IS *LUTZOMYIA SERRANA* (DIPTERA, PSYCHODIDAE, PHLEBOTOMINAE) PRESENT IN ECUADOR ?

DUJARDIN J.P.*, LE PONT F.** & MARTINEZ E.***

Summary :

We describe a multivariate metric comparison of three sandfly species showing strong differences in size, which questions the geographical distribution of one of them. Two species are represented by a single population (*L. robusta* and *L. guilvardae*) and one by two populations (*L. serrana*). All of them belong to the series *serrana* (Diptera, Psychodidae, Phlebotominae). The morphometric data confirm that *L. guilvardae* is a distinct species. However, they suggest that *L. robusta* and *L. serrana* in Ecuador are the same taxon, and that it is distinguishable from the population of *L. serrana* in Bolivia. A multilocus enzyme electrophoresis analysis comparing *L. serrana* in Bolivia and *L. robusta* in Ecuador adds further evidence that these two populations are distinct species. Thus, our data seem to indicate that, in Ecuador, the population previously identified as *L. serrana* is actually the same species as the allopatric population previously identified as *L. robusta*. Accepting *L. serrana* in Ecuador as small-sized *L. robusta*, the resulting geographic distribution of this latter becomes in closer agreement with ecology and epidemiology.

KEY WORDS : *Lutzomyia serrana*, *L. robusta*, *L. guilvardae*, Bergmann's rule, morphometry, isoenzymes.

Résumé : *LUTZOMYIA SERRANA* (DIPTERA, PSYCHODIDAE, PHLEBOTOMINAE) EST-IL PRÉSENT EN ÉQUATEUR ?

L'examen de la variabilité morphométrique de trois espèces de phlébotomes (Phlebotominae: *Lutzomyia*), décrites essentiellement sur la base de tailles très différentes, remet en cause la distribution géographique de l'une d'entre elles. Toutes trois appartiennent à la série *serrana* (sous-genre *Pifanomyia* Ortiz & Scorza). Deux de ces espèces (les plus "grandes") sont représentées chacune par un seul échantillon (*L. robusta*, *L. guilvardae*), la troisième (*L. serrana*) est composée de deux échantillons, l'un de Bolivie, l'autre d'Équateur. Puisque la taille fut l'un des critères principaux de la description de *L. guilvardae* et de *L. robusta*, notre analyse morphométrique tente d'éliminer son influence. Malgré cette correction, *L. robusta* et *L. guilvardae* conservent des différences métriques résiduelles très significatives, et sont ainsi validées a posteriori. De même, *L. robusta* est complètement distinct de l'échantillon bolivien de *L. serrana*. Toutefois, l'analyse morphométrique hors taille ne permet absolument pas de séparer *L. robusta* de l'échantillon équatorien de *L. serrana*. Ces relations sont renforcées par une étude isoenzymatique partielle (une seule comparaison), qui confirme une distance génétique importante entre *L. robusta* et l'échantillon bolivien de *L. serrana*. Nos analyses semblent donc bien valider *L. guilvardae* et *L. robusta*, mais elles jettent un doute sur l'échantillon équatorien de *L. serrana*. Ce dernier est en effet compatible avec une population de *L. robusta* en basse altitude. La distribution géographique nouvelle qui apparaît ainsi est plus conforme avec l'écologie (petites tailles en basse altitude) et l'épidémiologie.

MOTS CLÉS : *Lutzomyia serrana*, *L. robusta*, *L. guilvardae*, règle de Bergmann, morphométrie, isoenzymes.

INTRODUCTION

The series *serrana* (subgenus *Pifanomyia* Ortiz & Scorza) is defined by common morphological traits related to antenna and genitalia in males, to abdominal tergite VIII and spermathecae in females (Galati *et al.*, 1995). It contains one widely extent species, *Lutzomyia serrana* (Damasceno & Arouck, 1949), going from the Atlantic coast of Brazil to Mexico, and eight more local species (Fig. 1). Of them, *L. oresbia*

(Fairchild & Hertig, 1961) is limited to Central America, *L. odax* (Fairchild & Hertig, 1961), *L. ottolinai* (Ortiz & Scorza, 1963), *L. robusta* Galati, Caceres & Le Pont, 1995 and *L. guilvardae* Le Pont *et al.*, 1998 occupy limited geographic areas in South America, and *L. christophe* (Fairchild & Trapido, 1950), *L. orestes* (Fairchild & Trapido, 1950) and *L. novae* Gonzales and Garcia 1981 are insular species of Caribbean archipelago (Fig. 1).

