Morphometric and Phenetic Studies of Five Geographical Populations of *Lutzomyia whitmani* (Diptera: Psychodidae) in Brazil

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ABSTRACT A morphometric survey examined adult specimens of *Lutzomyia whitmani* (Antunes & Coutinho) captured at 5 municipalities in southeastern and northeastern Brazil to compare the populations. The localities were Ilhéus (Bahia), Martinho Campos (Minas Gerais), Corte de Pedra (Bahia), Baturité (Ceará), and Amaraji (Pernambuco); all are known foci of American cutaneous leishmaniasis. Fifteen males and 15 females from each population were analyzed morphometrically for 42 and 37 characters, respectively. Statistical data alone were insufficient to discriminate among the 5 populations. Further analysis generated phenograms that indicated there were 2 spatial clusters: the 1st was composed of specimens from Ilhéus (Bahia) and Baturité (Ceará) and the 2nd of specimens from Martinho Campos (Minas Gerais), Corte de Pedra (Bahia), and Amaraji (Pernambuco). Although insufficient to define the taxonomic status of the populations studied, the results delineated the existence of biogeographical structuring within *L. whitmani.* Complementary studies on the susceptibility to *Leishmania braziliensis* infection in the 5 populations are in progress to clarify the relationship between the 2 biogeographical clusters and American cutaneous leishmaniasis transmission in those Brazilian regions.

KEY WORDS *Lutzomyia whitmani*, sand fly, phenetics, geographical variation, Brazil

Lutzomyia whitmani (Antunes & Coutinho) is a sand fly species in the subgenus *Nyssomyia* that is prevalent at foci of leishmaniasis in Minas Gerais and Bahia in southeastern Brazil (Mayrink et al. 1979, Rosa et al. 1988, Passos et al. 1993). The association of human cases with natural infections of *Leishmania* parasites (*braziliensis* complex) in *L. whitmani* has incriminated this species as a vector of human American cutaneous leishmaniasis in Brazil (Hoch et al. 1986, De Queiroz et al. 1994). The transmission of leishmaniasis has been associated with *L. whitmani* for many years in the southern and southeastern regions of Brazil (1930–1950). During that period, *L. whitmani* was restricted to sylvatic areas (Marzochi 1989). Later, this sand fly appeared in banana plantation and peridomestic habitats (Marzochi 1989), demonstrating its adaptability to environmental modifications by humans. Further field studies at municipalities within the metropolitan area of Belo Horizonte (Minas Gerais) confirmed the peridomestic transmission of leishmaniasis in periurban areas where *L. whitmani* is the predominant sand fly (Passos et al. 1993).

Recently, Rangel et al. (1996) observed the existence of 2 geographically isolated forms of *L. whitmani*

Fig. 1. Distribution of localities used for sand fly captures in Brazil.

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Table 1. Morphometric data (in microns) of *L. whitmani* male characters expressed as mean ± SE

