Taxonomic revision of phlebotomine sand fly species in the series davisi and panamensis of the subgenus Psychodopygus Mangabeira, 1941 (Diptera: Psychodidae: Phlebotominae)

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Several species of the subgenus Psychodopygus Mangabeira, 1941 are known to be leishmaniosis vectors in Brazil. Some of them are morphologically similar, which makes their identification quite difficult concerning epidemiological studies. The aim of the current work is to study the morphology of adult specimens of the subgenus Psychodopygus, in accordance with the morphological similarity and still taking into account the epidemiological importance of some species. Thus 11 species have been studied, including four subspecies of adult specimens deposited in the phlebotomine collection of Centro de Pesquisas René Rachou-Fiocruz. Morphological characters found in the literature and new features observed in this study were recorded in a taxonomic discussion format. These characters make it easy to separate such species. Four taxa, previously considered as subspecies, were raised to the category of species.

Key words: Psychodopygus - Psychodidae - Phlebotominae

Certain members of the Psychodidae family are notorious vectors of leishmaniosis (Ward 1977), bartonellosis (Schultz 1968), and arboviroses (Tesh et al. 1974), diseases that have brought about many problems for humans living in Neotropical and other regions (Young 1979). The subfamily Phlebotominae includes all the Psychodidae of medical significance. Some authors consider the phlebotomine to be a separate family of their own, the family Phlebotomidae (Williams 1993).

From the perspective of leishmaniosis control, the study of the taxonomy and geographical distribution of species of phlebotomine is of great importance, due to their role in disease transmission, the prevalence of which has increased significantly in the past few decades. This is a result of constant alterations in the natural environment, caused mainly by the increasing process of urbanization, which has generated various endemic sites of concentration in non-forest regions, conferring a new and previously unknown feature on the epidemiology of this disease (Ryan et al. 1987). Some species in this subgenus are anthropophilic and identified as vectors of leishmaniosis, since several species are anthropophilic and identified as vectors of this disease (Ryan et al. 1987). Some species in this sub-
genus share morphological similarities, which bring about errors in their identification, leading, in some areas, to an incorrect epidemiological understanding of the disease. With the aim of helping to correct specific identification of the vectors, which is a pre-requisite for application of opportune strategies of prevention and control, in this paper we review and give new diagnostic characters for the separation of 11 species in this medically important subgenus allocated in the series davisi and panamensis.

MATERIALS AND METHODS

The material used for the taxonomic revision of species in the subgenus Psychodopygus belong to the scientific collection of phlebotomines located at Centro de Pesquisas René Rachou-Fiocruz, Belo Horizonte, state of Minas Gerais.

In the present work, 11 species were studied, including four subspecies of the subgenus Psychodopygus, according to the classification proposed by Young and Duncan (1994) who group them in four series: squamiventris, guyanensis, davisi, and panamensis.

The investigation of adult specimens was accomplished with an optical microscope, besides consulting the literature on this species. The priority in this revision was to observe structures proposed by Cipa Group (1991). Based on the analyzed structures, all the observable features in the specimens were noted in the form of a taxonomic evaluation. Thus, a characterization of closely related species was carried out in order to facilitate their recognition. Based on the analyzed structures, all the observable features in the specimens were noted in the form of a taxonomic evaluation. Thus, a characterization of closely related species was carried out in order to facilitate their recognition.

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The updated geographical distribution of every species involved in this study can be found in the publication of Aguiar and Medeiros (2003).

