Biota Neotropica 17(3): e20170337, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
article

Bioprospection and characterization of the amylolytic activity by filamentous fungi from Brazilian Atlantic Forest

Paula Zaghetto de Almeida[1]*, Marita Gimenez Pereira*[2]*, Caio Cesar de Carvalho*[2]*, Paulo Ricardo Heinen*[1]*,*
Luciana Sobrani Ziotti[2]*, Josana Maria Messias*[3]*, João Atilio Jorge*[2] *&*
Maria de Lourdes Teixeira de Moraes Polizeli[2*]

[1]*Universidade de São Paulo, Faculdade de Medicina de Ribeirão Preto, Departamento de Bioquímica e Imunologia, Ribeirão Preto, SP, Brazil*
[2]*Universidade de São Paulo, Faculdade de Filosofia, Ciências e Letras, Departamento de Biologia, Ribeirão Preto, SP, Brazil*
[3]*Universidade de São Paulo, Faculdade de Filosofia, Ciências e Letras, Departamento de Química, Ribeirão Preto, SP, Brazil*
**Corresponding author: Maria de Lourdes Teixeira de Moraes Polizeli, e-mail: polizeli@ffclrp.usp.br*

ALMEIDA, P. Z., PEREIRA, M. G., CARVALHO, C. C., HEINEN, P. R., ZIOTTI, L. S., MESSIAS, J. M., JORGE, J. A., POLIZELI, M. L. T. M. **Bioprospection and characterization of the amylolytic activity by filamentous fungi from Brazilian Atlantic Forest.** Biota Neotropica. 17(3): e20170337. http://dx.doi.org/10.1590/1676-0611-BN-2017-0337

Abstract: Filamentous fungi are widely diverse and ubiquitous organisms. Such biodiversity is barely known, making room for a great potential still to be discovered, especially in tropical environments - which are favorable to growth and species variety. Filamentous fungi are extensively applied to the production of industrial enzymes, such as the amylases. This class of enzymes acts in the hydrolysis of starch to glucose or maltooligosaccharides. In this work twenty-five filamentous fungi were isolated from samples of decomposing material collected in the Brazilian Atlantic Forest. The two best amylase producers were identified as *Aspergillus brasiliensis* and *Rhizopus oryzae*. Both are mesophilic, they grow well in organic nitrogen-rich media produce great amounts of glucoamylases. The enzymes of *A. brasiliensis* and *R. oryzae* are different, possibly because of their phylogenetical distance. The best amylase production of *A. brasiliensis* occurred during 120 hours with initial pH of 7.5; it had a better activity in the pH range of 3.5-5.0 and at 60-75°C. Both fungal glucoamylase had wide pH stability (3-8) and were activated by Mn^{2+}. *R. oryzae* best production occurred in 96 hours and at pH 6.5. Its amylases had a greater activity in the pH range of 4.0-5.5 and temperature at 50-65ºC. The most significant difference between the enzymes produced by both fungi is the resistance to thermal denaturation: *A. brasiliensis* glucoamylase had a T_{50} of 60 minutes at 70ºC. The *R. oryzae* glucoamylase only had a residual activity when incubated at 50°C with a 12 min T_{50}.

Keywords: *Amylase, Filamentous fungi, Aspergillus brasiliensis, Rhizopus oryzae, Glucoamylase, Bioprospection*

Bioprospecção e caracterização da atividade amilolítica de fungos filamentosos da Mata Atlântica Brasileira

Resumo: Fungos filamentosos são organismos amplamente diversificados e ubíquos. Esta biodiversidade ainda é pouco caracterizada, desta forma, há um grande potencial a ser descoberto, sobretudo em biomas tropicais, que favorecem o crescimento e diversificação de espécies. Fungos filamentosos são extensivamente utilizados para a produção industrial de enzimas, como as amilases. Esta classe de enzimas atua na hidrólise do amido em glicose ou maltooligossacarídeos. Neste trabalho 25 cepas de fungos filamentosos foram isoladas a partir de amostras de material em decomposição coletados na Mata Atlântica Brasileira. As duas cepas que produziram mais amilases foram identificadas como *Aspergillus brasiliensis* e *Rhizopus oryzae*. Ambos os fungos são mesofílicos, crescem bem em meio de cultivo rico em nitrogênio orgânico, e produziram grande quantidade de glucoamilase. As enzimas de *A. brasiliensis* e *R. oryzae* possuem características distintas, possivelmente devido à distância filogenética das espécies. A produção de amilase mais expressiva de *A. brasiliensis* ocorreu em 120 horas de cultivo e pH inicial de 7,5; possui maior atividade em temperaturas entre 60-75ºC e pH entre 3,5-5,0. Ambas glucoamilases fúngicas obtiveram ampla estabilidade de pH (3-8) e foram ativadas por Mn^{2+}. A melhor produção de *R. oryzae* ocorreu em 96 horas de cultivo e pH 6,5. Suas amilases são mais ativas na faixa de pH de 4,0-5,5 e temperatura entre 50-60ºC. A diferença mais significativa dentre as enzimas produzidas pelos fungos selecionados é a resistência à desnaturação térmica, tendo a glucoamilase de *A. brasiliensis* um T_{50} de 60 minutos a 70ºC, já a glucoamilase de *R. oryzae* somente obteve atividade residual quando incubada a 50°C, com um T_{50} de apenas 12 minutos.

Palavras-Chave: *Amilase, Fungos filamentosos, Aspergillus brasiliensis, Rhizopus oryzae, Glucoamilase, Bioprospecção*

Introduction

Fungi are widely diverse and distributed in all terrestrial ecosystems. They are decomposers, mutualists or pathogens with crucial roles in the cycling of nutrients (Tedersoo et al. 2014). According to Blackwell (2011), it is estimated that there are about 5.1 million species of fungi around the world. Up to the present date, the dictionary of fungi counts fewer than 100,000 species (Kirk et al. 2008).

Fungus diversity is spread along a latitudinal gradient. Therefore, equatorial and tropical forests, such as the Brazilian Atlantic forest, hold a great part of this diversity (Shi et al. 2014; Tedersoo et al. 2014). This forest has one of the most diverse and threatened biotas of the world, which remains with just 11.4% to 16% of the original coverage (Ribeiro et al. 2009; Joly et al. 2014).

Filamentous fungi are known as great enzyme producers, like amylases. Starch is the main reserve carbohydrate in plants and the second most abundant carbohydrate in nature. It is present in corn, potato, rice and wheat, what accounts for a great part of the human diet. Starch is enzymatically hydrolyzed to maltose, glucose and oligosaccharide syrup (Vielle & Zeikus 2001). Amylases are widely applied in industries such as textile, paper and cellulose, detergent, baking and beverage. They account for about 30% of total enzymes commercialized (Vielle & Zeikus 2001; Van Der Maarel 2002; Souza & Magalhães, 2010). Enzymatic hydrolysis is more specific than is chemical hydrolysis and has a theoretical efficiency of 100% (Ballesteros et al. 2002).

In this research, 25 filamentous fungi present in decaying material from the Brazilian Atlantic forest were isolated. The two strains with bigger secretion of enzymes had the production optimized and the amylases of crude extract were characterized. They showed characteristics with possible industrial application.

Material and Methods

1. Isolation of fungi

The fungi were isolated from decaying materials collected in the Atlantic forest in Peruíbe, SP, Brazil (Table 1). The samples were stored in sterile tubes, diluted in the ration of 1:1000 or 1:10000 and plated in oatmeal agar (2% oatmeal flour, 2% bacteriological agar, 0.5% glucose) containing traces of pentabiotic (benzathine benzylpenicillin, procain benzylpenicillin, potassium benzylpenicillin, dihydrostreptomycin sulfate, streptomycin sulfate). The Petri plates were incubated at 30°C for seven days. The strains grown were purified through successive streaking.

1.1 Selection of strains

The isolated strains were grown in conical tubes containing oatmeal medium. An aqueous suspension of conidia was obtained by scraping the surface of each one of these cultures with 10 mL distilled water. A volume of 1 mL of the conidia suspension was inoculated in 25 mL Adams modified medium (AM) as described by Peixoto et al. (2003) during 72 hours at 30°C. Then, the cultures were vacuum-filtered and dialyzed overnight in distilled water at 4°C for removal of residual reducing sugars. After this process, the amylolytic activity was measured.

1.2 Enzymatic assay

Amylolytic activity was determined with 3,5-Dinitrosalicylic acid (DNS) (Miller 1959). The assay was composed of 50 µL crude extract and 50 µL 1% soluble starch in 50 mM sodium citrate buffer, pH 5.5. The mixture was incubated at 60°C for 5 min, interrupted by the addition of 100 µL DNS reagent, and boiled for 5 minutes. After cooling, 1 mL distilled water was added and the assay was read at 540 nm in SpectraMax Plus 384 Microplate Reader. The blank was consisted the enzyme inactivated by DNS prior to the addition of the substrate. One unit of enzyme activity was defined as that catalyzing the conversion of 1 µmol glucose, per minute, in the assay conditions.

1.3 Optimization of culture

The cultures were carried out in 125 mL Erlenmeyer flasks containing 25 mL of one of the following liquid media: AM (Adams 1990, Peixoto et al. 2003); Khanna (Khanna et al. 1995), Segato Rizzatti (SR, Rizzatti et al. 2001) or Vogel (Vogel 1964). The pH of the medium was adjusted to 6.5 and it was supplemented with 1% soluble starch, as carbon source. A volume of 10^5 spores per mL of culture medium was inoculated and the incubation occurred in bacteriological incubator (static condition) or shaker (100 rpm) for 3 days, at 30ºC.

In order to determine the best temperature for fungal growth, cultures were held at 25, 30, 35 and 40ºC during 72 hours in the culture medium previously selected (AM and SR). In order to determine the effect of the initial pH in the enzymatic production, a pH range of 6.5 to 8.5 was tested for *Aspergillus brasiliensis* and 5.5 to 7.5 for *Rhizopus oryzae*. Time course was performed up to 144 hours to select the time of higher enzymatic secretion.

Table 1. Georeferencing means of isolated fungi and amylase activity

Collected material	Temperature (°C)	GPS precision (m)	Altitude (m)	Latitude	Longitude	Isolated fungi (Amylase U/mL)
Lichen	30	15	11	S-24°22.405’	H047°03.951’	1/9A (0); 1A (0); 1B (0); 1C (4.4);1D (0.3)
Flower	29	15	11	S-24°22.405’	H047°03.951’	2A (0.2); 2B (0.2)
Branch	30	15	11	S-24°22.405’	H047°03.951’	3A (0.5)
Green Leaf	26	11	34	S-24°26.462’	H047°04.050’	5A (0.4)
Bracken	26	16	34	S-24°26.462’	H047°04.050’	6A (0.4); 6B (3.5)
Lichen	26	16	34	S-24°26.462’	H047°04.050’	7A (0.5)
Leaf litter	26	16	34	S-24°26.462’	H047°04.050’	8A (4.2); 8B (0.5); 8C (0.3)
Leaf	26	11	2	S-24°24.462’	H047°04.050’	9A (0.5); 9B (0);
Lichen stone	29	9	2	S-24°26.054’	H047° 03.437’	10A (0);10B (0.1)
Sand	29	10	0	S-24°26.038’	H047°03.450’	11A (0)
Fruit	29	9	34	S-24°25.987’	H047°03.440’	13A (0)
Mushroom	29	14	5	S-24°25.462’	H047°03.440’	14A (0.5); 14B (0.7); 14C (0)
Atemoya	29	17	25	S-24°25.916’	H047°03.445’	15A (0.3)

1.4 Characterization of the crude extract

In order to determine the pH stability, the crude extract was incubated in McIlvaine buffer between 2.5 and 8.0 during 24 hours at 25°C (McIlvaine 1921). The resistance to thermal denaturation was determined at 50, 60 and 70 °C during 10 to 300 minutes.

In order to estimate the most favorable temperature zone and pH for enzymatic activity, an experimental design 2^2 was proceeded with three central point repetitions at $p<0.5\%$. The results were analyzed with the Statistica 12 software. The points were composed of the variation of pH in sodium citrate buffer 50 mM, range 3.5 to 7.5, and the temperature in a range of 40 to 70°C.

Several carbon sources (starch, wheat bran, glucose, maltose, barley bagasse, ground corn and oatmeal flour), at 1% concentration, were added to culture media of both fungi aiming to determine the type of amylase synthetized in optimal conditions of the microorganism development. After incubation, the samples were collected, dialyzed and incubated with 1% starch in sodium citrate buffer, for 20 minutes. The enzymatic assays were stopped by boiling. The end-products of the enzymatic assays were applied in a Thin-Layer Chromatography (TLC) and a solution of 1 mg/mL glucose, maltose, maltotriose, maltotetraose, and maltopentose was carried as control. The chromatography was developed in a solution of n-butanol, ethanol and distilled water (5:3:2). After the plates were developed and dried, they were sprayed with a solution containing 18 mL methanol, 2 mL sulfuric acid, and 0.04 g orcinol, and it was revealed at 100°C until the spots came clear.

The following compounds were added in the enzymatic assays at concentrations of 2 and 5 mM to determine the effect on the activity: NH_4F, NaH_2PO_4, $ZnCl_2$, $AlCl_3.6H_2O$, $AgNO_3$, KH_2PO_4, $CuSO_4.5H_2O$, BaCl, NH_4Cl, $CoCl_2.6H_2O$, $MgCl_2.6H_2O$, $Zn(NO_3)_2.6H_2O$, KCl, $Pb(C_2H_3O_2)_2.3H_2O$, NaBr, CaCl, NaCl, $MnCl_2.4H_2O$, β-mercaptoethanol and EDTA.

1.5 Fungi identification and deposit

The fungi 6B and 8A were identified using the regions of ITS1-ITS4 (Forward 5'-TCCGTAGGTGAACCTTGCGG-3' – Reverse 5'-TCCTCCGCTTATTGATATGC-3'), Calmodulin (Forward 5'-GCCGACTCTTTGACYGARGAR-3' – Reverse 5'-TTTYTGCATCATRAGYTGGAC-3') and β-tubulin (Forward 5'-GGTAACCAAATGGTGCTGCTTTC-3' – Reverse 5'-ACCCTCAGTGTAGTGACCCTTGGC-3') (White et al. 1990, Glass & Donaldson 1995, Balajee et al. 2009). The DNA extraction occurred according to Maki et al (2001). The sequencing was carried out at the blood center of Ribeirão Preto-SP. The sequences were aligned on BioEdit and blasted on GenBank database. The strains identified were added to the Filamentous Fungi Collection from the Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (FFCLRP-USP), and catalogued as CFF124 to *A. brasiliensis* and CFF 132 to *R. oryzae*. The unidentified fungi were cryopreserved for future access.

1.6 Phylogenetic analysis

The Phylogenetic analysis with *Rhizopus oryzae* and *Aspergillus brasiliensis* was based on the regions of Internal Transcribed Spacer (ITS) 1 and 4. A multiple alignment of sequences was obtained with the MUSCLE 3.6 software (Edgar 2004). Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 6 (Tamura et al. 2013). The phylogenetic trees were based on the Maximum Likehood using the Neighbor-Joining method with 500 robustness non-parametric bootstrap replicates. The similarity rate between the sequences was shown in percentage considering the number of conserved bases and the total number of aligned bases.

Results

2. Selection and identification

Twenty-five strains of filamentous fungi were isolated from samples of organic material in decomposition (Table 1). The strains were cultivated for 3 days in AM liquid medium. The amylolytic activities of the crude extracts were measured and are expressed on Table 1. Two strains stood out: the fungus 6B (3.5 U/mL) and the fungus 8A (4.2 U/mL).

The regions ITS 1-4 were amplified and sequenced for the identification of fungi (Table 2). The fungus 8A was identified as *Rhizopus oryzae* Went & Prinsen Geerligs. The fungus 6B showed a great similarity to the fungus *Aspergillus brasiliensis* Varga, Frisvad & Samson and *Aspergillus niger* van Tiegh. Hence, the amplification of the fragment of β-tubulin and calmodulin genes was performed, concluding the identification as *Aspergillus brasiliensis*.

2.1 Optimization of culture

The fungi selected were cultured in Khanna, AM, SR and Vogel media for 3 days, at 30°C, either in stirring at 100 rpm or in static condition, with initial pH adjusted to 6.5. The inoculum was composed of 10^5 spores per mL of culture medium. *A. brasiliensis* had a greater secretion in the SR medium under static fermentation, which saves more energy in comparison with the agitation condition (Figure 1). The SR medium contains yeast extract (0.45%) as the main source of nitrogen and it was the only medium tested with peptone (0.02%). The salt compositions of the culture medium are monobasic potassium phosphate in low concentration (0.015%), 0.012% magnesium sulfate heptahydrate and 0.05% monobasic ammonium phosphate. The latter can be also considered as a source of inorganic nitrogen.

R. oryzae had the best secretion of amylases in AM medium under agitation. AM medium has the biggest amount of yeast extract between the media tested (0.8%). In the Vogel medium, its activity was very low, suggesting a low assimilation of inorganic nitrogen. Besides carbon and nitrogen sources, the AM medium is composed by only two salts: monobasic potassium phosphate (0.3%) and magnesium sulfate heptahydrate (0.05%), both in a much higher concentration than the SR medium.

The fungi were grown in several temperatures in the previously standardized medium conditions for 3 days. The extracellular amylolytic activity was greater at 30°C for both fungi, suggesting that both are to be considered as mesophilic (Figure 2A).

In order to determine the ideal initial pH, the culture media were adjusted in the range 6.5 - 8.5 to the fungus *A. brasiliensis,* and in the range of 5.5 to 7.5 for *R. oryzae*. The initial pH of 7.5 favored the production

Table 2. Homologies found with the nucleotide blast tool from NCBI (National Center for Biotechnology Information) to the fungi 6B and 8A

Fungi	Sequence	Homology	Score	Valor E	Identity (%)
6B	ITS 1-4	*Aspergillus brasiliensis/ Aspergillus niger*	894/ 893	0.0/ 0.0	100 /100
	β-tubulin	*Aspergillus brasiliensis*	819	0.0	100
	Calmodulin	*Aspergillus brasiliensis*	1201	0.0	100
8A	ITS 1-4	*Rhizopus oryzae*	791	0.0	100

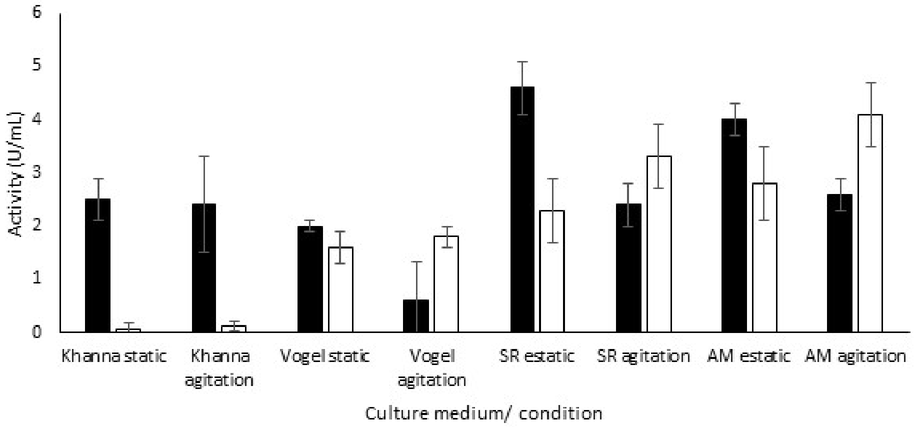


Figure 1. Production of amylase in several culture media, under static and agitation conditions. Symbols: (■) *A. brasiliensis*; (□) *R. oryzae*.

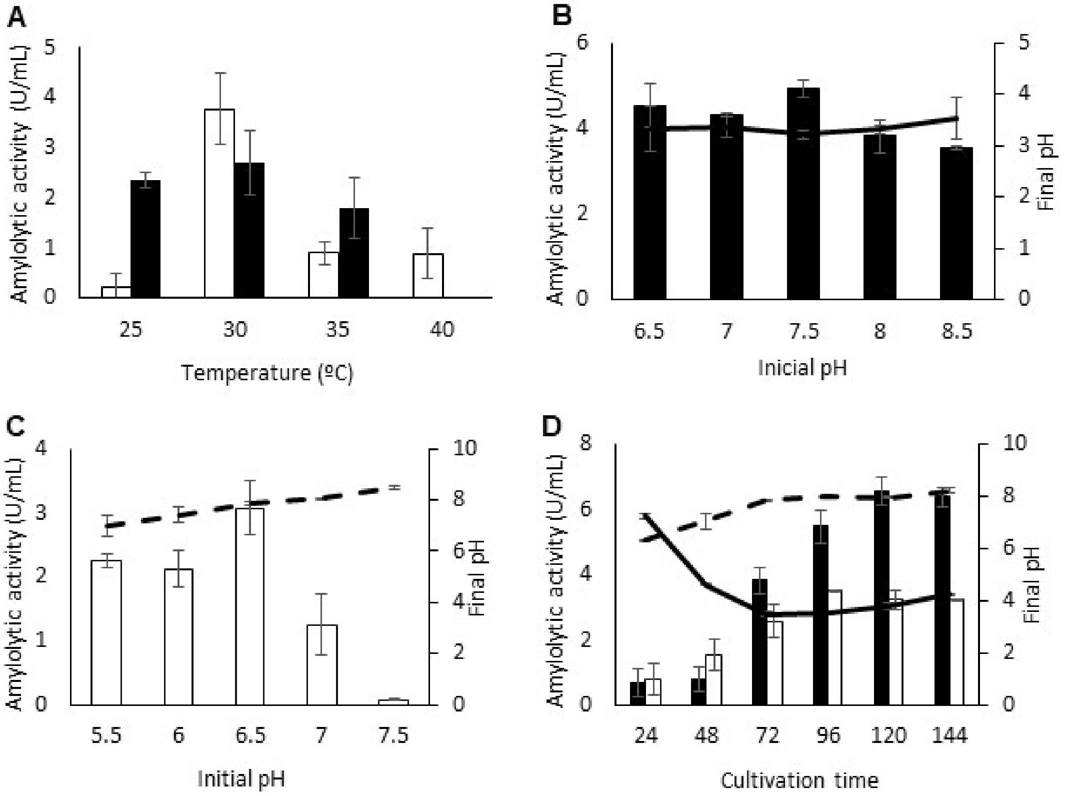


Figure 2. Determination of the effect of temperature (A), pH (B, C) and time of culture on amylase activity (U/mL) of *A. brasiliensis* (■), and *R. oryzae* (□). Final pH of *A. brasiliensis* (-); *R. oryzae* (- -).

of amylases by *A. brasiliensis* (Figure 2B). The final pH in all conditions tested was much lower than the initial one (around 3.5). In contrast, the best initial pH to *R. oryzae* was 6.5 (Figure 2C). The final pH in every condition was higher than was it in the initial one (about 8.0).

