Biogeographical aspects of the occurrence of *Nyssomyia neivai* and *Nyssomyia intermedia* (Diptera: Psychodidae) in a sympatric area of the Brazilian savannah

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Nyssomyia intermedia *and* Nyssomyia neivai *constitute a species complex associated with* Leishmania *transmission. The aim of this study was to analyse the ecological profiles of the* Ny. intermedia *and* Ny. neivai *populations in a sympatric area in the Brazilian savannah along the banks of the Velhas River. Captures were performed from July 2003-June 2005 in two distinct environments: a gallery forest with various degrees of anthropogenic modification and animal shelters. A total of 20,508* Ny. neivai *(86%) and* Ny. intermedia *(14%) sandflies were collected. The difference between the proportions of the sandflies that were collected (*Ny. neivai*/*Ny. intermedia*) per bank was significant. The right bank presented a greater number of sandflies (65%) and more preserved vegetation. The abundance of* Ny. neivai *was higher than that of* Ny. intermedia *on both banks. The results demonstrate that anthropic activities can affect the sandfly populations in this area, thereby leading to a reduction in species abundance. Nevertheless, the environments with higher levels of antropogenic modification displayed sandfly population numbers that favour the* Leishmania *transmission cycle.*

Key words: *Nyssomyia intermedia* - *Nyssomyia neivai* - Brazilizian savannah - Velhas River

Brazil has a diversified and widely distributed sandfly fauna and leishmaniasis represents a significant public health problem (MS 2006). According to Andrade Filho et al. (2001), the adaptability of sandflies to the human environment may lead to an increase in the number of leishmaniasis cases due to devastation of natural areas and the consequent destruction of the wild habitat of sandflies.

Savannah vegetation, known as *Cerrado* in Brazil, occupies an area of over two million km², thereby representing 22% of the national territory. The *Cerrado* biome is rich in both species biodiversity and abundance and represents a top priority in terms of conservation. Despite the ecological significance of this biome, it has been understudied, especially with respect to its insect fauna (Myers et al. 2000, WWF 2011).

Deforestation may be observed in the Brazilian *Cerrado* as in other biomes. Studies suggest that approximately 20% of the *Cerrado* vegetation remains relatively pristine (WWF 2011). Deforestation also affects the gallery forests on *Cerrado* riverbanks due to the establishment of ranches and farms.

Nyssomyia intermedia and *Nyssomyia neivai* constitute a species complex associated with *Leishmania* transmission (Andrade Filho et al. 2003). One of these two species has always predominated in all of the municipalities of the state of Minas Gerais (MG), where they occur in sympatry (Andrade Filho et al. 2007). These species are found along rivers, which may act as geographical barriers to their dispersion (Andrade Filho et al. 2007).

Collections performed on the left bank of the Velhas River demonstrated the predominant occurrence of *Ny. intermedia* over *Ny. neivai* and other species in the municipality of Lassance in 1997 (unpublished data). In contrast, *Ny. neivai* predominated in captures performed on the left bank of the river in 2002 in a village in the municipality of Corinto, which is approximately 8 km from Lassance (unpublished data). *Ny. neivai* and *Ny. intermedia* were recorded in sympatry in the municipalities of Pirapora and Arinos, which are located approximately 75 km and 360 km north of Lassance, respectively (Andrade Filho et al. 2007).

Research involving the sandfly fauna in various localities in the *Cerrado* biome, including Lassance and Corinto, is scarce. Knowledge of seasonal and other behavioural aspects of the sandfly species that occur in this region is of great importance for the entomology and biodiversity of the *Cerrado* biome. These data may provide information regarding the *Leishmania* vector species necessary for the control of cutaneous and visceral forms of leishmaniasis.

Twenty-four cases of American tegumentary leishmaniasis (ATL) were recorded in Lassance and 37 in Corinto in both urban and rural areas between 1986-1999. In the latter municipality during the same period, one autochthonous case of visceral leishmaniasis (VL) was also reported (Department of Health of the State of Minas Gerais). Between 2001-2011, seven cases of ATL were recorded in Lassance and five were observed in Corinto, though there were no reported cases of VL.

The aim of this study was to analyse the ecological profiles of the *Ny. intermedia* and *Ny. neivai* populations in Lassance and Corinto.

