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Article in Journal of Medical Entomology · September 2004
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MORPHOLOGY, SYSTEMATICS, EVOLUTION

Taxonomy and Distribution of the Series pia of the Lutzomyia verrucarum Group (Diptera: Psychodidae), with a Description of Lutzomyia emberai n. sp.

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ABSTRACT A new species of phlebotomine sand fly, Lutzomyia emberai n. sp., is described and illustrated from specimens collected in a dwelling of the Emberá Indian community, situated in the foothills of the Serranía del Baudó on the Colombian Pacific coast. The morphological characteristics of L. emberai n. sp. suggest that it belongs to the series pia of the group verrucarum, easily differentiated from the other members of this group by diagnostic characters on the palps, labro-opharynx, thorax, and spermathecal ducts. The discovery of this new phlebotomine raises to seven the number of species in the series pia, including Lutzomyia pia (Fairchild & Hertig, 1961); Lutzomyia reclusa Fernandez & Rogers, 1991; Lutzomyia suapiensis Le Pont, Torrez-Espejo & Dujardin, 1997; Lutzomyia tihuiiensis Le Pont, Torrez-Espejo & Dujardin, 1997; Lutzomyia tocaniensis Le Pont, Torrez-Espejo & Dujardin, 1997; Lutzomyia limafalcaoae (Wollf & Galati, 2002); and Lutzomyia emberai Bejarano, Duque & Velez, 2004, n. sp. The taxonomy, distribution, and medical importance of this series are reviewed.

KEY WORDS Phlebotominae, taxonomy, Lutzomyia emberai n. sp., Colombia

The Lutzomyia verrucarum group (Diptera: Psychodidae) is an informal taxonomic assemblage that includes 40 species of Neotropical phlebotomines (Bejarano et al. 2003a), several of which are involved in the transmission of Leishmania spp. and Bartonella bacilliformis (Scorza and Anez 1984, Young et al. 1987, Travi et al. 1990, Bonfante-Garrido et al. 1991, Pérez et al. 1994, Martínez et al. 1999). This group has been divided into four species based on the number and distribution of spines on the style of the masculine genitalia, i.e., series verrucarum, serrana, townsendi, and pia (Theodor 1965, Kreutzer et al. 1990, Galati 1995). Galati et al. (1995) consider there to be a fifth series of species (series evansi) based on the absence of papillae (sensilla in rosettes) on flagellomere III.

The series verrucarum includes the species that possess two medial and two distal spines, and incorporates Lutzomyia verrucarum (Townsend 1913); Lutzomyia evansi (Nunez-Tovar, 1924); Lutzomyia colombiana (Ristorcelli & Van Ty, 1941); Lutzomyia ovallesi (Ortiz 1952); Lutzomyia nunetzovari (Ortiz 1954); Lutzomyia nevesi (Damasceno & Arouck 1956); Lutzomyia andina Osorno, Osorno & Morales, 1972; Lutzomyia disiuncta Morales, Osorno & Osorno, 1974; Lutzomyia moralesi Young, 1979; L. aulari Feliciangeli, Ordóñez & Manzanilla, 1984; Lutzomyia deorsa Perez, Osuguku, Monje & Young, 1991; Lutzomyia maranonensis Galati, Caceres & Le Pont, 1995; Lutzomyia cajamarcensis Galati, Caceres & Le Pont, 1995; Lutzomyia antioquensis (Wollf & Galati 2002); and Lutzomyia falcata (Brazil & Andrade Filho, 2002).

The series serrana, characterized by the presence of two distal spines and a slender medial spine, is comprised of Lutzomyia serrana (Damasceno & Arouck, 1949); Lutzomyia christophlei (Fairchild & Trapido, 1950); Lutzomyia orestes (Fairchild & Trapido, 1950); Lutzomyia oesbia (Fairchild & Hertig, 1961); Lutzomyia odax (Fairchild & Hertig, 1961); Lutzomyia ottoliani (Ortiz & Scorza, 1963); Lutzomyia diazi Gonzalez & Garcia, 1981; Lutzomyia novoeae Gonzalez & Garcia, 1981; Lutzomyia robusta Galati, Caceres & Le Pont, 1995; and Lutzomyia guilvardae Le Pont, Martinez, Torrez-Espejo & Dujardin, 1998.

