimeron seta. Katatergite and anatergite

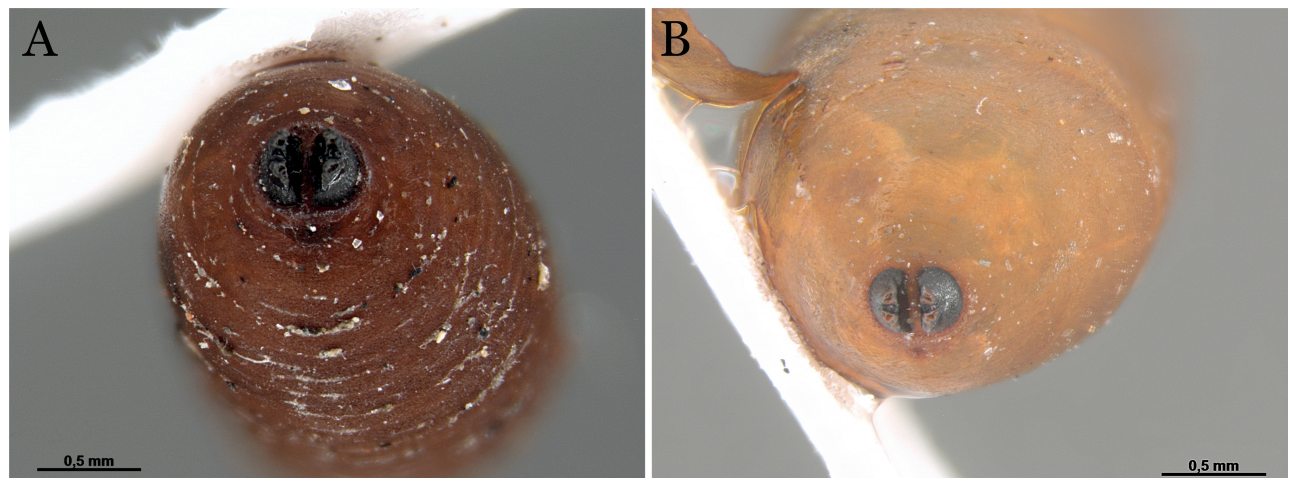


Fig. 5. Puparium, posterior view. A, *Leucostoma aciostre*. B, *Leucostoma aterrimum*.

bare. Wing: subequal to thorax + abdomen length. Ratio of wing length/maximum wing width 2.3–2.6. Costal spine not differentiated. Costal vein without breaks, beyond intersection with Sc vein without ventral setulae. Cell r_{4+5} with long petiole, petiole more than three times r-m length, ending before wing apex. Base of vein R_{4+5} with 1 dorsal and ventral setae. Crossvein dm-cu slightly curved. Calypters rounded, large, reaching end of sytergite 1+2. Legs: Fore coxa with many anterodorsal setae. Fore femur with row of posterodorsal and posteroventral seta. Fore tibia with one posteroventral seta. Mid coxa with row of setae on anterior surface. Mid femur with rows of anteroventral and posteroventral setae on basal half, two posterodorsal preapical setae. Mid tibia with three anterior, two posterodorsal and two ventral setae. Hind coxa with row of anterodorsal setae. Hind femur with rows of anterodorsal, anteroventral and posteroventral setae on basal half, two anteroventral preapical setae. Hind tibia with three to four anteroventral setae, row

of anterodorsal short setae with two strong, three to two posterodorsal setae. Tarsal claws longer than fifth tarsomere.

Abdomen: Elliptical, slightly pointed at apex. Abdomen fully covered with long setulae. Median marginal setae and lateral marginal setae on all tergites, row of marginal setae on tergite 4 and 5. Abdomen 1.5–1.7 times as long as wide, 1.1–1.2 times as long as thorax in dorsal view. Sternites partially covered by tergites. Sternite 5 plate-like, posterior margin slightly invaginated.

