

## Past and present land use influences on tropical riparian zones: an isotopic assessment with implications for riparian forest width determination

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**Abstract:** In this article, by using carbon stable isotopes, we assessed the past and present land use influences that riparian areas are subject within agricultural landscapes. Emphasis is given to the understanding of the effects of the 2012 Brazilian Forest Act on such areas. We selected five riparian areas within a highly C<sub>4</sub> dominated agricultural landscape. Three of them had 30 meters native riparian forest buffer (NRFB) and two of them had 8 meter and no NRFB. We used three 100 meter-transects located 5, 15 and 30 meters relative to stream channel to obtain soil samples (0 – 10 cm). All riparian areas presented soil carbon isotopic signatures that are not C<sub>3</sub> (native forests) irrespective of having or not 30 meters NRFB. Two cases presenting less than 30 meters NRFB had higher C<sub>4</sub> derived carbon contribution. All of the other three areas that followed the 30 meters NRFB presented, to some degree, C<sub>4</sub> derived carbon, which was attributed to C<sub>4</sub> organic matter deposition originated from cultivated areas and, in one case, to the persistence of former exotic grasses. With the 2012 Forest Act allowing narrower buffers (< 30 meters), we expect C<sub>4</sub> contributions to soil organic matter to remain high in riparian areas and streams within agricultural landscapes dominated by C<sub>4</sub> plants where 30 meter NRFB is no longer required. Such contributions will likely continue to have detrimental effects on stream water quality and biota.

**Keywords:** Watershed; Soil degradation; Organic matter; Sediment; Carbon cycling.

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**Resumo:** Neste artigo, ao utilizar isótopos estáveis de carbono, nós avaliamos as influências presentes e pretéritas do uso da terra a que as áreas ripárias estão sujeitas quando situadas dentro de paisagens agrícolas. Ênfase é dada ao entendimento dos efeitos do Código Florestal de 2012 em tais áreas. Nós selecionamos cinco áreas ripárias em uma paisagem agrícola altamente dominada por plantas C<sub>4</sub>. Três delas apresentam faixa ripária de floresta nativa (FRFN) de 30 metros de largura e as outras duas apresentam FRFN de 8 e 0 m (i.e. sem FRFN). Nós utilizamos três transectos de 100 metros localizados a 5, 15 e 30 metros de distância do canal fluvial para obter amostras de solo (0 – 10 cm). Todas as áreas ripárias apresentaram assinaturas isotópicas do carbono do solo que não são C<sub>3</sub> (floresta nativa)

independentemente de apresentarem ou não FRFN de 30 metros. Os dois casos em que FRFN era menor que 30 m apresentaram maior contribuição de carbono oriundo de plantas  $C_4$ . Todas as outras três áreas com FRFN de 30 m também apresentaram, em algum grau, carbono oriundo de plantas  $C_4$ . Todas as outras três áreas com FRFN de 30 m também apresentaram, em algum grau, carbono oriundo de plantas  $C_4$  que foi atribuído à deposição de matéria orgânica de plantas  $C_4$  originada das áreas cultivadas e, em um caso, à persistência de gramíneas exóticas pré-existentes. Com o Código Florestal de 2012 permitindo FRFN mais estreitas (< 30 metros), nós esperamos que a contribuição de plantas  $C_4$  para a matéria orgânica permaneça alta em áreas ripárias e rios dentro de paisagens agrícolas dominadas por plantas  $C_4$  onde a FRFN de 30 m não é mais uma obrigação. Tais contribuições irão, provavelmente, continuar a ter efeitos prejudiciais à qualidade de água dos rios e à sua biota.

**Palavras-chave:** *Bacia hidrográfica; Degradação do solo; Matéria orgânica; Sedimento; Ciclagem de carbono.*

## Introduction

Riparian ecosystems generally perform many important ecological processes. When these streamside ecosystems are under forest cover these areas are considered to be important due to a series of reasons: (i) protection of stream banks against erosion and bank sliding (Abernethy & Rutherford 2000); (ii) reduction of erosion and input of soil particles into the stream (Lowrance et al. 1986, Verstraeten et al. 2006, Pires et al. 2009); (iii) shading and reduction of the water temperature (important for fish reproduction) (Imholt et al. 2013); (iv) riparian forests provide nutrients and carbon to aquatic communities (Lowrance et al. 1985); and they might also increase the input of coarse woody debris to the stream channel, which is important in creating habitat diversity within the stream environment (De Paula et al. 2011). These attributes of riparian forests are especially important in watersheds dominated by upland agricultural fields, where soil disturbance by cultivation and use of fertilizers and agrochemicals are frequent.

Several studies have shown that the width of the riparian forest is an important characteristic regarding some of the aforementioned attributes (Wenger, 1999, Zhang et al., 2010). Using meta-analysis, Zhang et al. (2010) showed that in order to perform these processes, at least a width of 20 m is required, although, depending on the attributes in view, different widths might be required (for more details, see Wenger 1999, Sparovek et al. 2002, Hawes & Smith 2005, Yuan et al. 2009). However, if all these important processes are to be achieved in a single place at the same time, the process that requires a wider buffer might be the one that should be adopted.

In Brazil, riparian areas are protected by law according to the Forest Act that is the Brazilian federal legislation that regulates the presence and distribution of the minimal native forest cover within rural private properties. This Act was originally created in 1934 and it was reformulated in 1965. Such Act stipulated that surface water bodies should have a riparian buffer around them to guarantee soil, water, biodiversity resources conservation and ecosystem processes. In the case of small streams (> 10 meters wide), the riparian buffer should present a minimum of 30 m and springs should have a 50-meter buffer. These two buffers are denominated '*permanent preservation areas*' in the Brazilian Forest Act and should be under the native vegetation cover (i.e. riparian forests) in order to guarantee their conservation goal.

In 2012, the Brazilian Congress approved a series of changes in the aforementioned Forest Act allowing the decrease of the width of riparian buffers needed to be restored in rural properties in cases where the law had not been

followed. This led to the reduction, in many cases, of the riparian buffers around small streams. For instance, the riparian buffer width in the 2012 Forest Act might be of only 5 meters instead of the 30 meters previously established in the 1965 Forest Act.

In São Paulo State, the most economically developed state of Brazil, rural areas are intensively used and currently dominated by  $C_4$  plants such as sugarcane and tropical forage grass species (Rudorff et al. 2010, Adami et al. 2012), whereas forest remnants are generally dominated by  $C_3$  plants. These two types of photosynthetic pathways generate different carbon isotopic composition (Farquhar et al. 1989).  $C_3$  plants generally have a  $\delta^{13}C$  around -28‰, which is lower compared to -12‰ which is the  $\delta^{13}C$  average value for  $C_4$  plants (Farquhar et al. 1989). Therefore, carbon isotopic composition of the soil organic matter is highly influenced by vegetation cover (Zhang et al. 2015). This fact allows the use of carbon stable isotopes to track the source of organic matter ( $C_3$  versus  $C_4$ ) and relate it to land-cover (Martinelli et al. 1996).

In this study, we used carbon isotopic signature of the surface soil organic matter in order to investigate past and present land use influences on riparian areas of small agricultural watersheds in Southeast Brazil. Although the findings of this study are specific to the studied watersheds, we advocate here that the carbon isotopic signature of soil organic matter can be used as a proxy of the Forest Act compliance anywhere in the country in cases where the original forest was replaced by  $C_4$  plants (forage grasses, sugarcane and corn). We chose five small watersheds to conduct this study; three of them were in compliance with the Forest Act regarding the width of the riparian forest; and in two of these watersheds the riparian forest width was less than 10 meters.

## Material and Methods

### Study areas

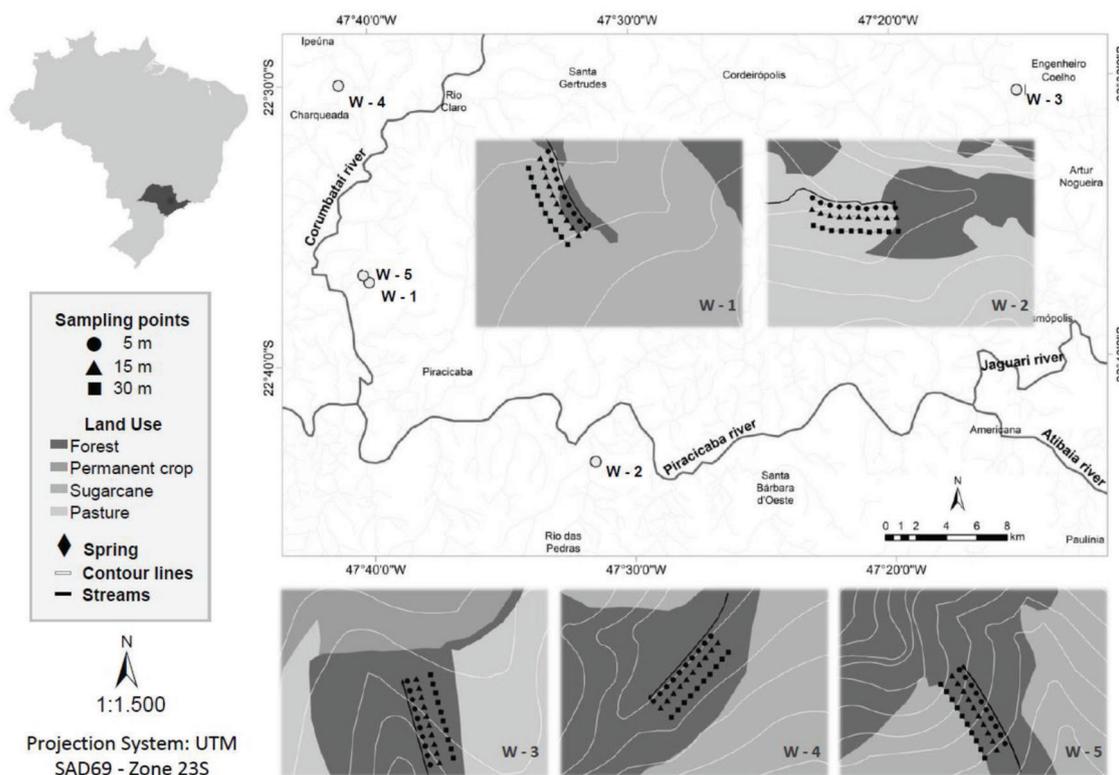
The selection of the areas was based on finding agricultural areas dominated by  $C_4$  crops with possible influence of  $C_4$ -derived carbon on riparian areas, which, at least initially, would be expected to present a dominance of  $C_3$  signal when under forest. Five first order streams and their respective watersheds belonging to the Piracicaba river basin (Southeast Brazil) were selected (Table 1; Figure 1). This is an important agricultural region of the country mainly due to the extensive sugarcane fields, with several sugar-ethanol mills in the region.

According to the Köppen classification, the climate is subtropical (Cwa), with a distinct dry season (April to September; mean monthly temperature of 20°C and an average

Carbon indicates past/present land use influences

**Table 1.** Location and land use in the riparian areas of the five small watersheds.

Watershed	Municipality	Coordinates		Land-use	Riparian forest buffer width (m)
1	Piracicaba	22 36' 44.82" S	47 40' 19.76" O	Sugarcane	~ 8
2	Piracicaba	22°43'32.03" S	47°31'29.11" O	Pasture	Absent
3	Limeira	22°30'33" S	47°15'12" O	Citrus/Pasture	30
4	Piracicaba	22°29'54.49" S	47°41'1.67" O	Sugarcane	30
5	Piracicaba	22 37' 02.91" S	47 40' 04.76" O	Sugarcane	30



**Figure 1.** Five riparian areas within the five small watersheds (W). Dark areas within W-1 to 5 indicate forest (C<sub>3</sub>) cover whereas lighter colours indicate C<sub>4</sub> cover. Black circles, triangles and quadrats indicate soils sampling points in linear transects located 5, 15 and 30 meters relative to stream channel. Scale on the bottom left corner refers to watersheds images whereas scale on the right, above W-5 image, refers to the map.

relative humidity of around 70%) and wet season (October to March; mean monthly temperature of around 24.4 °C and relative humidity around 80%). The annual rainfall in the region is approximately 1,400 mm, and the mean annual temperature is approximately 22°C.

All five small watersheds are within the domain of the Atlantic Forest biome. According to local farmers, the watersheds of streams 1 and 5 were converted to cornfields around the 1930s and after 20 years, corn was converted to sugarcane. Although these two watersheds are very close geographically, they present very different riparian forest cover patterns, being around 5 to 10 meters in the former, and 30 meters wide in the latter. In the watershed 2, pasture has been established for at least 13 years without any riparian forest left. Watersheds of streams 3 and 4, in turn, were covered by pasture and pasture and sugarcane, respectively, for at least 10 years with riparian forest buffer of 40 meters or more (Figure 1). In the watershed 3, interviews with local farmers revealed that the riparian area had been under pasture (*Andropogon bicornis* L.) until 1988. After

that, forest restoration practices took place and the area was recovered by native forest again as it is at the present moment.

Soils in four of the five riparian areas are generally classified as Ultisols (Udults); the only exception is in the riparian area of watershed 4 in which the soil is classified as an Oxisol (Udox). The topography is generally formed by gentle slopes (~8%) in areas under Ultisol soil type, and under gentle to flat slopes (~3%) in the watershed 4.

### Sampling design

In order to investigate the presence of carbon from C<sub>4</sub> plants, three 100-m transects were established parallel to the stream channels at a distance of 5, 15 and 30-m from to the stream channel (Figure 1). Each transect started from the spring to 100 meters downstream. Soil samples were obtained every 10 meters in each transect totaling 10 soil samples per transect. The sampling procedure was made by using Dutch augers to collect soil samples from 0 to 10 cm soil layer as

adopted elsewhere (Powers & Schlesinger 2002). Soil samples were collected from April to June 2011.

### Soil analysis

Soil samples were previously air-dried and then sieved to <2 mm in order to remove rocks, roots, leaves and charcoal. A sub-sample of 10 g of each sample was obtained using a Jones splitter. Afterwards, it was homogenized and milled. Finally, 5 mg of this sub-sample was weighed and packed separately into tin capsules.

The  $^{13}\text{C}:^{12}\text{C}$  ratio was determined using a mass spectrometer, Delta Plus from Finnigan Mat, and the isotopic ratio was reported as  $\delta^{13}\text{C}$  (‰) notation using the following equation:

$$\delta^{13}\text{C}(\text{‰}) = 1000 * [(R_{\text{sample}} - R_{\text{standard}})/(R_{\text{standard}})]$$

Where:

$\delta^{13}\text{C}$ : is the abundance of  $^{13}\text{C}$  in the sample

R sample: is the sample  $^{13}\text{C}:^{12}\text{C}$  ratio

R standard: is the standard  $^{13}\text{C}:^{12}\text{C}$  ratio

The precision for carbon isotope was obtained by running an internal standard and it was 0.2‰.

We assumed that organic matter derived from  $\text{C}_3$  plants has  $\delta^{13}\text{C}$  value equal to -27.9‰ which is the lowest  $\delta^{13}\text{C}$  measured in our five study areas, and plant organic matter derived from  $\text{C}_4$  plants has a  $\delta^{13}\text{C}_4$  as -11.3‰ based on a broad isotopic analysis carried out in Brazil available elsewhere (see Assad et al. 2013). The percentage contribution from  $\text{C}_4$ -derived carbon is given by the following isotope dilution equation:

$$\text{C}_4(\%) = (\delta^{13}\text{C}_{\text{soil}} - \delta^{13}\text{C}_3) / (\delta^{13}\text{C}_4 - \delta^{13}\text{C}_3)$$

Where:

$\delta^{13}\text{C}_{\text{soil}}$  is the carbon isotopic composition of the soil organic matter;

$\delta^{13}\text{C}_3$  is the carbon isotopic composition of  $\text{C}_3$  vegetation (-27.9‰); and

$\delta^{13}\text{C}_4$  is the carbon isotopic composition of  $\text{C}_4$  vegetation (-11.3‰).

### Results

The two riparian area with low or null forest cover (watersheds 1 and 2) had soil  $\delta^{13}\text{C}$  values predominantly higher than -17‰, some reaching values as high as -12‰, denoting a high contribution of  $\text{C}_4$  derived organic matter to the soil (Figure 2a, b). In the watershed 1, even the 5-m sampling points that are located within the riparian forest remnant had approximately 75% of organic carbon derived from  $\text{C}_4$  plants, suggesting that the entire riparian zone had influence of  $\text{C}_4$  plants (Table 2). In the riparian area of the watershed 2, the  $\delta^{13}\text{C}$  of the soil near the spring resembled those values of  $\text{C}_3$  vegetation, being lower than -24‰. After that, a marked downstream increase in the soil  $\delta^{13}\text{C}$  values in all three transects were observed, with values predominantly higher than -18‰, denoting a clear dominance of  $\text{C}_4$  derived carbon (Figure 2b). In these transects, with the exception of the spring, the average  $\text{C}_4$  derived carbon contribution was approximately 70% (Table 2).

In the watersheds 3, 4 and 5 forest covers more than 30 m of the riparian area (Figure 2b to 2d). Although there is the dominance of  $\text{C}_3$  vegetation cover, only the watershed 5 soil showed a clear soil  $\delta^{13}\text{C}$  values resembling those typical of  $\text{C}_3$  vegetation cover. In this watershed, soil samples collected along the 5-m transect line had  $\delta^{13}\text{C}$  values varying from approximately -28‰ to -25‰, typically within the  $\text{C}_3$  plants range values (Figure 2e). Soil  $\delta^{13}\text{C}$  values were higher in the other two transects of watershed 5, but with few values higher than -22‰, evidencing a low contribution of  $\text{C}_4$  plants to the soil in this watershed (Table 2). In the riparian areas of watershed 3,  $\delta^{13}\text{C}$  values were lower than -24‰ from 0 to 20 meters, increasing to values varying from -24 to -18‰ in the middle portion of the transect (from 20 to 70 meters). In the final part of the transect (from 70 to 100 m), soil  $\delta^{13}\text{C}$  values decrease again to values lower than -24‰ (Figure 2). Therefore, there was a low  $\text{C}_4$  contribution to the soil organic matter in the portion closer to the spring and in the final portion of the transect. Finally, the soil  $\delta^{13}\text{C}$  of riparian areas of watershed 4 varied between -22 to -16‰ along the transect, suggesting a mixture in different proportions of  $\text{C}_3$  and  $\text{C}_4$  vegetation (Figure 2d). Lower  $\delta^{13}\text{C}$  values were observed in the 5m-transect line with values varying from -22 to -20‰ in the first 60 meters, and increasing to approximately -18‰ in the final 40 meters of the transect, denoting an increase in the contribution of  $\text{C}_4$  derived organic matter in this last portion of the transect (Table 2). The soil  $\delta^{13}\text{C}$  values observed in the 15m-transect line varied from -18 to -16‰ in the entire transect, while values increased to approximately -16‰ in the 30m-transect line. The average  $\text{C}_4$  contribution along the 5m-transect line was 44%, increasing to 66 to 69 % in the 15m and 30m-transect lines, respectively (Table 2).

### Discussion

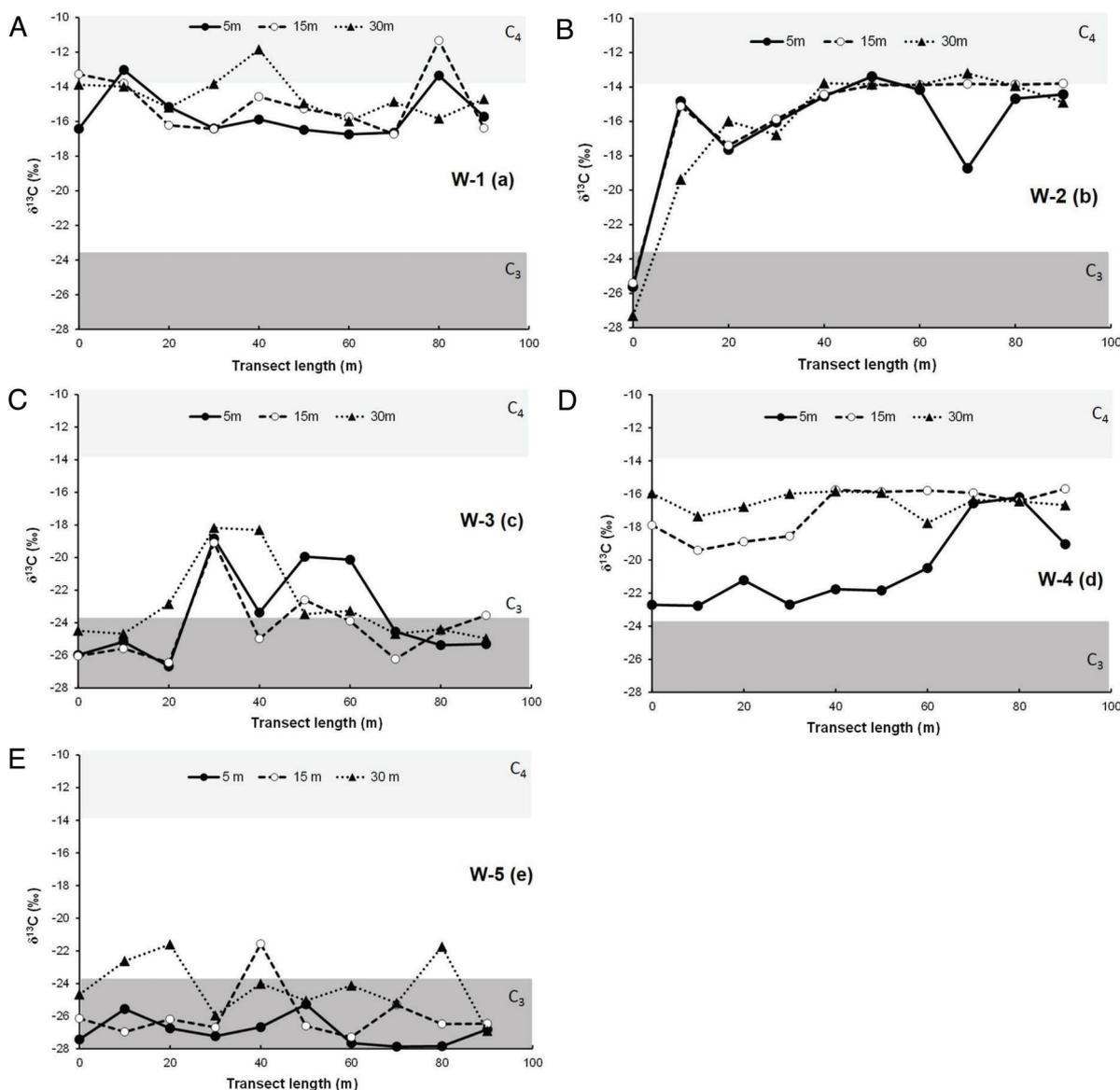
#### $\delta^{13}\text{C}$ variability within riparian areas

There was no forest left in the majority of riparian area of watershed 2 that since 2002 has been covered by  $\text{C}_4$  grass forage. Only around the spring, the  $\text{C}_3$  contribution to the soil increases due to the proximity of remnant forest immediately upslope the spring. In the rest of the transect, forest ( $\text{C}_3$ ) is practically absent and there is no litter layer on the soil surface. In this case, the vegetation cover and the soil organic matter have similar  $\delta^{13}\text{C}$  values, and this area is clearly in no compliance with the Forest Act.

The watershed 1 forest cover width is wider than watershed 2 forest cover, reaching an average of 8 meters. The soil  $\delta^{13}\text{C}$  in this area (5m-transect) does not reflect the  $\delta^{13}\text{C}$  of the forest, but instead the  $\delta^{13}\text{C}$  of a  $\text{C}_4$  vegetation, suggesting that this forest is a young secondary growth that did not yet contributed substantially to the soil organic matter (Marin-Spiotta et al. 2009) or that upland  $\text{C}_4$  influences within this area are substantial due to  $\text{C}_4$  organic matter deposition (Pires et al. 2009). The soil  $\delta^{13}\text{C}$  of the 15 m and 30 m-transect lines reflect the  $\text{C}_4$  vegetation type. This riparian area is not also complying with the Forest Act.

In the riparian area of watershed 5 (with a forest cover 30 meters wide), the  $\delta^{13}\text{C}$  of the soil showed an unequivocal  $\text{C}_3$  signal. Therefore, there is a full compliance of this riparian area with the Forest Act. On the other hand, it seems also that the 30m-riparian buffer zone has been effective in avoiding the

Carbon indicates past/present land use influences



**Figure 2.**  $\delta^{13}\text{C}$  variation throughout the 5, 15 and 30 m transects in the W-1 (a), W-2 (b), W-3 (c), W-4 (d) and W-5 (e). The top rectangles in the light grey indicates the expected area for  $\text{C}_4$  signals while the bottom rectangles in dark grey indicates the expected area for  $\text{C}_3$  signals. Middle rectangle in white indicates a region of a likely mixture of both photosynthetic types.

**Table 2.** Contribution (%) of  $\text{C}_4$  derived carbon in each of the transects within the five small watersheds (W).

W	Transects					
	5 m		15 m		30 m	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
1	74	8	78	11	81	7
2	69	22	73	22	70	26
3	26	17	22	13	30	15
4	44	15	66	9	69	4
5	6	5	11	10	22	11

\*S.D.: standard deviation

entrance of upland  $\text{C}_4$  carbon in the riparian given the higher contribution of  $\text{C}_4$  derived organic matter in the 30-m transect.

The riparian area of the watershed 3 is also covered with forest 30 m wide; however, this area was entirely covered by  $\text{C}_4$  forage grasses until  $\sim 25$  years ago when it was left for recover. Based on the soil  $\delta^{13}\text{C}$  values, it seems that the  $\text{C}_3$  vegetation replaced the old  $\text{C}_4$  forage only in the first and final part of the transect. It seems that in the middle, the signal of  $\text{C}_4$  vegetation of the old pasture still remains in the soil. Alternatively, the origin of this  $\text{C}_4$  material present in the middle of the transect could be generated upland by soil erosion and further deposition in this area. Several studies have documented the strong deposition rates of overland-flow derived materials within riparian areas, especially in flow convergence zones (i.e. depressions) (Lowrance et al. 1986, Cooper et al. 1987, Cavalcanti & Lockaby 2005, Cavalcanti & Lockaby 2006, Schoonover et al. 2006, Pires et al. 2009, Kreutzweiser et al. 2009, Mamoli et al. 2012). Moreover, there is the fact that tussocks of this grass were observed in the forest during our

field campaigns, suggesting that this C<sub>4</sub> signal might be derived from this persistent grass species (Guarantini et al. 2008, Griscom et al. 2009, Brancalion et al. 2009). The same is true for the riparian area of the watershed 4 that also has a large forest area wider than 30 meters, but still shows a clear C<sub>4</sub> sign in the soil, which could be generated upland or be a remnant of the old pasture.

Another point that has to be mentioned is related to riparian forest age. The younger the riparian forest, the weaker would be the C<sub>3</sub> signal in soil organic matter (Marin-Spiotta et al. 2009). Provided that most of riparian forests presented here are riparian forest remnants (watersheds 1, 4 and 5), no *in situ* influence (i.e. soil organic matter replacement by direct land use in the area) was responsible for our results. Even at watershed 3, which has a ~25 years old riparian forest, the course of recovery towards a C<sub>3</sub> signal can be seen in most parts of the transects. This is in accordance with findings that show that soils dominated by C<sub>4</sub> organic matter became dominated by C<sub>3</sub> organic matter within approximately 10 years after reforestation (Cook et al. 2014).

#### *Soil organic matter: proportion of C<sub>4</sub> derived carbon*

A study carried out in the same municipality of the present study (i.e. Piracicaba, São Paulo State, Brazil) showed that 50 years after a land-use conversion from forest (C<sub>3</sub>) to sugarcane (C<sub>4</sub>), the soil under sugarcane still had approximately 40% of soil organic matter derived from forest (Vitarello et al. 1989). In other areas, a similar persistence of the C<sub>3</sub> vegetation in the soil organic matter has been observed. For instance, Roscoe et al. (2001) assessed the replacement of a C<sub>3</sub> Cerrado-type vegetation physiognomy by an exotic grass *Brachiaria* spp. (C<sub>4</sub>). They found that higher replacement occurred at the first soil horizon (i.e. A horizon) where about 36% of the soil organic C was still derived from the C<sub>3</sub> vegetation after 23 years of pasture cultivation (Roscoe et al. 2001). Our results, in turn, show that after approximately 13 to 60 years under C<sub>4</sub> plants, a proportion of 25 – 30% C<sub>3</sub> derived carbon still can be found in the riparian areas of watersheds 1 and 2. Differences between the present study and the one by Vitarello *et al.* (1989) might be related to soil characteristics such as texture (Roscoe et al. 2001) or other factors affecting soil carbon turnover rate (Powers & Schlesinger 2002; Telles et al. 2003) such as soil moisture that might be typically higher in riparian soils compared to upland soils (Luizão et al. 2004). Moreover, the long-term persistence of C<sub>3</sub> signals in the riparian areas of watersheds 1 and 2 might be attributed to highly recalcitrant organic matter or organic matter that is tightly bound to clay soil particles (Roscoe et al. 2001, Powers & Schlesinger 2002, Telles et al. 2003, Alcântara et al. 2004, Marin-Spiotta et al. 2009).

#### *Implications for riparian forest buffer width determination*

The fact that riparian areas of watersheds 1 and 2, and even watersheds 3 and 4 showed clear contributions of C<sub>4</sub> derived carbon, may provide evidence that, in some cases, even riparian forest of 30 meters width or more are under strong influence of the upslope C<sub>4</sub> cultivated areas. As a consequence, 30 meters riparian forest buffers or narrower widths (as currently allowed in 2012 Forest Act) do not appear to be appropriate to conserve soil and water. Indeed, a similar finding has been

found in a nearby area where even 30 meters riparian buffers did not suffice to retain soil particles originated from sugarcane fields (Pires et al. 2009).

If the five cases presented here represent the variety of conditions by which riparian areas are subject in southeast São Paulo and other highly intensive agricultural landscapes elsewhere, it appears that, in most cases, riparian forest buffers of 30 meters or narrower might not be enough with regards to water resources protection. Thus, based on the 2012 Brazilian Forest Act, we expect that riparian areas with riparian forest buffers 5 to 15 meters wide will, in most cases, not be effective buffers to protect soil and water quality, the main original purpose of the Forest Act. Consequently, we expect the increases in suspended and bed load and their associated materials enhancing stream siltation, which in turn, alter light regime and stream environment with detrimental effects on stream water quality and biota.

## Conclusion

We have shown that soil stable carbon isotopic composition can be a useful tool to investigate compliance with the Forest Act in riparian areas that were or are currently under the influence of a C<sub>4</sub> vegetation type. As pasture soils in Brazil have approximately 200 million ha covered with exotic African C<sub>4</sub> grasses, and an additional 24 million ha of corn and sugarcane crops together (Martinelli et al. 2010), the isotopic approach could be used to study several of these areas. However, for future studies it is imperative that soil sampling has to include deeper soil layers to exclude the influence of C<sub>4</sub> carbon generated upland (i.e. outside of the riparian area).

Finally, narrower riparian forest buffers (< 30 meters) currently allowed in the 2012 Forest Act will likely contribute to keep the detrimental effects of soil erosion with consequences connected to stream water quality in agricultural landscapes. In other words, the already high stream suspended load and siltation with consequences for stream morphology, which shapes stream biota and nutrient uptake, are highly expected to remain in such landscapes where 30 native riparian forest buffer is no longer required.

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## Biology of *Boeckella poopoensis* Marsh, 1906 (Copepoda, Calanoida) in natural conditions in temporary saline lakes of the central Argentina

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VIGNATTI, A.M., CABRERA, G.C., ECHANIZ, S.A. **Biology of *Boeckella poopoensis* Marsh, 1906 (Copepoda, Calanoida) in natural conditions in temporary saline lakes of the central Argentina.** Biota Neotropica. 16(2): e20150063. <http://dx.doi.org/10.1590/1676-0611-BN-2015-0063>

**Abstract:** *Boeckella poopoensis* Marsh, 1906 is the dominant copepod in saline lakes from northern Patagonia to southern Peru. It is a tolerant species, which has been registered at salinities between 20 and 90 g.L<sup>-1</sup>, and is important because it integrates the diet of flamingos and fishes of commercial and sport interest. The aims of this study were to analyze the characteristics of populations of *B. poopoensis* in the central region of Argentina and to establish their relationships with environmental parameters. Monthly samples during 2007 were taken in four temporary lakes of La Pampa province. Environmental parameters and density, size, biomass, number and size of eggs were determined. The salinity ranged between 13.38 and 32.90 g.L<sup>-1</sup>. In three lakes that had previously continuously contained water, *B. poopoensis* was registered throughout the whole study. In the fourth lake, which was filled in January, it was present only when salinity exceeded 15 g.L<sup>-1</sup>. The population of the lake that was filled differed from that of the other lakes in terms of the density and biomass of adults and copepodites. The number of ovigerous females represented a higher percentage of the population during the colonization of the lake that had been dried and these produced the highest number of eggs. In the three lakes in which *B. poopoensis* was always recorded, its characteristics were more influenced by the availability of food than by temperature or salinity. It was found that the strategies of the species vary throughout the hydroperiod; at the beginning, thrives when the salinity rises and impedes the presence of less tolerant species. At this point, the production of relatively small eggs is high, allowing rapid colonization. When the lakes become relatively stable, *B. poopoensis* allocates more energy to reach larger sizes and although egg production is not so high, they are larger, allowing it to maintain stable populations.

**Keywords:** *Boeckella poopoensis*, halophilic copepods, saline lakes, temporary lakes.

VIGNATTI, A.M., CABRERA, G.C., ECHANIZ, S.A. **Biología de *Boeckella poopoensis* Marsh, 1906 (Copepoda, Calanoida) en condiciones naturales en lagos salinos temporarios del centro de Argentina.** Biota Neotropica. 16(2): e20150063. <http://dx.doi.org/10.1590/1676-0611-BN-2015-0063>

**Resumen:** *Boeckella poopoensis* Marsh, 1906 es el copépodo dominante en lagos salinos desde el norte de Patagonia hasta el sur del Perú. Es una especie halotolerante registrada con salinidades entre 20 y 90 g.L<sup>-1</sup> y es importante dado que integra la dieta de flamencos y de peces de interés comercial o deportivo. El objetivo del estudio fue analizar características de poblaciones de *B. poopoensis* en la región central de Argentina y establecer sus relaciones con los parámetros ambientales. Durante 2007 se tomaron muestras mensuales en cuatro lagos temporarios de la provincia de La Pampa. Se determinaron parámetros ambientales y la densidad, espectro de tallas, biomasa, número y tamaño de los huevos. La salinidad varió entre 13,38 and 32,90 g.L<sup>-1</sup>. En tres lagos que habían contenido agua en forma continua, *B. poopoensis* se registró durante todo el estudio. En el cuarto, que se llenó en enero, sólo estuvo presente cuando la salinidad superó 15 g.L<sup>-1</sup>. La densidad y biomasa de los adultos y copepoditos de la población del lago que se llenó difirieron de las de los otros. El número de hembras ovígeras representó un mayor porcentaje del total de la población durante la colonización del lago que había estado seco y fueron las que produjeron el mayor número de huevos. En los tres lagos en los que *B. poopoensis* se registró siempre, sus características fueron influidas más por la disponibilidad de alimento que por la temperatura o salinidad. Este estudio mostró que las estrategias de la especie varían a lo largo del hidropériodo; al principio, prospera cuando la salinidad aumenta e impide la presencia de especies menos tolerantes. En este momento, la producción de abundantes huevos relativamente pequeños permite una rápida colonización. Cuando los lagos alcanzan una relativa estabilidad, *B. poopoensis* destina más energía a alcanzar tamaños mayores y, aunque la producción de huevos no es tan elevada son más grandes, permitiéndole mantener poblaciones estables.

**Palavras-chave:** *Boeckella poopoensis*, copépodos halófilos, lagos salinos, lagos temporarios.

## Introduction

In South America, shallow temporary lakes are abundant and are located mainly in tropical and subtropical latitudes of the Andes (Hurlbert et al. 1986, Williams et al. 1995, De los Ríos & Crespo 2004), in the center and northwest of the Pampa Plains (Quirós 1997) and in the Patagonian plateau (Soto et al. 1994, Campos et al. 1996).

The zooplankton structure of these lakes is regulated mainly by salinity (De los Ríos & Crespo 2004, De los Ríos 2005, Vignatti 2011) and therefore, they have low species richness (De los Ríos-Escalante 2010, Vignatti 2011). Lakes frequently contain calanoid centropagid copepods, which generally reach high densities (Soto & Zúñiga 1991, Modenutti et al. 1998a, b). Among these, *Boeckella poopoensis* Marsh, 1906, is one of the predominant halophilic species, which has been registered in lakes with salinities between 20 and 90 g.L<sup>-1</sup> (Hurlbert et al. 1984, 1986, Bayly 1993, Williams et al. 1995; Zúñiga et al. 1999; Acosta et al. 2003, De los Ríos & Crespo 2004, De los Ríos 2005, Locascio de Mitrovich et al. 2005). However, its tolerance range might be higher, since has also been found in a water body in the central region of Argentina, with a salinity close to 116 g.L<sup>-1</sup> (Echaniz 2010).

*Boeckella poopoensis* has a very wide geographical distribution, from the north of the Patagonian plateau, in Argentina and Chile, to the south of Peru (Menu-Marque et al. 2000). Since it is part of the diet of the flamingo *Phoenicopterus chilensis* Molina, 1782 (Locascio de Mitrovich et al. 2005, Battauz et al. 2013), De los Ríos-Escalante (2010) proposed that the migration of these birds might have contributed to the wide distribution of this copepod in the South American saline ecosystems.

*Boeckella poopoensis* has been found in both clear and turbid (organic and inorganic) environments, with a water transparency between 0.08 and 1.53 m and a chlorophyll-*a* concentration between 0.07 and 218.62 mg.m<sup>-3</sup> (Echaniz 2010, Echaniz & Vignatti 2010, Vignatti 2011).

Given that copepods provide a significant proportion of the total zooplankton biomass (Margalef 1983) and since *B. poopoensis* is a species that reaches a large size, its contribution to the biomass of zooplankton in South American shallow lakes is relevant (Locascio de Mitrovich et al. 2005, Echaniz et al. 2013). It is an ecologically important species, because besides being part of the diet of at least one species of flamingo (Locascio de Mitrovich et al. 2005), it is one of the food items of fishes with commercial and sport interest in the central region of Argentina, such as the silverside *Odontesthes bonariensis* (Cuvier & Valenciennes 1835) (Sagretti & Bistoni 2001, Escalante 2002, Grosman 2002).

Although some information is known concerning some aspects of the biology of *B. poopoensis* in natural conditions, including its important morphometric plasticity (De los Ríos-Escalante et al. 2011), information on *B. poopoensis* in general is scarce. Therefore, the objectives of this study were to analyze the population characteristics (density, biomass and size range), particularly of ovigerous females, including their size range, and the number and size of eggs, in four hypo-mesosaline (Hammer 1986) shallow lakes of the province of La Pampa (Argentina), and to establish relationships with the principal environmental parameters.

## Material and Methods

### Study area

Between January and December 2007, monthly samples were taken in four shallow lakes located in different regions of the

province of La Pampa, Argentina (Figure 1): Chadilauquen (Cha) (64°19'W, 35°24'S), San José (SJ) (63°55'W, 36°21'S), Utracán (Ut) (64°36'W, 37°17'S) and El Carancho (EC) (65°03'W, 37°27'S). In all cases, these are temporary ecosystems that are principally fed by rainfall and to a lesser extent by groundwater contributions. The lake basins are arheic and water loss occurs mainly by evaporation or infiltration (Vignatti 2011).

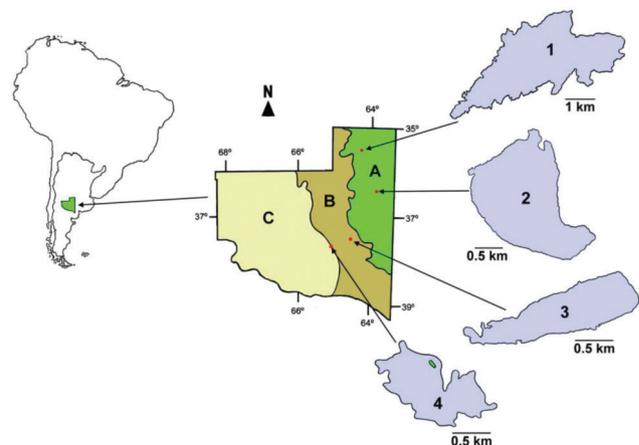
Although three lakes (Cha, SJ and Ut) had contained water for a relatively long time, EC was dry until January, when it was filled with torrential summer rains and could only be sampled from February onwards.

The lakes were surrounded by fields dedicated to cereal and oil cultivation and to extensive livestock cultivation. Among the avifauna, flamingos (*P. chilensis*) were present in all four lakes. Only Ut and EC were partially covered by the rhizomatous herbaceous macrophyta *Ruppia cirrhosa* (Petagna, Grande), which is characteristic of saline lakes (Vignatti 2011).

### Field and laboratory work

Samples were taken at three sites, along the major axis of each lake. The pH (Corning® PS-15 peachimeter), transparency (22 cm diameter Secchi disk), dissolved oxygen concentration and water temperature (Lutron® DO 5510 oxymeter) were determined *in situ* and water samples for the physicochemical determinations were taken and kept in the dark and refrigerated until analysis. At each site, qualitative zooplankton samples were taken by vertical and horizontal tows with a net 22 cm in diameter and 0.04 mm mesh aperture and quantitative samples were taken with a Schindler-Patalas plankton trap of 10 L with a 0.04 mm mesh aperture. Samples were anesthetized with CO<sub>2</sub> and kept refrigerated to avoid loss of eggs and deformation of the specimens (José de Paggi & Paggi 1995). After making the measurements, all samples were fixed with 5% formalin.

The dissolved solid concentration (salinity) was determined by the gravimetric method with the drying at 104°C of 50 mL of previously filtered water. The concentration of chlorophyll-*a* was determined by extraction with 90% (v/v) aqueous acetone



**Figure 1.** Location and maps of the four lakes studied. 1: Chadilauquen (Cha); 2: San José (SJ); 3: Utracán (Ut) and 4: El Carancho (EC). Phyto-geographic regions of La Pampa province: Pampa Plains (A); Thorny Forest (B) and Monte (C).

**Figura 1.** Ubicación geográfica y croquis de los cuatro lagos estudiados. 1: Chadilauquen (Cha); 2: San José (SJ); 3: Utracán (Ut) y 4: El Carancho (EC). Regiones fitogeográficas de la provincia de La Pampa: Llanura Pampeana (A); Espinal (B) y del Monte (C).

and subsequent reading in a spectrophotometer (APHA 1992, Arar 1997), total Kjeldahl nitrogen (TKN) was analyzed by the Kjeldahl method and total phosphorus (TP) using the ascorbic acid method, previous digestion with potassium persulfate. The content of suspended solids was determined with Microclar FFG047WPH fiberglass filters, which were dried at 103–105°C to constant weight and later calcined at 550°C (EPA 1993).

The taxonomic determination of the species was performed following Menu-Marque & Locascio de Mitrovich (1998) and Bayly (1992a, b). The counts of adults and copepodites were made in 5 mL Bogorov chambers under a stereomicroscope (20X) and those of nauplii, in a Sedgewick-Rafter chamber. Aliquots were taken with a 5 mL Russell subsampler and 1 mL micropipettes, respectively. The number of aliquots was determined using the Cassie formula (Dowing & Rigler 1984). The species density was expressed as: i) nauplii, by integrating the sum of the different stages; ii) the sum of adults (including ovigerous females) and the different copepodites stages and, iii) the ovigerous females as a separate group.

Measurements of specimens and eggs were performed using a light microscope equipped with a 10X Leitz ocular micrometer. To determine the number of eggs, the ovigerous sacs were dissected and the eggs were directly counted under the microscope. Biomass was estimated by applying dry weight/body length regression equations (McCauley 1984, Dumont et al. 1975). The total biomass was calculated as the product of individual mean biomass and total density.

A parametric ANOVA test (F), Tukey pairwise comparisons, nonparametric Kruskal-Wallis test (H) and Mann-Whitney pairwise comparisons were applied to determine significant differences between the environmental and biological parameters. In order to examine relationships between environmental factors and the features of *B. poopoensis*, nonparametric correlation coefficients of Spearman ( $r_s$ ) (Sokal & Rohlf 1995, Zar 1996, Pereyra et al. 2004) and Principal Components Analysis (PCA) (Pérez López 2004) were performed, which considered only adults and copepodites. We used Past (Hammer et al. 2001) and InfoStat (Di Rienzo et al. 2010) software.

Because no significant differences in physical, chemical and biological parameters of the three sites at each sampling were found, we used mean values.

## Results

### Limnological characterization of lakes

The salinity of lakes differed ( $H = 37.48$ ;  $p = 0.00$ ) and was higher in Cha, SJ and Ut, but was relatively stable, ranging

from 7.0 to 8.6 g.L<sup>-1</sup> (Table 1). In EC, the mean salinity was lower, but its range of variation was close to 15 g.L<sup>-1</sup>, and rose from a minimum of 5.7 g.L<sup>-1</sup> immediately after filling (February), to a maximum of 20.7 g.L<sup>-1</sup> (December). The water temperature of the four lakes did not differ and ranged from a minimum of 4.9°C in EC and SJ (June and July respectively), to a maximum of 25.1°C in Cha (January). The mean concentration of dissolved oxygen differed ( $H = 8.15$ ;  $p = 0.04$ ) and was slightly higher in Ut and EC (Table 1).

Water transparency varied ( $H = 16.89$ ;  $p = 0.00$ ), exceeding 1 m in Ut and EC and being less in Cha and SJ, and correlations between transparency and inorganic ( $r_s = -0.68$ ;  $p = 0.00$ ) or organic ( $r_s = -0.69$ ;  $p = 0.00$ ) suspended solid concentrations were found. The inorganic suspended solids were different ( $H = 20.11$ ;  $p = 0.00$ ), between four and nine times more abundant in the two lakes with a lower transparency (Table 1). The organic suspended solid concentrations were also different ( $H = 12.3$ ;  $p = 0.01$ ), and were much higher in SJ.

The nutrient concentration of the water was high and differed (TP:  $H = 7.87$ ;  $p = 0.05$  and TKN:  $H = 25.07$ ;  $p = 0.00$ ), and was slightly reduced in EC. The phytoplankton chlorophyll-*a* concentration differed ( $H = 13.59$ ;  $p = 0.00$ ), and was between three to four times higher in SJ than in the other lakes (Table 1).

### *Boeckella poopoensis*: density, biomass and size range

In Cha, SJ and Ut, *B. poopoensis* was registered throughout the sampling period, whereas it was only found in the last three months of study in EC.

The mean density and biomass of adults and copepodites were different ( $H = 8.59$ ;  $p = 0.04$  and  $H = 7.74$ ;  $p = 0.05$ ) and lower values of both parameters were recorded in the EC population. A higher mean annual density and biomass was reached by the population of SJ (Table 2). The maximum mean length of adults and copepodites was registered in SJ and the minimum in Cha (Table 2); however, the difference was not significant.

A correlation was observed between *B. poopoensis* adult and copepodite density and biomass and salinity ( $r_s = 0.64$ ;  $p = 0.00$  and  $r_s = 0.64$ ;  $p = 0.00$ ), chlorophyll-*a* concentration ( $r_s = 0.37$ ;  $p = 0.01$  and  $r_s = 0.35$ ;  $p = 0.02$ ), organic suspended solids ( $r_s = 0.53$ ;  $p = 0.00$  and  $r_s = 0.54$ ;  $p = 0.00$ ) and water transparency ( $r_s = -0.54$ ;  $p = 0.00$  and  $r_s = -0.54$ ;  $p = 0.00$ ). The adult and copepodite size did not correlate with any of the environmental parameters.

**Table 1.** Mean values and standard deviation of the environmental parameters determined in the studied lakes.

**Tabla 1.** Valores medios y desvíos estándar de los parámetros ambientales determinados en los lagos estudiados.

	Cha	SJ	Ut	EC
Salinity (g.L <sup>-1</sup> )	26.16 (± 2.13)	30.82 (± 2.67)	32.90 (± 2.69)	13.38 (± 4.02)
Temperature (°C)	16.58 (± 7.05)	16.45 (± 7.10)	16.08 (± 7.17)	15.04 (± 7.32)
DO (mg.L <sup>-1</sup> )	8.46 (± 1.1)	8.87 (± 1.8)	10.07 (± 2)	10.12 (± 1.3)
Transparency (m)	0.76 (± 0.26)	0.78 (± 0.41)	1.15 (± 0.29)	1.28 (± 0.36)
Inorg. susp. solids (mg.L <sup>-1</sup> )	18.27 (± 24.23)	16.50 (± 15.28)	4.30 (± 3.77)	2.77 (± 2.48)
Org. susp. solids (mg.L <sup>-1</sup> )	6.72 (± 4.95)	14.15 (± 10.05)	5.00 (± 1.89)	4.35 (± 3.45)
TP (mg.L <sup>-1</sup> )	7.02 (± 3.55)	5.59 (± 1.29)	7.21 (± 3.75)	4.6 (± 1.9)
TKN (mg.L <sup>-1</sup> )	15.94 (± 6.06)	18.58 (± 4.27)	13.03 (± 4.87)	7.79 (± 2.46)
Chl- <i>a</i> (mg.m <sup>-3</sup> )	1.73 (± 1.25)	4.88 (± 4.52)	1.22 (± 0.92)	1.89 (± 3.7)

**Table 2.** Mean, minimum and maximum density, biomass and specimen size, and egg number of *Boeckella poopoensis* in the four studied lakes. **Tabla 2.** Promedio, mínimos y máximos de densidad, biomasa y tamaños de los ejemplares y número de huevos de *Boeckella poopoensis* en los cuatro lagos estudiados.

		Cha	SJ	Ut	EC
<b>Adults + copepodites density (ind.L<sup>-1</sup>)</b>	<b>Mean</b>	<b>75.39</b>	<b>167.5</b>	<b>113.13</b>	<b>4.24</b>
	Min.-max.	2.1–182.3	30.3–460.3	16.0–251.7	0–26.3
<b>Adults + copepodites biomass (µg.L<sup>-1</sup>)</b>	<b>Mean</b>	<b>797.44</b>	<b>2240.56</b>	<b>1181.65</b>	<b>64.22</b>
	Min.-max.	15.9–1654.5	274.2–6757.7	208.0–2311.8	0–356.3
<b>Adults + copepodites size (µm)</b>	<b>Mean</b>	<b>1031.45</b>	<b>1174.2</b>	<b>1148.83</b>	<b>1052.9</b>
	Min.-max.	698.7–1297.2	931.8–1348.5	919.4–1376.7	635.3–1362.9
<b>Ovigerous females size (µm)</b>	<b>Mean</b>	1560.95	1802.12	1578.89	1910.00
	Min.-max.	1390.0–1677.5	1593.1–2010.0	1454.2–1642.5	1700.0–2130.0
<b>Nauplii density (ind.L<sup>-1</sup>)</b>	<b>Mean</b>	<b>49.87</b>	<b>162.86</b>	<b>216.58</b>	<b>81.94</b>
	Min.-max.	9.23–87.50	49.17–286.33	26.33–1013.33	35.00–153.00
<b>Nauplii biomass (µg.L<sup>-1</sup>)</b>	<b>Mean</b>	<b>42.13</b>	<b>136.46</b>	<b>194.68</b>	<b>63.45</b>
	Min.-max.	7.39–78.75	33.08–287.47	21.80–864.58	26.76–110.33
<b>Nauplii size (µm)</b>	<b>Mean</b>	<b>355.58</b>	<b>350.09</b>	<b>366.88</b>	<b>344.30</b>
	Min.-max.	202.0–514.8	200.2–529.1	214.2–557.4	171.6–500.5

The mean density and biomass of nauplii also differed ( $H = 17.99$ ;  $p = 0.00$  and  $H = 16.77$ ;  $p = 0.00$ , respectively) and were both higher in Ut (Table 2). The nauplii size differed ( $H = 11.38$ ;  $p = 0.01$ ) and was slightly larger in Ut. Correlations were only observed between the density and biomass of nauplii and salinity ( $H = 0.65$ ;  $p = 0.00$  and  $H = 0.62$ ;  $p = 0.00$ , respectively).

The density, biomass and size of adults and copepodites recorded during the annual cycle fluctuated differently between the lakes, although for three water bodies (Cha, SJ and Ut), lower densities and biomasses were registered during the autumn (April and May).

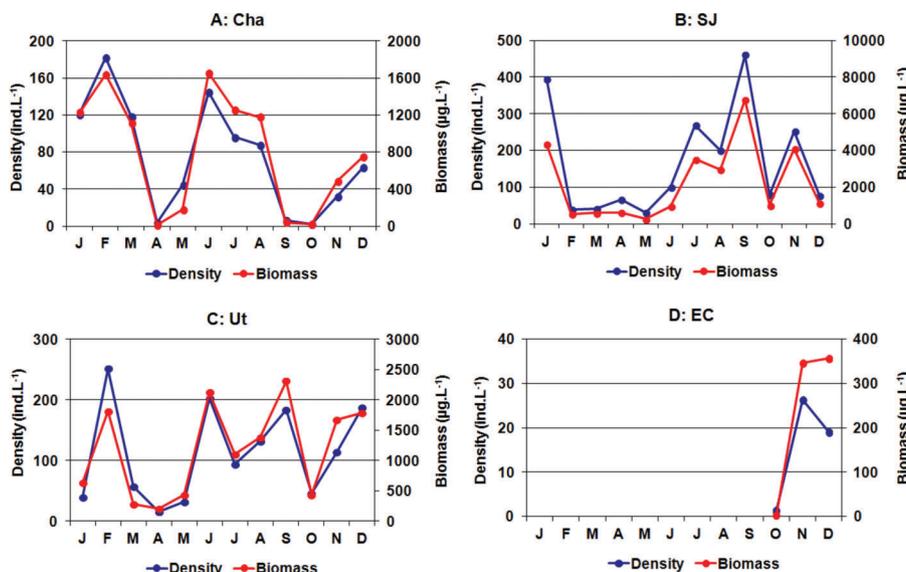
In Cha, two density and biomass peaks were recorded; the first occurred in summer (February) when the adults and copepodites reached a density of 182.3 ind.L<sup>-1</sup> and a biomass of 1,637.3 µg.L<sup>-1</sup>, although specimens of higher mean length (1,297 µm) were registered in November. The second peak occurred during the winter (June) and although the abundance was much lower (145 ind.L<sup>-1</sup>), the biomass was as high as

during the summer peak (1,654.4 µg.L<sup>-1</sup>), due to the presence of larger animals that reached a mean of 1,218 µm (Figure 2A).

In SJ, the adults and copepodites reached a maximum density and biomass (460.3 ind.L<sup>-1</sup> and 6,757.7 µg.L<sup>-1</sup>) in early spring (September). Despite the high biomass, the maximum sizes were observed in November (1,348.5 µm). A second peak was observed in January (395.3 ind.L<sup>-1</sup>), but the biomass was significantly lower than that in September, due to the presence of specimens with a smaller mean length (1,137.5 µm) (Figure 2B).

In Ut, density and biomass showed fluctuated greatly throughout the annual cycle. The highest density of 251.7 ind.L<sup>-1</sup> was recorded in summer (February), but the highest biomass, 2,312 µg.L<sup>-1</sup>, was observed in early spring (September), due to the presence of large specimens (Figure 2C).

Finally, in EC, where *B. poopoensis* was recorded from October, adults and copepodites reached a maximum density in November (26.3 ind.L<sup>-1</sup>), but because the monthly mean size increased and reached a maximum in December (1,363 µm), the greatest biomass was calculated in this month (356.3 µg.L<sup>-1</sup>) (Figure 2D).



**Figure 2.** Monthly variation of the density and biomass of adults and copepodites of *B. poopoensis* in the four studied lakes.

**Figura 2.** Variación mensual de la densidad y la biomasa de los adultos y copepodites de *B. poopoensis* en los cuatro lagos estudiados.

The fluctuations in nauplii density and biomass during the annual cycle also did not show a pattern that was common to all lakes, except for a slight decrease in spring in Cha, SJ and Ut (Figure 3). However, a strong summer peak of density and biomass was registered in Ut, which reached 1,013.33 ind.L<sup>-1</sup>. The biomass of nauplii followed an identical pattern to that of the density and was not influenced by size variations. Because the body length was slightly higher during winter, a correlation was found between size and the water temperature ( $r_s = -0.58$ ;  $p = 0.00$ ).

#### Ovigerous females: proportions, size and fecundity

The mean number of ovigerous females in each population differed ( $H = 10.41$ ;  $p = 0.02$ ) and the density in SJ (8.56 ind.L<sup>-1</sup>) was twice that in Cha (4.06 ind.L<sup>-1</sup>) and 16 times higher than that in EC (0.5 ind.L<sup>-1</sup>). However, the percentage of the total density in Ut, Cha and SJ was 1.90, 3.15 and 5.63%, respectively, and in EC was 14.01%, with a strong peak in October, when the ovigerous females represented 37.5% of the total density.

The ovigerous females recorded in Cha and Ut were similar in size but differed from those of EC and SJ ( $H = 20.2$ ;  $p = 0.00$ ) because the latter were larger, at about 1,800-1,900  $\mu\text{m}$  (Table 2). Significant differences were also found in the mean number of eggs per female ( $H = 24.02$ ;  $p = 0.00$ ) and in this case, the females of EC produced a much higher number of eggs, more than 20 per female (Table 2). No significant correlations were found between environmental variables and the characteristics of ovigerous females.

Although the size of ovigerous females was not significantly correlated with the water temperature, the size spectrum of females with eggs throughout the annual cycle (Figure 4), showed some seasonality, because during the warmer months, specimens tended to be smaller, and larger individuals were recorded in winter especially in SJ and Ut (Figure 4). The size of individuals in Ut remained relatively more constant than in the other lakes, as it varied by only 188  $\mu\text{m}$ , whereas in EC and SJ, the difference exceeded 410  $\mu\text{m}$  (Table 2).

The mean egg number per female was higher in larger specimens ( $r_s = 0.71$ ;  $p = 0.00$ ) and differed between lakes ( $H = 26.97$ ;  $p = 0.00$ ). The minimum number was observed in Ut, where the specimens showed a mean of only 2.45 ( $\pm 1.53$ ) eggs, compared with 21.67 ( $\pm 14.51$ ) eggs in EC (Figure 5).

The highest mean number of eggs per female, 37.3, was recorded in October in EC, and this number decreased to 8.8 in December, at the end of the study (Figure 6). In SJ, a higher mean number of eggs per female was recorded during the winter months, with a maximum of nine in May, whereas the number in the other lakes never exceeded five (Figure 6).

The size of the eggs produced by females throughout the annual cycle in the four lakes ranged between 100 and 230  $\mu\text{m}$ . Significant differences were found ( $F = 1,378$ ;  $p = 0.00$ ), since in EC smaller eggs were recorded in EC (mean: 112.92  $\pm 17.34$   $\mu\text{m}$ ), whereas in the other lakes, the egg diameter exceeded 160  $\mu\text{m}$ . Excluding EC, the mean egg size was not significantly different, since in the three lakes (Cha, SJ and Ut) smaller eggs were approximately 100–120  $\mu\text{m}$  and larger eggs were 220–230  $\mu\text{m}$  in size (Figure 7).

In order to compare the biology of *B. poopoensis* in a situation of relative stability against colonization at the beginning of a hydroperiod, a Principal Component Analysis (PCA) analysis was conducted for the three lakes in which the species was recorded throughout the year. The density, size and biomass of adults and copepodites were more influenced by component 1, which was particularly determined by chlorophyll-a and organic suspended solid concentrations (Figure 8), which implies the availability of food. In contrast, environmental conditions determined by salinity and especially by temperature (component 2) had a very limited influence on the density, biomass and the total size of adults and copepodites (Figure 8).

## Discussion

Throughout the study, the halophilic (De los Rios & Crespo 2004) and eurihaline character of *B. poopoensis* (Echaniz & Vignatti 2011, Echaniz et al. 2013) was evidenced, since it was recorded in a relatively wide range of salinities. The

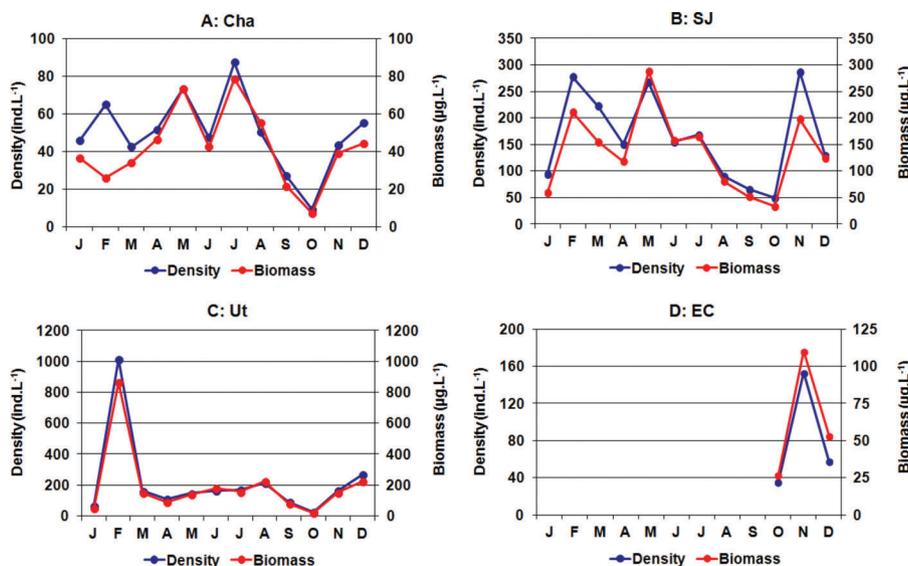
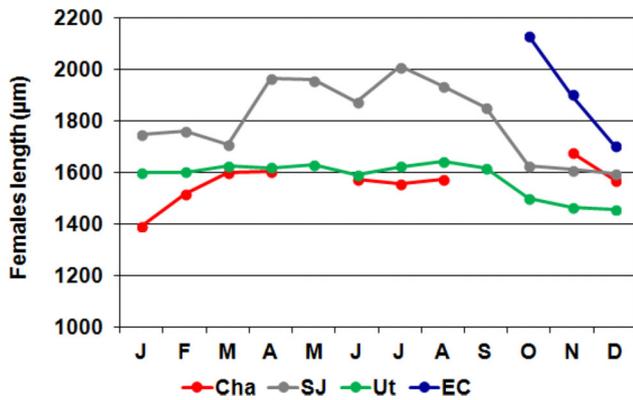


Figure 3. Monthly variation of the density and biomass of nauplii of *B. poopoensis* in the four studied lakes.

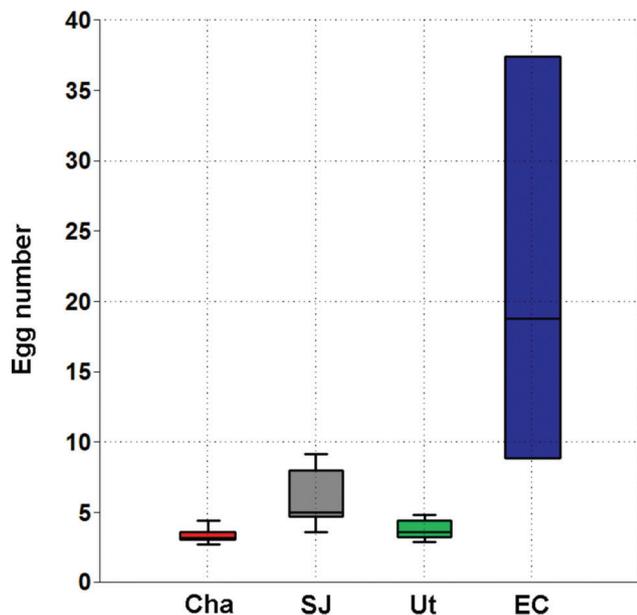
Figura 3. Variación mensual de la densidad y la biomasa de los nauplios de *B. poopoensis* en los cuatro lagos estudiados.



**Figure 4.** Monthly variation of the mean size of ovigerous females of *B. poopoensis* in the four studied lakes.

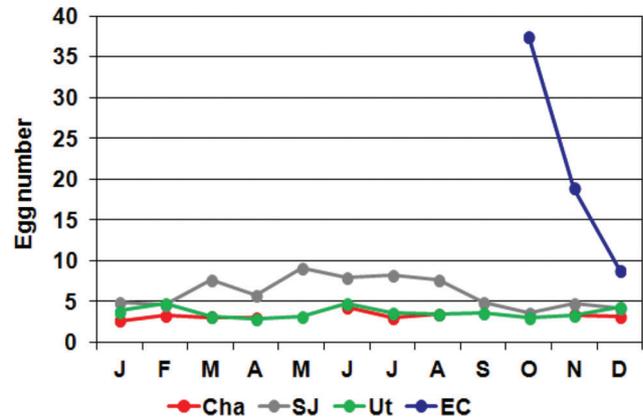
**Figura 4.** Variación mensual del tamaño medio de las hembras ovígeras de *B. poopoensis* en los cuatro lagos estudiados.

species was always present in three lakes (Cha, SJ and Ut), but was recorded only from October in EC. This might be because the salinity was relatively low until October, since the species appeared when the salinity exceeded  $15 \text{ g.L}^{-1}$ . It should be noted that the four studied shallow lakes are temporary and show wide variations in water level (Vignatti 2011, Echaniz et al. 2013), but during the study, salinity showed a relatively different behavior in the different lakes. Because Cha, SJ and Ut had contained water since 2001, the concentration of their dissolved solids was higher, so that they can be categorized as mesosaline lakes (Hammer 1986), and therefore, were relatively stable. The high salinity of the lakes probably prevented the establishment of other species that might compete and displace *B. poopoensis*, thus allowing it to thrive and maintain a stable population. However, in EC, the reverse situation was found; EC had remained completely dry since 2004 and in February



**Figure 5.** Mean egg number per ovigerous female of *B. poopoensis* in the four lakes studied.

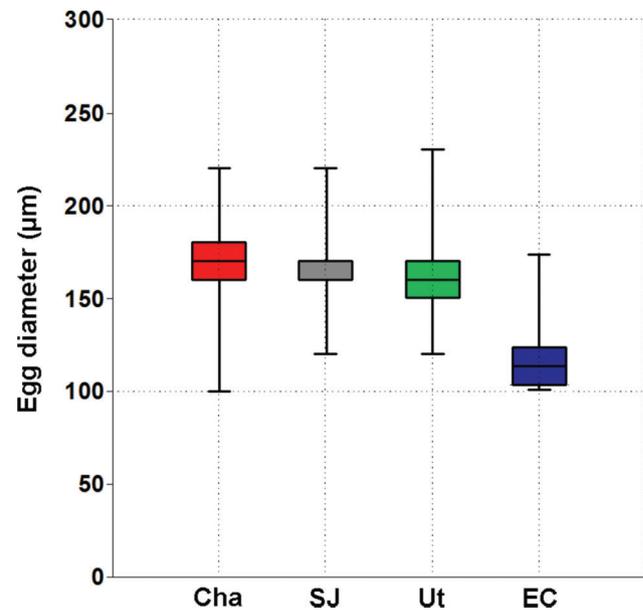
**Figura 5.** Número medio de huevos por hembra ovígera de *B. poopoensis* en los cuatro lagos estudiados



**Figure 6.** Variation in the number of eggs of *B. poopoensis* throughout the annual cycle in the four studied lakes.

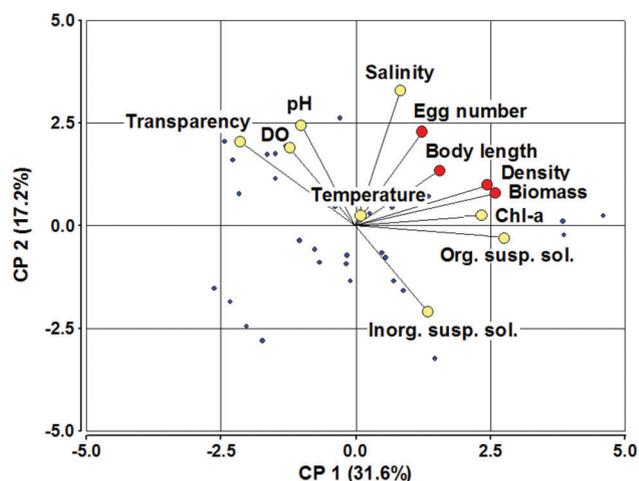
**Figura 6.** Variación en el número de huevos de *B. poopoensis* a través del ciclo anual en los cuatro lagos estudiados.

2007, it reached a depth of 1.6 m, due to torrential summer rain typical for the region (Cano 1980). Although the lake then became hyposaline (Hammer 1986), the redissolution of salts from sediments caused a three-fold increase in the saline concentration, without large fluctuations in the depth, and thus, the salinity reached the mesosaline level in the last month of the study (Vignatti et al. 2012). Salinity is an abiotic factor that can determine the zooplankton assemblage of South American aquatic ecosystems (Hurlbert et al. 1986, Williams et al. 1995, De los Ríos & Crespo 2004, Vignatti 2011, Battauz et al. 2013), and therefore, one reason why *B. poopoensis* was recorded as present from October onwards, might be due to the recorded presence of *Boeckella gracilis* until the previous month, which is a much less salinity-tolerant species but a potential competitor of *B. poopoensis*. In these environments,



**Figure 7.** Comparison of the size of the eggs of *B. poopoensis* throughout the annual cycle in the four studied lakes.

**Figura 7.** Comparación del tamaño de los huevos de *B. poopoensis* a través del ciclo anual en los cuatro lagos estudiados.



**Figure 8.** Biplot of the Principal Component Analysis, including only the three more stable lakes: Cha, SJ and Ut. Body length, density and biomass corresponds to that of adults and copepodites.

**Figura 8.** Resultados del Análisis de Componentes Principales que incluye sólo los tres lagos más estables: Cha, SJ and Ut. El largo del cuerpo, la densidad y la biomasa corresponden a los adultos y copepoditos.

which generate high physiological stress to organisms, species such as *B. poopoensis* might possess an adaptive advantage, since their ability to tolerate increased salinity allows them to find refuge against competition or predation by some fish (Herbst 2001, Santangelo et al. 2008), but not by flamingos (Battauz et al. 2013). Therefore, *B. poopoensis* could be considered a typical species for when temporary lakes have become relatively stable and not for the period of succession that occurs when a new hydroperiod begins. This conclusion is supported by the fact that *B. poopoensis* was observed at a low densities in EC, whereas its density in the remaining lakes was several times higher.

The analysis of the biology of *B. poopoensis* in a situation of relative hydrological stability found in the three lakes that had contained water for a long time, allowed the conclusion to be reached that the availability of food had a greater influence on the density, size and biomass of adults and copepodites than environmental conditions such as salinity or temperature. In the case of the size, this situation might be because the copepods have indirect development with many instars, which implies that they must allocate a significant amount of energy to reach maturity and reproduce. Since its growth in natural populations is mainly limited by the availability of food (Peterson & Hutchings 1995, Webber & Roff 1995, Havens et al. 2014), if the size is small, especially the ovigerous females, this might indicate a shortage of food resources and, on the contrary, a large size would be an indicator of better nutrition during development (Vignatti 2011). This could explain why larger ovigerous females were registered in SJ, the lake with greater availability of food, a situation similar to that reported by Lin et al. (2013).

Although the influence of water temperature on the size of epicontinental calanoid copepods continues to be discussed, it is thought to be very low, with the effect of predation being more important (Havens et al. 2014). In the three more stable lakes, water temperature had a very limited influence on the density, biomass and total size of adults and copepodites. The

low influence of salinity might be because, given the halophyllic nature of *B. poopoensis*, the parameter fluctuations that occurred in the three stable lakes were not sufficient to produce significant changes in the biological parameters measured. Despite the small influence of temperature on *B. poopoensis*, the greatest egg production occurred in the three more stable lakes during the winter, which was reflected by a slight increase in the number of nauplii. This agrees with the results of numerous laboratory studies with marine calanoid that report that fertility increases with decreasing water temperature (Landry 1978, Johnson 1980, Jiménez-Melero et al. 2005) or with data for *Sinodiaptomus (Rhinediaptomus) indicus*, a freshwater calanoid, in which ovisac and egg production decreases significantly with increasing temperature (Dilshad Begum et al. 2012).

It is known that a higher concentration of food in the environment produces more rapid postembryonic development, a larger size at maturity (Ban 1994) and increased egg production per female (Durbin et al. 1983, Kimmerer & McKinnon 1987, Peterson et al. 1991, Tourangeau & Runge 1991, McKinnon & Ayukai 1996), so that a greater number of eggs might indicate that females devote more energy to their production (Vignatti 2011). Considering the three most stable lakes, larger ovigerous females and more eggs per female were recorded in SJ, which might relate to the relatively greater availability of food in this lake. However, the number of eggs per female in EC was four times higher in the period following when the species was first recorded, allowing the efficiency of *B. poopoensis* to colonize an environment where was not previously present, to be monitored.

Considering that *B. poopoensis* was recorded in the province of La Pampa in lakes with salinities over  $40 \text{ gL}^{-1}$ , the concentration of dissolved solids observed in the lakes in this study was not a limiting factor for the growth and reproduction of *B. poopoensis*. Furthermore, this study allowed the different strategies that are used by a species that faces two different environmental situations to be analyzed in the hypo-mesosaline range (Hammer 1986). This situation is very frequent in saline temporary lakes, depending on the hydroperiod in which an ecosystem is found. If a lake is created by filling a basin with lowly mineralized water, a succession involving the replacement of species with a low tolerance to salinity by more tolerant species occurs, which include *B. poopoensis* (Vignatti 2011). When a species newly appears, the production of relatively small size eggs from females is very high, which allows the ecosystem to be rapidly colonized. However, once lakes have become relatively stable, which typically involves high salinities due to the redissolution of solutes from sediments, and in the presence of adequate amounts of food, *B. poopoensis* expends more energy in attaining a larger size, and although egg production per female is not so high, they are relatively large and allow a stable population to be maintained.

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## Fragment edge and isolation affect the food web: effects on the strength of interactions among trophic guilds

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**Abstract:** Habitat loss and fragmentation are processes that may affect communities by changing species interactions. These changes occur because the strength of linkages between species is not exclusively dependent on predator and prey traits. Species interaction changes also depend on the spatial context in which they take place. We used structural equation modelling to evaluate effects of these processes at patch-scale on top-down and bottom-up controls in food webs in Atlantic Forest. The model was composed of multiple species, and trophic guilds responded differently to fragment edge and isolation. Changes in bottom-up and top-down controls were mainly related to intermediate predator interactions. Efforts to restore connectivity among fragments should help recover the equilibrium of the trophic interactions by benefiting intermediate predators.

**Keywords:** *Atlantic Forest; bird; Brazil; connectivity; landscape; mammal; patch-scale; patch size; predator-prey interaction; trophic cascade.*

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**Resumo:** A perda e fragmentação de habitats podem afetar as comunidades através das mudanças nas interações entre espécies. Isso ocorre porque a força das ligações entre espécies não depende exclusivamente das características das espécies envolvidas, mas mudam dependendo do contexto espacial em que elas ocorrem. Usamos modelagem de equações estruturais para avaliar efeitos destes processos sobre o controle top-down e bottom-up nas teias alimentares em Mata Atlântica, na escala das manchas de habitats. O modelo foi composto com várias espécies. As guildas tróficas responderam de forma diferente ao efeito do isolamento e da borda dos fragmentos. Mudanças nos controles bottom-up e top-down foram principalmente relacionadas com as interações envolvendo os predadores intermediários. Esforços para restaurar a conectividade entre os fragmentos devem ajudar na recuperação do equilíbrio das relações tróficas, beneficiando predadores intermediários.

**Palavras-chave:** *aves; Brasil; cascata trófica; conectividade; escala ao nível da mancha; interação predador-presa; Mata Atlântica; mamíferos; paisagem; tamanho da mancha.*

### Introduction

Understanding how habitat loss and fragmentation affect the structure and dynamics of communities is vital for theoretical and applied conservation given the accelerated rate of deforestation around the world. Empirical evidence suggests that fragmentation and habitat loss affect communities by altering species interactions (Sinclair et al. 2003). Species interactions are disrupted because the strength of linkages between trophic levels is not exclusively dependent on predator and prey traits. They also change depending on

the spatial context in which the interactions take place (Morrison et al. 1992, Didham et al. 1996, Henle 2004, Terborgh et al. 2010). Thus, habitat fragmentation changes this context and consequently, modifies the interactions between species.

In addition, species in food webs do not respond uniformly to fragmentation. Many derived features of demography, optimal foraging, and life history have been suggested to influence sensitivity to fragmentation (Henle et al. 2004, Meyer et al. 2008). In this respect, top predators seem to be more vulnerable to fragmentation than other trophic guilds

(Pauly et al. 1998, Dobson et al. 2006), and several traits have been suggested to cause this pattern. First, top vertebrate predators are usually large and tend to require more resources (area and food), and consequently, they are more likely to have their habitat destroyed. Second, large predators (e.g. felines) are often in high conflict with humans, leading to purposeful hunting by people (Stewart 1985, Carey and Peeler 1995, Sick, 1997, Marks & Canning 1999). Lastly, large predators have low densities and more unstable population dynamics (Henle et al. 2004). Thus, the top levels are the first to lose species. The top-down cascade hypothesis was proposed as a possible mechanism to explain the consequences of the disappearance of top predators in communities (Crooks & Soulé 1999, Wangchuk 2004). The extinction of top predators may have overwhelming effects that propagate across more than one trophic link in a food web, resulting in inverse patterns in abundance or biomass in the trophic levels involved, e.g. an increase in smaller predators (mesopredators) and a decrease in primary consumers (Paine 1966, Holt & Loreau 2002, Ives et al. 2005). On the other hand, a bottom-up cascade describes the alteration in food-web components by the reduction of primary producers or the input of limiting nutrients to an ecosystem (Pace et al. 1999). Theoretically, the balance between top-down (predator acting as a major force in controlling community structure) and bottom up dynamics (primary producer or primary consumer acting as a major force in controlling community structure) explains the structure and function of the ecosystem (Frank et al. 2005).

Although the existence of trophic cascades is well accepted (Pace et al. 1999), their prevalence in terrestrial food webs is still debated. Previous studies suggest that trophic cascades may be restricted to less diverse communities (Strong 1992). Conversely, in highly diverse communities, food webs are diffuse, involving numerous species at both producer and consumer levels. Diffuse webs are redundant and create weak links between trophic levels, where the addition or removal of species results in minor adjustments elsewhere in the system and consequently, weak trophic cascades (Terborgh & Feeley 2010). However, recent empirical studies (Beschta & Ripple 2009, 2011) demonstrated the existence of strong trophic cascades in highly diverse communities.

However, critics pointed out that published examples of terrestrial trophic cascades generally involve smaller subsets of the food web (often single species per trophic level) when compared to cascades that occur in aquatic habitats (Nee et al. 1997, Bascompte & Sole 1998, Rushton et al. 2000, Nakagiri et al. 2001, Schneider 2001, Swihart et al. 2001, Prakash & De Roos 2002, Kondoh, 2003, Nakagiri & Tainaka 2004).

In our study, we used structural equation modelling (SEM) with multiple species at each level to evaluate the effect of forest fragmentation on top-down and bottom-up trophic cascades using a patch scale approach. This method allows the inclusion of interactions within food webs and the conditions for coexistence of species within trophic levels (Holt et al. 2010). Furthermore, SEM evaluates multiple direct and indirect pathways that operate simultaneously among trophic levels (Iriondo 2003). For a better understanding of the SEM process, two different types of causal relationships can be described: direct causal relationship, where one variable directly causes an effect on the other, and indirect causal relationship, where one variable causes an effect on another through a third variable.

We tested two hypotheses about how fragmentation and habitat loss affect the strength of trophic cascades in fragmented forest habitats by considering four patch metrics (i.e. area, shape complexity, connectivity, and isolation) that together describe the spatial attributes of individual patches in fragmented landscape. *Hypothesis 1: Area and isolation effects:* smaller and more isolated fragments are expected to harbour the lowest species diversities, due to population collapse arising from limited resources in small patches and impaired extinction rescue in isolated ones (Brown & Kodric-Brown 1977, Fahrig 2003, Ewers & Didham 2006). Thus, a lower diversity, indicated by fewer redundant links and less reticulate food webs, should strengthen trophic cascades. In addition, area and isolation are expected to differentially influence the different trophic levels. As a result, top predators are not expected to occur in small and isolated fragments (Schoener & Spiller 2010). *Hypothesis 2: Edge effect:* Fragments with complex shapes (i.e. a patch with irregular geometry) have a higher edge: core ratio (Ewers & Didham 2006). Additionally, shapes with a high complexity may decrease the amount of core habitat available. As a result, the top-down effect of specialist predators on their prey that live only in core habitats also decreases. These effects decrease the per capita effect of predators, i.e. the individual risk of predation decreases. However, the opposite can occur if generalist predators are incorporated in the food web. These predators are matrix tolerant or live primarily in the matrix, but readily occupy forested habitats.

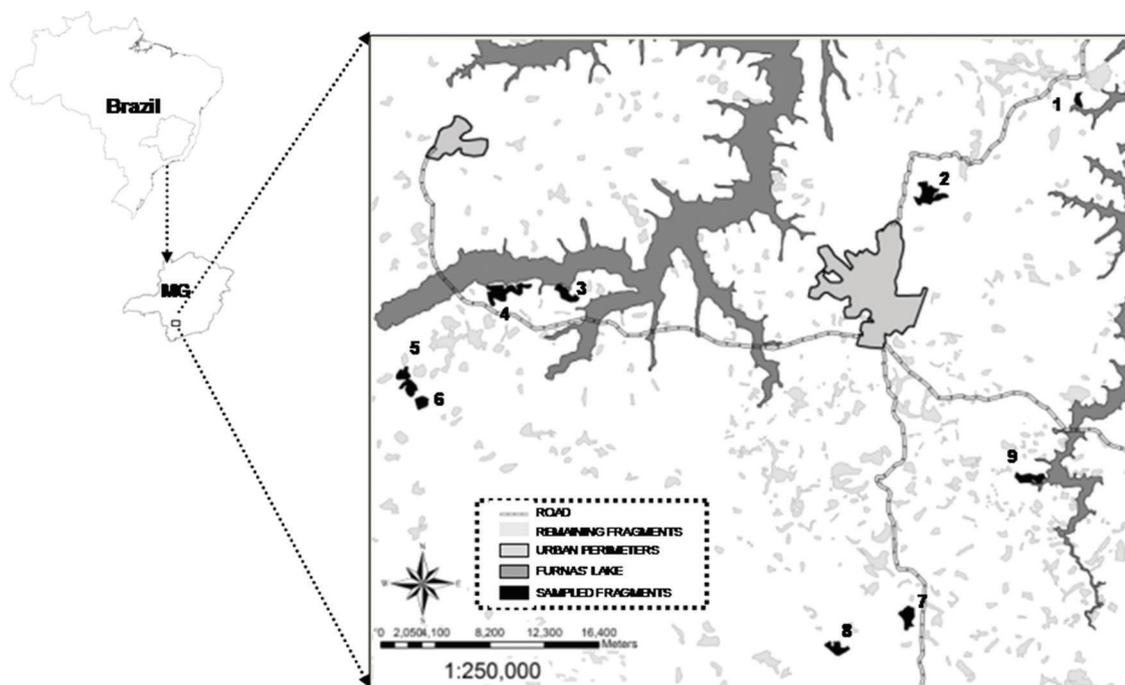
Specifically, we addressed the two following questions: i) What is the influence of patch metrics on food webs, considering both direct and indirect effects? ii) Is there evidence for trophic cascades in forest fragment habitats? If so, what patch metrics are most relevant in this process?

## Materials and methods

### 1 Study area and landscape analysis

We conducted this study in nine fragments of Atlantic Forest from January to November 2011 in Alfenas, Minas Gerais, south-eastern Brazil (21° 25' 48.03" S, 45° 56' 51.76" W; 880 m a.s.l.; Figure 1, Appendix 1). The average annual temperature and relative humidity are 23°C and 70%, respectively with an evenly distributed annual rainfall averaging 1600 mm/year. The original vegetation is classified as seasonal semideciduous forest. (Drummond et al. 2005, Martins et al. 2006). However, this vegetation has been drastically reduced in this region (3% forest cover, Capobianco 2001) and consist of small and sparse fragments at several successional stages (97% of fragments are < 50 ha, Hasui E. unpublished data). The landscape matrix is mainly composed of coffee plantations, but also has pastures and plantations growing sugar cane, limens, tangerines, garlic, bananas, tomatoes, potatoes, and rice (Fundação SOS Mata Atlântica & Instituto Nacional de Pesquisas Espaciais 2010, IBGE 2010).

In order to analyse the patch metrics, we conducted a preliminary analysis to select fragments for sampling. We digitally processed multispectral images generated by the CCD sensor of the CBERS-2B satellite, which has a 20 m resolution. We identified old growth forest fragments and water bodies within a 30 km radius of Alfenas, using the spectral mixture model satellite (Souza 2006). This model expresses the amount of shade, soil, green vegetation, and nonphotosynthetic vegetation within each



**Figure 1.** Location of fragments studied (dark coloured) in the surroundings of Alfenas, Minas Gerais (MG). The inset shows the fragmented landscape with the sampled fragments in black.

pixel of the image and enables the separation of forest and non-forest pixels (i.e. pasture, plantation, and water classes). Among forests, it is also possible to discriminate forest regeneration and secondary forests based on the structure, texture and colour of the pixel sets belonging to each patch. Then, we calculated the following patch metrics using the Fragstats program: AREA, SHAPE, ENN, and PROX (McGarigal et al. 2002).

**AREA** (Area) equals the patch area ( $m^2$ ), converted to hectares.

**SHAPE** (Shape Index) is calculated from the ratio of the perimeter to area of the patch. The perimeter for the most compact patch of the same patch area has a circular form, and the SHAPE index is equal to one. This value increases without limit as the shape becomes more complex.

**ENN** (Euclidean Nearest-Neighbour Distance) is defined as the shortest straight-line distance (in meters) between the focal patch and its nearest neighbour patch. This nearest neighbour was not sampled, to guarantee the independence of sampled data. The index varies from zero to infinity. High values indicate isolated patches.

**PROX** (Proximity Index) also quantifies patch connectivity. However, this index considers the size and proximity of all patches within a specified search radius (i.e. 1000 m in our study). If the focal patch has no neighbours of the same type within the radius, PROX equals zero, increasing as patches become closer and more contiguous.

For fieldwork and further analysis, we selected nine fragments (Appendix 1) that were at least 2 km distant from each other that had similar altitude, water availability, and were at a similar successional stage (i.e. were old growth forests). By choosing fragments with these characteristics, we hoped to eliminate possible effects of water availability and successional stages on species richness. Where possible, we chose patches that increased the range of patch metrics in the sample design.

## 2 Sampling methods and statistical analysis

We used a standardised sampling protocol in the nine fragments, taking the same number of samples during the same period (from January to November 2011) regardless of patch metrics. We used three methods to sample species: 1) pitfall traps to assess rodent biomass, 2) playbacks to measure owl species richness, and 3) interviews of local residents to determine species richness of medium and large mammals.

Rodents were collected using five pitfall traps (Corn 1994) arranged along a single line transect, spaced at 30 m intervals between each other and 50 m from the edge of the fragment. Each trap consisted of four 30 l buckets buried to ground level and connected by a 4 m fence guide (50 cm high). We collected samples from January to March of 2011, leaving the buckets open for 15 continuous days and checking them daily. We weighed rodents and noted where they were captured, but we did not identify them to the species level. Each specimen collected was euthanized and subsequently frozen.

We obtained owl species richness estimates using playbacks (Boscolo et al. 2006) and visual identification of species whenever possible. Eight owl species, known to occur in the study area (Sigrist 2006), were selected a priori: *Glaucidium minutissimum*, *Glaucidium brasilianum*, *Strix virgata*, *Strix hylophila*, *Pulsatrix koeniswaldiana*, *Bubo virginianus*, *Athene cucularia*, and *Megascops choliba*. They inhabit forested habitat (Amaral 2007), but the degree of forest dependence varies between species. We classified the owls as medium-sized predators, because they mostly eat small mammals, and occasionally other vertebrates and/or insects (Amaral 2007). Furthermore, they respond very well to playback of conspecific vocalisations (Galeotti and Pavan 1993). Playback sequences consisted of a 1 min of vocalisation, followed by a 2 min interval of silence, and then a repeat of the playback if an owl responded so as to identify the species with higher certainty (Johnson et al. 1981). Vocalisations were played

in increasing order of species size to reduce interference by larger owls with smaller ones. Vocalisations were played between 6:00p.m. and 10:00p.m., when owls are most active. Playbacks were played at three points along the transect in each fragment, at a minimum distance of 50 m from the edge and 300 m from each other to ensure sample independence. Playbacks were conducted twice (Bibby et al. 1992).

We obtained estimates of mammal species richness using interviews that were composed of open questions about mammal species occupancy in fragments, accompanied by species pictures (Michalski & Peres 2005). We divided the species into two classes based on prey weight consumption: medium- (typically weighing < 1 kg) and large (mostly 1 to 10 kg) (Konecny 1989, Costa et al. 2004, Gatti et al. 2006, Moreno et al. 2006, Azevedo & Murray 2007). To assess the frequency of type I or type II errors in the interviews, local informants were asked to identify which species were present in the patch from a selection of mammal pictures, including species known not to occur in the study area as a negative control. We obtained estimates of mammal species richness using interviews, five per fragment, that were usually long-term workers or landowners familiar with mammals inhabiting fragments.

For statistical analysis, we used structural equation modelling (program AMOS 5.0, Arbuckle 2003) to test hypotheses about the influences of patch metrics on food webs. We used this test because it allows one to test a set of regression equations simultaneously. The individual causal mechanisms between predators and their prey were represented in a path diagram (Didham et al. 1996).

We tested four hypotheses based on patch metrics (area, shape, connectivity, and isolation) that potentially explain top-down and bottom-up controls in predator-prey relationships.

1. Patch size (AREA). Larger predators only occupy large fragments and are expected to decrease the impact of mesopredators (Henle et al. 2004). In contrast, large predators would be rare in smaller fragments due to reduced carrying capacity (Terborgh et al. 2010). As a result, we expected a stronger trophic cascade effect of top-predator reduction in smaller fragments.
2. Shape complexity (SHAPE). We hypothesised that fragments with more complex shape are more prone to edge effects, which increase the loss of top predators restricted to forested areas. On the other hand, species that are not exclusively from forest habitat, using the matrix as a second habitat, are benefited. The reason is that, with the absence of top predators, they are released from predation control and are not negatively affected by edge effects (Laurence & Bierregard 1997).
3. Connectivity (PROX). We expected that top predators' vulnerability to extinction increases in less connected fragments (Michalski & Peres 2005). Thus, more connected fragments should have more reticulated food webs and less evidence of trophic cascades.
4. Isolation (ENN). Isolation affects metapopulation source-sink dynamics, as the increase of isolation stops the movement of species between habitats. This should destabilise the trophic dynamics, as the diversity of each level will be poor as will the interaction between them (Holt 1984). Thus, we expected lower species richness in isolated fragments and consequently, increased evidence of trophic cascades (Schoener & Spiller 2010).

We log-transformed the variables to obtain data homoscedasticity before performing further statistical analyses. All model parameters were estimated using maximum likelihood and the models were compared with the Akaike Information Criterion (AIC). Models with  $\Delta AIC > 2$  indicated differences in likelihood. We also used chi-squared likelihood tests to assess the robustness of models. A non-significant result in this test is equivalent to an appropriate model, i.e. no evidence to reject the null hypothesis that the proposed model is adequate. However, it is generally recommended that the chi-squared test be interpreted with caution and complemented with other goodness-of-fit measures, when data depart from multivariate normality and sample sizes are small (Bollen 1989, Loehlin 1992, Bollen & Long 1993). Therefore, we also calculated the Bentler and Bonett's normed-fit index (NFI), Bentler's comparative fit index (CFI), and/or the Goodness of Fit index (GFI). Values of GFI range from 0 to 1, and those higher than 0.9 indicate an acceptable fit (Bollen 1989).

The intensity of the relationships tested are given by P values (significant or not) as well as the coefficients that accompany every relationship established in the analysis. The higher the coefficient the greater the influence of one variable on the other.

## Results

We found four owl species: *Strix virgata*, *Pulsatrix koeniswaldiana*, *Athene cunicularia*, and *Megascops choliba*. Two of them are specialists (*Strix virgata* and *Pulsatrix koeniswaldiana*), restricted to forested habitats, but both were found in only one fragment. Due to their low abundance, we excluded them from further analysis. So, we just included in further analysis the species that also occur in altered environments, such as at the forest edge, and with low environmental sensitivity (Appendix 2). We found seven medium-large mammal species at different trophic levels: three top predators and four intermediate predators (Appendix 3).

All models seemed to be appropriate and none could be rejected (Tables 1 and 2). However, considering goodness-of-fit, half the models were below 0.9, suggesting that they miss important predictors and/or interaction pathways. Thus, patch metrics appeared to be influencing bottom-up control in the food web, through the intrinsic characteristics of the fragments (Figure 2). The most robust models with bottom-up control supported the hypotheses of isolation (ENN) and edge influence (SHAPE), but these effects were different among trophic guilds (Tables 1 and 3, Figure 2). Isolation positively affected the lowest trophic level (rodents, Appendix 4) and negatively affected intermediate and top predators (mammals, Appendix 3). Species richness of generalist owl predators (Appendix 2), which live primarily in the matrix, but can also occur in forested habitats, increased with the edge length/complexity.

The strength of bottom-up dynamics was affected by isolation. There was a higher biomass of the lowest trophic level (rodents) in more isolated fragments, positively influencing the level above. However, this effect was not propagated up to the third level (Figure 2).

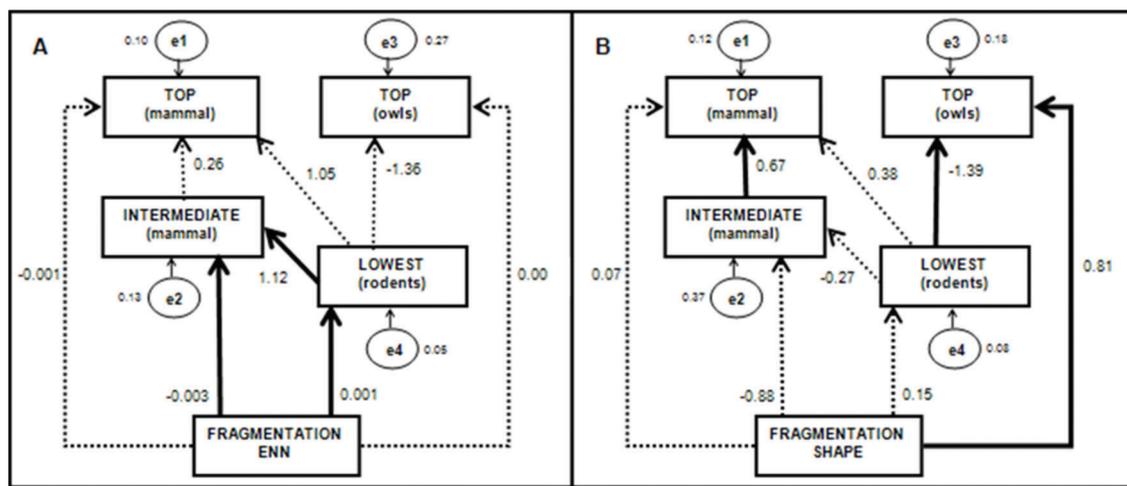
Unlike bottom-up effects, patch characteristics had a weak influence on top-down processes. The best models were composed of connectivity and shape, but the direct effects of these variables on trophic guilds were not significant (Table 2 and 4, Figure 3). Nevertheless, the positive effect of top

**Table 1.** Structural equation models for food webs with bottom-up control, ranked from best to worst according to Akaike’s information criterion (AIC).  $\Delta$  AIC represents the difference in AIC from one model to the one with the lowest AIC value. Path diagrams for the best two models (edge effect and isolation influence) are shown in Figure 2. A chi-squared value with  $P > 0.05$  means acceptable fit. Goodness-of-Fit index (GFI)  $> 0.9$  indicates an acceptable fit of the model to the data.

HYPOTHESIS OF <i>BOTTOM-UP</i> CONTROL	AIC	$\Delta$ AIC	GFI	Chi-squared	P
Edge effect (SHAPE)	26.671	0	0.969	0.671	0.715
Isolation influence (ENN)	26.980	0.309	0.956	0.980	0.613
Patch size influence (AREA)	28.762	2.091	0.895	2.762	0.251
Connectivity influence (PROX)	29.974	3.303	0.865	3.974	0.137

**Table 2.** Structural equation models for food webs with top-down control.  $\Delta$ AIC is the difference between one model to the model with the lowest AIC value. Path diagrams for the best two models (edge effect and connectivity influence) are shown in Figure 3. A chi-squared value with  $P > 0.05$  means acceptable fit. Goodness of Fit index (GFI)  $> 0.9$  indicates an acceptable fit of the model to the data.

HYPOTHESIS OF <i>TOP-DOWN</i> CONTROL	AIC	$\Delta$ AIC	GFI	Chi-squared	P
Edge effect (SHAPE)	27.519	0	0.935	1.519	0.468
Connectivity influence (PROX)	28.480	0.961	0.904	2.480	0.289
Patch size influence (AREA)	28.865	1.346	0.893	2.865	0.239
Isolation influence (ENN)	29.229	1.710	0.883	3.229	0.199



**Figure 2.** Bottom-up diagram representing the direct and indirect effects of patch metrics on the structure of food webs. Circles indicate error from other factors not considered (e1-e4). Continuous and discontinuous arrows connecting the boxes show significant ( $P > 0.05$ ) and not significant causal effects, respectively. The numbers next to the arrows indicate correlation values between variables through unstandardized coefficients of path analysis. (A) Direct and indirect effects of the model with bottom-up control on the connectivity of fragments. (B) Direct and indirect effects of the model with bottom-up control on the shape of fragments.

predators on the lower level suggests that the absence of top predators was benefiting the intermediate levels (mammals) and the hypotheses of top-down control of the lowest level (rodents) are supported by the intermediate levels (mammals) and owl predators, which had a negative effect on prey biomass.

### Discussion

Our results suggest that only bottom-up dynamics greatly differ in fragmented landscapes. The biomass of the lowest level (rodents) was higher in more isolated fragments, resulting in positive effects in the levels above. However, this effect was not propagated up through the third level, probably because each

trophic guild responded differently to isolation, with intermediate predators being more vulnerable to this factor. Thus, the loss of intermediate species in highly isolated fragments may influence the upper level by reducing prey availability.