L. robusta and *L. guilvardae* have been described as separate species mainly on the basis of clear-cut size differences from *L. serrana*. In Ecuador, *L. serrana* and *L. robusta* are separated by the Andes. Both taxa show a high degree of anthropophily and aggressiveness, biting day and night inside dwellings where transmission of leishmaniasis and bartonellosis occurs. *L. robusta* is a larger species than the coastal *L. serrana*,

* UMR IRD-CNRS 9926, Av. Agropolis, 911 BP 5045, 34032 Montpellier, France.

** IRD La Paz, CP 9214, La Paz, Bolivia.

*** Universidad Mayor San Andres, La Paz, Bolivia.

Correspondence : Jean-Pierre Dujardin.

Fig. 1. – Map of South and Central America showing composition and geographical extension of the known members of the series *serrana*. Numbers 1 and 2 show the location of *L. serrana* samples (dashed zone): 1, Union Berea (Nor Yungas province, Department of La Paz, Bolivia), also the collecting site of *L. guilvardae* (open circle) and 2, La Tablada (Esmeraldas province, Ecuador) on the Pacific coast. *L. robusta* (black circle) was collected at Zumba (Zamora Chinchipe province, Ecuador).

occurring at higher altitudes (900 m) in the Amazonian foothills of the Zamora Chinchipe province (Le Pont *et al.*, 1994; Galati *et al.*, 1995). Incidentally, its metric differences with *L. serrana* accord with “Bergmann’s rule”, which predicts an increasing of size with altitude, i.e. lower temperatures (Daly, 1985).

In other geographic areas, *L. serrana* is anthropophilic but shows less aggressivity and endophily. In the subandean area of Bolivia, it may be found in close sympatry with *L. guilvardae*. This latter is also a larger species than *L. serrana*, as described for male specimens (females are cryptic). These two species in Bolivia have no known epidemiological importance.

The global size differences among *L. robusta*, *L. serrana* and *L. guilvardae* could suggest that their shape divergence, whenever described, results from passive allometric variation within the same species. In the hypothesis that one group is a larger or smaller representative of the other, it is expected that allometric changes alone are responsible for possible differences in shape. This would justify to remove the allometric influence on shape changes, i.e. to evidence possible

shape differences unexplained by size variation (growth). We applied the technique combining the orthogonal projection method (Burnaby, 1966) and the common principal analysis (Flury, 1984; Airolidi & Flury, 1988; Klingenberg, 1996). We also performed a partial analysis based on multilocus enzyme electrophoresis which was possible only between two populations, the (Ecuadorian) *L. robusta* and the Bolivian sample of *L. serrana*.

MATERIALS AND METHODS

INSECTS

Our metric comparison concerns 37 male specimens. Bolivian specimens of *L. serrana* (ten males, “*serrana* YU”) and *L. guilvardae* (12 males) were collected together in the same light trap during the dry season of the year 1995 at Union Bera (Nor Yungas province, Department of La Paz, Bolivia) in the lowlands of the Yungas (600 m a.s.l.). Also eight specimens of Ecuadorian *L. serrana* were collected at La Tablada (Esmeraldas province, Ecuador) on the Pacific coast (“*serrana* PC”) in 1991. During the same year, *L. robusta* (7 males) were collected at Zumba, in the Amazonian foothills of Chinchipe province in Ecuador (900 m a.s.l.).

The isoenzymatic comparison between “*serrana* YU” (ten males) and *L. robusta* (seven males) used additional specimens of the same origin than above.

Lack of additional, live (or frozen), material made it impossible to perform other isoenzymatic comparisons. The remaining species of the series *serrana* could not be joined to the present analysis because of their rarity.

MORPHOMETRY

Using a camera lucida, we measured six non overlapping features belonging to three different anatomical structures: (i) the head: AIII – third antennal segment, and EP – epipharynx (ii) the wing: WW – wing width and WL – wing length (iii) the genitalia: GP – genital pump and LL – lateral lobe.