Time-course of amylase production was carried until 144 hours (Figure 2D). The period of 120 hours had the greater amylolytic secretion to *A. brasiliensis*. It was also noted that the pH of the culture medium began decreasing on the second day and stabilized after 72 hours. On the other hand, the culture of 96 hours was more favorable to *R. oryzae*. The pH of the culture medium began increasing on the second day and stabilized after 72 hours.

2.2 Optimum pH and temperature

An experimental design was performed to determine the optimum pH and temperature for the enzymatic hydrolysis according the points in Table 3. The experimental design was composed of 11 assays with 3 repetitions at the central point. According to the results, the enzymatic activity of the crude extract of *A. brasiliensis* was determined by the following equation:

$$\text{Activity (U/mL)} = 5.55 - 1.99.\ \text{pH} - 1.\ (\text{T°C.pH}) - 1.42.\ (\text{T°C})^2 - 1.14.\ (\text{pH})^2$$

The surface (Figure 3A) plot shows that the ideal assay temperature was in the range of 60 - 75°C, and the pH of 3.5 - 5. Replacing the values correspondent to 70°C (0.943) and pH 4 (-0.977), the activity of 6 U/mL was obtained. An assay in triplicate was carried out in the same parameters and an activity of 6.7 was obtained with a standard variation of ± 0.7, as expected to the model, with an r^2 of 0.96 and a calculated F 18.2 times higher than the tabulated F.

The enzymatic activity of the crude extract of *R. oryzae* can be determined by the following equation:

$$\text{Activity (U/mL)} = 2.52 - 0.68.\ \text{pH} - 0.96.\ (\text{T°C})^2 - 0.68.\ (\text{pH})^2$$

According to the surface plot (Figure 3B), the ideal assay temperature was in the range of 50-65°C and the pH of 4.0-5.5. Replacing the values of 60°C (0) and pH 4.0 (-0.977), 2.54 U/mL was obtained. An assay in the same conditions obtained 2.94 ± 0.58 U/mL, as expected to the model, with an r^2 of 0.9 and a calculated F 5.9 times higher than the tabulated F.

2.3 Stability and effect of compounds

The crude extracts were incubated in McIlvaine buffers for 24 hours (Figure 4A) at 25°C. The amylase of *A. brasiliensis* kept the activity above 90% in the range of pH 3.0-6.5 and above 88% in the range of pH 7.0-8.0. The amylase of *R. oryzae* kept 100% of the activity in the range of pH 3.5-8.0. At pH 3.0, it kept 91% of its activity and 75% at pH 2.5.

Table 3. Points used at the experimental design for the crude extract of *A. brasiliensis* and *R. oryzae*

	-1.41	**-1**	**0**	**+1**	**+1.41**
pH	3.5	4.09	5.5	6.91	7.5
Temperature	40	45.82	60	74.18	80

The thermal denaturation of amylases of both fungi was analyzed at several times and temperatures (Figure 4B). Amylases of *A. brasiliensis* were completely stable at 50°C, for 120 minutes. The enzyme was completely stable at 60°C, for 100 minutes. However, at 70°C the half-life was 32 minutes. *R. oryzae* amylases, among all assayed conditions, just had residual activity after exposure at 50°C, with a half-life of 12 minutes.

The influence of several compounds upon the amylolytic activity was tested (Table 4). The Al^{3+} ions had little influence upon *A. brasiliensis* but, in contrast, at 5 mM, it completely inhibited the *R. oryzae* amylase. Ag^{2+} decreased the amylase of both fungi extracts, but had a greater effect over *R. oryzae*, with no residual activity at the highest concentration. The amylase activity of *R. oryzae* was 55% inhibited with 5 mM Cu^{2+} but increased 9% in *A. brasiliensis*. The 2 mM Ba^{2+} decreased the activity in *A. brasiliensis* and a slight increase (7%) was observed with 5 mM of this compound; the opposite happened with *R. oryzae*, which was activated at 2 mM and inhibited at 5 mM. On the other hand, Co^{2+} ions had little effect over *A. brasiliensis,* but decreased 42% at 5 mM in *R. oryzae*. Both extracts were not affected by Pb^{2+}. The stronger enzymatic activation was obtained with 5 mM Mn^{2+} for *A. brasiliensis* and at 2 mM for *R. oryzae*. The glucoamylases of both extracts were not considerably influenced by β-mercaptoethanol and EDTA.

2.4 Hydrolysis products of amylase on starch revealed by TLC

Amylases produced by both fungi in culture media supplemented with several carbon sources (1% starch; wheat bran; maltose; glucose; barley bagasse; ground corn and oatmeal flour) were assayed with starch and the

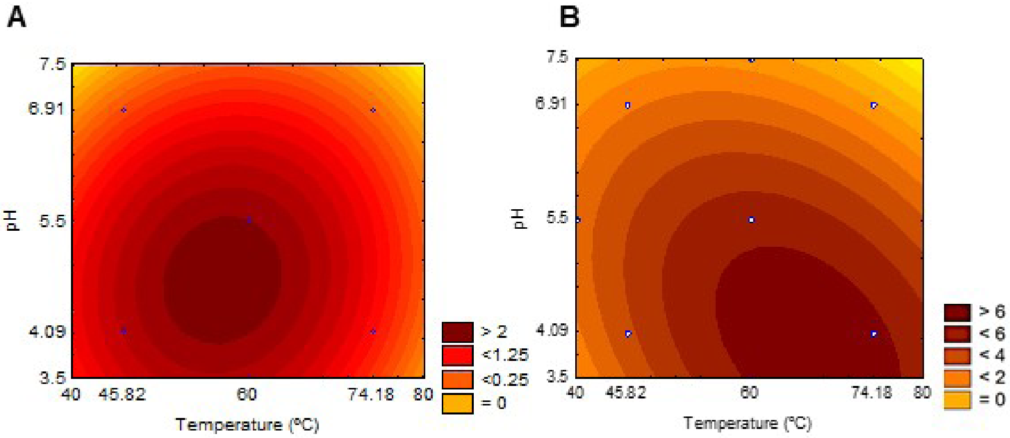


Figure 3. Zone of optimum pH and temperature to (A) *A. brasiliensis*; (B) *R. oryzae.*

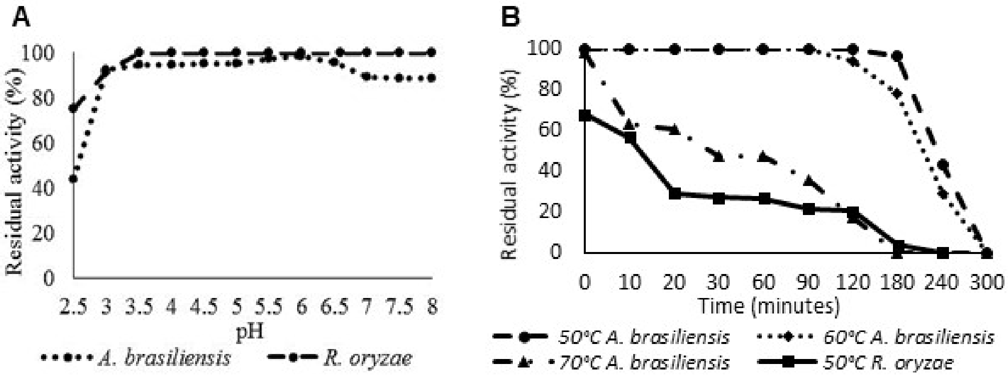


Figure 4. Stability of glucoamylases to pH, after 24 hours, at 25°C (A) and thermostability in optimal conditions of enzymatic assay (B) of *A. brasiliensis* and *R. oryzae*.

Table 4. Effect of compounds at enzymatic activity of *A. brasiliensis* and *R. oryzae*.

Compounds	Residual Activity (%)			
	A. brasiliensis		***R. oryzae***	
	Concentration		**Concentration**	
	2mM	**5mM**	**2mM**	**5mM**
None (control)	100	100	100	100
NH_4F	84	100	126	102
NaH_2PO_4	92	94	118	98
$ZnCl_2$	93	103	139	80
$AlCl_3.6H_2O$	93	94	93	0
$AgNO_3$	81	58	24	0
KH_2PO_4	102	97	97	72
$CuSO_4.5H_2O$	93	109	107	45
BaCl	55	107	114	66
NH_4Cl	104	104	109	102
$CoCl_2.6H_2O$	113	99	102	58
$MgCl_2.6H_2O$	97	101	117	97
$Zn(NO_3)_2.6H_2O$	94	100	110	79
KCl	91	105	102	86
$Pb(C_2H_3O_2)_2.3H_2O$	105	105	104	107
NaBr	99	101	127	94
CaCl	105	107	115	104
NaCl	101	102	115	88
$MnCl_2.4H_2O$	160	176	155	136
β-mercaptoethanol	100	100	100	100
EDTA	100	97	100	89

100% is equivalent to 5.9 U/mL to *A. brasiliensis* and 3.9 U/mL to *R. oryzae*

end-products were applied in TLC. The crude extract of *A. brasiliensis* in all sources enabled a great release of glucose and a small amount of oligosaccharides during the hydrolysis (Figure 5A), signaling for a higher secretion of glucoamylase. There was the production of glucoamylases even in the culture with glucose as an only carbon source, suggesting that it is a constitutive enzyme. The fungus *R. oryzae* also secreted more glucoamylase in all media, but there was the release of some more oligosaccharides, in small quantities, suggesting also the presence of α-amylases (Figure 5B). The glucoamylase of *R. oryzae* is also constitutive.

2.5 Phylogenetic analysis

The phylogenetic comparison of species close to *R. oryzae* and *A. brasiliensis* was based on the regions of ITS 1-2 (Internal Transcribed Spacer). The sequences were obtained on GenBank and aligned with MUSCLE 3.6. From the alignment, an unrooted dendrogram that shows the proximity of species was built (Figure 6). The analysis showed similarity between the sequences of *A. nidulans* (Eidam) G. Winter and *A. fumigatus* Fersen of 64.5%; 69.8% between *A. brasiliensis* and *A. awamori*; 71.1% between *A. oryzae* (Ahlb.) Cohn and *A. flavus* Link. The high rate of similarity between the species of *Aspergillus* can be observed with Castrillo et al. (2012), where *A. brasiliensis* and *A. awamori* belong to the same clade, and with Geiser et al. (2000), who demonstrate that *A. oryzae* and *A. flavus* form a paraphyletic group.

R. oryzae and *R. delemar* Boidin ex Wehmer & Hanzawa have a high rate of similarity (72.9%) and constitute a monophyletic group. *R. niveus* M. Yamaz. is closer to *R. oryzae* and *R. delemar,* with a high bootstrap value (100). Between the fungi studied, *Rhizopus microsporus var.*

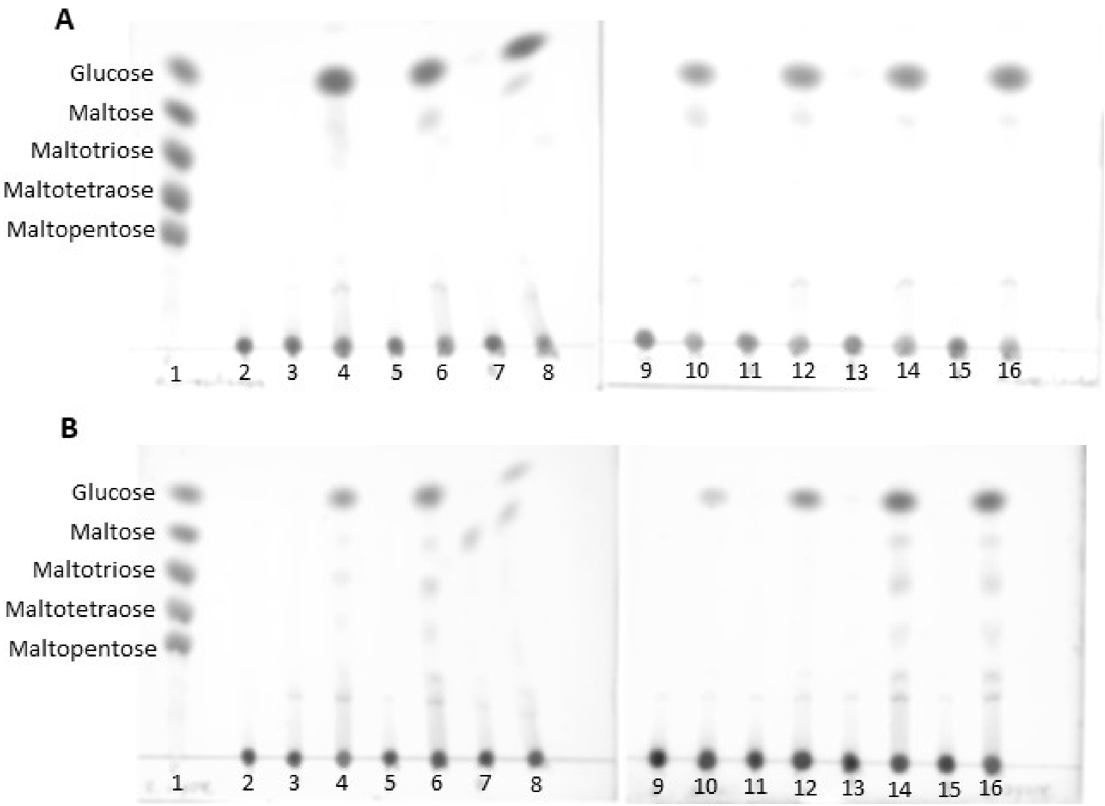


Figure 5. Hydrolysis products of *A. brasiliensis* (A) and *R. oryzae* (B) in culture with diverse carbon sources. The samples were applied in the following order: 1- controls; 2-substrate (1% starch); 3- crude extract of the culture with starch; 4- hydrolysis product of assay with starch extract; 5- wheat bran culture; 6- hydrolysis wheat bran extract; 7-maltose culture; 8- hydrolysis maltose extract; 9- glucose culture; 10- hydrolysis glucose extract; 11- barley bagasse culture; 12- hydrolysis barley bagasse extract; 13- ground corn culture; 14- hydrolysis ground corn extract; 15- oatmeal flour culture; 16- hydrolysis oatmeal flour extract.

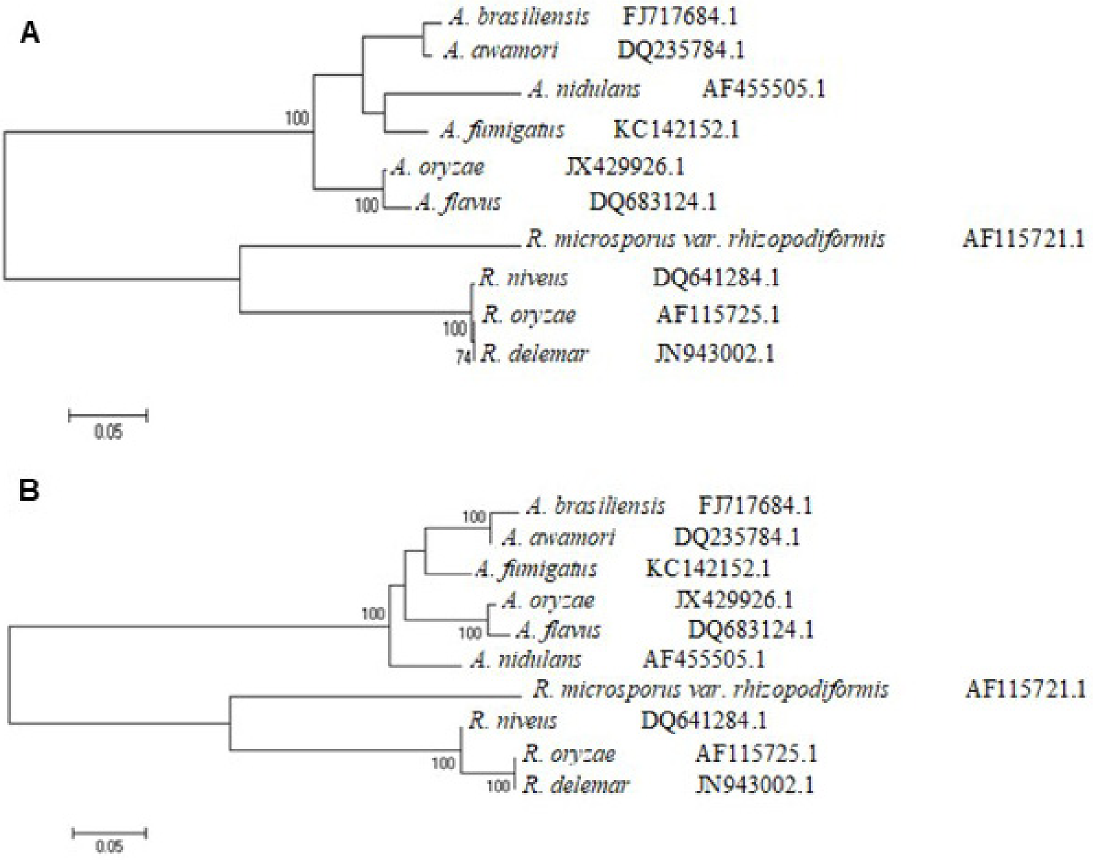


Figure 6: Molecular Phylogenetic analysis **(A) by Maximum Likelihood method.** The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura et al. (2013) model. The tree with the highest log likelihood (-2874.0908) is shown. The numbers show the bootstrap value analysis of 500 repetitions and the percentage higher than 70% of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 10 nucleotide sequences. There was a total of 776 positions in the final dataset. Evolutionary analyses were conducted in MEGA6. The species names are followed by the GenBank accession number.
(B) by Neighbor-Joining method. The optimal tree with the sum of branch length = 1.00924206 is shown. The percentage of replicate trees in which the associated taxa is clustered together in the bootstrap test (500 replicates) with the percentage higher than 70% are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tajima & Nei (1984) method and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 2). The analysis involved 10 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There was a total of 776 positions in the final dataset. Evolutionary analyses were conducted in MEGA6. The species names are followed by the GenBank accession number.

rhizopodiformis (Cohn) Schipper & Stalpers was the most singular of the species, forming an isolated taxon from other fungi of the genera; it also has the lowest rate of similarity (49.6% if compared to *R. delemar*). However, *R. rhizopodiformis* has the necessary apomorphy to be classified close to other *Rhizopus*. Liou et al. (2007) had similar results when they showed linages of *R. rhizopodiformis* among the main glucoamylase-producing species, what demonstrates the need for more information on this species.

Discussion

According to Domsch et al. (2007), the fungus distribution is related with the weather, soil, vegetation and the kind of organic matter. Fungi of the genera *Aspergillus* and *Rhizopus* are frequently isolated from the Atlantic forest biome (Costa et al. 2012, Schoenlein-Crusius et al. 2006, Tauk Tornisielo et al. 2005, Schoenlein-Crusius & Milanez 1998). The isolation methodology was not intended to recover a maximum number of species, but strains that were easily cultivated in laboratory conditions and prospective amylase producers.

The amylases have several applications in industry, and each of these bioprocesses requires enzymes with diverse characteristics of pH, temperature and stability. The importance of bioprospection lies in the discovery of novel enzymes, with unique features, that can contribute to a more efficient process (Dhali et al. 2016, Singh et al. 2014).

Coutinho & Reilly (1997) divided the glucoamylases following their evolutionary history. Fungi of *Aspergillus* genera are part of the most derivative group of glucoamylases, with a high efficient design. They have a well-developed starch-binding domain and a longer linker region. Fungi from the genera *Rhizopus* are part of a group with a more primitive glucoamylase structure, which reflects in its efficiency and stability.

The time-course of amylase production showed distinct characteristics of *A. brasiliensis* and *R. oryzae*. The pH of crude extract differed due to the production of secondary metabolites, probably the production of glycolic and citric acid by *A. brasiliensis* and ammonia *by R. oryzae*, as previously observed in *R. oligosporus* Saito when in nitrogen-rich culture media (Varga et al. 2007, Sparringa & Owens 1999).

When compared to fungi from the same genera, *A. brasiliensis* activity occurs in a more acidic pH and higher temperature than most of the others. Gudi et al. (2013) described that the glucoamylase of *A. niger* shows an optimum pH in the range of *A. brasiliensis* (4.5-5.5) and temperature below (65ºC). *A awamori*, a species close to *A. brasiliensis*, has a better activity in higher pH (range 4.8/5.8) and in lower temperatures (range 40/42-48ºC) (Prakasham et al. 2007, Pestana & Castilho 1985). *A. fumigatus* showed a similar optimum temperature (Silva & Peralta 1998). The great temperature stability is also an interesting characteristic of the *A. brasiliensis* glucoamylase. The same enzyme from *A. niger* has an inferior stability (half-life of 45 minutes at 50°C) like *A. awamori* (40% of residual activity after 30 minutes at 50°C) (Gudi et al. 2013, Pestana & Castilho 1985). *A. fumigatus* showed similar stability of temperature (Silva & Peralta 1998). The pH stability results were similar to the *A. niger* glucoamylases and superior to the stability of *A. awamori* (Nakaz.) (range pH 6.0-9.0) (Gudi et al. 2013, Pestana & Castilho 1985).

The *R. oryzae* strain isolated in this work produced glucoamylases with pH and temperature of activity with characteristics similar to the same species strain of Roch-chui & Hang (1990). *R. delemar* has glucoamylases that act better in pH within the range of *R. oryzae* (4.5) and lower optimum temperature (40°C) (Soccol et al. 1994). *R. niveus* has the activity pH in a higher range (4.5-6) and the same optimum temperature than *R. oryzae* (Saha & Ueda 1983). The tolerance from thermal denaturation of the glucoamylase in the absence of the substrate was extreme low. The pH stability results were again like the strain in the work of Roch-chui & Hang (1990). *Rhizopus microsporus* var. *rhizopodiformis*, a thermo-tolerant strain, showed lower stability of pH (approximately 80% of residual activity in the range of 2.5-7.5, after only 2 hours of incubation) (Peixoto et al. 2003).

