Studies on Populations of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera: Psychodidae: Phlebotominae) in Brazil

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Studies were performed on five Brazilian populations of Lutzomyia longipalpis*: Salvaterra (PA), São José do Ribamar (MA), Canindé (CE), Natal (RN) and Gruta da Lapinha, Lagoa Santa (MG). No morphological differences were observed that could distinguish between these populations. Homogeneity tests showed that the allopatric populations display a certain heterogeneity and that the sympatric populations, with different patterns of spots, are homogeneous. The Student-Newman-Keuls test, represented by Euler-Venn diagrams, showed a disjunction between the populations from the north/northeast and the one from Gruta da Lapinha. Genetic distances between the four populations (excluding the Canindé population) were within the range of intrapopulational differences. The Gruta da Lapinha population displayed a heterozygotic deficiency that could be a consequence of high levels of inbreeding due to cryptic habits of living in a small cave. These results do not favor the hypothesis of a* L. longipalpis *species complex in Brazil, and the species should be considered high polymorphic.*

Key words: *Lutzomyia longipalpis* - Phlebotominae - taxonomy - morphology - Brazil

Lutzomyia longipalpis has a broad distribution in Brazil and has been recorded in North, Central, and South America, from Mexico to Argentina (Young & Duncan 1994).

This sand fly species is considered a vector for visceral leishmaniasis, caused by *Leishmania (Leishmania) chagasi*, and in Brazil is frequently found associated with human dwellings and domestic animal shelters, in periurban areas, and on farms (Badaró 1995). Considering its occurrence in different geographical regions of Brazil, there may be barriers to the migration of populations. This possibility was mentioned by Mangabeira (1969), who first described morphological variations in *L. longipalpis* males, comparing specimens from the States of Ceará (CE) and Pará (PA) in Brazil. Males from PA had a single pair of pale spots on the fourth tergite, while those from CE had an additional pair of spots on the third tergite. Mangabeira (1969) commented that these two forms could be found in different ecological habitats and suggested that they might represent two different species.

A study by Ward et al. (1985) on the distribution of two morphological forms of *L. longipalpis* indicated that males with one pair of spots have appeared from Mexico to Southern Brazil and that the two-spotted form is concentrated more in northeast Brazil. The two forms occur sympatrically in some Brazilian States: Maranhão (MA), Piauí (PI), Ceará (CE), and Rio Grande do Norte (RN). Intermediate forms occur in areas where the two forms (with one and two spots, respectively) occur sympatrically in northeast Brazil (Ward et al. 1988, Mukhopadhyay et al. 1998).

Lanzaro et al. (1993) traced the isoenzymatic profiles of populations of *L. longipalpis* reared in the laboratory, originally from Costa Rica, Colombia, and Brazil. Some 27 enzymes were assayed, and the results showed multiple genetic polymorphisms, with hybridization between populations resulting in sterile males. This finding suggested that the Costa Rican population was different from those of Brazil and Colombia. According to Warburg et al. (1994) the different clinical manifestations caused by *L. (L.) chagasi* in Costa Rica, Colombia and Brazil were probably due to different concentrations of maxadilan in the saliva of

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the *L. longipalpis* populations. Differences were also found between the populations in the nucleotide sequence for maxadilan, revealing a polymorphism in the Costa Rican population.

Recently, Yin et al. (1999) examined microscopically the brain cells of fourth instar sandfly larvae of *L. longipalpis* of Brazil (Jacobina and Gruta da Lapinha), Colombia and Costa Rica. Differences of G-banding and/or position of the centromere on chromosome 4 distinguished four putative sibling species from Brazil, Colombia and Costa Rica. The karyotype of the population from Jacobina showed an apparently plesiomorphic pattern of G-banding.

Lanzaro et al. (1999) reported variation in the primary DNA and inferred amino acid sequences of maxadilan. Differences were found within and among natural field populations as well as among sibling species. Results indicated a high degree of divergence in the salivary peptide maxadilan in populations of the Brazil, Colombia and Costa Rica.

Mutebi et al. (1999) analyzed eleven populations of *L. longipalpis* from different areas of Brazil. Genotypic frequencies within populations were in close compliance to Hardy-Weinberg expectations, suggesting there are non sympatric species among these populations. The levels of genetic distance between pairs of populations were very low, consistent with local populations within a single sand fly species. Estimate of effective migration rates among all populations were low, suggesting that gene flow is restricted among populations, which is probably the reason for the observed genetic substructuring.

Other works on genetic variation and the implication on the taxonomic status of *L. longipalpis* have been extensively done with results pointing either for a single species or for a complex species (Ward et al. 1983, Lane & Ward 1984, Lane et al. 1985, Ward et al. 1988, Lanzaro et al. 1998, Mukhopadhyay et al. 1997, 1998, Dujardin et al. 1997, Munstermann et al. 1998, Mutebi et al. 1998).

Although morphology is a traditional tool in taxonomic studies, it has been overlooked in relation to *L. longipalpis*, which has primarily been the target of biochemical studies. Even, species has been proposed as a possible complex of cryptic species, there is a lack of detailed knowledge of its morphological characters which might define different populations or indicate some degree of intrapopulational heterogeneity.

In order to clarify the taxonomic status of *L. longipalpis*, the current article presents the results of morphological and morphometric observations of Brazilian populations displaying different patterns of spots on the abdominal tergites. Biochemical tests were also performed to complement the morphological analysis.

MATERIALS AND METHODS

Study samples - Specimens were obtained from the following areas: North Brazil - Salvaterra, Marajó Island (PA); Northeast Brazil - São José do Ribamar, São Luís Island (MA); Canindé (CE); Natal (RN); and Southeast Brazil - Gruta da Lapinha, Lagoa Santa (MG). These populations are representative of three different pale-spotted patterns (Fig. 1).

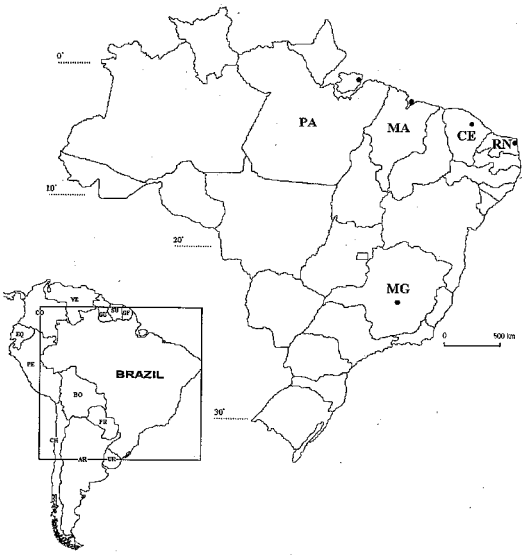

Fig. 1: localities where of *Lutzomyia longipalpis* specimens were collected : Pará (PA), Marajó Island, Salvaterra; Maranhão (MA), São Luís Island, São José do Ribamar; Ceará (CE), Canindé; Rio Grande do Norte (RN), Natal; Minas Gerais (MG), Lagoa Santa, Gruta da Lapinha.

Sand fly capture - Performed in peridomiciliary areas, in domestic animal shelters, using miniature CDC light traps. Specimens from Lagoa Santa were also caught in the cave at Gruta da Lapinha using CDC light traps.

Morphology and morphometry - Morphometric studies were used to verify intrapopulational homogeneity and variety among populations. In populations with different patterns of pale spots, heterogeneity was tested based on the correlation of morphometry with pale-spotted patterns. We examined 40 specimens (20 males and 20 females) from each site and routinely used 52 morphological and morphometric features to identify the sandflies (Young & Duncan 1994), along with other characters recommended by the CIPA Group – Computer Aided Identification of Phlebotomine Sandflies of the Americas (Bermudes et al. 1991).

For morphometry, we measured the length and/or width of structures (Table I).

Statistical analysis - Homogeneity, parametric (Levene, ANOVA, and Student-Newman-Keuls or SNK), and non-parametric tests (Kruskall-Wallis) were applied to the populations in which different patterns of spots occurred sympatrically (MA, CE and RN) and in the allopatric populations (PA, MA, CE, RN and MG). The SNK results were displayed graphically using Euler-Venn diagrams and interpreted according to the Intuitive Set Theory (Abe & Papavero 1991). Tests were performed using SPSS for Windows, with a significance level of 5%. When the statistical F value was not significant and the variances among populations were statistically significant, the Kruskall-Wallis test was applied.

