ECOLOGY, BEHAVIOR AND BIONOMICS

Species Richness and Distribution of Blackflies (Diptera: Simuliidae) in the Chapada Diamantina Region, Bahia, Brazil

Victor L Landeiro[1], Mateus Pepinelli[2], Neusa Hamada[1]

[1]*Entomologia, Instituto Nacional de Pesquisas da Amazônia, Manaus, AM; vllandeiro@gmail.com; nhamada@inpa.gov.br;* [2]*Univ. Federal de São Carlos, São Carlos, SP; mateuspepi@yahoo.com.br*

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Riqueza e Distribuição de Espécies de Borrachudos (Diptera: Simuliidae) na Região da Chapada Diamantina, BA

RESUMO - A família Simuliidae é taxonomicamente uma das mais conhecidas entre os insetos aquáticos no Brasil. No entanto, faltam informações relativas à sua distribuição em muitas regiões do Brasil. Foram coletadas larvas e pupas de borrachudos em 50 riachos na região da Chapada Diamantina, BA, localizados em três regiões geográficas distintas, 20 em Lençóis, 18 em Mucugê e 12 em Rio de Contas. Foram analisadas a riqueza e distribuição de espécies da área da Chapada Diamantina como um todo, bem como em cada uma das três áreas geográficas distintas. Coletamos 20 espécies, das quais três ainda não foram descritas e duas foram descritas após este estudo. As três áreas são distintas em relação às variáveis ambientais, principalmente devido às diferenças de altitude e pH da água. Quatro espécies foram coletadas apenas em Rio de Contas, enquanto outras quatro apenas em Mucugê. Dezesseis espécies foram encontradas em Mucugê e quinze em Rio de Contas, enquanto em Lençóis encontramos apenas doze espécies, mesmo com o maior esforço de amostragem exercido naquela região. Rio de Contas parece ser a região que abriga a maior riqueza de espécies. A riqueza de espécies foi relacionada a fatores ambientais apenas quando avaliada em pequena escala espacial (ou seja, quando cada área foi analisada separadamente). Apesar de não ter sido realizado um teste causal, os resultados aqui obtidos corroboram outros estudos que mostraram que o pH e altitude são fatores importantes com os quais a riqueza e a distribuição de borrachudos podem estar associadas.

PALAVRAS-CHAVE: Riacho neotropical, cerrado, altitude, pH

ABSTRACT - Taxonomically, blackflies (Simuliidae) are among the best-known aquatic insects in Brazil. However, information on their distribution is lacking for many regions. We sampled simuliids in 50 streams in the Chapada Diamantina region, State of Bahia, located in three distinct geographical areas, 20 at Lençóis, 18 at Mucugê, and 12 at Rio de Contas. We analyzed simuliid species richness and distribution in the Chapada Diamantina area as a whole, as well as in each of the three distinct geographical areas. We collected 20 species, three of which were not yet described and two were described after our sampling. The three areas are distinct in relation to environmental variables, mainly owing to differences in altitude and water pH. Four species were restricted to Rio de Contas, while four other species were restricted to Mucugê. Sixteen species were present in Mucugê and fifteen in Rio de Contas. Only twelve species were present in Lençóis, despite the higher sampling effort. Rio de Contas appears to be the area that harbors the highest species richness. Species richness was related to environmental factors only when evaluated on a small spatial scale (i.e. when each area was analyzed separately). Although we have not tested for causal relationships, our findings agree with other studies that showed that pH and altitude are important factors with which species richness and species distribution appear to be associated.

KEY WORDS: Neotropical stream, Brazilian savanna, altitude, pH

The question of what factors contribute to variation in species composition at local (alpha diversity) and regional (beta diversity) scale has been of great interest to biologists (Legendre *et al* 2005, Tuomisto & Ruokolainen 2006). This variation might originate from niche differences between species and from the variation in environmental factors among sites (Bocard *et al* 1992). The study of environmental processes controlling species distribution can provide insights into the mechanisms structuring species assemblages. The knowledge and data acquired from such studies can be used to predict species distribution, help to identify factors that can affect the biodiversity in a given area, and provide useful information for conservation planning (Begon *et al* 2006).