UNIVARIATE ANALYSIS

Separate analyzes of each character were limited to the computing of their mean, standard deviation, minimum and maximum values.

MULTIVARIATE ANALYSIS

To remove a common allometric trend among this set of characters, we used the common principal components model (Flury, 1984; Airolidi & Flury, 1988). In

this model, a single component is taken to account for common allometric variation, provided the coefficients of the eigenvector are large and positive, and the correlations between this single component and each variable are positive and significant (Klingenberg & Zimmerman, 1992). In the space of the log-transformed measurements, conspecific individuals are expected to be found along the straight line defined by this single component. Metric variation orthogonal to this direction is allometry-free by construction, and describes allometry-free shape differences among individuals (Burnaby, 1966).

Using the six measurements described above, or all the five combinations of five of them, the total sample was not compatible with the hypothesis of a common allometric axis (i.e. with the model of a common principal component). We therefore explored the 15 possible combinations of four measurements, and identified seven sets compatible with a common allometric axis model, we used one of them to illustrate the metric relationships between samples (a3, ww, pg, ll). The influence of within-group allometries was then removed by using residuals of an orthogonal projection of the data onto the first common principal component (Burnaby, 1996) – i.e., all the common principal components except the first one (Klingenberg, 1996). The resulting “allometry-free” variables were submitted to a canonical variate analysis (CAV), using four groups (“*serrana* YU”, “*serrana* PC”, *L. robusta* and *L. guilvardae*).

Size was compared using the first common principal axis, which represented a putative common axis of growth, or a common axis of allometric changes, some of them attributable to growth, other to evolutionary allometry.

Statistical significance of these analyses was estimated by the Wilks statistics (Wilks, 1932). Calculations used the following packages: STATA (Computing Resource Center, 1992), JMP (SAS Institute Inc. 1995) and ADE (Chessel & Dolédec, 1992).

ISOENZYME ELECTROPHORESIS

Eight enzymes were analyzed by isoenzyme electrophoresis on cellulose acetate for ten specimens of Bolivian *L. serrana* and six ones of *L. robusta*: PGM (phosphoglucumutase, EC 2.7.5.1), AP (alkaline phosphatase, EC 3.1.3.1), HK (hexokinase, EC 2.7.1.1), ALDH (aldehyde dehydrogenase 1.2.1.5), ME (malic enzyme, EC 1.1.1.40), IDH (isocitrate dehydrogenase, EC 1.1.1.42), MDH (malate dehydrogenase, EC 1.1.1.37) and GPI (glucose phosphate isomerase, EC 5.3.1.9). Migration and revelation techniques were derived from Dujardin & Tibayrenc (1985) and Richardson *et al.* (1986).

RESULTS

MORPHOMETRY

Metric properties for each character are given Table I. Multivariate analyses were illustrated with Figs 2 and 3. On the first common principal component (CPC-1), individuals were plotted according to their relative global size differences (Fig. 2). In the size-free canonical variate analysis (Fig. 3), three groups were distinguished on the first canonical factor (76 % contribution) and two groups on the second one (20 %). They did not reproduce the taxonomic classification, since *L. robusta* and “*serrana* PC” (Ecuador) largely overlapped and constituted one group (see “e” and “r”, Fig. 3). Thus, in the discriminant space representing 96 % of total size-independent variation, *L. guilvardae* represented an isolated group (see “g”, Fig. 3), “*serrana* YU” and “*serrana* PC” were clearly distinguished (see “b” and “e”, Fig. 3), but this latter (“*serrana* PC”) was not different from *L. robusta* (see “e” and “r”, Fig. 3). Size-free differentiation patterns could distinguish *L. guilvardae* from the remaining samples, but disclosed two unexpected results, (i) the important divergence between Bolivian (“*serrana* YU”) and Ecuadorian (“*serrana* PC”) samples of *L. serrana*, and (ii) the lack of separation of the Ecuadorian samples of *L. robusta* and *L. serrana* (“*serrana* PC”).