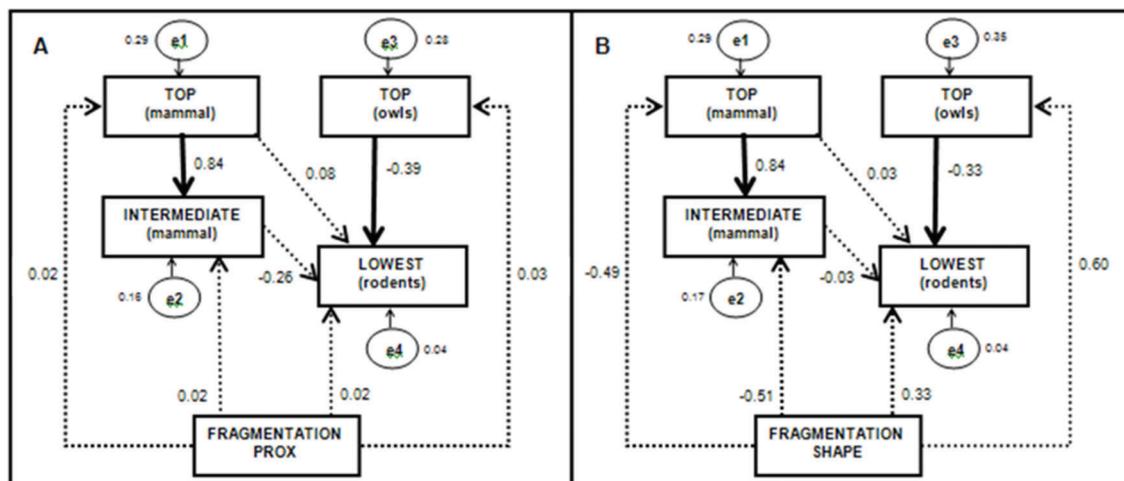
There are several processes that may decrease the richness of intermediate predators with increasing isolation. Since distance acts as a filter, more isolated fragments are less likely to receive immigrants from the nearby patch(es) (Fahrig 2013). In addition, isolation can also affect extinction rates. Populations in fragments near others are less prone to extinction because individuals from other fragments can supply the population with individuals from source habitats (“rescue effect”, Brown and Kodric-Brown 1977, see also Wolfe et al. 2015). Thus, lower species richness in isolated fragments is often attributed to lower immigration and higher extinction

**Table 3.** Influence of edge effect (SHAPE) and isolation (ENN) on each trophic level with bottom-up control. Unstandardized path coefficients are listed for the direct effects of each metric. Unstandardized indirect effects are the products of path coefficients for variables (each trophic level). Total effects are the sum of direct and indirect effects.

	TROPHIC LEVEL	DIRECT	INDIRECT	TOTAL
ENN	Top (large-sized mammals)	-0.001	0	-0.001
	Top (owls)	0	-0.001	-0.001
	Intermediate (medium-sized mammals)	-0.003	0.001	-0.002
	Lowest (rodents)	0.001	0	0.001
SHAPE	Top (large-sized mammals)	0.066	-0.555	-0.489
	Top (owls)	0.806	-0.202	0.604
	Intermediate (medium-sized mammals)	-0.877	-0.039	-0.915

**Table 4.** Influence of edge effect (SHAPE) and connectivity (PROX) on each trophic level with top-down control. Unstandardized path coefficients are listed for direct effects for each metric. Unstandardized indirect effects are the products of path coefficients for variables (each trophic level). Total effects are the sum of direct and indirect effects.

	TROPHIC LEVEL	DIRECT	INDIRECT	TOTAL
PROX	Top (large-sized mammals)	0.016	0	0.016
	Top (owls)	0.033	0	0.033
	Intermediate (medium-sized mammals)	0.021	0.013	0.034
	Lowest (rodents)	0.015	-0.021	-0.006
SHAPE	Top (large-sized mammals)	-0.489	0	-0.489
	Top (owls)	0.604	0	0.604
	Intermediate (medium-sized mammals)	-0.505	-0.41	-0.915
	Lowest (rodents)	0.328	-0.182	0.145



**Figure 3.** Top-down diagram representing the direct and indirect effects of patch metrics on the structure of food webs. Circles indicate error form other factors not considered (e1-e4). Continuous and discontinuous arrows connecting the boxes show significant ( $P > 0.05$ ) and not significant causal effects, respectively. The numbers next to the arrows indicate correlation values between variables through unstandardized coefficients of path analysis. (A) Direct and indirect effects of the model with top-down control on the connectivity of fragments. (B) Direct and indirect effects of the model with top-down control on the shape of fragments.

rates. However, it does not explain the positive or lack of responses of top predators (mammals and owls) to isolation. The literature is also full of these conflicting results (see review in Ewers & Didham 2006) and the reason may be related to the interspecific difference in their dispersal ability in the matrix or habitat preferences. For instance, in our study, most owl species sampled in fragments (e.g. *Athene cucularia* and

*Megascops choliba*) are matrix-based (i.e. live primarily in the matrix, but can also occur in the forest). These species are completely independent of, or respond positively to habitat loss and fragmentation *per se*, but cause considerable mortality on their prey (rodents; Ryall & Fahrig 2006).

From a trophic perspective, some of the persistent species in isolated fragments were imbalanced, and others, such as forest

dependent owls, were probably missing. This suggests a lack of linkages in the food web or a change in the strength of bottom-up and top-down dynamics. Therefore, isolated fragments may be less efficient or productive than habitats with higher species richness. Furthermore, reductions in species richness can weaken interspecific actions (e.g. predation or herbivory), which would in turn weaken trophic dynamics (Otto et al. 2008).

While the loss of linkages can explain an overall weakening in trophic dynamics, it does not explain the distinct response of bottom-up and top-down dynamics to isolation. We found that bottom-up dynamics were mediated by isolation, while top-down dynamics remained unchanged: top predators were not affected (mammals) or benefited (owls) by isolation and fragment edge. Top predators maintained consumption and strengthened top-down control of the level below. However, this was not propagated to the lowest level, because intermediate predators are sensitive to high isolation (Virgós & García 2002). Predation pressure is directly influenced by predator richness, with lower richness leading to lower predation pressure (Charnov et al. 1976). In this situation, rodent populations could have higher growth rates, since they have partly escaped from predation pressure and in turn, from predator control. In addition, rodents have short life cycles and can keep stable populations even in small patch sizes or in edge habitats (Jordão et al. 2010). Thus, rodents would expand until self-regulation because of resource constraints. However, self-regulation is improbable, because rodents can also use resources from the matrix (McInville & Keith 1974, Crooks & Soulé 1999, Wangchuk 2004).

The absence of evidence for trophic cascades in forest fragments in our study is in disagreement with Terborgh & Feeley (2010), who found that top predators were entirely absent from smaller islands. Nevertheless, from the functional perspective, species persistence in fragmented habitat is also imbalanced: there is a higher biomass of rodents, lower richness of intermediate mammal predators, and higher richness of generalist owl predators (matrix-based). These alterations could have influenced bottom-up and top-down controls, mainly related to intermediate predator linkages. Under the weakened top-down control in rodents, vegetation should eventually change in composition and/or structure as a consequence of over-consumption of seeds, making plant recruitment in these fragments severely deficient (Terborgh & Feeley 2010). Conversely, the weakened bottom-up control between intermediate and top predators should lead to top predators avoiding more isolated fragments, not because they are unable to colonise them, but because their prey are scarce

there. One possible implication is that scarce energy can limit the number of trophic levels or the number of species within each trophic level (Zanden et al. 2006).

Linkages between basic food web research and restoration are weak. Nonetheless, they should be effective in nudging ecosystems toward a desired state, in maximising ecosystem services and supporting biodiversity (Dobson et al. 1997). Identifying the main cause of disturbances in predator-prey interactions can help in interventions by removing or managing impacts. Thus, efforts to restore the connectivity between isolated fragments should restore the equilibrium of trophic linkages by benefiting intermediate predators (Clewel et al. 2004, Zanden et al. 2006).

These results concerning the impact of habitat loss and fragmentation on trophic interactions had limitations because our study design was restricted to patch level. There are still many facets in the knowledge that remain unclear. Edge effect is one of them. The matrix-based owl species found in patches may be the result of the increase of the edge in the fragment per unit of core area. This can boost incursion of generalist predators into the fragment. Typically, top predators have higher dispersal ability than intermediate species (Konecny 1989). Thus, top predators can easily predate opportunistically, without being limited or regulated by the abundance of their prey within a single fragment (Marsh & Trenham 2001, Virgós & García 2002, Terborgh et al. 2010).

We also do not know whether the matrix structure obscured the patch size effect on trophic interactions (Cook et al. 2002) or whether it was because of the interference of the area by the nearby patch(es) (Fahrig 2013). To resolve these doubts, we recommend a landscape-scale approach in future research, with the inclusion of matrix structure and habitat amount in the analysis. Over larger spatial scales (Pardini et al. 2010), the absence of patch size effects can also be related to the high level of deforestation in our study area (3% forest cover). To support this explanation, future studies should examine this relationship between patch size and trophic interactions along the regional gradient of landscape degradation.

In summary, fragment isolation and edge complexity interfere directly with trophic interactions by changing species richness and biomass within each trophic level, and also by changing the strength of trophic linkages. Contrary to our expectations, a species' vulnerability to patch features was not related to body size or trophic position. Regardless of the absence of trophic cascades, food webs in more isolated fragments had missing or weakened trophic linkages and need to be restored.

**Appendix 1.** Patch metrics for nine forest fragments sampled in Alfenas, Minas Gerais, south-eastern Brazil. SAD69: geodetic reference system. UTM: System that describes spatial positions using distance units. Zone 23K: latitudinal zone in which the study area lies.

FRAGMENT	SAD69/ UTM Zone 23K	AREA (ha)	SHAPE	PROX	ENN (m)
1	411100.70 - 7639788.51	15.30	1.47	5.27	361.25
2	402977.76 - 7615160.99	81.54	2.12	0.00	1068.27
3	383754.52 - 7630631.59	42.93	1.71	1.79	296.99
4	379121.94 - 7626339.45	96.03	2.46	1.66	424.26
5	379823.41 - 7625366.80	63.54	2.17	32.63	94.87
6	399598.25 - 7613649.98	27.72	1.22	16.16	201.25
7	403980.90 - 7635268.39	49.68	1.47	2.23	276.59
8	386710.82 - 7630526.75	38.43	1.81	0.88	496.59
9	408792.94 - 7621760.03	47.00	1.87	1.28	268.38

Appendix 2. Sensitivity, body size, and occurrence of the studied owl species sampled in each fragment.

SPECIES	SENSITIVITY/HABITAT									
	(Stotz et al. 1996)			BODY SIZE (cm)			FRAGMENT			
	1	2	3	4	5	6	7	8	9	
<i>Glaucidium minutissimum</i> (Wied, 1830)						13	0	0	0	0
<i>Glaucidium brasiliatum</i> (Gmelin, 1788)						17	0	0	0	0
<i>Strix virgata</i> (Cassin, 1849)						35	0	0	0	0
<i>Strix hylophila</i> (Temminck, 1825)						36	0	0	0	0
<i>Pulsatrix koeniswaldiana</i> (Bertoni & Bertoni, 1901)						42	0	0	0	0
<i>Bubo virginianus</i> (Gmelin, 1788)						55	0	0	0	0
<i>Athene cucularia</i> (Molina, 1782)						23	0	0	0	0
<i>Megascops choliba</i> (Vieillot, 1817)						20-24	0	1	1	1

Appendix 3. Frequency of occurrence of species in fragments studied obtained by interview.

SPECIES	TROPIC LEVEL								
	1	2	3	4	5	6	7	8	9
<i>Panthera onca</i> (Linnaeus, 1758)	0.6	0	0	0	0.6	0.6	0	0	0
<i>Puma concolor</i> (Linnaeus, 1771)	0.8	0	0.4	0.4	0	0	0	0	0.4
<i>Leopardus pardalis</i> (Lineu, 1758)	0.8	0	0.6	0.6	0.6	0.6	0.6	0.6	0.8
<i>Puma yagouaroundi</i> (Geoffroy, 1803)	0	0	0	0	0.6	0.6	0	0	0
<i>Cerdocyon thous</i> (Linnaeus, 1766)	0.8	0	0.4	0.4	0.8	0.8	0.8	0.8	0.6
<i>Nasua nasua</i> (Linnaeus, 1766)	1.0	0	0.4	0.4	0.6	0.6	0.6	0.6	0.4
<i>Procyon cancrivorus</i> (Cuvier, 1798)	0.6	0	0	0	0	0	0	0	0

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## Life-history features of a rapids-dwelling loricariid catfish from Atlantic forest streams, Brazil

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**Abstract:** Spatial distribution, suitable spawning sites, and sexual dimorphism were investigated in armored catfish *Neoplecostomus microps* in the Macaé River from March 2004 to March 2005. Individuals of *N. microps* (n=290) were limited to fast-flowing stretches, and the distribution was related to ontogenetic development. Larvae in post-flexion occurred in the marginal vegetation, and adults only in gaps between boulders and stones in the river channel. Juveniles occurred in both environments. Spawns were found in the natural habitat in rapid stretches, with the eggs attached to the lower surface of stones in openings formed in the riverbed. The number of eggs per stone ranged from 62 to 375, with significant differences among the mean sizes of the eggs. Sexual dimorphism was noticed to *N. microps*. The anal and urogenital papillae are separated in males and merged in females and there is an epidermal growth along the pectoral-fin spine of males.

**Keywords:** *Neoplecostomus microps*, Loricariidae, distribution, spawn, sexual dimorphism.

BRITO, M.F.G., LAZZAROTTO, H., CARAMASCHI, E.P. **Atributos da história de vida de um cascudo de corredeiras de riachos de Mata Atlântica, Brasil.** *Biota Neotropica*. 16(2): e20150068. <http://dx.doi.org/10.1590/1676-0611-BN-2015-0068>

**Resumo:** Distribuição espacial, locais favoráveis à desova e dimorfismo sexual foram estudados no cascudo *Neoplecostomus microps* no rio Macaé de março 2004 a março 2005. Espécimes de *N. microps* estiveram restritos a trechos de águas rápidas, e sua distribuição foi relacionada ao desenvolvimento ontogenético. Larvas em pós-flexão ocorreram somente na vegetação marginal e adultos somente em frestas entre rochas e pedras no canal do rio. Juvenis ocorreram em ambos ambientes. Desovas foram encontradas no ambiente natural em trechos de corredeira, com os ovos aderidos a superfície ventral de rochas em aberturas formadas com o leito do rio. O número de ovos/pedra variou de 62 a 375, com diferenças significativas entre os valores médios dos ovos. Dimorfismo sexual foi verificado em *N. microps*. As papilas anal e urogenital são separadas em machos e colabadas em fêmeas, e há uma projeção epidérmica na nadadeira peitoral de machos.

**Palavras-chave:** *Neoplecostomus microps*, Loricariidae, distribuição, desova, dimorfismo sexual.

### Introduction

For fishes that inhabit regions of river rapids, the current speed and the complexity of the flow around obstacles and close to the riverbed are some of the most important factors affecting their distribution (Vannote et al. 1980, Allan 1995, Matthews 1998). One of the most successful groups occupying fast-flowing habitats in the Neotropical region is the family Loricariidae (Reis et al. 2003). They show several adaptations for moving in strong current or preventing them from being washed away. In some species, the elongated pectoral fins help to keep the fish fixed in place by friction, also acting as a hydrofoil, using the strength of the current to hold the fish against the substrate (Webb 1989).

Their suckermouth functions as an organ of adherence, fixing the fish to the substrate and avoiding drifting (Hora 1930). The choice of a favorable breeding site in this type of environment requires caution, because the spawn must be protected from the water flow, and, in some cases, by means of parental care (Balon 1975).

These features are recognized in several species of loricariids, including the genus *Neoplecostomus*, small-sized rapid-dwelling fishes with little-known life histories (Ferraris-Jr. 2003). The genus is currently represented by 14 species (Andrade & Langeani 2014). The armored catfish *Neoplecostomus microps* (Steindachner 1877) occurs in the Paraíba do Sul River basin and adjacent coastal rivers (Langeani 1990,

Buckup 2007) in southeastern Brazil. Information about the diet and reproductive dynamics of *N. microps* in mountain-streams of the upper Paraíba do Sul River basin was presented by Braga et al. (2008). However, these authors did not report information on the spatial distribution and spawning sites. In the present study, we present the first records of *N. microps* spawns in natural habitats and describe sexual dimorphic characters of this species. The patterns of spatial distribution of the different-sized individuals in a mesohabitat and microhabitat scales are also described and discussed.

## Material and Methods

The Macaé River is a seventh-order river located in northern Rio de Janeiro State, southeastern Brazil (22°21' 22°28' S; 42°27' 42°35' W). The basin of this river lies within the region with the highest rate of endemism for fishes of the Atlantic Forest (MMA 2000), and is one of the largest drainages in the State of Rio de Janeiro, draining directly into the Atlantic Ocean from altitudes up to 1,700 m. The river channel at upper and middle stretches of the Macaé River and its tributaries is characterized by sequences of pools and riffles. The streambed consisted of stones and boulders, and occasionally gravel. In the lower portion, the river flows predominantly on sandy and muddy substrate.

The longitudinal distribution of *N. microps* was assessed by sampling in 12 locations equally distributed throughout the upper, middle and lower stretches of the Macaé River and in major tributaries. The fish sampling was performed bimonthly between March 2004 and March 2005.

To assess the species' distribution in the mesohabitat, the occurrence of individuals in riffles and pools was evaluated. In the riffles, the current was fast (Global Flow Probe FP101) ( $1.29 \pm 0.32 \text{ m s}^{-1}$ ) and water depths (graduated scale) were typically less than 30 cm. The pools were located between stretches of rapids, had mainly sandy bottom, and commonly contained deposits of leaves and detritus. The pools were always deeper than the runs and riffles, with a slower current ( $0.24 \pm 0.13 \text{ m s}^{-1}$ ). Two microhabitats were evaluated within each mesohabitat, the river channel and the marginal region. In fast-flowing stretches, marginal microhabitats presented mainly live vegetation of Zingiberaceae. On the other hand, the substrate in the river channel was composed exclusively by medium to large-sized rocks and boulders.

The specimens were collected using sieves (40 x 60 cm; mesh sizes 5 mm and 500  $\mu\text{m}$ ), drift net (50 cm diameter; mesh 500  $\mu\text{m}$ ) and conical net (1.5 m x 1 m; mesh 5 mm) in the river channel. In this last method, rocks on the riverbed are picked up and moved, exploiting the current to carry fish, benthic invertebrates, leaves and debris toward the net, which is below the disturbed area, retaining all organisms. The fish longer than 3 cm were collected with 5 mm mesh (sieve and conical net) and fixed in 10% formalin; juveniles and larvae caught with 500  $\mu\text{m}$  mesh (sieve and drift net) were fixed in 4% buffered formalin (Nakatani et al. 2001). Environmental parameters were measured using YSI 30 (temperature and electrical conductivity) and YSI 55 (dissolved oxygen). Voucher specimens of adults were deposited in the Museu Nacional do Rio de Janeiro under number MNRJ 39018.

The specimens collected were measured and then dissected ( $n = 219$ ) for confirmation of the sex and reproductive phase (Brown-Peterson et al. 2011). A Student's T test was used to evaluate differences in SL between genders.

The number of size classes of *N. microps* was determined by the Sturges rule, where  $K = 1 + 3.222 \times \log n$ , where  $n$  is the sample size. The interval between the classes was generated based on  $H = R / K$ , where  $R$  is the amplitude of standard length, and  $K$  is the number of classes (Sturges 1926). The sizes of the specimens collected in the marginal region and in the channel were compared using Student's T test (Zar 1996).

During the collections, stones and boulders larger than 30 cm were moved and had their undersides examined, to locate spawns. Each spawn was photographed, and some eggs were removed and immediately fixed in 4% buffered formalin for subsequent measurement on the diameters. Then, the stones were repositioned in the original position and their two major axes were measured from the insertion point on the riverbed. Dissection of eggs with embryos in advanced stage of development and comparison with larvae confirmed that the spawns belong to *N. microps*.

The photographs taken in the field were used for egg counting. Unhatched eggs and empty shells were included in the counts. The diameters of the eggs collected were measured with a stereoscopic microscope fitted with a micrometer lens.

The Shapiro-Wilk test was applied to verify normality of the egg-diameter data for each stone. Because the data did not indicate a normal distribution, they were submitted to the nonparametric Kruskal-Wallis test to compare the egg diameter of different stones. In cases where there was a significant difference, the post-hoc H test of multiple comparisons was performed (Zar 1996).

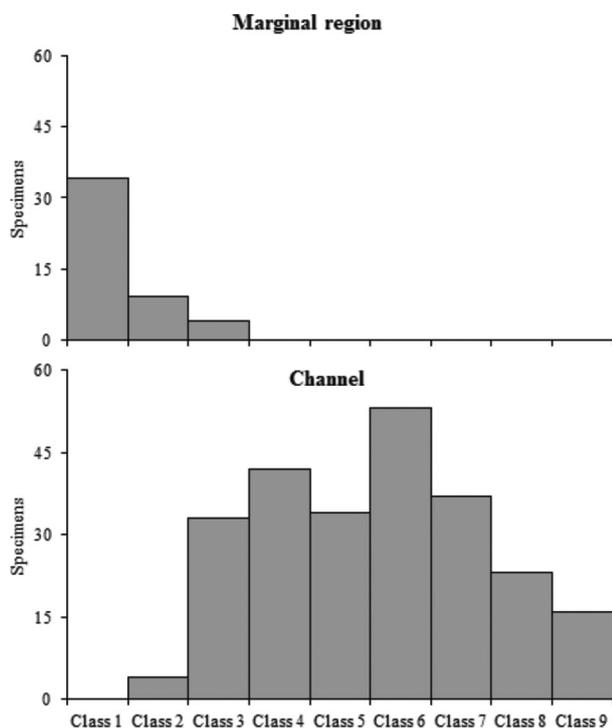
## Results

*Neoplecostomus microps* occurred in the middle and upper stretches of the Macaé River and tributaries, at altitudes between 50 and 1090 m. Specimens were captured only in fast-flowing habitats. At the capture sites, water temperature ranged from 11.6 to 25.3°C, electrical conductivity from 12.2 to 73.2  $\mu\text{S cm}^{-1}$  and dissolved oxygen from 7.45 to 10.78  $\text{mg L}^{-1}$ .

A total of 290 specimens of *N. microps* were collected, 242 of them in the river channel, 47 in the river margin and one in the drift. Of this total, 117 were males, 102 females and 71 were of indeterminate sex (all of them below 30 mm SL). The standard length (SL) ranged from 7.5 mm to 99.0 mm. Males were generally larger than females (mean SL 66.6 mm vs. 57.9 mm, respectively;  $p < 0.001$ ). The largest female measured 80.0 mm, and the largest male 99.0 mm. The smallest female and smallest male found to be reproductively active were 58.0 mm and 64.0 mm, respectively.

Nine size classes were determined, with intervals of 10.25 mm between the classes. The distribution of sizes in the rapids showed a difference related to ontogenetic development (t test,  $t = 10.71$ ,  $p < 0.0001$ ). Post-flexion larvae and juveniles from 7.50 mm to 32 mm SL (classes 1 to 3, Figure 1) occurred almost exclusively in the marginal vegetation. Juveniles and adults with standard lengths between 27 mm and 99 mm (classes 2 to 9) were reported in rapids, in gaps between stones and boulders on the riverbed (Figure 1). Classes 2 and 3 were the only ones with specimens collected in both marginal and channel microhabitats (Figure 1).

During the analysis, external morphological differences were observed, suggesting sexual dimorphism. The dissections confirmed that the differences were of sexual nature, even in juveniles. Males of *N. microps* show an epidermal growth along



**Figure 1.** Histogram of the size classes of *Neoplecostomus microps* collected in the marginal region and channel of the Macaé River, Rio de Janeiro State, from March 2004 to March 2005.

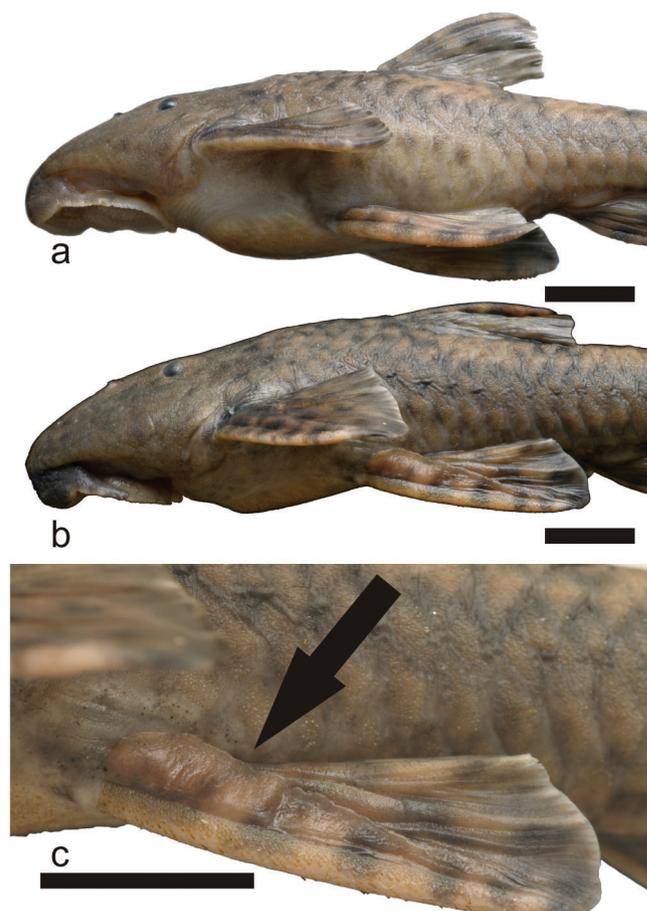
the pelvic-fin spine, forming a flap that projects toward the side of the body (Figures 2b and 2c); this flap was observed in specimens from 32 mm SL. No females showed this epidermal expansion (Figure 2a).

Another difference observed between the sexes was in the genital area. In *N. microps* females, the anal and urogenital pores were very close, with the middle of the papillae merged, giving the appearance of a single opening (Figure 3a). The males showed a separation between the anal and urogenital pores, featuring two distinct and defined openings (Figure 3b).

Spawns were found in two sites in the Macaé River basin, in November 2004 and January 2005, during the rainy season, exclusively in rapid stretches. Of the total of eight spawns found, six were in the upper Macaé River, at 850 m altitude (site RM3), and two in the Boa Esperança River (site RBE), a left tributary of the middle Macaé River, at 651 m altitude (Table 1). The characteristics of the sites were similar, with the fast-flowing portion associated with stones and boulders (i.e. headwaters).

Eggs of *N. microps* were found attached to the undersides of eight stones, in cavities formed in the river bottom. The number of eggs per stone ranged from 62 to 375 (Table 1). Eggs in different stages of development were found on the same stone (Figures 4a, 4b, 4c and 4f). The egg colors ranged from yellow-gold, in eggs full of yolk in early development (Figure 4d), to brown, in eggs with pigmented embryos (Figure 4e) in an advanced stage of development.

The mean diameter of eggs was 4.0 mm, with significant differences in the mean diameter of the eggs on different stones (KW = 37.84,  $p < 0.001$ ). The greatest variation in diameter occurred in stone VIII, and eggs on stone III differed in diameter from the eggs of the other stones (Table 1). The stone



**Figure 2.** Lateral view of specimens of *Neoplecostomus microps*: a – female; b – male; c – detail of the epidermal growth (arrow) along the pelvic-fin spine of a male *Neoplecostomus microps*. Bar = 1 cm.

with spawn V attached to the riverbed could not be removed, and therefore the number and sizes of the eggs were not determined. The current speed at the location of each spawn-bearing stone ranged from 0.97 m/s to 1.97 m/s (Table 1). The largest stone with a spawn corresponded to spawn IV, and the smallest stone had spawn VIII (Table 1).

## Discussion

*Neoplecostomus microps* occurred only in fast-flowing stretches with stones and boulders, typical of the upper and middle stretches of the Macaé River. *Neoplecostomus microps* was not recorded in the lower portion of the Macaé River, where the flow is slow and the substrate is sand and mud. This species has its life cycle restricted to the rapids. In this environment, *N. microps* of smaller size classes occur in the marginal vegetation and specimens of larger classes were captured in the river channel. Although *N. microps* is known to occur in altered habitats, the vegetation specificity where young individuals were found (almost exclusively Zingiberaceae) highlights the importance of the native vegetation in the life cycle of this species. In addition, silting caused by deforestation could alter substrate composition, rapids dynamics and spawning sites, thus affecting the species' longitudinal and spatial distribution. This spatial segregation can be related to limited or even absent swimming

**Table 1.** Spawns of *Neoplecostomus microps* in the Macaé River (RM3) and Boa Esperança River (RBE), number of eggs on stone (N), number of eggs measured (M), maximum, minimum and mean diameter of eggs, mean current speed at the stone site, stone size (SS), and results of the H test (H) for significance among the sizes of eggs. NM = Not measured, \*  $p < 0.05$ ; \*\*  $p < 0.005$ ; \*\*\*  $p < 0.001$ .

Spawn	Site	N	M	Max (mm)	Min (mm)	Mean (mm)	Vel (m/s)	SS (cm)	H
I	RM3	375	24	4.3	3.6	3.9	1.10	63 X 51	I x III**
II	RM3	240	25	4.3	3.5	4.0	1.46	43 X 44	II x III**
III	RM3	177	15	4.4	4.0	4.2	1.30	37 X 32	III x I**III x II**III x IV***III x VI***III x VII*
IV	RM3	103	16	4.0	3.6	3.9	1.97	74 X 56	IV x III***
V	RBE	NM	NM	NM	NM	NM	0.97	61 X 36	NM
VI	RBE	62	18	4.0	3.6	3.8	1.13	36 X 35	VI x III***VI x VIII*
VII	RM3	70	14	4.3	3.8	4.0	1.31	47 X 34	VII x III*
VIII	RM3	112	24	4.5	3.5	4.0	1.28	36 X 20	VIII x VI*

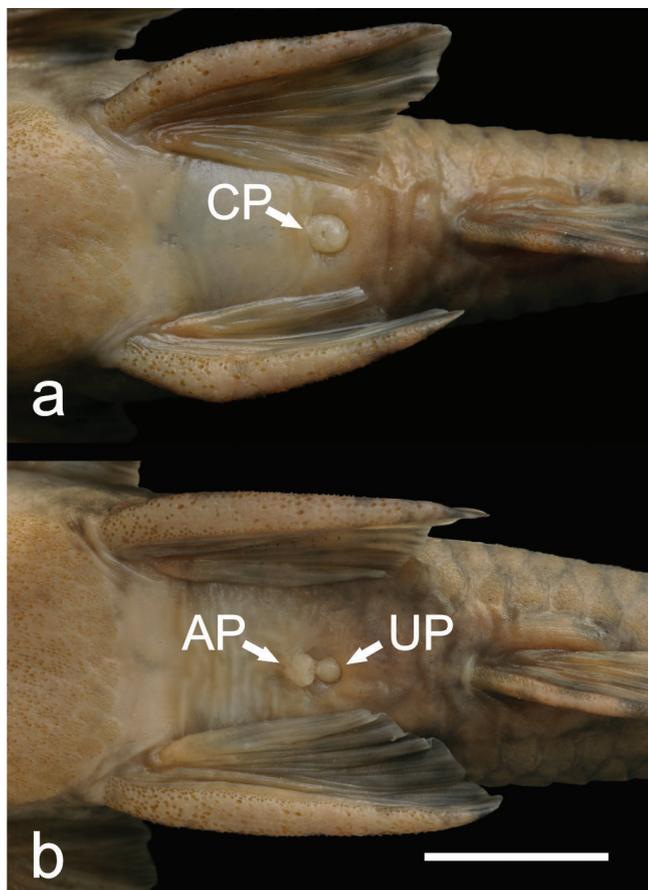
performance of juveniles to persist in rapids due to higher water velocity (Nikolsky 1963). The presence of juveniles in margin and a rare capture in drift may be associated to short period of drift, as observed to other rapids-dwelling catfishes (Brown & Armstrong 1985). This spatial segregation would also avoid intraspecific competition in species with the same preferences at different stages of life (Arratia 1983, Garutti & Figueiredo-Garutti 2000).

The care given to offspring implies both modifications in the parents' bodies to protect or carry their young, and a

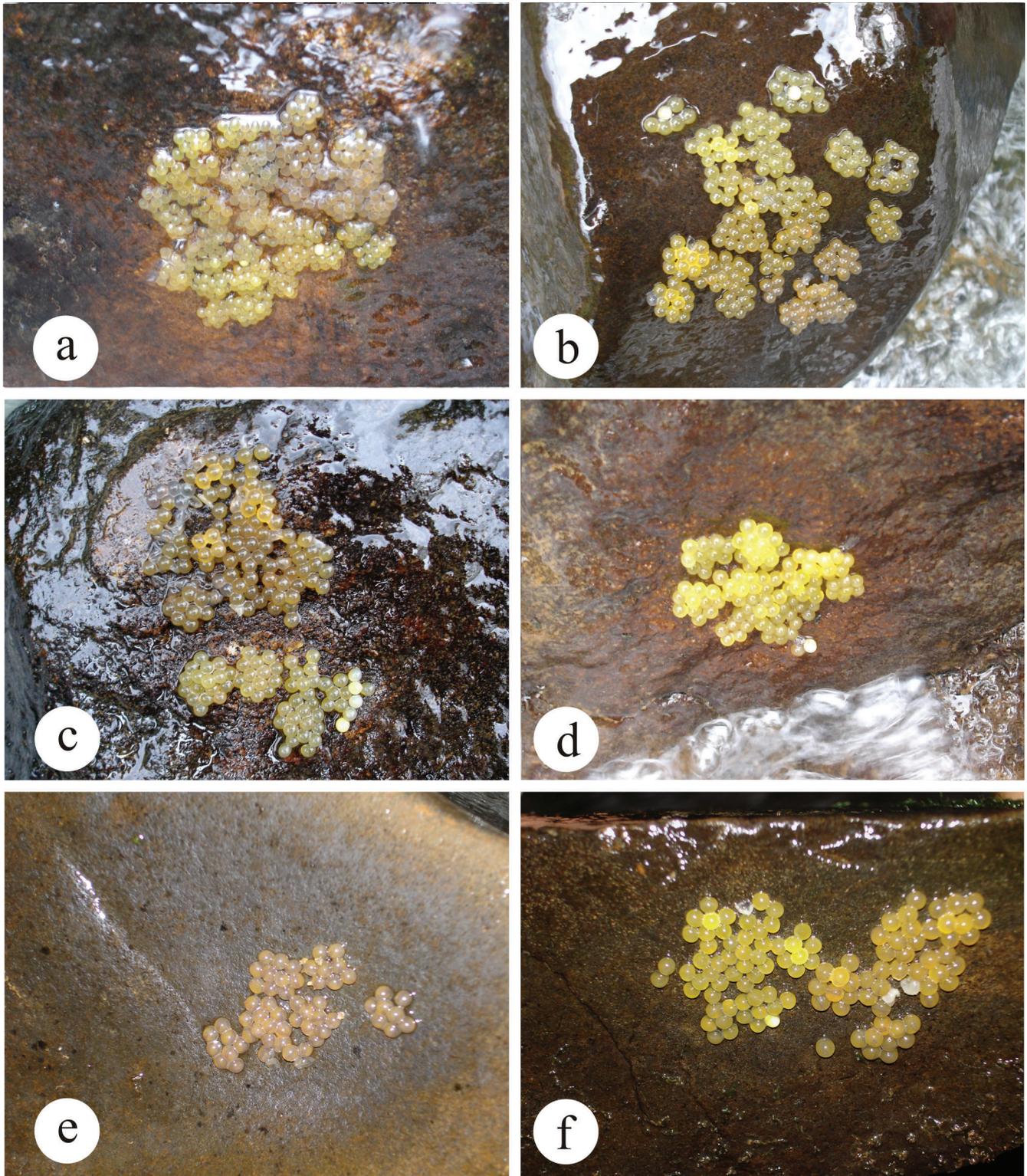
greater investment in egg quality, i.e., a larger amount of yolk. The spawns of *N. microps* found in the natural environment had a small number of large, adherent eggs. This seems to be a common character for Loricariidae that provide care for the offspring, represented by species that lay their eggs in cavities (Moodie & Power 1982, Sabaj et al. 1999, Cruz & Langeani 2000) and by species that carry their eggs attached to the body (Menezes 1949, Taylor 1983, Schmidt 2001). Furthermore, larger oocyte size is associated with larger embryo/fry size, which increases the chance of survival of the offspring in unstable environments (Nikolsky, 1963).

The presence of *N. microps* eggs with different sizes and in different stages of development on the same stone suggests that the same female spawned more than once or that more than one female spawned at that site. This variation in egg size may be associated with age (larger females produce larger oocytes), asynchronous spawning (reduction in the size of oocytes from previous spawnings), nutrition (proper diet during gonadal maturation provides nutrient transfer) and genetics (quality of oocytes) (Brooks et al. 1997, Einum & Fleming 2002, Kunz 2004). Reproduction with different partners, as recorded for other Loricariidae, both in the natural environment (Moodie & Power 1982 - *Loricaria uracantha*) and in captivity (Sabaj et al. 1999 - *Ancistrus* spp.), also appears to occur in *N. microps*. Considering the low fecundity (44 to 54 oocytes found by Braga et al. 2008; 32 to 55 oocytes found by Alves et al. unpublished) and the relatively high numbers of eggs (up to 375) found in *N. microps* nests, it is probable that the male mates with more than one female.

Apparently, one factor in choosing a stone is the presence of cavities or gaps (formed between the stone and riverbed) appropriate for the surface, size and position relative to the current. Larger stones may also have a stronger support on the river bottom and thus have a smaller surface available for laying eggs. This may be observed in spawns IV and VIII, with similar numbers of eggs but on stones of different sizes. Another important factor was the shape of the stone. Round stones may provide a smaller surface for laying eggs than flatter stones, as well as being potentially less stable in strong current. Hiding the spawn in a hole or in a cavity does not mean that the offspring will be actively protected by the parent. However, the location and the environmental disturbances were significant factors in determining the spawning sites. The choice of larger stones for spawning in the rapids demonstrates the caution of *N. microps* in avoiding stones that can be displaced



**Figure 3.** Detail of the ventral region of specimens of *Neoplecostomus microps*, showing the merged anal and urogenital pores (CP) in females (A), and the distinct anal pore (AP) and urogenital pore (UP) in males (B). Bar = 1 cm.

Life-history features of *Neoplecostomus microps*

**Figure 4.** Spawns of *Neoplecostomus microps* found in the Macaé River at RM3: a – spawn I; b – spawn II; c – spawn III; d – spawn IV; e – spawn VII; f – spawn VIII. Yellow-gold eggs in early development and brown eggs in advanced stage of development. Mean size of eggs = 4.0 mm

in a flash flood, risking the spawn of being crushed or predated if the stone is dislocated and the surface with the eggs is exposed. In addition, the position of the cavity in relation to the current may prevent sediments from accumulating on the eggs (Moodie & Power 1982, Cruz & Langeani 2000). Nests

located in hydraulically protected areas can survive adverse conditions (i.e. flash floods) and assure the survival of the offspring (Constanz 1985).

Considering the larger size of males, low fecundity (Braga et al. 2008, Alves et al. unpublished), large eggs, and care in

selecting the spawning site, probably the males of *N. microps* perform this function, as observed for most Loricariidae with this pattern of reproduction.

Sexual dimorphism has been detected in *Neoplecostomus* species where the males have developed odontodes and an extension in the head region (Zawadzki et al. 2008, Roxo et al. 2012, Andrade & Langeani 2014). In *N. microps*, the sexual dimorphism observed in the genital area and in the pelvic fins had been poorly reported in literature. The distinctive papillae in male differ from the unique papilla in females, noted in immature specimens but most visible in adult. Other distinction between the sexes documented here is the epidermal projection on the pelvic-fin spine, present in males, even immature ones. Females did not express this modification in any life stage.

The identification of permanent external dimorphism is important for biological studies, because it eliminates the need for dissection to determine the sex of an individual. In the case of species such as *N. microps*, and of species that are targets of monitoring and conservation, population-monitoring protocols that allow determination of the sex ratio without killing individuals will be useful.

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## Composition and distribution of diatom assemblages from core and surface sediments of a water supply reservoir in Southeastern Brazil

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FAUSTINO, S.B., FONTANA, L., BARTOZEK, E.C.R., BICUDO, C.E.M., BICUDO, D.C. **Composition and distribution of diatom assemblages from core and surface sediments of a water supply reservoir in Southeastern Brazil.** Biota Neotropica. 16(2): e20150129. <http://dx.doi.org/10.1590/1676-0611-BN-2015-0129>

**Abstract:** Fresh water biodiversity is an increasing concern due to growing human impact. Herein, we report a long-term survey (ca. 90 years) of sedimentary diatoms and the modern flora from surface sediments and their biodiversity changes along a eutrophication gradient. Study was carried out in one of the most important water supply reservoirs (Guarapiranga Reservoir) of São Paulo Metropolitan Region, Brazil. Results are based on 75 core subsamples (subfossil assemblages from core) previously dated by <sup>210</sup>Pb and 14 samples from surface sediments (modern assemblages). Overall, 84 taxa were reported, belonging to 30 genera, 71 species and eight non-typical varieties, besides five probable new taxa. Results expanded two new additions for the Brazilian diatom flora (*Chamaepinnularia submuscularia* and *Stauroneis acidoclinata*) and 30 infrageneric taxa for the state of São Paulo. 47.6% of total taxa inventoried were accounted exclusively for the subfossil assemblages indicating a significant biodiversity change over time. Access to past oligotrophic conditions and to contemporary mesotrophic regions of the Guarapiranga Reservoir accounted for these new additions representing 25% of the total diatom flora. Decline in the total species number along the trophic state gradient occurred for subfossil and modern assemblages. This pattern was even clearer when considering the changes in species richness over time. *Eumotia* with 21 taxa was the far most represented genera particularly in the oligotrophic phase. During the transitional period (1947-1974), richness gradually declined. With the onset (in the 1970s) and the major eutrophication period (since ca. 1990) occurred a drastic reduction in richness and the replacement of oligotrophic to eutrophic species. Human management also caused abrupt changes in richness. Marked decline occurred (1933) associated with hydrological impacts (water discharge increase) with the initial use of the reservoir as a public water supply. Unlike, sudden increase occurred probably associated with the application of algacide to control cyanobacterial blooms. Present findings highlight the need for surveying the diatom assemblages in protected environments or in less degraded conditions for biodiversity assessment. Furthermore, reinforce the use of paleolimnological approach as in many cases the only tool to assess biodiversity changes encompassing time scales relevant to human-induced degradation and pre-anthropogenic impacts.

**Keywords:** Bacillariophyta, biodiversity change, eutrophication, Guarapiranga Reservoir, paleolimnology, species richness.

FAUSTINO, S.B., FONTANA, L., BARTOZEK, E.C.R., BICUDO, C.E.M., BICUDO, D.C. **Composição e distribuição das diatomáceas de perfil sedimentar e sedimentos superficiais em reservatório de abastecimento no Sudeste do Brasil.** Biota Neotropica. 16(2): e20150129. <http://dx.doi.org/10.1590/1676-0611-BN-2015-0129>

**Resumo:** A biodiversidade de águas continentais vem se tornando uma preocupação crescente devido ao grande aumento do impacto antropogênico nesses ambientes. Nosso objetivo foi documentar o inventário e a mudança da biodiversidade das comunidades de diatomáceas subfósseis ao longo de 90 anos e das diatomáceas recentes de sedimentos superficiais ao longo de um gradiente de eutrofização. O estudo foi realizado em um dos mais importantes reservatórios de abastecimento público (represa Guarapiranga) da Região Metropolitana de São Paulo, Brasil. Baseou-se na análise de 75 subamostras de um perfil sedimentar (comunidades subfósseis) previamente datado pelo <sup>210</sup>Pb e em 14 amostras de sedimento superficial

(comunidades recentes). Ao todo, identificamos 84 táxons, distribuídos em 30 gêneros, 71 espécies e oito variedades não típicas, além de cinco prováveis novidades taxonômicas. Os resultados acresceram duas novas citações para a flórmula diatomológica do Brasil (*Chamaepimularia submuscularis* e *Stauroneis acidoclinata*) e 30 táxons infragêneros para o Estado de São Paulo. Desse total, 47,6% foram identificados exclusivamente para as comunidades subfósseis indicando uma mudança significativa da biodiversidade ao longo do tempo. O acesso às condições oligotróficas pretéritas e às regiões mesotróficas recentes da represa foi responsável pelas novas adições que representaram 25% do total da diatomoflórula. Declínio do número total de espécies ao longo do gradiente de estado trófico foi observado para as comunidades de diatomáceas subfósseis e modernas. Esta tendência foi ainda mais evidente com base na alteração da riqueza de espécies ao longo do tempo. *Eunotia* com 21 táxons foi o gênero mais representado particularmente na fase oligotrófica da represa. Durante o período de transição (1947-1974) houve diminuição gradativa da riqueza. Com o início da eutrofização (década de 1970) e do período de maior eutrofização (desde 1990) ocorreu drástica redução da riqueza e substituição de espécies oligotróficas por eutróficas. Mudanças abruptas de riqueza de diatomáceas também foram observadas devido ao manejo da represa. Declínio marcado ocorreu (1933) associado a impactos hidrológicos (aumento da vazão) com o início do uso do reservatório para abastecimento público. De forma diferente, aumento repentino de riqueza ocorreu associado à aplicação de algicida para controlar as florações de cianobactérias. Os presentes resultados salientam a necessidade de levantamentos das comunidades de diatomáceas de ambientes protegidos ou em condições menos degradadas em estudos que visem o acesso à biodiversidade. Ademais, reforçam o uso da paleolimnologia como a única ferramenta, em muitos casos, que possibilita avaliar as mudanças da biodiversidade em escalas relevantes para acessar a degradação induzida pelo homem e períodos pré-impactados.

**Palavras-chave:** Bacillariophyta, mudança de biodiversidade, eutrofização, represa Guarapiranga, paleolimnologia, riqueza de espécies.

## Introduction

Despite covering just 0.8% of the Earth's surface, freshwater ecosystems are considered hotspots for biodiversity supporting ~6% of all described species (Dudgeon et al. 2006). However, the growing anthropogenic impacts in the last century has led to growing threats to fresh water biodiversity as well as to the largely "unknown" diversity worldwide (Strayer & Dudgeon 2010). Decreases in biodiversity are so widespread that they are now considered a form of global change (Gregory-Eaves & Beisner 2011). Therefore, information on biodiversity changes in long timescales has become an important issue of freshwater ecology and conservation.

Given long-term community data are sparse and usually span no more than five years, the paleolimnological approach has recently been highlighted as an emerging field for biodiversity science (Gregory-Eaves & Beisner 2011). Lake sediments integrate organisms over time and space, different habitats, providing whole-lake, annual to multi-annual assemblage information more efficiently than neolimnological studies (Bennion 1995, Gregory-Eaves & Beisner 2011). They can provide valuable information about past and contemporary environmental conditions, having good records of biodiversity (Froyd & Willis 2008, Liu et al. 2012, Davidson et al. 2013) and floristic changes (Schmidt et al. 1990).

Among the biological groups preserved in the sediments, diatoms have been widely used because of their taxonomic distinction, abundance, good preservation in sediments and their rapid response to environmental changes (Reid 2005, Bennion & Simpson 2011). The use of diatoms as indicators of environmental changes require high taxonomic precision (Birks 1994), since misidentifications can modify the interpretation in obtaining reliable data on modern diatom biodiversity (Buczkó & Magyari 2007, Wetzel & Ector 2014). Also relevant is the auto-ecological knowledge of diatom species in order to use them as modern analogues in quantitative paleo-environmental reconstruction using sediment records of past communities (Birks 1994). Moreover, past communities are very often the only available tool to

provide information on natural biodiversity before human impacts, such as cultural eutrophication. For those purposes, floristic surveys in long time series are considered crucial (Schmidt et al. 1990), though rarely available in the world, especially in tropical regions

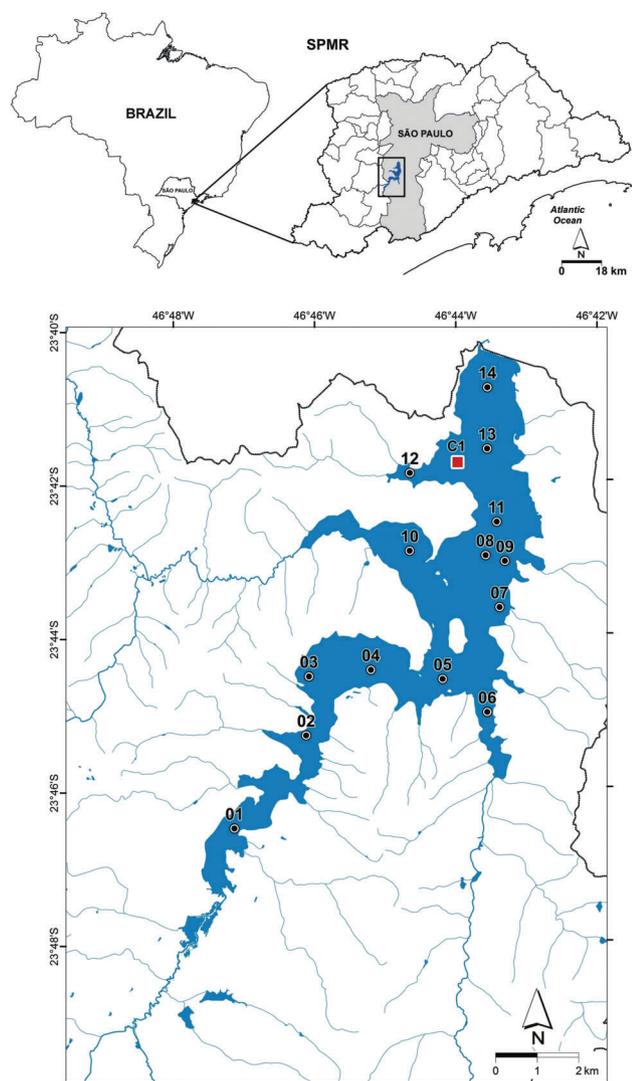
There has been an increasing knowledge of the diatom flora in some regions of Brazil (e.g. Ferrari & Ludwig 2007, Melo et al. 2010, Wetzel et al. 2010, Santos et al. 2011, Bartozek et al. 2013). However, only recently taxonomical studies of surface sediments have begun (Fontana & Bicudo 2009, 2012, Silva & Bicudo 2014), including the addition of new species (Wengrat et al. 2015, Almeida et al. 2015).

Despite the studies on paleoenvironmental reconstruction using diatoms (e.g. Costa-Böddeker et al. 2012, Fontana et al. 2014), to our knowledge floristic and taxonomical studies of sedimentary diatoms in long timescale in Brazil and probably in tropical regions have not been published. We presently documented the floristic survey and the spatial-temporal distribution of diatoms from the surface sediments (modern flora) and core (ca. 90 years, subfossil flora) of the Guarapiranga Reservoir along a spatial and temporal eutrophication gradient. The subfossil diatom assemblage was taxonomically studied and revised based on the ecological study of Fontana et al. (2014). The Guarapiranga Reservoir is one of the most important public water supplies for the metropolitan region of São Paulo. Present study expands the knowledge of biodiversity changes, taxonomical and ecological information of tropical diatoms, contributing to their use in water quality bioassessment and paleoenvironmental reconstruction.

## Material and Methods

Guarapiranga Reservoir is a strategic public water supply located in one of the most urbanized cities worldwide, the São Paulo Metropolitan Region (SPMR), in the state of São Paulo, southeastern Brazil (23°41'S, 46°43'W) (Figure 1). The SPMR is one of the most important financial, commercial and industrial

## Diatoms from core and surface sediments



**Figure 1.** Map showing the location of the state of São Paulo in Brazil and the city of São Paulo metropolitan region (SPMR) with location of Guarapiranga Reservoir. Enlarged map of the reservoir with sampling sites: solid circles for surface sediments (1 to 14) and solid red square for core (C<sub>1</sub>).

centers in Brazil, and one of the most densely populated areas of the country, with nearly 20 million inhabitants (IBGE 2014). The reservoir has an area of 36.18 km<sup>2</sup>, mean and maximum depth of 7 and 13 m, respectively, and a water volume of 253 × 106 m<sup>3</sup> (Mozeto et al. 2001). It was built in 1906-1909 for energy production, and dam construction flooded a large portion of Atlantic Forest habitat (Whatley & Cunha 2006). In 1927, the city of São Paulo began to use the reservoir as a public water supply, and today the reservoir is the main water source for the city, supplying drinking water to about 25% of the population. Fontana et al. (2014) inferred the major ecological shifts (using diatoms and geochemical proxies) in the water body over the last 90 years related to multiple stressors, mainly the influence of forest flooding and eutrophication. The reservoir was oligotrophic from 1919 to 1947 and the onset of eutrophication occurred in the mid-1970s. By the early 1980s the reservoir had become eutrophic, in response to an explosive increase in human population in its watershed. Severe cultural eutrophication has

persisted since 1990. Further information on the major shifts of the reservoir is available in Fontana et al. (2014).

Core was retrieved by divers from the northern area of the basin, close to the dam (Figure 1). The core chronology was determined by <sup>210</sup>Pb dating as detailed in Fontana et al. (2014). Divers collected a 75-cm core in February 2010 using acrylic tube that was sectioned at 1 cm intervals. In total, 75 subsamples (slices) were examined (subfossil diatom flora). In addition, 14 samples of surface sediments (modern diatom flora) were collected in August/2011, using a gravity corer (UWITEC), and the first 2 cm of the sediments were saved for diatom analysis. Sampling stations for surface sediments covered the trophic spatial gradient of the reservoir (Figure 1). Limnological characteristics of these sites are provided in Table 1 (according to AquaSed database Project). Subsurface samples in the limnetic zone were taken with a van Dorn sampler in the dry (August/2011) and rainy seasons (March/2011). Water temperature (°C), pH and conductivity (μS cm<sup>-1</sup>) were measured in the field using standard electrodes (Horiba U-53). The analytical procedure for dissolved oxygen (DO, mg L<sup>-1</sup>), ammonium (N-NH<sub>4</sub> μg L<sup>-1</sup>), nitrate (N-NO<sub>3</sub> μg L<sup>-1</sup>), soluble reactive silica (SRS, mg L<sup>-1</sup>), total nitrogen (TN, μg L<sup>-1</sup>) and total phosphorus (TP, μg L<sup>-1</sup>) followed Standard Methods (APHA 2005). Chlorophyll *a* (μg L<sup>-1</sup>), corrected for phaeophytin, was measured using 90% ethanol (Sartory & Grobbelaar 1984). The Trophic State Index (TSI) was calculated according to Lamparelli (2004). For details, see Wengrat & Bicudo (2011). Sampling sites 1 to 5 were considered mesotrophic, and 6 to 11 and 13 to 14 eutrophic, while site 12 was classified as supereutrophic (Table 1).

Diatom samples were oxidized according to standard procedures (Battarbee et al. 2001), using concentrated hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 35%) and hydrochloric acid (HCl 37%). Oxidized subsamples were rinsed with deionized water and permanent slides were prepared using Naphrax as mounting medium. Optical observations, measurements and micrographs were taken at a magnification of 1000 × with a Zeiss Axioskop 2 plus microscope equipped with DIC and phase contrast, with an Axiocam ERc5s high-resolution digital camera. Micrographs were digitally manipulated and plates containing LM images were created using CorelDraw X6. Morphometric information is provided for all taxa (L: length; W: width; D: diameter; M: mantle height; S: striae; AS: apical striae; MS: median striae; F: fibulae, A: areolae; AC: alar canals; MF: mantle fultoportulae) as well as temporal and spatial distribution in the reservoir. Descriptions are presented for the new records for Brazil, and comments are provided when relevant (e.g. poorly known species worldwide or in Brazil). Taxonomy and nomenclature followed classic works and new publications (e.g., Husted 1950, Krammer 2000, Metzeltin et al. 2005, Lange-Bertalot et al. 2011) and the on-line catalogue of valid names (site of California Academy of Sciences 2012). The classification systems followed Medlin & Kaczmarek (2004) for supra-ordinal taxa and Round et al. (1990) for subordinal taxa, except for genera published subsequently to this work. To account for the species distribution in Brazil and the state of São Paulo, literature with illustration or sufficient taxonomic description of the species was considered. Sediment samples were deposited at the “Herbário Científico do Estado Maria Eneyda P. Kauffmann Fidalgo” (SP), Brazil. Finally, to determine species richness (Magurran 2004) diatom was quantified to standardize the analytical procedure among samples. Enumeration was made at a magnification of

Table 1. Water chemistry data for Guarapiranga Reservoir (water subsurface mean values for summer and winter), according to AquaSed database Project. Abbreviation in Material and methods.

Sites	Temperature (°C)	pH	Conductivity ( $\mu\text{S cm}^{-1}$ )	DO ( $\text{mg L}^{-1}$ )	N-NH <sub>4</sub> ( $\mu\text{g L}^{-1}$ )	N-NO <sub>3</sub> ( $\mu\text{g L}^{-1}$ )	TN ( $\mu\text{g L}^{-1}$ )	TP ( $\mu\text{g L}^{-1}$ )	SRS ( $\text{mg L}^{-1}$ )	Chlorophyll <i>a</i> ( $\mu\text{g L}^{-1}$ )	TSI	Trophic State
01	18.6	6.6	41.8	6.2	75.6	91.6	692.6	25.0	2.5	4.7	53.3	Mesotrophic
02	19.6	7.0	50.4	6.6	152.9	89.6	791.1	25.4	2.4	16.2	56.4	Mesotrophic
03	19.6	6.9	60.9	7.1	162.6	87.2	889.2	28.2	2.2	13.1	56.7	Mesotrophic
04	19.6	7.1	66.0	6.6	129.4	104.8	878.0	25.9	2.2	18.8	57.6	Mesotrophic
05	18.2	7.3	90.6	6.2	349.0	174.7	1479.3	41.5	2.4	16.5	58.3	Mesotrophic
06	18.1	8.1	131.5	6.8	1027.8	194.7	2003.8	105.9	2.5	21.7	61.7	Eutrophic
07	18.6	7.5	105.5	8.3	456.7	213.1	1077.8	53.3	2.4	23.5	60.3	Eutrophic
08	18.3	8.0	118.0	7.4	354.1	339.6	1484.3	47.5	3.0	34.7	60.6	Eutrophic
09	18.8	7.9	115.5	6.4	294.6	381.8	1037.0	54.6	3.5	37.2	61.1	Eutrophic
10	21.8	7.6	123.0	8.4	1071.2	343.8	2285.0	65.1	2.9	35.6	61.3	Eutrophic
11	18.0	8.0	124.0	7.1	169.7	413.0	766.1	52.7	2.8	39.1	61.3	Eutrophic
12	18.0	7.9	123.0	3.6	1017.2	306.2	1446.2	187.2	3.2	52.4	65.3	Supereutrophic
13	23.5	7.9	108.5	11.4	157.5	193.1	1301.2	48.7	1.9	38.0	61.0	Eutrophic
14	23.4	7.7	105.5	12.7	64.9	265.8	1107.2	38.3	1.9	30.5	59.8	Eutrophic

1000 × using a Zeiss Axioskop 2 microscope, and at least 400 valves were counted per slide (Battarbee et al. 2001).

## Results and discussion

### Taxonomy and ecological preferences

Below are presented the taxonomical aspects of the species identified in this study and their ecological preferences. Taxa preceded by one asterisk represent new records for the state of São Paulo, and those preceded by two asterisks are first citations for Brazil. The infrageneric taxa commonly reported in Brazilian literature are shown in Table 2.

#### Aulacoseiraceae Crawford

##### *Aulacoseira* Thwaites

\**Aulacoseira granulata* Ehrenberg var. *australiensis* Moro, Arquivos de Biologia e Tecnologia 34(2), p. 353-359, 1991.

Figs. 5-7.

M: 11.0-20.0  $\mu\text{m}$ ; D: 16.0-23.0  $\mu\text{m}$ ; S: 8-9 in 10  $\mu\text{m}$ ; A: 8-11 in 10  $\mu\text{m}$ .

It differs from *Aulacoseira granulata* (Ehrenberg) Simonsen var. *granulata* mainly due to its greater diameter (18.0-31.0  $\mu\text{m}$ ) and the presence of visible rimoportulae in LM in valve surface (Moro 1991). No ecological information was found in literature. This variety occurred in 2% of all samples in eutrophic conditions for subfossil and modern assemblages. This is the first report for the state of São Paulo.

*Aulacoseira pusilla* (Meister) Tuji & Houk, Bulletin of the National Science Museum, Series B (Botany), Tokyo, 30 (2), p. 38, 2004. Figs. 10-11.

M: 2.0-2.6  $\mu\text{m}$ ; D: 5.2-6.6  $\mu\text{m}$ ; inconspicuous striae.

In Brazil, this species was commonly confused with *A. alpigena* (e.g. Brassac et al. 1999, Ludwig et al. 2005), *A. distans* (Ehrenberg) Simonsen (e.g. Ludwig et al. 2004, Raupp et al. 2006) or *A. muzzanensis* (e.g. Morandi et al. 2006). It is considered a cosmopolitan and eutrophic species (Houk & Klee 2007, Taylor et al. 2007, Tuji & Williams 2007). Presently distributed in 68% of the core samples (from oligo- to eutrophic phases). Our data expanded its ecological range from oligotrophic to mesotrophic conditions.

*Aulacoseira tenella* (Nygaard) Simonsen, Bacillaria 2, p. 63, 1979.

Figs. 12-13 girdle view, Figs. 14-15 valve view.

M: 2.0- 3.0  $\mu\text{m}$ ; D: 5.0-8.0  $\mu\text{m}$ ; S: 8-9 in 10  $\mu\text{m}$ .

This species was reported from oligotrophic to oligo-mesotrophic and slightly acidic to neutral waters (Siver & Kling 1997, Wetzel 2011). Distributed in 60% of all samples from oligotrophic to mesotrophic (subfossil samples) and from mesotrophic to eutrophic conditions (modern samples).

#### Orthoseiraceae Kützing

##### *Orthoseira* Thwaites

*Orthoseira roseana* (Rabenhorst) O'Meara, Proceedings of the Royal Irish Academy, 2 p. 255, pl. 26, 1875.

Figs. 16-18.

D: 13.1-17.3  $\mu\text{m}$ ; M: observed only in valve view; S: 16-18 in 10  $\mu\text{m}$ ; A: 18-22 in 10  $\mu\text{m}$ .

Distributed in 41% of the subfossil samples in past oligotrophic conditions.

#### Stephanodiscaceae Glezer & Makarova

##### *Cyclotella* (Kützing) Brébisson

**Table 2.** Valve dimensions and trophic state range distribution based on literature and in this study for the species recorded in Guarapiranga Reservoir that are commonly reported in Brazil.

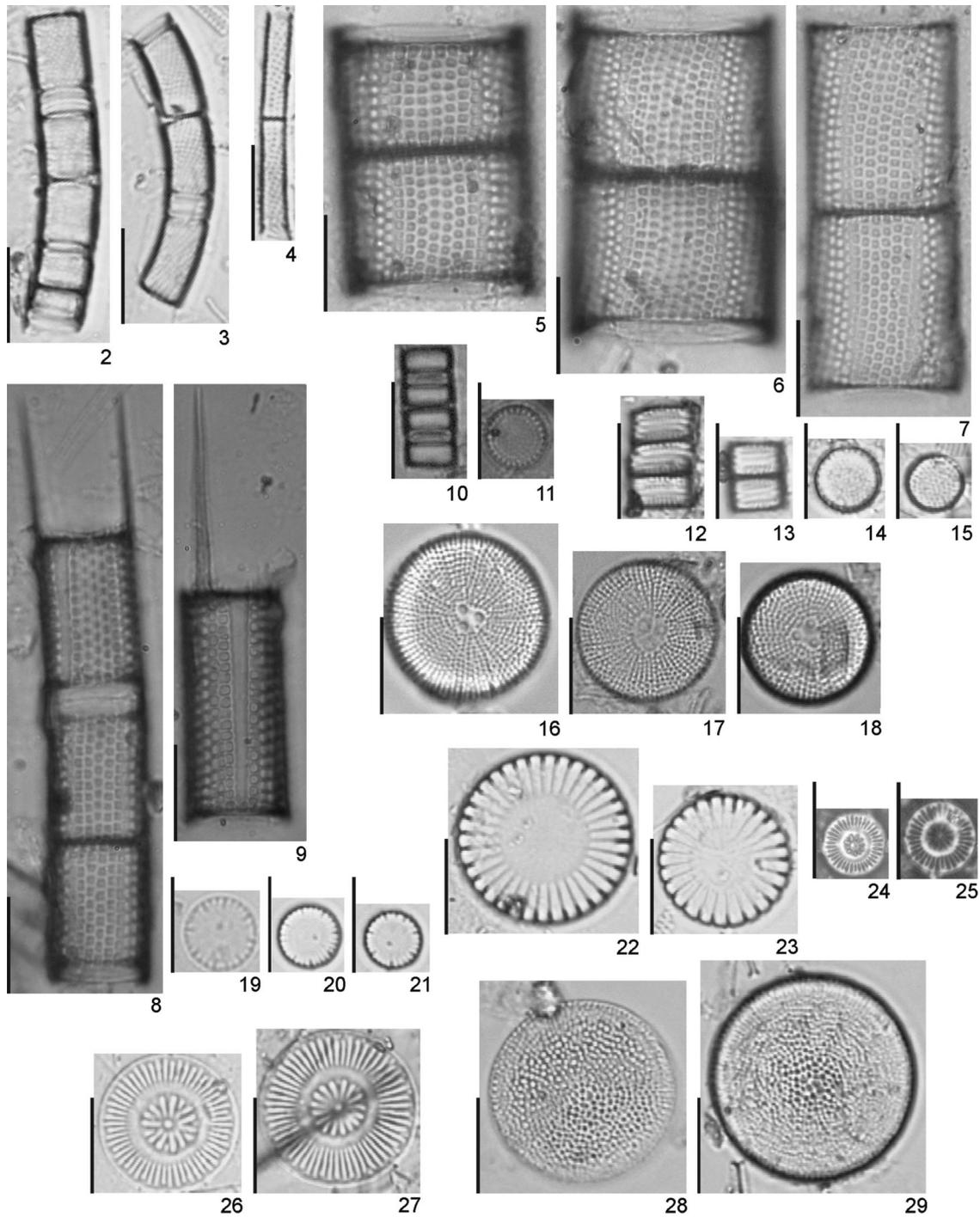
Taxon	Dimension ( $\mu\text{m}$ ) and striae (in 10 $\mu\text{m}$ )	Ecology (literature)	Distribution in samples (S: subfossil; M: modern diatoms)
<b>AULACOSEIRACEAE</b>			
<i>Aulacoseira ambigua</i> (Grunow) Simonsen (Figs. 2-3)	M: 4.0-10.0; D: 5.0-6.6; S: 10-13; A: 10-16	Oligotrophic to eutrophic <sup>1,6</sup>	Meso to supereutrophic conditions in 89% of samples (S, M)
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen var. <i>angustissima</i> (Müller) Simonsen (Fig. 4)	M: 10.0-20.0; D: 2.0-3.3; S: 10-11; A: 10-12	Mesotrophic to eutrophic <sup>3</sup>	Eutrophic conditions in 26% of samples (S, M)
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen var. <i>granulata</i> (Figs. 8-9)	M: 9.9-19.3; D: 5.2-12.6; S: 8-10; A: 9-10	Eutrophic <sup>1</sup>	Oligo to super-eutrophic in 70% of samples (S, M)
<b>STEPHANODISCACEAE</b>			
<i>Cyclotella meneghiniana</i> Kützing (Figs. 22-23)	D: 13.8-22.0; S: 7-9	Eutrophic <sup>3</sup>	Meso to super-eutrophic conditions in 79% of samples (S, M)
<i>Discostella pseudostelligera</i> (Hustedt) Houk & Klee (Figs. 24-25)	D: 5.0-8.0; S: 13-19; MF: 7-12	Eutrophic <sup>1</sup>	Mesotrophic to super-eutrophic conditions in 37% of samples (S, M)
<i>Discostella stelligera</i> (Cleve and Grunow) Houk & Klee (Figs. 26-27)	D: 9.7-17.5; S: 13-16	Oligotrophic to eutrophic <sup>4,5</sup>	Mesotrophic to super-eutrophic conditions in 64% of samples (S, M)
<i>Spicaticribrbra rudis</i> (Tremarin et al.) Tuji et al. (Figs. 28-29)	D: 15.3-22.6; S: 18-20; A: 16-18	Mesotrophic <sup>7</sup>	Mesotrophic to eutrophic conditions in 34% of samples (S, M)
<b>FRAGILARIACEAE</b>			
<i>Asterionella formosa</i> Hassal (Figs. 30-31)	L: 42.5-60.0; W: 2.2-2.6; inconspicuous striae	Eutrophic <sup>1</sup>	Eutrophic conditions in 41% of samples (S)
<b>EUNOTIACEAE</b>			
<i>Eunotia camelus</i> Ehrenberg (Figs. 50-51)	L: 24.6-61.5; W: 4.6-7.0; S: 9-11	-	Oligo to mesotrophic conditions in 25% of samples (S, M)
<i>Eunotia rabenhorstii</i> var. <i>monodon</i> Cleve & Grunow in Van Heurck (Figs. 62-63)	L: 11.2-23.6; W: 5.0-8.1; S: 12-13	-	Oligotrophic to mesotrophic conditions in 21% of samples (S)
<i>Eunotia zygodon</i> Ehrenberg (Fig. 70)	L: 66.0-76.2; W: 20.0; S: 12	-	Oligotrophic conditions in 36% of samples (S)
<b>CYMBELLACEAE</b>			
<i>Cymbopleura naviculiformis</i> (Auerswald) Krammer (Figs. 84-85)	L: 34.4-41.3; W: 9.3-10.0; S: 11-14	Tolerant <sup>2</sup>	Mesotrophic conditions in 14% of samples (M)
<i>Encyonema silesiacum</i> (Bleisch) Mann (Figs. 86-87)	L: 35.0-40.0; W: 6.0-7.5; S: 7-10	Tolerant <sup>2</sup> to indifferent <sup>1</sup>	Mesotrophic to supereutrophic conditions in 46% of samples (S, M)
<b>GOMPHONEMATAACEAE</b>			
<i>Gomphonema laticollum</i> Reichardt (Figs 99-100)	L: 34.6-51.2; W: 12.0-13.3; S: 11-13	-	Oligo to mesotrophic conditions in 12% of samples (S)
<b>ACHNANTHIDIACEAE</b>			
<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki (Figs. 107-109)	L: 13.7-14.6; W: 2.6-3.3; inconspicuous striae	Oligotrophic to eutrophic <sup>3</sup>	Oligo to eutrophic conditions in 65% of samples (S, M)
<b>STAURONEIDACEAE</b>			
<i>Capartogramma crucicula</i> (Grunow ex Cleve) Ross (Fig. 149)	L: 30.0; W: 9.4; inconspicuous striae	-	Oligotrophic conditions in 1% of samples (S)
<b>CATENULACEAE</b>			
<i>Amphora copulata</i> (Kützing) Schoeman & Archibald (Figs. 161-162)	L: 28.0-36.3; W: 8.0-10.0; S: 8-13	Eutrophic <sup>1</sup> to tolerant <sup>2</sup>	Oligotrophic to eutrophic conditions in 19% of samples (S, M)

<sup>1</sup>van Dam et al. (1994); <sup>2</sup>Hofmann (1994); <sup>3</sup>Moro & Fürstenberger (1997); <sup>4</sup>Yang & Dickman (1993); <sup>5</sup>Potapova & Charles (2007); <sup>6</sup>Stenger-Kovacs et al. (2007); <sup>7</sup>Fontana et al. (2014); - : unavailable information.

\**Cyclotella atomus* Hustedt, Archiv für Hydrobiologie, Supplement, p. 12, pl. 9, figs. 1-4, 1937. Figs. 19-21.

D: 6.3-8.0  $\mu\text{m}$ ; S: 10-12 in 10  $\mu\text{m}$ .

This species differs from *C. meneghiniana* Kützing due to the marginal area with short striae and distinct fuloportulae at every third, fourth or fifth (seldom sixth to seventh) appearing as thicker striae than the others (shadowlines), and the presence of a single,



**Figures 2-29.** Modern and subfossil diatom flora of Guarapiranga Reservoir. Brazil. 2-3. *Aulacoseira ambigua*. 4. *Aulacoseira granulata* var. *angustissima*. 5-7. *Aulacoseira granulata* var. *australiensis*. 8-9. *Aulacoseira granulata* var. *granulata*. 10-11. *Aulacoseira pusilla*. 12-13. *Aulacoseira tenella* girdle view. 14-15. *Aulacoseira tenella* valve view. 16-18. *Orthoseira roseana*. 19-21. *Cyclotella atomus*. 22-23. *Cyclotella meneghiniana*. 24-25. *Discostella pseudostelligera*. 26-27. *Discostella stelligera*. 28-29. *Spicaticribra rudis*. Scale bars: 10 μm.

seldom two, subcentral fultoportulae (Hakansson & Clarke 1997). Furthermore, *C. meneghiniana* populations can reach larger diameters (6-35 μm) than *C. atomus* (6-10 μm) (Lowe 1975).

This taxon was probably only reported in Brazil by Moro & Fürstenberger (1997) and Cavalcante et al. (2013). It is considered a planktonic, acidophilic to alcalinophilic, and a eutrophic species (van Dam et al. 1994, Moro & Fürstenberger 1997, Kiss et al. 2012). In this study, the species occurred in 79% of all samples in modern

assemblages from mesotrophic to supereutrophic conditions. First report for the state of São Paulo.

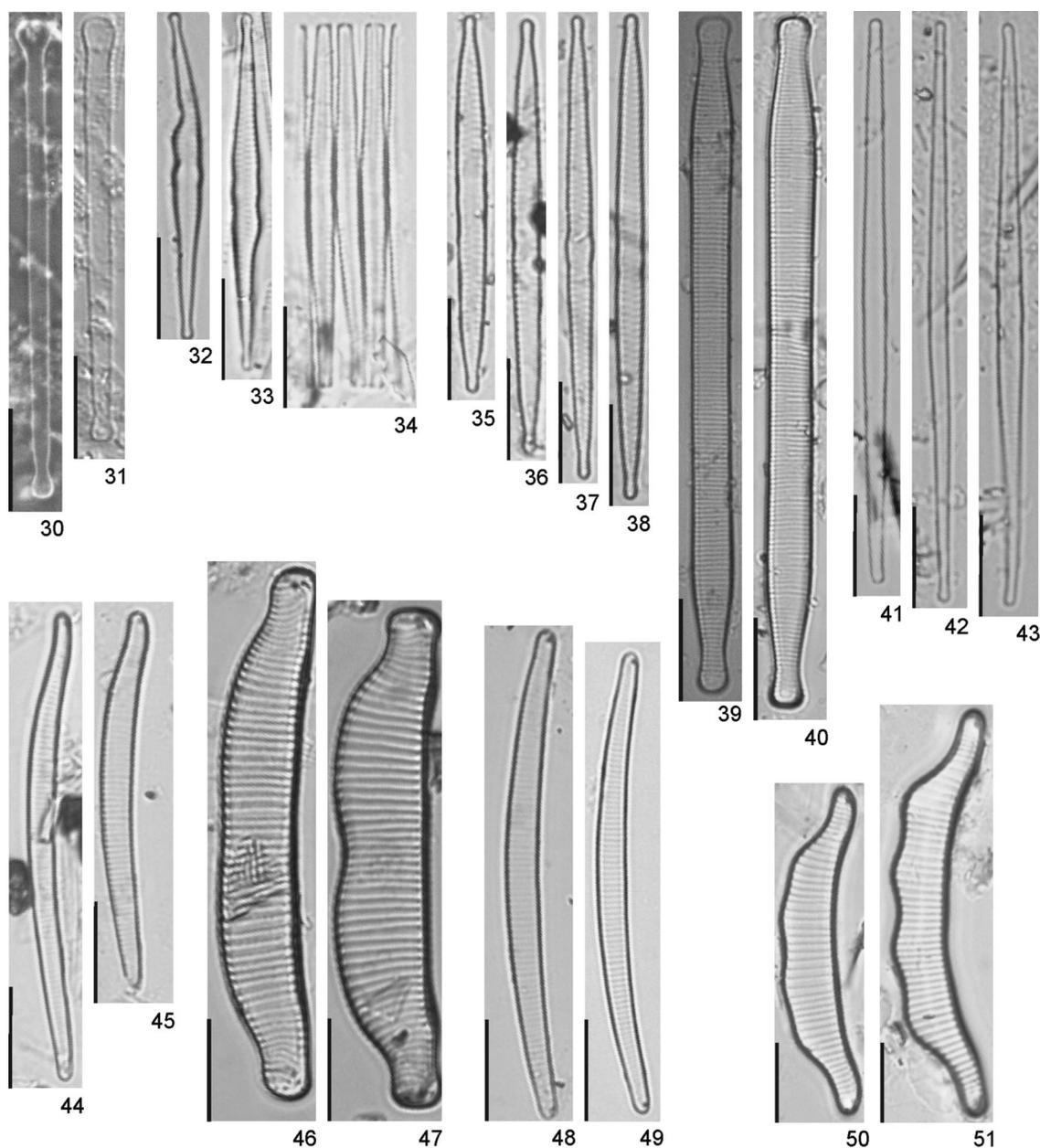
Fragilariaceae Greville

*Fragilaria* Lyngbye

\**Fragilaria crotonensis* Kitton, Hardwicke's Science-Gossip 5, p. 109-110, pl. 3, 1869.

Figs. 32-34.

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**Figures 30-51.** Modern and subfossil diatom flora of Guarapiranga Reservoir, Brazil. 30-31. *Asterionella formosa*. 32-34. *Fragilaria crotonensis*. 35-38. *Fragilaria fragilarioides*. 39-40. *Fragilaria rolandschmidtii*. 41-43. *Fragilaria* sp. 1. 44-45. *Actinella lange-bertalotii*. 46-47. *Eunotia bidens*. 48-49. *Eunotia bilunaris*. 50-51. *Eunotia camelus*. Scale bars: 10 µm.

L: 32.5-76.6 µm; W: 2.6-4.0 µm; S: 17-19 in 10 µm.

*F. crotonensis* forms long raft-like chains, where the cells are joined along the valve faces (Crawford et al. 1985). It is usually found in mesotrophic to eutrophic conditions (van Dam et al. 1994, Hofmann 1994). In this study, the species was well distributed in subfossil and modern assemblages in 81% of all samples from oligo- to eutrophic conditions. Although widely distributed in ecological studies, this is the first taxonomical report for the state of São Paulo.

*Fragilaria fragilarioides* (Grunow) Chohnoky, Nova Hedwigia 5, p. 168, 1963. Figs. 35-38.

L: 22.7-48.7 µm; W: 2.6-3.3 µm; S: 12-16 in 10 µm.

This species occurred in 100% of the modern assemblages in samples ranging from mesotrophic to super-eutrophic conditions.

*Fragilaria rolandschmidtii* Metzeltin & Lange-Bertalot, Iconographia Diatomologica 5, p. 236, pl. 1, fig. 14-16, 1998. Figs. 39-40.

L: 66.1-110.0 µm; W: 3.6-4.6 µm; S: 20-22 in 10 µm.

This species was proposed from Amazonian material. Metzeltin & Lange-Bertalot (1998) mentioned its close resemblance with *Fragilariforma javanica* (Hustedt) Wetzel, Morales & Ector. It differs by the absence of spines and sternum, two difficult characteristics to observe in LM. This taxon has several features that are distinguished from *Fragilaria sensu stricto* such as narrow or absent sternum, striae, and apical pore fields, and needs ultrastructural studies to be transferred into to genus *Fragilariforma* (P.D. Almeida, personal communication in October 2015).

This species has been mistakenly reported in Brazil as *F. javanica* (e.g. Ferrari & Ludwig 2007), and this is the first report

with the correct name. It was distributed in 19% of all samples from subfossil assemblages from oligo to mesotrophic conditions.

***Fragilaria* sp. 1**

Figs. 41-43.

Valves linear with weakly convex margin; ends slightly capitated; central area indistinct, bilaterally swelling. L: 53.8-58.7 µm; W: 2.0-2.6 µm; inconspicuous striae.

This taxon resembles *Fragilaria aquaphus* Lange-Bertalot & Ulrich in relation to the valve shape. However, *F. aquaphus* presents smaller valve dimensions (L: 22-45 µm; W: 1.5-2.4 µm) and more capitated apices in smaller individuals (Lange-Bertalot & Ulrich 2014). Furthermore, additional studies on ultra-structures, such as rimoportulae, are necessary to evaluate if it is a new species. The taxon was commonly reported in modern flora (64%) in mesotrophic to eutrophic conditions.

Eunotiaceae Kützing

***Actinella* Lewis**

\****Actinella lange-bertalotii*** Kociolek, Studies on Diatoms 148, pl. 46-50, 99-103, 2001. Figs. 44-45.

L: 37.0-46.0 µm; W: 2.4-3.3 µm; S: 19-20 in 10 µm.

The species was reported in acidic and black waters in the Amazon basin (Melo et al. 2010). It occurred in 21% of modern assemblage samples in mesotrophic condition. This is the first report for the state of São Paulo.

***Eunotia* Ehrenberg**

***Eunotia bidens*** Ehrenberg, Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin, p. 413, figs. 1-4, 1841 (1843). Figs. 46-47.

L: 44.6-53.7 µm; W: 8.0-9.0 µm at the swellings, 4.4-5.6 µm at the apices; S: 10-11 in 10 µm.

Reported in oligo-mesotrophic waters (van Dam et al. 1994). It was presently well reported (41% samples) in subfossil assemblages during past oligotrophic conditions.

***Eunotia bilunaris*** (Ehrenberg) Schaarschmidt, Magyar Novenytani Lapok 5, p. 159, 261-268, 1881. Figs. 48-49.

L: 41.2-50.0 µm; W: 2.6-3.0 µm; S: 19-20 in 10 µm.

Tolerant to indifferent (van Dam et al. 1994, Hofmann 1994). It occurred in modern assemblages (21% of all samples) in mesotrophic waters.

\****Eunotia desmogonioides*** Metzeltin & Lange-Bertalot, Iconographia Diatomologica 11, p. 27, pl. 5: figs. 1-7, pl. 6: figs. 1-4, 2002 Fig. 52.

L: 115.7-126.5 µm; W: 4.0-6.0 µm; S: 13-16 in 10 µm.

Distributed in 23% of all samples and only in subfossil assemblage in oligotrophic past conditions. First report for the state of São Paulo.

\****Eunotia elephas*** Ehrenberg, Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin, p. 414, pl. 1: fig. 5, 1841 (1843). Fig. 53.

L: 113.5 µm; W: 25.0 µm; S: 15 in 10 µm.

The species was identified based on Ehrenberg's type material available in Metzeltin & Lange-Bertalot (2007) and Silva et al. (2012). It was scarcely distributed in only 1% of samples from subfossil assemblages during past oligotrophic conditions with other acidophilic *Eunotia* species. First report for the state of São Paulo.

\****Eunotia metamonodon*** Lange-Bertalot, Diatoms of Europe 6, p. 153, pl. 217: figs. 1-10, pl. 218: figs. 1-7, 2011.

Fig. 54.

L: 156.6-185.0 µm; W: 18.0-19.7 µm; S: 5-6 in 10 µm.

This species is very similar to *Eunotia monodon* Ehrenberg on the valve outline. However, *E. metamonodon* usually presents populations with higher dimensions (L: 40.0-250.0 µm, W: 10.0-15.0 µm; S: 7-11 in 10 µm) than *E. monodon* (L: 35.0-90.0 µm, W: 7.0-15.0 µm; S: 8-12 in 10 µm; Patrick & Reimer 1966).

It occurred in 36% of all samples from subfossil assemblages during past oligotrophic conditions. First report for the state of São Paulo.

***Eunotia monodon*** Ehrenberg, Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin, p. 414, pl. 2, fig. 7, 1841 (1843).

Fig. 55.

L: 76.0-88.0 µm; W: 7.0-8.0 µm; S: 10-11 in 10 µm.

Reported in oligotrophic (van Dam et al. 1994) and hypereutrophic waters (Faria et al. 2010). This species was exclusively distributed in 36% of all samples from subfossil assemblages during past oligotrophic conditions.

\****Eunotia cf. paludosa*** Grunow, Verhandlungen der kaiserlich-königlichen zoologisch-botanischen Gesellschaft in Wien 12, p. 368, pl. 7, 1862. Figs. 65-66.

Valves weakly arched; ventral margins slightly concave; dorsal margins convex; ends weakly protracted and dorsally reflexed; terminal nodules close to the poles; terminal raphe fissure short. L: 26.9-32.5 µm; W: 2.5-3.6 µm; S: 16-17 in 10 µm.

*Eunotia paludosa* presents higher striae density (E: 18-25 in 10 µm) and more protracted ends (Lange-Bertalot et al. 2011) than the individuals observed in this study. According to these authors, this is an acidophilic species. It was reported in 7% of samples of subfossil assemblages during past oligotrophic conditions. First report for the state of São Paulo.

***Eunotia pseudosudetica*** Metzeltin, Lange-Bertalot & García-Rodríguez, Iconographia Diatomologica 15, p. 57, pl. 24, fig. 15-18, 2005. Figs. 58-60.

L: 33.1-45.3 µm; W: 5.6-6.8 µm; S: 10-11 in 10 µm.

The taxon was reported by Faria et al. (2010) in a hypereutrophic reservoir. In this study, it is well distributed, occurring in 71% of modern assemblages in samples ranging from meso to supereutrophic conditions.

\****Eunotia rabenhorstiana*** (Grunow) Hustedt var. ***rabenhorstiana*** Süßwasser-Diatomeen aus dem Albert National Park in Belgisch-Kongo, p.72, 1949. Fig. 61.

L: 86.1-127.5 µm; W: 5.5-5.8 µm; S: 15-23 in 10 µm.

The species occurred in 33% of all samples and only during past oligotrophic conditions. First report for the state of São Paulo.

\****Eunotia rabenhorstii*** var. ***triodon*** Cleve & Grunow, Synopsis des Diatomées de Belgique, pl. 35, fig. 12A, 1881. Fig. 64.

L: 20.0-34.2 µm; W: 5.5-7.9 µm; S: 12-13 in 10 µm.

This variety occurs in 17% of all samples from past oligotrophic conditions. First report for the state of São Paulo.

\****Eunotia superbidens*** Lange-Bertalot, Diatoms of Europe 6, p. 229, pl. 81: figs. 1-10, pl. 82: figs. 1-6, 2011.

Fig. 56-57.

L: 57.0-67.0  $\mu\text{m}$ ; W: 11.0-11.7  $\mu\text{m}$  at the swellings, 6.5-10.0  $\mu\text{m}$  at the apices; S: 9-11 in 10  $\mu\text{m}$ .

It was reported in 41% of all samples for subfossil assemblages in past oligotrophic conditions. First report for the state of São Paulo.

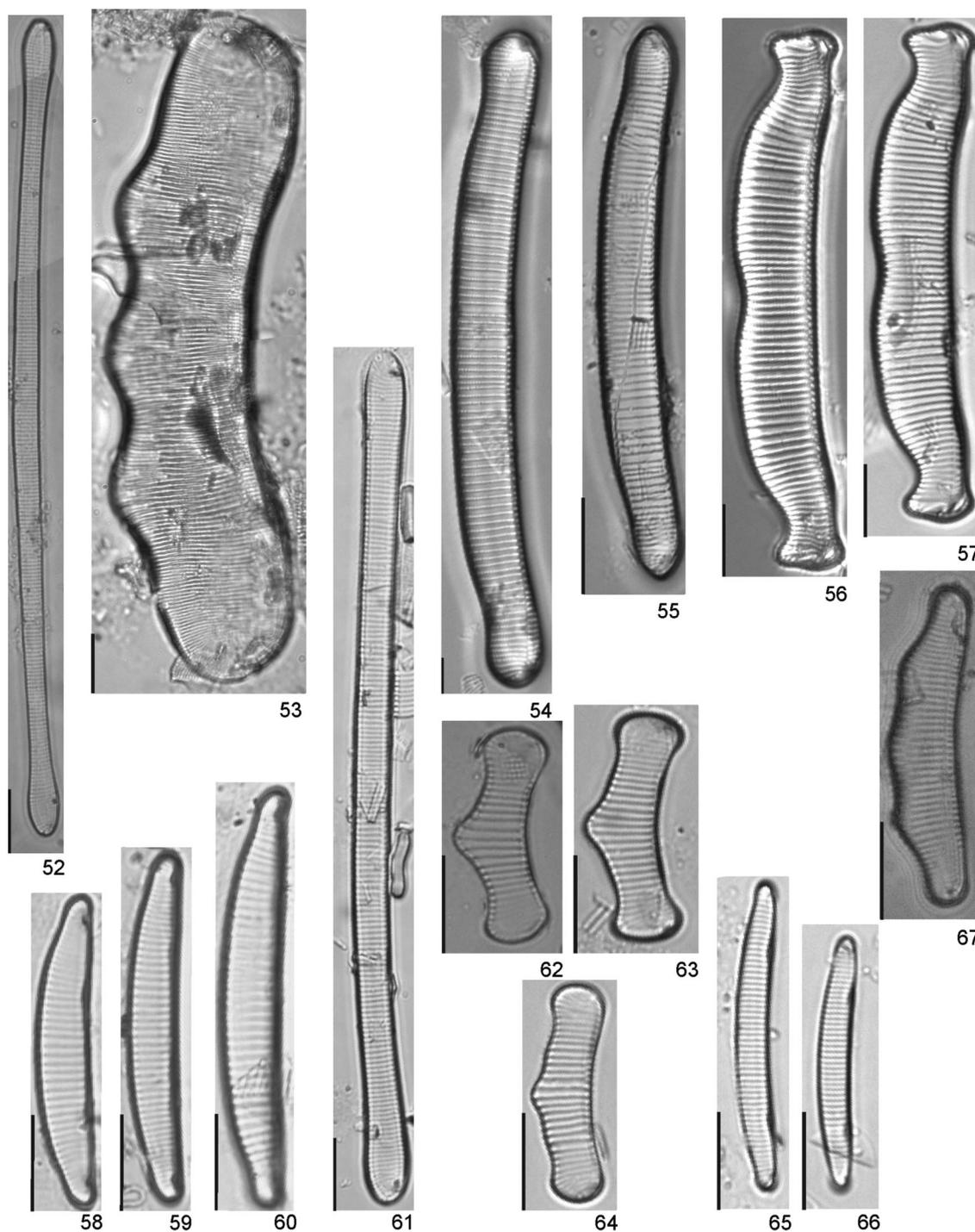
\**Eunotia tridentula* Ehrenberg, Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin, p. 414, pl.2: fig. 14, 1841 (1843).

Fig. 67.

L: 29.6-34.6  $\mu\text{m}$ ; W: 5.8-7.5  $\mu\text{m}$ ; S: 13-18 in 10  $\mu\text{m}$ .

The species was found in 9% of the samples for the subfossil assemblages during past oligotrophic conditions. However, Faria et al. (2010) reported this taxon in a hypereutrophic reservoir. First report for the state of São Paulo.

\**Eunotia trigibba* Hustedt, Atlas de Diatomaceen-kunde, pl. 286, fig. 16-18, 1913.



**Figures 52-67.** Modern and subfossil diatom flora of Guarapiranga Reservoir, Brazil. 52. *Eunotia desmogonioides*. 53. *Eunotia elephas*. 54. *Eunotia metamonodon*. 55. *Eunotia monodon*. 56-57. *Eunotia superbidens*. 58-60. *Eunotia pseudosudetica*. 61. *Eunotia rabenhorstiana* var. *rabenhorstiana*. 62-63. *Eunotia rabenhorstii* var. *monodon*. 64. *Eunotia rabenhorstii* var. *triodon*. 65-66. *Eunotia* cf. *paludosa*. 67. *Eunotia tridentula*. Scale bars: 10  $\mu\text{m}$ .

Figs. 68-69.

L: 41.3-46.8  $\mu\text{m}$ ; W: 11.0-13.1  $\mu\text{m}$ ; S: 8-9 in 10  $\mu\text{m}$ .

It was reported in 11% of samples for the subfossil assemblages in past oligotrophic conditions. First report for the state of São Paulo.

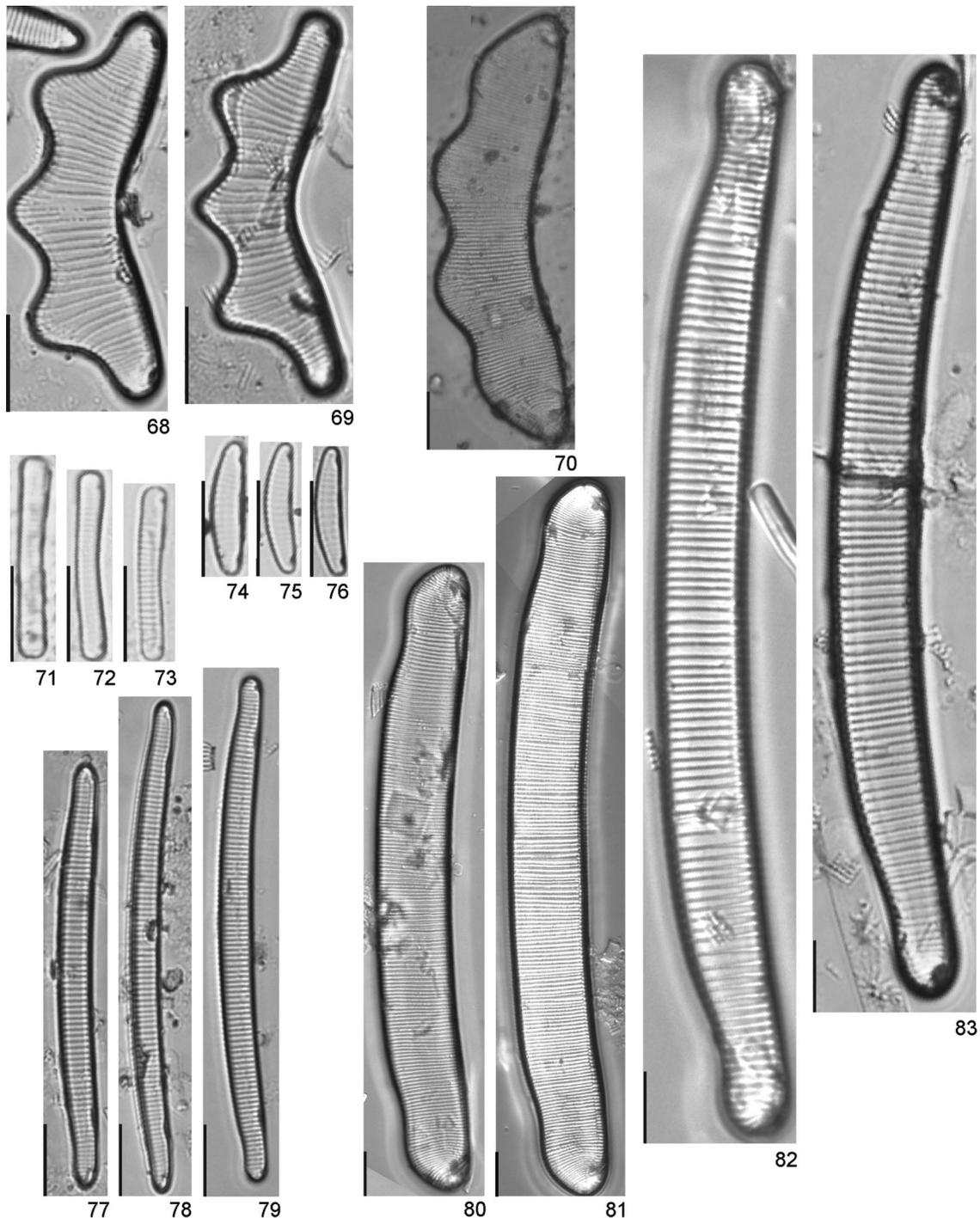
\**Eunotia tukanorum* Wetzel & D. Bicudo, Nova Hedwigia 91(1-2), p. 58, pls. 173-234 and 253, 2010.

Figs. 71-73.

L: 11.3-28.0  $\mu\text{m}$ ; W: 2.0-2.6  $\mu\text{m}$ ; S: 18-21 in 10  $\mu\text{m}$ .

*Eunotia tukanorum* is a planktonic species proposed by Wetzel et al. (2010) for Negro River (North Brazil), whose waters are generally oligotrophic and characterized by the presence of humic acids due to decomposition of flooded vegetation during the rainy season. In other regions of Brazil, the species has been reported as *E. asterionelloides* Hustedt in the plankton of oligotrophic and slightly acidic rivers (Laux & Torgan 2011), and in plankton and periphyton of a pond in the South region (Bicca et al. 2011).

In this study, the species was reported in subfossil assemblages in oligotrophic condition, period characterized by flooded vegetation



**Figures 68-83.** Modern and subfossil diatom flora of Guarapiranga Reservoir, Brazil. 68-69. *Eunotia trigibba*. 70. *Eunotia zygodon*. 71-73. *Eunotia tukanorum*. 74-76. *Eunotia* sp.1. 77-79. *Eunotia valida*. 80-81. *Eunotia* sp. 2. 82-83. *Eunotia* sp. 3. Scale bars: 10  $\mu\text{m}$ .

during the reservoir construction. Fontana et al. (2014) registered dominance of *E. tukanorum* during this phase along with the high increase in water discharge associated with the initial use of the reservoir as a public water supply. It was also reported for modern assemblages in mesotrophic conditions. This is a common species in the study area, occurring in 56% of all samples. So far, the occurrence of this species is restricted to tropical and subtropical regions and seems to be mainly associated with oligotrophic and slightly acidic environments. Although the species was cited in Fontana et al. (2014), this is the first taxonomical register for the state of São Paulo.

\**Eunotia valida* Hustedt, Die Süßwasser-Flora Mitteleuropas. Heft. 10, 2, p. 178, pl. 229, 1930.

Figs. 77-79.

L: 64.0-92.5 µm; W: 5.0-6.0 µm; S: 12-13 in 10 µm.

This is a frequent species in Holarctic regions, occasionally occurring in paleo and neotropical regions, and in oligotrophic to dystrophic waters with moderately low pH and conductivity (Lange-Bertalot et al. 2011). Indeed, this species occurred in 36% of all samples in subfossil assemblages during past oligotrophic conditions and associated with other acidophilic *Eunotia* species. This is the species first report in the state of São Paulo.

#### *Eunotia* sp. 1

Figs. 74-76.

Valves slightly arched, ventral margins straight to slightly concave, dorsal margins convex; rounded valve ends, sometimes deflected to the ventral margin; striae parallel; terminal nodule conspicuous and close the poles. L: 13.7-14.6 µm; W: 2.5-3.4 µm; S: 13-15 in 10 µm.

This taxon resembles two species. However, *Eunotia rhomboidea* Hustedt often presents heteropolar valves, higher striae density (16-18 in 10 µm) and rhomboid frustules in girdle view (Furey 2011, Furey et al. 2011), and *E. botuliformis* Wild, Nörpel & Lange-Bertalot presents less conspicuous ventral polar nodules, slightly heteropolar valves and higher striae density (15-19 in 10 µm; Lange-Bertalot 1993; Silva & Bicu 2014). The identification of this taxon depends on further populational studies.

It was distributed in 36% of all samples mainly in subfossil assemblages from oligotrophic conditions, but also in recent mesotrophic conditions.

#### *Eunotia* sp. 2

Figs. 80-81.

Ventral margin of valves concave, dorsal margin convex; valve ends slightly protracted; striae parallel, slightly spaced; terminal nodule moderately distant from poles. L: 123.0-174.3 µm; W: 11.4-15.0 µm; MS: 10-12 in 10 µm; AS: 14-15 in 10 µm.

This taxon resembles *Eunotia indica* Grunow in valve outline. However, this species presents a soft inflation in the middle and ends broadly protracted, mostly becoming obliquely wedge-shaped. Furthermore, *E. indica* presents smaller populations (L: 38.0-100.0 µm; W: 8.8-11.0 µm; Lange-Bertalot et al. 2011). The taxon was reported in 27% of samples in subfossil assemblages during past oligotrophic conditions with other acidophilic *Eunotia* species. It is probably a new species and needs further study.

