Journal of the American Mosquito Control Association, 14(3):277–282, 1998

GENETIC POLYMORPHISM OF MORPHOLOGICAL AND BIOCHEMICAL CHARACTERS IN A NATAL, BRAZIL, POPULATION OF *LUTZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE)

JABA MUKHOPADHYAY,[1] KASHINATH GHOSH,[1] ALFREDO C. R. AZEVEDO,[2] ELIZABETH F. RANGEL[2] AND LEONARD E. MUNSTERMANN[1]

ABSTRACT. The phlebotomine sand fly, *Lutzomyia longipalpis,* is the vector of visceral leishmaniasis in the New World. Variability in its tergal spot morphology has led to conflicting interpretations of the species status of the various forms. An *L. longipalpis* field population from eastern Brazil was found with three co-occurring morphological variations—1-spot, 2-spot, and an intermediate form. Genetic profiles were established for each form. Fifteen isoenzyme loci provided the data matrix for comparison of genetic variation among the forms. Spot patterns and isoenzyme frequencies fit Hardy-Weinberg expectations, and no significant differences in isoenzyme frequencies were associated with morphological phenotype. The spot phenotype appears to be a polymorphic character not related to genetic isolation or differentiation at the species level.

KEY WORDS *Lutzomyia longipalpis,* phlebotomine sand flies, systematics, morphology, isoenzymes, leishmaniasis, genetic variability, cryptic species

INTRODUCTION

In the Western Hemisphere, visceral leishmaniasis is caused by the protozoan, *Leishmania chagasi* Cunha and Chagas, and is always associated with the occurrence of the phlebotomine sand fly, *Lutzomyia longipalpis* Lutz and Neiva (Grimaldi et al. 1989). The suggestion that this sand fly is a cryptic species complex raised serious concerns of species identification and their roles in the transmission of leishmaniasis. *Lutzomyia longipalpis* occurs from southern Mexico to northern Argentina in discontinuous, patchy distributions (Young and Duncan 1994). However, the morphological variation in eastern Brazil was the 1st evidence cited for the presence of cryptic species within a species complex (Lane 1986). In addition, the spot morphology was postulated to be a marker of epidemiological significance when Ward et al. (1985, 1988) observed that the predominance of the 2-spot form occurred in the same regions of northeastern Brazil with a high incidence of leishmaniasis. Ward et al. also discovered intermediate forms in areas where the 1-spot and 2-spot forms occurred sympatrically and commented that the presence of intermediate forms indicated that this character is a genetic polymorphism in the species and cannot reliably differentiate sexually isolated populations (Ward et al. 1988).

Protein electrophoretic analysis of *L. longipalpis* has demonstrated substantial intraspecies variability at local levels (Morrison et al. 1995, Munstermann et al. 1998) as well as substantial genetic differentiation among colonies originating from different geographic areas (Lanzaro et al. 1993). Comparison of a broader array of laboratory strains with a field population (Mukhopadhyay et al. 1997) has led to a limitation of the systematic inferences initially proposed by Lanzaro et al. (1993) on the basis of genetic comparisons of 3 laboratory colonies. However, the multiple-species model that Lanzaro et al. proposed may yet be realized in some regions as more geographically dispersed field populations are analyzed.

Recently, we discovered the presence of 3 co-occurring morphological phenotypes (1-spot, 2-spot, intermediate form) of *L. longipalpis* in a visceral leishmaniasis endemic area in eastern Brazil, near the city of Natal. Since 1989, a dramatic increase in cases of visceral leishmaniasis has occurred in the urban and peri-urban zones of Natal (Jeronimo et al. 1994). In the present report, *L. longipalpis* genetic data and analyses are provided, 1st, for comparing isoenzyme profiles of the sand flies in this city with other populations in eastern Brazil and, 2nd, to lend factual support to the suggestion by Ward et al. (1988) that spot morphology is an intraspecific, polymorphic character. The analytical approach we followed serves as a model for relating morphological or physiological polymorphisms with underlying genetic relationships (including *L. longipalpis* from other regions of its distribution).

MATERIALS AND METHODS

Sand flies were obtained from Selmo Marinho, near Natal, Rio Grande do Norte, in eastern coastal Brazil by personnel of the Entomology Department of Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro. Flies were collected by mouth aspiration from the walls of domestic animal shelters. Female flies were retained by FIOCRUZ for other studies; the males were frozen in liquid nitrogen in the field

[1] Yale University School of Medicine, Laboratory of Epidemiology and Public Health, 60 College Street, New Haven, CT 06520-8034.

