

Notas Científicas

Description of the genital atrium of three *Lutzomyia* species in the subgenus *Psathyromyia* (Diptera, Psychodidae, Phlebotominae) from the Central Brazilian Amazon

Descripción del atrio genital de tres especies de Lutzomyia del subgénero Psathyromyia (Diptera, Psychodidae, Phlebotominae) de la Amazonía Central Brasileña

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SUMMARY

Phlebotomine sand flies are responsible for the transmission of the etiological agents of several diseases, including protozoan parasites that cause leishmaniasis. In the *Lutzomyia* França genus, the females of some species are morphologically similar, and the morphological characters normally used to identify them are unreliable. The aim of this study was to distinguish between females of cryptic *Lutzomyia* França species from the subgenus *Psathyromyia* Barretto by analyzing the armature in the genital atrium (AGA). The species studied were *Lutzomyia dendrophyla* Mangabeira, *L. scaffi* Damasceno & Arouck and *L. shannoni sensulato* Dyar. Lab-reared females were dissected to isolate the genital atrium and spermathecae. The AGA of each species was then photographed, and the arrangement of spines described. We conclude that the AGA can be a useful tool for distinguishing between female cryptic species of the subgenus *Psathyromyia*.

Key words: morphology; taxonomy; phlebotomine.

RESUMEN

Los flebotomíneos (Diptera, Psychodidae, Phlebotominae) son responsables por la transmisión de agentes etiológicos de varias enfermedades, incluyendo protozoarios parásitos que causan las leishmaniasis. En el género *Lutzomyia* França, las hembras de algunas especies tienen morfologías semejantes y los caracteres morfológicos utilizados para identificarlas no son confiables. El objetivo de este estudio fue distinguir las hembras de especies crípticas del subgénero *Psathyromyia* Barretto analizando la armadura del área genital (AGA). Las especies estudiadas fueron *Lutzomyia dendrophyla* Mangabeira-Filho, *Lutzomyia scaffi* Damasceno & Arouck y *Lutzomyia shannoni sensulato* Dyar. Las hembras criadas en laboratorio fueron diseccionadas para aislar la genitalia y la espermateca, las armaduras fueron fotografiadas y la distribución de las espinas fue descrita. Concluimos que la AGA puede ser una herramienta útil para distinguir las hembras crípticas del subgénero *Psathyromyia*.

Palabras clave: morfología, taxonomía, flebótomo.

Phlebotomine sand flies are insects of medical and veterinary importance, because they can transmit etiological agents that cause leishmaniasis, bartonellosis, and arboviruses (e.g. Guerra *et al.*,

2006. *Cad.SaúdePública* 22: 2319-2327; Cohnstaedt *et al.*, 2011. *Am. J. Trop. Med. Hyg.* **84**: 913-922; Comer *et al.*, 1994. *J. Med. Entomol.* **31**: 850-854). In the Amazon region, these insects exhibit high species

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richness and high indices of local diversity (Alves *et al.*, 2012. *Rev. Bras. Entomol.* **56**: 220-227; Oliveira *et al.*, 2013. *Uakari.* **9**: 55-59). Several species of sandflies, are considered cryptic (e.g. Arrivillaga & Feliciangeli 2001. *J. Med. Entomol.* **38**: 783-790; De La Riva *et al.*, 2001. *Mem Inst. Oswaldo Cruz.* **96**: 1089-1094; Dujardin *et al.*, 2005. *Infect. Genet. Evol.* **5**: 362-365; Pinto *et al.*, 2010. *J. Med. Entomol.* **47**: 16-21; Freitas *et al.*, 2015. *Paras. Vectors.* **8**: 448).