Terminalia (Fig. 7B): Tergite 6 partially fused to sytergosternite 7+8. Sternite 6 symmetric, V-shaped, connected by a membrane with tergite 6 and sytergosternite 7+8 on both sides. Epandrium arched, with setae; epandrium anterior process narrow. Bacilliform sclerite thin, elongate. Hypandrium with hypandrial apodeme slightly concave, with rounded apex in ventral view; hypandrial arms not fused dorsally; hypandrial central plate extended posteriorly,

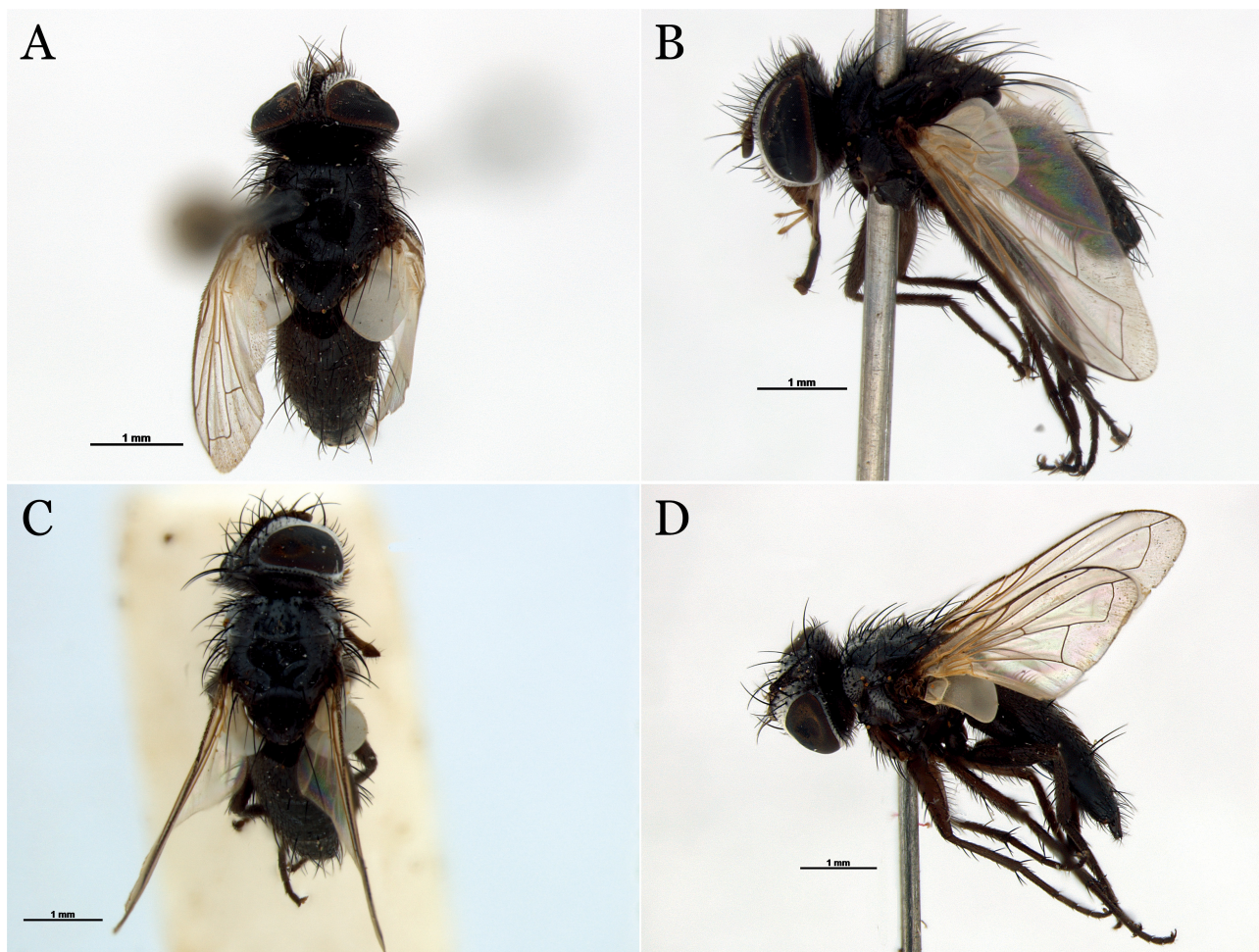


Fig. 6. *Leucostoma aterrimum*. A, Male, dorsal view. B, Male, lateral view. C, Female, dorsal view. D, Female, lateral view. Male from Andes, SP, Brazil, Female from São Paulo, SP, Brazil.

ending as two elongated pointed arms. Pregonite small, hard to differentiate, fused with hypandrium and postgonite; with sensory pits. Postgonite short and elongated, pointed apically. Phallapodeme rod-like, slightly enlarged in both ends, subequal to hypandrium length. Phallic guide not distinguished. Ejaculatory apodeme small, rod-shaped. Epiphallus not differentiated. Basiphallus as curved tube, with two anterior dorsal projections, fused with distiphallus. Distiphallus as a flattened ribbon, short. Syncercus fused, elongated, and pointed distally, the tip directed anteriorly in lateral view. Surstylus triangular, short.