#### *Eunotia* sp. 3

Figs. 82-83.

Valves slightly arched, dorsal margin convex, ventral margin slightly concave; valve ends rounded to rounded-rostrate; terminal nodule close to apices; striae transapical, parallel throughout valve.

L: 135.7-155.0 µm; W: 13.0-13.5 µm; S: 7-8 in 10 µm.

This taxon resembles *Eunotia longicollis* Metzeltin & Lange-Bertalot in valve shape. However, *E. longicollis* presents terminal nodule moderately distant from poles and populations with smaller individuals, ranging from 60.0-80.0 µm long, 7.0-7.7 µm width and 8-11 striae in 10 µm (Metzeltin & Lange-Bertalot 1998). It is probably a new species and needs further study.

The taxon was reported in 36% of all samples in subfossil assemblages in past oligotrophic conditions.

#### Cymbellaceae Greville

##### *Encyonema* Kützing

*Encyonema minutum* (Hilse) Mann, The Diatoms: Biology and morphology of the genera, p. 667, 1990.

Figs. 88-89.

L: 16.9-18.6 µm; W: 5.3-6.6 µm; S: 9-12 in 10 µm.

This species is reported in oligo- to mesotrophic conditions (van Dam et al. 1994, Hofmann 1994). However, in this study it occurred in 14% of samples of modern assemblages and in eutrophic condition.

#### Gomphonemataceae Kützing

##### *Gomphonema* Ehrenberg

*Gomphonema brasiliense* Grunow, Naturwissen Beiträge zur Kenntnis der Kaukasusländer, p. 110, pl. 3-4, 1878.

Figs. 92-93.

L: 23.1-27.3 µm; W: 6.8-7.3 µm; S: 11-12 in 10 µm.

Eutrophic species (van Dam et al. 1994), currently found in 14% of all samples in recent mesotrophic conditions.

\**Gomphonema curvipedatum* Kobayasi ex Osada, Atlas of Japanese diatoms based on electron microscopy 1, p. 10, pl. 122, fig. 1-13, 2006.

Figs. 94-96.

L: 25.6-34.6 µm; W: 4.4-6.0 µm; S: 14-16 in 10 µm.

This species was previously cited for Brazil and identified as *Gomphonema* sp. 1 by Silva et al. (2010) and Bertolli et al. (2010). It is characterized by the presence of cuneate and slightly curved apices, unlike *Gomphonema hawaiiense* Reichardt, whose apices are attenuated. The examined population is in agreement with Kobayasi et al. (2006). Ecological information is not available in the literature. Currently, it was found in 14% of all samples in recent mesotrophic conditions. It is the first report for the the state of São Paulo.

*Gomphonema gracile* Ehrenberg, Die Infusionsthierchen als vollkommene Organismen, p. 217, pl. 18, fig. 3, 1838.

Figs. 97-98.

L: 43.7-54.6 µm; W: 6.6-8.6 µm; S: 11-14 in 10 µm.

Distributed in mesotrophic to eutrophic waters (van Dam et al. 1994, Hofmann 1994). In this study it occurred in only 3% of all samples from subfossil assemblages in oligo to eutrophic conditions.

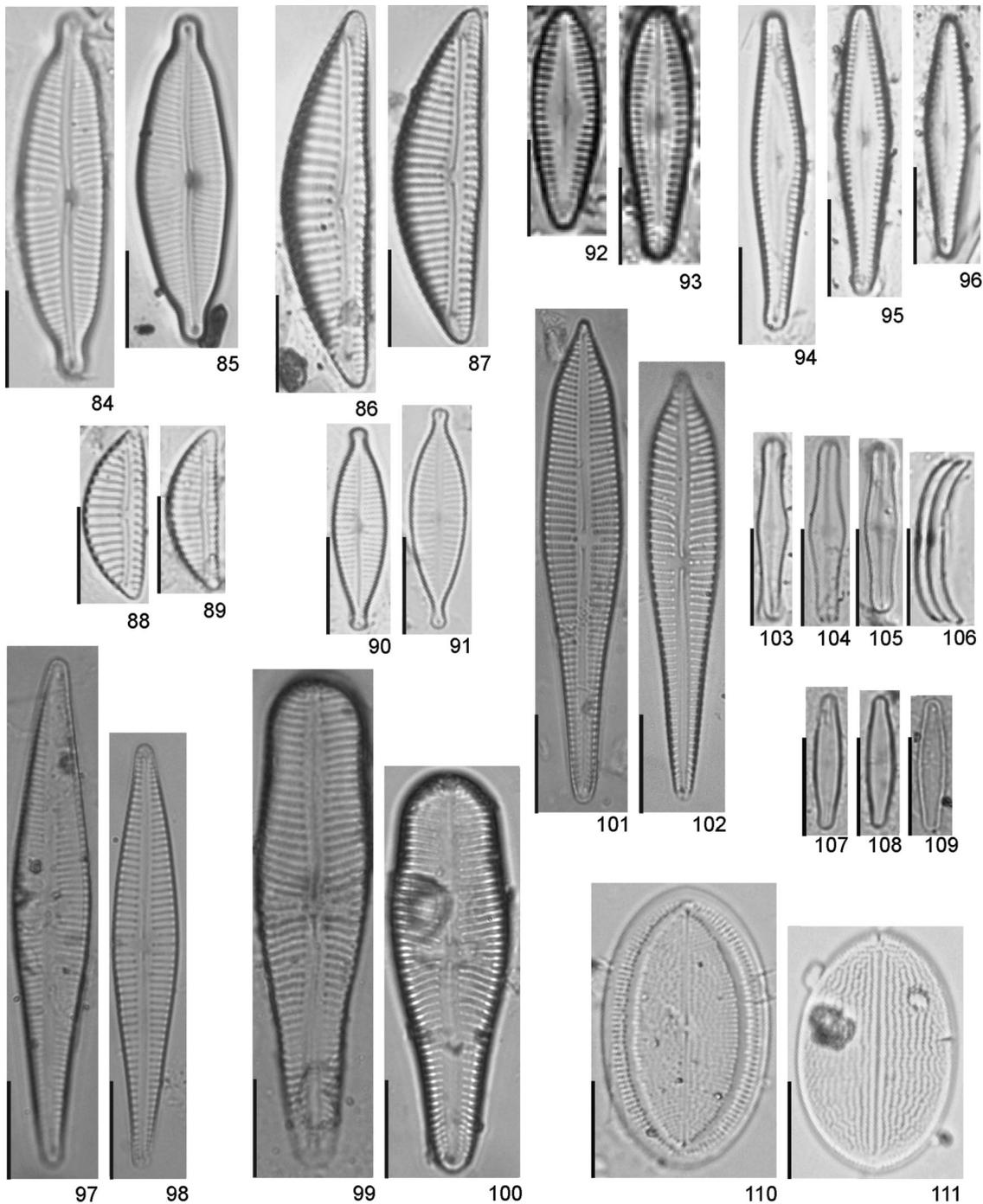
*Gomphonema lagenula* Kützing, Die Kieselschaligen Bacillarien oder Diatomeen, p. 85, pl. 30, fig. 60, 1844.

Figs. 90-91.

L: 16.2-23.2 µm; W: 5.6-6.0 µm; S: 14-16 in 10 µm.

Present in oligotrophic waters (van Dam et al. 1994). Our findings expand its range distribution, from oligo to eutrophic conditions. It occurred in 27% of all samples for subfossil and modern assemblages.

\**Gomphonema neoapiculatum* Lange-Bertalot, Reichardt & Metzeltin, Iconographia Diatomologica 5, p. 120, pl. 157, figs. 6-9, 1998. Figs. 101-102.



**Figures 84-111.** Modern and subfossil diatom flora of Guarapiranga Reservoir, Brazil. 84-85. *Cymbopleura naviculiformis*. 86-87. *Encyonema silesiacum*. 88-89. *Encyonema minutum*. 90-91. *Gomphonema lagenula*. 92-93. *Gomphonema brasiliense*. 94-96. *Gomphonema curvipedatum*. 97-98. *Gomphonema gracile*. 99-100. *Gomphonema laticollum*. 101-102. *Gomphonema neoapiculatum*. 103-105. *Achnanthisdium catenatum* valve view. 106. *Achnanthisdium catenatum* lateral view. 107-109. *Achnanthisdium minutissimum*. 110. *Cocconeis placentula* var. *lineata*. raphe valves. 111. *Cocconeis placentula* var. *lineata* rapheless valves. Scale bars: 10  $\mu$ m.

L: 44.4-51.2  $\mu$ m; W: 7.5-8.0  $\mu$ m; S: 9-13 in 10  $\mu$ m.

According to Metzeltin & Lange-Bertalot (1998) *Gomphonema neoapiculatum* has apiculate ends while *Gomphonema apiculatum* Ehrenberg presents rounded-cuneate to slightly apiculate ends.

This species was mistakenly reported as *Gomphonema apiculatum* by Faria et al. (2010) in a hypereutrophic reservoir in the State of Paraná. In this study, it occurred in 14% of all samples in contemporary eutrophic conditions. Therefore, this is first record in

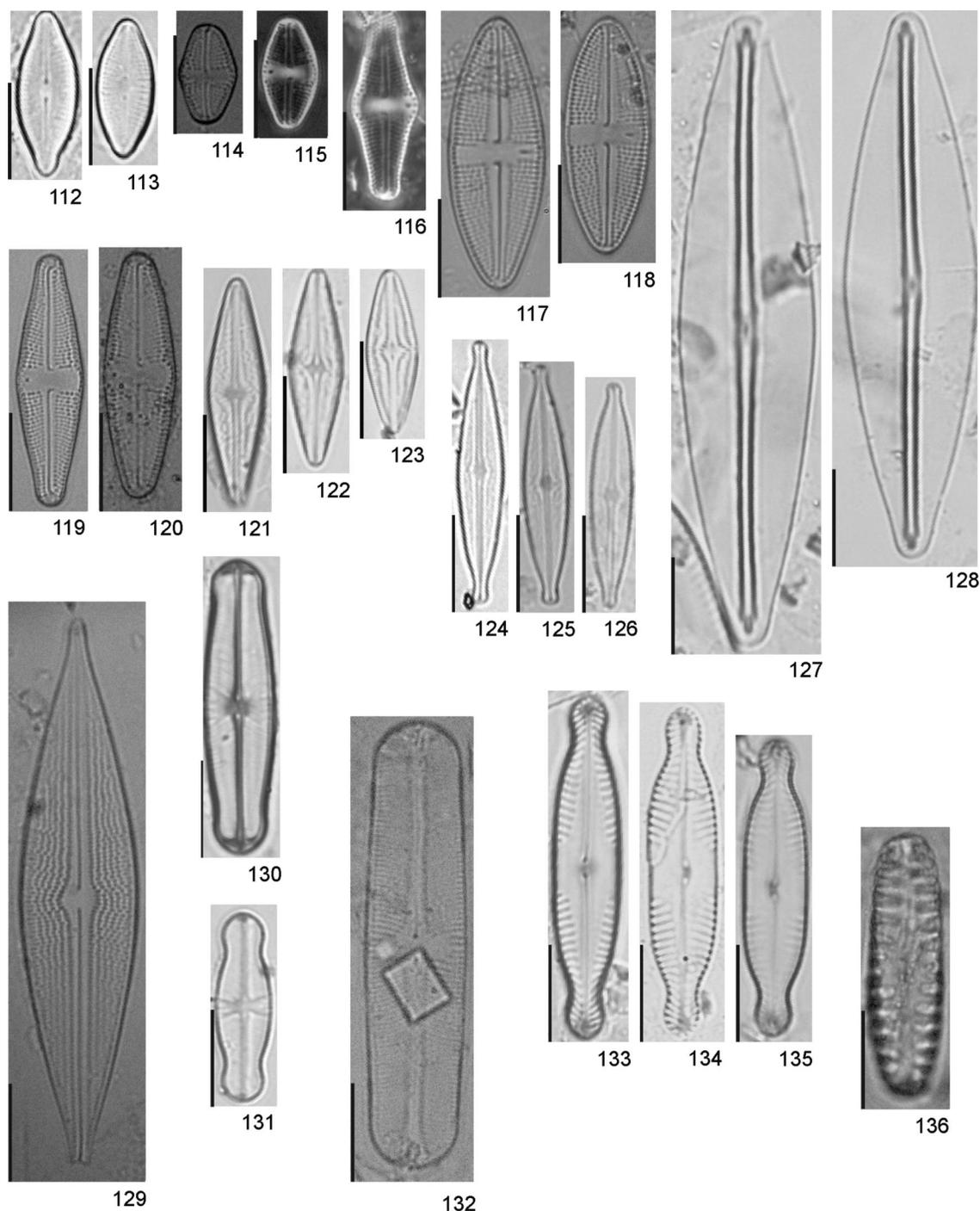
Brazil with the correct name and the first citation for the State of São Paulo.

Cocconeidaceae Kützing

*Cocconeis* Ehrenberg

*Cocconeis placentula* var. *lineata* (Ehrenberg) Van Heurck, Synopsis des Diatomées de Belgique, p. 133, 1885. Fig. 110 raphe valves, Fig. 111 rapheless valves.

## Diatoms from core and surface sediments

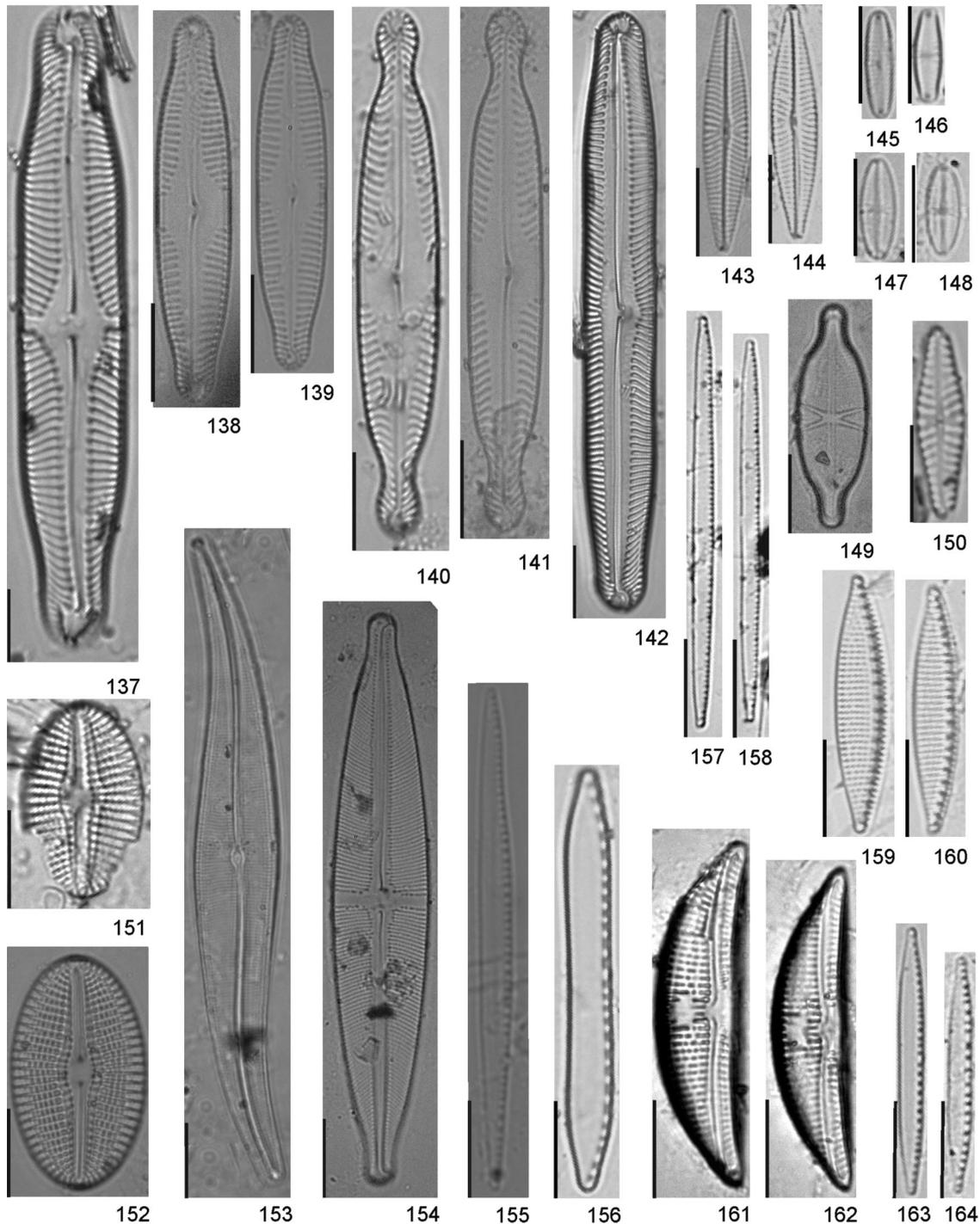


**Figures 112-136.** Modern and subfossil diatom flora of Guarapiranga Reservoir, Brazil. 112-113. *Diademsis confervacea*. 114-115. *Luticola acidoclinata*. 116. *Luticola hustedtii*. 117-118. *Luticola isabelae*. 119-120. *Luticola simplex*. 121-123. *Brachysira brebissonii*. 124-126. *Brachysira microcephala*. 127-128. *Frustulia saxonica*. 129. *Brachysira serians* var. *acuta*. 130. *Sellaphora capitata*. 131. *Sellaphora ventraloconfusa*. 132. *Sellaphora rectangularis*. 133-135. *Pinnularia brauniana*. 136. *Pinnularia borealis*. Scale bars: 10  $\mu$ m.

L: 20.6 -30.6  $\mu$ m; W: 14.0-18.0  $\mu$ m; S: 18-19 in 10  $\mu$ m in the raphe valve, 26-28 in the rapheless valve; A: 20-22 in 10  $\mu$ m in the raphe valve, 14-16 in 10  $\mu$ m  $\mu$ m in the rapheless valve.

Ehrenberg (1843) first described the species *Cocconeis lineata*. Subsequently, based on the similarity between *C. placentula* Ehrenberg var. *placentula* and *C. lineata*, Van Heurck (1885) proposed the combination *C. placentula* var. *lineata* (Ehrenberg)

van Heurck. Recently, Romero & Jahn (2013) analyzed Ehrenberg's type-material (1843) and complementary culture samples, and concluded that the morphometric differences in valves support the recognition of *C. lineata*. However, according to Potapova & Spaulding (2013) further studies are necessary to determine whether and how individual species within *C. placentula sensu lato* may be distinguished using morphological characters. Therefore, we followed AlgaBase (Guiry in Guiry & Guiry 2016).



**Figures 137-164.** Modern and subfossil diatom flora of Guarapiranga Reservoir. Brazil. 137. *Pinnularia divergens* var. *mesoleptiformis*. 138-139. *Pinnularia subgibba* var. *lanceolata*. 140-141. *Pinnularia subanglica*. 142. *Pinnularia* sp. 1. 143-144. *Navicula notha*. 145-146. *Chamaepinnularia submusculata*. 147-148. *Eolimna minima*. 149. *Capartograma crucicula*. 150. *Hippodonta avittata*. 151. *Diploneis ovalis*. 152. *Diploneis subovalis*. 153. *Gyrosigma acuminatum*. 154. *Stauroneis acidoclinata*. 155. *Nitzschia gracilis*. 156. *Nitzschia terrestris*. 157-158. *Nitzschia fruticosa*. 159-160. *Nitzschia amphibia*. 161-162. *Amphora copulata*. 163-164. *Nitzschia palea* var. *debilis*. Scale bars: 10  $\mu$ m.

This taxon is widely reported in Brazilian literature (as *C. placentula* var. *lineata*), suggesting it is a cosmopolitan species. It is common in eutrophic environments (Hofmann 1994, van Dam et al. 1994). In this study, it occurred in 21% of all samples including past oligotrophic condition and modern meso to eutrophic conditions, suggesting a broader trophic spectrum distribution.

#### Achnanthidiaceae Mann

##### *Achnanthidium* Kützing

*Achnanthidium catenatum* (Bily & Marvan) Lange-Bertlot, *Iconographia Diatomologica* 6, p. 277, 1999.

Fig. 103-105 valve view. Fig. 106 lateral view.

L: 14.4-20.0  $\mu$ m; W: 3.2-3.7  $\mu$ m; inconspicuous striae.

*Achnantheidium catenatum* is very similar to *Achnantheidium minutissimum* Kützing on valve view, but easily recognized in girdle view due to the "C" shaped-valves. Furthermore, *A. catenatum* presents a widened central portion resulting in an undulated valve margin (Hlúbikova et al. 2011). Bicudo et al. (2009) first registered this species in the state of São Paulo. However, this species was probably previously misidentified as *A. minutissimum*. *Achnantheidium catenatum* is an indicator of organic pollution (Berthon et al. 2011). In a paleoreconstruction of eutrophication of a Brazilian reservoir, this species highlighted the onset of a marked eutrophication phase (Costa-Böddeker et al. 2012). In the present study, it was very frequent occurring in 71% of all samples from oligo to eutrophic conditions for subfossil and modern assemblages. According to Fontana et al. (2014), this species achieved higher abundances during the major cultural eutrophication phase of Guarapiranga Reservoir. Overall, this species is probably an indicator of an environmental shift particularly associated with the eutrophication process.

#### Diadesmidaceae Mann

##### *Diadesmis* Kützing

*Diadesmis confervacea* Kützing, Bacillarien oder Diatomeen, p. 109, pl. 30, fig. 8, 1844.  
Figs. 112-113.

C: 15.0-18.0 µm; L: 6.0-6.6 µm; S: 22-29 in 10 µm.

Registered in eutrophic waters (van Dam et al. 1994). Presently, this species had broader distribution from recent mesotrophic to supereutrophic conditions, occurring in 43% of all samples.

##### *Luticola* Mann

\**Luticola acidoclinata* Lange-Bertalot, Iconographia Diatomologica 2, p. 76, pl. 24; figs. 24-26, pl. 104, figs. 10-16, 1996.  
Figs. 114-115.

L: 10.3-12.0 µm; W: 6.0-6.6 µm; S: 19-23 in 10 µm; central area surrounded by 4 areolae.

Levkov et al. (2013) provided a thorough discussion of this species' taxonomical problem. The authors considered *L. intermedia* (Hustedt) Levkov, Metzeltin & Pavlov the most similar species to *L. acidoclinata* and pointed out some subtle differences. In *L. acidoclinata*, the striae has a coarser appearance (8-22 striae in 10 µm), central area is surrounded by 3-4 areolae with strongly shortened central striae. *L. intermedia* presents 22-24 striae in 10 µm, and central area bordered with 5 areolae. In addition, *L. acidoclinata* inhabits oligotrophic, slightly acidic and cold habitats in temperate zones, while *L. intermedia* occurs in rich waters in tropical/subtropical regions. Presently rare and only registered in 1% of all samples for subfossil assemblages from oligo to mesotrophic conditions. This is the first citation for the state of São Paulo.

\**Luticola hustedtii* Levkov, Metzeltin & Pavlov, Diatoms of Europe 7, p. 131, pl. 24, fig. 49, pl. 166, fig. 24-37, pl. 168, fig. 7-26, pl. 170, fig. 1-7, 2013.  
Fig. 116.

L: 19.4 µm; W: 6.8 µm; S: 23 in 10 µm.

This species might be misidentified as *Luticola lagerheimii* (Cleve) Mann, since both species have rhombic valves expanded in the middle. However, both are easily distinct in their valve size, shape of the central area, position of the stigma and the shape of the areolae (Levkov et al. 2013). The latter authors reported its distribution in several localities from Brazil, Indonesia, Congo,

Tanzania, New Caledonia and Yowa (USA) (Levkov et al. 2013). Presently it was found in only 1% of all samples during past oligotrophic conditions. This is the first citation for the state of São Paulo.

\**Luticola isabelae* Metzeltin & Levkov, Diatoms of Europe 7, p. 140, pl. 106, fig. 6, pl. 108, fig. 1-21, pl. 109, fig. 1-20, 2013.  
Figs. 117-118.

L: 13.3-28.7 µm; W: 6.6-10.0 µm; S: 21-24 in 10 µm.

This species resembles *Luticola muticoides* (Hustedt) Mann, however the second species has linear-elliptic valves with finally punctate and higher striae density (26-30 in 10 µm, Levkov et al. 2013). No ecological information was found. It is a common species occurring in 41% of all samples only in subfossil assemblages in past oligotrophic conditions. This is the first citation for the state of São Paulo.

\**Luticola simplex* Metzeltin, Lange-Bertalot & García-Rodríguez, Iconographia Diatomologica 15, p. 117, pl. 87, fig. 1-9, 2005.  
Figs. 119-120.

L: 25.6-26.0 µm; W: 6.8-8.0 µm; S: 22-23 in 10 µm.

*Luticola simplex* occurs in subtropical regions of South America (Levkov et al. 2013). Ecological information was not found in literature. This study indicates a broad distribution range, occurring in 8% of all samples from subfossil assemblages (oligotrophic period) and modern assemblages (in eutrophic condition).

#### Amphipleuraceae Rabenhorst

##### *Frustulia* Rabenhorst

*Frustulia saxonica* Rabenhorst, Die Algen Sachsens, p. 50, pl. 7, fig. 1, 1853.  
Figs. 127-128.

L: 57.5-65.6 µm; W: 14.0-14.6 µm; inconspicuous striae.

Reported in oligotrophic waters (van Dam et al. 1994). Presently found in 47% of all samples and for a broader ecological range, including subfossil assemblages for oligotrophic to eutrophic conditions.

#### Brachysiraceae Mann

##### *Brachysira* Kützing

*Brachysira brebissonii* Ross, Journal of the Marine Biological Association of the United Kingdom 66(3), p. 607, 1986.  
Figs. 121-123.

L: 18.0-24.6 µm; W: 5.3-6.2 µm; S: 22-24 in 10 µm.

Distributed in oligotrophic waters (van Dam et al. 1994, Hofmann 1994). Presently found in 21% of all samples in modern mesotrophic condition.

*Brachysira microcephala* (Grunow) Compère, Bulletin du Jardin Botanique National de Belgique, p. 26, fig. 94, 1986.  
Figs. 124-126.

L: 23.7-28.6 µm; W: 4.0-5.0 µm; inconspicuous striae.

According to Wolfe & Kling (2001), this species was traditionally named *Anomoeoneis vitrea* (before the resurrection of *Brachysira*). However, a detailed study by Compère, in 1988, showed that *Navicula microcephala* is the earliest available basionym for the diatom equivalent to *A. vitrea*, and thus the correct name within *Brachysira* is *B. microcephala*. Furthermore, Lange-Bertalot & Moser (1994) did not consider Compère's observations of the lectotype for *B. microcephala* and published another name for the same diatom: *B. neoexilis*

(Wolfe & Kling 2001). Therefore, *B. microcephala* was registered in Brazil as *Brachysira vitrea* in the state of Paraná (Tavares & Valente-Moreira 2000; Ludwig et al. 2005) and as *B. neoexilis* in the state of São Paulo (Silva & Bicudo 2014).

*Brachysira microcephala* is a highly polymorphic (ranging from lanceolate to capitate forms) and cosmopolitan diatom distributed in clean and circumneutral to slightly acidic waters (Denys & Straaten 1992, Wolfe & Kling 2001). It is commonly found in periphyton and metaphyton worldwide (Czarnecki et al. 1995, Potapova & Charles 2003, Ibarra et al. 2009), and in pristine environments in paleolimnological studies (Grenier et al. 2006). In the present study it occurred in 42% of all samples, including subfossil (oligotrophic conditions) and modern assemblages (mesotrophic to eutrophic conditions).

\**Brachysira serians* var. *acuta* (Hustedt) Vyverman, Bibliotheca Diatomologica 22, p. 49, pl. 36, figs. 3 a-d, pl. 132, fig D, 1991. Fig. 129.

L: 57.5 µm; W: 12.5 µm; S: 25 in 10 µm.

It differs from the typical variety due to the end shapes, more acute in *B. serians* var. *acuta*. Registered in oligotrophic waters by Rodrigues (1984). In this study, var. *acuta* occurred in 30% of all samples for subfossil assemblages in past eutrophic conditions. This is the variety's first report for the state of São Paulo.

#### Sellaphoraceae Mereschkowsky

##### *Sellaphora* Mereschkowsky

\**Sellaphora capitata* Mann & McDonald, Phycologia 43(4), p. 477, figs 4j-l, figs 38-42, 2004. Fig. 130.

L: 12.3-27.8 µm; W: 5.2-6.6 µm; S: 18-22 in 10 µm; central area: 45-55% of the valve width.

*Sellaphora capitata* differs from *S. pupula* (Kützing) Mereschkowsky by presenting subcapitated ends, sinuous raphe, lower striae density (16-22 in 10 µm) that are strongly radiated across the valve surface (Mann et al. 2004). This is a poorly known species in Brazil, and was probably only recorded for the southern region of Brazil by Santos et al. (2011). No ecological information is available. It presently occurred in 21% of all samples in modern assemblages from mesotrophic to eutrophic conditions. This is the species' first citation for the state of São Paulo.

\**Sellaphora rectangularis* (Gregory) Lange-Bertalot & Metzeltin, Iconographia Diatomologica 2, p. 102, pl. 25, figs. 10-12, pl. 125, fig. 7, 1996. Fig. 132.

L: 47.0 µm; W: 10.6 µm; S: 17 in 10 µm; central area: 71% of the valve width.

*Sellaphora rectangularis* belongs to the group '*pupula*' (Mann et al. 2008), but differs from *S. pupula* (Kützing) Mereschkowsky by presenting linear elliptical valves, with broadly rounded poles, and parallel valve edge or slightly convex. Can be confused with *Sellaphora laevis* (Kützing) Mann, however this has grooves enclosing the raphe system and polar bars absent.

Reported in mesotrophic waters (van Dam et al. 1994). In Brazil this taxon was recorded for the Central-Western (Delgado & Souza 2007) and Southern regions (Santos et al. 2011). In our data, it was a common species occurring in 44% of all samples in subfossil assemblages during past oligotrophic conditions. This is the species' first citation for the state of São Paulo.

\**Sellaphora ventraloconfusa* (Lange-Bertalot) Metzeltin & Lange-Bertalot, Iconographia Diatomologica 5, p. 212, 1998. Fig. 131.

L: 16.1-19.4 µm; W: 5.6-5.8 µm; inconspicuous estriae; central area: 71% of the valve width.

The species has probably only been cited by Santos et al. (2011) for the Southern region of Brazil. Ecological information is not available in literature. In the present study, it was registered in 14% of all samples in recent mesotrophic conditions. This is the species' first report for the state of São Paulo.

#### Pinnulariaceae Mann

##### *Pinnularia* Ehrenberg

\**Pinnularia brauniana* (Grunow) Mills, Index of Genera and species of Diatomaceae and their synonyms, p. 1273, 1934. Figs 133-135.

L: 31.2-36.6 µm; W: 6.5-7.3 µm; S: 10-13 in 10 µm.

Reported in oligotrophic waters (van Dam et al. 1994). In this study, it was registered in 14% of all samples in recent mesotrophic to eutrophic conditions. This is the species' first register for the state of São Paulo.

*Pinnularia borealis* Ehrenberg, Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin, p. 420 (132), pl. ½, fig. 6, pl. 4/1, fig. 5, 1843. Fig. 136.

L: 27.5-29.9 µm; W: 7.5-8.0 µm; S: 6-7 in 10 µm.

Registered in oligo-mesotrophic waters (Hofmann 1994, van Dam et al. 1994). Presently distributed in 7% of all samples in past oligotrophic phase of the reservoir.

*Pinnularia divergens* Smith var. *mesoleptiformis* Krammer & Metzeltin, Iconographia Diatomologica 5, p. 170, pl. 173, fig. 1-4, 1998. Fig. 137.

L: 70.6-74.0 µm; W: 11.3-12.0 µm; S: 9-11 in 10 µm.

*Pinnularia divergens* var. *mesoleptiformis* differs from the type variety in presenting valves with more pronounced undulations, more linear and subcapitated ends (Frenguelli 1933, Metzeltin & Lange-Bertalot 1998). This variety is poorly known in Brazil, and was only registered in Southern region by Rocha & Bicudo (2008). No ecological information is available. It was rare and occurred in only 1% of all samples in subfossil assemblages in past oligotrophic conditions.

*Pinnularia subanglica* Krammer, Diatoms of Europe 1, p. 108, 223, pl. 78, fig. 7; pl. 84, fig. 107, 2000. Figs. 140-141.

L: 53.3-56.6 µm; W: 5.3-8.6 µm; S: 9-11 in 10 µm.

According to Krammer (2000), this is a benthonic species, with preference for highly oxygenated waters. This species was found in Brazilian planktonic samples (Rocha & Bicudo 2008). It occurred in 20% of all samples for subfossil assemblages in past oligotrophic conditions.

*Pinnularia subgibba* Krammer var. *lanceolata* Gaiser & Johansen, Diatom Research 15(1), p. 117, fig. 90-91, 2000. Figs. 138-139.

L: 37.5-40.6 µm; W: 7.2-8.1 µm; S: 11-13 in 10 µm.

*Pinnularia subgibba* var. *lanceolata* belongs to the *P. gibba* Ehrenberg and *P. gibbiformis* Krammer complexes, differing from the first by not having capitated ends and, from the second, by having larger length/width ratio and central area

with fascia (Gaiser & Johansen 2000). Rocha & Bicudo (2008) registered this taxon in the plankton, metaphyton and periphyton in São Paulo, but ecological information was unavailable. In the present study, the taxon was rare, and occurred in 1% of all samples of subfossil assemblages in the past oligotrophic conditions of the reservoir.

#### ***Pinnularia* sp. 1**

Fig. 142.

Valves elliptic-lanceolate; margin straight to slightly convex; axial area asymmetric; central area asymmetric, proximal ends in hooked shape, deflected to the same side; robust striae, radiate in the center becoming slight convergent towards the ends. L: 82.6 µm; W: 10.0 µm; S: 11 in 10 µm.

The taxon resembles *Pinnularia toscana* Krammer, however the later has wider valve (17.0-20.0 µm) and bent raphe (Krammer 2000). It occurred in 21% of all samples for subfossil assemblages only during past oligotrophic conditions.

#### ***Chamaepinnularia* Lange-Bertalot & Krammer**

\*\**Chamaepinnularia submusvicola* (Krasske) Lange-Bertalot, Bibliotheca Diatomologica 38, p. 27, pl. 3, fig. 1-4, 1998. Figs. 145-146.

Valves lanceolate; rounded to attenuate-rounded ends; raphe sternum filiform and narrow; raphe straight; central area slightly lanceolate; striae radiate throughout the entire valve. L: 10.0-11.3 µm; W: 2.5-2.8 µm; inconspicuous striae.

It differs from *Chamaepinnularia mediocris* (Krasske) Lange-Bertalot because the second species presents slightly swollen valves in the median portion (Metzeltin & Witkowski 1996). No ecological information was available in literature. In this study, *C. submusvicola* was found in 7% of all samples in recent mesotrophic conditions. This is the first report of species for Brazil.

#### Diploneidaceae Mann

##### ***Diploneis* (Ehrenberg) Cleve**

***Diploneis ovalis* (Hilse) Cleve, Acta Societatis pro Fauna et Flora Fennica 8(2), p. 44, pl. 2, 1891. Fig. 151.**

L: 21.3 µm; W: 13.1 µm; S: 11 in 10 µm; A: 16 in 10 µm.

Registered in oligotrophic waters (van Dam et al. 1994, Hofmann 1994). It was sparsely distributed occurring in 1% of all samples in past oligotrophic conditions.

***Diploneis subovalis* Cleve, Kongliga Svenska Vetenskapsakademiens Handlingar, Series 4 26(2), p. 96, pl. 1, fig. 27, 1894. Fig. 152.**

L: 27.5 µm; W: 15.5 µm; S: 11 in 10 µm; 16 in 10 µm.

No ecological information was found. It was rare, occurring in 1% of all samples in past oligotrophic conditions.

#### Naviculaceae Kützing

##### ***Navicula* Bory**

***Navicula notha* Wallace, Notulae Naturae Bacillarien order Diatomeen, p. 4, pl. 1, figs. 4A-D, 1960. Figs. 143-144.**

L: 26.9-43.3 µm; W: 5.0-7.3 µm; S: 14-17 in 10 µm.

Distributed in oligotrophic to mesotrophic waters (van Dam et al. 1994). In this study, it occurred in 33% of all samples in all trophic state gradients in subfossil and modern assemblages.

##### ***Eolimna* Lange-Bertalot & Schiller**

***Eolimna minima* (Grunow in Van Heurck) Lange-Bertalot & Schiller, Bibliotheca Diatomologica 38, p. 153, 1998. Figs. 147-148.**

L: 8.6-10.6 µm; W: 3.4-4.0 µm; inconspicuous striae.

Eutrophic to tolerant species (van Dam et al. 1994, Hofmann 1994). The species was found in 43% of all samples of modern assemblages from mesotrophic to eutrophic conditions.

##### ***Hippodonta* Lange-Bertalot, Witkowski & Metzeltin**

**\**Hippodonta avittata* (Cholnoky) Lange-Bertalot, Metzeltin & Witkowski, Iconographia Diatomologica 4, p. 253, pl. 1, figs. 30-34, 1996.**

Fig. 150.

L: 17.6-20.3 µm; W: 5.0-5.3 µm; S: 10-12 in 10 µm.

Species reported in mesotrophic to eutrophic waters (van Dam et al. 1994). It was found in 14% of all samples of modern assemblages in mesotrophic conditions. This is the species' first citation for the state of São Paulo.

#### Pleurosigmales

##### ***Gyrosigma* Hassall**

**\**Gyrosigma acuminatum* (Kützing) Rabenhorst, Die Süßwasser-Diatomeen (Bacillarien.): für Freunde der Mikroskopie, p. 47, pl. 5, fig. 5a, 1853.**

Fig. 153.

L: 82.8 µm; W: 10.7 µm; inconspicuous striae.

Reported in eutrophic waters (Luchini & Verona 1972). Our data expanded its distribution for oligotrophic conditions. It was found in 7% of all samples in past oligotrophic phase of the reservoir. Although cited in ecological studies, this is the species' first taxonomical citation for the state of São Paulo.

#### Stauroneidaceae Mann

##### ***Stauroneis* Ehrenberg**

**\*\**Stauroneis acidoclinata* Lange-Bertalot & Werum, Iconographia Diatomologica 13, p. 173, pl. 42, figs. 1-12, 2004. Fig. 154.**

Valves linear-lanceolate; subcapitate ends; proximal ends of the raphe slightly curved to the same side; axial area linear; central broad area with stauros slightly larger near valve margin; striae radiate and absent in the stauro. L: 69.8-73.8 µm; W: 12.0-12.3 µm; S: 19-20 in 10 µm; A: 23-25 in 10 µm.

The studied population presented larger valves dimensions and lower striae density than proposed in Werun & Lange-Bertalot (2004; L: 35-60 µm; W: 8.5-10.5 µm, 25-30 striae in 10 µm). According to these authors, *S. acidoclinata* was found in a fountain in Germany with low conductivity water, associated with acidophilic *Eunotia* species. Further ecological information was not found. In this study, the species also occurred with other abundant *Eunotia* species and in 24% of all samples during past oligotrophic phase of the reservoir. First citation for Brazil.

#### Bacillariaceae Ehrenberg

##### ***Nitzschia* Hassall**

***Nitzschia amphibia* Grunow, Verhandlungen der kaiserlich-königlichen zoologisch-botanischen Gesellschaft in Wien 12, p. 574, pl. 12, fig. 23, 1862. Figs. 159-160.**

L: 17.3-28.0 µm; W: 4.4-6.0 µm; S: 15-16; F: 9-10 in 10 µm.

Considered a eutrophic species (van Dam et al. 1994). Our data reported the species in 25% of all samples of modern assemblages from mesotrophic to super-eutrophic conditions.

\**Nitzschia fruticosa* Hustedt, Die Diatomeenflora des Flußsystems der Weser im Gebiet der Hansestadt Bremen 34(3), 349, pl. 1, 1957. Figs. 157-158.

L: 40.3-44.0 µm; W: 1.9-2.2 µm; inconspicuous striae; F: 15-17 in 10 µm.

*Nitzschia fruticosa* Hustedt is characterized by the presence of stellate colonies. Although no colonies were found in sediment samples, further analyses of planktonic materials showed typical stellate colonies, allowing its identification.

*Nitzschia fruticosa* was registered in Brazil for planktonic samples in pond and rivers in southern Brazil (Moro & Fürstenberger 1993, Laux & Torgan 2011). No ecological information was available in literature. In the present study, the species was reported in 59% of all samples for subfossil assemblages in a broad environmental range from past oligotrophic to eutrophic conditions, although mainly in the eutrophic phase. This is the first taxonomical report of the species in the state of São Paulo.

*Nitzschia gracilis* Hantzsch ex Rabenhorst, Hedwigia 2, p. 40, pl. 6, fig. 8, 1860. Fig. 155.

L: 51.2-52.2 µm; W: 2.8-3.4 µm; inconspicuous striae; F: 9-11 in 10 µm.

Distributed in mesotrophic to eutrophic waters (van Dam et al. 1994). It occurred in modern assemblages in 36% of all samples from eutrophic to super-eutrophic conditions.

*Nitzschia palea* var. *debilis* (Kützing) Grunow, Beiträge zur Kenntniss der Arctischen Diatomeen. Kongliga Svenska-Vetenskaps Akademiens Handlingar 17(2), p. 96, 1880. Figs. 163-164.

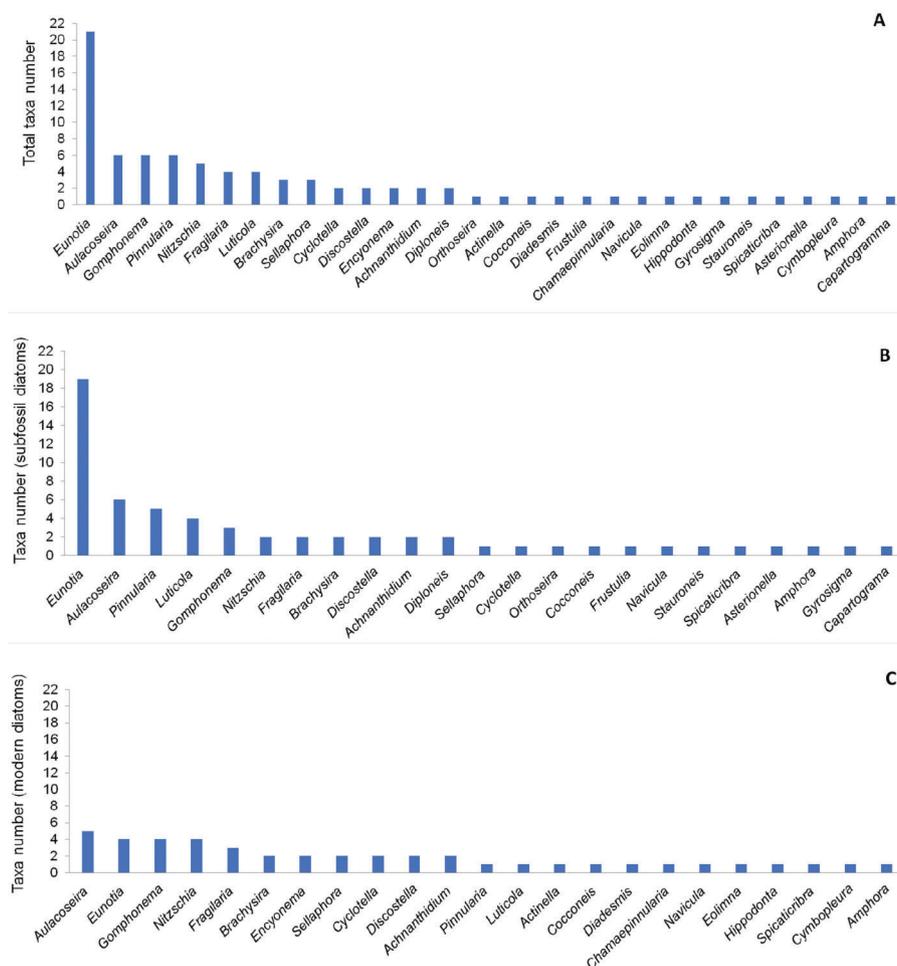
L: 22.0-28.4 µm; W: 2.0-2.6 µm; inconspicuous striae; F: 9-11 in 10 µm.

No ecological information was found. The species was widespread in modern assemblages, occurring in 93% of all samples in mesotrophic to supereutrophic conditions.

\**Nitzschia terrestris* (Petersen) Hustedt, Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin 8(9), p. 386, 1934. Fig. 156.

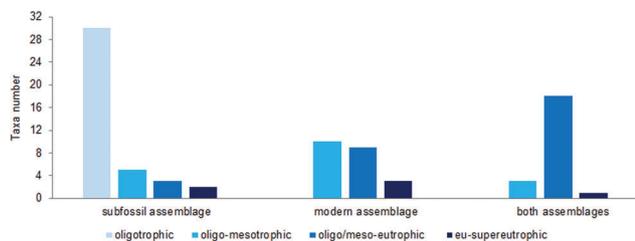
L: 19.0-45.0 µm; W: 4.2-5.0 µm; inconspicuous striae; F: 6-10 in 10 µm.

This species is not well known in Brazil, and was registered in the periphyton by Santos et al. (2011). Ecological information was not found. Currently, the species occurred in 30% of all samples in subfossil assemblages in past oligotrophic phase of the reservoir and in modern assemblages in mesotrophic conditions. Although



**Figure 165.** Taxa number per genera in Guarapiranga Reservoir. (A) for both subfossil and modern diatom assemblages; (B) for subfossil diatom (core sediment) assemblage; (C) for modern diatom assemblage (surface sediment).

## Diatoms from core and surface sediments

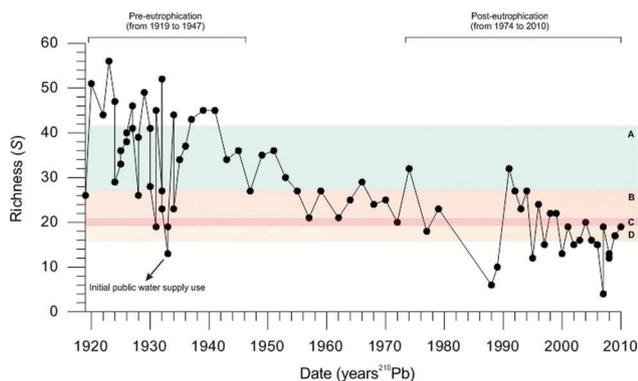


**Figure 166.** Taxa number according to trophic state gradient exclusively found in subfossil assemblages, modern assemblages and in both assemblages.

the species was cited in a paleolimnological study (Costa-Böddeker et al. 2012), this is the first taxonomical report for the state of São Paulo.

### Richness and eutrophication gradient

The subfossil and modern diatom flora of Guarapiranga comprised 84 infrageneric taxa from which 47.6% were exclusively from the subfossil assemblages. *Eunotia* was by far the most represented genus in species number, reaching a 3.5 times greater number than the second ranking genera *Aulacoseira*, *Gomphonema* and *Pinnularia* (Figure 165A), and this trend was mostly accounted by the distribution of subfossil diatoms (Figure 165B). *Eunotia* was mainly found during the initial oligotrophic phase of the reservoir (ca. 1919-1932), characterized by flooded vegetation (Atlantic Forest) for the reservoir construction. This early phase probably had a well-illuminated water column, oligotrophic and acidic waters due to the dissolved humic substances originated from the decomposition of vegetation (Fontana et al. 2014), favouring this commonly benthic/periphytic genus usually abundant in acidic oligotrophic waters (Krammer & Lange-Bertalot 1991, van Dam et al. 1994, Wetzel et al. 2010, Lange-Bertalot et al. 2011). Differently, modern assemblages presented the species number more uniformly distributed among the genera (Figure 165C).



**Figure 167.** Diatom species richness recorded over 90 years (subfossil assemblage) and in modern assemblages in surface sediments (colored horizontal zones) of the Guarapiranga Reservoir. Brackets indicate past limnological phases. For modern assemblages - A: richness under contemporary mesotrophic conditions (sites 01 to 05), B: richness in eutrophic conditions (sites 06 to 11), C: richness in supereutrophic conditions (site 12), D: richness under eutrophic conditions (sites 13-14).

Concerning diatom distribution according to trophic state range (Figure 166), the species number for the oligotrophic condition was markedly higher in the subfossil assemblages, where this phase was uniquely represented in Guarapiranga Reservoir. Furthermore, a decline in the total species number along the trophic state gradient was observed for subfossil and modern assemblages (Figure 166). This pattern was even clearer when considering the changes in species richness (number of species per sample) over time following trophic state changes in the reservoir (Figure 167). Thus, during pre-eutrophication period (up to ca. 1947), richness presented high values, achieving its highest figure over time and space in the reservoir history. However, a marked decline occurred around 1933 associated with physical and hydrological impacts when started the use of the reservoir as a public water supply and the water discharge had a seven-fold increase (Fontana et al. 2014). According to these authors, this change led to dominance of *Eunotia tukanorum*, a typical planktonic species of the genus, and disappearance of benthic species. During the transitional period with moderate cultural eutrophication (ca. 1947-1974) richness gradually declined. With the onset of eutrophication in the 1970s (Fontana et al. 2014) until 1990 there was a drastic decline in richness and the replacement of oligotrophic to eutrophic species mainly *Aulacoseira granulata* var. *granulata*, *Cyclotella meneghiniana* and *Nitzschia fruticosa* (Fontana et al. 2014). The major eutrophication period occurred particularly after ca. 1990 (Fontana et al. 2014) when an unexpected increase in richness was observed. During this period, population rapidly increased in drainage basin with expansion of slum dwellings without adequate sewage treatment (Whately & Cunha 2006). Consequently, gastroenteritis infection in local population became frequent and the agency in charge of the public water supply started using copper sulphate in 1991 to control cyanobacterial blooms (Beyruth 2000). The sudden increase in richness was very probably associated with the control of cyanobacterial dominance and the abrupt opening of new resources for other algal assemblages, including diatoms. With the intensification of urbanization and eutrophication, richness continued declining over time. Considering the space gradient, richness in modern assemblages never achieved values corresponding to the oligotrophic phase of subfossil assemblages although higher values were also achieved in contemporary mesotrophic conditions of the upstream sites 01 to 05 (Fig. 167A). Towards downstream richness decreased along the trophic state gradient (Figs. 167B, C, D).

Overall, the change in richness from oligotrophic to super-eutrophic phases led to a sharp reduction of the oligotrophic species, which represented 23% of the total number of diatom species in the reservoir mainly represented by *Eunotia* (15 species and probably 3 new species). Losses in algal biodiversity due to cultural eutrophication has been scarcely reported in Brazil (e.g. Crossetti et al. 2008) given the paucity of long-term monitoring data, and the lack of information before the onset of eutrophication. In this regard, the statement by Davidson et al. (2013) that our understanding of the relationship between anthropogenic impacts and lake biodiversity changes is typically based on contemporary data sets and space-for-time substitution holds true for Brazilian ecosystems. Therefore, in many cases paleolimnological approach can offer a unique tool to assess biodiversity changes encompassing time scales relevant to human-induced degradation and since pre-anthropogenic impacts.

## Final remarks

This survey encompassing subfossil diatom (ca. 90 years) and modern assemblages in surface sediments along an eutrophication gradient allowed the following conclusions:

- Overall, 84 infrageneric taxa were reported belonging to 71 species and eight non-typical varieties, besides five identified only to the genus level. From those, 47.6% were accounted exclusively for the subfossil assemblages indicating a significant biodiversity change over time.
- Our results expanded two new additions for Brazilian diatom flora (*Chamaepinnularia submuscicola* and *Stauroneis acidoclinata*), 30 infrageneric taxa for the state of São Paulo and four probable new species.
- Access to past oligotrophic conditions and mesotrophic regions of Guarapiranga Reservoir allowed a significant number of new additions to the Brazilian and the state of São Paulo diatom floras, which accounted for 25% of the total reservoir flora.
- Human-induced eutrophication led to a sharp decline in the oligotrophic species number, mainly of *Eunotia* and to a drastic reduction in species richness mainly since the major eutrophication period in ca. 1990.
- Abrupt changes in richness were also caused by human management as the increase in the reservoir's water discharge and the application of algacide to control cyanobacterial blooms.
- Present findings highlight the need for surveying diatom assemblages in protected environments or in less degraded conditions (considering space and time) for biodiversity assessment, and reinforce the use of paleolimnological approach in many cases as the only tool to access baseline conditions of degraded fresh waters (Smol 2008, Gregory-Eaves & Beisner 2011). These issues are crucial given freshwater ecosystems have been experiencing far greater declines in biodiversity than those recorded in terrestrial ecosystems and constitute a valuable natural resource (Dundgeon et al. 2006). Furthermore, cultural eutrophication is considered a common scenario worldwide and one of the most pervasive environmental issues affecting freshwater ecosystems (Smol 2008, Davidson & Jeppesen 2013, Cumming et al. 2015).

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## Longitudinal distribution of the ichthyofauna in a tributary of Tietê River with sources on the Basaltic Cuestas of São Paulo, Southeastern Brazil

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**Abstract:** This work aimed to study the composition and distribution of fish species at four sites along Cabeça River, an affluent of Corumbataí River, tributary of Tietê River, intending to better understand the influence of local geomorphology on the occurrence of the species along the river. Using gill nets, trawl net, cast net and sieves, five samples were taken from each site every two months. These data were analyzed from constancy of occurrence, similarity index of Jaccard, rarefaction analysis and descriptive statistical analysis of richness. 4,532 fish individuals belonging to six different orders, 17 families and 65 species were captured. Differences in composition, richness (diversity), abundance and frequency were observed at these sites along the river. The sample site near the mouth of Cabeça River, was richer (49 species) and more abundant than the other three upstream sites, which showed fewer species and equivalent amounts each other (between 22 and 29 species). Moreover, this downstream site had the highest percentage of accessory species, whereas the other samples had a greater number of constant species. Cluster analysis from the Jaccard similarity showed that each site had distinct species composition. Cabeça River basin drains a rugged terrain in the state of São Paulo, between the Basaltic Cuestas and Peripheral Depression geomorphologic provinces. In this scenario, waterfalls and rapids influence the distribution, composition and richness of fish species.

**Keywords:** *Neotropical fish, upper Paraná River, richness of species, composition, waterfalls.*

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**Resumo:** Objetivou-se com este trabalho estudar a composição e distribuição das espécies de peixes em quatro locais ao longo do Rio Cabeça, afluente do Rio Corumbataí, tributário do Rio Tietê, visando uma melhor compreensão das influências da geomorfologia local sobre a ocorrência das espécies ao longo do rio. Usando redes de emalhar, rede de arrasto, tarrafa e peneiras, foram realizadas cinco coletas em cada ponto, uma a cada dois meses. Os dados foram analisados utilizando-se constância de ocorrência, índice de similaridade de Jaccard, análise de rarefação e uma análise estatística descritiva de riqueza. Foram coletados 4.532 indivíduos de peixes pertencentes a seis ordens, 17 famílias e 65 espécies. Diferenças na composição, riqueza (diversidade), abundância e frequência foram observadas nos pontos ao longo do rio. O ponto quatro, perto da foz do Rio Cabeça, apresentou maior riqueza (49 espécies) e maior abundância, e os outros três pontos apresentaram valores menores e mais próximos entre si (entre 22 e 29 espécies). Além disso, o ponto quatro teve a maior porcentagem de espécies acessórias em comparação aos outros pontos, que apresentaram maior número de espécies constantes. A análise de agrupamento a partir da similaridade de Jaccard mostrou que cada ponto teve composição de espécies diferenciada em relação aos demais. A bacia do Rio Cabeça drena uma área caracterizada por relevo acidentado no interior do estado de São Paulo, na região de transição entre as províncias geomorfológicas Cuestas Basálticas e Depressão Periférica. Nesse cenário, cachoeiras e corredeiras ao longo de seu curso interferem na distribuição, composição e riqueza das espécies de peixes.

**Palavras-chave:** *Peixes neotropicais, Alto Rio Paraná, Riqueza de espécies, composição, cachoeiras.*

## Introduction

The Tietê River rises in the Planalto Atlântico geomorphologic province, Serra do Mar region, and drains toward interior São Paulo State, crossing other provinces and flowing to the Paraná River in the border with Mato Grosso do Sul state. In its middle course, at the Barra Bonita reservoir region, the Tietê River receives waters from the Piracicaba River, from which the Corumbataí River is an affluent. Numerous sources of Corumbataí River and part of its tributaries, such as Passa Cinco and Cabeça rivers are at the top of the Cuestas Basálticas geomorphologic province (Tavares et al., 2007), and these small rivers flow toward the geomorphologic province called Depressão Periférica, a region that lies between the Precambrian flanges of Planalto Atlântico and escarpment edges of the Cuestas Basálticas western plateau. In the Corumbataí drainage, rivers and streams flow through a rugged terrain whose waterfalls and rapids influence the distribution of fish species. Bennemann et al. (1995), Casatti (2005) e Oliveira & Garavello (2003) found that the rivers of the upper Parana River basin regularly have a natural tendency to increase in species richness from source toward the river mouth.

The river continuum concept of Vannote et al. (1980) states that features such as diversity, primary productivity, biotic relationships, and the relative importance of allochthonous material change in predictable ways over the course of a river. However, changes in the river channel may affect the characteristics of "continuity", for example: occurrence of rapids and falls that may restrict the movement of fish and influence the distribution of species in different portions of the drainage. Thus, such natural barriers may generate patterns of discontinuity in the composition and distribution of ichthyofauna (Balon & Stewart, 1983).

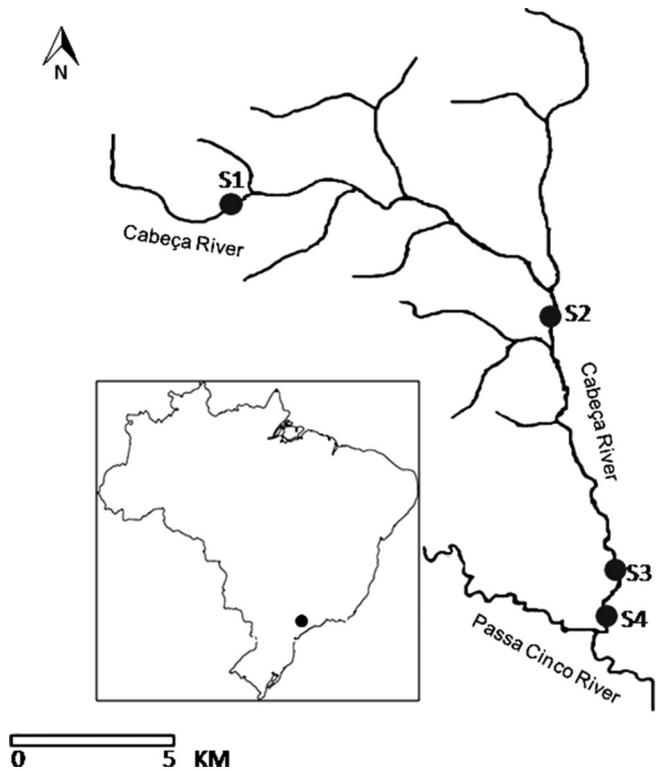
The Cabeça River, main tributary of the Passa Cinco River, has an irregular relief with not only rapids but also large waterfalls, consequently hampering the passage of fishes from downstream to upstream. Thus, this physical feature of river drainage may promote a fish distribution different from the expected for rivers in plane areas, where species richness and composition may change gradually toward the mouth. So, an inventory of fish species is presented at different sites of the drainage of the Cabeça River aiming to evaluate their longitudinal distribution, and how the local geomorphology may act on the fish communities.

## Material and Methods

### Area of study

The headwaters of Cabeça River are located at 740 meters of altitude, in Itirapina municipality, São Paulo State, south-eastern Brazil. It is a small river of the Tietê River system that flows 28 km from source to mouth on Passa Cinco River, at an altitude of 520 meters. It belongs to Corumbataí River basin in a drainage area of 211 km<sup>2</sup>, according Cetra & Petrere (2006).

From maps prepared by IBGE, satellite images (Google Earth 2010) and travels for recognition, we choose four sample sites, each one with 200 meters long. Site 1 is the closest to the spring; site 2 is in the midst and sites 3 and 4 are near the mouth with the Passa Cinco River, close to each other, but separated by Altarugio Waterfall. They were purposely chosen to study



**Figure 1.** Drainage of the Cabeça River showing the location of the four sampling sites. Scale map: 1:250.000 kilometers.

the influence of the waterfall in the distribution of fish populations (Figure 1).

### Characterization of sampling sites

Each sampling site had been characterized from the following data: geographic coordinates (Datum SAD 69), river depth and width, water speed and riparian forest. Due to the fact of each site has 200 meters in length, its physical characteristics could vary along the riverbed. The measurements were obtained in 5 locations (one every 45 meters approximately) of each sampling site. After this procedure, averages express the characteristics of the entire reach where fish were collected.

Width and depth were measured with a tape of 30 meters and the exact depth was measured from right and left banks and the center of channel. Thus, we obtained an average for each of the five sampling locations within each stretch of 200 meters and an overall average. Water velocity was measured from the time check that a float (5 cm diameter Styrofoam ball) covered a distance of three meters. This procedure was also performed at the five locations within the 200 meters stretch of the four sample sites.

### Sampling and identification of ichthyofauna

Between June 2010 and April 2011, five samplings were performed at each site. The sites 1 and 2 were sampled in June, August, October, December/2010 and February/2011 and sites 3 and 4 in September, November/2010 and January, March and April/2011.

**Table 1.** Environmental characterization of the four sampling sites on the Cabeça River, Corumbataí River basin.

Sample Site	Geographical Coordinates (UTM)	Depth (m)	Width (m)	Water velocity (m/s)	Environmental Characteristics
1	23K 215774 7531283	0.90	5.06	0.48	Narrower than other sites, comparatively deep, voluminous and fast water – features of rivers that drain steeper reliefs. Little aquatic or terrestrial vegetation.
2	23K 224851 7526385	1.03	10.50	0.34	The same depth of site 1, but twice the average width, more open water and a few deeper pools separated by rapids. Some trees and aquatic vegetation in some reaches.
3	23K 226563 7515176	1.36	14.26	0.10	The widest and deepest site, slow waters, few rapids and shallow reaches. Aquatic vegetation and submerged structures can be found as logs and rocks and the riparian vegetation is sparse.
4	23K 226675 7514907	0.90	13.72	0.34	As wide as the third site, with shallow and fast rapids. Despite of abundant riparian forest, there is no aquatic vegetation.

Fish were collected with gill nets of 1.5 to 4.0 cm among adjacent nodes, trawling (2 mm mesh), cast net (2 cm between nodes) and sieves (2 mm mesh). In each sampling, active collecting (trawls, cast nets and sieve) was made for a period of two hours and set of gill nets remained in the water from nightfall to sunrise. The sampled material was fixed in 10% formalin, preserved in 70% ethanol, and added to the fish collection of the Laboratório de Ictiologia Sistemática of the Departamento de Ecologia e Biologia Evolutiva (LISDEBE-UFSCar).

The species identification was based on comparative material housed in LISDEBE and on specialized systematic and taxonomy bibliography. Biological classification to each species is in accord to Buckup, Menezes & Ghazzi (2007).

### Data analysis

The constancy of occurrence (C) was obtained following the expression:  $C = (p / P) \times 100$ , where "p" is the number of samples in which the species was recorded, and "P" the total number of samples. The species of each site were classified according to Dajoz (1983) as: constant, if  $C \geq 50\%$ ; accessory, if  $25\% < C < 50\%$ , and occasional, if  $C \leq 25\%$ .

Jaccard similarity index was calculated by using the expression:  $Q = [C / (A + B - C)] \times 100$ , available in Magurran (1991), where: "A" is the number of species in a sample; "B" the number of species from another sample and "C" is the number of species common to both samples "A" and "B". From these results, a similarity dendrogram was obtained using the software PAST version 2.03.

For comparison of richness among the sampling sites we calculated the averages and standard deviation and performed a rarefaction analysis according to Krebs (1999), all using the PAST software, version 2.03.

## Results

### Environmental features of the sampling sites

Measurements and observations of the four sites showed the features of each site and the results are in Table 1.

### Characterization of ichthyofauna

Were sampled 4,532 individuals of fish belonging to six orders, 17 families and 65 species (Table 2). Characiformes was the most abundant and most diverse: presented 35 species in seven families (Erythrinidae, Characidae, Crenuchidae, Parodontidae, Curimatidae, Anostomidae and Prochilodontidae). Siluriformes, 21 species in five families (Callichthyidae, Loricariidae, Trichomycteridae, Heptapteridae and Pimelodidae). Gymnotiformes, two species in two families (Sternopygidae and Gymnotidae). Cyprinodontiformes, two species in a family (Poeciliidae). Perciformes, four species in a family (Cichlidae). And Synbranchiformes presented a single species of the family Synbranchidae.

In site 1, 408 individuals were sampled, belonging to 22 species. In site 2, the 1218 individuals belonged to 29 species. In site 3, the 668 individuals belonged to 25 species. In site 4, 49 species and 1476 individuals (Table 2) were sampled.

The rarefaction analysis (Figure 2) shows that, for standard samples, site 4 is richest than sites 1, 2 and 3. For a 408 individuals sampling: site 1 had 22 species; site 2, 25 species; site 3, 23 species; and site 4, 35 species.

Regarding to constancy of species, site 1 had 15 constant species (68.2%), 3 accessory species (13.6%) and 4 occasional species (18.2%). In site 2, 20 species were constant (69.0%), 4 accessory (13.8%) and 5 occasional (17.2%). Site 3, 12 species were constant (48%), 8 accessory (32%) and 5 occasional (20%). Site 4, 19 constant species (38.8%), 15 accessory (30.6%) and 15 occasional species (30.6%) (Table 2).

The similarity dendrogram showed different species composition in each of the sampled sites (Figure 3). Samples taken at sites 1, 2, and 3 were more similar to each other and in relation to themselves. Instead, site 4 they were less similar to themselves and relative to the other sites. The cophenetic correlation coefficient ( $r = 0.9484$ ) showed a low degree of distortion of the dendrogram relative to the data similarity matrix.

### Species Distribution

Fish species were distributed in the sample sites in the following way: *Cyphocharax vanderi*, only at site 1;

**Table 2.** Taxonomic list of species registered in Cabeça River, number of individuals of each species (N) and CO = constancy of species occurrence (A = accessory, C = constant and O = occasional).

	Site 1		Site 2		Site 3		Site 4	
	N	CO	N	CO	N	CO	N	CO
<b>Superorder Ostariophysi</b>								
<b>Characiformes</b>								
<b>Erythrinidae</b>								
<i>Hoplias intermedius</i> (Günther 1864)	2	A	7	C	6	C	4	A
<i>Hoplias malabaricus</i> (Bloch, 1794)	1	O	2	A	1	O	2	A
<b>Characidae</b>								
<i>incertae sedis</i>								
<i>Astyanax altiparanae</i> Garutti & Britski, 2000	31	C	37	C	2	A	586	C
<i>Astyanax cf. bockmanni</i> Vari & Castro, 2007			1	O	134	C	63	C
<i>Astyanax fasciatus</i> (Cuvier, 1819)					7	A	28	A
<i>Astyanax paranae</i> Eigenmann, 1914	9	A	34	C				
<i>Bryconamericus stramineus</i> Eigenmann, 1908					34	C	14	C
<i>Bryconamericus turiuba</i> Langeani et al., 2005					222	C	252	C
<i>Hyphessobrycon anisitsi</i> (Eigenmann, 1907)	6	C	1	O		1	O	
<i>Hyphessobrycon bifasciatus</i> Ellis, 1911			13	C	2	A		
<i>Oligosarcus pintoii</i> Campos, 1945	46	C	2	O	34	C		
<i>Piabina argentea</i> Reinhardt, 1867					71	C	12	C
<i>Salminus brasiliensis</i> (Cuvier, 1816)					1	O	2	A
<b>Characinae</b>								
<i>Roebooides</i> sp.							18	C
<b>Triporthetinae</b>								
<i>Triporthetus cf. signatus</i> (Garman, 1890)							4	A
<b>Serrasalminae</b>								
<i>Serrasalmus maculatus</i> Kner, 1858							1	O
<b>Cheirodontinae</b>								
<i>Odontostilbe</i> sp. 1							42	O
<i>Odontostilbe</i> sp. 2	12	A	6	A		12	A	
<i>Serrapinnus heterodon</i> (Eigenmann, 1915)	25	C	48	C		5	A	
<i>Serrapinnus notomelas</i> (Eigenmann, 1915)	1	O	40	C	3	A	2	O
<b>Aphyocharacinae</b>								
<i>Aphyocharax dentatus</i> Eigenmann e Kennedy, 1903							2	A
<b>Crenuchidae</b>								
<i>Characidium gomesi</i> Travassos, 1956			47	C	3	A	1	O
<i>Characidium zebra</i> Eigenmann, 1909	39	C	31	C	65	C	72	C
<b>Parodontidae</b>								
<i>Apareiodon affinis</i> (Steindachner, 1879)					7	A	9	A
<i>Parodon nasus</i> Kner, 1859					3	C	9	C
<b>Curimatidae</b>								
<i>Cyphocharax modestus</i> (Fernández-Yépez, 1948)	11	C	6	C				
<i>Cyphocharax nagelii</i> (Steindachner, 1881)							50	C
<i>Cyphocharax vanderi</i> (Britski, 1980)	1	O						
<i>Steindachnerina insculpta</i> (Fernández-Yépez, 1948)							3	C
<b>Anostomidae</b>								
<i>Leporinus aff. friderici</i> (Bloch, 1794)							2	A
<i>Leporinus lacustris</i> Campos, 1945							1	O
<i>Leporinus obtusidens</i> (Valenciennes, 1837)							2	O
<i>Leporinus octofasciatus</i> Steindachner, 1915							1	O
<i>Schizodon nasutus</i> Kner, 1858							1	O
<b>Prochilodontidae</b>								
<i>Prochilodus lineatus</i> (Valenciennes, 1836)							2	O
<b>Siluriformes</b>								
<b>Callichthyidae</b>								
<i>Corydoras aeneus</i> (Gill, 1858)	17	C	558	C				
<i>Corydoras flaveolus</i> Ihering, 1911							55	C
<i>Hoplosternum littorale</i> (Hancock, 1828)			2	A				

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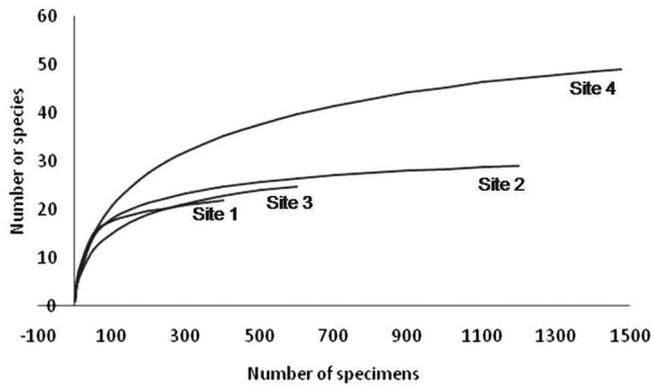
Table 2. Continued.

	Site 1		Site 2		Site 3		Site 4	
	N	CO	N	CO	N	CO	N	CO
<b>Loricariidae</b>								
<b>Loricariinae</b>								
<i>Rineloricaria latirostris</i> (Boulenger, 1900)					3	C	9	C
<b>Hypostominae</b>								
<i>Hypostomus albopunctatus</i> (Regan, 1908)							12	C
<i>Hypostomus ancistroides</i> (Ihering, 1911)	17	C	18	C	14	C	6	C
<i>Hypostomus hermanni</i> (Ihering, 1905)							9	C
<i>Hypostomus nigromaculatus</i> (Schubart, 1964)					3	O	7	C
<i>Hypostomus paulinus</i> (Ihering, 1905)							28	C
<i>Hypostomus cf. strigaticeps</i> (Regan, 1908)			26	C	13	A	87	C
<i>Hypostomus</i> sp.							9	A
<b>Hypoptopomatinae</b>								
<i>Corumbataia cuestae</i> Britski, 1997	53	C	65	C				
<b>Trichomycteridae</b>								
<b>Trichomycterinae</b>								
<i>Trichomycterus</i> sp.	14	C	12	C				
<b>Heptapteridae</b>								
<i>Cetopsorhamdia iheringi</i> Schubart e Gomes, 1959					1	O	3	A
<i>Imparfinis cf. borodini</i> Mees e Cala, 1989							4	A
<i>Imparfinis mirini</i> Haseman, 1911	5	C	40	C				
<i>Phenacorhamdia tenebrosa</i> (Schubart 1964)							1	O
<i>Pimelodella</i> sp.							2	A
<i>Rhamdia quelen</i> (Quoy e Gaimard, 1824)	5	C	3	C			15	C
<b>Pimelodidae</b>								
<i>Pimelodus maculatus</i> Lacépède, 1803					2	A		
<i>Pimelodus microstoma</i> Steindachner, 1877					2	O	4	A
<b>Gymnotiformes</b>								
<b>Sternopygidae</b>								
<i>Eigenmannia</i> sp.							2	O
<b>Gymnotidae</b>								
<i>Gymnotus</i> sp.	16	C	9	C			1	O
<b>Cyprinodontiformes</b>								
<b>Poeciliidae</b>								
<i>Phalloceros harpagos</i> Lucinda, 2008	40	C	45	C				
<i>Poecilia reticulata</i> Peters, 1859			68	C	11	C	2	O
<b>Perciformes</b>								
<b>Cichlidae</b>								
<i>Australoheros</i> sp.			4	A				
<i>Geophagus brasiliensis</i> (Quoy e Gaimard, 1824)	56	C	88	C	24	C	17	O
<i>Oreochromis niloticus</i> (Linnaeus, 1758)			5	A				
<i>Tilapia rendalli</i> (Boulenger, 1897)	1	O	3	O				
<b>Synbranchiformes</b>								
<b>Synbranchidae</b>								
<i>Synbranchus marmoratus</i> Bloch, 1795			1	O				
Total of species	22		29		25		49	
Total of individuals	408		1218		668		1476	
Mean of species by sample	15		20.4		13.2		23.8	
Standard deviation	2.12		1.51		3.5		4.6	

*Hoplosternum littorale* occurred only at site 2; and *Pimelodus maculatus* only at Site 3. Site 4 had 21 exclusive species: *Roebooides* sp., *Triportheus cf. signatus*, *Serrasalmus maculatus*, *Odontostilbe* sp.1, *Aphyocharax dentatus*, *Cyphocharax nagelii*, *Steindachnerina insculpta*, *Leporinus aff. friderici*, *Leporinus lacustris*, *Leporinus octofasciatus*, *Leporinus obtusidens*, *Schizodon nasutus*, *Prochilodus lineatus*, *Corydoras flaveolus*,

*Hypostomus albopunctatus*, *Hypostomus hermanni*, *Hypostomus paulinus*, *Hypostomus* sp., *Imparfinis cf. borodini*, *Phenacorhamdia tenebrosa*, *Pimelodella* sp., and *Eigenmannia* sp.

Only seven species appeared at all sites: *Hoplias intermedius*, *Hoplias malabaricus*, *Astyanax altiparanae*, *Serraspinus notomelas*, *Characidium zebra*, *Hypostomus ancistroides* and *Geophagus brasiliensis*. And 15 species appeared only in



**Figure 2.** Rarefaction curves for the four sampling sites on the Cabeça River, Corumbatai basin.

three sites upstream of the waterfall Altarugio, namely: *Astyanax paranae*, *Hyphessobrycon bifasciatus*, *Oligosarcus pintoii*, *Cyphocharax modestus*, *Cyphocharax vanderi*, *Corydoras aeneus*, *Hoplosternum littorale*, *Corumbataia cuestasi*, *Trichomycterus* sp, *Imparfinis mirini*, *Pimelodus maculatus*, *Phallosseros harpagos*, *Oreochromis niloticus*, *Tilapia rendalli* and *Synbranchus marmoratus*.

## Discussion

Because of the rugged terrain typical of the Cuestas Basálticas relief, there are several waterfalls and rapids in the Cabeça River drainage which influence the distribution of fish species. The Altarugio Waterfall located near the confluence of the Cabeça and Passa Cinco rivers difficult upstream migration of individuals and shoals. The highest number of species recorded in the lower course of the river (site 4), but not in the nearby site 3, attest the difficulty of fish movement upstream through the Altarugio Waterfall. The sampled site 4 contained 49 of the 65 fish species, representing 75.3% of species richness and the other three sites located upstream of the waterfall, had 42 species which together represent 64.6% of the total.

The similarities among the studied sites of Cabeça River basin reflect the differences in species composition, as well as the average and the total species richness registered in each site. Greatest similarities can be seen among the reaches to the upstream of the Altarugio fall. Considering the similarity among the samples of each site; the site 4 with 30.6 % of occasional species, presented greater variation in similarity among themselves, due to a higher occurrence of different species during the sampling period. Unlike of site 4, the other three sites are more similar among themselves, perhaps by restricting fish in such sites, showed higher occurrence of constant species, where fish populations are temporally more stable. This fact was also confirmed by the rarefaction analysis in which we note that the curves of the sites 1, 2 and 3 tend to stabilize with fewer samples than that of the downstream site 4, which apparently receives different species with greater intensity that upstream stretches. Similarly, Perez Jr. & Garavello (2007) noted that of the 13 species found upstream to waterfall of Pântano River, 11 were constant (84.6%), against a lower proportion (43.9% of 41 species sampled downstream of the waterfall) at not isolated sites.

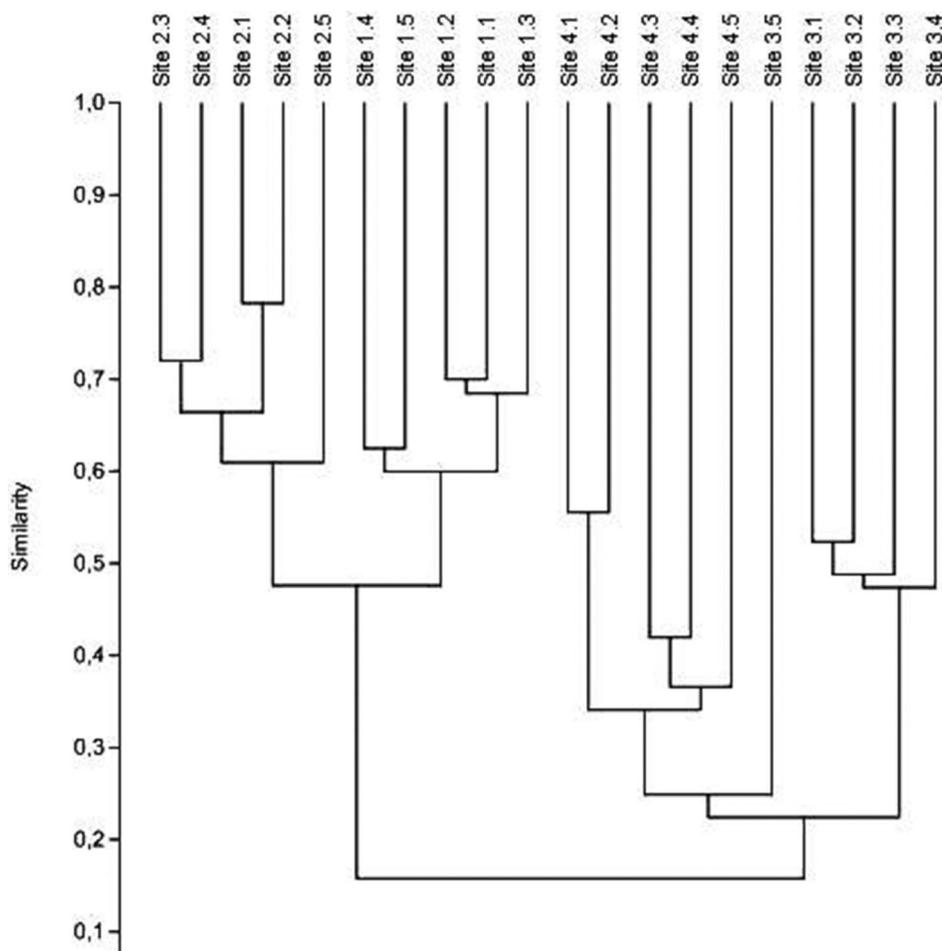
Langeani et al. (2005) has studied fish populations in different environments of Santa Barbara River (tributary of the

Tietê River), and stated that its proximity to major drainages could explain the higher number of rare species (occasional), usually found in larger rivers and that could temporarily explore smaller environments for feeding and reproduction. In fact, studies conducted in small rivers and streams from upper Paraná River basin attested the fact that the fish fauna in lower portions of the drainages are influenced by downstream greater rivers, as discussed by Pavanelli & Caramaschi (1997) when studied tributaries of the Paraná River and Oliveira et al. (2009) in tributaries of the Mogi-Guaçu River.

Although this hypothesis of stability versus temporal variability should be tested in medium and long term, the following discussion reinforces that the waterfall Altarugio acts as an important factor in the structure of fish assemblages, especially in terms of composition and ichthyofauna distribution. This becomes evident by comparison of the species richness among the closely located sites 3 and 4, and the difference of 24 more species recorded downstream from the waterfall. This waterfall influence was also observed in others studies, as by Perez Jr. & Garavello (2007), studying the Pântano River and showing large varieties of species among the sites separated by Pântano waterfall, where only 13 species out of 63 were recorded. Also, Jonck & Aranha (2010) have studied the aquatic population of two pools, separated by a waterfall in a Atlantic Rainforest stream and have concluded that there is a strong influence of the obstacle, the waterfall, especially to the organisms that spend all their life cycle in water.

On the other hand, gentler slope rivers, as Cabaceiras Stream that were studied by Oliveira & Garavello (2003), have more free movement of shoals toward the upper reaches of the basin, especially during the rainy season, when midsized and migratory species can be found, such as the *Leporinus* aff. *friderici*. In the Cabeça River, species known as migratory fish were registered only at site number 4. At this site, the occurrence of some species, such as anostomids *Leporinus obtusidens*, *Schizodon nasutus*, *Leporinus octofasciatus*, *Leporinus lacustris* and *Leporinus* aff. *friderici*, and prochilodontid *Prochilodus lineatus*, showed the difficulty for upstream fish movement imposed by Altarugio Waterfall. *Salminus brasiliensis*, belonging to the genus characterized by Sato et al. (2003) as migrant, captured to the upstream of site 4, is the only evidence of large fish toward the upper reaches of the basin. This may have happened in the wettest periods when water can flow fast enough to allow the passage of migratory large fish. So, Altarugio Waterfall may hamper the fish movement but occasionally some individuals or shoals may reach the waterfall upstream stretches.

During sampling at site 4 in January 2011, there was a moment of "spawning" on the Cabeça River, when the shoals moving upstream, became gathered below the Altarugio waterfall. There, 541 individuals of *Astyanax altiparanae* were collected. On the other hand, upstream from the waterfall (site 3), on the same day, only one individual of this species was captured. The other two sites (1 and 2), regardless of the water level or time of spawning, no changes were observed on the abundance of individuals, reinforcing that natural barriers influence the migration of individuals and shoals. Additionally, species with less vagility may be restricted to some river portions because the waterfalls influence. Evidence of this can be reported by the occurrence of *Corydoras aeneus*, in large shoals above the waterfall Altarugio, and absence of this species at site 4, where only *Corydoras flaveolus* was recorded.



**Figure 3.** Dendrogram of similarity (Jaccard's index) of the samples obtained in each of the four sites at Cabeça River (example: 1<sup>st</sup> sampling at site 1 = site 1.1).

Thus, site 4 proved to be important for regional ichthyofauna. In studies such as of Carmassi et al. (2009), Gomiero & Braga (2006), Cetra & Petrere Jr. (2006), in rivers that comprise Corumbataí River basin and, sometimes, Cabeça River, never so many species were found at a single site as found in this research.

The physical features of Cabeça River channel indicate large amount of water at sites near the river's source where it is narrower and relatively deep what leads to higher quantities of exploitable habitat by fish. Discussions of the capacity of an environment support a certain number of species due to its physical conformations can explain the occurrence of many species due to these varieties of habitats. Langeani et al. (2005), who has studied a river in southeastern Brazil has also showed that slower and deepwater environments support more species than the areas of rapids, mainly because their increased complexity and quantity of habitat. The study of Cabeça River revealed different situations from those discussed previously, such as the species-environment relationship at sites 3 and 4. At site 3, waters are deeper, slower with submerged vegetation (grasses and swamp lilies) as well as submerged vegetal debris and rocks, however this site have had fewer species than the stretches to downstream, which has shallower and fast waters and no submerged vegetation.

The analysis of fish composition and distribution along the Cabeça River showed that small rivers that drain the Cuestas

Basálticas province can furnish shelter to great amount of the native species of the Tietê River basin. The rivers in this area frequently present great altitudinal differences in relatively short extensions, which characterize them to have much rapids and waterfalls. As showed here, in this scenario the fish assemblage distribution along the river may be hardly affected by these geographical accidents. Although the study of fish assemblage in the Cabeça River allows elucidating some of the factors that influence the ichthyofauna, there are still much to investigate for a better understanding in what extension the historical factors represented by waterfalls and geomorphological features and the ecological factors represented by environmental variability and biological interactions affect the structure of fish communities.

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## Buffer zone use by mammals in a Cerrado protected area

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PAOLINO, R.M., VERSIANI, N.F., PASQUALOTTO, N., RODRIGUES, T.F., KREPSCHI, V.G., CHIARELLO, A.G. **Buffer zone use by mammals in a Cerrado protected area.** *Biota Neotropica*. 16(2): e20140117. <http://dx.doi.org/10.1590/1676-0611-BN-2014-0117>

**Abstract:** Habitat loss and degradation is threatening mammals worldwide. Therefore, Protected Areas (PA) are of utmost importance to preserve biodiversity. Their effectiveness, however, depends on some management strategies such as buffer zones, which prevent/mitigate the impact of external threats and might increase the amount of available habitat for wildlife existing within reserves. Nevertheless, how intensively terrestrial mammals use buffer zones remains little studied, particularly in the Neotropical region. Aiming to analyse the use of a buffer zone (5 km wide) by medium and large-sized mammals, we modelled the occupancy probabilities of five species of conservation concern including local (interior and buffer zone) as a site covariate, simultaneously controlling for imperfect detection. Data collection was made with camera traps from April to September 2013 in a 9000 ha Cerrado PA ("interior") and in its surrounding area (39721.41 ha; "buffer zone"). This PA (Jataí Ecological Station) is immersed in a landscape where sugarcane plantations predominate in the northeastern of the state of São Paulo. We also conducted an inventory to compare the number and composition of species between interior and buffer zone. A total of 31 mammal species (26 natives) was recorded via camera traps and active search for sightings, vocalizations, tracks and signs. Occupancy estimates for *Myrmecophaga tridactyla*, *Leopardus pardalis* and *Pecari tajacu* were numerically higher in interior. On the other hand, *Chrysocyon brachyurus* had the highest occupancy in buffer zone, while the largest predator, *Puma concolor*, used both areas similarly. However, as the confidence intervals (95%) overlapped, the differences in occupancy probabilities between interior and buffer were weak for all these species. Additionally, regarding only the species recorded by cameras, the observed and estimated richness were similar between interior and buffer zone of the PA. Our data demonstrated that the buffer zone is indeed used by medium and large-sized mammals, including conservation-dependent ones. The lack of enforcement of current legislation regarding buffer zones is therefore a real threat for mammals, even when protection is guaranteed in the interior of protected areas.

**Keywords:** *Medium and large-sized mammals, camera trapping, detection probability, occupancy, Jataí Ecological Station.*

PAOLINO, R.M., VERSIANI, N.F., PASQUALOTTO, N., RODRIGUES, T.F., KREPSCHI, V.G., CHIARELLO, A.G. **Uso da zona de amortecimento de uma Unidade de Conservação de Cerrado por mamíferos.** *Biota Neotropica*. 16(2): e20140117. <http://dx.doi.org/10.1590/1676-0611-BN-2014-0117>

**Resumo:** A perda e degradação de habitat têm ameaçado os mamíferos no mundo todo. Dessa forma, a criação de Unidades de Conservação (UC) faz-se cada vez mais fundamental para sua preservação. Entretanto, estratégias de manejo são necessárias para que as UCs cumpram plenamente seus objetivos, tal como o estabelecimento de uma zona de amortecimento ao seu redor, a qual aumenta a área de habitat protegido e visa servir como barreira a impactos negativos externos. Contudo, o uso das zonas de amortecimento por mamíferos terrestres permanece pouco estudado, particularmente no Neotrópico. Com o objetivo de analisar o uso de uma zona de amortecimento (5 km de extensão) por mamíferos de médio e grande porte, nós modelamos a probabilidade de ocupação de cinco espécies de interesse conservacionista, incluindo local (interior e zona de amortecimento) como uma covariável de sítio, controlando, simultaneamente, as imperfeições na detecção. Os dados foram coletados por meio de armadilhamento fotográfico de abril a setembro de 2013 em uma UC de Cerrado de 9000 ha e em

39721,41 ha do seu entorno. A área de estudo (Estação Ecológica de Jataí) está imersa em uma paisagem de matriz canavieira no nordeste do estado de São Paulo. Nós também realizamos um inventário a fim de comparar a riqueza e a composição de espécies no interior e na zona de amortecimento. Foi registrado um total de 31 espécies (26 nativas), incluindo aquelas amostradas apenas por identificação de vestígios, de vocalizações e por observação direta. As estimativas de ocupação para *Myrmecophaga tridactyla*, *Leopardus pardalis* e *Pecari tajacu* foram numericamente maiores no interior, enquanto *Chrysocyon brachyurus* obteve a maior estimativa de ocupação na zona de amortecimento. Já o maior predador, *Puma concolor*, utilizou ambas as áreas de forma semelhante. Entretanto, como houve sobreposição entre os intervalos de confiança (95%), as diferenças nas probabilidades de ocupação entre interior e zona de amortecimento foram fracas para as cinco espécies analisadas. Além disso, considerando apenas as espécies registradas por armadilhamento fotográfico, a riqueza observada e a estimada foram similares no interior e na zona de amortecimento da UC. Nossos dados demonstraram que a zona de amortecimento é de fato utilizada por várias espécies de mamíferos de médio e grande porte, incluindo aquelas prioritárias para a conservação. Portanto, a falta de cumprimento da legislação vigente em relação às zonas de amortecimento é uma ameaça real para as espécies de mamíferos, mesmo quando a proteção é garantida no interior das UCs.

**Palavras-chave:** Mamíferos de médio e grande porte, armadilha fotográfica, probabilidade de detecção, ocupação, Estação Ecológica de Jataí.

## Introduction

The persistence of several species worldwide has been threatened by current levels of anthropogenic disturbances in natural environment to the point that the rate of species extinction is already comparable to mass extinction events of the geological past (Dirzo et al. 2014). Among mammals, one in each four species is already threatened, mainly due to habitat loss and degradation. Furthermore, the effect of hunting or gathering species for food, medicines or fuel in natural areas of the Neotropics affect 64% of the large-bodied mammals, which have large home ranges and depend on a large amount of area to maintain viable populations (Schipper et al. 2008). However, the maintenance of large natural areas depends primarily on public policies that regulate land use and protection, together with community commitment (Watson et al. 2014).

The replacement of native vegetation by agriculture, pastures and urban areas is a top concern in the Neotropics, where deforestation rates are high (Schipper et al. 2008). In Brazil, notwithstanding its conservation importance, the Cerrado has already lost between 48% and 55% of its original area (Klink & Machado 2005; IBGE 2010). This domain is the most biodiverse savannah in the world (Klink & Machado 2005), comprising 251 mammalian species, 31 of which are endemic (Paglia et al. 2012). It is thus considered a biodiversity hotspot and a priority region for conservation (Myers et al. 2000). Nevertheless, there is a relatively small number of Cerrado protected areas, which covers only 4.1% of its original area (Klink & Machado 2005).

Protected areas (PA) are recognized as the most effective strategy for *in situ* conservation, maintaining not only the biodiversity and ecosystem services, but also historical and cultural values (Chape et al. 2005, Xavier et al. 2008). However, the conservation of viable populations in the long term inside PA depends on their surroundings, since species are not restricted to the interior of the reserves (Hjert 2006, Vynne et al. 2014). Thus, the establishment of buffer zones around them are important management strategies for adequately achieving the objectives of a PA, as well as to mitigate isolation problems (Morsello 2001).

A buffer zone is defined as the immediacy of a PA where the land uses are subjected to specific norms and restrictions in order to protect and increase the conservation value of a PA (Lynagh

& Urich 2002), providing supplementary habitat and resources for larger wild animal populations (Morsello 2001). The establishment of a buffer zone prevents and/or mitigates direct and indirect anthropogenic-derived impacts from outside of the PA such as fire, erosion, alien species invasion, noise disturbance and edge effects (Vitalli et al. 2009, Vynne et al. 2014). Moreover, this strategy contributes to avoid that the immediate surroundings of a PA act as ecological traps for wildlife (i.e., road kills, trapping and poaching) (van der Meer et al. 2015). Additionally, buffer zones have the potential to mitigate conflicts between local communities and wildlife (Hjert 2006).

Many species of medium and large-sized mammals use both the interior of PA and its surroundings as part of their home range such as *Chrysocyon brachyurus*, *Puma concolor* and *Leopardus pardalis* (Mantovani 2001). The buffer zone is suitable habitat for several species, even though there are cases in which animal abundances decrease from the PA perimeter towards the buffer zone limit because of the concomitant increase of human activities and occurrence of domestic animals (Jotikapukkana et al. 2010). Mantovani (2001) and Jotikapukkana et al. (2010) are some of the very few studies that assess the occurrence of wildlife in buffer zones and the importance of these areas (Massara et al. 2012). Furthermore, the Brazilian law on buffer zones lacks clarity and objectivity that hamper their practical application (Vitalli et al. 2009). The establishment of biologically meaningful and efficient buffer zones is an intricate issue further complicated by lack of information derived directly from wildlife studies occurring in this zones (Tambosi 2008).

In order to improve the knowledge of species use of buffer zones, occupancy modeling based on camera trap data can be used to compare the occurrence of a given species among different sites such as the interior and the buffer zone of a PA (Mackenzie et al. 2002, Bailey et al. 2004, O'Connell 2011). This approach has the differential of dealing with imperfect detection, because individuals may not be detected due to sampling limitations or low population density, which is common issue for several species of medium and large-sized tropical mammals (Mackenzie et al. 2002, O'Connell et al. 2006). Furthermore, occupancy models are a powerful tool to analyze the influence of landscape covariates and habitat changes on species occurrence (Nicholson & Manen 2009, Cove 2011).

Hence, this study analyse the use of a buffer zone by five species of conservation concern through occupancy modelling, assessing whether these species are using similarly interior and buffer zone of a Cerrado PA. Additionally, we compare species richness and composition of medium and large-sized mammals between interior and buffer zone of the UC. We expected that (1) species would use the buffer zone, but less than the interior of the PA; and that (2) richness in the interior of the PA would be higher than in the buffer zone.

## Materials and Methods

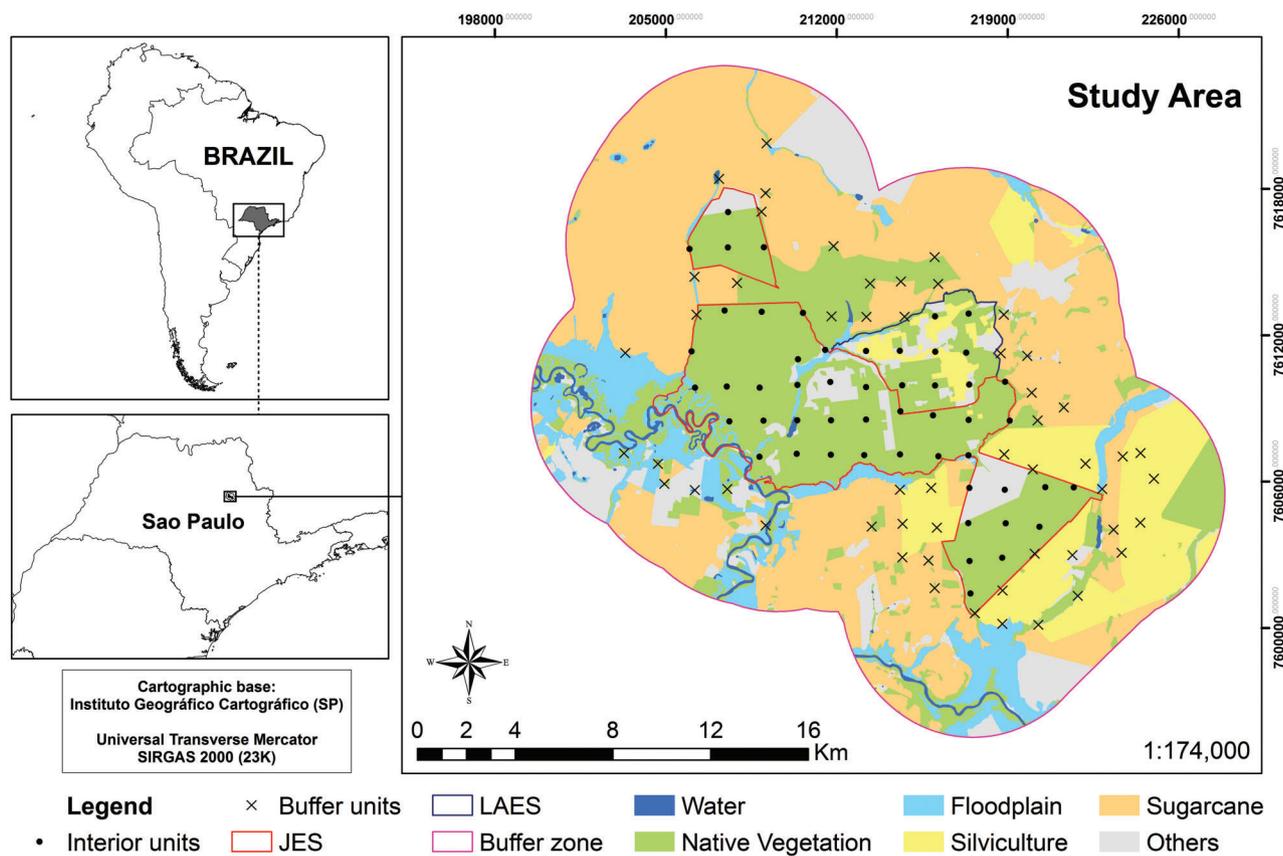
### 1. Study Area and Landscape Characterization

This study was carried out in the Jataí Ecological Station (JES; 9013 ha) and in the Luiz Antônio Experimental Station (LAES; 2009 ha), which are adjacent to each other (Figure 1). These two areas are located in the Luiz Antônio municipality, in the northeastern portion of the state of São Paulo (21° 30' S, 47° 50' W). According to Köppen classification, the climate of this area is Aw (tropical humid) (CEPAGRI 2014). The predominant vegetation in JES is the Cerradão (wooded Cerrado; 60.7%), followed by areas of Cerrado in regeneration (19.5%) and Atlantic Semi-deciduous forest (13.6%) (Toppa 2004).

