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Source: Florida Entomologist, 98(2) : 446-450

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.098.0209>

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Bionomics of the black fly *Simulium guianense* (Diptera: Simuliidae) in northeast Brazil

Cláudio Rabelo dos Santos-Neto^{1*}, Neusa Hamada¹, and Sheyla R. M. Couceiro²

Abstract

Simulium guianense Wise (Diptera: Simuliidae) is the main vector of the nematode *Onchocerca volvulus* (Leuckart) (Spirurida: Onchocercidae), which causes onchocerciasis in the Amazonian focus of the disease. We present the first report of the presence of *S. guianense* in the Parnaíba River basin, its oviposition behavior, and its number of instars. Immatures were found in high density on bedrock, on leaves of Podostemaceae and other aquatic plants, and on branches and leaves of riparian vegetation. Larvae and pupae were collected in 9 rivers: 8 in the state of Piauí and 1 in the state of Ceará. Six instars were determined for a population in Piauí. Oviposition behavior was of the dabbling type. Ants (*Solenopsis* sp.; Hymenoptera: Formicidae) preyed on larvae and pupae stranded when the river receded.

Key Words: aquatic insect; oviposition behavior; instar; predation

Resumo

Simulium guianense Wise (Diptera: Simuliidae) é o principal vetor de *Onchocerca volvulus* (Leuckart) (Spirurida: Onchocercidae), que causa oncocercose no foco amazônico da doença. Nós registramos *S. guianense* pela primeira vez na bacia hidrográfica do rio Parnaíba, o seu comportamento de oviposição e o seu número de estádios larvais. Imaturos foram encontrados em alta densidade sobre rochas, folhas de Podostemaceae e de outras plantas aquáticas, além de galhos e folhas da vegetação ripária. Larvas e pupas foram coletadas em nove rios, oito no estado do Piauí e um no estado do Ceará. Seis estádios larvais foram determinados para uma população do Piauí. Fêmeas depositavam seus ovos sobre o substrato sem pousar sobre ele. Larvas e pupas expostas após o nível do rio descer foram predadas por formigas (*Solenopsis* sp.; Hymenoptera: Formicidae).

Palavras Chave: insetos aquáticos; comportamento de oviposição; estádios larvais; predação

Simulium guianense Wise (Diptera: Simuliidae) is widely distributed in Brazil (Adler & Crosskey 2014), but it is more frequent in the northern portion of the country where it is the main vector of the nematode *Onchocerca volvulus* (Leuckart) (Spirurida: Onchocercidae) in the Amazon region. Despite the medical importance of this species (Shelley et al. 1997), its biology and ecology are poorly known. Two studies on this species were done in the Brazilian state of Amazonas, one providing water physical-chemical characteristics of the rivers where larvae and pupae were collected (Gomes & Py-Daniel 2002) and the other identifying the microalgae in the larval stomach contents (Gomes et al. 2002). Villamizar et al. (2011) provided the first information on the habitat of *S. guianense* immatures in the onchocerciasis area of Venezuela. Several studies on anthropophilic activity of this species have been done in Brazil (e.g., Shelley et al. 1997) and Venezuela (e.g., Grill et al. 2001).

Because *S. guianense* has wide geographical distribution and some populations are zoophilic whereas others are anthropophilic, it is suspected that the species is a complex of 2 or more sibling species (i.e., morphologically similar but reproductively isolated species) (Shelley et al. 1997). A cytotoxic study conducted with 4 populations of *S. guianense* in Brazil indicated the presence of 4 cytotypes, according to geographical region (Charalambous et al. 1996).

The number of instars is not fixed in Simuliidae, in contrast, for example, to Chironomidae and Culicidae, which are 2 families of aquatic Diptera in which there are 4 instars. In Simuliidae, the number of instars may vary depending on the species; also, larval development can be related to environmental conditions such as food availability and the temperature of streams (Crosskey 1990). Water temperature can affect the population of black flies, reducing the development period, the number of instars, and the size of the larva at maturity (Crosskey 1990). However, some studies conducted in Brazil have indicated no change in either the number of instars or the larval size, considering either the season (Alencar et al. 2001) or the geographical distribution (Alvan-Aguilar & Hamada 2003).