AIII	EP	WW	WL	GP	LL
Bolivian <i>L. Serrana</i>					
ME (SD)					
238 (11)	192 (8)	1,458 (40)	456 (21)	92 (4)	199 (5)
MN-MX					
221-255	180-206	1,380-1,540	410-480	87-100	192-210
Ecuadorian <i>L. Serrana</i>					
ME (SD)					
237 (7)	181 (13)	1,405 (119)	407 (37)	95 (6)	212 (14)
MN-MX					
229-250	159-200	1,230-1,620	370-475	85-100	188-230
<i>L. robusta</i>					
ME (SD)					
244 (11)	202 (9)	1,561 (93)	454 (23)	90 (6)	226 (7)
MN – MX					
229-256	187-216	1,450-1,700	430-490	80-100	217-236
<i>L. guilvardae</i>					
ME (SD)					
285 (11)	217 (12)	1,711 (90)	538 (41)	95 (4)	218 (11)
MN-MX					
270-305	190-234	1,530-1,810	475-600	87-100	207-245

Table I. – Metric properties for some head, wing and genitalia characters, all given in microns. ME, mean value; SD, standard deviation; MN, minimum; MX, maximum. AIII – third antennal segment, EP – epipharynx, WW – wing width, WL – wing length, GP – genital pump and LL – lateral lobe.

Fig. 2. – Range of variation of each group along the first common principal component, representing allometric change of size common to the four groups. Each box shows the group median separating the 25th and 75th quartiles, with the 10th and 90th quartiles shown as lines on the right and left sides of the box.

ISOENZYMES

Since ME, IDH and MDH each showed two distinct loci (*Me1*, *Me2*, *Idb1*, *Idb2*, *Mdb1*, *Mdb2*), a total of 11 loci was counted. *Pgm*, *Ap* and *Gpi* each showed four alleles. Two alleles were disclosed at the following loci: *Me1*, *Me2*, *Idb1*, *Idb2*, *Mdb1*, *Mdb2*, whereas no variation was apparent at *Hk* or *Aldb*. Allelic frequencies, observed and expected heterozygosities of Bolivian *L. serrana* and *L. robusta* (Ecuador) are detailed in Table II.

	<i>L. serrana</i> (Bolivia)	<i>L. robusta</i>
N	10	6
Observed (expected) H	0.233 (0.199)	0.266 (0.399)
Rate of gene polymorphism	0.36	0.73
Allelic frequencies		
<i>Pgm</i> -1	0.10	0.25
<i>Pgm</i> -2	0.60	0.67
<i>Pgm</i> -3	0.20	0.08
<i>Pgm</i> -4	0.10	0.00
<i>Ap</i> -1	0.00	0.17
<i>Ap</i> -2	0.15	0.42
<i>Ap</i> -3	0.45	0.42
<i>Ap</i> -4	0.40	0.00
<i>Hk</i>	1.00	1.00
<i>Aldb</i>	1.00	1.00
<i>Me1</i> -1	0.30	0.25
<i>Me1</i> -2	0.70	0.75
<i>Me2</i> -1	1.00	0.75
<i>Me2</i> -2	0.00	0.25
<i>Idb1</i> -1	0.00	0.17
<i>Idb1</i> -2	1.00	0.83
<i>Idb2</i> -1	1.00	0.58
<i>Idb2</i> -2	0.00	0.42
<i>Mdb1</i> -1	1.00	0.67
<i>Mdb1</i> -2	0.00	0.33
<i>Mdb2</i> -1	1.00	0.00
<i>Mdb2</i> -2	0.00	1.00
<i>Gpi</i> -1	0.15	0.33
<i>Gpi</i> -2	0.60	0.67
<i>Gpi</i> -3	0.20	0.00
<i>Gpi</i> -4	0.05	0.00

Table II. – Allelic frequencies at 11 loci for Bolivian *L. serrana* and for *L. robusta* (Zumba, Ecuador). Alleles are numbered according to the decreasing rate of migration on the gel. N, number of specimens. Expected H, expected heterozygosity (Nei, 1987). Rate of gene polymorphism, the proportion of loci presenting genetic variation.

Fig. 3. – Size-free canonical variate analyses comparing *Lutzomyia serrana* from Bolivia ("b"), *L. serrana* from the Pacific coast of southern Ecuador ("e"), *L. robusta* in the Amazonian area of southern Ecuador ("r") and *L. guilvardae* from Bolivia ("g"). Between brackets, percentage contribution of canonical factors to total variation. Polygons enclose specimens of each group.

The locus *Mdh2* was diagnostic between them (no shared alleles). The Nei's standard genetic distance was 0.20 ± 0.14 (Nei, 1987).