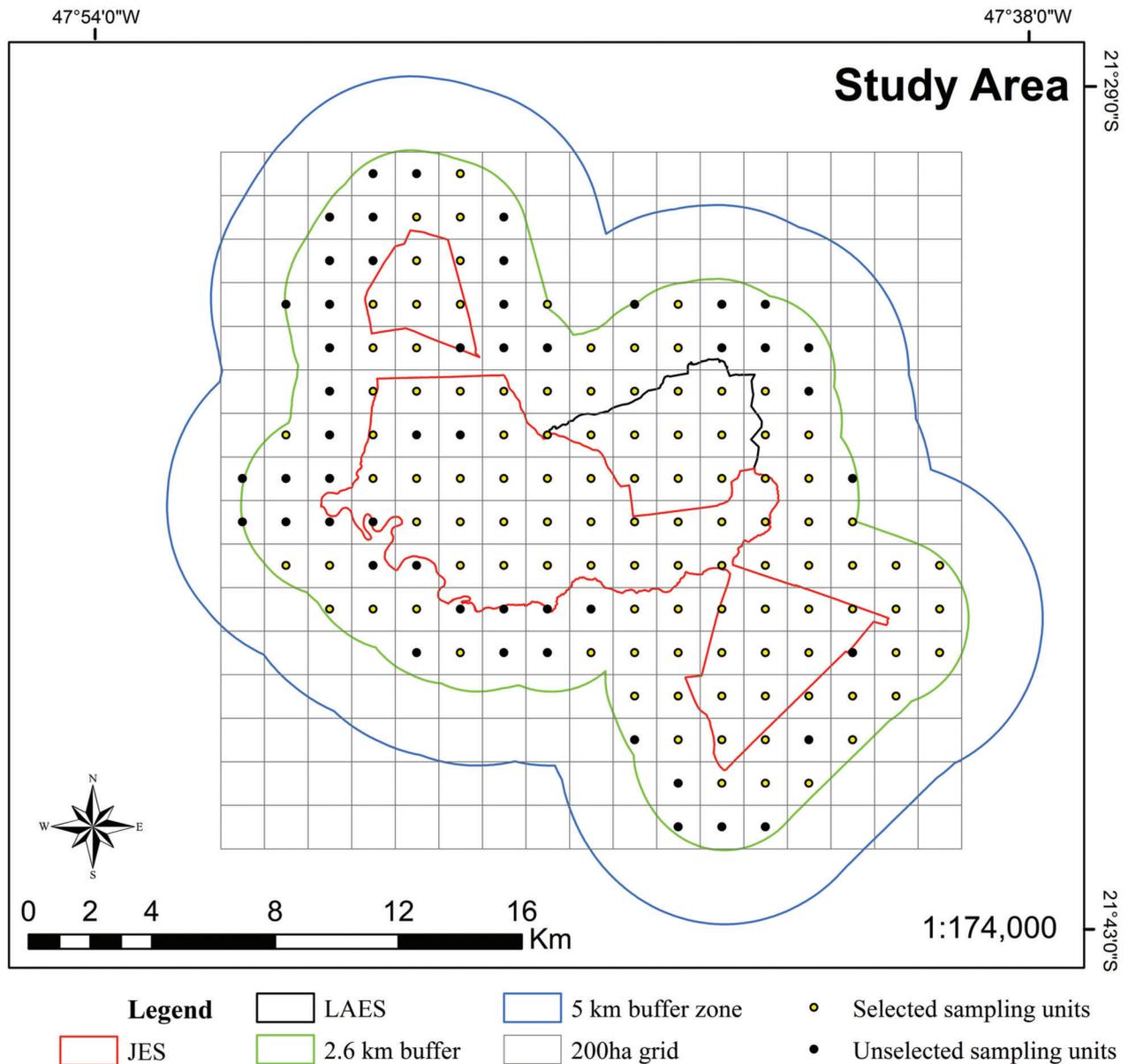
Land use and land cover map classification of the PAs (JES and LAES) and their buffer zone (Figure 1) were done in a

1:20000 scale, based on ortophotomosaics from IGC (Instituto Geográfico e Cartográfico do Estado de São Paulo) and field data, using QuantumGIS 2.2.0 and ArcGIS 10.1 (ESRI 2002). We defined the buffer zone as a 5 km buffer around the perimeter of JES and LAES following the definition given by JES Management Plan (Leonel et al. 2010). In addition, as camera traps were placed up to 2.6 km away from the perimeter of JES and LAES (Figures 1 and 2) and considering that some medium and large-sized mammals have large home ranges (Mantovani 2001, Oliveira 1994), the 5 km buffer includes the influence zone of the outermost points sampled. Therefore, our 5 km buffer comprises almost all, and surpasses in parts, the “official” buffer zone of JES. For this reason, we discuss our results as representative of the buffer zone of this reserve.

We made an accuracy analysis (Kappa concordance analysis) of our map of land cover type and use by comparing the final map with Google Earth images and the achieved overall accuracy was of 90%. Our mapping showed that the whole study area (50744.1 ha: JES + LAES + 5 km buffer) is mainly covered by sugarcane plantations (40.6%), followed by native vegetation (Cerradão, Cerrado *stricto sensu*, Semi-deciduous Atlantic forest, Deciduous Atlantic forest and riverine forests), which covers about a third of this area (Table 1). Examining separately PA and the buffer zone, however, we see a contrasting scenario. While the interior of these two PA are mainly covered by native vegetation (77.3%), only 13.2% of the buffer zone



**Figure 1.** Location and limits of the study areas in the state of São Paulo, Brazil: Jataí Ecological Station (JES), Luiz Antônio Experimental Station (LAES) and their buffer zone (5 km from the perimeter of the reserves). The land use and cover map (1:20000) is illustrated for the entire study area with the location of sampling units (52 in interior and 53 in buffer zone). Cartographic bases: Instituto Florestal do estado de São Paulo and IBGE (2008). Projection: Transverse Mercator. Datum: SIRGAS 2000.



**Figure 2.** Sampling design of this study with the limits of the study areas (JES = Jataí Ecological Station; LAES = Luiz Antônio Experimental Station), the 2.6 km buffer, the 5 km buffer zone, the grid of 200 ha squares and the potential and selected sampling units.

retains native vegetation. Sugarcane is, by far, the predominant land cover type in the buffer zone (51.8%), followed by silviculture (*Pinus* spp. and *Eucalyptus* spp.; 13.5%). Nonetheless, it is important to highlight that although the buffer zone is predominantly “anthropic”, the amount of natural habitats remaining in the buffer zone (native vegetation plus floodplains) is quite significant (9322 ha) (Figure 1 and Table 1).

## 2. Camera trapping and Data collection

We surveyed mammals in the study area using camera traps (Reconyx<sup>®</sup>, digital model HC 500) and active search for signs (footprints, scats, scratches) during the 2013 dry season (April-September). We restricted the inventory to only one season aiming to satisfy the closure assumption in single-season occupancy studies (no extinction and colonization of our study species at

sampling points during our “sampling season”) (Mackenzie et al. 2006). We distributed camera traps (sampling points) overlying a grid of square cells (200 ha each or 1.4 x 1.4 km wide) over the study area. The center of each square was a potential point for sampling. Within the JES and LAES, we sampled 52 of the 54 squares that covered the entire area of these two reserves (Figure 2). We discarded two cells because they were inaccessible. Since the buffer zone is a much larger area than the interior, we randomly sampled 53 squares from the total of 97 squares available (Figure 2). Thus, we divided our sampling effort similarly between interior and buffer. Given logistical and safety issues, all randomly selected points in sugarcane plantations or pasture were relocated to the nearest available native vegetation, where cameras were oriented to aim at the originally chosen land cover type (either sugarcane or pasture). In some cases, we placed the camera as close as possible to the original point because we

**Table 1.** Landscape characterization (hectares and %) within the protected areas (JES and LAES: “interior”) and outside these protected areas (“5 km buffer zone”). We also show the landscape represented by the area of influence of each camera trap location (the 200 ha surrounding all sampled points = “camera traps”). Native vegetation = Cerradão, Cerrado *stricto sensu*, Atlantic Semi-deciduous, deciduous and riverine forests; Silviculture = *Pinus* spp. and *Eucalyptus* spp.; Others = Water, Grassland, Regenerating area, Orange plantation, Pasture and Urban areas.

	Area (ha)	Native Vegetation (%)	Floodplain (%)	Sugarcane (%)	Silviculture (%)	Others (%)
Interior	11021.91	77.31	2.64	0.00	5.38	14.61
5 km buffer	39722.19	13.24	10.23	51.83	13.51	11.20
Total	50744.10	27.16	8.58	40.57	11.74	11.94
Camera traps	21006.74	46.56	5.53	21.02	16.62	10.27

were unable to access the exact location of all sampling points. Thus, the average minimum distance between two adjacent points was 1.31 km for the interior and 1.15 km for the buffer.

Every month, 18 camera traps were placed in the study area. For each sampling point, a single camera trap was set on a tree trunk 40-60cm above the ground and programmed to monitor continuously (24hd) during 30 consecutive days, recording date and time of each photograph. Due to some logistical problems, 105 of 108 sampling points were sampled, totalling an effort of 3150 camera trap days, half of which in JES/LAES and half in the buffer zone. There were no relevant differences in sampling effort between camera traps since 98% of them worked on average 30 days (average sampling effort = 29.8 days, SD = 0.76).

The 105 sampled points were distributed in native vegetation (62.9%), silviculture (20%), sugarcane (5.7%) and in other land cover types (11.7%). Thus, the land cover represented by the exact location of cameras differed from the percentage of area covered by each land type in the whole landscape (Table 1). However, this contrast is smaller when we consider the landscape inside the 200 ha circle surrounding all camera trap locations (Table 1).

During the setup and removal periods, we actively searched for mammal tracks, walking 200 m in unpaved roads existing nearest to each camera trap station, totalling 21 km of sampling throughout the entire study. The data collected during the active searches for signs, direct observation and vocalizations was complementary to the inventory. We photographed signs and footprints and identified the species with the help of field guides (Becker & Dalponte 1999, Borges & Walfrido 2004, Carvalho Jr. & Luz 2008, Mamede & Albo 2008, Moro-Rios et al. 2008, Miranda et al. 2009).

### 3. Data analysis

For comparative purposes, the pool of medium and large-sized mammal species that could possibly occur in the region was compiled based on the current Checklist of Brazilian Mammals (Paglia et al. 2012), together with other general sources of information for mammals (Emmons & Feer 1997, Eisenberg & Redford 1999, Kasper et al. 2009, IUCN 2014), and also from Mammalian Species ([www.asnjournals.org/loi/mmssp](http://www.asnjournals.org/loi/mmssp)). The resulting list totalled 39 species of medium to large-sized mammals (approximately  $\geq 2$  kg) with terrestrial or scansorial habit. Since our sampling methods did not represent arboreal species (primates, sloths and porcupines), these were not included in statistical analyses. Moreover, occupancy modelling and analysis of species richness were carried out using solely camera trapping data.

Among the recorded species, we selected five to model occupancy and detection probabilities (Mackenzie et al. 2006)

in order to assess if they were occupying differently the interior of the PA and the buffer zone. Selection of the species was based on their conservation status and habitat requirements. The selected species included the top predator still extant in the region, the puma (*Puma concolor*), a mesopredator, the ocelot (*Leopardus pardalis*), a large omnivore, the maned wolf (*Chrysocyon brachyurus*), a large frugivore, the collared peccary (*Pecari tajacu*), and a large myrmecophage, the giant anteater (*Myrmecophaga tridactyla*). All these species are threatened with extinction in the state of São Paulo (classified as Vulnerable), except the collared peccary, which is classified as Near Threatened (Bressan et al. 2009).

We organized presence data into detection/non detection matrices, one for each species, considering six sampling occasions of five days each (6 occasions x 5 days = 30 days). We modelled occupancy ( $\psi$ ) and detection ( $p$ ) probabilities of each species in MARK 8.0 (White & Burnham 1999), assessing the effect of explanatory variables. It is important to assess detection to improve the estimation of the key parameter of interest here ( $\psi$ ). We choose one site covariate for modelling occupancy and detection [“local”: interior (0) or buffer (1)]; and three site covariates [mean temperature (Temp), cumulative rainfall (Precip), distance from the nearest unpaved road (DRD)] and one survey-specific covariate [sampling date (Julian date beginning at our first sampling day) to incorporate time variation] for detection. Correlation tests (Spearman and Pearson tests) were performed to investigate the collinearity between covariates in R (R Core Team 2014); all tested correlations were weak (correlation coefficients  $< 0.6$ ).

A set consisting of candidate models was constructed from all possible additive combinations of up to two covariates for detection parameter ( $p$ ). For this set of models  $\psi$  was estimated as null or with “local” covariate (Doherty et al. 2012). The most explanatory model or models was/were selected using the Akaike Information Criterion corrected for small-sample bias (AICc) (Burnham & Anderson 2002, MacKenzie et al. 2006) and associated model weights ( $\omega_i$ , Burnham & Anderson 2002). This approach is considered the best method for achieving useful estimates of cumulative variable weights ( $w_{cum}$ ) since it allows constructing a balanced model set (Doherty et al. 2012).

We used the parametric bootstrap approach based on 10000 bootstrap resamples to evaluate the goodness of fit of global model (the most parameterized model with all additive covariates in  $\psi$  and  $p$ ) in program PRESENCE v.6.2 (MacKenzie & Bailey 2004; Hines 2006). The global model is considered an adequate description of the data, implying that there is no spatial autocorrelation between sampled points, if the estimated overdispersion parameter ( $c$ ) is approximately equal to one (Burnham & Anderson 2002), which was the case here (see Results).

In order to evaluate the sampling effort and to compare species richness between interior and buffer zone, we computed rarefaction curves with incidence data (presence/absence of native species only) using EstimateS version 9.1.0 (Colwell 2013). We analysed the randomized curve of “observed” species ( $S_{est}$ ) as well as the curve of “estimated” richness (first order Jackknife estimator) separately for interior and buffer zone.

## Results

Our camera trap data indicated that all five selected species were using both interior and buffer zone. Collared peccary and giant anteater were mostly detected in the interior (77% and 67% of sampling points, respectively) while maned wolf and puma were more frequent in the buffer zone (80% and 60%, respectively). Ocelots were registered at 26 sites in total, half in the interior and half in the buffer zone.

Regarding occupancy and detection modeling, the ‘goodness of fit’ test using the global model showed good fit with no evidence of overdispersion for those species (Table 2). From the best ranked model that contained “local” covariate for  $\psi$  for each species, giant anteater had the highest occupancy probability for interior ( $\psi = 0.73$ ), followed by ocelot ( $\psi = 0.63$ ) and collared peccary ( $\psi = 0.50$ ) (Table 2). For these species, occupancy probabilities were numerically higher in the interior than in the buffer zone, but the difference was not strong since there was overlap between the 95% confidence intervals of the estimates for “local” covariate (Figure 3). However, for giant anteater, the overlap was smaller, indicating a higher use within the protected area (Table 2 and Figure 3). On the other hand, the second-ranked model for puma showed higher occupancy probability in the buffer ( $\psi = 0.64$ ) than in the interior ( $\psi = 0.41$ ), but its 95% confidence intervals overlapped, indicating that this species used both areas similarly (Table 2 and Figure 3).

Although “local” was also important for maned wolf, the best-ranked model with this covariate did not converge (Table 2) and its confidence intervals included “zero”. This may have happened because maned wolf was detected by cameras trap almost four times more frequently in the buffer zone (24 sites) than in the interior (6 sites). Then, considering the best-ranked converged model with a reliable occupancy estimates [ $\psi$  (local),  $p(\text{DRD} + \text{Temp})$ ], maned wolf had the highest occupancy estimate in the buffer ( $\psi = 0.79$ ; Figure 3) among all species of interest in this study.

Local was the most important variable for modeling variation in detection probability for collared peccary and maned wolf ( $w_{cum} = 0.86$  and  $w_{cum} = 0.94$ , respectively). We found a negative relationship for *P. tajacu* ( $\beta = -1.98$ ; 95% CI = -2.84 to -1.11) and a positive relationship for *C. brachyurus* ( $\beta = 2.00$ ; 95% CI = 1.07 to 2.93) between buffer zone and detection of these species at occupied sites. For puma and ocelot, the most important variable affecting detection was “distance to the nearest unpaved road”, since this covariate had a combined explanatory power of 99% for each species. For these two felids, detection was lower for camera traps located further from unpaved roads. For giant anteater, no model with covariates for detection was better ranked than the null model.

In regard to the inventory, we recorded 31 species of medium and large-sized mammals in the study area, 26 native and five exotic species. The use of multiple methods was important because some species were recorded solely by one

method of survey (Table 3). Twenty-six species occurred in the interior of JES and LAES, while 25 in the buffer zone. Among native species, five were solely recorded in interior (*Cavia aperea*, *Cuniculus paca*, *Didelphis albiventris*, *Sapajus nigritus* and *Tapirus terrestris*) while two exotic and two natives species were observed only in the buffer zone (*Bos taurus*, *Felis catus*, *Callicebus nigrifrons* and *Procyon cancrivorus*, respectively). Considering native species that occurred both in interior and in buffer zone, 10 species were mostly recorded in interior, while six were more frequent in buffer zone (*Cabassous unicinctus*, *Cerdocyon thous*, *C. brachyurus*, *Puma yagouaroundi*, *Mazama gouazoubira* and *Hydrochoerus hydrochaeris*) (Table 3).

Considering only native mammal species recorded by camera traps ( $n = 21$ ), the observed richness was similar between interior ( $n = 19$ ) and buffer zone ( $n = 18$ ), as there was overlap in the 95% confidence intervals of the resulting rarefaction curves (Figure 4). In addition, interior and buffer shared 60% of native species ( $n = 16$ ) (Table 3). The rarefaction curves (both interior and buffer zone) did not reach asymptotes. Nevertheless, they showed a stabilization trend (Figure 4), indicating a satisfactory survey effort. The estimates of total mammal richness (1<sup>st</sup> order Jackknife estimator) suggested, however, that there might be additional species in the study area that were not recorded during our sampling. Regarding this, these curves indicated that the number of additional species might be higher in buffer zone than in interior of the PA (Figure 4).

## Discussion

According to our results, *M. tridactyla*, *P. tajacu*, *L. pardalis* and *P. concolor* all used similarly the interior and the buffer zone, when we consider the confidence intervals of the estimated occupancy parameter. The large amount of continuous native vegetation within JES and LAES (77%), probably makes the area within their perimeters a highly favourable environment for these species for several reasons, including thermoregulation (Camilo-Alves & Mourão 2006), shelter and feeding (Oliveira 1994, Emmons & Feer 1997, Murray & Gardner 1997, Medri & Mourão 2005). However, large mammals inhabiting the Cerrado might not be able to survive in the long term solely within protected areas (Massara et al. 2012). The amount of native vegetation in buffer zone are thus important sources of additional resources for some species, and for providing dispersal routes.

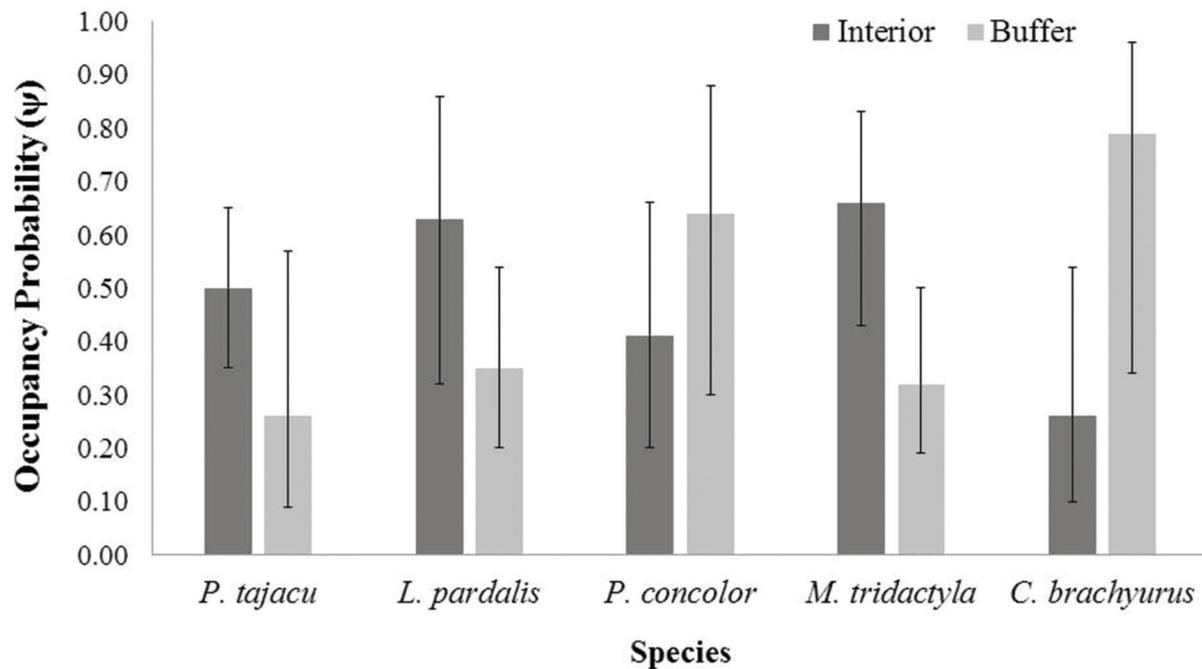
Moreover, some species seem capable of using land cover types common in the buffer. The giant anteater, for example, used pine and eucalyptus plantations in the buffer zone. These production areas represent 13% of buffer and might be considered as complementary habitats, at least for this threatened species. Other authors have already found the giant anteater making extensive use of planted forests in northern Brazil (Kreutz et al. 2012, Timo et al. 2014), as well as soybean plantations and disturbed grassland in central Brazil (Shaw et al. 1987). According to some studies, forest plantations can catalyse the natural regeneration in their understory, making them more attractive habitats for wild species and contributing to biodiversity conservation in general (Viani et al. 2010).

Occurrence of mammals in buffer zones was also reported in a multi-species agroforestry system in Indonesia, where this type of land-use was identified as a good strategy to extend the habitat for mammalian populations (Salafsky 1993). This author highlights, however, that this system may not be as

Buffer zone use by mammals in a Cerrado protected area

**Table 2.** Model selection analysis (AIC weight  $\leq 2$ ) and occupancy probabilities ( $\psi$ ) with their respective 95% confidence intervals (CI) for five species of mammals in the interior and buffer zone of Iataí Ecological Station and Luis Antônio Experimental Station, São Paulo, Brazil. Temp = mean temperature; Local = interior or buffer zone; DRD = distance from the nearest unpaved road; Precip = cumulative rainfall; k = number of parameters in the model;  $\hat{c}$  = overdispersion parameter. \* For this model, numerical convergence was not achieved due to the low number of records in the interior of the protected areas (JES and LAES) and, thus, the occupancy estimates are not reported.

Species	Model	k	$\Delta AICc$	Akaike weight	Deviance	$\psi$ (local) (CI)		Naive Occupancy	$\hat{c}$
						Interior	Buffer		
<i>Pecari tajacu</i>	{ $\psi(\cdot), p(\text{Temp} + \text{Local})$ }	4	0	0.392	327.57	0.48 (0.33-0.63)		0.286	1.09
	{ $\psi(\text{Local}), p(\text{Temp} + \text{Local})$ }	5	0.91	0.248	326.27	0.50 (0.35-0.65)	0.26 (0.09-0.57)		
<i>Leopardus pardalis</i>	{ $\psi(\text{Local}), p(\text{DRD} + \text{Temp})$ }	5	0	0.43	277.33	0.63 (0.32-0.86)	0.35 (0.20-0.54)	0.248	0.73
	{ $\psi(\cdot), p(\text{DRD} + \text{Temp})$ }	4	0.8	0.288	280.34	0.43 (0.28-0.60)			
<i>Puma concolor</i>	{ $\psi(\cdot), p(\text{DRD} + \text{Local})$ }	4	0	0.537	324.9	0.52 (0.33-0.71)		0.286	1.39
	{ $\psi(\text{Local}), p(\text{DRD} + \text{Local})$ }	5	0.83	0.355	323.52	0.41 (0.20-0.66)	0.64 (0.30-0.88)		
<i>Myrmecophaga tridactyla</i>	{ $\psi(\text{Local}), p(\cdot)$ }	3	0	0.156	391.97	0.66 (0.43-0.83)	0.32 (0.19-0.50)	0.371	1.08
	{ $\psi(\text{Local}), p(\text{Local})$ }	4	0.25	0.138	390.07	0.73 (0.40-0.91)	0.29 (0.17-0.45)		
	{ $\psi(\text{Local}), p(\text{Precip})$ }	4	1.3	0.082	391.11	0.66 (0.44-0.83)	0.32 (0.19-0.50)		
	{ $\psi(\text{Local}), p(\text{DRD})$ }	4	1.41	0.077	391.23	0.71 (0.42-0.89)	0.32 (0.19-0.50)		
<i>Chrysocyon brachyurus</i>	{ $\psi(\text{Local}), p(\text{Precip} + \text{Local})$ }	5	1.53	0.073	389.14	0.73 (0.41-0.91)	0.29 (0.17-0.45)		
	{ $\psi(\cdot), p(\text{Temp} + \text{Local})$ }	4	0.00	0.256	281.34	0.69 (0.39-0.89)		0.286	1.25
	{ $\psi(\cdot), p(\text{Local})$ }	3	1.09	0.149	284.59	0.67 (0.38-0.87)			
	{ $\psi(\cdot), p(\text{DRD} + \text{Local})$ }	4	1.38	0.129	282.71	0.70 (0.37-0.90)			
	{ $\psi(\text{Local}), p(\text{Temp} + \text{Local})$ };*	5	1.87	0.101	281.00	-			



**Figure 3.** Occupancy estimates ( $\psi$ ) for five species of medium and large-sized mammals in the Jatai Ecological Station and Luiz Antônio Experimental Station interior and in the buffer zone with their respective 95% confidence intervals (vertical lines). For *C. brachyurus*, the estimates are from the ninth-ranked model [ $\psi$  (local),  $p$ (DRD + Temp)] because the best-ranked model including “local” as a covariate had no numeric convergence for the interior and the confidence interval included “zero” (see Table 2).

suitable for animals when it is not surrounding a forested core area. In Thailand, even relatively rare large mammals used a buffer zone. Nonetheless, their occurrence was negatively affected by domestic animals and human presence (Jotikapukkana et al. 2010).

Our results from the two largest carnivores, puma and maned wolf, also reinforce the importance of buffer zone, since they were mostly recorded and detected in the surrounding of the PA. As both species are wide-ranging, it is rather expected that they might use areas much larger than the protected areas. However, this finding also indicate, apart from the high capacity of movement and dispersal (Oliveira 1994, Mantovani 2001), a relatively high tolerance of these species to anthropogenic habitat alterations (Van Dyke 2008; Massara et al. 2012). These results are very interesting, because they suggest that the buffer zone, although disturbed, can still provide favorable habitat and food resources for these large carnivores. Similarly, the generalist puma and maned wolf have been recorded outside of Emas National Park, another large Cerrado protected area (Silveira 2004; Vynne et al. 2010; Vynne et al. 2014). Our results support therefore the notion that buffer zones are potential usable area for these large carnivores, corroborating other authors (Massara et al. 2012; Miotto et al. 2012).

In addition, detection probabilities for puma and maned wolf were higher in buffer zone than inside the PA, while detection of collared peccary was lower in the buffer zone. Puma and maned wolf have a high movement capacity and travel long distances (Oliveira 1994, Massara et al. 2012). Thus, their higher use of the buffer zone may increase their detection probability. On the other hand, distance from the nearest road explained better the detection of wild cats. This result is expected since felids, in general, have been detected

preferentially along unpaved roads, while species such as *P. tajacu* avoid roads and have been recorded with higher frequency in forest interiors (Trolle & Kéry 2005, Goulart et al. 2009, Srbeek-Araujo & Chiarello 2013). Moreover, although we did not assess the relative impact of poaching, the collared peccary is a traditional target in Brazil (Cullen et al. 2000), and it might also be disturbed by domestic dogs (*Canis familiaris*), present in human settlements that are common outside protected areas.

Records from the inventory also stressed the need for buffer zone conservation, for example through the use of biodiversity friendly agricultural practices. Whereas some species were restricted to the interior, the majority was recorded in both interior and buffer zone and others were restricted to the buffer. Our study area is currently harbouring 61.5% ( $n = 24$ ) of all native medium and large-sized non-arboreal mammals species that could potentially occur in the study region ( $n = 39$ ), according to the literature. We made an important record of the lowland tapir (*Tapirus terrestris*; Rodrigues et al. 2014), a species threatened with extinction regionally (Bressan et al. 2009) and globally (IUCN 2014). This species had not been seen in JES for more than 50 years (Lyra-Jorge 2007) and its last sign was a skeleton found in the middle 1980's (Talamoni et al. 2000). However, apart from us, an unpublished camera trap study carried out in JES between 2009 and 2010 detected tapirs inside JES recently (Enrique Queralt, personal communication). Although this species was recorded only in the interior, it has large home-range requirements to maintain a viable population (Medici et al. 2012). For this reason, native vegetation of the buffer zone may be fundamental to its conservation. Another important record was the marsh deer, *Blastocerus dichotomus*, which is critically endangered in São Paulo state (Bressan et al. 2009). This deer was reintroduced in JES and surrounding areas in 1998 (Figueira et al. 2005) and

## Buffer zone use by mammals in a Cerrado protected area

**Table 3.** Species list of the medium and large-sized mammals observed in the Jataí Ecological Station (JES), Luiz Antônio Experimental Station (LAES) and their buffer, the record type, the local where the species was recorded (interior or buffer), the number of sites and of records of each species. Type of record: CT – camera trapping; DO – direct observation; FO – footprint; VO – vocalization. <sup>A</sup> In some, but not all photos of *Dasybus* spp., *D. novencinctus* was clearly identified, but in others the identification is possible only to the genus level; <sup>B</sup>Exotic species and <sup>C</sup> Domesticated exotic species.

ORDER/Family/Species	Record type	Local			
		Interior		Buffer Zone	
		Sites	Records	Sites	Records
<b>DIDELPHIMORPHIA</b>					
<b>Didelphidae</b>					
<i>Didelphis albiventris</i>	CT	3	3	0	0
<b>PILOSA</b>					
<b>Myrmecophagidae</b>					
<i>Myrmecophaga tridactyla</i>	CT, FO, DO	32	52	19	39
<i>Tamandua tetradactyla</i>	CT, DO	3	3	1	1
<b>CINGULATA</b>					
<b>Dasypodidae</b>					
<i>Cabassous unicinctus</i>	CT, DO	1	1	2	2
<i>Dasybus</i> spp. <sup>A</sup>	CT, FO	11	15	9	16
<i>Euphractus sexcinctus</i>	CT, FO, DO	3	3	1	1
<b>PRIMATES</b>					
<b>Cebidae</b>					
<i>Sapajus nigritus</i>	DO	1	1	0	0
<i>Callicebus nigrifrons</i>	VO	0	0	2	2
<b>LAGOMORPHA</b>					
<b>Leporidae</b>					
<i>Sylvilagus brasiliensis</i>	CT, FO, DO	13	23	13	18
<i>Lepus europaeus</i> <sup>B</sup>	CT, FO, DO	10	13	20	35
<b>CARNIVORA</b>					
<b>Canidae</b>					
<i>Canis familiaris</i> <sup>C</sup>	CT, FO, DO	7	14	24	64
<i>Cerdocyon thous</i>	CT, FO, DO	10	10	30	123
<i>Chrysocyon brachyurus</i>	CT, FO, DO	17	19	35	69
<b>Felidae</b>					
<i>Felis catus</i> <sup>C</sup>	CT	0	0	1	1
<i>Leopardus pardalis</i>	CT, FO	15	29	14	34
<i>Puma yagouaroundi</i>	CT, FO	2	4	8	9
<i>Puma concolor</i>	CT, FO, DO	22	53	22	45
<b>Mustelidae</b>					
<i>Eira barbara</i>	CT, FO, DO	16	26	7	12
<b>Mephitidae</b>					
<i>Conepatus semistriatus</i>	CT, FO, DO	13	17	13	19
<b>Procyonidae</b>					
<i>Nasua nasua</i>	CT	6	7	2	40
<i>Procyon cancrivorus</i>	FO	0	0	1	1
<b>PERISSODACTYLA</b>					
<b>Tapiridae</b>					
<i>Tapirus terrestris</i>	CT, FO	4	4	0	0
<b>ARTIODACTYLA</b>					
<b>Tayassuidae</b>					
<i>Pecari tajacu</i>	CT, FO, DO	30	245	10	36
<b>Cervidae</b>					
<i>Mazama gouazoubira</i>	CT, FO, DO	24	68	32	73
<i>Blastocercus dichotomus</i>	FO	4	4	1	1
<b>Bovidae</b>					
<i>Bos taurus</i> <sup>C</sup>	CT, FO, DO	0	0	5	28
<b>Suidae</b>					
<i>Sus scrofa</i> <sup>B</sup>	CT, FO	10	22	5	6

Continued on next page

Table 3. Continued.

ORDER/Family/Species	Record type	Local			
		Interior		Buffer Zone	
		Sites	Records	Sites	Records
<b>RODENTIA</b>					
<b>Cuniculidae</b>					
<i>Cuniculus paca</i>	CT	3	6	0	0
<b>Caviidae</b>					
<i>Cavia aperea</i>	DO	2	2	0	0
<i>Hydrochoerus hydrochaeris</i>	CT, DO	1	1	3	3
<b>Dasyproctidae</b>					
<i>Dasyprocta azarae</i>	CT, FO, DO	19	74	5	13

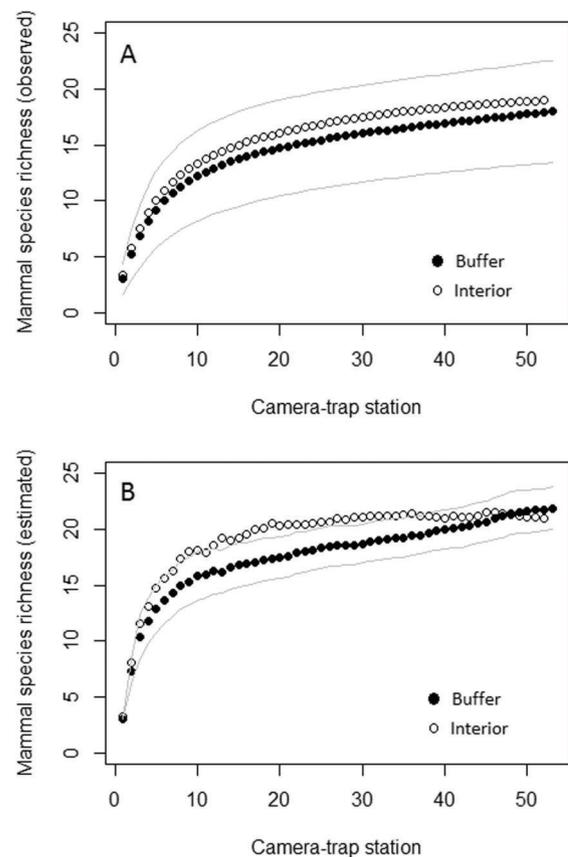
seems to be still using both interior and buffer zone. The continued presence of the marsh deer suggests, therefore, an enduring population. Hence, further investigation is necessary to ascertain current conservation status of marsh deer and lowland tapir in the region.

Considering the Management Plan of JES (Leonel et al. 2010) and previous inventories carried out in the study area (Gargaglioni et al. 1998, Talamoni et al. 2000, Lyra-Jorge 2007), some species were expected to occur but were not detected by us, such as *Galictis* sp., that may have not been recorded due to its swiftness and relatively small body size, and *Leopardus tigrinus*, that has been rarely recorded by camera traps, perhaps due to its natural rarity (Emmons & Feer 1997). Low abundance or local extinction may also explain the lack of records of the red brocked deer (*Mazama americana*), since this species is vulnerable to environmental changes (Duarte et al. 2012). Species such as *Ozotoceros bezoarticus*, *Panthera onca*, *Priodontes maximus* and *Tayassu peccary* are possibly extinct locally since they have not been observed there in the last decades (Gargaglioni et al. 1998, Bressan et al. 2009, Duarte et al. 2012, Keuroghlian et al. 2013). They are very sensitive to habitat loss and fragmentation, disappearing from degraded areas (Chiarello 1999, Cullen et al. 2000, Morato et al. 2013).

We consider that our sampling effort was sufficient to record most of the common and abundant species and possibly some of the rarest ones as well, still occurring in the study area. Although generalisations about this are tricky, camera trapping studies carried out elsewhere suggest that rare species begin to “appear” in inventories after sampling efforts in the range of 1000-2000 camera trap days (Tobler et al. 2008). However, although the buffer area is almost four times larger than the interior, the sampling effort was roughly the same for both areas. This difference may, therefore, explain why the curve of total estimated richness for the interior tended to stabilize, while that of the buffer did not. This is in accord with the classical species-area relationship (MacArthur & Wilson 1967), which indicates that larger sampled areas should hold higher diversity.

This species-area relationship could partially explain the fact that buffer zone was almost as rich as the interior of the PA. This similarity probably also results from the close proximity of JES, which may act as a source of individuals, and from the existence of a large floodplain (the Mogi-Guaçu river floodplain that has 4064 ha) in the buffer zone. Moreover, despite being proportionally less covered by native vegetation than the PA,

the buffer zone has approximately 5260 ha of native vegetation distributed in several fragments. These remnants of the buffer zone are close to or structurally linked to the riparian vegetation distributed along the Mogi-Guaçu River. The preservation of these riparian forests is, therefore, of utmost importance, considering the overwhelming predominance of sugarcane plantations in the buffer area.



**Figure 4.** A. Rarefaction curves for observed richness of native species recorded by camera trapping in the Jataí Ecological Station (JES) and Luiz Antônio Experimental Station (LAES) interior and buffer zone. B. Estimated richness of native species recorded by camera trapping in JES and LAES interior and buffer zone by the Jackknife 1<sup>st</sup> order estimator. The grey lines represent the 95% confidence intervals for the observed richness in the interior and the estimated richness in the buffer zone respectively.

Overall, our study contributes with relevant information that highlight the importance of buffer zones for wildlife. As more biological data are obtained, the closer we get to a biologically sound extend of a buffer zone (Tambosi 2008, Alexandre et al. 2010, Massara et al. 2012). Definitions about this should be clearly stated in the Management Plan of protected areas, as stated by law 9985/2000 (Brasil, 2000). If the PA does not have a Management Plan, the CONAMA Resolution n° 428/2010 states that the buffer zone should be at least three kilometres wide. This extent is very close to the buffer zone we assessed, since our camera traps were located up to 2.6 km from the perimeter of the closest PA. We did not expect finding comparable levels of mammal species richness in a buffer zone heavily dominated by intensive agriculture and silviculture. Furthermore, as we discussed above, this minimum extent was indeed very important for the set of species we studied more closely, including threatened ones, as the differences in occupancy status between interior and buffer zone were less contrasting than we anticipated. Put together, these findings might be seen as good news for the conservation of our study species. In addition, our results may help managers of protected areas not only to delimit the range of buffer zone, but also to define what type of activity might be allowed inside it.

Nevertheless, we stress the need for a cautionary look at these findings. What we got here was just an instantaneous snapshot of a truly dynamic process, of which we still have a lot to study. Is the buffer zone by itself able to sustain “independent” mammal populations in the long term? Or, alternatively, is it a sink area for individuals coming from inside the PA? What portion of the “resident” populations living inside JES and LAES also inhabits (and perhaps is dependent from), the surroundings of these PA? How occupancy and detection vary at different distances from PA? These, of course, are key questions that we must start addressing as soon as possible to qualify our findings. Notwithstanding these, our main findings do highlight the need of researchers, rural inhabitants and managers of protected areas, to urgently start looking more closely and deeply to the role played by buffer zones, and to the need to effectively implement management strategies to these important zones.

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## Cultural keystone species of fauna as a method for assessing conservation priorities in a Protected Area of the Brazilian semi-arid

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**Abstract:** The Cultural Keystone Species (CKS) method of identification has been used to define culturally important species. The objective of this study was to identify and characterize CKS vertebrates in the communities surrounding the Environmental Protection Area of Chapada do Araripe and define conservation priorities using semi-structured interviews and free listings. Interviews were conducted with 246 people; 53 species were identified and then included in categories of use: food, medicinal, handicraft and symbolic. Food preference was identified for *Mazama gouazoubira*, *Penelope superciliaris*, *Dasyprocta prymnolopha* and *Dasytus novencinctus*. As medicinal resources, *Salvator merianae* and *M. gouazoubira* represented 51.85% of treatment recommendations. For use in handicrafts, *M. gouazoubira*, which is also the only species recorded for symbolic use, stands out. Use values (UV) varied from 0.03 to 1.34, and *M. gouazoubira* had the highest UV (1.34) and a 0.74 projection. Regarding cultural importance, *M. gouazoubira* was the species with the highest UV and projection, and was indicated as a CKS to be prioritized in research and conservation studies.

**Keywords:** Ethnozoology, *Mazama gouazoubira*, Protected Area, Chapada do Araripe, Caatinga.

BONIFÁCIO, K.M., FREIRE, E.M.X., SCHIAVETTI, A. **Espécie-Chave Cultural de fauna como método de designação de prioridades para conservação em Área Protegida do Semiárido brasileiro.** *Biota Neotropica*. 16(2): e20140106. <http://dx.doi.org/10.1590/1676-0611-BN-2014-0106>

**Resumo:** O método para identificação de Espécie-Chave Cultural (ECC) tem sido utilizado para definir espécies importantes culturalmente. Nesse sentido, para identificar e caracterizar ECC de vertebrados em comunidades do entorno da Área de Proteção Ambiental da Chapada do Araripe, e definir prioridades de conservação, utilizou-se formulário semiestruturado e listagem livre. Foram entrevistadas 246 pessoas; identificaram-se 53 espécies que foram incluídas nas categorias de uso: alimentar, medicinal, artesanal e simbólico. Identificou-se preferência alimentar para *Mazama gouazoubira*, *Penelope superciliaris*, *Dasyprocta prymnolopha* e *Dasytus novencinctus*. Como recurso medicinal *Salvator merianae* e *M. gouazoubira* representaram 51.85% das indicações de tratamento. No uso artesanal, destaca-se *M. gouazoubira*, que também foi a única espécie registrada para o uso simbólico. Os valores de uso (VU) encontrados variaram entre 0.03 e 1.34, sendo *M. gouazoubira* com o VU mais alto (1.34) e 0.74 de saliência. Em relação à importância cultural, *M. gouazoubira* foi a espécie que apresentou maior VU, saliência e indicada como ECC, para receber prioridades em ações e estudos para conservação.

**Palavras-chave:** Etnozoologia, *Mazama gouazoubira*, Área Protegida, Chapada do Araripe, Caatinga.

### Introduction

Several animal species play a significant role in cultural systems and people commonly identify with these species for cultural or economic reasons (Lohani 2010, Ferreira et al. 2013). Studies in ethnozoology conducted in different cultural contexts, such as in rural (Torres et al. 2009) and indigenous communities (Martínez 2013), have shown that a species is considered important when a utility value is assigned to it.

From a conservation point of view, the way people interact with locally important biological resources must be taken into account, since it is dynamic and susceptible to various influences (economic and socio-cultural factors, for example), a fact that is discussed in specialized literature (Alves 2009, Alves & Dias 2010, Santos-Fita et al. 2012, Hunt 2013). Thus, understanding the issues arising from this relation (social and ecological elements) may be useful to guide conservation strategies of wildlife resources at local level.

With this perspective, the concept of keystone species has been used with human populations in order to identify and describe the cultural importance of biological species in order to understand the particularities of cultural systems (social, economic, cultural and ecological) that affect its maintenance regarding conservation purposes (Platten & Henfrey 2009).

The cultural keystone species method (CKS), designed by Cristancho & Vining (2004) and adapted by Garibaldi & Turner (2004), is a social model that proposes a categorization of species of local importance based on the intensity of established interactions of local communities with local species. CKS may be defined as "a species with a high cultural relevance to a particular social group, defining the identity of a certain place (Garibaldi & Turner 2004) and which is critical to the stability of this group over time" (Cristancho & Vining 2004). It differs from ecological keystone species because it interacts with local people, although it may also play a part in ecological systems.

The first study assessing the importance of a biological species using the CKS method was conducted by Cristancho & Vining (2004) with indigenous peoples of the Colombian Amazon. They defined that CKS would correspond to species that were present in daily life, had some kind of use and were unique in their function (non-redundant species). However, Garibaldi & Turner (2004) evaluated that the results obtained in this study did not allow for a comparative analysis of the species, and proposed a measure of quantitative importance: the Identification Index of Cultural Influence (ICI), which is based on the sum of proportions assigned to each variable. Thus, a CKS would be the one with the highest ICI.

The CKS method is recent and has been employed in the identification of plant species that maintain the traditional way of life of communities (Cristancho & Vining 2004, Garibaldi & Turner 2004, Assis et al. 2010), in mediating conflicts regarding the use of natural resources (Garibaldi 2009) and in the understanding of the dynamic relation between the availability of plant species and cultural change (Barnes 2008). Despite the relevance of this method to guide biological conservation initiatives, there are no published studies using the CKS method to propose conservation priorities for animals in Brazil. The Araripe National Forest (Floresta Nacional do Araripe – FLONA) presents a high diversity of vertebrates with utility potential, with 19 mammal and five reptile species (Nascimento et al. 2015); thus, the knowledge of CKS occurrences in this Protected Area may be useful to define biocultural conservation priorities of animal species used mainly for wild game purposes.

In this study, CKS refers to species that, within a cultural context and a temporal scale, are locally and/or ecologically important, but not necessarily linked to the cultural identity of a group. That's because the same species may have different historical interaction with local people who reflect on its potential use and local communities' way of life (Alves 2012). This definition takes into account that the cultural importance attached to a species by the local community may vary according to the type of social group studied, the availability and the seasonality of the species in earlier or current situations (Medeiros et al. 2013).

Assuming that the value assigned by an informant to each evaluation variable indicates more precisely the factors that affect the survival of the species assessed, one of the main contributions of the CKS approach is its effectiveness in translating information of certain cultural elements so that it is understandable to decision makers (Garibaldi & Turner 2004). Albuquerque & Medeiros (2013) argue that the relation between people and the use of

natural resources is influenced by biological (species are selected by intrinsic factors; a higher amount of biomass, for example) and cultural factors (tradition of use) that interact with each other. Therefore, understanding this dynamic may interfere with the selection of priorities for the conservation of cultural biodiversity. The basic assumption of Biocultural Diversity is that the relation between human species and the environment is mediated by culture (Loh & Harmon 2005).

In this context, this study's objective was to identify and characterize CKS vertebrates in communities surrounding the Environmental Protection Area (EPA) of Chapada do Araripe and propose conservation strategies through the intersection of local and scientific knowledge, with a bioecological perspective. Specifically, this study aimed to identify: 1) Which and how many animal species are known by the communities of the EPA of Chapada do Araripe; and 2) Which animal species are culturally important and subject to conservation and management actions. Answers to these questions may support management and conservation measures for wildlife, aligned with the objectives of this Protected Area (Conservation Unit - CU - Brazil).

## Material and methods

### 1. Study Area

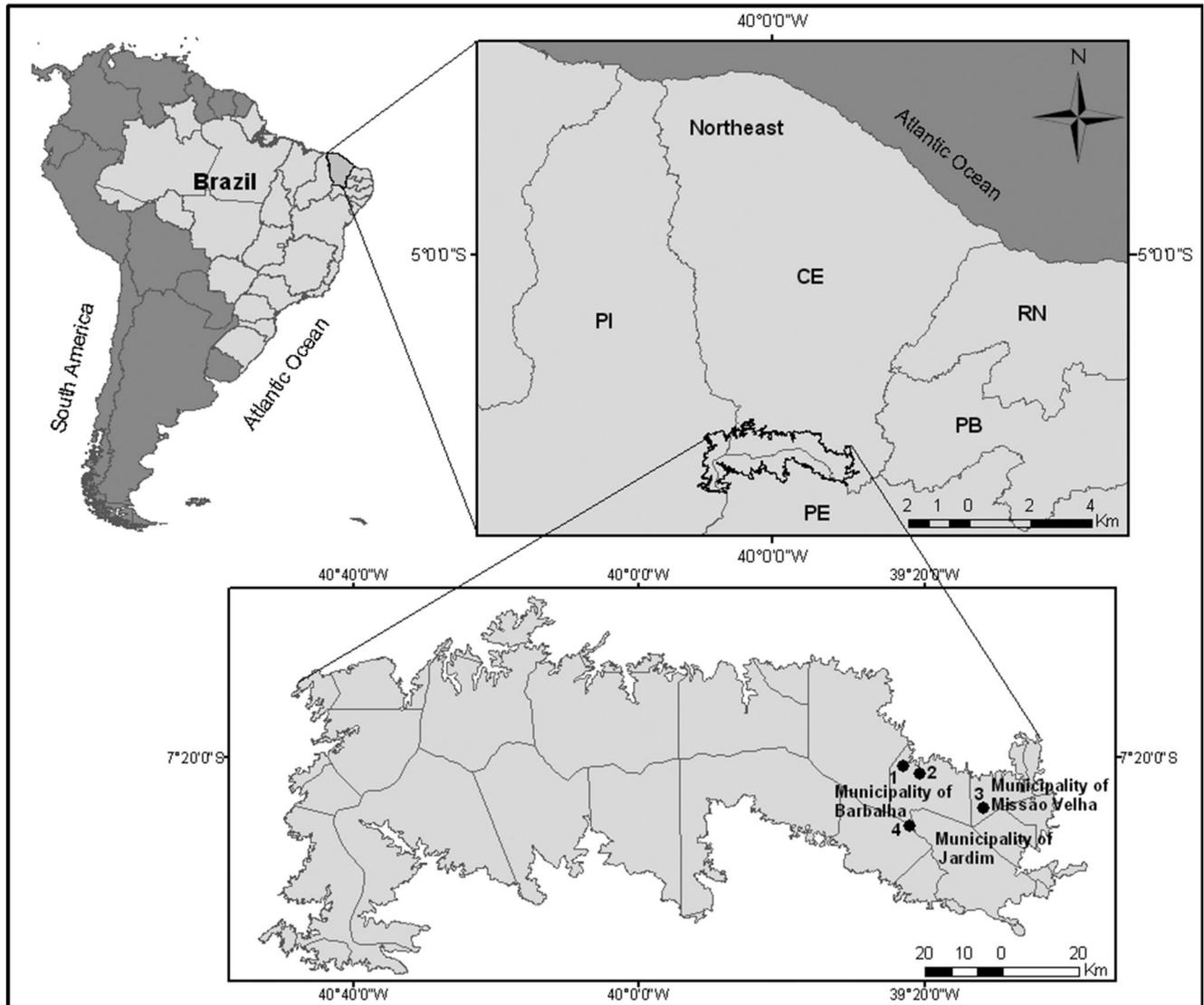
The study was conducted in four communities in the surroundings of the EPA of Chapada do Araripe, Ceará, Brazil (7°42'42" and 7°50'28" S, 39°17'04" and 40°35'23" W), (Figure 1): Caldas (7°22'43"S, 45°21'01"W and 787 m) and Farias (7°29'39"S, 45°22'01"W and 693 m), from the municipality of Barbalha; Novo Horizonte (7°29'39"S, 45°22'01"W and 837 m), from Jardim; and Banco de Areia (7°26'23"S, 45°12'40"W and 917 m), from Missão Velha.

This Federal Protected Area has an extension of 972.590.45 hectares and covers the states of Ceará, Piauí and Pernambuco (CNUC 2011). In climatological terms, it is part of the semi-arid climate domain, with an average rainfall of 1,000 mm per year and average temperatures between 23°C and 25°C (Pernambuco 2007).

Local vegetation is diverse, with some areas of humid forests transitioning to "cerradão", "carrasco" and "cerrado" (Ribeiro-Silva et al. 2012). In general, the *cerradão* is a forest formation with tall, dense and bushy trees; the *carrasco* it is a high xerophytic dense shrub and *cerrado* it is a savanna formation with low and sparse trees (Roberts & Araújo 2013 Araújo et al. 1999).

The EPA of Chapada do Araripe is considered an area of extreme priority for the conservation of biodiversity in Caatinga areas (including forest formations and non-forests), that are still little known from a biological point of view (MMA 2007). The Caatinga domain, an exclusively Brazilian ecosystem of the semi-arid region, comprises an ample variety of vegetation formations characterized according to factors such as altitude, floristic composition and soil composition (Albuquerque et al. 2012). This domain has suffered strong extractivist pressure especially from the conversion of natural areas into pastures or plantations (Oliveira et al. 2007). Originally with 827,934 sq. km, it is the least protected Brazilian biome, with 7.6%, the equivalent of 62,697 sq. km, of its original area in Conservation Units (CUs), of which 1.2% is of Integral Protection and 6.4% of Sustainable Use (CNUC 2015).

According to the National Register of Protected Areas (CNUC 2011), this EPA does not yet have management tools that allow territory usage planning.



**Figure 1.** EPA Chapada do Araripe location and the four studied communities in their surroundings, in the state of Ceará, Brazil. 1: Caldas Community; 2: Farias community; 3: Banco de Areia Community; 4: Novo Horizonte Community.

## 2. Data collection

Data collection occurred in March 2012 and January to February 2013. Information was collected with semi-structured interviews and free listings, with the consent of the interviewees with the free and informed consent (FIC) form approved by the Research Ethics Committee (REC) of the Federal University of Rio Grande do Norte (UFRN), REC/UFRN no. 227/12.

The sample universe included men and women over 18 years of age with a minimum residence of 10 years in the locality; only one member of each family was interviewed in accordance with previously established criteria. The Snowball technique (Bernard 1996) was used to select respondents, which consists of selecting research participants based on recommendation of others.

A total of 246 people were interviewed, with ages varying between 20 and 90, with an average of  $50 (\pm 17.91)$  years. The interviewees practice subsistence farming, mainly cultivating beans, manioc and corn. Among the most important activities for the local economy are the extraction of pequi fruit (*Caryocar coriaceum* Wittm.) and field beans (*Dimorphandra*

*gardneriana* Tul.) (Sousa Júnior et al. 2013). General data of the four communities studied is summarized in Table 1.

There was a difference between the number of men ( $n=173$ ) and women ( $n=73$ ) due to the fact that women refused to participate in the study when their husbands were present during the visits, explaining that the knowledge about animals belonged to the men. According to Torres et al. (2014), the gender variable reflects differences in the production of knowledge and, therefore, in the amount of known animals, and the type of information provided may differ between men and women. Unpublished research conducted by the same authors of the present study in the same communities found significant differences in the number of species cited between men and women, verifying that the men know more animals than the women (men =  $10.35 \pm 4.88$  animals; women =  $10.24 \pm 4.67$ ;  $U = 6811$ ;  $p = 0,697$ ). This may be explained by the fact that the men participate in activities that offer a more direct interaction with the environment, such as hunting and farming (Pfeiffer & Butz 2005).

The semi-structured interview contained four questions regarding interviewees' profile data and seven related to

**Table 1.** General data of the four studied communities in the surroundings of the EPA Chapada do Araripe, Ceará (NE Brazil).

Characteristics	Caldas	Farias	Novo Horizonte	Banco de Areia
Municipality	Barbalha	Barbalha	Jardim	Missão Velha
Total number of families*	142	116	272	116
Total number of interviewees	57	56	82	51
<b>Number of interviewees</b>				
<i>Gender</i>				
Men	40	41	55	37
Women	17	15	27	14
<i>Age (mean in years)</i>	54.1	64.62	51.91	45.56
<i>Occupation (%)</i>				
Agriculture	14.13	32.14	31.70	52.94
Retired	38.02	46.42	21.95	13.72
<i>Time of residency (mean)</i>	46.12	60.62	48.17	39.78

\*Population estimated by the local health center in 2012.

knowledge about the local fauna (vernacular names and uses). To collect information on the known animal species, a free listing was consolidated with the help of the following guiding question: *Which animals of leather, hull and feather exist in the mountains?* This classification was defined based on the specific vocabulary of the local population and was used by the researcher to facilitate understanding by the interviewees. The percentage of mentions was calculated relative to the total number of respondents in order to identify the species of major and minor importance locally.

We confirmed the species listed in the interviews using techniques recommended by Alves & Souto (2010), such as displaying an album to each respondent containing photos of the animals that occur in the study area, with the list of species documented in the National Forest of Araripe Management Plan (IBAMA 2004) as a reference, and with animal parts donated by respondents and/or collected by the researcher.

The taxonomic classification and nomenclature of reptile, bird and mammal species mentioned in this study followed, respectively, the rules of the Brazilian Society of Herpetology (Bérnils et al. 2010), of the Brazilian Committee of Ornithological Registration (CBRO 2011) and of the annotated List of Mammals of Brazil (Paglia 2012).

### 3. Data analysis

To evaluate sampling efforts in each community, the cumulative curve of species (Cowell & Coddington 1994) from 1000 randomizations was used, plotting the number of interviews (x-axis) and the number of ethnospecies (y-axis) on the matrix; the richness of ethnospecies was estimated by the Chao 2 diversity estimator with the help of the EstimateS 8.2.0 software (Cowell 2009). The nonparametric estimator Chao 2 is based on the richness of species found in only one sample as well as in exactly two samples, and requires only the use of a qualitative data matrix (presence or absence) (Magurran 2011). Due to these features, Chao 2 is indicated for ethnozoological studies (Alves et al. 2012).

The use value (UV) of animal species mentioned is calculated with the formula (modified from Rossato et al., 1999)  $UV = \sum U/n$ , where  $UV$  = index of the species' use value;  $U$  = number of mentions per ethnospecies;  $n$  = number of respondents. This formula was used to identify the

most important species. The mentions of use were analyzed by calculating the Fidelity Level (FL, Friedman et al. 1986), obtained by:  $FL = Ip / Iu \times 100\%$ , where  $FL$  = fidelity level;  $Ip$  = number of informants who suggest use of a particular animal as the main purpose, and  $Iu$  = total number of respondents who mentioned the animal for any purpose. According to this index, the greater the consensus among respondents regarding the use mentioned for a species, the higher the FL, where 100% is the maximum value that the species can reach. To verify the level of importance of each species for the communities studied, the Smith's Saliency Index (Smith S) was calculated using the Anthropac 4.0 software (Borgatti 1996). The Saliency Index takes into account the frequency with which a species is mentioned and the amount of times it was mentioned in a certain order (Smith 1993). The species with the highest saliency value represents the species most mentioned. The possible relationship between UV and Smith's Saliency Index was tested by Pearson correlation ( $r$ ), using SPSS Software.

Regarding the assessment of CKS, the analysis resorted to an important timeframe connected to the Protected Area in order to enable all respondents to think in the same timeframe when mentioning the three most important animal species to them: *Of the animals that you mentioned earlier (leather, hull and feather animals), which were the most used (good for use) when there was open forest around the mountain? Name at least three most important animals in your order of preference (Dos bichos que você citou [de couro, de casco e de pena] quais foram os mais usados [bom de serventia] quando a mata da serra era aberta? Diga o nome de pelo menos três mais importantes em uma ordem do mais preferido).* The period in question refers to the years between 1947 and 1965, the period in which hunting, as a subsistence resource, was not prohibited. Moreover, people had free access to Serra do Araripe to cultivate cassava (*Manihot sculenta*, Cranz) and raise cattle, which provided a more direct contact with wildlife resources, with the consequent recognition of animals of local importance. After 1965, the forest was "closed", restricting the use of natural resources (plants and animals) by local people.

Although 15.85% of the interviewed have not experienced the key event, these people took as reference the recent environment and cultural information acquired to report potentially useful animal species. In addition, the residence

time more than 10 years in the location is considered sufficient for a minimum knowledge of the local fauna and their uses (Gehara 2009, Lopes et al. 2009). According to Donnell et al. (2010), when the use of a resource is associated with a landmark event for the local community, this relation can help the interviewee to more easily remember the most important cultural significance resource and its type of use.

After mapping of the three species, a CKS indicator called ICI was developed (Garibaldi & Turner 2004), which calculates the sum of the weighted average of values from 0 to 3 (0 - no, 1 - little, 2 - reasonably; 3 - high), assigned by all respondents to each variable, adapted from Garibaldi & Turner (2004). In this study, CKS assessment variables considered appropriate to calculate ICI were: use, popularity, availability and history. The end result led to an animal species system, in which the highest ICI value elects the animal species that fits the CKS category. Species with an ICI > 10 were established as a top priority for conservation; species with an ICI between 9 – 10, as a high priority; species with an ICI between 5 – 8, as relevant for conservation; and species with an ICI ≤ 5 were considered low priority.

## Results and discussion

### 1. Ethnospecies richness

In free listings, 53 animal ethnospecies were mentioned, distributed in three taxonomic categories: Birds, Reptiles and Mammals, comprising 50 genera and 31 families (Table 2). Among the species mentioned, the groups that had a greater representation regarding richness were birds, with 34 species (64.15%) and mammals with 17 species (32.07%). As for families, Columbidae (14 spp.), Tynamidae (12 spp.) and Emberezidae (3 spp.) showed a higher number of species mentioned for the bird group. Among mammals, Felidae (4 spp.) represented the family with the highest number of species mentioned, followed by Dasypodidae and Canidae (3 spp. each). The reported richness of species corresponds to those with some kind of known use in the study area. The number of species recorded in this study proved to be compatible with what is mentioned in other ethnozoological studies in semi-arid regions of Northeastern Brazil, which ranged from 38 (Barbosa et al. 2011) to 81 species (Alves et al. 2012). The predominance of a greater richness of birds is probably due to the abundance and/or variety of this group in the studied area, a fact also noted by Torres et al. (2009) for the Caatinga. According to the Management Plan (IBAMA 2004), the National Forest of Araripe has 193 bird species and 37 mammal species. On the other hand, in another semi-arid area, "Tupinambá of Olivença", inhabited by indigenous communities (Bahia), a different result was found: of the 42 species mentioned, 30 belonged to mammals and 7 to birds (Pereira & Schiavetti 2010). According to these authors, the greater diversity of mammals mentioned may be related to the availability and/or preference for these animals, but also to energy benefits, as the study reveals a richness of hunted species. A greater taxonomic richness for mammals (n = 16) was also reported by Altrichter (2006) in the arid area of Argentine Chaco. In both studies, hunting is a deeply rooted and widespread custom in the population. In the case of arid regions, due to poverty coupled with social and economic marginalization, much of the diet of rural communities is still sustained by wild meat consumption,

and mammals provide 92% of the protein demand (Altrichter 2006).

According to the cumulative curve of ethnospecies mentions, the number of interviews was satisfactory to conclude the richness of locally known animal species, since there was a stabilization of the number of mentioned ethnospecies (n = 53) around the 244<sup>th</sup> interview. The richness indicated by the Chao 2 estimator was 52 species, with 95% confidence interval, ranging from 51.99 to 52.09 (Figure 2).

### 2. Relative importance of ethnospecies: uses and cultural salience

Of the 53 ethnospecies animals listed, 15 belonging to 11 families were recorded with some value to the communities studied: mammals (9 spp.; 60%) and birds (5 spp.; 33.3%) represent the zoological groups with the highest number of mentioned species (Table 3). Together, these animals fall into four categories of use: food (15 spp./1359 mentions/89.05% of the total of mentions), medicinal (8 spp./102 mentions/6.68%), handicraft (4 spp./52 mentions/3.40%) and symbolic (2 spp./15 mentions/0.98%). The consensus among the interviewees' answers on known uses is detailed in Table 3. In different studies conducted in the Caatinga (Costa-Neto 2000, Oliveira et al. 2010b, Melo et al. 2014) on the use of wildlife, birds and mammals are among the most mentioned taxa in number of species, which demonstrates the cultural importance given to these animals. The highest number of species in the food and medicinal categories was a pattern observed by Alves (2009), while reviewing research on zotherapeutic practices in semi-arid environments, mainly explained by the role of wildlife as a protein source in different parts of the world.

### 3. Categories of Use

**3.1. Food resource.** In studied communities, obtaining meat for food was recorded as the primary use of wildlife, indicating a preference for the following animals (Table 4): common deer, *Mazama gouazoubira* G. Fischer, 1814 (n=237 mentions/17.43%, FL = 77.21%); guan, *Penelope supercilialis* Temminck, 1815 (n=211/15.52%; FL = 98.11%); agouti, *Dasyprocta prymnolopha* Wagler, 1831 (n=205/15.8%; FL = 98.5%); and common armadillo, *Dasypus novemcinctus* (Linnaeus, 1758) (n=195/14.34%; FL = 94.41%). The significant number of mentions of the common deer (*M. gouazoubira*) may be related to its size (amount of biomass obtained in hunting activity), to the appreciable flavor and tenderness of the meat, or to it being considered "healthy". Santos-Fita et al. (2012), in a similar study with rural communities in Mexico, also found *Mazama* sp. among the animals with most mentions for consumption due to palatability and the nutritional value of its meat. On the other hand, Altrichter (2006), in Argentine Chaco, registered unanimous consumption of "tatu-bola", *Tolypeutes matacos* (Desmarest, 1804), justified by the quality of the meat, and a secondary preference for *M. gouazoubira*. According to the same author, this preference may be due to the disappearance of high-productivity mammals (>10 kg) in habitats near communities.

The preference for wild animals whose protein supply is considerable (> 10 kg) constitutes a tendency in other regions of Brazil (Pereira & Schiavetti 2010, Barbosa et al. 2011, Alves et al. 2012) and of Latin America (Quijano-Hernández & Calme 2002, Hurtado-Gonzales & Bodmer 2004, Gil & Gíascon 2012).

**Table 2.** Animal species mentioned by EPA Chapada do Araripe communities, Ceará (NE Brazil).

Family/ Species	Local Name / N° of citations	Conservation Status	
		MMA	IUCN
<b>BIRDS</b>			
<b>Tinamidae</b>			
<i>Crypturellus noctivagus zabele</i> (Spix, 1825)	“zabelê” (yellow-legged tinamou)/21	VU	NT
<i>Crypturellus parvirostris</i> (Wagler, 1827)	“nambu” (Small-billed tinamou)/73	—	LC
<i>Nothura maculosa</i> (Temminck, 1815)	“corduniz” (spotted nothura)/65	—	LC
<b>Accipitridae</b>			
<i>Rupornis magnirostris</i> (Gmelin, 1788)	“gavião” (hawk)/23	—	LC
<b>Falconidae</b>			
<i>Caracara plancus</i> (Miller, 1777)	“carcará” (southern caracara)/18	—	—
<b>Cracidae</b>			
<i>Penelope superciliaris</i> (Temminck, 1815)	“jacu” (guan)/ 211	—	LC
<b>Carimidae</b>			
<i>Cariama cristata</i> (Linnaeus, 1766)	Sariema/ 102	—	LC
<b>Columbidae</b>			
<i>Columbina minuta</i> (Linnaeus, 1766)	“rolinha comum”(common turtle dove)/48	—	LC
<i>Columbina talpacoti</i> (Temminck, 1811)	“rolinha caldo de feijão” (ruddy ground dove)/17	—	LC
<i>Columbina squamatta</i> (Lesson, 1831)	“rolinha cascaval” (scaled dove)/17	—	—
<i>Leptotila verreauxi</i> Bonaparte, 1855	“jurití” (white-tipped dove)/ 70	—	LC
<i>Zenaida auriculata</i> (Des Murs, 1847)	“ribeirão” (eared dove)/ 10	—	LC
<b>Psittacidae</b>			
<i>Eupsittula cactorum</i> (Kuhl, 1820)	“guinguirro” (cactus parakeet)/24	—	LC
<i>Forpus xanthopterygius</i> (Spix, 1824)	“pacu” (Blue-winged parrotlet)/ 6	—	—
<b>Cuculidae</b>			
<i>Crotophaga ani</i> Linnaeus, 1758	“anu preto” (smooth-billed ani)/2	—	—
<i>Guira guira</i> (Gmelin, 1788)	“anu branco” (guira cuckoo)/1	—	LC
<i>Piaya cayana</i> (Linnaeus, 1766)	“alma de gato” (squirrel cuckoo)/7	—	LC
<b>Strigidae</b>			
<i>Glaucidium brasilianum</i> (Gmelin, 1788)	“caburé” ferruginous pygmy owl)/12	—	LC
<i>Megascops choliba choliba</i> (Vieillot, 1817)	“coruja” (owl)/12	—	LC
<b>Nyctibiida</b>			
<i>Nyctibus grisésus</i> (Gmelin, 1789)	“mãe da lua”/12	—	—
<b>Trochilidae</b>			
<i>Chlorostilbon lucidus</i> (Shaw, 1812)	“bizunga”/12	—	—
<i>Eupetomena macroura</i> (Gmelin, 1788)	“tesourão” (swallow-tailed hummingbird)/2	—	LC
<b>Bucconidae</b>			
<i>Nystalus maculatus</i> (Gmelin, 1788)	“fura-barreiro” (puffbird)/2	—	LC
<b>Picidae</b>			
<i>Veniliornis passerinus</i> (Linnaeus, 1766)	“pica-pau-pequeno” (woodpecker)/14	—	—
<b>Tyrannidae</b>			
<i>Fluvicola negenta</i> (Linnaeus, 1766)	“lavadeira”/5	—	—
<i>Pitangus sulphuratus</i> (Linnaeus, 1766)	“bem-te-vi” (great kiskadee)	—	LC
<b>Pipridae</b>			
<i>Antilophia bokermanni</i> Coelho & Silva, 1998	“soldadinho-do-araripe” (Araripe manakin)/20	CR	CR
<b>Corvidae</b>			
<i>Cyanocorax cyanopogon</i> (Wied, 1821)	“cancão” (white-naped jay)/33	—	LC
<b>Troglodytidae</b>			
<i>Pheugopedius genibarbis</i> (Swainson, 1838)	“chorró” (moustached wren)/6	—	LC
<b>Turdidae</b>			
<i>Turdus leucomelas</i> Vieillot, 1818	“sabiá comum” (pale-breasted thrush)/34	—	LC
<i>Turdus rufiventris</i> (Vieillot, 1818)	“sabiá peito amarelo” (rufous-bellied thrush)/13	—	LC
<b>Thraupidae</b>			
<i>Sicalis flaveola</i> (Linnaeus, 1776)	“canário da terra” (saffron finch)/8	—	LC
<b>Parulidae</b>			
<i>Setophaga fusca</i> (Statius Muller, 1776)	“papo de fogo” (lackburnian warbler)/3	—	LC
<i>Myiothlypis flaveola</i> Baird, 1865	“canário comum” (common canary)/9	—	LC

Continued on next page

Table 2. Continued.

Family/ Species	Local Name / N° of citations	Conservation Status	
		MMA	IUCN
<b>MAMMALIA</b>			
<b>Didelphidae</b>			
<i>Didelphis albiventris</i> (Lund, 1840)	“cassaco” (white-eared Opossum)/27	—	LC
<b>Dasyproctidae</b>			
<i>Cabassous unicinctus</i> (Linnaeus, 1758)	“china” (southern naked-tailed armadillo)/69	—	LC
<i>Dasyypus novemcinctus</i> (Linnaeus, 1758)	“tatu comum” (common armadillo)/195	—	LC
<i>Euphractus sexcinctus</i> (Linnaeus, 1758)	“peba” (six-banded armadillo)/194	—	LC
<b>Canidae</b>			
<i>Cerdocyon thous</i> (Linnaeus, 1766)	“raposa” (fox)/82	—	LC
<i>Nasua Nasua</i> (Linnaeus, 1766)	“coati”/20	—	LC
<i>Procyon cancrivorus</i> (G. Cuvier, 1798)	“guaxinim” (raccoon)/19	—	LC
<b>Mustelidae</b>			
<i>Conepatus semistriatus</i> (Boddaert, 1785)	“gambá” (possum)/51	—	LC
<i>Galictis vittata</i> (Schreber, 1776)	“furão” (ferret)/15	—	LC
<b>Felidae</b>			
<i>Leopardus tigrinus</i> Shreber, 1775	“gato do mato; lagartcheiro” (oncilla; tiger cat)/45	EN	VU
<i>Leopardus wiedii</i> (Schinz, 1821)	“gato maracajá” (margay cat)/7	VU	—
<i>Panthera onca</i> Linnaeus, 1758	“onça pintada” (spotted jaguar)/45	VU	NT
<i>Puma concolor</i> (Linnaeus, 1771)	“onça vermelha” (red jaguar)/130	VU	LC
<i>Mazama gouazoubira</i> (G. Fischer, 1814)	“veado comum” (common deer)/237	—	LC
<b>Caviidae</b>			
<i>Galea spixii</i> (Wagler, 1831)	“preá” (guinea pig)/40	—	LC
<b>Dasyproctidae</b>			
<i>Dasyprocta prymnolopha</i> (Wagler, 1831)	“cutiá” (agouti)/205	—	LC
<b>Myrmecophagidae</b>			
<i>Tamandua tetradactyla</i> (Linnaeus, 1758)	“tamanduá” (anteater)/113	—	LC
<b>REPTILIA</b>			
<b>Iguanidae</b>			
<i>Iguana iguana</i> (Linnaeus, 1758)	“camaleão” (chameleon)/38	—	—
<b>Teiidae</b>			
<i>Salvator merianae</i> (Duméril & Bibron, 1839)	“teíu” (black and white tegu)/88	—	LC

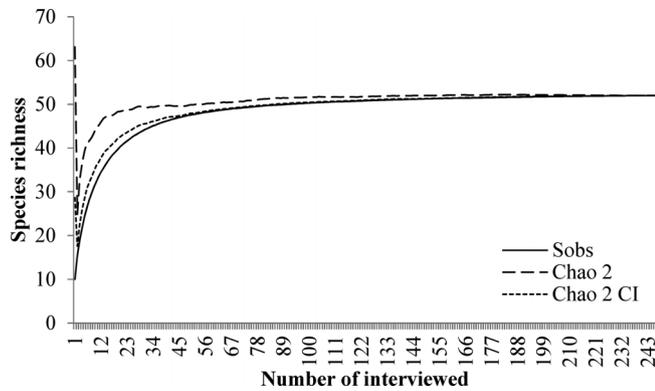
Legend: Categories of the Red List of IUCN (2014.1): DD – Data deficient, LC – Least Concern, NT – Near Threatened, VU – Vulnerable, CR – Critically endangered. Categories of Brazilian Red List (MMA, 2008): CE – Critically endangered, E – Endangered, VU – Vulnerable.

The low consensus (low FL) regarding the common deer, *M. gouazoubira*, compared to other species used as food, is due to the fact that respondents assigned different uses (more than two) for this species besides its main use. This study recorded multiple uses for common deer, including handicraft, symbolic and medicinal uses. Other studies have verified this versatility of the common deer in local communities (Altrichter 2006, Gehara et al. 2009).

Small species also substantially contribute to the amount of meat used for food. The guan (*P. superciliaris*) was appointed as a preference in the communities of Caldas (52 mentions/17.16%) and Novo Horizonte (74 mentions/16.12%); agouti, *D. prymnolopha* (40 mentions/15.62%) in Banco de Areia; and common armadillo, *D. novemcinctus* (52 mentions/15.24%) in Farias, suggesting that these species must be hunted with some frequency. In addition, related preferences reside in the taste and flavor of the meat and possibly in the availability of these animals, as well as their being easy to capture. These results are in agreement with studies that show that the choice of local species hunted for food is influenced by availability, richness and size (Alves et al. 2012). On the other hand, they differ from

Altrichter (2000), who studied the indigenous people of Costa Rica, where it was found that consumption of a species reflects the appreciation of the meat and not its availability.

In this study we found that women in the studied communities avoid a group of animals as a food resource: peba, *Euphractus sexcinctus* (Linnaeus, 1758); china, *Cabassous unicinctus* (Linnaeus, 1758); agouti (*D. prymnolopha*); cavy, *Galea spixii* (Wagler, 1831); anteater, *Tamandua tetradactyla* (Linnaeus, 1758); juriti, *Leptotila verreauxi* Bonaparte, 1855; and common armadillo (*D. novemcinctus*). This is due to temporary taboos, especially due to shrewishness (“carrego”), which suggests that respondents believe that avoiding these animals preserves their health. Temporary taboos are defined as food bans during certain life periods, such as during menstruation, pregnancy and postpartum, since they are considered offensive (Colding & Folke 2001). Jorgenson (1995) found a rejection, regardless of gender, of the consumption of *D. novemcinctus* among indigenous people from Mexico, motivated by the very fat and tasteless meat. From a conservation point of view, the restriction on the use of animals may promote availability of these species over time.



**Figure 2.** Cumulative curve of ethnospecies of the EPA Chapada do Araripe's fauna mentioned by 246 interviewees. Sobs: 53; Expected richness: Chao 2 = 52, stabilization curve, n = 244 interviews. CI: 95% confidence interval.

Respondents unanimously stated that the consumption of "peba", *E. sexcinctus*, increased for women in times of menses, at the risk of causing diseases such as leg swelling, blood thinning followed by an increased menstrual flow and unpleasant odor. A respondent from the Banco de Areia community (L.A.B.,

58 years old) also pointed out that this restriction is lifted at the age of 60, at the postmenopausal stage. This species was cited by Souza & Alves (2014), in the Atlantic Forest area of Paraíba, as restrictive for human consumption due to the omnivorous habit of the species. "Peba", *E. sexcinctus*, is considered a generalist animal, whose diet includes mortal remains (Alves et al. 2012), which suggests that the shrewishness from peba meat may be due not only to the fat, but also to the lack of selection of the animal's diet.

**3.2. Medicinal resource.** The belief in the healing properties of fauna was the second largest use reported by respondents of the communities studied (Table 4). Eight ethnospecies (2 birds, 5 mammals and 1 reptile) which can be used to treat 18 kinds of diseases (Table 5) were mentioned. The parts mentioned by respondents were horn, leather, tail, shin, liver, fat (lard), urine, feces and feather, with lard being the most used as zootherapeutic (6 recommendations for use; 37.5% of recommendations), used to treat 10 kinds of diseases, especially respiratory (asthma, otitis, sore throat) and muscle diseases (rheumatism and swelling in the legs). This finding is consistent with the work of Ferreira et al. (2009) and Vasconcelos-Neto et al. (2012), which refer to lard as the raw material most used in the treatment and cure of various diseases; this was also recorded by Martínez (2013) for the communities of Toba, Argentina.

**Table 3.** Fidelity Level of animal species with use value mentioned by surrounding communities of EPA Chapada do Araripe, Ceará (NE Brazil).

Family/Species	Local name	Main use	FL (%)
<b>BIRDS</b>			
<b>Tinamidae</b>			
<i>Crypturellus noctivagus zabele</i> (Spix, 1825)	zabelê (yellow-legged tinamou)	medicinal	100
<i>Crypturellus parvirostris</i> (Wagler, 1827)	nambu (small-billed tinamou)	food	100
<i>Nothura maculosa</i> (Temminck, 1815)	corduniz (spotted nothura)	food	100
<b>Cracidae</b>			
<i>Penelope superciliosa</i> (Temminck, 1815)	jacu (guan)	food	98.11
<b>Columbidae</b>			
<i>Leptotila verreauxi</i> Bonaparte, 1855	juriti (white-tipped dove)	food	100
<b>MAMMALIA</b>			
<b>Dasypodidae</b>			
<i>Cabassous unicinctus</i> (Linnaeus, 1758)	china (southern naked-tailed armadillo)	food	100
<i>Dasypus novemcinctus</i> (Linnaeus, 1758)	tatu comum (common armadillo)	food	94.41
<i>Euphractus sexcinctus</i> (Linnaeus, 1758)	peba (six-banded armadillo)	food	97.48
<b>Canidae</b>			
<i>Cerdocyon thous</i> (Linnaeus, 1766)	raposa (fox)	medicinal	72.72
<b>Mustelidae</b>			
<i>Conepatus semistriatus</i> (Boddaert, 1785)	gambá (skunk)	medicinal	100
<b>Cervidae</b>			
<i>Mazama gouazoubira</i> (G. Fischer, 1814)	veado comum (common deer)	food	77.21
<b>Caviida</b>			
<i>Galea spixii</i> (Wagler, 1831)	preá (guinea pig)	food	100
<b>Dasyproctidae</b>			
<i>Dasyprocta prymnolopha</i> (Wagler, 1831)	cutia (agouti)	food	98.5
<b>Myrmecophagidae</b>			
<i>Tamandua tetradactyla</i> (Linnaeus, 1758)	tamanduá (anteater)	medicinal	100
<b>REPTILIA</b>			
<b>Teiidae</b>			
<i>Salvator merianae</i> (Duméril & Bibron, 1839)	teiú (black and white tegu)	medicinal	100

**Table 4.** Animal species and their respective categories of use, utilized parts, cited by the inhabitants of the communities surrounding the EPA Chapada do Araripe (NE Brazil).

Family/Species	Local name	N° of citations	Category of use	Part used
<b>BIRDS</b>				
<b>Tinamidae</b>				
<i>Crypturellus noctivagus zabele</i> (Spix, 1825)	zabelê (yellow-legged tinamou)	21	medicinal	feather
<i>Crypturellus parvirostris</i> (Wagler, 1827)	nambu (Small-billed tinamou)	73	food	meat
<i>Nothura maculosa</i> (Temminck, 1815)	corduniz (spotted nothura)	65	food	meat
<b>Cracidae</b>				
<i>Penelope superciliaris</i> (Temminck, 1815)	jacu (guan)	211	food, medicinal	meat, fat
<b>Columbidae</b>				
<i>Leptotila verreauxi</i> Bonaparte, 1855	juriti (White-tipped dove)	70	food	meat
<i>Zenaida auriculata</i> (Des Murs, 1847)	ribaçã (Eared dove)	10	food	meat
<b>MAMMALIA</b>				
<b>Dasypodidae</b>				
<i>Cabassous unicinctus</i> (Linnaeus, 1758)	china (Southern naked-tailed armadillo)	69	food	meat
<i>Dasyypus novemcinctus</i> (Linnaeus, 1758)	tatu comum (Common armadillo)	195	food, medicinal	meat, tail
<i>Euphractus sexcinctus</i> (Linnaeus, 1758)	peba (Six-banded armadillo)	194	food	meat
<b>Canidae</b>				
<i>Cerdocyon thous</i> (Linnaeus, 1766)	raposa (Fox)	82	medicinal, handicraft	fat, leather
<b>Mustelidae</b>				
<i>Conepatus semistriatus</i> (Boddaert, 1785)	gambá (Skunk)	51	medicinal	fat
Family/Species	Local name	N° of citations	Category of use	Part used
<b>Cervidae</b>				
<i>Mazama gouazoubira</i> (G. Fischer, 1814)	veado comum (common deer)	237	food, medicinal, handicraft symbolic	meat, leather, fat, liver, feces, horn, paw
<b>Dasyproctidae</b>				
<i>Dasyprocta prymnolopha</i> (Wagler, 1831)	cutia (Agouti)	205	food, handicraft	meat, leather
<b>Myrmecophagidae</b>				
<i>Tamandua tetradactyla</i> (Linnaeus, 1758)	tamanduá (Anteater)	113	handicraft	leather
<b>REPTILIA</b>				
<b>Teiidae</b>				
<i>Salvator merianae</i> (Duméril & Bibron, 1839)	Teiú (Black and white tegu)	88	medicinal	fat

**Table 5.** Animal species known and/or used for medicinal purposes by communities surrounding the EPA Chapada do Araripe, Ceará (NE Brazil).

Family/Species/Local name	Citations	Use value	Salience	Used part	Indications
<b>BIRDS</b>					
<b>Tinamidae</b>					
<i>Crypturellus noctivagus zabele</i> (Spix, 1825) “yellow-legged tinamou”	4	0.02	0.03	feather	snake bite
<b>Cracidae</b>					
<i>Penelope superciliaris</i> Temminck, 1815 “guan”	1	0.86	0.43	lard (= fat)	the flu
<b>MAMMALIA</b>					
<b>Dasypodidae</b>					
<i>Dasypus novemcinctus</i> (Linnaeus, 1758) “common armadillo”	1	0.81	0.51	tail	otitis
<i>Euphractus sexcinctus</i> (Linnaeus, 1758) “six-banded armadillo”	2	0.8	0.5	lard	ringworm, feet fissure
<b>Canidae</b>					
<i>Cerdocyon thous</i> (Linnaeus, 1766) “fox”	11	0.04	0.19	lard	“puxado” (= asthma), rheumatism
<b>Mustelidae</b>					
<i>Conepatus semistriatus</i> (Boddaert, 1785) “skunk”	6	0.02	0.11	urine	rheumatism, eyes, back pains
				lard	rheumatism, otitis
<b>Cervidae</b>					
<i>Mazama gouazoubira</i> (G. Fischer, 1814) “common deer”	33	1.34	0.74	lard	swelling (= edema), rheumatism
				brine (= meat secretion)	children walking too early in life
				leather feces	snake bite asthma
				marrow (= oil)	otitis, “moquidão” (= deafness)
				horn	toothache, teeth appearing in children
				liver	asthma
<b>REPTILIA</b>					
<b>Teiidae</b>					
<i>Salvator merianae</i> (Duméril & Bibron, 1839) “black and white tegu”	43	0.17	0.29	lard	otitis, sore throat, cough

In the studied communities, there was a smaller number of vertebrate species used for medicinal purposes than in other areas of the caatinga biome. Examples of this include the works of Alves et al. (2011) and Alves et al. (2012), which documented the use of 47 and 15 species respectively. As in other ethnozoological works done in different biomes, the number of species with medicinal use in this study was also low. Yirga

et al. (2011), for example, reported the use of 66 species in Ethiopia; Benítez (2011) reported the use of 26 species in Granada Province, Spain; Bagde & Shampa (2013), in communities adjacent to the Pench National Park in India, documented the medicinal use of 30 species. The few records of medicinal use found in this study may be a reflection of the recovery of species by type of use between men and women,

since in this study 70.32% of respondents were men; however, knowledge of men and women regarding medicinal species did not differ greatly ( $n = 101$  mentions of use, being 52 mentions by men and 49 by women). Still regarding gender, women tend to know about the use of species that are more closely related to the medical category, while men show a better knowledge of species in the food category; however, this knowledge may vary from region to region, and it may be similar or different (Lucena et al. 2012).

The most mentioned species used as a medicinal resource in the studied communities was the tiú, *Salvator merianae* (Duméril & Bibron, 1839): 4 diseases, 43 mentions (FL = 100%), which, together with the common deer, *M. gouazoubira* (10 diseases, 33 mentions), accounted for 51.85% of treatment indications (Table 2). Alves et al. (2010) also reported the indication of tiú lard, *S. merianae*, in the treatment of four different types of diseases. In the Argentine Chaco, the lard of this species was equally important as medicine (Altrichter 2006), which demonstrates the wide geographic distribution of tiú, *S. merianae*, and its relevance for therapeutic purposes. A pharmacological study by Ferreira et al. (2010) depicts that oil derived from the fat of *S. merianae* has anti-inflammatory action. For the species *M. gouazoubira*, therapeutic indications or purposes recorded in this study were higher when compared to the indications documented in other ethnozoological research as similar to the *Mazama* gender, a record demonstrating the medicinal potential of this species in the communities studied. Gehara et al. (2009) in their study with residents of Ibitipoca State Park in Minas Gerais, Brazil, found medical reports only of horn scraping, which is indicated for colic. The use of horn scraping was recorded in this study, but with other reported therapeutic indications, such as for toothache and tooth eruption in children. It is noteworthy that the medical efficiency of therapeutic indications of common deer (*M. gouazoubira*) products are yet unproven.

In the communities studied, 62.5% of different species ( $n = 5$ ) were prescribed to treat more than one disease: the lard of common deer, *M. gouazoubira*; possum, *Conepatus semistriatus* (Boddaert, 1785); and foxes, *Cerdocyon thous* (Linnaeus, 1776) was recommended for rheumatism; common deer shin, *M. gouazoubira*, possum lard, *C. semistriatus*, tiú lard, *S. merianae* and common armadillo tail, *D. novemcinctus*, for otitis. In other studies, these species have records of therapeutic indications (Alves & Alves 2011, Ferreira et al. 2012, Martínez 2013). However, except for the use of lard of *S. merianae*, there is no pharmacological evidence for the effectiveness of other uses. The pharmacy hypothesis (Begossi 2012), in which species for medicinal use are not the most important for consumption, is not confirmed for the studied communities, probably due to the few existing resources to be exploited in the region. In the case studied, maximizing the use of exploited species should be stressed, since that may be a key-point for the maintenance of local species.

It is worth noting that more than one product of the same species was indicated to treat the same disease, with differences on how to use it. In the Caldas community, possum (*C. semistriatus*) urine, was indicated to treat rheumatism: "... three drops in the joints once a day until getting better, or drink a tablespoon once a day, any time" (N. B., 52 years old); while in the Novo Horizonte community, lard was recommended for this purpose: "one boils the lard, melt it, lays on a glass, let it cool and spread in the joints" (B. A., 45 years old). These results

support the concept of utilitarian redundancy (Albuquerque & Oliveira 2007), which assumes that one disease may be treated by more than one animal species. The medicinal use of animals for the same purpose suggests that different species may share similar medicinal properties (Ahmed & Ahmed 2011). Ferreira et al. (2013) emphasize that the inclusion of a species in treating more than one disease can trigger a mechanism to reduce the impact on other animals with medicinal purposes.

**3.3. Handicraft resource.** Regarding handicraft use, five species were mentioned for this purpose (Table 4): common deer, *M. gouazoubira* (45 mentions); agouti, *D. prymnolopha* (2); anteater, *T. tetradactyla* (4); and fox, *C. thous* (1).

The body parts mentioned were leather, horn, tail, feet and nail. Leather was the product most often used for handicraft purposes (65.38% mentions for this use), particularly in the manufacture of musical instruments (tambourine, drum and bass drum), household items (chair upholstery, stools), personal utensils (belt, knapsack, doublet, blanket), and making of "reios" (belts) for use in flour mills. Vasconcelos-Neto et al. (2012), in the Paraíba semi-arid, recorded the use of anteater leather, *T. tetradactyla*, in the production of tambourines and drums.

Out of all the species used for handicraft ( $n = 5$ ), the common deer, *M. gouazoubira*, stood out as the most used in the four communities, with 86.53% mentions for the uses mentioned above. Gil & Guisacón (2012), in Mayan communities, Mexico, reported the use of *Mazama* sp. leather for upholstery (chair seats), handbags, belts and shoes. Altrichter (2006) also documented the use of *M. gouazoubira* leather by peasants of the Argentine Chaco.

**3.4. Symbolic resource.** The common deer, *M. gouazoubira*, was the only species recorded in the symbolic use category in the communities studied with 15 mentions (Table 4), of which 46.66% occurred in the community of Novo Horizonte, supporting the hypothesis of a more diverse and deeper knowledge of the use of *M. gouazoubira* in this community. According to reports from hunters ( $n = 8$ ), the horn and the paw of the common deer have a great power to bring good fortune; in hunting activities, they're said to draw prey. They often carry these items on themselves as a protection amulet or keep them at home to expel bad mojo. This practice still persists in the minds of these people, a fact confirmed in opportune occasions observing the use of horn keychains for car keys, and paws kept at homes in the Banco de Areia and Farias communities ( $n = 2$  observations at each site), during the study period. Recent acquisitions of these amulets by the lead researcher of this study, in a period between six months and one year, in the communities of Farias and Banco de Areia, denote that this species is still exploited in the region. The use of the common deer paw as an amulet (against bad mojos) was also recorded in other sites in the same state where the study was conducted (Ferreira et al. 2009, Teles et al. 2013), which confirms the spread of this practice in the EPA region of Chapada do Araripe.

#### 4. Use value and Smith's Salience Index

The use value (UV) analysis of the 15 species mentioned indicated an UV between 0.03 and 1.34, which shows considerable variation in the use and/or knowledge of the species in studied communities (Table 4). For most species, a low UV ( $< 0.5$ ) was found; only one species had a very high

UV, greater than 1.0: the common deer, *M. gouazoubira* (1.34). Four other species showed a UV higher than 0.5: guan, *P. superciliaris* (UV=0.86); agouti, *D. prymnolopha* (0.84); common armadillo, *D. novemcinctus* (0.81); and peba, *E. sexcinctus* (0.80), thus representing species of greatest cultural relevance. By using the UV as a parameter, these species have had their cultural importance investigated in the semiarid region of Paraíba, Brazil (Barbosa et al. 2011) and in Mexico (Gil & Guiascón 2012).

A comparison of the UV of *M. gouazoubira* recorded in this study with other ethnozoological studies conducted in Brazil revealed that, in these studies, this species showed a very low UV, as in studies conducted by Souto et al. (2011, 2012) in the Caatinga biome, UV = 0.03 and 0.04, respectively; and by Alves & Rosa (2007), UV = 0.25 among medicinal animal dealers, also in the same biome. The difference between the use values found in this and in other studies may be explained by the environmental context of the use of the common deer. Local communities, due to daily contact with biological resources, developed a system of knowledge in which a more familiar resource will have greater use versatility; on the other hand, among sellers in public markets, knowledge on the use of biological resources tends to be more general due to the number of species used with different objectives. In addition, knowledge of sellers is restricted to the species most sought after by people.

The general Smith's Index identified that the species with the greatest value of salience (0.74), which is also the one with the highest UV, is the common deer (*M. gouazoubira*), confirming the cultural importance of this species to the studied local communities.

Pearson's Correlation test showed high correlation between the UV and the Smith's Index (S):  $r = 0.87$ ,  $p < 0.001$ . Therefore, the common deer, *M. gouazoubira* is, *a priori*, the most culturally important species (according to Cristancho & Vining 2004, Garibaldi & Turner 2004), and a priority for conservation. Note that, not all cultural keystone species will have the same characteristics in different cultures. Therefore, to clearly identify a cultural keystone species is necessary to define the indicators based on the cultural system studied (Platten & Henfrey 2009) and understand it in a systemic perspective.

At the challenge to clearly identify the cultural key species, it is recommended that decision makers have very carefully before including them in conservation strategies (Power et al. 1996). Nuñez & Simberloff (2005) point out the presence or insertion of invasive species in local communities by using the CKS method. According to the authors, these species can acquire important value to people after his introduction as to replace the key role of native species. In this regard, Sampaio & Schmidt (2013) points out that biological invasions are recognized as the second leading cause of biodiversity loss on the planet. The occurrence of invasive alien species in protected areas has led to consequences such as alteration of species composition and ecosystem processes and, in extreme cases, the local extinction of species.

Of the animal species mentioned as having use value in the studied communities (n = 15), only the Zabele bird, *Crypturellus noctivagus* (Wied, 1820), is listed in the National List of Brazilian endangered Fauna Species and is considered "VU" (vulnerable) (Piacentini & Straube 2008). In IUCN's Official endangered Species list (2014), the species *C. noctivagus* is evaluated as "NT" (Near threatened); other recorded species (93.33%) are in the category "LC" (Least concern).

## 5. CKS from the surrounding communities of EPA Chapada do Araripe

Five ethnospecies were mentioned as locally preferred: common deer, *M. gouazoubira*; guan, *P. superciliaris*; agouti, *D. prymnolopha*; common armadillo, *D. novemcinctus*; and peba, *E. sexcinctus*. The value of the general Identification Index of Cultural Influence (ICI) obtained for the species *M. gouazoubira* (ICI = 9.68) was the highest among the evaluated ethnospecies, indicating that this is a cultural keystone species, and can be considered as a priority for conservation and management (Table 6). Three species obtained ICI between 5.92 and 8.02, indicating that they are relevant for conservation, and only one species, peba, *E. sexcinctus*, showed a low ICI value (5.0), suggesting that this species is of minor importance to the studied communities, and, therefore, is considered of lower priority regarding conservation of cultural biodiversity. This result confirms the position of Albuquerque & Medeiros (2013) in stating that, within a cultural system, the choice and the use of a species is a result of the cultural and biological factors in which a local community is inserted.

### 5.1. Characterization of CKS *M. gouazoubira*.

Worldwide, cervidae play an important role in local communities due to its importance as a hunting resource (Pinder & Leeuwenberg 1997). The common deer, *M. gouazoubira*, which occurs in all biomes of Brazil (Duarte et al. 2012), is widely used in the EPA Chapada do Araripe but is not emphasized as an essential component in the diet of local communities, given that other wild species also have a similar role as food. The high value attributed to this species is notable for its history and multitude of uses, because, according to interviewees, in the 1930s, hunting, in association with farming of cassava, was driven by the need to guarantee a protein source for families, exemplified by F. N.'s testimony, a resident of the community of Farias (65 years old): "*The mountain was everybody's; food for poverty*". This practice of subsistence hunting was intense until the year of 1965, when the National Forest of Araripe ("FLONA Araripe") was created and hunting was forbidden in Brazil by the Forest Code (Federal Law no. 5197/67). However, it happens to the present day, as the uses found in the studied communities show. According to Melo et al. (2014), the social practice of mammalian use still persists in the EPA Chapada do Araripe, probably due to rooted traditions, poverty and lack of stricter inspections.

Another characteristic that has helped to strengthen the high cultural value of the species *M. gouazoubira* is the intensified connection of residents of the studied communities with wildlife resources, which makes it a remarkable and very popular animal for the local people. All four studied communities have a territorial overlap with the National Forest of Araripe, thus respondents demonstrated having a refined ecological knowledge about this cervidae, including behavioral aspects ("smart animal"), taxonomic ("looks like a goat"), food preferences ("where there's fruit, he eats") and spatial distribution ("you only find it in the deep forest"). Expressions like "has much; is rare to see" suggest that *M. gouazoubira* is very common in the EPA and in the National Forest. The common deer, *M. gouazoubira*, is a small deer, with an average height of 50 cm, which is always associated to and dependent on forests for shelter and food, being markedly frugivorous (Pinder & Leeuwenberg 1997).

As for availability, according to respondents, *M. gouazoubira* is the most abundant when compared to other species since the creation of the National Forest of Araripe. The presence of this

**Table 6.** Average rating of animal species for the evaluation variables of a CKS (adapted from Garibaldi & Turner 2004). 3 = very; 2 = reasonable; 1 = little; 0 = none.

Variables / CKS evaluation questions	Species / Common name / Average results				
	<i>M.</i> <i>gouazoubira</i> “common deer”	<i>P.</i> <i>superciliaris</i> “guan”	<i>D.</i> <i>prymnolopha</i> “agouti”	<i>D.</i> <i>novemcinctus</i> “common armadillo”	<i>E.</i> <i>sexcinctus</i> “six-banded armadillo”
1 <b>Uses</b> Is the species an important part of the diet and/or does it have multiple uses? (A espécie é importante componente da dieta e/ou tem múltiplos usos?)	3.16	1.98	1.97	2.0	1.0
2 <b>Popularity</b> Is the species recognized in all its details? (A espécie é reconhecida em detalhes?)	2.80	3.0	3.0	2.92	3.0
3 <b>Availability</b> Does the species still exist? Is it easily seen? (A espécie ainda existe? É fácil de ver?)	3.0	3.0	2.99	1.0	1.0
4 <b>History</b> Does the species stand out in popular stories? (A espécie é destacada em narrativa popular?)	0.72	0.04	0.01	0	0
<b>TOTAL = ICI</b>	9.68	8.02	7.97	5.92	5.0

deer is recognized by its footprints on trails/roads commonly used by respondents in agricultural activities, extraction and/or displacement from one community to another, a fact confirmed during fieldwork. The availability of the species *M. gouazoubira* in the study area can be justified by the fact that this species is the most abundant in South America, occupying forest areas and open fields, cerrado and caatinga, and it can be found in agricultural and anthropic areas (Duarte et al. 2012).

Regarding the historical aspects of *M. gouazoubira*, it proved to be restricted to those attending and/or having attended the National Forest of Araripe during extractive and/or hunting activities. However, due to oral stories, exploits and experienced hunting adventures were fairly shared by all communities. These stories are kept alive in people’s memories. There are reports that the capture of this species is regulated by a symbolic universe and tactics as well as teamwork are needed in order to slaughter it. Hunting is a type of recreational activity that depends on a hunter’s various skills to succeed, as well as on refined knowledge of the environment and particular circumstances of the target species, as highlighted by Kaltenborn et al. (2013).