Therefore, from environmental samples from the Brazilian Atlantic Forest, it was possible to isolate filamentous fungi strains that produced high amounts of amylases with distinct biochemical characteristics. These differences reflect upon the evolutionary history of *R. oryzae*, a basal filamentous fungus that alkalinizes the culture media during growth and secretes a less stable glucoamylase, with half-life of 12 minutes at 50°C; *A. brasiliensis,* a derivative species that produces a highly stable, glucoamylase with half-life of 1 hour at 70°C, and an acidic one, with great activity at pH 3.5, so that it has interesting characteristics for future large-scale applications.

Acknowledgements

This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho de Desenvolvimento Científico e Tecnológico (CNPq), National System for Research on Biodiversity (Sisbiota-Brazil, CNPq 563260/2010-6/FAPESP nº 2010/52322-3). J.A.J. and M.L.T.M.P. are Research Fellows of CNPq. P.Z.A. was recipient FAPESP Fellowship (2013/01437-3) and M.G.P., C.C.C., P.R.H., L.S.Z., J.M.M. were recipient of CNPq and CAPES. We thank Ricardo Alarcon and Mariana Cereia for technical assistance and Abilio Borghi for language review.

Authors Contributions

Paula Zaghetto de Almeida, Maria de Lourdes Polizeli, Marita Gimenez Pereira: substantial contribution in the work conception and design.

Paula Zaghetto de Almeida, Maria de Lourdes Polizeli, Caio Cesar Carvalho, Paulo Ricardo Heinen, Luciana Sobrani Ziotti, Josana Maria Messias: contribution in data acquisition.

Paula Zaghetto de Almeida, Maria de Lourdes Polizeli, Marita Gimenez Pereira, Caio Cesar Carvalho, Paulo Ricardo Heinen, Luciana Sobrani Ziotti, Josana Maria Messias: contribution to the data analysis and interpretation.

Paula Zaghetto de Almeida, Maria de Lourdes Polizeli: contribution to the work writing.

Paula Zaghetto de Almeida, Maria de Lourdes Polizeli, Marita Gimenez Pereira, João Atílio Jorge: contribution to the critical review adding intellectual content.

Conflict of Interests

The authors declare no conflict of interests.

References

ADAMS, P. R. 1990. Mycelial amylase activities of thermophilic species of *Rhizomucor, Humicola* and *Papulaspora*. Mycopathologia 112: 35-37.

BALAJEE S.A., BADDLEY J.W., PETERSON S.W., NICKE D., VARGA J., BOEY A., LASS-FLÖRL C., FRISVAD J.C., SAMSON R.A. 2009. *Aspergillus alabamensis*, a new clinically relevant species in the section *Terrei*. Eukaryot. Cell. 8:713-722.

BALLESTEROS I., OLIVA J.M., NEGRO M.J., MANZANARES P. & BALLESTEROS M. 2002. Enzymatic hydrolysis of steam exploded herbaceous agricultural waste (*Brassica carinata*) at different particle sizes. Process Biochem. 38:187-192.

BLACKWELL M. 2011. The Fungi: 1, 2, 3… 5.1 million species? Am. J. Bot. 98 (3):426-438.

CASTRILLO M.L., FONSECA M.I., BICH G.A., JERKE G. & HORIANSKI MA. 2012. Taxonomy and phylogenetic analysis of *Aspergillus* section Nigri isolated from yerba mate in misiones (Argentina). J. Basic Appl. Genet. 23:19-27.

COSTA M.O., MOTTA C.M.S. & MALOSSO E. 2012. Diversity of filamentous fungi in different systems of land use. Agroforest. Syst. 85:195-203.

COUTINHO P.M. & REILLY P.J. 1997. Glucoamylase structural, functional and evolutionary relationships. Proteins: Struct. Funct. Genet. 29:334-347.

DHALI, R., DEY, A., CHATTOPADHYAY, A. N., SAHA, P., MUKHOPADHYAY, S. K., ROY, P., & CHATTERJEE, S. 2016. Isolation, characterization and study of amylase activity of microorganisms from arctic soil sample. AOBR, 3:5-15.

DOMSCH K.H., GAMS W. & ANDERSON T. 2007. Compendium of soil fungi. San Francisco: IHW–Verlag ed.

EDGAR R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–7.

GLASS N.L. & DONALDSON G.C. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl. Environ. Microbiol. 61: 1323-1330.

GEISER D.M., DORNER J.W., HORN B.W., TAYLOR J.W. 2000. The phylogenetics of mycotoxin and sclerotium production in *Aspergillus flavus* and *Aspergillus oryzae*. Fungal Genet. Biol. 31:169–79.

GUDI S.K., CHANDRNSEKHAN G., RATHER G., CHAMDRA M.G.S., MANGAMURI U.K., PODHA S.E. &, CHOI Y.L. 2013. Glucoamylase from a newly isolated *Aspergillus niger* FME: detergent-mediated production, purification, and characterization. J. Korean Soc. Appl. Biol. Chem. 56:427−433.

JOLY C.A., METZGER J.P. & TABARELLI M. 2014. Experiences from the Brazilian Atlantic Forest: ecological findings and conservation initiatives. New Phytol. 204 (3):459-473.

KHANNA P., SUNDARI S.S & KUMAR N.J. 1995. Production, isolation and partial purification of xylanase from an *Aspergillus* sp. World J. Microbiol. Biotechnol. 11:242-243.

KIRK P.M., CANNON P.F., MINTER D.W. & STALPERS J.A. 2008. 10th ed. Dictionary of the fungi. Wallingford: CABI Press.

LIOU G.Y., CHEN S.R., WEI Y.H., LEE F.L., FU H.M., YUAN G.F., & STALPERS J.A. 2007. Polyphasic approach to the taxonomy of the *Rhizopus stolonifer* group. Fungal Biol. 111:196–203.

MAKI, C. S.; TEIXEIRA, F. F.; PAIVA, E. & PACCOLAMEIRELLES, L. D. 2001. Analyses of genetic variability in *Lentinula edodes* through mycelia responses to different abiotic conditions and RAPD molecular markers. Braz. J. Microbiol., 32(3):170-175.

MILLER G.L. Use of dinitrosalicylic acid reagent for determination o reducing sugars. 1959. Anal. Chem. 31:426-428.

MCILVAINE T.C. 1921. A buffer solution for colorimetric comparison. J. Biol. Chem. 49:183-186.

PEIXOTO S.C., JORGE J.A., TERENZI H.F. & POLIZELI M.L.T.M. 2003. *Rhizopus microsporus* var. *rhizopodiformis*: a thermotolerant fungus with potential for production of thermostable amylases. Int. Microbiol. 6:269-273.

PESTANA F. & CASTILHO F.J. 1985. Glucoamylase production by *Aspergillus awamori* on rice flour medium and partial characterization of the enzyme. MIRCEN J. Appl. Microbiol. Biotechnol. 1:225-237.

PRAKASHAM R.S., SUBBA C.H.R., SREENIVAS R.R., SARMA P.N. 2007. Enhancement of acid amylase production by an isolated *Aspergillus awamori.* J. Appl. Microbiol. 102:204–211.

RIBEIRO M.C., METZGER J.P., MARTENSEN A.C., PONZONI F.J., HIROTA, M.M. 2009. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biol. Cons. 142(6):1141-1153.

RIZZATTI A.C.S, JORGE J.A., RECHIA C.G.V & POLIZELI M.L.T.M. 2001. Purification and properties of a thermostable extracellular β-D-xylosidase produced by thermotolerant *A. phoenicis*. J. Ind. Microbiol. Biotechnol. 26:156-160.

ROCH-CHUI Y. & HANG Y.D. 1990. Amylolytic enzyme production by *Rhizopus oryzae* grown on agricultural commodities. World J. Microb. Biot. 6:15-18.

SAHA B.C., UEDA S. 1983. Raw starch adsorption, elution and digestion behavior of *Rhizopus niveus*. J. Ferm. Technol. 61:67–72.

SHI L.L., MORTIMER P.E., SLIK J.F., ZOU X.M., XU J., FENG W.T., & QIAO, L. 2014. Variation in forest soil fungal diversity along a latitudinal gradient. Fungal Divers. 64(1): 305-315.

SCHOENLEIN-CRUSIUS I.H & MILANEZ A.I. 1998. Fungos microscópicos da Mata Atlântica de Paranapiacaba, São Paulo, Brasil. Braz. J. Bot. 21:73-79.

SCHOENLEIN-CRUSIUS I.H., MILANEZ A.I., TRUFEM S.F., PIRES-ZOTTARELLI C.L., GRANDI R.A.P., SANTOS M.L., & GIUSTRA, K.C. 2006. Microscopic fungi in the Atlantic Rainforest in Cubatão, São Paulo, Brazil. Braz. J. Microbiol. 37:267-275.

SILVA W.B. & PERALTA R.M. 1998. Purification and characterization of a thermostable glucoamylase from *Aspergillus fumigatus*. Can. J. Microbiol. 44:493.497.

SOCCOL C.R., MARIN B., RAIMBAULT M. & LEBEAULT J.M. 1994. Breeding and growth of *Rhizopus* in raw cassava by solid state fermentation. Appl. Microbiol. Biotechnol. 41:330-336.

SOUZA, P.M & MAGALHÃES P.O. 2010 Application of microbial α-amylase in industry – A review. Braz. J. Microbiol. 41:850-861.

SINGH, S., SINGH, S., BALI, V., SHARMA, L., & MANGLA, J. 2014. Production of fungal amylases using cheap, readily available agriresidues, for potential application in textile industry. BioMed Res. Int. 2014:1-9.

SPARRINGA R.A. & OWENS J.D. 1999. Causes of alkalization in tempe solid substrate fermentation. Enzyme Microb. Technol. 25:677–681.

TAJIMA F. & NEI M. 1984. Estimation of evolutionary distance between nucleotide sequences. Mol. Biol. Evol. 1:269-285.

TAMURA K., PETERSON D., PETERSON N., STECHER G., NEI M. & KUMAR S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30:2725-2729.

TAUK TORNISIELO S.M., GARLIPP A., RUEGGER M., ATILLI D.S., & MALAGUTTI M. 2005. Soilborne filamentous fungi in Brazil. J. Basic Microbiol. 45:72-85.

TEDERSOO L., BAHRAM M., PÕLME S., KÕLJALG U., YOROU N. S., WIJESUNDERA R., RUIZ L. V., VASCO-PALACIOS A. M., THU P. M., SUIJA A., SMITH M.E., SHARP C., SALUVEER E., SAITTA A., ROSAS M., RIIT T., RATKOWSKY D., PRITSCH K., PÕLDMAA K., PIEPENBRING M., PHOSRI C., PETERSON M., PARTS K., PÄRTEL K., OTSING E., NOUHRA E., NJOUONKOU A.J., NILSSON R. H., MORGADO L.N., MAYOR J., MAY T.W., MAJUAKIM L., LODGE D.J., LEE S.S., LARSSON K.H., KOHOUT P., HOSAKA K., HIIESALU I., HENKEL T.W., HAREND H., GUO L.D., GRESLEBIN A., GRELET G., GEML J., GATES G., DUNSTAN W., DUNK C., DRENKHAN R., DEARNALEY J, KESEL A., DANG T., CHEN X., BUEGGER F., BREARLEY F.Q., BONITO G., ANSLAN S., ABELL S., ABARENKOV K. 2014. Global diversity and geography of soil fungi. Science 346 (6213):1256688.

VAN DER MAAREL M.J.E.C., VAN DER VEEN B., UITDEHHAG J.C.M., LEEMHUIS H. & DIJKHUIZEN L. 2002. Properties and applications of starch-converting enzymes of the α-amylase family. J. Biotechnol. 94:137–155.

VARGA J., KOCSUBÉ S., TÓTH B. FRISVAD J.C., PERRONE G., SUSCA A., MEIJER M. & SAMSON R.A. 2007. *A. brasiliensis* sp. nov., a biseriate black *A.* species with worldwide distribution. Int. J. Syst. Evol. Micr. 57:1925 -1932.

VIELLE C. & ZEIKUS G.J. 2001. Hyperthermophilic enzymes: sources, uses and molecular mechanisms for termostability. Microbiol. Mol. Biol. R. 65:1- 43.

VOGEL H.J. 1964. Distribution of lysine pathways among fungi: evolutionary implications. Am. Nat. 98:435-446.

WHITE T.J., LEE S. & TAILOR S. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics in PCR protocols: a guide to methods and applications. California: Academic Press Inc. San Diego; pp 315-322.

Received: 23/02/2017
Revised: 26/05/2017
Accepted: 14/07/2017

Biota Neotropica 17(3): e20170323, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
article

Diversity of ants and mites in the diet of the Brazilian frog *Chiasmocleis leucosticta* (Anura: Microhylidae)

Mariana S. Lopes*[1]*, Ricardo S. Bovendorp*[1]*, Gilberto José de Moraes*[1]*, Alexandre Reis Percequillo*[1] *&

Jaime Bertoluci*[1]

[1]Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, Brazil
**Corresponding Author: Dr. Jaime Bertoluci, e-mail: jaime.bertoluci@usp.br*

LOPES, M. S., BOVENDORP, R. S., DE MORAES, G. J., PERCEQUILLO, A. R., BERTOLUCI, J. **Diversity of ants and mites in the diet of the Brazilian frog *Chiasmocleis leucosticta* (Anura: Microhylidae)**. Biota Neotropica. 17(3): e20170323. http://dx.doi.org/10.1590/1676-0611-BN-2017-0323

***Abstract*:** We describe here the diet of the microhylid frog *Chiasmocleis leucosticta* based on the stomach contents of 72 individuals (47 males and 25 females) collected in pitfall traps at the Reserva Florestal de Morro Grande, state of São Paulo, southeastern Brazil. We identified 1,981 food items distributed in 13 prey categories of arthropods, mainly ants, mites and collembolans. Formicidae was the most abundant and frequent prey category, including 16 genera from seven subfamilies, and data on ant availability in the habitat suggest that *C. leucosticta* selects ants actively. The second main prey category was Acari, predominantly represented by mites of the suborder Oribatida. This is the first work identifying mites to the family level in the diet of a Microhylidae. There was no statistical difference between males and females regarding diet composition.

***Keywords*:** *Acari, Anuran, Atlantic Rainforest, Food habits, Formicidae, Myrmecophagy.*

Diversidade de formigas e ácaros na dieta do anuro brasileiro *Chiasmocleis leucosticta* (Anura: Microhylidae)

***Resumo*:** Descrevemos neste trabalho a dieta do anuro *Chiasmocleis leucosticta*, pertencente à família Microhylidae, com base no conteúdo estomacal de 72 indivíduos (47 machos e 25 fêmeas) coletados em armadilhas-de-queda na Reserva Florestal de Morro Grande, SP. Foram identificados 1981 itens alimentares, distribuídos em 13 categorias de artrópodes, especialmente formigas, ácaros e colêmbolos. Formicidae foi a categoria de presas mais abundante e frequente, incluindo 16 gêneros pertencentes a sete subfamílias. Os dados de disponibilidade de formigas no ambiente sugerem que *C. leucosticta* seleciona formigas ativamente. A segunda categoria de presas mais relevante foi Acari, predominantemente representada por ácaros da subordem Oribatida. Este é o primeiro trabalho que identifica os ácaros ao nível de família na dieta de um Microhylidae. Não houve diferença estatística entre machos e fêmeas quanto à composição da dieta.

***Palavras-chave*:** *Acari, Anuros, Mata Atlântica, Hábitos alimentares, Formicidae, Mirmecofagia.*

Introduction

The anuran family Microhylidae is composed of 13 subfamilies and 612 species (Frost 2017), comprising about 8% of the global anuran diversity (Sá et al. 2012), and distributed worldwide, except in Europe (Vitt & Caldwell 2013, Pough et al. 2016). Microhylids are extremely diversified in habits, and fossorial and semifossorial species tend to be myrmecophagous, feeding on ants and termites (Vitt & Caldwell 2013). The gastrophrynine genus *Chiasmocleis* (Méhelÿ, 1904) is widely distributed and the most speciose genus among tropical microhylids (Van Sluys et al. 2006, Forlani, 2010). Currently, the genus comprises 29 species, which are spread from Panama to Southern Brazil (Frost 2017), including 11 species found in the Atlantic rainforest of southeastern Brazil.

Information about the food habits of *Chiasmocleis* species is scarce, ants being the most common item of the diet (Duellman 1978, Schlüter & Salas 1991; Caramaschi & Cruz 2001, Van Sluys et al. 2006).

Chiasmocleis leucosticta (Boulenger, 1888) is found in Brazilian Atlantic rainforest areas from São Paulo to Santa Catarina states (Cruz et al. 1997). Similarly to the other species of the genus, *C. leucosticta* is fossorial and an explosive breeder, characterized by large aggregations in temporary water bodies during a very short period of the year (Van Sluys et al. 2006). Thereby, due to their fossorial habits and annual breeding pattern, individuals are rarely found, and natural history data about the species are rare.

We describe here the diet of *Chiasmocleis leucosticta* based on stomach contents and test if there are dietary differences between the sexes.

Material and Methods

This study was conducted with individuals of *C. leucosticta* collected accidentally in pitfalls traps used to sample arthropods in a study on rodent ecology (Bovendorp 2013) in the Reserva Florestal de Morro Grande, Cotia municipality, state of São Paulo, southeastern

Brazil (23º39'–23º48' S, 46º55'– 47º01' W). Climate in this area is Cfb of Koeppen, warm temperate and humid, and mean annual rainfall is 1339 mm (Metzger et al. 2006). The reserve locates in a continuum of montane rainforest, between the Atlantic and the Semideciduous forests (Aragaki & Mantovani 1998, Catharino et al. 2006), occupying an area of 9,400 ha; vegetation is composed by a mosaic of secondary forests in different stages of conservation and mature forests (Metzger et al. 2006).

Sampling occurred monthly (five days) between January 2008 and January 2010. Two additional samples were performed in July 2010 (16 days) and January 2011 (13 days). Traps (400 mL plastic cups) were buried at soil level and filled with 200 mL 92% alcohol in three areas with different successional stages in a grid consisting of six lines with 100 m separated by 20 m (six equidistant traps in each line), totalling 36 traps per area.

All frogs had their snout-vent length (SVL) measured with digital callipers to the nearest 0.5 mm. Animals were dissected, and food items were determined to the lowest possible taxonomic level under stereo microscopes (Motic K-700L and Zeiss Discovery V20) with the aid of dichotomy keys (Rafael et al. 2012, for insect orders, Fernández 2003, for subfamilies and genera of Formicidae). Mites were mounted in Hoyer´s medium on microscope slides and identified to family using keys provided in Krantz & Walter (2009). Total number of each prey category was counted, and frequency of occurrence was calculated by the ratio between the number of stomachs with the prey category and the total number of stomachs (empty stomachs were excluded).

All results were compared between males and females with t-student test (Zar 1999). The sexes were determinate by the presence or the absence of ovaries and oviducts. Voucher material (frogs and stomach contents) was deposited in the herpetological collection of the Laboratório de Zoologia de Vertebrados, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo (acronym VESALQ), but mites were deposited in the mite reference collection of the Departamento de Entomologia e Acarologia of the same institution.

Results

We analyzed 72 individuals of *C. leucosticta* (47 males and 25 females). Only one stomach was empty, which was excluded from the analysis. We identified 1,981 food items distributed in 13 prey categories (Table 1). The diet was composed solely of arthropods, mainly ants, mites and collembolans. Formicidae was the more abundant and frequent prey category. Furthermore, we found a high diversity of ants in the diet (16 genera from seven subfamilies), Myrmicinae being the most abundant and frequent ant subfamily, mostly represented by the genera *Pheidole* and *Solenopsis* (Table 2).

The second main prey category was Acari, predominantly represented by the suborder Oribatida, with 33 individuals (Table 3). Other prey categories appeared in low abundances (one to five individuals). Crustaceans and hemipterans were identified only in males, while non-ant hymenopterans and carabid larva were found only in females. The presence of plant material in the stomachs was interpreted as accidental ingestion.

There was no statistical difference between males and females regarding diet composition (t = 0.5022, p > 0.05).

Discussion

In this study, only one stomach (1.4%) was empty. The proportion of empty stomachs is highly variable in *Chiasmocleis* species whose diet was determined, varying from 19.3% in *C. capixaba* (Van Sluys et al. 2006) to 82.3% in *C. ventrimaculata* (Schluter & Salas 1991).

Our results suggest that *Chiasmocleis leucosticta* is an ant and mite specialist anuran (sensu Toft 1981), as many other Neotropical [*C. antipes, C. bassleri* (Duellman 1978), *C. ventrimaculata* (Schluter & Salas 1991), *C. capixaba* (Van Sluys et al. 2006), *C. albopunctata* (Araújo et al. 2009), *Hamptophryne boliviana* (Schluter & Salas 1991), *Elachistocleis ovalis* (Solé et al. 2002), and *E. bicolor* (Berazategui et al. 2007, Araujo et al. 2009)], and Australian [e.g., *Cophyxalus* spp. (Williams et al. 2006, Hoskin & Aland 2011] microhylids. Data on arthropod availability at Reserva

Table 1. Food items of the diet of the microhylid frog *Chiasmocleis leucosticta*. N = total number of prey by category; F = frequency of prey categories.

Prey categories		**Males (n = 47)**		**Females (n = 24)**	
		N	**F**	**N**	**F**
ARACHNIDA					
Acari		34	0.447	18	0.542
Araneae		7	0.149	2	0.083
CRUSTACEA		3	0.064	–	–
HEXAPODA					
Collembola		16	0.191	8	0.208
Diptera		1	0.021	2	0.083
Hemiptera					
	Heteroptera	2	0.043	–	–
	Sternorryncha	8	0.149	2	0.083
Hymenoptera					
	Formicidae	498	0.830	328	0.917
	Remains of Formicidae (number of ant heads)	740	0.894	272	0.833
	Other (no-ant)	–	–	4	0.167
Insect larvae					
	Carabeidae	–	–	1	0.042
	Diptera	6	0.064	2	0.042
	undetermined	1	0.021	1	0.042
PLANT MATERIAL			0.213		0.250
UNIDENTIFIED			18	0.255	7
Total		**1334**		**647**	

Table 2. Subfamilies and genera of Formicidae found in the diet of the microhylid frog *Chiasmocleis leucosticta*. N = total number of ants; F = frequency of ants.