Oviposition behavior by female black flies can be classified into 4 types, depending on how the eggs are placed and the timing (continued or interrupted) in which they are laid (Crosskey 1990). In Brazil, the dabbling technique (with eggs placed singly or in groups with interrupted timing) has been reported for 2 species of *Simulium* (*Etecmnaspis*) (Gorayeb 1981; Hamada 1998). One species of *Simulium* (*Inaequalium*) was observed laying eggs in the terrestrial environment on riparian vegetation in a splash zone of a water fall in southern Brazil (Moreira & Sato 1996). Improved knowledge of the distribution of this species is important for understanding the potential for onchocerciasis disper-

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sion and to serve as a basis for other studies, such as phylogeographic and ecological analyses. The present study aimed to expand the geographical records of this species in Brazil and to provide biological information on the immature stages and on the oviposition behavior observed in Piauí State. Information on the biology and distribution of this species may assist in understanding its population dynamics and help in detecting the presence of potential sibling species.

The numbers of instars reported for black fly species of South America are: 6 (e.g., Coscarón-Arias & Bramardi 1996), 7 (e.g., Alencar et al. 2001; Alvan-Aguillar & Hamada 2003), and 8 (e.g., Coscarón-Arias & Bramardi 1996). Thus, we tested the hypothesis that the number of instars of *S. guianense* could be 6, 7 or 8.

Materials and Methods

The sampled area (Table 1) is located in a transition zone between deciduous Caatinga/Cerrado and Carnaúba/Caatinga riparian forest, where the annual precipitation is between 800 and 1,600 mm (Giulietti et al. 2004). Sampling was done in the northern portion of Piauí State and in the western portion of Ceará State (both in northeastern Brazil), from 27 May to 8 Jun 2011 (at the beginning of the dry season). The width of rivers sampled was estimated visually. Temperature, pH, and electrical conductivity were measured using portable equipment (waterproof pH meter, conductivity meter, and thermometer; Oakton Instruments, Vernon Hills, Illinois, USA). The geographical position was obtained with a GPS (Garmin Map 76Cx; Garmin, Olathe, Kansas, USA).

Oviposition behavior was observed in one stretch of the Jacareí River, located in Piracuruca Municipality, Piauí State, while we were sampling for black flies. Two females were collected for species identification. Larvae used to estimate the number of instars came from the same river, which was the only place where oviposition was observed, thereby allowing the collection of 1st instars. Predation of larvae and pupae was observed in the Longá River, Batalha Municipality, Piauí, during one afternoon.

Larvae and pupae were collected directly from the substrate by using tweezers. The larvae and pupae were fixed in Carnoy's solution (acetic acid and absolute alcohol in a proportion of 3:1) and in absolute ethanol, respectively. Both fixatives were changed at least 4 times. Pupae containing pharate adults were kept alive in plastic containers with moist filter paper until adult emergence; exuviae and their respective adults were fixed in 80% ethanol for species identification in the laboratory. Vouchers of the analyzed specimens were deposited in the Invertebrate Collection of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil.

To estimate the number of instars, the following measurements were taken: total body length (TL), lateral length of the head capsule (LLH, a line drawn from the base of the labral fan stalk to the posterior limit of the head, passing through the ocelli), and the width of the frontoclypeal apotome (WFA) (as in Alencar et al. 2001). The measurements were taken under a stereomicroscope (Leica M165C model; Leica, Wetzlar, Germany) with a camera attached (DFC420 model; Leica, Wetzlar, Germany), using a self-assembly program (LAS version 3.7). A Pearson analysis was used to evaluate possible correlations between the measured structures (TL, LLH, WFA) and to eliminate correlated metrics.

To test the hypothesis that the number of instars of *S. guianense* could be 6, 7, or 8, we considered the following steps: a) determination of the size of the 1st and last instars, which were easily recognized by their structural characteristics (Fig. 1); b) analysis of the distribution frequency of the intermediate measurements to estimate the intermediate stages, testing the hypothesis that *S. guianense* has 6 (4 intermediate), 7 (5 intermediate), or 8 (6 intermediate) instars; c) performing 3 ANOVAs with an a posteriori Tukey test to determine if the number of instars of *S. guianense* was 6, 7, or 8, using the measured structures of the 1st and last instars and the sizes of the intermediate instars, which were estimated by frequency distribution analysis; d) finally, we chose the analysis where no overlapping instars were observed.