Neotropical biodiversity is inadequately studied because many species remain unknown to science and the inherent lack of distributional information of many taxonomic groups (Bini *et al* 2006). In the Neotropical region, the taxonomic resolution of many groups of aquatic insects is especially low (Melo & Froehlich 2001, Sites *et al* 2003). Consequently, previous studies focused at the family or generic levels due to the difficulty in obtaining reliable species-level identifications. However, some groups are relatively well studied in neotropical streams, such as the family Simuliidae (Coscarón & Arias 2007, Hamada *et al* 2002); 91 species were reported to Brazil (Hernández *et al* 2007). This attribute makes Simuliidae good candidates for testing hypothesis in studies of community structure (Adler & McCreadie 1997).

The Simuliidae have a worldwide distribution and are rarely absent from a stream (Malmqvist *et al* 1999, Hamada *et al* 2002, McCreadie *et al* 2005); despite the nearly ubiquitous distribution, many environmental variables are known to affect species distribution, such as water temperature and pH, stream width and depth, and stream substrate (Hamada *et al* 2002, Coscarón & Arias 2007). Indeed, many of these variables are used as predictors of species assemblages (Hamada et al 2002, McCreadie *et al* 2005, McCreadie & Adler 2006, Illésová *et al* 2007).

Recently, several areas around the world have been considered hotspots of biodiversity and designated as priority areas for conservation efforts (Myers *et al* 2000). Brazil's *cerrado* (central-Brazilian savanna) is one of these biodiversity hotspots. However, suitable information on the taxonomy and geographical distribution of cerrado resident species is lacking (Bini *et al* 2006). Great parts of the poorly studied cerrado areas are located at the north of the biome. One of these areas is the Chapada Diamantina, located in the central of Bahia state. In 1985 the Brazilian government created the Chapada Diamantina National Park, a 152,000-km^2 conservation area. This area comprises the transition between the *cerrado* and *caatinga* (Northeast Brazilian thorny scrub vegetation) biomes, which some authors classify as *cerrado* and others as *caatinga* (Walter 2006). The Chapada Diamantina area comprises a vast network of fast-flowing streams, which provide excellent habitat for the development of simuliid larvae.

Our study is intended to provide a comprehensive inventory of simuliid species occurring in the Chapada Diamantina region (Bahia state), a *cerrado* area in northeastern Brazil. We also evaluated species richness and species distribution in three distinct geographical areas within the Chapada Diamantina region (Lençóis, Mucugê and Rio de Contas), defined in accordance with the geographical positions of the sampling sites. Sites at north were considered inside the area of Lençóis County, sites at middle were considered inside the area of Mucugê County, and sites at south were included in the Rio de Contas County.

Material and Methods

Immature stages of simuliids were sampled from 50 streams between 21 July and 12 August, 2005 (Fig 1), which were selected in accordance with their accessibility by roads and with an attempt to cover a broad range of the Chapada Diamantina region. Based on geographical coordinates we divided our sampling sites in three distinct geographical areas, in which 20 streams were located in the north of Chapada Diamantina, 18 in the middle, and 12 in the south. Hereafter we refer to these three areas as Lençóis, Mucugê and Rio de Contas, respectively. Lençóis, Mucugê and Rio de Contas are tourist-destination counties situated in each area (Fig 1); however, some of the streams sampled were situated inside the area of other municipalities. Rio de Contas is the only area where none of the sampled streams is located within Chapada Diamantina National Park (CDNP).

Larvae and pupae of simuliids were collected from each stream on all available substrates (e.g. macrophytes, leaf litter and stones), following the sampling technique described by McCreadie & Colbo (1991). Each stream was sampled once for approximately 40-50 min; however, in some instances, when we identified a possible new species and the quantity of larvae and pupae was low, the sampling time was extended to almost 4h, aiming to obtain as much material as possible to attend the requirements for description of all life-stages of new species (larva, pupa and adult). It could be argued that this procedure might result in a greater chance to find more species than could be found in sites in which we used a less-intensive sampling effort. However, our field experience, and that of others (McCreadie & Colbo 1991), reveals that virtually all species present in a given site are collected after a 40-50 min collecting-effort. On the other hand, the higher effort applied in some sites led to a higher abundance; therefore, we only used presence-absence data in the analysis.

