Effects of timber harvest on phlebotomine sand flies (Diptera: Psychodidae) in a production forest: abundance of species on tree trunks and prevalence of trypanosomatids

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The Amazon forest is being exploited for timber production. The harvest removes trees, used by sand flies as resting sites, and decreases the canopy, used as refuges by some hosts. The present study evaluated the impact of the timber harvest, the abundance of sand flies, and their trypanosomatid infection rates before and after selective logging. The study was accomplished in terra-firme production forest in an area of timber harvest, state of Amazonas, Brazil. Sand fly catches were carried out in three areas: one before and after the timber harvest, and two control areas, a nature preservation area and a previously exploited area. The flies were caught by aspiration on tree trunks. Samples of sand flies were dissected for parasitological examination. In the site that suffered a harvest, a larger number of individuals was caught before the selective extraction of timber, showing significant difference in relation to the number of individuals and their flagellate infection rates after the logging. The other two areas did not show differences among their sand fly populations. This fact is suggestive of a fauna sensitive to the environmental alterations associated with selective logging.

Key words: environmental impacts - Phlebotominae - Trypanosomatidae - Central Amazon

Phlebotomine sand flies (Diptera: Psychodidae) are insects of medical and veterinary importance, as they can transmit leishmaniasis, bartonellosis, and arboviruses. In Amazonia, these insects have a high richness of species (Young & Duncan 1994) and high indices of local diversity in terra-firme forest (Barrett et al. 1996). In spite of this, the ecological studies on Amazonian species of phlebotomine sand flies are still scarce and almost restricted to the *Leishmania* (Kinetoplastida: Trypanosomatidae) vectors. This fact may be explained in part because of the diurnal resting places of a considerable part of the forest phlebotomine species are unknown. Among the few species of phlebotomine that can be easily found in the forest, are those that rest in the base of tree-trunks. Systematic collections on trunk trees, relating species of trees, and phlebotomine sand flies were done by Geoffroy et al. (1985) in the French Guyana and by Cabanillas and Castellón (1999) in Brazilian Central Amazonia. Although the predominant species in both papers was *Lutzomyia umbratilis* Ward & Frahia, some differences occurred in the faunistic composition of the other species of sand flies, showing a different characterization of specific fauna for each region.

In Costa Rica, Memmott (1991) found that 97% of the phlebotomines caught on tree trunks were composed of four species. To the author (Memmott 1991, 1992), the bases of trees are mating and resting places, swarming place, and source of vertebrate hosts.

Amazonia has been exploited intensively in relation to timber. The harvest removes a substrate (bases of trees) used as resting places by sand flies and opens up the canopy used by vertebrate hosts. The females of phlebotomines need to engorge on vertebrate blood to develop their eggs, so alterations in the macrofauna may have reflections in the fauna of those insects. The adults of sand flies need vegetable sugars (Cameron et al. 1995) and the larvae are dentrivores or feed on small organisms in the soil (Hanson 1968), and probably will be affected by the changes of edaphic fauna and microclimatic alterations (Rutledge & Ellenwood 1975).

Although the direct contact between man and phlebotomine foci on the bases of trees is an important source for human infection, the variation of the infection rates of females in time and space has not been systematically studied. The effects of timber harvest on the infection rates of females of those species are unknown. According to Walsh et al. (1993) deforestation results in inevitable effects on vector-borne disease, which in tropical rain forests have rarely been adequately documented. Each environmental change by natural phenomena or through human intervention alters the ecological balance and context within which vectors and their parasites breed develop and transmit diseases (Patz et al. 2000). The present study evaluated the effect of the timber harvest on the abundance of sand flies and their flagellate infection rates. This study is probably the first to evaluate the logging effects on tree-inhabiting sand fly fauna in Amazonia.

MATERIALS AND METHODS

The Mil Madeireira production forest is an area of 806 km^2 and mainly of terra-firme vegetation under sustained management located north of the Amazon River

Financial support: Inpa, PPI 1-3060
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FACP was financed by CNPq
Received 25 January 2007
Accepted 11 June 2007

in the municipalities of Silves and Itacoatiara, in the state of Amazonas between 2º43'S and 3º04'S and 58º31'W and 58º57'W. The management plan is based on compartments of 2000 ha, one or more of which is selectively logged annually.