Female: Differs from male as follows. Head with only strong silver pruinosity and dichoptic. Ocellar setae stronger. Frontal setae stronger, with 5–7 setae, the posterior laterocline. Two proclinate fronto-orbital setae. Inner and outer verticals stronger, the latter directed laterally. Thorax with stronger silver pruinosity; in presutural scutum delimiting two

diverging median black stripes and two triangular lateral black spots. Three katapisternal setae. Calypters slightly shorter. Fore tibia with three anterodorsal and one posteroventral. Mid tibia with four anterior setae. Tarsal claws shorter and more curved, pulvilli shorter. Abdomen with silver pruinosity band stronger on tergite 3, weak on tergite 4. Abdomen longer and narrower, tapering gradually from base to apex; abdomen 2.3–2.5 times as long as wide, 1.5–1.7 times as long as thorax in dorsal view. Tergite 5 anterior part telescopically inserted in abdomen.

Terminalia (Fig. 4B): Sternite 5 plate-like. Tergite 6 strongly modified as two pincer structures; each posterior projection elongated, with setae modified as spines; not fused dorsally; not fused to sternite 6. Sternite 6 subquadrate, posteriorly deeply invaginated. Tergite 7 divided in two separated pieces, with anterior elongation, and with a setulose lobe posteriorly. Sternite 7 as an elongated plate, with two anterior projections;

