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Description of *Trichophoromyia macrisae*, a new phlebotomine sand fly species (Diptera: Psychodidae) from Manu Biosphere Reserve, Peru

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Abstract

Phlebotomine sand flies are a highly diverse group of insects capable of transmitting *Leishmania* parasites. Therefore, the identification of vector species is important to understand local leishmaniasis dynamics. Among the Neotropical sand fly species, the genus *Trichophoromyia* is predominantly found in the Amazon. A new species of this genus, *Trichophoromyia macrisae* **sp. nov.**, is described. Specimens were collected using Katchy light traps in forested areas of Manu Biological Station, located within the Manu Biosphere Reserve, in southern Peru. Morphological description was based on 10 male specimens. The disposition of setae in the gonocoxite and the shape of the paramere allows *Th. macrisae* **sp. nov.** to be distinguished from other species of genus that have aedeagal ducts > 4 times the length of the sperm pump. The description of *Th. macrisae* **sp. nov.** brings the number of species of *Trichophoromyia* in the Americas to 47 and in Peru to 15.

Key words: Phlebotominae, South America, UV light trap, new species

Introduction

The subfamily Phlebotominae consists of over 1,060 species worldwide and in Peru at least 150 species have been reported, including 55 species that were described from material collected in the country (Galati & Rodrigues 2023; Cáceres & Galati 2001; Shimabukuro *et al*. 2017; Valdivia *et al*. 2021). Phlebotomine sand flies are vectors of *Leishmania* parasites, the causative agents of the leishmaniases. Particularly, in the Americas, 252,998 cases of cutaneous and mucosal leishmaniasis were registered between 2017 and 2022, with Brazil, Colombia and Peru reporting the majority of the cases (Pan American Health Organization 2023).

The identification of vector species in transmission foci is important to understand dynamics of leishmaniasis and design prevention strategies (Davies *et al*. 2000). In Peru, 33,436 cutaneous and mucosal cases were reported between 2017 and 2022, with an annual average of 6,687 cases (Pan American Health Organization 2023). The department of Madre de Dios has the highest incidence rate of leishmaniasis in Peru, with 472.49 cutaneous cases/100,000 people representing 16.32 % of cases for the country, followed by the department of Cusco with an incidence of 50.37 cases/100,000 people, accounting for 13.26 % of cases in 2021 (Ministerio de Salud de Perú 2022). These regions are characterized for having high phlebotomine species richness, reporting at least 54 sand fly species in Madre de Dios and 53 in Cusco (Valdivia *et al*. 2021; Cáceres *et al*. 2000).

Phlebotomine sand flies are classified in several tribes, subtribes, and genera (Galati 2018). Among the Neotropical sand flies, the genus *Trichophoromyia* is predominantly found in the Amazon basin and the taxonomic identification of the majority of the species is only possible based on male characters. In relation to the epidemiological importance of the genus, recent interest in its diversity and ecology has incriminated some species as biological and suspected vectors of *Leishmania*, particularly the species *Th. ubiquitalis* (Mangabeira), *Th. velascoi* (Le Pont & Desjeux), *Th. auraensis* (Mangabeira), *Th. ininii* (Floch & Abonnenc) and *Th. brachipyga* (Mangabeira) (Santos & Silveira 2020).

Herein we described a new species of *Trichophoromyia* from Manu Biological Station, located within the Manu Biosphere Reserve in southern Peru, in the departments of Madre de Dios and Cusco.

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Methods

Phlebotomine sand flies were sampled between March and April 2023 in forested areas of Manu Biological Station, formerly known as Villa Carmen (Coordinates: -12.8955, -71.4038), located between Madre de Dios and Cusco. Sand flies were collected using modified Katchy UV light traps (Charope Inc., Walpole MA, USA), as part of a study comparing the efficacy of Katchy UV light traps. Traps were set up 1.5 m above ground and operated overnight between 18:00 and 6:00 hours.

Specimens were cleared in 10% KOH for 10 min at approximately 60 ºC and then immersed in saturated liquid phenol. Permanent mounts were made on glass slides with a mixture of Canada balsam and liquid phenol. The terminology and morphological characters included in the description follows Galati *et al*. (2017). Species-discriminant characters were measured with a calibrated eyepiece micrometer in a phase contrast microscope (Alphaphot-2 model YS2-T Nikon, Tokyo, Japan). All measurements are expressed in micrometers (µm). The initial value represents the holotype, followed by the mean or range of countable characters ± 1 standard deviation for the paratypes shown in parentheses. Photographs were taken using an inverted microscope (model IX73, Olympus, Tokyo, Japan).