Results

Out of 29 sites sampled in the study area, 9 had *S. guianense*: 8 in Piauí and 1 in Ceará State (Fig. 2). In total, 264 larvae from 1 river (Jacareí) were measured (LLH, TL, WFA). We verified the relationship between WFA, TL, and LLH, and all 3 measurements were highly correlated (Fig. 3). We therefore only considered LLH values in determining the number of instars. Measurements of the intermediate stages are shown in Fig. 4. The ANOVA and Tukey tests indicated that *S. guianense* has 6 instars, because the analysis with 7 or 8 instars resulted in overlapping instars (Fig. 5).

Simulium guianense is reported here for the first time in the states of Piauí and Ceará, in the Parnaíba River hydrographic basin (Table 1). Immatures of *S. guianense* were collected in rivers with an average width of 55 m (SD = 31.9; $n = 9$), average water temperature of 28.4 °C (SD = 1.28; $n = 9$), pH of 7.28 (SD = 2.31; $n = 9$), and electrical conductivity of 157.3 $\mu\text{S}/\text{cm}$ (SD = 76.1; $n = 9$) (Table 1).

Immatures were distributed in high density (approximately 30 larvae per cm^2) on different substrates such as twigs, branches, macrophytes (including Podostemaceae), and rock. Despite the high density

Table 1. Sites where *Simulium guianense* (Diptera: Simuliidae) were collected in Piauí (PI) and Ceará (CE) States, Brazil, and some habitat characteristics of the sampled site.

| State | Municipality, river name, and geographical coordinates | River width (m) | Water | | |
|-------|---|-----------------|------------------|-----|--|
| | | | Temperature (°C) | pH | Conductivity ($\mu\text{S}/\text{cm}$) |
| PI | Batalha, Longá River, 04°12'19.9"S, 42°14'10.6"W | 70 | 29.6 | 7.3 | 71 |
| PI | Batalha, river without name, 04°03'38.1"S, 41°57'19.0"W | 8 | 29.8 | 7.6 | 204 |
| PI | Piracuruca, Cabra Bom River, 04°0'9.7"S, 41°41'43.0"W | 10 | 28.1 | 6.6 | 48 |
| PI | São João da Fronteira, Genipapo River, 04°0'13.1"S, 41°26'54.1"W | 13 | 28.0 | 7.3 | 216 |
| PI | Brasileira, Piracuruca River, 04°05'02.7"S, 41°33'22.6"W | 40 | 29.5 | 7.2 | 169 |
| PI | Piracuruca, Jacareí River, 03°43'58.7"S, 41°40'54.2"W | 15 | 28.4 | 7.1 | 209 |
| PI | São Miguel do Tapuio, São Nicolau River, 05°48'34.8"S, 41°53'19.8"W | 20 | 29.4 | 7.8 | 98 |
| PI | Aroases, Sambito River, 06°13'8.2"S, 41°51'5.6"W | 23 | 26.5 | 7.3 | 101 |
| CE | Viçosa do Ceará, Pirangi River, 03°33'31.8"S, 41°21'56.9"W | 18 | 26.1 | 7.3 | 213 |

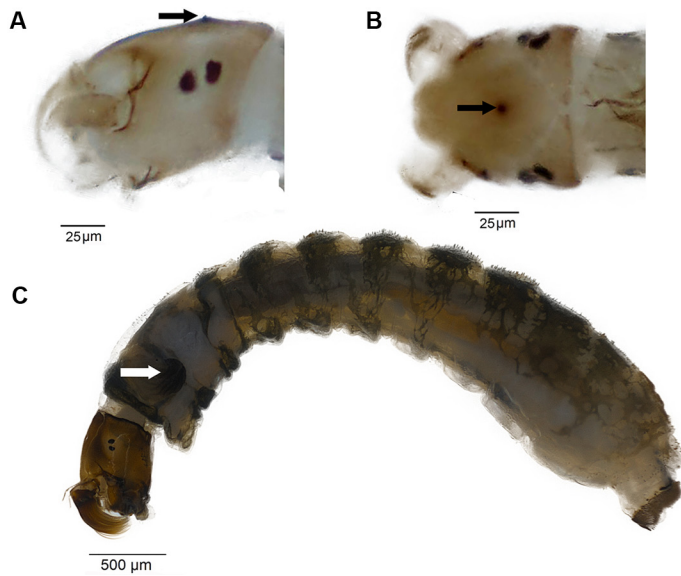


Fig. 1. *Simulium guianense* larvae. First instar, arrows indicate the egg-burster (A and B); the last instar is characterized by the presence of a completely formed gill histoblast (C).

of *S. guianense* immatures, adults were not observed in anthropophilic activity during the fieldwork.