Isoenzymatic studies - Adult male sandflies captured in the field were analyzed by agarose as in Rosa-Freitas et al. (1990) and cellulose acetate as in Dujardin and Tibayrenc (1985) for allozyme gel electrophoresis. Nine enzymes were assayed for four populations (PA, MA, RN and MG): malic enzyme (ME), phosphogluconate dehydrogenase (GPD), glucose-6-phosphato isomerase (GPI), phosphoglucomutase (PGM), glicerol 3-phosphate isomerase (∝-GPD), hexokinase (HK), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH) and mannose 6-phosphate isomerase (MPI), comprising a total analysis of 10 loci, considering that two loci were scorable for the MDH enzyme. Specimens from CE population were not in enough number to allow isoenzyme analysis.

TABLE I

Morphological and morphometric characters: *Lutzomyia longipalpis*

Morphological	Morphometric
Head	Male and female
Palpal formula	Length of frontal head
Spines of the pharynx	Length of head
Striae of the pharynx	Length of labrum-epipharynx
Labial fork	Length of 1st, 2nd, 3rd, 4th and 5th palpal segments
Proximal prolongation of ascoids	Length of clipeo
Terminal part of proximal ascoids	Minimal distance between eyes
Distal prolongation of ascoids	Length of F_1, F_2, F_{13} and F_{14}
Teeth of cibarium	
Teeth in the lacinea (arrangement)	
Number of apicolateral teeth hypopharynx	
Thorax	Male and female
Colour pronotum	Width of wing
Colour pre-scutum	Length of R_5, *alfa*, *beta*, *gama* and *delta* veins of wing
Colour scutum	Length of fore, mid and hind femur
Colour post-scutellum	Length of fore, mid and hind tibia
Colour katepisternum	Length of fore, mid and hind tarsomere
Colour anepimeron	
Colour katepimeron	
Colour coxa	
Abdomen	Male
Pale spots on abdominal tergites	Length of coxite
Setae tuffs on coxite	Width of coxite
Number of setae of the coxite	Length of lateral lobe
Differentiated setae of coxite	Width of lateral lobe
Differentiated setae of paramere	Length of genital pump
Shape and setae on style	Length of piston
Number of spines style	Length of genital filament
Distribuition of insertion style spines	Length of style
	Distance between the setae and apical part of paramere
	Female
Aspect of the spermatheca body and individual ducts	Length of spermatheca body
Axis of spermatheca head	Width of spermatheca body
	Length of individual duct of spermatheca
	Length of cercus

Genotype frequencies were obtained directly by band counting. Based on these gene frequencies, heterozygosity estimates, fits to Hardy-Weinberg equilibrium, levels of genetic distance (D, Nei 1978), levels of inbreeding for each population (F_{IS}, Nei 1977), and the effect of geographical subdivision on the genetic structure of the whole population (F_{ST}, Nei 1977) were calculated using the Biosys-1 Computer Program (Swofford & Selender 1981). Significance levels of F_{ST} estimates were evaluated by the Waples (1987) chi-squared test: $\chi^2= 2NF_{ST}(k-1)$; DF=(k-1)(s-1), where N is the total number of individual sampled, k is the number of alleles at the locus, and s is the number of populations. The significance levels of F_{IS} were evaluated according to Li and Horvitz (1953): $\chi^2= F_{IS}{}^2N(k-1)$; DF= k(k-1)/2. The F_{ST} used to estimate the number of migrants (Nm) among populations per generation (Slatkin 1987) was according to the formula: $F_{ST}=1/ (1+4Nm)$.

RESULTS

Morphology - Using structural characters of the head, thorax, and abdomen of both sexes we could detect no significant morphological differences among the five populations studied. We calculated the percentages of qualitative and quantitative morphological characters for males and females from the population samples of *L. longipalpis* from PA, MA, RN, CE and MG (Tables II, III).

Variations occurred in the males in both the formula of the palpus and the number of spots on the abdominal tergites. The most frequent formula was 1.2.4.3.5, occurring in 100% of the specimens from the PA and CE populations. As for the number of spots on the tergites, the MG population was the only one in which we observed no variation with a constant feature of one pair of spots. This aspect was also observed in the other populations, except for MA. In the latter there was an equal proportion of the intermediate (the pair of spots on

TABLE II

Percentage of variations found in morphological characters in males *Lutzomyia longipalpis* from Salvaterra (Marajó Island, PA), São José do Ribamar (São Luís Island, MA), Canindé (CE), Natal (RN) and Lagoa Santa (Gruta da Lapinha, MG), Brazil

Character	Variation	%				
		PA	MA	CE	RN	MG
Palpal formula	1.2.4.3.5	100	90	100	85	85
	1.4.2.3.5	-	-	-	10	10
	1.(2.4).3.5	-	10	-	5	5
Spines of the pharynx	Present with uniform distribution	100	100	100	100	100
Striae of the pharynx	Present	100	100	100	100	100
Labial fork	Present	100	100	100	100	100
Proximal prolongation of ascoids	Rudimental	100	100	100	100	100
Terminal part of proximal of ascoids	Rounded	100	100	100	100	100
Distal prolongation of ascoids	Reaching or not surpassing the end of the flagellomere	100	100	100	100	100
Colour pronotum	Well pigmented	100	100	100	100	100
Colour pre-scutum	Well pigmented	100	100	100	100	100
Colour scutum	Well pigmented	100	100	100	100	100
Colour post-scutellum	Well pigmented	100	100	100	100	100
Colour katepisternum	Lightly pigmented	100	100	100	100	100
Colour anepimeron	Lightly pigmented	100	100	100	100	100
Colour katepimeron	Lightly pigmented	100	100	100	100	100
Colour coxa	Lightly pigmented	100	100	100	100	100
Pale spots on abdominal tergites	One pale spot	95	-	55	10	100
	Intermediate form	-	50	5	65	-
	Two pale spots	5	50	40	25	-
Setae tuffs on coxite	Basal	100	100	100	100	100
Differentiated setae of coxite	Undifferentiated setae	100	100	100	100	100
Number of setae of the coxite	Four	100	100	100	100	100
Differentiated setae of paramere	Dorsal curved setae	100	100	100	100	100
Shape of the style	Simple	100	100	100	100	100
Subterminal setae on style	Present	100	100	100	100	100
Number of spines style	Four	100	100	100	100	100
Distribuition of insertion style spines	1/1/1/1	100	100	100	100	100

TABLE III

Percentage of variations found in morphological characters in females *Lutzomyia longipalpis* from Salvaterra (Marajó Island, PA), São José do Ribamar (São Luís Island, MA), Canindé (CE), Natal (RN) and Lagoa Santa (Gruta da Lapinha, MG), Brazil

Character	Variation	%				
		PA	MA	CE	RN	MG
Palpal formula	1.2.4.3.5	63	26	90	39	37
	1.4.2.3.5	11	42	-	28	37
	1.(2.4).3.5	26	32	10	33	26
Number of horizontal teeth of cibarium	8	90	89	55	94	89
	10	10	11	45	6	11
Number of vertical teeth of cibarium	10	-	-	-	14	-
	11	-	-	-	21	17
	12	52	68	44	-	33
	13	12	16	6	30	50
	14	12	16	31	7	-
	15	12	-	6	14	-
	16	12	-	13	-	-
	17	-	-	-	-	-
Spines of the pharynx	Present with uniform distribution	100	100	100	100	100
Striae of the pharynx	Present	100	100	100	100	100
Labial fork	Present	100	100	100	100	100
Teeth in the lacinea	4	-	7	-	5	-
	5	32	32	-	21	7
	6	47	20	24	26	33
	7	16	7	29	47	47
	8	5	28	47	-	13
	9	-	7	-	-	-
Teeth internal in the lacinea	15	-	-	-	-	-
	17	-	-	8	-	17
	18	-	25	25	11	-
	19	-	-	17	39	17
	20	15.5	50	17	11	-
	21	23	25	17	22	17
	22	15.5	-	17	-	-
	23	23	-	-	11	49
	24	23	-	-	-	-
Number of apicolateral teeth	13	-	-	6	-	-
of the hypopharynx	14	8	62	19	-	33
	15	42	25	19	63	17
	16	25	13	31	31	42
	17	8	-	25	6	8
	18	8	-	-	-	-
	20	8	-	-	-	-
Proximal prolongation of ascoids	Rudimental	100	100	100	100	100
Terminal part of proximal ascoids	Rounded	100	100	100	100	100
Distal prolongation of ascoids	Reaching or not surpassing the end of the flagellomere	22	31	50	10	100
	Reaching and surpassing the end of the flagellomere	78	69	50	90	-
Colour pronotum	Well pigmented	100	100	100	100	100
Colour pre-scutum	Well pigmented	100	100	100	100	100
Colour scutum	Well pigmented	100	100	100	100	100
Colour post-scutellum	Well pigmented	100	100	100	100	100
Colour katepisternum	Lightly pigmented	100	100	100	100	100
Colour anepimeron	Lightly pigmented	100	100	100	100	100
Colour katepimeron	Lightly pigmented	100	100	100	100	100
Colour coxa	Lightly pigmented	100	100	100	100	100
Aspect spermathecae bodies	Formed by annulations	100	100	100	100	100
Aspect of annulations of the spermathecae bodies	No imbricated	100	100	100	100	100
Aspects individual ducts	Smooth non-sclerotized	100	100	100	100	100
Width of individual ducts	Uniform	100	100	100	100	100
Axis of spermathecae head	Not curved	100	100	100	100	100