Results

Trichophoromyia macrisae sp. nov.

Description—Male—Holotype. Insect predominantly brown with the pleura pale.

Head: length 333.33 (328.98 ± 9.94; *n*=9), width 323.53 (319.85 ± 8.98; *n*=8). Clypeus length 97.5 (87.78 ± 6.67; *n*=9), width 67.5 (70.28 ± 3.63; *n*=9). Eye length 202.5 (196.95 ± 8.64; *n*=9), width 115 (95.28 ± 6.9; *n*=9). Interocular distance 112.5 (126.94 ± 4.29; *n*=9). Interocular and interantennal sutures unconnected. Flagellomere lengths: FI 222.5 (213.61 ± 11.46; *n*=9), FII 122.5 (108.75 ± 7.4; *n*=9), FIII 117.5 (106.94 ± 5.42; *n*=9), FIV 115 (105.56 ± 6.59; *n*=9), FV 110 (106.39 ± 5.88; *n*=9), FVI 105 (101.11 ± 4.17; *n*=9), FVII 102.5 (98.61 ± 4.86; *n*=9), FVIII 102.5 (96.11 ± 4.35; *n*=9), FIX 102.5 (94.17 ± 3.54; *n*=9), FX 90 (88.89 ± 4.70; *n*=9), FXI 87.5 (84.58 ± 7.13; *n*=9), FXII 67.5 (71.39 ± 5.46; *n*=9), FXIII 65 (60.28 ± 4.91; *n*=9), FXIV 62.5 (62.22 ± 2.64; *n*=9). Preapical papilla present on FI and FII but absent on FIII. Presence of basal, median and apical papillae on FXII-FIV (Fig 1).

Ascoids long with a rudimentary proximal spur and with the distal projection reaching or extending slightly beyond the base of the subsequent flagellomere. Ascoidal formula FI-FXI 2, FXII-FXIV 0; internal and external ascoids arise before the middle of the segment, except for FI. Ascoids implanted at or almost at the same level; for FI the external ascoid is implanted slightly more proximal than the internal. Palpal formula: 1.4.2.3.5. Length of the palpal segments: PI 30 (29.72 ± 3.41; *n*=9), PII 85 (81.39 ± 6.97; *n*=9), PIII 125 (121.67 ± 7.4; *n*=9), PIV 60 (56.11 ± 3.97; *n*=9), PV 142.5 (136.11 ± 6.26; *n*=9). Newstead´s sensilla present in the inner margin of the second palpal segment, and scattered on the inner margin of the third palpal segment. Simple setae present on PIII: 1 (1; *n*=9), and PIV: 4 (4; *n*=9). Labial suture united in furca. Pharynx unarmed, widest part of pharynx with some grooves (Fig. 1).

***Cervix*:** Cervical sclerite with two sensilla. Ventro-cervical sensilla absent.

Thorax: length 549.02 (534.86 ± 17.75; *n*=9). Pleura with three proepimeral setae (3–5; *n*=9) and 15 (12–17; *n*=9) upper anapisternal setae. Post alar, paratergital, lower anepisternal, anepimeral, metaepisternal, metaepimeral setae absent. Setae absent from the anterior katepisternum margin. Suture between mesepimerum and metaepisternum absent. Metafurca with united vertical arms and long horizontal arms.

Wing length 1825 (1858.33 ± 61.24; *n*=9), width 558.82 (532.68 ± 33.96; *n*=9). Length of vein sections: R5 1175 (1163.89 ± 56.06; *n*=9), *alpha* 519.61 (523.97 ± 41.91; *n*=9), *beta* 245.1 (266.88 ± 16.64; *n*=9), *gamma* 215.69 (214.6 ± 15.84; *n*=9), *delta* 352.94 (331.15 ± 39.45; *n*=9), *pi* 117.65 (120.92 ± 16.26; *n*=9) (Fig. 2A).