The study was realized in three different areas: (a) Compartment N, area with timber harvest during the study where the sand flies collections were accomplished before and after the timber harvest, (b) a preservation area (PA), as control area, and (c) a second control area, Compartment A, where previous exploitation had been made. In Compartment N, the selected trees were those not destined to the harvest of 1999. Ten trees were selected in each study area, and all of them identified by the keys of Ribeiro et al. (1999). The selection of the trees was based on previous collections to verify the constancy of sand fly populations on the base of these trees. Only trees with a good number of sand flies were chosen.

The trees had similar size, with height over 20 m and girth > 50 cm (Table I). During the catches, three people worked simultaneously in each area, during the morning, in the interval between 9:00-11:00 AM, when sand flies are known to be present on the trunk of trees, near the roots (Arias & Freitas 1982). Samples were obtained by sweeping the tree base, using a CDC trap attached to a conventional gauze insect cage as aspirator (Killick-Kendrick 1987), during at least 10 min per tree. The cage was replaced after the collection from each tree. Six systematic collections were made, between July and November of 1999, a dry period, when the Mil Madeireira company usually starts the timber exploitation. The capture effort in collecting sand flies was three days per collection. The N Compartment was logged at the end of September of 1999. The three areas were sampled simultaneously for three days for each of three collections before and three collections after the harvest in compartment N.

Portions of the female sand flies from each sample were dissected individually in a drop of sterile saline solution on a microscope slide and the digestive tract examined with a phase contrast microscope to detect the presence of flagellates. Identification and classification of the sand flies follows Young and Duncan (1994).

The Kruskall-Wallis test was used to compare the population of sand flies and the trypanosomatid rates among the studied areas. In order to compare the effects of the timber harvest on the sand fly phlebotomine populations and their trypanosomatid infection rates, we used the Mann-Whitney Rank Sum test (Zar 1974).

TABLE I

List of selected trees for the systematic collections of phlebotomine sand flies in the N and A compartments and preservation area , Madeireira Mil, Itacoatiara, AM, Brazil

Areas	No.	Tree species	Family
Compartment N	1	*Sloanea* sp.1	Elaenocarpaceae
	2	*Caryocar pallidum* A.C.Sm.	Caryocaraceae
	3	*Eschweirela micrantha* (Berg) Myers	Lecythidaceae
	4	*Lecythis prancei* S.A.Mori	Lecythidaceae
	5	*Lycania* sp.	Chrysobalanaceae
	6	*Micropholis* sp.	Sapotaceae
	7	*Cryssophyllum amazonicum* T.D.Penn.	Sapotacea
	8	*Caryocar pallidum* A.C.Sm.	Caryocaraceae
	9	*Crysophyllum manaosense* T.D.Penn.	Sapotaceae
	10	*Caryocar pallidum* A.C.Sm.	Caryocaraceae
Preservation area	1	*Licania* sp.	Chrysobalanaceae
	2	*Iryanthera lancifolia* Ducke	Myristicaceae
	3	*Cariniana micrantha* Ducke	Lecythidaceae
	4	*Eschweirela micrantha* (Berg) Myers	Lecythidaceae
	5	*Iryanthera lancifolia* Ducke	Myristaceae
	6	*Eschweirela pseudodecolorans* S.A.Mori	Lecythidaceae
	7	*Licania* sp.	Chrysobalanaceae
	8	*Licania impressa* Prance	Chrysobalanaceae
	9	Not identified	Lauraceae
	10	*Caryocar pallidum* A.C.Sm.	Caryocaraceae
Compartment A	1	*Pouteria flavilatex* T.D.Penn.	Sapotaceae
	2	*Eschweirela coriacea* (DC.) Mart. ex Berg	Lecythidaceae
	3	*Eschweirela micrantha* (Berg) Miers	Lecythidaceae
	4	*Scleronema micrantha* Ducke	Bombacaceae
	5	*Sextonia rubra* (Mez) van der Werff	Lauraceae
	6	*Goupia glabra* Ducke	Goupiaceae
	7	*Glycydendron amazonicum* Ducke	Euphorbiaceae
	8	*Eschweirela coriacea* (DC.) Mart. ex Berg	Lecythidaceae
	9	*Sclerobium melanocarpum* Ducke	Leguminosae
	10	*Vatairea paraensis* (DC.) Mart. ex Berg	Leguminosae

Slonaea sp.1: probably an undescribed species.

