

## *Lutzomyia diamantinensis* sp. nov., a new phlebotomine species (Diptera: Psychodidae) from a quartzite cave in Diamantina, state of Minas Gerais, Brazil

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*A new species of Brazilian phlebotomine sandfly found in Brazil, municipality of Diamantina, state of Minas Gerais, is described based on males and females collected in a quartzite cave. The body of spermathecae is continuous to the individual duct, lanky and tapering at the end, with conical shaped, not striated and presenting the head with dense setae. The male presents gonostyle with four spines and a small subterminal seta and gonocoxite with one group of persistent setae. The paramere is simple with a group of small setae on the dorsal apex. The morphological features of this new species permit its inclusion in the migonei group.*

Key words: *Lutzomyia diamantinensis* sp. nov. - sandfly - cave - Diamantina

Sandflies (Diptera: Psychodidae) are small insects belonging to the Phlebotominae subfamily, many of them known vectors of *Leishmania* to humans and other mammals (Lane 1993). They often take shelter in tree trunks, animal burrows under leaves on the ground, in the canopy of trees, in cracks in rocks and in caves (Young & Duncan 1994).

Caves are ecotones very different from the external environment and present in limestone, sandstone and quartzite formations. Sandflies are constantly found among the insects that live in or visit the caves or their adjacent environments and new species of this group have been described from these ecotypes (Alves et al. 2008, Carvalho et al. 2010, 2011). In Brazil, studies on the phlebotomine fauna in caves are very scarce. With the recent expansion of ecotourism and the lack of management programs for access to these environments, valuable geological, paleontological and/or biological information may have been lost due to predatory human exploitation of these caves.

This study sought to contribute to knowledge of Brazilian biodiversity, by describing a new species of phlebotomine sandfly captured in a cave known as Gruta do Salitre, located in the municipality of Diamantina, state of Minas Gerais (MG), Brazil.

### MATERIALS AND METHODS

The new species is described based on 10 females and 10 males. The sandflies were mounted on slides in Berlese fluid, measured with a binocular Olympus CH-2 microscope fitted with a micrometer objective and the drawings

were done with the help of a *camera lucida*. All measurements are in micrometers. The nomenclature and classification is that proposed by Young and Duncan (1994).

### RESULTS

*Lutzomyia diamantinensis* sp. nov. Barata,  
Serra-e-Meira and Carvalho  
(Figs 1-8)

*Male - Holotype* - Sandfly of medium size, measurement ca.  $2.967 (2.953 \pm 173.9; n = 9)$  in length, general colour light brown.

*Head (Fig. 1)*:  $621 (581 \pm 10.5; n = 9)$  long and  $326 (300 \pm 13.9; n = 7)$  wide. Head length/head width ratio  $1.90: 1 (1.93 \pm 0.08; n = 7)$ . Clypeus  $119 (126 \pm 8.0; n = 10)$  long; clypeus length/head length ratio  $0.19: 1 (0.22: 1 \pm 0.01; n = 9)$ . Eye  $153 (148 \pm 6.0; n = 8)$  long and  $99 (89 + 6.5; n = 8)$  wide; eye length/head length  $0.25: 1 (0.26: 1 \pm 0.01; n = 8)$ . Interocular distance  $122 (126 \pm 5.4; n = 9)$ . Labrum-epipharynx (LE)  $218 (217 \pm 7.0; n = 9)$ . LE/head length  $0.35: 1 (0.37 \pm 0.01; n = 9)$ . Antenna with simple and short ascoid. Antennomere lengths: AIII  $272 (275 \pm 10.6; n = 9)$ ; AIV  $139 (129 \pm 5.2; n = 9)$ ; AV  $136 (132 \pm 4.4; n = 9)$ ; AXV < AXVI (AXV < AXVI;  $n = 5$ ). Antennal formula: 2/III-XIII (2/III-XIII;  $n = 4$ ). AIII, AIV, AV, AXII, AXIII, AXIV, AXV, AXVI with papilla; ratios: AIII/head length  $0.44: 1 (0.47: 1 \pm 0.01; n = 9)$ ; AIII/LE  $1.25: 1 (1.27: 1 \pm 0.04; n = 9)$ . Palpal formula 1.4.2.3.5 [1.4.2.3.5;  $n = 7$  and 1.(2,4).3.5;  $n = 1$ ]. Palpomere lengths: P1  $44 (43 \pm 2.4; n = 9)$ ; P2  $136 (129 \pm 6.6; n = 9)$ ; P3  $163 (157 \pm 3.8; n = 8)$ ; P4  $112 (123 \pm 3.6; n = 8)$ ; P5  $391 (402 \pm 16.4; n = 7)$ . Newstead spines implanted in the median region of the third palpomere.