Character	Population				
	Ilhéus	Martinho Campos	Corte de Pedra	Baturité	Amaraji
1. Head length	328.5 ± 11.9	337.6 ± 13.9	333.3 ± 11.6	330.1 ± 6.4	333.9 ± 1.8
2. Clypeus	82.1 ± 3.7[a,c]	93.3 ± 7.8[b,e,g]	84.8 ± 5.1[f]	85.9 ± 7.1[h]	90.1 ± 4.7[d]
3. Interocular distance	74.1 ± 13.7[a]	86.4 ± 10.6[b]	86.4 ± 13.2[b]	90.7 ± 10.8[b]	89.1 ± 7.9[b]
4. Total length of palpus	450.1 ± 28.4[a]	413.2 ± 17.5[b]	406.1 ± 19.9[b]	404.9 ± 27.0[b]	408.9 ± 38.6[b]
5. Palpal I length	38.4 ± 3.3[a]	37.9 ± 3.7	39.5 ± 2.1[b]	35.7 ± 4.1	38.4 ± 3.3
6. Palpal II length	114.1 ± 5.6	113.0 ± 6.7	112.0 ± 7.4	109.9 ± 7.1	107.2 ± 2.4
7. Palpal III length	131.2 ± 7.9[a,c]	122.7 ± 5.8[b]	126.9 ± 5.9	122.7 ± 7.2[d]	126.8 ± 5.5
8. Palpal IV length	51.2 ± 4.1[a]	48.5 ± 4.7	48.6 ± 5.8	43.7 ± 4.1[b]	48.0 ± 6.5
9. Palpal V length	112.5 ± 16.7[a]	92.0 ± 13.6[b]	78.8 ± 11.2[b]	91.1 ± 16.2[b]	76.0 ± 22.1[b]
10. Labrum length	233.1 ± 15.7[b]	242.1 ± 13.3[b]	242.1 ± 12.6[b]	213.3 ± 13.7[a]	234.7 ± 11.2[b]
11. Flagellomere I	70.4 ± 5.4[a]	68.8 ± 5.1	65.1 ± 2.8[b]	66.1 ± 4.7	66.7 ± 3.9
12. Flagellomere II	62.4 ± 3.3	63.5 ± 2.1	62.4 ± 3.3	61.9 ± 4.7	62.9 ± 2.8
13. Flagellomere III	230.4 ± 15.8[a,c]	245.3 ± 12.0[b]	252.3 ± 13.1[d,e]	241.6 ± 10.1	263.8 ± 12.0[f]
14. Flagellomere IV	104.0 ± 6.8	109.3 ± 7.2	107.2 ± 6.6	104.5 ± 4.7	106.7 ± 4.9
15. Flagellomere V	106.1 ± 5.6	110.9 ± 5.1[a]	107.7 ± 5.9	103.5 ± 3.6[b]	107.2 ± 5.1
16. Flagellomere VI	105.1 ± 6.7[a]	111.5 ± 5.6[b,c]	107.7 ± 5.9	102.9 ± 2.8[d]	106.1 ± 4.7
17. Flagellomere VII	105.1 ± 5.9	109.9 ± 4.7[a,c]	107.7 ± 5.9[e]	101.9 ± 3.6[b,f]	103.5 ± 3.7[d]
18. Flagellomere VIII	102.4 ± 4.5	107.2 ± 5.1[a,c]	105.1 ± 5.1	100.3 ± 5.1[b]	100.3 ± 5.1[d]
19. Flagellomere IX	100.3 ± 4.1[a]	106.3 ± 5.8[b,c,e]	106.5 ± 4.7	98.1 ± 6.4[d]	99.2 ± 5.9[f]
20. Flagellomere X	98.1 ± 4.7	104.0 ± 4.6[a,c]	100.3 ± 5.9	96.0 ± 5.4[b]	93.5 ± 10.0[d]
21. Flagellomere XI	96.5 ± 4.7	100.0 ± 6.4	98.7 ± 4.9	96.0 ± 4.8	95.2 ± 5.9
22. Flagellomere XII	93.1 ± 6.0	93.8 ± 5.2	94.9 ± 5.1	92.7 ± 5.3	93.0 ± 8.5
23. Flagellomere XIII	88.6 ± 6.6	84.0 ± 6.7	88.0 ± 5.4	86.5 ± 6.0	84.0 ± 6.0
24. Maximum width of wing	493.9 ± 32.5	491.7 ± 40.7	481.1 ± 33.4	466.1 ± 45.9	499.7 ± 13.5
25. R-5 length	1,107.7 ± 39.5	1,137.4 ± 62.6	1,155.6 ± 49.2	1,129.1 ± 33.5	1,147.2 ± 57.4
26. α length	452.6 ± 31.8	477.6 ± 44.8	475.2 ± 33.3	436.3 ± 52.8[a]	482.3 ± 29.0[b]
27. β length	243.2 ± 17.6[a]	273.4 ± 13.8[b]	266.9 ± 25.4[b]	273.1 ± 19.1[b]	271.4 ± 18.9[b]
28. χ length	191.5 ± 14.6[a]	168.0 ± 10.8[b,c]	176.7 ± 18.4	190.9 ± 17.9[d]	181.7 ± 18.7
29. δ length	227.4 ± 32.1	240.0 ± 27.7	241.4 ± 32.6	204.3 ± 39.3[a]	246.9 ± 31.7[b]
30. Coxa length	283.2 ± 12.4[b]	262.9 ± 24.3[a]	284.8 ± 9.5[b]	286.9 ± 10.8[b]	288.5 ± 8.8[b]
31. Femur length	657.6 ± 35.9	664.6 ± 57.9	667.4 ± 39.8	668.8 ± 29.0	663.2 ± 33.0
32. Tibia length	916.3 ± 147.1	1,047.4 ± 183.6	1,075.4 ± 157.0	906.1 ± 134.3	894.4 ± 154.6
33. Tarsus length	1,102.9 ± 109.4	1,184.6 ± 135.2	1,196.9 ± 115.8	1,081.7 ± 95.6	1,109.6 ± 110.8
34. Genital filament + pump length	627.7 ± 48.6	659.2 ± 38.6[b]	650.1 ± 42.6[d]	596.3 ± 45.7[a,c,e]	651.2 ± 48.4[f]
35. Pump length	140.8 ± 5.1	143.5 ± 7.1	144.5 ± 9.8	136.5 ± 8.8	142.4 ± 6.2
36. Genital filament	486.9 ± 47.0	515.7 ± 38.0[b]	505.6 ± 37.8[d]	459.7 ± 46.2[a,c,e]	508.8 ± 45.9[f]
37. Lateral lobe length	260.3 ± 11.3	270.9 ± 13.5	266.7 ± 12.3	261.3 ± 25.9	272.5 ± 11.1
38. Lateral lobe width	30.9 ± 5.1	32.5 ± 2.1	32.0 ± 0.0	32.5 ± 3.7	31.5 ± 2.1
39. Paramere length	177.1 ± 11.7[a]	171.2 ± 12.8	170.1 ± 10.7	157.3 ± 18.3[b]	170.7 ± 11.6
40. Coxite length	297.1 ± 14.5	304.0 ± 12.5[b]	301.3 ± 13.1[d]	282.7 ± 18.1[a,c,e]	300.8 ± 14.8[f]
41. Coxite width	74.7 ± 7.8[b]	74.7 ± 5.8[d]	64.0 ± 5.2[a,c]	69.9 ± 7.7	70.4 ± 10.1
42. Style length	136.5 ± 5.6	134.4 ± 6.2	132.8 ± 9.5	127.5 ± 10.2[a]	137.6 ± 12.9[b]