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MATERIALS AND METHODS

Study area - The municipalities of Lassance (17º53'12"S 44º34'39"W) and Corinto (18º22'51"S 44º27'23"W) are located in the region of the upper and middle reaches of the São Francisco River, MG, on the banks of the Velhas River. Lassance covers an area of 3.214 km^2, which includes 6,554 inhabitants, whereas Corinto covers an area of 2.541 km^2 and has 24,678 inhabitants. The main economic activities of the region are agriculture, cattle breeding and fishing. The mean annual temperature is approximately 23ºC (Fig. 1).

Collections - Captures were performed over two 12-month periods, the first of which extended from July 2003-June 2004, while the second was from August 2004-July 2005. Due to operational problems, the study site locations and the sampling methodology varied between the two periods.

First period - Samplings were performed monthly over two consecutive nights using 16 Falcão light traps (Falcão 1981) installed in two environments: the gallery forest and animal shelters. The traps operated from 06:00 p.m.-06:00 a.m. The total sampling time per trap was 288 h and 4.608 total hours of sampling were conducted. The traps were hung at a radius of 15 m from each other at each collection site. Collections were performed on both banks of the Velhas River in both muncipalities. Eight light traps were used in each area, with four being placed on each bank of the river.

Muncipality of Corinto - The sampled sites were situated in a fishing village and on farms approximatly 8 km from Lassance. Four traps were hung in the gallery forest on each bank {collection sites A [17º58.141'S 44º32.392'W, 494 m above sea level (a.s.l.)] and B (17º58.115'S 44º32.581'W, 503 m a.s.l.) (Supplementary data)}. The average width of the river was 198 m.

Muncipality of Lassance - The sampled sites were located on fishermens' small holdings situated in the rural area approximately 3 km from Lassance. On the left bank, one trap was installed in a hen house, one in a cattle shelter and two were hung in the gallery forest [collection site C (17º54.859'S 44º34.410'W, 505 m a.s.l.) (Supplementary data)]. On the right bank, one trap was hung in a hen house, one in a goat shelter and two were hung in the gallery forest [collection site D (17º54.739'S 44º34.460'W, 509 m a.s.l.) (Table I)]. The average width of the river was 149 m.

Second period - Twelve Falcão light traps were hung each month over two consecutive nights (from August 2004-June 2005) in two types of environments: the gallery forest and animal shelters. The traps were exposed from 06:00 p.m.-06:00 a.m. The total sampling time per trap was 288 h and a total of 1.728 h of sampling were conducted. The traps were hung in a radius of 15 m at all collection points.

Collections were performed exclusively in Lassance in two distinct areas with three traps on each bank of the river. One of the collection areas was the same as in the first period (sites C and D) (Supplementary data).

The other collection sites [collection sites E (17º51.935'S 44º32.869'W, 517 m a.s.l.) and F (17º51.928'S 44º33.008'W, 519 m a.s.l.) (Supplementary data)] were located further north in Lassance. On the left bank, one trap was hung in a hen house and two were hung in the gallery forest. On the right bank, three traps were hung in the galery forest (Supplementary data). The average width of the river in this area was 166 m. The distance between collection areas A, B and C, D was 6.9 km and the distance between sites C, D and E, F was 5.8 km. All collection points were situated in the same hypsometric range (from 494-571 m a.s.l.).

Phlebotomine identification - The collected sandflies were identified according to the classification proposed by Galati (2003). The morphological characters used to distinguish males from females of *Ny. intermedia* and *Ny. neivai* were described by Marcondes et al. (1997) and Andrade Filho et al. (2003).

Description of the sampled areas - A summarised description of the areas was produced during the field collections. The areas were characterised according to the degree of human modification. The aspects observed concern the vegetation and the presence of both dwellings and animal shelters (Supplementary data).

Climatic data - Climatic data were obtained from the National Institute of Meteorology (Ministry of Agriculture, Livestock and Supply, 5th District of Meteorology, Pirapora e Corinto Stations).

Analyses - The data on the *Ny. intermedia* and *Ny. neivai* populations were analysed in relation to the banks of the Velhas River, precipitation, humidity, temperature, season of the year and the degree of anthropogenic modification of the areas.

The data were organised using Excel 97/2003, which was employed to calculate descriptive statistics. GraphPad Prism 4.0 was used for statistical analyses. The chi-square two-sided degree of freedom = 1 test was used to compare the proportions of specimens per species collected on each bank of the river.

RESULTS

The enviromental characterisation demonstrated that the study areas presented different degrees of anthropogenic modification. The right bank of the Velhas River was more preserved, exhbiting medium and large-sized trees and fewer human dwellings and animal shelters. The left bank was more modified, showing the presence of human dwellings and animal shelters (Supplementary data).