*Ventrocervical sensillae*: present.

*Thorax*: proepimeral setae present, 3-2 [(3-2;  $n = 3$ ), (3-3;  $n = 2$ ), (2-2;  $n = 3$ ), (2-1;  $n = 1$ )] and anepisternal superior setae present, 5-5 [(5-5;  $n = 2$ ), (6-4;  $n = 3$ ), (4-3;  $n = 1$ ), (4-4;  $n = 1$ ), (6-5;  $n = 1$ ), (6-6;  $n = 1$ )]; setae on

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the anterior region of the katepisternum present. Wing (Fig. 3) measurement 1.835 ( $1.762 \pm 52.0$ ;  $n = 9$ ) long and 442 ( $434 \pm 22.1$ ;  $n = 9$ ) at maximum width. Length/width ratio 4.2: 1 ( $4.1: 1 \pm 0.14$ ;  $n = 9$ ). Length of the vein sections: R<sub>5</sub> 1.145 ( $1.118 \pm 39.1$ ;  $n = 9$ ); alpha 331 ( $327 \pm 31.6$ ;  $n = 9$ ); beta 262 ( $256 \pm 15.7$ ;  $n = 9$ ); gamma 235 ( $284 \pm 12.3$ ;  $n = 9$ ); delta 110 ( $106 \pm 238$ ;  $n = 9$ ). The specimens used to describe the male had their legs damaged, except for one specimen which showed the following measures: anterior leg: femur 704; tibia 966; tarsomeres I 607 and tarsomeres II + III + IV + V 607; posterior leg: femur 800; tibia 1.325 tarsomere I 828 and tarsomeres II + III + IV + V 828.

**Abdomen:** terminalia (Fig. 5): gonostyle 119 ( $119 \pm 4.8$ ;  $n = 9$ ) long, with four spines: one apical, one upper external, implanted near of the apical, one lower external and one internal spine implanted on the same level than the lower external. Subterminal seta present. Abdominal tergites without papillae. Gonocoxite 207 ( $202 \pm 7.4$ ;  $n = 9$ ) long and 68 ( $69 \pm 5.5$ ;  $n = 9$ ) wide, with a group of 10-12 setae. Paramere broad at the base and narrowing to the apex where it shaped a head consisting of a group of small bristles remnants on the dorsal face of the same. Lateral lobe rounded 235 ( $219 \pm 7.8$ ;  $n = 9$ ) long and 20 ( $23 \pm 3.5$ ;  $n = 9$ ) wide. Lateral lobe/gonocoxite ratio 1.14: 1 ( $1.09 \pm 0.03$ ;  $n = 9$ ). Conical aedeagus. Genital filament (Fig. 6) 634 ( $635 \pm 39.0$ ;  $n = 9$ ) long and genital pump 153 ( $150 \pm 5.9$ ;  $n = 9$ ). Genital filament/genital pump ratio 4.14: 1 ( $4.24 \pm 0.33$ ;  $n = 9$ ). Type of genital filaments slender and unchanged at the end.