3rd tergite is smaller than the other in the 4th tergite) and two-spotted form, while in the RN population the intermediate form occurred more frequently.

Variations occurred in the females in some quantitative morphological characters: the number of horizontal and vertical teeth on the cibarium, the number of external and internal teeth on the maxilla, and the number of teeth on the hypopharynx, which normally display considerable variation within the same species. Variations were also observed in qualitative characters: the formula of the palpus and the relationship of the distal prolongation of the ascoids. As in males, the most frequent palpus formula in females was 1.2.4.3.5. With regard to the distal prolongation of the ascoids, the only population in which we observed no variation was the one of MG, in which this character reached the middle of the flagellomere and did not extend beyond its extremity. In the other populations, except for the one of CE, the predominant feature was the distal prolongation reaching the middle of the flagellomere and extending beyond its extremity.

Morphometrics. Homogeneity in relation to geographical origin - In order to verify the homogeneity of the population samples, we applied the tests as shown in Tables IV and V. The results suggest that there is not a total homogeneity among the samples. Therefore, we applied the SNK test in order to identify possible heterogeneous populations. In order to better evaluate the correlation between populations, we considered characters whose means displayed statistically significant differences, and the results are shown graphically.

In the illustration of the SNK test results, each diagram represents the character's mean for each population. Union, disjunction, and intersection were the operations performed among the sets. The elements of a set (sub-sets or populations) make up the universe of the *L. longipalpis* population analyzed for each character. When the sub-sets are analyzed separately, the CE and MG populations were the ones that generally reached extreme values, establishing a disjunction between the two. The disjunction became more evident when the union of the PA, MA, CE and RN populations was performed to form the North/Northeast set, thereby revealing, on the basis of 13 male and 19 female characters, the separation between them now considered two sets (North/Northeast and MG - Figs 2, 3). The union of the sub-sets occurred when the differences between the means obtained for the populations in the morphometric studies were not significant. In the opposite case, disjunction occurred. The intersections indicated that at the 5% level, the test was not capable of separating populations based on sharing of values by two or more populations.

Morphometrics. Homogeneity in relation to number of spots - In order to verify the populations' homogeneity with regard to variations in the spot patterns, we applied statistical tests as shown in Tables VI, VII, and VIII. Analyses of the population samples from RN, CE and MA proved inconsistent with the populations' heterogeneity from the taxonomic point of view. In the RN population (Table VIII), only the lengths of the piston and genital pump displayed significant values at the 5% level. Likewise, in the CE population (Table VII), the only significant value was for the length of the F_{14} antennal segment. No character in the MA population (Table VI) was considered significant. Although the Salvaterra population displayed variation in the spot pattern, it was not possible to perform the homogeneity test for variance, because one of the patterns (two pairs of spots) was only found in one individual.

Isoenzymatic studies - Gene frequencies for the ten allozyme loci in the four populations is given in Table IX. Six loci were polymorphic (i.e., the frequency of the more common allele was less than 0.95). Heterozygosity values ranged from 0.149 (RN) to 0.215 (PA). The mean number of individuals analyzed per locus and the mean number of alleles per locus are shown in Table X. All of the populations were in Hardy-Weinberg equilibrium, except for MG, which showed a heterozygote deficiency at two loci (ME: F_{IS}=0.402, χ^2=21.01, $p<0.001$; GPI: F_{IS}=0.474, χ^2=14.83, $p<0.005$). It is also worth noting that three other loci (MPI, PGM and IDH), showed high positive F_{IS} values for MG population, even thought these were not significant. None of the loci was diagnostic (Ayala 1983) for any particular population, leading to low overall D levels (Table XII). The genetic distances were used to build a UPGMA dendrogram of the four populations (Fig. 4). However, despite the low D levels, we observed that the three northern populations (PA, MA and RN) were very similar, differing from the southernmost MG population. Furthermore, we observed that the northern population shared some unique alleles that were not present in the MG population, and vice versa. To facilitate the analysis and to test the legitimacy of the differences between the northern populations and the southern one, the frequencies of the former were grouped and analyzed as one single population. Differences between these two groups in terms of allele frequencies were confirmed by a contingency table (Table XIII). Differences in allele frequencies were also detected by the mean fixation index value (Table XI) revealing a moderate level of genetic structuring

TABLE IV

Homogeneity of quantitative characteres in males of *Lutzomyia longipalpis* populations from Salvaterra (Marajó Island, PA), São José do Ribamar (São Luís Island, MA), Canindé (CE), Natal (RN) and Lagoa Santa (Gruta da Lapinha, MG), Brazil

Character	Number of specimens	Minimum maximum (µm)	Mean±standard deviation (µm)	ANOVA p	Levene test p	Kruskall-Wallis p
Length of frontal head	100	290–380	329.9±16.3	**0.021**	0.185	-
Length of head	100	430–550	488.7±21,2	**0.034**	0.091	-
Length of labrum-epipharynx	100	240–320	276.0±18.8	**0.0**	**0.004**	-
Length of 1st palpal segment	100	40–55	48.1±3.2	**0.006**	0.956	-
Length of 2nd palpal segment	100	130–200	152.0±12.6	**0.006**	**0.0**	-
Length of 3rd palpal segment	100	165–250	193.5±14.6	0.0003	**0.0**	-
Length of 4th palpal segment	100	145–220	168.6±13.0	0.062	**0.0**	0.059
Length of 5th palpal segment	98	305–550	462.6±45.0	**0.039**	**0.022**	-
Length of clipeo	100	140–190	158.4±8.8	0.151	0.319	-
Minimal distance between eyes	97	80–140	117.9±14.1	**0.004**	**0.008**	-
Length of F_1 antennal segment	100	260–390	317.0±27.0	**0.0**	**0.0**	-
Length of F_2 antennal segment	99	110–170	140.2±12.2	**0.0**	**0.0**	-
Length of F_{13} antennal segment	96	50–65	56.7±3.6	**0.0**	0.057	-
Length of F_{14} antennal segment	96	55–75	67.3±3.6	**0.0003**	0.433	-
Width of wing	100	525–775	681.7±58.6	**0.0**	**0.0**	-
Length of R_5 veins of wing	100	1300–1775	1515.5±112.1	**0.0**	**0.0**	-
Length of *alfa* veins of wing	100	300–500	393.3±43.6	**0.0**	0.339	-
Length of *beta* veins of wing	100	225–425	318.8±35.7	**0.0**	**0.105**	-
Length of *gama* veins of wing	100	325–550	442.0±48.7	**0.0**	**0.033**	-
Length of *delta* veins of wing	100	0–175	76.5±31.9	**0.001**	0.575	-
Length of fore femur	100	750–1025	861.2±53.4	**0.0**	**0.002**	-
Length of mid femur	100	750–1000	875.5±48.4	**0.0001**	0.081	-
Length of hind femur	98	875–1125	998.7±60.0	**0.0005**	**0.006**	-
Length of fore tibia	100	825–1100	954.7±62.3	**0.0**	**0.001**	-
Length of mid tibia	100	1050–1425	1220.5±89.9	**0.0**	**0.0**	-
Length of hind tibia	98	1325–1825	1560.9±108.9	**0.0**	**0.008**	-
Length of fore tarsomere	100	450–600	520.5±34.5	**0.0**	**0.033**	-
Length of mid tarsomere	100	525–750	643.0±44.1	**0.0**	0.795	-
Length of hind tarsomere	96	675–950	787.7±53.5	**0.0**	0.799	-
Length of coxite	100	360–450	411.0±16.0	**0.0022**	**0.0**	-
Width of coxite	100	100–150	136.5±11.0	0.078	0.12	-
Length of lateral lobe	100	380–490	423.8±23.6	**0.0**	**0.0**	-
Length of genital pump	100	130–190	154.1±9.7	**0.0101**	0.222	-
Length of piston	100	100–170	126.8±11.0	**0.0152**	**0.048**	-
Length of genital filament	99	390–570	469.5±39.6	**0.0**	**0.001**	-
Length of style	100	190–230	214.5±9.4	**0.0**	**0.0**	-
Distance between the setae and apical part of paramere	100	90–140	121.6±8.4	**0.0**	**0.0**	-