The sampled larvae and most pupae were fixed in 95% ethanol, while mature pupae that were close to emergence were isolated in plastic tubes containing a small piece of humid filter paper in order to obtain adults (Hamada *et al* 2006; more details of these procedures can be found in Pepinelli *et al* 2005). Specimens collected were identified to the species level and will be deposited in the collection of the Instituto Nacional de Pesquisas da Amazônia (INPA).

Data analysis. As in other studies (Hamada *et al* 2002, McCreadie *et al* 2005), we assumed that the species found in each stream represent all local occurrences (i.e. no species were missing) at the sampling time (21 July to 12 August, 2005). Due to the fact that our sampling differed from stream to stream we only used presence-absence data in our analyses.

In order to evaluate species richness in the sampled area, we constructed a species accumulation (rarefaction) curve using the moment-based method (Colwell *et al* 2004). We also fit an exponential model to the curve to predict how many species could be found if we had collected in more streams, using the formula S = (1/z)$\log_n$(1+zax), where S is the number of species, and *a*, *z*, and *x* are the coefficients of the exponential model (Colwell *et al* 2004).

Principal components analysis was used to evaluate the similarity among the three sampled areas as regarding to their environmental characteristics (Legendre & Legendre 1998). We also tested the correlation between species richness and the first principal component obtained to observe the relationship among species richness and environmental factors. This correlation was also tested for each area separately. All analyses were conducted using the R package (R Development Core Team 2007) and the *vegan* library (Oksanen *et al* 2007).