In the subgenus *Psathyromyia*, of the genus *Lutzomyia*, females of some species have morphologically similar external structures, and similar spermathecal characters e.g., *Lutzomyia dendrophyla*, *L. scaffi* and *L. shannoni sensulato*. Among these species, similar females are distinguished only by discrete chromatic characters of the pleura of the thorax, and these characters usually disappear when specimens are clarified for slide mounting (Young & Duncan 1994. *Mem. Am. Entomol. Inst.* **54**: 1-881). In the Amazon, these groups of species are also difficult to identify because they share the same resting place: tree trunks (Pessoa *et al.*, 2007. *Mem. Inst. Oswaldo Cruz.* **102**: 593-599).

According Valenta *et al.*, 1999 (*Ann. Trop. Med. Parasitol.* **93**: 389-399), the genital atrium is a structure with a thinchitinous membrane bearing an armature of small spines. The armature in the genital atrium (AGA) is located between the arms of the furca (Fig. 1). Armature morphology is consistent for individuals of the same species, but different species exhibit variations in the size, shape, number, and arrangement of spines and spine comb-sets. The size and width of ewach AGA is showed in the Table I.

Madulo-Leblond *et al.*, 1991 (*Parasitol.* **33**: 387-391) noted that the AGA of the female *Phlebotomus papatasi* Scopoli exhibited spines of a different length than those found in the female *P.*

dubosqi Neveu-Lemaire. Spine length was used as a diagnosis character to distinguish between the two species. Sandfly AGA was used subsequently as a taxonomic tool to assess African and Asian species of the genus *Phlebotomus* Rondani, and was applied successfully to the identification of subgenera *Phlebotomus* Rondani & Brete, *Larroussious* Nitzulescu, and *Paraphlebotomus* Theodor (Killick-Kendrick *et al.*, 1994. *Ann. Trop. Med. Parasitol.* **88**: 433-437; Killick-Kendrick *et al.*, 1997. *Ann. Trop. Med. Parasitol.* **91**: 417-428; Pessonnet *et al.*, 1994. *Ann. Trop. Med. Parasitol.* **88**: 539-542; Depaquitet *et al.*, 1998. *Bull. Soc. Path. Exot.* **91**: 346-352; Kakarsulemankhel 2003. *Parassitol.* **45**: 103-118).

In South America, Valenta *et al.*, 1999 (*Ann. Trop. Med. Parasitol.* **93**: 389-399) used the AGA to assess the genus *Lutzomyia*, and identified useful taxonomic characters for distinguishing females of the verrucarum group from other species of sandfly in Venezuela. In Brazil, Farias *et al.*, 2014 (*Acta Amaz.* **45**: 81-88) described the AGA of 16 species of sand fly from the North and Northeast regions, and found significant differences between sibling species, such as *L. longipalpis* Lutz & Neiva, *L. cruzi* Mangabeira, and *L. umbratilis* Ward & Fraiha.

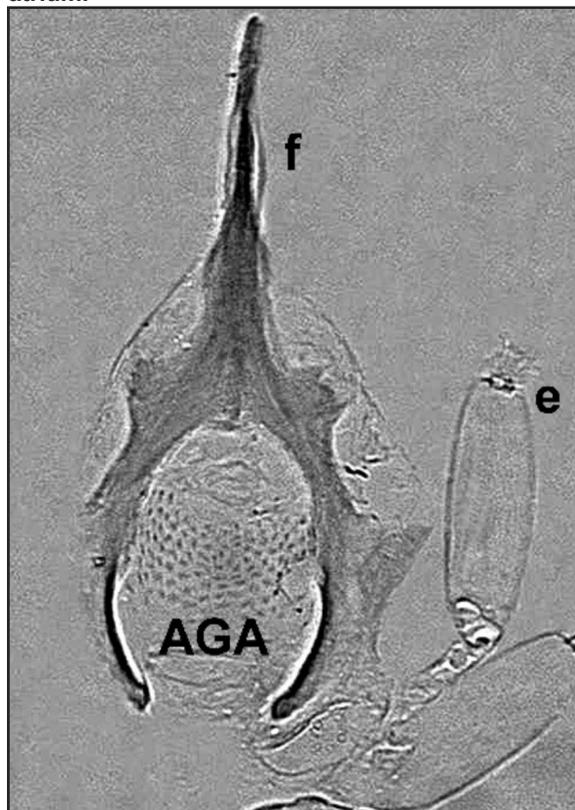
The aim of this study was to describe the AGA of the species *L. dendrophyla*, *L. scaffi*, and *L. shannoni* s.l. These species have very similar females and share the same ecotypes in the Central Brazilian Amazon.