RESULTS

Distribution of phlebotomine and flagellate infections by sampling plot and tree species - On the 30 chosen trees, 5222 phlebotomine sand flies were collected, representing 11 species of the genus *Lutzomyia*. *L. (Nyssomyia) umbratilis* was the predominant species with 78.14% of the collected specimens, followed by three species of the subgenus *Psathyromyia*, with 18.47% of the specimens (Table II).

Significantly more sand flies were collected in N Compartment with 2763 specimens (H = 9508, p = 0.009; Kruskal-Wallis) than in PA, with 1512 flies or A Compartment, with 947 specimens (Table III).

In N Compartment, the trees with higher numbers of collected sand flies were (by individual tree) *Caryocar pallidum* and *Eschweirela micrantha*, and the trees with less sand flies were *Sloanea* sp. 1 and *Lecythis prancei* (Table III). In PA, the trees with the highest number of collected sand flies were: *Cariniana micrantha* and *Iryanthera lancifolia;* and the trees with the smallest number of sand flies were *E. micrantha* e *I. lancifolia* (Table III). In A Compartment, the trees with the higher number of sand flies collected were *E. micrantha* and *Pouteria flavilatex*, and the trees with less sand flies were *Goupia glabra* and *Glycydendron amazonicum* (Table III). The sand fly species with higher relative frequencies in the three areas were *L. umbratilis*, followed by *L. dendrophyla* and *L. shannoni* (Table II).

A total of 562 females of sand flies (27.04%) of eight species were dissected. Overall, flagellates were found in 64 (13.8%) of *L. umbratilis*, 17 (35.4%) of *L. shannoni*, and 4 (15.1%) of *L. rorotaensis* (Table VI). The N Compartment had more infected phlebotomine sand flies (H = 14.42; p < 0.001) than A Compartment and PA (Table VI). Trypanosomatid infection rates in flies collected in different tree species in the three plots are shown in Table IV.

Populations of phlebotomine sand flies before and after the timber harvest - In N Compartment more sand flies were collected in July to September (pre-harvest) than in October to November (post-harvest) (T = 66; p = 0.047 Mann-Whitney) (Table V). In the control plots A Compartment and PA, there were no significant differences between samples collected in the two periods (T = 74, p = 0.170, T = 81, p = 0.365, respectively).

Infection rates of trypanosomatids in phlebotomine sand flies, before and after the timber harvest - In the N Compartment 61 infected females were found before the timber harvest and only 14 after the harvest (U = 64, p = 0.031; Mann-Whitney) (Table VI). The rates of infected phlebotomine sand flies collected in A Compartment and PA were not significantly different before and after the harvest in N Compartment (U = 70, p = 0.095 and U = 75, p = 0.193; Mann-Whitney, respectively).

DISCUSSION

Almost 60 species within 29 families of trees have been indicated as a resting place of phlebotomine sand flies: Central Amazonia of Brazil, Itacoatiara municipality, Amazonas (present study) and Adolpho Ducke Forest Reserve in Manaus municipality, Amazonas (Cabanillas & Castellón 1999); Panama (Rutledge & Mosser 1971, Chaniotis et al. 1972,1974, Rutledge & Elenwood 1975, Christensen & Vasquez 1983, Memmott 1991); French Guiana (Geofroy et al 1985); and Peru (Parrot 1935).

Those species of trees showed some common characteristics and the most outstanding is that all the tree species concerned reach the canopy of the forest. Ready et al. (1986) analyzed some characteristics in 120 trees (simple trunk with buttresses, bark furrowed or scaly smooth, foliage in part of upper canopy or under storey only) in the state of Pará, Brazil and they observed that *L. umbratilis* had a preference for large trees whose branches interlock in the upper canopy. In the present study, relative abundances in sand fly species (Table III) were similar to those observed elsewhere (Barrett et al. 1991, Geoffroy et al. 1985, Cabanillas & Castellón 1999); where *L. umbratilis*, followed by some species of the subgenus *Psathyromyia* and *L. rorotaensis* were the predominant species on tree trunks. The low frequencies of the other species registered on tree bases in

TABLE II

Phlebotomine sand fly species caught on the base of trees in the N and A compartments and the preservation area, Mil Madeireira, Itacoatiara, AM, Brazil, during July 7 to November 25, 1999

