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ARTIGO

Ecology of Phlebotomine sandflies (Diptera, Psychodidae) in Brazilian Atlantic Forest

Ecologia de flebotomíneos (Diptera, Psychodidae) na Floresta Atlântica do Brasil

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Abstract *The phlebotomine sandfly fauna of a primary forest reserve at Morretes (eastern Paraná State) was studied, using CDC-like light traps, one night per month, at canopy and ground level, between April 1995 and March 1996. A total of 3,106 insects were collected, identified as belonging to nine species.* Lutzomyia ayrozai *and* Lu. geniculata *were predominant, seven other species also being present. Monthly mean temperature, rainfall and the temperature of the collection night significantly influenced the numbers of* Lu. ayrozai *while the two first factors influenced the numbers of* Lu. geniculata*, besides the collected quantities of females of the two species. The influence of the factors on* Lu. ayrozai *numbers was more immediate than in those of* Lu. geniculata*. Numbers of both species and of the females of* Lu. geniculata *collected in different seasons, but not at the different heights, varied significantly. Differences between the behaviour of* Lu. ayrozai *in Morretes and in other regions could be attributed to environmental differences and/or to regional variations in the species, which could constitute species complexes. Hourly variations of collections were different in the species and seasons.*

Key-words: *Phlebotominae. Fauna. Seasonal variation. Psychodidae. Height influence.* Lutzomyia*.*

Resumo *A fauna de flebotomíneos de uma reserva de floresta primária em Morretes (leste do Estado do Paraná) foi estudada, utilizando armadilhas luminosas tipo CDC, uma noite por mês, no nível do solo e da copa, entre abril de 1995 e março de 1996. Um total de 3.106 insetos foi coletado.* Lu. ayrozai *e* Lu. geniculata *foram predominantes, além de outras sete espécies. A temperatura média mensal, a pluviosidade e a temperatura na noite de coleta influenciaram significativamente as quantidades de* Lu. ayrozai *e os dois primeiros fatores influenciaram* Lu. geniculata*, além das quantidades de fêmeas coletadas de ambas as espécies. A influência do aumento dos fatores nas quantidades de* Lu. ayrozai *foi mais imediata que nas de* Lu. geniculata*. As quantidades coletadas de ambas as espécies e das fêmeas de* Lu. geniculata *nas estações, mas não nas alturas, foram significativamente diferentes. As diferenças entre os comportamentos de* Lu. ayrozai *em Morretes e em outras regiões poderiam ser atribuídas a diferenças ambientais e/ou a variações regionais na espécie, que poderia constituir um complexo de espécies. As variações horárias nas coletas foram diferentes nas espécies e estações.*

Palavras-chaves: *Phlebotominae. Fauna. Variação estacional. Psychodidae. Influência da altura.* Lutzomyia*.*

The study of the seasonal variation in populations of phlebotomine sandflies is very important to understand their biology and possible epidemiological importance. Studies of this variation have been done[7 11], and some of which have provided data on the risk of transmission of parasites to man[26].

The numbers of insects collected at several heights above ground level can help identify to define their hosts and the transmission cycle of parasites, as shown by Williams[46 47]. The study of hourly variations in the activity of phlebotomine sandflies can be useful in clarifying their biology and determining their relationship to possible reservoirs of parasites and man biting. This variation has been studied in several regions[23 38 43 45 46]. Information on seasonal and hourly variation of sandfly activity is useful for control of transmission of parasites to man.

Human[16 28 40] and canine[29] infections by *Leishmania* Ross, 1903 have been detected in western Paraná. *L. braziliensis* Vianna, 1911 (*L.* for *Leishmania*, to distinguish from *Lu.* for *Lutzomyia*) was isolated from sentinel animals in the Ribeira River Valley, São Paulo[18 21], and *L. enrietti* was isolated from rodents in this region[32] and near Curitiba, Paraná[44]. Sand fly vectorial ecology was reviewed[17] in the State of São Paulo, including the important endemic region in the Ribeira River Valley. Additionally, three authoctonous

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human cases of dermal leishmaniasis, one of them due to *L. braziliensis* Vianna, 1911, were observed at Piçarras, in eastern Santa Catarina State[42], about 150 km south of Morretes. A preliminary study of this focus indicated a high prevalence of dermal leishmaniasis in the human population (M. Steindel- pers. commun.). The occurrence of leishmaniasis in Piçarras indicates the possibility of natural undetected transmission of *Leishmania* in the forested areas between the Ribeira Valley and Piçarras, which would justify studies on the phlebotomine fauna in this region.

The phlebotomine sandfly fauna of the western region of Paraná was previously studied[4 10 12 13 19 43]. However, except for the findings of *Lu. shannoni* and *Lu. lanei* at São José dos Pinhais[35] and *Lu. monticola* Costa Lima, 1932 at Curitiba (both ca. 910 m a. s. l.)[30], no information on the fauna of the eastern region of this state has been published.