The series townsendi consists of species that present three distal spines and an isolated basal spine and includes Lutzomyia townsendi (Ortiz 1959); Lutzomyia spinicrassa Morales, Osorno & Munoz, 1969; Lutzomyia longiflocosa Osorno, Morales, Osorno & Munoz, 1970; Lutzomyia quasitownsendi Osorno, Osorno & Munoz, 1969; Lutzomyia sauroidea Osorno, Morales & Osorno, 1972; Lutzomyia amilcar Arredondo, 1984; Lutzomyia youngi Feliciangeli & Murillo, 1987; Lutzomyia nadianae Feliciangeli, Arredondo & Ward, 1992; and Lutzomyia torvida Young, Morales & Ferro, 1994.

In the series pia the males possess five spines distributed between the medial and distal regions of the style. The taxonomic determination of species in this...
last series is based principally on female morphological characteristics, the males of certain species being unknown (Le Pont et al. 1997). A new Colombian species belonging to the series *pia* is described in the present article. In addition, information published since 1994 on the taxonomy, biogeography, and medical importance of the series is reviewed and a dichotomous key presented for species identification.

The type specimens were collected with a CDC-type light trap inside a dwelling of the Emberá Indian community of El Brazo, Bahía Solano (06° 13’ N, 77° 24’ W), Department of Chocó. This locality is situated at 100 m above sea level in the foothills of the Serranía del Baudó mountain range on the Colombian Pacific coast, a region considered to be one of the most biodiverse in the world (García-Kirchbride 1986). Ecologically, it is classified as very humid tropical forest (Holdridge 1967). The mean relative humidity, temperature and annual precipitation for the municipality of Bahía Solano are 90%, 25.6°C, and 4982 mm, respectively. Phlebotomines were collected during an eco-epidemiological study of leishmaniasis.

The description is based on morphological characters suggested by Bermudez et al. (1991), Galati (1995), and Valenta et al. (1995). The type material will be deposited in the Colección de Vectores y Hospedadores Intermediarios de Enfermedades Tropicales (VHET), Programa de Estudio y Control de Enfermedades Tropicales (PECET), at the Universidad de Antioquia in Medellín, Colombia.

**Taxonomy and Distribution**

**Series *pia* Galati, 1995**


**Female.** (Figs. 1–6). Cibarium with four to six horizontal teeth, one to two rows of vertical teeth and small lateral teeth, these which are occasionally difficult to see. Cibarial arch complete. Pharynx with striations or very rudimentary spines at its apex. Fifth palpomere relatively short, subequal to, or shorter than the sum of the lengths of the third and fourth palpomere. Newstead’s spine on the third palpomere. Flagellomere II with simple ascoids and papillae. Sacc-like spermatheca striated, with a prominent apical ring with almost straight sides. Spermathecal ducts thick, common duct usually striated. Common duct generally exceeding individual ducts in length.

**Male.** (Figs. 7–12) (including characters present in both sexes). Style with five large spines arranged between medial and distal regions, with a subapical seta. Coxite with a tuft of setae in the basal or medial region. Paramere simple with simple setae between medial and apical region.

**Lutzomyia pia** (Fairchild & Hertig, 1961) (Figs. 1–12)

*Phlebotomus pius* Fairchild & Hertig, 1961: 248 (male holotype, Santa Clara, El Volcan, Chiriquí, Panama; allotype female, Palo Santo, Chiriquí, Panama).