Species	Male	Female	Total	%
Lutzomyia (*Nyssomyia*) *umbratilis* Ward & Frahia	2415	1663	4078	78.14
L. (*Psathyromyia*) *dendrophyla* (Mangabeira)	396	135	531	10.17
L. (*Psathyromyia*) *shannoni* (Dyar)	146	139	285	5.45
L. (*Psathyromyia*) *scaffi* (Damasceno & Arouk)	80	16	96	1.83
L. (*Psathyromyia*) *abonnenci* (Floch & Chassignet)	27	13	40	0.76
L. (*Psathyromyia*) *lutziana* (Costa Lima)	9	5	14	0.26
L. (*Psychodopygus*) *davisi* (Root)	0	2	2	0.04
L.(*Lutzomyia*) *spathotrichia* Martins, Falcão & Silva	30	18	48	0.91
L. rorotaensis (Group Oswaldoi) (Floch & Abbonnenc)	26	64	90	1.72
L. (*Pintomyia*) *christenseni* Young & Duncan	6	19	25	0.48
L. (*Viannamyia*) *furcata* (Mangabeira)	9	4	13	0.24
Total	3144	2078	5222	100

TABLE III

Relative frequencies of phlebotomine sand flies on the selected species of trees of N and A compartments and preservation area (PA), Mil Madeireira, Itacoatiara, AM, Brazil, during July 7 to November 25, 1999

	Species							
Tree species	1	2	3	4	5	6	%	Total
N Compartment								
Sloanea sp.	67,5	18,0	8,2	2,7	0,0	3,6	100	111
Caryocar pallidum	85,5	8,6	3,4	0,5	0,2	1,8	100	407
Eschweirela micrantha	93,0	2,2	3,0	0,6	0,6	0,6	100	276
Lecythis prancei	75,2	15,9	6,3	0,0	0,0	2,6	100	113
Lycania sp.	69,0	8,6	9,9	9,9	0,0	2,6	100	152
Micropholis sp.	68,5	12,6	8,3	2,6	1,1	6,9	100	300
Cryssophyllum amazonicum	66,7	23,0	6,3	2,0	0,5	1,5	100	192
Caryocar pallidum	83,4	9,4	5,7	0,4	0,1	1,0	100	599
Crysophyllum manaosense	79,5	11,9	7,8	0,0	0,0	0,8	100	244
Caryocar pallidum	84,5	8,2	5,1	1,7	0,5	0,0	100	369
Total								2763
PA								
Licania sp.	83,0	4,9	4,1	1,8	0,0	6,2	100	272
Iryanthera lancifolia	77,9	12,2	6,9	0,8	2,2	0,0	100	231
Cariniana micrantha	87,0	5,0	2,5	1,7	0,5	3,3	100	362
Eschweirela micrantha	78,9	0,0	0,0	1,7	1,7	10,7	100	57
Iryanthera lancifolia	75,0	7,3	10,3	4,4	1,5	1,5	100	69
Eschweirela pseudodecolorans	82,9	15,8	1,3	0,0	0,0	0,0	100	77
Licania sp.	77,7	15,0	0,8	0,8	0,8	4,9	100	122
Licania impressa	71,5	12,2	13,1	1,6	1,6	0,8	100	123
Tree 9	90,6	2,0	3,4	0,0	0,0	3,0	100	101
Caryocar pallidum	81,5	5,1	8,3	0,0	0,0	5,1	100	98
Total								1512
A Compartment								
Pouteria flavilatex	87,0	2,6	5,2	2,6	0,5	2,1	100	195
Eschweirela coriacea	92,2	5,7	1,5	0,0	0,0	0,6	100	207
Eschweirela micrantha	89,0	0,0	2,5	0,8	1,8	5,9	100	120
Scleronema micrantha	49,3	25,4	11,9	0,0	1,5	11,9	100	69
Sextonia rubra	41,3	30,7	0,0	0,0	0,0	28,0	100	77
Goupia glabra	43,8	12,5	0,0	0,0	0,0	43,7	100	16
Glycydendron amazonicum	82,6	6,9	0,0	0,0	0,0	10,5	100	29
Eschweirela coriacea	76,2	17,9	0,0	4,7	0,0	1,2	100	84
Lerobium melanocarpum	69,4	9,7	0,0	18,0	0,0	2,9	100	72
Vatairea paraensis	75,6	1,3	3,8	0,0	3,8	15,5	100	78
Total								947

1: *Lutzomyia umbratilis*; 2: *L. dendrophyla*; 3: *L. shannoni*, 4: *L. scaffi*; 5: *L. abonnenci*; 6: other species of sand flies (*L. rorotaensis; L. spathotrichia; L. christenseni; L. lutziana; L. davisi; L. furcata*); Tree 9: tree not identified, family Lauraceae.