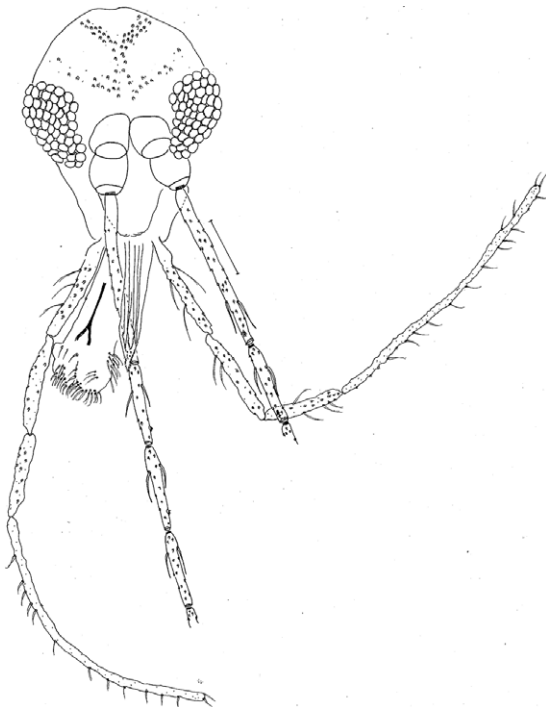


Fig. 1: *Lutzomyia diamantinensis* sp. nov. (paratype male 90.048). Head, frontal view. Bar = 100  $\mu$ m.

**Female - Allotype** - Sandfly of medium size, measuring ca. 3.422 ( $3.084 \pm 243.2$ ;  $n = 9$ ) in length. Colouration as in the male.

**Head (Fig. 2):** 759 ( $706 \pm 33.1$ ;  $n = 9$ ) long and 386 ( $363 \pm 10.6$ ;  $n = 7$ ) wide. Head length/head width ratio 1.96: 1 ( $1.96 \pm 0.06$ ;  $n = 7$ ). Clypeus 146 ( $135 \pm 5.2$ ;  $n = 9$ ) long; clypeus length/head length ratio 0.19: 1 ( $0.19: 1 \pm 0.01$ ;  $n = 7$ ). Eye 187 ( $172 \pm 10.0$ ;  $n = 8$ ) long and 126 ( $113 \pm 7.1$ ;  $n = 8$ ) wide; eye length/head length 0.25 ( $0.24: 1 \pm 0.01$ ;  $n = 7$ ). Interocular distance 133 ( $142 \pm 5.1$ ;  $n = 8$ ). LE 330 ( $297 \pm 16.8$ ;  $n = 7$ ). LE/head length 0.42: 1 ( $0.42 \pm 0.01$ ;  $n = 7$ ). Antenna with simple ascoid which almost reaches the apex of flagellomeres. Antennal formula: 2/III-XIV, 1/XV (2/III-XIV, 1/XV;  $n = 6$ ). Antennomere (lost in the allotype) lengths: AIII ( $247 \pm 15.0$ ;  $n = 8$ ); AIV ( $112 \pm 7.5$ ;  $n = 8$ ); AV ( $114 \pm 6.9$ ;  $n = 8$ ); (AXV < AXVI;  $n = 7$ ). AIII, AIV, AV, AX, AXI, AXII, AXIII, AXIV, AXV and AXVI with papilla; ratios: AIII/head length (0.35:  $1 \pm 0.01$   $n = 6$ ); AIII/LE (0.83:  $1 \pm 0.02$ ;  $n = 6$ ). Palpal formula 1.4.2.3.5 ( $n = 10$ ). Palpomere lengths: P1 51 ( $48 \pm 4.0$ ;  $n = 9$ ); P2 167 ( $148 \pm 8.6$ ;  $n = 9$ ); P3 187 ( $174 \pm 11.6$ ;  $n = 9$ ); P4 146 ( $131 \pm 13.6$ ;  $n = 9$ ); P5 469 ( $425 \pm 40.7$ ;  $n = 9$ ). Newstead spines implanted in the median region of the third palpomere. Cibarium with four posterior (horizontal) teeth well