Significant values in bold ($p<0.05$)

among the populations and leading to the estimated number of 3.6 migrants per generation.

DISCUSSION

L. longipalpis has been found in both the periphery of large cities and in rural areas of Brazil. This sand fly species occurs under different geographical conditions and is distributed throughout various regions of Brazil. Geographical barriers could be preventing the migration of specimens from one region to another that would account for observed morphological and biochemical variation.

The lack of a standard to describe the species, the dissociation between the sexes, the large amount of synonymy and lack of knowledge exchange between taxonomists are factors impeding the proper identification of sand fly species.

When first Lutz and Neiva described sandflies in Brazil in 1912, they reported their difficulties in classifying the species, due to the limited number

TABLE V

Homogeneity of quantitative characteres in females of *Lutzomyia longipalpi* Salvaterra (Marajó Island, PA), São José do Ribamar (São Luís Island, MA), Canindé (CE), Natal (RN) and Lagoa Santa (Gruta da Lapinha, MG), Brazil

Character	Number of specimens	Minimum maximum (µm)	Mean±standard deviation (µm)	ANOVA p	Levene test p	Kruskall-Wallis p
Length of frontal head	100	280–370	328.5±18.7	**0.0247**	**0.043**	-
Length of head	100	420–550	492.5±25.4	**0.0084**	**0.005**	-
Length of labrum-epipharynx	100	290–390	339.0±19.7	**0.0**	0.489	-
Length of 1st palpal segment	100	45–60	51.1±4.1	**0.0**	**0.024**	-
Length of 2nd palpal segment	100	145–195	163.9±11.4	**0.0**	0.404	-
Length of 3rd palpal segment	99	170–230	197.6±13.5	**0.0**	**0.005**	-
Length of 4th palpal segment	99	145–195	166.8±10.6	**0.0243**	**0.0**	-
Length of 5th palpal segment	94	285–579	452.6±47.6	**0.0**	0.486	-
Length of clipeo	100	120–190	163.0±12.3	**0.0**	**0.021**	-
Minimal distance between eyes	96	80–150	129.2±11.4	**0.0397**	0.556	-
Length of F_1 antennal segment	100	200–300	251.7±23.0	**0.0**	**0.0**	-
Length of F_2 antennal segment	100	100–140	114.5±10.7	**0.0**	**0.001**	-
Length of F_{13} antennal segment	79	50–65	55.6±3.3	**0.0**	**0.01**	-
Length of F_{14} antennal segment	100	60–75	66.7±3.3	**0.0358**	0.505	-
Width of wing	100	600–875	725.7±60.2	**0.0**	**0.0**	-
Length of R_5 veins of wing	100	1325–1875	1584.3±134.1	**0.0**	**0.001**	-
Length of *alfa* veins of wing	100	325–575	423.7±49.0	**0.0**	**0.001**	-
Length of *beta* veins of wing	100	250–425	331.2±38.2	**0.0001**	0.179	-
Length of *gama* veins of wing	100	325–625	459.0±68.9	**0.0**	0.083	-
Length of *delta* veins of wing	100	0–75	87.7±37.6	**0.0**	0.158	-
Length of fore femur	91	700–975	841.2±55.9	**0.0**	0.097	-
Length of mid femur	92	750–1000	863.3±55.9	**0.0**	0.394	-
Length of hind femur	90	825–1150	996.1±71.2	**0.0**	**0.024**	-
Length of fore tibia	91	700–1000	838.7±71.7	**0.0**	0.239	-
Length of mid tibia	91	900–1300	1081.3±86.7	**0.0**	**0.029**	-
Length of hind tibia	90	1150–1725	1438.8±122.6	**0.0**	0.086	-
Length of fore tarsomere	90	400–675	465.0±43.0	**0.0**	0.122	-
Length of mid tarsomere	91	500–700	564.5±45.6	**0.0**	0.529	-
Length of hind tarsomere	90	625–875	738.0±56.8	**0.0**	0.88	-
Length of spermatheca body	95	10–15	13.4±1.5	**0.0018**	0.694	-
Width of spermatheca body	95	25–40	30.1±3.2	**0.0**	**0.0**	-
Length of individual duct spermateca	66	150–230	196.1±19.3	**0.0**	0.187	-
Length of cercus	99	140–170	157.1±7.6	**0.0**	0.225	-

Significant values in bold ($p<0.05$)

of specimens collected for comparative studies. The authors described the males and females of *L. longipalpis*, providing only an overall description of the body and the alar and palpal indexes. In 1924, Nuñez-Tovar in Venezuela described the male of *Phlebotomus otamae*, and in 1934 Galliard described the female of *P. almazani*, with the two being placed in synonymy (Dyar & Nuñez-Tovar 1926/27, Fairchild & Hertig 1958).

L. longipalpis has a wide distribution in the Americas and is adapted to different ecological systems, and Mangabeira (1969) thus discussed the ability of this sand fly species to survive in such diverse habitats as the States of PA and CE, Brazil. Besides its geographical distribution, the author suggested *L. longipalpis* as a complex of cryptic species and considered different pale-spotted patterns on the abdominal tergites. Dujardin et al. (1997), studying the morphometrics of wing veins in *L. longipalpis* males from Nicaragua, Colombia, Bolivia and Brazil, suggested that Bolivian populations with one and two pairs of pale spots

