Phlebotomine sand flies from Bolivia: Morphological description of Pintomyia (Pifanomyia) veintemillasi n. sp. from the sub-Andean region

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Abstract

Pintomyia (Pifanomyia) veintemillasi n. sp., is described as a new anthropophilic sand fly species based on morphological diagnostic characteristics, which allow differentiating it from the closely related species *Pintomyia (Pifanomyia) nevesi* and *Pintomyia (Pifanomyia) maranonensis* within the Evansi series. This species was collected in the Marimonos mountain range, at around 900 m altitude in the Municipality of Palos Blancos, Sud Yungas Province, Department of La Paz, Bolivia, and was misidentified in the past as *Pi*. (*Pif*.) *nevesi.*

Background

Phlebotomine sand flies are natural vectors of around 30 kinetoplastids of the genus *Leishmania*, secondarily of the bacteria *Bartonella bacilliformis*, and of some arboviruses. The medical importance of sand flies is mostly related to their ability to transmit to humans at least 20 well-known *Leishmania* species, causing different clinical forms of leishmaniasis, with around of two million people affected worldwide every year [1, 2].

For neotropical sandflies, in the 90s, Duncan and Young published what was considered the most complete compilation of the American phlebotomine sand flies at the time [1, 3]. Later on, Galati proposed a new classification based on phylogenetic analysis which elevated to genus most of the subgenera proposed in the earliest classification [1, 2] and is currently, the most accepted approach. According to Galati, the current list of American sandflies, includes 546 species (17 fossil) distributed in 23 genera and 20 subgenera, 3 groups of species, and 30 series of species [2]. The taxonomic groups and series proposed are frequently used by sand fly taxonomists in the New World despite are not familiar in the Old World and thus, are not always recognized.

The genera *Pintomyia* Costa Lima, 1932 includes the subgenera *Pintomyia* Ortiz & Scorza 1963 with 8 species, and *Pifanomyia* Ortiz & Scorza 1963 with 72 species. Most of species the subgenera *Pifanomyia* are distributed in the Andean region [2]. Within this subgenera, *Pintomyia* (*Pifanomyia*) *nuneztovari anglesi* Le Pont & Desjeux, 1984 (Syn. *Pintomyia* (*Pifanomyia*) *nuneztovari* Ortiz, 1954) has been incriminated in the transmission of zoonotic leishmaniasis in domestic environments in the sub-Andean area of Bolivia, as the proven vector of *Leishmania amazonensis* and a suspected vector of *Leishmania braziliensis* [1, 2]. This subgenus has been divided in seven series of sand fly species. Among them, the Evansi series Galati 2003, comprises four species including *Pintomyia* (*Pifanomyia*) *nevesi* Damasceno & Arouck, 1956 [2], that is widely present in South America and is distributed from the foothills of Andes mountains of Colombia, Ecuador, Peru, and Bolivia to the lowlands in the five Brazilian states of Para, Acre, Rondonia, Maranhao, and Mato Grosso [2]. In the past, this species was included in the Verrucarum group [3].

Pintomyia (Pif.) nevesi is a sylvatic species, originally described in 1956 from two male specimens collected in a station on the Capim River in the state of Para, Brazil [4]. Unfortunately, in the original

description, some measurements were imprecise, making the morphological identification of specimens difficult and raising doubts as to their true identity.

Similarly, a few decades ago, from a collection of 12 specimens from the sub-Andean foothills of La Paz, Bolivia, a female was identified as *Pi.* (*Pif.*) *nevesi* [5]. But lacking information on the males that were collected and given the distance and difference in altitude compared to the type-locality of *Pi.* (*Pif.*) *nevesi* in Brazil, the report from Bolivia raised controversy.