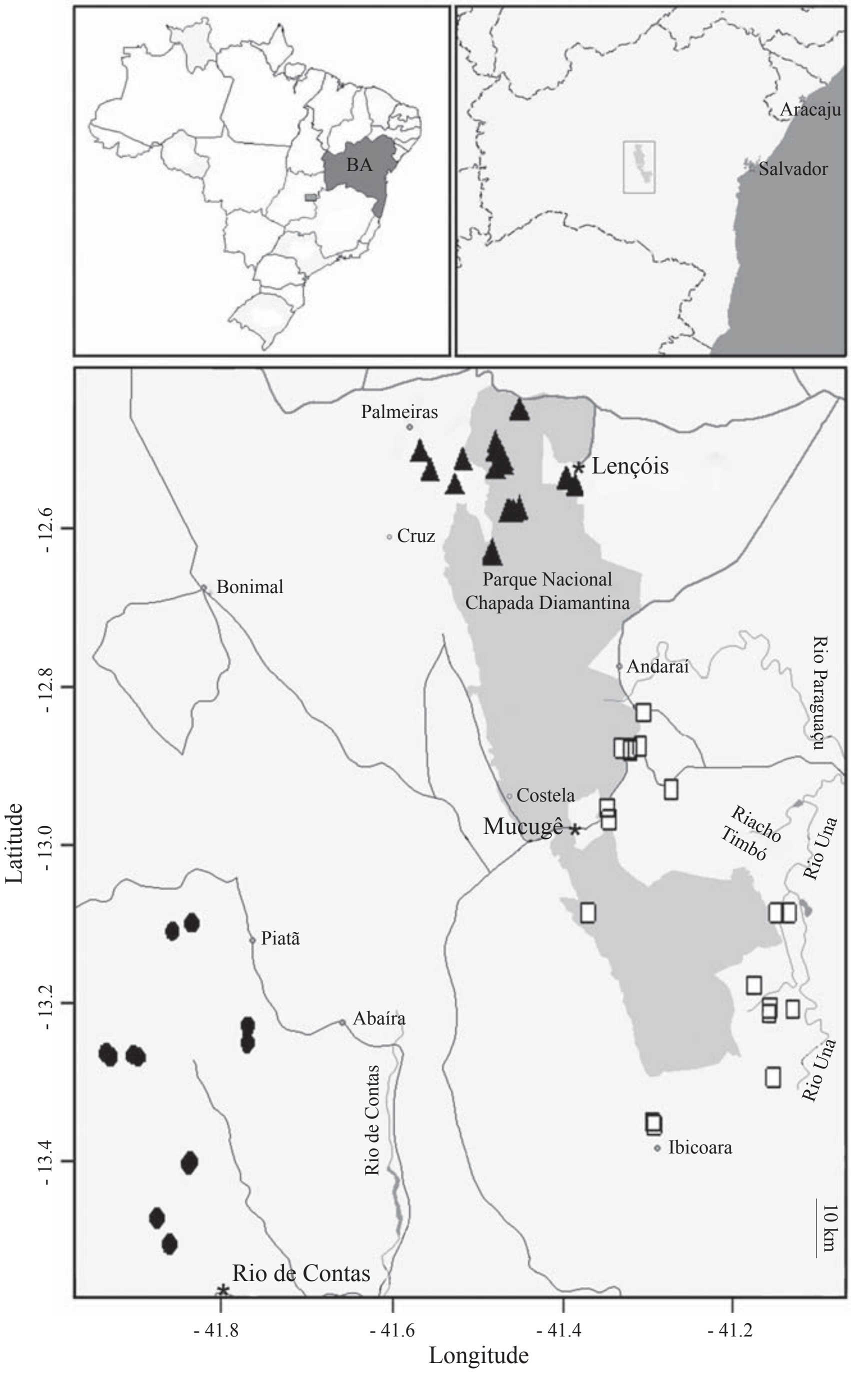

Fig 1 Outline of the area sampled, showing the geographical position of each sampled site. Top left = map of Brazil, top right = Bahia state, below = sampled area, in which the shaded area is the limits of the Parque Nacional Chapada Diamantina. Twelve streams are near Rio de Contas County (●), 18 near Mucugê County (□) and 20 near Lençóis County (▲). Symbols for streams that are very close together might be overlapping.

Results

Twenty species were collected (Table 1), three of them are new species described and two were described only after our samplings. Eleven species occurred in the Lençóis area, 16 in Mucugê and 15 in Rio de Contas. *Simulium subnigrum* Lutz was the most common species, occurring in 62% of the sampled streams. *Simulium jefersoni* Hamada, Hernández, Luz & Pepinelli, *S. papaveroi* Coscarón, *S. spinibranchium* Lutz, *S. incrustatum* Lutz and *S. margaritatum* Pepinelli, Hamada & Luz occurred in more than 20% of the sampled streams. In contrast to these frequently collected species, *S. rubrithorax* Lutz, *S. lobatoi* Luna Dias, Hernández, Maia-Herzog & Shelley, *Simulium* (*Inaequalium*) sp2 [undescribed] e *Lutzsimulium hirticosta* Lutz occurred in only one stream each (Fig 2). In general, the most common species in the streams of the Chapada Diamantina region as a whole were also the most common in each region separately. The only exceptions were certain uncommon species in Rio de Contas (Fig 2).

The average number of species per stream was three (SD = 1.7); 33 streams had three or fewer species. Eight species was the maximum richness recorded for any stream, this one located in Mucugê. This is a high number of species relative to other studies conducted in Brazil (Hamada *et al* 2002, Pepinelli *et al* 2005).

By fitting the exponential model to the species accumulation curve, it was predicted that only three or four more species would be found if the sampling effort were doubled ($z = 0.197$; $a = 5.158$; Fig 3) (Table 2). However, when each region was analyzed separately (Fig 4), Rio de Contas potentially harbored the greatest number of species. The Lençóis region, despite receiving the greatest sampling effort (20 streams), was the region with the lowest number of species.

Principal components analysis revealed that there is an environmental separation of the three areas, as indicated by the first principal component. This first component accounts for 39% of the total variation and is related to altitude and water pH ($r = -0.85$ e $r = -0.60$, respectively (Fig 5), which had the highest values in Rio de Contas (Altitude: $df = 47$; $F = 19.68$, $P < 0.0001$; pH: $df = 47$, $F = 17.78$, $P < 0.0001$, Anova) (Fig 6). The correlations between species richness and the first principal component revealed no relationship between species richness and environmental factors ($r = -0.165$, $P = 0.25$). However, when these correlations were calculated for each area separately, the species richness of Rio de Contas and Mucugê were related to environmental factors (0.629, -0.467, respectively, $P < 0.05$). The correlation between Lençóis species richness and the first principal component was very low ($r = -0.052$, $P = 0.82$).

Table 1 Species found in 50 streams in Chapada Diamantina, Brazil, together with the region and the number of streams in which each species occurred.