During the present study, phlebotomine sandflies collected in Atlantic forest at Morretes, in the eastern, low-lying part of Paraná, were studied, in order to obtain information on seasonal abundance, hourly variation in the activity and the influence of height above ground level on the collections.

MATERIAL AND METHODS

Two CDC-like light traps[37] were hung in a primary forest situated within IAPAR (Agronomic Institute of Paraná) reserve in the municipality of Morretes (25° 28'37" S 48° 50' 04" W, 10 m a. s. l.). They were separated by a horizontal distance of 5 m and were installed at heights of 1.5 and 7 m above ground level, designated respectively as *ground* and *canopy* stations. Collections were made at 200 m from the border of the forest. The vegetation is that of a rain forest, in the transition between tropical and subtropical types [31]. Collections were made from April 1995-March 1996. Due to the observations of better efficiency of light traps at new moon[6 39], relative respectively to sandflies and Culicidae, collections were done in this phase of the lunar cycle.

Monthly temperature, precipitation and relative humidity data were obtained from the Meteorological System of Paraná (SIMEPAR), and the daily precipitation from IAPAR, at Morretes. A thermometer situated between the traps measured the temperature on each collection night.

The numbers of sandflies in each sample were transformed to the modified geometric mean (Williams mean- M_w)[9], to prevent excessive influence of some large samples. ANOVA with two variables was used to check the possible influence of sampling height and seasonalities. The correlation between the numbers collected during each month and the climatic conditions was tested. The significance of r was tested by the t test, at the 1% and 5% significance levels[24].

Light traps were utilised from 18.00-06.00. Collection chambers of traps were replaced at two hour intervals, the change being completed within 2-3 min. Sandflies were prepared for identification by NC method[34].

RESULTS

The numbers of sandflies collected in the canopy and at ground level in each month are shown in Table 1, together with climatic data. Results indicate that the numbers of *Lu. ayrozai* (Barretto & Coutinho, 1940) collected appeared to show a correlation with monthly mean temperatures and rainfall, and to a lesser degree to the temperature on the night of the collection. The same relationship appeared also to occur in *Lu. geniculata* (Mangabeira, 1941), although there was a delay between their own rise and that observed in the collected quantities.

Statistical analysis of correlation between collections of *Lu. ayrozai* and *Lu. geniculata* and climatic conditions (Table 1) showed some significant results. *Lu. ayrozai* numbers were correlated to monthly temperature in the month of collection, rainfall and temperature in the night of collection, and female numbers only to the first and the last of the factors above. The total numbers and those of females of *Lu. geniculata* were correlated to monthly temperatures two and three months before collection and the last also to monthly rainfall three months before. These data confirm the aforementioned suspicion of the influence of temperature and rainfall in the preceding months in the numbers of *Lu. geniculata* collected.

Collections of both sexes and of females of *Lu. ayrozai* and *Lu. geniculata* from ground level and the canopy were correlated at a significance level of 0.1%. When insects from canopy and ground were analysed together, numbers of *Lu. ayrozai* were correlated to all the studied climatic conditions in the month; totals of *Lu. geniculata* were correlated to monthly rainfall three months before and numbers of females were correlated to monthly relative humidity and temperature of night collection three months before.

Table 2 shows the seasonal M_w for all species, combining samples of April, May and June for Autumn, and so on for the four seasons. No statistically significant correlation was found between the numbers of sandflies of any species and the temperature in any of the two-hour periods. The mean numbers collected in each period varied according to the season. *Lu. ayrozai* was collected during all the periods, but occurred most frequently around midnight. In contrast, *Lu. geniculata* was collected more frequently before midnight, and exclusively before this hour in winter and spring.

It was difficult to establish standards of variation for the less common species. *Lu. fischeri* was collected almost exclusively before midnight, generally after 20.00. However, it was collected in greater numbers between

Table 1 - Numbers of phlebotomine sandflies collected by CDC-like light traps at Morretes, Paraná, at canopy and ground levels.