REMARKS

This research revised and validated 11 species as belonging to the subgenus Psychodopygus. Three of them, Lutzomyia (Psychodopygus) davisi (Root, 1934), Lutzomyia (Psychodopygus) amazonensis (Root, 1934), and Lutzomyia (Psychodopygus) claustrei Abonnenc, Leger and Fauran, 1979, are allocated in the series davisi by Young and Duncan (1994), the other eight belong to the series panamensis. Four of these, considered as subspecies, are raised to the category of species: Lutzomyia (Psychodopygus) hirsuta (Mangabeira, 1942) (new status), Lutzomyia (Psychodopygus) nicaraguensis (Fairchild & Hertig, 1961) (new status), Lutzomyia (Psychodopygus) carrerai (new status) (Barretto, 1946), and Lutzomyia (Psychodopygus) thula Young. 1979 (new status). Beside these, we include in this review Lutzomyia (Psychodopygus) ayrozai (Barretto & Coutinho, 1940), Lutzomyia (Psychodopygus) paraensis (Costa Lima, 1941), Lutzomyia (Psychodopygus) yucumensis (Le Pont, Caillard, Tibayrenc, Desjeux, 1986), and Lutzomyia (Psychodopygus) panamensis (Shannon, 1926). We do not include Lutzomyia (Psychodopygus) llanosmartinsi (Fraiha & Ward, 1980) and Lutzomyia (Psychodopygus) recurva Young, 1973, which have a paramere with a characteristic arched dorsal arm and are easily distinguished. Moreover, we do not deal with Lutzomyia (Psychodopygus) fairchildi Barretto, 1966 and Lutzomyia (Psychodopygus) nootica Young, 1973, because the available material was not enough for comparison. However, new features observed in 11 species studied, which make easy to distinguish them, justify this work. Such characters, with other found in literature are presented in a taxonomic discussion, so that the most similar species are discussed together, thus simplifying the process of distinguishing among them.

TAXONOMIC DISCUSSION

Species in the series davisi

Lutzomyia (Psychodopygus) amazonensis
Lutzomyia (Psychodopygus) davisi
Lutzomyia (Psychodopygus) claustrei
Lutzomyia (Psychodopygus) panamensis

In this series are included three species were males show five well developed spines on style. It is possible to distinguish each one by the following features: L. (P.) amazonensis shows a lateral-inferior dilation in aedeagus in the shape of “cheeks” (Fig. 1), while in L. (P.) davisi, it is not dilated (Fraiha et al. 1980) (Fig. 2). Aedeagus in L. (P.) claustrei shows no distinctive characteristics. Genital filaments in L. (P.) amazonensis are shorter than those of L. (P.) davisi (Fraiha & Ward 1980b). Another morphological feature that is crucial to separate among the three species is the paramere: in L. (P.) amazonensis, the lateral arm is thinner and the tuft is practically inserted in its whole extent; in L. (P.) davisi, this lateral arm is thicker and the tuft is uniformly inserted in the distal margin; in L. (P.) claustrei, the lateral arm is very characteristic and notably stout, as well as the principal lobe, with a more leafy tuft than the two other species (Abonnenc et al. 1979) (Fig. 3).

Another morphological character as thorax pigmentation and disposition of the spines on the style were observed and may help in the distinction of the three species, mainly when analyzed with the other characters mentioned above. However, these features may not be used individually in the distinction of the species of this series, since they present variations in several of the specimens studied. Regarding the thorax pigmentation, the follow-
also not to be considered, because variations may occur inclusive at the same specimen. However, some specimens of L. (P) clastrei present the inferior external spine and the internal one, inserted at the same level while in L. (P) amazonensis and L. (P) davisi the internal spine is more basal than the external one.

Females of the three species mentioned may be differentiated by spermathecae, common, and individual sperm ducts. L. (P) davisi may be easily distinguished from, L. (P) amazonensis and L. (P) clastrei by the angle formed at the junction of the individual sperm ducts and by the chitinous area at the beginning of the rough part of the common sperm duct, that is absent in the two other species; furthermore L. (P) davisi shows the common sperm duct longer and more chitinous than those of the two other species; L. (P) amazonensis and L. (P) clastrei show basically the same size of common sperm duct, but they may be distinguished by the number of rings of the spermathecae, being approximately eight in L. (P) clastrei and about ten in L. (P) amazonensis, such as in L. (P) davisi. L. (P) amazonensis and L. (P) clastrei may also be distinguished by the length of the individual sperm ducts and the spermathecae: in L. (P) amazonensis, the formers are approximately as long as the spermathecae and in L. (P) clastrei they are shorter than spermathecae (Le Pont & Pajot 1980, Young & Rogers 1984).