[2] Instituto Oswaldo Cruz, Department of Entomology, Rio de Janeiro, Brazil.

Fig. 1. Location of a morphologically heterogeneous population (Natal) of the phlebotomine sand fly, *Lutzomyia longipalpis*, relative to homogeneous populations of the 1-spot form (Lapinha Caves) and the 2-spot form (Jacobina). Small circles indicate site records of *L. longipalpis* throughout Brazil.

for later transport to Yale University. In the sample of 123 male flies, one of us (K.G.) discovered it to consist of 3 morphological phenotypes with respect to 3rd and 4th tergite pale spots (note that this spot phenotype occurs only in males): 1) 1-spot, having a pair of round whitish spots with a diameter nearly the full width of the 4th abdominal tergite but no spot on the 3rd abdominal tergite, 2) 2-spot, having pairs of similar spots on the 3rd and 4th abdominal tergites, and 3) an intermediate form with a pair of 3rd-tergite spots somewhat variable in size, but smaller than normal, along with the normal spots on the 4th tergite. Before further analysis, males were separated into the 3 classes by microscopic inspection of flies on a chill plate. Of the 123 flies, all of the 1-spot flies (n = 8), all of the heterozygote flies (n = 33), and 38 of the 2-spot flies were subjected to electrophoresis. The remaining 2-spot male flies (n = 44) were retained as biochemical and morphological vouchers; 5 were deposited at the Yale University Museum of Natural History as museum vouchers.

Genetic profiles of the 3 classes were compared with data on field strains that were monomorphic for the 1-spot and 2-spot morphology. Genetic distance values were calculated from data taken from the 1-spot population extant at Lapinha Caves, Minas Gerais (Mukhopadhyay et al. 1997), and from a 2-spot population at Jacobina, Bahia (Mukhopadhyay et al. 1998), as external, geographic reference populations from eastern Brazil. The geographic origins and distributions of the strains are indicated in Fig. 1.

Electrophoretic methods: Each morphological type was analyzed separately for electrophoretic variation by separating the protein products of 15 enzyme loci on polyacrylamide gels. Preparation, buffer systems and protocols have been previously described (Munstermann 1988, Mukhopadhyay et al. 1997, Munstermann et al. 1998). Standard enzyme-specific histochemical staining of the gels followed Manchenko (1994).

Enzymes tested, locus abbreviations, and number assigned by the International Union of Biochemistry were as follows: aspartate transaminase (*Aat-2,* 2.6.1.10), adenylate kinase (*Ak,* 2.7.4.3), arginine kinase (*Ark,* 2.7.3.3), esterase (*Est-1,* 3.1.1.56), glycerol 3-phosphate dehydrogenase (*Gpd,* 1.1.1.8), glucosephosphate isomerase (*Gpi,* 5.3.1.9), hexokinase (*Hk,* 2.7.1.1), isocitrate dehydrogenase (*Idh-1, Idh-2,* 1.1.1.42), malate dehydrogenase (*Mdh-2,* 1.1.1.37), "malic" enzyme (*Me,* 1.1.1.40), mannose-6-phosphate isomerase (*Mpi,* 5.3.1.8.), phosphogluconate dehydrogenase (*Pgd,* 1.1.1.44), phosphoglucomutase (*Pgm,* 5.4.2.2), and trehalase (*Tre-1,* 3.2.1.28).

Data collection and analysis: Mpi, Pgm, Idh-1, and occasionally other enzymes produced a very low enzyme titre or low activity, and the bands on the gels were resolved by computer enhancement (Mukhopadhyay et al. 1997). Gene frequencies were summarized with the BIOSYS-1 computer

Table 1. Allele frequencies[1] in 3 morphological forms isolated from a heterogeneous field population of *Lutzomyia longipalpis* from Natal, Brazil.