Subfamilies	Genera	Males (n = 47)		Females (n = 24)	
		N	F	N	F
Cerapachyinae		6	0.064	1	0.042
	Acanthostichus	4	0.043	1	0.042
	Undetermined	2	0.021	–	–
Ecitoninae		3	0.043	75	0.167
	Labidus	3	0.043	75	0.167
Ectatomminae		50	0.340	40	0.333
	Gnamptogenys	50	0.340	40	0.333
Formicinae		43	0.085	47	0.250
	Brachemyrmex	–	–	17	0.021
	Paratrechina	9	0.043	2	0.042
	Undetermined	34	0.043	28	0.167
Heteroponerinae		4	0.064	3	0.125
	Heteroponera	4	0.064	3	0.125
Myrmicinae		372	0.596	139	0.667
	Acanthognatus	–	–	1	0.042
	Adelomyrmex	1	0.021	–	–
	Basiceros	2	0.043	–	–
	Crematogaster	–	–	10	0.042
	Cyphomyrmex	5	0.042	–	–
	Pheidole	306	0.383	86	0.500
	Solenopsis	40	0.234	40	0.250
	Strumigenys	4	0.064	–	–
	Wasmania	5	0.021	–	–
	Undetermined	9	0.128	2	0.042
Ponerinae		18	0.213	15	0,167
	Odontomachus	1	0.021	3	0.042
	Hypoponera	16	0.191	12	0.125
	Undetermined	1	0.021	–	–
Unidentified		1	0.021	1	0.042

Table 3. Mites found in the diet of the microhylid frog *Chiasmocleis leucosticta*. N = total number of mites; F = frequency of mites.

	Males (n = 47)		Females (n = 24)	
	N	F	N	F
Mesostigmata	**4**	**0.064**	**7**	**0.250**
Laelapidae	–	–	2	0.042
Macrochelidae	–	–	2	0.083
Podocinidae	4	0.064	1	0.042
Rhodacaridae	–	–	1	0.042
Veigaiidae	–	–	1	0.042
Oribatida	25	**0.298**	**8**	**0.250**
Prostigmata	–	–	**1**	**0.042**
Smarididae	–	–	1	0.042
Unidentified	**5**	**0.106**	**2**	**0.083**

Florestal do Morro Grande indicate that Formicidae was not the most abundant item along the year neither in number of individuals nor in biomass (Bovendorp 2013), which suggests that the prevalence of ants in the diet of *C. leucosticta* in this study is related to prey selection by *C. leucosticta.*

Myrmicinae ants dominated the diet of *C. leucosticta*, which can be explained by the higher diversity of genera and habits (arboreal, soil and leaf litter inhabitants, and some associated with plants, fungus and other ants) of this taxon when compared with other ant subfamilies (Fernández 2003). All Myrmicinae genera found in our study include leaf litter or epigean ants, except *Crematogaster*, an arboreal genus that can be associated with plants and other ants (Fernández 2003). *Crematogaster* ants were found only in one stomach, suggesting the predation event occurred when those were foraging in the leaf litter. Among Myrmicinae, *Pheidole* was the most speciose genus, followed by *Solenopsis*; *Pheidole* is the most common genus in the Neotropics and very abundant locally, comprising up to 70 species by locality, while *Solenopsis* includes about 90 species (Fernández 2003). These two genera were also the most abundant in the diet of the microhylid *Elachistocleis ovalis* (Solé et al. 2002).

Considering males and females together, Formicinae and Ectatomminae were the second most abundant food category in our study. Formicinae ants are characterized by the production of formic acid and by the presence of venom gland; *Brachemyrmix* is a common genus in the soil and leaf litter, and *Paratrechina* is a cosmopolitan genus in natural and disturbed habitats (Fernández 2003). Solé et al. (2002) found that although the genus *Paratrechina* is present in the diet of *E. ovalis*, in field experiments the frogs adopted defensive postures when these ants were offered to them as potential prey. The relatively high consumption of Formicinae in this study (10 frogs) indicates that this behavior may not be present in *C. leucosticta*.

The only Ectatomminae ants our frogs ingested belong to the genus *Gnamptogenys*, represented in the Neotropics by 81 species of predatory ants inhabiting the forest floor or the vegetation (Fernández 2003). Ecitoninae ants are the famous legionary ants, nomad predators that live in dense colonies, alternating stationary and migratory stages; ants in the genus *Labidus* impose heavy impact on invertebrate populations (Fernández 2003). Six out the nine ant genera consumed by the microhylid *Elachistocleis ovalis* (Solé et al. 2002) were also consumed by *C. leucosticta* (*Cyphomyrmex, Pheidole, Solenopsis, Wasmannia, Gnamptogenys,* and *Hypoponera*).

Mites have been rarely reported as food items to anurans. Duellman (1978) did not find mites in the diet of *Chiasmocleis anatipes*, *C. bassleri* and *C. ventrimaculata,* while Schluter & Salas (1991) reported two individuals *C. ventrimaculata* feeding on mites at night (but did not find them in the stomachs). The importance of mites in the diet of *C. leucosticta* may be explained by the fact that this group of organisms is the most abundant in the forest floor, especially the Oribatida (Franklin et al. 2001), which suggests that its presence could be due to the great abundance rather than a food preference. Mites of this group are mostly known for being mainly saprophagous and/or fungivorous, thus being more numerous in soil covered with abundant litter (Norton & Behan-Pelletier 2009). All other mite families found in this study are known mainly for their predatory habits, being typically soil inhabitants (Carrillo et al. 2015).

Some *Chiasmocleis* species are considered important mite predators in leaf litter communities, the level of acarophagy being negatively correlated to the anuran body size (Simon and Toft 1991). *Chiasmocleis leucosticta* is a small (male SVL = 18.3 ± 2.80 mm; female SVL = 23.5 ± 1.72 mm) fossorial frog that forages in the leaf litter, being included in Class 2 of Simon & Toft (1991), which could explain the presence and the relative abundance of mites in its diet. Mites were also well represented in the diet of the Amazonian microhilyd *Sincope antenori* (Simon & Toft 1991) and in the Atlantic forest microhylid *Chiasmocleis capixaba* (Van Sluys et al. 2006). To our knowledge this is the first work to identify mites to the family level in the diet of a Microhylidae frog.

Some dendrobatid frogs are able to remove alkaloids from their diets (especially from ants, bees, millipedes and mites) and turn them into chemical defenses (Saporito et al. 2004). Myrmicinae ants have several alkaloids, such as pyrrolidine, pyrrolizidine, piperidine, indolizidine, and quinolizidine decahydroquinoline (Daly et al. 1994a, 2000). Similarly, pumiliotoxins were also found in *Brachemyrmix* and *Paratrechina* ants (Saporito et al. 2004). Since most microhylids are ant-specialists, some studies have investigated whether microhylids are able to remove alkaloids from the diet. Berazategui et al. (2007) pointed out that the high consumption of Myrmicinae suggests that *Elachistocleis bicolor* is able to capture alkaloids. However, as toxicity is generally associated to diurnal activity and aposematism, the adaptive nature of this character in *E. bicolor*, a cryptic and nocturnal species, remains unclear. Additionally, Mebs et al. (2010) were unable to detect alkaloids in the skin of *Elachistocleis* sp. from Bolivia, but have detected them in the dendrobatid *Ameerega picta*, concluding that “myrmecophagy provides dendrobatids with alkaloids, which they sequester and store in their skin, but an alkaloid sequestering system is absent in the microhylid *Elachistocleis* sp.” This system of alkaloid sequestration was previously described for dendrobatids by Daly et al. (1994b), and it is probably absent in ant-specialized microhylids.

Some studies have been conducted to verify the presence of alkaloids in mites. Saporito et al. (2007) isolated more than 80 alkaloids from oribatid mites, and half of them are also present in the dendrobatid *Oophaga pumilio.* Oribatid mites are the main source of alkaloids to Dendrobatidae and Eleutherodactylidae (Raspotnig et al. 2011). For the same reason discussed above for ants, microhylids seem to be unable to sequester alkaloids from their mite prey.

Author Contributions

Mariana S. Lopes: conceptualization, methodology, laboratory work (dissections, ant determination), data analysis, validation, writing.

Ricardo S. Bovendorp: methodology, fieldwork (anuran collection), funding acquisition, validation, writing.

Gilberto José de Moraes: methodology, laboratory work (mite determination), resources, validation, writing.

Alexandre Reis Percequillo: methodology, funding acquisition, resources, project administration, supervision, validation, writing.

Jaime Bertoluci: conceptualization, methodology, funding acquisition, resources, project administration, supervision, validation, writing.

Conflicts of interest

The authors have declared that no conflicts of interest exist.

References

ARAGAKI, S. & MANTOVANI, W. 1998. Caracterização do clima e da vegetação de remanescente florestal no planalto paulista (SP). Publicações ACIESP 104:25–36.

ARAÚJO, M.S., BOLNICK, D.I., MARTINELLI, L.A., GIARETTA, A.A. & REIS, S.F. 2009. Individual-level diet variation in four species of Brazilian frogs. J. Anim. Ecol. 78:848–856.

BERAZATEGUI, M., CAMARGO, A. & MANEYRO, R. 2007. Environmental and seasonal variation in the diet of *Elachistocleis bicolor* (Guérin–Méneville 1838) (Anura: Microhylidae) from Northern Uruguay. Zool. Sci. 24:225–231.

BOVENDORP, R.S. 2013. História natural e ecologia de duas espécies de roedores simpátricas da tribo Oryzomyini (Cricetidae: Sigmodontinae) Floresta Atlântica [doctoral thesis]. Piracicaba: Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo.

CARAMASCHI, U. & CRUZ, C.A.G. 2001. A new species of *Chiasmocleis* Méhelÿ, 1904 from Brazilian Amazonia (Amphibia, Anura, Microhylidae). Bol Mus Nac *Rio de Janeiro,* N.S. Zoologia 469:1–8.

CARRILLO, D., MORAES, G.J. & PEÑA, J. 2015. Prospects for biological control of plant feeding mites and other harmful organisms. Cham: Springer. 328p.

CATHARINO, E.L.M., BERNACCI, L.C., FRANCO, G.A.D.C., DURIGAN, G. & METZGER, J.P. 2006. Aspectos da composição e diversidade do componente arbóreo das florestas da Reserva Florestal do Morro Grande, Cotia, SP. Biota Neotropica 6:1–28.

CRUZ, C.A.G., CARAMASCHI, U. & IZECKSOHN, E. 1997. The genus *Chiasmocleis* Mehely, 1904 (Anura, Microhylidae) in the Atlantic Forest of Brazil, with description of three new species. Alytes 15:49–71.

DALY, J.W., GARRAFFO, H.M., SPANDE, T.F., JARAMILLO, C. & RAND, A.S. 1994A. Dietary source of skin alkaloids of poison frogs (Dendrobatidae)? J. Chem. Ecol. 20:943–955.

DALY, J.W., SECUNDA, S.I., GARRAFFO, H.M., SPANDE, T.F., WISNIESKI, A. & COVER, J.F. 1994B. An uptake system for dietary alkaloids in poison frogs (Dendrobatidae). Toxicon 32:657–663.

DALY, J.W., GARRAFFO, H.M., JAIN, P., SPANDE, T.F., SNELLING, R.R., JARAMILLO, C. & RAND, A.S. 2000. Arthropod-frog connection: decahydroquinoline and pyrrolizidine alkaloids common to microsympatric myrmicine ants and dendrobatid frogs. J. Chem. Ecol. 26:73–85.

DUELLMAN, W.E. 1978. The Biology of an Equatorial Herpetofauna in Amazonian Ecuador. Misc. Publ. Mus. Nat. Hist. Univ. Kansas 65:1–352.

FERNÁNDEZ, F. (Ed.). 2003. Introducción a las hormigas de la región Neotropical. Bogotá: Instituto de Investigación de Recursos Biológicos Alexander von Humboldt. 398 p.

FORLANI, M.C. 2010. Variação morfológica do gênero *Chiasmocleis* Méhely 1904 (Anura, Microhylidae, Gastrophryninae) e suas implicações filogenéticas. [MSc dissertation]. São Paulo: Universidade de São Paulo.

FRANKLIN, E.N., MORAIS, J.W. & SANTOS, E.M.R. 2001. Density and biomass of Acari and Collembola in primary forest, secondary forest and polycultures in central Amazônia. Andrias 15:141–153.

FROST, D.R. 2017. Amphibian Species of the World: an Online Reference. Version 6.0 (accessed 2 March 2017). Electronic database accessible at http://research.amnh.org/herpetology/amphibia/index.html. American Museum of Natural History, New York, USA.

HOSKIN, C.J. & ALAND, K. 2011. Two new frog species (Microhylidae: *Cophixalus*) from boulder habitats on Cape York Peninsula, north-east Australia. Zootaxa 3027:39–51.

MEBS, D., JANSEN, M., KOHLER, G., POGODA, W. & KAUERT, G. 2010. Myrmecophagy and alkaloid sequestration in amphibians: a study on *Ameerega picta* (Dendrobatidae) and *Elachistocleis* sp. (Microhylidae) frogs. Salamandra 46:11–15.

METZGER, J.P., ALVES, L.F., GOULART, W., TEIXEIRA, A.M.G., SIMÕES, S.J.C. & CATHARINO, E.L.M. 2006. Uma área de relevante interesse biológico, porém pouco conhecida: a Reserva Florestal do Morro Grande. Biota Neotropica 6:1–33.

POUGH, F.H., ANDREWS, R.M., CRUMP, M.L., SAVITZKY, A.H., WELLS, K.D. & BRANDLEY, M.C. 2016. Herpetology. 4th ed. Sinauer Associates. 718 pp.

RAFAEL, J.A., MELO, G.A.R., CARVALHO, C.J.B, CASARI, S.A. & CONSTANTINO, R. (Eds.). 2012. Insetos do Brasil: Diversidade e Taxonomia. Ribeirão Preto: Holos Editora. 810p.

RASPOTNIG, G., NORTON, R.A. & HEETHOFF, M. 2011. Oribatid mites and skin alkaloids in poison frogs. Biol. Lett. 7:555–556.

SÁ, R.O., STREICHER, J.W., SEKONYELA, R., FORLANI, M.C., LOADER, S.P., GREENBAUM, E., RICHARD, S. & HADDAD, C.F.B. 2012. Molecular phylogeny of microhylid frogs (Anura: Microhylidae) with emphasis on relationships among New World genera. BMC Evol. Biol. 12:241–262.

SAPORITO, R.A., DONNELLY, M.A., NORTON, R.A., GARRAFFO, H.M., SPANDE, T.F. & DALY, J.W. 2007. Oribatid mites as a major source for alkaloids in poison frogs. Proc. Nat. Acad. Sci. USA 104:8885–8890.

SAPORITO, R.A., GARRAFFO, H.M., DONNELLY, M.A., EDWARDS, A.L., LONGINO, J.T. & DALY, J.W. 2004. Formicine ants: an arthropod source for the pumiliotoxin alkaloids of dendrobatid poison frogs. Proc. Nat. Acad. Sci. USA 104:8045–8050.

SCHLÜTER, A. & SALAS, A.W. 1991. Reproduction, tadpoles and ecological aspects of three syntopic microhylid species from Peru (Amphibia: Microhylidae). Stutt. Beitr. Naturkd. 458:1–17.

SIMON, M.P. & TOFT, C.A. 1991. Diet specialization in small vertebrates: mite-eating in frogs. Oikos 61:263–278.

SOLÉ, M., KETTERL, J., DI BERNARDO, M. & KWET, A. 2002. Ants and termites are the diet of the microhylid frog *Elachistocleis ovalis* (Schneider, 1799) at an Araucaria forest in Rio Grande do Sul, Brazil. Herp. Bull. 79:14–17.

TOFT, C.A. 1981. Feeding ecology of Panamanian litter anurans: patterns in diet and foraging mode. J. Herpetol. 15:139–144.

VAN SLUYS, M., SCHITTINI, G.M., MARRA, R.V., AZEVEDO, A.R.M., VICENTE, J.J. & VRCIBRADIC, D. 2006. Body size, diet and endoparasites of the microhylid frog *Chiasmocleis capixaba* in an Atlantic Forest area of southern Bahia state, Brazil. Braz. J. Biol. 66:167–173.

VITT, L.J. & CALDWELL, J.L. 2013. Herpetology: an introductory biology of amphibians and reptiles. Fourth edition. San Diego: Academic Press. 776p.

WILLIAMS, Y.M., WILLIAMS, S.E., ALFORD, R.A., WAYCOTT, M. & JOHNSON, C.N. 2006. Niche breath and geographical range: ecological compensation for geographical rarity in rainforest frogs. Biology Letters 2:532–535.

ZAR, J.H. 1999. Biostatistical Analysis. New Jersey: Prentice Hall. 929p.

Received: 17/01/2017
Revised: 19/06/2017
Accepted: 10/07/2017
Published online: 21/08/2017

Biota Neotropica 17(3): e20170353, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
short communication

Population dynamics of the brine shrimp *Artemia persimilis* Piccinelli & Prosdocimi, 1968 (Crustacea, Anostraca) in a hypersaline lake of the Central Pampa (Argentina)

Alicia M. Vignatti[1]*, Gabriela C. Cabrera[1] & Santiago A. Echaniz[1]

[1]Facultad de Ciencias Exactas y Naturales, Universidad Nacional de La Pampa. Avenida Uruguay 151. 6300.
Santa Rosa, La Pampa, Argentina
**Corresponding author: Alicia M. Vignatti, e-mail: aliciavignatti@cpenet.com.ar;*
aliciavignatti@exactas.unlpam.edu.ar

VIGNATTI, A. M., CABRERA, G. C., ECHANIZ, S. A. **Population dynamics of the brine shrimp *Artemia persimilis* Piccinelli & Prosdocimi, 1968 (Crustacea, Anostraca) in a hypersaline lake of the Central Pampa (Argentina).** Biota Neotropica. 17(3): e20170353. http://dx.doi.org/10.1590/1676-0611-BN-2017-0353

Abstract: The anostracan *Artemia* is one of the few organisms that can inhabit hypersaline lakes. In Argentina, this genus is represented by two species: the invasive *A. franciscana* Kellogg, 1906 and the autochthonous *A. persimilis* Piccinelli & Prosdocimi, 1968, the latter being the only one recorded in the province of La Pampa. Some of the biological aspects of *A. persimilis* are known based on laboratory studies, and data on its ecology in natural conditions are scarce. The aim of this work is to present information on the density, biomass, and population structure of *A. persimilis* in relation to environmental variables in Utracán, a hypersaline lake of the semiarid Central Pampa of Argentina. Water and zooplankton samples were taken monthly from May 2009 until August 2010. The mean density and biomass were 56.98 ind.L^{-1} (± 106.64) and 1.23 mg.L^{-1} (± 1.35), respectively. The species was negatively affected by salinity, and it was absent between November and February, when salinity exceeded 320 g.L^{-1}. However, when it was present, even during the months of higher salinity, a high proportion of naupliar stages was found, which could indicate that, in nature, *A. persimilis* can reproduce with high salinity. Although it has been indicated that *A. persimilis* could be displaced by *A. franciscana*, the highest tolerance of the former at low temperatures, which allowed the population to continue reproducing at values close to 3°C, would limit its displacement. However, an increase in the temperature of lakes as a result of global warming could modify this situation and allow the advance of the introduced species to the south.

Keywords: *Artemia persimilis, hypersaline lakes, Pampa Argentina*

Dinámica poblacional de *Artemia persimilis* Piccinelli & Prosdocimi, 1968 (Crustacea, Anostraca) en un lago hipersalino de la Pampa Central (Argentina)

***Resumen*:** Los anostracos del género *Artemia* son algunos de los pocos organismos que pueden habitar los lagos hipersalinos. En Argentina, este género está representado por dos especies: la introducida *A. franciscana* Kellogg, 1906 y la autóctona *A. persimilis* Piccinelli & Prosdocimi, 1968, esta última, la única especie registrada en la provincia de La Pampa. Algunos aspectos de la biología de *A. persimilis* basados en estudios de laboratorio son conocidos, pero la información sobre su ecología en condiciones naturales es muy escasa. El objetivo de este trabajo es presentar información sobre la densidad, biomasa y la estructura de la población de *A. persimilis* en relación con parámetros ambientales en Utracán, un lago hipersalino de la Pampa Central semiárida de Argentina. Se tomaron muestras mensuales de zooplancton y agua desde mayo de 2009 hasta agosto de 2010. La densidad y biomasa medias a lo largo del estudio fueron 56.98 ind.L^{-1} (± 106.64) y 1.23 mg.L^{-1} (± 1.35). La especie fue afectada negativamente por la salinidad, dado que no se registró entre noviembre y febrero, cuando la misma excedió los 320 g.L^{-1}. Sin embargo, cuando estuvo presente, aún en los meses de mayor salinidad se encontró una proporción de estadios naupliares elevada, lo que podría indicar que, en la naturaleza, *A. persimilis* puede reproducirse con alta salinidad. A pesar de que se ha indicado que *A. persimilis* podría ser desplazada por *A. franciscana*, la mayor tolerancia de la primera a bajas temperaturas, que permitió que a valores cercanos a 3°C la población continuara reproduciéndose, limitaría su desplazamiento. Sin embargo, un aumento de la temperatura de los lagos como consecuencia del calentamiento global podría modificar esta situación y permitir el avance de la especie introducida hacia el sur.

Palabras-clave: *Artemia persimilis, lagos hipersalinos, Pampa Argentina*

Introduction

The anostracan *Artemia* is one of the few organisms that can inhabit hypersaline lakes (total dissolved solids > 50 g.L^{-1}) because it has physiological mechanisms to withstand osmotic stress (Gajardo & Beardmore 2012). In Argentina, the genus *Artemia* is represented by two species: *Artemia franciscana* Kellogg, 1906 and *Artemia persimilis* Piccinelli & Prosdocimi, 1968 (Cohen 2012). The former is a species of North America that would have been introduced to South America by the migrations of birds (Muñoz et al. 2013), and its distribution is limited to the latitude of 33°S (Ruiz et al. 2008). *A. persimilis* is autochthonous, restricted to environments in Chile and Argentina (Clegg & Gajardo 2009), and at present is the only species recorded in the province of La Pampa (Vignatti et al. 2014, Echaniz et al. 2015).

The species of this genus have economic importance, since from the naupliar stage until adulthood they are used as live food in the breeding of several fish and crustaceans of commercial value, because they constitute a source of proteins, lipids, essential amino acids, and unsaturated fatty acids beneficial to the organisms in culture (Dhont & Lavens 1996, Dhont & Sorgeloos 2002). This has caused some biological aspects of *A. persimilis* to be studied in the laboratory (Pastorino et al. 2002, Mechaly et al. 2004, 2013, Sato et al. 2004, Medina et al. 2007), but there is little information about its geographical distribution and its ecology under natural conditions. In addition, increasing knowledge about the biology of the species is an important aspect, as its geographical distribution could be in decline, because it has been proven that *A. franciscana* tends to displace native species of the places it colonizes (Green et al. 2005) due to its higher phenotypic plasticity (Browne & Wanigasekera 2000, Clegg & Gajardo 2009, Vikas et al. 2012).