Species	Occurrences	Regions
Simulium subnigrum Lutz	31	Le, Mu, RC
S. jefersoni Hamada, Hernandez, Luz & Pepinelli	19	Le, Mu, RC
S. papaveroi Coscarón	17	Le, Mu, RC
S. incrustatum Lutz	12	Le, Mu, RC
S. margaritatum Pepinelli, Hamada & Luz	12	Le, Mu, RC
S. dekeyseri Shelley & Py-Daniel	11	Le, Mu, RC
S. (*Ectemnaspis*) sp.	10	Le, Mu, RC
S. stellatum Gil, Figueiró & Maia-Herzog	9	Le, Mu, RC
S. perflavum Roubaud	7	Le, Mu, RC
S. jujuyense Paterson & Shannon	5	Le, Mu, RC
S. scutistriatum Lutz	4	Mu
S. subpallidum Lutz	4	Mu, RC
S. inaequale Paterson & Shannon	2	Mu
S. spinibranchium Lutz	2	Mu, Le
Simulium (*Inaequalium*) sp1	2	RC
S. rubrithorax Lutz	1	Mu
S. lobatoi Luna Dias, Hernández, Maia-Herzog & Shelley	1	RC
S. hirtipupa Lutz	1	RC
Simulium (*Inaequalium*) sp2	1	RC
Lutzsimulium hirticosta Lutz	1	Mu

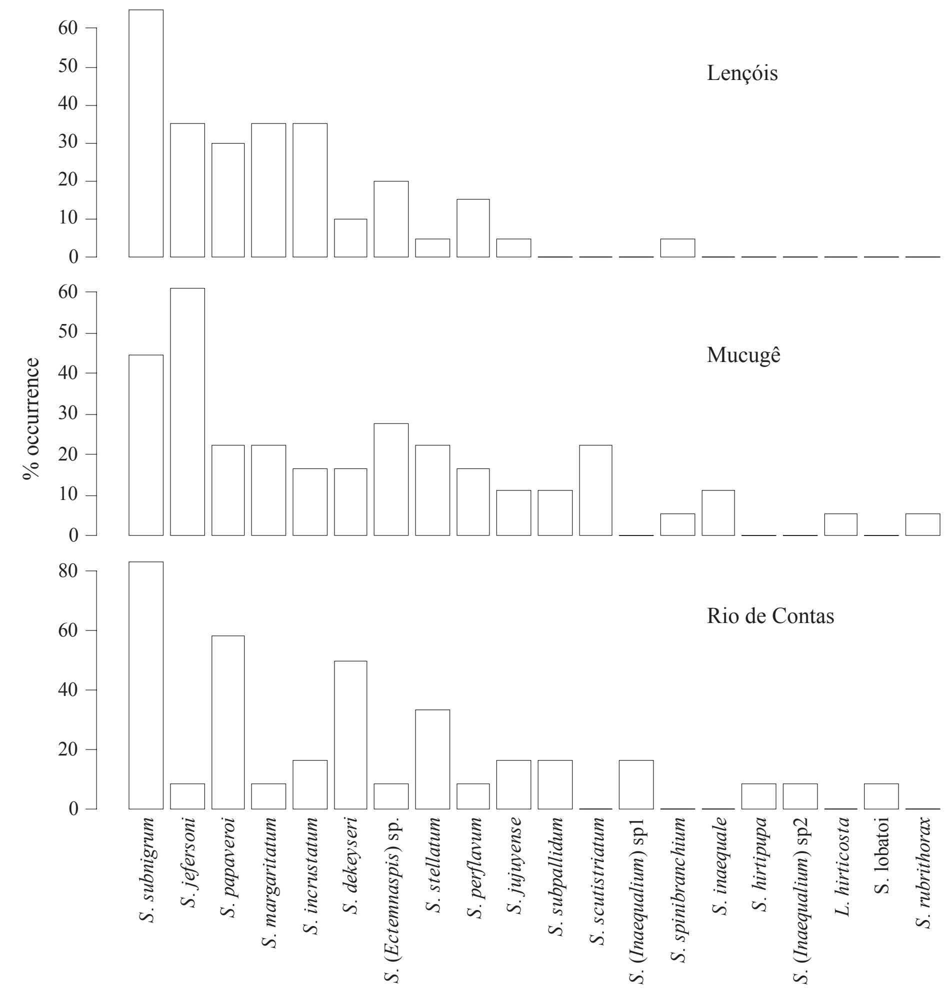

Fig 2 Percentage of species occurrence in each region (number of streams in which the species occurs versus the number of streams sampled). Notice that four species are restricted to Mucugê and another four species are restricted to Rio de Contas.