Itacoatiara may indicate that they use the trunks only as occasional resting sites or reflect low natural abundance.

The relative frequencies of collected sand flies were constants in the three studied areas, reinforcing the hypothesis of an intrinsic relationship between the sand fly fauna and the microenvironment of tree bases. These results suggest that there is not an obligate direct relation between the sand fly population and particular tree species. The nature of attraction of some species of phlebotomine sand flies to certain trees is not explained yet. Memmott (1991, 1992) did not find any significant difference in the turnover rate of *Lutzomyia* sand flies on trees with naturally abundant sand flies compared to trees on which sand flies had been experimentally transplanted. This indicated that the trees without flies were suitable for using as a resting site, but were apparently lacking in some other attribute important to the flies. She proposed that for sand fly aggregation, microenvironment of tree bases, the presence of vertebrate hosts, and branches interlocking in the upper canopy could be of importance. Rutledge and Ellenwood (1975) suggested that shading and rain exposure of the trunk and the amount of litter influence aggregation.

Flagellate infections - The high rates of trypanosomatids in dissected sand flies in this work were expected. Some previous work developed in Amazonas and Pará, Brazil, with similar sand fly fauna and leishmaniasis foci, demonstrated infection rates comparable to those reported here (Arias & Freitas 1978, Arias et al. 1985, Lainson et al. 1979, 1981, Freitas et al. 2002). In N Compartment and PA, the trees where we found more infected individuals were not those with the greatest num-

TABLE IV

Natural infection by Trypanosomatidae in sand flies on tree species at the N Compartment (CN), preservation area (PA), and A Compartment (CA), Mil Madeireira, Itacoatiara, AM, during July 7 to November 25, 1999

	CN		PA		CA		Total	
Tree species	NI	%	NI	%	NI	%	NI	%
Sloanea sp.	5	7.7	4	26.7	-	-	9	10.6
Caryocar pallidum	25	38.5	0	0.0	-	-	25	29.4
Eschweirela micrantha	22	33.8	1	6.7	0	0	23	27.0
Eschweirela pseudodecolorans	-	-	0	0.0	-	-	0	0.0
Eschweirela coriacea	-	-	-	-	2	40	2	2.3
Lecythis prancei	4	6.2	-	-	-	-	4	4.7
Licania impressa	-	-	2	13.3	-		2	2.4
Lycania sp.	0	0.0	5	33.3	-	-	5	5.9
Cryssophyllum amazonicum	4	6.2	-	-	-	-	4	4.7
Crysophyllum manaosense	3	4.6	-	-	-		3	3.5
Micropholis sp.	2	3.0	-	-	-	-	2	2.3
Pouteria flavilatex	-	-	-	-	3	60	3	3.5
Iryanthera lancifolia	-	-	2	13.3	-	-	2	2.3
Cariniana micrantha	-	-	1	6.7	-	-	1	1.2
Total	65	100	15	100	5	100	85	100

TABLE V

Descriptive analysis of populations of sand flies collected per day in the A and N Compartment and the preservation area, Mil Madeireira, Itacoatiara, AM, during July 7 to November 25, 1999

Area	Period	Mean	Total
N Compartment	July-September[a]	218,55 ± 172,00 (16-577)	1967
	October-November[a]	99,44 ± 99,74 (6-272)	895
Preservation area	July-September	7 1,22 ± 75,01 (5-201)	641
	October-November	96,77 ± 97,23 (27-321)	871
A Compartment	July-September	69,67 ± 127,82 (0-400)	627
	October-November	35,44 ± 25,65 (2-82)	319

a: there are significant differences.