Coxa 303.92 (288.67 ± 28.21; *n*=9), 313.73 (294.12 ± 16.26; *n*=9), 303.92 (294.12 ± 21.92; *n*=9); femur 794.12 (734.2 ± 40.85; *n*=9), 745.10 (703.7 ± 37.58; *n*=9), 803.92 (789.76 ± 29.05; *n*=9); tibia 1025 (941.67 ± 45.07; *n*=9), 1250 (1152.78 ± 55.12; *n*=9), 1375 (1341.67 ± 70.71; *n*=9); tarsomere I 637.25 (592.59 ± 29.05; *n*=9), 745.1 (687.36 ± 27.49; *n*=9), 803.92 (761.44 ± 44.66; *n*=9); tarsomeres II+III+IV+V 666.67 (647.06 ± 34.66; *n*=9), 696.08 (679.74 ± 36.68; *n*=9), 774.51 (727.67 ± 35.95; *n*=9). Metafemur without spines. Metatarsomere III with two verticils with spines, one median and one apical.

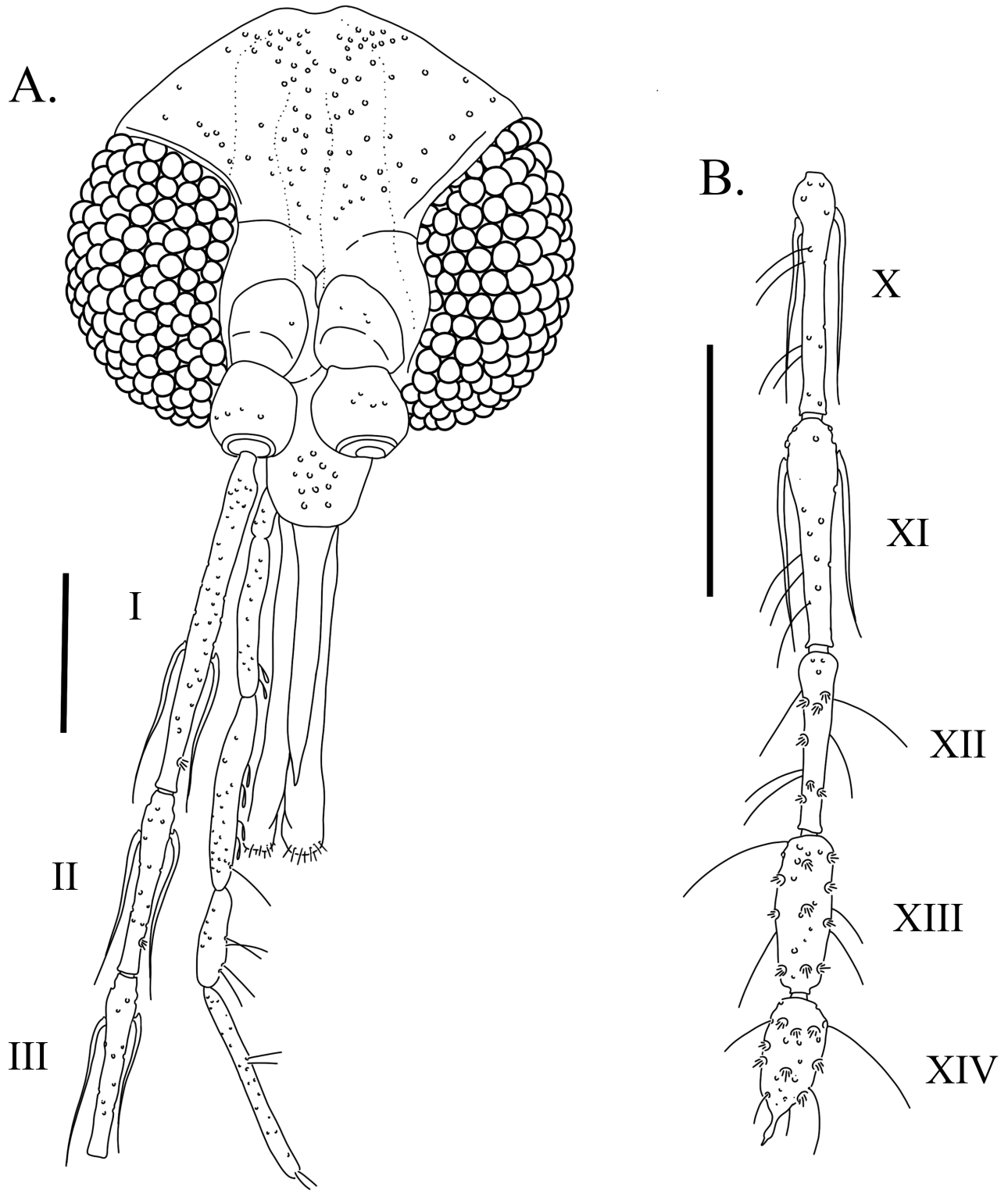

FIGURE 1. *Trichophoromyia macrisae* **sp. nov.** (male). **A.** head, frontal view ; **B.** antennal segments AX-AXIV. Bar: 100 µm.