The context of the results suggests that the common deer, *M. gouazoubira*, is an important component of the local culture of populations adjacent to the EPA Chapada do Araripe, not as a food source, but as an element embedded in cultural practice. Therefore, insertion of local knowledge is relevant to management decisions and wildlife conservation of the EPA Chapada do Araripe.

## Conclusions

For the communities studied in the vicinity of the EPA Chapada do Araripe, the species with the highest use value, the most mentioned and with the highest salience was the common deer, *M. gouazoubira*; therefore, this species should be prioritized in conservation actions and studies. This fact was also confirmed by the CKS method, which also indicated the species *M. gouazoubira* as worthy of conservation attention.

The use of the CKS method to establish priority species for conservation implies the incorporation of local relevant aspects still little explored in the decision-making processes, such as recovering the entire history of the species assessed regarding its cultural and biological aspects from the knowledge of local communities. In addition, the measurement of these attributes may minimize one of the criticisms of the prioritization method traditionally used, as it will provide the reduction of subjectivism in identifying the importance of priority species for conservation, because a quantitative index is used for its determination: ICI. The results of this study are unprecedented regarding the conservation of wild animals and present relevant issues for future work on the management and conservation of *M. gouazoubira* in the EPA Chapada do Araripe. For example, in the pressure-for-use scenario, it is important to know and understand how local communities are affecting and/or promoting the maintenance of animal populations and how these social actors can participate in conservation strategies.

One of the limitations to wildlife conservation is the lack of knowledge about a species’ distribution, biology and ecology. In the case of *M. gouazoubira*, the scarce information about the living conditions of its wild populations puts them in the Least Concern category on the IUCN List, limiting the possibility of working on conservation strategies. According to Duarte et al. (2012), among future actions aimed at the conservation of the species *M. gouazoubira*, studies on population estimates for understanding the dynamics of these populations and the expansion of protected areas in federal, state, and municipal government as a measure for effective and urgent conservation are included. According to the same authors, to date, there is no conservation program directed to this species in Brazil.

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## Taxonomy, morphology and distribution of Cymatosiraceae (Bacillariophyceae) in the littorals of Santa Catarina and Rio Grande do Sul

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GARCIA, M. Taxonomy, morphology and distribution of Cymatosiraceae (Bacillariophyceae) in the littorals of Santa Catarina and Rio Grande do Sul. Biota Neotropica. 16(2): e20150139. <http://dx.doi.org/10.1590/1676-0611-BN-2015-0139>

**Abstract:** Species of Cymatosiraceae (diatoms) studied from Santa Catarina and Rio Grande do Sul littorals: *Campylosira cymbelliformis*, *Cymatosira belgica*, *Cymatosirella minutissima*, *Plagiogrammopsis minima* and *Plagiogrammopsis vanheurckii* are presented with a morphological description, dimension data, distribution in the studied area and are illustrated in light microscope, and scanning and transmission electron microscopes. Superficial sand samples from the swash zone and plankton were collected from over 30 marine sandy beaches. *Cymatosirella minutissima* and *Plagiogrammopsis minima* are new recordings in Brazil widening their distribution to South America, previously being restricted to Europe. *Campylosira cymbelliformis* was the species most often observed in plankton samples and *Plagiogrammopsis minima* in sediment samples. *Cymatosira belgica* and *Plagiogrammopsis minima* were recorded as abundant species in Mariscal, Quatro Ilhas and Zimbros reaching a relative frequency of 9.6%. The study adds new morphological data to some taxa as the presence of rimoportula on *C. minutissima* and one row of poroids on copulae of *P. minima*, these features being in agreement with Cymatosiraceae diagnosis.

**Keywords:** biodiversity, psammic diatoms, sandy beaches, South Atlantic Ocean.

GARCIA, M. Taxonomia, morfologia e distribuição de Cymatosiraceae (Bacillariophyceae) nos litorais de Santa Catarina e Rio Grande do Sul. Biota Neotropica. 16(2): e20150139. <http://dx.doi.org/10.1590/1676-0611-BN-2015-0139>

**Resumo:** As espécies de Cymatosiraceae (diatomácea) encontradas no litoral de Santa Catarina e Rio Grande do Sul: *Cymatosira belgica*, *Campylosira cymbelliformis*, *Cymatosirella minutissima*, *Plagiogrammopsis minima* e *Plagiogrammopsis vanheurckii* são apresentadas com descrições morfológicas acompanhadas de dados referentes a dimensões, distribuição na área de estudo e ilustrações em microscopia óptica, e eletrônica de varredura e de transmissão. As amostras da areia superficial foram coletadas na região de varrido das ondas e de plâncton em mais de 30 praias arenosas. *Cymatosirella minutissima* and *Plagiogrammopsis minima* são registros novos para o Brasil ampliando sua distribuição para a América do Sul antes restritas à Europe. *Campylosira cymbelliformis* foi a espécie mais frequentemente observada nas amostras de plâncton and *Plagiogramma minima* in the sediment samples. *Cymatosira belgica* e *Plagiogrammopsis minima* são registradas como espécies abundantes em Mariscal, Quatro Ilhas e Zimbros alcançando a frequência relativa máxima de 9,6%. Este estudo adiciona novos dados morfológicos para alguns táxons com a presença de uma rimopórtula em *C. minutissima* e uma fileira de poróides nas cópulas de *P. minima*, estando estas características de acordo com a diagnose da Cymatosiraceae.

**Palavras-chave:** Biodiversidade, diatomáceas psâmicas, Oceano Atlântico Sul, praias arenosas.

### Introduction

The Cymatosiraceae family belongs to class Mediophyceae and according to Hasle et al. (1983) it presents eight genera mainly from the Northern Hemisphere: *Cymatosira* Grunow, *Campylosira* Grunow ex Van Heurck, *Plagiogrammopsis* Hasle, von Stosch & Syvertsen, *Brockmaniella* Hasle, von Stosch & Syvertsen, *Minutocellus* Hasle, von Stosch & Syvertsen,

*Leyanella* Hasle, von Stosch & Syvertsen, *Arcocellulus* Hasle, von Stosch & Syvertsen and *Papiliocellulus* Hasle, von Stosch & Syvertsen. Recently, Sabbe et al. (2010) e Dabek et al. (2013) described a new genus *Cymatosirella* Dabek, Witkowski & Sabbe with a known distribution to Europe and South Africa and *Pierrecomperia* Sabbe, Vyverman & Ribeiro which was confined to Europe until now.

The most important features in this family are the bipolar symmetry, heterovalvy (present in several genera as in *Campylosira* and *Cymatosira*), open girdle bands in a number of 4 or more, areolae occluded by an external cribrum, the chains organizational pattern, presence of fascia on the centre of the valve face reaching the valve margin, one rimoportula per valve located on the central position, ocelluli (a type of small ocello), pseudoseptum and the simple or complex spines that are ultra-structural details useful for their correct identification.

In Brazil, the family is represented by *Cymatosira atlantica* Frenguelli, *Cymatosira lorenziana* Grunow, *Cymatosira belgica* Grunow, *Campylosira cymbelliformis* (A. Schimdt) Grunow ex Van Heurck and *Plagiogramma vanheurcki* Grunow which were registered by Procopiak et al. 2006 to Paraná State (Brazil).

Regarding its presence in the Rio Grande do Sul State littoral, there are three taxonomic studies reporting Cymatosiraceae: one carried out with plankton samples from Tramandaí beach by Rosa (1982), another executed with associated diatoms on *Hypnea musciformis* (Wulfen) Lamouroux from rocks of Torres beach by Busellato-Toniolli (1986) and a third studied conducted on exposed sand sediments of the sandy beach of Praia Azul by Garcia-Baptista (1993). All authors have found only *Campylosira cymbelliformis* and Garcia-Baptista (1993) records the species as frequent in sand samples (present in 105 of the 189 samples studied) with an average number varying from 500 to 3,100 frustules/cm<sup>3</sup> while other studies have no data on its abundance or frequency.

More recently, Garcia (2013) described *Extubocellulus brasiliensis* M.Garcia as a new species for Science with a known distribution to a few sandy beaches of Santa Catarina State.

Cymatosiraceae genera are widespread in benthic habitats and particularly common on sediments, but they can occur occasionally in plankton samples. Some species present a higher abundance in sandy sediments such as

*Plagiogrammopsis minima* (Salah) Sabbe & Witkowski and *Cymatosirella minutissima* Sabbe et al. (2010) and others such as *Cymatosira belgica* are common in exposed mud samples of river deltas according to Manoylov & Dominy (2013) and (Hassan et al. 2009), silty sediments and in plankton samples Muylaert & Sabbe (1999). Hassan et al. (2009) showed their use for Paleosalinity reconstructions whereas *C. belgica* was related to marine or marine brackish environments in Argentina.

This paper aims at describing species of Cymatosiraceae showing the diversity of it from samples collected in Santa Catarina and Rio Grande do Sul State littorals with data on morphology, dimension, distribution in the studied area and illustrations in LM (Light Microscope), TEM (Transmission Electron Microscope) and SEM (Scanning Electron Microscope).

## Materials and Methods

The first centimeter of sediment was scraped from exposed portions (next to the sea water and at a midway point between the dunes and the sea water) of sandy beaches located in Santa Catarina and Rio Grande do Sul State littorals (Table 1). Almost all beaches have a general arc shape and samples were collected at their extremities and at their middle totaling at least 6 samples per beach studied. Two set of samples were studied: the first set of samples (121) was collected in December 1995 and in January 1996, and the second set of samples (182) was collected from September 2001 to July 2002.

The sediment was collected by using a 3.7 cm diameter polyvinyl chloride tube, which was pressed down vertically into the sediment to a one centimeter depth. The sample was kept in flasks with 20 ml of 3% Lugol solution; later 4% formaldehyde was added.

Plankton samples net (20 µm opening) were collected from Cassino beach (located in the Rio Grande do Sul littoral) on three dates: in March 2001, in October and in December 2005. These samples were fixed with formaldehyde 4%.

**Table 1.** Presence of Cymatosiraceae species in the studied beaches (x) and relative frequency of species observed in abundance.

Beaches	<i>C. cymbelliformis</i>	<i>C. belgica</i>	<i>C. minima</i>	<i>P. minima</i>	<i>P. vanheurckii</i>
Araçá	–	x	x	–	–
Bombas	–	–	–	x	–
Canto Grande	–	–	–	–	–
Cassino	x	x	–	–	–
Gamboá	–	x	–	x	–
Garopaba	–	–	–	x	–
Guarda do Embaú	–	–	–	x	–
Iró	x	–	–	–	–
Itapirubá	x	–	–	–	–
Mar Grosso	x	–	–	–	–
Mariscal	–	(3 %)	x	–	–
Navegantes	x	–	–	–	–
Penha	–	–	–	x	–
Perequê	x	x	–	–	–
Quatro Ilhas	–	x	–	(2 - 2.3%)	x
São Miguel	–	–	–	x	–
Siriú	–	–	–	x	–
Tramandaí	x	–	–	–	–
Zimbros	–	x	x	(9.6%)	–

In order to separate diatoms from sediment, about 2 gr of sediment and 2 mL from the fixative solution were taken from each flask, and to study plankton, a subsample of 2 mL were taken from each flask. The Simonsen (1974) technique was followed to clean all samples. Part of the material was mounted in Naphrax<sup>®</sup> (Brunel Microscopes Ltd., Chippenham, Wilt-shire, UK) and examined with a light microscope (BX40, Olympus, Tokyo) equipped with an digital camera (OPT14000, Plustek, Taipei).

For scanning electron microscopy, cleaned specimens were dried onto a stub, and coated with platinum at 40 mA for 100 seconds using a sputter coater (Bal-tec SCD 050, Balzers, FL) Stubs were observed using a JEOL (JSM-6060, JEOL, Tokyo) scanning electronic microscope at an accelerating voltage of 15 - 20kV with a working distance of 10 mm.

In two permanent slides for each sample, 400 valves were counted in order to calculate the relative frequency of each species (Schoeman, 1973). Species that achieve a higher count than the mean value were considered abundant.

The samples have been deposited in **ICN** and **PEL** herbaria of the Federal University of Rio Grande do Sul (UFRGS) and the Federal University of Pelotas (UFPel), respectively.

Beach classification follows Brown & McLachan (1990) and diatom morphology terminology follows Hasle et al. (1983).

## Results And Discussion

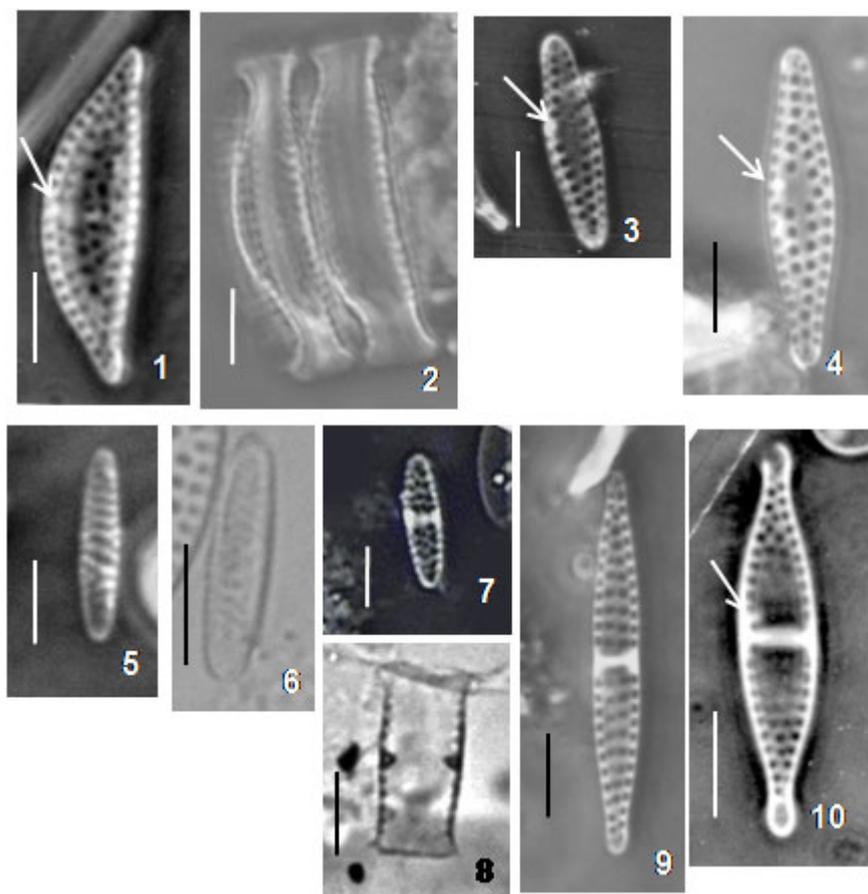
The distribution, general morphology and valve ultrastructure details of five Cymatosiraceae species are presented below:

*Campylosira cymbelliformis* (A. Schmidt) Grunow ex Van Heurck, Synopsis des Diatomées de Belgique, 158, 1885.

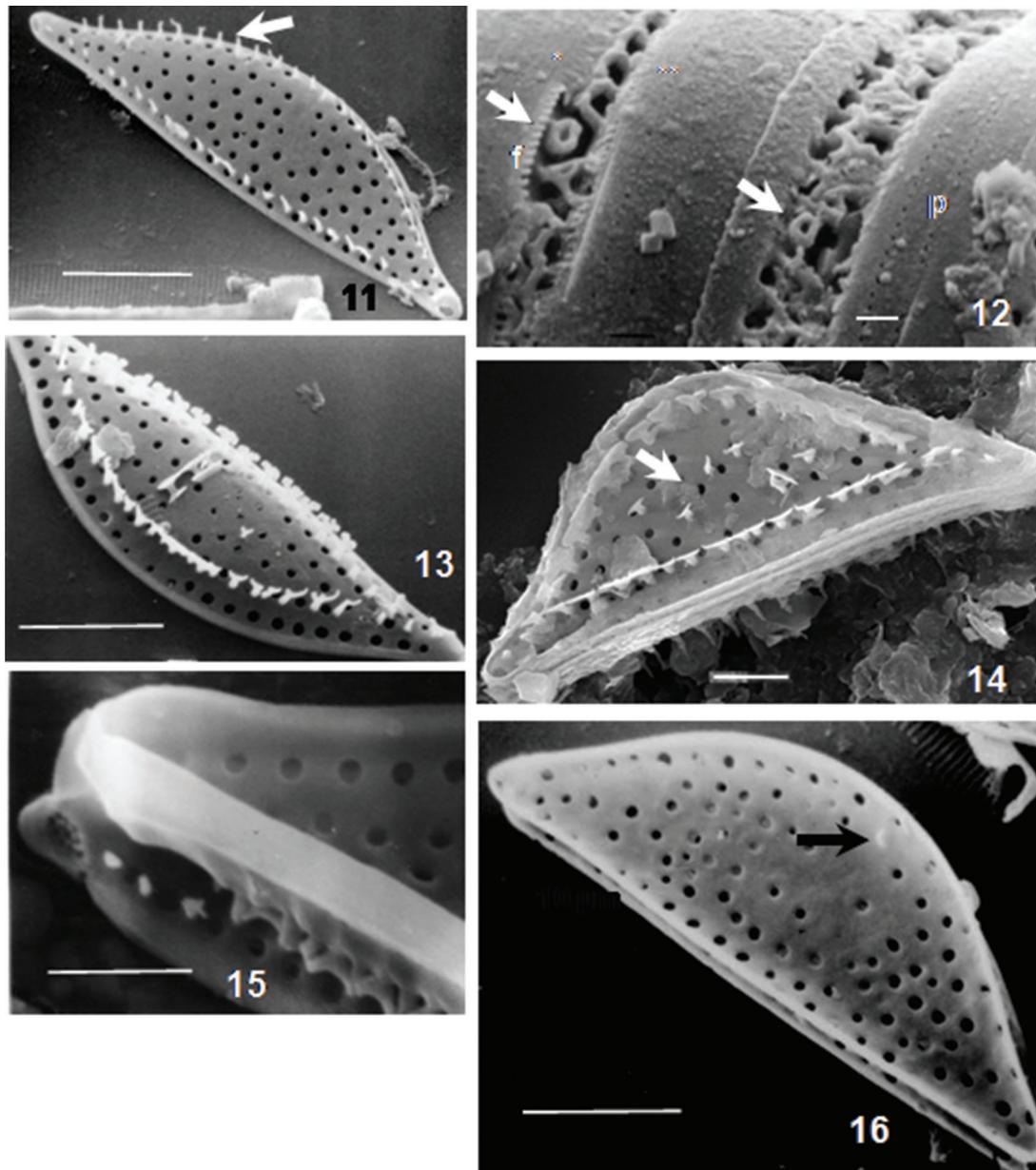
*Synedra cymbelliformis* A. Schmidt, Atlas der Diatomaceen-kunde. pl. 3, fig. 13. 1874.

### Figures 1-2, 11-16

Observations: Cells cymbelliform, 16 – 28  $\mu\text{m}$  long and 4.5 – 5.5  $\mu\text{m}$  wide ( $n = 20$ ), were observed as isolated (Figure 1) or in short colonies from 2 to 4 frustules (Figure 2). It was observed 3 of the 4 valve types as described by Hasle et al. (1983): two internal valves both with linking spines in a Tshape (Figure 13), one with rimoportula and another valve without rimoportula (Figure 12), and an end valve with simple non-linking spines and no rimoportula (Figure 11). The interior valves can present a rimoportula (Figures 12, 16) or not (Figure 12). The striae on the valve face are formed by round areolae 10 – 12 in 10  $\mu\text{m}$  more or less organized in rows leaving a semi-circular central area on the dorsal (curved) side (Figure 11). Between the valve face and the mantle there is a row of simple non-linking spines on the end valves (Figure 11) or bifurcate linking spines (Figure 13) on the internal valve. One ocelluli formed by several porelli is present on each valve apex, turned towards the straight margin of the valve



**Figures 1-10.** LM images. 1-2. *Campylosira cymbelliformis*. Fig. 1. Valva in valve view. Fig. 2. Chain with 2 frustules in girdle view. Figs 3-4. *Cymatosira belgica*. Valve views. Figs 5-6. *Cymatosirella minutissima*. Valve views. Figs 7-9. *Plagiogrammopsis minima*. Figs 7-8 Valve views. Fig. 9. Girdle view. Fig. 10. *Plagiogrammopsis vanheurckii*. Rimoportula position is indicated by arrows. Scales: 5  $\mu\text{m}$ .



**Figures 11-16.** SEM images of *Campylosira cymbelliformis*. Fig. 11. External view of an end valve without rimportula and several simple non-linking spines (arrowed). Scale: 5 $\mu$ m. Fig. 12. Colony with three frustules in girdle view showing valves with (\*) and without (\*\*) rimportula. Note also bands ornamented by a row of poroids 'p' with margin fringes 'f' and external rimportula aperture arrowed. Scale: 1 $\mu$ m. Fig. 13. External view of a concave valve without rimportula showing bifurcate linking spines between valve face and mantle. Scale: 5  $\mu$ m. Fig. 14. Spore or resting valve? with bifurcate linking spines (arrowed) on the valve face. Scale: 2  $\mu$ m. Fig. 15. Apex detail showing ocelluli in external view. Scale: 2  $\mu$ m. Fig. 16. Internal view of an interior valve with rimportula indicated by an arrow. Scale: 5  $\mu$ m.

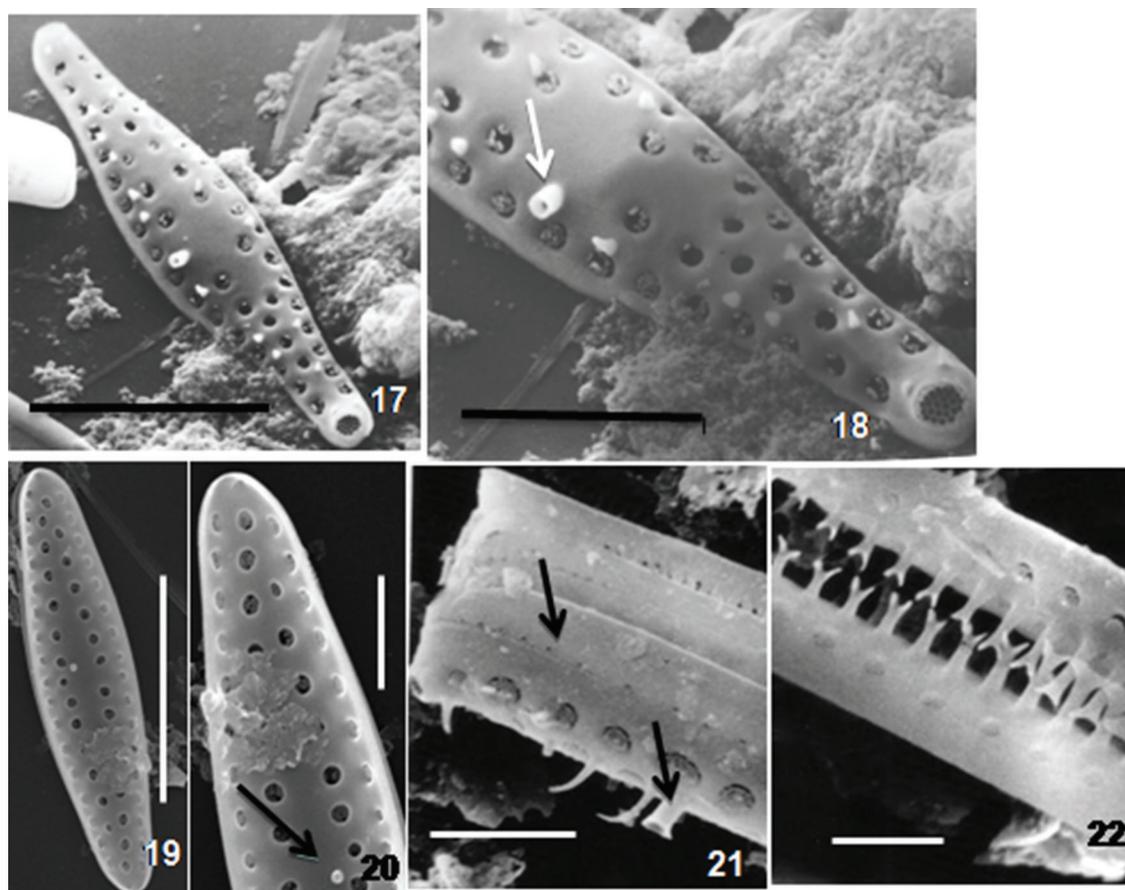
(Figures 11, 15) and cigulum with bands ornamented by rows of porelli and fringes at its margin (Figure 12). The cell illustrated in Figure 14 may represent spores or a resting stage with bifurcated linking spines on the valve face.

Distribution in the studied area: Brazil, Santa Catarina State: Garopaba (28°01'S; 48°37'W), sediment 13.V.2002 (PEL 23329); Itapirubá (28°25'S; 48°44'W), sediment 23.VI.2002 (PEL 22557); Mar Grosso (28°28'S; 48° 46'W), sediment 08.XII.2001 (PEL 22564); Mariscal (27°11'S; 48°29'W), sediment 07.I.2001 (PEL 22574); Navegantes (26°53'S; 48°38'W) sediment 19.XII.2001 (PEL

23333); Praia do Iró (28°28'S; 48°45'W), sediment 08.XII.2001 (PEL 22848); Perequê (27°09'S; 48°33'W), plankton 15.XII.1995 (ICN 91431), sediment 15.XII.1995 (ICN 91392), 22.I.1996 (ICN 91447). Rio Grande do Sul State: Cassino (32°12'S; 52°10'W), plankton XII.2005, Tramandaí (30°15'S; 50°30'W), plankton 29.XI.1995 (ICN 91430), 22.I.1996 sediment (ICN 91509).

Dimension and morphological data are in agreement with Hustedt (1939) and Hasle et al. (1983).

All beaches listed above can be classified as dissipative, with the exception of Perequê beach, which is a beach located in a bay.



**Figures 17-22.** *Cymatosira belgica*. Fig. 17. view of an external valve with linear lanceolate outline. Scale: 5  $\mu$ m. Fig. 18. Detail of Fig. 17 showing the rimportula (arrowed) and simple spines. Scale: 2.5  $\mu$ m. Fig. 19. Internal view of a valve with elliptical lanceolate outline. Scale: 5  $\mu$ m. Fig. 20. Detail of fig. 19 showing rimportula aperture internally (arrowed). Scale: 2  $\mu$ m. Fig. 21. Part of a valve in external view showing bands ornamented by one row of poroids, external tube of rimportula and areolae occluded by cribrum and simple spines. Scale: 2  $\mu$ m. Fig. 22. Part of two valves in girdle view showing bifurcate linking spines. Scale: 2  $\mu$ m.

*Cymatosira belgica* Grunow In Van Heurck, Synopsis des Diatomées de Belgique, Pr. 45, figs. 38-41, 1881.

#### Figures 3-4, 17-22

**Observations:** According to Hasle et al. (1983) this taxon forms a colony with distinct morphological cells inside and outside of the colony with at least three types of valves. During this study, external valves with simple non-linking spines (Figures 17, 21) and internal valves with bifurcate linking spines were observed (Figure 22). The valves are elliptical to linear lanceolate in shape, 15 - 18  $\mu$ m long and 3.5 - 4.5  $\mu$ m wide ( $n = 25$ ) (Figures 17, 19). Round areolae (10-14 in 10  $\mu$ m) are occluded by external cribrum (Figure 17) and organized in rows parallel to an apical axis (Figure 19). One central rimoportula is present inside the areolae row located on the valve face in all cells observed (Figures 18-20). The cingulum is composed of bands ornamented by rows of poroids (Figure 21).

**Distribution in the studied area:** Brazil, Santa Catarina State: Gamboa (27°57'S; 48°37'W), sediment 13.V.2002 (PEL 22575); Mariscal (27°11'S; 48°29'W), sediment 22.I.1996 (ICN 91479, ICN 91482, ICN 91483, ICN 91484), 07.I.2002 (PEL 22574, PEL 22569); Quatro Ilhas (27°09'S; 48°29'W), sediment 22.I.1996 (ICN 91468, ICN 91469, ICN 91470), 07.I.2002 (PEL 23336); Perequê (27°09'S; 48°33'W), sediment 15.XII.1995

(ICN 91447); Zimbros (27°12'S; 48°29'W), sediment 22.I.1996 (ICN 91485), 07.I.2002 (PEL 22622, PEL 23259); Rio Grande do Sul State: Cassino (32°12'S; 52°10'W), plankton III.2001, X.2005.

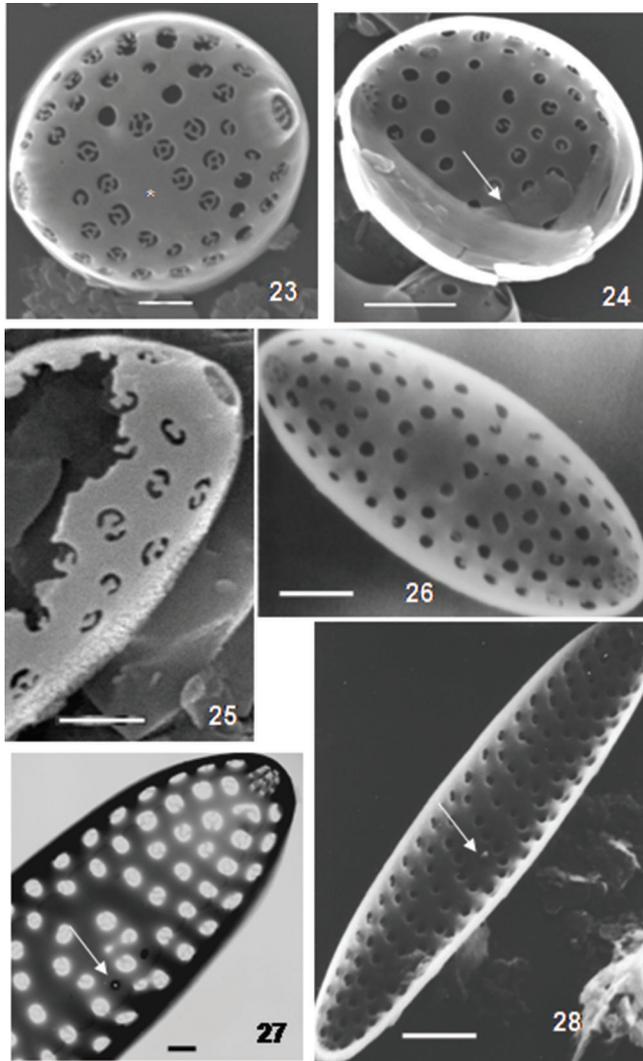
Dimensions and morphology are in agreement with Hasle et al. (1980). In our samples *C. belgica* was found in a wide range of sandy beaches (from dissipative sandy beaches [open] to bays [closed]) and in plankton. This distribution is in agreement with that found in the literature and cited in the introduction. Silva et al. 2010 found one valve of it in sediments from the Lagoa dos Patos mouth (31°59'18.60"S; 52°14'58.50"W) and in the Westerschelde estuary (The Netherlands) it occurs in silty sediments (low dynamic environments) and plankton samples (Muylaert & Sabbe, 1999).

*Cymatosirella minutissima* (Sabbe & Muylaert) Dabek, Witkowski & Sabbe, Phytotaxa, 121: 50. 2013.

*Cymatosira minutissima* Sabbe & Muylaert, Vie Milieu, 60: 246, figures 17-20, 28,31. 2010.

#### Figures 5-6, 23-28

**Observations:** The identification of this tiny taxon depends on Electronic Microscope observations (Figures 5, 6). The valves are round to elliptical lanceolate in shape, 3.0 - 9  $\mu$ m long and 1.5 - 3  $\mu$ m wide ( $n = 10$ ). The areolae are arranged irregularly or organized in lines (Figure 27) occluded by



**Figures 23-28.** *Cymatosirella minutissima*. Figs 23-26, 28. SEM images. Fig. 27. TEM image. Fig. 23. External view of a valve with round outline. Note the presence of a central area (\*). Scale: 0.5µm. Fig. 24. Internal view of a valve with round outline, bands are indicated. Scale: 1µm. Fig. 25. External view of part of a valve with elliptical outline. Scale: 0.5µm. Fig. 26. Internal view of a valve with elliptical outline. Scale: 1µm. Fig. 27. Part of a valve showing the presence of central circular area, rimoportula (indicated) and ocelluli with 10 porelli. Scale: 200 nm. Fig. 28. Part of a valve in internal view showing the presence of central circular area and rimoportula (indicated).

external cribra, 12 – 15 in 10 µm (Figures 23, 25). The valvar face is flat (Figures 23, 24, 26, 28). The central area is circular with one rimoportula sometimes present (Figures 27-28). Two ocelluli with 10 porelli (2 centrally located), open slightly laterally on the valve (Figure 23). The marginal spines were not observed and cingulum composed of several bands were not ornamented (Figure 24).

Distribution in the studied area: Brazil, Santa Catarina State: Araçá (27°08'S; 48°31'W), sediment 05.I.2002 (PEL 22618); Mariscal (27°11'S; 48°29'W), sediment 07.I.2002 (PEL 22574); Zimbros (27°12'S; 48°29'W), sediment 15. XII.1995 (ICN 91415, ICN 91416, ICN 91417).

Dimensions and morphology are in agreement with Dabek et al. (2013). During this study it was possible to show the presence of the rimoportula not observed by Sabbe et al. 2010. *Cymatosirella*

Dabek et al. was included in the Extubocelluloideae where rimoportula are occasionally observed and our findings are in agreement with the occasional occurrence of rimoportula in this family.

Among the species studied, it seems to present a more restricted distribution, but it may be related to its small size.

Our distribution data is not fully in agreement with Sabbe et al. 2010. The authors found *C. minutissima* on sandy areas (more dynamic) and we have it on beaches under distinct hydrodynamics such as Mariscal (an open, dissipative sandy beach) and Zimbros (bay).

*Plagiogramopsis minima* (Salah) Sabbe & Witkowski, Vie Milieu, 60: 246, figures 7-10, 32-35, 43-44. 2010.

*Plagiogramma minimum* Salah, Hydrobiologia, 7: 91, Pl.1, fig. 15. 1955.

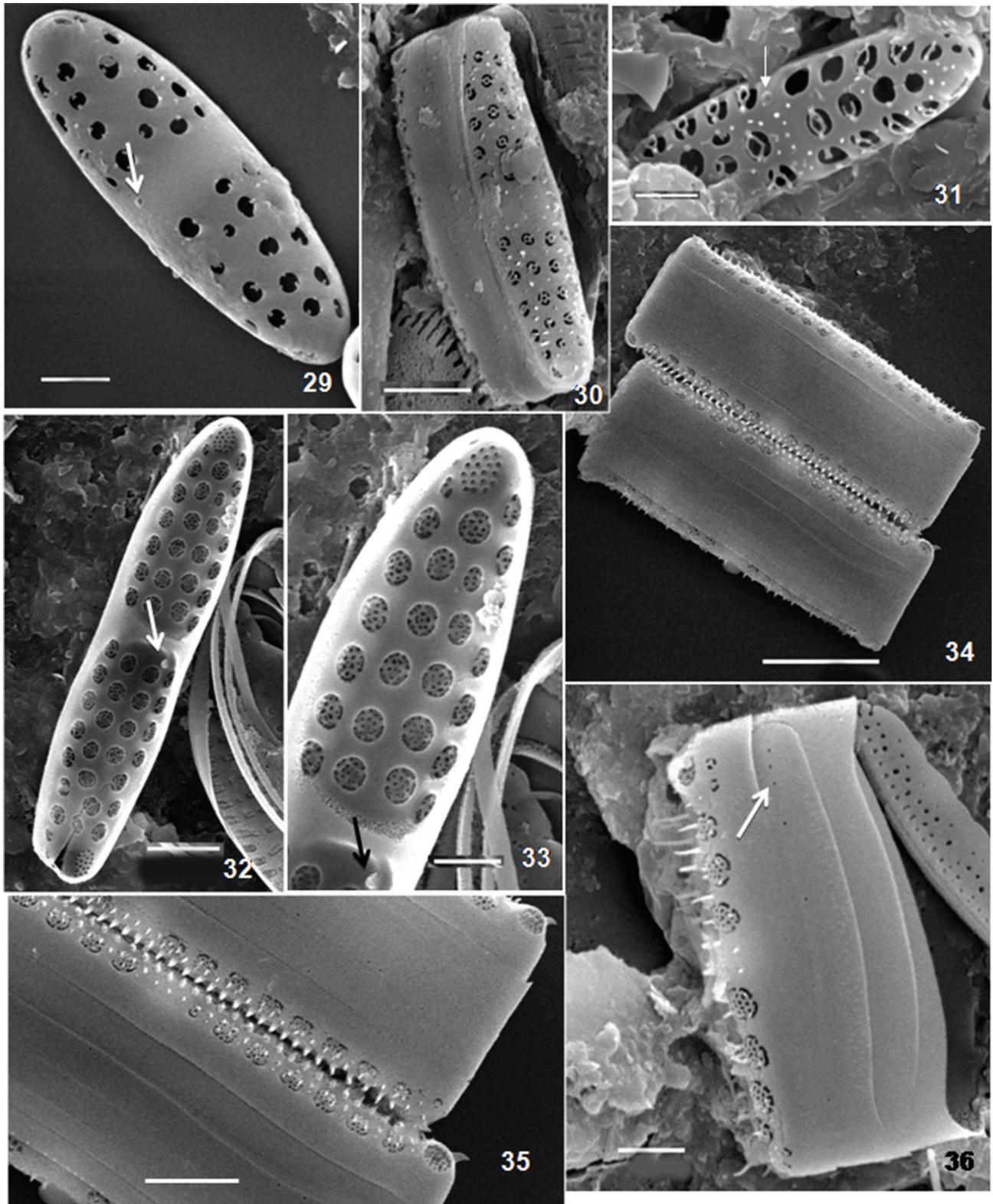
#### Figures 7 - 9, 29 – 36

Observations: The valves are lanceolate with rounded apices, 9.0 – 13.0 µm long and 1.5 - 2.5 µm wide (n = 22) (Figures 7, 9). The valves present a slightly twisted outline with ocelluli opening in opposite directions (Figure 30). At the center, the valve presents a fascia outside and a pseudoseptum inside (Figures 29, 30, 32, 33). Next to the pseudoseptum, at the valve edge, there is one rimoportula observable outside as a simple aperture (Figures 29, 31). The striae are composed of two areolae, one on the valve face and another on the mantle. Areolae are round (12-20 in 10 µm) and occluded by external cribrum that is, sometimes, covered by spinules (Figures 35 and 36). Granule-like spines can be observed scattered on the valve face (Figures 30, 31) and copulae present one row of poroids (Figure 36). Frustules are connected by short (Figure 35) simple spines although long simple spines are observable on valve face sometimes (Figure 36).

Distribution in the studied area: Brazil, Santa Catarina State: Bombas (27°08'S; 48°30'W), sediment 15.XII.1995 (ICN 91403, ICN 91404); Gamboa (27°57'S; 48°37'W), sediment 13.V.2002 (PEL 22575, PEL 23329); Jeremias (26°55'S; 48°38'W), sediment 02.XII.2001 (PEL 23683); Guarda do Embaú (27°54'S; 48°58'W), sediment 08.XII.2001 (PEL 22613); Mariscal (27°11'S; 48°29'W), sediment 22.I.1996 (PEL 91483, PEL 91484, PEL 91485); Penha ((26°47'S; 48°36'W), sediment 19.XII.2001 (PEL 22832, PEL 23348); Pinheira (27°84'S; 48° 58'W), sediment 08.XII.2001 (PEL 23257); Quatro Ilhas (27° 09'S; 48°29'W), sediment 07.I.2002 (PEL 23269, PEL 22838, PEL 23336), 22.I.1996 (PEL 91468, PEL 91469); São Miguel (26°49'S; 48°36'W), sediment 19.XII.2001 (PEL 22550); Siriú (27°58'S; 48°37'W), sediment 22.VI.2002 (PEL 22568, PEL 22590); Zimbros (27°12'S; 48°29'W), sediment 22.I.1996 (ICN 91485).

Dimensions and morphology are in agreement with Sabbe et al. (2010), although the number of areolae in 10 µm is somewhat higher. In the original description the rimoportula is located next to striae while in the Brazilian material is located always next to the pseudoseptum. Sabbe et al. 2010 found it in abundance in summer time as we have observed as well. They also refer to it as a species from sandy sediments (more dynamic areas), on the other hand we observed it on all types of sandy beaches (dissipative, intermediate and reflective) and abundant in low dynamic (Zimbros on January 1996) and dynamic beaches (Quatro Ihas on January 2002).

The presence of a row of poroids on copulae of *P. minima* was not observed by Sabbe et al. (2010) and this finding is in agreement with Cymatosiraceae diagnosis.



**Figures 29-36.** *Plagiogrammopsis minima*. Fig. 29. External view of a valve with areolae eroded and external aperture of the rimoportula indicated. Scale: 1  $\mu$ m. Fig. 30. External view of a frustule showing valve face with several eroded spines. Scale: 2  $\mu$ m. Fig. 31. Part of a valve in external view showing partially eroded areolae occlusion, spines and rimoportula aperture arrowed. Scale: 1  $\mu$ m. Fig. 32. General view of a valve in internal view. Internal rimoportula aperture is arrowed. Scale: 1  $\mu$ m. Fig. 33. Detail of fig. 32, note cribrum occlusion located outside of the areolae and internal rimoportula aperture arrowed next to the pseudoseptum. Scale: 1  $\mu$ m. Fig. 34. External general view of two frustules. Scale: 5  $\mu$ m. Fig. 35. Detail of fig. 34, showing in detail a row of short spines between valve face and mantle. Scale: 2  $\mu$ m. Fig. 36. External view of valve in girdle view showing open bands ornamented by a row of poroids and spinules on the cribra. Scale: 1  $\mu$ m.

*Plagiogrammopsis vanheurckii* (Grun.) Hasle, von Stosch & Syvertsen, *Bacillaria*, 6: 31, figures. 104-131. 1983.

*Plagiogramma vanheurckii* Grunow in Van Heurck, *Synopsis des Diatomées de Belgique*. pl. 36. fig. 4. 1881.

#### Figure 10

Observations: The cell is lanceolated in shape, 18 µm long and 3.5 µm wide (n=1), with long produced apices. Small round areolae are observed scattered or forming transapical rows on the valve face. At the middle of the valve there is a pseudoseptum and next to it and the valve margin one rimoportula is present (Figure 10). Only one isolated valve was observed.

Distribution in the studied area: Brazil, Santa Catarina State: Quatro Ilhas (27°09'S; 48°29'W), sediment 22.I.1996 (ICN 91467).

Morphological and dimension data are in agreement with Hasle et al. (1983). This taxon was registered to the marine beaches of Cabeçudas, Piçarras, Itajubá e Barra Velha by Valente-Moreira & Moreira-Filho (1978) and Ilha de Florianópolis por Corte-Real & Aguiar (1971). It seems nowadays a rarer species than some years ago, although the beaches cited above have not been included in this study.

## Final Discussion

Cymatosiraceae species were found attached to sand grains and plankton samples of sandy beaches of the South of Brazil. The few recordings to the Brazilian coast may be related to the diminute size of these diatoms.

Among the species studied, two are recorded in abundance on sediments, *Cymatosira belgica* at Mariscal and *Plagiogramma minima* at Quatro Ilhas and Zimbros, both in January 1996 (Table 1).

Regarding the occurrence of Cymatosiraceae species in plankton samples, *C. cymbelliformis* was registered to Tramandaí, Cassino, and Perequê and *Cymatosira belgica* to Cassino. These characteristic benthic species may be found in plankton, although never in abundance.

Based on beaches studied in this paper, *Campylosira cymbelliformis* is more likely to occur in shallow open coastal (dissipative) beaches such as Navegantes, Itapirubá, Mariscal in Santa Catarina State, and Tramandaí and Cassino, and also in Praia Azul according to Garcia-Baptista (1993), in Rio Grande do Sul State (Table 1).

*Cymatosira belgica* was abundant at an open sandy beach such as Mariscal and can be considered a common diatom in the Santa Catarina littoral but it is very rare in the Rio Grande do Sul littoral. Garcia-Baptista (1993) observed over a 128 sand samples from Praia Azul and has never observed it. It is herein for the first time recorded for the Rio Grande do Sul littoral at Cassino beach. Although Silva et al. (2010) has cited it to Lagoa dos Patos estuary, its occurrence in this area maybe is related to the proximity with the international port of Rio Grande.

The distribution of *Cymatosirella minutissima* and *Plagiogrammopsis minima* are amplified to South America, before they were restricted to Europe.

The ecological data for *Plagiogrammopsis minima* (the widest spread diatom in this study) and *Cymatosirella minutissima* are not fully in agreement with Sabbe et al. 2010 that found both species on sandy (more dynamic) areas. In Brazil, on the other hand they were observed in Mariscal (open) and Zimbros (closed) beaches.

Regarding morphological diagnostic features new data were added to some taxa as the presence of rimoportula on *C. minutissima* and one row of poroids on copulae of *P. minima*.

This study shows higher diversity of Cymatosiraceae present on Brazilian beaches than previously, especially on the beaches located along Santa Catarina State, area where there are diverse types of beaches. These distinct pattern distributions relight the necessity of more studies on the marine flora of Brazil.

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## Arctiinae (Lepidoptera: Erebiidae) in the state of Rio de Janeiro, Brazil

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**Abstract:** This study describes the composition and distribution of the Arctiinae species (Lepidoptera: Erebiidae) in Rio de Janeiro, generating the first list of Arctiinae species in the state. We assessed the variations in species composition and the different vegetation types in the municipalities of Rio de Janeiro. Data were collected primarily through surveys of museums and publication lists of species. We also conducted field sampling in March and September 2010. Of the 2,077 records covering 28 municipalities, 679 Arctiinae species were found in Rio de Janeiro state and were divided into two tribes and 220 genera. The location with the highest number of species recorded was Itatiaia, with 362 species, followed by Petrópolis and Angra dos Reis, with 320 and 306 species, respectively. Thirty-four percent ( $n = 230$ ) of the species recorded were exclusive, i.e., occurred in only one location. The lowest dissimilarity values were observed between neighboring municipalities with the same type of plant formation, such as Petrópolis and Teresópolis (19%) or Itatiaia and Resende (24%). Some municipalities slightly further apart geographically, but with the same type of plant formation, also showed low levels of dissimilarity, for example, Petrópolis and Resende (29%). However, most locations showed intermediate dissimilarity values of 40–60%, and in some cases, this figure rose to 96%. In the state of Rio de Janeiro, irrespective of its small geographical size and the predominance of a unique ecosystem, the Atlantic Forest shows a wide variation in relief, which may explain the high beta diversity values. Despite the large number of Arctiinae species recorded in Rio de Janeiro, few species were identified in areas with important forest remnants, such as Guapimirim, Nova Iguaçu, and Campos dos Goitacazes. Some regions of the northwestern part of the state were also subsampled. It is likely that new records of Arctiinae still exist in these locations, further increasing the list of Arctiinae species in Rio de Janeiro.

**Keywords:** *Beta diversity of moths, List of species, Atlantic Forest.*

NASCIMENTO, M.S., FERRO, V.G., MONTEIRO, R.F. **Arctiinae (Lepidoptera: Erebiidae) no estado do Rio de Janeiro, Brasil.** *Biota Neotropica*. 16(2): e20150112. <http://dx.doi.org/10.1590/1676-0611-BN-2015-0112>

**Resumo:** Arctiinae (Lepidoptera: Erebiidae) no estado do Rio de Janeiro, Brasil. O presente trabalho teve como objetivo descrever a composição e distribuição de espécies de Arctiinae (Lepidoptera: Erebiidae) no estado do Rio de Janeiro, gerando a primeira lista de espécies de Arctiinae para o estado. Avaliou-se também a variação na composição de espécies nos municípios e nas diferentes formações vegetais do estado. Os dados foram obtidos através de levantamentos em museus e de publicações de listas de espécies. Foram também realizadas coletas de campo nos meses de março e setembro de 2010. De um total de 2.077 registros, abrangendo 28 municípios, encontrou-se 679 espécies de Arctiinae para o estado do Rio de Janeiro, distribuídas em duas tribos e 220 gêneros. O município com o maior número de espécies foi Itatiaia, com 362 espécies, seguido de Petrópolis e Angra dos Reis, com 320 e 306 espécies, respectivamente. Trinta e quatro por cento ( $n = 230$ ) das espécies de Arctiinae registradas tiveram distribuição exclusiva, ou seja, ocorreram em apenas uma localidade. Os menores valores de dissimilaridade foram observados entre municípios próximos e localizados sob o mesmo tipo de formação vegetal, como Petrópolis e Teresópolis (19%) ou Itatiaia e Resende (24%). Alguns municípios mais afastados geograficamente, mas ainda localizados sob o mesmo tipo de formação vegetal, apresentaram também baixos valores de dissimilaridade, como Petrópolis e Resende (29%), por exemplo. Entretanto, a maior parte dos municípios apresentou valores intermediários de dissimilaridade, entre 40 e 60%, e em alguns casos esse valor chegou até a 96%. O estado do Rio de Janeiro, mesmo com sua pequena dimensão geográfica e predomínio de um único ecossistema, a Mata Atlântica, apresenta uma grande variação no seu relevo, o que pode explicar alguns valores altos de diversidade beta. Apesar da

grande quantidade de espécies de Arctiinae registrada para o estado do Rio de Janeiro, foram identificadas algumas localidades com remanescentes florestais importantes, como Guapimirim, Nova Iguaçu e Campos dos Goitacazes, e também algumas regiões do noroeste do estado ainda subamostradas. É bem provável que nessas localidades sejam encontrados novos registros de Arctiinae, aumentando ainda mais a lista de espécies de Arctiinae do estado do Rio de Janeiro.

**Palavras-chave:** *Diversidade Beta de mariposas, Lista de espécies, Mata Atlântica.*

## Introduction

The Atlantic Forest biome is the second most biodiverse forest complex in Brazil, behind only the Amazon Forest (Ab'Sáber 2005). Originally, the Atlantic Forest covered more than 1 million km<sup>2</sup> (SOS Mata Atlântica & INPE 2015). Today, the biome covers only about 8% of its original area, representing approximately 1% of the national territory (SOS Mata Atlântica & INPE 2015). Due to the extensive habitat loss and the high degree of endemism, the Atlantic Forest is considered a hotspot of biodiversity (Myers et al. 2000, Galindo-Leal & Câmara 2005) and is one of the most threatened forests in the world (Oliveira-Filho & Fontes 2000). The state of Rio de Janeiro, which had 100% of its area inside the Atlantic Forest, has only about 20% of its area covered by remnants of this biome today (SOS Mata Atlântica & INPE 2015). The Atlantic Forest in the state of Rio de Janeiro includes different vegetation types and can be divided into major floristic blocks, such as dense rainforest (lowland, hillside, and altitude forests), semi-deciduous forest (forests inland plateaus), and associated ecosystems (salt marshes and mangroves) (SOS Mata Atlântica & INPE 2015).

The Atlantic Forest is considered one of the most studied forests among Brazilian biomes, probably because of its proximity to major research centers. However, many gaps still exist, particularly in very diverse groups, such as insects (Lewinsohn & Prado 2004). The order Lepidoptera is among the major groups of invertebrates in terms of published inventories, but much of the work has focused on butterflies (Lewinsohn & Prado 2004). In the state of Rio de Janeiro, even with a high concentration of research centers and a large area of preserved Atlantic Forest, relatively few studies have been conducted on Lepidoptera fauna, except for the publications by Zikán & Zikán (1968), Monteiro et al. (2004) and the lists of Pieridae species in Monteiro et al. (2009) and of Lycaenidae in Duarte et al. (2009).

The Arctiinae subfamily, as well as other moth and butterfly families, has been the subject of many studies, as they are suitable for studies on environmental impact and change models, are easy to collect and identify, and have a short lifecycle (Lewinsohn et al. 2005). About 11,000 Arctiinae species exist in the world (Jacobson & Weller 2002), of which approximately 6,000 species occur in the Neotropics and 1,400 in Brazil (Ferro & Diniz 2010). In a recent study, Vincent & Laguerre (2014) published a catalog of the Neotropical Arctiini (except Ctenuchina and Euchromiina) in which presents an updated list of neotropical tiger moths, with 2,404 species of Arctiini. In Brazil, there are several studies about tiger moths, in different biomas like Savanas (Ferro & Diniz 2007, Ferro & Diniz 2010, Moreno et al. 2015), the Amazon Forest (Teston & Delfina 2010, Delfina & Teston 2013, Teston & Correa, 2015) and even in the Atlantic Forest (Ferro & Teston 2009, Ferro & Melo 2011, Ferro & Romanowski, 2012, Zenker et al. 2015). In general, these works address the richness, diversity and species composition of Arctiinae in certain areas, but there are few studies that compile lists of species for Brazilian states. Therefore, there have been no reviews that have summarized the existing knowledge

about this family in the state of Rio de Janeiro. Thus, we generated a list of Arctiinae species for the state of Rio de Janeiro and investigated the richness, composition, and species similarity in the different plant formations of the state.

## Materials and Methods

Arctiinae records for the state of Rio de Janeiro (i.e., the presence of a species in a given locality/municipality) were obtained predominantly from specimens deposited in the following Brazilian collections: (1) Entomological Collection of Instituto Oswaldo Cruz; (2) Museu Nacional, Universidade Federal do Rio de Janeiro; (3) Entomological Collection of Laboratório de Ecologia de Insetos, Universidade Federal do Rio de Janeiro; (4) Entomological Collection Padre Jesus Santiago Moure, Universidade Federal do Paraná; (5) Museu de Zoologia, Universidade de São Paulo; (6) Museu Paraense Emílio Goeldi; and (7) Vitor Becker's collection. Specimens with dubious provenance and identification were not considered. The collection labels of many specimens did not have the geographic coordinates and altitudes of the localities. In such cases, we obtained this information through the "Splink" information system, developed by the Reference Center on Environmental Information (<http://splink.cria.org.br/geoloc?criaLANG=pt>).

We also used data from published lists of Arctiinae species (Zikán & Zikán 1968, Monteiro et al. 2004) and samplings. The moths were sampled in protected areas in the municipalities of Casimiro de Abreu (Reserva Biológica União), Nova Iguaçu (Reserva Biológica Tinguá), and Teresópolis (Parque Nacional da Serra dos Órgãos). Samplings were conducted during the end of the rainy season (March 2010) and the late dry season (September 2010). These samplings were made during new-moon nights, using a 2-m-wide × 1.5-m-high white sheet and a 250 W mixed light lamp. The lamp was lit one hour after sunset and was connected to a power generator for two hours. The moths were identified through the literature (Watson & Goodger 1986, Piñas Rubio et al. 2000, Piñas Rubio & Manzano 2003) and by a comparison with the digital images of deposited species in the Becker's collection, whose identifications were confirmed by a comparison of the types.

The second order Jackknife and Chao 2 non-parametric estimators were used for estimating Arctiinae richness in the state of Rio de Janeiro. Moreover, the variation in species composition of these moths between different vegetation types was assessed using the Simpson's beta diversity index. This index was selected because it is less influenced by differences in species richness between samples (Koleff et al. 2003). Municipalities with Arctiinae records were categorized according to their predominant vegetation type, following the classification of SOS Mata Atlântica & INPE (2015). The vegetation types considered were: (1) altitude forests: formations of dense rainforests located at elevations above 200 m; (2) lowland forests: formations of dense rainforests located at low altitudes (up to 200 m), including salt marshes and floodplain forests, which are predominantly coastal

vegetation on sandy soil; and (3) mixed formations: municipalities that have both lowland forests and altitude forests. Statistical analyses were performed using R software (R Development Core Team 2009).

## Results

We obtained 2,077 Arctiinae records for the state of Rio de Janeiro, including 28 (30%) municipalities in the state. In total, 679 Arctiinae species were recorded for the state of Rio de Janeiro, which were divided into two tribes and 220 genera. The list of Arctiinae species in each municipality of the state of Rio de Janeiro is presented in Appendix 1. The estimations of second order Jackknife richness and Chao 2 show the existence of 916 and 1,037 Arctiinae species in the state, respectively. Therefore, according to the Chao 2 estimate, which is the most conservative index, our species list represented 74% of the expected Arctiinae fauna.

The location with the highest number of recorded species was Itatiaia, with 362 species (Table 1). The municipalities of Petrópolis, Angra dos Reis, Rio de Janeiro, and Teresópolis also showed high species richness of Arctiinae (320, 306, 239, and 207, respectively) (Table 1). The distribution of Arctiinae

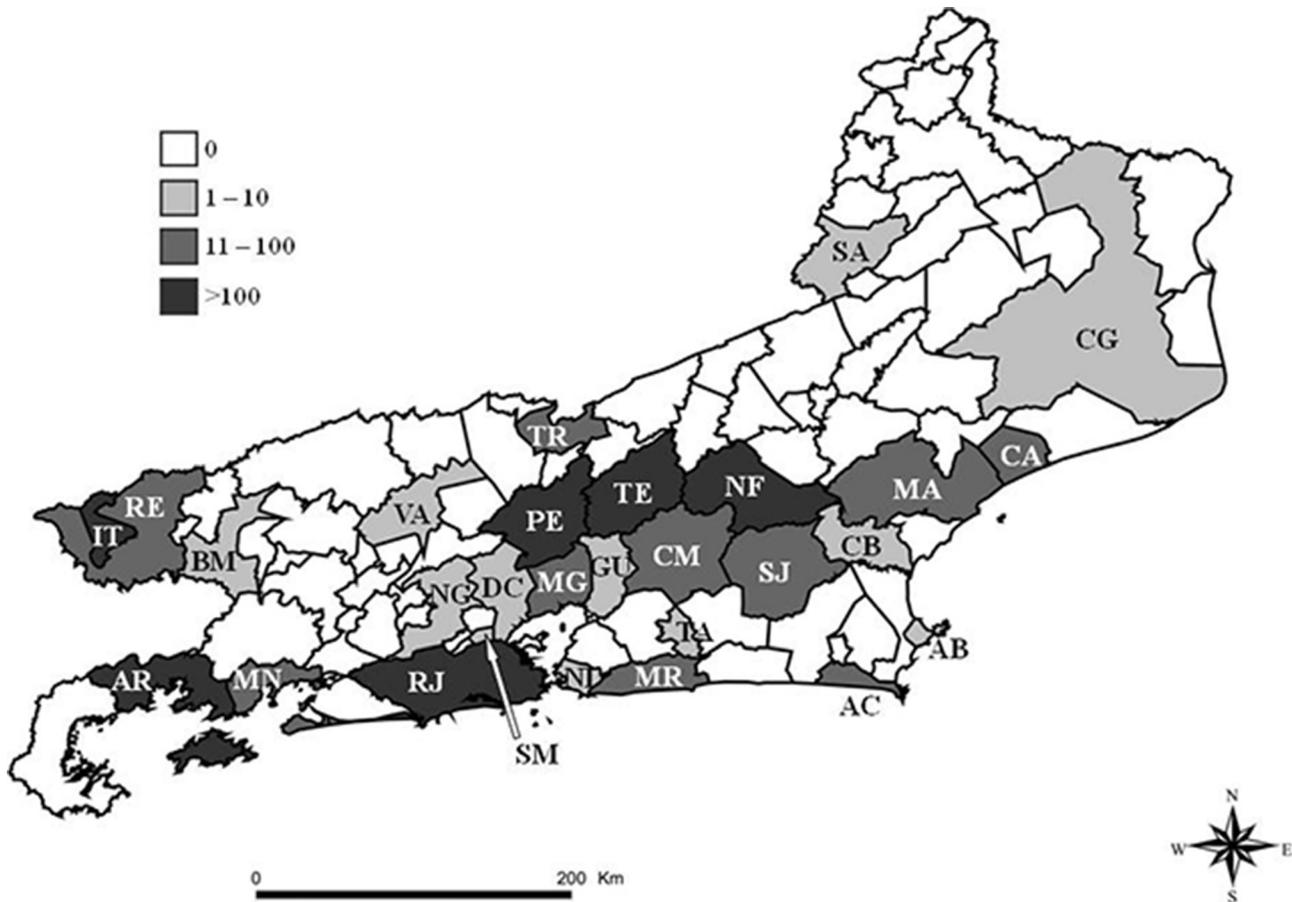
species in the municipalities of Rio de Janeiro was quite heterogeneous, as the three richest municipalities accounted for 86% of all Arctiinae species in the state. Of the 28 municipalities in the state with Arctiinae records, 46% (n = 13) had less than 15 species and only 21% (n = 6) had more than 100 species (Figure 1).

*Aclytia heber* (Cramer 1780) had the highest number of records throughout the study, occurring in 14 municipalities. *Ilipa tengyra* (Walker 1854) (12 municipalities), *Pionia lycooides* (Walker 1854) (11 municipalities), and *Dycladia lucetius* (Cramer 1782) (11 municipalities) were also very common in the state. Thirty-four percent (n = 230) of the Arctiinae species occurred in only one location. Itatiaia had the highest number of exclusive species (n = 96 or 26.5%).

Comparing the species composition of Arctiinae in different vegetation types in the state of Rio de Janeiro, the largest number of recorded species was observed in the altitude forests formations (574 species), followed by mixed formations (418 species), whereas lowland forests formations only presented 139 species. The dissimilarity of species was low within the three types of plant formations evaluated (Table 3). The smallest difference between the Arctiinae compositions was observed between the altitude forests and the mixed formations (Table 2).

**Table 1.** Arctiinae species richness in the municipalities of the state of Rio de Janeiro which had records of these moths and the percentage of exclusive species for each locality. Coordinates (in decimal degrees; datum SAD69), altitudes and vegetation types of each locality are also presented.

Municipality	Coordinates		Altitude (m)	Vegetation type	Richness	Exclusive species (%)
	LAT	LONG				
Angra dos Reis	-23,0067	-44,3181	6	Mixed formation	306	9.8
Armação dos Búzios	-22,7469	-41,8817	3	Lowland forest	4	0
Arraial do Cabo	-22,9661	-42,0278	8	Lowland forest	11	0
Barra Mansa	-22,5442	-44,1714	381	Altitude forest	1	0
Cachoeiras de Macacu	-22,4625	-42,6531	60	Mixed formation	91	9.9
Campos dos Goytacazes	-21,7542	-41,3244	13	Lowland forest	1	0
Carapebus	-22,1872	-41,6611	15	Lowland forest	32	0
Casimiro de Abreu	-22,4806	-42,2042	17	Lowland forest	8	12.5
Duque de Caxias	-22,7856	-43,3117	19	Lowland forest	1	0
Guapimirim	-22,5372	-42,9819	48	Altitude forest	5	0
Itatiaia	-22,4961	-44,5633	600	Altitude forest	362	26.5
Macaé	-22,3708	-41,7869	2	Lowland forest	32	0
Magé	-22,6528	-43,0406	5	Lowland forest	33	6.1
Mangaratiba	-22,9597	-44,0406	18	Mixed formation	47	4.3
Maricá	-22,9194	-42,8186	5	Lowland forest	41	7.3
Niterói	-22,8833	-43,1036	5	Lowland forest	3	0
Nova Friburgo	-22,2819	-42,5311	846	Altitude forest	171	8.2
Nova Iguaçu	-22,7592	-43,4511	25	Lowland forest	5	40.0
Petrópolis	-22,5050	-43,1786	809	Altitude forest	320	9.1
Resende	-22,4689	-44,4467	407	Altitude forest	55	5.5
Rio de Janeiro	-22,9028	-43,2075	2	Mixed formation	239	9.6
Santo Antônio de Pádua	-21,5394	-42,1803	86	Mixed formation	3	33.3
São João do Meriti	-22,4814	-43,222	19	Lowland forest	2	0
Silva Jardim	-22,6508	-42,3917	35	Lowland forest	51	21.6
Tanguá	-22,7303	-42,7142	20	Lowland forest	3	0
Teresópolis	-22,4122	-42,9656	871	Altitude forest	207	2.0
Três Rios	-22,1167	-43,2092	269	Altitude forest	41	0
Vassouras	-22,4039	-43,6625	434	Altitude forest	2	0
Total					679	33.9



**Figure 1.** Map of the state of Rio de Janeiro, indicating the number of records of Arctiinae species in each municipality. AR: Angra dos Reis; AB: Armação dos Búzios; AC: Arraial do Cabo; BM: Barra Mansa; CM: Cachoeiras de Macacu; CG: Campos dos Goytacazes; CA: Carapebus; CB: Casimiro de Abreu; DC: Duque de Caxias; GU: Guapimirim; IT: Itatiaia; MA: Macaé; MG: Magé; MN: Mangaratiba; MR: Maricá; NI: Niterói; NF: Nova Friburgo; NG: Nova Iguaçu; PE: Petrópolis; RE: Resende; RJ: Rio de Janeiro; SA: Santo Antônio de Pádua; SM: São João de Meriti; SJ: Silva Jardim; TA: Tanguá; TE: Teresópolis; TR: Três Rios; VA: Vassouras. Cities without records of Arctiinae species are not indicated by abbreviations on the map.

**Table 2.** Matrix of dissimilarity between vegetation types in the state of Rio de Janeiro using Simpson's beta diversity index.

	Altitude forest	Mixed formation
Mixed formation	0.22	-
Lowland forest	0.30	0.36

## Discussion

The Arctiinae fauna of the state of Rio de Janeiro compiled in this study represents 11% of the Arctiinae registered for the Neotropics (Jacobson & Weller 2002) and about 50% of the recorded fauna for Brazil (Ferro & Diniz 2010). There are few lists of Arctiinae species for Brazilian states (e.g., Ferro & Teston 2009), but the richness of these moths in the state of Rio de Janeiro is the largest ever recorded for a Brazilian state, with more than twice that observed in the state of Rio Grande do Sul (Ferro & Teston 2009) and 1.4 times greater than that of

Santa Catarina (Ferro et al. 2012). The richness of the Arctiinae species in the state of Rio de Janeiro presented here is quite close to that for the state of São Paulo's estimated richness (Brown Jr. & Freitas 1999), although the latter has approximately six times the area of the Rio de Janeiro (IBGE 2013). Indeed, the state of Rio de Janeiro has still areas well preserved from the Atlantic Forest and also has several research centers, these two facts can support this great diversity of Arctiinae found in the state.

The three municipalities with the highest level of Arctiinae species richness in this study (Itatiaia, Petrópolis, and Angra dos Reis) showed the highest richness values for Arctiinae ever recorded at a single site collection from Brazil (Ferro & Diniz 2007). According the Atlas of Forest Remnants of the Atlantic Forest (SOS Mata Atlântica & INPE 2015), these three municipalities are located in areas that have the largest remaining forest in this biome; thus, they are municipalities with large areas of preserved Atlantic Forest. Besides being well preserved, these locations are very well sampled, which probably explains the richness of Arctiinae in these locations,

especially Itatiaia, because of the insects that Zikán & Zikán (1968) collected for 36 years in the Parque Nacional do Itatiaia area and its surroundings. Previous surveys of butterfly families, such as Lycaenidae (Duarte et al. 2009) and Pieridae (Monteiro et al. 2009), also highlight Itatiaia as a municipality with the highest number of recorded species of these insects. The small number of records in Nova Iguaçu and Guapimirim can be explained by the lack of Lepidoptera surveys in these regions, as there are still well-preserved forest remnants in these municipalities, such as Reserva Biológica Tinguá and the lowest part of the of Parque Nacional da Serra dos Órgãos, respectively. Municipalities without Arctiinae records match those where no survey of Lepidoptera has been conducted, and most have little or no forested areas, as in several municipalities north and west of the state (SOS Mata Atlântica & INPE 2015).

In a study conducted with Arctiinae in the Atlantic Forest of southern Brazil, Ferro & Teston (2009) found that 33% of the species occurred in only one municipality, almost identical to the percentage observed in this study. The large number of species recorded exclusively in Itatiaia (26.5%) may reflect the extensive survey of Lepidoptera species (Zikán & Zikán 1968) or the high degree of endemism in the region.

The altitude forest had the highest number of Arctiinae records because it encompassed nine municipalities, including the best-sampled ones (Itatiaia and Petrópolis). Moreover, we must also consider the conservation status of these formations within the state, as the altitude forests represent the best preserved formation in the state of Rio de Janeiro, where the largest conservation areas in the state are concentrated (Itatiaia National Park and Serra dos Órgãos National Park). The mixed formation also encompassed municipalities that were well sampled, such as Angra dos Reis and Rio de Janeiro, which have areas in good condition within the state, such as Serra da Bocaina National Park and Tijuca National Park, respectively, which are protected. Moreover, lowland forests, including the sandbank formations, is one of the most degraded vegetation formations in the state, as it occurs in regions of high population density, high real estate value, accelerated deforestation, and forest fragmentation. In general, the remnants of sandbank formations in the state of Rio de Janeiro are located in patches of vegetation surrounded by urban or rural areas. The one large remaining sandbank conservation area in the state is the Restinga de Jurubatiba National Park.

After compiling existing records for Arctiinae moths in the State of Rio de Janeiro from Brazilian museum collections, data from the literature, and samplings, we obtained a list of 679 species. This species number was larger than any previously published list of Arctiinae in a Brazilian state. Although, several subsampled locations in the state lack surveys, such as Guapimirim, Nova Iguaçu, and Campos dos Goytacazes, which still have representative areas of Atlantic Forest vegetation. Our results indicate a wide variation in the Arctiinae dissimilarity values among the municipalities of the state of Rio de Janeiro. This pattern may reflect the low host specificity of the caterpillars and the small geographical size, although there is a large variation existing in relief of the state. If the results for the Arctiinae species represent a standard for other groups of Lepidoptera, or for insects as a whole, it is clear that host specificity is an issue that remains to be investigated.

**Appendix I.** List of Arctiinae species in the state of Rio de Janeiro. AR: Angra dos Reis; AB: Armação dos Búzios; AC: Arraial do Cabo; BM: Barra Mansa; CM: Cachoeiras de Macacu; CG: Campos dos Goytacazes; CA: Carapebus; CB: Casimiro de Abreu; DC: Duque de Caxias; GU: Guapimirim; IT: Itatiaia; MA: Macaé; MG: Magé; MN: Mangaratiba; MR: Maricá; NI: Niterói; NF: Nova Friburgo; NG: Nova Iguaçu; PE: Petrópolis; RE: Resende; RJ: Rio de Janeiro; SA: Santo Antônio de Pádua; SM: São João de Meriti; SJ: Silva Jardim; TA: Tanguá; TE: Teresópolis; TR: Três Rios; VA: Vassouras.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Achyta flavigutta</i> (Walker, 1854)	X				X						X												X					X
<i>Achyta gynamorpha</i> Hampson, 1898					X						X												X					X
<i>Achyta heber</i> (Cramer, 1780)	X				X		X				X												X					X
<i>Achyta jonesi</i> Rothschild, 1912																												
<i>Achyta punctata</i> Butler 1876																												
<i>Achyta reducta</i> Rothschild, 1912											X																	
<i>Achyta taeniata</i> Draudt, 1915											X																	
<i>Achyta terra</i> Schaus, 1896											X																	
<i>Aemilia pagana</i> (Schaus, 1894)											X																	
<i>Aethria andis</i> Schaus, 1901											X																	
<i>Aethria andromacha</i> (Fabricius, 1775)											X																	
<i>Aethria gracilis</i> (Möschler, 1877)											X																	
<i>Aethria haemorrhoidalis</i> (Stoll, 1790)											X																	
<i>Aethria melanobasis</i> (Druce, 1897)											X																	
<i>Aethria paula</i> Schaus, 1894											X																	

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Arctiinae of Rio de Janeiro, Brazil

Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Areva subfulgens</i> (Schaus, 1896)	X			X						X					X				X								X	X
<i>Areva trigenmis</i> Hübner, 1827										X						X					X							
<i>Argyrooides braco</i> (Herrich-Schäffer, 1855)	X																				X							
<i>Argyrooides ophiom</i> (Walker, 1854)																			X		X							
<i>Argyrooides sanguinea</i> Schaus, 1896																			X									
<i>Argyrooides variegata</i> Kaye, 1911																			X									
<i>Aristodaema hanga</i> (Herrich-Schäffer, 1854)	X									X									X									X
<i>Atyphopsis roseiceps</i> Druce, 1898	X																		X		X							
<i>Barittius acuminata</i> (Walker, 1856)	X									X									X		X							X
<i>Barsinella mirabilis</i> Butler, 1878																			X									
<i>Belennia eryx</i> (Fabricius, 1775)	X									X									X									
<i>Belennia inaurata</i> (Sulzer, 1776)	X							X																				
<i>Belennia ochriplaga</i> Hampson, 1901	X																		X									
<i>Bernathonomus minuta</i> Fragoso, 1953										X									X		X							X
<i>Bernathonomus piperita</i> (Herrich-Schäffer, 1855)										X									X		X							
<i>Bernathonomus punktata</i> (Reich, 1933)										X									X		X							
<i>Bertholdia albipuncta</i> Schaus, 1896	X									X									X		X							X
<i>Bertholdia almeidai</i> Travassos, 1950										X									X		X							X
<i>Bertholdia griseocens</i> Rothschild, 1909										X									X		X							X
<i>Bertholdia myosticta</i> Druce, 1897	X																		X									X
<i>Bertholdia pseudofumida</i> Travassos, 1950	X																		X									X
<i>Bertholdia soror</i> Dyar, 1901	X									X									X									X
<i>Bertholdia specularis</i> (Herrich-Schäffer, 1853)	X									X									X									X
<i>Brycea itaitayae</i> Zerny, 1924										X									X									X
<i>Callisthenia plicata</i> (Butler, 1877)										X									X									X
<i>Callopepla emarginata</i> (Walker, 1854)	X									X									X									X
<i>Callopepla flammula</i> (Hübner, 1832)	X									X									X									X
<i>Callopepla inachia</i> (Schaus, 1892)										X									X									X
<i>Callopepla similis</i> (Heylaerts, 1890)										X									X									X
<i>Calodesma amica</i> (Stoll, 1781)										X									X									X
<i>Calodesma collaris</i> (Drury, 1782)	X									X									X									X
<i>Calodesma contracta</i> (Walker, 1854)										X									X									X
<i>Calodesma itaitubae</i> Hering, 1925										X									X									X
<i>Calodesma quadrimaculata</i> Hering, 1925	X									X									X									X
<i>Calonotos fenestratus</i> (Klages, 1906)	X									X									X									X
<i>Calonotos phlegmon</i> (Cramer, [1775])	X									X									X									X
<i>Carales astur</i> (Cramer, 1777)										X									X									X
<i>Carales maculicollis</i> Walker, 1855										X									X									X
<i>Carathis byblis</i> (Schaus, 1892)	X									X									X									X
<i>Castrica phalaenoides</i> (Drury, 1773)	X									X									X									X
<i>Centronia melanimis</i> Hübner, 1827										X									X									X
<i>Ceramidia chloroplegia</i> Druce 1905	X									X									X									X

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Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Cercopimorpha dolens</i> (Schaus, 1905)												X							X								X	
<i>Cercopimorpha hoffmanni</i> Zerny, 1931											X																	X
<i>Cercopimorpha postflavia</i> Rothschild, 1912	X																		X									X
<i>Chetone isse</i> (Hübner, 1831)	X																			X								X
<i>Chionostia apicalis</i> (Zeller, 1874)										X																		
<i>Chrysoptera dycladotoides</i> (Heylaerts, 1890)																		X										
<i>Chrysoptera discoplaga</i> Schaus, 1905																												
<i>Chrysoptera croesus</i> Hampson, 1900																												
<i>Cissura decora</i> Walker, 1854																												
<i>Cisthene dives</i> Schaus, 1896																												
<i>Cisthene endoxantha</i> (Hampson, 1903)																												
<i>Cisthene fasciata</i> Schaus, 1896																												
<i>Cisthene rosacea</i> Schaus, 1905																												
<i>Cisthene ruficollis</i> (Hübner, 1824)																												
<i>Cisthene triplaga</i> Hampson, 1905																												
<i>Clemensia panthera</i> (Schaus, 1896)																												
<i>Clemensia quinqueferana</i> (Walker, 1893)																												
<i>Cloesia digna</i> Schaus, 1911																												
<i>Coreura fida</i> Hübner, 1827																												
<i>Coreura phoenicoides</i> (Druce, 1884)	X																											
<i>Correbidia assimilis</i> Rothschild, 1912	X																											
<i>Correbidia calopteridia</i> (Butler, 1878)	X																											
<i>Correbidia joinvillea</i> Schaus, 1921	X																											
<i>Cosmosoma achemon</i> (Fabricius, 1781)																												
<i>Cosmosoma auge</i> (Linnaeus, 1767)	X	X																										
<i>Cosmosoma brinkley</i> Rothschild, 1911																												
<i>Cosmosoma centralis</i> (Walker, 1854)	X																											
<i>Cosmosoma chrysis</i> (Hübner, 1827)	X																											
<i>Cosmosoma cingla</i> (Schaus, 1894)	X																											
<i>Cosmosoma durca</i> Schaus, 1896	X																											
<i>Cosmosoma elegans</i> Butler, 1876	X																											
<i>Cosmosoma festivum</i> (Walker, 1854)	X																											
<i>Cosmosoma leuconotum</i> Hampson, 1898	X																											
<i>Cosmosoma pheres</i> (Cramer, 1782)	X																											
<i>Cosmosoma plutona</i> Schaus, 1894	X																											
<i>Cosmosoma ratera</i> (Jones, 1914)	X																											
<i>Cosmosoma raseria</i> (Schaus, 1894)	X																											
<i>Cosmosoma regia</i> (Schaus, 1894)	X																											
<i>Cosmosoma remotum</i> (Walker, 1854)	X																											
<i>Cosmosoma restrictum</i> Butler, 1876	X	X																										
<i>Cosmosoma subflamma</i> (Walker, 1854)	X																											
<i>Cosmosoma telephus</i> (Walker, 1854)	X																											
<i>Cosmosoma teuthras</i> (Walker, 1854)	X																											

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Arctiinae of Rio de Janeiro, Brazil

Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Cratoplasis catharinae</i> (Rothschild, 1916)	X												X						X			X						X
<i>Cresera annulata</i> Schaus, 1894	X			X									X						X	X								X
<i>Cresera ilus</i> (Cramer, 1776)	X						X																					X
<i>Cresera optimus</i> (Butler, 1877)																			X									
<i>Cresera silvestrii</i> Travassos, 1956	X																		X									
<i>Ctenucha braganza</i> (Schaus, 1892)	X																		X	X								
<i>Ctenucha jonesi</i> Rothschild, 1912	X																		X									X
<i>Ctenucha mortia</i> Schaus, 1901	X																		X									X
<i>Ctenucha palmeira</i> Schaus, 1892	X																		X									
<i>Cyanopepla fastuosa</i> (Walker, 1854)	X																		X	X								X
<i>Cyanopepla jucunda</i> (Walker, 1854)	X																		X	X								X
<i>Cyanopepla julia</i> (Druce, 1883)																			X									X
<i>Cyanopepla orbona</i> (Druce, 1893)	X																		X									X
<i>Dasyphnix torquata</i> (Druce, 1883)	X																		X									X
<i>Delphyre albiventus</i> (Druce, 1898)	X																		X	X								X
<i>Delphyre arpi</i> Schaus, 1894	X																		X	X								X
<i>Delphyre brunnea</i> (Druce, 1898)	X																		X	X								X
<i>Delphyre dizona</i> Druce, 1898																			X									
<i>Delphyre flaviceps</i> (Druce, 1905)																			X									
<i>Delphyre flaviventralis</i> Hampson, 1901																			X									X
<i>Delphyre hamptoni</i> Rothschild, 1912																			X									X
<i>Delphyre pusilla</i> (Butler, 1878)	X																		X	X								X
<i>Delphyre pyroperas</i> Hampson, 1911	X																		X	X								X
<i>Delphyre testacea</i> Druce, 1884	X																		X	X								X
<i>Demolis albicostata</i> Hampson, 1901																			X									
<i>Demolis albitegula</i> (Rothschild, 1935)	X																		X									X
<i>Demolis flavithorax</i> Rothschild, 1909																			X									
<i>Desmidocnemis hypochryseis</i> Hampson, 1898																			X									
<i>Diarhabdosta mandana</i> Dyar, 1907	X																		X									X
<i>Dinia eagrus</i> (Cramer, 1779)	X																		X	X								X
<i>Dinia mena</i> (Hübner, 1827)	X																		X	X								X
<i>Diptilon doeri</i> Schaus, 1892																			X									
<i>Dixophlebia holophaea</i> Hampson, 1909																			X									
<i>Dixophlebia quadristrigata</i> (Walker, 1864)	X																		X									
<i>Dycladia correbioides</i> Felder, 1874																			X									
<i>Dycladia lucretius</i> (Cramer, 1782)	X																		X									
<i>Dycladia melaena</i> Hampson, 1898																			X									
<i>Dysschema amphissa</i> (Geyer, 1832)	X																		X	X								X
<i>Dysschema boisduvalii</i> (van der Hoeven e de Vriese, 1840)	X																		X	X								X
<i>Dysschema fantasma</i> (Butler, 1873)																			X	X								
<i>Dysschema hilarina</i> Weymer, 1914																			X									
<i>Dysschema hypoxantha</i> Hübner 1818	X																		X									X

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Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Dysschema indecisa</i> (Walker, 1854)										X																	X	
<i>Dysschema lucifer</i> (Butler, 1873)	X			X						X											X							X
<i>Dysschema luctuosa</i> Dognin 1919	X									X										X								X
<i>Dysschema marginata</i> (Guérin-Méneville, 1844)	X									X										X								X
<i>Dysschema neda</i> (Klug, 1836)	X									X										X								X
<i>Dysschema picta</i> (Guérin-Méneville, 1844)	X									X										X								X
<i>Dysschema sacrificata</i> (Hübner, 1831)	X									X										X								X
<i>Dysschema subpicalis</i> (Walker, 1854)	X			X						X										X								X
<i>Dysschema trapeziata</i> Walker 1865	X									X										X								X
<i>Echeta divisa</i> (Herrich-Schäffer, 1855)	X									X										X								X
<i>Echeta juno</i> (Schaus, 1892)	X									X										X								X
<i>Echeta minerva</i> (Schaus, 1915)	X									X										X								X
<i>Echeta rubr retina</i> Dognin, 1906	X									X										X								X
<i>Elysius chimaera</i> (Druce, 1893)	X									X										X								X
<i>Elysius cingulata</i> (Walker, 1856)	X									X										X								X
<i>Elysius conjunctus</i> Rothschild, 1910	X									X										X								X
<i>Elysius conspersus</i> Walker, 1855	X									X										X								X
<i>Elysius discoplaga</i> (Walker, 1856)	X									X										X								X
<i>Elysius itaunensis</i> Rego Barros, 1971	X									X										X								X
<i>Elysius meridionalis</i> Rothschild, 1917	X									X										X								X
<i>Elysius ordinaria</i> (Schaus, 1894)	X									X										X								X
<i>Elysius pyrostricta</i> Hampson, 1905	X			X						X										X								X
<i>Elysius sebrus</i> (Druce, 1899)	X									X										X								X
<i>Emurena fernandezi</i> Watson, 1975	X									X										X								X
<i>Emurena lurida</i> (Felder, 1874)	X									X										X								X
<i>Emurena luridoidea</i> (Rothschild, 1910)	X									X										X								X
<i>Epeitromulona hamata</i> Field, 1952	X									X										X								X
<i>Ephestris melaxantha</i> (Hübner, [1809])	X									X										X								X
<i>Epidesma crameri</i> Travassos 1938	X									X										X								X
<i>Epidesma josioidea</i> Zerny, 1931	X									X										X								X
<i>Epidesma obliqua</i> (Schaus, 1898)	X									X										X								X
<i>Epidesma ursula</i> Cramer 1782	X									X										X								X
<i>Episcea extravagans</i> Warren, 1901	X									X										X								X
<i>Episcepsis capysoides</i> Dognin, 1911	X									X										X								X
<i>Episcepsis endodasia</i> Hampson, 1898	X									X										X								X
<i>Episcepsis flavipuncta</i> Zerny, 1931	X									X										X								X
<i>Episcepsis gnoma</i> (Butler, 1877)	X									X										X								X
<i>Episcepsis lenaeus</i> (Cramer, 1780)	X									X										X								X
<i>Episcepsis thetis</i> (Linnaeus, 1771)	X									X										X								X
<i>Ernassa cruenta</i> (Rothschild, 1909)	X									X										X								X
<i>Ernassa gabriellae</i> Travassos, 1954	X									X										X								X
<i>Ernassa sanguinolenta</i> (Cramer, 1779)	X									X										X								X

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Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Euagra azurea</i> (Walker, 1854)	X									X																		X
<i>Eucereon acolum</i> Hampson, 1898	X																											X
<i>Eucereon albidia</i> Rothschild, 1912	X																											X
<i>Eucereon aoris</i> Möscher 1877	X																				X							X
<i>Eucereon apicalis</i> (Walker, 1856)	X																				X							X
<i>Eucereon aroa</i> Schaus, 1894	X																				X							X
<i>Eucereon atrigutta</i> Druce, 1905	X																				X							X
<i>Eucereon chalcodon</i> Druce, 1893	X																				X							X
<i>Eucereon confinis</i> (Herrich-Schäffer, 1855)	X																				X							X
<i>Eucereon costinotatum</i> Dognin, 1900	X																				X							X
<i>Eucereon discolor</i> Walker, 1856	X																				X							X
<i>Eucereon dorsipuncta</i> Hampson, 1905	X																				X							X
<i>Eucereon formosum</i> Dognin 1905	X																				X							X
<i>Eucereon ladasi</i> Schaus, 1892	X																				X							X
<i>Eucereon latifascia</i> Walker, 1856	X																				X							X
<i>Eucereon leucophaeum</i> (Walker, 1855)	X																				X							X
<i>Eucereon lithosoides</i> Rothschild, 1912	X																				X							X
<i>Eucereon lychnis</i> Zerny, 1931	X																				X							X
<i>Eucereon maja</i> Druce, 1884	X																				X							X
<i>Eucereon marcatum</i> Schaus, 1901	X																				X							X
<i>Eucereon metoides</i> Hampson, 1905	X																				X							X
<i>Eucereon nebulosum</i> Dognin, 1891	X																				X							X
<i>Eucereon nubilosa</i> Rothschild, 1912	X																				X							X
<i>Eucereon obscurum</i> (Möscher 1872)	X																				X							X
<i>Eucereon pallescens</i> Rothschild, 1912	X																				X							X
<i>Eucereon perplicatum</i> Draudt, 1917	X																				X							X
<i>Eucereon pilatii</i> (Walker, 1854)	X																				X							X
<i>Eucereon plumbicollum</i> Hampson, 1898	X																				X							X
<i>Eucereon pometinum</i> Druce, 1894	X																				X							X
<i>Eucereon pseudarchias</i> Hampson, 1898	X																				X							X
<i>Eucereon punctatum</i> (Guérin, 1844)	X																				X							X
<i>Eucereon quadricolor</i> (Walker 1855)	X																				X							X
<i>Eucereon rosa</i> (Walker, 1854)	X																				X							X
<i>Eucereon scyton</i> (Cramer, 1777)	X																				X							X
<i>Eucereon setosum</i> Sepp, 1848	X																				X							X
<i>Eucereon striatum</i> Druce, 1889	X																				X							X
<i>Eucereon sylvius</i> (Stoll, 1780)	X																				X							X
<i>Eucereon taperinhae</i> Dognin, 1923	X																				X							X
<i>Eucereon tarona</i> Hampson, 1898	X																				X							X
<i>Eucereon theophanes</i> Schaus, 1924	X																				X							X
<i>Eucereon velutinum</i> Schaus, 1896	X																				X							X
<i>Eucereon vestalis</i> (Schaus, 1892)	X																				X							X

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## Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Eucereon xanthura</i> Schaus, 1910	X									X									X	X	X						X	
<i>Eucereon zamorae</i> Dognin, 1894	X									X																		
<i>Euceriodes wernickei</i> Draudt, 1917	X			X						X										X								
<i>Euchaetes rizoma</i> (Schaus, 1896)	X									X																		
<i>Euchlaenidia neglecta</i> Rothschild, 1910	X									X										X							X	
<i>Euchlaenidia transcisa</i> (Walker, 1854)	X									X										X	X	X					X	
<i>Euclera diversipennis</i> (Walker, 1854)	X									X										X	X	X					X	
<i>Euclera meones</i> (Cramer, 1780)	X									X																	X	
<i>Eudemia ruficollis</i> (Donovan, 1798)	X									X																	X	
<i>Eugonostia angulifer</i> Schaus, 1899	X									X										X							X	
<i>Eupseudosoma aberrans</i> Schaus, 1905	X									X											X						X	
<i>Eupseudosoma grandis</i> Rothschild, 1909	X									X																	X	
<i>Eupseudosoma involuta</i> (Sepp, 1855)	X									X																	X	
<i>Eupseudosoma larissa</i> (Druce, 1890)	X									X																	X	
<i>Eurota hermione</i> (Burmeister, 1878)	X									X																	X	
<i>Eurota herrichi</i> Butler, 1876	X									X																	X	
<i>Eurota tisamena</i> Dognin, 1902	X									X																	X	
<i>Euryptidia basivitta</i> (Walker, 1854)	X									X											X	X					X	
<i>Euryptidia univittata</i> Hampson, 1900	X									X											X	X					X	
<i>Euthyone purpurea</i> (Jones, 1914)	X									X																	X	
<i>Euthyone simplex</i> (Walker, 1854)	X									X																	X	
<i>Evius hippia</i> (Stoll, 1790)	X									X											X	X					X	
<i>Galethalea pica</i> (Walker, 1855)	X									X											X	X					X	
<i>Gangamela saturata</i> Walker, 1864	X									X											X	X					X	
<i>Gorgonidia garleppi</i> (Druce, 1898)	X									X											X	X					X	
<i>Graphaea marmorea</i> Schaus, 1894	X									X											X	X					X	
<i>Gymnelia laennus</i> (Walker, 1854)	X								X	X											X	X					X	
<i>Gymnelia xanthogastra</i> (Perty, 1834)	X									X											X	X					X	
<i>Haemanota rubriceps</i> Hampson, 1901	X									X											X	X					X	
<i>Haemaphysbiella strigata</i> (Jones, 1914)	X									X											X	X					X	
<i>Halysidota cinctipes</i> Grote, [1866]	X									X											X	X					X	
<i>Halysidota cyclozonata</i> Hampson, 1901	X									X											X	X					X	
<i>Halysidota interlineata</i> Walker, 1855	X									X											X	X					X	
<i>Halysidota interstriata</i> Hampson, 1901	X									X											X	X					X	
<i>Halysidota pearsoni</i> Watson, 1909	X									X											X	X					X	
<i>Halysidota schausi</i> Rothschild, 1909	X									X											X	X					X	
<i>Halysidota tessellaris</i> (Smith, 1797)	X								X	X											X	X					X	
<i>Halysidota underwoodi</i> Rothschild, 1909	X									X											X	X					X	
<i>Heliciniidia nigrilinea</i> (Walker, 1856)	X									X											X	X					X	
<i>Helitura hecale</i> (Schaus, 1892)	X									X											X	X					X	
<i>Helitura kennedyi</i> Rothschild, 1912	X									X											X	X					X	
<i>Helitura phaeosoma</i> Druce, 1905	X									X											X	X					X	

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Arctiinae of Rio de Janeiro, Brazil

Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Heliura rhodophila</i> (Walker, 1856)												X																
<i>Heliura subplena</i> (Walker, 1854)	X			X						X												X						X
<i>Heliura suffusa</i> Lathy 1899	X			X						X												X						X
<i>Heliura tetragramma</i> (Walker, 1854)																						X						X
<i>Heliura zonata</i> Druce, 1905																						X						X
<i>Hemihyalea diminuta</i> (Walker, 1855)																												
<i>Herea metaxanthus</i> (Walker, 1854)				X																								
<i>Homoeocera acuminata</i> (Walker, 1856)																						X						
<i>Horama castrensis</i> Jones, 1908				X																								X
<i>Hyalarcia sericea</i> Schaus, 1901																												
<i>Hyalucerea costinotatum</i> Dognin, 1900																												
<i>Hyalucerea lemoulti</i> Schaus 1905																						X						
<i>Hyalucerea morosa</i> Schaus, 1910				X																								
<i>Hyalucerea mundula</i> (Berg, 1882)																												
<i>Hyalucerea sororia</i> Schaus, 1910																												
<i>Hyalurga fenestra</i> (Linnaeus, 1758)																												
<i>Hyalurga fenestrata</i> (Walker, 1855)	X											X																X
<i>Hyalurga leucophaea</i> (Walker, 1854)																												
<i>Hyalurga peritta</i> Hering, 1925																												
<i>Hyalurga sora</i> (Boisduval, 1870)	X																											
<i>Hyalurga subnormalis</i> Dyar, 1914	X																											
<i>Hyalurga syma</i> (Walker, 1854)	X																											
<i>Hyda basilutea</i> (Walker, 1854)																												
<i>Hyperandra appendiculata</i> (Henrich-Schäffer, [1856])	X																											
<i>Hypercompe abdominalis</i> (Walker, [1865])	X																											
<i>Hypercompe brasiliensis</i> (Oberthür, 1881)	X																											
<i>Hypercompe cuningunda</i> (Stoll, 1781)	X																											
<i>Hypercompe heterogena</i> Oberthür, 1881	X																											
<i>Hypercompe jaguarina</i> (Schaus, 1921)																												
<i>Hypercompe kennedyi</i> (Rothschild, 1910)																												
<i>Hypercompe kinkelini</i> (Burmeister, 1880)																												
<i>Hypercompe laeta</i> (Walker, 1855)																												
<i>Hypercompe magdalenae</i> (Oberthür, 1881)																												
<i>Hypercompe mus</i> (Oberthür, 1881)	X																											
<i>Hyperthaema albipuncta</i> Schaus, 1901																												
<i>Hyperthaema caroei</i> Jörgensen, 1935																												
<i>Hyperthaema hoffmannsi</i> Rothschild, 1909																												
<i>Hyperthaema signatus</i> (Walker, 1862)																												
<i>Hypidalia enervis</i> Schaus, 1894																												
<i>Hypocladia elongata</i> Druce, 1905																												
<i>Hypocristis jonesi</i> Schaus 1894																												
<i>Hypocrita bicolora</i> (Sulzer, 1776)																												

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Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Hyponerita pinon</i> (Druce, 1911)	X			X											X				X								X	
<i>Hyponerita tipolis</i> (Druce, 1896)	X																		X								X	
<i>Ichoria chalcomedusa</i> Druce, 1893						X													X		X						X	
<i>Ichoria trincta</i> (Herrich-Schäffer, 1855)	X			X		X									X				X		X						X	
<i>Idalus admirabilis</i> (Cramer, 1777)						X									X				X		X						X	
<i>Idalus agastus</i> Dyar, 1910																			X		X						X	
<i>Idalus albescens</i> (Rothschild, 1909)	X																		X									
<i>Idalus aleteria</i> (Schaus, 1905)	X																		X								X	
<i>Idalus carinosa</i> (Schaus, 1905)	X			X															X		X							
<i>Idalus citrina</i> Druce, 1890						X													X									
<i>Idalus daries</i> Druce 1894	X																		X									
<i>Idalus felderi</i> (Rothschild, 1909)	X														X				X		X						X	
<i>Idalus flavicostalis</i> (Rothschild, 1935)	X																		X								X	
<i>Idalus herois</i> Schaus, 1889	X																		X								X	
<i>Idalus idalia</i> (Hampson, 1901)	X																		X		X						X	
<i>Idalus lineosus</i> Walker, 1869	X																		X		X						X	
<i>Idalus metacrinis</i> (Rothschild, 1909)	X																		X		X						X	
<i>Idalus noiva</i> Jones 1914										X																		
<i>Idalus tybris</i> (Cramer, [1776])						X																						
<i>Idalus vitrea</i> (Cramer 1780)	X																										X	
<i>Ilipa tengyra</i> (Walker, 1854)	X			X			X								X				X		X						X	
<i>Illice batialis</i> Walker, 1859				X																								
<i>Illice blanda</i> Jones 1914				X																							X	
<i>Illice cryptopyra</i> Hampson 1903						X																						
<i>Illice ditrigona</i> Schaus 1899						X																						
<i>Illice orbonella</i> Hampson 1900						X																						
<i>Illice persimilis</i> Hampson 1903						X																						
<i>Illice petrovna</i> Schaus 1892						X																						
<i>Illice</i> sp.						X																						
<i>Isanthrene incendiaria</i> (Hübner, 1827)						X													X		X							
<i>Isanthrene melas</i> Cramer 1775																					X							
<i>Isanthrene pertexta</i> Draudt, 1917						X													X									
<i>Isanthrene ustrina</i> Hübner, 1827						X																					X	
<i>Ischnocampa lithosoides</i> (Rothschild, 1912)																												
<i>Ischnocampa lugubris</i> (Schaus, 1892)	X					X													X		X						X	
<i>Ischnocampa styx</i> Jones, 1914						X													X		X						X	
<i>Ischnocampa tristis</i> Schaus, 1889																												
<i>Ischnocampa semipalina</i> Felder, 1874																												
<i>Isia alcaumena</i> Berg, 1882																												
<i>Isia intricata</i> Walker, 1856	X																		X		X							
<i>Ixylasia semivittata</i> Hampson, 1905																			X		X							
<i>Ixylasia trogonoides</i> (Walker, 1864)						X													X		X							

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Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Lamprostola pascuala</i> (Schaus, 1896)				X						X																		
<i>Lamprostola unisfascia</i> (Rothschild, 1913)							X																					
<i>Lampruna rosea</i> Schaus, 1894												X																
<i>Lepidokirbyia vittipes</i> (Walker, 1855)	X																											X
<i>Lepidoneiva erubescens</i> (Butler, 1876)	X																											X
<i>Lepidozikania cinerascens</i> (Walker, 1855)	X																											X
<i>Lepidozikania similis</i> Travassos, 1949	X																											X
<i>Leucanopsis acuta</i> (Hampson, 1901)	X																											X
<i>Leucanopsis ahyssa</i> (Schaus, 1933)	X																											X
<i>Leucanopsis athor</i> (Schaus, 1933)	X																											X
<i>Leucanopsis bactris</i> Sepp, 1852																												X
<i>Leucanopsis biedata</i> (Schaus, 1941)																												X
<i>Leucanopsis cedon</i> (Druce, 1897)																												X
<i>Leucanopsis coniota</i> (Hampson, 1901)																												X
<i>Leucanopsis dallipa</i> (Jones, 1908)																												X
<i>Leucanopsis fuscosa</i> (Jones, 1908)																												X
<i>Leucanopsis leucanina</i> (Felder & Rogenhofer, 1874)	X																											X
<i>Leucanopsis mandus</i> (Herrich-Schäffer, [1855])																												X
<i>Leucanopsis oruba</i> (Schaus, 1892)	X																											X
<i>Leucanopsis perirrorata</i> (Reich, 1935)	X																											X
<i>Leucanopsis pseudomanda</i> (Rothschild, 1910)																												X
<i>Leucanopsis puberea</i> (Schaus, 1896)																												X
<i>Leucanopsis rhomboidea</i> (Sepp, [1852])																												X
<i>Leucanopsis sablona</i> (Schaus, 1896)																												X
<i>Leucanopsis squalida</i> (Herrich-Schäffer, [1855])																												X
<i>Leucanopsis strigulosa</i> (Walker, 1855)																												X
<i>Leucanopsis terola</i> (Schaus, 1941)																												X
<i>Leucanopsis umbrosa</i> (Hampson, 1901)																												X
<i>Leucanopsis valentina</i> Schaus 1924																												X
<i>Leucotmemis nexa</i> (Herrich-Schäffer, [1854])	X																											X
<i>Lomuna nigripuncta</i> (Hampson, 1900)																												X
<i>Lophocampa annulosa</i> (Walker, 1855)																												X
<i>Lophocampa atrimaculata</i> (Hampson, 1901)																												X
<i>Lophocampa catenulata</i> Hübner 1812																												X
<i>Lophocampa citrina</i> (Sepp, [1852])	X																											X
<i>Lophocampa modesta</i> (Kirby, 1892)	X																											X
<i>Lophocampa romolola</i> (Schaus, 1933)																												X
<i>Lophocampa ronda</i> (Jones, 1908)																												X
<i>Lophocampa texta</i> Herrich-Schäffer 1855																												X
<i>Loxophlebia brasiliensis</i> Rothschild, 1911																												X
<i>Loxophlebia flavinigra</i> Jones, 1908																												X
<i>Loxophlebia pyrgion</i> (Druce, 1884)																												X