A total of 21,050 sandfly specimens were collected. The majority of the specimens (20,508, 97%) were identified as *Ny. intermedia* (2,817, 13%) and *Ny. neivai* (17,691, 84%). The total sex ratio (M:F) was 1:1.05. The sex ratio for *Ny. intermedia* was 1:0.41 during the first year, 1:1.01 during the second year and 1:0.54 over the entire period. The *Ny. neivai* sex ratio was 1:1.06 during the first year, 1:2.08 during the second year and 1:1.16 for the entire period.

A greater number of sandflies was collected on the right bank than on the left bank during both periods. The

proportions of the *Ny. intermedia* and *Ny. neivai* specimens collected were significantly different during both periods (chi-squared test, $p < 0.0001$). *Ny. neivai* was the predominant species observed during both periods and for all of the study sites (Fig. 2, Tables I, II).

Comparing Supplementary data and Table I, it appears that the human modifications in the study area (changes in phyto-physiognomy and the presence of both animal shelters and human dwellings) might be associated with the decrease in the number of sandflies in this region. The difference between the proportions of *Ny. neivai* specimens on each bank was statistically significant, with a greater number observed on the right bank (Table I).

The number of *Ny. neivai* specimens predominated over that of *Ny. intermedia* during both periods; the ratio of *Ny. neivai* to *Ny. intermedia* was 7.54 for the first period and 2.99 for the second (Table II).

Regarding the seasonal variation in the first period, the peak in the number of insects collected was observed after the rainy periods (from February-May 2004), which coincided with periods of high relative humidity (83-74%) and high temperatures (23.3-23.7ºC). *Ny. neivai* was the most frequent and abundant species in all of the months studied, being collected at least twice as often as *Ny. intermedia* (Fig. 2, Table II) in this period.

In the second period, the sandfly distribution pattern was slightly different, with peaks occurring during different months (October 2004, March 2005 and June 2005) (Fig. 2). The highest peak was observed in March, when heavy rainfall (302 mm) and high humidity and temperatures occurred. Although *Ny. neivai* was the most abundant species, the total numbers of *Ny. neivai* and *Ny. intermedia* specimens collected were more similar during the second period. *Ny. intermedia* was more abundant than *Ny. neivai* only during August and November of 2004 (Fig. 2, Table II).

DISCUSSION

Although there have been several studies on sandflies in Brazil, their scope has generally been restricted to one phytogeographic zone according to Dias-Lima et al. (2003). Few studies have addressed the ecological features of the sandfly populations and a majority of the studies fo-

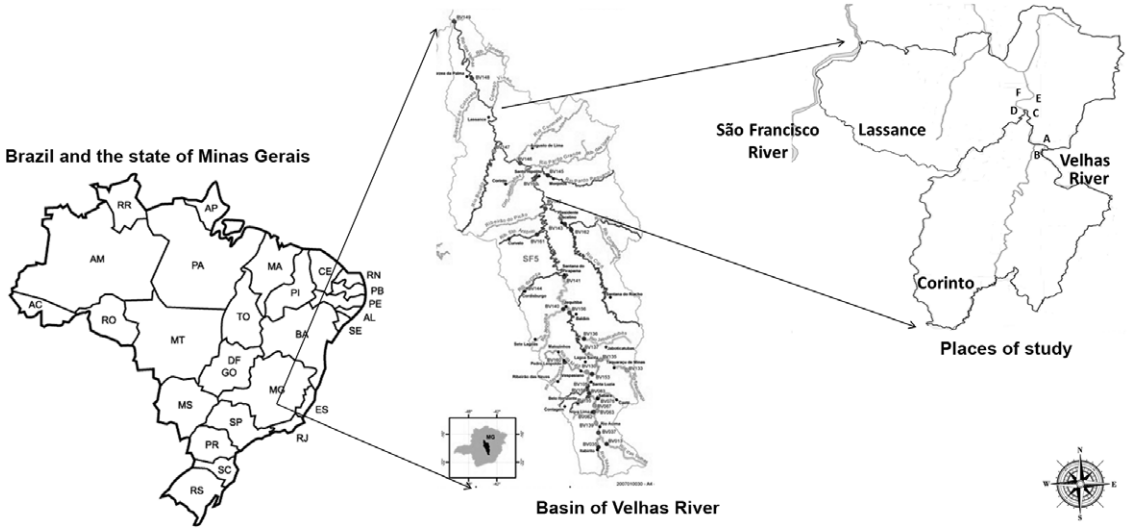

Fig. 1: map of the region bordering the municipalities of Corinto and Lassance, Minas Gerais, Brazil and location of collection points A-F according to Table I.