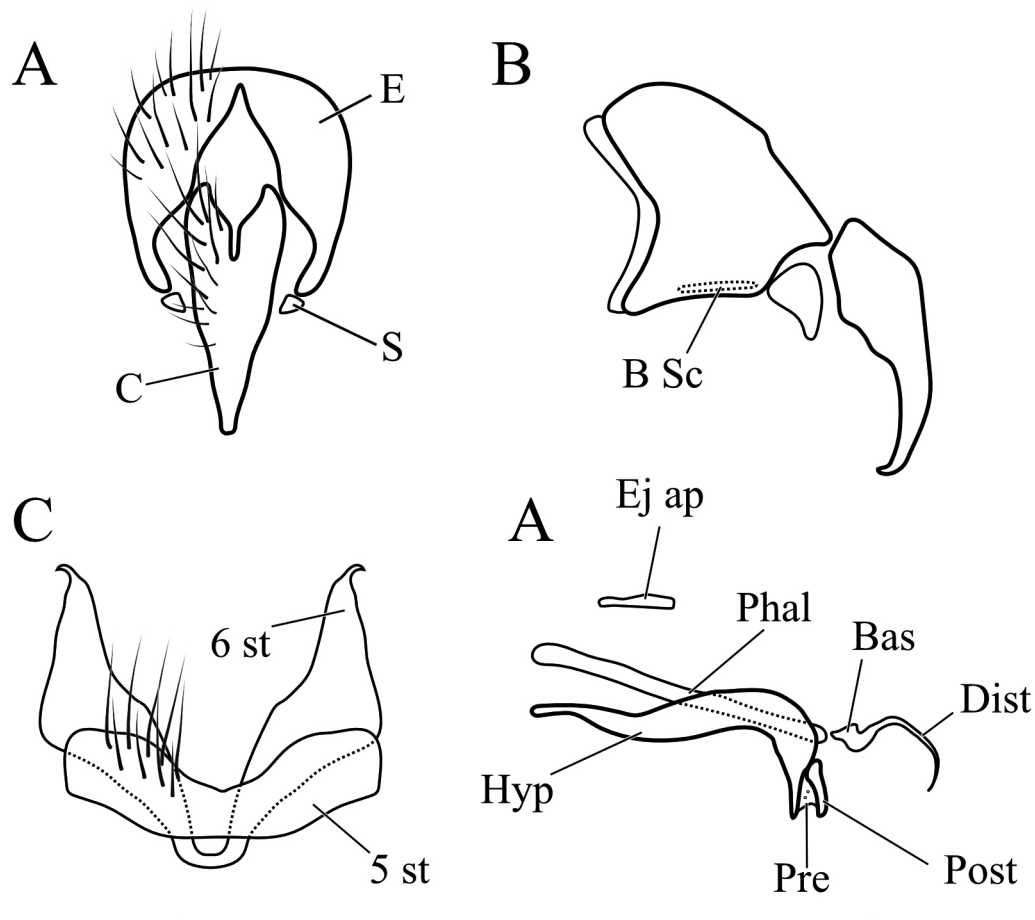


Fig. 7. *Leucostoma aterrimum*, male terminalia, scale bar = 0.1 mm. A, Epandrium, cercus and surstylus, posterior view, setulae represented only on the left side, B, Epandrium, cercus, surstylus and bacilliform sclerite, lateral view, C, Sternites 5 and 6, ventral view, setulae represented only on the left side, D, Lateral view, highlighting outer and inner structures (dashed lines). Abbreviations: B scl = bacilliform sclerite, bas = basiphallus, e = epandrium, c = syncercus, dist = distiphallus, ej ap = ejaculatory apodeme, hyp = hypandrium, post = postgonite, phapod = phallapodeme, pre = pregonite, s = surstylus, st = sternite.

posteriorly deeply invaginated medially, forming two lobes with setulae. Segment 8 fused; with a narrow-fused arch dorsally (probably part of tergite 8); ventral part elongated anteriorly, with a posterior bent piercer, projecting dorsally, then curving downwards (sternite 8). Epiproct and hypoproct not recognizable. Cerci elongate and thin, setulose posteriorly. Three spherical spermathecae.

Puparium (Fig. 5B): Elliptical, without horns or conspicuous spines. Posterior spiracle at the end of conical tubercle, with two sinuous spiracle openings.

Distribution: Nearctic: Canada, U.S.A. Neotropical: Argentina, Brazil (new record), Chile, Mexico, Peru (new record), Puerto Rico, Palearctic: “Europe” (type locality of *Musca aterrima*). Australasian & Oceanian: Hawaii (immigrant) (O’Hara et al. 2020).

Examined material: BRASIL: 1 ♀ (CEIOC), Rio de Janeiro, Jardim Botânico, vi.1936, H.S. Lopes; 1 ♀ (CEIOC), São Paulo, Cantareira, Horto Florestal, iii.1936, S. Lopes, col.; 1 ♂ (MZSP), São Paulo, Bebedouro, Andes, ii.1955, M Carrera col. PERU: 4 ♂, 5 ♀ (MZSP), Arequipa, Santa Rita, 10.vii.2015, G. Mamani col.

Host list

Hemiptera (Heteroptera), Rhopalidae

Liorhyssus hyalinus (Fabr.) (Arnaud, 1978)

DISCUSSION

Leucostomatini non-heteropteran hosts

There are only three Leucostomatini host records from insect orders other than Hemiptera: a Meloidae (Coleoptera) and a Tortricidae (Lepidoptera) from Chile (Cortés and Hichins 1969), and an Acrididae (Orthoptera) from Canada (Smith and Finlayson 1950). However, these records are possible mistakes, as most non-heteropteran hosts have been discredited (Dupuis 1953 1963).

For the orthopteran hosts, Dupuis (1963) mentioned that the described larva is not a Leucostomatini but an Acemyiini, which was later confirmed by Smith (1958); however, such a record was still incorrectly recorded in Arnaud (1978). For the Lepidoptera, Cortés and Hichins (1969) mentioned that the record is probably wrong since the host has been mismatched. For the Coleoptera, the original catalog record mentioned (Cortés and Hichins 1969) is Cortés, 1951. However, there are two catalog references as Cortés, 1951 (Cortés 1951a b), and no records for *Leucostoma* hosts. A record for the same

host was noted in Cortés (1948), where *Phasia curvipes* (Aldrich), another Phasiinae, had been obtained from *E. pilme*. However, no *Leucostoma* is mentioned. We also checked all the other Cortés’s publications mentioned in the catalog (Cortés and Hichins 1969), but found no *Leucostoma* records. Unfortunately, we were unsuccessful in checking and examining the original material. The record remains doubtful, and the original specimens need to be checked to confirm this record.

New host records, new distribution records and biological control relevance

Both host records herein are from Rhopalidae bugs. Rhopalids are phytophagous, presenting generalist habits or some preferences for certain plant families (Schaefer and Kotulski 2000). In general, they do not have major impacts on agriculture; few are of economic importance, but further investigation is necessary since they can be overlooked in agricultural settings due to their small size (Schaefer and Kotulski 2000; Fowles et al. 2015).