Fig. 2: *Lutzomyia diamantinensis* sp. nov. (allotype female 90.049). Head, frontal view. Bar = 100  $\mu$ m.

developed and individualized, not fused in their base. The anterior (vertical) teeth are few and situated below the posterior teeth, forming an arch (Fig. 7). The sclerotized area is well defined and the sclerotized arch is complete. Unarmed pharynx. Lacinia of the maxilla with five external teeth in a single longitudinal row.

*Ventricercal sensillae*: present.

*Thorax*: proepimeral setae present, 3-2 [(3-2; n = 4), (3-3; n = 3), (4-2; n = 2)] and anepisternal superior setae present, 5-4 [(5-4; n = 3); (6-4; n = 2); (8-6; n = 2); (8-7; n = 2)]; setae on the anterior region of the katepisternum present. Wing (Fig. 4) measurement 2.194 (2.065 ± 103.0; n = 9) long and 593 (558 ± 45.2; n = 9) at maximum width. Length/width ratio 3.70:1 (3.71: 1 ± 0.14). Length of the vein sections: R<sub>5</sub> 1.435 (1.314 ± 95.8; n = 9); alpha 483 (434 ± 62.0; n = 9); beta 304 (284 ± 15.8; n = 9); gamma 359 (324 ± 25.9; n = 9); delta 221 (189 ± 45.1; n = 9). The specimens used to describe the female had their legs damaged. Legs, anterior, median and posterior, respectively: femur 769 ± 60.0 (n = 4), 787 ± 36.5 (n = 3), 879 ± 28.7 (n = 3); tibia 893 ± 68.9 (n = 4), 1.072 ± 82.9 (n = 3), 1.139 ± 41.5 (n = 3); tarsomere I 584 ± 28.6 (n = 3), 621 ± 58.0 (n = 2), 791 ± 34.7 (n = 3); tarsomeres II + III + IV + V 717 ± 23.7 (n = 3), 677 ± 38.9 (n = 2), 810 ± 39.8 (n = 3).

*Abdomen*: spermathecae (Fig. 8): 58 (60 ± 4.7; n = 9) long by 10 (10; n = 9) at maximum width. The spermathecae is continuous with the individual duct, very long, narrow and tapering towards the end, with a conical shape, not striated and presenting a terminal knob with dense setae. The common sperm ducts are at least three times longer than the individual ducts. Both ducts

are not striated. The individual ducts have small invaginations. The common duct is wide. The individual duct 82 (80 ± 7.7; n = 5) long and the common duct 272 (323 ± 48.1; n = 3) long. Cercus 102 (108 ± 5.2; n = 9) long.

*Types* - Holotype male (90.055) and allotype female (90.049) collected with HP light trap in a limestone cave (Gruta do Salitre) in the locality of Monte Cristo, Diamantina, together with nine male (90.046-90.048, 90.056-90.060, 90.071) and nine female paratypes (90.050, 90.054, 90.063, 90.065-90.070). The type-material is deposited in the Coleção de Flebotomíneos of the René Rachou Research Centre/Oswaldo Cruz Foundation, Belo Horizonte (MG).

*Etymology* - The name *Lu. diamantinensis* sp. nov. has been given in honour of the municipality of Diamantina, an important historical city of colonial Brazil, type-locality of the new phlebotomine species.

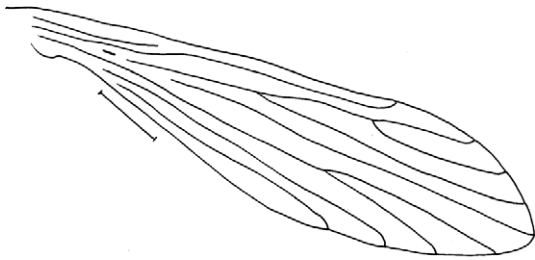


Fig. 3: *Lutzomyia diamantinensis* sp. nov. (paratype male 90.048). Wing. Bar = 250 µm.