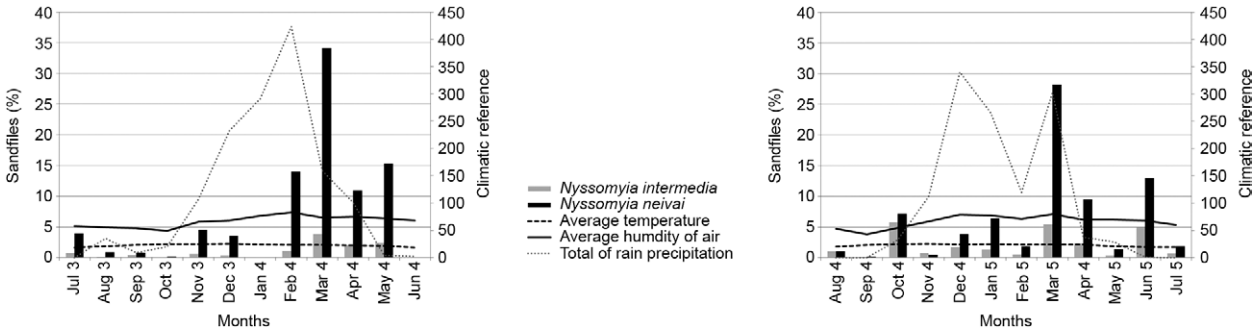

Fig. 2: month distribution of *Ny. neivai* and *Ny. intermedia* collected in the region of Corinto and Lassance, Minas Gerais, Brazil from July 2003-June 2004 (1st period) and from August 2004-July 2005 (2nd period).

cus principally on the importance of these insects in terms of the epidemiology of the leishmaniasis cycle.

However, some studies have found differences between sandfly populations in various geographical areas characterised by different degrees of anthropogenic change. Additionally, some authors have reported differences between the sandfly populations found in pastures, orchards, intradomiciliary and peridomiciliary areas and primary forests (Andrade Filho et al. 2001, de Luca et al. 2003).

Ny. intermedia and *Ny. neivai* occur in sympatry and represent the dominant species of sandflies in the study area, constituting approximately 100% of the sandflies collected in Corinto and Lassance (Fig. 1). Similar results were observed by Galati et al. (2010) in a study involving both species, also in a sympatric area, in which the two species accounted for a total of 97.9% of the phlebotomines captured.

Previous studies of the sandfly fauna have recorded 19 species in Lassance and Corinto and important vec-

TABLE I

Sandflies collections per bank of the Velhas River and period of study in the region of Lassance and Corinto, Minas Gerais, Brazil from July 2003-June 2005

	Left bank						
	First period			Second period			
Species/sex	Site A	Site C	Total	Site C	Site E	Total	Total
Nyssomyia intermedia/M	35	202	237	121	15	136	373
Ny.intermedia/F	59	395	454	114	23	137	591
Subtotal	94	597	691	235	38	273	964[a]
Nyssomyia neivai/M	1,294	1,732	3,026	159	24	183	3,209
Ny. neivai/F	1,432	1,570	3,002	87	21	108	3,110
Subtotal	2,726	3,302	6,028	246	45	291	6,319[a]
Total	2,820	3,899	6,719	481	83	564	7,283
	Right bank						
Species/sex	Site B	Site D	Total	Site D	Site F	Total	Total
Ny. intermedia/M	27	335	362	99	157	256	618
Ny. intermedia/F	107	878	985	75	175	250	1,235
Subtotal	134	1,213	1,347	174	332	506	1,853[a]
Ny. neivai/M	1,076	3,820	4,896	543	845	1,388	6,284
Ny. neivai/F	1,364	3,076	4,440	233	415	648	5,088
Subtotal	2,440	6,896	9,336	776	1,260	2,036	11,372[a]
Total	2,574	8,109	10,683	950	1,592	2,542	13,225

a: $p < 0.0001$.