Means followed by no letter or the same letter were not significantly different ($P > 0.05$).

in Brazil, specific to distinct regions, and suggested the existence of a species complex. A retrospective examination of a large number of specimens of *L. whitmani*, captured at different Brazilian localities during the last 40 yr (Phlebotomine Reference Collection at our laboratory), revealed subtle variability in a number of morphological characters. To clarify this variability, field specimens of *L. whitmani* were captured at 5 municipalities located in the southeastern and northeastern areas in Brazil and submitted to detailed morphometric study. These municipalities presented similar ecological conditions, presence of American cutaneous leishmaniasis with peridomestic transmission, and abundance of *L. whitmani.* Our investigation describes the existence of biogeographical clusters among those 5 populations, based on morphometry.

Materials and Methods

Lutzomyia whitmani were captured at night by Shannon (Shannon 1939) and CDC (Sudia and Chamberlain 1962) traps at the Brazilian municipalities of Ilhéus, Bahia (14° 47′ 2″ S, 39° 02′ 58″ W); Martinho Campos, Minas Gerais (19° 19′ 54″ S, 45° 14′ 13″ W); Corte de Pedra, Bahia (13° 26′ 23″ S, 59° 39′ 36″ W); Baturité, Ceará (04° 19′ 43″ S, 38° 53′ 05″ W); and Amaraji, Pernambuco (08° 22′ 59″ S, 35° 27′ 09″ W) (Fig. 1).