Morphological Remarks. The females of the Colombian populations of *L. pia* examined all had numerous sharp pointed lateral teeth on either side of the horizontal teeth (Fig. 2). These structures are difficult to observe with the 40× objective but are easily identifiable at 100×. The lateral teeth are not mentioned in any of the descriptions of this species available to date (Fairchild and Hertig 1961, Forattini 1973, Young 1979, Murillo and Zeledón 1985, Young and Duncan 1994), so specimens from populations in other countries should be examined. Furthermore, in the Colombian specimens examined here the number of horizontal teeth varied between four and six. This was also noted by Young (1979) in studying specimens from Panama and Costa Rica.

*Lutzomyia reclusa* Fernandez & Rogers, 1991

*Lutzomyia reclusa* Fernandez & Rogers, 1991: 129 (male holotype, El Monte, Catache, Santa Cruz, Cajamarca, Peru), Young and Duncan 1994: 715 (key, figures, distribution, references), Balard et al. 1999: (distribution, habitats, references), Williams 1999: 482 (geographical distribution), Cáceres and Villaseca 2002: 23 (listed), Bejarano et al. 2003a: 90 (systematics).

Distribution. PERU: Cajamarca (Fernandez and Rogers 1991).

**Lutzomyia suapiensis** Le Pont, Torrez-Espejo & Dujardin, 1997


Distribution. BOLIVIA: La Paz (Le Pont et al. 1997); PERU: Cusco (Cáceres et al. 2001).

**Lutzomyia tihuiliensis** Le Pont, Torrez-Espejo & Dujardin, 1997


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Fig. 7–12. Male of *L. pia* Fairchild & Hertig, 1961. (7) Head. Scale line, 200 μm. (8) Flagellomere II. (9) Palpomere 3. (10) Genital pump and filaments. (11) Terminalia. Scale lines, 100 μm. (12) Wing. Scale line, 1000 μm.
Distribution. BOLIVIA: La Paz (Le Pont et al. 1997); PERU: Cusco (Cáceres and Galati 2001).

*Lutzomyia tocaniensis* Le Pont, Torrez-Espejo & Dujardin, 1997


Distribution. BOLIVIA: La Paz (Le Pont et al. 1997); PERU: Cusco (Cáceres and Galati 2001).

*Lutzomyia limafalcaoae* Wolff & Galati, 2002

*Pintomyia limafalcaoae* Wolff & Galati, 2002: 317 (male holotype and female allotype, Savanitas, Montebello, Antioquia, Colombia).


*Lutzomyia emberai* Bejarano, Duque & Velez, 2004, new species (Figs. 13–19)

Type Locality. El Brazo, Bahía Solano, Chocó, Colombia.

Holotype Female (measurements in micrometers). Medium-sized phlebotomine, length 2,378.3.
Head. Pale brown. Height of head from vertex to the tip of the clypeus 408, width 436.8. Length of clypeus 109.2, bearing 12 setae. Length of labro-epipharynx 268.8. Hypopharynx with 16–17 lateral apical teeth. Lacinia of maxilla with six longitudinal external and 22 internal teeth. Length of the eyes 237.6, width 144. Interocular distance 139.2, equivalent to 8.42 facet diameters. Interocular suture incomplete. Ratio of length of labro-epipharynx/height of head 0.66. Labrum longer than flagellomere I, reaching the medial part of flagellomere II and third palpomere. Length of the flagellomeres: I 255.6, II 114.7, III 113.7, IV 113.7, V 112.3, VI 108.9, VII 107.4, VIII 106.9, IX 107.4, X 107.4, XI 101.1, XII 88.9, XIII 68, XIV 63.2. Ratio of the length of flagellomeres I/II + III 1.11. Paired ascoids present on flagellomeres I to XIII, those of flagellomere II inserted at almost the same level and exceeding the apex of the segment. Papilla on the distal fifth of the internal border of flagellomere II. Length of the palpomeres: first 54.9, second 89.1, third 146.4, fourth 66.6, fifth 132. Fifth palpomere shorter than sum of lengths of third and fourth palpomeres. Palpul formula 1.4.2.5.3. Approximately 26 Newstead’s spines, distributed between the proximal 0.34 and 0.84 of the internal border of the third palpomere. Pharynx unarmed, frayed and with fine striations in its apical portion. Length of pharynx 206.4, maximum width 84. Cibarial armature formed by four long horizontal teeth distributed equidistantly and ~40 vertical teeth arranged in two very irregular transverse rows. Numerous small lateral teeth also present at each side of the horizontal teeth. Pigmented area very faint and subtriangular. Cibarial arch complete.