TABLE VI

Relative frequency of Trypanosomatidae infection in sand flies pre-harvest of trees (during July and September) at the N Compartment post-harvest (during October and November), and respective trypanosomatid frequencies in the A Compartment and the preservation area (PA) control groups, Mil Madeireira, Itacoatiara, AM, Brazil

	Before selective timber logging											
	A Compartment			N Compartment			PA			Total		
Sand fly species	DF	IF	%	DF	IF	%	DF	IF	%	DF	IF	%
L. umbratilis	38	3	7.9	172	41	23.8	42	3	7.1	252	47	18.7
L. shannoni	2	0	0	13	8	61.5	4	3	75.0	19	12	63.2
L. rorotaensis	7	2	28.6	2	2	100.0	0	0	0.0	9	4	44.5
Total	47	5	10.4	187	61	32.6	46	6	13	280	63	22.5
	After selective timber logging											
L. umbratilis	28	1	3.6	77	9	11.7	117	7	5.1	222	17	7.65
L. shannoni	1	0	0.0	19	5	26.3	9	1	11.1	29	6	20.7
L. rorotaensis	1	0	0.0	7	0	0.0	1	0	0.0	9	0	0
Total	30	1	3.3	103	14	13.6	127	7	5.5	260	23	8.85

DF: dissected females; IF: infected females.

ber of sand flies (Tables III, IV). In Mil Madeireira, the most abundant populations of sand flies probably contained a higher proportion of younger females not yet exposed to vertebrate reservoirs of trypanosomatids (cf. Ready et al. 1986)

Impact of the timber harvest on sand fly populations - In N Compartment, the sand fly populations suffered a significant decrease after the selective timber harvest realized in October 1999. In the other areas (A Compartment and PA) the number of sand flies collected remained relatively constant. Although the logging impact in N Compartment negatively affected sand fly abundance, medically important species were still able to exploit the modified environment. Travi et al. (2002) working in a tropical dry forest in Northern Colombia, came to a similar conclusions, comparing sand fly abundance and diversity of a natural forest reserve with a highly degraded area.

There are few studies on the impact of changes in the vegetation on arthropods, especially medically important insects. Travi et al. (2002) compared the abundance of phlebotomine sand flies in Colombia, in degraded and preserved dry forests. Wilson et al. (2002) demonstrated replacement of forest species by savannah complex species, as effects of deforestation on black fly populations, using cytological probes for African black flies. Studies using other insects as biodiversity indicators in Amazonia have been tested, especially with butterflies and ants. Moutinho (1998) and Carvalho and Vasconcelos (1999) showed a reduction on the number of collected species of ants, between natural forest areas and degraded areas, in northern Pará and Central Amazonia. In Manaus, Brazil, Brown (1991) found an increase of Lepidoptera species in secondary forests, compared with primary forests. Similar comparison has been made with scarabaeid beetles in primary forest, near Manaus, that suffered fragmentation, showing a decrease in the abundance and diversity of these insects (Klein 1989). In studies of environment impacts in Marabá, Pará, Oliveira et al. (1999), showed a drastic difference in the ground invertebrate macrofauna, between a primary and a secondary forest. The results of our work suggest tree-inhabiting sand flies are sensitive to selective logging of even moderate intensity.

Rates of natural infection of trypanosomatids in phlebotomine sand flies, before and after the selective timber harvest - The rate of females infected by trypanosomatids in N Compartment, suffered a significant decrease after the selective timber harvest. The other areas (A Compartment and PA) did not show difference between sand fly infection rates as shown in the period of sand fly collection in the N Compartment area. The decrease of infected sand flies in N Compartment after the harvest had a direct relation with the decrease of sand fly population of that area. The timber harvest may have caused immediate high mortality or migration to areas near the impacted area with better conditions of life for these insects. The subsequent renovation of early emerged nuliparous flies may explain the lower infection rates after the harvest. As an alternative hypothesis, the harvest may cause an impact on the mammalian hosts fauna such as the two-toed sloth, causing their migration to areas less impacted and thus directly influencing the sand flies and their infection rates.

Although the logging impact in N Compartment had negatively affected sand fly abundance and the rates of infected females, the populations of *L. umbratilis* and other species were still capable of maintaining the life cycle of local trypanosomatids.

ACKNOWLEDGEMENTS

To the Late Dr Raul Guerra de Queiroz, adviser of the first author. To Dr Mike Hopkins (Sapeca), by the plant identification. The Mil Madeireira directors by help facilities.

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