Later, Dujardin & Le Pont (2004) examined the intraspecific variation of the traditional morphometric traits used for the identification of sand flies [6]. The comparison of metric characteristics was carried out from 12 species from Bolivia between conspecific populations belonging to the same eco-region and between conspecific populations from different eco-regions. Unfortunately, when comparing the populations of *Pi.* (*Pif.*) *nevesi* from the sub-Andean and Amazonian regions, anomalies forced these specimens to be excluded from this study.

Afterwards, a molecular study was carried out by Beati et al. (2004) [6] to identify seven sand fly species from the Verrucarum group (*sensu* Young & Duncan, 1994) from Peru, where two females and one male *Pi.* (*Pif.*) *nevesi* from the sub-Andean region of San Martin, were included. However, the female specimens were eliminated from the study, due to significantly different sequences in comparison to clearly identified male *Pi.* (*Pif.*) *nevesi*.

Considering the dubious identity of sub-Andean *Pi.* (*Pif.*) *nevesi* from Bolivia and Peru, the identity of past Bolivian sand fly collections identified as *Pi. nevesi* was thoroughly revised. Based on the identification key of the species of the Evansi series [2], we provide both male and female descriptions of a new species close to *Pi. nevesi* and the taxonomic characteristics of the related species.

Materials And Methods

Entomological collections were carried out, both, at the ground and canopy level, using CDC light-traps as well as protected human bait, in a subtropical humid forest, in 1982. Both male and female individuals of the species of interest were initially sorted based on the similarity of the pleura pigmentation. Sand fly specimens were sorted using a dissecting microscope and placed in 70% ethanol. For species identification, sand flies were treated using the Abonnenc technique [8] and mounted in Euparal media. Ten to 12 specimens of each sex and its related species were used for the morphometric analysis. The measurements are given in mm (a = average). The classification, characters nomenclature, genera, and subgenera name abbreviations followed Galati [2] and Galati et al. [9].

Results

Pintomyia (*Pifanomyia*) *veintemillasi* Martinez, Leon, Mihalca, Dujardin & Le Pont n. sp. (Figs. 1-17)

Type material and depository

Male Holotype: Marimonos station, in the Marimonos mountain range, Bolivia. Municipality of Palos Blancos (15°35’02’S-67°15’07’W), altitude 900m, Sud Yungas province, Department of La Paz (CBF La Paz), Bolivia.

Female allotype: *idem* holotype (CBF, La Paz).

Paratypes. 5 males and 5 females, collected from the same locality, *in* coll. CBF, La Paz, Bolivia; UPAMETROP/IINSAD, La Paz, Bolivia; MNHN, Paris, France.

Description

Male Holotype. Sand fly of small size, generally gray in color, mesonotum and abdominal tergites light brown, measuring 2 mm from the tip of the labrum to the end of the gonocoxite.

Head. Length 0.298 (0.294-0.324) including the clypeus; maximum width 0.286 (0.284-0.321). Head length/head width ratio 1.04. Interocular distance 0.101 (0.85-0.101) equal to the diameter of 5.5 facets. Labrum-epipharynx (LE) length 0.192 (0.181-0.214) from the edge of the clypeus. Antennal flagellomeres: fI 0.171 (0.160-0.201), fII + fIII = 0.077 + 0.087. Ratio fI / LE = 0.89 (a = 0.91). Short ascoids, only visible on the first flagellomeres. Third flagellomere without sensilla in rosette in the preapical region. Palpus: total length 0.591 (0.548-0.673), respective lengths of the palpomeres: P1 0.030 (0.025-0.039); P2 0.105 (0.102-0.127); P3 0.114 (0.108-0.125); P4 0.079 (0.077-0.095); P5 0.263 (0.202-0.311); palpal formula: 1-4-(2-3)-5 or 1-4-(3-2)-5. Cibarium armed with a row of tiny, sharp, slightly sclerotized teeth of irregular size, and an anterior, discontinuous row of dot-shaped denticles. Sclerotized arch complete, well chitinized; pigmented patch, triangular, striated, extending anteriorly. Narrow pharynx, length 0.140, maximum width 0.037, with posterior denticulate scales.