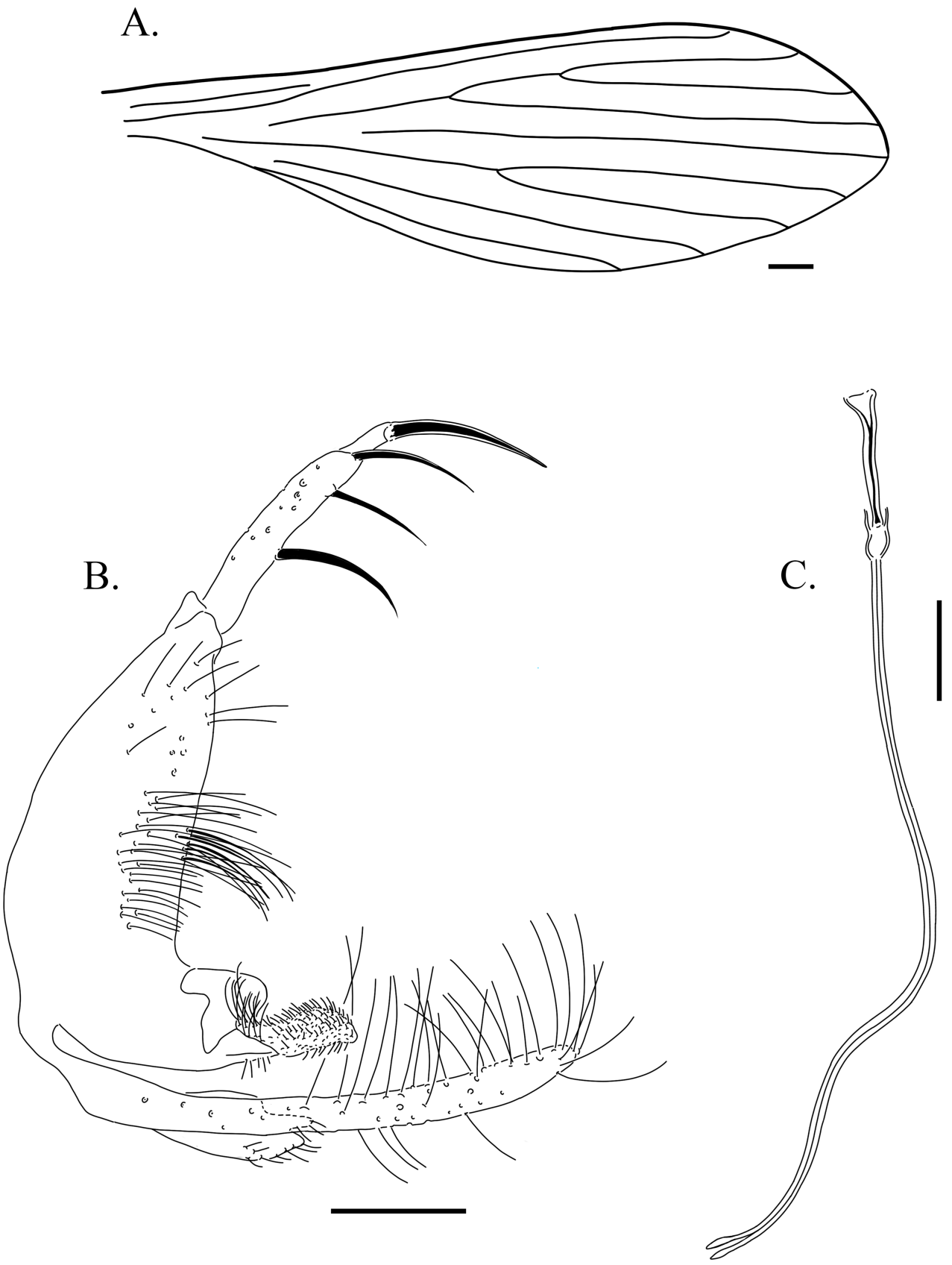

FIGURE 2. *Trichophoromyia macrisae* **sp. nov.** (male). **A.** wing ; **B.** genitalia, lateral view ; **C.** sperm pump and aedeagal ducts. Bar: 100 µm.