Regarding the cibarium, the three species may be differentiated, mainly by the vertical teeth. However, it was observed that this character may present variation between specimens of the same species. Even so, this character may be useful to distinguish the females of these species, mainly when observed with other characters. The disposition and length of the vertical teeth present the following tendency: in L. (P) amazonensis, are small and uniformly distributed into one to three transversal rows (Fig. 6); in L. (P) davisi, most of the vertical teeth are small, but with a double longitudinal row with larger vertical teeth (Fig. 5); in L. (P) clastrei, vertical median teeth are remarkably larger than the other ones and they generally form one or two longitudinal rows smaller than those in L. (P) davisi (Fig. 4).

**Species in the series panamensis**

*Lutzomyia (Psychodopygus) ayrozai*
*Lutzomyia (Psychodopygus) paraensis*
*Lutzomyia (Psychodopygus) hirsuta* (New Status)
*Lutzomyia (Psychodopygus) nicaraguensis* (New Status)
*Lutzomyia (Psychodopygus) carrerae* (New Status)
*Lutzomyia (Psychodopygus) thula* (New Status)
*Lutzomyia (Psychodopygus) yucumensis*
*Lutzomyia (Psychodopygus) panamensis*

The species listed above belong to the group, were males show style with three strong spines and an atrophied one and the basal part of the paramere without an arched dorsal arm, but it shows a wide basal lobe, with one or two tufts of long bristles. This lobe shows a lateral digitiform prolongation, also called lateral arm. Females are very similar as for the cibarium and spermathecae (Martins et al. 1978). A taxonomic discussion of this group is yielded below.
gonocoxite, with a round distal end of the principal lobe, covered with few slightly curved and thick bristles (around 20) and turned towards the genitalia apex (Fig. 7). Lateral arm is slightly strangulated in its base, covered with straight, curved, and thin bristles, inserted in the superior and external side of the appendix. Still in paramere, just ahead the lateral arm insertion, about five straight, thin and short bristles are inserted on the inferior side of the principal lobe of the paramere (Barretto & Coutinho 1940).

The spines in the style are slightly different displayed when compared to the similar species: one apical; the external superior inserted in the distal end; the external inferior and the atrophied internal inserted in the middle of the structure (Barretto 1966). Genital filaments are thin and very long, with internally dilated ends.

Females may be distinguished mainly by the cibarium (Fig. 8), with four short horizontal teeth inclined towards the center at an angle of 45° and are frequently difficult to be visualized (Christensen & Fairchild 1971). Vertical teeth present approximately the same size and form a regular arch at the base of horizontal teeth. Spermatheca has 10-12 rings, with individual sperm ducts never exceeding the size of spermatheca and common sperm ducts with the apical third having a very visible striation.

The most closely related species of *L. (P.) ayrozai* is *L. (P.) paraensis*. The two males can be easily distinguished: *L. (P.) paraensis* has a very peculiar thorax pigmentation – mesonotum, first urotergite, procoxae, and pleurae just above these latter dark-brown, sharply contrasting with the rest of the body (Costa Lima 1941). Moreover, in the paramere, a little evident pigmented crest may be observed externally on its middle part (Fig. 9), from which three

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**Fig. 4:** *Lutzomyia (Psychodopygus) claustrei*. Arrow showing the disposition of vertical teeth in the cibarium.

**Fig. 5:** *Lutzomyia (Psychodopygus) davisi*. Arrow showing the disposition of vertical teeth in the cibarium.

**Fig. 6:** *Lutzomyia (Psychodopygus) amazonensis*. Arrow showing the disposition of vertical teeth in the cibarium.
spines and the internal one closely inserted at the distal third (Barretto 1966).

*L. (P.) paraensis* females: characterized by common sperm duct which presents an sclerotized area as an inverted “V-shape” in the junction between smooth and rough parts (Fig. 10); it is also present in *L. (P.) yucumensis* and *L. (P.) carrerae*, but distinguishable from the latter by thorax pigmentation (Young 1979, Le Pont et al. 1986) which is completely brown, and also by a very peculiar cibarium with four horizontal teeth which are separated by a curved projection that extends until the pigmented area. Those horizontal teeth are a little inclined towards the center or not inclined at all, differently from that of *L. (P.) ayrozai*. Vertical teeth form two longitudinal rows, separated by the equal space among horizontal teeth, which arise at the exact site of their insertion. Each row is followed by other vertical teeth, all of them of the same size (Fairchild & Hertig 1951, Martins et al. 1973).