Thorax. Length 0.429. Unpigmented paratergite. Clear pleuras, except for the basal region of the katepisternum and katepimeron, slightly pigmented. Upper anepisternal bristles: 6+7 (from 5 to 10 per pleura) and proepimeral bristles: 2+4 (2 to 4 per pleura). Wings: length 1.385 (1.385-1.560), maximum width 0.405 (0.376-0.440). Length/width ratio 3.41. Wing indices: *alpha* 0.305 (0.289-0.361), *beta* 0.153 (0.136-0.174), *gamma* 0.202 (0.200-0.244), *delta* 0.099 (0.079-0.151); *alpha/beta* ratio 1.99 (a = 2.06). Legs lengths of the femur, tibia, and basitarsus, respectively: front legs 0.505-0.530-0.325; middle legs 0.549-0.660-0.420 and hind legs 0.580-0.815-0.490.

Abdomen. Length 1.097 including the gonocoxite. Tergal papillae present from 3rd to 7th tergite. Second sternite with 7 to 9 bristles on each apical region. Gonocoxite: length 0.172 (0.172-0.193), maximum width 0.055, without perennial bristles. Gonostyle length 0.104 (0.100-0.114) bearing 4 strong spines: an apical spine, an upper external spine inserted in the distal third, and the spines inferior and internal implanted in mid-segment; presence of a fine spiniform, subterminal bristle. Paramere: length 0.150 (0.145-0.157), measured from the dorsal margin; rectangular base, then posteriorly finger-shaped, garnished with erect bristles, curved anteriorly. Aedeagus conical, well sclerotized, with the tip reaching the finger-shaped part of the paramere. Lateral lobe without perennial bristles, similar in size to that of the

gonocoxite; length 0.177 (0.161-0.188); sub-median lamella, without particularities. Genital pump (GP) length 0.111 (0.100-0.119; a = 0.109); genital filaments (GF) with finely striated apical third, and smooth apex, length 0.434 (0.427-0.490; a = 0.448), duct/pump ratio GF/GP 3.90 (3.90-4.38; a = 4.12).

Female allotype. Sand fly identical in coloration to that of the male, measuring 2.45 mm from the tip of the labrum to the end of the cerci.

Head. Length including clypeus 0.363 (0.337-0.363), maximum width 0.347 (0.325-0.348); Head length/head width ratio 1.04. Interocular distance 0.129 (0.108-0.129), equal to the diameter of 6 facets. Labrum-epipharynx (LE) length 0.296 (0.275-0.296) from edge of clypeus; maxillary laciniae: 6 external teeth and 23 internal. Antennas: length of flagellomeres, fI 0.178 (0.166-0.183), fII + fIII = 0.083 + 0.084; ratio fI / LE = 0.60 (a = 0.61). In fIII, absence of papilla in rosette in preapical region. Ascoids strong and short, well staggered, not reaching the apical third. Palpus: total length 0.771 (0.636-0.771). The palp segments measuring respectively: P1 0.039 (0.033-0.040); P2 0.149 (0.134-0.154); P3 0.146 (0.134-0.146); P4 0.102 (0.083-0.102); P5 0.335 (0.236-0.335); Palpal formula: 1-4-(2-3)-5, segments 2 and 3 subequal; Newstead's sensilla not visible. Cibarium with 4 equidistant acute horizontal teeth of equal size; a row of 9 to 12 vertical teeth, and several lateral, dot shaped, grouped teeth. Very distinct sclerotized area, thickened anteriorly, triangular, narrowed at the level of the chitinous arch; this last, rounded and continuous from one edge of the cibarium to the other, surpassing it laterally. Pharynx: with the most posterior scales, denticulate; length 0.165, maximum width 0.070.