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Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Lycomorhodes aracia</i> Jones 1914										X																		
<i>Lycomorhodes dichroa</i> Dognin, 1912										X																		
<i>Lycomorhodes epatra</i> Schaus, 1905										X																		
<i>Lycomorhodes strigosa</i> (Butler, 1877)				X						X																	X	
<i>Lycomorhodes suspecta</i> (Felder, 1875)				X						X																	X	
<i>Machadoia xanthosticta</i> (Hampson, 1901)				X						X																	X	
<i>Macrocneme aurifera</i> Hampson 1914										X																	X	
<i>Macrocneme cyanea</i> Butler 1876										X																	X	
<i>Macrocneme indistincta</i> Butler, 1876										X																	X	
<i>Macrocneme lades</i> (Cramer, 1776)				X						X																	X	
<i>Macrocneme pelotas</i> Dietz, 1994				X						X																	X	
<i>Macrocneme sura</i> Schaus, 1901										X																	X	
<i>Mallodeta clavata</i> (Walker, 1854)				X						X																	X	
<i>Mallodeta consors</i> (Walker, 1854)				X						X																	X	
<i>Mallodeta sortita</i> Walker 1854				X						X																	X	
<i>Mazaeras conferta</i> Walker, 1855				X						X																	X	
<i>Mazaeras francki</i> Schaus, 1896				X						X																	X	
<i>Mazaeras janeira</i> (Schaus, 1892)				X						X																	X	
<i>Mazaeras melanopyga</i> (Walker, 1869)				X						X																	X	
<i>Melese asana</i> Druce, 1884										X																	X	
<i>Melese amastris</i> Druce 1884				X						X																	X	
<i>Melese babosa</i> (Dognin, 1894)				X						X																	X	
<i>Melese castrena</i> Schaus, 1905				X						X																	X	
<i>Melese dorothea</i> (Stoll, 1782)				X						X																	X	
<i>Melese hebetis</i> Rothschild, 1909				X						X																	X	
<i>Melese incertus</i> Walker 1855				X						X																	X	
<i>Melese ocellata</i> Hampson, 1901				X						X																	X	
<i>Melese peruviana</i> Rothschild, 1909				X						X																	X	
<i>Mellamastus nero</i> (Weymer, 1907)				X						X																	X	
<i>Mesothen desperata</i> (Walker, 1856)				X						X																	X	
<i>Mesothen inconspicua</i> (Kaye, 1911)				X						X																	X	
<i>Mesothen pyrrihina</i> Jones, 1914				X						X																	X	
<i>Mesothen rogenhoferi</i> Schaus 1892				X						X																	X	
<i>Metallosia chrysois</i> Hampson, 1900				X						X																	X	
<i>Metalobosia ciprea</i> (Schaus, 1896)				X						X																	X	
<i>Metalobosia varda</i> (Schaus, 1896)				X						X																	X	
<i>Metamya aenetus</i> (Schaus, 1896)				X						X																	X	
<i>Metaxanthia threnodes</i> Druce 1905				X						X																	X	
<i>Metaxanthia vespiformis</i> Druce 1899				X						X																	X	
<i>Mirandisca harpalyce</i> (Schaus, 1892)				X						X																	X	
<i>Munona iridescens</i> Schaus 1894				X						X																	X	
<i>Napata albiplaga</i> (Walker, 1854)				X						X																	X	

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Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Napata atricincta</i> Hampson, 1901																						X						
<i>Napata jynx</i> (Hübner, 1832)	X																				X							X
<i>Napata leucotela</i> Butler, 1876	X																											
<i>Napata quadrimaculata</i> (Möschler, 1872)						X																						X
<i>Napata splendida</i> (Herrich-Schäffer, [1854])						X																						
<i>Napata terminalis</i> (Walker, 1854)																					X							
<i>Napata walkeri</i> (Druce, 1889)																					X							
<i>Neidalia orientalis</i> Rothschild, 1933						X															X							
<i>Neonerita dorsipuncta</i> Hampson 1901	X			X																	X							X
<i>Neotrichura nigripes</i> Heylaerts 1890	X			X																	X							X
<i>Neozatrophes schausi</i> (Rothschild, 1909)						X																						
<i>Neozatrophes tessila</i> Druce, 1893																												X
<i>Nepe coelestina</i> (Cramer, 1782)	X																											
<i>Neritos leucoplagia</i> Hampson 1905	X																											
<i>Neritos onytes</i> (Cramer, 1777)						X																						
<i>Neritos psamas</i> (Cramer, 1779)						X																						
<i>Neritos repanda</i> Walker, 1855						X																						X
<i>Nodozana corea</i> Schaus 1896	X					X																						
<i>Nodozana jucunda</i> Jones, 1914						X																						X
<i>Nodozana rhodosticta</i> (Butler, 1878)						X																						X
<i>Notophyson heliconides</i> Swainson 1833	X																											
<i>Notophyson tiresias</i> Cramer 1776	X																											X
<i>Nyridela acroxantha</i> Perty 1834	X					X															X							X
<i>Odozana cocciniceps</i> Jones 1908						X																						
<i>Odozana domina</i> Schaus 1896						X																						
<i>Odozana obscura</i> (Schaus, 1896)						X																						X
<i>Opharus basalis</i> Walker 1856	X					X																						
<i>Opharus bimaculata</i> (Dewitz 1877)	X					X																						
<i>Opharus intermedia</i> Rothschild 1909																												X
<i>Opharus flavimaculata</i> Hampson 1901																												
<i>Opharus notata</i> (Schaus 1892)						X																						X
<i>Opharus procroides</i> Walker, 1855	X					X																						X
<i>Opharus rema</i> (Dognin, 1891)	X					X																						X
<i>Ordishia rutilus</i> Stoll, 1782						X																						X
<i>Ormetica chrysomelas</i> (Walker, 1856)	X					X																						X
<i>Ormetica fulgurata</i> (Butler, 1876)	X					X																						X
<i>Ormetica neira</i> (Schaus, 1905)	X					X																						X
<i>Ormetica ochreomarginata</i> (Joicey & Talbot, 1918)						X																						X
<i>Ormetica taniata</i> Schaus 1910																												
<i>Ormetica xanthia</i> Hampson 1901																												X
<i>Pachydota affinis</i> Rothschild, 1909																												
<i>Pachydota albiceps</i> (Walker, 1856)	X					X																						X

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Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Pachydota ducasa</i> Schaus, 1905	X									X									X									
<i>Pachydota punctata</i> Rothschild 1909	X									X									X									
<i>Pachydota saduca</i> (Druce, 1895)	X																		X									
<i>Paracles affinis</i> (Rothschild, 1910)										X									X								X	
<i>Paracles alonia</i> Schaus 1933										X									X									
<i>Paracles bilinea</i> (Schaus, 1901)										X									X									
<i>Paracles brunnea</i> (Hübner, [1831])										X									X									
<i>Paracles costata</i> (Burmeister, 1878)										X									X									
<i>Paracles fusca</i> (Walker, 1856)										X									X								X	
<i>Paracles variegata</i> (Schaus, 1896)	X																		X								X	
<i>Paraethria triseriata</i> (Herrich-Schäffer, [1855])										X									X								X	
<i>Parathyrus cedonulli</i> (Stoll 1781)	X									X									X									
<i>Paranerita inaequalis</i> Rothschild, 1909	X									X									X								X	
<i>Pareuchaetes aurata</i> (Butler, 1875)	X									X									X									
<i>Pareuchaetes insulata</i> (Walker, 1855)	X									X									X									
<i>Parevia sisenna</i> Druce 1899																			X									
<i>Parevia vulmaria</i> Schaus, 1924	X									X									X								X	
<i>Pelochyta arontes</i> (Stoll 1782)										X									X									
<i>Pelochyta cinerea</i> (Walker, 1855)	X									X									X								X	
<i>Pelochyta pallida</i> (Schaus, 1901)										X									X								X	
<i>Phaegoptera albescens</i> Travassos 1955										X									X								X	
<i>Phaegoptera albimacula</i> (Jones, 1908)										X									X								X	
<i>Phaegoptera chorima</i> Schaus, 1896										X									X								X	
<i>Phaegoptera depicta</i> Herrich-Schäffer, [1855]										X									X								X	
<i>Phaegoptera flavopunctata</i> Herrich-Schäffer, [1855]										X									X								X	
<i>Phaegoptera granifera</i> Schaus, 1892	X									X									X								X	
<i>Phaegoptera histrionica</i> Herrich-Schäffer, [1853]	X									X									X								X	
<i>Phaegoptera nexa</i> (Herrich-Schäffer, 1855)										X									X								X	
<i>Phaegoptera pseudocatenata</i> Travassos, 1955										X									X								X	
<i>Phaegoptera punctularis</i> Herrich-Schäffer, [1855]										X									X								X	
<i>Phaegoptera schaefferi</i> Herrich-Schäffer, [1855]										X									X								X	
<i>Phaegoptera superba</i> (Druce, 1911)	X									X									X								X	
<i>Phaeomolis lineatus</i> Druce 1884	X									X									X								X	
<i>Phaeomolis polystria</i> Schaus 1905										X									X								X	
<i>Phaloe cruenta</i> (Hübner, 1823)										X									X								X	
<i>Pheia albisigna</i> (Walker, 1854)										X									X								X	
<i>Pheia catherina</i> (Schaus, 1892)	X									X									X								X	
<i>Pheia crocata</i> (Herrich-Schäffer, 1854)	X									X									X								X	
<i>Pheia elegans</i> (Druce, 1884)										X									X								X	
<i>Pheia picta</i> (Walker, 1854)	X									X									X								X	
<i>Philoros colombina</i> Draudt 1915										X									X								X	
<i>Philoros rubriceps</i> (Walker, 1854)	X									X									X								X	

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Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Phoenicoprocta andlis</i> Schrottky, 1909																				X								
<i>Phoenicoprocta haemorrhoidalis</i> (Fabricius, 1775)				X																X								
<i>Phoenicoprocta steinbachi</i> Rothschild 1911	X	X																			X							
<i>Phoenicoprocta teda</i> (Walker, 1854)	X									X																		X
<i>Phoenicoprocta vacillans</i> (Walker, 1856)	X									X																		
<i>Ptilopleura sanguipuncta</i> Hampson, 1898							X																					
<i>Ptonia elongata</i> (Dognin, 1890)	X									X																		
<i>Ptonia lycoides</i> (Walker, 1854)	X							X		X																		X
<i>Polioptastea ockendeni</i> Rothschild, 1909	X									X																		X
<i>Pronola magniplaga</i> Schaus, 1899										X																		
<i>Pseudactyia pseudodelphire</i> Rothschild 1912										X																		X
<i>Pseudapistostia umber</i> Cramer 1775										X																		X
<i>Pseudohyaloleucera vulnerata</i> (Butler, 1875)	X									X																		X
<i>Pseudomyia tipulina</i> (Hübner, 1812)	X									X																		X
<i>Pseudophaloe tellina</i> (Weymer, 1895)	X									X																		X
<i>Pseudopompilia mimica</i> Druce 1898	X									X																		X
<i>Pseudosphex fulvisphex</i> (Druce, 1898)	X									X																		X
<i>Pseudosphex ichneumonea</i> Herrich-Schäffer (1854)	X									X																		X
<i>Pseudosphex jonesi</i> Kaye 1911	X									X																		X
<i>Pseudosphex melanogen</i> Dyar 1910	X									X																		X
<i>Pseudosphex rubripalpus</i> Hampson 1901	X									X																		X
<i>Pseudotesellarcia brunneincta</i> (Hampson, 1901)	X									X																		X
<i>Pseudotesellarcia ursina</i> Schaus 1892	X									X																		X
<i>Psilopleura pentheri</i> Zerny 1912	X									X																		X
<i>Psilopleura sanguipuncta</i> Hampson 1898	X									X																		X
<i>Psychophasma erosa</i> (Herrich-Schäffer, (1858))	X									X																		X
<i>Psychotrichos elongatus</i> Schaus, 1905	X									X																		X
<i>Psychotrichos zeus</i> Schaus, 1894	X									X																		X
<i>Puritus pilumnia</i> (Stoll, 1780)	X									X																		X
<i>Rezia cardinale</i> (Hampson, 1898)	X									X																		X
<i>Rezia erythrarchos</i> (Walker, 1854)	X									X																		X
<i>Rhipha flavoplagiata</i> Rothschild 1911	X									X																		X
<i>Rhipha persimilis</i> Rothschild 1909	X									X																		X
<i>Rhipha strigosa</i> (Walker, 1854)	X									X																		X
<i>Rhipha subflammans</i> (Rothschild, 1909)	X									X																		X
<i>Rhodographa phaeoplaga</i> Schaus, 1899	X									X																		X
<i>Rhynchopyga meisteri</i> (Berg, 1883)	X									X																		X
<i>Rhynchopyga subflamma</i> Druce 1884	X									X																		X
<i>Robinsonia dewitzi</i> Gundlach, 1881	X									X																		X
<i>Robinsonia lefaiivrei</i> Schaus 1895	X									X																		X
<i>Robinsonia spitzii</i> Rothschild 1933	X									X																		X
<i>Roeselia bifiliferata</i> Walker 1862	X									X																		X

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Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA
<i>Roeselia nepheloleuca</i> Hampson										X																		
<i>Roeselia perangulata</i> Hampson 1900										X																		
<i>Roeselia polyodonta</i> Schaus 1905										X																		
<i>Romualdia elongata</i> (Felder, 1874)	X									X															X			
<i>Romualdia opharina</i> (Schaus, 1921)	X									X															X			
<i>Sarosa pompilina</i> Butler 1876	X									X																		
<i>Saurita attenuata</i> Hampson, 1905	X									X																	X	
<i>Saurita carmanina</i> Druce, 1833	X									X																		
<i>Saurita cassandra</i> (Linnaeus, 1758)	X			X						X																	X	
<i>Saurita erythrogya</i> Hampson, 1898	X			X						X																		
<i>Saurita intricata</i> (Walker, 1854)	X			X						X																	X	
<i>Saurita melaniifera</i> Kaye, 1911	X									X																		
<i>Saurita nigripalpia</i> (Hampson, 1898)	X									X																		
<i>Saurita pellucida</i> (Schaus, 1892)	X									X																		
<i>Saurita phoenicosticta</i> Hampson 1898	X									X																	X	
<i>Saurita sericea</i> (Herrich-Schäffer, [1854])	X									X																		
<i>Saurita triangulifera</i> (Druce, 1898)	X									X																		
<i>Scaptius pseudoprumala</i> (Rothschild, 1935)	X																											
<i>Scaptius sanguistrigata</i> (Doggin, 1910)	X																										X	
<i>Sciopsyche tropica</i> Walker 1854	X									X																	X	
<i>Selenarcia elissa</i> (Schaus, 1892)	X									X																	X	
<i>Selenarcia flavidorsata</i> Watson, 1975	X									X																	X	
<i>Sermylea transversa</i> (Walker, 1854)	X									X																	X	
<i>Sphecosoma abdominalis</i> Schaus 1905	X																											
<i>Sphecosoma besasa</i> Schaus 1924	X																											
<i>Sphecosoma melissa</i> Schaus, 1896	X									X																	X	
<i>Sthenognatha gentilis</i> Felder, 1874	X									X																	X	
<i>Sutonocrea lobifer</i> (Herrich-Schäffer, [1855])	X									X																		
<i>Sutonocrea reducta</i> (Walker, 1856)	X									X																	X	
<i>Sychesia dryas</i> (Cramer, 1775)	X									X																	X	
<i>Sychesia erubescens</i> Jordan, 1916	X									X																	X	
<i>Symphlebia abdominalis</i> (Herrich-Schäffer, [1855])	X									X																	X	
<i>Symphlebia catenata</i> (Schaus, 1905)	X									X																	X	
<i>Symphlebia distincta</i> (Rothschild, 1933)	X									X																	X	
<i>Symphlebia doncasteri</i> Rothschild 1910	X									X																	X	
<i>Symphlebia indistincta</i> (Rothschild, 1909)	X									X																	X	
<i>Symphlebia lophocampoides</i> Felder, 1874	X									X																	X	
<i>Symphlebia muscosa</i> (Schaus, 1910)	X									X																	X	
<i>Symphlebia nejia</i> Schaus 1905	X									X																	X	
<i>Symphlebia perflua</i> (Walker, 1869)	X									X																	X	
<i>Symphlebia suamus</i> (Druce, 1902)	X									X																	X	
<i>Syntomeida austera</i> Doggin 1902	X									X																	X	

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Arctiinae of Rio de Janeiro, Brazil

Appendix I. Continued.

Species	AR	AB	AC	BM	CM	CG	CA	CB	DC	GU	IT	MA	MG	MN	MR	NI	NF	NG	PE	RE	RJ	SA	SM	SJ	TA	TE	TR	VA	
<i>Syntomeida melanthus</i> Cramer 1780																X			X										
<i>Talara barema</i> Schaus, 1896	X																												
<i>Talara ditis</i> (Butler, 1878)						X	X																						
<i>Talara niveata</i> Butler, 1878						X	X																						
<i>Tessela sertata</i> (Berg, 1882)				X															X										
<i>Tessellarcia semivaria</i> (Walker, 1856)																			X										
<i>Theages pseuscanturum</i> Schaus, 1910																			X										
<i>Thysanopyrmyna haemorrhoides</i> (Schaus, 1905)	X																		X										
<i>Thysanopyrmyna pyrhopya</i> (Walker, 1865)	X																		X										
<i>Timalus clavipennis</i> Druce 1897	X																		X										
<i>Tipulodes ima</i> Boisduval, 1832	X																		X										
<i>Trichomelia celenna</i> Schaus, 1892	X																		X										
<i>Trichura cerberus</i> (Pallas, 1772)	X																		X										
<i>Trichura coarctata</i> Drury 1773	X																		X										
<i>Trichura cyanea</i> Schaus, 1892	X																		X										
<i>Trichura dixanthia</i> Hampson, 1898	X																		X										
<i>Trichura fulvicaudata</i> Lathry 1899	X																		X										
<i>Tricypha nigrescens</i> Rothschild, 1909																			X										
<i>Tricypha ochrea</i> Hampson 1901																			X										
<i>Uetheisa ornatrix</i> (Linnaeus, 1758)	X																		X										
<i>Virbia brevilinea</i> (Walker, 1854)																			X										
<i>Virbia divisa</i> (Walker, 1864)				X															X										
<i>Virbia fasciata</i> Rothschild 1910				X															X										
<i>Virbia punctata</i> Druce 1911				X															X										
<i>Virbia zonata</i> Felder 1874				X															X										
<i>Viviennea euricosivai</i> Travassos 1954																			X										
<i>Viviennea flavicincta</i> (Herrich-Schäffer, [1855])																			X										
<i>Viviennea moma</i> (Schaus, 1905)	X																		X										
<i>Viviennea salma</i> (Druce 1896)																			X										
<i>Viviennea superba</i> Druce 1883																			X										
<i>Viviennea zonana</i> (Schaus, 1905)																			X										
<i>Wanderbiltia wanderbilti</i> Rego Barros 1958																			X										
<i>Xantholopha purpurascens</i> Schaus, 1899																			X										
<i>Xanthophaeina levis</i> (Druce, 1899)	X																		X										

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## Arctiinae moths (Lepidoptera, Erebiidae) of the Emas National Park, Goiás, Brazil

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**Abstract:** Arctiinae are a species-rich subfamily of moth, with approximately 1,400 species in Brazil and 723 recorded in the Cerrado biome. A list of species of these moths was compiled during three years of sampling in four vegetation types within the Emas National Park. A total of 5,644 individuals belonging to 149 species were collected. About 67% of these species are new records for the Emas National Park, 31% for the State of Goiás and 9% for the Cerrado biome. Cerrado *sensu stricto* and semideciduous forests have higher species richness, followed by *campo cerrado* and *campo sujo*. The vegetation type with the highest number of exclusive species was the semideciduous forest, followed by cerrado *sensu stricto*, *campo cerrado* and *campo sujo*. The high species richness and the high proportion of new species records for Goiás and Cerrado reinforce the importance of the Emas National Park region as a center of diversity for this group of moths. The conservation of areas not yet cleared around the Park, including the creation of new protected areas, and the establishment of ecological corridors between these areas and the Park would be strategies to preserve the fauna of these moths.

**Keywords:** *light trap*, *Lithosiini*, *Arctiini*.

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**Resumo:** Arctiinae é uma das subfamílias de mariposas mais ricas em espécies. Já foram registradas cerca de 1400 espécies no Brasil e 723 no bioma Cerrado. Uma lista de espécies destas mariposas foi compilada de três anos de amostragens realizadas em quatro fitofisionomias do Parque Nacional das Emas. Um total de 5.644 indivíduos, pertencentes a 149 espécies foram coletados. Cerca de 67% das espécies representam novos registros para o Parque Nacional das Emas, 31% para o Estado de Goiás e 9% para o bioma Cerrado. Cerrado *sensu stricto* e mata estacional semidecídua apresentaram maior riqueza de espécies, seguidas por campo cerrado e campo sujo. A fitofisionomia que apresentou maior número de espécies exclusivas foi a mata estacional semidecídua, seguida por cerrado *sensu stricto*, campo cerrado e campo sujo. A grande riqueza de espécies e a alta proporção de novos registros de espécies para Goiás e para o Cerrado reforçam a importância da região do Parque Nacional das Emas como um centro de diversidade para esse grupo de mariposas. A conservação das áreas ainda não desmatadas no entorno do Parque, incluindo a criação de novas unidades de conservação, e o estabelecimento de corredores ecológicos entre essas áreas e o Parque seriam estratégias para preservar a fauna dessas mariposas.

**Palavras-chave:** *armadilha luminosa*, *Lithosiini*, *Arctiini*.

## Introduction

The biodiversity knowledge is still insufficient due the Linnean and Wallacean shortfalls (Bini et al., 2006). The first is related to the lack of taxonomists, since a significant proportion of species have not been described for many taxa, mainly the rich tropical invertebrate groups. The second is related to the limited knowledge of species occurrences, since, for the majority of taxa, geographical distributions are poorly understood and contain many gaps. The best way to reduce the

Wallacean shortfall is to invest in biodiversity inventories and publish lists of species. The results of these inventories, i.e. the species lists, provide support for actions on conservation and management, which are especially important in areas undergoing rapid environmental degradation (Lewinsohn et al. 2005). They are also important in macroecological and evolutionary studies.

Arctiinae moths are a species-rich subfamily of Lepidoptera (Heppner 1991). There are approximately 11,000 species worldwide, with 6,000 in the Neotropics (Watson & Goodger 1986)

and 1,400 in Brazil (Ferro & Diniz 2010). According to Ferro et al. (2010), 723 Arctiinae species have been recorded in the Brazilian Cerrado. However, the number of species occurring in this biome should be much higher, because less than 60% of the one degree latitude/longitude cells that cover the area of the Cerrado have sampling records of Arctiinae, and only one of these cells was adequately sampled (Ferro et al. 2010).

According to the new classification of Zahiri et al. (2012), Arctiinae moths consist of the tribes Arctiini, Lithosiini, Amerilini and Syntomini, with the two latter not occurring in the Neotropics (Heppner 1991, Hauser & Boppré 1997). The vast majority of Lithosiini species have a small body size (Weller et al. 2009). The moths of this tribe mainly feed on mosses, lichens and algae (Weller et al. 2009) and are generally more associated with vegetation types in early stages of succession (Hilt & Fiedler 2006). The Arctiini tribe are composed of small to medium sized moths whose larvae feed on a wide variety of plants, including grasses, herbs, shrubs and trees (Weller et al. 2009). Furthermore, Arctiini moths can explore different vegetation types because they are polyphagous (Singer & Bernays 2009) and can therefore inhabit a wide variety of terrestrial habitats.

The Cerrado is a biodiversity hotspot for conservation priorities (Myers et al. 2000). Only 2% of its area is legally protected (Klink & Machado 2005) and the Arctiinae fauna is not known properly, even in this area. The Cerrado biome is composed by a wide variety of vegetation types ranging from open areas with no shrub element to areas with a high density of tall trees (Oliveira-Filho & Ratter 2002). The vegetation types that comprise the cerrado *sensu lato* are *campo limpo*, *campo sujo*, *campo cerrado*, *cerrado sensu stricto* and *cerradão*. These five vegetation types represent a gradient of vegetation increasing in woody plant density and decreasing in herbaceous density (Oliveira-Filho & Ratter 2002). The Cerrado also contains forest formations, which are not considered to belong to the cerrado *sensu lato* (Oliveira-Filho & Ratter 2002). The Arctiinae moths respond to these differences in vegetation, and the fauna of the forest formations differs from the savanna formations (Ferro & Diniz 2007, Moreno et al. 2014).

We present a list of Arctiinae moth species of the Emas National Park, one of the largest conservation units of the Cerrado biome. The park includes the Brazilian Long Term Ecological Research Network. We have performed sampling in different vegetation types of the park and measured the observed and extrapolated richness of species across the study area and in each vegetation type. We have also measured the exclusive species of each vegetation type and the number of species that are new records for the Park, the State of Goiás and for the Cerrado biome.

## Material and Methods

Specimens of Arctiinae were sampled in the Emas National Park (ENP), located between the cities of Mineiros and Chapadão do Céu, in the State of Goiás, Central West of Brazil (17°49'-18°28'S and 52°39'-53°10'W) (Figure 1). The ENP covers 132,941 ha and approximately 80% of this area consists of grasslands (*campos limpos* and *campos sujos*). Approximately 15% of the ENP area is composed of *campo cerrado* and *cerrado sensu stricto* and 5% of the remaining area consists of *campos úmidos*, *veredas* and forests (França et al.

2007). The climate is Aw in Koppen classification, i.e. the climate is tropical humid with three to six months of dry winter and rainy summer, which imposes a strong seasonality in the vegetation (Ramos-Neto & Pivello 2000). The annual precipitation ranges from 1,200 to 2,000 mm, concentrated between September and March, with an average annual temperature of 24.6°C (Ramos-Neto & Pivello 2000).

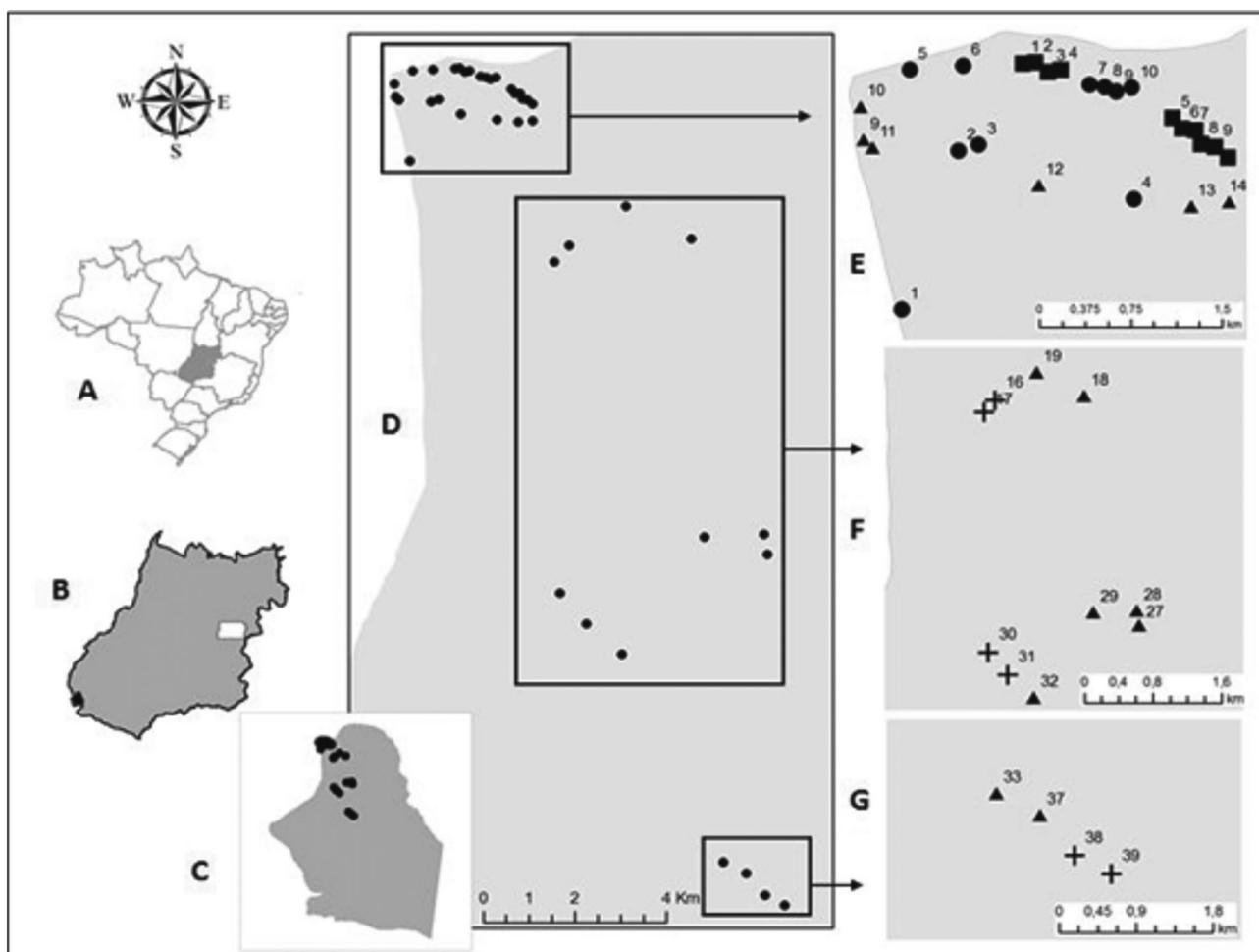
Sampling units consisted of 40 plots of 10x10 m, distributed in four vegetation types: semideciduous forest (n = 10 plots); *cerrado sensu stricto* (n = 10 plots); *campo cerrado* (n = 14 plots); *campo sujo* (n = six plots) (Figure 1). In each plot, the moths were collected from dusk until dawn through a Luiz de Queiroz light trap (Silveira-Neto & Silveira 1969) equipped with a 15 W black lamp. The traps were suspended 1.5 m above the ground in the center of each plot. The minimum distance between plots was 100 m to minimize the capture of species from surrounding vegetation types. This distance corresponds to the radius of attraction of a 125 W lamp (Muirhead-Thompson 1991), a power eight times greater than that we used in this study. Sampling was restricted to periods of new and waning lunar phases due to the radius of attraction of the traps being greater (Yela & Holyoak 1997). Sampling was conducted for three years during both the dry (June to July 2010, July 2011 and July 2012) and rainy seasons (December 2010 to February 2011, November 2011 and December 2012). The moths were collected in all plots over two non-consecutive nights in order to increase the representativeness of the fauna, totaling 12 nights of sampling in each plot (2 in each dry season and 2 in each rainy season) and 84 nights of sampling in total. On each sampling night, we set eight light traps, two in each vegetation type. Plots sampled in the same night were the most distant possible to avoid pseudoreplication. Each plot was sampled once at the new moon and once during the waning moon.

Arctiinae individuals were identified by comparison with digital images of the identified species of the V.O. Becker Collection (where the identification was confirmed by comparison with types) and through the literature (Hampson, 1898, 1900, 1901, 1914, Watson & Goodger 1986, Piñas-Rubio et al. 2000, Piñas-Rubio & Manzano 2003). All individuals were deposited in the Zoological Collection of the Federal University of Goiás (Goiânia, Brazil).

We used three non-parametric species richness estimators (first and second order Jackknife and second order Chao) to better estimate the total richness of Arctiinae in the study area and in each vegetation type. These three estimators are based on incidence (presence/absence) of species in assemblages (Melo 2004).

## Results

We recorded 5,644 Arctiinae individuals during our sampling; belonging to 149 species, 73 genera, two tribes (Arctiini and Lithosiini) and nine subtribes (Arctiina, Callimorphina, Cisthenina, Ctenuchina, Euchromiina, Eudesmiina, Lithosiina, Pericopina and Phaegopterina). Of the 149 species sampled, 117 were identified to the species level (78%), 16 at the genus level (10%) and 16 at the tribal level (10%) (Appendix 1). The subtribe with the highest number of species was Phaegopterina (46 species, 30% of the total), followed by Euchromiina (30, 20%), Ctenuchina (26, 17%), Cisthenina (13, 8%), Lithosiina (7, 4%), Arctiina



**Figure 1.** Map of Brazil showing Goiás State in black (a); map of Goiás State showing the Emas National Park (ENP) in black (b); area of the ENP showing the sample plots (black points, c); smaller scale of c (d); smaller scale of the squares indicated in d (e, f, g). The symbols indicate the vegetation type: semideciduous forest (■), cerrado *sensu stricto* (●), *campo cerrado* (▲) and *campo sujo* (+). The numbers represent the code of sample plots.

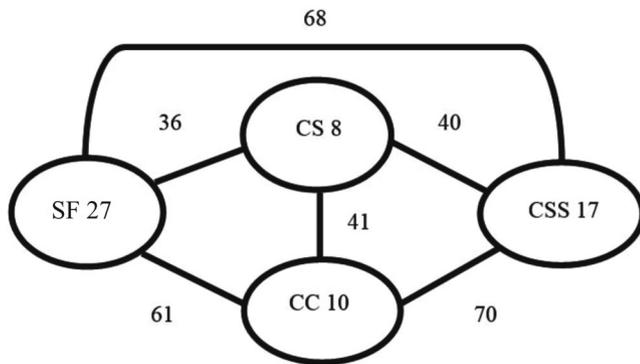
**Table 1.** Observed (Obs. rich.) and extrapolated richness (first order Jackknife, second order Jackknife and second order Chao) and percentage of extrapolated richness sampled in each phytophysionomy (Phyto) and also in the total study area (ENP). In the first column, CS means *campo sujo*, CC *campo cerrado*, CSS *cerrado sensu stricto*, SF semideciduous forest and ENP, Emas National Park.

Phyto	Obs. rich.	Jackk 1 ± DP	Jackk1 rich. sampled	Jackk 2 ± DP	Jackk2 rich. sampled	Chao 2 ± DP	Chao 2 rich. sampled
CS	51	75.8 ± 10.28	68%	86.6 ± 16.3	63%	83.4 ± 15.1	65%
CC	86	114.8 ± 9.82	75%	128.7 ± 18.5	67%	117 ± 14.4	74%
CSS	98	132.2 ± 12.08	74%	150.5 ± 20.1	65%	140.4 ± 18.3	69%
SF	98	132.2 ± 12.08	74%	141 ± 18.5	70%	123 ± 10.4	80%
<b>ENP</b>	<b>149</b>	<b>190.9 ± 9.46</b>	<b>78%</b>	<b>214.1 ± 22.1</b>	<b>69%</b>	<b>197.6 ± 19.8</b>	<b>75%</b>

(5, 3%), Pericopina (4, 2%), Callimorphina and Eudesmiina (both with 1 species, 0.6%) (Appendix 1).

It was estimated by first order Jackknife, second order Jackknife and second order Chao, that the sample region has a

richness of approximately 190, 214 and 197 species respectively (Table 1). Fourteen species (9%) were new records for the Cerrado, 47 (31%) were new records for the State of Goiás and 101 (67%) were new records for the ENP (Appendix 1). Cerrado



**Figure 2.** Venn diagram indicating the number of species that were sampled only in a vegetation type (numbers within the ellipses) and the number of shared species between vegetation types (numbers next to the lines). *Campo sujo* (CS), *campo cerrado* (CC), *cerrado sensu stricto* (CSS) and semideciduous forest (SF).

*sensu stricto* and semideciduous forest vegetation types had the highest species richness (98), followed by *campo cerrado* with 86 and *campo sujo* with 51 (Appendix 1).

About 41% of the sampled species ( $n = 62$ ) occurred in only one vegetation type, 16% (25 species) occurred in two vegetation types, 18% (27 species) in three and 23% (35 species) occurred in all vegetation types (Appendix 1). The semideciduous forest had the highest number of exclusive species (27), followed by *cerrado sensu stricto* (17), *campo cerrado* (10) and *campo sujo* (eight) (Figure 2, Appendix 1). *Campo cerrado* and *cerrado sensu stricto* had more shared species and *campo sujo* and semideciduous forest had the least shared species (Figure 2, Appendix 1). Most species that occurred in three vegetation types (88.8%) were shared between *campo cerrado*, *cerrado sensu stricto*, and semideciduous forest.

Some species occurred in only one season (58 species occurred only in the dry and 30 species only in the rainy season) and the others species in both seasons ( $N = 61$ ). The dry showed more species ( $N = 119$ ) than the rainy season ( $N = 91$ ) (Appendix 1).

## Discussion

The ENP Arctiinae fauna represents approximately 20% of the species recorded for the Cerrado (Ferro et al. 2010) and 10% of species recorded from Brazil (Ferro & Diniz 2010). The previous Cerrado Arctiinae richness (Ferro & Diniz 2010) is replaced by 737 species, with the addition of 14 new records for the biome. The fauna of the ENP is the second richest locality in the Cerrado, after Brasília (222 species, Ferro & Diniz 2010). The richness observed in the ENP (149 species) was similar to the other intensively sampled areas in the Cerrado, such as Vilhena (136) and Chapada dos Guimarães (129) (Ferro & Diniz 2010), and Rain Forest sites, such as São José dos Ausentes (121) (Ferro & Romanowski 2012), La Selva Biological Station (148) (Brehm 2007), and São Bento do Sul (162) (Ferro et al. 2012). However, Hilt & Fiedler (2005) observed a significantly greater tiger moth richness in Ecuador (287 species).

This high richness, the large number of new distribution records (including 14 for the Cerrado), and the existence of at

least another 40 species in the study area (according to the first order Jackknife estimator), reinforces the importance of conserving the ENP and its surroundings. Despite being well preserved, the ENP is a large fragment surrounded by extensive monocultures of soybean, corn, cotton and sugarcane matrix. These monocultures can act as a barrier to the dispersal of individuals and hence cause problems associated with small, isolated populations, such as inbreeding, genetic drift and increased susceptibility to future stochastic events. Furthermore, the use of insecticides on these crops can cause increased mortality of insects at the edges of the park, affecting, for example, pollination of entomophilous plants. Moreover, the invasion of alien species such as *Brachiaria* can reduce the natural vegetation due to competition among species (Almeida-Neto et al. 2010), for example, which results in a lower availability of host plants. All of these factors can affect the assemblages of insect herbivores and pollinators. Thus, among the priority actions for the conservation of the ENP (and its surroundings) Arctiinae fauna are the creation of new protected areas in their surroundings and the creation of ecological corridors between protected areas for the fauna of the region (Rodrigues et al. 2002).

The semideciduous forest had the highest number of exclusive species in relation to other vegetation types. This result can be explained by the fact that this vegetation type has a different microclimate, plant species and soil types than *cerrado sensu lato* (Oliveira-Filho & Ratter 2002, Ruggiero et al. 2002).

Both seasons presented exclusive species, but this was more evident in the dry season. The dry presented more Arctiinae species than the rainy season, as found for other Lepidoptera species (Morais et al. 1999, Pinheiro et al. 2002). In Cerrado biome, the dry winter season is marked by adverse conditions, like low humidity and cold temperature (Ramos-Neto & Pivello 2000). Also, the vegetation faces a water deficit and a reduction in nutritional quality in this season (Ramos-Neto & Pivello 2000, Pinheiro et al. 2002) and it can affect the Arctiinae species, as they depend on the plants, both in larval (herbivorous) and in adult (pollinator) phases. Thus, we believe that the more Arctiinae species found in the winter dry season must be due to a temporarily enemy-free space (Jeffries & Lawton 1984, Morais et al. 1999). In this period, the predators and parasitoids should be less abundant than in the rainy season (Morais et al. 1999) and it should enable more Arctiinae species to coexist in the severe dry season.

The Cerrado biome has diminished in recent decades mainly due to agricultural expansion (Klink & Machado 2005). Many species may have been lost in this process, including species not yet known to science. According to Ferro et al. (2010), much of the biome has not yet been inventoried. Thus, studies that generate lists of species, especially in places rarely or never sampled regarding the fauna, are urgent and very important to understand the biodiversity. Furthermore, these data improve our estimation of the geographical distribution and the status (e.g. rare, endemic, threatened) of species. These data, therefore, can be analyzed by niche modeling and guide future conservation strategies, such as the location of new conservation units. However, these estimates will be much more accurate if natural history data are included and if the species identification is correct.

## Arctiinae moths of the Emas National Park, Brazil

**Appendix 1.** List of Actiinae moth species sampled in four phytophysiognomies of the Emas National Park (ENP), in the dry and rainy seasons. CS means campo sujo, CC campo cerrado, CSS cerrado sensu stricto and SF semideciduous forest. Species with symbols <sup>#S\*</sup> are new records for Cerrado, <sup>#S</sup> for Goiás State and <sup>#</sup> for ENP.

Species	Phytophysiognomy				Season	
	CS	CC	CSS	SF	Dry	Rainy
Arctiinae						
Arctiini						
Arctiini sp.1		X	X	X	X	
Arctiini sp.2			X		X	
Arctiini sp.3			X	X		X
Arctiini sp.4		X				X
Ctenuchiini sp.1	X	X				X
Arctiina						
<i>Hypercompe mus</i> (Oberthür, 1881) <sup>#S</sup>			X			X
<i>Paracles phaeocera</i> (Hampson, 1905) <sup>#</sup>		X	X		X	X
<i>Paracles</i> sp.1	X	X	X	X	X	X
<i>Paracles</i> sp.2			X		X	
<i>Pseudalus limona</i> Schaus, 1896 <sup>#</sup>	X	X	X	X		X
Callimorphina						
<i>Utetheisa ornatrix</i> (Linnaeus, 1758)	X	X	X	X	X	X
Ctenuchina						
<i>Aclytia flavigutta</i> (Walker, 1854) <sup>#S</sup>	X	X	X	X	X	X
<i>Aclytia heber</i> (Cramer, 1780) <sup>#</sup>	X	X	X	X	X	X
<i>Aclytia</i> sp.1		X	X	X	X	
<i>Argyrooides braco</i> (Herrich-Schäffer, [1855]) <sup>#</sup>				X	X	
<i>Cercopimorpha postflavia</i> Rothschild, 1912 <sup>#S</sup>				X	X	
<i>Correbidia calopteridia</i> (Butler, 1878) <sup>#S</sup>				X	X	
<i>Correbidia</i> sp.1				X	X	
<i>Delphyre discalis</i> (Druce, 1905) <sup>#</sup>	X	X	X	X	X	X
<i>Delphyre dizona</i> (Druce, 1898) <sup>#</sup>	X	X	X	X	X	X
<i>Episcepsis klagesi</i> Rothschild, 1911 <sup>#S</sup>			X		X	
<i>Episcepsis lenaeus</i> (Cramer, 1780) <sup>#</sup>			X		X	
<i>Episcepsis thetis</i> (Linnaeus, 1771) <sup>#S</sup>			X	X	X	
<i>Eucereon albidia</i> Rothschild, 1912 <sup>#S*</sup>			X	X	X	
<i>Eucereon arenosun</i> Butler, 1877 <sup>#S</sup>	X					X
<i>Eucereon dorsipuncta</i> Hampson, 1905 <sup>#</sup>			X	X	X	
<i>Eucereon pseudarchias</i> Hampson, 1898 <sup>#S</sup>				X	X	
<i>Eucereon setosum</i> (Sepp, [1830]) <sup>#S</sup>		X	X	X	X	X
<i>Eucereon</i> sp.1		X	X	X	X	X
<i>Heliura rhodophila</i> (Walker, 1856) <sup>#</sup>			X			X
<i>Heliura tetragramma</i> (Walker, 1854) <sup>#</sup>	X	X	X	X	X	X
<i>Napata leucotela</i> Butler, 1876 <sup>#</sup>	X				X	
<i>Philoros rubriceps</i> (Walker, 1854) <sup>#</sup>	X	X	X		X	X
<i>Pseudohyaloleucerea vulnerata</i> (Butler, 1875) <sup>#S</sup>				X	X	
<i>Pseudosphex discoplaga</i> (Schaus, 1905) <sup>#</sup>			X		X	
<i>Pseudosphex fulvisphex</i> (Druce, 1898) <sup>#S</sup>		X	X	X	X	
<i>Pseudosphex nivaca</i> (Jones, 1914)	X	X	X	X	X	X
Euchromiina						
<i>Autochloris enagrus</i> (Cramer, 1780) <sup>#S*</sup>		X	X		X	X
<i>Cosmosoma achemon</i> (Fabricius, 1781) <sup>#</sup>	X	X	X	X	X	X
<i>Cosmosoma auge</i> (Linnaeus, 1767) <sup>#</sup>		X		X	X	
<i>Cosmosoma nigriscens</i> Rothschild, 1911 <sup>#</sup>		X			X	
<i>Cosmosoma rasera</i> Jones, 1914 <sup>#</sup>		X	X	X	X	X
<i>Cosmosoma theuthras restrictum</i> Butler, 1876 <sup>#</sup>	X	X	X	X	X	X
<i>Cosmosoma</i> sp.1		X	X	X	X	X
<i>Cosmosoma</i> sp.2	X				X	
<i>Cosmosoma</i> sp.3	X		X	X	X	X
<i>Dycladia lucetius</i> (Stoll, 1781)	X	X	X	X	X	X
<i>Erruca hanga</i> (Herrich-Schäffer, [1854]) <sup>#S</sup>	X					X
<i>Eurota histrio</i> (Guérin, 1843) <sup>#</sup>			X		X	

## Appendix 1. Continued.

Species	Phytophysiognomy				Season	
	CS	CC	CSS	SF	Dry	Rainy
<i>Eurota nigricincta</i> Hampson, 1907 <sup>#S</sup>	x				x	
<i>Hyda basilutea</i> (Walker, 1854) <sup>#</sup>		x	x		x	x
<i>Lepidoneiva erubescens</i> (Butler, 1876)	x	x	x	x	x	x
<i>Macrocne me aurifera</i> Hampson, 1914 <sup>#S*</sup>	x	x	x	x	x	x
<i>Nyridela acroxantha</i> (Perty, 1833) <sup>#</sup>		x		x	x	x
<i>Nyridela chalciope</i> (Hübner, [1827]) <sup>#</sup>			x			x
<i>Pheia albisigna</i> (Walker, 1854)	x	x	x	x	x	x
<i>Pheia gaudens</i> (Walker, 1856) <sup>#</sup>		x			x	
<i>Pheia haematosticta</i> Jones, 1908	x	x	x	x	x	x
<i>Pheia haemopera</i> Schaus, 1898	x	x	x	x	x	x
<i>Pheia seraphina</i> (Herrich-Schäffer, 1854)	x	x	x	x	x	x
<i>Pheia</i> sp.1	x		x			x
<i>Phoenicoprocta baeri</i> Rothschild, 1911	x	x	x	x		x
<i>Phoenicoprocta</i> sp.1		x	x	x		x
<i>Poliopastea plumbea</i> Hampson, 1898 <sup>#S</sup>	x	x	x		x	x
<i>Poliopastea</i> sp.1				x	x	
<i>Saurita attenuata</i> Hampson, 1905 <sup>#S*</sup>				x	x	x
<i>Sphecosoma aenetus</i> (Schaus, 1896) <sup>#S*</sup>			x			x
Pericopina						
<i>Dysschema boisduvalli</i> (van der Hoeven & de Vriese, 1840) <sup>#</sup>	x					x
<i>Dysschema sacrificata</i> (Hübner, [1831]) <sup>#</sup>		x	x	x		x
<i>Hyalurga fenestra</i> (Linnaeus, 1758) <sup>#</sup>			x		x	
<i>Hyalurga partita</i> (Walker, 1854) <sup>#S*</sup>				x	x	
Phaegopterina						
<i>Agaraea semivitrea</i> Rothschild, 1909 <sup>#</sup>				x		x
<i>Amaxia dyuna</i> Schaus, 1896 <sup>#S</sup>	x	x	x	x	x	x
<i>Amaxia kennedyi</i> (Rothschild, 1909) <sup>#S</sup>				x	x	
<i>Bertholdia detracta</i> Seitz, 1921 <sup>#</sup>	x				x	
<i>Biturix diversipes</i> (Walker, 1855) <sup>#S*</sup>		x			x	
<i>Carales astur</i> (Cramer, 1777) <sup>#</sup>				x	x	
<i>Cresera affinis</i> (Rothschild, 1909) <sup>#</sup>				x	x	
<i>Cresera ilioides</i> (Schaus, 1905) <sup>#S*</sup>		x			x	
<i>Cresera optima</i> (Butler, 1877) <sup>#S</sup>				x	x	x
<i>Echeta juno</i> (Schaus, 1892) <sup>#S*</sup>				x		x
<i>Elysius hermia</i> (Cramer, 1777) <sup>#</sup>			x		x	
<i>Elysius joiceyi</i> Talbot, 1928 <sup>#</sup>	x	x	x			x
<i>Eupseudosoma grandis</i> Rothschild, 1909 <sup>#S</sup>	x	x	x	x	x	
<i>Eupseudosoma involuta</i> (Sepp, [1855]) <sup>#</sup>			x	x	x	
<i>Halysidota sannionis</i> (Rothschild, 1909) <sup>#S</sup>		x	x	x	x	x
<i>Hyperandra appendiculata</i> (Herrich-Schäffer, [1856]) <sup>#</sup>				x	x	
<i>Hyperthaema</i> sp.1		x	x	x	x	x
<i>Hyperthaema</i> sp.2		x	x		x	x
<i>Hyponerita lavinia</i> (Druce, 1890) <sup>#S</sup>	x					x
<i>Idalus agricus</i> Dyar, 1910 <sup>#S*</sup>		x	x	x	x	
<i>Idalus carinosa</i> (Schaus, 1905)	x	x	x	x	x	x
<i>Idalus citrina</i> Druce, 1890 <sup>#S</sup>	x	x	x	x	x	x
<i>Idalus dares</i> Druce, 1894 <sup>#</sup>		x			x	
<i>Idalus lineosus</i> Walker, 1869 <sup>#</sup>		x	x		x	x
<i>Lepidokirbyia vittipes</i> (Walker, 1855) <sup>#</sup>		x	x	x	x	x
<i>Leucanopsis rosetta</i> (Schaus, 1896) <sup>#</sup>	x	x	x	x		x
<i>Leucanopsis squalida</i> (Herrich-Schäffer, [1855]) <sup>#S</sup>		x	x			x
<i>Leucanopsis strigulosa</i> (Walker, 1855) <sup>#S</sup>	x	x	x	x	x	x
<i>Lophocampa annulosa</i> (Walker, 1855) <sup>#S</sup>			x		x	
<i>Lophocampa atrimaculata</i> (Hampson, 1901) <sup>#S*</sup>				x	x	
<i>Lophocampa citrina</i> (Sepp, [1852]) <sup>#</sup>		x	x	x	x	x
<i>Mazaeras francki</i> Schaus, 1896 <sup>#</sup>		x				x
<i>Melese incertus</i> (Walker, 1855) <sup>#</sup>		x	x		x	

## Appendix 1. Continued.

Species	Phytophysionomy				Season	
	CS	CC	CSS	SF	Dry	Rainy
<i>Melese paranensis</i> Dognin, 1911 <sup>#S</sup>				X	X	X
<i>Neritos atta</i> Schaus, 1920 <sup>#S</sup>		X	X	X	X	
<i>Neritos flavimargo</i> Joicey & Talbot, 1916		X	X		X	
<i>Neritos hampsoni</i> Rothschild, 1909 <sup>#S</sup>			X	X	X	X
<i>Neritos sanguipuncta</i> Schaus, 1901 <sup>#</sup>				X	X	
<i>Pareuchaetes aurata</i> (Butler, 1875) <sup>#</sup>	X	X	X	X	X	X
<i>Pelochyta arontes</i> (Stoll, 1782) <sup>#</sup>				X	X	
<i>Psychophasma erosa</i> (Herrich-Schäffer, [1858]) <sup>#</sup>		X		X	X	
<i>Rhipha pulcherrima</i> (Rothschild, 1935) <sup>#</sup>	X	X	X	X		X
<i>Rhipha strigosa</i> (Walker, 1854) <sup>#S</sup>			X		X	X
<i>Robinsonia dewitzi</i> Gundlach, 1881 <sup>#S</sup>		X			X	
<i>Scaptius submarginalis</i> (Rothschild, 1909) <sup>#S*</sup>				X	X	
<i>Viviennea salma</i> (Druce, 1896) <sup>#S</sup>		X	X		X	X
Lithosiini						
Lithosiinii sp. 1			X	X		X
Lithosiinii sp. 2		X	X	X	X	X
Lithosiinii sp. 3		X	X	X	X	
Lithosiinii sp. 4	X	X			X	
Lithosiinii sp. 5			X		X	
Lithosiinii sp. 6				X	X	
Lithosiinii sp. 7				X	X	
Lithosiinii sp. 8		X	X	X	X	X
Lithosiinii sp. 9				X	X	
Lithosiinii sp. 12			X			X
Lithosiinii sp. 14	X	X	X	X	X	X
Cisthenina						
<i>Barsinella mirabilis</i> Butler, 1878			X	X	X	X
<i>Cisthene dives</i> (Schaus, 1896) <sup>#</sup>		X	X	X	X	
<i>Cisthene ruficollis</i> (Schaus, 1896) <sup>#S</sup>		X				X
<i>Cisthene subruba</i> (Schaus, 1905) <sup>#</sup>	X	X	X	X	X	X
<i>Cisthene triplaga</i> (Hampson, 1905) <sup>#</sup>	X	X	X	X	X	X
<i>Cisthene</i> sp.1		X	X	X	X	X
<i>Cisthene</i> sp.2		X	X	X		X
<i>Cisthene</i> sp.3			X	X		X
<i>Illice croesus</i> Hampson, 1914 <sup>#S</sup>		X			X	
<i>Illice griseola</i> (Rothschild, 1913) <sup>#S*</sup>				X	X	
<i>Odozana domina</i> (Schaus, 1896)		X	X	X	X	X
<i>Odozana obscura</i> (Schaus, 1896)	X	X	X	X	X	X
<i>Talara grisea</i> Schaus, 1896 <sup>#</sup>		X	X	X	X	X
Eudesmiina						
<i>Antona fallax</i> (Butler, 1877) <sup>#</sup>			X			X
Lithosina						
<i>Agylla argentea</i> (Walker, 1863) <sup>#S*</sup>	X	X	X	X	X	X
<i>Agylla marcata</i> (Schaus, 1894) <sup>#S</sup>	X	X			X	X
<i>Agylla</i> sp.1	X	X	X	X	X	X
<i>Apistosia judas</i> Hübner, [1819] <sup>#</sup>				X		X
<i>Metalobosia diaxantha</i> Hampson, 1914 <sup>#</sup>				X	X	X
<i>Nodozana jucunda</i> Jones, 1914	X	X	X	X	X	X
<i>Parablavia sadima</i> (Schaus, 1896)	X	X	X	X	X	X

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## Hidden impacts of the Samarco mining waste dam collapse to Brazilian marine fauna – an example from the staurozoans (Cnidaria)

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**Abstract:** The collapse of the Fundão tailings dam at Mariana (State of Minas Gerais, Brazil) started a huge human tragedy and likely the most serious environmental disaster in recent Brazilian history. The dam had contained waste from processing iron ore from mines owned by Samarco, a joint venture company of the Brazilian Vale S.A. and the Anglo-Australian BHP Billiton Ltd. Following ineffective attempts to contain the disaster, after 16 days the mud flood reached the sea, where its impact is expected to affect thousands of marine fauna and flora species. Here, we provide an example of one of these species, the cnidarian *Kishinouyea corbini* Larson 1980 (Staurozoa), emblematic because it is extremely rare, poorly studied, and its known distribution overlaps the threatened area on the Brazilian coast. Based on this case, we discuss the need for efforts to monitor and minimize the possible impacts of this socio-environmental crime, as well as to identify and punish all responsible players in this tragedy, including negligent licensing and supervisory state agencies, in order to prevent future similar tragedies.

**Keywords:** *Espírito Santo, Rio Doce, mud, Staurozoa, Kishinouyea corbini*.

MIRANDA, L.S., MARQUES, A.C. **Impactos ocultos do colapso da barragem de resíduos da mineradora Samarco para a fauna marinha brasileira – um exemplo em estaurozoários (Cnidaria)**. *Biota Neotropica*. 16(2): e20160169. <http://dx.doi.org/10.1590/1676-0611-BN-2016-0169>

**Resumo:** O colapso da barragem de rejeitos de Fundão, em Mariana (Minas Gerais, Brasil) iniciou uma enorme tragédia humana e, provavelmente, o mais grave desastre ambiental da história recente do Brasil. A barragem continha rejeitos do processamento de minério de ferro de minas de propriedade da Samarco, uma empresa controlada pela brasileira Vale S.A. e pela anglo-australiana BHP Billiton Ltda. Apesar de tentativas ineficazes para conter o desastre, após 16 dias a lama atingiu o mar, onde provavelmente afetará milhares de espécies da fauna e flora marinhas. Este ponto de vista fornece um exemplo de uma dessas espécies, o cnidário *Kishinouyea corbini* Larson 1980 (Staurozoa), emblemática pois é extremamente rara, insuficientemente estudada e sua distribuição conhecida para a costa brasileira sobrepõe a área ameaçada pelo desastre. Com base neste caso, discutimos a necessidade de esforços para monitorar e minimizar os possíveis impactos desse crime socioambiental, bem como para identificar e punir todos os responsáveis por esta tragédia, incluindo agências estatais de fiscalização e licenciamento negligentes, a fim de evitar futuras tragédias semelhantes.

**Palavras-chave:** *Espírito Santo, Rio Doce, lama, Staurozoa, Kishinouyea corbini*.

On November 5, 2015, the collapse of the Fundão tailings dam near the Rio Doce at Mariana (State of Minas Gerais, Brazil) started a huge human tragedy and likely the most serious environmental disaster in recent Brazilian history. The Fundão retention pond had contained waste from processing iron ore from mines owned by Samarco, a joint venture company of the Brazilian Vale S.A. and the Anglo-Australian BHP Billiton Ltd. Although the consequences of this release of some 50 million cubic meters of mud and toxic sludge are still unfolding, the spill has caused 19 fatalities, buried the subdistrict of Bento Rodrigues, and

dislodged numerous families, and its passage downstream has erased the riverine fishery and compromised access to clean water and food for hundreds of thousands of residents in riverside communities.

The environmental damage is also catastrophic. The mud traveled about 650km along the Rio Doce Basin, among the most important South-American river basins, causing a massive mortality of its biota, mostly buried and suffocated by the mud. Riverside areas were also affected, and may be exposed to heavy metals and other toxic substances in the muddy waste (according

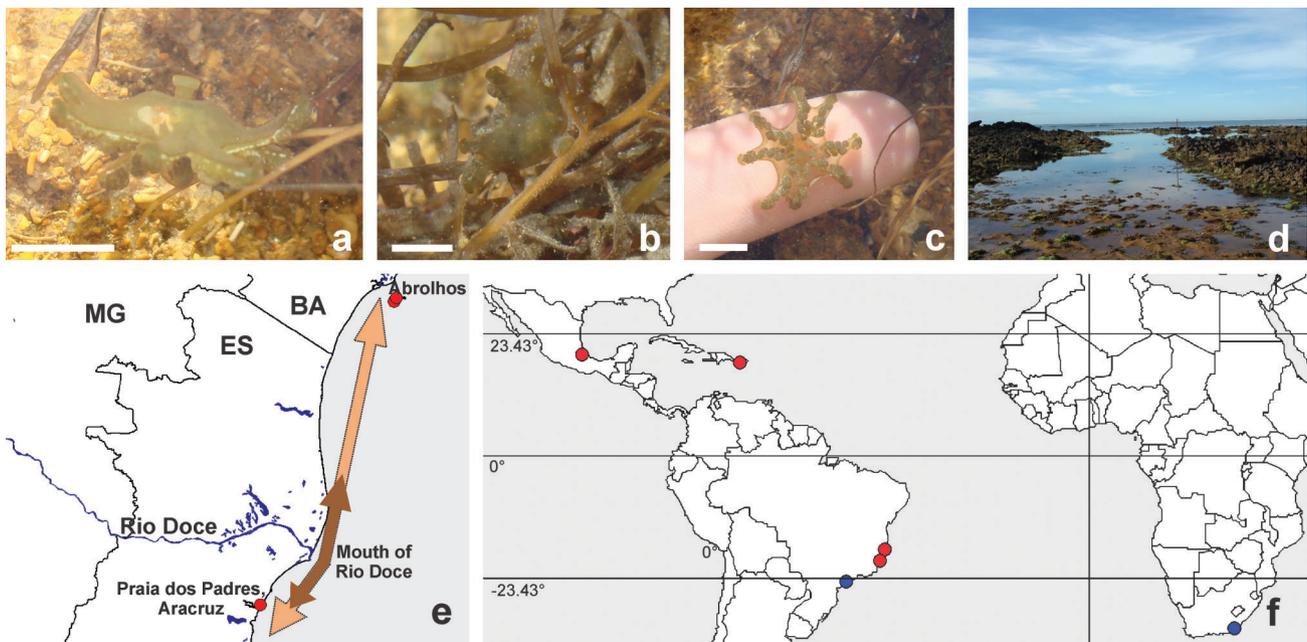
to the United Nations Office of the High Commissioner for Human Rights, <http://www.ohchr.org/>), which are often bioaccumulated in the food chain (Wang 2002), triggering manifold damage to organisms (Bryan 1971).

Following ineffective attempts to contain the disaster, after 16 days the mud flood reached the sea, where its impact is expected to be equally devastating and long-lasting. The regime of ocean currents, winds, and rains is driving the mud tailings southward and northward from the mouth of the Rio Doce, in the State of Espírito Santo (ES), reaching a total area of about 7,000km<sup>2</sup> (January, 2016; including areas with higher and lower concentration of the mud plume, <http://www.ibama.gov.br/publicadas>, IBAMA – NUGEO/SP). Beaches at Linhares (ES), one of the most affected municipalities, have been closed. According to the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) and the Chico Mendes Institute for Biodiversity Conservation (ICMBio), the denser mud has already affected the marine protected area of Comboios Biological Reserve, in Regência (ES), and the mud plume has reached two other federal conservation units, the Environmental Protection Area of Costa das Algas and the Wildlife Refuge of Santa Cruz, located in the municipalities of Aracruz, Fundão, and Serra (ES) (<http://www.icmbio.gov.br/portal/>). In early January 2016, IBAMA and ICMBio voiced suspicions that the mud could have reached the Marine National Park of Abrolhos, in the Abrolhos Archipelago, State of Bahia (BA), a coral reef region that is among the most biodiverse areas in the South Atlantic Ocean (Werner et al. 2010). ICMBio is concerned by the potential decrease of light

incidence over Abrolhos' coral reefs caused by water turbidity, in addition to mud sedimentation over the biota.

It is evident that the marine fauna has already been affected by the tragedy, including critically endangered species in Brazil, such as the Leatherback turtle (*Dermodochelys coriacea*; see <http://www.iucnredlist.org/>). However, thousands of unknown and poorly studied species are also threatened by the disaster. Indeed, recent estimates indicated that 91% of species in the ocean still await description (Mora et al. 2011), which dramatically worsens the consequences of marine impacts. In addition, rare species are likely to play critical roles even in diverse ecosystems, being important under future unfavorable conditions, such as habitat degradation and climate change (Mouillot et al. 2013). Here, we provide an example of one of these species, emblematic because it is extremely rare, poorly studied, and its known occurrence overlaps the impacted area on the Brazilian coast.

The cnidarian *Kishinouyea corbini* Larson 1980 (Figure 1a-c) was the first species of the class Staurozoa recorded for Brazil, exactly from the coast of Espírito Santo (Grohmann et al. 1999). Although these tiny benthic stalked jellyfish live in the intertidal zone, they are cryptic and hard to find, often camouflaged on *Sargassum* sp. (Figure 1b, Grohmann et al. 1999). The diet of stalked jellyfishes consists basically of small crustaceans, such as copepods and amphipods (Zagal, 2004), but there are no information concerning the trophic relationships (prey and predators) for *K. corbini* from Brazil. Specimens from the Espírito Santo coast are deposited in the zoological collections of Museum of Zoology of the University of São Paulo (MZUSP



**Figure 1.** (a) Lateral view of *Kishinouyea corbini* in the field; (b) Lateral view of *K. corbini*, attached and camouflaged on *Sargassum* sp.; (c) Oral view of *K. corbini* on the forefinger of the researcher; (d) Collection site of *K. corbini*, on Praia dos Padres, Aracruz (ES), during low tide; (e) Area threatened by the mud tailings overlaps the known distribution of *K. corbini* (in red): darker arrows indicate higher concentration of mud and lighter arrows indicate lower concentration of mud plume (Source: IBAMA – NUGEO/SP; January, 2016); (f) Global distribution of *K. corbini* (in red) and *Lucernariopsis capensis* (in blue), staurozoan species recorded in Brazil. Photos: A.C. Morandini. Scale: 5.0 mm.

**Figura 1.** (a) Vista lateral de *Kishinouyea corbini* no ambiente; (b) Vista lateral de *K. corbini*, fixa e camuflada no *Sargassum* sp.; (c) Vista oral *K. corbini* no dedo indicador do pesquisador; (d) Local de coleta de *K. corbini*, na Praia dos Padres, Aracruz (ES), durante a maré baixa; (e) Área ameaçada pela lama de rejeitos sobrepõe a área de distribuição conhecida de *K. corbini* (em vermelho): setas escuras indicam maior concentração de lama e setas claras indicam menor concentração da pluma (Fonte: IBAMA – NUGEO/SP; Janeiro, 2016); (f) Distribuição mundial de *K. corbini* (em vermelho) e *Lucernariopsis capensis* (em azul), espécies de Staurozoa registradas para o Brasil. Fotos: A.C. Morandini. Escala: 5,0 mm.

CNID 1563-1565) and Federal University of Rio de Janeiro (UFRJ-DZ-IB 1-50 to 1-53). There is only one other record of *K. corbini* for the Brazilian coast, in Recife de Viçosa, Abrolhos (BA), whose specimens are deposited in the National Museum of Rio de Janeiro (MNRJ 2472-2473; see Oliveira et al. 2016). However, in fact, the only known established population of the species was located in Praia dos Padres, Aracruz (ES) (Figure 1d, e), an area impacted by the mud plume (Figure 1e). Even though there may be other populations not yet recorded for the species, since *K. corbini* has been also recorded from Puerto Rico (Capriles & Martínez 1970, Larson 1980) and Mexico (Lechuga & Alamo 2005) (Figure 1f), the molecular identity of all these materials has never been investigated and the possibility of cryptic species cannot be discarded. We also do not know if these populations are isolated or interdependent, making it difficult to estimate the effect of an impact of this magnitude.

The only other staurozoan known on our coast, *Lucernariopsis capensis* Carlgren 1938, was recorded only once, at Itanhaém, State of São Paulo (SP) (Figure 1f), in 1985 (Miranda et al. 2012). This fact could be related to the increasing pollution of the southern coast of SP (Miranda et al. 2012), since staurozoan populations seem to be vulnerable to anthropic impacts. For instance, once locally abundant populations of *Haliclystus auricula* Clark 1863 have now disappeared, probably due to contamination and pollution of their habitat (Mayer 1910, Berrill 1962). This vulnerability is intensified by the relatively low genetic diversity found in populations of Staurozoa (Miranda et al. 2010), in the small number of species that have been intraspecifically investigated.

Staurozoa is a small group of animals, with about 50 species, whose individuals usually live camouflaged on seaweed (Larson 1980, Daly et al. 2007). Most species occur in temperate and polar waters (Mills & Hirano 2007), and there are only two tropical species, one of them *K. corbini* (Capriles & Martínez 1970, Larson 1980, Grohmann et al. 1999, Lechuga & Alamo 2005). Consequently, the impact on the only known population of the species on the Brazilian coast will hamper researches on the group, such as physiological and evolutionary studies about staurozoan adaptations associated with the diversification of the group in warmer waters.

The competent authorities have made only timid efforts to monitor and minimize the possible impacts of this socio-environmental crime, as well as to identify and punish all responsible players in this tragedy, including negligent licensing and supervisory state agencies. Descriptions of the consequences of the disaster will inevitably underestimate the devastating biological reality, since numerous species that are barely studied (including endemic species from the Espírito Santo coast, e.g. the cnidarian *Hydrocoryne iemanja* Morandini et al. 2009) or even completely unknown occur in the region, such as the example provided above. It is essential to organize and intensify, immediately and effectively, studies of the environmental impacts, and to investigate the responsibilities associated with the environmental licensing process, the lack of a contingency plan, the supervisory failures, and the negligence and even possible deceptive practices of those responsible for authorizing and operating the Fundão dam. The immediate result of a diligent investigation will, at least, increase our ability to prevent future similar tragedies. Most important, the Samarco tragedy should not be seen as an isolated case: it is one of the consequences of the abandonment of environmental values by Brazilian society, with economic development disconnected from conservation/sustainability and social responsibilities. Sufficiently serious in itself, the microcosm of the Mariana dam is, unfortunately, one small

facet of the ongoing environmental catastrophe that is unfolding in the most mega-diverse country in the world.

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## Taxonomy of *Aspidosperma* Mart. (Apocynaceae, Rauvolfioideae) in the State of Pará, Northern Brazil

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PEREIRA, A.S.S., SIMÕES, A.O., SANTOS, J.U.M. Taxonomy of *Aspidosperma* Mart. (Apocynaceae, Rauvolfioideae) in the State of Pará, Northern Brazil. Biota Neotropica. 16(2): e20150080. <http://dx.doi.org/10.1590/1676-0611-BN-2015-0080>

**Abstract:** *Aspidosperma* Mart. is one of the most important genera from Apocynaceae Juss. occurring in Brazil. It is present in many plant formations and has species with both medicinal and economic value. From a taxonomic point of view, many of its species are difficult to identify, especially those occurring in the Amazonian region. In order to complement the information already available with regard to the taxonomy and distribution of the genus, the goal of this work was to provide a taxonomic study of the *Aspidosperma* species occurring in the state of Pará, northern Brazil. This study was based on the analysis of vouchers from the BHCB, HBRA, IAC, IAN, INPA, MG, R, RB and UEC herbaria, as well as scanned images of vouchers from Brazilian and foreign herbaria, and specimens collected in the field. Species identification was confirmed by analysis of protologues and types (or images). We present taxonomic descriptions, illustrations and one identification key for the 20 species of *Aspidosperma* found in Pará, with the Baixo Amazonas Mesoregion presenting the largest number of species (17), and the Metropolitana de Belém Mesoregion presenting the smallest number of species (3). *Aspidosperma eteanum*, *A. oblongum*, *A. salgadense* (endemic of Pará) and *A. sandwithianum* are accepted as species here. *Aspidosperma cuspa* is a new record for Pará. The fruits of *Aspidosperma eteanum* and *A. salgadense*, and the seeds of *A. eteanum*, have been described and illustrated for the first time.

**Keywords:** *Aspidospermeae*, diversity, Eastern Amazon, floristic, morphology.

PEREIRA, A.S.S., SIMÕES, A.O., SANTOS, J.U.M. Taxonomia de *Aspidosperma* Mart. (Apocynaceae, Rauvolfioideae) no Estado do Pará, Brasil. Biota Neotropica. 16(2): e20150080. <http://dx.doi.org/10.1590/1676-0611-BN-2015-0080>

**Resumo:** Dentre os gêneros de Apocynaceae Juss. ocorrentes no Brasil, *Aspidosperma* Mart. é um dos mais importantes. Está presente em várias formações vegetais e apresenta espécies com valor medicinal e econômico. Do ponto de vista taxonômico, várias de suas espécies são de difícil identificação, principalmente as que ocorrem na Amazônia. Com o intuito de complementar as informações disponíveis sobre a taxonomia e a distribuição do gênero, este trabalho teve por objetivo realizar estudos taxonômicos com as espécies de *Aspidosperma* ocorrentes no estado do Pará, Brasil. Este estudo foi baseado na análise de exsicatas dos herbários BHCB, HBRA, IAC, IAN, INPA, MG, R, RB e UEC, assim como de imagens digitalizadas de exsicatas de herbários nacionais e estrangeiros, e de materiais coletados. A identificação das espécies foi confirmada por meio de consulta aos protólogos e tipos (ou imagens). São apresentadas descrições, ilustrações e uma chave de identificação para as 20 espécies de *Aspidosperma* encontradas no Pará, com a Mesorregião do Baixo Amazonas apresentando um maior número de espécies (17), e a Mesorregião Metropolitana de Belém apresentando um menor número de espécies (3). As espécies *Aspidosperma eteanum*, *A. oblongum*, *A. salgadense* (endêmica do Pará) e *A. sandwithianum* são aqui aceitas. *Aspidosperma cuspa* é uma nova ocorrência para o Pará. Os frutos de *Aspidosperma eteanum* e *A. salgadense*, e as sementes de *A. eteanum*, estão sendo descritos e ilustrados pela primeira vez.

**Palavras-chave:** *Aspidospermeae*, diversidade, Amazônia Oriental, florística, morfologia.

## Introduction

Apocynaceae Juss. is one of the five families of the order Gentianales, and is one of the ten largest Angiosperm families in the world (APG III 2009, Rapini 2012, Morokawa et al. 2013). According to the most recent classifications, the family is divided into five subfamilies: Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae and Asclepiadoideae (Endress & Bruyns 2000, Endress et al. 2007, 2014). Apocynaceae has a cosmopolitan distribution, comprising of 366 genera and ca. 5100 species (Matozinhos & Konno 2011, Nazar et al. 2013, Endress et al. 2014). In Brazil, Apocynaceae is the tenth largest Angiosperm family, with members belonging to subfamilies Rauvolfioideae, Apocynoideae and Asclepiadoideae (Kinoshita & Simões 2005, Rapini et al. 2010).

Among the Brazilian genera of Apocynaceae, *Aspidosperma* Mart. (Rauvolfioideae) is one of the most important, being present in many plant formations and is comprised of species with medicinal and economic value (Duarte 1970, Marcondes-Ferreira 1988). *Aspidosperma* has a Neotropical distribution, occurring from Mexico to Argentina (except for Chile) (Marcondes-Ferreira & Kinoshita 1996, Marcondes-Ferreira 1999). According to Marcondes-Ferreira (1999), about 44 species are currently recognized in the genus. Thirty-nine of these are reported to occur in Brazil (10 endemic), with the greatest concentration found in the Amazon (25 species) (Marcondes-Ferreira 1999, Koch et al. 2015).

*Aspidosperma* was created by Martius (1824a) with five species. The genus is characterized by an arboreal or shrubby habit, latex whitish to reddish, leaves alternate, rarely opposite or whorled, flowers with the style-head fusiform to globose, anthers free from the style-head, follicles woody and seeds winged (Woodson 1951, Marcondes-Ferreira 1988, Simões & Kinoshita 2002, Kinoshita & Simões 2005, Morokawa et al. 2013). In the Brazilian Amazon region, most species of *Aspidosperma* are popularly known as “carapanaúba” or “araracanga”, whereas in other regions of the country they are mainly known as “peroba”, “guatambu” or “pereiro” (Koch et al. 2015).

Some species of the genus have medicinal value, such as *Aspidosperma ramiflorum* Müll.Arg., which has antileishmanial properties, and *A. excelsum* Benth., which has antimalarial properties (Pereira et al. 2007, Oliveira et al. 2009). Other species have economic importance in industry and timber production, and are used as ornamental plants (Lorenzi 2008, 2009, Silva 2013). For example, the wood of the “perobas”, *Aspidosperma cylindrocarpon* Müll.Arg. and *A. polyneuron* Müll.Arg., are widely used in construction and in landscaping (Lorenzi 2008, 2009).

Regarding to group's taxonomy, some of the most relevant work was done by de Candolle (1844), Müller-Argoviensis (1860), Schumann (1895), Pichon (1947), Woodson (1951), Marcondes-Ferreira (1988) and Marcondes-Ferreira & Kinoshita (1996). The latter proposed the infrageneric division that is currently accepted for *Aspidosperma*, dividing the genus in two subgenera – *A.* subgenus *Aspidosperma* (comprising nine sections) and *A.* subgenus *Coutinia* (Vell.) Marc.-Ferr. (without further subdivisions) (Marcondes-Ferreira & Kinoshita 1996). Although *Aspidosperma* has been a widely studied genus, many of its species are difficult to identify, especially those occurring in the Amazonian region, because some of them are very similar to each other and have overlapping morphological features (Woodson 1951, Marcondes-Ferreira 1988).

These issues justify the importance of taxonomic studies on *Aspidosperma* to better understand the genus, particularly the Amazonian species. The Brazilian Amazon in the state of Pará is an important area to study *Aspidosperma*, as it is the second largest state in this region, and according to “Lista de Espécies da Flora do Brasil” (Koch et al. 2015), it is where many of the Amazonian species from this genus occur (17) (Souza et al. 2002, IBGE 2008, Pará 2007, 2010). Furthermore, *Aspidosperma* is the most represented genus of Apocynaceae family in Pará (Koch et al. 2015). In this context, the goal of this study was to provide a taxonomic study of the *Aspidosperma* species occurring in the state of Pará, Northern Brazil.

## Material and Methods

### 1. Study area

The study area was located in the state of Pará, Brazil, which has 1,248,042 km<sup>2</sup>, and is the second largest state in the country (Pará 2010). It is located in the North Region of Brazil and in the Amazon Biome (IBGE 2004). The dominant climate is equatorial, with an average temperature of 25 °C and annual rainfall ranging from 1000 to 4500 mm (Brasil 1999, IBGE 2004). According to IBGE (1990), the state of Pará is geographically divided into six Mesoregions: Baixo Amazonas, Marajó, Metropolitana de Belém, Nordeste Paraense, Sudeste Paraense and Sudoeste Paraense (Figure 1).

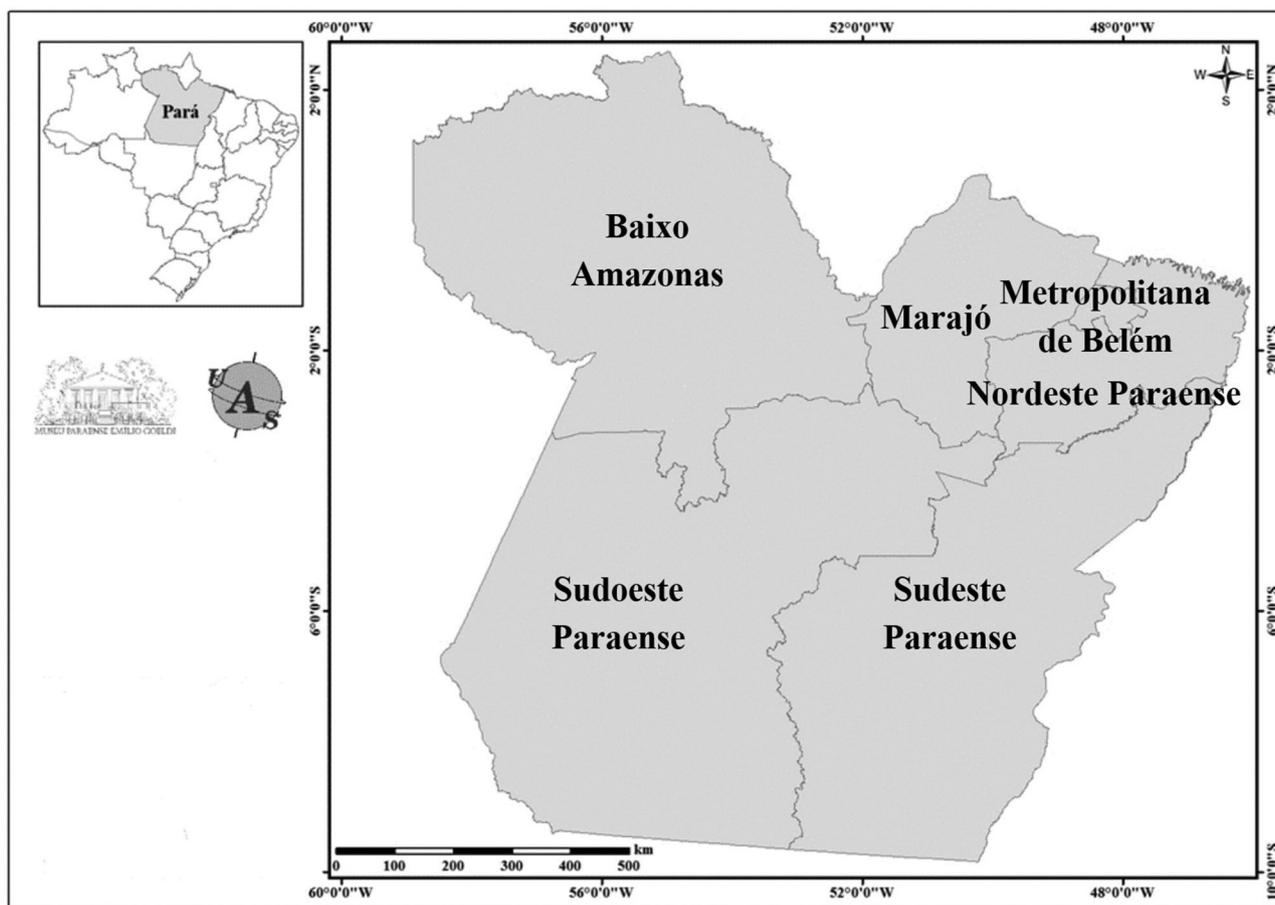
In Pará, there is a predominance of the forest physiognomy, with the Dense Ombrophilous Forest phytocological region covering most of the region (Baixo Amazonas, Marajó, Metropolitana de Belém, Nordeste Paraense, Sudeste Paraense and Sudoeste Paraense) (IBGE 1990, 2008). There are also areas of Open Ombrophilous Forest (Sudeste Paraense and Sudoeste Paraense), and to a lesser extent, Semideciduous Forest (Sudoeste Paraense), Deciduous Forest (Sudoeste Paraense), regions of Campinarana (Marajó, Sudeste Paraense and Sudoeste Paraense) and Savannah (Baixo Amazonas and Sudeste Paraense) (IBGE 1990, 2008).

### 2. Taxonomic treatment

Herbarium vouchers, scanned images of vouchers from Brazilian and foreign herbaria and specimens collected in the field were analysed in this study. Collections from the BHC, HBRA, IAC, IAN, INPA, MG, R, RB and UEC herbaria (acronyms according to Thiers (continuously updated)), which hold relevant collections of Amazonian plants, were examined. Online collections from COL, F, INCT - Herbário Virtual da Flora e dos Fungos (speciesLink Network (UFRN, USP)), K, NY and P herbaria were also examined. The botanical material used was derived from specimens collected in Pará (material examined) and, in general, from specimens collected in the rest of the Amazon, especially in the Brazilian Amazon (additional material).

Plant specimens were collected throughout the year of 2014, from February to September, in Marajó, Metropolitana de Belém and Nordeste Paraense Mesoregions. Specimens were collected and herborized following the usual techniques described by Fidalgo & Bononi (1984), and vouchers are deposited in the Herbarium of the Museu Paraense Emílio Goeldi (MG).

Species identification was confirmed through the analysis of protologues and types, or through images of types available in



**Figure 1.** The State of Pará and its Mesoregions, Northern Brazil. Source: MPEG-UAS.

**Figura 1.** O Estado do Pará e suas Mesorregiões, Brasil. Fonte: MPEG-UAS.

the database “JSTOR Global Plants” (Ithaka 2015), or using the online collections from C, F, G, K, NY, P, U, US and W herbaria. Spelling of scientific names and information on protologues were checked in the original publications, in the guide “Taxonomic Literature” (Stafleu & Cowan 1981) and in the databases “Lista de Espécies da Flora do Brasil” (JBRJ 2015), “Tropicos” (Missouri Botanical Garden 2015) and “World Checklist of Selected Plant Families” (WCSP 2015). Author names are abbreviated according to Brummitt & Powell (1992).

Vegetative structures and follicles were measured with a digital caliper and a ruler, and described with the help of a stereomicroscope. Flowers were rehydrated (when obtained from herbarium specimens), stored in bottles containing 70% ethanol, dissected, measured with graph paper and described with the help of a stereomicroscope. The terminology used to characterize the vegetative and reproductive structures was based on the works of Woodson (1951), Lawrence (1973), Radford et al. (1974), Rizzini (1977), Marcondes-Ferreira (1988), Ribeiro et al. (1999), Gomes e Cavalcanti (2001), Gomes (2008) and Morokawa et al. (2013). Genus and species descriptions were prepared with the data obtained from analyses on examined material and additional material. Information with regard to geographic distribution, phytoecological regions, phenology and common names was obtained from herbarium specimens, data from field notes and literature.

## Results and Discussion

*Aspidosperma* Mart., Flora 7(1): 135. 1824. *nom. cons.*  
Type: *Aspidosperma tomentosum* Mart.

Tree, 2-48 m high; trunk straight or tortuous. Branches cylindrical or angular, suberous or not, sparsely to densely lenticellate, velutinous to glabrous, with or without cataphylls covering the buds; latex whitish, orange or reddish. Leaves simple, alternate or, more rarely, subopposite, congested at apex or arranged along the branches; petiole velutinous to glabrous; blade membranaceous to coriaceous, flat or bullate, margin straight or revolute, concolorous or discolorous, venation craspedodromous, brochidodromous, eucamptodromous or reticulodromous, adaxial surface dull or lustrous, greenish, brownish or blackish, velutinous to glabrous, primary vein flat, prominulous or prominent, secondary veins impressed, prominulous or prominent, abaxial surface dull, whitish, yellowish, greenish or brownish, velutinous to glabrous, primary vein flat or prominent, secondary veins impressed, prominulous or prominent. Inflorescences leaf-opposed, axillary, supra-axillary or terminal, corymbiform dichasia, fascicles or panicles, velutinous to tomentose. Flower buds with corolla lobes twisted or not. Flowers subsessile or pedicellate. Calyx gamosepalous, campanulate, without colletes; lobes 5 or, more rarely, 6-7, subequal, lanceolate or ovate, apex acuminate, acute or obtuse. Corolla gamopetalous, tubular or salverform, white, yellow or orange; lobes erect, patent or deflexed, oblong, filiform, lanceolate,

ovate or obovate, apex acute or obtuse. Stamens included; anthers free, positioned above the style-head, lanceolate or ovate, apex acuminate, apiculate or acute, base cordate. Ovary superior, hemisyncarpous, 2-carpellate, ovoid or globoid, tomentose to glabrous; style cylindrical; style-head main body oblong or globose, with 2 apical appendages oblong, filiform, ovate or inconspicuous. Follicles 2 or 1 by abortion, flat, falciform, dolabriform, pyriform or suborbicular, smooth, sulcate, verrucose or spinescent, sessile or stipitate, mucronate or not, lenticels conspicuous or inconspicuous, woody, yellow, brown or black, velutinous to glabrous. Seeds oblong, ovate or orbicular, winged, yellow, glabrous; seminal nucleus basal, central, lateral or apical, with or without radial lines.

The information about the protologues of *Aspidosperma* were checked in their original publications. In the databases “Tropicos” (Missouri Botanical Garden 2015) and “World Checklist of Selected Plant Families” (WCSP 2015), this information is conflicting. The diagnosis of *Aspidosperma* (*A. bicolor* Mart. (now a synonym of *A. pyriform*), *A. macrocarpon* Mart., *A. pyriform* Mart., *A. refractum* Mart. (now a synonym of *A. pyriform*) and *A. tomentosum* Mart. (type)), was first published by Martius (1824a) in “Flora”, and published again by the same author in “Nova Genera et Species Plantarum” (Martius 1824b, Stafleu & Cowan 1981, Marcondes-Ferreira 1988). Stafleu & Cowan (1981) highlighted that Zuccarini should not be cited as co-author of *Aspidosperma*, since he helped Martius (1824b) only with the organization of the work and did not participate on the original publication (Martius 1824a). This interpretation was later followed by Marcondes-Ferreira (1988) in his study of *Aspidosperma* and is the interpretation accepted in the present study.

Based on the infrageneric division of *Aspidosperma* established by Marcondes-Ferreira & Kinoshita (1996), only species from *Aspidosperma* subgenus *Aspidosperma* occur in the state of Pará. Of the nine sections of *Aspidosperma* subgenus *Aspidosperma*, seven occur in Pará: *Aspidosperma* (*A. macrocarpon*, *A. multiflorum* A.DC., *A. pyriform* and *A. subincanum* Mart.), *Nobilia* (Woodson) Marc.-Ferr. (*A. album* (Vahl) Benoist ex Pichon, *A. araracanga* Marc.-Ferr., *A. desmanthum* Benth. ex Müll.Arg., *A. eteanum* Markgr., *A. sandwithianum* Markgr. and *A. spruceanum* Benth. ex Müll.Arg.), *Polyneura* (Woodson) Marc.-Ferr. (*A. cuspa* (Kunth) S.F.Blake ex Pittier and *A. darienense* Woodson ex Dwyer), *Inundata* Marc.-Ferr. (*A. inundatum* Ducke), *Schultesia* Marc.-Ferr. (*A. schultesii* Woodson), *Rigida* (Woodson) Marc.-Ferr. (*A. rigidum* Rusby) and *Excelsa* Marc.-Ferr. (*A. carapanauba* Pichon, *A. discolor* A.DC., *A. excelsum* Benth., *A. oblongum* A.DC. and *A. salgadense* Markgr.) (Marcondes-Ferreira & Kinoshita 1996).

In Pará, 20 species of *Aspidosperma* were found, with the Baixo Amazonas Mesoregion presenting the largest number of species (17), and the Metropolitana de Belém Mesoregion presenting the smallest number of species (3) (IBGE 1990). *Aspidosperma araracanga* and *A. excelsum* are the only species of the genus occurring in all the Mesoregions of Pará (IBGE 1990). We accept *Aspidosperma eteanum*, *A. oblongum*, *A. salgadense* (endemic of Pará) and *A. sandwithianum* as species in this study. *Aspidosperma cuspa* is a new record for Pará. Furthermore, the fruits of *Aspidosperma eteanum* and *A. salgadense*, and the seeds of *A. eteanum*, have been described and illustrated for the first time.

#### Key to species of *Aspidosperma* Mart. (Apocynaceae, Burseroideae) in the State of Pará, Northern Brazil.

- 1.Branches angular ..... 16. *A. salgadense*
- 1'.Branches cylindrical ..... 2
- 2.Branches with cataphylls covering the buds; leaves congested at apex of branches ..... 3
- 3.Leaves discolorous; flower buds with corolla lobes not twisted; corolla tubular; ovary tomentose..... 20. *A. subincanum*
- 3'.Leaves concolorous; flower buds with corolla lobes twisted; corolla salverform; ovary glabrous ..... 4
- 4.Leaves membranaceous; flowers 6-6.5 mm long; calyx glabrous externally; corolla lobes filiform..... 12. *A. multiflorum*
- 4'.Leaves chartaceous; flowers 14-23 mm long; calyx tomentose externally; corolla lobes oblong..... 14. *A. pyriform*
- 2'.Branches without cataphylls covering the buds; leaves arranged along the branches ..... 5
- 5.Branches sparsely lenticellate ..... 6
- 6.Calyx with 6-7 lobes ..... 5. *A. darienense*
- 6'.Calyx with 5 lobes ..... 7
- 7.Venation reticulodromous; inflorescences in panicles ..... 4. *A. cuspa*
- 7'.Venation craspedodromous, brochidodromous or eucamptodromous; inflorescences in corymbiform dichasia or fascicles ..... 8
- 8.Venation craspedodromous ..... 9
- 9.Corolla pubescent externally, lobes lanceolate; ovary tomentose; seminal nucleus with radial lines ..... 18. *A. schultesii*
- 9'.Corolla glabrous externally, lobes filiform; ovary glabrous; seminal nucleus without radial lines ..... 10
- 10.Corolla tubular, lobes 1-1.5 mm long ..... 8. *A. eteanum*
- 10'.Corolla salverform, lobes 2.5-6.5 mm long ..... 11
- 11.Leaves with 39-41 pairs of secondary veins, prominent on both surfaces ..... 2. *A. araracanga*
- 11'.Leaves with 21-34 pairs of secondary veins, not prominent on both surfaces ..... 12
- 12.Leaves bullate, adaxial surface dull, with primary vein tomentose ..... 17. *A. sandwithianum*
- 12'.Leaves flat, adaxial surface lustrous, with primary vein glabrous ..... 13
- 13.Leaves dark green on adaxial surface, with secondary veins prominent; calyx glabrescent to glabrous internally ..... 1. *A. album*
- 13'.Leaves olive green or brown on adaxial surface, with secondary veins impressed or prominulous; calyx pubescent at apex internally ..... 14
- 14.Leaves with secondary veins impressed on both surfaces ..... 6. *A. desmanthum*
- 14'.Leaves with secondary veins prominulous on both surfaces ..... 19. *A. spruceanum*
- 8'.Venation brochidodromous or eucamptodromous ..... 15

15. Leaves bullate; inflorescences in fascicles; calyx pubescent at base internally .... 3. *A. carapanauba*  
 15'. Leaves flat; inflorescences in corymbiform dichasia; calyx pubescent at apex to glabrous internally ..... 16  
 16. Leaves concolorous; corolla glabrous externally; follicles not mucronate..... 15. *A. rigidum*  
 16'. Leaves discolorous; corolla tomentose externally; follicles mucronate ..... 17  
 17. Venation eucamptodromous, with 10-13 pairs of secondary veins ..... 11. *A. macrocarpon*  
 17'. Venation brochidodromous, with 22-25 pairs of secondary veins ..... 18  
 18. Leaves with marginal vein; corolla salverform; anthers lanceolate; ovary tomentose ..... 10. *A. inundatum*  
 18'. Leaves without marginal vein; corolla tubular; anthers ovate; ovary glabrous ..... 9. *A. excelsum*  
 5'. Branches densely lenticellate ..... 19  
 19. Leaves elliptic or ovate, base cuneate or oblique; anthers lanceolate; follicles spinescent..... 7. *A. discolor*  
 19'. Leaves oblong, base revolute; anthers ovate; follicles verrucose ..... 13. *A. oblongum*

1. *Aspidosperma album* (Vahl) Benoist ex Pichon, Bull. Mus. Natl. Hist. Nat. sér. 2 19(4): 367. 1947. (Figure 2a-e).

Trees 15-30 m high; trunk straight. Branches cylindrical, suberous, sparsely lenticellate, pubescent to glabrous, without cataphylls; latex reddish. Leaves alternate, arranged along the branches; petioles 1.2-1.9 cm long, tomentose; blade 7-8.9 x 2.5-3.4 cm, coriaceous, flat, obovate, apex acute, obtuse or retuse, base cuneate or oblique, margin revolute, discolorous, venation craspedodromous, adaxial surface lustrous, dark green, glabrous, primary vein flat, secondary veins prominent, tertiary veins conspicuous, abaxial surface dull, white, tomentose along the primary vein, primary vein prominent, secondary veins prominulous, 22-24 pairs, tertiary veins conspicuous. Inflorescences 5.7-6.5 cm long, terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes twisted. Flowers 5.2-6.5 mm long; pedicellate, pedicel 0.9-1 x 0.5-1 mm, tomentose. Calyx 2-2.5 x 1.5-1.8 mm, tomentose externally, glabrescent to glabrous internally; lobes 5, 1.5 x 1-1.3 mm, ovate, apex acute. Corolla 3.5-5.8 x 1-1.2 mm, salverform, yellow, glabrous externally, pubescent to glabrescent below the anthers internally; lobes 2.5 x 0.5 mm, erect, filiform, apex acute. Stamens 1-1.5 mm long; filaments 0.5-1 mm long, pubescent to glabrescent; anthers 0.5 mm long, ovate, apex apiculate. Carpels 1-1.2 mm long; ovary 0.5 x 0.5 mm, globoid, glabrous; style 0.3-0.5 mm long; style-head 0.2 mm long, main body globose, with 2 oblong apical appendages. Follicles 11.7-13 x 7.1-7.3 cm, suborbicular, sulcate, stipitate, mucronate, lenticels inconspicuous, brown, pubescent. Seeds 7.3-9 cm diam., orbicular; seminal nucleus lateral, without radial lines, nucleus 1.8-2 cm diam.

**Material examined:** BRAZIL, PARÁ: Almeirim, Monte Dourado, Cova da Onça, 28.VIII.1979, fl., N.T. Silva 5157 (INPA, MG); Almeirim, região do Jarí, Planalto A, km 11, 2.

VIII.1969, fl., N.T. Silva 2569 (IAN, NY-scanned image); Breves, perto do Igarapé Arapijô, transecto para inventário florestal, Q 4-70, 7-30.VII.1956, st., J.M. Pires et al. 5059 (IAN); Breves, transecto para inventário florestal, 20-6 (sp. n° 3), 7-30.VII.1956, fr., J.M. Pires et al. 5290 (IAN); Breves, transecto para inventário florestal, 70-80 (sp. n° 7a), 7-30.VII.1956, fr., J.M. Pires et al. 5454 (IAN); São Sebastião da Boa Vista, Sítio Campina on River Pracuabamirim, 18.X.1984, st., G.L. Sobel et al. 4669A (NY-scanned image).

**Additional material:** BRAZIL, AMAPÁ: Oiapoque, beira do caminho, 5.X.1949, fl., G.A. Black 49-8406 (IAN); Rio Araguari, on forested island in river, downriver from Porto Platon, 21.IX.1961, fr., J.M. Pires et al. 51149 (IAN, MG); Rio Araguari, upland plant between camps 6 and 7, 12.IX.1961, fl., J.M. Pires et al. 50882 (IAN, RB).