In the central semi-arid region of Argentina, the presence of *A. persimilis* was registered in some hypersaline lakes. In Guatraché, located to the southeast of the province of La Pampa, the species was found at a time when the concentration of dissolved solids was higher than 400 g.L^{-1}, the highest salinity reported for this species in the literature (Vignatti et al. 2014). The population dynamics of *A. persimilis* have been described in La Amarga, but it is a lake with unique characteristics, because it belongs to a river system that is inactive due to strong anthropogenic influence (Echaniz et al. 2015).

Given the scarcity of information on the population dynamics of the species under natural conditions and the influence of environmental factors, the objectives of this work were to know the main limnological parameters, density, biomass, and population structure of *A. persimilis* and to establish relationships between the biotic and environmental variables in Utracán, a typical hypersaline lake in the Central Pampa of Argentina.

Materials and methods

1. Study area

Utracán (37°16´N, 64°35´W) is a shallow lake located in the central region of the province of La Pampa (Figure 1) in a protected area of the municipality of the city of General Acha. It is located in a dune landscape, surrounded by natural vegetation, with grasslands and native forest (Figure 2).

Utracán Lake is fed principally by rainfall and groundwater inputs. It is in a region where the average annual rainfall is around 600 mm (Casagrande et al. 2006), with peaks in summer and early autumn (Vergara & Casagrande, 2012). However, the rains during 2009 and 2010 only reached 312 and 499 mm respectively (http://www.policia.lapampa.gov.ar/contenidos/ver/lluvias). Since the lake is located in an arheic basin, water outlets are usually produced by evaporation.

Fish have never been recorded in Utracán, probably due to the high salinity and lack of connection to other aquatic ecosystems. In its basin only livestock farming is done. During the period included in this work, only scarce flamingoes (*Phoenicopterus chilensis* Molina, 1782) were spotted sporadically in the lake's avifauna.

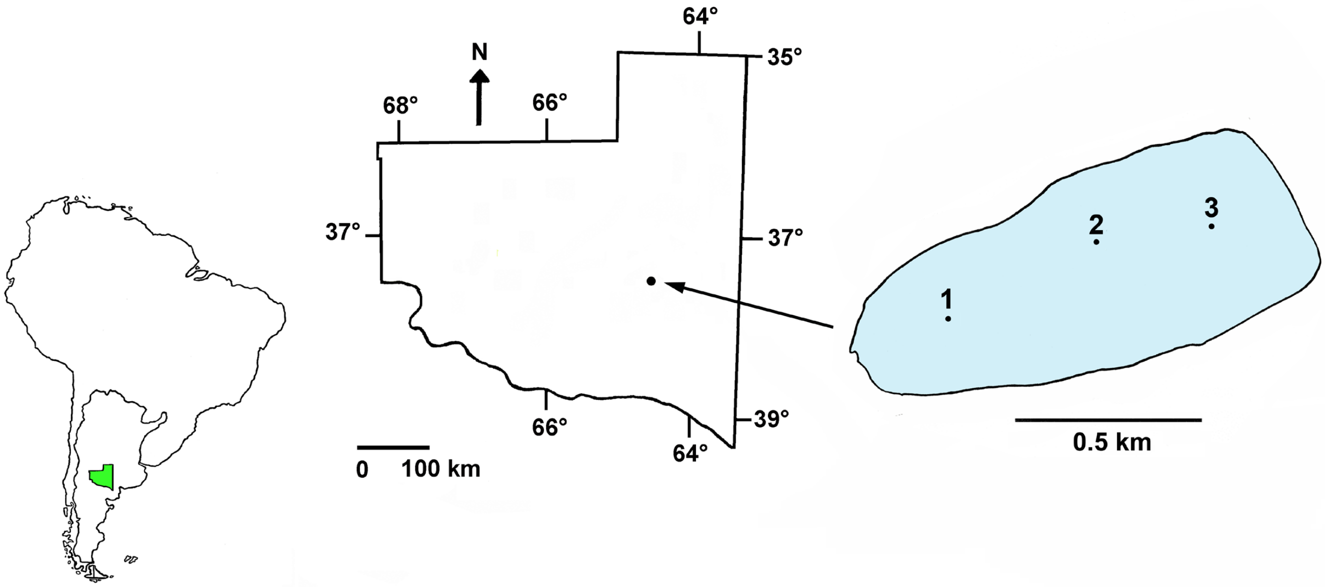


Figure 1. Geographic location of Utracán Lake. The light blue area is the extension of the lake in May 2009. 1, 2, and 3: sampling sites

2. Field and laboratory work

Samples were collected monthly from May 2009 until August 2010. Between May and October 2009 the sampling was conducted in the three stations along the longest axis of the lake (Figure 1), totaling 18 samples. Subsequently, the station located in the west of the lake (Figure 1, point 1), disappeared when the water level decreased, which meant that only samples at sites 2 and 3 were taken (for a total of 22 samples).

Water temperature, dissolved oxygen concentration (oximeter Lutron® OD 5510), water transparency (Secchi disc), and pH (digital pH meter Cornning® PS 15) were determined in each station.

Salinity was determined by means of the gravimetric method with drying at 104°C. Chlorophyll *a* concentration (Chl-*a*) was measured by extraction with aqueous acetone and spectrophotometry (Arar 1997, APHA 1992), the total phosphorus (TP) by the ascorbic acid method after digestion with acidic persulfate, and the Kjeldahl total nitrogen (TKN) by the digestion method with sulfuric acid and colorimetric determination with Nessler reagent (APHA 1992).

Quantitative zooplankton samples were collected in each site. Because of the shallow depth, these samples were taken with graduated containers that integrated the water column and were filtered through a 0.04 mm mesh-size net. A qualitative sample was also taken with a similar net. All the samples were anesthetized with CO_2 to avoid contractions that may deform the individuals collected and were kept refrigerated until fixation with formaldehyde 4%.

Counts to determine the density of zooplankton were made in Bogorov chambers under a stereomicroscope. The stages of *A. persimilis* were determined in Sedgwick Rafter chambers under a microscope at 40–100X following the criteria of Cohen et al. (1999). These authors grouped the development of this species into five periods. The first, or naupliar period, is characterized by the presence of a naupliar, polygonal and frontal eye, segmentation sketches in the post-mandibular region, and a thin middle indentation in the posterior region which, towards the end of the period, becomes a deep proctodeal invagination. The metanaupliar period is characterized by the presence of protuberances corresponding to compound eyes. On each side of the naupliar eye, thoracic segments delimited by furrows and protuberances correspond to the first pairs of phyllopods. The third period, the post-metanaupliar, is characterized by the development of the peduncle of the compound eyes, which become prominent and pigmented, and by the presence of seven to eleven thoracic segments, whose phyllopods are progressively acquiring setae and, as a result, their functions of locomotion and feeding. During this period the delimitation of the abdomen occurs. During the fourth or post-larval period, the antennae gradually reach the shape, size, and characteristics that distinguish males and females. In both sexes the genital region and related structures reach their full development and each furcal branch (uropod) carries two setae and a thorn or three setae. During the fifth and final adult period, the male penises reach the typical morphology of the species, with a mound with spines in the inner angle of its basal portion and spines in the eversible portion. The female's ovaries show oocytes and the ovisacs acquire the typical triangular form. The posterior end of the abdomen of both sexes carries a pair of furcal branches (uropods), each with three to five feathery silks.

The biomass was determined by drying to constant weight of all specimens (previously washed) of 5 mL subsamples, obtained with a Russell subsampler.

To examine relations between environmental factors and *A. persimilis* attributes, Spearman correlation coefficients were calculated (Sokal & Rohlf 1995, Zar 1996) and principal components analysis (PCA) (Pérez López 2004) was performed. We used Infostat software (Di Rienzo et al. 2010).

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Results

1. Environmental parameters

The depth was always reduced. It was at maximum at the beginning of the study, and, after fluctuating markedly by evaporation and the influence of relatively small precipitations, the lake was dried in September 2010 (Figure 3). The mean salinity was higher than 230 $g.L^{-1}$ (Table 1) and also varied widely and abruptly, from a maximum of 424.7 $g.L^{-1}$, registered in February 2010, to a minimum of 99.2 $g.L^{-1}$, the following month (Figure 3). Significant negative correlation was found between both parameters (r_s = -0.91; p < 0.0001).

Figure 2. Utracán Lake in February 2010

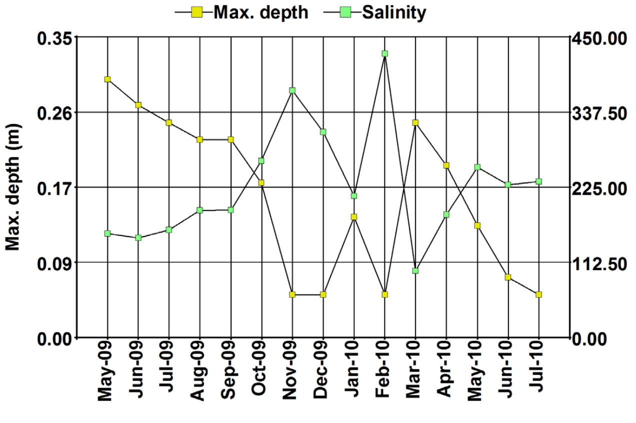


Figure 3. Monthly variation of the maximum depth and salinity in Utracán Lake between May 2009 and August 2010

Table 1. Main limnological parameters, total density of *Artemia persimilis*, and stages (mean values ± standard deviation) in saline lakes of La Pampa in which the species was recorded.

	Utracán (2009–2010)	Guatraché (2011–2012)*	La Amarga (2007)**
Water temperature (°C)	16.53 ± 7.61	23.78 ± 10.87	14.58 ± 7.99
Salinity (g.L^{-1})	238.05 ± 92.34	345.40 ± 48.25	115.51 ± 19.02
pH	9.21 ± 0.09	7.73 ± 0.23	7.82 ± 0.53
Transparency (m)	0.11 ± 0.06	> 0.2	1.54 ± 0.15
Chlorophyll-*a* (mg.m^{-3})	39.16 ± 22.69	8.39 ± 6.97	1.65 ± 1.16
Dissolved oxygen (mg.L^{-1})	8.11 ± 3.07	N/D	6.83 ± 1.23
Total density (ind.L^{-1})	56.98 ± 106.64	9.60 ± 16.12	1.56 ± 2.17
Nauplii density (ind.L^{-1})	11.59 ± 33.68	2.15 ± 1.12	0.46 ± 0.63
Metanauplii density (ind.L^{-1})	16.10 ± 38.54	1.22 ± 1.50	0.82 ± 1.35
Postmetanauplii density (ind.L^{-1})	22.87 ± 43.04	5.77 ± 11.80	0.08 ± 0.08
Postlarvae density (ind.L^{-1})	3.78 ± 6.48	1.08 ± 1.57	0.14 ± 0.16
Adults density (ind.L^{-1})	2.73 ± 3.95	1.30 ± 2.05	0.05 ± 0.06

*Vignatti et al. 2014; ** Echaniz et al. 2015

The water temperature varied between a minimum of 3.43 °C (July 2009) and a maximum of 31.2 °C (February 2010) (Figure 4). The dissolved oxygen concentration ranged from values close to 1.5 mg.L^{-1} in November and February to a maximum of 12 mg.L^{-1} in April. Negative correlation was found between both parameters (r_s = - 0.72; p = 0.0054).

Chl-*a* mean concentration was close to 38 mg.m^{-3} (Table 1) and was very variable, although it did not show a seasonal pattern. The concentration was less than 20 mg.m^{-3} on three occasions but had a peak of 101.26 mg.m^{-3} in January (Figure 4) and no significant correlation was found with salinity (r_s = 0.33; p = 0.2189) and water temperature (r_s = 0.10; p = 0.6984). The transparency of the water was always highly reduced and highly variable, since it fluctuated between 0.05 m, registered on five occasions, and a maximum of 0.25 m, recorded in March and no seasonal pattern was detected. A positive correlation was found between this variable and maximum depth of the lake (r_s = 0.93; p< 0.0001) but a negative one between water transparency and salinity (r_s = -0.89; p< 0.0001). The correlation between water transparency and Chl-*a* concentration was not significant (r_s = -0.40; p = 0.1352).

2. *Artemia persimilis* biology

The species was recorded over 11 months, from the beginning of the study until October 2009, when the salinity fluctuated between 155.5 and 264.5 g.L^{-1}, and between March and July 2010, when the salinity fluctuated between 99.2 and 228.4 g.L^{-1}. It was not registered between November 2009 and February 2010, when the concentration of dissolved solids exceeded 320 g.L^{-1}, nor in August 2010, when salinity exceeded 380 g.L^{-1}, just before the drying of the lake. The species' mean density and biomass throughout the study were 56.98 ind.L^{-1} (± 106.64) and 1.23 mg.L^{-1} (± 1.35), respectively, and correlation was found between both parameters (r_s= 0.94; p= 0.000). Since the two biological parameters were lower on the occasions when the concentration of dissolved solids was higher, a correlation was found between total population density and biomass and salinity (r_s = -0.75; p = 0.0008 and r_s = -0.67; p = 0.0049), but not with other environmental parameters.

The PCA analysis, whose first two components explained 67.1% of the variance (Figure 5), also showed that the salinity was the environmental factor that has the greatest influence on the density and biomass of *A. persimilis* and also showed certain influence of this species on concentration of phytoplankton chlorophyll-*a* and therefore, on the water transparency.

In both years, the density peaks were recorded during the autumn and were 180.75 ind.L^{-1} in June 2009 and 399.3 ind.L^{-1} in April 2010 (Figure 6), when the naupliar stages predominated in the population (Figure 7). However, during 2009 the maximum biomass was recorded during the winter, in August, when it reached 2.56 mg.L^{-1}, when the adult specimens predominated in the population. In 2010 the biomass peak was higher, since it reached 4.88 mg.L^{-1} and coincided with that of density. The lowest densities were recorded in the months prior to the recordings of the maximum salinities, 16.33 ind.L^{-1} in October 2009 and only 0.83 ind.L^{-1} in July 2010, times at which the population was dominated by postlarvae and adults (Figure 7).

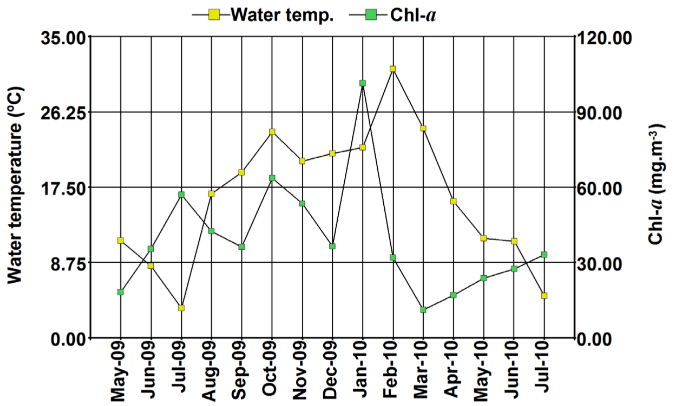


Figure 4. Monthly variation of the water temperature and phytoplankton chlorophyll-*a* concentration in Utracán Lake between May 2009 and August 2010

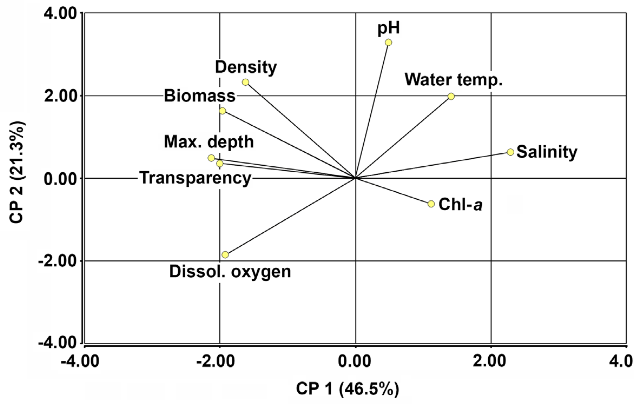


Figure 5. Biplot with results of Principal Component Analysis

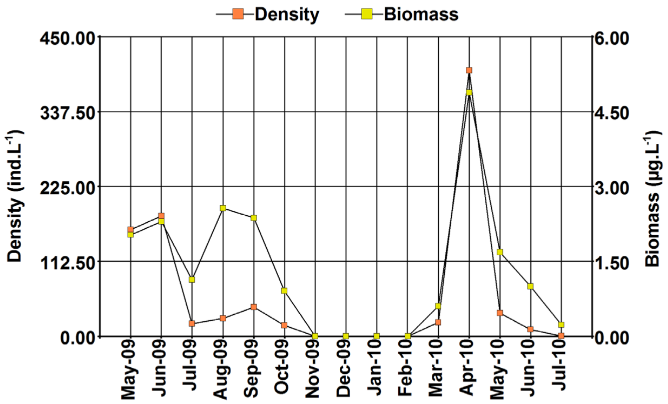


Figure 6. Variation of density and total biomass of *Artemia persimilis* in Utracán Lake during the study

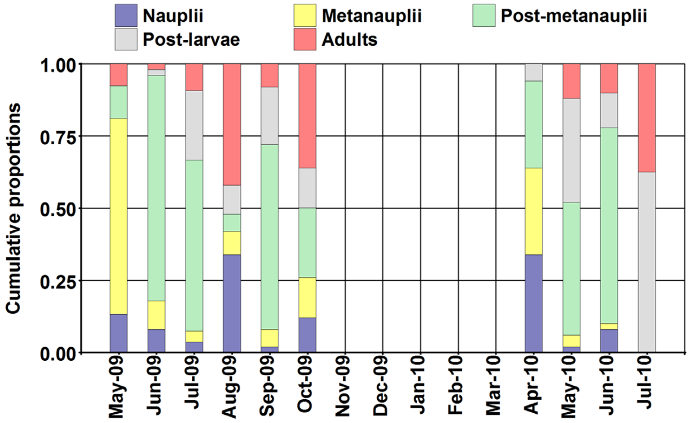


Figure 7. Proportions of the contribution of each stage to the total density of *A. persimilis* in Utracán Lake between May 2009 and July 2010

Discussion

Artemia persimilis was present for most of the sampling occasions; however, the negative effect of salinity was evident, since the species was absent between November and February, when the salinity was close to 370 g.L^{-1}.

Later recolonization may have been due to the hatching of cysts deposited in the egg bank of the sediments, since during all occasions a large number of dormant eggs were observed in the borders (Vignatti & Echaniz, pers. observ.)

The situation in Utracán is different from that found in Guatraché, another pampean lake, where the species was recorded when the salinity was higher than 400 g.L^{-1}, after which, due to an increase in the concentration of dissolved solids, it was not found (Vignatti et al. 2014). In La Amarga, the third hypersaline lake studied in La Pampa, *A. persimilis* was found at all times, probably because the maximum salinity remained within the tolerance range of the species (Echaniz et al. 2015).

In both Utracán and Guatraché populations (Vignatti et al. 2014), a relatively high proportion of naupliar stages was found in the months of higher salinity, although the population later disappeared from the water column. This could indicate that, in the wild, *A. persimilis* can reproduce up to these limits of salinity. On the other hand, in both lakes a relatively high proportion of larval stages were found during the colder months, which could indicate that the low temperatures would not affect reproduction.

Whereas in Guatraché and La Amarga the average density was reduced, in Utracán it was almost 6 and 35 times higher, respectively (Table 1). Considering that the concentrations of phytoplankton chlorophyll-a that were determined in the former two lakes were considerably lower than those of Utracán (Table 1), the greater density of the species in the latter could be due to the greater availability of food, given the greater quantities of phytoplankton present in this lake.

Although information on the geographic distribution of the *Artemia* genus in Argentina is scarce, in the few saline lakes in the north of the country where specimens were found, they corresponded to *A. franciscana* (Ruiz et al. 2008). The southernmost register corresponded to Las Tunas Lake in the province of Cordoba, where it could have come because of dispersal by birds (Muñoz et al. 2013). At present, all records to the south of 33 ° latitude, both in the center of the country and in Patagonia, correspond to *A. persimilis* (Ruiz et al. 2008, Vignatti et al. 2014, Echaniz et al. 2015).

As previously mentioned, it is thought that *A. franciscana* could be in geographical range expansion due to its great colonizing ability (Clegg & Gajardo, 2009), which may displace the native species (Green et al. 2005). One possible limitation to *A. franciscana*'s progress towards the south would be its lower tolerance to low temperatures (Amat et al. 2004, Vignatti et al. 2014), to the point that it was not recorded in the Great Salt Lake of Utah with temperatures below 3 °C (Wurtsbaugh & Gliwicz 2001). In Argentina, where there are few studies that cover annual cycles in saline lakes of this species, there is only the record in winter (July) in Mar Chiquita Lake, when the water temperature was 10.7 °C (Pilati et al. 2016).

In Utracán, located much to the south, *A. persimilis* was recorded in July, with a temperature only above 3°C. Considering that a high proportion of juvenile stages were counted at that time, the species' reproduction may not be affected by the low temperature, which could constitute an effective defense against possible invasions of their congeneric species. However, considering that climate change is a particularly important phenomenon in Argentina's arid diagonal (D'Ambrosio et al. 2016), this situation could change drastically, since a small increase in the mean water temperature of the lakes would allow *A. franciscana* to colonize lakes located farther south than its present distribution, which could potentially lead to local extinctions of the native species. This necessitates new studies on both species in saline lakes located between 33° and 37° S, in what can be considered the actual transition zone between the distributions of *A. franciscana* and *A. persimilis*.

Acknowledgements

We thank the municipality of the city of General Acha for allowing entrance to the property where Utracán Lake is located and to the anonymous reviewers who, with their work, improved this article.

Author Contributions

Alicia María Vignatti: substantial contribution in the concept and design of the study; performing field and laboratory work; contribution to manuscript preparation and critical revision.

Gabriela Cecilia Cabrera: performing laboratory work; contribution to manuscript preparation and critical revision.

Santiago Andrés Echaniz: substantial contribution in the concept and design of the study; performing field and laboratory work; contribution to manuscript preparation and critical revision.

Conflicts of interest

The authors declares that they have no conflict of interest related to the publication of this manuscript.

References

AMAT, F., COHEN, R.G., HONTORIA, F. & NAVARRO, C. 2004. Further evidence and characterization of *Artemia franciscana* (Kellogg, 1906) populations in Argentina. J. Biogeogr. 31:1735–1749. DOI 10.1111/j.1365-2699.2004.01123.x.