***Abdomen*:** length 1625 (1508.33 ± 58.63; *n*=9). Setae on tergites not forming two bands. Tergal papillae absent from the second to seventh tergite.

Terminalia: gonocoxite length 362.75 (367.1 ± 17.06; *n*=9), width 127.45 (133.99 ± 12.01; *n*=9), ornamented with a median cluster of long and thick setae, with 24 setae (24–28; *n*=9) inserted in dorsal position and 9 setae (8–11; *n*=9) inserted in ventral position. Shorter setae extend towards the base without a clear transition. Dispersed thinner long setae are observed in the median-apical region. Gonostyle length 205.88 (203.16 ± 6.54; *n*=9) with four spines: terminal spine 125 (127.22 ± 4.75; *n*=9), external superior spine 97.5 (96.94 ± 5.7; *n*=9), external inferior spine 87.5 (84.86 ± 4.26; *n*=9), internal spine 105 (108.89 ± 5.61; *n*=9). Subterminal setae absent (Fig. 2B)

Paramere with an apical-dorsal lobe, arranged just posterior to its middle, which is endowed with a concavity between its base and the apical third. Its base is slightly wider than the concave area and has elongate setae, with tips curved towards the apex of the paramere. The area of the apical third is clearly wider than the concave area and densely covered with shorter setae. Dorsal margin length 150 (145 ± 5.86; *n*=9); ventral margin length 232.5 (234.44 ± 7.48; *n*=9). Parameral sheath subtriangular with rounded apex, dorsal margin length 67.5 (60.28 ± 6.05; *n*=9), ventral margin length 80 (71.11 ± 9.77; *n*=9). Epandrial lobe length 401.96 (418.3 ± 15.5; *n*=9), width 29.41 (35.95 ± 4.9; *n*=9). Cercus length 212.5 (211.11 ± 11.33; *n*=9). Sperm pump length 167.5 (159.17 ± 7.18; *n*=9); ejaculatory apodeme length 135 (126.67 ± 7.4; *n*=9); aedeagal ducts length 774.51 (831.88 ± 71.90; *n*=9); ratio of aedeagal duct/sperm pump 4.62 (5.23 ± 0.4; *n* = 9); apex of aedeagal ducts slightly expanded (Fig. 2C).

***Type locality*:** Holotype male: Peru, Cusco, Pillcopata, Manu Biological Station. Coordinates: -12.8955, -71.4038, Altitude: 550 m.a.s.l., 16-IV-2023, Méndez-Cardona S. Coll.

***Type material*:** Holotype permanent mount on a microscope slide deposited at Museo de Historia Natural Universidad Nacional Mayor de San Marcos UNMSM, Lima, Peru. Nine paratype males as follows: 1 male (Museo de Historia Natural UNMSM), six males (INS09120–25 Laboratorio de Entomología, Instituto Nacional de Salud, Bogotá, Colombia), two males (Florida State Collection of Arthropods FSCA, Gainesville, FL, USA). Paratype males same data as holotype, but collected between March and April 2023.

***Etymology*:** The name *Th. macrisae* is given in homage to María Cristina Carrasquilla Ferro for her contributions in the field of medical entomology in Colombia and her role as a mentor in the professional development of the first author.

Discussion

The new species described belongs to the genus *Trichophoromyia* as it is a medium-sized phlebotomine sand fly, with dark brown coloration, long antennal ascoids with short posterior spurs, and a relatively short PV, shorter than the combined length of PIII and PIV (Young & Duncan 1994). It is characterized by the presence of papilla in the first and second flagellomere, the absence in the third flagellomere and the presence of Newstead's sensilla on the second palpal segment (Galati 2018). In the thorax, the ventro-cervical sensilla are absent. Male´s genitalia has a stylus with four spines inserted at different levels, including an isolated proximal spine (Young & Duncan 1994), and a terminal spine that arises from a projection in the stylus. The stylus is also characterized by the absence of subterminal seta. The gonocoxite has one or more groups of setae, and the parameral sheath is pigmented and rounded with subequal width and length. Species of the genus *Trichophoromyia* are divided into two groups depending on the ratio between the length of the aedeagal ducts and the sperm pump (i. Aedeagal ducts ≤ 3 times the length of the sperm pump, ii. Aedeagal ducts > 4 times the length of the sperm pump) (Galati 2018). *Trichophoromyia macrisae* sp. nov. belongs to the second group. The description of *Th. macrisae* brings the number of species of *Trichophoromyia* in the Americas to 47 and in Peru to 15 (Shimabukuro *et al*. 2017; Rodrigues *et al*. 2023; Cavalcante *et al*. 2024).