	Month												
Canopy	Apr/95	May/95	Jun/95	Jul/95	Aug/95	Sep/95	Oct/95	Nov/95	Dec/95	Jan/96	Feb/96	Mar/96	Total
Lu. ayrozai	300	17	1	8	0	0	19	60	62	166	29	135	797
Lu. geniculata	210	58	1	30	0	0	0	0	1	11	6	33	350
Lu. fischeri	43	94	2	7	2	0	9	21	27	3	2	4	214
Lu. pascalei	0	2	0	1	0	1	4	1	9	3	1	2	24
Lu. shannoni	14	2	0	0	0	0	0	0	0	0	0	0	16
Lu. monticola	9	2	0	0	0	0	0	0	1	0	0	0	12
Lu. lanei	4	0	0	1	0	0	0	0	1	0	0	0	6
Lu. migonei	0	0	0	0	0	0	0	0	1	0	0	0	1
B. nitzulescui	1	0	0	0	0	0	0	0	0	0	0	0	1
Not identified	3	0	0	0	2	0	1	2	0	2	0	0	10
Total	584	177	4	47	4	1	33	84	102	185	38	174	1431
Ground													
Lu. ayrozai	416	0	0	8	4	0	5	166	112	70	31	123	935
Lu. geniculata	353	31	1	26	1	0	1	6	1	11	10	79	520
Lu. fischeri	73	12	3	4	1	0	5	16	17	7	0	2	140
Lu. pascalei	2	0	4	1	3	0	2	3	10	2	2	2	31
Lu. monticola	14	0	0	0	0	0	0	0	2	0	0	0	16
Lu. lanei	12	0	0	0	1	0	0	0	0	0	0	0	13
Lu. shannoni	12	0	0	0	0	0	0	0	0	0	0	0	12
B. nitzulescui	1	0	0	0	0	0	0	0	0	0	0	0	1
Not identified	1	1	0	0	0	0	0	0	4	0	1	0	7
Total	884	44	8	39	10	0	13	191	146	90	44	206	1675
Monthly rainfall	98.9	36.7	74.5	145.6	74.2	141.8	118.6	193.6	300	342.8	306.6	259.6	-
M. mean temp.	22	18.8	18	18.2	18	18	19	22.4	24	25.2	24.7	23.2	-
Monthly rel. hum.	83	87	89	88	87	88	87	83	80	73	80	83	-
Temp. night coll.	20	13.7	14.1	15.9	18.2	16.7	15	20.2	23.8	20.9	23.1	21.9	-

Coll. collection; hum. humidity; m. monthly; rel. relative; temp. temperature.

Table 2 - Mean quantities (M_w) of sandflies collected by CDC-like light traps at canopy and ground levels in the seasons at Morretes (PR).

Height	Canopy				Ground			
Species/ season	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
Lu. Geniculata[a b]	28.20	2.14	2.96	13.19	27.30	8.42	2.04	20.94
Lu. Ayrozai[a]	21.13	1.08	31.58	87.00	6.47	3.33	47.38	64.56
Lu. Fischeri[a]	22.23	1.88	16.02	2.91	14.67	2.42	11.24	1.88
Lu. Shannoni[a]	2.56	0.00	0.00	0.00	1.35	0.00	0.00	0.00
Lu. Monticola[a]	2.11	0.00	0.26	0.00	1.47	0.00	0.44	0.00
Lu. Lanei[a]	0.71	0.26	0.59	0.00	1.35	0.26	0.00	0.00
Lu. Pascalei	0.44	0.59	2.42	1.88	1.47	0.59	4.09	2.00
B. nitzulescui	0.26	0.00	0.00	0.00	0.26	0.00	0.00	0.00
Not identified	0.59	0.44	0.44	0.44	0.59	0.00	0.71	0.26
Total	79.25	6.83	73.90	107.28	70.03	11.43	72.38	93.64

a: significant for seasons and not for levels, at 5%. b: significant influence of season, but not of level, at 5%, on the quantity of females.

18.00-20.00, in April. *Lu. shannoni* was collected only in April and May, most frequently after midnight. *Lu. monticola* and *Lu. lanei* showed similar patterns, and *Lu. pascalei* showed a more uniform distribution with respect to its diurnal activity and seasonal abundance.

DISCUSSION

The sandfly fauna of the area studied includes species previously found in São Paulo State, more northern areas and western Paraná. This is the first report of *Lu. ayrozai* and *Lu. geniculata* in Paraná.

The females of *Lu. ayrozai* and *Lu. geniculata* were redescribed[15]. *Lu. ayrozai* and *Lu. hirsuta* were the commonest species collected in forested areas of Rio de Janeiro, on human bait[6] and *Lu. barrettoi* (Mangabeira,

1942) and these in light traps[6]. *Lu. ayrozai* was the commonest species collected by light traps in a forested area of southern São Paulo[21] and has been collected, together with *Lu. geniculata*, in five of the seven American dispersion centres defined by Martins & Morales-Farias[36].