The relationship between *Leucostoma* and *Harmostes*, is not unprecedented, as Yonke and Walker (1970) described *Leucostoma acirostre* on adult *Harmostes reflexulus* (Say). *Harmostes* is a Rhopalid genus known from 32 species distributed throughout the Americas. The genus is classified into two subgenera: *Harmostes* and *Neoharmostes* (Coreoidea Species File Online 2024). The genus’ distribution was recently expanded to southern Brazil (Ramos and Barcellos 2021), and although information for other regions is scarce they are suspected to be well-distributed throughout the Neotropics (Göllner-Scheiding 1978; Melo and Montemayor 2015). *H. reflexulus* is distributed in North America, while *H. parafraterculus*, studied herein, is distributed in South America (Coreoidea Species File Online 2024). In this way, it seems that the tachinid could adapt to the local species depending on where it inhabits. Additionally, we found some specimens of *Harmostes gravidator* (Fabr.) coexisting with *H. parafraterculus* in São Paulo, but cannot confirm if *L. acirostre* was using *H. gravidator* as a host. Nonetheless, this possibility cannot be excluded, as many tachinids use different species, and even families, as hosts (Stireman et al. 2017). Even though *Harmostes* did not present risks to agricultural crops, knowledge about their parasitoids can reveal important aspects about the biodiversity and natural history of these organisms.

The other *Leucostoma* species, *L. aterrimum*, has already been reported in South America in Chile and Argentina (O’Hara et al. 2020). Here we present the first records from Brazil and Peru. All specimens from

Peru were reared from *Liorhysus hyalinus* collected on quinoa crops. Quinoa is an important crop that originates from the Andes highlands with constantly expanding production and consumption in more than 50 countries (Murphy and Matanguihan 2015). *L. hyalinus* is one of the quinoa pests, having been reported causing damage in different South American countries (Chorbadjian et al. 2021; Cruces et al. 2022). This host association was already observed (Arnaud 1978) for *L. aterrimum* in Hawaii and in Mississippi, as well as host for *Leucostoma simplex* in Hawaii. The species *L. aterrimum* is also an exotic species in Hawaii (Nishida 1992).

As mentioned, some *Leucostoma* species seem to present a wide distribution range, probably because they follow the distribution of their hosts, many of which are crop pests with synanthropic distribution. Little is known about their original distribution, how fast they spread and if they are using new local species as hosts. This is also true for other Phasiinae, such as in the genus *Trichopoda* (Dios et al. 2021). The species *L. acirostre* is likely widespread in South America since we found a female record from Cassilândia, MS, Brazil on the iNaturalist platform (2024). The photo shows the unique long proboscis and the characteristic terminalia, which helps confirm the species. Knowing that some *Leucostoma* species use important pests and other widespread hemipterans as hosts adds to the understanding of their distribution. However, little has been explored regarding their potential for biological control or their potential to be invasive species, making more studies necessary.

Indirect oviposition on host plants

Dupuis (1963 1973) already noticed the strong ecological correlation between the heteropteran plant host and the Leucostomatini flies, as he collected many adults from the same plant, and not from other flowering plants more commonly visited by Phasiinae (such as umbellifers). The same behavior was observed and photographed herein, and *Leucostoma acirostre* was collected on *Bidens pilosa* L. (Asteraceae), a host plant of *Harmostes parafraterculus*. Such finding corroborates the correlation between the heteropteran host plant and the fly.

Furthermore, Dupuis (1953) mentioned that all literature records of Leucostomatini eggs being injected into the host (Pantel 1910; Townsend 1936; Otten 1943) have no observational evidence, but are inferences based on egg and female morphology. He also commented that he has dissected many hosts of *Leucostoma analis* (Meigen) – now a synonym of *Leucostoma tetraptera* (Meigen) – but could not find a single egg inside the

host.

Regarding observations of females ovipositing on plants, this work presents the first record of this behavior in Phasiinae. A similar behavior was observed in other tachinids, *i.e.*, Goniini with their microtype eggs attached to leaves (Stireman et al. 2006). Likewise, in the more closely related Oestrophasiini, species of *Cenosoma* Wulp are known to pierce plants to inject eggs (Santis and Nihei 2022), which could be similar to the behavior observed in *Leucostoma*. It is possible that larvae hatch and waits for their hosts, or actively seeks them out like some Dexiinae and Tachininae (Stireman et al. 2006). Males of *L. acirostre* were also observed resting on the same host plant (Fig. 3), probably waiting for females.