Based on the arrangement of setae in the median region of the gonocoxite, *Th. macrisae* **sp. nov.** is closely related to *Th. auraensis*, *Th. rufreitasi* Oliveira, Teles, Medeiros, Camargo & Pessoa and *Th. velezbernali* Posada-López, Galvis-Ovallos & Galati (Galati 2018). In *Th. auraensis* and *Th. macrisae* **sp. nov.**, the setae inserted in the ventral and dorsal positions of the median cluster have a similar width and length and extend towards the base. In contrast to *Th. rufreitasi* and *Th. velezbernali*, in which the setae of the ventral position are shorter and narrower than those in dorsal position (Oliveira *et al*. 2015; Posada-López *et al*. 2018). In relation to the paramere, *Th. auraensis* has long setae throughout the length of the dorsal lobe of the paramere, while *Th. macrisae* has long setae restricted to the basal expansion (Fig. 3B).

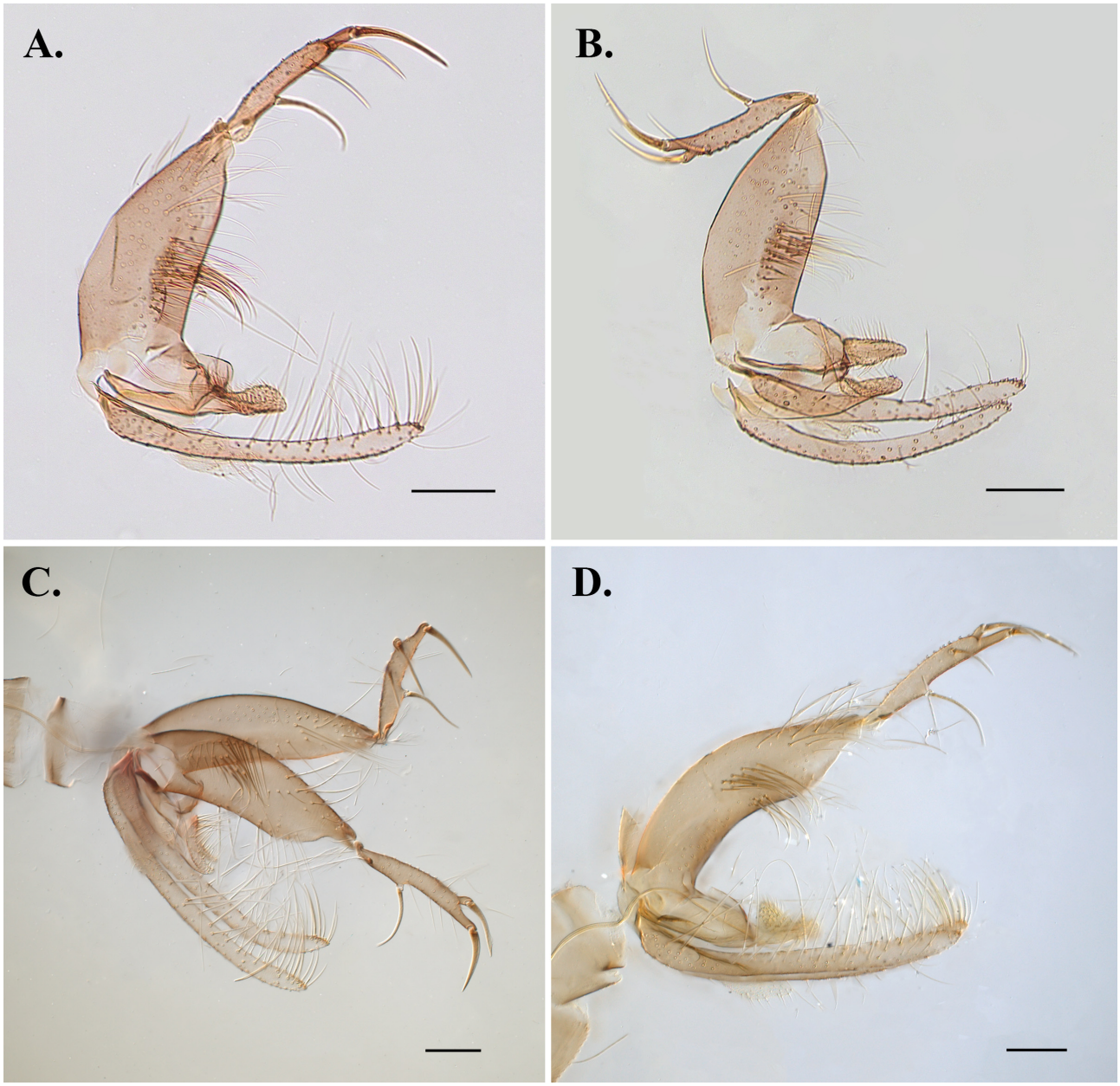

FIGURE 3. A. genitalia of *Trichophoromyia macrisae* **sp. nov.** (holotype); **B.** genitalia of *Trichophoromyia auraensis* from Manu Biological Station, Peru; **C.** genitalia of *Trichophoromyia sinuosa* (holotype) from FSCA; **D.** genitalia of *Trichophoromyia velascoi* from FSCA. Bar: 100 µm.

Trichophoromyia macrisae also resembles *Th. velascoi* and *Th. sinuosa* (Young & Duncan). These three species have a paramere with a dorsal lobe forming a concavity. In the new species, the apical expansion is narrower than the basal expansion (Fig. 3A). In contrast, the basal section of the dorsal lobe in *Th. velascoi* is wider than the apical section (Fig. 3D). In *Th. sinuosa*, the dorsal lobe extends towards the middle of the paramere with a concave shape between symmetrical expansions, giving the apex of the dorsal lobe a digitiform aspect (Fig. 3C). In relation to the gonocoxite, *Th. macrisae* has a median cluster with a dorsal and ventral group of setae extending towards the base. This is different to *Th. velascoi* that has a distinct inner and external cluster of setae restricted to the median region (Galati 2018) (Fig. 3D). Regarding *Th. sinuosa*, the disposition of setae in the gonocoxite represents a difficulty in its identification. We observed mounted specimens of *Th. sinuosa* at the FSCA. Particularly, the holotype shows a median cluster with few shorter setae extending towards the base in accordance with the description and key of Young and Duncan (1994), but contrasting with their illustration that shows an additional group of setae. Galati´s

key (Galati 2018) follows Young and Duncan´s illustration and includes *Th. sinuosa* in the same group with *Th. velascoi* and *Th. cellulana* (Young), according to the disposition of setae in the gonocoxite. Therefore, further revision of the morphology of *Th. sinuosa* is suggested.

Trichophoromyia macrisae **sp. nov.** was found sympatrically with *Th. nemorosa*, *Th. auraensis* and *Trichophoromyia* sp. The composition of *Trichophoromyia* species is consistent with a previous study in the same municipality (Pérez & Ogusuku 1995). Most species of *Thichophoromyia* females cannot be identified due to the great similarity between the spermathecae of the majority of the species in this genus. In this study, a great abundance of *Trichophoromyia* males, including *Th. macrisae* **sp. nov.,** was captured along with females of *Trichophoromyia* sp. near abandoned crops of guava (*Psidium guajaba*), cassava (*Manihot esculenta*), papaya (*Carica papaya*), corn (*Zea maiz)* and banana (*Musa paradisiaca*). *Trichophoromyia macrisae* **sp. nov.** was also captured in lower abundances in the secondary forest and in the peridomicile of Manu Biological Station, Pillcopata, Cusco. Additionally, *Trichophoromyia* females were occasionally captured in the intradomicile. Further research on the role in leishmaniasis transmission of these species is warranted, considering the presence of the different species in anthropic habitats in this study and the detection of *Th. auraensis* naturally infected with *Le.* (*Viannia*) *braziliensis* and *Le.* (*V.*) *lainsoni* in Peru (Valdivia *et al*. 2012).

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