	Natal population		
Locus (R_f)[2]	One-spot	Intermediate	Two-spot
Aat-2			
0.67	0.375	**0.578**[3]	**0.568**
0.45	**0.625**	0.422	0.432
n	8	32	38
Ak			
1.36	0.063	0.016	0.026
1.26	**0.938**	**0.938**	**0.961**
1.12	0	0.047	0.013
n	8	32	38
Gpd			
1.63	0.063	0.016	0.013
1.41	**0.938**	**0.984**	**0.987**
n	8	32	38
Gpi			
0.48	0.125	0.031	0.026
0.39	**0.875**	**0.969**	**0.947**
0.30	0	0	0.013
0.22	0	0	0.013
n	8	32	38
Hk			
0.82	**0.938**	**0.953**	**0.934**
0.73	0.063	0.047	0.066
n	8	32	38
Idh-2			
0.84	0	0.016	0.013
0.79	0	0.016	0
0.68	**1.000**	**0.953**	**0.974**
0.50	0	0.016	0.013
n	8	32	38
Mdh-2			
0.72	0	0.016	0.026
0.61	**1.000**	**0.953**	**0.934**
0.50	0	0.031	0.026
0.42	0	0	0.013
n	8	32	38
Me			
1.05	0.063	0.016	0
0.97	**0.875**	**0.984**	**0.961**
0.93	0.063	0	0.013
0.90	0	0	0.026
n	8	32	38
Mpi			
1.00	0.125	0	0.017
0.90	0.188	0.080	0.133
0.80	0.188	0.380	**0.383**
0.75	**0.375**	**0.440**	0.283
0.60	0.063	0.060	0.150
0.50	0.063	0.020	0.033
0.40	0	0.020	0
n	8	25	30
Pgm			
1.12	0.188	0.094	0.149
1.08	0	0	0.014
1.04	**0.813**	**0.875**	**0.770**
0.87	0	0.031	0.068
n	8	32	37

[1] Monomorphic alleles—*Ark*$^{0.91}$, *Est-1*$^{0.93}$, *Idh-1*$^{0.61}$, *Pgd*$^{1.04}$, and *Tre-1*$^{1.12}$.

[2] R_f = ratio of the distance an enzyme-generated band migrates from the gel origin to the distance migrated for the same enzyme in the *Aedes aegypti* reference standard.

[3] Frequencies in bold are the most common allele in each locus–population combination.

Table 2. Contingency chi-square values for 10 polymorphic enzyme loci comparing the gene frequencies in the 1-spot, 2-spot, and intermediate morphological groups isolated from a field population of *Lutzomyia longipalpis* collected in Natal, Brazil.

Locus	Chi-square	df	*P*-value
Aat-2	2.28	2	0.320
Ak	3.12	4	0.538
Gpd	1.78	2	0.410
Gpi	5.72	6	0.456
Hk	0.24	2	0.889
Idh-2	1.96	6	0.923
Me	10.26	6	0.114
Mdh-2	2.16	6	0.905
Mpi	17.60	12	0.129
Pgm	4.60	6	0.596

program (Swofford and Selander 1981) to display average heterozygosities, Nei's unbiased genetic distance coefficients, and the unweighted pair-group method (UPGMA) dendrogram. The program also tested the gene frequencies among the 3 morphological classes for significant differences by the HETXSQ option (heterogeneity chi-square).

RESULTS

In the Natal field sample of 123 male flies, 8 1-spot, (no spot on 3rd tergite), 33 intermediate, and 82 2-spot forms were discerned. The spot on the 4th tergite did not vary its phenotype among the 3 classes. A reasonable genetic model describing these phenotypes was the following: the spot morphology on the 3rd tergite is controlled by a single codominant gene with 3 phenotypes, no spot, spot, and intermediate-sized spot (heterozygote). The 3rd tergite phenotypes were in close agreement with the Hardy–Weinberg expectations (expected number: no spot = 4.9, 1-spot = 78.9, and intermediate-sized spot [heterozygote] = 39.2), where $p_{no\ spot} = 0.199$ and $q_{spot} = 0.801$ ($\chi^2 = 3.32$, $P < 0.30$).

Allele frequencies of 15 enzyme loci for the 3 morphological classes are described by Table 1. A considerable range in genetic variability was observed, with 5 showing no variability (monomorphic) and, at the other extreme, one locus—*Mpi* with 7 alleles. With the exception of *Mpi*, the major alleles were present in similar frequencies in the 3 classes of spot phenotypes. A heterogeneity χ^2 test for enzyme frequency differences among the 3 morphological classes revealed no significant differences (Table 2). Rare alleles unique to a single morphological class were expected as a consequence of sampling probabilities. These alleles included the following: 1) 2-spot, $n = 38$—*Gpi*$^{0.22}$, *Gpi*$^{0.30}$, *Mdh-2*$^{0.42}$, *Me*$^{0.90}$, and *Pgm*$^{1.08}$; 2) intermediate form, $n = 32$—*Idh-2*$^{0.79}$ and *Mpi*$^{0.40}$; and 3) 1-spot, $n = 8$—none. No significant deviation from the Hardy–Weinberg equilibrium occurred within each group. When the Natal sample was analyzed

Table 3. Genetic variability at 15 loci in 3 morphological forms of a field population of *Lutzomyia longipalpis* from Natal, Brazil.