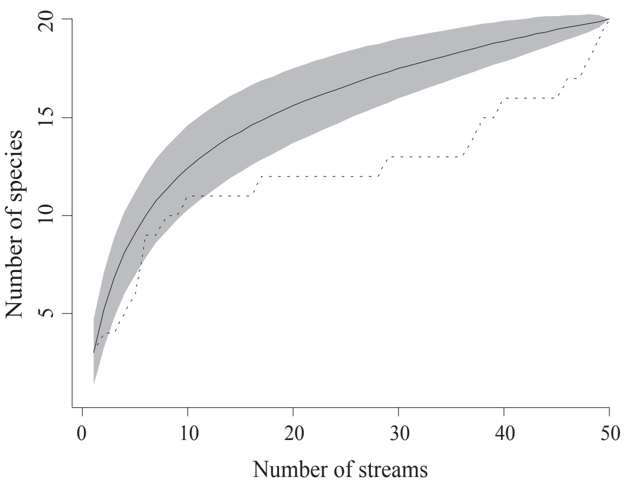

Fig 3 Species accumulation (rarefaction) curve for the whole Chapada Diamantina area. Shaded area = confidence interval (2 SD). Dotted line = number of species found in the order that the streams were sampled (collector method).

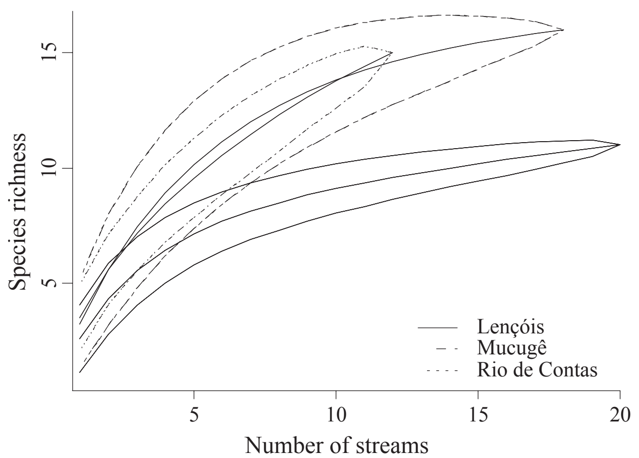

Fig 4 Species accumulation (rarefaction) curve for each region of the Chapada Diamantina area. Small lines = confidence intervals (2 SD). Coefficients of the exponential models: Lençóis (z = 0.312, a = 5.006); Mucugê (z = 0.174, a = 5.403); Rio de Contas (z = 0.137, a = 4.074).

Table 2 Number of species observed and the expected species richness in each region if additional samples were collected.

Richness	Observed	Expected			
Number of streams	*	20	30	50	100
Lençóis	11	11.0	12.3	13.9	16.1
Mucugê	16	16.8	19.1	22.1	26.0
Rio de contas	15	17.5	20.5	24.2	29.2

*20 streams in Lençóis, 18 in Mucugê and 12 in Rio de Contas

Discussion

The recently described *S. jefersoni* and *S. margaritatum* were among the most common species found in our samplings (Hamada *et al* 2006, Pepinelli *et al* 2006), pointing to the extent of our ignorance of the simuliid fauna in the Chapada Diamantina. Our data suggest that Rio de Contas potentially harbors the greatest number of species. Unfortunately, the Rio de Contas area is not protected, as it is not included within the boundaries of Parque Nacional Chapada Diamantina.

It is a well established fact in ecology that the number of species increases as the collecting effort increases (Gotelli & Colwell 2001). However, there are regions that naturally support fewer species and a large number of samples would not incur an increase in species richness. On the other hand, ecologists also recognize that there is a relationship between the number of species encountered and the size of the sampled area (Gleason 1922, He & Legendre 1996). A possible explanation for the lower number of species in Lençóis may be the result of a species-area relationship because, even though we sampled more streams in that area, the Lençóis region is the smallest geographical area (Fig 1).