APHA. 1992. Standard Methods for the Examination of Water and Wastewater. 18th edition. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF), Washington, DC.

ARAR, E.J. 1997. In Vitro Determination of Chlorophylls a, b, c + c and Pheopigments in Marine and Freshwater Algae by Visible Spectrophotometry. Method 446.0. U.S. Environmental Protection Agency.

BROWNE, R. & WANIGASEKERA, G. 2000. Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*. J. Exp. Mar. Bio. Ecol. 244: 29-44.

CASAGRANDE, G., VERGARA, G. & BELLINI, Y. 2006. Cartas agroclimáticas actuales de temperaturas, heladas y lluvia de la provincia de La Pampa (Argentina). Rev. Fac. Agronomía UNLPam 17 (1/2): 15-22.

CLEGG, J.S. & GAJARDO, G. 2009. Two highly diverged New World Artemia species, *A. franciscana* and *A. persimilis*, from contrasting hypersaline habitats express a conserved stress protein complement. Comp. Biochem. Phys. A. 153:451–456. doi: 10.1016/j.cbpa.2009.04.613.

COHEN, R., RODRÍGUEZ GIL, S. & VÉLEZ, C. 1999. The post-embryonic development of *Artemia persimilis* Piccinelli & Prosdocimi. Hydrobiologia. 391(1-3): 63-80.

COHEN, R.G. 2012. Review of the biogeography of *Artemia* Leach, 1819 (Crustacea: Anostraca) in Argentina. Int. J. Artemia Biol. 2: 9-23.

D'AMBROSIO, D.S., CLAPS, M.C. & GARCÍA, A. 2016. Zooplankton diversity of a protected and vulnerable wetland system in southern South America (Llancanelo area, Argentina). Int. Aquat. Res. 8:65–80. DOI 10.1007/s40071-016-0125-2.

DHONT, J. & LAVENS, P. 1996. Tank production and use of ongrown *Artemia*. Pp. 164-195. In P. Lavens & P. Sorgeloos (eds.). Manual on the Production and Use of Live Food for Aquaculture. FAO Fisheries Technical Paper 361, Rome.

DHONT, J. & SORGELOOS, P. 2002. Applications of *Artemia*. In: Abatzopoulos T. J, Beardmore J. A, Clegg J. S, Sorgeloos P, eds, *Artemia* basic and applied biology. Kluwer Academic Publishers, Dordrecht: 251-277.

DI RIENZO, J.A., CASANOVES, F., BALZARINI, M.G., GONZÁLEZ, L.M., TABLADA, C. & ROBLEDO, W. 2010. InfoStat (versión 2010). Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

ECHANIZ, S.A., CABRERA, G.C. & VIGNATTI, A.M. 2015. Limnological parameters and population structure of *Artemia persimilis* Piccinelli and Prosdocimi, 1968 (Crustacea, Anostraca) in La Amarga, a hypersaline lake of La Pampa (Argentina). Res. Zool. 5(2): 25-31. (p-ISSN: 2325-002X; e-ISSN: 2325-0038). DOI: 10.5923/j.zoology.20150502.01.

GAJARDO, G.M., & BEARDMORE, J. 2012. The brine shrimp *Artemia*: adapted to critical life conditions. Front. Phys. 3:1-8.

GREEN, A.J., SÁNCHEZ, M.I., AMAT, F., FIGUEROLA, J., HONTORIA, F., RUIZ, O. & HORTAS, F. 2005. Dispersal of invasive and native brine shrimps *Artemia* (Anostraca) via waterbirds. Limnol. Oceanogr., 50(2): 737–742.

MECHALY, A.S., ANGELETTI, S., DE LOS RÍOS-ESCALANTE, P. & CERVELLINI, P. M. 2013. A review of the biology and ecology of *Artemia persimilis* Piccinelli & Prosdocimi, 1968 (Crustacea: Anostraca) as basis for its management. Int. J. Artemia Biol. 3(1): 12-19.

MECHALY, A.S., CERVELLINI, P.M. & BAMBILL, G.A. 2004. Experiencias preliminares con *Artemia persimilis* (Crustacea, Anostraca) como potencial alimento vivo en acuicultura. AquaTIC. 21: 1-7.

MEDINA, G.R., GOENAGA, J., HONTORIA, F., COHEN, G. & AMAT, F. 2007. Effects of temperature and salinity on prereproductive life span and reproductive traits of two species of Artemia (Branchiopoda, Anostraca) from Argentina: *Artemia franciscana* and *A. persimilis*. Hydrobiologia. 579: 41–53.

MUÑOZ, J., AMAT, F., GREEN, A., FIGUEROLA, J. & GÓMEZ, A. 2013. Bird migratory flyways influence the phylogeography of the invasive brine shrimp *Artemia franciscana* in its native American range. PeerJ 1:e200; DOI 10.7717/peerj.200.

PASTORINO, X.I., MARSCHOFF, E. & COHEN, R.G. 2002. Reproductive and brood cycles of *Artemia persimilis* Piccinelli and Prosdocimi from Colorada Chica Lake (Province of La Pampa, República Argentina), under laboratory conditions. Hydrobiologia. 486: 279–288.

PÉREZ LÓPEZ, C. 2004. Técnicas de Análisis Multivariado de datos. Pearson Educación, Madrid. 676 p.

PILATI, A., CASTELLINO, M. & BUCHER, E.H. 2016. Nutrient, chlorophyll and zooplankton seasonal variations on the southern coast of a subtropical saline lake (Mar Chiquita, Córdoba, Argentina). Ann. Limnol. - Int. J. Lim. 52: 263–271. DOI: 10.1051/limn/2016014

RUIZ, O., AMAT, F., SAAVEDRA, C., PAPESCHI, A., COHEN, R., BAXEVANIS, A., KAPPAS, I., ABATZOPOULOS, T. & NAVARRO, J. 2008. Genetic characterization of Argentinean Artemia species with different fatty acid profiles. Hydrobiologia. 610: 223–234. DOI 10.1007/s10750-008-9437-6

SATO, N.E, MALLO, J.C. & FENUCCI, J.L. 2004. Calidad de los quistes de *Artemia persimilis* (Piccinelli & Prosdocimi) (Crustacea: Branchiopoda) de diferentes zonas de Argentina, como alimento en acuicultura. Rev. Biol. Mar. Oceanog. 39: 79-92.

SOKAL, R. & ROHLF, F. 1995. Biometría. Principios y métodos estadísticos en la investigación biológica. Ed. Blume, Barcelona.

VERGARA, G. & CASAGRANDE, G. 2012. Estadísticas agroclimáticas de la Facultad de Agronomía, Santa Rosa, La Pampa, Argentina. Rev. Fac. Agronomía UNLPam 22 (1): 3-74.

VIGNATTI, A.M., CABRERA, G.C., PILATI, A. & ECHANIZ, S.A. 2014. Biology of *Artemia persimilis* Piccinelli and Prosdocimi, 1968 (Crustacea: Anostraca) at the highest salinities reported for the species under natural conditions. Int. J. Artemia Biol. 4(1): 38-43.

VIKAS, P., SAGESHKUMAR, N., THOMAS, P., CHAKRABORTY, K. & VIJAYAN, K. 2012. Aquaculture related invasion of the exotic *Artemia franciscana* and displacement of the autochthonous *Artemia* populations from the hypersaline habitats of India. Hydrobiologia. 684: 129-142.

WURTSBAUGH, W.A. & GLIWICZ, Z.M. 2001. Limnological control of brine shrimp population dynamics and cyst production in the Great Salt Lake, Utah. Hydrobiologia. 466: 119–132.

ZAR, J.H. 1996. Biostatistical analysis. 3º Ed. Prentice Hall, New Jersey.

Received: 19/04/2017
Revised: 01/09/2017
Accepted: 08/09/2017
Published online: 28/09/2017

Biota Neotropica 17(3): e20160291, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
article

The community of Diptera (Insecta) colonizing axils of *Alocasia macrorrhizos* (L.) G.Don (Araceae), with records of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in urban areas of Manaus, Amazonas

Ruth Leila Ferreira-Keppler[1], Ulisses Gaspar Neiss[2], Sharlene Roberta da Silva Torreias[1] & Claudimir Menezes Campos[1]*

[1]*Instituto Nacional de Pesquisas da Amazônia, Coordenação de Biodiversidade, Manaus, AM, Brazil*
[2]*Departamento de Polícia Técnico-Científica, Instituto de Criminalística, Manaus, AM, Brazil*
**Corresponding author: Ruth Leila Ferreira-Keppler, e-mail: ruth@inpa.gov.br*

FERREIRA-KEPPLER, R. L., NEISS, U. G., TORREIAS, S. R. da S., CAMPOS, C. M. **The community of Diptera (Insecta) colonizing axils of *Alocasia macrorrhizos* (L.) G.Don (Araceae), with records of *Aedes aegypti* (L.) and Aedes albopictus (Skuse) in urban areas of Manaus, Amazonas.** Biota Neotropica. 17(3): e20160291. http://dx.doi.org/10.1590/1676-0611-BN-2016-0291

Abstract: *Alocasia macrorrhizos* (L.) G.Don grows and proliferates in shaded areas in several forest fragments in urban zones. The adult plant has axils that accumulate rain water (phytotelmata) and serve as breeding sites for several families of aquatic insects. The objective of this study was to determine the composition of the entomofauna associated with water accumulations in axils of *A. macrorrhizos* in urban areas of Manaus, Brazil. Individuals of *A. macrorrhizos* were sampled in five forest fragments, between April/2005 and March/2007 in urban areas of Manaus, totaling 184 sampling units. A manual suction hose was used for the removal of entomofauna. A total of 1,941 immatures of Diptera were collected representing the following families: Culicidae (96.5%), Chironomidae (2%), Psychodidae (0.7%), Ephydridae (0.7%) and Corethrellidae (0.1%). Five Culicidae species were identified: *Wyeomyia melanocephala* Dyar & Knab, 1906 (91.7%), *Wy. ypsipola* Dyar, 1922 (4.3%), *Aedes albopictus* (Skuse, 1894) (2.3%), *Ae. aegypti* (Linnaeus, 1762) (1.5%) and *Johnbelkinia longipes* (Fabricius, 1805) (0.1%). The members of Culicidae were the most abundant, confirming the importance of this group in communities associated with phytotelmata, with a positive relation to the volume of water ($p < 0.05$). The occurrence of *Ae. aegypti* and *Ae. albopictus* in axils of *A. macrorrhizos* demonstrates the potential of this Araceae as a breeding site of these medically important species. However, the real contribution of this Araceae in the maintenance and dispersion of populations of these two species of mosquitoes throughout the year should be further studied.

Keywords: *Culicidae, forest fragments, mosquitoes, phytotelmata, vectors*

Comunidade de Diptera (Insecta) colonizando axilas de *Alocasia macrorrhizos (L.) G.Don* (Araceae), com registros de *Aedes aegypti* (L.) e *Aedes albopictus* (Skuse) na área urbana de Manaus, Amazonas

Resumo: *A. macrorrhizos* (Araceae) é cultivada e se prolifera naturalmente em locais sombreados em diversos fragmentos de mata na zona urbana de Manaus. A planta adulta apresenta axilas que acumulam água da chuva (phytotelmata) e servem de criadouro para diversas famílias de insetos aquáticos. O objetivo do trabalho foi conhecer a composição da entomofauna associada às axilas de *A. macrorrhizos*, na área urbana de Manaus, Amazonas, Brasil. Entre 2005 e 2007, indivíduos de *A. macrorrhizos* foram amostrados em cinco fragmentos urbanos de mata, totalizando 184 unidades amostrais. Uma mangueira de sucção manual foi utilizada para coletar a fauna associada. Um total de 1.941 imaturos de Diptera foram coletadas, representadas pelas seguintes famílias: Culicidae (96,5%), Chironomidae (2%), Psychodidae (0,7%), Ephydridae (0,7%) e Corethrellidae (0,1%). Foram identificadas cinco espécies de Culicidae: *Wyeomyia melanocephala* Dyar & Knab, 1906 (91,7%), *Wy. ypsipola* Dyar, 1922 (4,3%), *Aedes albopictus* (Skuse, 1894) (2,3%), *Ae. aegypti* (Linnaeus, 1762) (1,5%) e *Johnbelkinia longipes* (Fabricius, 1805) (0,1%). Os representantes de Culicidae foram os mais abundantes confirmando a importância desse grupo nas comunidades associadas a fitotelmatas, com relação positiva com o volume de água por planta ($p < 0.05$). A ocorrência de *Ae. aegypti* e *Ae. albopictus* nas axilas de *A. macrorrhizos* demonstra o potencial dessa Araceae como criadouro para estas espécies de importância médica. Contudo, a real contribuição dessa Araceae na manutenção e dispersão populacional dessas duas espécies de mosquitos ao longo do ano precisa ser melhor estudada.

Palavras-chave: *Culicidae, fitotelmata, fragmentos florestais, mosquitos, vetores.*

Introduction

Plant structures capable of storing rain water, such as modified leaves, stem holes, leaf axils, flowers, open fruits and fallen leaves are known by the term phytotelmata (Fish 1983). Among the best-known phytotelmata are the axils of plants. Plants with these structures that occur in South America include: *Phenakospermum* Endl. ("bananeira-brava": Strelitziaceae), *Musa* L. (Musaceae), *Bambusa* Schreb. (Poaceae), *Mauritia* L. (Arecaceae), *Heliconia* L. (Heliconiaceae), *Calathea* G. Mey. (Maranthaceae), *Guzmania* Ruiz & Pavón and *Vriesea* Lindl. (Bromeliaceae), *Alocasia* (Schott) G. Don (Araceae) (Kitching 2000) and *Eryngium* (Apiaceae) (Campos 2010). Some of these plants have been used as ornaments, such as giant taro or "tajá" (Araceae), a plant from Asia that is well distributed through Venezuela, Colombia and Brazil. In Brazil, 12 genera and 55 species of Araceae are known in Reserva Florestal Ducke, which is adjacent to the city of Manaus (Ribeiro et al. 1999).

The fauna associated with phytotelmata is mainly composed of insects, with more than 70 families and 11 orders recorded so far, with Diptera as the most abundant order (Greeney 2001). In these environments aquatic larvae of Diptera are considered to be an ecologically important group because it occupies several aquatic ecosystems and contributes to the diet of other animals in these environments (Kitching 2000, 2001).

Many families of Diptera have medical or veterinary importance because they are involved in the transmission of etiological agent. The habitats of the immature forms of Culicidae have therefore received attention. Ecological and epidemiological studies show the increasing number of recipients in urban areas as a result of waste discarded by modern society (Soares et al. 2008). Consequently, providing sites for mosquitoes of *Aedes (Stegomyia)* Theobald, in urban areas (Cunha et al. 2002), being responsible for transmitting dengue, Chikungunya, zika virus and yellow fever throughout the Americas, Africa and Asia (Marcondes & Ximenes 2016, Lopes et al. 2014).

The capacity of Araceae (genera *Alocasia*, *Colocasia* and *Xanthosoma*) to hold immature forms of aquatic insects, mainly Diptera, in the water stored in their axils has been studied in forest areas in Venezuela (Delgado & Machado-Allison 2006). Members of Sabethini (Culicidae) were the most frequent and abundant (Machado-Allison et al. 1986, Delgado & Machado-Allison 2006). Some species have the capacity to detect favorable habitats for oviposition, with varying degrees of specificity as to the host plant, and this can be specific to only one plant or to several plant species with similar structures within a specific area (Kitching 2000). We also need to consider that the axil size, volume of water, number of leaves, and quantity of debris are all variables that influence the structure of the communities associated with phytotelmata (Ambruster et al. 2002, Melnychuk & Srivastava 2002, Ospina-Bautista et al. 2004, Torreias et al. 2010).

Forest fragments in urban or suburban areas of Manaus offer favorable places for the development of several insect species in public and private areas. These environments, if not monitored, may become locations used by city residents for disposal of artificial recipients – the main breeding sites of *Aedes aegypti* (L., 1762) (Lopes et al. 1985, Consoli & Lourenço-de-Oliveira 1994). In addition, many of these fragments have secondary forest, where populations of *Alocasia macrorrhizos* (L.) G.Don are abundant and grow naturally in these uninhabited areas (personal observations).

The objective of the present study was to determine the composition of entomofauna associated with the axils of *A. macrorrhizos* and to verify the potential of this species of ornamental plant as a breeding site for *Ae aegypti* and *Ae. albopictus* (Skuse, 1894) in urban zones of Manaus, Brazil.

Material and Methods

Samples were collected in urban areas of the municipality of Manaus, Amazonas state. The region in which the city is located has two seasons during the year: the rainy season (December to May) being March the rainiest month, and the dry season (June to November) being August the driest month (Fisch et al. 1998). *Alocasia macrorrhizos* (Figure 1a) is grown and proliferates naturally in shaded areas, mainly on wet and acidic soils. The adult plant has leaves up to one-meter long, thus having large leaf axils that can store a significant quantity of water (Gómez Zuluaga 2003).

Sampling was done where more individuals of *A. macrorrhizos* were available, in five forest fragments: Area 1: *Campus* I of the Instituto Nacional de Pesquisas da Amazônia / INPA (03º 05' 46.97" S, 59º 59' 23.58" W), Area 2: *Paraiba Street* (03º 05' 31.92" S, 60º 00' 30.19" W), Area 3: *Parque Municipal do Mindu* (03º 04' 41.6" S, 60º 00' 29.5" W), Area 4: *Franceses Road* (03º 04' 25" S, 60º 02' 10" W), Area 5: *Passeio do Mindu* (03º 05' 12.4" S, 60º 00' 33" W).

Areas 1 and 3 have plant species that are characteristic of primary forest that survived cutting mainly due to resprouting from the original stem, as well as species introduced by gardening. These areas make a major contribution to the conservation of the natural and cultural resources in Manaus municipality. By contrast, forest fragments in Areas 2, 4 and 5 are characterized by heavy anthropic intervention, with pioneer species characteristic of secondary vegetation. These areas have rainwater retained in permanently waterlogged soil, favoring the development of palms, "bananeira-brava" and large individuals of giant taro (more than 1 m in height).

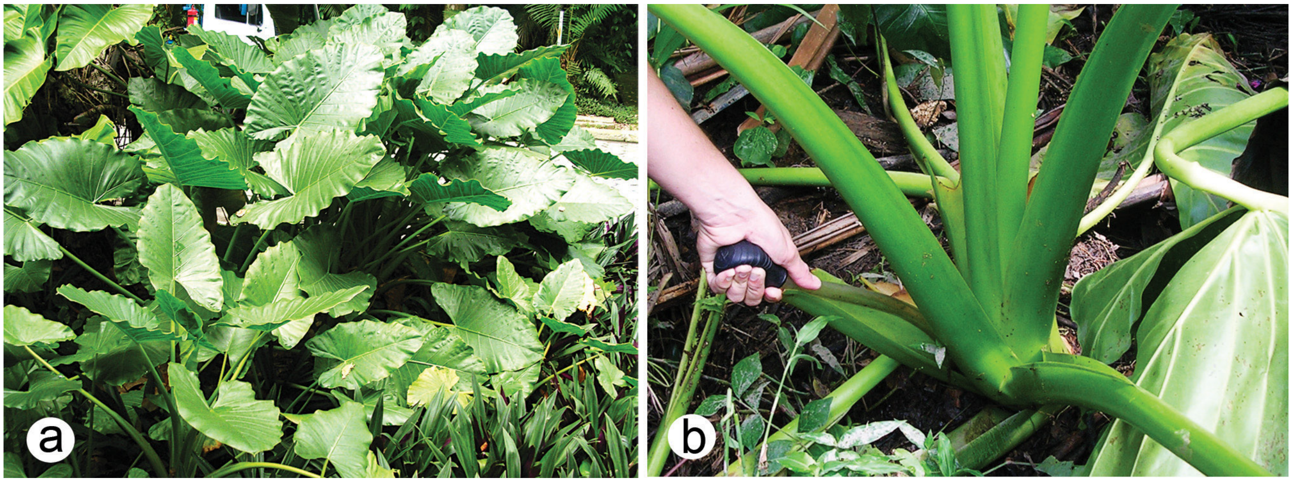


Figure 1. a) Group of *A. macrorrhizos*, located at INPA *Campus I*, Manaus, AM; b) Manual suction hose used to collect aquatic entomofauna.

Campus I of INPA (Area 1) and *Parque do Mindu* (Area 3) were considered to be the sites with the lowest anthropic impact because, despite having masonry constructions, they had a broad cover of primary vegetation and ornamental plants. Area 2 (Paraiba Street) was considered to be the most impacted because it has lowland vegetation that is exposed to sewage disposal and the waste dumping. Areas 4 and 5 have secondary vegetation fragments of approximately 1 ha each and low canopy vegetation, which partially covers Araceae plants. These are found grouped on sites covered by *Selaginella* (Pteridophyta).

Sampling periods corresponded to transition months between wet and dry periods in the hydrological regime: April to July 2005, January to August 2006, and March 2007, for a total of 13 months. A manual suction hose was used for the removal of the entomofauna (Figure 1b) and all of the liquid content was transferred to plastic 500 mL recipients. To ensure the removal of any specimens from the bootom, a second wash was performed on each leaf axil, with distilled water, and the liquid deposited in another container properly labeled. All material was transported to the Laboratório de Insetos Aquáticos da Coordenação de Biodiversidade (CBio/INPA). Fourth-instar larvae and pupae of Culicidae were individualized to obtain adults and to help in species-level identification (Lane 1953a, b, Motta & Lourenço-de-Oliveira 2000). The remaining samples were fixed in 80% ethanol for identification at the family level. Mosquito genera were abbreviated according to Reinert (2009). The positivity index for each species of Culicidae was estimated by dividing the number of positive sampled axils for a given species by the total *A. macrorrhizos* axils investigated (Gomes 1998). The positivity index is usually used in studies with Culicidae and is important because it reveals the percentage frequency of occurrence of a particular species on the sampled sites throughout the investigated period (Resende et al. 2013).

In order to characterize the phytotelmata environment, we measured pH with a Warterproof PC 300, Oakton Instruments and the volume of water retained in *A. macrorrhizos* axils using a graduated cylinder. Such parameters were selected for having influence phytotelmata communities structuring (Yanoviak 1999, Kitching 2001). Simple linear regression (Ln (x+1)) was used to evaluate relationships between total mosquito abundance and the total amount of water (mL), and also between total mosquito abundance and pH values (significance level, $p < 0.05$) (Zar 1996). The collected material is deposited in the Coleção de Invertebrados do Instituto Nacional de Pesquisas da Amazônia – INPA, Manaus, Brazil.