Predation of immatures by ants (*Solenopsis* sp.; Hymenoptera: Formicidae) was observed in the Longá River (Fig. 6). At this site, we observed that the river water level went down very fast (observation at the beginning of the dry season), approximately 5 cm in 12 h, leaving larvae and pupae, which were using the bedrock as a substrate, exposed to this predator (Fig. 7). The rapid decrease in the water level of this river made it possible to observe many larvae confined to puddles of water formed in depressions in the rock (Fig. 8).

In a stretch of the Jacarei River, females of *S. guianense* were observed in oviposition activity in the morning (8:00 am), using as substrate submerged and/or partially submerged vegetation (Figs. 9 and 10). Because observation was not done in the afternoon, we do not know if this activity also occurs during this period of the day. Females hovered about 30 cm over the water, flying down to deposit small groups of eggs (8–40 eggs) over the substrate without landing on it.

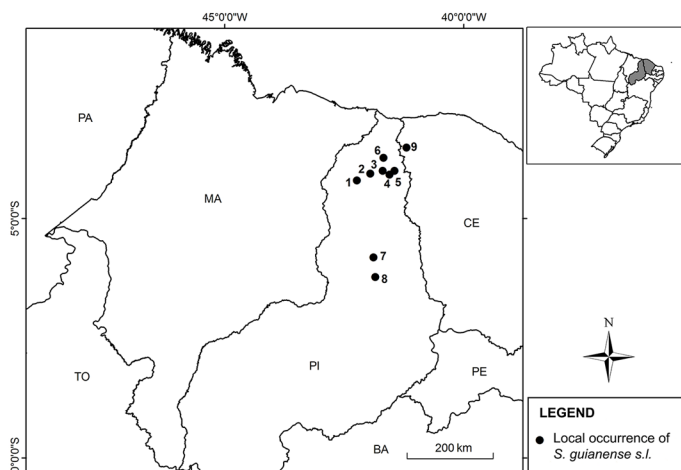


Fig. 2. Map of sampling sites with *Simulium guianense* in the northeast region of Brazil.

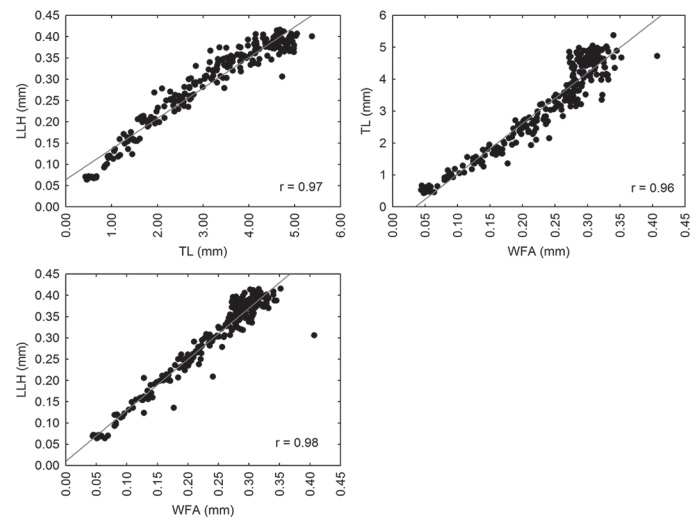


Fig. 3. Relationship between width of the frontoclypeal apotome (WFA), total body length (TL), and lateral length of the head capsule (LLH) of *Simulium guianense* larvae collected in the Jacarei River, Piracuruca Municipality, Piauí, Brazil, in Jun 2011.