Fig. 4: *Lutzomyia diamantinensis* sp. nov. (paratype female 90.050). Wing. Bar = 250 µm.



Fig. 5: *Lutzomyia diamantinensis* sp. nov. (holotype 90.055). Terminalia. Bar = 100 µm.

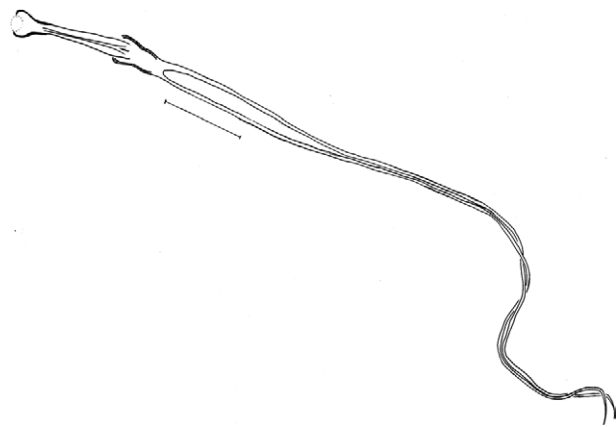


Fig. 6: *Lutzomyia diamantinensis* sp. nov. (paratype male 90.047). Genital pump and filaments. Bar = 100 µm.

**DISCUSSION**

The morphological features of the new species permit its inclusion in the *migonei* group. According to the classification proposed by Young and Duncan (1994) this group presents 24 species: *Lutzomyia lenti* (Mangabeira 1938), *Lutzomyia carmelinoi* Ryan, Frahia, Lainson & Shaw 1986, *Lutzomyia edwardsi* (Mangabeira 1941), *Lutzomyia paca*e (Floch & Abonnenc 1943), *Lutzomyia gruta* Ryan 1986, *Lutzomyia rabelloi* Galati & Gomes 1992, *Lutzomyia migonei* (França 1920), *Lutzomyia cortezezzii* (Brèthes 1923), *Lutzomyia sallesi* (Galvão & Coutinho 1939), *Lutzomyia corumbaensis* Galati, Nunes, Oshiro & Rego 1989, *Lutzomyia tupynambai* (Mangabeira 1942), *Lutzomyia bahiensis* (Mangabeira & Sherlock 1961), *Lutzomyia callipyga* Martins & Silva 1965, *Lutzomyia costalimai* (Mangabeira 1942), *Lutzomyia petropolitana* Martins & Silva 1968, *Lutzomyia willamsi* (Damasceno, Causey & Arouck 1945), *Lutzomyia termitophila* Martins, Falcão & Silva 1964, *Lutzomyia sericea* (Floch & Abonnenc 1944), *Lutzomyia andersoni* Le Pont & Desjeux 1988, *Lutzomyia evandroi* (Costa Lima & Antunes 1936), *Lutzomyia firmatoi* (Barretto, Martins & Pellegrino 1956), *Lutzomyia baculus* Martins, Falcão & Silva 1965, *Lutzomyia dubitans* Sherlock 1962 and *Lutzomyia walkeri* (Newstead 1914). Other species described later were added to the above list, part of the *migonei* group: *Lutzomyia cerradincola* Galati, Nunes, Oshiro & Dorval 1995, *Lutzomyia aldafalcao*e Santos, Andrade Filho & Honer, 2001, *Lutzomyia vaniae* (Galati, Fonseca

& Marassá 2007) and *Lutzomyia spelunca* (Carvalho, Brazil, Sanguinette & Andrade-Filho 2011) (Galati et al. 1995, 2007, Santos et al. 2001, Carvalho et al. 2011).