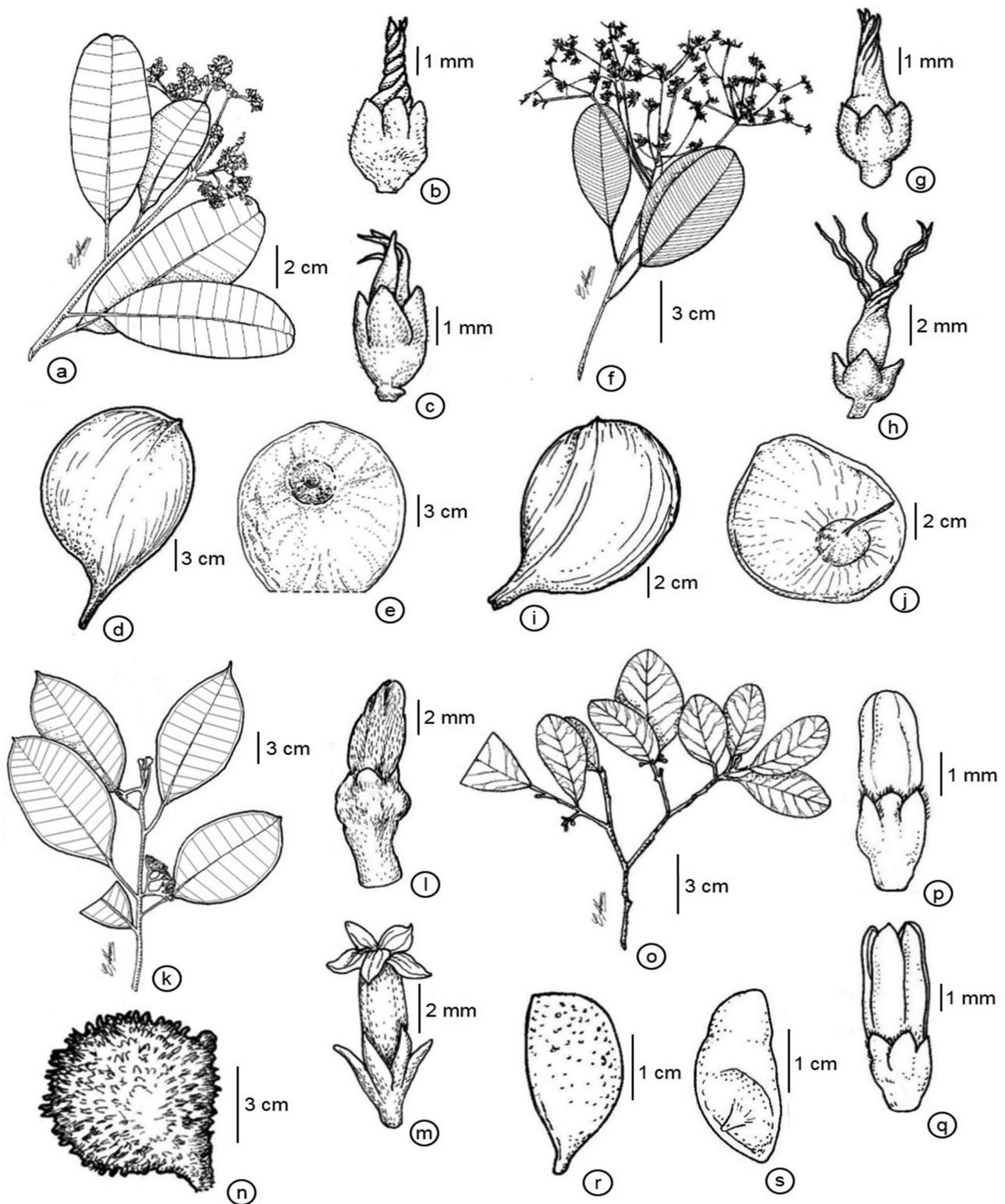
**Phytogeological region and phenology:** Dense Ombrophilous Forest (terra firme forest). Flowers and fruits collected from August to January.

**Common names:** araracanga, araracanga-vermelha.

*Aspidosperma album* occurs in Bolivia, Brazil, Colombia, French Guiana, Guyana, Suriname and Venezuela (Missouri Botanical Garden 2015). In the state of Pará, this species was found in Baixo Amazonas and Marajó Mesoregions (IBGE 1990). In "Livro Vermelho da Flora do Brasil" (Rapini et al. 2013), *Aspidosperma album* was assigned to the Data Deficient category (DD), but in "Lista de Espécies da Flora e da Fauna Ameaçadas do Pará" (Pará 2007), it was listed as a Vulnerable species (VU). According to Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma album* belongs to section *Nobilia*. Among the studied species, *Aspidosperma album* shares morphological features with both *A. desmanthum* and *A. spruceanum*, especially relating to the flower, but differs from these other two species by the dark green color of the leaf blade and prominent secondary veins on the adaxial surface, and the suborbicular follicles.

2. *Aspidosperma araracanga* Marc.-Ferr., Revista Brasil. Bot. 14(2): 127. 1991. (Figure 2f-j).

Trees 2.5-44 m high; trunk straight. Branches cylindrical, not suberous, sparsely lenticellate, pubescent to glabrous, without cataphylls; latex orange to reddish. Leaves alternate, arranged along the branches; petioles 1.8-3.5 cm long, tomentose to glabrous; blade 9.7-10.8 x 3.9-4.8 cm, chartaceous, flat, oblong, elliptic or obovate, apex acuminate, acute or obtuse, base acute, cuneate or oblique, margin revolute, discolorous, venation craspedodromous, adaxial surface lustrous, olive green, glabrous, primary vein prominulous, secondary veins prominent, tertiary veins conspicuous, abaxial surface dull, light green, glabrous, primary vein prominent, secondary veins prominent, 39-41 pairs, tertiary veins conspicuous. Inflorescences 7.7-10.5 cm long, terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes twisted. Flowers 8-10.5 mm long; pedicellate, pedicel 1 x 1 mm, tomentose. Calyx 2-2.5 x 1.5 mm, tomentose externally, glabrescent internally; lobes 5, 1-1.5 x 1 mm, ovate, apex acute. Corolla 7-10 x 2 mm, salverform, yellow, glabrous externally, tomentose below the anthers internally; lobes 5.4-6.5 x 0.5 mm, erect, filiform, apex acute. Stamens 2-2.5 mm long; filaments 1.5-2 mm long, tomentose; anthers 0.5 mm long, ovate, apex acute. Carpels 2-2.5 mm long; ovary 0.5 x 0.5 mm, globoid, glabrous; style 1-1.5 mm long; style-head 0.5 mm long, main body oblong or globose, with 2 oblong apical appendages. Follicles 10.2 x 8 cm, suborbicular, sulcate, stipitate, mucronate,



**Figure 2.** a-e. *Aspidosperma album*. f-j. *A. araracanga*. k-n. *A. carapanauba*. o-s. *A. cuspa*: a, f, k, o. flowering branch; b, g, l, p. flower bud; c, h, m, q. flower; d, i, n, r. follicle; e, j, s. seed. a-e. J.M. Pires et al. 50882; J.M. Pires et al. 51149. f-j. J. Huber 3848; J.M. Pires 11911; R. Romero-Castañeda 1131. k-n. M.G. Silva & A. Pinheiro 4300; M.G. Silva & C. Rosário 4847. o-s. G.S. Pinheiro & J.F.V. Carvalho 674; P.O. Rosa et al. 249.

**Figura 2.** a-e. *Aspidosperma album*. f-j. *A. araracanga*. k-n. *A. carapanauba*. o-s. *A. cuspa*: a, f, k, o. ramo florido; b, g, l, p. botão floral; c, h, m, q. flor; d, i, n, r. folículo; e, j, s. semente. a-e. J.M. Pires et al. 50882; J.M. Pires et al. 51149. f-j. J. Huber 3848; J.M. Pires 11911; R. Romero-Castañeda 1131. k-n. M.G. Silva & A. Pinheiro 4300; M.G. Silva & C. Rosário 4847. o-s. G.S. Pinheiro & J.F.V. Carvalho 674; P.O. Rosa et al. 249.

lenticels inconspicuous, yellow or brown, pubescent. Seeds 7-8 cm diam., orbicular; seminal nucleus lateral, without radial lines, nucleus 2 cm diam.

**Material examined:** BRAZIL, PARÁ: Almeirim, Monte Dourado, Perimetral, 23.I.1980, st., N.T. Silva 5420 (MG); Belém, I.IX.1903, fl., J. Huber 3848 (RB); Belém, IPEAN, Área de Pesquisas Ecológicas do Guamá (APEG), 8.VIII.1968, fl., J.M. Pires 11911 (RB); Belém, IPEAN, Reserva Mocambo, L-12-17, árvore n° 18, 8.VIII.1968, fl., J.M. Pires & N.T. Silva 11909 (IAN); Bragança, terreno baldio na Rua Edgar Cordeiro de Souza, frente ao portão do Instituto Federal do Pará, 2001-2002, fl., U. Mehlig 1398 (HBRA); Breves, perto do Igarapé Arapijó, transecto para inventário florestal, Q 1-76, 7-30. VII.1976, st., J.M. Pires et al. 5083 (IAN); Cachoeira do Arari, Rio Camará, Fazenda Gurupatuba, 8.VIII.1950, fl., G.A. Black 50-9924 (NY-scanned image); Colares, 18.VIII.1913, fl., A. Ducke s.n. (RB 13333); Maracanã, área do centro de treinamento, 4.VII.1977, fl., E. Oliveira 6650 (MG, NY-scanned image); Melgaço, Caxiuanã, Rio Caxiuanã, pt. 02, 9.IX.1983, st., B. Pena 1027 (INPA); Oriximiná, Baixo Trombetas, 14.IX.1910, fl., A. Ducke s.n. (RB 13332); Oriximiná, Lago Salgado (Rio Trombetas), 25.IV.1917, fr., A. Ducke s.n. (RB 22441); Santarém, Reserva Curuá-Una, Parque Fenológico, árvore n° 152, 19.IV.1999, st., M.R. Cordeiro 3811 (IAN); São Sebastião da Boa Vista, Sítio Campina on River Pracuabamirim, 17.X.1984, st., G.L. Sobel et al. 4638 (NY-scanned image); Tucuruí, Transgoiânia, área nuclear n° 4, III.1981, st., P. Lisboa et al. 3017 (MG); Vitória do Xingu, Usina Hidrelétrica Belo Monte, 29.V.2012, fl., L.C. Antônio PSACF 276 (MG).

**Additional material:** BRAZIL, AMAZONAS: Parintins, Lago José-Assú, 18.IX.1932, fl., A. Ducke s.n. (RB 24572); MATO GROSSO: Gaúcha do Norte, Fazenda Pontal, propriedade de Silvino Perotto, 16.VIII.2000, fl., N.M. Ivanauskas 4354 (UEC); RONDÔNIA: Vicinity of Santa Bárbara, 15 km east of km 117, Porto Velho to Cuiabá highway, 16.VIII.1968, fl., G.T. Prance & J.F. Ramos 7016 (MG). COLOMBIA, BOLÍVAR: Palotal, 4.VII.1948, fr., R. Romero-Castañeda 1131 (COL-scanned image).

**Phytoecological region and phenology:** Dense Ombrophilous Forest (terra firme forest). Flowers and fruits collected from June to April.

**Common names:** araracanga, aracandea.

*Aspidosperma araracanga* occurs in Brazil, Colombia, Peru and Venezuela (Marcondes-Ferreira 1991, Missouri Botanical Garden 2015). In the state of Pará, this species was found in all Mesoregions (IBGE 1990). According to Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma araracanga* belongs to section *Nobilia*. *Aspidosperma araracanga* resembles *A. desmanthum*, especially flower features, but is distinguished from *A. desmanthum*, and from the other species in section *Nobilia* occurring in Pará, by the number of secondary veins (39-41 pairs), with the veins being prominent on both leaf surfaces.

**3. *Aspidosperma carapanauba*** Pichon, Bull. Mus. Natl. Hist. Nat. sér. 2 19(4): 365. 1947. (Figure 2k-n).

Trees 20-38 m high; trunk tortuous. Branches cylindrical, suberous, sparsely lenticellate, tomentose to pubescent, without cataphylls; latex whitish. Leaves alternate, arranged along the branches; petioles 2.4-2.9 cm long, tomentose to pubescent; blade 9.4-17.1 x 4.1-7.8 cm, subcoriaceous to coriaceous, bullate, oblong, elliptic or ovate, apex acuminate or acute, base cuneate or oblique, margin revolute, discolorous, venation brochidromous, with

marginal vein 2-4 mm from the margin, adaxial surface dull or lustrous, brown or black, pubescent along the primary vein, primary vein flat, secondary veins impressed, tertiary veins conspicuous, abaxial surface dull, yellow, velutinous, primary vein prominent, secondary veins prominent, 13-22 pairs, tertiary veins inconspicuous. Inflorescences 2.4-4.8 cm long, axillary or supra-axillary, fascicled, tomentose. Flower buds with corolla lobes not twisted. Flowers 12.5-13.5 mm long; pedicellate, pedicel 3.5-4 x 1.5-2 mm, tomentose. Calyx 3-3.5 x 3.5 mm, tomentose externally, pubescent at base internally; lobes 5, 2-3 x 1.2-2 mm, ovate, apex acute. Corolla 6-7.5 x 2.5-3 mm, tubular, white, tomentose externally, pubescent to glabrescent along the tube internally; lobes 1.5-2.5 x 1 mm, erect, ovate, apex acute. Stamens 2.5-4 mm long; filaments 1.7-3 mm long, pubescent to glabrescent; anthers 0.8-1 mm long, ovate, apex acute. Carpels 2 mm long; ovary 1 x 1.5 mm, globose, tomentose; style 0.8 mm long; style-head 0.2 mm long, main body oblong, with 2 inconspicuous apical appendages. Follicles 5.5-6.5 x 4.6-5 cm, dolabriform, spinescent, sessile or stipitate, mucronate, lenticels inconspicuous, brown, pubescent. Seeds not observed.

**Material examined:** BRAZIL, PARÁ: Almeirim, região do Jarí, estrada do Munguba, km 12, 11.VIII.1969, fl., N.T. Silva 2642 (IAN); Almeirim, região do Jarí, Monte Dourado, serra de 1,40 m de altura, a 3 km da margem, 3.XII.1967, fl., E. Oliveira 3799 (IAN); Almeirim, região do Rio Jarí, Monte Dourado, Planalto A, 9.IX.1968, fl., N.T. Silva 912 (IAN); Almeirim, região do Jarí, Planalto Monte Dourado, 22.I.1968, fl., E. Oliveira 3945 (IAN); Almeirim, região do Rio Jarí, Monte Dourado, Planalto, 2.II.1968, fr., E. Oliveira 4076 (IAN).

**Additional material:** BRAZIL, AMAPÁ: Mazagão, área do Felipe 03, 0°40'S, 52°18'W, 3.VI.1983, fl., N.T. Silva 5296 (MG); MATO GROSSO: Aripuanã, km 238 da BR-174, núcleo Juina, área urbana, 17.I.1979, fr., M.G. Silva & A. Pinheiro 4300 (MG, RB, UEC); Guarantã, adjacências do Hotel Floresta Amazônica, 7.VI.1997, fl., G.F. Árbocz et al. 4000 (UEC); km 330 da rod. BR-174, margem da Fazenda Vale do Tucaná, 10.VI.1979, fl., M.G. Silva & C. Rosário 4847 (MG, RB).

**Phytoecological region and phenology:** Dense Ombrophilous Forest (terra firme forest). Flowers and fruits collected from August to February.

**Common names:** carapanaúba, carapanaúba-preta.

According to Koch et al. (2015), *Aspidosperma carapanauba* is a species endemic to Brazil, and occurs in the states of Amapá, Amazonas, Mato Grosso, Pará and Rondônia. In the state of Pará, this species was found only in Baixo Amazonas Mesoregion (IBGE 1990). As stated by the classification from Marcondes-Ferreira & Kinoshita (1996), it belongs to section *Excelsa*. Although seeds were not observed here, Marcondes-Ferreira (1988) described them as nearly orbicular (ca. 4.5 cm diam.) with a lateral seminal nucleus (ca. 2.5 cm diam.). *Aspidosperma carapanauba* was one of the most easily recognized species, and is distinguished from the other species in section *Excelsa* occurring in Pará by the larger petioles (2.4-2.9 cm long) and leaf blades (9.4-17.1 x 4.1-7.8 cm), bullate leaves, fasciculate inflorescences and larger flowers (12.5-13.5 mm long).

**4. *Aspidosperma cuspa*** (Kunth) S.F.Blake ex Pittier, Man. Pl. Usual. Venez.: 110. 1926. (Figure 2o-s).

Trees 4-27 m high; trunk straight. Branches cylindrical, not suberous, sparsely lenticellate, glabrescent to glabrous, without cataphylls; latex whitish. Leaves alternate, arranged along the branches; petioles 0.4-0.6 cm long, pubescent to glabrescent;

blade 2.7-6 x 1.4-2.6 cm, chartaceous, flat, oblong or obovate, apex acute or obtuse, base cuneate, attenuate or oblique, margin revolute, discolorous, venation reticulodromous, adaxial surface dull, olive green, glabrous, primary vein prominulous, secondary veins prominent, tertiary veins conspicuous, abaxial surface dull, light green, pubescent, primary vein prominent, secondary veins prominulous, 9-12 pairs, tertiary veins inconspicuous. Inflorescences 1.5-11.3 cm long, leaf-opposed or axillary, paniced, tomentose. Flower buds with corolla lobes not twisted. Flowers 4.5-5 mm long; sessile or pedicellate, pedicel 1 x 0.5 mm, glabrous. Calyx 1.3-1.5 x 1.2 mm, glabrous on both surfaces, except at margin; lobes 5, 1 x 0.5-0.7 mm, ovate, apex acute. Corolla 3-3.5 x 1.2 mm, tubular, yellow, glabrous externally, tomentose below the anthers internally; lobes 1-1.3 x 0.6 mm, erect, oblong, apex obtuse. Stamens 2.2-2.7 mm long; filaments 1.5-2 mm long, tomentose; anthers 0.7 mm long, lanceolate, apex acute. Carpels 1.6 mm long; ovary 0.7 x 0.5 mm, ovoid, glabrous; style 0.5 mm long; style-head 0.4 mm long, main body globose, with 2 oblong apical appendages. Follicles 2.5-3 x 1.3-1.5 cm, dolabriform, smooth, stipitate, mucronate, lenticels conspicuous, yellow, pubescent. Seeds 2.2-2.4 x 0.9-1 cm, oblong or ovate; seminal nucleus basal or apical, without radial lines, nucleus 1.1 x 0.8 cm.

**Material examined:** BRAZIL, PARÁ: Marabá, Estreito-Marabá, km 2, 9.IV.1974, fr., G.S. Pinheiro & J.F.V. Carvalho 674 (IAN).

**Additional material:** BRAZIL, GOIÁS: 12°15'S, 46°45'W, 21.VI.1979, fr., L.C.O. Filho 29 (RB); MARANHÃO: São Francisco do Maranhão, 21.V.2009, fr., L. Rodrigues et al. s.n. (UFRN 9480-scanned image); MATO GROSSO: Barra do Garças, Fazenda Taquaral, Cabeceira do Rio Pindaíba, 25.X.2003, fl., C. Fernandes-Bulhão et al. 358CFB (UEC); Barra do Garças, Serra do Taquaral, mata em grotão, 25.XI.1997, fl., L.C. Bernacci & G.F. Árbocz 2616 (IAC, UEC); Santo Antônio de Leverger, 25.III.1982, fr., A.L. Prado et al. 327 (UEC); MINAS GERAIS: Araguari, Funil I, Capim Branco I, 15.XII.2006, fl., P. O. Rosa et al. 249 (UEC).

**Phytoecological region and phenology:** Savannah. Flowers and fruits collected from April to September.

**Common names:** guatambuzinho (Koch et al. 2015).

According to Marcondes-Ferreira (1988), *Aspidosperma cuspa* has the widest distribution of the genus, occurring from Haiti to Paraguay, in many plant formations. *Aspidosperma cuspa* is a new record for the state of Pará, being found only in Sudeste Paraense Mesoregion (IBGE 1990). In the classification from Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma cuspa* belongs to section *Polyneura*. It can be easily differentiated from the other species that occur in Pará by the reticulodromous venation, paniculate inflorescence and smaller seeds (2.2-2.4 cm long) with basal or apical seminal nucleus.

**5. *Aspidosperma darienense*** Woodson ex Dwyer, Ann. Missouri Bot. Gard. 53(1): 104. 1966. (Figure 3a-d).

Trees 10-38 m high; trunk tortuous. Branches cylindrical, not suberous, sparsely lenticellate, pubescent, without cataphylls; latex whitish. Leaves alternate, arranged along the branches; petioles 0.7-1.1 cm long, tomentose to pubescent; blade 8.9-21 x 3.5-7.8 cm, coriaceous, flat, oblong or obovate, apex acuminate, acute or obtuse, base cuneate, margin revolute, discolorous, venation brochidodromous, with marginal vein 1 mm from the margin, adaxial surface lustrous, dark brown, glabrous, primary vein prominulous, secondary veins impressed, tertiary veins conspicuous, abaxial surface dull, light brown, pubescent,

primary vein prominent, secondary veins impressed, 30-38 pairs, tertiary veins inconspicuous. Inflorescences 3.2-4 cm long, supra-axillary, corymbiform dichasia, tomentose. Flower buds with corolla lobes not twisted. Flowers 6.5-8.5 mm long; sessile or pedicellate, pedicel 1-2.5 x 1 mm, tomentose. Calyx 2.3-2.5 x 2-2.5 mm, tomentose to pubescent externally, glabrous internally; lobes 6-7, 1.5-2 x 1.5-2 mm, ovate, apex obtuse. Corolla 4.5-6 x 1.5 mm, salverform, white, tomentose externally, tomentose along the corolla internally; lobes 2.5-3 x 1 mm, erect, lanceolate, apex acute or obtuse. Stamens 1.5-2 mm long; filaments 0.5-1 mm long, tomentose; anthers 1 mm long, lanceolate, apex acuminate. Carpels 1.7 mm long; ovary 0.7 x 0.8 mm, globose, pubescent at apex; style 0.8 mm long; style-head 0.2 mm long, main body oblong, with 2 inconspicuous apical appendages. Follicles 7.4-10.2 x 4.4-4.6 cm, falciform or dolabriform, smooth, sessile, mucronate, lenticels conspicuous, brown, glabrous. Seeds 6.7-7 x 4.3-5.2 cm, ovate; seminal nucleus central, without radial lines, nucleus 2.9 cm diam.

**Material examined:** BRAZIL, PARÁ: Almeirim, Monte Dourado, próximo a marina da SION em Munguba, 26.IX.1986, fr., M.J. Pires & N.T. Silva 1390 (INPA, MG); Almeirim, Monte Dourado, Bom Futuro, 10.III.1981, fr., N.T. Silva 5273 (INPA, MG); Almeirim, região do Rio Jarí, Monte Dourado, Planalto B, entre Pilão e Repartimento, 28.X.1968, fl., N.T. Silva 1325 (IAN, NY-scanned image).

**Additional material:** BRAZIL, AMAPÁ: Rio Araguari, upriver, 20 minutes from camp 5 toward camp 4, 8.IX.1961, fr., J.M. Pires et al. 50756 (BHCB, IAN, MG); Rio Araguari, vic. camp 12, 28.IX.1961, fl., J.M. Pires et al. 51291 (IAN, MG); Rio Oiapoque, about 0.5 km south of mouth of Rio Muturá, 21.IX.1960, fl., H.S. Irwin et al. 48412 (BHCB, MG). COLOMBIA, NORTE DE SANTANDER: San José de Cúcuta, Aguacalera, Hacienda San Roque, 26.V.1970, fr., R.J. Mejía 4913 (COL-scanned image). ECUADOR, PASTAZA: Pozo petrolero Villano 2 de ARCO, 1-18.XII.1991, fl., F. Hurtado 2868 (UEC).

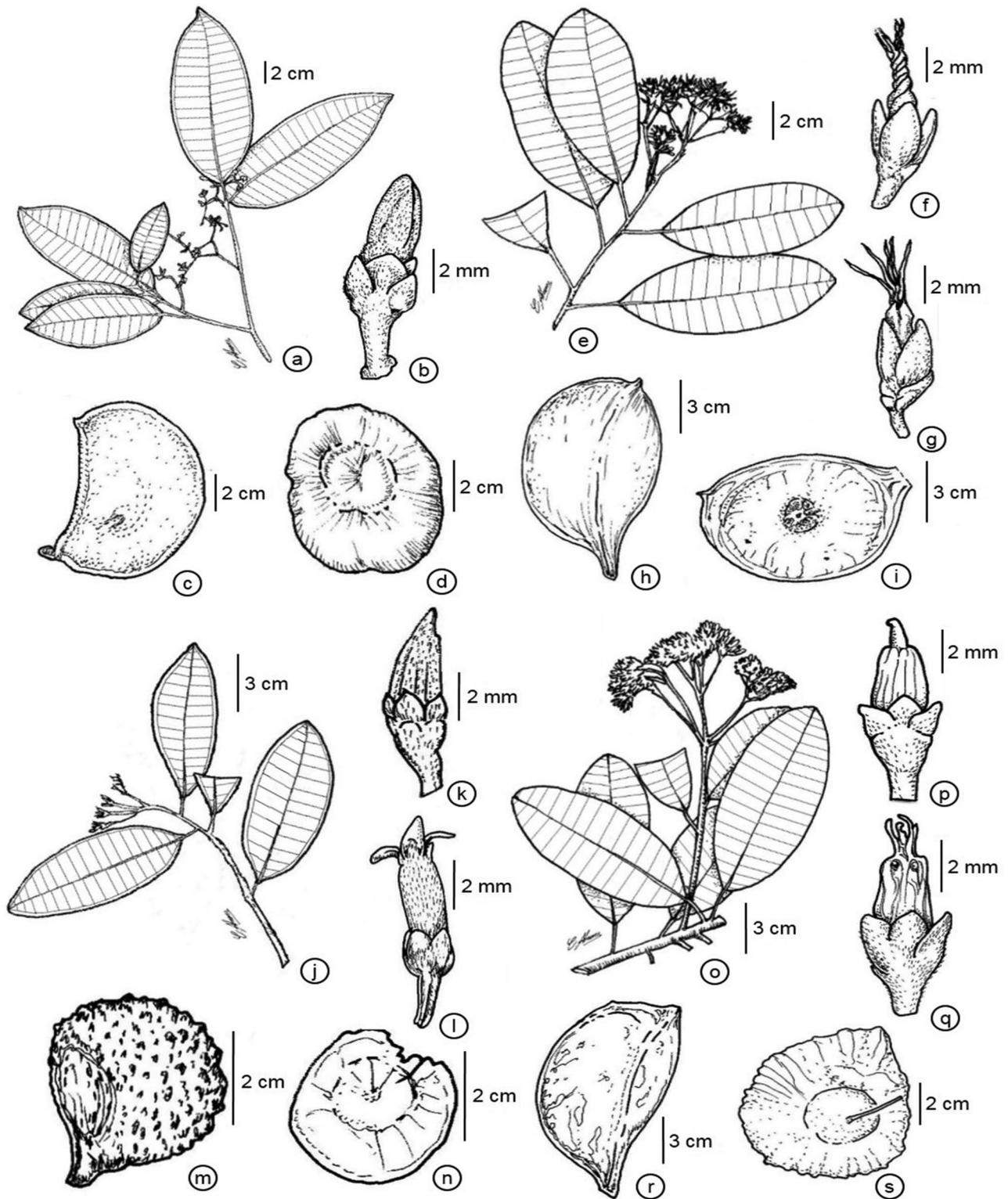
**Phytoecological region and phenology:** Dense Ombrophilous Forest (terra firme and várzea forests). Flowers and fruits collected from September to March.

**Common names:** araracanga-da-várzea, araracanga, garlipo.

*Aspidosperma darienense* occurs in Brazil, Colombia, Ecuador, French Guiana, Panama and Suriname (Missouri Botanical Garden 2015). In the state of Pará, this species was found only in Baixo Amazonas Mesoregion (IBGE 1990). In "The IUCN Red List of Threatened Species 2015.2" (IUCN 2015), *Aspidosperma darienense* was assigned to the Endangered category (EN). According to Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma darienense* belongs to section *Polyneura*. This species is easily distinguished from the other species analysed by the calyx, which has 6-7 lobes. In fact, according to Marcondes-Ferreira (1988) this feature distinguishes *Aspidosperma darienense* from all other species in the genus, as well as almost all other species from Apocynaceae.

**6. *Aspidosperma desmanthum*** Benth. ex Müll.Arg. in C.F.P. von Martius & auct. suc. (eds.), Fl. Bras. 6(1): 51. 1860. (Figure 3e-i).

Trees 6-35 m high; trunk straight. Branches cylindrical, suberous, sparsely lenticellate, pubescent to glabrescent, without cataphylls; latex reddish. Leaves alternate, arranged along the branches; petioles 0.6-2.1 cm long, pubescent to glabrescent; blade 7.2-11.5 x 2.7-5.5 cm, coriaceous, flat, elliptic or obovate, apex acute, retuse or emarginate, base cuneate or oblique, margin straight or revolute, discolorous, venation craspedodromous,



**Figure 3.** a-d. *Aspidosperma darriense*. e-i. *A. desmanthum*. j-n. *A. discolor*. o-s. *A. eteanum*: a, e, j, o. flowering branch; b, f, k, p. flower bud; g, l, q. flower; c, h, m, r. follicle; d, i, n, s. seed. a-d. N.T. Silva 1325; M.J. Pires & N.T. Silva 1390; R.J. Mejía 4913. e-i. M. Pacheco et al. 164; R. Souza 10348. j-n. A.P. Duarte 8179; H.S. Irwin et al. 17160; R.L. Fróes 33480. o-s. A. Ducke s.n. (RB 22445); E. Oliveira 4033.

**Figura 3.** a-d. *Aspidosperma darriense*. e-i. *A. desmanthum*. j-n. *A. discolor*. o-s. *A. eteanum*: a, e, j, o. ramo florido; b, f, k, p. botão floral; g, l, q. flor; c, h, m, r. foliculo; d, i, n, s. semente. a-d. N.T. Silva 1325; M.J. Pires & N.T. Silva 1390; R.J. Mejía 4913. e-i. M. Pacheco et al. 164; R. Souza 10348. j-n. A.P. Duarte 8179; H.S. Irwin et al. 17160; R.L. Fróes 33480. o-s. A. Ducke s.n. (RB 22445); E. Oliveira 4033.

adaxial surface lustrous, olive green or brown, glabrous, primary vein flat, secondary veins impressed, tertiary veins inconspicuous, abaxial surface dull, light green, glabrous, primary vein prominent, secondary veins impressed, 21-26 pairs, tertiary veins inconspicuous. Inflorescences 6.1-10.3 cm long, terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes twisted. Flowers 5-11 mm long; pedicellate, pedicel 0.2-2.3 x 0.5-1 mm, tomentose. Calyx 2-3.5 x 2-3 mm, tomentose externally, pubescent at apex internally; lobes 5, 2-2.5 x 1-1.2 mm, lanceolate or ovate, apex acute. Corolla 3.5-8 x 1.5-2 mm, salverform, yellow, glabrous externally, tomentose below the anthers internally; lobes 2.5-5 x 0.3 mm, erect, filiform, apex acute. Stamens 1.5-2.5 mm long; filaments 1-2 mm long, tomentose; anthers 0.5 mm long, ovate, apex acute. Carpels 1.5 mm long; ovary 0.5 x 0.7 mm, globoid, glabrous; style 0.7 mm long; style-head 0.3 mm long, main body oblong, with 2 oblong apical appendages. Follicles 10.4-10.6 x 6.8-7.2 cm, dolabriform, sulcate, stipitate, mucronate, lenticels inconspicuous, yellow, pubescent to glabrescent. Seeds 6.9-7.3 cm diam., orbicular; seminal nucleus central, without radial lines, nucleus 1.5-2 cm diam.

**Material examined:** BRAZIL, PARÁ: Itaituba, Rio Tapajós, 1.IX.1916, fl., A. Ducke s.n. (RB 22439); Itaituba, Rio Tapajós, Pimental, 5.II.1917, st., A. Ducke s.n. (RB 22442); Itaituba, Rio Tapajós próximo a boca do Rio Rato, margem direita do Rio Tapajós, s.d., fr., I.L. do Amaral et al. 3738 (INPA); Jacareacanga, Parque Nacional do Tapajós, Ilha do Pacú no Tapajós, 30.XI.1978, fr., M.G. Silva & C. Rosário 4031 (MG, NY-scanned image).

**Additional material:** BRAZIL, ACRE: Cruzeiro do Sul, nos arredores do acampamento do projeto RADAM, próximo do aeroporto novo, 11.II.1976, st., D.P. Monteiro & C. Damião 321 (MG); AMAZONAS: Manaus, XI.1942, fr., A. Ducke 1156 (MG); Manaus, Distrito Agropecuário, Fazenda Porto Alegre, reserva 3402 (Cabo Frio) of the WWF/INPA MCS project, 02°25'25"S, 59°54'38"W, 28.I.1989, fl., M. Pacheco et al. 164 (NY-scanned image); Manaus, Reserva Florestal Ducke, Manaus-Itacoatiara, km 26, 23.IV.1995, fl., P. A.C.L. Assunção & E.C. Pereira 190 (MG, RB); MATO GROSSO: Aripuanã, Rio Juruena, Fontanilha, arredores do aeroporto, 18.VII.1977, fl., M.G. Silva 3375 (MG); Expedition base camp, 12°49'S, 51°46'W, just north of base camp, 29. IX.1968, fr., R. Souza 10348 (RB); RONDÔNIA: Basin of Rio Madeira, Rio Laje on road Guajará-Mirim to Ribeião, 3. VIII.1968, fl., G.T. Prance et al. 6741 (MG).

**Phytocological region and phenology:** Dense Ombrophilous Forest (terra firme forest). Flowers and fruits collected from September to November.

**Common names:** araracanga, piquiá-marfim-do-roxo, quina-da-mata.

*Aspidosperma desmanthum* occurs in Belize, Bolivia, Brazil, Costa Rica, Guatemala, Mexico, Nicaragua and Panama (Missouri Botanical Garden 2015). In the state of Pará, this species was found only in Sudoeste Paraense Mesoregion (IBGE 1990). In "Lista de Espécies da Flora e da Fauna Ameaçadas no Estado do Pará" (Pará 2007), *Aspidosperma desmanthum* was listed as a Vulnerable species (VU). According to Marcondes-Ferreira & Kinoshita (1996), it belongs to section *Nobilia*. Among the studied species, *Aspidosperma desmanthum* was one of the most difficult to distinguish. This species shares morphological features with both *Aspidosperma album* and *A. araracanga*, mainly flower structures, but differs from them by the impressed secondary veins on both leaf surfaces and dolabriform follicle. *Aspidosperma desmanthum*

also shares morphological features with *A. eteanum*, especially leaf traits, but differs from *A. eteanum* by the impressed secondary veins on the abaxial surface, salverform corolla, length of corolla lobes (2.5-5 mm long) and orbicular seeds. *Aspidosperma desmanthum* is most often confused with *A. spruceanum*, which was highlighted by Marcondes-Ferreira (1988), but can be distinguished from *A. spruceanum* by the impressed secondary veins on both leaf surfaces.

**7. *Aspidosperma discolor*** A.DC. in A.P.de Candolle, Prodr. 8: 398. 1844. (Figure 3j-n).

Trees 6-28 m high; trunk tortuous. Branches cylindrical, not suberous, densely lenticellate, pubescent to glabrescent, without cataphylls; latex whitish. Leaves alternate, arranged along the branches; petioles 1-1.2 cm long, pubescent; blade 6.6-8.3 x 2-3.5 cm, chartaceous to subcoriaceous, flat, elliptic or ovate, apex acuminate or acute, base cuneate or oblique, margin revolute, discolorous, venation brochidodromous, with marginal vein 1 mm from the margin, adaxial surface lustrous, olive green, glabrous, primary vein flat, secondary veins prominent, tertiary veins inconspicuous, abaxial surface dull, light green, pubescent, primary vein prominent, secondary veins prominent, 16-22 pairs, tertiary veins inconspicuous. Inflorescences 3.5-4.5 cm long, terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes not twisted. Flowers 6.5-8.5 mm long; pedicellate, pedicel 1.7-3 x 0.7 mm, tomentose. Calyx 2-2.5 x 1-2 mm, tomentose externally, pubescent to glabrous at apex internally; lobes 5, 1-1.5 x 1-2 mm, ovate, apex acute or obtuse. Corolla 6.5-7 x 1.5-2 mm, tubular, white, tomentose externally, pubescent below the anthers internally; lobes 2-3.5 x 0.3 mm, patent, ovate, apex acute. Stamens 3-5 mm long; filaments 2-4 mm long, pubescent; anthers 1 mm long, lanceolate, apex acute. Carpels 1.3-2.8 mm long; ovary 0.7-1 x 1-1.2 mm, globoid, tomentose; style 0.3-1.5 mm long; style-head 0.3 mm long, main body oblong, with 2 oblong apical appendages. Follicles 4-4.5 x 3.5-3.7 cm, dolabriform or suborbicular, spinescent, stipitate, mucronate, lenticels inconspicuous, brown, pubescent. Seeds 3-3.5 cm diam., orbicular; seminal nucleus central, without radial lines, nucleus 1.7 cm diam.

**Material examined:** BRAZIL, PARÁ: Canaã dos Carajás, área da Mina do Sossego, 25.XI.2009, fl., R.D. Ribeiro et al. 1387 (MG, RB).

**Additional material:** BRAZIL, GOIÁS: Goiânia, Alexânia, 13.VII.1964, fr., A.P. Duarte 8179 (RB); Luziânia, Fazenda Suindara do Alagado, ponto 14, 08.XI.2002, fl., G. Pereira-Silva et al. 6967 (UEC); MARANHÃO: Santa Quitéria, Fazenda Marflora, 7.IX.1993, fr., B.A.S. Pereira 2519 (UEC); MATO GROSSO: Barra do Garças, Serra do Taquaral, mata em grotão, 24.XI.1997, fl., L.C. Bernacci & G.F. Árbocz 2593 (IAC, UEC); Drainage of the upper Rio Araguaia, lower slopes, Serra Azul, ca. 85 km S of Xavantina, 15.VI.1966, fr., H.S. Irwin et al. 17160 (NY-scanned image, RB); MINAS GERAIS: Presidente Olegário, região do Vale do Rio Paranaíba, próximo das Cabeceiras do Rio São Francisco, 7.IX.1957, fl., R.L. Fróes 33480 (IAN).

**Phytocological region and phenology:** Savannah. Flowers and fruits collected from November to June.

**Common names:** carapanaúba, peroba, peroba de gomo.

*Aspidosperma discolor* occurs in Brazil, Suriname and Venezuela (Missouri Botanical Garden 2015). In the state of Pará, this species was found only in Sudeste Paraense Mesoregion (IBGE 1990). According to Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma discolor* belongs to section

*Excelsa*. It shares morphological features with both *Aspidosperma oblongum* and *A. salgadoense*, mainly flower structures, but differs from the former by the elliptic or ovate leaf blades with cuneate or oblique base, lanceolate anthers and spinescent follicles, and from the latter by the cylindrical branches and tomentose ovary.

**8. *Aspidosperma eteanum*** Markgr., Notizbl. Bot. Gart. Berlin-Dahlem 12: 297. 1935. (Figure 3o-s).

Trees 12-38 m high; trunk straight. Branches cylindrical, suberous or not, sparsely lenticellate, pubescent, without cataphylls; latex orange to reddish. Leaves alternate, arranged along the branches; petioles 1-2 cm long, pubescent; blade 7.5-12.2 x 2.9-5 cm, coriaceous, flat, oblong or ovate, apex acuminate or acute, base cuneate or oblique, margin straight or revolute, discolorous, venation craspedodromous, adaxial surface lustrous, dark brown, glabrous, primary vein flat, secondary veins impressed, tertiary veins inconspicuous, abaxial surface dull, light brown, glabrous, primary vein prominent, secondary veins prominulous, 24-26 pairs, tertiary veins inconspicuous. Inflorescences 6.2-7.8 cm long, axillary or terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes twisted. Flowers 8-9 mm long; pedicellate, pedicel 1.5-3 x 0.7-1 mm, tomentose. Calyx 2.5-3 x 2.5-3 mm, tomentose externally, pubescent at apex internally; lobes 5, 1.5-2.5 x 1.7-2 mm, ovate, apex acute. Corolla 5-6 x 2 mm, tubular, yellow, glabrous externally, tomentose below the anthers internally; lobes 1-1.5 x 0.3 mm, erect, filiform, apex acute. Stamens 2-3 mm long; filaments 1-2 mm long, tomentose; anthers 1 mm long, ovate, apex acuminate or acute. Carpels 1.5 mm long; ovary 0.6 x 0.7 mm, globose, glabrous; style 0.6 mm long; style-head 0.3 mm long, main body oblong, with 2 oblong apical appendages. Follicles 8-9.5 x 6.5 cm, dolabriform, sulcate, stipitate, mucronate, lenticels inconspicuous, yellow or brown, pubescent. Seeds 6.5 x 6 cm, ovate; seminal nucleus lateral, without radial lines, nucleus 2.5 cm diam.

**Material examined:** BRAZIL, PARÁ: Almeirim, Monte Dourado, 28.I.1968, fr., E. Oliveira 4033 (IAN); Almeirim, Serra de Almeirim, 24.VIII.1918, fl., A. Ducke s.n. (RB 22445); Altamira, margem direita do Rio Ariri (serraria Banach), 28.VIII.1986, fl., A.T.G. Dias et al. 103 (MG); Aveiro, região do Tapajós, Boa Vista, 12.IX.1932, fl., P. Capucho 432 (F-scanned image); Conceição do Araguaia, range of low hills ca. 20 km west of Redenção, near Corrego São João and Troncamento Santa Teresa, 11.II.1980, fr., T. Plowman et al. 8703 (F-scanned image, IAN, INPA, MG); Concórdia do Pará, Cristo Rei, 15.I.2002, fr., M.R. Cordeiro MC-19-10 (100.471) (IAN); Marabá, Rio Itacaiunas, afl. do Rio Tocantins, Serra Buritirama (B 5), região com minério de manganês, árvore 5-26-13, VIII.1970, st., J.M. Pires & R.P. Belem 12736 (IAN); Melgaço, Estação Científica Ferreina Penna, trilha central, 24.VI.2004, fl., J. Oliveira et al. 868 (MG); Oriximiná, Lago Salgado (Baixo Trombetas), 22.IV.1917, st., A. Ducke s.n. (RB 22444); Pau D'Arco, Santana do Araguaia, 21.VIII.1998, st., J. Grogan 605 (IAN); Porto de Moz, Rio Perí, afl. do Xingu, região onde foi feito um levantamento estatístico florestal pelo IAN, SPVEA e FAO, 29.XI.1955, fl., R.L. Fróes 32430 (IAN); São João do Araguaia, Parque da Serra dos Martírios (Andorinhas), 18.IX.2002, st., M.R. Cordeiro MC-29-18 (100.587) (IAN); Tucuruí, estrada para Repartimento, km 25, 4.VI.1980, fr., M.G. Silva & C. Rosário 5364 (INPA, MG); Vitória do Xingu, Usina Hidrelétrica Belo Monte, 23.X.2012, fl., C. Faveri PSACF 756 (MG).

**Additional material:** BRAZIL, AMAPÁ: Mazagão, área do Felipe, 3.VII.1984, fl., N.T. Silva 5381 (MG); AMAZONAS: Manaus, Reserva Florestal Ducke, Manaus-Itacoatiara, km 26, 29.V.1995, fl., P.A.C.L. Assunção & E.C. Pereira 201 (MG); RORAIMA: Alto Alegre, Estação Ecológica de Maracá, Grade do PPBio, trilha N01, segmento 1000-1100, 18.V.2012, fr., R.O. Perdiz et al. 1361 (INPA).

**Phytocological region and phenology:** Dense Ombrophilous Forest (terra firme forest). Flowers and fruits collected from June to February.

**Common names:** araracanga, araracanga-preta, jararacanga, muirapyranga.

According to Woodson (1951), *Aspidosperma eteanum* is a species endemic to Brazil, and occurs in the states of Amapá, Amazonas, Maranhão, Pará and Roraima (Missouri Botanical Garden 2015). In the state of Pará, this species was found in Baixo Amazonas, Marajó, Nordeste Paraense, Sudeste Paraense and Sudoeste Paraense Mesoregions (IBGE 1990). Marcondes-Ferreira (1988) reduced *Aspidosperma eteanum* to be synonymous with *A. desmanthum* (section *Nobilia*), but we decided to keep it as an accepted species due to observed differences between the specimens of the two species. *Aspidosperma eteanum* resembles *A. desmanthum*, especially leaf traits, but differs from *A. desmanthum* by the prominulous secondary veins on the abaxial surface, tubular corolla, length of corolla lobes (1-1.5 mm long) and ovate seeds.

**9. *Aspidosperma excelsum*** Benth., J. Bot. (Hooker) 3: 245. 1841. (Figure 4a-e).

Trees 18-48 m high; trunk tortuous. Branches cylindrical, not suberous, sparsely lenticellate, pubescent to glabrous, without cataphylls; latex whitish. Leaves alternate, arranged along the branches; petioles 1.2-1.9 cm long, tomentose; blade 7-8.9 x 2.5-3.4 cm, coriaceous, flat, elliptic or obovate, apex acute, obtuse or retuse, base cuneate or oblique, margin revolute, discolorous, venation brochidodromous, without marginal vein, adaxial surface lustrous, dark brown, glabrous, primary vein flat, secondary veins impressed, tertiary veins inconspicuous, abaxial surface dull, light brown, tomentose along the primary vein, primary vein prominent, secondary veins impressed, 22-24 pairs, tertiary veins inconspicuous. Inflorescences 5.7-6.5 cm long, supra-axillary or terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes not twisted. Flowers 6.2-7.5 mm long; pedicellate, pedicel 1.5-2 x 1 mm, tomentose. Calyx 2.5-3 x 2.5 mm, tomentose externally, pubescent at apex internally; lobes 5, 1.5-3 x 1-1.5 mm, ovate, apex acute or obtuse. Corolla 5.5-6.2 x 1.5 mm, tubular, white, tomentose externally, tomentose below the anthers internally; lobes 1-1.5 x 0.3 mm, patent, ovate, apex acute. Stamens 3-4 mm long; filaments 2.5-3.2 mm long, tomentose; anthers 0.5-0.8 mm long, ovate, apex acute. Carpels 2.9-3.9 mm long; ovary 0.5-1 x 0.5 mm, ovoid, glabrous; style 2-2.5 mm long; style-head 0.4 mm long, main body oblong, with 2 oblong apical appendages. Follicles 4.5-6 x 4-5 cm, dolabriform, spinescent, stipitate, mucronate, lenticels inconspicuous, brown, pubescent. Seeds 4-5 cm diam., orbicular; seminal nucleus central, without radial lines, nucleus 1.2 cm diam.

**Material examined:** BRAZIL, PARÁ: Almeirim, região do Rio Jarí, Planalto de Monte Alegre, 5.IX.1968, fl., E. Oliveira 4841 (IAN, NY-scanned image); Altamira, Xingu River, Assurini indians, 13.VI.1986, st., W. Balée 2491 (NY-scanned image); Belém, Jardim Botânico da Amazônia Bosque Rodrigues Alves, canteiro 75, árvore n° 2270, 24.IX.2014, fr., A.S. de S. Pereira 97 (MG); Breves, local onde foi feito um inventário

florestal, X-XI.1957, fr., J.M. Pires & N.T. Silva 6645 (IAN); Concórdia do Pará, Sítio São José, 13.II.2002, st., M.R. Cordeiro MC-24-02 (100.510) (IAN); Gurupá, 19.VIII.1918, st., A. Ducke s.n. (MG 17224); Ipixuna, 7.VII.1966, st., A.P. Duarte 9804 (RB); Itaituba, 27.VI.1972, st., J.B. Rodrigues 53 (R); Jacareacanga, Rio Tapajós, estrada das cachoeiras, lugar Periquito, 7.XII.1915, st., A. Ducke s.n. (MG 15860); Mãe do Rio, Vila Mãe do Rio, 26.III.1998, st., N.A. Rosa 5744 (MG); Marituba, mata da Cia. Pirelli, Fazenda Uribóca, 12.IX.1958, fl., J.M. Pires 7141 (IAN); Moju, campo experimental da Embrapa Amazônia Oriental no km 30 da rodovia PA-150, margem do ramal atrás do 3° pátio da exploração, 13.II.1998, st., G.C. Ferreira & J.C. Freitas 422 (IAN); Monte Alegre, Macau airstrip, 1 1/2 hrs. upstream from Lageira airstrip, on Rio Maicurú, 28.VII.1981, st., J.J. Strudwick et al. 3632 (MG); Oriximiná, 3.III.1915, fr., A. Ducke s.n. (MG 15703); Pau D'Arco, Marajoara, s.d., st., J. Grogan 22 (IAN); Santa Bárbara, entrada do Genipaúba, 9.V.2013, fr., R.C.S. Trindade & M.R.C. Ferreira 1 (MG, UEC); Santana do Araguaia, 100 km south of Redenção on road (PA-150) to Barreiras dos Campos, Fazenda Inajaporã between Rio Inajazinho and Rio Inajá, 19.II.1980, fr., T. Plowman et al. 8943 (INPA, MG); Santarém, Reserva Curuá-Una, Parque Fenológico, árvore n° 173, 14.VI.1999, st., M.R. Cordeiro & N.J. Moraes 3900 (IAN); Tucuruí, Transgoiânia, área nuclear n° 4, III.1981, st., P. Lisboa et al. 2451 (MG).

**Additional material:** BRAZIL, AMAZONAS: Manaus, Cachoeira Baixa do Tarumã, margem inundada ao pé da cachoeira, 9.IX.1940, fl., A. Ducke 624 (MG); Manaus, 24.XII.1942, fr., A. Ducke 1163 (NY-scanned image); Manaus, estrada da Reserva Florestal Ducke, 7.III.1958, fr., Pessoal do C.P.F. s.n. (RB 125179).

**Phytoecological region and phenology:** Dense Ombrophilous Forest (terra firme forest). Flowers and fruits collected from September to May.

**Common names:** carapanaúba, carapanaúba-amarela, carapanaúba-do-baixio, carapanaúba-da-terra-firme, kina, pepembyra.

*Aspidosperma excelsum* is a widely distributed species, occurring in Bolivia, Brazil, Colombia, Costa Rica, French Guiana, Guyana, Panama, Peru, Suriname and Venezuela (Missouri Botanical Garden 2015). In the state of Pará, this species was found in all Mesoregions (IBGE 1990). According to Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma excelsum* belongs to section *Excelsa*. It shares morphological features with *Aspidosperma oblongum*, especially flower structures, but can be differentiated from *A. oblongum* by the glabrous ovary, spinescent follicles and leaves without a marginal vein.

**10. *Aspidosperma inundatum*** Ducke, Arch. Jard. Bot. Rio de Janeiro 3: 245. 1922. (Figure 4f-j).

Trees 10-18 m high; trunk straight. Branches cylindrical, not suberous, sparsely lenticellate, pubescent to glabrescent, without cataphylls; latex whitish. Leaves alternate, arranged along the branches; petioles 1-1.5 cm long, pubescent; blade 7.2-8.5 x 3-5 cm, chartaceous to subcoriaceous, flat, oblong or elliptic, apex acuminate or acute, base cuneate or oblique, margin revolute, discolorous, venation brochidodromous, with marginal vein 1.2 mm from the margin, adaxial surface lustrous, dark brown, glabrous, primary vein flat, secondary veins impressed, tertiary veins inconspicuous, abaxial surface dull, light brown, pubescent to glabrescent, primary vein prominent, secondary veins prominent, 23-25 pairs, tertiary veins inconspicuous. Inflorescences 6.1-7 cm long, axillary or supra-axillary, corymbiform dichasia, tomentose.

Flower buds with corolla lobes not twisted. Flowers 17.5-18.5 mm long; pedicellate, pedicel 2-3 x 1 mm, tomentose. Calyx 3-3.5 x 2.1-2.5 mm, tomentose externally, pubescent at apex internally; lobes 5, 2-2.1 x 2 mm, ovate, apex acute. Corolla 15-15.7 x 2 mm, salverform, white, tomentose externally, pubescent along the corolla internally; lobes 9.7-10 x 2 mm, erect or deflexed, oblong or lanceolate, apex acute or obtuse. Stamens 5.5-6 mm long; filaments 3.5-4 mm long, tomentose; anthers 2 mm long, lanceolate, apex apiculate or acute. Carpels 3.5-3.8 mm long; ovary 2-2.3 x 1-1.5 mm, ovoid, tomentose; style 1 mm long; style-head 0.5 mm long, main body oblong, with 2 filiform apical appendages. Follicles 8-8.5 x 6 cm, dolabriform, smooth, sessile, mucronate, lenticels conspicuous, black, glabrous. Seeds 7.1-7.5 x 6.1-5.7 cm, oblong; seminal nucleus central, without radial lines, nucleus 3.6 cm diam.

**Material examined:** BRAZIL, PARÁ: Breves, Antônio Lemos, Rio Amazonas, Igarapé Pixuna, beira do Rio Tajapurú, 19.VII.1948, fl., G.A. Black 48-2946 (IAC, IAN); Gurupá, silva ab Amazonum fluvio inundata, 13.VIII.1918, fl., A. Ducke s.n. (NY 297971-scanned image, RB 15814); Oriximiná, Lago Salgado, Rio Trombetas, 9.IX.1927, fl., A. Ducke s.n. (RB 21808).

**Additional material:** BRAZIL, AMAPÁ: Macapá, Rio Carapanã Branca, 6.I.1977, fr., E. Oliveira 6530 (MG).

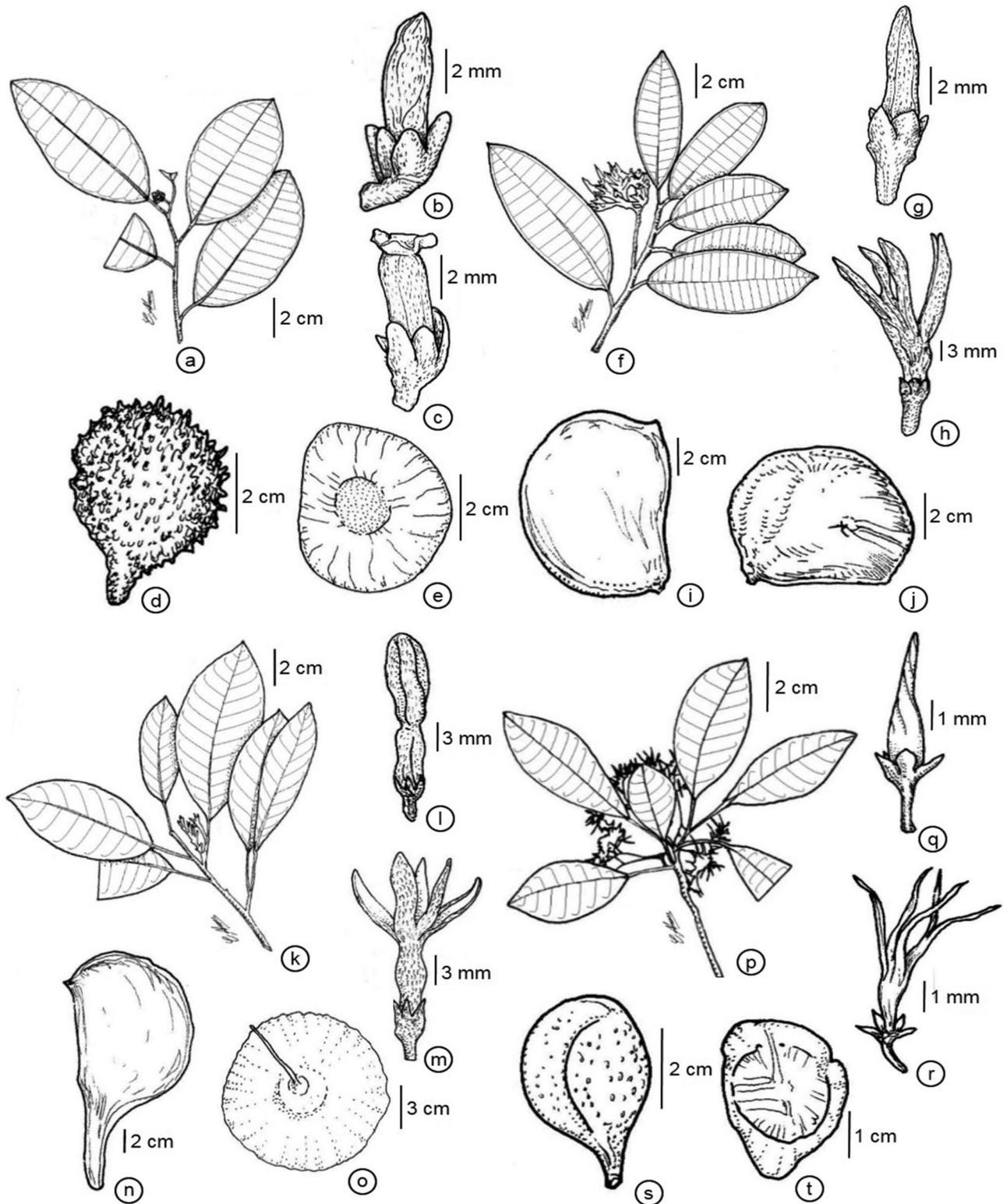
**Phytoecological region and phenology:** Dense Ombrophilous Forest (várzea forest). Flowers and fruits collected from July to January.

**Common names:** araracanga, jacamiramane, maparaná.

According to Koch et al. (2015), *Aspidosperma inundatum* is a species endemic to Brazil, and occurs in the states of Amapá, Amazonas, Mato Grosso and Pará. In the state of Pará, this species was found in Baixo Amazonas and Marajó Mesoregions (IBGE 1990). In Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma inundatum* is placed in the monospecific section *Inundata*. *Aspidosperma inundatum* was one of the most easily recognized species, and can be differentiated by the larger flowers (17.5-18.5 mm long) and the sessile black follicles with conspicuous lenticels.

**11. *Aspidosperma macrocarpon*** Mart., Flora 7(1): 136. 1824. (Figure 4k-o).

Trees 3-35 m high; trunk straight. Branches cylindrical, not suberous, sparsely lenticellate, velutinous to tomentose, without cataphylls; latex whitish. Leaves alternate, arranged along the branches; petioles 1-4.5 cm long, velutinous to tomentose; blade 9-20.1 x 7-15 cm, chartaceous, flat, oblong or ovate, apex acute or obtuse, base cuneate or oblique, margin straight or revolute, discolorous, venation eucamptodromous, adaxial surface dull or lustrous, dark brown, velutinous, tomentose or pubescent, primary vein prominulous or prominent, secondary veins prominulous, tertiary veins conspicuous, abaxial surface dull, light brown, velutinous to tomentose, primary vein prominent, secondary veins prominent, 10-13 pairs, tertiary veins conspicuous. Inflorescences 5-7 cm long, axillary or terminal, corymbiform dichasia, velutinous to tomentose. Flower buds with corolla lobes not twisted. Flowers 15-20 mm long; pedicellate, pedicel 2-2.5 x 1 mm, tomentose. Calyx 2.5-3 x 3-3.5 mm, tomentose externally, pubescent at apex internally; lobes 5, 1.5 x 1 mm, ovate, apex acute. Corolla 15-16 x 2 mm, salverform, white, tomentose externally, pubescent to glabrescent along the corolla internally; lobes 10-12 x 4 mm, erect or deflexed, oblong or obovate, apex obtuse. Stamens 5-6 mm long; filaments 3.5-4.5 mm long, pubescent to glabrescent; anthers 1.5 mm long, ovate,



**Figure 4.** a-e. *Aspidosperma excelsum*. f-j. *A. inundatum*. k-o. *A. macrocarpon*. p-t. *A. multiflorum*: a, f, k, p. flowering branch; b, g, l, q. flower bud; c, h, m, r. flower; d, i, n, s. follicle; e, j, o, t. seed. a-e. A. Ducke 624; A. Ducke 1163; Pessoa do C.P.F. s.n. (RB 125179). f-j. A. Ducke s.n. (NY 297971, RB 15814); G.A. Black 48-2946. k-o. C.A. Cid et al. 1378; C.R. Sperling et al. 6248; U.N. Maciel & M.R. Cordeiro 120. p-t. A.M. Miranda et al. 5310; G.A. Black 47-1740.

**Figura 4.** a-e. *Aspidosperma excelsum*. f-j. *A. inundatum*. k-o. *A. macrocarpon*. p-t. *A. multiflorum*: a, f, k, p. ramo florido; b, g, l, q. botão floral; c, h, m, r. flor; d, i, n, s. folículo; e, j, o, t. semente. a-e. A. Ducke 624; A. Ducke 1163; Pessoa do C.P.F. s.n. (RB 125179). f-j. A. Ducke s.n. (NY 297971, RB 15814); G.A. Black 48-2946. k-o. C.A. Cid et al. 1378; C.R. Sperling et al. 6248; U.N. Maciel & M.R. Cordeiro 120. p-t. A.M. Miranda et al. 5310; G.A. Black 47-1740.

apex apiculate. Carpels 3.7 mm long; ovary 1 x 1 mm, globose, glabrous; style 2 mm long; style-head 0.7 mm long, main body oblong, with 2 oblong apical appendages. Follicles 8.2-12 x 7-10.4 cm, dolabriform, smooth, stipitate, mucronate, lenticels inconspicuous, brown, velutinous to pubescent. Seeds 8.2-10 cm diam., orbicular; seminal nucleus central, without radial lines, nucleus 4 cm diam.

**Material examined:** BRAZIL, PARÁ: Almeirim, Monte Dourado, área do Ideal, 14.X.1980, fl., N.T. Silva 5448 (INPA, MG); Altamira, região Garotire, 8.VIII.1962, fl., N.T. Silva 786 (IAN); Belterra, beira do Rio Tapajós, Pindobal, 27.X.1947, fr., G.A. Black 47-1827 (IAC, IAN); Belterra, margem da estrada Pindobal-Porto Novo, 9.XII.1978, fl., R. Vilhena et al. 182 (MG, NY-scanned image); Faro, Terra Santa, 4.VII.1975, st., S. Assunção & D. Coelho 35 (INPA); Monte Alegre, Rio Maicuru, mata entre Caá-ussú e localidade de Balança, 16.IX.1953, fl., R.L. Fróes 30242 (IAN); Óbidos, 20.X.1919, fl., A. Ducke s.n. (RB 11402); Oriximiná, Rio Trombetas, margem da estrada, mineração Santa Patricia, 8.VII.1980, fr., C.A. Cid et al. 1378 (INPA, MG); Parauapebas, Serra dos Carajás, 10 km east of AMZA camp N-1 and 5-10 km along the entrance road to Azul, an abandoned manganese exploration camp, 19.VI.1982, fl., C.R. Sperling et al. 6248 (MG); Santarém, beira da estrada que liga Belterra a Porto Novo, 4.XII.1978, fr., M.G.A. Lobo et al. 108 (MG, NY-scanned image); Santarém, estrada de Belterra, 6.X.1962, fl., A. P. Duarte 7016 (RB); Santarém, Porto Novo-Aramanai, 2.XII.1978, fr., U.N. Maciel & M.R. Cordeiro 120 (MG); São Geraldo do Araguaia, morro 3, 15.VI.1995, fr., M.N. Bastos & M.R. Cordeiro 2015 (IAN, MG).

**Additional material:** BRAZIL, MATO GROSSO: Cuiabá, X.1914, st., J.G. Kuhlmann 1227 (R); Entroncamento das rodovias, Cuiabá-Santarém e Porto Velho, arredores do aeroporto, 4.II.1979, fr., M.G. Silva & A. Pinheiro 4432 (MG); Sacaré, campo base do RADAMBRAZIL, folha SD 21-YC, rodovia BR-416 entre Pontes de Lacerda e Mato Grosso (ex Vila Bela), 2.VIII.1978, fr., J.M. Pires & M.R. Santos 16347 (MG); RONDÔNIA: Basin of Rio Madeira, Serra dos Murales, 14 km NNW of junction of Rios Madeira and Abunã, 14.VII.1968, fl., G.T. Prance et al. 6030 (MG).

**Phytocological region and phenology:** Dense Ombrophilous Forest (terra firme forest) and Savannah. Flowers and fruits collected from June to December.

**Common names:** marajussara, muirajuçara, muirajussara, peroba, peroba-branca, peroba-mico.

The information about the protologue of *Aspidosperma macrocarpon* was checked in its original publication. The guide "Taxonomic Literature" (Stafleu & Cowan 1981), also states that *Aspidosperma macrocarpon* was first described in "Flora" by Martius (1824a) and not in "Nova Genera et Species Plantarum" by the same author (Martius 1824b). In the databases "Tropicos" (Missouri Botanical Garden 2015) and "World Checklist of Selected Plant Families" (WCSP 2015), this information is conflicting. *Aspidosperma macrocarpon* occurs in Bolivia, Brazil, Peru and Venezuela (Missouri Botanical Garden 2015). In the state of Pará, this species was found in Baixo Amazonas, Sudeste Paraense and Sudoeste Paraense Mesoregions (IBGE 1990). According to Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma macrocarpon* belongs to section *Aspidosperma*. This species can be easily differentiated from the other species in section *Aspidosperma* occurring in Pará by the larger follicles (8.2-12 x 7-10.4 cm)

with inconspicuous lenticels and branches without cataphylls covering the buds.

**12. *Aspidosperma multiflorum* A.DC.** in A.P.de Candolle, Prodr. 8: 397. 1844. (Figure 4p-t).

Trees 2-22 m high; trunk straight. Branches cylindrical, not suberous, densely lenticellate, pubescent to glabrescent, with cataphylls; latex whitish. Leaves alternate, congested at apex of branches; petioles 2-3 cm long, pubescent to glabrous; blade 5.5-6.3 x 3.5-4.5 cm, membranaceous, flat, elliptic, apex acuminate or acute, base attenuate or oblique, margin straight, concolorous, venation eucamptodromous, adaxial surface dull, light brown, pubescent to glabrous along the primary vein, primary vein flat, secondary veins prominulous, tertiary veins inconspicuous, abaxial surface dull, light brown, pubescent to glabrous along the primary vein, primary vein flat or prominent, secondary veins prominulous, 14-18 pairs, tertiary veins conspicuous. Inflorescences 4-5.3 cm long, terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes twisted. Flowers 6-6.5 mm long; pedicellate, pedicel 1-1.3 x 0.2 mm, glabrous. Calyx 0.5-1 x 1 mm, glabrous externally, pubescent at apex internally; lobes 5, 0.5-0.8 x 0.3-0.5 mm, ovate, apex acute. Corolla 5 x 0.5 mm, salverform, orange, glabrous externally, pubescent along the corolla internally; lobes 3.5-4 x 0.2 mm, erect or deflexed, filiform, apex acute. Stamens 1.8-2 mm long; filaments 1.3-1.5 mm long, pubescent; anthers 0.5 mm long, ovate, apex acute. Carpels 1.6 mm long; ovary 0.5 x 0.5 mm, globose, glabrous; style 0.8 mm long; style-head 0.3 mm long, main body oblong, with 2 ovate apical appendages. Follicles 3-4 x 2-3 cm, pyriform or suborbicular, smooth, stipitate, mucronate or not, lenticels conspicuous, brown, glabrous. Seeds 3-3.5 x 2.2-2.5 cm, ovate; seminal nucleus central, without radial lines, nucleus 1.5-2 cm diam.

**Material examined:** BRAZIL, PARÁ: Belterra, beira do Rio Tapajós, 24.X.1947, fr., G.A. Black 47-1740 (IAC, IAN); Belterra, estrada Porto Novo-Pindobal, 7.XII.1978, fl., M.G. A. Lobo et al. 204 (IAN, MG); Canaã dos Carajás, 27.XII.2000-6.I.2001, fr., L.C.B. Lobato et al. 2618 (MG); Faro, Campina do Jupiry (Lago de Faro), 25.I.1927, st., A. Ducke s. n. (RB 148804); Monte Alegre, 11.III.1923, st., A. Ducke s. n. (RB 22436); Santarém, região Rio do Tapajós, IX.1957, fl., R. L. Fróes 33564 (IAN); Santarém, região do Planalto de Santarém, onde foi feito um levantamento estatístico florestal pelo IAN, SPVEA e FAO, 28.X.1954, fl., R.L. Fróes 31179 (IAN, RB); Santarém, Taperinha, Paraná do Ituqui, região do Planalto de Santarém, 3.XII.1954, fl., R.L. Fróes 31205 (IAN, R); Santarém, Vila de Alter do Chão, praia arenosa do Rio Tapajós, depois do Lago Jacundá, em direção ao Lago do Pindobal, 4.I.1992, fr., L.V. Ferreira & A.L.K.M. Albernaz 73 (INPA); Tucuruí, approx. 30-35 km from Tucuruí, 25 km on old BR-422, then 5-10 km E on old railroad bed., 31.X.1981, fl., D.C. Daly et al. 1114 (INPA, MG).

**Additional material:** BRAZIL, MARANHÃO: Approx. 40 km E of Barra do Corda toward Presidente Dutra, 10.X.1980, fl., D.C. Daly et al. D568 (MG); MATO GROSSO: Nobres, BR-163 Cuiabá-Sinop a 145 km ao S de Sinop, próximo a Lucas do Rio Verde, 17.IX.1985, fl., C.A.C. Ferreira et al. 6074 (MG); PIAUÍ: Guadalupe, margem da barragem de Boa Esperança, 22.XI.2005, fl., A.M. Miranda et al. 5310 (RB); RONDÔNIA: Vicinity of Santa Bárbara, 15 km east of km 117, Porto Velho to Cuiabá highway, 12.VIII.1968, fl., G.T. Prance & J.F. Ramos 6882 (MG).

**Phytocological region and phenology:** Savannah. Flowers and fruits collected from August to April.

**Common names:** muirajussára-hy.

According to Koch et al. (2015), *Aspidosperma multiflorum* is a species endemic to Brazil, and occurs in more than half of the states. In the state of Pará, this species was found in Baixo Amazonas and Sudeste Paraense Mesoregions (IBGE 1990). In Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma multiflorum* is placed in section *Aspidosperma*. This species resembles *Aspidosperma pyriforme* especially fruit morphology, but differs from *A. pyriforme* by the membranaceous leaves, smaller flowers (6-6.5 mm long), filiform corolla lobes and externally glabrous calyx.

**13. *Aspidosperma oblongum*** A.DC. in A.P.de Candolle, Prodr. 8: 399. 1844. (Figure 5a-e).

Trees 13-42 m high; trunk tortuous. Branches cylindrical, not suberous, densely lenticellate, pubescent to glabrescent, without cataphylls; latex whitish. Leaves alternate, arranged along the branches; petioles 0.7-2.1 cm long, glabrous; blade 5-8.1 x 2.3-3 cm, chartaceous, flat, oblong, apex acuminate or acute, base revolute, margin straight or revolute, discolorous, venation brochidodromous, with marginal vein 1 mm from the margin, adaxial surface lustrous, black, glabrous, primary vein flat, secondary veins prominent, tertiary veins inconspicuous, abaxial surface dull, light brown, pubescent, primary vein prominent, secondary veins prominent, 24-26 pairs, tertiary veins inconspicuous. Inflorescences 6.1-14 cm long, terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes not twisted. Flowers 7-8.1 mm long; pedicellate, pedicel 2.7-3.5 x 0.5-0.7 mm, tomentose. Calyx 1.8-2 x 1.5-2 mm, tomentose externally, pubescent at apex internally; lobes 5, 0.8-1 x 0.5-0.7 mm, ovate, apex acute or obtuse. Corolla 4.5-4.7 x 1.5 mm, tubular, white, tomentose externally, pubescent to glabrescent along the corolla internally; lobes 1-1.5 x 0.5 mm, patent, ovate, apex acute. Stamens 2.5-3 mm long; filaments 2-2.2 mm long, pubescent to glabrescent; anthers 0.5-0.8 mm long, ovate, apex apiculate. Carpels 1.4-2.1 mm long; ovary 0.5-0.7 x 0.7 mm, ovoid, tomentose; style 0.5-1 mm long; style-head 0.4 mm long, main body oblong or globose, with 2 oblong apical appendages. Follicles 4.4-6 x 3.2-4 cm, suborbicular, verrucose, stipitate, not mucronate, lenticels inconspicuous, brown, pubescent. Seeds 4 cm diam., orbicular; seminal nucleus central, without radial lines, nucleus 2 cm diam.

**Material examined:** BRAZIL, PARÁ: Almeirim, região do Rio Jarí, estrada entre Planalto B e Braço, 12.IV.1969, fr., N.T. Silva 1855 (IAN); Almeirim, região do Rio Jarí, Monte Dourado, Planalto B, 21.X.1968, fl., N.T. Silva 1285 (IAN); Belterra, 25.VII.1947, st., G. Black 47-1075 F25 (IAC, IAN, UEC); Itaituba-Santarém, árvore n° 29-3-13, XII.1972, fr., J. M. Pires 13820 (IAN); Melgaço, Floresta Nacional de Caxiuanã, local onde será construída a Estação Científica "Ferreira Penna", 2-15.II.1991, st., A.S.L. da Silva et al. 2313 (MG); Melgaço, Floresta Nacional de Caxiuanã, trilha que dá acesso a área do projeto ESECAFLOR, 2.IX.2014, fl., A.S. de S. Pereira et al. 96 (MG); Santarém, Lago Cuçari, região do Planalto de Santarém, onde foi feito o levantamento estatístico florestal pelo IAN, SPVEA e FAO, 13.V.1955, st., R.L. Frões 31817 (IAN); Tucuruí, A.N.5, próx. Rio Macoari, margem direita do Rio Tocantins, 10.IV.1981-29.V.1981, st., U.N. Maciel et al. 649 (MG); Vitória do Xingu, Sítio Pimental, 26. IV.2013, fl., F.A. Raul PSACF 1077 (MG).

**Additional material:** BRAZIL, AMAPÁ: Rio Araguari, camp 13, 5.X.1961, fl., J.M. Pires et al. 51510 (RB); AMAZONAS: Manaus, Reserva Florestal Ducke, Q III, árv.

352, 30.VII.1964, fr., W. Rodrigues & Osmarino 5980 (RB); Manaus-Itacoatiara km 31, 17.IX.1962, fl., A.P. Duarte & Gilbert 6924 (RB). FRENCH GUIANA: s.d., fr., R. Benoist 963 (P-scanned image); Caiena, s.d., fl., Martin s.n. (P 4205125-scanned image).

**Phytoecological region and phenology:** Dense Ombrophilous Forest (terra firme forest). Flowers and fruits collected from September to April.

**Common names:** carapanaúba, carapanaúba-branca, carapanaúba-legítima.

*Aspidosperma oblongum* occurs in Brazil, French Guiana, Guyana, Suriname and Venezuela (Missouri Botanical Garden 2015). In the state of Pará, this species was found in Baixo Amazonas, Marajó, Sudeste Paraense and Sudoeste Paraense Mesoregions (IBGE 1990). Marcondes-Ferreira (1988) reduced *Aspidosperma oblongum* to be synonymous with *A. discolor* (section *Excelsa*), but based on observed differences between the specimens of the two species, we decided to recognize *A. oblongum* as an accepted species. *Aspidosperma oblongum* shares morphological features with both *A. discolor* and *A. excelsum*, mainly flower structures, but differs from the former by the oblong leaf blades with revolute base, ovate anthers and verrucose follicles, and from the latter by leaves with a marginal vein, tomentose ovary and verrucose follicles.

**14. *Aspidosperma pyriforme*** Mart., Flora 7(1): 136. 1824. (Figure 5f-j).

Trees 3-12 m high; trunk straight. Branches cylindrical, not suberous, densely lenticellate, glabrescent to glabrous, with cataphylls; latex whitish. Leaves alternate, congested at apex of branches; petioles 0.7-2 cm long, tomentose, pubescent or glabrescent; blade 6-9.7 x 2.5-4 cm, chartaceous, flat, oblong, elliptic or ovate, apex acuminate, acute or obtuse, base attenuate or oblique, margin revolute, concolorous, venation eucamptodromous, adaxial surface dull or lustrous, dark brown, pubescent to glabrescent, primary vein flat, secondary veins prominent, tertiary veins inconspicuous, abaxial surface dull, dark brown, velutinous to glabrescent, primary vein prominent, secondary veins prominent, 12-17 pairs, tertiary veins conspicuous. Inflorescences 3.4-6.5 cm long, terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes twisted. Flowers 14-23 mm long; pedicellate, pedicel 1-5 x 0.5-1 mm, pubescent. Calyx 0.5-1 x 1.8 mm, tomentose externally, glabrous internally; lobes 5, 2-3 x 0.5-0.7 mm, lanceolate, apex acuminate. Corolla 12-15 x 1.2 mm, salverform, white, pubescent externally, pubescent at base internally; lobes 10-13 x 3 mm, deflexed, oblong, apex acute. Stamens 2-2.5 mm long; filaments 1-1.5 mm long, pubescent; anthers 1 mm long, ovate, apex acute. Carpels 2 mm long; ovary 0.7 x 0.5 mm, globoid, glabrous; style 1 mm long; style-head 0.3 mm long, main body globose, with 2 oblong apical appendages. Follicles 4.3-5 x 2-2.5 cm, pyriform or suborbicular, smooth, stipitate, mucronate or not, lenticels conspicuous, brown, glabrous. Seeds 3.5-6 x 2.2-4.7 cm, ovate; seminal nucleus central, without radial lines, nucleus 1.5-2.2 cm diam.

**Material examined:** BRAZIL, PARÁ: Belterra, Pindobal, 23. XII.1956, fr., J.M. Pires et al. 6512 (IAN); Faro, Campina do Jupiry, 17.VI.1926, st., A. Ducke s.n. (RB 21801); Monte Alegre, Parque Estadual, base da Serra do Ererê, 18.IV.2006, fr., A.E.S. Rocha 470 (MG); Santarém, centro de treinamento da FAO, 5. X.1962, fr., A.P. Duarte 7015 (INPA, RB); Santarém, Lago Cuçari, região do Planalto de Santarém, onde foi feito o levantamento estatístico florestal pelo IAN, SPVEA e FAO,

V.1955, fr., R.L. Fróes 31825 (IAN, RB); Santarém, Rio Curuá-Una, região do Planalto de Santarém, onde foi feito o levantamento estatístico florestal pelo IAN, SPVEA e FAO, 14. XI.1954, fr., R.L. Fróes 31394 (IAN).

**Additional material:** BRAZIL, BAHIA: Canudos, estrada em direção a Jeremoabo, ca. 17,8 km de Canudos, 9°53'18"S, 38°51'54"W, 28.VI.2002, fr., L.P. Queiroz et al. 7261 (RB); Jeremoabo, ca. 23 km E de Canudos na estrada para Jeremoabo (BR-235), 26.VIII.1996, fr., L.P. Queiroz & N.S. Nascimento 4647 (INPA); Rui Barbosa, trilha para o Pátio das Orquídeas, 12° 18'7"S, 40°29'16"W, 12.XI.2004, fl., A. Rapini & L.P. Queiroz 1156 (INPA); MATO GROSSO: Rio Juruena, beira do rio, 15. VII.1977, fr., M.G. Silva & J. Maria 3357 (MG); Xavantina-Cachimbo road, cerrado c. 0.5 km W of km 264, expedition base camp, 20.III.1968, fr., D. Philcox & A. Ferreira 4594 (RB); RONDÔNIA: Vilhena, estrada que vai para Aripuanã, local Fazenda Flor da Serra, a 15 km de Vilhena, 22.V.1984, fr., C.S. Rosário et al. 475 (MG); Médiçi, Picadão que confina a 7ª e a 8ª linha, margem esquerda da BR-429, 24.III.1986, fr., N.A. Rosa et al. 4985 (MG).

**Phytocological region and phenology:** Savannah. Flowers and fruits collected from March to December.

**Common names:** mirajussara, perobinha.

The information about the protologue of *Aspidosperma pyrifolium* was checked in its original publication. The guide "Taxonomic Literature" (Stafleu & Cowan 1981), also states that *Aspidosperma pyrifolium* was first described in "Flora" by Martius (1824a) and not in "Nova Genera et Species Plantarum" by the same author (Martius 1824b). In the databases "Tropicos" (Missouri Botanical Garden 2015) and "World Checklist of Selected Plant Families" (WCSP 2015), this information is conflicting. *Aspidosperma pyrifolium* occurs in Bolivia, Brazil and Paraguay (Missouri Botanical Garden 2015). In the state of Pará, this species was found only in Baixo Amazonas Mesoregion (IBGE 1990). According to Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma pyrifolium* belongs to section *Aspidosperma*. This species shares morphological features with *Aspidosperma multiflorum*, mainly fruit morphology, but differs from *A. multiflorum* by the chartaceous leaves, larger flowers (14-23 mm long), ovate corolla lobes and externally tomentose calyx.

**15. *Aspidosperma rigidum*** Rusby, Mem. New York Bot. Gard. 7: 323. 1927. (Figure 5k-o).

Trees 15-20 m high; trunk tortuous. Branches cylindrical, not suberous, sparsely lenticellate, glabrescent to glabrous, without cataphylls; latex whitish. Leaves alternate, arranged along the branches; petioles 1.7-2 cm long, glabrous; blade 6.1-9 x 3.7-5.5 cm, chartaceous to coriaceous, flat, elliptic, apex acuminate, base attenuate or oblique, margin straight or revolute, concolorous, venation eucamptodromous, adaxial surface dull or lustrous, dark brown, glabrous, primary vein flat, secondary veins impressed, tertiary veins inconspicuous, abaxial surface dull, dark brown, glabrous, primary vein prominent, secondary veins prominulous, 12-15 pairs, tertiary veins inconspicuous. Inflorescences 4-5 cm long, axillary or terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes not twisted. Flowers 10-13 mm long; pedicellate, pedicel 2.7-4.2 x 0.2-0.5 mm, tomentose. Calyx 1.2-1.5 x 1.5 mm, tomentose externally, glabrous internally; lobes 5, 1-1.2 x 0.5-1 mm, ovate, apex acute. Corolla 4-8 x 1-1.2 mm, salverform, yellow, glabrous externally, pubescent along the tube internally; lobes 2-5 x 1 mm, erect or deflexed, lanceolate, apex acute. Stamens 1.6-3 mm long; filaments 1-2.2 mm long,

pubescent; anthers 0.6-0.8 mm long, ovate, apex acute. Carpels 1.5 mm long; ovary 0.5 x 0.5 mm, globose, glabrous; style 0.7 mm long; style-head 0.3 mm long, main body globose, with 2 oblong apical appendages. Follicles 5-7.5 x 3.9-7.8 cm, suborbicular, smooth, sessile or stipitate, not mucronate, lenticels inconspicuous, black, glabrous. Seeds 6-7 cm diam. orbicular; seminal nucleus central, without radial lines, nucleus 1.5 cm diam.

**Material examined:** BRAZIL, PARÁ: Alenquer, mata de Bom Futuro, 25.II.1953, fr., R.L. Fróes 29400 (IAN); Alenquer, Rio Curuá, estrada Urapurú-Água Branca, área de castanheira, 4. III.1953, fr., R.L. Fróes 29472 (IAN); Breu Branco, Rio Tocantins near Igarapé Cajazeirinha, approx. 30 km N of Itupiranga, 29. XI.1981, fl., D.C. Daly et al. 1610 (INPA, MG); Itaituba, árvore n° 76-2-6, XII.1972, fl., J.M. Pires 13822 (IAN); Monte Alegre, Colônia Japonesa Assaizal, 22.IX.1953, fl., R.L. Fróes 30320 (IAN); Monte Alegre, Colônia Japonesa Assaizal, 22.IX.1953, fr., R.L. Fróes 30306 (IAN); Oriximiná, Comunidade Jauari, casa Irineu, 21.II.2010, fl., D.R. Oliveira et al. 216 (UEC); Parauapebas, Serra dos Carajás, Rio Parauapebas, control point at entrance to Serra Norte, ca. 39 km east of AMZA camp N-5, 23. VI.1982, fr., C.R. Sperling et al. 6318 (MG); Vitória do Xingu, CBVL, 21.II.2013, fl., T.C.S. Silva PSACF 960 (MG); Xinguara, Rio Vermelho, região do Tocantins, ao longo da margem do rio, 23.IV.1951, fr., R.L. Fróes 26988 (IAN, USP-scanned image).

**Additional material:** BRAZIL, AMAZONAS: Boca do Solimões, Paranã do Careiro, 7.VI.1948-2.VIII.1948, fl., A. Ducke 2132 (IAC, IAN, R); RONDÔNIA: Rio Jaru, BR-29, 21.IX.1962, fl., A.P. Duarte 7017 (INPA). PERU, HUÁNUCO: Pachitea, Codo de Pozuzo, trail S of settlement toward main river, 9°40'S, 7°25'W, 18.X.1982, fl., R.B. Foster 9274 (F-scanned image).

**Phytocological region and phenology:** Dense Ombrophilous Forest (terra firme forest). Flowers and fruits collected from June to April.

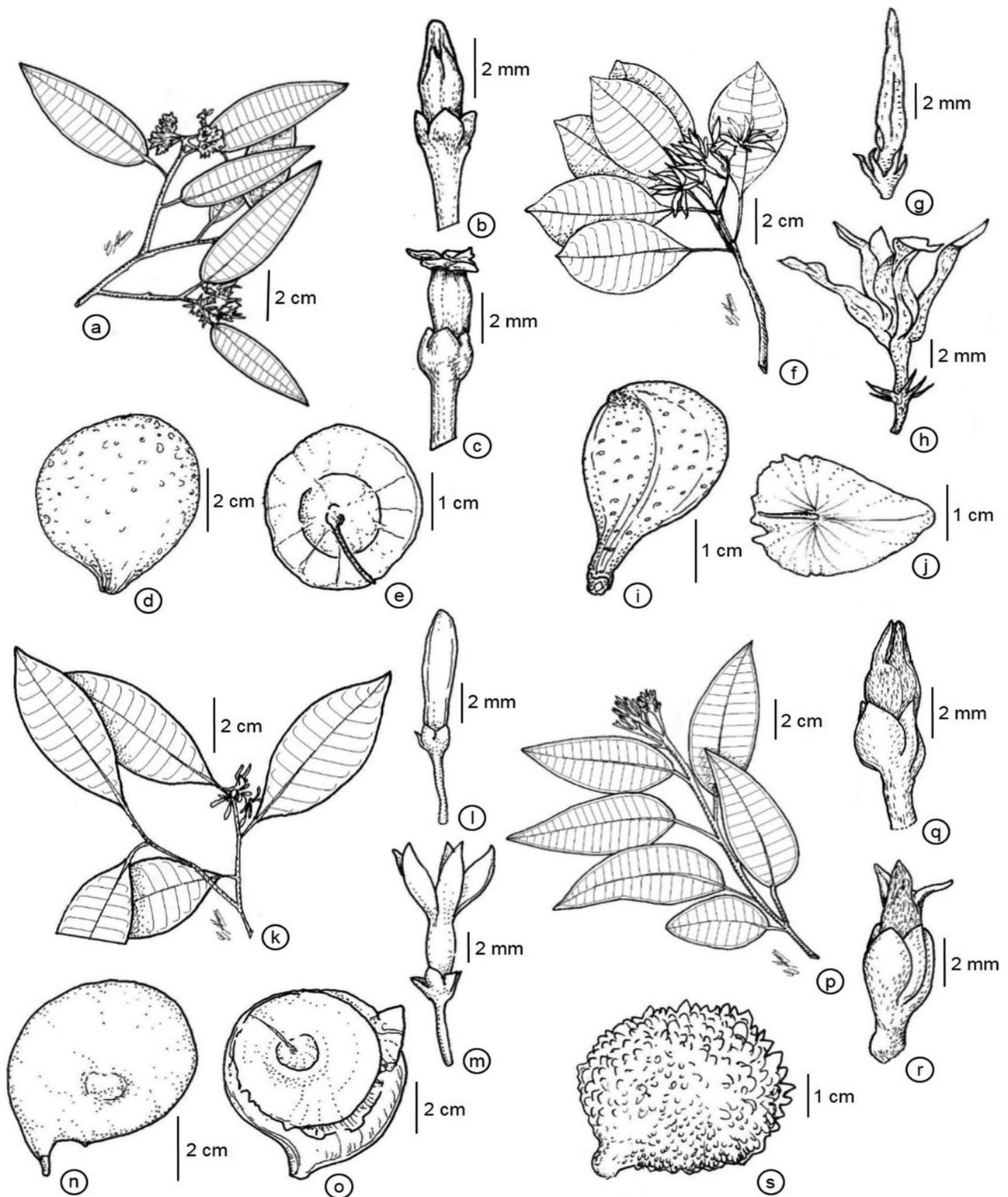
**Common names:** carapanaúba, carapanaúba-do-baixio.

*Aspidosperma rigidum* occurs in Bolivia, Brazil, Colombia, Costa Rica, Ecuador, Peru and Venezuela (Missouri Botanical Garden 2015). In the state of Pará, this species was found in Baixo Amazonas, Sudeste Paraense and Sudoeste Paraense Mesoregions (IBGE 1990). According to Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma rigidum* belongs to monospecific section *Rigida*. *Aspidosperma rigidum* can be distinguished from the other species occurring in Pará by the elliptic leaves with acuminate apex and the non-mucronate black follicles with inconspicuous lenticels.

**16. *Aspidosperma salgadense*** Markgr., Notizbl. Bot. Gart. Berlin-Dahlem 12(115): 554. 1935. (Figure 5p-s).

Trees 8-25 m high; trunk tortuous. Branches angular, not suberous, sparsely lenticellate, pubescent to glabrous, without cataphylls; latex whitish. Leaves subopposite or alternate, arranged along the branches; petioles 1-2 cm long, tomentose; blade 5.5-12.5 x 3.5-4.8 cm, chartaceous, flat, oblong or ovate, apex acute, base revolute, margin revolute, discolorous, venation brochidodromous, with marginal vein 1 mm from the margin, adaxial surface dull or lustrous, dark brown, glabrous, primary vein flat, secondary veins prominent, tertiary veins inconspicuous, abaxial surface dull, light brown, tomentose along the primary vein, primary vein prominent, secondary veins prominent, 20-22 pairs, tertiary veins inconspicuous. Inflorescences 5.6-6 cm long, terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes not twisted. Flowers 6.5-7.5 mm long; pedicellate, pedicel 1.7-2.2 x 1 mm, tomentose. Calyx 2.5-2.7 x 2-2.5 mm, tomentose externally, pubescent at base and/

Aspidosperma Mart. (Apocynaceae) in Pará



**Figure 5.** a-e. *Aspidosperma oblongum*. f-j. *A. pyriformium*. k-o. *A. rigidum*. p-s. *A. salgadense*: a, f, k, p. flowering branch; b, g, l, q. flower bud; c, h, m, r. flower; d, i, n, s. follicle; e, j, o. seed. a-e. Martin s.n. (P 4205125); N.T. Silva 1285; N.T. Silva 1855; R. Benoist 963. f-j. A. Rapini & L.P. Queiroz 1156; L.P. Queiroz et al. 7261; M.G. Silva & J. Maria 3357. k-o. A. Ducke 2132; R.B. Foster 9274; R.L. Fróes 26988. p-s. L.C. Antônio PSACF 266; R.L. Fróes 30286.

**Figura 5.** a-e. *Aspidosperma oblongum*. f-j. *A. pyriformium*. k-o. *A. rigidum*. p-s. *A. salgadense*: a, f, k, p. ramo florido; b, g, l, q. botão floral; c, h, m, r. flor; d, i, n, s. folículo; e, j, o. semente. a-e. Martin s.n. (P 4205125); N.T. Silva 1285; N.T. Silva 1855; R. Benoist 963. f-j. A. Rapini & L.P. Queiroz 1156; L.P. Queiroz et al. 7261; M.G. Silva & J. Maria 3357. k-o. A. Ducke 2132; R.B. Foster 9274; R.L. Fróes 26988. p-s. L.C. Antônio PSACF 266; R.L. Fróes 30286.

or at apex internally; lobes 5, 1.5-2 x 1 mm, ovate, apex acuminate or acute. Corolla 3.5-5.5 x 1.5 mm, tubular, white, tomentose externally, tomentose along the tube internally; lobes 1.5-2 x 0.3 mm, patent, ovate, apex acute. Stamens 2-2.8 mm long; filaments 1-1.8 mm long, tomentose; anthers 1 mm long, lanceolate, apex acute. Carpels 1 mm long; ovary 0.5 x 0.7 mm, globose, glabrous; style 0.2 mm long; style-head 0.3 mm long, main body oblong, with 2 oblong apical appendages. Follicles 4.3-6 x 3.2-5 cm, dolabriform, spinescent, stipitate, not mucronate, lenticels inconspicuous, brown, pubescent. Seeds not observed.

**Material examined:** BRAZIL, PARÁ: Aveiro, região do Tapajós, Boa Vista, 24.IX.1932, fl., Capucho 457 (IAN); Itaituba, árvore n° 38-4-25, XII.1972, st., J.M. Pires 13821 (IAN); Marabá, IX.1970, fl., J.M. Pires & R.P. Belem 12644 (IAN); Monte Alegre, Rio Maicurú, 3:30 hrs por canoa de motor de poupa mais 1:30 hrs de caminhada acima da pista de pouso do Lageiro, 30.VII.1981, st., J. Jangoux & B.G.S. Ribeiro 1575 (MG); Monte Alegre, Rio Maicurú, caminho de Caá-ussú à localidade Balança, 16.IX.1953, fl., R.L. Frões 30286 (IAN); Monte Alegre, Rio Maicurú, entre Caá-ussú e localidade Balança, 15.IX.1953, fl., R.L. Frões 30271 (IAN, NY-scanned image, R); Santarém, Rio Curuá-Una, região do Planalto de Santarém, onde foi feito o levantamento estatístico florestal pelo IAN, SPVEA e FAO, 14.IX.1954, fl., R.L. Frões 31382 (IAN); Vitória do Xingu, Travessão 55, 26.V.2012, fr., L.C. Antônio PSACF 266 (MG); Vitória do Xingu, Balança-km 9,5, 1. II.2013, fl., E. Takanohashi PSACF 919 (MG).

**Phytocological region and phenology:** Dense Ombrophilous Forest (terra firme forest). Flowers and fruits collected from May to September.

**Common names:** carapanaúba.

According to Woodson (1951), *Aspidosperma salgadense* is a species endemic to Brazil, and occurs only in the state of Pará in Baixo Amazonas, Sudeste Paraense and Sudoeste Paraense Mesoregions (IBGE 1990, Missouri Botanical Garden 2015). Marcondes-Ferreira (1988) reduced *Aspidosperma salgadense* to be synonymous with *A. discolor* (section *Excelsa*), but we recognize *A. salgadense* as an accepted species due to observed differences between the specimens of the two species. *Aspidosperma salgadense* resembles *A. discolor*, especially flower structures, but differs from *A. discolor* by the angular branches and, mainly by the glabrous ovary, a feature that has already been highlighted by Markgraf (1935) in the protologue of *A. salgadense*.

**17. *Aspidosperma sandwithianum*** Markgr., Notizbl. Bot. Gart. Berlin-Dahlem 12(115): 561. 1935. (Figure 6a-e).

Trees 12-40 m high; trunk straight. Branches cylindrical, suberous, sparsely lenticellate, velutinous to glabrous, without cataphylls; latex orange to reddish. Leaves alternate, arranged along the branches; petioles 1-3 cm long, tomentose; blade 10.1-37.5 x 3-9.8 cm, coriaceous, bullate, oblong or obovate, apex acute, obtuse or retuse, base cuneate or oblique, margin revolute, discolorous, venation craspedodromous, adaxial surface dull, olive green or brown, tomentose along the primary vein, primary vein flat, secondary veins impressed, tertiary veins inconspicuous, abaxial surface dull, white or yellow, tomentose, primary vein prominent, secondary veins prominent, 26-34 pairs, tertiary veins inconspicuous. Inflorescences 6.5-15 cm long, axillary or terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes twisted. Flowers 6-8 mm long; pedicel 1-1.5 x 1 mm, tomentose. Calyx 2-2.5 x 2-2.2 mm, tomentose externally, pubescent at apex internally; lobes

5, 1.6-2 x 1 mm, ovate, apex acute or obtuse. Corolla 4.5-6.2 x 1.2 mm, salverform, yellow, glabrous externally, tomentose below the anthers internally; lobes 2.5-3.2 x 0.3 mm, erect, filiform, apex acute. Stamens 2 mm long; filaments 1.5 mm long, tomentose; anthers 0.5 mm long, ovate, apex apiculate or acute. Carpels 1.3 mm long; ovary 0.3 x 0.3 mm, globose, glabrous; style 0.8 mm long; style-head 0.2 mm long, main body globose, with 2 oblong apical appendages. Follicles 10-12 x 8.5-10 cm, suborbicular, sulcate, stipitate, mucronate, lenticels inconspicuous, brown, velutinous. Seeds 6-7 cm diam., orbicular; seminal nucleus lateral, without radial lines, nucleus 2-2.5 cm diam.

**Material examined:** BRAZIL, PARÁ: Almeirim, Monte Dourado, Cova da Onça, 0°45'S, 52°45'W, 15.VIII.1979, fl., N. T. Silva 5259 (MG); Almeirim, região do Jarí, estrada entre Tinguelim e Braço, 29.V.1970, fl., N.T. Silva 3172 (IAN); Almeirim, região do Jarí, estrada entre Planalto A e Tinguelim, km 21, 24.XI.1969, st., N.T. Silva 2855 (IAN); Belém, IPEAN, Reserva Mocambo, L-8-14, árvore n° 95, 5.VIII.1968, fl., J.M. Pires & N.T. Silva 11907 (IAN); Belém, IPEAN, Reserva Mocambo, L-8-14, árvore n° 95, 17.VII.1968, fl., J.M. Pires & N. T. Silva 11892 (IAN); Belém, Mocambo, Embrapa Forest Reserve, ca. 10 km from Belém, transect 2, 1°30'S, 47°59'W, 13.XI.1984, st., A. Gentry 48924 (IAN); Belém, Reserva Mocambo (EMBRAPA), árvore 18-16-8, 11.I.1983, st., R. Lima & R.P. Bahia 7 (MG); Belterra, 6.VIII.1947, st., G.A. Black 47-1150 (IAC); Breves, perto do Igarapé Arapijô, transecto para inventário florestal, Q 4-53, 7-30.VII.1956, st., J.M. Pires et al. 5060 (IAN); Marabá, Rio Itacaiunas, afl. do Rio Tocantins, Serra Buritirama (B 4), região com minério de manganês, árvore 7.9.3, VIII.1970, st., J.M. Pires & R.P. Belem 12699 (IAN); Melgaço, Floresta Nacional de Caxiuanã, local onde será construída a Estação Científica "Ferreira Penna", 2-15.II.1991, st., A.S.L. da Silva et al. 2350 (MG); Moju, 25.VIII.1975, st., N. T. Silva 3961 (IAN).

**Additional material:** BRAZIL, RORAIMA: Paracaima, as proximidades da divisa com a Venezuela, km 11-2 do marco BV-9, Cordilheira do Paracaima, 19.IX.1979, fr., N.A. Rosa & O.C. Nascimento 3503 (MG). FRENCH GUIANA, RÉMIRE-MONTJOLY: Haut Oyapock, Mt. St. Marcel, layon camp Coulevre-Mt. St. Marcel, 27.III.1976, fr., C. Sastre 4564 (P-scanned image).

**Phytoecological region and phenology:** Dense Ombrophilous Forest (terra firme forest). Flowers and fruits collected from May to September.

**Common names:** araracanga, araracanga-branca, cabeça-de-arara, canoinha.

*Aspidosperma sandwithianum* occurs in Brazil, French Guiana, Guyana and Suriname (Missouri Botanical Garden 2015). In the state of Pará, this species was found in Baixo Amazonas, Marajó, Metropolitana de Belém, Nordeste Paraense and Sudeste Paraense Mesoregions (IBGE 1990). In "Livro Vermelho da Flora do Brasil" (Rapini et al. 2013), *Aspidosperma sandwithianum* was assigned to the Data Deficient category (DD), but in "Lista de Espécies da Flora e da Fauna Ameaçadas no Estado do Pará" (Pará 2007), it was listed as a Vulnerable species (VU). Marcondes-Ferreira (1988) reduced *Aspidosperma sandwithianum* to be synonymous with *A. spruceanum* (section *Nobilis*), but we found both taxa to have differences significant enough to be recognized as distinct species. Therefore, *Aspidosperma sandwithianum* is accepted here at the species level. *Aspidosperma sandwithianum* resembles *A. spruceanum*, mainly flower features, but differs from *A. spruceanum* by the bullate

leaves, tomentose primary vein and impressed secondary veins on the adaxial surface, and suborbicular follicles.