Nevertheless, more studies are necessary to understand how these females oviposit on the plant, and if larvae hatch and actively search for hosts. Collecting host plants that have been visited by *Leucostoma* females and keeping them under observation would be ideal. Furthermore, exploring if such behavior is restricted to a few species, the whole genus, or even the whole tribe is important. More data on the larvae of *L. acirostre* could help compare its morphology with other tachinid larvae that also actively seek hosts. Some *Leucostoma* larvae have different external spines (Dupuis 1963), which could be indicative of active host finding. Understanding Leucostomatini oviposition is key to understanding the evolution of different oviposition strategies in Tachinidae, as well as for studies aiming to implement biological control strategies.

CONCLUSIONS

Recognizing changes in the behavior associated with host infection is key to understanding the evolution of parasitoid flies. That is also essential for exploring and developing biological control strategies. The evidence of a shift from ovipositing directly on the host to ovipositing onto the hemipteran host plant is new and important for unraveling such strategy in the tribe. New studies are necessary to understand if that is an isolated behavior or more common among the other tribe members. The details of such an infection are also important for future works. The data presented herein documents how little is known about the distribution of these flies and their host range, especially in biodiverse regions such as the Neotropics. Such information is also important for estimating the original distribution and understanding if and how these flies are capable of invading other environments.

Acknowledgments: We thank MZSP and all its employees for material and infrastructure. We also thank the curators of the institutions where the studied material is deposited, Dr. Carlos José Einicker Lamas, Dr. Jane Costa and Dr. Torsten Dikow. Special thanks to all members of the Alto da Boa Vista Community Garden for their support and for keeping the host plants alive, so we could observe host specimens. Financial support from São Paulo Research Foundation – FAPESP grant 2022/14482-6 to RVPD is gratefully acknowledged. We thank Marcelo Domingos de Santis for the discussion on tachinid larvae structures and life habits, and Dr. Aline Barcellos for helping identify species of *Harmostes*. Special thanks to both anonymous reviewers and the editor for important comments and contributions to this manuscript.

Authors' contributions: RVPD: conceptualization and discussion, original manuscript writing, illustrations, photographs, and editing; TR and GT: conceptualization and discussion, manuscript writing and reviewing.

Competing interests: The authors declare no competing interest.

Availability of data and materials: All examined material is deposited at the MZUSP.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