The species belonging to this group have in common antennas with simple ascoids, which in females almost reaching the base of the subsequent flagellomere, that are short in this group, the ascoids of males in this group may have varying size, usually shorter than the female, fifth palpomere longer than the sum of the third and fourth together. The males have four spines on the gonostyle, which present a pre-apical setae and one group of long bristles on the gonocoxite, except *Lu. edwardsi* which presents two tufts of bristles. The paramere of this group presents various forms and may be single, bifurcated or with a tooth-shaped process on the ventral part and may also present at its back side, a row of bristles or a spiniform bristle. Tip of filaments ducts may be modified or not. Lateral lobe is unarmed. The females have four horizontal teeth and a row of vertical teeth on the cibarium, spermathecae annulated or not, ducts varying in length. Other features are very variable and common to the other groups and/or genus and are not criteria that define this particular group (Young & Duncan 1994).

*Lu. diamantinensis* can be distinguished from other species by the following morphological characteristics: in males, although the paramere resembles that of *Lu. tupynambai* and *Lu. petropolitana*, it differs in not presenting an internal suture and having a boot like shape (Fig. 5), the gonocoxite present a dense tuft of long bristles (10-

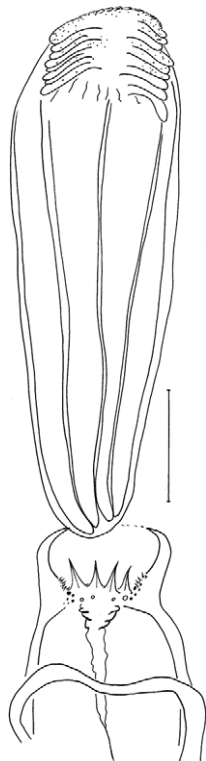


Fig 7: *Lutzomyia diamantinensis* sp. nov. (allotype 90.049). Pharynx and cibarium. Bar = 100 µm.

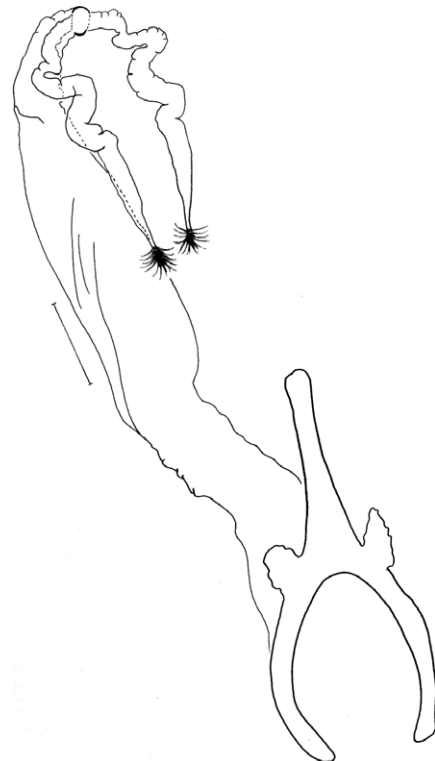


Fig 8: *Lutzomyia diamantinensis* sp. nov. (paratype female 90.050). Spermathecae. Bar = 100 µm.



12). The arrangement of the gonostyle spines is similar to that of the other species of the group, with one apical, one upper external, implanted near the apical, one lower external and one internal spine implanted at the same level as the lower external one. The subterminal seta is present. The females have spermathecae similar to those of *Lu. gruta*, but are distinguished by the very wide and long common duct, at least three times of the individual ducts. The spermathecae are very peculiar, very long, narrow and tapering towards the end, conical shape, not striated and presenting a terminal knob with dense setae.

The association of the sexes of *Lu. diamantinensis* was established by the simultaneous catches of males and females in the usual places of collection (the photic and aphotic zones of Gruta do Salitre) and in the absence of any unidentifiable species. Extragenital and genital features were also used for the correlation of the two sexes.

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