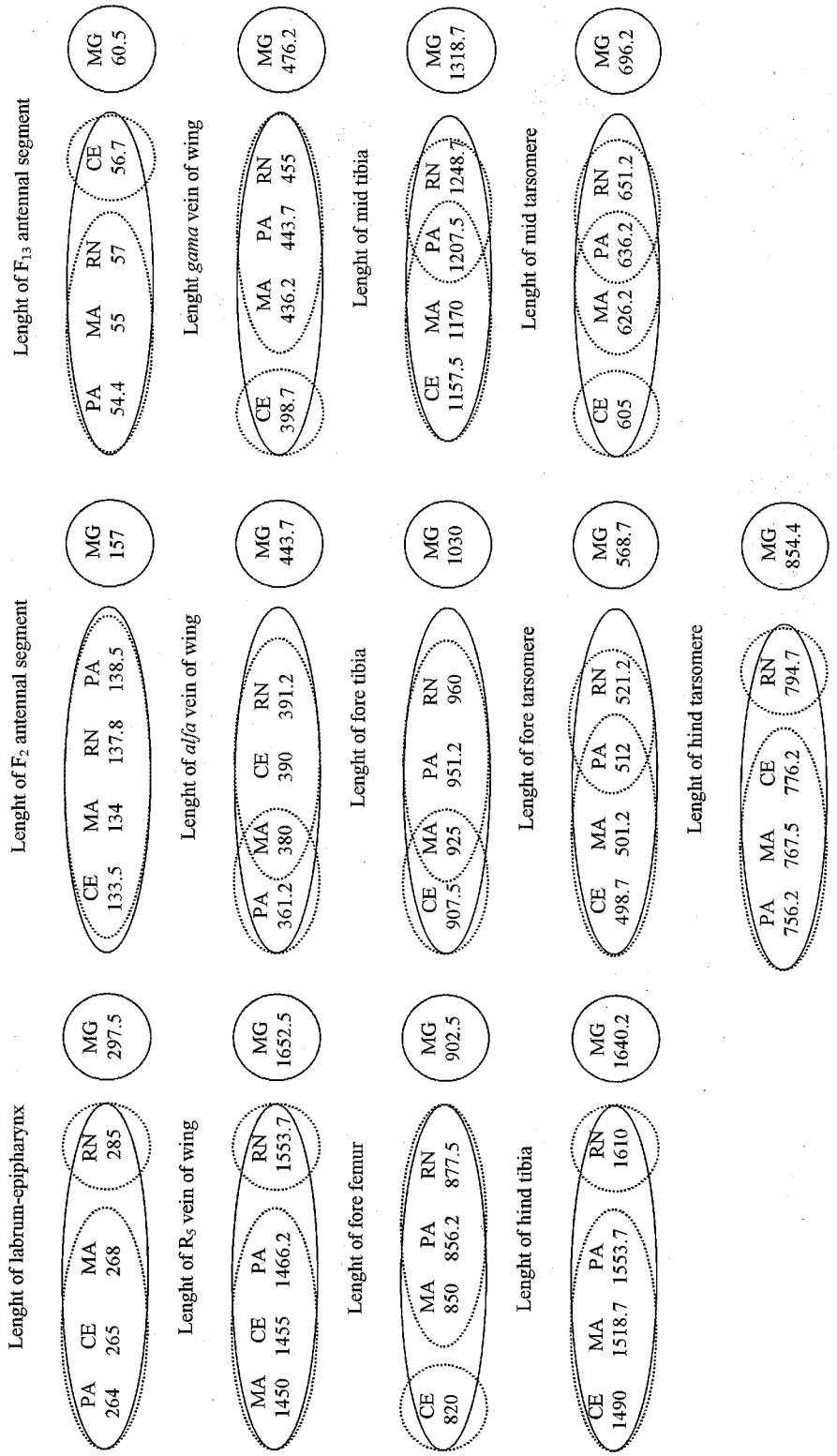

Fig. 2: Euler-Venn diagram according to Student-Newman-Keuls comparing male populations of *Lutzomyia longipalpis* from Gruta da Lapinha (MG) and North/Northeast populations (PA, MA, CE, RN). Continuous line: analysis of the populations as two groups - North/Northeast (PA, MA, CE, RN) and Gruta da Lapinha (MG). Interrupted line: analysis of individual populations, PA: Pará; MA: Maranhão; CE: Ceará; RN: Rio Grande do Norte; MG: Minas Gerais.

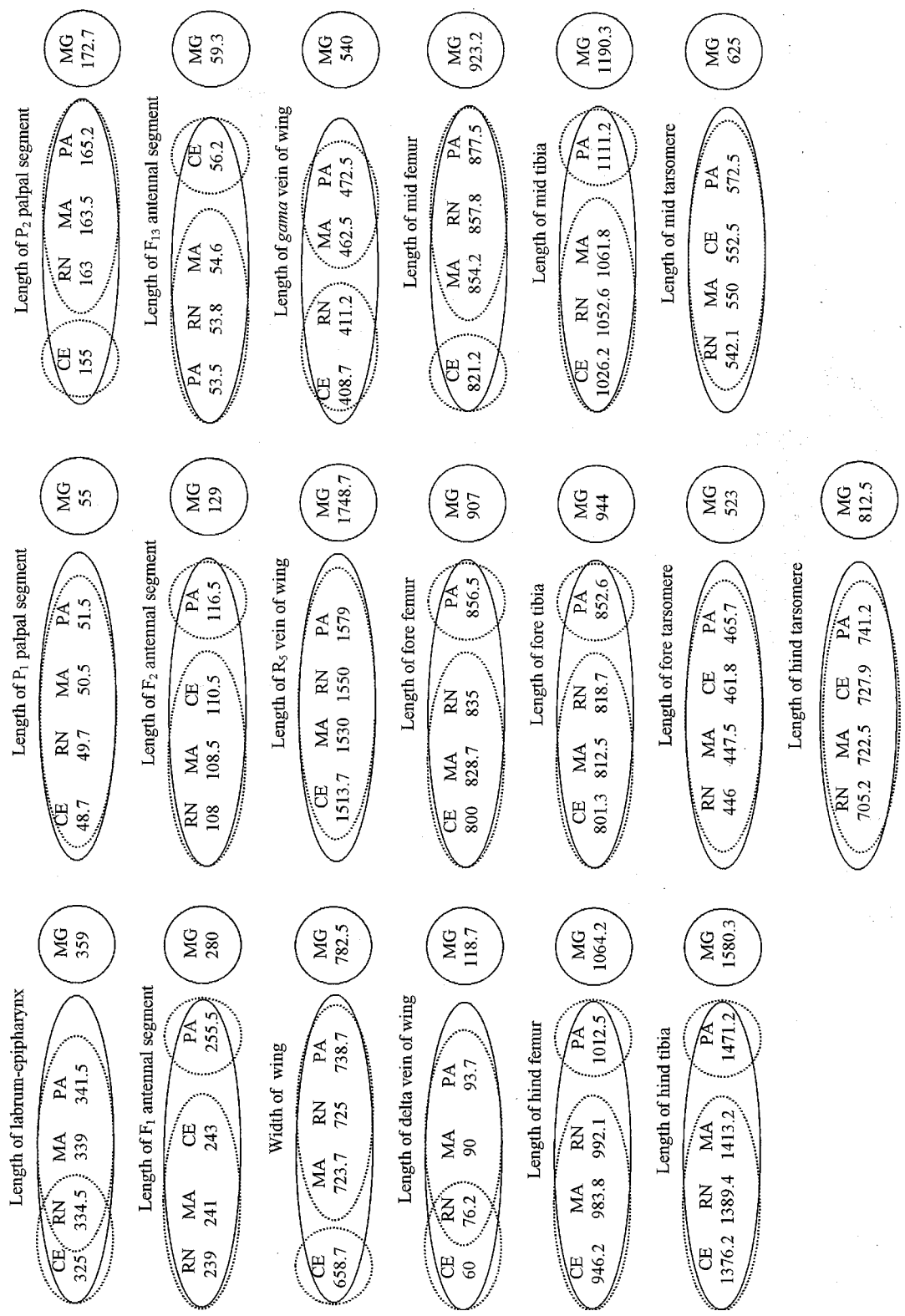

Fig. 3: Euler-Venn diagram according to Student-Newman-Keuls comparing female populations of *Lutzomyia longipalpis* from Gruta da Lapinha (MG) and North/Northeast populations (PA, MA, CE, RN). Continuous line: analysis of populations as two groups - North/Northeast (PA, MA, CE, RN) and Gruta da Lapinha (MG). Interrupted line: analysis of individual populations, PA: Pará; MA: Maranhão; CE: Ceará; RN: Rio Grande do Norte; MG: Minas Gerais.