REFERENCES

- Arnaud PH Jr. 1978. A host-parasite catalog of North American Tachinidae (Diptera). United States Department of Agriculture. Miscellaneous Publication **1319**:1–860.
- Blaschke JD, Stireman JO III, O'Hara JE, Cerretti P, Moulton JK. 2018. Molecular phylogenetics and piercer evolution in the bug-killing flies (Diptera: Tachinidae: Phasiinae). *Syst Entomol* **43**:218–238. doi:10.1111/syen.12272.
- Chorbadjian RA, Ahumada MI, Urrea F, Elgueta M, Gilligan TM. 2021. Biogeographical patterns of herbivore arthropods associated with *Chenopodium quinoa* grown along the latitudinal gradient of Chile. *Plants* **10**:2811. doi:10.3390/plants10122811.
- Coreoidea Species File Online. 2024. Version 5.0/5.0. Available at: <http://Coreoidea.SpeciesFile.org>. Accessed 12 Jul. 2024.
- Cortés R. 1948. Sobre algunos taquinidos chilenos y sus hospedes. *Revista de la Universidad Católica de Chile* **33**:119–1, 25.
- Cortés R. 1951a. Sobre tres especies de taquinidos chilenos (Dipt., Tachinidae). *Agricultura Técnica (Chile)* **10**:59–65.
- Cortés R. 1951b. Nuevos generos de Tachininae chilenos con cerdas facio-orbitales (Diptera Tachinidae). *Rev Chil Entomol* **1**:249–262
- Cortés R, Hichins N. 1969. Distribución geográfica y huéspedes conocidos de los taquinidos de Chile (Diptera: Tachinidae). Ediciones de la Universidad de Chile, Santiago, 92 pp. + [8] pls.
- Cruces L, de la Peña E, De Clercq P. 2022. Thermal Biology of *Liorhyssus hyalinus* (Hemiptera: Rhopalidae) and *Nysius simulans* (Hemiptera: Lygaeidae), Fed on the Milky Stage of Maize Grains. *J Insect Sci* **22**:1. doi:10.1093/jisesa/ieac034.
- Cumming JM, Wood DM. 2017. Adult morphology and terminology. *In: Manual of Afrotropical Diptera*. Vol. 1. Introductory chapters and keys to Diptera families (eds AH Kirk-Spriggs & BJ Sinclair), pp. 89–133. Suricata 4. South African National Biodiversity Institute Graphics & Editing, Pretoria.
- Dios RVP, Nihei SS. 2020. Taxonomic revision of the genus *Trichopoda* Berthold, 1827 (Diptera: Tachinidae: Phasiinae), with emphasis on the Neotropical fauna. *Zootaxa* **4870**:1–104. doi:10.11646/zootaxa.4870.1.1.
- Dios RVP, Ziegler J and Zeegers T. 2021. The American genus *Trichopoda* (Diptera: Tachinidae) in Europe – Decades of a misidentified invasive species. *Contributions to Entomology (Beiträge zur Entomology)* **71**:221–225. doi:10.21248/contrib.entomol.71.2.221-225.
- Dios RVP, Nihei SS. in prep. Cladistic analysis of Phasiinae (Diptera: Tachinidae) based on morphological characters. *Zool. J. Linn. Soc.*
- Dupuis C. 1953. Contributions à l'étude des Phasiinae cimicophages (Diptera, Larvaevoridae) XV. Données sur les Leucostomatina et, en particulier, *Leucostoma analis* (Meigen) s. str. *Ann Parasitol Hum Comp* **28**:64–97.
- Dupuis C. 1963. Essai monographique sur les Phasiinae. *Memoires du Museum National d'Histoire Naturelle* **A**:1–461.
- Dupuis C. 1973. Contributions à l'étude des Phasiinae cimicophages (Diptera Tachinidae) 38-Inféodation à la plante nourricière de l'hôte chez *Phania pseudofunesta* (Villen.) et *Dionaea aurifrons* (Mg.) avec des remarques nomenclatoriales et taxinomiques [Study of Phasiinae cimicophages (Diptera Tachinidae). *Ann Parasitol Hum Comp* **48**:359–376.
- Fallén CF. 1815. Beskrifning öfver några Rot-fluge Arter, hörande till släkterna *Thereva* och *Ocyptera*. *Kongliga Vetenskaps Academiens Nya Handlingar, Ser* **3**:229–240.
- Fowles TM, Coscarón MC, Panizzi AR, Carroll SP. 2015. Chapter 20: Scentless Plant Bugs (Rhopalidae). *In: Panizzi AR and Grazia J (eds) True Bugs (Heteroptera) of the Neotropics*. Springer: Entomology in focus, pp. 607–636.
- Gaponov SP. 2003. Morphology of Eggs of Tachinidae (Diptera). Voronezh, Russian: Voronezh State University, 87 pp.
- Göllner-Scheiding U. 1978. Revision der Gattung *Harmostes* Burm., 1835 (Heteroptera, Rhopalidae) und einige Bemerkungen zu den Rhopalinae. *Mitt Zool Mus Berlin* **54**:257–311. doi:10.1002/mmz.19780540205.
- Guimarães JH. 1971. Family Tachinidae (Larvaevoridae). *In: Papavero N (ed) A Catalogue of the Diptera of Americas South of the United States*, vol. 104. Departamento de Zoologia, Secretaria da Agricultura, São Paulo, pp. 1–333.
- Guimarães JH. 1977. Host-parasite and parasite-host catalogue of South American Tachinidae (Diptera). *Arq Zool* **28**:1–131.
- iNaturalist. Available at: <https://www.inaturalist.org>. Accessed on 12th Jul. 2024.
- Murphy KS, Matanguihan J. 2015. *Quinoa: Improvement and sustainable production*. John Wiley and Sons, New Jersey, USA.
- Meigen JW. 1803. Versuch einer neuen Gattungs Eintheilung der europäischen zweiflügeligen Insekten. *Magazin für Insektenkunde* **2**:259–281.
- Meigen JW. 1824. Systematische Beschreibung der bekannten europäischen zweiflügeligen Insekten. *Vierter Theil. Schulz-Wundermann, Hamm*. xii + 428 pp. + pls. 33–41.