The most different environmental factors among the three areas were pH and altitude. Samples from Rio de Contas were in areas of higher altitude and in streams of higher pH. Several studies have shown that ecoregions are good biota predictors. McCreadie & Adler (2006) observed that pH is among the variables that contributes most to ecoregion separation in Simuliidae. However, the contribution of variables in explaining and predicting assemblage structures may decrease as the spatial scale increases (Mykrä *et al* 2007). That is, if the beta diversity among ecoregions is high (high turnover), and there is no environmental gradient crossing ecoregions, then the environmental variables have low correlations with assemblage structure. This relationship between spatial extent and environmental factors is illustrated by the fact that species richness is high only when each area is analyzed separately (i.e., when spatial extent is low).

In a similar study of simuliids carried out in central Amazonia, in which 58 streams were sampled, a total of

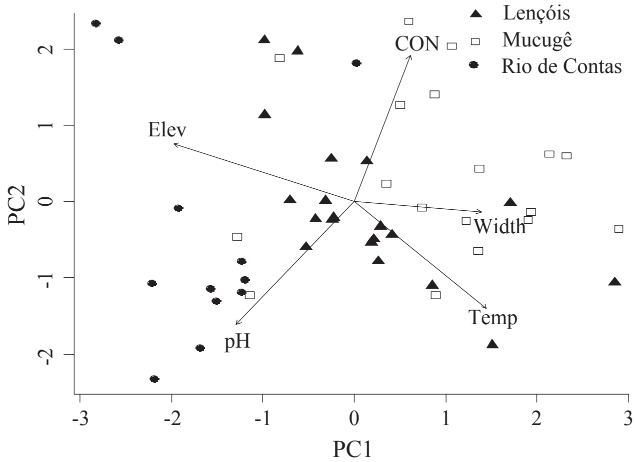

Fig 5 Biplot of the first and second principal components of environmental variables. Percentages of explanation for each component = 39.4% and 28.4%, respectively.

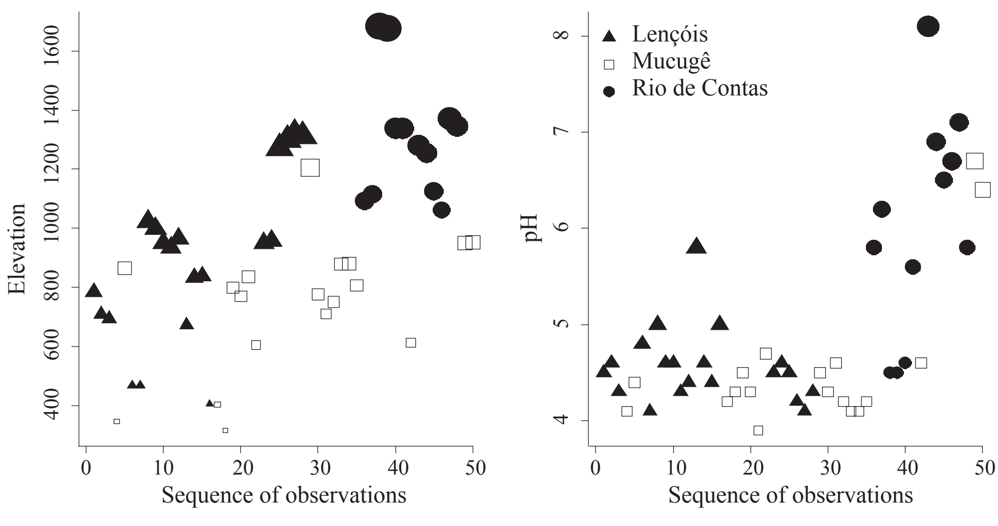

Fig 6 Altitude and pH values measured in each stream sampling site. The X axis is the order in which the samples were collected. Symbol size is proportional to the magnitude of the original value.

only 11 species were reported, with a maximum of just four species per stream (Hamada *et al* 2002). In other areas of the world, the number of Simuliidae species may be higher. For example, Malmqvist *et al* (1999) found 39 species in 56 streams in Sweden with a maximum of 14 species in a single site. But in general, the species number per stream rarely exceeds 10 (Adler *et al* 2004, Hamada *et al* 2002).

Our study provides new information on simuliid species distribution in a little-studied region that is considered to be of high biological interest (Myers *et al* 2000). The information provided herein will be useful for future studies on the ecology, epidemiology and conservation biology of simuliids from this environmentally important area.

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