Results

A total of 1,941 immatures of Diptera were collected in 184 sampling units. As expected in communities of phytotelmata, the members of family Culicidae were the most abundant, being 96.5% of all individuals, followed by Chironomidae (2%), Psychodidae (0.7%), Ephydridae (0.7%) and Corethrellidae (0.1%) (Table 1 and 2). Of the total of 184 samples analyzed, 147 had immature insect in their axils, with an average of 10.5 (SE = 16.8) individuals per plant.

Wyeomyia melanocephala Dyar & Knab, 1906 was the most abundant species (91.7%), followed by *Wy. ypsipola* Dyar, 1922 (4.3%) (Table 2). These species occurred in all of the sampling sites. They had high rates of positivity index (78.3% and 24.5%, respectively). *Johnbelkinia longipes* (Fabricius, 1805), a wild mosquito, was found in only one sample (Table 2). Immatures of *Ae. albopictus* and *Ae. aegypti* was found in four areas with low abundance (Table 2) and low index of positivity (Table 3).

The volume of water in the plants ranged from a minimum of 10 to a maximum of 210 mL (average of 40 mL), and the abundance of Culicidae larvae was positively related to the water volume ($p < 0.05$, Figure 2). The pH varied from 6 to 7.2, and did not influence the abundance of mosquitoes ($r^2 = 0.0286$, $y = -2.43970427 + 0{,}644322259*x$, $p > 0.05$).

Discussion

Two Culicidae tribes were found in this study: Sabethini and Aedini. Sabethini species are known by their strong association with phytotelmata formed by the axils of plants such as bromeliads, Araceae, Rapataceae and Heliconiaceae, and also phytotelmata formed in bamboo internodes, fallen husks of fruits and fallen flower bracts of palms (Navarro et al. 2007, Kitching 2000). In these environments, females lay their eggs in isolation and larvae usually develop in confined spaces.

The two mosquito species most abundant and frequent, *Wy. melanocephala* and *Wy. ypsipola*, occurred in all of the sampling sites, with high rates of positivity index, indicating a strong affinity with the phytotelmata studied in the urban area. This corroborates the studies by Machado-Allison et al. (1986) and Delgado & Machado-Allison (2006), who recorded these two species as being the most frequent and abundant larvae collected in *A. macrorrhizos* in a forest in Venezuela. In urban areas, *Wy. melanocephala* was also recorded colonizing Zingiberaceae axils, which have a similar

Table 1. Abundance of Diptera families collected in *A. macrorrhizos* (n = 184) in urban areas of Manaus, AM, 2005 to 2007. Caption: Area 1: *Campus I* – INPA; Area 2: Paraiba Street; Area 3: Parque do Mindu; Area 4: "Franceses" Road; Area 5: "Passeio do Mindu".

Families	Area 1	Area 2	Area 3	Area 4	Area 5	Total
Culicidae	771	613	184	184	121	1,873
Chironomidae	0	40	0	0	0	40
Psychodidae	0	8	0	4	1	13
Ephydridae	10	1	1	0	1	13
Corethrellidae	0	2	0	0	0	2
Total	781	664	185	188	123	1,941

Table 2. Abundances and percentages of total (%) of Culicidae species collected in *A. macrorrhizos* (n = 184) in urban areas of Manaus, AM, 2005 to 2007. Area 1: *Campus I*/INPA; Area 2: Paraiba Street; Area 3: Parque do Mindu; Area 4: "Franceses" Road; Area 5: "Passeio do Mindu".

Species	Area 1 (%)	Area 2 (%)	Area 3 (%)	Area 4 (%)	Area 5 (%)	Total (%)
Wy. melanocephala	696 (37.2)	557 (29.7)	174 (9.3)	172 (9.2)	118 (6.3)	1,717 (91.7)
Wy. ypsipola	54 (2.9)	8 (0.4)	5 (0.3)	10 (0.5)	3 (0.2)	80 (4.3)
Ae. albopictus	14 (0.7)	27 (1.4)	1 (0.1)	2 (0.1)	0	44 (2.3)
Ae. aegypti	7 (0.4)	21 (1.1)	1 (0.1)	0	0	29 (1.5)
Jo. longipes	0	0	3 (0.2)	0	0	3 (0.2)
Total	771 (41.2)	613 (32.7)	184 (9.8)	184 (9.8)	121 (6.5)	1,873 (100)

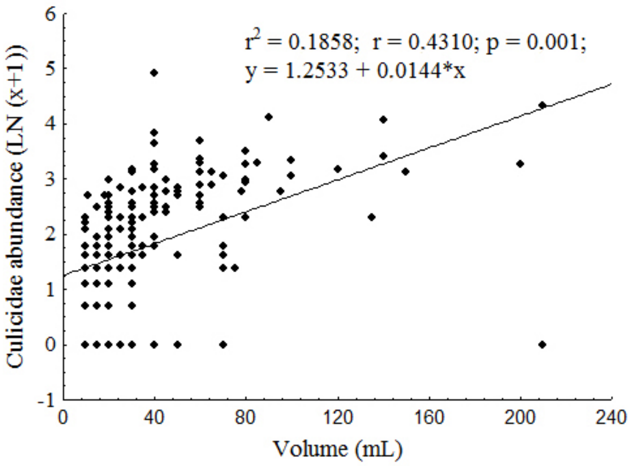


Figure 2. Simple linear regression (Ln (x+1)) of Culicidae abundance and the volume of water (mL) retained in the axils of *A. macrorrhizos*, Manaus, AM.

Table 3. Positivity index (frequency of occurrence) of Culicidae species collected in *A. macrorrhizos* (n = 184) in urban areas of Manaus, AM, 2005 to 2007.

Culicidae species	Positivity index
Wy. melanocephala	78.26
Wy. ypsipola	24.45
Ae. albopictus	8.15
Ae. aegypti	6.52
Jo. longipes	0.54

ways of retaining water (Machado-Allison et al. 1986, Consoli & Lourenço-de-Oliveira 1994).

Wyeomyia specimens are often found in bromeliads, bamboo internods and tree holes located in forest fragments in Brazil (Marques & Forattini 2008, Torreias et al. 2010, Ceretti-Junior et al. 2014). It is known that adult females are eclectic in the search for hosts. However, detailed studies of feeding behavior and biology of the group are still incipient (Forattini 2002). In Brazil, the genus *Wyeomyia* may be involved in the natural transmission of arbovirus *Ilheus*, causing fever and encephalitis (Lopes et al. 2014). However, the species of this genus are not yet listed as important vectors of viral agents.

On the field, observations were made regarding the feeding habit that corroborated with the results by Machado-Allison et al. (1986): *Wy. melanocephala* larvae uses the siphon as a supporting organ to fix itself in the deepest part of the axil and filter organic matter particles. By contrast, *Wy. ypsipola* larvae stays at the bottom searching for prey (smaller larvae of Culicidae and Ephydridae, which are common in these Araceae), directing its mouth apparatus to the surface. This species has a bucal apparatus that is well developed for predatory habits; however, if prey is lacking, it can also feed on filtered organic matter (Machado-Allison et al. 1986).

We found *Jo. longipes* in only one sample (with three individuals) in June 2006. Anthropic alterations, including use of ornamental plants for landscaping purposes, may offer a stimulus for the presence of these species, which previously were found exclusively in wild areas (Consoli & Lourenço-de-Oliveira 1994). Specimens of *Johnbelkinia* Zavortink, appear to be specific to phytotelmata formed by axils of Araceae (Navarro et al. 2007), as reported by Machado-Allison et al. (1986) and Delgado & Machado-Allison (2006), who recorded larvae of *Jo. ulopus* (Dyar & Knab) only in axils of the aroids plants *Alocasia*, *Colocasia* and *Xanthosoma* in Venezuela.

Regarding Aedini, we identified members of *Ae. albopictus* and *Ae. aegypti*. Immatures of *Ae. albopictus* and *Ae. aegypti* were collected in areas with low anthropic impact (Areas 1 and 3) and also in an area with high impact (Area 2), but always in shaded areas provided by well-developed leaves of large Araceae located a few meters from human constructions and residential areas. In Brazil, *Ae. albopictus* has been previously found in phytotelmata, such as holes in trees, bamboo internodes (Gomes et al. 1992, Muller & Marcondes 2010) and bromeliads (Marques et al. 2001, Mocellin et al. 2009).

Ae. albopictus is only considered to be a primary vector for the spread of dengue and yellow fever in some regions of Asia; additionally, many studies show the capacity of this species to serve as a potencial vector for several arboviruses (Alencar et al. 2008), including dengue, Chikungunya and zika virus (Marcondes & Ximenes 2016). Thus, the possibility of local transmission of all these arboviruses by this insect cannot be discarded, indicating the need for studies of their breeding sites, especially in urban areas. *Aedes albopictus* can easily adapt to rural areas as well as urban areas, becoming a zoonotic bridge in both places. Therefore, due to the wide ecological valence and the susceptibility of this species to the main arboviruses registered in Brazil, it is important that this mosquito is also included in epidemiological monitoring and control programs together with *Ae. aegypti* (Alencar et al. 2008, Marcondes & Ximenes 2016).

Ae. aegypti is the only known vector of dengue, Chikungunya and zika virus in urban areas in many Brazilian States. The occurrence of *Ae. aegypti* tends to indicate a public health problem. This species has synanthropic behavior and usually occupies anthropic areas (Forattini 2002). In urban areas, it usually breeds in discarded recipients; however, studies show that this species uses natural breeding sites in urban and rural areas with a great variety of microorganisms and organic matter for the development of its immature forms, such as domesticated bromeliads whether or not they are used for decorative purposes (Cunha et al. 2002, Delgado & Machado-Allison 2006, Gonçalves & Messias 2008).

In the present study, *Ae. aegypti* and *Ae. albopictus* was found in low number and with sporadic nature of occurrence, as shown by the low index of positivity for both species. As demonstrated in other studies in urban areas (Mocellin et al. 2009; Santos et al. 2011), this kind of habitat (axils of plants) does not seem to play an important role as breeding site for the larvae of these mosquitoes, being the water held in manmade containers the main oviposition sites for *Ae. aegypti* and *Ae. albopictus* females (Soares et al. 2008). These two species of mosquitoes were more abundant in Areas 1 and 2, especially Area 2, considered to be the most impacted anthropically, with greater discarding of domestic waste, which may have contributed to the higher number of *Ae. aegypti* and *Ae. albopictus* in this sample site in comparison with the other sample areas. The record of these species colonizing axils of *A. macrorrhizos* is important, since this small population of mosquitoes can serve as a reservoir for the introduction of these species in nearby residences.

Other organisms were collected in the axils of *A. macrorrhizos*, including Chironomidae, Psychodidae, Ephydridae and Corethrellidae larvae, some of which inhabited the axils with Culicidae. These families are very common in these environments, but according to the literature they had low number of species when compared with other studies of phytotelmata in forest areas in Central Amazon (Neiss 2007, Torreias & Ferreira-Keppler 2011).

Chironomidae were found in only one sample (Area 2). Nevertheless, after Culicidae, this is the second most abundant and diverse group in phytotelmata, colonizing various species of plants that are capable of storing water (Kitching 2000) in addition to breeding in sites formed by mycotelmata (Ferreira et al. 2001, Serpa-Filho et al. 2007). In this environment, chironomid larvae have broad feeding plasticity (preying, filtering-collecting, scraping and collecting).

Information on Corethrellidae is relatively scarce in the Brazilian Amazon. According to Borkent (2008) there are 12 species recorded from this area. The females of *Corethrella* Coquillett, the only extant genus of the family, are hematophagous, feeding exclusively on frog blood. The immature forms are generalist predators which develop in still waters, lakes, bamboo internodes and plant axils (Borkent 2008). *Corethrella* was collected only once in the present study. Larvae of the genus was also found in the bromeliad *Guzmania brasiliensis* Ule, in forest areas in the Amazon (Torreias & Ferreira-Keppler 2011).

Ephydridae larvae were found in the axils of Araceae, usually with little water and high quantities of organic matter. These organisms are detritivorous (Delgado & Machado-Allison 2006) and feed on fine sediments from allocthonous components that, in these locations, depend on retained rain water, leading to a diet with extrinsic characteristics. Larvae of Ephydridae has already been registered in bamboo internodes (Sanchez & Liria 2009), and has been previously found in *A. macrorrhizos*, as related by Delgado & Machado-Allison (2006). It seems that this family mainly inhabits places with plenty of organic matter where they feed on debris.

The positive relationship between immature mosquitoes and water volume, corroborate other studies on Culicidae that use phytotelmic habitats (Yanoviak 1999, Torreias et al. 2010). In *A. macrorrhizos*, as in other phytotelmic habitats, the increase in the volume and persistence of water are essential factors to maintain an aquatic community in these ecosystems (Srivastava & Lawton 1998, Kitching 2000, Ospina-Bautista et al. 2004, Yanoviak et al. 1999 and 2006).

Through this study it was possible to verify the potential of *A. macrorrhizos* as breeding sites for *Ae. aegypti* and *Ae. albopictus*, as well as other wild Culicidae. The results show that giant taro (Araceae), commonly used as ornamental plants in urban areas of Manaus, can be used sporadically by epidemiologically important mosquitoes species. However, the real contribution of this Araceae in the maintenance and dispersion of populations of these two species of mosquitoes throughout the year should be further studied.

Acknowledgments

We thank Dr. Monique Albuquerque Motta, FIOCRUZ – RJ, for helping in the identification of genus *Wyeomyia*. Financial support was provided by CNPq process 480105/2007-3 and 306081/2013-0, MCTI/INPA, and FAPEAM - Fundação de Amparo à Pesquisa do Estado do Amazonas Edital 020/2013 – PAPAC. The second and fourth author thanks PCI/MCTI/INPA/CNPq for the fellowships provided.

Author's Contributions

Ruth L. Ferreira-Keppler: substantial contribution in the concept and design of the study; contribution to manuscript preparation.

Ulisses G. Neiss: contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Sharlene Roberta S. Torreias: contribution to data analysis and interpretation; contribution to manuscript preparation.

Claudimir M. Campos: contribution to data collection.

Conflicts of Interest

The authors declares that they have no conflict of interest related to the publication of this manuscript.

References

ALENCAR, C.H.M., ALBUQUERQUE, L.M., AQUINO, T.M.F., SOARES, C.B., RAMOS JÚNIOR, A.N., LIMA, J.W.O. & PONTES, R.J.S. 2008. Potencialidades do *Aedes albopictus* como vetor de arboviroses no Brasil: um desafio para a atenção primária. Rev. APS. 11:459-467.

AMBRUSTER, P., HUTCHINSON, R.A. & COTGREAVE, P. 2002. Factors influencing community structure in a South American tank bromeliad fauna. Oikos. 96:225–234.

BORKENT, A. 2008. The frog-biting midges of the world (Corethrellidae: Diptera). Zootaxa. 1804:1–456.

CAMPOS, R. E. 2010. *Eryngium* (Apiaceae) phytotelmata and their macro-invertebrate communities, including a review and bibliography. Hydrobiologia. 652:311–328.

CERETTI-JÚNIOR, W., MEDEIROS-SOUSA, A.R., MULTINI, L.C., URBINATTI, P.R., VENDRAMI, D.P., NATAL, D., MARQUES, S., FERNANDES, A., OGATA, H. & MARRELLI, M.T. 2014. Immature mosquitoes in bamboo internodes in municipal parks, city of São Paulo, Brazil. J. Am. Mosq. Control. Assoc. 30(4):268–74.

CONSOLI, R.A.G.B. & LOURENÇO-DE-OLIVEIRA, R. 1994. Principais Mosquitos de Importância Sanitária no Brasil. Editora FIOCRUZ, Rio de Janeiro.

CUNHA, S.P., ALVES, J.R.C., LIMA, M.M., DUARTE, J.R., BARROS, L.C.V. & SILVA, J.L. 2002. Presença de *Aedes aegypti* em Bromeliaceae e depósitos com plantas no Município do Rio de Janeiro, RJ. Rev. Saúde Pública. 36:244–255.

DELGADO, L. & MACHADO-ALLISON, C.E. 2006. La comunidad de insectos acuáticos asociados a *Alocasia macrorrhiza* en Venezuela. Composición de la fauna y aspectos de su historia natural. Entomotropica. 21:105–115.

FERREIRA, R.L.M., OLIVEIRA, A.F., PEREIRA, E.S. & HAMADA, N. 2001. Occurence of larval Culicidae (Diptera) in water retained in *Aquascypha hydrophora* (Fungus: Stereaceae) in Central Amazonia, Brazil. Mem. Inst. Oswaldo Cruz. 96:1165–1167.

FISCH, G., MARENGO, J.A. & NOBRE, C.A. 1998. Uma revisão geral sobre o clima da Amazônia. Acta Amazon. 28:101–126.

FISH, D. 1983. Phytotelmata: Flora and Fauna. In Phytotelmata: Terrestrial Plants as Host for Aquatic Insect Communities (J.H. Frank & L.P. Lounibos, eds). Plexus, New Jersey, p.1–28.

FORATTINI, O.P. 2002. Culicidologia Médica. Vol. 2: Identificação, Biologia, Epidemiologia. Editora Universidade de São Paulo, São Paulo.

GOMES, A.C. 1998. Medidas dos níveis de infestação urbana por *Aedes* (*Stegomyia*) *aegypti* e *Aedes* (*Stegomyia*) *albopictus* em programas de Vigilância Entomológica. Inf. Epidemiol. SUS. 7:49−57.

GOMES, A.C., FORATTINI, O.P., KAKITANI, I., MARQUES, G.R., MARQUES, C.C. & MARUCCI, D. 1992. Microhabitats de *Aedes albopictus* (Skuse) na região do Vale do Paraíba, Estado de São Paulo, Brasil. Rev. Saúde Pública. 26:108–118.

GÓMEZ ZULUAGA, M.H.G. 2003. Una revisión sobre el Bore (*Alocasia macrorrhiza*). In Agroforestería para la Producción Animal en América Latina – II. Memorias de la segunda conferencia electrónica (agosto de 2000 - marzo de 2001) (M.D. Sánchez & M.R. Méndez, eds). Roma: Organización de las Naciones Unidas para la agricultura y la alimentación, p.203–212.

GONÇALVES, K.S. & MESSIAS, M.C. 2008. Ocorrência de *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) (Insecta, Diptera, Culicidae) em bromélias, no município do Rio de Janeiro (Rio de Janeiro, Brasil). Biota Neotrop. 8(1):235–237. http://www.biotaneotropica.org.br/v8n1/pt/fullpaper?bn01508012008+pt.

GREENEY, H.F. 2001. The insects of plant-held waters: a review and bibliography. J. Trop. Ecol. 17:241-260.

KITCHING, R.L. 2000. Food webs and container habitats: the natural history and ecology of phytotelmata. Cambridge University Press, Cambridge.

KITCHING, R.L. 2001. Food webs in phytotelmata: "bottom-up" and "top-down" explanations for community structure. Ann. Rev. Entomol. 46:729–760.

LANE, J. 1953a. Neotropical Culicidae. Vol. 2. Tribe Culicini*, Deinocerites, Uranotaenia, Mansonia, Orthopodomyia, Aedeomyia, Aedes, Psorophora, Haemagogus,* tribe *Sabethini, Trichoprosopon, Wyeomyia, Phoniomyia, Limatus* and *Sabethes*. Editora USP, São Paulo.

LANE, J. 1953b. Neotropical Culicidae. Vol. 1. Dixinae, Chaoborinae and Culicinae, tribes Anophelini, Toxorhynchitini and Culicini (Genus Culex only). Editora USP, São Paulo.

LOPES, J., ARIAS, J.R. & CHARLHWOOD, J.D. 1985. Estudos ecológicos de Culicidae (Diptera) silvestres criados em pequenos recipientes de água em mata e em capoeira no Município de Manaus-AM. Cienc. Cul. 37:299–1311.

LOPES, N., NOZAWA, C.& LINHARES, R.E.C. 2014. Características gerais e epidemiologia dos arbovírus emergentes no Brasil. Rev. Pan-Amaz Saude. 5(3):55–64.

MACHADO-ALLISON, C.E., BARRERA, R.R., DELGADO, L., GÓMEZ-COVA, C. & NAVARRO, J.C. 1986. Mosquitos (Diptera: Culicidae) e los Fitotelmata de Panaquire, Venezuela. Acta Biol. Venez. 12(2):1–12.

MARCONDES, C.B. & XIMENES, M.F.F.M. 2016. Zika virus in Brazil and the danger of infestation by *Aedes (Stegomyia)* mosquitoes. Rev. Soc. Bras. Med. Trop. 49(1):4–10.

MARQUES, G.R.A.M & FORATTINI, O.P. 2008. Culicídeos em bromélias: diversidade de fauna segundo influência antrópica, litoral de São Paulo. Rev. Saúde Pública. 42(6):979–85.

MARQUES, G.R.A.M., SANTOS, R.L.C. & FORATTINI, O.P. 2001. *Aedes albopictus* em bromélias de ambiente antrópico no Estado de São Paulo, Brasil. Rev. Saúde Pública. 35:243–248.

MELNYCHUK, M.C. & SRIVASTAVA, D.S. 2002. Abundance and vertical distribution of a bromeliad-dwelling zygopteran larva, *Mecistogaster modesta*, in a Costa Rican rainforest (Odonata: Pseudostigmatidae). Int. J. Odonat. 5(1):81–97.

MULLER, G.A. & MARCONDES, C.B. 2010. First report of oviposition of *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) through holes in bamboos. Ent. News. 121:102–103.

MOCELLIN, M.G., SIMÕES, T.C., NASCIMENTO, T.F.S., TEIXEIRA, M.L.F., LOUNIBOS, L.P. & OLIVEIRA, R.L. 2009. Bromeliad-inhabiting mosquitoes in an urban botanical garden of dengue endemic Rio de Janeiro - Are bromeliads productive habitats for the invasive vectors *Aedes aegypti* and *Aedes albopictus*? Mem. Inst. Oswaldo Cruz. 104:1171–1176.

MOTTA, M.A. & LOURENÇO-DE-OLIVEIRA, R. 2000. The subgenus *Dendromyia* Theobald: a review with redescription of four species (Diptera: Culicidae). Mem. Inst. Oswaldo Cruz. 95:649–683.

NAVARRO, J.C., LIRIA, J., PIÑANGO, H. & BARRERA, R. 2007. Biogeographic area relationships in Venezuela: A Parsimony analysis of Culicidae - Phytotelmata distribution in National Parks. Zootaxa. 1547:1–19.