Thorax. Proscutum, scutum, and lower part of the pleura dark brown. Pronotum, preepisternum, anepisternum, scutellum, postscutellum, halteres, coxae, legs, and upper part of the pleura pale brown. Length of thorax from anterior of proscutum to posterior of scutellum 648. Pleura with 21 upper and six lower anepisternal setae. Length of wing from start of the costal vein 1757.8, maximum width 600. Length of the principal wing veins: R1 (α) 595.2, R1 (ε) 720, R3 (θ) 1004.5, R4 1214.4, R5 + R6 (β) 201.6, R8 + R9 + R10 (γ) 184.8. Distance from bifurcation R1 + R3 to termination of R1 (δ) 237.6, distance from bifurcation R1 + R3 + R4 to bifurcation M1 + M2 (π) 96. δ and π positive. Wing index α/β 2.95. Length of the femur, tibia and basitarsus: anterior leg 710.4, 823.2, 518.4; median leg 667.2, 1065.6, 652.8; and posterior leg 506.4, 1310.4, 729.6, respectively. Length of tarsomeres II, III, IV and V: anterior leg 266.4, 192, 163.2, 115.2; median leg 292.8, 208.8, 177.6, 112.8; and posterior leg 314.4, 224.4, 187.2, 115, respectively. Femur unarmed.

Abdomen. Color pale brown. Length of abdomen 1432.7. Cercus simple, length 105.6. Spermatheca sac-like, with little incomplete transverse striations and a prominent, smooth apical ring with straight sides. Terminal button short and wide with short filaments. Length of apical ring, including the terminal button, 15.5, width 12. Individual and common ducts with little transverse striations. Length of common duct 78.7, width 14.6. Length of individual ducts 108.4, width 7.8. Ratio of lengths common duct/individual duct 0.73. Total length of genital atrium 149.7. Internal width of genital atrium 50.5. Genital furca strongly sclerotized. Length of furca 87.5. Ratio between the length of the furca and internal width 1.73. Ratio between total atrium length and length of the furca 1.71.

Material Examined. HOLOTYPE: one female, El Brazo, Bahia Solano, Chocó, COLOMBIA, 13-X-93, CDC light trap, P. Duque, VHET. PARATYPE: one female, same data.

Etymology. The name of the new species refers to the Emberá Indian Community that inhabits the area where the type specimens were collected. The Emberá occur in groups from Panama to Ecuador, along the Pacific Coast and western cordillera of the Colombian Andes.

Distribution. COLOMBIA: Chocó.

Taxonomic observations. The presence of striated, sac-like spermathecae, long, thick and striated spermathecal ducts, complete cibarial arch, cibarium with four horizontal teeth, two rows of vertical teeth and small lateral teeth, simple asciids, femur and pharynx without spines, suggest that L. emberai n. sp. belongs to the verrucarum group. The new species exhibits the typical spermatheca of females of the series pia, which are characterized by the presence of a smooth, differentiated apical ring with almost straight sides. L. emberai n. sp. can be distinguished easily from other species of this series by the length of the individual ducts, which exceeds that of the common duct. In L. emberai n. sp. the ratio of the lengths of the common/individual ducts is ~0.73, whereas in the other species the ratio is ≥2.

The characteristic of a fifth palpomere shorter than the third also permits L. emberai n. sp. to be separated from the other members of the series pia. The palpul formula of L. emberai n. sp. is 1.4.2.5.3., whereas that of other species is 1.4. (2.3).5, the fifth palpomere being subequal to the sum of the lengths of the third and fourth palpomeres. In addition to the previous characters, L. emberai n. sp. can be differentiated from L. tocaniensis, L. suapiensis and L. tihuiihiensis by the color of pleura. These are totally pigmented in the new species, unpigmented in L. tocaniensis and partially pigmented in L. suapiensis and L. tihuiihiensis. L. emberai n. sp. also differs from L. pia in the length of the labro-epipharynx <300 versus >350 in the latter species.