TABLE II

Nyssomyia neivai/*Nyssomyia intermedia* ratios in the region of Corinto and Lassance, Minas Gerais, Brazil in both banks of the Velhas River, by month from July 2003-June 2004 (1st period) and from August 2004-July 2005 (2nd period)

First period Month	Jul 3	Aug 3	Sep 3	Oct 3	Nov 3	Dec 3	Jan 4	Feb 4	Mar 4	Apr 4	May 4	Jun 4	Total
Total	5.36	8.32	2.00	2.31	7.50	10.30	0.00	12.56	8.98	5.20	6.38	3.50	7.54
Left bank	0.56	0.86	1.11	2.00	5.55	6.63	0.00	24.57	10.29	9.09	8.06	6.00	8.72
Right bank	6.15	12.67	5.00	2.42	12.62	19.59	0.00	8.25	8.54	4.12	4.97	1.00	6.93
Second period Month	Aug 4	Sep 4	Oct 4	Nov 4	Dec 4	Jan 5	Feb 5	Mar 5	Apr 5	May 5	Jun 5	Jul 5	Total
Total	0.97	1.20	1.23	0.61	2.22	5.00	3.69	5.18	4.04	3.91	2.63	2.64	2.99
Left bank	0.62	0.60	0.16	0.55	0.21	0.96	0.00	6.29	1.10	1.25	0.52	1.20	1.07
Right bank	1.58	3.00	1.72	2.00	4.56	11.06	3.93	4.99	5.98	5.43	3.60	3.83	4.02

tor species implicated in the transmission of *Leishmania* have been collected (Saraiva et al. 2008). The putative and proven vectors collected included *Ny. intermedia, Ny. neivai, Nyssomyia whitmani* (Antunes & Coutinho 1939), *Lutzomyia longipalpis* (Lutz & Neiva 1912) *and Migonemyia migonei* (França 1920) and both *Ny. neivai* and *Evandromyia sallesi* (Galvão & Coutinho 1939) were found to be infected by *Leishmania infantum chagasi* in this area (Saraiva et al. 2009).

Some authors have reported alternating dominance of one of the two species in places where they occur in sympatry (Andrade Filho et al. 2003, 2007). However, *Ny. neivai* was found to be the most abundant species in both of the periods of collection and all of the locations analysed in this study (Table I, Chart 2). Therefore, based on the hypothesis of competition between the two species, it is possible to infer that *Ny. neivai* displays greater a ability to exploit the investigated habitats.

The sex ratio varied during the two study periods for both species. It is difficult to explain this variation, as there are many factors that might affect the number of males and females collected. Among these factors, physiological status may represent the factor that contributes most to this variation.

In the present study, the observed abundance of *Ny. neivai* suggests that it is one of the species that is most likely to act as the primary vector of ATL, although the values obtained for *Ny. intermedia* suggest that it also is associated with *Leishmania* transmission in this region. Both species have been found to be naturally infected with *Leishmania* and are associated with ATL foci (Forattini et al. 1972, Rangel et al. 1984, Casanova et al. 1995, Córdoba-Lanus et al. 2006, Marcondes et al. 2009, Saraiva et al. 2009, 2010, Pita-Pereira et al. 2009, Margonari et al. 2010, Oliveira et al. 2011), thereby demonstrating the great epidemiological importance of these species.

Lutz and Neiva (1912) have reported that *Ny. intermedia* presents a synanthropic habit, also affirmed by Rangel et al. (1986), who described the highly anthropophilic behaviour of leishmaniasis in the state of Rio de Janeiro, Brazil. In the state of São Paulo, *Ny. neivai* was predominantly collected with a Disney trap using a dog as bait and blood meal identification revealed the preference of this species for human blood, followed by that of birds (Casanova 2001).

Comparison of the environmental data (Table I) and the data on the relative abundance of species (Tables I, II) demonstrated the more frequent occurrence of *Ny. neivai* compared to *Ny. intermedia* in all of the studied environments. The difference between the proportions of the specimens of the two species collected on the left and right banks was statistically significant ($p < 0.0001$). The left bank displayed a smaller number of collected specimens, with a lower relative abundance of *Ny. neivai* being recorded in both study periods (Table I). These data might support the hypothesis that anthropic modifications have both reduced the abundance of specimens and modified the population structure of this insect group in the region and period studied.

The pattern of seasonal variation on the two banks was similar (Fig. 2), with the period of the greatest occurrence of sandflies coinciding with the late summer and early spring. This period corresponds to the end of the rainy season and is characterised by high relative humidity in the studied region. The seasonal pattern is mainly determined by the species *Ny. neivai* and *Ny. intermedia*. *Ny. neivai* was collected in greater numbers in 21 of the 24 months sampled on both banks (Fig. 2, Table II).