Population	Mean *n* per locus (±SEM)	Average heterozygosity		Mean no. alleles per locus (±SEM)	% of polymorphic loci[1]
		Direct count (±SEM)	Hardy–Weinberg (±SEM)		
1-spot	7.5 (0.4)	0.175 (0.064)	0.166 (0.060)	1.9 (0.3)	53.3
Intermediate	28.7 (1.3)	0.110 (0.040)	0.127 (0.051)	2.3 (0.4)	66.7
2-spot	31.8 (2.4)	0.145 (0.055)	0.148 (0.057)	2.5 (0.4)	66.7

[1] A locus is considered polymorphic if more than 1 allele was detected.

as a single population, the alleles at all loci were found to be in equilibrium.

Table 3 shows the average heterozygosity, mean number of alleles and the percentage of polymorphic loci in each of the forms. The values are high as compared with the laboratory populations (Mukhopadhyay et al. 1997) but are typical of those seen in other field populations of *L. longipalpis* (Munstermann et al. 1998, Mukhopadhyay et al. 1998).

Nei's unbiased genetic distance indices (*D*) were calculated from data presented in Table 1 for comparison with the additional field populations of Jacobina (100% 2-spot morphology) and Lapinha (100% 1-spot morphology). The index values to the 3rd decimal place were 0.001 between Natal 1-spot and 2-spot forms and 0 for the Natal combinations, reflecting no genetic differentiation among the morphological forms. The *D* matrix values were depicted as a UPGMA dendrogram (Fig. 2) which illustrated the identity of the 3 morphological forms as well as indicating the very close relationship (genetic distance <0.05) among the 3 widely separated geographic strains of eastern Brazil.

DISCUSSION

Knowledge of sand fly biology is relatively limited in contrast to that of mosquitoes and black flies; this has had a negative impact on the development of a sound taxonomy for phlebotomine sand flies at the species and infraspecies level (Lane 1986). Since the early days of the 20th century, most advances in the taxonomy of sand flies have followed from studies on their role in the transmission of disease. Because the sand fly genera *Lutzomyia* and *Phlebotomus* are the only 2 genera known to transmit *Leishmania* pathogenic to man, most studies have focused on these groups. Recent observations on geographic and local variation in behavior and morphology have led to speculations concerning presence of sibling species complexes in several of the species in these genera. However, without a broadly based knowledge of the sand fly biology as well as its broad scale and microhabitat distributions, evaluation of the variation in taxonomic and evolutionary terms has been problematic (Lane 1986).

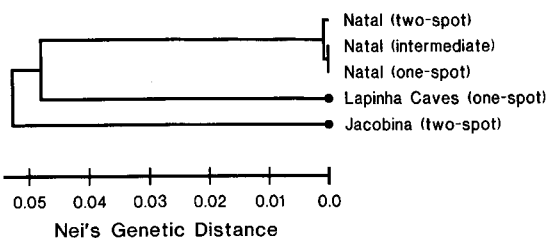

Fig. 2. Nei's (1978) unbiased genetic distance indices depicted in the form of a UPGMA dendrogram for 3 morphological forms of *Lutzomyia longipalpis* field collected from Natal, Brazil, and 2 morphologically homogeneous forms field collected from Lapinha Caves and Jacobina, Brazil.

Application of some population genetic principles in association with comparisons of multiple variable loci can give evidence in specific instances where the presence of a species complex is suspected. In the present study, these principles have been applied to the case of a morphological character that previously had been presented as symptomatic of the presence of a species complex in *L. longipalpis*.

We have shown that the tergal morphology is not correlated with a distinctive electrophoretic profile. In the morphologically heterogeneous population of Natal, no significant differences were discerned in pairwise comparisons among the 1-spot, intermediate and 2-spot forms, nor did evidence of population substructuring (2 or more sympatric species) appear.