It has recently been postulated that the species pia could represent an ancestral lineage within the verrucarum group (Bejarano et al. 2003a). The hypothesis of the ancestral condition is based on analyses of morphological (Galati 1995) and molecular systematics (Rojas et al. 2002). If correct this hypothesis would also validate the suggestion of Theodor (1965) that reduction of the number of spines of the style is an evolutionary tendency. Theodor (1965) considered the presence of five spines on the style to be a primitive condition, suggesting that the reduction in their number occurred independently in several species groups of the subfamily Phlebotominae. Under this scheme, the series serrana whose members possess
three spines would correspond to the most advanced lineage within the group verrucarum, the series verrucarum and tozensendi with four spines would be intermediate and the series pia with five spines would represent the most primitive lineage. Nevertheless, further molecular studies that include other five-spined Lutzomyia species are needed to permit evaluation of the monophyly and robustness of the evolutionary relationship between the series pia and the verrucarum group.

Key for the Species of the Series pia

[Adapted from Le Pont et al. (1997) and Wolff and Galati (2002).]

1. Female ........................................... 2
1° Male ........................................... 7
2. Common sperm duct clearly longer than individual ducts, ratio of the lengths of the common/individual ducts ≥2. Fifth palpomere longer than third palpomere .................. 3
2°. Common sperm duct shorter than individual ducts, ratio of the lengths of the common/individual ducts ≤0.73. Fifth palpomere shorter than third palpomere. .................. 5
3. Pleura completely pigmented .................. 4
3° Pleura pale or partially pigmented ........... 5
4. Common and individual sperm duct striated. Labro-epipharynx longer than 350 \(\mu\)m. .................................. L. pia (Fairchild & Hertig, 1961)
4°. Common and individual sperm duct smooth. Labro-epipharynx shorter than 310 \(\mu\)m. .................................. Lutzomyia limafalcaoae (Wolff & Galati, 2002)
5. Pleura all pale .................................. Lutzomyia tocaniensis
Le Pont, Torrez-Espejo & Dujardin, 1997
5°. Pleura partially pigmented .................... 6
6. Labro-epipharynx equal to, or shorter than, 310 \(\mu\)m. Second palpomere shorter than or equal to 150 \(\mu\)m. ..................... Lutzomyia suapiensis
Le Pont, Torrez-Espejo & Dujardin, 1997
6°. Labro-epipharynx equal to, or longer than, 350 \(\mu\)m. Second palpomere longer than or equal to 170 \(\mu\)m. ..................... L. tihuiilenis
Le Pont, Torrez-Espejo & Dujardin, 1997
7. Coxite with compact tuft of setae inserted at basal half of structure .................. L. reclusa
Fernandez & Rogers, 1991
7°. Coxite with diffuse tuft of setae inserted at middle third of structure .................. 8
8. Dorsal margin of the paramere almost straight, with setae covering the apical half of structure. ........ L. pia (Fairchild & Hertig, 1961)
8°. Dorsal margin of the paramere curve, with setae restricted to apical fifth of structure. ........ L. limafalcaoae (Wolff & Galati, 2002)

Biogeography

The species of the series pia are restricted to northwestern South America, with the exception of L. pia, which occurs from Costa Rica to Bolivia. L. pia presents the widest altitudinal range within the series, occurring from 100 to 1900 m a.s.l. (Pérez et al. 1991, Alexander et al. 1995), whereas L. suapiensis, L. tihuiilenis, and L. tocaniensis are found from 1000 to 1500 m a.s.l in Bolivia and Peru (Le Pont et al. 1997, Cáceres et al. 2001). The known distributions of the other species are based on single records. L. reclusa has been recorded at 1360 m a.s.l. in Peru (Fernandez and Rogers 1991). L. limafalcaoae is endemic to the Central Cordillera of the Andes in Colombia, where it lives in small caves at 2,060 m a.s.l. (Wolff and Galati 2002). L. emberai n. sp. is present in the foothills of the Serranía del Baudó on the Colombian Pacific coast at 100 m a.s.l.