**Fig. 7:** *Lutzomyia (Psychodopygus) ayrozai*. Arrow showing the principal lobe of the paramere.

**Fig. 8:** *Lutzomyia (Psychodopygus) ayrozai*. Arrow showing the cibarium.

**Fig. 9:** *Lutzomyia (Psychodopygus) paraensis*. Arrow showing the pigmented crest of the paramere.

**Fig. 10:** *Lutzomyia (Psychodopygus) paraensis*. Arrow showing the inverted “V-shaped” in the junction between smooth and rough portions of the common sperm duct.

Spiniform bristles arise almost parallel to the paramere, while in other similar species this region is covered by short, thin, down-turned spiniform bristles. They are in higher number and never inserted in the crest (Martins et al. 1973). The principal lobe of the paramere has three or four thick irregular rows of bristles which apexes exceed the top of lateral arm (Barretto 1946). The lateral arm is broadened and slightly curved at the distal part, where a tuft of short bristles is inserted. In contrast to *L. (P.) ayrozai*, *L. (P.) paraensis* lateral lobe is slightly longer than coxite. Style shows one apical spine, two external
L. (P.) hirsuta and L. (P.) nicaraguensis are two species that belong to the group under discussion and are easily distinguished. Males show a characteristic paramere, were basal lobe shows a row of very wide bristles uniformly inserted in the distal end, and they usually exceed the lateral arm length (Mangabeira 1942). Females present the junction between individual and common sperm ducts wider, with an increasing diameter of the individual duct from the proximal to the distal region (Young 1979). Distinction between males of these species is based on the paramere features, such as the presence of bristles at the principal lobe, inserted on the posterior margin, which are more numerous and not very “spatula-shaped” in L. (P.) nicaraguensis. In contrast, L. (P.) hirsuta, shows less numerous and “leaf-shaped” bristles (Fig. 11). Only in L. (P.) nicaraguensis, at the dorsal-basal region of paramere, there is a strong spine-shaped bristle inserted in a prominent tubercle (Fairchild & Hertig 1961). A ventral tuft, inserted in a more basal region of paramere is also different in both species, consisting of more numerous bristles in the latter species.

The lateral arm in L. (P.) nicaraguensis is external and straight, with a thin spine at the distal region and a bristle inserted in its implantation. In L. (P.) hirsuta, however, it is more internally inserted with no bristle in its implantation. Style in L. (P.) nicaraguensis shows the external inferior spine inserted in a median position, while, in the other species this same bristle is inserted at the distal third (Barretto 1966).

Females may be easily separated by procoxae pigmentation, which is as dark as the mesonotum and the other coxae are pale in L. (P.) nicaraguensis and, in L. (P.) hirsuta, all coxae are pale (Young 1979) (Fig. 12). The cibarium is very similar in both species, however vertical teeth may distinguish them: in L. (P.) nicaraguensis, they form two longitudinal rows, and in L. (P.) hirsuta are more irregular. Common sperm duct is also very similar between these two species, however, it is partially rough in L. (P.) hirsuta and presents incomplete lines, while in L. (P.) nicaraguensis it is formed by distinct punctuations.

According to Lane (1988), the use of the subspecies concept would mirror a more philosophical part of the specialist than a biological particularity of the sand fly. The concept of subspecies refers to a differently known geographical population (Futuyma 2002); nevertheless, it is quite difficult or, yet, impossible, to know if those populations would mate in case of contact among them, so that such concept is strictly arbitrary, once many subspecies are taken into account with no biological evidence to support such fact (Lane 1988).

Thus, we believe that there are no biological and ecological data that prove the hypothesis of L. hirsuta and L. nicaraguensis as subspecies. Moreover, morphological differences between both species are very clear, mainly in males, which would be enough for a reproductive isolation between them, in case of sympatry.