**18. *Aspidosperma schultesii*** Woodson, Ann. Missouri Bot. Gard. 38(2): 168. 1951. (Figure 6f-j).

Trees 12-30 m high; trunk tortuous. Branches cylindrical, suberous, sparsely lenticellate, glabrescent to glabrous, without cataphylls; latex whitish. Leaves alternate, arranged along the branches; petioles 2.5-4.3 cm long, glabrescent to glabrous; blade 7.6-15 x 3.1-7 cm, chartaceous, flat, elliptic or obovate, apex acute, obtuse or retuse, base cuneate, attenuate or oblique, margin revolute, discolorous, venation craspedodromous, adaxial surface dull, dark brown, glabrous, primary vein prominulous, secondary veins impressed, tertiary veins inconspicuous, abaxial surface dull, light brown, pubescent to glabrous, primary vein prominent, secondary veins impressed, 18-20 pairs, tertiary veins inconspicuous. Inflorescences 9-11 cm long, terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes twisted. Flowers 11-20 mm long; pedicellate, pedicel 1.5-4 x 0.5 mm, tomentose. Calyx 1.5-2 x 1.5-2 mm, tomentose externally, glabrous internally; lobes 5, 1-1.5 x 1 mm, ovate, apex acute. Corolla 13-15 x 1.5-2 mm, salverform, yellow, pubescent externally, pubescent along the corolla internally; lobes 6-10 x 1.5-3 mm, patent, lanceolate, apex acute. Stamens 1.7-3 mm long; filaments 1-2 mm long, tomentose; anthers 0.7-1 mm long, ovate, apex acute. Carpels 1.5 mm long; ovary 0.7 x 0.8 mm, globose, tomentose; style 0.5 mm long; style-head 0.3 mm long, main body globose, with 2 oblong apical appendages. Follicles 8-12.3 x 6-9.4 cm, suborbicular, smooth or sulcate, stipitate, mucronate, lenticels inconspicuous, black, glabrous. Seeds 6-7.8 cm diam. orbicular; seminal nucleus central, with radial lines, nucleus 2.5 cm diam.

**Material examined:** BRAZIL, PARÁ: Almeirim, Monte Dourado, estrada Perimetral (COMARA), 19.VI.1984, fl., N.T. Silva 5375 (INPA, MG); Jacareacanga, Rio São Manoel, posto do índios Caiabi, 7.I.1952, fr., J.M. Pires 3860 (IAN); Novo Progresso, Serra do Cachimbo, BR-163, Cuiabá-Santarém highway, km 872, 6.XI.1977, st., G.T. Prance et al. P24968 (MG, NY-scanned image).

**Additional material:** BRAZIL, AMAZONAS: Apuí, floresta na beira do Rio Sucunduri acampamento, 26.VI.2006, fl., C. E. Zartman et al. 5562 (INPA); Carauari, Juruá I, VII.1980, st., A.S.L. Silva et al. 908 (MG); Manaus, Reserva Florestal Ducke, Manaus-Itacoatiara, km 26, 9.III.1994, fl., A. Vicentini & C.F. Silva 404 (MG); Manaus, Reserva Florestal Ducke, Manaus-Itacoatiara, km 26, 1.VIII.1995, st., A.A. Oliveira & P. A.C.L. Assunção 2807 (IAN); Tefé, estrada do Luc 3, Porto Urucú, 16.VII.1991, fl., A.S. Tavares et al. 414 (INPA); MATO GROSSO: Aripuanã, km 245 da BR-174, projeto Juina, linha 4, 19.I.1979, fr., M.G. Silva & A. Pinheiro 4323 (MG); Vila Bela da Santíssima Trindade, 58 km S of Rondônia state line on BR-364 from Vilhena to Cáceres, 2.XI.1985, fr., W. Thomas et al. 4775 (INPA); RONDÔNIA: Road Vilhena to Colorado, 28 km from Vilhena (20 km from BR-364), forest at Rio Vermelho, 28.X.1979, fr., J.L. Zarucchi et al. 2814 (INPA).

**Phytoecological region and phenology:** Dense Ombrophilous Forest (terra firme forest). Flowers and fruits collected from January to November.

**Common names:** araracanga-rana.

*Aspidosperma schultesii* occurs in Brazil, French Guiana, Peru and Venezuela (Missouri Botanical Garden 2015). In the state of Pará, this species was found in Baixo Amazonas and Sudoeste Paraense Mesoregions (IBGE 1990). According to Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma*

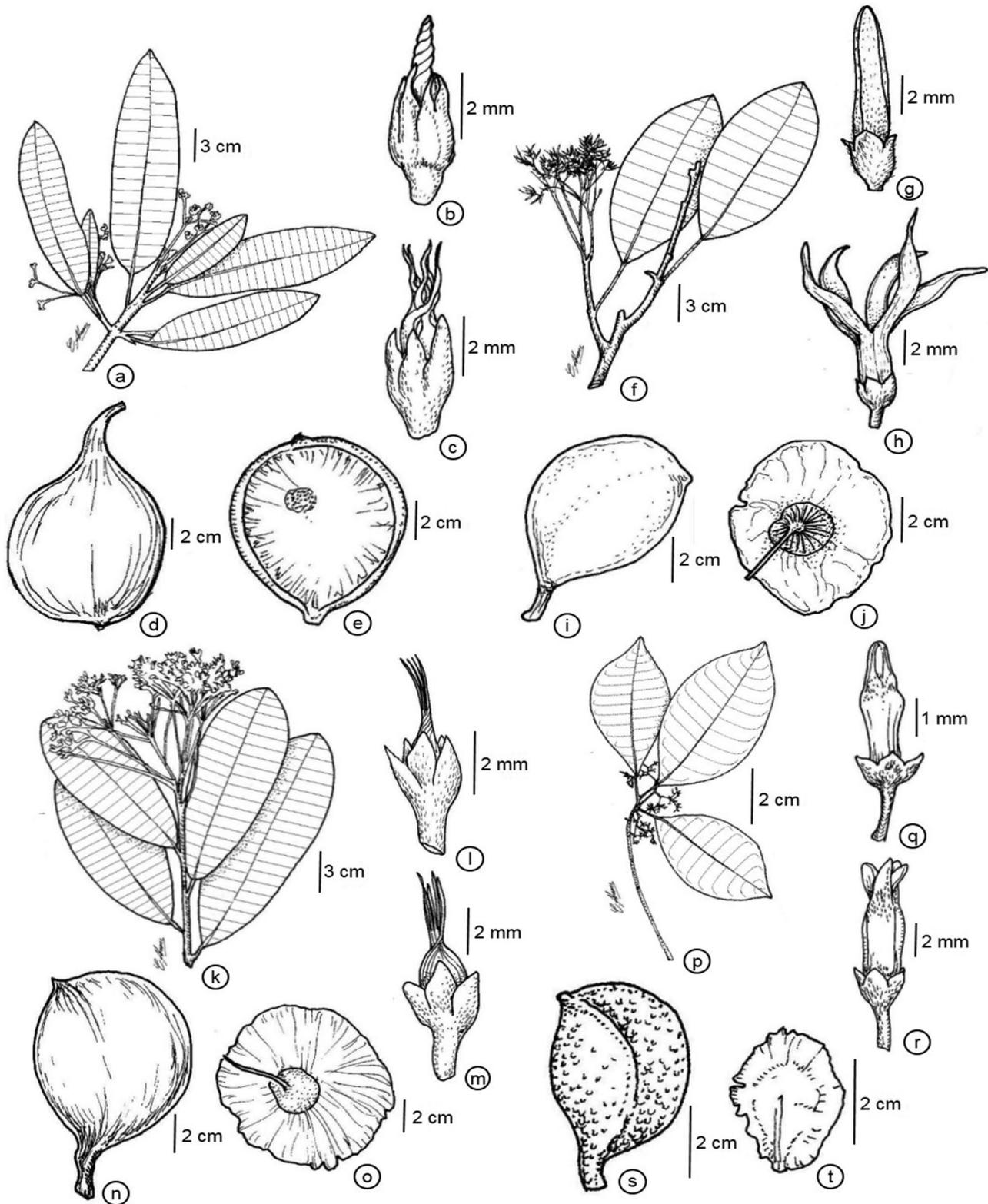
*schultesii* belongs to monospecific section *Schultesia*. *Aspidosperma schultesii* was one of the most easily recognized species, and can be differentiated from the other species occurring in Pará by the larger flowers (11-20 mm long), mucronate black follicles with inconspicuous lenticels and seminal nucleus with radial lines. *Aspidosperma schultesii* is the only species from the state with this last feature.

**19. *Aspidosperma spruceanum*** Benth. ex Müll.Arg. in C.F.P. von Martius & auct. suc. (eds.), Fl. Bras. 6(1): 52. 1860. (Figure 6k-o).

Trees 6-38 m high; trunk straight. Branches cylindrical, suberous, sparsely lenticellate, tomentose to glabrescent, without cataphylls; latex reddish. Leaves alternate, arranged along the branches; petioles 1-2.9 cm long, tomentose to glabrescent; blade 7.2-16.2 x 3.1-6.1 cm, coriaceous, flat, oblong or obovate, apex acute or obtuse, base cuneate or oblique, margin revolute, discolorous, venation craspedodromous, adaxial surface lustrous, olive green or brown, glabrous, primary vein flat or prominulous, secondary veins prominulous, tertiary veins conspicuous, abaxial surface dull, white, tomentose to glabrous, primary vein prominent, secondary veins prominulous, 25-28 pairs, tertiary veins conspicuous or inconspicuous. Inflorescences 8.5-10 cm long, terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes twisted. Flowers 8.5-9 mm long; pedicellate, pedicel 1.7-2 x 0.5-0.7 mm, tomentose. Calyx 2-2.5 x 2-3 mm, tomentose externally, pubescent at apex internally; lobes 5, 1.5-1.7 x 1 mm, ovate, apex acute. Corolla 6.5-7 x 1.5 mm, salverform, yellow, glabrous externally, tomentose below the anthers internally; lobes 3.5-4 x 0.3 mm, erect, filiform, apex acute. Stamens 2.5-3 mm long; filaments 2-2.5 mm long, tomentose; anthers 0.5 mm long, ovate, apex acute. Carpels 1.4-2.4 mm long; ovary 0.3 x 0.5 mm, globose, glabrous; style 0.7-1.7 mm long; style-head 0.4 mm long, main body globose, with 2 oblong apical appendages. Follicles 10.1-14 x 6-10.1 cm, dolabriform, sulcate, stipitate, mucronate or not, lenticels inconspicuous, brown, velutinous to glabrescent. Seeds 5.8-6.6 cm diam., orbicular; seminal nucleus lateral, without radial lines, nucleus 1.6-3.2 cm diam.

**Material examined:** BRAZIL, PARÁ: Almeirim, região do Rio Jarí, Monte Dourado, Planalto A, 9.IX.1968, fl., N.T. Silva 918 (IAN, NY-scanned image); Oriximiná, Porto Trombetas, próximo a área industrial, 9.X.1986, fl., E. Soares 221 (INPA); Porto de Moz, R. Xingu, margem esquerda do rio, região onde foi feito um levantamento estatístico florestal pelo IAN, SPVEA e FAO, 18.IX.1955, fr., R.L. Frões 32377 (IAN); Santarém, Reserva Florestal de Curuá-Una, Planalto Alto II a 1,5 km do Flanco, 7.X.1963, fr., Tressel 22 (INPA); Santarém, região do Rio Curuá-Una, Baixo Amazonas, VI.1957, fl., R.L. Frões 33893 (IAN); Tucuruí, estrada de ferro Santa Rosa, 1. VI.1981, fr., L.Coêlho, 1843 (INPA).

**Additional material:** BRAZIL, AMAZONAS: Barcelos, ilha periodicamente inundável, 8.IX.1962, st., A.P. Duarte 7007 (RB); Manaus, Reserva Florestal Ducke, próximo ao Igarapé Sempre Viva, 26.IX.1957, fr., E. Ferreira 109-57 (INPA); Manaus, Rio Tarumã-mirim, 19.X.1943, fr., A. Ducke 1419 (R); Maués, along Rio Apoquitaua, just above mouth of Rio Pacoval, 27.VII.1983, fr., J.L. Zarucchi 3210 (INPA); Rio Negro, boca do Rio Branco, 2.VII.1976, fl., L.F. Coêlho 538 (MG); Santa Isabel do Rio Negro, margem inundada do rio, 9.VI.1937, st., A. Ducke 511 (R); Santa Isabel do Rio Negro, Tapurucuará, Rio Negro, beira do rio, 7.II.1959, fr., P. Cavalcante 547 (MG); MARANHÃO: São Luiz, Sítio Tambaú, transectum 2-2-11, 1.VIII.1980, st., M.G. Silva 5594 (MG); RIO DE JANEIRO:



**Figure 6.** a-e. *Aspidosperma sandwithianum*. f-j. *A. schultesii*. k-o. *A. spruceanum*. p-t. *A. subincanum*: a, f, k, p. flowering branch; b, g, l, q. flower bud; c, h, m, r. flower; d, i, n, s. follicle; e, j, o, t. seed. a-e. C. Sastre 4564; J.M. Pires & N.T. Silva 11907. f-j. J.L. Zarucchi et al. 2814; N.T. Silva 5375; W. Thomas et al. 4775. k-o. E. Ferreira 109-57; J.G. Kuhlmann s.n. (P 4204499); L.F. Coêlho 538. p-t. A.T.G. Dias 398; C.F.P. von Martius 262; G.T. Prance et al. 9494.

**Figura 6.** a-e. *Aspidosperma sandwithianum*. f-j. *A. schultesii*. k-o. *A. spruceanum*. p-t. *A. subincanum*: a, f, k, p. ramo florido; b, g, l, q. botão floral; c, h, m, r. flor; d, i, n, s. foliculo; e, j, o, t. semente. a-e. C. Sastre 4564; J.M. Pires & N.T. Silva 11907. f-j. J.L. Zarucchi et al. 2814; N.T. Silva 5375; W. Thomas et al. 4775. k-o. E. Ferreira 109-57; J.G. Kuhlmann s.n. (P 4204499); L.F. Coêlho 538. p-t. A.T.G. Dias 398; C.F.P. von Martius 262; G.T. Prance et al. 9494.

Sumaré, 6.VII.1926, fr., J.G. Kuhlmann s.n. (P 4204499-scanned image).

**Phytoecological region and phenology:** Dense Ombrophilous Forest (terra firme forest). Flowers and fruits collected from February to October.

**Common names:** araracanga, araracanga-amarela, pau-amarelo, piquiá-marfim, piquiá-marfim-do-branco.

*Aspidosperma spruceanum* is a widely distributed species, occurring in Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama and Suriname (Missouri Botanical Garden 2015). In the state of Pará, this species was found in Baixo Amazonas and Sudeste Paraense Mesoregions (IBGE 1990). According to Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma spruceanum* belongs to section *Nobilia*. Among the species studied, *Aspidosperma spruceanum* was one of the most difficult to recognize, as it shares morphological features with both *A. album* and *A. sandwithianum*, especially flower morphology. However, *Aspidosperma spruceanum* differs from the former by the olive green or brown color of the leaf blade on the adaxial surface and from the latter by the flat leaves and glabrous primary vein on the adaxial surface. Additionally, *Aspidosperma spruceanum* is differentiated from both species by the prominent secondary veins on the adaxial surface and dolabriform follicles. *Aspidosperma spruceanum* is most often confused with *A. desmanthum*, which was mentioned by Müller-Argoviensis (1860) in the protologue of *A. spruceanum*, and by Marcondes-Ferreira (1988), but can be distinguished from *A. desmanthum* by the prominent secondary veins on both leaf surfaces.

**20. *Aspidosperma subincanum* Mart., Flora 21(2): 82. 1838. (Figure 6p-t).**

Trees 6-20 m high; trunk straight. Branches cylindrical, not suberous, densely lenticellate, glabrescent to glabrous, with cataphylls; latex whitish. Leaves alternate, congested at apex of branches; petioles 0.9-2 cm long, tomentose to glabrescent; blade 5.3-19.5 x 2.8-10.2 cm, chartaceous, flat, elliptic, apex acuminate or acute, base attenuate or oblique, margin straight, discolorous, venation eucamptodromous, adaxial surface dull, olive green, pubescent to glabrescent, primary vein flat, secondary veins prominent, tertiary veins inconspicuous, abaxial surface dull, light green, tomentose, primary vein prominent, secondary veins prominent, 17-22 pairs, tertiary veins conspicuous. Inflorescences 4-6 cm long, terminal, corymbiform dichasia, tomentose. Flower buds with corolla lobes not twisted. Flowers 5-7 mm long; pedicellate, pedicel 1.3-1.5 x 0.5 mm, tomentose. Calyx 1-1.2 x 1-1.5 mm, tomentose externally, glabrous internally; lobes 5, 1 x 0.5 mm, ovate, apex acute. Corolla 4-5.2 x 1 mm, tubular, white, pubescent at apex externally, pubescent along the tube internally; lobes 1-1.5 x 0.7 mm, patent, ovate, apex acute. Stamens 2-2.5 mm long; filaments 1.5-2 mm long, pubescent; anthers 0.5 mm long, ovate, apex apiculate or acute. Carpels 1.8 mm long; ovary 0.5 x 0.5 mm, ovoid, tomentose; style 1 mm long; style-head 0.3 mm long, main body oblong, with 2 filiform apical appendages. Follicles 4-7.5 x 2-3.8 cm, pyriform or suborbicular, smooth, stipitate, mucronate or not, lenticels conspicuous, brown, pubescent. Seeds 3 x 5 cm, ovate; seminal nucleus central, without radial lines, nucleus 2 cm diam.

**Material examined:** BRAZIL, PARÁ: Alenquer, Sete Varas airstrip on Rio Curuá, 0°95'S, 54°92'W, 4.VIII.1981, fr., J.J. Strudwick et al. 4045 (MG); Altamira, Rio Xingu, morro atrás do Lago do Muruci, 20.X.1986, fl., A.T.G. Dias 398 (MG); Canaã dos Carajás, 27.XII.2000-6.I.2001, fr., L.C.

B. Lobato et al. 2587 (MG); Monte Alegre, estrada de Caissú para a localidade Balança, 15.IX.1953, st., R.L. Fróes 30238 (IAN, INPA, R); Monte Alegre, Serra do Ererê, parte do Mirante, 1°55'S, 54°15'W, 6.XI.1987, fl., C.A.C. Ferreira 9503 (INPA, MG, RB); Monte Alegre, região do Igarapé da Formosa, 30.IX.1953, fr., R.L. Fróes 30450 (IAN); Óbidos, Flota de Trombetas, 0°58'10"S, 55°31'03"W, IV.2008, fr., L.C. B. Lobato & C.A.S. Silva 3397 (MG); Pau D'Arco, Marajoara, 10.X.1997, fl., J. Grogan 24 (IAN, INPA, MG); São Geraldo do Araguaia, morro 3, 15.VI.1995, fr., M.N. Bastos & M.R. Cordeiro 2173 (IAN, MG).

**Additional material:** BRAZIL, MARANHÃO: Presidente Dutra, roadside & capoeira approx. 10 km E of Barra do Corda, 10.X.1980, fl., D.C. Daly et al. D541 (MG); MATO GROSSO: Cuiabá, s.d., fr., C.F.P. von Martius 262 (K-scanned image); Chapada dos Guimarães, behind Refeitório, about 1 km from Buriti, 26.X.1973, fl., G.T. Prance et al. 19422 (INPA); Drainage of the upper Rio Araguaia, Serra Azul, ca. 85 km S of Xavantina, 17.VI.1966, fr., H.S. Irwin et al. 17354 (RB); RORAIMA: Dormida, foothills of Serra da Lua, 25. I.1969, fr., G.T. Prance et al. 9494 (NY-scanned image).

**Phytoecological region and phenology:** Dense Ombrophilous Forest (terra firme forest) and Savannah. Flowers and fruits collected from April to November.

**Common names:** guatambu, pau-pereira, pereiro.

The information about the protologue of *Aspidosperma subincanum* was checked in its original publication. The guide "Taxonomic Literature" (Stafleu & Cowan 1981), also states that *Aspidosperma subincanum* was first described in "Flora" by Martius (1838) and not in "Herbarium florum brasilienses" by the same author (Martius 1837-1841). In the database "Tropicos" (Missouri Botanical Garden 2015) this information is conflicting. *Aspidosperma subincanum* occurs in Bolivia and Brazil (Missouri Botanical Garden 2015). In the state of Pará, this species was found in Baixo Amazonas, Sudeste Paraense and Sudoeste Paraense Mesoregions (IBGE 1990). According to Marcondes-Ferreira & Kinoshita (1996), *Aspidosperma subincanum* belongs to section *Aspidosperma*. *Aspidosperma subincanum* can be differentiated from the other species in section *Aspidosperma* occurring in Pará by the tubular corolla, tomentose ovary and non-twisted corolla lobes.

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## Isotopic variation among Amazonian floodplain woody plants and implications for food-web research

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**Abstract:** Isotopic variation within food sources adds uncertainty to models intended to reconstruct trophic pathways. Understanding this variation is pivotal for planning sampling protocols for food-web research. This study investigates natural variation in C and N stable isotopes among plant species in two western Amazon flooded forests with contrasting watershed biogeochemistry (white-water várzea-forest and black-water igapó-forest). Our objectives were to compare  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of leaves and fruits between sites; assess the magnitude of within-site variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of leaves (várzea: 28 spp., igapó: 10 spp.) and fruits (várzea: 22 spp., igapó: 22 spp.); determine within-plant variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of leaf, wood and fruit tissues; and test whether inter-specific variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  influence the results of a mixing model predicting the contribution of terrestrial C sources to an aquatic consumer. Mean  $\delta^{13}\text{C}$  values of leaves and fruits were not statistically different between the two sites despite regional differences in biogeochemistry and floristic composition. In contrast, mean  $\delta^{15}\text{N}$  of leaves and fruits were significantly lower at the várzea than at the igapó site. The high floristic diversity of both forests was reflected in large within-site interspecific variation in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Paired comparisons revealed that  $\delta^{13}\text{C}$  of wood and fruits and  $\delta^{15}\text{N}$  of fruits were generally greater than values obtained for leaves from the same plant. The predicted contribution of different carbon sources to the consumer biomass changed between models as a function of source variability. We discuss implications of source variation for designing sampling protocols, interpreting isotopic signatures, and establishing trophic links between plants and consumers. Our findings highlight the importance of *in situ* sampling to establish reliable primary production baselines for local food webs.

**Keywords:** Stable isotopes, Igapó, Várzea, Fruits, Food web, Mixing model.

CORREA, S.B., WINEMILLER, K., CÁRDENAS, D. **Variación isotópica entre plantas leñosas de planicies de inundación del Amazonas e implicaciones para la investigación de red tróficas.** Biota Neotropica. 16(2): e20150078. <http://dx.doi.org/10.1590/1676-0611-BN-2015-0078>

**Resumo:** La variación isotópica dentro de fuentes alimenticias añade incertidumbre a los modelos destinados a reconstruir redes tróficas. La comprensión de esta variación es fundamental para la planificación de protocolos de muestreo para la investigación de redes alimentarias. Este estudio investiga la variación natural en isótopos estables de C y N entre especies de plantas en dos bosques inundables de la Amazonía noroccidental con biogeoquímica de cuenca contrastantes (várzea-bosques de aguas blanca e igapó-bosques de aguas negras). Nuestros objetivos fueron comparar las señales isotópicas de  $\delta^{13}\text{C}$  y  $\delta^{15}\text{N}$  de hojas y frutos entre bosques; evaluar la magnitud de la variación en la composición isotópica de hojas (várzea: 28 spp., Igapó: 10 spp.) y frutas (várzea: 22 spp., igapó: 22 spp.) a nivel local; determinar la variación en la composición isotópica de madera, hojas y frutas dentro de una planta; y evaluar si la variación inter-específica en los valores de  $\delta^{13}\text{C}$  y  $\delta^{15}\text{N}$  ejerce influencia sobre los resultados de modelos que predicen la contribución de fuentes de alimentos terrestres a un consumidor acuático. Los valores promedio de  $\delta^{13}\text{C}$  en hojas y frutos no fueron estadísticamente diferentes entre los dos tipos de bosque a pesar de las diferencias regionales en biogeoquímica y composición florística. Por el contrario, los valores promedio de  $\delta^{15}\text{N}$  en hojas y frutos fueron significativamente más bajos en la várzea que en el igapó. La alta diversidad florística de los dos bosques se reflejó en una gran variación inter-específica a nivel local en  $\delta^{13}\text{C}$  y  $\delta^{15}\text{N}$ . Comparaciones pareadas revelaron que los valores de  $\delta^{13}\text{C}$

de madera y frutas y  $\delta^{15}\text{N}$  de frutas fueron generalmente mayores que los valores obtenidos para las hojas de la misma planta. Los resultados sobre la contribución de diferentes fuentes de carbono a la biomasa del consumidor cambió de un modelo a otro en función a la variabilidad isotópica en las fuentes alimenticias. Discutimos las implicaciones de la variación en las fuentes alimenticias para el diseño de los protocolos de muestreo, la interpretación de las señales isotópicas, y el establecimiento de relaciones tróficas entre plantas y consumidores. Nuestros resultados destacan la importancia de la toma de muestras *in situ* para establecer líneas de base fiables de producción primaria para las redes tróficas locales.

**Palabras clave:** *Isótopos estables, Igapó, Várzea, Frutas, Red trófica, Modelos de mezcla.*

## Introduction

The use of stable isotopes in ecology has increased steadily during the past three decades, and ecological applications are diverse in scope and scale (Dawson et al. 2002, Fry 2006, West et al. 2006). Stable isotope analysis has become a primary tool in food-web studies to depict production sources supporting consumers and to analyze food-web structure (Boecklen et al. 2011, Layman et al. 2012). Use of stable isotopes in food-web research relies on natural variation in the abundance of stable isotopes within components of the ecosystem (Peterson & Fry 1987, Fry 2006).

Variation in the ratio of carbon stable isotopes ( $^{13}\text{C}/^{12}\text{C}$  and indicated by  $\delta^{13}\text{C}$ ) in plants is commonly used to trace the origin of food assimilated by animals. Differential assimilation of the lighter  $^{12}\text{C}$  isotope during photosynthesis, results in plants having  $\delta^{13}\text{C}$ -depleted tissues relative to atmospheric  $\text{CO}_2$ . Local abiotic factors (e.g., irradiance, elevation, atmospheric humidity, soil moisture, salinity, and N availability), as well as plant morphological traits, physiological processes, and genotype, influence  $^{13}\text{C}$  discrimination to varying degrees (reviewed by Dawson et al. 2002, and Bowling et al. 2008). As a result, plants have distinctive  $\delta^{13}\text{C}$  signatures that can be traced as they are transferred to herbivores and other consumers at higher levels in the food web (DeNiro & Epstein 1978, Fry & Sherr 1984, McCutchan et al. 2003).

Nitrogen isotopic ratios ( $^{15}\text{N}/^{14}\text{N}$  as indicated by  $\delta^{15}\text{N}$ ) are commonly used to estimate trophic position of consumers in food webs because  $\delta^{15}\text{N}$  accumulates incrementally from the base to the top of a food chain (Post 2002).  $\delta^{15}\text{N}$  signatures of plants are largely influenced by the  $\delta^{15}\text{N}$  of N sources (Handley & Raven 1992). Plants are generally  $\delta^{15}\text{N}$  depleted relative to the soils in which they grow (Amundson et al. 2003), as most plants absorb inorganic forms of N from the soil. Spatial and temporal variation in N sources, as well as symbiotic associations and physiological mechanisms of N assimilation and allocation, cause variation in plant  $\delta^{15}\text{N}$  isotopic signatures (reviewed by Evans 2001, Dawson et al. 2002). Differences among co-occurring species usually range from 0 to 10‰ (Evans 2001), with differences of  $\sim 1\text{‰}$  being considered biologically significant (Handley & Raven 1992).

Lowland floodplains of the Amazon Basin support large expanses of highly diverse forests that flood up to 10 m and as long as 8 months each year (Junk & Piedade 2010). Fruiting phenology of most trees and liana species in flooded forests is synchronized with the annual flood (Parolin et al. 2004). This resource pulse attracts a diverse suite of frugivores, from birds, bats and monkeys to turtles and fishes (Goulding 1980, Barnett et al. 2005, Haugaasen & Peres 2007, 2008, Correa & Winemiller 2014). The high predictability of the flood pulse in the Amazon favors consumers capable of exploiting

floodplain resources, therefore riparian terrestrial and aquatic food webs are subsidized by energy inputs from the flooded forest (Junk et al. 1997, Oliveira et al. 2006a, Junk & Piedade 2010). Amazonian floodplain forests cover an extensive area, have high floristic diversity and provide important resources for diverse consumer taxa. Nonetheless, natural variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in flooded forest plants has seldom been investigated, especially among samples from the same locality (e.g., Martinelli et al. 1991, 1992, 1994). Given that local abiotic factors as well as plant traits cause isotopic variation, how much variation in isotopic ratios is expected among species within a local plant community? What is the range of variation of tissues within an individual plant? How does this variation influence our ability to link basal producers and consumers in local food webs?

Stable isotope analysis of relative contributions of floodplain carbon sources to Amazon fishes rarely have accounted for natural variability in local plant communities (e.g., Oliveira et al. 2006a,b). Moreover, isotopic ratios of  $\text{C}_3$  terrestrial plants usually are assessed from very limited samples of leaves or composite sample of tree parts without consideration of variation within and among species or tissue types within a single plant (e.g., Araujo-Lima et al. 1986, Forsberg et al. 1993). Very little is known on isotopic variation between vegetative and reproductive tissues, despite the importance of fruit as food for large suites of invertebrates and vertebrates in both temperate and tropical ecosystems (Jordano 2000).

Here we assessed between-site, within-site and within-plant variation in C and N stable isotopes of woody plants in Amazonian flooded forests and investigated how such variation affects interpretations of trophic links between primary producers and aquatic consumers that feed heavily on foods derived from the flooded forest. Our objectives were to: 1) compare  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of woody plants from forests with contrasting watershed biogeochemistry and floristic composition (i.e., neutral, nutrient-rich white water várzea forest and acidic, nutrient-poor black water igapó forest; Worbes 1997); 2) assess the magnitude of within-site variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of leaves and fruits; 3) determine within-plant variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of leaf and fruit tissues; and 4) test the influence of isotopic variation on a mixing model predicting the relative contribution of C sources to the biomass of a fruit-eating fish species. We hypothesized that 1) woody plants of white-water and black-water flooded forests have different  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures; 2) within-site variation in  $\delta^{13}\text{C}$  in single floodplain forest sites is smaller than the variation previously reported at a regional scale in the central Amazonia (Martinelli *et al.* 1991); 3) non-photosynthetic tissues, particularly fruits, have different isotopic signatures than leaves from the same plant; and 4) isotopic variation changes the predicted contribution of different C sources to the consumer biomass.

## Materials and Methods

### 1. Study sites

We sampled floodplain forests in two areas of the western Amazon (southern Colombia) with distinct biogeochemistry but similar climate. The first forest (*várzea*, which refers to a forest inundated by sediment-laden white water (Sioli 1984)) is located on the floodplain between the Amazon and Loreto-Yacu rivers. Sampling was conducted during the middle of the rising-water season (February to March, 2007) in the evergreen flooded forest surrounding three interconnected lakes within the floodplain (hereafter these will be referred to as the “Tarapoto Lakes complex”). Sampled lakes included Lago Shaman (3°47' S, 70°24'W), Lago Tarapoto (sampled at Poza de Soraida, 3° 49'S, 70° 28'W), and Cocha Larga (3° 49'S, 70° 28'W). Rainfall is estimated at 3325 mm year<sup>-1</sup>, average monthly precipitation ranges from 180 ± 80 mm to 370 ± 100 mm, and average monthly temperature ranges from 23–28 °C (1973–2004; Duque et al. 1997). The annual flooding regime imposes changes in water level of up to 12 m (Duque et al. 1997). Water in these lakes, although dark in color, have physicochemical properties similar to those of other upper white-water Amazonian floodplain lakes (pH ranges from 6.2–6.9, conductivity ranges from 78.1–95.2 μS cm<sup>-1</sup>, and Secchi disk transparency ranges from 0.4–0.8 m; Sioli 1984). Soil in the floodplain is composed of fine sand over sedimentary rocks formed from a sandy base (Botero 1999). Vegetation corresponds to a seasonally flooded, medium-height forest over alluvial deposits of Andean origin (Botero 1999). Plant species richness in this forest has not been estimated.

The second forest (*igapó*, which refers to a forest inundated by acidic water, darkly stained with dissolved humic compounds and containing negligible amounts of dissolved salts (Sioli 1984)) is located in the lower Apaporis River, a large black-water river that drains the southwestern portion of the ancient Guyana Shield. Sampling was conducted during the high-and falling-water seasons (from late May to early October 2009) in the evergreen flooded forest along the river and in an adjacent oxbow lake (Lago Taraira, 1°09'S, 69° 29'W). Rainfall is estimated at 3836 ± 486.3 mm year<sup>-1</sup>, average monthly precipitation ranges from 258 ± 92.3 mm to 370 ± 100 mm, and average monthly temperature ranges from 22.5–27.3 °C (Correa 2003). The annual flooding regime causes a 9-m fluctuation in water level, flooding the forest for up to 7-8 months of each year (Correa 2008). Water in the Apaporis has physicochemical properties similar to those of other black-water rivers in the Amazon Basin (pH ranges from 5.2–6.5, conductivity ranges from 5.9–7.4 μS cm<sup>-1</sup>, and Secchi disk transparency ranges from 0.6–1.3 m; Sioli 1984). Soil in the floodplain is characterized as infertile white clay (Defler & Defler 1996). The vegetation corresponds to a seasonally flooded, medium-height forest growing on sand deposits and a Precambrian rock basement (Hoorn 2006). Plant species richness in the flooded forest is estimated at 215 species, 65% of which are not shared with other forest types in the area (Clavijo et al. 2009).

### 2. Sample collection

We collected samples for analysis of δ<sup>13</sup>C and δ<sup>15</sup>N stable isotope ratios from 20 to 30 species of woody plants, at each site. Species included trees, shrubs, vines, and hemi-epiphytes that were common at each site, most of which were bearing

fruits at the time when sampling was conducted. We conducted sampling inside the flooded forest and along the forest edge using a canoe; samples from the canopy were obtained by climbing. In the *várzea* site, samples of fruits, leaves, and trunk wood (which included bark and sapwood) were collected from individual plants in order to assess within-plant and inter-specific variation. We included wood samples in our within-plant comparisons to use it as a reference since wood is reported to be <sup>13</sup>C-enriched relative to leaves (Badeck et al. 2005). We also collected samples of fruits and leaves in the *igapó* site. Except for one shrub species (*Symmeria paniculata*), none of the sampled species were shared between the two sites. Botanical vouchers for all species were deposited in the Colombian Amazon Herbarium (COAH), Instituto Amazónico de Investigaciones Científicas-Sinchi, Colombia. Taxonomic identifications follow APG III (Haston et al. 2009).

We choose the frugivorous fish species *Mylossoma duriventre* (Serrasalmidae, Characiformes) because it is highly abundant in *várzea* forests and fruits are the most important component of its diet during the flood season (S.B. Correa, unpublished results). We collected samples of fish, seston, leaves of four C<sub>3</sub> aquatic macrophytes species (*Eichornia crassipes*, *Pistia stratiotes*, *Ceratopteris pteridoides*, and *Phyllanthus fluitans*), and leaves of a C<sub>4</sub> grass species (*Paspalum repens*) for isotopic analysis. We sampled fish with gill nets deployed within the flooded forest. Immediately after euthanasia, we removed a sample of approximately 2 cm<sup>2</sup> of muscle tissue from the dorsal region of the flank below the dorsal fin. Scales and skin were removed before preservation. We sampled seston with a plankton net, filtered each sample onto pre-combusted Whatman GF/F filters and dried it at 60° C for 24 h. We collected several seston samples, however all but one sample degraded due to improper storage after the samples were dried.

We preserved samples in NaCl immediately after collection. This is an effective preservation technique for remote field settings where ice is not available, and has been shown to have a negligible effect on δ<sup>13</sup>C and δ<sup>15</sup>N of fish muscle tissue (Arrington & Winemiller 2002). We rinsed samples twice with deionized water, soaked them over a 48 h period and dried them at 60°C for 24 h (fish muscle) or 48 h (plant tissue). We grinded dried samples to a fine powder using an electric grinder (Wing-L-Bug, Pike Technologies). We weighed samples to the nearest 0.1 mg using an analytical balance (Adventurer, Ohaus) and send them to the Analytical Chemistry Laboratory, Institute of Ecology, University of Georgia, for analysis of carbon and nitrogen percentage composition and stable isotope ratios. Carbon and nitrogen isotope values represent the deviation from the isotopic ratios of Pee Dee Belemnite limestone and atmospheric nitrogen, respectively, in delta notation: δ<sup>13</sup>C or δ<sup>15</sup>N = ((R sample / R standard)-1) x 1000. Precision of the isotopic analysis was ≤ 0.11‰ measured as the standard deviation among bovine reference samples. Because all samples were collected while the forest was flooded, isotopic variation due to seasonality was not expected. Likewise, within-site spatial variation was expected to be low because sampling was confined to a single floodplain location containing relatively consistent plant composition.

### 3. Statistical analyses

We compared mean values of δ<sup>13</sup>C and δ<sup>15</sup>N of leaves and fruits between sites using anova and within-sites using t-tests.

We compared paired tissue samples taken from a single individual plant with non-parametric Wilcoxon signed rank tests in order to test the null hypothesis that isotopic signatures ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) are equal between tissues. In the event of tied ranks,  $P$ -values were calculated from the exact null distribution using the COIN package of R (Hothorn et al. 2008). We conducted Spearman's rank correlation to determine between tissue correlations in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measured from the same plants. Statistical analyses were conducted in R (version 2.15.1, R-Core-Development-Team 2012).

To demonstrate the importance of accounting for isotopic interspecific- and within-plant variability in food-web research, we ran a series of mixing models to assess the relative contribution of forest plant material to the biomass of a primarily fruit-eating fish (Appendix 1; standard length ranging from 86 to 145 mm;  $N = 24$ ). Plant material included in the models were forest fruits and leaves of 20 woody plant species (both tissues collected from the same individuals) (Appendix 2), leaves of four  $\text{C}_3$  aquatic macrophyte species ( $N = 8$ ), leaves of a  $\text{C}_4$  grass ( $N = 2$ ), and seston ( $N = 1$ ). These sources represent the main primary production sources in Amazonian floodplains (Benedito-Cecilio et al. 2000, Oliveira et al. 2006b).

We used MixSIR (Version 1.0.4, Moore & Semmens 2008), a software package that allows for inclusion of isotopic variation of consumers, sources, and trophic fractionation rather than relying only on mean values as input. We followed a similar approach to that of Moore and Semmens (2008) in which we manipulated ranges of variation in mixing models. Because we were mostly interested in assessing the influence of source variation on model results, we only changed variation for sources and did not vary fractionation values or consumer isotopic ratios. Model 1: included 4 sources (fruits,  $\text{C}_3$  aquatic macrophyte leaves,  $\text{C}_4$  grass leaves, seston) and one standard deviation (SD) per source. Model 2: resembled model 1, but we increased the isotopic variability of each source from 1 SD to 2 SD. Model 3: resembled model 2, but we eliminated isotopic variation in fruits (SD = 0). Model 4: resembled model 1, but we replaced fruits with woody plant leaves. Model 5: resembled model 4, but we eliminated isotopic variability in leaves (SD = 0). Model 6: resembled model 1, but we eliminated isotopic variation in all sources (SD = 0). Although unrealistic, we included models without isotopic variation of sources (SD = 0) to demonstrate the various effects of isotopic variation on model results as other popular software packages build mixing models on mean values without accounting for variation (e.g., IsoSource, Phillips & Gregg 2003). Fractionation values used in mixing models ( $N = 4.39 \pm 0.05$ ;  $C = 0.06 \pm 0.08$ ) follow those in German & Miles (2010) calculated from plasma of a tropical herbivorous fish.

## Results

### 1. Between-site isotopic variation

Despite differences in biogeochemistry and floristic composition, the two sites had similar mean  $\delta^{13}\text{C}$  for both leaves and fruits. Mean  $\delta^{13}\text{C}$  of leaves from the várzea (mean  $\pm$  SD,  $-32.05 \pm 2.10\text{‰}$ ) was only marginally lower than the mean from the igapó (mean  $\pm$  SD,  $-30.63 \pm 1.86\text{‰}$ ; t-test =  $-2.00$ ,  $df = 36$ ,  $P = 0.06$ ). Fruits from both sites had similar mean  $\delta^{13}\text{C}$  (mean  $\pm$  SD, várzea:  $-31.14 \pm 2.45\text{‰}$ ; igapó:  $-30.18 \pm 1.91\text{‰}$ ;

t-test =  $-1.44$ ,  $df = 42$ ,  $P = 0.16$ ). Leaves and fruits from the várzea, however, had greater  $\delta^{13}\text{C}$  variation (Figure 1).

For both leaves and fruits, mean  $\delta^{15}\text{N}$  was lower for the várzea (mean  $\pm$  SD, leaves:  $1.64 \pm 1.41\text{‰}$ ; fruits:  $2.33 \pm 1.27\text{‰}$ ) than igapó (mean  $\pm$  SD, leaves:  $4.43 \pm 1.02\text{‰}$ ; fruits:  $3.92 \pm 1.53\text{‰}$ ; leaves-t-test =  $-6.67$ ,  $df = 36$ ,  $P < 0.0001$ ; fruits-t-test =  $-3.77$ ,  $df = 42$ ,  $P < 0.0001$ ; respectively). Fruits from the várzea revealed less variation in  $\delta^{15}\text{N}$  relative to those from the igapó (Fig 2c,g).

C:N ratios of leaves were higher in the várzea than in the igapó (mean  $\pm$  SD,  $28.17 \pm 7.79$  and  $19.16 \pm 4.91$ , respectively; t-test =  $3.41$ ,  $df = 36$ ,  $P < 0.001$ ). No differences in C:N ratios of fruits, however, were detected between sites (mean  $\pm$  SD,  $38.55 \pm 16.62$  and  $40.79 \pm 19.94$ , respectively; t-test =  $-0.41$ ,  $df = 42$ ,  $P = 0.69$ ).

### 2. Within-site interspecific variation

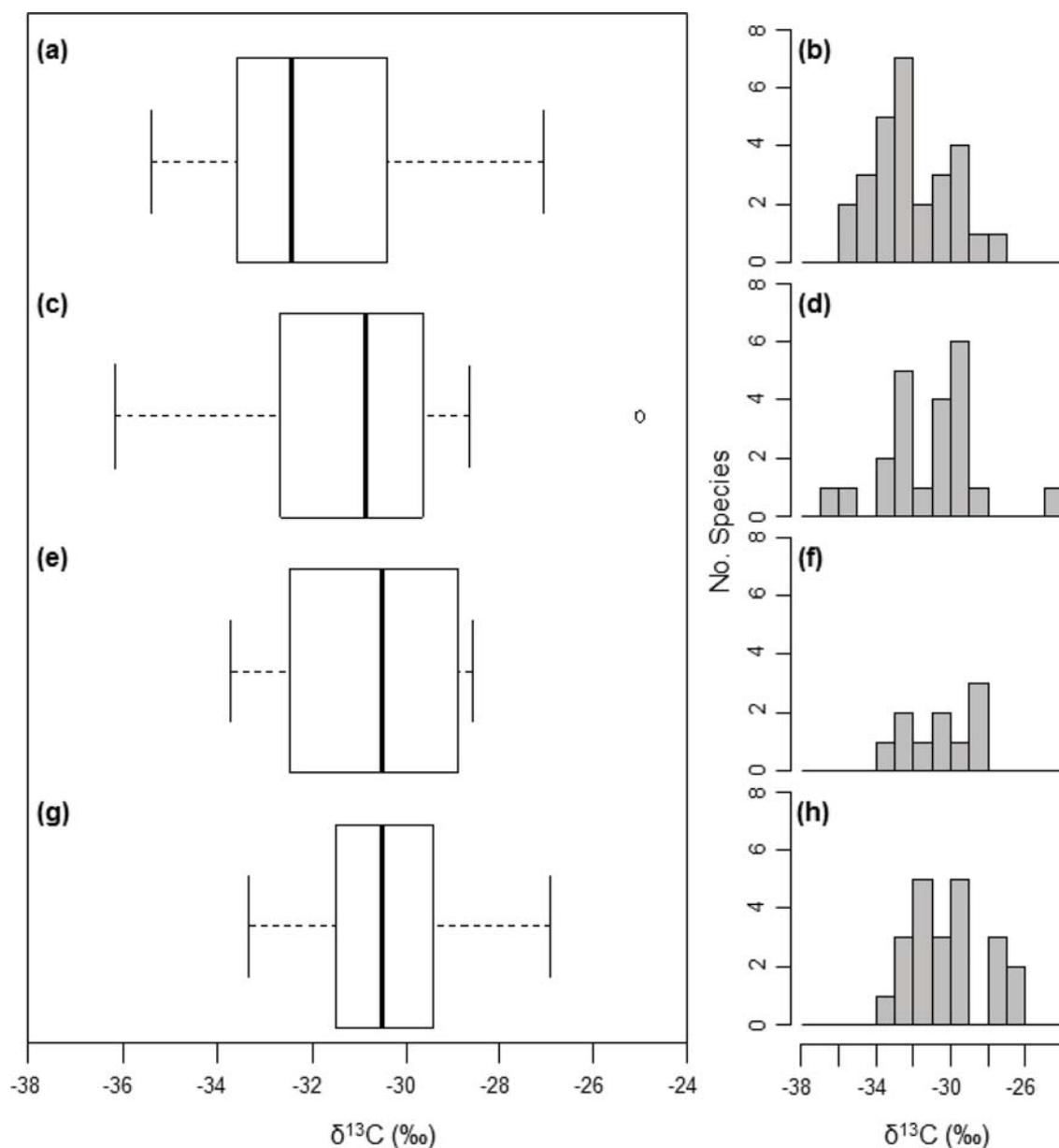
Within each site,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of both leaves and fruits was variable among species. In the várzea,  $\delta^{13}\text{C}$  of leaves of 28 species (Appendix 2) ranged  $8.4\text{‰}$  (Figure 1a,b), whereas half of the species had values that ranged at least  $3\text{‰}$  (Figure 1a).  $\delta^{13}\text{C}$  of fruits of 22 species (Appendix 2) revealed greater variation than leaves (range =  $11.2\text{‰}$ , Figure 1c,d), and half of the species had fruit samples that varied by at least  $3\text{‰}$  (Figure 1c). The range in  $\delta^{13}\text{C}$  of leaves from 10 species from the igapó site (Appendix 3) was  $5.1\text{‰}$  (Figure 1e,f), with half of the species varying by at least  $3.3\text{‰}$  (Figure 1e).  $\delta^{13}\text{C}$  values of fruits from 22 species (Appendix 3) ranged  $6.4\text{‰}$  (Figure 1g,h), with half of the species varying by at least  $2.1\text{‰}$  (Figure 1g).

$\delta^{15}\text{N}$  of leaves from the várzea ranged  $5.1\text{‰}$  (Figure 2a,b), with half of the species ranging by at least  $1.8\text{‰}$  (Figure 2a).  $\delta^{15}\text{N}$  of fruits ranged  $4.1\text{‰}$  (Figure 2c,d), with half of the species ranging by at least  $2.2\text{‰}$  (Figure 2c).  $\delta^{15}\text{N}$  of leaves from the igapó ranged  $3.2\text{‰}$  (Figure 2e,f), with half of the species varying  $< 1\text{‰}$  (Figure 2e).  $\delta^{15}\text{N}$  of fruits was more variable with a range of  $5.8\text{‰}$  (Figure 2g,h), and fruits of half of the species varied by at least  $1.6\text{‰}$  (Figure 2g).

### 3. Within-plant isotopic variation

Paired comparisons of different tissues sampled from individual plants from the várzea site showed that, as expected,  $\delta^{13}\text{C}$  of wood was generally  $^{13}\text{C}$ -enriched relative to leaves (Wilcoxon signed rank test,  $W = 131$ ,  $n = 18$ ,  $P < 0.05$ ), with an average difference of  $1.02\text{‰}$ . Wood of seven species, however, was  $^{13}\text{C}$ -depleted by  $\leq 0.5\text{‰}$  relative to leaves, and wood of one species was  $^{13}\text{C}$ -depleted by  $\leq 1.8\text{‰}$  relative to leaves (Figure 3a).  $\delta^{13}\text{C}$  of wood was positively and significantly correlated with that of corresponding leaves when all taxa were analyzed (Spearman's rank correlation,  $\rho = 0.66$ ,  $P < 0.05$ ).

$\delta^{15}\text{N}$  of wood generally was  $^{15}\text{N}$  enriched relative to leaves; however, the difference ( $0.73\text{‰}$ ) was not statistically significant (Wilcoxon signed rank test,  $W = 126$ ,  $Z = 1.76$ ,  $n = 18$ ,  $P = 0.08$ ). Only a few species had leaves that were  $^{15}\text{N}$  enriched relative to wood. In three of these species, leaf signatures were enriched as much as nearly  $2\text{‰}$  (Figure 3b).  $\delta^{15}\text{N}$  of wood was not correlated with that of corresponding leaves (Spearman's rank correlation,  $\rho = 0.22$ ,  $P = 0.39$ ). Wood C:N ratios were consistently greater than those of leaves across all species



**Figure 1.** Boxplot and frequency distributions of  $\delta^{13}\text{C}$  of co-occurring plant species from two western Amazonian flooded forest sites with different biogeochemistry: Tarapoto Lakes complex (várzea): (a, b) leaves (28 species), (c, d) fruits (22 species); Lower Apaporis River (igapó): (e, f) leaves (10 species), (g, h) fruits (22 species). The box indicates the distribution of 50% of the species.

(Wilcoxon signed rank test,  $W = 171$ ,  $n = 18$ ,  $P < 0.0001$ ), with an average difference of 14.7 (1.4 – 75.3).

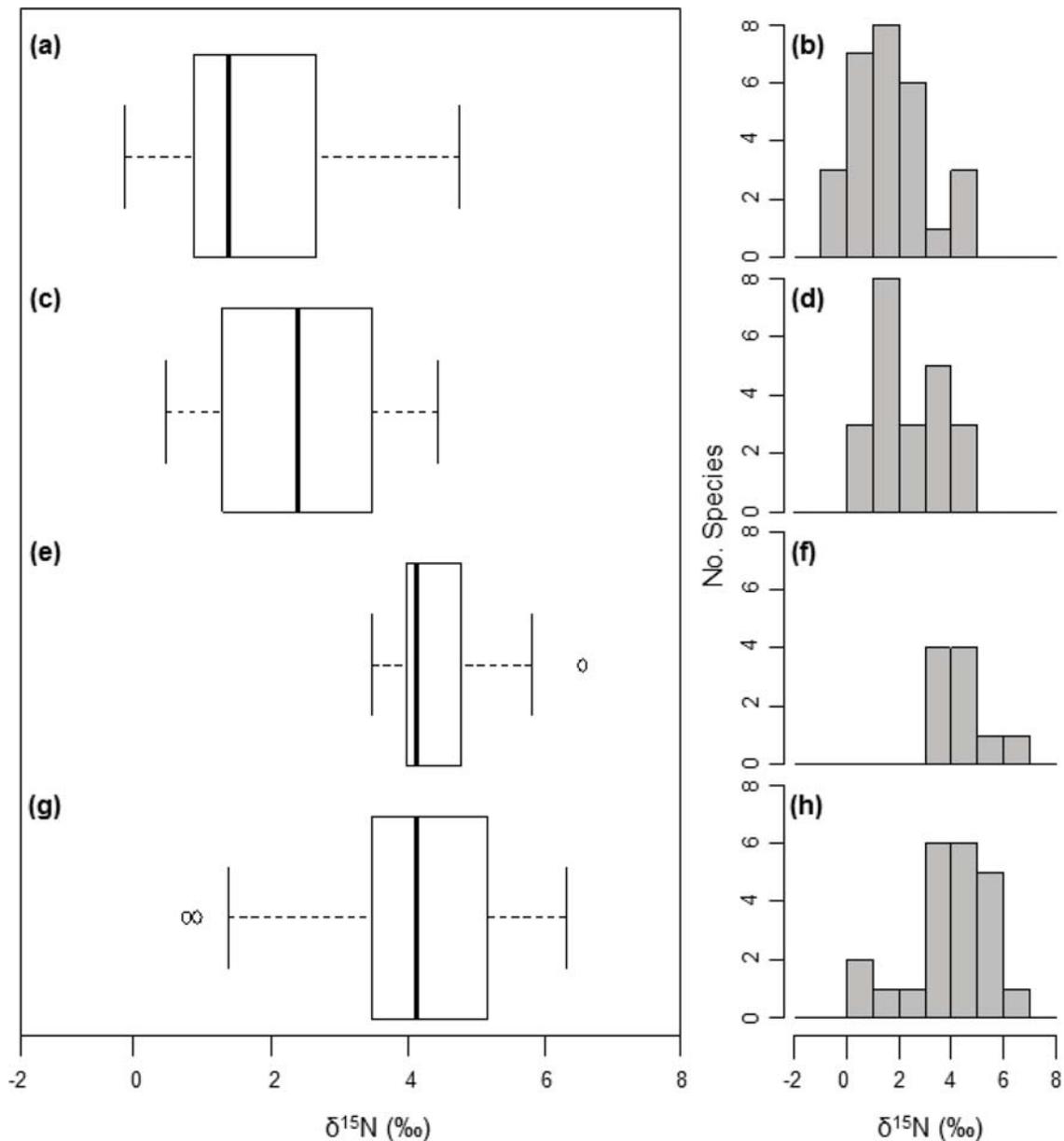
$\delta^{13}\text{C}$  of fruits generally was  $^{13}\text{C}$  enriched relative to leaves (Wilcoxon signed rank test,  $W = 161$ ,  $n = 20$ ,  $P < 0.04$ ), with an average difference of 0.68‰. Four species, however, have  $^{13}\text{C}$  enriched leaves relative to fruits; in one of them, the difference was nearly 3‰ (Figure 4a).  $\delta^{13}\text{C}$  of fruits was positively correlated with that of corresponding leaves (Spearman's rank correlation,  $\rho = 0.82$ ,  $P < 0.0001$ ).

$\delta^{15}\text{N}$  of fruits generally was  $^{15}\text{N}$  enriched relative to leaves (Wilcoxon signed rank test,  $W = 179$ ,  $n = 20$ ,  $P < 0.005$ ), with a difference of 1.02‰. A few species had leaves that were  $^{15}\text{N}$  enriched relative to fruits; in two species, the difference was nearly 1.5‰ (Figure 4b).  $\delta^{15}\text{N}$  of fruits was weakly correlated

with that of corresponding leaves (Spearman's rank correlation,  $\rho = 0.44$ ,  $P = 0.055$ ). Fruit C:N ratios generally were greater than those of leaves from the same plant (Wilcoxon signed rank test,  $W = 173$ ,  $n = 20$ ,  $P < 0.01$ ), with an average difference of 9.9 (difference values ranged from -6.9 to 44.9). A few species, however, had fruits with lower C:N ratios than leaves (Appendix 2).

#### 4. Influence of plant isotopic variation on estimates of plant contributions to consumers

Estimated relative contributions of primary production sources to *Mylossoma duriventre* in the várzea of the Tarapoto Lake's complex differed among models as a function of



**Figure 2.** Boxplot and frequency distributions of  $\delta^{15}\text{N}$  of co-occurring plant species from two western Amazonian flooded forest sites with different biogeochemistry: Tarapoto Lakes complex (várzea): (a, b) leaves (28 species), (c, d) fruits (22 species); Lower Apaporis River (igapó): (e, f) leaves (10 species), (g, h) fruits (22 species). The box indicates the distribution of 50% of the species.

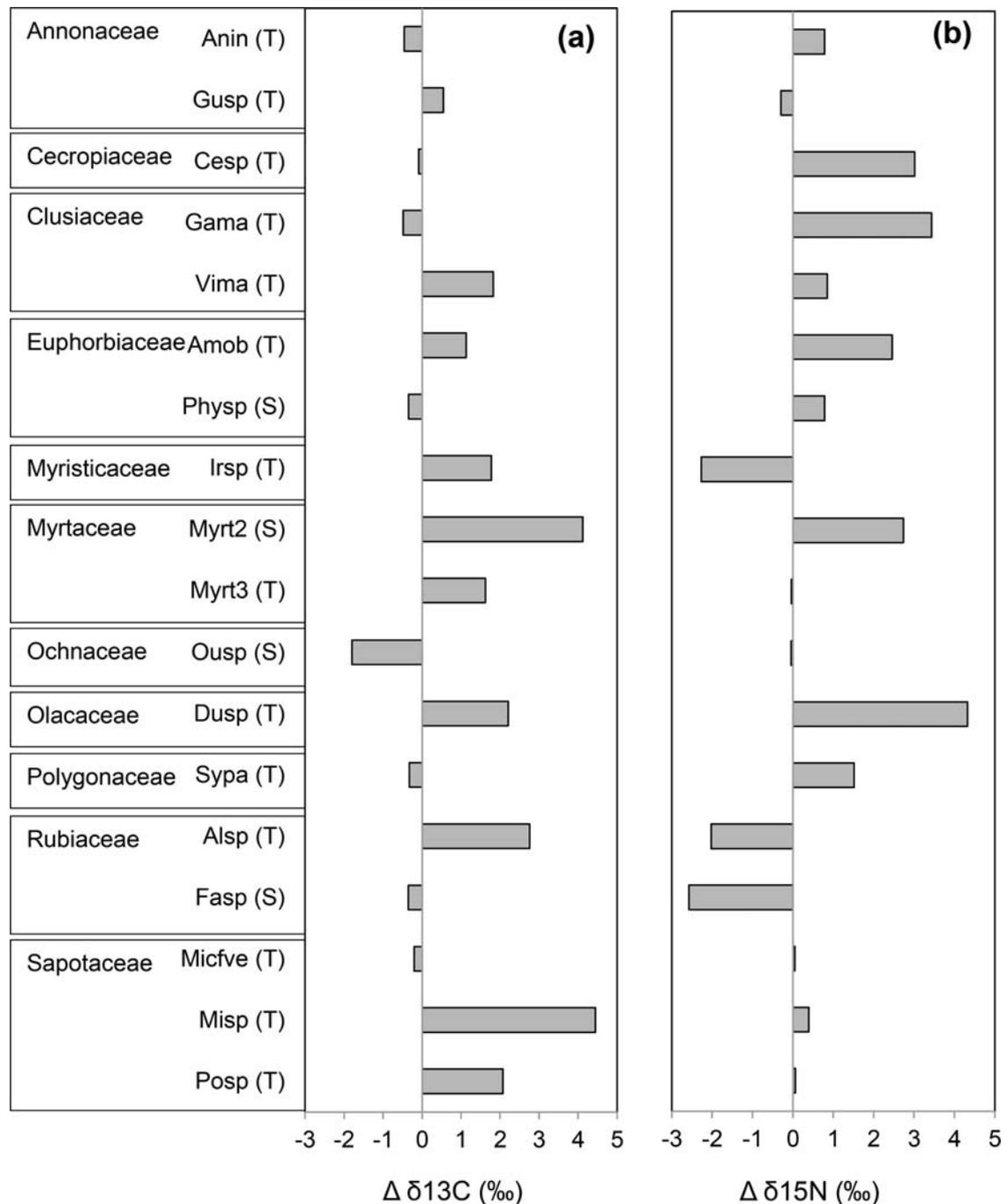
source variability. Fruits made the highest contribution to *M. duriventris*'s biomass according to MixSIR models that account for variation in isotopic signatures (1 and 2 SD). The model with increased variability (2 SD), however, reduced the predicted median contribution of fruits by 15% while increasing the median contribution of a  $\text{C}_4$  aquatic grass by 2%. When variability in isotopic ratios of fruit was eliminated from the model ( $\text{SD} = 0$ ), the predicted median contribution of fruit shifted to zero and  $\text{C}_3$  aquatic macrophytes became the most important food source. Model fit, however, was low (Table 1). Similar patterns were found in models in which fruits were replaced by leaves (Table 1). A model without variation in isotopic ratios of all sources failed to find a feasible solution for source contribution to the consumer (results not shown).

## Discussion

### 1. Between-site variation

Contrary to our expectation, the two flooded forest sites analyzed in this study had similar mean values for leaves as well as for fruits, despite significant within-site variation in  $\delta^{13}\text{C}$ . The two sites have similar climatic regimes (precipitation, humidity, temperature, and hydrological cycle) but different biogeochemistry. The várzea forest in the Tarapoto Lakes complex grows over fine-sand sediments accumulated over sedimentary rocks of Andean origin (Botero 1999) while the igapó forest in the lower Apaporis River grows on white-clay soil (Defler & Defler 1996) derived from Miocene sand deposits overlying a Precambrian rock basement (Hoorn 2006). In a study of basin-wide variation in  $\delta^{13}\text{C}$  of Amazon *terra firme*

## Isotopic variation Amazonian flooded forests

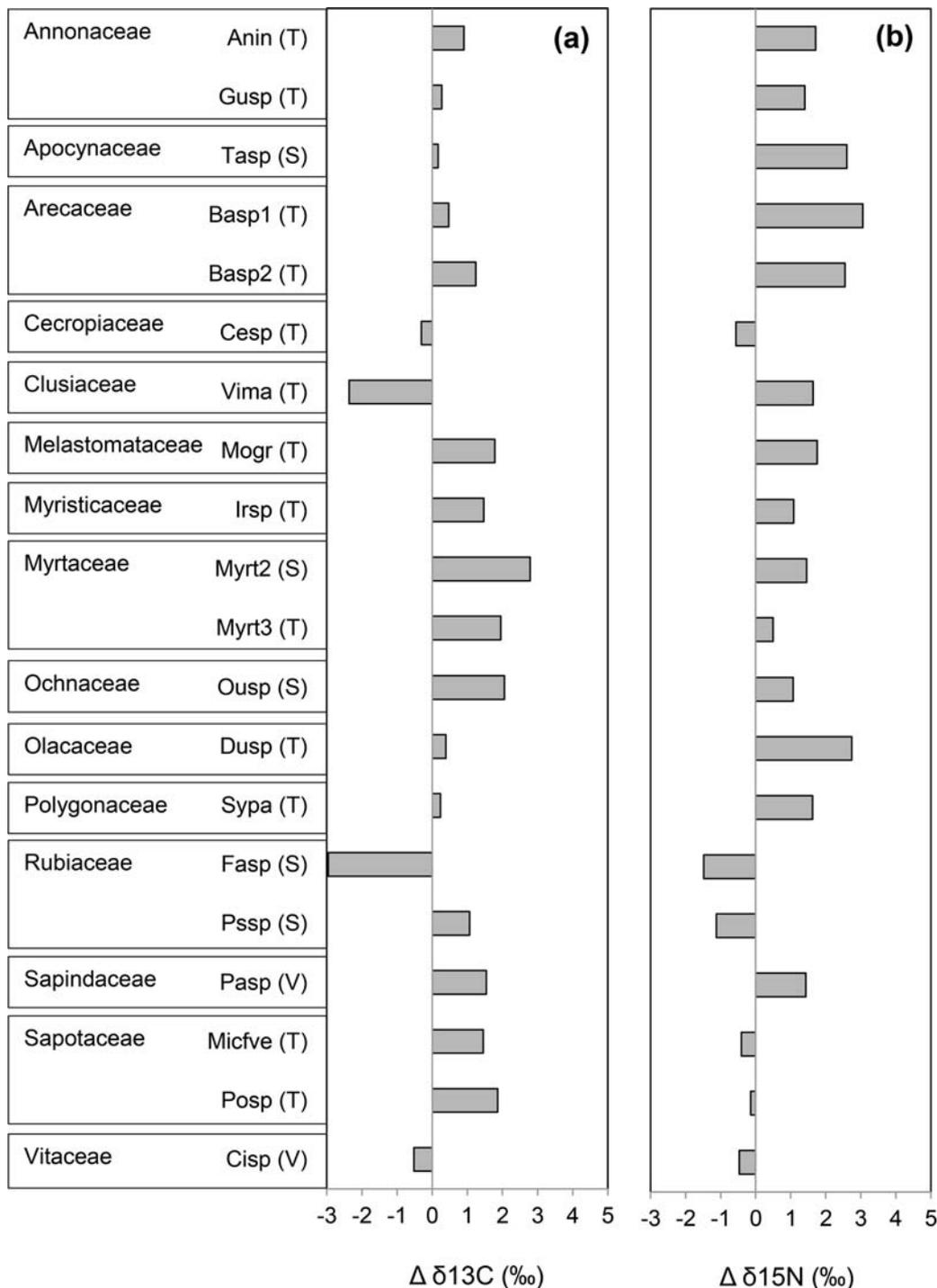


**Figure 3.** Isotopic variation between trunk wood and leaf tissue of 18 co-occurring plant species from the flooded forest (várzea) of the Tarapoto Lakes complex, western Amazonia. (a)  $\Delta\delta^{13}\text{C}$  (Wood  $\delta^{13}\text{C}$  - Leaf  $\delta^{13}\text{C}$ ), (b)  $\Delta\delta^{15}\text{N}$  (Wood  $\delta^{15}\text{N}$  - Leaf  $\delta^{15}\text{N}$ ). Positive values indicate that wood has heavier isotopic signatures with respect to leaves from the same individual plant. Species codes follow those in Appendix 2. Plant growth habit is indicated in parenthesis (T = tree, S = shrub).

forests, Fyllas *et al.* (2009) found that soil fertility (Ca, K, Mg, and P) was the best predictor of foliar  $\delta^{13}\text{C}$ . In contrast to *terra firme* forests of the Amazon, soil characteristics in flooded-forests may not have a strong influence on plant  $\delta^{13}\text{C}$ . The isotopic composition of plants in additional flooded forest sites, however, should be examined before making a generalization. Mean foliar  $\delta^{13}\text{C}$  values in these two flooded forests are also congruent with those of *terra firme* forests in the Amazon Basin (Table 2), despite the great differences in

floristic composition of flooded and non-flooded forests even within a given locality (Campbell *et al.* 1986, Haugaasen & Peres 2006, Clavijo *et al.* 2009).

Woody plants from the igapó of the lower Apaporis had higher mean  $\delta^{15}\text{N}$  values for leaves (2.8‰) and fruits (1.6‰) than plants from the várzea. Differences in mean annual precipitation (MAP) and mean annual temperature (MAT) have been identified as main factors contributing to inter-site variation in site-averaged foliar  $\delta^{15}\text{N}$  at a global scale



**Figure 4.** Isotopic variation between fruits (pulp and seeds) and leaves of 20 co-occurring plant species from the flooded forest (várzea) of the Tarapoto Lakes complex, western Amazonia. (a)  $\Delta\delta^{13}\text{C}$  (Fruit  $\delta^{13}\text{C}$  - Leaves  $\delta^{13}\text{C}$ ), (b)  $\Delta\delta^{15}\text{N}$  (Fruit  $\delta^{15}\text{N}$  - Leaves  $\delta^{15}\text{N}$ ). Positive values indicate that fruits had heavier isotopic signatures with respect to leaves from the same individual plant. Species codes follow those in Appendix 2. Plant growth habit is indicated in parenthesis (T = tree, S = shrub, V = vine).

(Handley et al. 1999, Amundson et al. 2003). The two forest sites analyzed in this study, however, have very high and similar MAP (3.3 m and 3.8 m in the várzea and igapó, respectively). MAT in the igapó site (25°C) falls within the range of mean monthly temperature (23 – 28°C) registered at the várzea site. Thus it is unlikely that such relatively small differences in precipitation and temperature are causing the observed differences in isotopic signatures.

However, in addition to climate, plant  $\delta^{15}\text{N}$  vary as a function of soil  $\delta^{15}\text{N}$  (Handley & Raven 1992). In a study of soil chemistry in Amazon flooded forests sampled during the dry season, Furch (1997) found no significant differences in total N content between a várzea and an igapó forest site. Nitrogen content, however, was higher in the humic layer in the várzea soil, but the top mineral layer (0-10 m) in the igapó site contained twice the amount of N than the same layer in the

**Table 1.** Median and 5th–95th percentile ranges (in parentheses) of source contributions to biomass of a flooded-forest frugivorous fish (*Mylossoma duriventre*) estimated from MixSIR mixing models with uninformative priors.  $10 \times 10^6$  interactions were carried out per model. † < 1000 posterior draws indicates poor model fit. MIR = maximum importance ratio < 0.01 indicates good model fit (Moore and Semmens 2008). SD = standard deviation. NA = not applicable.

Model variability	Fruits	Leaves	C <sub>3</sub> aquatic macrophytes	C <sub>4</sub> aquatic grass	Seston	MIR
1) 1SD	0.82 (0.78–0.86)	NA	0.01 (0.00–0.02)	0.16 (0.13–0.20)	0.01 (0.00–0.03)	0.003
2) 2SD	0.67 (0.58–0.76)	NA	0.03 (0.00–0.11)	0.18 (0.12–0.24)	0.10 (0.01–0.22)	0.000
3) Fruit SD = 0	0 (0.00–0.01)	NA	0.83 (0.82–0.85)	0.17 (0.15–0.18)	0 (0.00–0.01)	0.042†
4) 1SD	NA	0.76 (0.73–0.79)	0.01 (0.00–0.03)	0.23 (0.20–0.25)	0.01 (0.00–0.03)	0.004
5) Leaf SD = 0	NA	0 (0.00–0.01)	0.83 (0.82–0.85)	0.16 (0.15–0.17)	0 (0.00–0.01)	0.044†

**Table 2.** Foliar  $\delta^{13}\text{C}$  (mean  $\pm$  SD) reported from single forest sites in the Amazon Basin. *N* = number of samples analyzed, followed by the number of species in parenthesis. Forest layer: C = canopy, U = understory, LC = lower canopy, UC = upper canopy. Source: 1 = Medina and Minchin (1980), 2 = Ometo et al. (2006), 3 = Martinelli et al. (1998).

$\delta^{13}\text{C}$	<i>N</i>	Forest layer	Forest type	Region	Country	Source
-32.1 $\pm$ 2.1‰	28 (28)	C & U	Várzea	Western Amazonia	Colombia	This study
-30.6 $\pm$ 1.9‰	24 (10)	C & U	Igapó	Western Amazonia	Colombia	This study
-28.7 $\pm$ 1.7‰	5 (5)	UC	Terra-firme	Western Amazonia	Venezuela	1
-34.3 $\pm$ 1.5‰	5 (5)	LC	Terra-firme	Western Amazonia	Venezuela	1
-30.5 $\pm$ 1.4‰	4 (4)	UC	Caatinga	Western Amazonia	Venezuela	1
-33.4 $\pm$ 1.5‰	9 (9)	LC	Caatinga	Western Amazonia	Venezuela	1
-35.2 $\pm$ 1.2‰	11 (11)	U	Caatinga	Western Amazonia	Venezuela	1
-31.9 $\pm$ 2.2‰	40 (n/a)	C & U	Terra-firme	Central Amazonia (Roraima)	Brazil	2
-32.2 $\pm$ 2.5‰	133 (n/a)	C & U	Terra-firme	Central Amazonia (Amazonas)	Brazil	2
-32.6 $\pm$ 2.7‰	42 (n/a)	C & U	Terra-firme	Central Amazonia (Amazonas)	Brazil	2
-32.8 $\pm$ 2.2‰	150 (n/a)	C & U	Terra-firme	Central Amazonia (Pará)	Brazil	2
-32.1 $\pm$ 2.6‰	280 (n/a)	C & U	Terra-firme	Central Amazonia (Pará)	Brazil	2
-32.1 $\pm$ 1.5‰	208 ( $\approx$ 100)	C	Terra-firme	Southern Amazonia (Rondônia)	Brazil	3

várzea site. Soils of various natural ecosystems have increased  $^{15}\text{N}$  enrichment along the vertical profile (Handley & Raven 1992). Thus, perhaps igapó plants are using N from a deeper  $^{15}\text{N}$  enriched pool. Lastly, differences in watershed biogeochemistry may also influence differences in plant  $\delta^{15}\text{N}$  between our sampled sites. For instance, terra firme forest growing on white sand, which are N limited, have significantly lower foliar  $\delta^{15}\text{N}$  relative to forests growing on other soil types in the Amazon region, including várzea forests (Martinelli et al. 1999). Analysis of the isotopic composition of várzea and igapó soils from multiple sites would help elucidate causes of inter-site differences in plant  $\delta^{15}\text{N}$ .

## 2. Interspecific isotopic variation

Natural variation in  $\delta^{13}\text{C}$  among flooded forest plants has seldom been investigated, especially among samples from the same locality. Contrary to our expectation, isotopic variation among plants at our two western Amazon sites (várzea and igapó) was comparable to the variation reported from composite samples collected from várzea forests along a 1,800-km stretch of the central Amazon River (Martinelli et al. 1991). At a regional scale, a longitudinal gradient of  $^{13}\text{C}$  enrichment in foliar tissue of trees and understory plants occurs as a result of greater recycling of  $^{13}\text{C}$ -depleted biogenic  $\text{CO}_2$  in the western portion of the Amazon Basin. In general, tree

leaves from the easternmost site are enriched as much as 3‰ and understory leaves as much as 4‰, with respect to leaves from the westernmost site (Martinelli et al. 1991). At a local scale, foliar  $\delta^{13}\text{C}$  values of half the species we sampled at a single forest site fell within a range of 3‰.

The variation in foliar  $\delta^{13}\text{C}$  among co-occurring species revealed in the present study is comparable to levels of interspecific within-site variation found in other *terra firme* forests in the Amazon Basin (e.g., 9 to 12‰ at four forest sites (see Figure 1 in Ometto et al. 2006), 8‰ at a forest site in southern Amazonia (Martinelli et al. 1998)). One of the explanations for large within-site variation in  $\delta^{13}\text{C}$  is the “canopy effect” that causes leaves closer to the forest floor to have more  $^{13}\text{C}$ -depleted  $\delta^{13}\text{C}$  signatures relative to leaves in the upper canopy. Two hypotheses have been postulated to explain this pattern. One idea is that light availability influences  $\text{CO}_2$  concentration within leaf intercellular spaces (Farquhar et al. 1982), and the other proposes vertical stratification in the isotopic composition of forest  $\text{CO}_2$  (Medina & Minchin 1980). Contrary to patterns described for *terra firme* forests, some of the lowest  $\delta^{13}\text{C}$  values in the two flooded forests we sampled (< -33‰) came from tree leaves collected from the crown (Appendices 1 and 2). Martinelli et al. (1994) hypothesized that proximity to the river may promote better mixing of atmospheric and  $^{13}\text{C}$ -depleted biogenic  $\text{CO}_2$  that, in conjunction with better light penetration, may prevent the occurrence of a

strong “canopy effect” in flooded forests. Since the samples analyzed in this study included partially submerged species growing at different heights (e.g., small and large trees, shrubs, and vines) and under different light conditions (e.g., inside the forest and along the edge), the variation depicted by these data sets are examples of the natural distribution of  $\delta^{13}\text{C}$  among woody plants at a single forest site. Larger sample sizes within each stratum would be needed to test for effects of plant height or distance from the river’s edge.

The two flooded forest sites analyzed in this study also revealed significant variation in  $\delta^{15}\text{N}$  among co-occurring species. Such ranges are similar to those reported for foliar  $\delta^{15}\text{N}$  among non- $\text{N}_2$ -fixing woody plants in a várzea forest (ca. 5.5‰, Figure 2 in Kreibich et al. 2006) and a *terra firme* forest (5.5‰, Ometto et al. 2006) sampled during the dry season near Manaus in the central Amazon Basin. Wider ranges of variation in foliar  $\delta^{15}\text{N}$  among non- $\text{N}_2$ -fixing woody plants have been reported in other tropical *terra firme* forests, including three sites within the Amazon Basin (7–8‰, Ometto et al. 2006).

Within-site variation in foliar  $\delta^{15}\text{N}$  is partially explained by the presence of  $\text{N}_2$ -fixing species. Although  $\text{N}_2$ -fixers generally have  $\delta^{15}\text{N}$  signatures slightly lower than that of atmospheric  $\text{N}_2$  (i.e., from –1 to –2‰, Shearer & Kohl 1991), non- $\text{N}_2$ -fixers also can have low  $\delta^{15}\text{N}$  (e.g. Domenach et al. 1989, Roggy et al. 1999, Kreibich et al. 2006). Samples from the várzea of the Tarapoto Lakes complex did not include legume species, and only three legume species were sampled in the igapó of the lower Apaporis River. Foliar  $\delta^{15}\text{N}$  of those three species, however, had values > 3‰ suggesting that these are most likely non- $\text{N}_2$ -fixers. Access to alternative N pools due to mycorrhizal associations and differential root depth can cause  $\delta^{15}\text{N}$  variation among non- $\text{N}_2$ -fixing plants (Handley & Raven 1992, Högberg 1997). In a várzea forest site near Manaus, Kreibich et al. (2006) found a non- $\text{N}_2$ -fixing tree species with high ectomycorrhizal density having lower  $\delta^{15}\text{N}$  than co-occurring non- $\text{N}_2$ -fixing species without mycorrhiza. Roggy et al. (1999) found longer roots and more depleted  $\delta^{15}\text{N}$  signatures among non- $\text{N}_2$ -fixing pioneer species compared with primary-forest species in a *terra firme* rainforest site in French Guiana. In flooded forests, root length can reach up to 30 m in some species (Worbes 1997), but the relationship between root length and foliar  $\delta^{15}\text{N}$  remains to be examined in these forests.

### 3. Within-plant isotopic variation

As expected, wood and fruit had different isotopic signatures than paired leaf samples. Generally, non-photosynthetic tissues in  $\text{C}_3$  plants are  $^{13}\text{C}$ -enriched relative to leaves (Cernusak et al. 2009). Paired wood and leaf samples of trees in a *terra firme* forest in the southwestern Amazon revealed variation in  $\delta^{13}\text{C}$  (range = –0.85 to +9‰,  $n = 33$  trees; Martinelli et al. 1998) larger than that observed in the várzea of the Tarapoto Lakes complex. Although the pattern of  $^{13}\text{C}$  enrichment in wood relative to leaves appears to be widespread across diverse taxa and ecosystems, causal mechanisms remain poorly understood (Bowling et al. 2008), and a combination of processes likely contribute to this pattern (Cernusak et al. 2009).

Within-plant variation in  $\delta^{15}\text{N}$  is usually < 3‰ between leaves and roots from plants in temperate deciduous forests

and tallgrass prairies, while desert plants can have up to 7‰ individual variation (Evans 2001). Greater  $^{15}\text{N}$ -enrichment of trunk wood (i.e., bark and sapwood) relative to leaves was apparent for most of the plant species analyzed in the Tarapoto Lakes várzea; however, this pattern may not be repeated in other ecosystems. For example, lower  $\delta^{15}\text{N}$  values in trunk wood relative to those of leaves were found among a  $\text{N}_2$ -fixing legume (*Prosopis glandulosa*) in the Sonora Desert (Shearer et al. 1983). Differential N values among tissues are the result of internal N cycling within plants (Shearer et al. 1983).

Our results regarding a generalized  $^{13}\text{C}$  enrichment in fruit relative to leaves in a várzea forest represent a significant contribution to the little existing evidence for isotopic variation between fruits and leaves among woody plants. A recent study of mangrove forests revealed that fruits of most species had  $^{13}\text{C}$ -enriched signatures and lower C:N ratios relative to leaves (Figure 3 and 7 in Muzuka & Shunula 2006). Under experimental conditions,  $\delta^{13}\text{C}$  signatures seeds of an evergreen tree (*Lithocarpus densiflorus*) from the western United States, were less negative (> 2‰) than leaves (Kennedy et al. 2004). Among herbaceous crops,  $\delta^{13}\text{C}$  signatures of chickpea pods and seeds were enriched by at least 3.1 and 4.7‰, respectively, relative to leaves under experimental conditions (Behboudian et al. 2000); and soybean pods were enriched by 1 to 2‰, relative to somatic tissues (Yoneyama et al. 2000). Varied enrichment in these herbaceous species and among várzea plants suggests that higher  $\delta^{13}\text{C}$  of fruits relative to leaves is species specific.

Mechanisms causing variation in  $\delta^{13}\text{C}$  of fruits and leaves within an individual plant remain poorly understood. Movement of compounds with differential isotopic composition inside the plant (Kennedy et al. 2004), respiratory differences among tissues, and recycling of  $\text{CO}_2$  inside the pod (Behboudian et al. 2000) are hypothesized as possible causes for the differences in  $\delta^{13}\text{C}$  between seeds and leaves. Fruit age also could influence isotopic variation, although, Handley and Scrimgeour (1997) found that  $\delta^{13}\text{C}$  of fruits from one shrub species increased as they matured, but for a different shrub species in the same field values declined by 3‰ during maturation. Sampling larger samples of fruits and leaves at multiple developmental stages and from a greater number of species would clarify these relationships.

Information concerning variation in  $\delta^{15}\text{N}$  of fruit and leaves is even more limited. Patterns of  $^{15}\text{N}$  enrichment in fruits relative to leaves are consistent across three dominant shrub species in a successional field in Scotland (Handley & Scrimgeour 1997). In contrast to the patterns of enrichment in fruits observed among most várzea plants, fruits of *P. glandulosa* in the Sonora Desert had slightly lower  $\delta^{15}\text{N}$  signatures than leaves (Shearer et al. 1983). Soybean pods also had lower  $\delta^{15}\text{N}$  than vegetative parts (Yoneyama et al. 2000).

### 4. Implications of plant isotopic variation for food-web research

Requirements for multiple samples from species, individuals, and tissues will obviously be determined by the research question and spatiotemporal scale of the study.  $\delta^{15}\text{N}$  of leaves in our igapó forest were enriched by an average of 2.8‰ with respect to leaves from the várzea site. Because  $\delta^{15}\text{N}$  usually increases 2–4‰ per trophic level (Post 2002, Vanderklift & Ponsard 2003, German & Miles 2010), establishing a reliable

baseline is essential for estimation of consumer trophic positions.

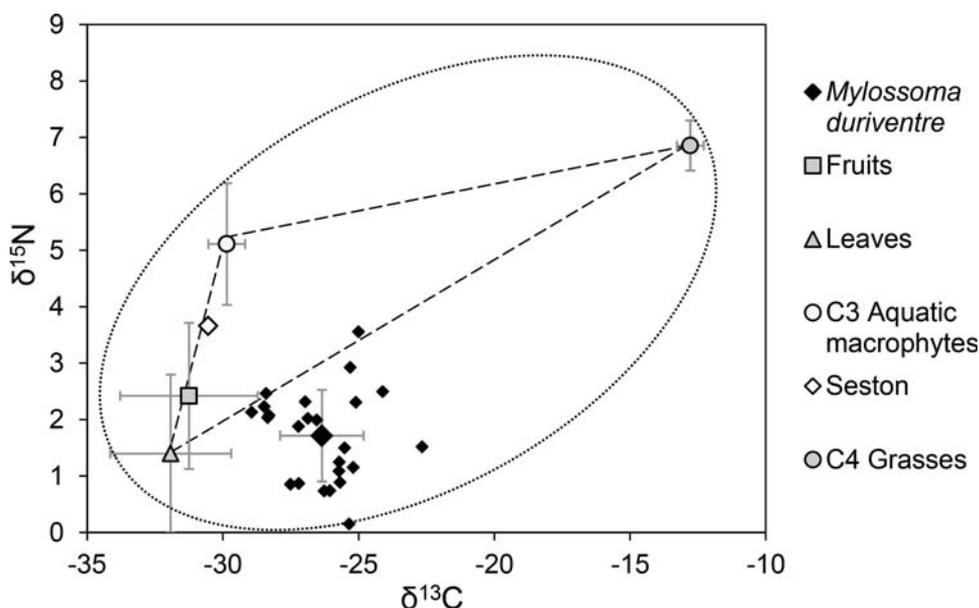
In addition to between-site differences in  $\delta^{15}\text{N}$ , our isotopic analysis from multiple species showed wide inter-specific variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  at a given location. Samples encompassing plant taxa with diverse physiological attributes (e.g.,  $\text{N}_2$ -fixers- and non- $\text{N}_2$ -fixers), growth forms, and micro-habitats (e.g., understory, canopy, inside forest, and edge) are needed in order to characterize natural within-site isotopic variation. This is especially true in species-rich ecosystems, such as tropical forests. For example, including only  $\text{N}_2$ -fixers or only non- $\text{N}_2$ -fixers could produce different mean  $\delta^{15}\text{N}$  values for macrophytes at the base of the food web (e.g., Roggy et al. 1999, Kreibich et al. 2006). In our study,  $\delta^{15}\text{N}$  showed significant between- and within-site variation, and therefore *in situ* data should be used to establish primary production baselines for local food webs.

Large within-site variation in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  observed at both sites in this study also emphasizes the need to use mixing models that can account for isotopic variation in basal production sources suspected of supporting consumer biomass (e.g., MixSIR, Moore & Semmens 2008). As expected, the results of a mixing model predicting the relative contribution of different food sources to a fruit-eating fish were largely influenced by isotopic variation. The mixing model that used mean source values without variability failed to find feasible source contributions to *Mylossoma duriventre* at our várzea site. This is because the fish fell outside the mixing polygon connecting mean source values (Figure 5; Phillips & Gregg 2003). The magnitude of variation for a particular source (e.g., none, 1 SD, 2SD) greatly influenced model results for this consumer. Stomach contents analysis revealed that fruit is the most important food for *M. duriventre* during the flood season (Appendix 1). Models that accounted for variability in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of fruit confirmed that fruit accounts for a large

fraction of *M. duriventre*'s diet. Fish frugivory in Amazonian flooded forest is facilitated by high fruit production as most plants have their fruiting phenology synchronized with the flood season (Parolin et al. 2004). When only mean isotopic ratios were included, the model predicted that  $\text{C}_3$  aquatic macrophytes had the greatest contribution; however, aquatic macrophytes were not found in stomachs. Aquatic macrophytes may contribute to fish diets during the dry season, particularly in white-water rivers where macrophytes are abundant. Fruits have heavier  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  than leaves which allowed for fairly good estimates of contributions of these sources to consumer biomass.

## 5. Conclusions

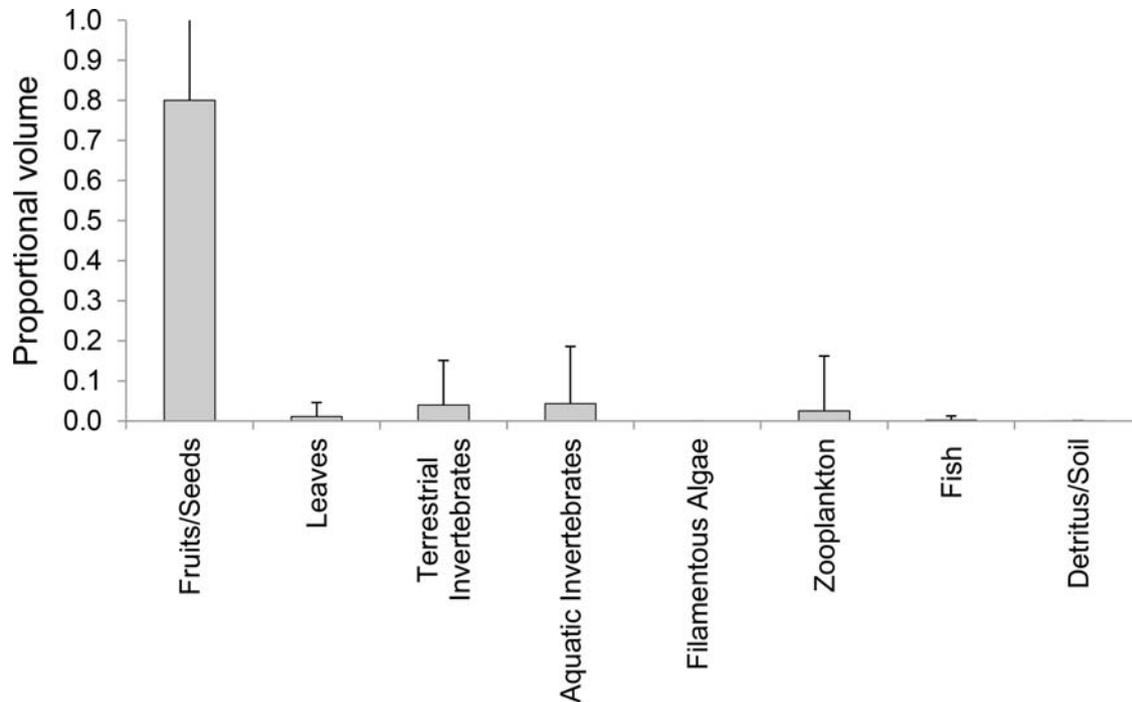
Within-source isotopic variation increases uncertainty in mixing model results (Boecklen et al. 2011, Fry 2013), and understanding of this variation is pivotal for sampling protocols for food-web research. Here we demonstrated that mean  $\delta^{15}\text{N}$  but not  $\delta^{13}\text{C}$  differ between riparian plants from várzea and igapó forests of the Amazon. Within-site inter-specific variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was large, and fruits had heavier  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  than leaves of the same plant. Large between-site variation in  $\delta^{15}\text{N}$  emphasizes the need for *in-situ* sampling in order to establish reliable baselines for local food webs. Large interspecific variation in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of forest plants influenced the outcome of MixSIR models predicting contribution of terrestrial food sources to an aquatic consumer in a local food web. Differences in isotopic signatures among tissues within plants increased the ability of MixSIR models to correctly identify the most important food source. Thus, local inter-specific and among-tissue isotopic variation should be considered in sampling of basal production sources and modelling their relative contribution to consumer biomass.



**Figure 5.** Carbon and nitrogen stable isotope ratios (mean  $\pm$  SD) of a frugivorous fish and potential food sources (abundant primary producers) in the flooded forest (várzea) of the Tarapoto Lakes complex, western Amazonia. Small black diamonds represent isotopic signatures of individual fish. Fish positions were adjusted to account for trophic fractionation. Dashed polygon connects mean source values; dotted oval encloses sources within approximately one standard deviation of the mean.

## Supplementary material

**Appendix 1.** Diet of *Mylossoma duriventre* assessed from the volumetric analysis of stomach contents of 55 individuals caught during the rising waters season of 2007 in the flooded forest (várzea) of the Tarapoto Lakes complex, western Amazon Basin.



**Appendix 2.** Isotopic and elemental ratios of leaves and fruits of woody plants ( $n = 28$  and  $22$  species, respectively) in the flooded forest (várzea) of the Tarapoto Lakes complex, western Amazon Basin. Species: indet. = indeterminate. Growth habit: Pl = palm, S = shrub, T = tree, Li = liana. Tissue: L = leaf, F = fruit (pulp and seed/s), S = seed only, P = pulp only. Maturity: M = mature, R = ripe, I = immature. \*Species included in mixing models assessing the relative contribution of different food sources to a frugivorous fish.

Family	Species	Code	Growth habit	Tissue	Maturity	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N Ratio
Annonaceae	Annonaceae indet.	Anin*	T	L	M	-29.52	1.64	27.46
				P	I	-28.62	3.35	50.80
	<i>Guatteria</i> sp.	Gusp*	T	L	M	-32.97	0.46	24.90
Apocynaceae	<i>Tabernaemontana</i> sp.	Tasp*	S	F	I	-32.70	1.87	44.95
				L	M	-29.73	1.16	18.37
				F	I	-29.55	3.76	23.41
Arecaceae	<i>Blepharodon</i> sp.	Blsp	Li	L	M	-32.21	4.73	14.19
	<i>Bactris</i> sp.1	Basp1*	Pl	L	M	-34.08	0.34	36.96
	<i>Bactris</i> sp.2	Basp2*	Pl	S	n/a	-33.61	3.40	74.68
Clusiaceae	<i>Garcinia macrophylla</i>	Gama	T	L	M	-33.90	0.85	28.64
				F	R	-32.65	3.40	58.16
				F	I	-30.76	1.60	44.30
Euphorbiaceae	<i>Amanoa oblongifolia</i>	Amob	T	L	M	-30.57	1.21	31.17
	<i>Croton bilocularis</i>	Crbi	T	L	M	-30.19	-0.28	30.72
	<i>Phyllanthus</i> sp.	Physp	S	F	I	-29.25	1.15	18.66
Hypericaceae	<i>Vismia macrophylla</i>	Vima*	T	L	M	-32.64	1.21	22.59
				F	n/a	-32.96	1.04	33.59
				F	n/a	-35.33	2.68	28.56

## Isotopic variation Amazonian flooded forests

## Appendix 2. Continued.

Family	Species	Code	Growth habit	Tissue	Maturity	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N Ratio
Melastomataceae	<i>Mouriri grandiflora</i>	Mogr*	T	L	M	-34.31	2.47	30.63
				F	R	-32.53	4.23	75.48
Myristicaceae	<i>Tococa guianensis</i>	Togu	S	L	M	-31.47	2.71	24.52
	<i>Iryanthera</i> sp.	Irsp*	T	L	M	-33.47	0.82	27.78
				F	I	-32.01	1.91	39.80
Myrtaceae	Myrtaceae indet. 1	Myrt1	T	L	M	-34.61	2.52	34.35
	Myrtaceae indet. 2	Myrt2*	T	L	M	-35.41	-0.33	34.69
				F	R	-32.62	1.13	41.10
	Myrtaceae indet. 3	Myrt3*	T	L	M	-33.01	2.18	42.51
				F	I	-31.06	2.68	46.04
Ochnaceae	<i>Ouratea</i> sp.	Ousp*	S	L	M	-27.03	3.32	36.02
				F	R	-24.97	4.40	30.93
Olacaceae	<i>Dulacia</i> sp.	Dusp*	T	L	M	-33.69	-0.11	32.21
				F	R	-33.30	2.63	31.01
Polygonaceae	<i>Symmeria paniculata</i>	Sypa*	S	L	M	-29.84	2.56	36.45
				S	I	-29.60	4.18	43.34
Rubiaceae	<i>Alibertia</i> sp.	Alsp	T	L	M	-32.12	4.65	15.98
	<i>Faramea</i> sp.	Fasp*	S	L	M	-33.21	2.88	26.70
				F	I	-36.17	1.39	21.54
	<i>Psychotria</i> sp.	Pssp*	S	L	M	-30.89	4.21	15.00
				F	R	-29.83	3.08	20.18
Sapindaceae	<i>Paullinia</i> sp.	Pasp*	Li	L	M	-32.07	0.19	33.89
				F	I	-30.53	1.62	46.97
Sapotaceae	<i>Micropholis</i> cf. <i>venulosa</i>	Micfve*	T	L	M	-31.39	1.42	38.85
				P	I	-29.93	1.01	43.52
	<i>Micropholis</i> sp.	Misp	T	L	M	-35.22	1.24	27.23
	<i>Pouteria</i> sp.	Posp*	T	L	M	-32.82	0.67	29.62
				F	I	-30.96	0.52	22.72
Urticaceae	<i>Cecropia</i> sp.	Cesp*	T	L	M	-28.70	1.44	18.23
				F	I	-29.01	0.88	22.84
Vitaceae	<i>Cissus</i> sp.	Cisp*	Li	L	M	-29.48	0.75	15.63
				F	I	-30.00	0.29	19.10

**Appendix 3.** Isotopic and elemental ratios of leaves and fruits of woody plants (n = 10 and 22 species, respectively) in the flooded forest (igapó) of the lower Apaporis River, western Amazon Basin. Values represent mean  $\pm$  SD. Species: indet. = indeterminate. Growth habit: H = hemi-epiphyte, Pl = palm, S = shrub, T = tree, Li = liana. Tissue: L = leaf, F = fruit (pulp and seeds), S = seed only, P = pulp only. Maturity: M = mature, Y = young, R = ripe.

Family	Species	Growth habit	Tissue	Maturity	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N Ratio	N
Apocynaceae	<i>Marsdenia rubrofusca</i>	Li	L	Y & M	-28.85 $\pm$ 0.80	4.76 $\pm$ 0.48	17.22 $\pm$ 4.55	4
			S	R	-26.97	5.35	18.29	1
Aquifoliaceae	<i>Ilex</i> sp.	S	F	R	-31.43 $\pm$ 0.96	4.29 $\pm$ 0.39	42.36 $\pm$ 10.70	5
Arecaceae	<i>Bactris riparia</i>	Pl	P	R	-32.83	5.62	47.70	1
	<i>Astrocaryum jauari</i>	Pl	P	R	-29.57 $\pm$ 0.28	5.15 $\pm$ 0.17	33.89 $\pm$ 2.02	2
Chrysobalanaceae	Indet.	T	L	Y	-32.47	6.59	23.66	1
Ebenaceae	<i>Diospyros poeppigiana</i>	T	S	n/a	-31.14	2.67	43.82	1
Euphorbiaceae	<i>Alchornea discolor</i>	T	F	R	-27.60	1.24	19.13	1
	<i>Mabea nitida</i>	T	L	M	-33.69	3.93	22.90	1
Fabaceae	<i>Inga</i> sp.1	T	L	Y	-30.14 $\pm$ 0.16	3.45 $\pm$ 0.41	15.12 $\pm$ 1.24	2
			P	R	-30.45 $\pm$ 1.14	0.77 $\pm$ 0.24	22.43 $\pm$ 1.96	3
	<i>Inga</i> sp.2	T	L	Y	-31.60	3.41	12.53	1

## Appendix 3. Continued.

Family	Species	Growth habit	Tissue	Maturity	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C:N Ratio	N
	<i>Macrolobium acaciifolium</i>	T	L	Y	-28.61	5.83	13.72	1
			S	R	-27.89	4.02	29.21	1
			F	R	-30.52	4.26	34.80	1
Lauraceae	<i>Nectandra egensis</i>	T	L	M	-30.86 ± 0.57	4.11 ± 0.52	26.66 ± 3.37	3
			F	R	-29.78 ± 1.11	3.82 ± 1.21	65.67 ± 23.03	3
Malpighiaceae	<i>Byrsonima japurensis</i>	T	F	R	-32.60 ± 0.23	3.97 ± 0.91	71.85	3
Melastomataceae	<i>Miconia splendens</i>	T	F	n/a	-29.38	4.14	33.01	1
	<i>Tococa coronata</i>	S	F	R	-30.54	6.35	24.09	1
Moraceae	<i>Ficus americana</i>	H	L	Y & M	-32.46 ± 0.23	4.07 ± 0.63	24.48 ± 3.43	2
			F	R	-31.86 ± 0.87	4.37 ± 0.46	47.63 ± 7.18	3
Myrtaceae	<i>Eugenia</i> sp.	T	F	R	-33.32	0.60	85.96	1
	<i>Psidium densicomum</i>	S	L	Y & M	-29.07 ± 1.98	4.20 ± 0.82	17.60 ± 5.01	6
Ochnaceae	<i>Quiina amazonica</i>	S	F	R	-26.88	3.22	49.51	1
Polygonaceae	<i>Symmeria paniculata</i>	S	L	Y	-28.57 ± 1.26	3.99 ± 1.06	17.69 ± 2.17	3
Rubiaceae	<i>Psychotria</i> cf. <i>lupulina</i>	S	F	R	-31.46 ± 1.67	4.86 ± 0.69	23.86 ± 4.97	4
	<i>Bothriospora corymbosa</i>	S	F	R	-29.51 ± 0.76	5.45 ± 0.68	22.24 ± 1.14	4
	<i>Genipa americana</i>	T	F	R	-29.40 ± 0.57	3.59 ± 4.27	43.09 ± 17.53	2
	<i>Amaioua guianensis</i>	T	F	R	-32.18	3.41	78.12	1
Sapindaceae	<i>Matayba guianensis</i>	T	F	R	-31.02 ± 1.14	3.72 ± 1.08	44.95 ± 6.41	4
Simaroubaceae	<i>Simaba orinocensis</i>	T	P	R	-27.68	5.46	15.76	1

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