Little is known about the ecological preferences of these species. Nevertheless, almost all the members of the series coexist sympatrically, with up to five species of the verrucarum group (Alexander et al. 1992) present in the Andes. L. suapiensis, L. tihuiilenis and L. tocaniensis are the only members of the series that have been found in sympatry in Bolivia and Peru (Le Pont et al. 1997, Cáceres et al. 2001).

The latitudinal and altitudinal distribution of the series pia suggests that it is of Andean origin. The speciation and distribution of the series could be the result of geological and climatic events that affected the flora and fauna of the region (Bejarano et al. 2003a). One of the most plausible explanations is the isolation of ancestral populations in humid forest refugia during the Quaternary period (Young 1979, Haffer 1982). This process was driven by climatic changes characterized by cyclical periods of glaciation and thawing (Peltier 1994) that caused several species to become extinct, whereas others survived unchanged and some populations diverged at the subspecies and species level. Young (1979) suggested that the majority of Colombian phlebotomine species originated in the cis-Andean region (east of the Andes), where L. pia has been recorded. This author also suggested that some Lutzomyia species could have crossed the Andes via the River Marañon Valley in southern Ecuador and northern Peru during interglacial periods, when primary forests occupied the now dry valleys (Young 1979). Some evidence for this last route comes from the finding of pia series females in the Marañón region of Peru (Cáceres et al. 1995).

Although less probable, a trans-Andean origin (i.e., one west of the Andes) should also be considered for some species of the series such as L. emberai n. sp. given its presence on the Pacific Coast of Colombia. However, it is also possible that L. emberai n. sp. and L. pia reached the Pacific region by means of inter-Andean valleys during humid periods when the forested zones coalesced. Additionally, the occurrence of L. pia in Panama and Costa Rica could have been facilitated by the appearance of the Isthmus of
Panama that permitted dispersion of species between South and Central America. The exploration of little-studied areas within the Andes could provide new records that contribute to the knowledge of the genesis of the series pia.

Medical Importance

To date, none of the taxa that comprise the series pia have been directly incriminated in the transmission of pathogenic agents to man. Nevertheless, several species exhibit anthropophilic tendencies throughout their distribution (Zeledón et al. 1985, Le Pont et al. 1989, Alexander et al. 1992, Barreto et al. 1997, Cáceres et al. 2001, Agudelo et al. 2002). L. suapiensis, L. tihuiliensis, and L. tocaniensis are recognized by their anthropophilic behavior in Leishmania-endemic areas of Bolivia and Peru (Le Pont et al. 1997, Cáceres et al. 2001). The strongest evidence for a possible vectorial role for L. pia is from Colombia, where in addition to biting man it has been shown to be susceptible to infection with Leishmania braziliensis (Warburg et al. 1991). Experimentally infected females presented a high percentage of parasites in the stomodeal valve and the anterior midgut, suggesting that they are potentially capable of transmitting the parasite by bite (Killick-Kendrick 1990). Despite the anthropophily of L. pia, slight behavioral differences have been observed among Colombian populations. Warburg et al. (1991) found that L. pia in Valle del Cauca in the Western Cordillera did not attempt to bite immediately after settling, whereas Zuleta (2002) observed that in Valle de Aburrá (central Cordillera) it began to bite as soon as it landed on human skin. These subtle bionomic differences merit more detailed investigation, given that they could reflect evolutionary divergences within the species.

Acknowledgments

We are grateful to Eunice A. Bianchi Galati (Universidade de São Paulo—USP) for valuable comments and suggestions on the manuscript. We are equally grateful to the anonymous reviewers for useful comments.

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