- Melo MC, Montemayor SI. 2015. Biodiversity of the scentless plant bugs (Hemiptera: Rhopalidae) in southern South America. *J Nat Hist* **50**:163–200. doi:10.1080/00222933.2015.1073810.
- Nishida GM (ed). 1992. Hawaiian terrestrial arthropod checklist. Bishop Museum Press, Honolulu, USA.
- Nihei SS, Alvarez-Garcia DM, Gudín FM, Santis MD, Dios RVP. 2024. Tachinidae in Catálogo Taxonômico da Fauna do Brasil. PNUD. Disponível em: <http://fauna.jbrj.gov.br/fauna/faunadobrasil/13141>. Accessed 5 Apr. 2024.
- O'Hara JE, Henderson SJ, Wood DM. 2020. Preliminary checklist of the Tachinidae of the world. Version 2.1. PDF document, 1039 pages. Available at: <http://www.nadsdiptera.org/Tach/WorldTachs/Checklist/Worldchecklist.html>. Accessed 21 Mar. 2024.
- Otten E. 1943. Beobachtungen über *Leucostoma anale* Mg. Parasit von *Spilostethus pandurus* Scop. in Nordafrika (Hemiptera Heteroptera: Lygaeidae und Diptera: Phasiidae). *Arbeiten über physiologische und angewandte Entomologie aus Berlin-Dahlem* **10**:136–138.
- Pantel J. 1910. Recherches sur les diptères a larves entomobies. I. Caractères parasitiques aux points de vue biologique, éthologique et histologique. *La Cellule* **26**:25–216 + 5 pls.
- Ramos T, Barcellos A. 2021. *Harmostes* Burmeister, 1835 (Hemiptera, Heteroptera, Rhopalidae): new records, with an emphasis in the southernmost region of Brazil. *Check List* **17**:1475–1486. doi:10.15560/17.6.1475.
- Reinhard HJ. 1956. A Synopsis of the Tachinid Genus *Leucostoma* (Diptera). *J Kans Entomol Soc* **29**:155–168.
- Santis MD, Nihei SS. 2022. Phylogenetic analysis of the tribe Dufourini (Diptera: Tachinidae) using a total evidence approach based on adult and immature stages. *Arthropod Syst. Phylogeny* **80**:1–38. doi:10.3897/asp.80.e69618.
- Schaefer CW, Kotulski J. 2000. Chapter 9: Scentless Plant Bugs (Rhopalidae). In: Schaefer CW and Panizzi AR (eds). *Heteroptera of economic importance*. Florida: CRC Press, pp. 309–320.
- Smith RW, Finlayson TU. 1950. Larvae of dipterous parasites of nymphal and adult grasshoppers. *Can J Res* **28**:81–117. doi:10.1139/cjr50d-007.
- Smith RW. 1958. Parasites of nymphal and adult grasshoppers (Orthoptera: Acrididae) in western Canada. *Can J Zool* **36**:217–262. doi:10.1139/z58-022.
- Stireman JO III, O'Hara JE, Wood DM. 2006. Tachinidae: evolution, behavior, and ecology. *Annu Rev Entomol* **51**:525–555. doi:10.1146/annurev.ento.51.110104.151133.
- Stireman JO III, Dyer LA, Greeney HF III. 2017. Specialised generalists? Food web structure of a tropical tachinid-caterpillar community. *Insect Conservation Diversity* **10**:367–384. doi:10.1111/icad.12238.
- Townsend CHT. 1892. Notes on North American Tachinidae, with descriptions of new genera and species. Paper V; Paper VI. *Can Entomol* **24**:64–70, 77–82; 165–172.
- Townsend CHT. 1915. New western and southwestern Muscoidea. *J N Y Entomol Soc* **23**:216–234.
- Townsend CHT. 1936. Manual of Myology in twelve parts. III: Oestroid classification and habits: Gymnosomatidae to Tachinidae. Ch. Townsend & filhos. São Paulo, p. 255.
- Tschorsnig HP. 2017. Preliminary host catalogue of Palaearctic Tachinidae (Diptera). pdf, approximately 470 pp. Available at: www.nadsdiptera.org/Tach/WorldTachs/CatPalHosts/Home.html. Accessed 21 Mar. 2024.
- Villers C de. 1789. Caroli Linnaei entomologia, faunae Suecicae descriptionibus aucta; DD. Scopoli, Geoffroy, de Geer, Fabricii, Schrank, &c. speciebus vel in systemate non enumeratis, vel nuperrime detectis, vel speciebus Galliae australis locupletata, generum specierumque rariorum iconibus ornata. Tome tertius. Piestre et Delamolliere, Lugduni [= Lyon], 657 pp. + pls. 7–10.
- Yonke TR, Walker DL. 1970. Field history, parasites, and biology of *Harmostes reflexulus* (Say) (Hemiptera: Rhopalidae). *J Kansas Entomol Soc* **43**:444–450.
- Ziegler J. 1998. Die Morphologie der Puparien und der larvalen Cephalopharyngealskelette der Raupenfliegen (Diptera, Tachinidae) und ihre phylogenetische Bewertung. *Studia dipterologica Supp* **3**:1–244.