Sand flies were mounted on microscope slides in Canada balsam (for males) or Berlese medium (for females). In total, 3,550 specimens were identified as *L. whitmani*, using specific descriptions and taxonomic keys (Antunes and Coutinho 1939, Young and Duncan 1994). Fifteen males and 15 females from each population were selected, based on the completeness and

Table 2. Morphometric data (in microns) of *L. whitmani* female characters expressed as mean ± SE

Character	Population				
	Ilhéus	Martinho Campos	Corte de Pedra	Baturité	Amaraji
1. Head length	390.9 ± 13.8	411.7 ± 33.5[a,c]	382.9 ± 34.8[b]	381.9 ± 16.1[d]	394.7 ± 8.9
2. Clypeus	118.9 ± 5.9[a]	129.1 ± 7.9[b,d]	121.6 ± 11.4[f]	110.9 ± 12.8[c,e,g]	123.7 ± 5.1[h]
3. Interocular distance	109.9 ± 7.7[a,c]	120.5 ± 5.6[b]	117.3 ± 8.4	116.3 ± 4.1	123.2 ± 10.4[d]
4. Total length palpus	591.4 ± 25.0	578.1 ± 35.8	592.5 ± 40.2	597.9 ± 27.1	586.3 ± 16.2
5. Palpal I length	44.3 ± 4.1[b]	37.3 ± 5.8[a]	43.2 ± 4.1[b,c]	48.0 ± 4.3[b,d]	47.5 ± 2.1[b]
6. Palpal II length	162.7 ± 9.4	168.0 ± 9.1[a]	163.7 ± 9.5	160.0 ± 6.8	156.8 ± 4.1[b]
7. Palpal III length	180.3 ± 9.5	173.3 ± 8.4	176.5 ± 11.5	172.3 ± 6.7	173.3 ± 5.8
8. Palpal IV length	61.7 ± 8.5	62.4 ± 7.5	59.7 ± 5.1	65.6 ± 3.3	61.9 ± 3.7
9. Palpal V length	144.6 ± 6.6	140.8 ± 8.4	146.1 ± 13.0	152.0 ± 15.7	148.3 ± 9.6
10. Labrum length	364.3 ± 23.0	372.3 ± 40.0	362.7 ± 29.6	363.7 ± 14.4	361.1 ± 10.4
11. Pharynx length	182.7 ± 4.9	185.7 ± 15.5	176.4 ± 7.0	183.8 ± 9.4	179.1 ± 6.7
12. Flagellomere I	70.4 ± 3.3[a]	66.1 ± 5.6	65.1 ± 5.9[b]	68.3 ± 4.1	68.8 ± 4.1
13. Flagellomere II	64.5 ± 2.1	64.0 ± 4.3	65.1 ± 4.1	64.5 ± 2.1	64.5 ± 2.1
14. Flagellomere III	228.8 ± 12.0	226.7 ± 17.0	233.6 ± 13.2	225.1 ± 10.4	231.5 ± 16.1
15. Flagellomere IV	96.5 ± 2.1	98.1 ± 7.1	98.1 ± 6.4	95.5 ± 3.7	99.7 ± 5.9
16. Flagellomere V	96.53 ± 3.7	99.7 ± 5.9	99.2 ± 5.9	94.9 ± 2.8	99.7 ± 5.1
17. Flagellomere VI	97.6 ± 3.3	100.3 ± 5.1[a]	98.1 ± 2.8	94.9 ± 2.8[b]	99.2 ± 4.1
18. Flagellomere VII	98.1 ± 3.7	101.9 ± 7.1[b]	100.3 ± 4.1[d]	94.9 ± 2.8[a,c]	99.2 ± 4.0
19. Flagellomere VIII	96.5 ± 3.7	100.0 ± 5.2[a]	97.1 ± 4.1	94.4 ± 3.3[b]	98.1 ± 3.7
20. Flagellomere IX	95.5 ± 3.7	100.0 ± 6.8[a]	98.7 ± 3.9	94.4 ± 3.3[b]	98.7 ± 3.9
21. Flagellomere X	94.4 ± 3.3	96.0 ± 5.4	94.9 ± 5.1	94.4 ± 3.3	97.1 ± 2.8
22. Flagellomere XI	93.7 ± 3.7	95.4 ± 2.2	95.5 ± 4.7	92.8 ± 5.1	96.6 ± 3.8
23. Flagellomere XII	90.7 ± 3.9	90.2 ± 3.7	91.2 ± 5.9[a]	84.3 ± 10.4[b]	90.9 ± 4.0
24. Flagellomere XIII	86.0 ± 5.6	85.3 ± 5.6	85.9 ± 4.7	80.5 ± 10.7	87.3 ± 4.3
25. Maximum width of wing	633.1 ± 24.9[b]	635.2 ± 49.9[d]	610.7 ± 40.1	592.5 ± 15.8[a,c]	619.2 ± 22.6
26. R-5 length	1,336.5 ± 56.3	1,402.1 ± 124.4[a]	1,349.3 ± 64.5	1,308.3 ± 49.0[b]	1,364.3 ± 54.3
27. α length	580.8 ± 48.7[b]	604.8 ± 85.4[b]	614.9 ± 56.1[b]	522.1 ± 30.4[a]	619.2 ± 39.0[b]
28. β length	294.4 ± 25.9	312.5 ± 55.3	282.7 ± 31.2	317.9 ± 60.4	293.3 ± 23.2
29. χ length	232.5 ± 22.7[b]	203.2 ± 38.5[a,c]	210.1 ± 18.5	235.2 ± 24.3[d]	226.7 ± 20.4
30. δ length	312.5 ± 45.7	321.6 ± 84.9	341.3 ± 51.6[b]	264.5 ± 51.8[a,c]	351.5 ± 28.4[d]
31. Coxa length	327.2 ± 22.2[a,c]	300.8 ± 28.2[b]	306.7 ± 18.8[d]	316.3 ± 10.8	318.9 ± 10.8
32. Femur length	740.3 ± 37.4	740.8 ± 37.4	733.5 ± 40.6	748.8 ± 34.6	747.2 ± 35.7
33. Tibia length	926.9 ± 160.4	940.3 ± 190.6	873.6 ± 200.9	940.8 ± 205.5	922.9 ± 169.6
34. Tarsus length	1,125.5 ± 108.3	1,144.0 ± 139.9	1,138.1 ± 125.7	1.103.5 ± 128.7	1,130.7 ± 121.7
35. Cercus length	126.5 ± 4.9[b]	125.5 ± 4.8[d,h]	131.9 ± 4.2[a,c,e]	131.6 ± 3.5[g,i]	122.8 ± 6.6[f,j]
36. Spermathecae length	47.5 ± 2.3[b,h]	39.4 ± 10.2[a,c,e]	40.7 ± 4.9[g,i,k]	51.3 ± 4.7[d,j]	48.7 ± 4.7[f,l]
37. Spermathecae width	10.6 ± 1.0[b,h]	7.2 ± 1.0[a,c,e]	8.0 ± 1.4[g,i,k]	10.2 ± 1.1[d,j]	10.7 ± 0.9[f,l]