Sand fly collections carried out in solid ground forest located at the Experimental Farm of the Federal University of Amazonas, on BR - 174 high way, Km 38 (2° 38'59.46 "S, 60° 3'16:47" W). Live sand flies were taken to the entomological facility at the Leônidas & Maria Deane Institute. The samples taken, the females who were with

Table I. Length and width, in μm of the females of armature of genital atria, of *Lutzomyia scaffi*, *L. shannoni* and *L. dendrophyla*.

	<i>L. scaffi</i>		<i>L. shannoni</i>		<i>L. dendrophyla</i>	
	Lenght	width	leght	width	lenght	width
Maximum	202,6	61,3	192	34,6	186,6	42,6
Minimum	186,6	26,6	176	8	173,3	10,6
Median	200	37	184	12	176	32
Standard deviation	4,1	7,1	6,0	5,6	1,7	4,6

Fig. 1. Photomicrograph of the genital furca *Lutzomyia (Psathyromyia) scaffi* – f: furca; e: spermathecae; AGA: armature in the genital atrium.



eggs or engorged were separated into individual tubes oviposition. For all three species, the first generation of laboratory reared females was obtained according to the methodology described by Killick-Kendrick & Killick-Kendrick 1991 (*Parasitol.* **33**: 315-320) and Pessoa *et al.*, 2008 (*Zootaxa.* **1740**: 1-14). The identification of cryptic species females was confirmed by identification of the reared males

morphological characters. The identification key and nomenclature used were Young & Duncan 1994 (*Amer. Entomol. Inst.* **54**: 1-881) proposals.

To obtain the furca of each species studied, we separated the last three abdominal segments and placed them overnight in 10% KOH. The samples were then washed in 10% acetic acid and water, and placed in glycerin for dissection under a stereoscopic microscope. The segments were separated using fine needles to open them ventrally and expose the furca within the atrium (Killick-Kendrick *et al.*, 1994. *Ann. Trop. Med. Parasitol.* **88**: 433-437). The atria were then mounted in Berlese's fluid. The images were obtained using a Leica DM 1000 optical microscope with a 100x objective. Images were recorded with a JVC - 3 CCD digital camera and a computer, using Auto Montage 4.0 (Syncroscopy).

In this study, we slide mounted nine furcae from each of the species studied. We did not find any intraspecific differences in the AGA of all individuals examined. In *L. scaffi*, there were three to six spines in each comb, the combs formed horizontal strips, and spine-sets were abundant across the membrane (Fig. 2A, 3A). In *L. shannoni* s.l., spine clusters in the middle of the membrane formed combs of three to six short spines, while spines on the lateral edges of the membrane were large and ungrouped (Fig. 2B, 3B). In *L. dendrophyla*, spines were large and clustered in sets of one to three; grouped and ungrouped spines were present across the membrane (Fig. 2C, 3C).

In this study, the species examined showed specific differences in AGA distinguished at specific levels. Valenta *et al.*, 1999 (*Ann. Trop. Med. Parasitol.* **93**: 389-399) distinguished between important species of the verrucarum group that are involved in the

Fig. 2. The armature in the genital atria of A) *Lutzomyia scaffi*, B) *L. shannoni sensu lato*, C) *L. dendrophyla*.