TABLE VI

Homogeneity of quantitative characters related to pale spots (two spots and intermediate form) in *Lutzomyia longipalpis* males from São José do Ribamar (MA), Brazil

Characters	Intermediate form		Two spots		ANOVA	Levene test	Kruskall-Wallis
	Number of specimens	Mean±standard deviation (µm)	Number of specimens	Mean±standard deviation (µm)	p	p	p
Length of frontal head	10	327.0±8.2	10	327.0±11.6	1.000	0.416	-
Length of head	10	484.0±11.7	10	483.0±13.4	0.861	0.948	-
Length of labrum-epipharynx	10	267.0±6.7	10	269.0±8.8	0.574	0.366	-
Length of 1st palpal segment	10	47.5±2.6	10	48.0±2.6	0.673	0.548	-
Length of 2nd palpal segment	10	148.0±4.8	10	150.5±6.0	0.318	0.575	-
Length of 3rd palpal segment	10	189.0±5.2	10	188.0±6.7	0.714	0.41	-
Length of 4th palpal segment	10	161.5±6.3	10	164.5±6.9	0.320	0.787	-
Length of 5th palpal segment	10	445.0±26.0	10	458.0±38.7	0.389	0.219	-
Length of clipeo	10	155.0±8.5	10	156.0±7.0	0.777	0.548	-
Minimal distance between eyes	9	124.4±12.4	10	122.0±4.2	0.563	**0.022**	0.462
Length of F_1 antennal segment	10	310.0±9.4	10	305.0±8.5	0.229	0.602	-
Length of F_2 antennal segment	10	135.0±5.3	10	133.0±4.8	0.388	0.207	-
Length of F_{13} antennal segment	10	54.5±2.8	10	55.5±1.6	0.343	0.263	-
Length of F_{14} antennal segment	10	66.0±3.2	10	65.5±2.8	0.714	0.512	-
Width of wing	10	675.0±40.8	10	667.5±26.5	0.632	0.278	-
Length of R_5 veins of wing	10	1457.5±65.7	10	1442.5±40.9	0.547	0.166	-
Length of *alfa* veins of wing	10	377.5±27.5	10	382.5±20.6	0.651	0.288	-
Length of *beta* veins of wing	10	312.5±17.7	10	315.0±21.1	0.777	0.648	-
Length of *gama* veins of wing	10	437.5±46.0	10	435.0±26.9	0.884	0.064	-
Length of *delta* veins of wing	10	72.5±32.2	10	70.0±15.8	0.828	0.125	-
Length of fore femur	10	855.0±32.9	10	845.0±28.4	0.476	0.256	-
Length of mid femur	10	880.0±32.9	10	867.5±29.0	0.379	0.333	-
Length of hind femur	10	990.0±31.6	10	1000.0±33.3	0.500	0.8	-
Length of fore tibia	10	937.5±29.5	10	912.5±39.5	0.126	0.331	-
Length of mid tibia	10	1180.0±42.2	10	1160.0±63.7	0.418	0.21	-
Length of hind tibia	10	1535.0±80.1	10	1502.5±79.5	0.374	0.602	-
Length of fore tarsomere	10	500.0±11.8	10	502.5±24.9	0.777	0.224	-
Length of mid tarsomere	10	622.5±27.5	10	630.0±28.4	0.556	0.865	-
Length of hind tarsomere	10	770.0±45.3	10	765.0±45.9	0.809	0.928	-
Length of coxite	10	414.0±5.2	10	412.0±7.9	0.511	0.246	-
Width of coxite	10	140.0±10.5	10	145.0±8.5	0.258	**0.037**	0.345
Length of lateral lobe	10	416.0±7.0	10	414.6±6.9	0.530	1.000	-
Length of genital pump	10	156.0±8.4	10	153.0±4.8	0.342	0.107	-
Length of piston	10	128.0±10.3	10	126.0±7.0	0.618	0.229	-
Length of genital filament	10	451.0±23.8	10	445.0±24.6	0.586	0.874	-
Length of style	10	210.0±0.0	10	210.0±0.0	-	-	-
Distance between the setae and apical part of paramere	10	119.0±3.2	10	120.0±0.0	0.331	**0.037**	0.317

Significant values in bold ($p<0.05$)

TABLE VII

Homogeneity of quantitative characters related to pale spots (one, two spots and intermediate form) in *Lutzomyia longipalpis* males from Canindé (CE), Brazil

Characters	One spot		Intermediate form		Two spots		ANOVA	Levene test	Kruskall-Wallis
	Number of specimens	Mean±standard deviation (µm)	Number of specimens	Mean±standard deviation (µm)	Number of specimens	Mean±standard deviation (µm)	p	p	p
Length of frontal head	11	324.5±21.5	1	310	8	318.8±10.5	0.32	0.053	-
Length of head	11	480.0±28.6	1	470	8	481.1±16.2	0.939	0.284	-
Length of labrum-epipharynx	11	257.3±32.2	1	250	8	265.0±11.2	0.32	0.053	-
Length of 1st palpal segment	11	46.4±4.3	1	45	8	46.3±2.2	0.911	0.121	-
Length of 2nd palpal segment	11	147.3±15.1	1	140	8	140.6±8.1	0.464	0.332	-
Length of 3rd palpal segment	11	183.2±10.9	1	175	8	184.4±9.2	0.876	0.506	-
Length of 4th palpal segment	11	166.4±6.4	1	160	8	165.6±5.8	0.979	0.649	-
Length of 5th palpal segment	11	475.9±26.4	1	490	8	457.5±24.5	0.129	0.653	-
Length of clipeo	11	155.5±7.8	1	160	8	161.3±7.8	0.124	0.124	-
Minimal distance between eyes	11	124.5±9.9	1	120	8	116.3±13.2	0.184	0.68	-
Length of F1 antennal segment	11	301.8±20.8	1	270	8	293.8±11.1	0.113	0.132	-
Length of F2 antennal segment	11	138.4±6.4	1	130	8	130.0±8.7	0.302	0.916	-
Length of F13 antennal segment	11	58.2±3.2	1	55	8	55.0±2.5	0.056	0.105	-
Length of F14 antennal segment	11	70.0±2.1	1	70	8	66.3±2.2	**0.008**	0.33	-
Width of wing	11	611.4±26.9	1	600	8	621.9±31.7	0.718	0.78	-
Length of R5 veins of wing	11	1459.1±51.4	1	1400	8	1456.3±67	0.977	0.243	-
Length of *alfa* veins of wing	11	386.4±41.8	1	425	8	390.6±27.8	0.362	0.148	-
Length of *beta* veins of wing	11	284.1±24.5	1	300	8	303.1±34.1	0.466	0.377	-
Length of *gama* veins of wing	11	406.8±26.3	1	375	8	390.6±37.4	0.515	0.452	-
Length of *delta* veins of wing	11	52.3±32.8	1	25	8	59.4±17.4	0.789	0.084	-
Length of fore femur	11	831.8±47.8	1	775	8	809.4±21.4	0.588	**0.02**	0.707
Length of mid femur	11	838.6±48.1	1	800	8	837.5±33.1	0.993	0.163	-
Length of hind femur	11	961.4±52.6	1	900	8	956.3±42.8	0.862	0.707	-
Length of fore tibia	11	915.9±44.3	1	875	8	900.0±33.1	0.742	0.377	-
Length of mid tibia	11	1181.8±71.6	1	1075	8	1134.4±46.7	0.441	0.621	-
Length of hind tibia	11	1509.1±74.1	1	1375	8	1471.9±71.2	0.632	0.278	-
Length of fore tarsomere	11	506.8±28.4	1	475	8	490.6±17.4	0.538	0.616	-
Length of mid tarsomere	11	613.8±35.9	1	600	8	593.8±34.8	0.325	0.782	-
Length of hind tarsomere	11	788.6±32.6	1	750	8	762.5±37.5	0.431	0.756	-
Length of coxite	11	400.9±17.8	1	390	8	396.3±7	0.511	0.096	-
Width of coxite	11	129.1±15.6	1	140	8	137.5±6.6	0.422	**0.006**	0.654
Length of lateral lobe	11	404.5±12.3	1	390	8	400.0±10	0.192	0.191	-
Length of genital pump	11	148.2±10.3	1	150	8	155.0±7.1	0.326	**0.008**	0.443
Length of piston	11	119.1±13.1	1	120	8	128.8±7.8	0.175	0.071	-
Length of genital filament	11	428.2±27.2	1	440	8	446.3±29.6	0.474	0.442	-
Length of style	11	210.0±11.3	1	200	8	206.3±7	0.688	0.789	-
Distance between the setae and apical part of paramere	11	112.7±8.6	1	120	8	115.0±7.1	0.433	0.273	-

Significant values in bold ($p<0.05$)

TABLE VIII

Homogeneity of quantitative characters related to pale spots (one, two spots and intermediate form) in *Lutzomyia longipalpis* males from Natal (RN), Brazil