Discussion

The number of instars is not known for most Brazilian black fly species, but most of the species for which this parameter has been analyzed have 7 instars (e.g., Alencar et al. 2001; Alvan-Aguiar & Hamada 2003). *Simulium guianense* in the Jacarei River in Piauí State had 6 instars, which is similar to some species in Argentina and in the USA (e.g., Ross & Merritt 1978; Coscarón-Arias & Bramardi 1996).

The association of *S. guianense* larvae and pupae with Podostemaceae has been reported in the literature (e.g., Gomes & Py-Daniel 2002). In our study, this association was also observed, but immatures were also found in high density on other substrates provided by riparian vegetation and other aquatic macrophytes. Of the 9 rivers in which *S. guianense* was collected in the study area, only 1 (the Longá River) had larvae and pupae in high density on bedrock. This is not a common substrate for this species and may indicate the scarcity of

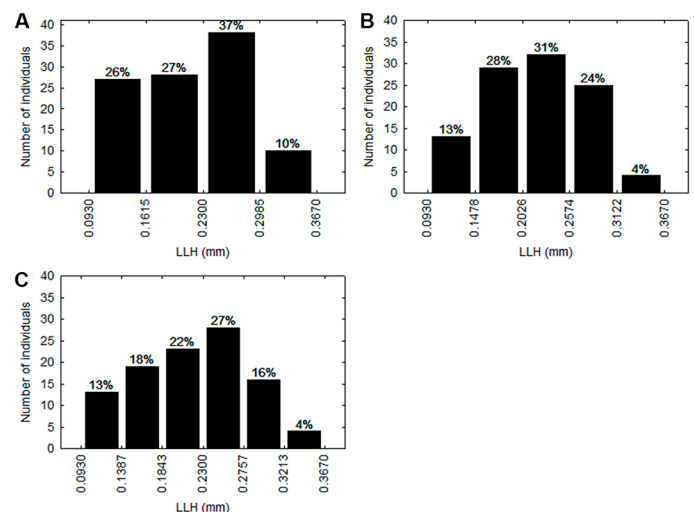


Fig. 4. Histogram with lateral length of the head capsule (LLH) values to establish 4 (A), 5 (B), and 6 (C) intermediate instars of *Simulium guianense* larvae collected in the Jacarei River, Piracuruca Municipality, Piauí, Brazil, in Jun 2011.

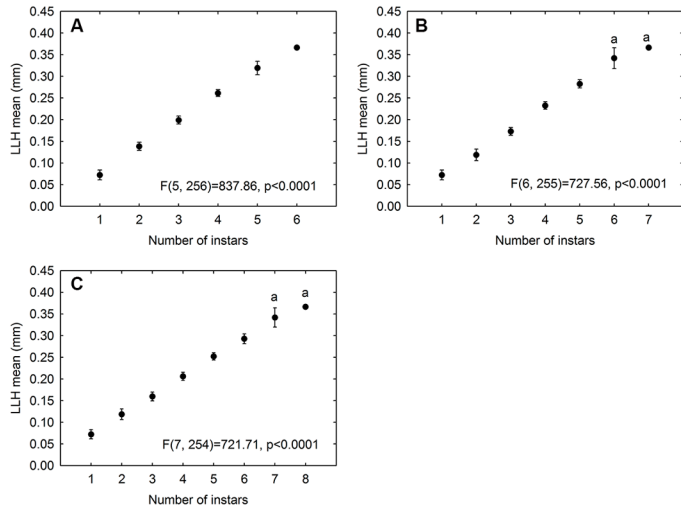


Fig. 5. Evaluation of the number of instars of *Simulium guianense* collected in the Jacareí River in the municipality of Piracuruca, Piauí, Brazil in Jun 2011. Instars are distinguished by the mean LLH (lateral length of the head capsule). Panel A has larvae grouped into 6 instars, Panel B into 7 instars, and Panel C into 8 instars. Vertical bars denote 95% confidence intervals and equal lowercase letters indicate lack of statistical difference between instars. The last 2 instars are statistically indistinguishable in Panels B and C, meaning that 6 instars (Panel A) represents the correct number.



Fig. 8. Larvae of *Simulium guianense* confined to puddles formed by depressions in the bedrock in the Longá River, Batalha Municipality, Piauí, Brazil, in Jun 2011.