Means followed by no letter or the same letter were not significantly different ($P > 0.05$).

integrity of specimens, for morphometric analysis of characters previously found important in separating genera, subgenera, and species in phlebotomine sand flies (Martins et al. 1978, Young and Duncan 1994) (Tables 1 and 2).

Morphometric data were compared by one-way analysis of variance. When a statistically significant difference was found for a given character, data were analyzed further by the all pairwise multiple comparison procedure (Bonferroni *t*-test) using SigmaStat statistical software (Jandel 1995).

For phenetic analysis, each population (A–E) was considered as 1 operational taxonomic unit. A mean value (M1) was determined for each character in a given operational taxonomic unit. A mean value (M2) of the means M1s for the 5 operational taxonomic units together was calculated for each character. Based on the relationship between M1 and M2, each character was coded as "0" (zero) for M1 < M2 or "1" (1) for M1 ≥ M2. A phenogram was constructed by the unweighted pair-group method analysis according to Sneath and Sokal (1973) after determination of the

Table 3. Association coefficients between *L. whitmani* populations (males, upper right half; females, lower left half)

Population	Ilhéus	Martinho Campos	Corte de Pedra	Baturité	Amaraji
Ilhéus	—	0.23	0.26	0.64	0.47
Martinho Campos	0.21	—	0.69	0.26	0.57
Corte de Pedra	0.43	0.56	—	0.38	0.59
Baturité	0.72	0.16	0.32	—	0.35
Amaraji	0.40	0.59	0.48	0.29	—