Two studies of Bolivian *L. longipalpis* compared similar genetic features, but the conclusions differed somewhat from one another. The first was that of Bonnefoy et al. (1986), who failed to find an association between size heterogeneity and isozyme profile in Bolivian samples of *L. longipalpis*. A more recent study (Dujardin et al. 1997) compared 1-spot and 2-spot populations separated by a distance of 800 km with 9 electrophoretic loci. Only

3 of the 9 loci exhibited variability (cellulose acetate electrophoresis), but together with size characters, the authors concluded that the populations were genetically "isolated by distance"—differences that "possibly have taxonomic significance." On the basis of variability recovered in the present study and in other populations of *L. longipalpis* (Morrison et al. 1995; Mukhopadhyay et al. 1997, 1998; Munstermann 1998), these differences probably reflect founder effects in a species with relatively low vagility (Morrison et al. 1993). None of these studies noted differences between male and female sand flies with respect to gene frequencies.

The 3 Natal forms are in Hardy–Weinberg equilibrium for both the spot phenotype and the enzyme polymorphisms. This equilibrium provides support for a hypothesis of panmixis in the Natal population. The data also lend credence to the hypothesis promoted by Ward et al. (1988) and Santos et al. (1991) to explain both their difficulties in establishing pure lines of 1-spot and 2-spot laboratory colonies and the frequent appearance of males "intermediate" between the 2 forms.

Presentation of historical details of the 1-spot/2-spot discourse is warranted because of the role of their discovery in fostering the concept of species complexes in sand flies. When Mangabeira (1969) noted the tergal spot morphological differences in male *L. longipalpis* from Pará and Ceará states in Brazil, as a classical morphologist, he felt that the 2 forms represented different species or varieties. The spot phenotype became the sole morphological marker for identification and separation of field-collected male specimens. To evaluate these differences in terms of the biological species concept, Ward et al. (1983, 1988) initiated crosses between different spot forms. They noticed partial reproductive isolation in sympatric and allopatric 1-spot/2-spot populations, prompting them to suggest the presence of a species complex.

Some of the data did not fit this motif very well, however. For example, although the yield of hybrid (1-spot × 2-spot) flies was low, backcross progeny were recovered in high numbers (26%–48%) when backcrossed to 1-spot flies. In addition, a lower survivorship occurred in crosses between strains of same spot morphology from different (allopatric) origins; yet, allopatric populations with males of different phenotypes were sexually compatible (Ward et al. 1988). Finally, the intermediate spot morphology was recovered from some of the allopatric crosses.

Several alternative explanations are possible for the reduced hatching rate in the hybrid crosses. In sand flies, even in the parental reference crosses, individual to individual female and trial to trial variation in progeny generation can be very large (Ghosh et al. 1997; L. Munstermann, unpublished). Possibly, the lower survivorship in crosses between the allopatric strains may be the effect of genetic factors not associated with spot morphology. Because of the long larval development period and sensitivity to an unfavorable laboratory environment, changes in humidity, nutrition or mating success can influence survival rates greatly.

ACKNOWLEDGMENTS

This study was supported by NIH grant AI-34521, Genetics and Biogeography of Sand Fly Disease Vectors.

REFERENCES CITED

Bonnefoy, S., M. Tibayrenc, F. Le Pont, J.-P. Dujardin, P. Desjeux and F. J. Ayala. 1986. An isozymic study of *Lutzomyia longipalpis* (Diptera, Psychodidae), the vector of visceral leishmaniasis in the "Yungas" (Bolivia). Cah. ORSTOM Ser. Entomol. Med. Parasitol. 24:213–217.

Dujardin, J.-P., E. M. Torrez, F. Le Pont, D. Hervas and D. Sossa. 1997. Isozymic and metric variation in the *Lutzomyia longipalpis* complex. Med. Vet. Entomol. 11: 394–400.

Ghosh, K. N., J. Mukhopadhyay, H. Guzman, R. B. Tesh and L. E. Munstermann. 1998. Interspecific hybridization and genetic variability in strains of phlebotomine sand flies. Med. Vet. Entomol. (in press).

Grimaldi, G., Jr., R. B. Tesh and D. McMahon-Pratt. 1989. A review of the geographic distribution and epidemiology of leishmaniasis in the New World. Am. J. Trop. Med. Hyg. 41:687–725.