Lu. geniculata, considered to be either a synonym of *Lu. guyanensis* (Floch & Abonnenc, 1941)[21 22] or a valid species[50], is also common in the eastern region of São Paulo[21 22] and several other parts of South and Central America[27 47 48], but it has not yet been found in collections from the Municipality of Piçarras and the Island of Santa Catarina (CB Marcondes: unpublished data). Although *Lu. geniculata* was not found at Serra dos Órgãos (Teresópolis, ca. 850 m a. s. l.)[2 3 6 7] and Itaguaí (ca. 35 m a. s. l.)[5], it was collected at Parati (ca. 5 m a. s. l.), in an area similar to Itaguaí, although closer to the sea[1]. These *Psychodopygus* species therefore seem to have a patchy distribution in SE Brazil, including the possible replacement of *Lu. hirsuta* by *Lu. geniculata* at higher latitudes and lower elevations. Although some specimens of *Lu. geniculata* were collected biting man near houses at Parati[1], it seems to be a typical primary forest species that occurs only near the sea. *Lu. ayrozai* is common in secondary forest at Florianópolis (Santa Catarina State), but *Lu. geniculata* was not yet found in this state (CB Marcondes: unpublished results). This could indicate that the southern limit of distribution of the last species is somewhere between Morretes and Florianópolis or can be due to a poor adaptation to secondary forests. *Lu. geniculata* is an infrequent man-biter in Belize[47] and it has been taken on human bait at Curiche and some other localities in Colombia[49]. No flagellates were found in 19 dissected females of *Lu. geniculata* from Belize[48].

Several specimens of *Lu. pascalei* were collected at Morretes and this species was also very common in light trap collections made in southern São Paulo[21]. *Lu. barrettoi*, a very similar species, was very common in light traps[7] at Serra dos Órgãos (Rio de Janeiro) and was not collected by this method[2 6] at Morretes and at São Paulo[21].

The numbers of *Lu. fischeri* collected in the canopy and at the ground level did not significantly differ at Morretes, possibly because the distance between the two stations was too small. The numbers collected at Rio de Janeiro in the canopy appear to be greater than those collected at ground level, although these were not analysed statistically[2]. This species seems to be highly anthropophilic[5 14].

Lu. neivai (Neiva, 1926) and *Lu. intermedia* (Lutz & Neiva, 1912), may be predominant in forested regions, respectively, in São Paulo[13] and Rio de Janeiro[41] States. *Lu. intermedia s. l.* was found in forested areas of the Ribeira River Valley[18 20 21 22] in southern São Paulo, a similar environment situated close to Morretes. No specimens of this species complex [33] were collected at Morretes. This could be attributed to environmental characteristics or to the restriction of the present study to one area in the forest. *Lu. neivai* has been found only in houses and in the border of forested areas at Florianópolis, and some in patches of secondary forest at Piçarras (CB Marcondes: unpublished results).

The sandfly fauna in forested areas at Pariqüera Açu[20] and Cananéia[21], about 120km to the north-east of Morretes, was much more complex than that observed in Morretes. Possibly the study of other biotopes at Morretes will reveal the presence of other species.

During the present study, *Lu. ayrozai* was found at ground and canopy level, without significant differences in numbers between the two stations. Sandflies of this species are common man-biters in some forest environments[2 6 21 38] and can be collected within and outside the forest in southern São Paulo,[20] occasionally even near houses[21]. Insects of this species were collected exclusively at ground level at Rio de Janeiro[6], almost exclusively at this level at Colombia[38] and principally at this level at Southern São Paulo State [21]. Due to its occurrence in both levels, it was considered to be acrodendrophilic[21]. *Lu. geniculata* was collected only at ground level at Belize[47], unlike the results of the Morretes study. The rarity of human infection by *L. naiffi* Lainson & Shaw, 1989 in the Amazon basin could be due to the low degree of anthropophily shown by *Lu. ayrozai*[25], although many specimens of the species were collected on human bait in the Ribeira Valley[21], constituting additional evidence of regional variation of the species.

The regional variations in the behaviour of *Lu. ayrozai* and *Lu. geniculata* could be due to unperceived environmental differences among the localities studied, but could also indicate that species complexes, rather than single species, are involved. The wide geographical distributions of these species, like those of *Lu. shannoni* (Dyar, 1929), *Lu. whitmani* (Antunes and Coutinho, 1939) and *Lu. intermedia s. l.*, indicates the need for studies on their taxonomy and ecology. The possible importance of such conditions in the transmission of parasites should be studied by dissection for parasites and analysis of the parous rate of the insects. The highest months of biting activity for *Lu. ayrozai* near Manaus were different, according to the bait animals used[8]. This illustrates the influence of the collection method on the results and the need to use several methods to study the biology of sandflies.

Lu. ayrozai was mostly collected around midnight, the hourly variation in the biting activity being similar to that observed in forest in Rio de Janeiro[7] and São Paulo[21]. The diel rhythm of some species can vary between different sites, as observed for *Lu. trapidoi* (Fairchild & Hertig, 1952) in two regions of Colombia[38 45].

There are no precise data on man biting activity at Morretes, and the seasonal fluctuation in the female numbers could give indications of this activity.

The occurrence of *Brumptomyia nitzulescui* in the trap hung in the *canopy* is curious, since this species is probably associated with armadillos. Several specimens of this species also were collected under similar conditions in Florianópolis (CB Marcondes: unpublished data).

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