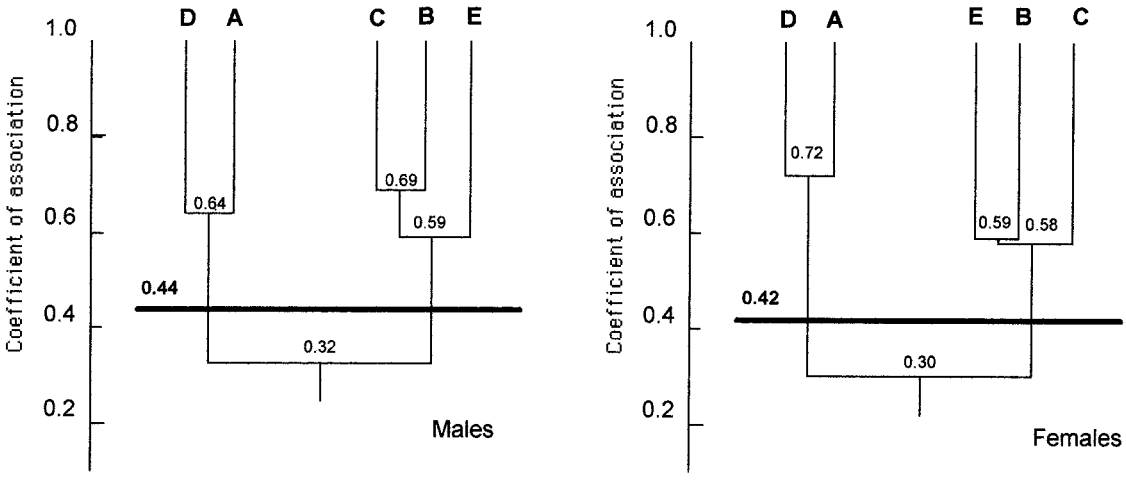

Fig. 2. Phenogram depicting the similarity among 5 Brazilian populations of males and females of *L. whitmani* obtained by unweighted pair-group method analysis. A, Ilhéus; B, Martinho Campos; C, Corte de Pedra; D, Baturité; E, Amaraji.

association coefficients by the simple matching method.

Results and Discussion

The morphometric analysis confirmed the existence of variability within several characters among populations. Significant differences were observed in 27 out of 42 male (64%) and 19 of 37 (54%) female characters. Some characters discriminated one population from the remaining populations. For males, 4 characters (interocular distance, total length of palpus, palpomer V length and β length of wing section) discriminated the population of Ilhéus from the other 4. The population of Martinho Campos presented smaller femur length and the population of Baturité smaller labrum length when compared with the others. For females, smaller palpal I length and the length of wing vein section distinguished populations from Martinho Campos and Baturité, respectively. Other characters statistically varied, but were not discriminative of a unique population and are marked with letter coding in the list of characters analyzed (Tables 1 and 2).

Morphometric data were analyzed by phenetic methods. Character states were coded as "0" or "1" for the 42 male and 37 female characters. The association coefficients between each pair of localities are indicated for males and females in the upper and lower triangular matrix, respectively (Table 3).

The phenograms obtained for males and females indicated 2 groups: one composed of populations from Ilhéus and Baturité and the other of populations from Martinho Campos, Corte de Pedra, and Amaraji (Fig. 2). Placement of populations from Ilhéus and Baturité in the same group is not surprising, because Rangel et al. (1996) could not detect significant morphological differences between *L. whitmani* populations from these 2 localities. More recently, Ready et al. (1997) published a cladogram based on the comparative analysis of mitocondrial DNA sequences (haplotypes) of *L. whitmani* and found that Ilhéus and Corte de Pedra were distinct. These findings are consistent with the morphometric phenograms, in which these 2 populations are found in 2 different clusters.

Our results are not sufficient to define the taxonomic status of the specimens studied, but indicate the existence of biogeographical populations of *L. whitmani.* Complementary studies of susceptibility to *Leishmania braziliensis* infection in the 5 populations are in progress to clarify the relationship between biogeographical clusters and American cutaneous leishmaniasis transmission in those Brazilian regions.

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