Jeronimo, S. M. B., R. M. Oliveira, S. Mackay, R. M. Costa, J. Sweet, E. T. Nascimento, K. G. Luz, M. Z. Fernandes, J. Jernigan and R. D. Pearson. 1994. An urban outbreak of visceral leishmaniasis in Natal, Brazil. Trans. R. Soc. Trop. Med. Hyg. 88:386–388.

Lane, R. P. 1986. Recent advances in the systematics of phlebotomine sandflies. Insect Sci. Appl. 7:225–230.

Lanzaro, G. C., K. Ostrovska, M. V. Herrero, P. G. Lawyer and A. Warburg. 1993. *Lutzomyia longipalpis* is a species complex: genetic divergence and interspecific hybrid sterility among three populations. Am. J. Trop. Med. Hyg. 48:839–847.

Manchenko, G. P. 1994. Handbook of detection of enzymes on electrophoretic gels. CRC Press, Boca Raton, FL.

Mangabeira, O. 1969. Sobre a sistemática e biologia dos *Phlebotomus* do Ceará. Rev. Bras. Malariol. Doencas Trop. 21:3–26.

Morrison, A. C., C. Ferro, A. Morales, R. B. Tesh and M. L. Wilson. 1993. Dispersal of the sand fly *Lutzomyia longipalpis* (Diptera: Psychodidae) at an endemic focus of visceral leishmaniasis in Colombia. J. Med. Entomol. 30:427–435.

Morrison, A. C., L. E. Munstermann, C. Ferro, R. Pardo and M. Torres. 1995. Ecological and genetic studies of *Lutzomyia longipalpis* in a central Colombia focus of visceral leishmaniasis. Bol. Dir. Malariol. Saneamiento Ambiental 35(Suppl. 1):235–248.

Mukhopadhyay, J., K. N. Ghosh, E. F. Rangel and L. E. Munstermann. 1998. Genetic variability in biochemical characters of Brazilian populations of the *Leishmania* vector, *Lutzomyia longipalpis* (Diptera: Psychodidae). Am. J. Trop. Med. Hyg. (in press).

Mukhopadhyay, J., E. F. Rangel, K. N. Ghosh and L. E. Munstermann. 1997. Patterns of genetic variability in colonized strains of *Lutzomyia longipalpis* (Diptera:

Psychodidae) and its consequences. Am. J. Trop. Med. Hyg. 57:216–221.

Munstermann, L. E. 1988. Biochemical systematics of nine nearctic *Aedes* mosquitoes (subgenus *Ochlerotatus, annulipes* group B), pp. 133–147. *In:* M. W. Service (ed.). Biosystematics of haematophagous insects. Clarendon, Oxford, United Kingdom.

Munstermann, L. E., A. C. Morrison, C. Ferro, R. H. Pardo and M. Torres. 1998. Genetic structure of local populations of *Lutzomyia longipalpis* (Diptera: Psychodidae) in central Colombia. J. Med. Entomol. 35:82–89.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Santos, M. C. dos, P. Williams and M. Ferreira. 1991. Changes in sex ratio during attempts to establish a laboratory colony of *Lutzomyia longipalpis* (Diptera: Psychodidae). Parassitologia 33(Suppl. 1):169–176.

Swofford, D. L. and R. B. Selander. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281–283.

Ward, R. D., A. Phillips, B. Burnet and C. B. Marcondes. 1988. The *Lutzomyia longipalpis* complex: reproduction and distribution, pp. 257–269. *In:* M. W. Service (ed.). Biosystematics of haematophagous insects. Clarendon, Oxford, United Kingdom.

Ward, R. D., A. L. Ribeiro, P. D. Ready and A. Murtaugh. 1983. Reproductive isolation between different forms of *Lutzomyia longipalpis* (Lutz and Neiva) (Diptera: Psychodidae), the vector of *Leishmania donovani chagasi* Cunha and Chagas and its significance to kala-azar distribution in South America. Mem. Inst. Oswaldo Cruz 78:269–280.

Ward, R. D., A. L. Ribeiro, L. Ryan, A. L. Falcao and E. F. Rangel. 1985. The distribution of two morphological forms of *Lutzomyia longipalpis* (Lutz & Neiva) (Diptera: Psychodidae). Mem. Inst. Oswaldo Cruz. 80:145–148.

Young, D. G. and M. A. Duncan. 1994. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae). Mem. Am. Entomol. Inst. No. 54. Associated Publishers, Gainesville, FL.