Thorax. Length 0.574. Pigmentation identical to that of the male. Upper anepisternal bristles: 7+11 (7 to 12 per pleura) and proepimeral bristles 5+6 (2 to 6 per pleura). Wings: length 1.760 (1.649-1.795), maximum width 0.525 (0.485-0.545); Length/width ratio 3.35. Wing indices: *alpha* 0.434 (0.410-0.491), *beta* 0.195 (0.187-0.224), *gamma* 0.310 (0.237-0.320), and *delta* 0.186 (0.175-0.227), *alpha/beta* ratio 2.22 (a = 2.16). Legs lengths of the femur, tibia, and basitarsus, respectively: front legs 0.660-0.630-0.375; middle legs 0.673-0.775-0.450 and hind legs 0.725-0.990-0.545.

Abdomen. Length 1.225. Second sternite with 8-10 bristles on each apical half. Spermatheca, like a pear-shaped sac, finely wrinkled transversely, head deeply invaginated in the spermatheca, with pluri-lobed apex (Table 2), fan-shaped; Importantly, the head is slightly offset from the axis of the spermatheca, and therefore most often emerges laterally after mounting. Common duct and individual ducts not measurable, but a long common duct presence.

Etymology: Dedicated to Dr. Felix Veintemillas for his great contribution to the research and control of infectious and parasitic diseases in Bolivia, the main Bolivian reference on leishmaniasis in the mid-20th century.

Taxonomic discussion

Specimens of the new species *Pi. veintemillasi* were collected through entomological surveys carried out, both, at the ground and canopy level, using CDC light-traps as well as protected human bait. Misidentified

as *Pi. (pif.) nevesi, Pi. (pif.) veintemillasi* was the most anthropophilic species at the canopy level and the third most anthropophilic on the ground, after *Psyhodopygus carrerai carrerai* (vector of *Leishmania braziliensis*) and *Psychodopygus hirsutus hirsutus* (identified naturally infected by flagellates) [10]. Two species of *Pifanomyia* were collected in sympatry [10]; both presented similar size and a gray pigmentation: *Pintomyia* (*Pifanomyia*) *serrana* Damasceno & Arouck, 1949 (Serrana series) and *Pi.* (*Pif.*) *nuneztovari* Ortiz, 1954 (*incertae sedis*), syn. *Pi.* (*Pif.*) *nuneztovari anglesi* Le Pont & Desjeux 1984 [2].

Following Galati [2], the new species, *Pi.* (*Pif.*) *veintemillasi*, belongs to the subgenus *Pifanomyia*, Evansi series, according to the following morphological characteristics: palp P5 to be larger in length than P3 in both sexes, which is a relevant feature of the subgenus *Pifanomyia*; in the males, presence of short ascoids, reaching half of the segment, a strong 4-spines gonostyle, and the presence of a preapical bristle; a gonocoxite without tuft, and a simple paramere; in the females, ascoids reaching the subapical region of the segment, an hypopharynx with deep apicolateral teeth, and a short row of external teeth for the maxillae; a cibarium with 4 horizontal teeth, the vertical teeth fitting into one or two transverse rows; a complete chitinous arch, and a narrow, triangular, pigmented area; spermathecae with a long common duct, vesicular body, without apical ring, transversely wrinkled, and well individualized head. Finally, the absence of sensilla in rosette on the 3rd flagellomer fIII in *Pi.* (*Pif.*) *veintemillasi* confirms it belongs to the Evansi series.