L. (P.) carrerai and Lutzomyia (Psychodopygus) thula are easily distinguished from the other species of the same series. Males show paramere with thin bristles displayed in numerous rows and thorax pigmentation completely pale. Females are separated from others of the same group by a combination of cibarium and spermatheca features together with a pale yellowish thorax. Distinction between males of these species has only been possible by comparing them with females and observing their geographical distribution, since they are morphologically similar, with no peculiar feature for distinction. However we believe that such as L. (P.) hirsuta and L. (P.) nicaraguensis, L. (P.) carrerai, and L. (P.) thula present no biological and ecological data that prove the hypothesis of subspecies.

To distinguish females an association of features can be used: the length of the labrum with the combination of the length of the pedicel and the first flagellomere. In L. (P.) carrerai the labrum is shorter than the sum of the pedicel and the first flagellomere. In contrast, the labrum of L. (P.) thula is much longer and is greater than the sum.
of the pedicel + flagellomere I (Young 1979). As for the common sperm duct, in \( L. (P.) \) \( thula \) there is no inverted “V”, formed by striation in the junction between the smooth and rough portions, which is normally observed in \( L. (P.) \) \( carrerae \). This inverted “V” weakly extends towards the apex of the sperm common duct, becoming Y-shaped, which separates \( L. (P.) \) \( carrerae \) from \( L. (P.) \) \( yucumensis \) and \( L. (P.) \) \( paraensis \) (Le Pont et al. 1986). Another feature that may be used for distinction of these species is maxillary palpus length: much longer in \( L. (P.) \) \( thula \) than in \( L. (P.) \) \( carrerae \), but for the males, morphometry is needed or further studies to detect possible differences.

\( L. (P.) \) \( yucumensis \) is easily distinguished from others of the same group by the paramere. Bristles inserted in the margin of the principal lobe are curved and leaf-shaped, not exceeding the lateral arm, form a posterior tuft, becoming “paint-brush-shaped”. Lateral arm is long and thin, with a low number of bristles inserted both at the distal part and at the appendix base. Another feature used for distinction is the lateral lobe, which is very long, exceeding coxite length. Aedeagus is long and the distal part is dark. Females are more closely related to the species \( L. (P.) \) \( carrerae \), being easily separated by thorax pigmentation, which shows a dark mesonotum in \( L. (P.) \) \( yucumensis \), contrasting with a pale colored mesonotum of \( L. (P.) \) \( carrerae \). Concerning common sperm duct, it has already been distinguished in the discussion above on \( L. (P.) \) \( carrerae \).

Cibarium shows four horizontal teeth, the two median ones are larger and distant from each other. In the space between median horizontal teeth two longitudinal and irregular rows of vertical teeth arise, becoming divergent in the anterior position. On each side of the cibarium, anterior to the horizontal teeth, there is a thick row of short vertical teeth (Le Pont et al. 1986, Young 1979).

\( L. (P.) \) \( panamensis \) is the only species of the subgenus \( Psychodopygus \) in which the males show the basal lobe of paramere with a double tuft of bristles (Fig. 13): one proximal tuft with long, strong, and curved bristles and another distal one with short and thin bristles. Lateral arm, which arises from the basal region of the distal lobe, is curved towards the coxite with two subterminal strong spines, thorax with a very dark mesonotum, contrasting with the pleura, style shows an apical spine, an upper external and a lower external spine inserted at the distal third and the internal-atrophied spine implanted between the apical and the upper external spines.

Females are distinguished among other characteristics by a very characteristic cibarium, with four long horizontal teeth. The distance between the two median horizontal teeth is greater than that from the median to the lateral ones. Vertical teeth are large and irregular (Fig. 14). Spermatheca shows an asymmetric distal segment and 12 nearly overlapping rings, longer than the individual sperm ducts (Shannon 1926, Barretto 1946, 1966, Fairchild & Hertig 1951).

This taxonomic revision presents the difficulties of distinguishing some species of the subgenus \( Psychodopygus \) due to their morphological similarities, mainly concerning females. However using the main morphological features described in the literature as well as adding some new characteristics it was possible to distinguish them, providing an accurate and specific identification. Nevertheless, not all species of the subgenus \( Psychodopygus \) were studied in this work, but virtually all species reviewed here are present in several places in Brazil, and many of them are part of the fauna of endemic regions for leishmaniosis, making necessary and important to accurately and specifically identify these species.

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