Fig. 6. Ant (*Solenopsis* sp.), indicated by arrow, preying on larvae of *Simulium guianense* in the Longá River, Batalha Municipality, Piauí, Brazil, in Jun 2011.



Fig. 9. Female of *Simulium guianense* together with egg masses on a macrophyte, in the Jacareí River, Piracuruca Municipality, Piauí, Brazil, in Jun 2011.

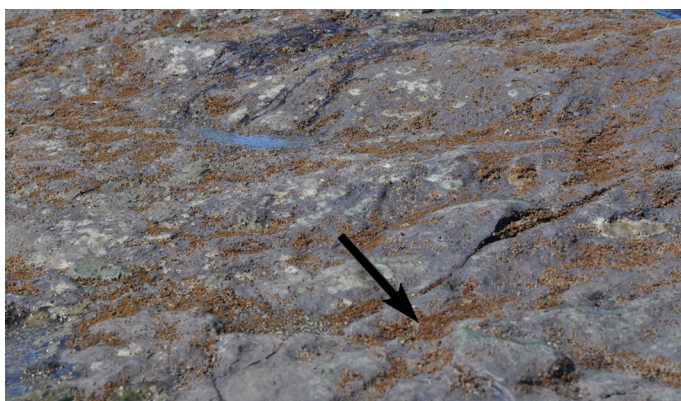


Fig. 7. Pupae of *Simulium guianense*, indicated by arrow, on bedrock in the Longá River, Batalha Municipality, Piauí, Brazil, in Jun 2011.



Fig. 10. Group of eggs of *Simulium guianense* on a leaf of an aquatic macrophyte in the Jacareí River, Piracuruca Municipality, Piauí, Brazil, in Jun 2011.

the other substrates, considering the observed high larval and pupal densities at this site. In a study in the state of Amazonas, Gomes & Py-Daniel (2002) attributed the use of submerged rocks and roots as substrates for *S. guianense* larvae and pupae to environmental changes due to the construction of a dam, which nearly caused the disappearance of Podostemaceae in their study area.

The water characteristics in the study area in the northeast region of Brazil were somewhat different from those in *S. guianense* habitats in Amazonian rivers (Gomes & Py-Daniel 2002). In the Amazon region, the water pH was acidic (5.4–6.7) and electrical conductivity was much lower (8.2–3.2 $\mu\text{S}/\text{cm}$) than we observed in northeastern Brazil. However, water temperature was similar in both regions (27.8–30.0 °C, in Amazonia) (Gomes & Py-Daniel 2002). These differences suggest either that these variables do not limit the populations of *S. guianense* or that this species is composed of sibling species with different habitat requirements. Molecular and cytotoxic studies of these populations will help to evaluate this problem.

During the sampling period, ants (*Solenopsis* sp.) preyed on larvae and pupae of *S. guianense* in the Longá River when the water level in the river fell rapidly, leaving them exposed. Ants in this genus have been observed preying on larvae and pupae of *Simulium perflavum* Roubaud in a similar situation in the Amazon region (Hamada 1998). Another consequence of the rapid loss of water by the system at the beginning of the dry season was the trapping of larvae in puddles in depressions in the bedrock. *Simulium guianense* may be subjected to a high mortality rate during this time of the year.

Simulium guianense females used the dabbling technique (Crosskey 1990), laying their eggs in groups without landing on the substrate, similar to the behavior of *Simulium rorotaense* Floch & Abonnence (Gorayeb 1981) and *S. perflavum* (Hamada 1998).

The present study extends the distribution of *S. guianense* to the states of Piauí and Ceará in the northeastern region of Brazil, in the Parnaíba River drainage. Previously, the only state in the northeastern region reported with this species was Maranhão, in the Tocantins River (a large tributary of the Amazonas River drainage) (Charalambous et al. 1996; Adler & Crosskey 2014).

Acknowledgments

We thank Ranyse B. Querino and Paulo V. Cruz for help with the field work, and RBQ for the photographs. This paper was written as part of the project “Diversidade críptica em *Simulium* (*Thyrsopelma*) (Diptera: Simuliidae) e relações entre suas espécies” (CNPq, processo n. 475663/2012-8). CRSN received a fellowship from PCI/INPA/MCTI and NH is a CNPq research fellow. P. M. Fearnside reviewed the English.

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