The morphologically similar species in this series are *Pi.* (*Pif.*) *nevesi* and *Pi.* (*Pif.*) *maranonensis* Galati et al., 1995 [11], noting the latter is only present on the Ecuador-Peru border. *Pi. (Pif.) veintemillasi* is different from *Pi.* (*Pif.*) *nevesi* based on larger size and pigmentation of the lower part of the pleura. Significantly shorter genital filaments in the new species shed light on the doubts that puzzled about the criteria for identifying *Pi.* (*Pif.*) *nevesi* [6]: a genital filaments/genital pump (GF/GP) ratio = 4.12 notably shorter than ratio= 4.60 established for *Pi.* (*Pif.*) *nevesi* (Table 1). In the *Pi.* (*Pif.*) *veintemillasi* female (Table 2), the labrum-epipharynx (LE) is larger; the spermatheca has a purse-like shape and is finely wrinkled, with a multi-lobed head, opening out laterally, while in *Pi.* (*Pif.*) *nevesi*, it is oblong, with a fine head emerging at the apex. *Pi. (Pif.). veintemillasi* is only present in Bolivia in the forest regions of the sub-Andean cordillera (300-1400m altitude) belonging to the wide basin of the high tributaries of the Amazon, whereas *Pi.* (*Pif.*) *nevesi* has been found far from this area in the lowland region.

The difference is more evident with *Pi.* (*Pif.*) *maranonensis,* which has a pigmented paratergite; the male has a tuft of bristles at the gonocoxite, and the GF/GP ratio = 4.73; the female of this species has a rounded spermatheca, with a head strongly invaginated to mid-body, and emerging laterally (Tables 1-2).

A female of *Pi.* (*Pif.*) *nevesi* was identified by Velasco & Martins (1974) from the foothills of La Paz, in Huacakarita (altitude 800m), approximately 50 km from Marimonos in the sub-Andean region [5]. Its metric data correspond to *Pi.* (*Pif.*) *veintemillasi,* confirming the presence of a cryptic species that is distinct from *Pi.* (*Pif.*) *nevesi.*

As a final remark, in Bolivia, on the La Paz-Riberalta transect, *Pi.* (*Pif.*) *veintemillasi* and *Pi.* (*Pif.*) *nevesi* presented an allopatric distribution, confined respectively to the sub-Andean (300-1400m) and the Amazonian regions (130-200m), separated by 400 km of the Beni plain.

In Peru, in the region of San Martin, with a geographical configuration similar to that of the department of La Paz in Bolivia, *Pi.* (*Pif.*) *veintemillasi* could represent the species, similar to *Pi.* (*Pif.*) *nevesi*, reported by Beati et al. [7]. Taxonomical studies through more recent entomological collections should give light to the possible presence of this species in Peru and neighboring countries.

This morphological description was based on mounted biological material, without available specimens for molecular studies, nevertheless, will be develop further field surveys in sub-Andean region, to collect specimens for molecular studies needed to complete the genetic identity of this new species in comparison with similar taxa.

Conclusions

Pintomyia (*Pif.*) *veintemillasi,* is described as a new sub-Andean sand fly, that was during decades misidentified as *Pi.* (*Pif.*) *nevesi* from the Amazon lowlands, due to its similar morphology. The high anthropophily of this species, could be related to an eventual vector role.

Abbreviations

CBF (Colección Boliviana de Fauna); MNHN (Museum National d'Histoire Naturelle); UPAMETROP/IINSAD (Unidad de Parasitología, Medicina Tropical y Medio Ambiente; Instituto de Investigación en Salud y Desarrollo); LEMMT (Medical Entomology & Tropical Medicine Laboratory, Universidad San Francisco de Quito).

Declarations

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Availability of data and materials

The data that support the description are available from the corresponding author.

Author contributions

JPD, conceptualization, analyzing data. EM, RL, FLP: entomological collections, species identification. EM, RL, ADM, FLP: wrote the paper. EM, RL, ADM, JPD, FLP: edited and approved the final manuscript.

Ethics approval and consent to participate

Non applicable

Consent for publication

Non applicable

Competing interests

The authors declare no conflicts of interest.