Characters	One spot		Intermediate form		Two spots		ANOVA	Levene test	Kruskall-Wallis
	Number of specimens	Mean±standard deviation (µm)	Number of specimens	Mean±standard deviation (µm)	Number of specimens	Mean±standard deviation (µm)	p	p	p
Length of frontal head	2	335.0±7.1	13	334.6±19.8	5	344.0±11.4	0.601	0.334	-
Length of head	2	485.0±21.2	13	492.3±24.2	5	506.0±8.9	0.397	0.192	-
Length of labrum-epipharynx	2	270.0±14.1	13	288.5±15.2	5	284.0±5.5	0.218	0.323	-
Length of 1st palpal segment	2	52.5±3.5	13	48.8±3.0	5	48.0±2.7	0.215	0.993	-
Length of 2nd palpal segment	2	147.5±3.5	13	155.4±8.5	5	156.0±8.2	0.437	0.488	-
Length of 3rd palpal segment	2	195.0±0.0	13	198.5±13.0	5	198.0±10.4	0.931	0.188	-
Length of 4th palpal segment	2	170.0±0.0	13	169.6±12.8	5	174.0±14.3	0.809	0.293	-
Length of 5th palpal segment	2	497.5±3.5	13	458.5±50.8	4	471.3±81.1	0.648	0.107	-
Length of clipeo	2	150.0±14.1	13	157.7±9.3	5	160.0±7.1	0.442	0.3	-
Minimal distance between eyes	2	130.0±0.0	12	122.5±15.4	5	116.0±20.7	0.578	0.332	-
Length of F_1 antennal segment	2	325.0±7.1	13	316.9±19.3	5	332.0±11.0	0.4397	0.097	-
Length of F_2 antennal segment	2	140.0±0.0	12	138.3±9.4	5	140.0±7.1	0.697	0.125	-
Length of F_{13} antennal segment	1	55.0±0.0	12	57.7±2.6	5	56.0±2.2	0.338	**0.01**	0.433
Length of F_{14} antennal segment	1	65.0±0.0	12	68.2±2.5	5	69.0±2.2	0.353	0.061	-
Width of wing	2	700.0±70.7	13	719.2±37.0	5	740.0±13.7	0.379	**0.032**	0.551
Length of R_5 veins of wing	2	1462.5±123.7	13	1559.6±57.3	5	1575.0±46.8	0.104	0.129	-
Length of *alfa* veins of wing	2	375.0±3.5	13	392.3±41.3	5	395±20.9	0.793	0.087	-
Length of *beta* veins of wing	2	327.5±3.5	13	355.8±34.1	5	340.0±13.7	0.351	**0.011**	0.63
Length of *gama* veins of wing	2	437.5±17.7	13	455.8±37.0	5	460.0±45.4	0.779	0.4	-
Length of *delta* veins of wing	2	87.5±17.7	13	96.2±32.0	5	75.0±46.8	0.535	0.16	-
Length of fore femur	2	850.0±35.4	13	880.8±37.0	5	880.0±27.4	0.514	**0.722**	-
Length of mid femur	2	862.5±53	13	890.4±38.9	5	885.0±22.4	0.614	0.228	-
Length of hind femur	2	975.0±70.7	13	1021.2±57.6	5	1025.0±35.4	0.513	0.444	-
Length of fore tibia	2	925.0±70.7	13	965.4±41.5	5	960.0±37.9	0.481	0.517	-
Length of mid tibia	2	1187.5±53	13	1255.8±54.2	5	1255.0±32.6	0.216	0.568	-
Length of hind tibia	2	1512.5±123.7	13	1619.2±87.3	5	1625.0±61.2	0.255	0.499	-
Length of fore tarsomere	2	500.0±0.0	13	528.8±24.7	5	510.0±22.4	0.155	0.157	-
Length of mid tarsomere	2	612.5±17.7	12	661.5±33.3	5	640.0±28.5	0.109	0.364	-
Length of hind tarsomere	2	737.5±53	13	806.3±47.8	5	790.0±13.7	0.132	0.057	-
Length of coxite	2	410.0±14.1	13	414.6±15.1	5	410.0±10.0	0.783	0.634	-
Width of coxite	2	130.0±14.1	13	137.7±11.7	5	132.0±8.4	0.783	0.634	-
Length of lateral lobe	2	425.0±21.2	13	443.8±24.3	5	442.0±16.4	0.556	0.744	-
Length of genital pump	2	155.0±7.1	13	146.2±7.7	5	156.0±55	**0.037**	0.685	-
Length of piston	2	125.0±7.1	13	118.5±8.0	5	130.0±0.0	**0.017**	**0.011**	-
Length of genital filament	2	475.0±7.1	13	503.8±21.8	4	480.0±53.5	0.25	**0.029**	0.228
Length of style	2	215.0±7.1	13	220.0±8.2	5	214.0±5.5	0.295	0.813	-
Distance between the setae and apical part of paramere	2	120.0±14.1	13	124.6±5.2	5	128.0±4.5	0.282	0.0	0.481

Significant values in bold ($p<0.05$)

TABLE IX

Gene frequencies of ten loci for *Lutzomyia longipalpis* specimens from four populations (Salvaterra - PA, São José do Ribamar - MA, Natal - RN, Gruta da Lapinha - MG) in Brazil

		Population							
	Locus	Salvaterra	χ^2 (DF)	São José do Ribamar	χ^2 (DF)	Natal	χ^2 (DF)	Gruta da Lapinha	χ^2 (DF)
	α-GPD								
(N)		35		33		36		31	
A		0.014	0.007 (1)	0.030	0.032 (1)	0.014	0.007 (1)	0.032	0.034 (1)
B		**0.986**		**0.970**		**0.986**		**0.968**	
	HK								
(N)		31		32		28		42	
A		**1.000**		**0.984**	0.008 (1)	**0.982**	0.009 (1)	**1.000**	
B		0.000		0.016		0.018		0.000	
	IDH								
(N)		33		33		38		50	
A		0.000		0.000		0.000		0.140	
B		**1.000**		**1.000**		**1.000**		**0.790**	6.704 (3)
C		0.000		0.000		0.000		0.070	
	MDH-1								
(N)		32		28		28		34	
A		0.016		0.036		0.018		0.029	
B		**0.969**	0.33 (3)	**0.964**	0.038 (1)	**0.982**	0.009 (1)	**0.971**	0.031 (1)
C		0.016		0.000		0.000		0.000	
	MDH-2								
(N)		35		28		26		34	
A		0.014	0.07 (1)	0.000		0.019	0.01 (1)	0.000	
B		**0.986**		**1.000**		**0.981**		**1.000**	
	ME								
(N)		38		49		32		65	
A		0.000		0.020		0.000		0.062	
B		**0.908**	1.728 (1)	**0.969**	0.049 (3)	**0.969**	0.033 (1)	**0.838**	24.52 (3)
C		0.092		0.010		0.031		0.100	
	MPI								
(N)		40		61		41		64	
A		0.000		0.049		0.000		0.250	
B		0.000		0.025		0.012		0.047	
C		0.038		0.033		0.061		**0.320**	
D		0.063	8.803 (10)	0.016	33.941 (28)	0.037	14.06 (15)	0.000	19.412 (15)
E		0.363		**0.311**		0.317		0.203	
F		**0.525**		0.270		**0.415**		0.156	
G		0.013		0.180		0.159		0.023	
H		0.000		0.049		0.000		0.000	
	PGD								
(N)		28		26		26		35	
A		0.054		0.019		0.000		0.000	
B		**0.946**	0.09 (1)	**0.981**	0.01 (1)	**0.981**	0.01 (1)	**0.986**	0.07 (1)
C		0.000		0.000		0.019		0.014	
	GPI								
(N)		36		37		34		33	
A		0.000		0.014		0.000		0.000	
B		0.028		0.000		0.029		0.045	
C		**0.958**	0.068 (3)	**0.932**	0.194 (3)	**0.971**	0.031 (1)	**0.939**	13.991 (3)
D		0.000		0.000		0.000		0.015	
E		0.000		0.054		0.000		0.000	
F		0.014		0.000		0.000		0.000	
	PGM								
(N)		52		66		52		75	
A		0.000		0.038		0.077		0.000	
B		0.000		0.000		0.000		0.047	
C		**0.596**		**0.462**		**0.683**		**0.667**	
D		0.394	0.877 (3)	0.356	4.637 (15)	0.212	3.428 (10)	0.000	11.468 (3)
E		0.010		0.015		0.000		0.287	
F		0.000		0.121		0.010		0.000	
G		0.000		0.008		0.019		0.000	

N: number of specimens analysed; DF: degrees of freedom

TABLE X

Genetic variability of four populations of *Lutzomyia longipalpis* from Salvaterra (Marajó Island, PA), São José do Ribamar (São Luís Island, MA), Canindé (CE), Natal (RN) and Lagoa Santa (Gruta da Lapinha, MG), Brazil

Population	Mean sample size per locus	Mean number of alleles per locus	Percentage of polymorphic loci[a]	Mean heterozigosity	
				Ho	He
Salvaterra	36.0	2.4	40	0.138	0.156
	(2.1)	(0.4)		(0.057)	(0.067)
São José do Ribamar	39.3	3.0	30	0.163	0.183
	(4.5)	(0.7)		(0.076)	(0.091)
Natal	34.1	2.6	20	0.144	0.149
	(2.6)	(0.5)		(0.073)	(0.077)
Gruta da Lapinha	46.3	2.6	50	0.162	0.215
	(5.1)	(0.5)		(0.063)	(0.081)

a: a locus is considered polymorphic if the frequency of the more common allele was less than 0.95; Ho: heterozigosity observed; He: Hardy-Weinberg expected; (standard errors).