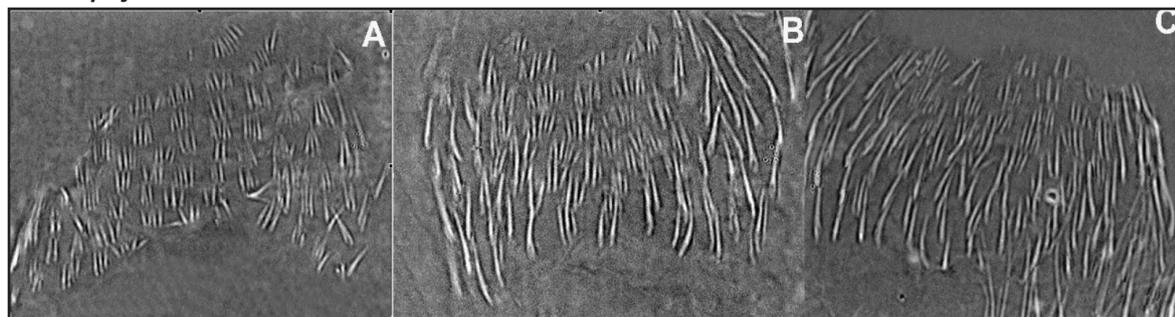
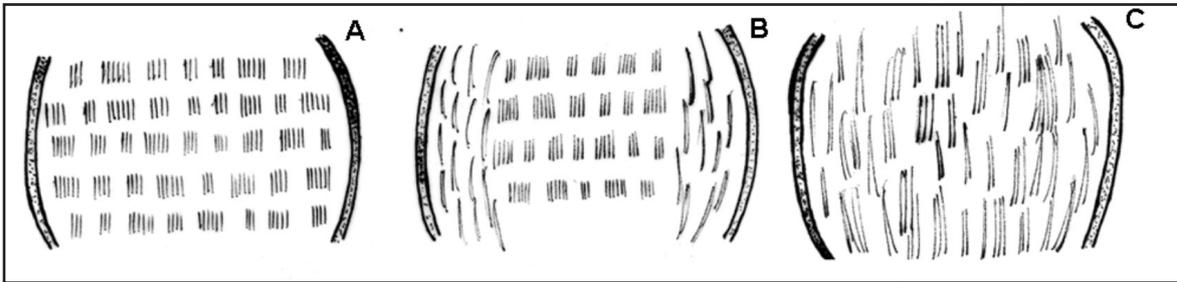


Fig. 3. The schematic graphs of the genital atria of A) *L. scaffi*, B) *L. shannoni sensu lato*, C) *L. dendrophyla*.



transmission of leishmaniasis. Farias *et al.*, 2014 (*Acta Amaz.* **45**: 81-88) distinguished *L. lenti* (Mangabeira) from *L. carmelinoi* Ryan, Fraiha, Lainson & Shaw, and also distinguished *L. longipalpis* (Lutz & Neiva) from *L. cruzi* (Mangabeira) species that are vectors of *Leishmania infantum*, and have very similar females. This study has shown that there are morphological differences in the atria of *L. dendrophyla*, *L. shannoni* s.l., and *L. scaffi*. Two of these species are important in vector competence studies: *L. dendrophyla* may be infected by *Leishmania amazonensis* (Ryan *et al.*, 1986. *Trans. R. Soc. Trop. Med. Hyg.* **80**: 164-165); *L. shannoni* s.l. has been incriminated as a vector of vesicular stomatitis virus (Comer *et al.*, 1990. *Am. J. Trop. Med. Hyg.* **42**: 483-490; 1991. *Parassitol.* **33**: 151-158; 1994. *J. Med. Entomol.* **31**: 850-854; Corn *et al.*, 1990. *Am. J. Trop. Med. Hyg.* **42**: 476-482). This study concludes that morphological differences in the AGA can be adopted as important taxonomic

characters for differentiating between cryptic species of the subgenus *Psathyromyia* in the Central Brazilian Amazon.

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