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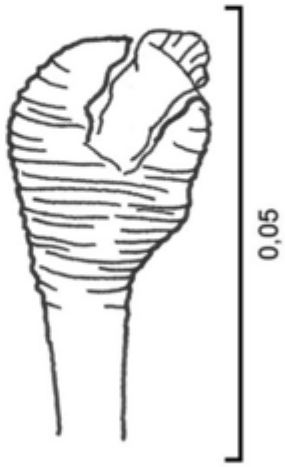
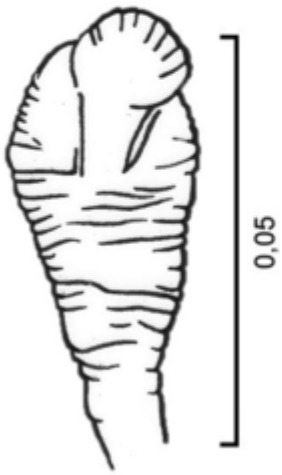
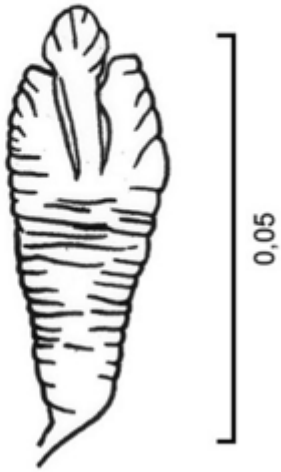
Tables

Table 1. Male taxonomical characters between *Pi. (Pif.) veintemillasi* n. sp. and related species.

Character	*Pi. (Pif.) maranonensis* Zumba (Ecuador) 900 m	*Pi. (Pif.) veintemillasi* n. sp. Marimonos (Bolivia) 900 m	*Pi. (Pif.) nevesi* Orthon river (Bolivia) 144 m
Pleura	- Gray - Pigmented paratergite - Superior anepisternum and posterior anepimeron pigmented - Base of katepimeron and katespisternum pigmented	- Light color - Clear paratergite - Base of katepimeron and katespisternum pigmented	- Light color - Clear paratergite
Gonocoxite	- Tuft, 2-4 bristles	- Naked, without perennial bristles	- Naked, without perennial bristles
GF	a = 0.525 (0.490 – 0.560)	a = 0.448 (0.487 – 0.490)	a = 0.519 (0.495 – 0.543)
GP	a = 0.110 (0.104 – 0.118)	a = 0.109 (0.100 – 0.119)	a = 0.112 (0.106 – 0.117)
GF/GP	a = 4.73 (4.43 – 5.38)	a = 4.12 (3.90 – 4.38)	a = 4.60 (4.44 – 4.86)

Measurements for 10 specimens of each population (a, average; GF, genital filaments; GP, genital pump)

Table 2. Female taxonomical characters between *Pi. (Pif.) veintemillasi* n. sp. and related species.

Character	*Pi. (Pif.) maranonensis*	*Pi. (Pif.) veintemillasi* n. sp.	*Pi. (Pif.) nevesi*
Pleura	*Idem*, males	*Idem*, males	*Idem*, males
Labrum-Epipharinx LE	a = 0.345 (0.330 – 0.375) n = 12	a = 0.284 (0.275 – 0.296) n = 11	a = 0.255 (0.233 – 0.266) n = 12
Spermatheca			

a, average; n, number of measured individuals. Scale 0.05.

Figures

Figure 1

Pintomyia (Pif.) veintemillasi n. sp. Male. 1, Head frontal view; 2, Antennomer fII; 3, Cibarium and pharynx; 4, Sternite 2; 5, Genitalia profile; 6, Paramere and aedeagus, in lateral view; 7, Genital pump and genital filaments; 8, Wing. Scales, in mm.

Figure 2

Pintomyia (Pif.) veintemillasi n. sp. Female. 9, Head frontal view; 10, Antennomer fII; 11, Cibarium and pharynx; 12, Cibarium; 13, laciniae of the maxillae; 14, Sternite 2; 15, Spermatheca; 16, rapid view of a complete genitalia; 17, Wing. Scales, in mm.