TABLE XI

Fixation indexes for 10 isoenzymatic loci of populations of *Lutzomyia longipalpis* from Salvaterra (Marajó Island, PA), São José do Ribamar (São Luís Island, MA), Canindé (CE), Natal (RN) and Lagoa Santa (Gruta da Lapinha, MG), Brazil

Locus	F_{IS}	F_{ST}
α-GPD	-0.027	0.003
HK	-0.011	0.006
IDH	0.260	0.089 *b*
MDH-1	-0.027	0.0
MDH-2	0.023	0.011
ME	0.333	0.024 *a*
MPI	0.192	0.076 *b*
PGD	-0.023	0.005
GPI	0.251	0.004
PGM	0.141	0.087 *b*
Mean	0.181	0.065

a: p<0.01, *b*: p<0.001

were different lineages. Our studies did not permit us to ascribe taxonomic importance to spot patterns as a character. The character's variation may be associated with the number of papules comprising these spots (Lane & Ward 1984), varying from one individual to another and thus conferring different morphological profiles associated with this character. Mukhopadhyay et al. (1998), in studies on *L. longipalpis* populations from Natal, Brazil, with morphological variations (one and two spots and an intermediate form), observed that spot patterns and isoenzymatic frequencies fit Hardy-Weinberg expectations, and that no significant differences in isoenzymatic frequencies were associated with morphological phenotype. This demonstrates that the Natal population is panmictic.

Homogeneity tests showed that the populations analyzed (Salvaterra - PA, São José do Ribamar - MA, Canindé - CE, Natal - RN and Gruta da

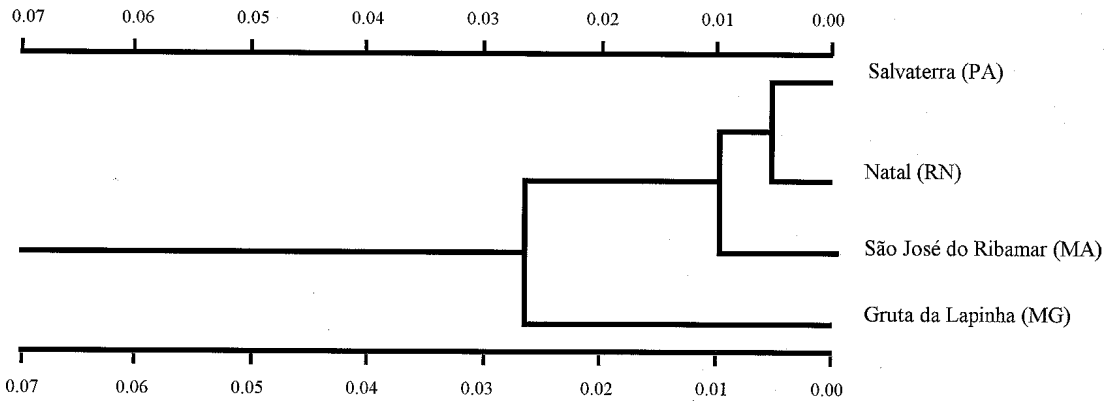

Fig. 4: UPGMA dendrogram derived from Nei's standard genetic distance among four populations of *Lutzomyia longipalpis* from Brazil. PA: Pará; MA: Maranhão; CE: Ceará; RN: Rio Grande do Norte; MG: Minas Gerais.

TABLE XII

Pairwise Nei's genetic distance among *Lutzomyia longipalpis* populations from Salvaterra (Marajó Island, PA), São José do Ribamar (São Luís Island, MA), Canindé (CE), Natal (RN) and Lagoa Santa (Gruta da Lapinha, MG), Brazil

Populations	1	2	3	4
1 Salvaterra	*****	0.004	0.007	0.037
2 Natal		*****	0.005	0.026
3 São José do Ribamar			*****	0.032
4 Gruta da Lapinha				*****

TABLE XIII

Studies on the homogeneity among populations of *Lutzomyia longipalpis* from North/Northeast and Gruta da Lapinha, Southeast Brazil

Locus	Allelos number	Chi-square	DF	Probability
α-GDP	2	0.82	1	0.367
HK	2	0.93	1	0.335
IDH	3	46.88	2	0.0
MDH1	3	0.48	2	0.788
MDH2	2	1.55	1	0.213
ME	3	14.4	2	0.001
MPI	8	172.48	7	0.0
PGD	3	2.13	2	0.345
GPI	6	6.55	5	0.256
PGM	7	166.14	6	0.0
		412.34	29	0.0

DF: degrees of freedom

Lapinha - MG) display a certain degree of heterogeneity, but no disjunction pattern was observed that could suggest the existence of distinct populations. The heterogeneity became more evident when the union of the north/northeast populations (PA, MA, CE and RN) was performed. This new population proved to be disjunctive from that of Gruta da Lapinha (MG), based on 13 male and 19 female characters. This fact could suggest that the Gruta da Lapinha population is the most heterogeneous in relation to the other populations.

Mukhopadhyay et al. (1998), studying *L. longipalpis* from Northeast and Southeast Brazil, suggested *L. longipalpis* in Brazil as a single species. They used 15 enzymatic loci and observed short distances and absence of diagnostic loci.

The allozyme data showed that the four populations of *L. longipalpis* (PA, MA, RN and MG) analyzed should be considered members of the same biological species. No diagnostic locus was detected among the four populations studied, and the Nei's D levels were thus very low, ranging from 0.004 to 0.037. These values are well within the range observed for comparisons between conspecific populations (Ayala 1983, Thorpe & Sole-Cava 1994).

Gene flow levels among the four Brazilian populations (Nm=3.6) agree well with the values reported for other *L. longipalpis* populations from Costa Rica and Honduras (Nm=3.6 and 3.0, respectively, Mutebi et al. 1998).

However, the population structure analysis showed that the populations displayed a moderate (F_{ST}=0.065) degree of genetic structuring (Wright 1978), confirming similar observations of a broader study in Brazil (Mutebi et al. 1999). The three northern populations (PA, MA and RN) were clearly more similar, differing from Gruta da Lapinha in the Southern. Since the geographical distance between the two most separate northern populations, Salvaterra and Natal (1.550 km) is about the same as the distance between Natal and Gruta da Lapinha (1.770 km), it is unlikely that the differentiation patterns follow the isolation-by-distance model. On the other hand, despite these similar geographical distances, the three northern populations are situated in the latitudinal range between 0º and 6ºS, while the Gruta da Lapinha population is located at 20ºS. This may suggest that the observed genetic structuring is a consequence of adaptation to different climatic conditions. Adaptation to different climatic and ecological conditions has been proposed to explain the differentiation between *L. whitmani* populations in Brazil (Ready et al. 1998).

The Gruta da Lapinha population (consisting exclusively of sand flies with one pale-spotted pattern) showed a significant heterozygote deficiency for two allozyme loci.

It is important to emphasize the close relationship between the results obtained from morphological, morphometric, and isoenzymatic analyses, showing a peculiar profile for the Gruta da Lapinha population as compared to the North/Northeast ones. The morphological and genetic differences noted between the North/Northeast (PA, MA, CE and RN) and Gruta da Lapinha (MG) populations may result from a latitudinal or even altitudinal variation, since the analysis of other populations

from the Brazilian plateau review a high similarity the Lapinha Cave population (Mutebi et al. 1999).

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