DISCUSSION

In the original descriptions of *L. robusta* (Galati *et al.*, 1995) and *L. guilvardae* (Le Pont *et al.*, 1998), size differences from *L. serrana* were disclosed by univariate analyses on raw data. We used multivariate analyses on log-transformed data. The logarithmic transformation allows discrimination on the basis of proportional differences rather than absolute differences (Pimentel, 1992). The multivariate approach allows to remove the statistical (not biological) correlation between morphological traits, and provides a way to extract size heterogeneity out of the total variation (see Materials & Methods).

The sample sizes used here were small and could question our results. However, this study compared different species, i.e. groups among which dispersion matrices are less likely to overlap than for conspecific populations (Marcus, 1990). In agreement with these considerations, the observed metric divergence between only seven specimens of *L. robusta* and ten ones of "*serrana* YU" was confirmed by multilocus enzyme electrophoresis.

The first common principal component reproduced the known size differences between *L. serrana*, *L. robusta* and *L. guilvardae*. Removal of size as a differentiation factor was then intended to reveal patterns of variation that would be less influenced by growth or environmental factors – hence more related to evolutionary processes (Hutcheson *et al.*, 1995) –, which is particularly useful for studies of geographic variation where ecological, climatic or altitudinal differences are apparent (Claridge & Gillham, 1992). In our samples, there was obvious evidence for such differences, since altitudes of collecting sites varied from sea level to 900 meters a. s. l., extending from Bolivia to Ecuador in various ecoclimatical environments.

Both size-in and size-free canonical variate analyses confirmed the morphological differences between the sympatric Bolivian *L. serrana* ("*serrana* YU") and *L. guilvardae*, bringing new arguments to consider the last one as a valid species. However, they failed to distinguish *L. robusta* and the coastal Ecuadorian *L. serrana* ("*serrana* PC"), questioning their taxonomic status. On the other hand, the "*serrana* PC" - *L. robusta* group was clearly distinguished from the Bolivian *L. serrana* (Fig. 3). In agreement with these morphometric comparisons, genetic differences between *L. robusta* and the Bolivian *L. serrana* ("*serrana* YU") were compatible with two species (Kreutzer, 1990;

Lanzaro & Warburg, 1995; Dujardin *et al.*, 1996). Nei's genetic distance value (0.20) was indeed much higher than the highest genetic distance (0.08) found between supposed geographic conspecific populations of sand flies (Dujardin *et al.*, 1997).

The recently described species, *L. guilvardae*, was collected in the same light trap as *L. serrana*, which discarded the hypothesis of environmental influence on size or the probability of different cohorts of the same species (Ogusuku *et al.*, 1993).

These results, assimilating the Ecuadorian *L. serrana* as small-sized *L. robusta*, were consistent with ecological considerations. According to the "Bergmann's rule" (Marcondes *et al.*, 1998), the altitudinal differences in Ecuador between the allopatric *L. serrana* and *L. robusta* could account for size differences within one single species, and suggested that the so-called *L. serrana* on the Pacific Coast of Ecuador could be actually small-sized *L. robusta*.

Our data also agreed with epidemiological considerations. Both Ecuadorian *L. serrana* and *L. robusta* are highly aggressive and endophilic sand flies, respectively in the coastal province of Manabi (Gomez *et al.*, 1994) and in the leishmaniasis focus of Zumba (Le Pont *et al.*, 1994), and both are candidate vectors of bartonellosis in the same regions: on the Pacific coast (Maldonado, 1931) and at Zumba (Cooper *et al.*, 1996; Caceres *et al.*, 1997). In Bolivia however, *L. serrana* is poorly endophilic and has no role in parasite transmission.

In sum, isoenzymatic and metric comparisons confirmed the validity of *L. robusta* as a distinct species from the Bolivian *L. serrana*. However, metric comparisons between *L. robusta* and the Ecuadorian *L. serrana* showed lack of consistent differences, suggesting the presence of *L. robusta* on the Pacific coast. Given the epidemiological importance of these species in Ecuador, further samples will need to be analyzed before any taxonomic revision is justified. As far, our data suggest that *L. robusta* is covering a larger geographic area than expected, actually corresponding to the endemic region for leishmaniasis and bartonellosis.

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