Several studies have shown that anthropic modifications may favour populations of some species that colonise anthropised environments, as was observed for *Lu. longipalpis* in urban areas of Brazil. However, urban areas display reduced levels of species richness compared to wild areas. For example, some studies on the sandfly populations in the metropolitan region of Belo Horizonte have demonstrated a higher frequency of *Ny. whitmani* in green areas close to urban zones, while *Lu. longipalpis* is most frequent in urban areas. Therefore, we may infer that urbanisation alters species dynamics, thereby favouring those species that are most adapted to anthropically modified environments (Souza et al. 2004, Resende et al. 2006, Carvalho et al. 2010, Saraiva et al. 2010).

The left bank present a higher degree of anthropic modification and a slightly higher species richness and diversity than the right bank (Saraiva et al. 2008). This pattern can be explained by the wider range of habitats present in an environment characterised by a greater degree of anthropic change, though that is less drastic compared to the process of urbanisation. We assume that anthropogenic interference destabilises ecological communities and redirects the succession process. Intermediate disturbance in ecosystems leads to intermediate successional stages, associated with higher rates of biodiversity (Begon et al. 2007).

It is necessary to emphasise that it is impossible to conclude that the river acts as a geographical barrier between the *Ny. neivai* and *Ny. intermedia* populations, although this has been established in other regions (Marcondes et al. 1998), as the environmental differences alone might explain the results and genetic analysis was not performed.

In a previous study, the São Francisco River was found to play an important role in the speciation of *Lu. longipalpis*, leading to the development of sibling species (Coutinho-Abreu et al. 2008). In that study, the authors analysed sequences of the cytochrome *b* gene. Thus, we believe that the present study highlights the need for analysis of the genetic structure of the populations of these two sandfly species on the left and right banks of the Velhas River to assist in the interpretation of the ecological patterns that were observed.

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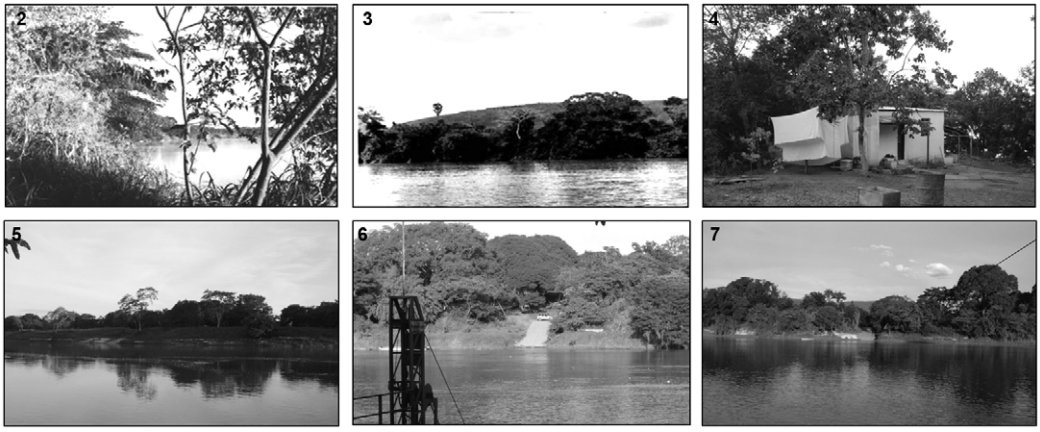
Environmental characteristics of the banks of the Velhas River in the region of Lassance and Corinto, Minas Gerais, Brazil

Municipality	Places	Characteristics	Figure
Corinto	A	Left bank: gallery forest with high level of anthropogenic modification. There were smallholder farmers and fishermen ranches.	2
	B	Right bank: gallery forest, relatively well preserved. Presence of large and medium size trees.	3
Lassance	C	Left bank: gallery forest with high level of anthropogenic modification. There were smallholder farmers and fishermen ranches.	4
	D	Right bank: gallery forest relatively well preserved. Presence of large and medium size trees.	5
	E	Left bank: gallery forest with medium level of anthropogenic modification. Only one human dwelling with a shelter for hens.	6
	F	Right bank: gallery forest relatively well preserved. Presence of large and medium trees.	7



Figs 2-7: enviromental characteristis of the collection places according to Table I. 2: place A, Corinto, left bank; 3: place B, Corinto, right bank; 4: place C, Lassance, left bank; 5: place D, Lassance, right bank; 6: place E, Lassance, left bank; 7: place F, Lassance, right bank.