NEISS, U.G. 2007. Estrutura da comunidade de macroinvertebrados aquáticos associados a *Mauritia flexuosa* Linnaeus (Arecaceae), fitotelmata, na Amazônia Central, Brasil. Dissertação de Mestrado, Instituto Nacional de Pesquisas da Amazônia, Manaus.

OSPINA-BAUTISTA, F., ESTÉVEZ-VARÓN, J.V., BETANCUR, J. & REALPE-REBOLLEDO, E. 2004. Estructura y composición de la comunidad de macro invertebrados acuáticos asociados a *Tillandsia turneri* Baker (Bromeliaceae) en un bosque alto andino colombiano. Acta Zool. Mex. 20(1):153–166.

REINERT, J.F. 2009. List of abbreviations for currently valid generic-level taxa in family Culicidae (Diptera). Eur. Mosq. Bull. 27:68–76.

RESENDE, M.C., SILVA, I.M., ELLIS, B.R. & EIRAS, A.E. 2013. A comparison of larval, ovitrap and MosquiTRAP surveillance for *Aedes (Stegomyia) aegypti*. Mem. Inst. Oswaldo Cruz. 108(8):1024-1030.

RIBEIRO, J.E.L.S., HOPKINS, M.J.G., VICENTINI, A., SOTHERS, C.A., COSTA, M.A.S., BRITO, J.M., SOUSA, M.A., MARTINS, L.H.P., LOHMANN, L.G., ASSUNÇÃO, P.A.C.L., PEREIRA, E.C., SILVA. C.F., MESQUITA, M.R. & PROCÓPIO, L.C. 1999. Flora da Reserva Ducke: Guia de identificação das plantas vasculares de uma floresta de terra-firme na Amazônia Central. Editora INPA, Manaus.

SANCHEZ, E. & LIRIA, J. 2009. Relative abundance and temporal variation of macroinvertebrates in a Venezuelan cloud forest habitat. Int. J. Trop. Insect Sci. 29(1):3–10.

SERPA-FILHO, A., FERREIRA, R.L.M., & BARBOSA, U.C. 2007. Ocorrência de *Polypedilum (Tripodura) amataura* Bidawid-Kafka, 1996 (Diptera; Chironomidae) em *Aquascypha hydrophora* (berk.) Reid (Fungi; Stereaceae), com descrição da pupa na Amazônia Central, Brasil. Acta Amazon. 37:151–156.

SOARES, V.A.R.C., RODRIGUES, W.C. & CABRAL, M.M.O. 2008. Estudo de áreas e depósitos preferenciais de *Aedes albopictus* (Skuse, 1894) e *Aedes aegypti* (Linnaeus, 1762) no município de Paracambi – Rio de Janeiro, Brasil. EntomoBrasilis. 1(3):63–68.

SRIVASTAVA, D.S. & LAWTON, J.H. 1998. Why more productive sites have more species: an experimental test of theory using tree-hole communities. American Naturalist. 116:770–787.

TORREIAS, S.R.S., FERREIRA-KEPPLER, R.L., GODOY, B.S. & HAMADA, N. 2010. Mosquitoes (Diptera, Culicidae) inhabiting foliar tanks of *Guzmania brasiliensis* Ule (Bromeliaceae) in central Amazonia, Brazil. Rev. Bras. Entomol. 54(4):618–623.

TORREIAS, S.R.S. & FERREIRA-KEPPLER, R.L. 2011. Macroinvertebrates inhabiting the tank leaf terrestrial and epiphyte bromeliads at Reserva Adolpho Ducke, Manaus, Amazonas. Braz. Arch. Biol. Technol. 54(6):1193–1202.

YANOVIAK, S.P. 1999. Community structure in water-filled tree holes of Panama: efects of hole height and size. Selbyana. 20:106–115.

YANOVIAK, S.P.; LOUNIBOS, L.P.; WEAVER, S.C. 2006. Land use affects macroinvertebrate community composition in phytotelmata in the Peruvian amazon. Ann. Entomol. Soc. Am. 99(6):1172–1181.

ZAR, J.H. 1996. Biostatistical Analysis. 3 ed. Upper Saddle River. New Jersey: Prentice Hall.

Received: 28/10/2016
Revised: 20/06/2017
Accepted: 07/07/2017
Published online: 14/08/2017

Biota Neotropica 17(3): e20160314, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
point of view

Spotlight on *Plasmodium falciparum* evolutionary system in the southeastern Atlantic forest

Gabriel Zorello Laporta[1,2*]
[1]*Universidade Federal do ABC, Centro de Engenharia, Modelagem e Ciências Sociais Aplicadas, Av. dos Estados, 5001, Santo André, SP, Brazil.*

[2] *Faculdade de Medicina do ABC, Setor de Pós-graduação, Pesquisa e Inovação, Av. Lauro Gomes, 2000, Santo André, SP, Brazil.*
**Corresponding author: Gabriel Zorello Laporta, e-mail: gabriel.laporta@ufabc.edu.br*

LAPORTA, GZ. **Spotlight on *Plasmodium falciparum* evolutionary system in the southeastern Atlantic forest.** Biota Neotropica. 17(3): e20160314. http://dx.doi.org/10.1590/1676-0611-BN-2016-0314

Abstract: Malaria elimination is now set to occur in Brazil until 2030. While this achievement is feasible, as it is for other endemic regions worldwide, it is important to recognize resistance of parasites and vectors against anti-malarial interventions. Resistance against drugs and insecticides can lead to discontinuities of malaria transmission, known as residual malaria transmission. Herein, we described a novel phenomenon that is occurring in a residual malaria transmission scenario in the southeastern Atlantic forest. This novel phenomenon does not belong to what is known and therefore we decided to explain it based on an evolutionary perspective. Although it shall not be viewed as a threat to public health, the phenomenon has important aspects that should be highlighted. Specifically, it represents an adaptation of *P. falciparum* among vectors and hosts in the southeastern Atlantic forest. Knowledge about this phenomenon could be of importance, including to the on-going malaria elimination programs.

Keywords: *Anopheles, Biological Evolution, Malaria, Plasmodium falciparum, Rainforest.*

Destaque para o sistema evolutivo de *Plasmodium falciparum* no sudeste da Mata Atlântica

Resumo: A eliminação da malária pode ocorrer no Brasil antes de 2030. Embora seja viável no Brasil e em outras regiões endêmicas do mundo, reconhece-se a importância da resistência de parasitos e vetores às intervenções. A resistência contra drogas e inseticidas pode levar à malária residual. Aqui, descrevemos um novo fenômeno que está ocorrendo em um cenário residual de transmissão de malária no sudeste da Mata Atlântica. Esse fenômeno não faz parte do conhecimento atual e, portanto, propomos uma perspectiva evolutiva para explicá-lo. Não sugerimos uma nova ameaça à saúde pública; entretanto, ressaltam-se aspectos importantes sobre o fenômeno. Especificamente, o fenômeno representa uma adaptação de *P. falciparum* entre vetores e hospedeiros no sudeste da Mata Atlântica. O conhecimento sobre essa adaptação pode ser importante para os programas de eliminação da malária em andamento.

Palavras-chave: *Anopheles, Evolução Biológica, Floresta Tropical, Malária, Plasmodium falciparum.*

Malaria eradication is the permanent reduction to zero of the incidence of infection worldwide. In contrast, elimination is the permanent reduction to zero of the incidence of *Plasmodium* infection on a country or regional scale. A new perspective on, and enthusiasm for, malaria elimination in this century have together overcome the failure of the first malaria eradication program (1950 - 1969) (Cotter et al. 2013). The current support appears to be justified, because the malaria burden is declining in endemic countries, 18 of which have reached the goal of elimination between 2007-2015 (WHO 2016). The on-going malaria elimination programs adopted by many endemic countries are making headway, with a general understanding that elimination is possible in Brazil until 2030 (Ferreira & Castro 2016).

Interventions (e.g., drug administration on infected individuals, vector control management) can control malaria transmission and thus have strong impact on these malaria elimination programs (Cotter et al. 2013). Resistant fraction of parasite and vector populations, however, can persist in the environment of transmission even after intensive malaria control programs (Haji et al. 2013, Barbosa et al. 2014, Guyant et al. 2015, Waltmann et al. 2015). This strong selective pressure on *Plasmodium* parasite and *Anopheles* mosquito populations had been documented in many situations in the past and have been still occurring nowadays (Najera et al. 2011, Haji et al. 2013, Waltmann et al. 2015). The mechanism of resistance is evolutionary-based, because it favors adaptation of *Plasmodium* transmission among vectors and hosts, leading to rare and discontinuous cycles (Corey et al. 2016, Chang et al. 2016). Such discontinuity in transmission dynamics is represented by persistent transmission post-intervention and occurs when frequent malaria outbreaks are replaced by rare randomly-distributed malaria clusters in an endemic region, i.e., residual malaria transmission (Killeen 2014). Residual malaria transmission is often overlooked because there is neither a strong nor an obvious impact on public health.

The southeastern Atlantic forest is historically known to hold a residual malaria transmission recognized as 'bromeliad-malaria'. The main characteristics are: very few annually reported *Plasmodium vivax* cases among residents and high abundance of the primary vector *Anopheles cruzii*, a forest-loving bromeliad-mosquito species (Gadelha 1994). This paradigmatic scenario has been challenged recently, resulting in a new puzzle to be solved (Maselli et al. 2014, Sallum et al. 2014, Laporta et al. 2015). While these authors recognize that systematic studies and specific funding are needed, an effort towards solving that puzzle is made and thus a novel theoretical view is hypothesized in the present work.

Setting the Scene: a New Puzzle

Plasmodium falciparum is the most threatening malaria-parasite because of the magnitude of deaths attributable to this species. In many parts of the world, however, scientists are observing high prevalence of sub-microscopic *P. falciparum* infections among asymptomatic residents (Elbadry et al. 2015, Tiedje et al. 2017). For instance, we investigated blood samples for *Plasmodium falciparum* contamination in a center of blood donation in hospital of the University of São Paulo. As a result, we found 10.6% prevalence of *P. falciparum* subclinical infections among residents living in the southeastern Atlantic forest (Maselli et al. 2014). On the one hand, this evidence represented an institutional incident among scientists and directorial board of blood donation center (Mendrone et al. 2014). On the other hand, it allowed thinking more theoretically and concluding that an interesting phenomenon related to evolution of malaria transmission system was taking place (Sallum et al. 2014).

Given the hypothesis of locally *P. falciparum* transmission in the southeastern Atlantic forest, we tested field collected anophelines throughout that region for malaria parasites. We found 4.4% (21/480) of *P. falciparum*-infected anophelines and 99% similarity of the *P. falciparum* 18S rDNA fragments shared with *P. falciparum* sequences previously isolated from wild monkeys in Amazonia (Laporta et al. 2015). With the real-time quantitative curves from Laporta et al. 2015, we estimated the average number of *P. falciparum* sporozoites per anopheline (Figure 1).

The scientific puzzle-solving game is now setup. It has the following pieces of evidence: 1) high prevalence of *P. falciparum* subclinical infections among residents of the southeastern Atlantic forest, 2) widespread distribution of *P. falciparum*-infected anophelines in the same region, 3) close relationship between *P. falciparum* found in anophelines with non-human primates, and 4) low number of *P. falciparum* sporozoites in anophelines. One could see all these pieces of information as totally independent from each other. On the contrary, other would view all of them connected on a web of causal mechanisms.

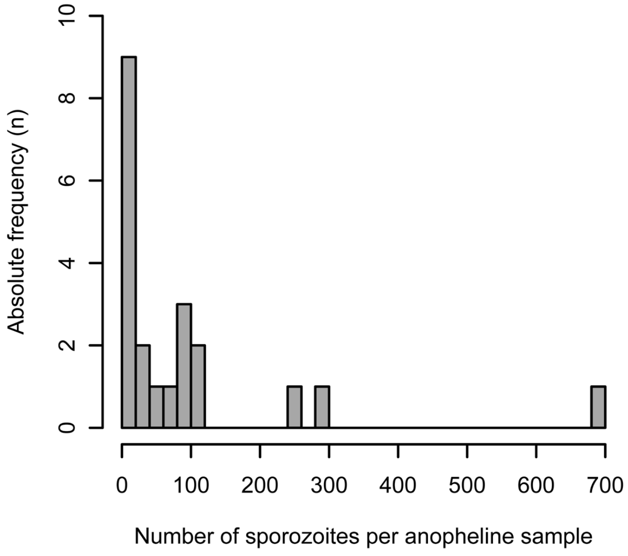


Figure 1. Number of *P. falciparum* sporozoites per anopheline according to the real-time quantitative curves from Laporta et al. 2015.

Considering the assumption that these evidences are interconnected, the next step is very straightforward: to propose an explanation that combines it all in a single theoretical hub. Acknowledging that a malaria transmission system is flexible and persistent, the working hypothesis herein is the evolution pathways of such a system under anti-malarial intervention.

Working Hypothesis: Malaria Transmission System Under Anti-malarial Intervention

Mechanisms of resistance to anti-malarial interventions are still a threat to the programs of malaria elimination today. For instance, the spread of artemisinin-resistant *P. falciparum* in the Greater Mekong subregion, Asia is challenging the current triumph, i.e., artemisinin combination therapy, in the so-called malaria elimination era (Imwong et al. 2017). The Lancet Infectious Diseases' editorial ("Is malaria elimination within reach?"; 2017) is not more optimistic than results by Imwong et al. (2017).

When a malaria transmission system is resistant to anti-malaria interventions, it means that clinical cases of malaria will keep continuing. Other possibilities are that anti-malarial interventions can kill the most pathogenic and virulent fractions of the malaria parasites involved, resulting in sub-microscopic (Elbadry et al. 2015, Tiedje et al. 2017) and/or sub-clinical infections (Maselli et al. 2014). Individuals had few clinical symptoms and sub-microscopic *P. falciparum* infections in Central America (Elbadry et al. 2015) and Africa (Tiedje et al. 2017), meaning that transmission is still occurring despite anti-malarial interventions. Sub-clinical *P. falciparum* infections in Maselli et al. (2014) are yet more complex, because 100% of tested individuals had no symptoms. The pathways that malaria transmission systems create to resist against anti-malaria interventions should be less looked from a medical view and more studied from an evolutionary perspective.

In ecological systems, one important property is resilience of fundamental processes for a system under disturbance (Holling 1973). Resilience has two dimensions: 1) stochastic, variations on the behavior of a system under disturbance without losing the deterministic dimension, 2) deterministic, achievement of the goals of such a system (Holling 1973). Deterministic dimension of a malaria transmission system is to persist on parasite transmission among vectors and hosts. When an anti-malaria intervention collides with such a deterministic dimension, malaria transmission system's stochastic dimension finds new pathways for keeping up the transmission goal. The compounds of a malaria transmission system are the key to understand its resilience. For instance, while parasite's asexual reproduction is made within host, parasite's sexual reproduction inside mosquito produces important genetic variability for malaria transmission (Talman et al. 2004). It seems therefore that biology of mosquito vectors should contribute decisively to the behavior of stochastic dimension of malaria transmission system.

Biology of Malaria Vectors and the Transmission of Parasites among Hosts

Several *Anopheles* species in many parts of the African continent, Western Pacific, and throughout the Amazon, can transmit malaria parasites to humans (Massey et al. 2016). Biology of each of these anopheline species defines important features of malaria epidemiology. For instance, forested anopheline species are highly associated with transmission of

Plasmodium in forested landscapes in the Western Pacific. Anopheline species associated with open and deforested areas are abundant and make thousands of people sick annually in urbanized zones in Sub-Saharan Africa (Guerra et al. 2006). *Anopheles darlingi* is associated with forest fringe, resulting in the current paradigm of malaria transmission called 'the frontier malaria' in Amazon (Barros & Honório 2015). *Plasmodium falciparum* transmission in the southeastern Atlantic forest (Maselli et al. 2014, Laporta et al. 2015) could also be explained by means of biology of the main malarial vector in this system, *An. cruzii*.

Anopheles cruzii is a forest-loving species that inhabits the southeastern Atlantic forest. Beyond its importance as a vector of *P. vivax* to humans, it is related to transmission of *Plasmodium simium* among monkeys and/or humans (Pina-Costa et al. 2014). Recently, Brasil et al. (2017) suggested that transmission of *P. simium* to humans may have been neglected because it seems more important than previously thought. Additionally, out of twenty-one (4.4%) *P. falciparum*-infected anophelines found in the southeastern Atlantic forest, eighteen (3.75%) were *An. cruzii* (Laporta et al. 2015). Considering that *An. cruzii* can interchangeably transmit *P. vivax* to humans and *P. simium* to monkeys and/or humans, it is possible that *P. falciparum* circulation in the southeastern Atlantic forest is helped by vector contact with both humans and monkeys.

Beyond the vector ability of *An. cruzii*, this species seems to live longer than other anophelines. *Anopheles cruzii* females had been recaptured up to two months after the release date (Santos 2001). This means that a malaria parasite can have a long period for completing its development inside this mosquito (Poulin 2011). Availability of such a long period means that transmission will be exponentiated. But, what would happen if a long period for parasite development is combined with efficient anti-malarial interventions? Considering the efficient anti-malarial interventions in the southeastern Atlantic forest (Smith 1952) and the current low number of sporozoites found in this mosquito species (Figure 1), it is possible that a selection of a less efficient parasite occurred.

Plasmodium falciparum Evolutionary System in the Southeastern Atlantic Forest

Oocytes of *P. falciparum* were commonly found in *An. cruzii* females and other anophelines during malaria epidemics period that reached the cities of Blumenau, Joinville, and Florianopolis in the 1940s (Smith 1952). *Plasmodium falciparum* was considered eliminated in the southeastern Atlantic forest after the malaria eradication programme in the 1950-60s (Gadelha 1994, Griffing et al. 2015). A long gap lasted for approximately 40 years (1970 – 2010) in when *P. falciparum* circulation had not been documented in this region. It is either possible that this parasite remained in silent cycles inside the forest with monkeys as a reservoir or that it was re-introduced by migration of infected humans from endemic counties of Amazon without being noticed. Anyway, a cornerstone publication was made in 2008 suggesting that *Alouatta* monkeys could be reservoirs of *P. falciparum* in the southeastern Atlantic forest (Duarte et al. 2008). Following, a high proportion of *P. falciparum* was found throughout the southeastern Atlantic forest, mainly in *An. cruzii* mosquitoes, and infecting high fractions of asymptomatic human hosts and potentially circulating among reservoir monkeys (Maselli et al. 2014, Laporta et al. 2015).

Notwithstanding the above, having a *P. falciparum* adapted to monkeys would be an idiosyncrasy. Not only an idiosyncrasy, but this adaptation has not yet been proved or published. The most comprehensive study on interactions among human malaria parasites on non-human primates by Liu et al. (2010) showed that *P. falciparum* is not infecting great apes in Africa nowadays. Great apes have infections with species closely related to *P. falciparum*; for instance, gorillas can be infected by *P. praefalciparum* (Liu et al. 2010). Then, there is not a scientific consensus that it can occur species permeability (Molina-Cruz et al. 2016). In other words, there is not a strong support for the parasite being transmitted in both directions: 1) from humans to apes, and 2) from apes to humans. This is because infections found in nature so far, apes with *P. falciparum*, were not effective (Loy et al. 2016).

Now we are set into the most important step of this puzzle solving game. While *P. falciparum* should not circulate among monkeys and humans, a novel system of *P. falciparum* transmission is taking place in the southeastern Atlantic forest. In such malaria transmission system, two mechanisms may be contributing to the *P. falciparum* circulation: 1) Elevated host tolerance (asymptomatic infected humans; Maselli et al. 2014), 2) Inefficient vector adaptation (low number of *P. falciparum* sporozoites in *An. cruzii*; Laporta et al. 2015, Figure 1). These mechanisms could be further related to the evolution of *P. falciparum* resilience.

Open Remarks for New Opportunities

A close adaptation of *P. falciparum* to its vector could occur. This adaptation was discussed by Sallum et al. (2014) and exemplified by Molina-Cruz et al. (2016). An adaptation would be selected in the environment if *P. falciparum* could not cause symptoms in human hosts (as evidentiated in Maselli et al. 2014); because then it would be invisible to the surveillance system, which is based mainly on symptomatic malaria individuals. For instance, São Paulo State has not reported any autochthonous case of *P. falciparum* in the southeastern Atlantic forest since 1980 (Couto et al. 2010, São Paulo 2017).

The average number of sporozoites in the glands of an infective anopheline is expected to be 1,000 (Ross 1910). We estimated a low mean number (95) of *P. falciparum* sporozoites in 18 *An. cruzii* and 3 other anophelines in the southeastern Atlantic forest (Figure 1). This low number in the mosquito's glands would lead to an absence of viable sporozoites to be inoculated into humans and thus no transmission should occur. However, we also observed a high prevalence of *P. falciparum* subclinical infections in humans (Maselli et al. 2014). What is the underlying mechanism behind these evidences?

The high prevalence of *P. falciparum* subclinical infections in humans could be triggered by the low number of sporozoites in *An. cruzii* in the southeastern Atlantic forest, evidentiated in Laporta et al. 2015 (Figure 1). Exposure to an irrelevant load of inoculum of *P. falciparum* could help in decreasing the recovery time among susceptible hosts. These exposures with a very low load of *P. falciparum* along time could serve as a natural immunization for residents in the southeastern Atlantic forest.

Adaptation of *P. falciparum* to sylvatic transmission among exclusively asymptomatic humans is hypothetically suggested as belonging to evolution of *P. falciparum* resilience. Resilience in such case is defined as the capacity of an infectious disease to adapt to an environment under a disturbance regime represented by an eradication programme.

To prove evolution of resilience is not trivial. It needs data and evidences within a profound temporal framework, such as shown in Figure 2, which is not always available, particularly in the southeastern Atlantic forest, a neglected malaria region.

Figure 2 is a hypothetical temporal framework that represents steps of malaria transmission system. Prior to the 1870s, during the pre-biological invasion phase, the only known malaria-parasite was *P. simium* in non-human primates, and the prevalent mosquitoes were identified as *Kerteszia* species. From approximately 1890-1910, massive European immigration to southern Brazil occurred (Carvalho Filho & Monasterio 2012), a biological invasion that resulted in forest clearing, the emergence of *Nyssorhynchus* species and a notable increase in *P. falciparum* and *P. vivax*. This invasion resulted in a subsequent acute malaria epidemic that lasted for approximately 20 years (1930-1950). During this time, both *Kerteszia* and *Nyssorhynchus* species transmitted both human parasites at an unprecedented scale (Smith 1950). By the 1950s the worldwide malaria

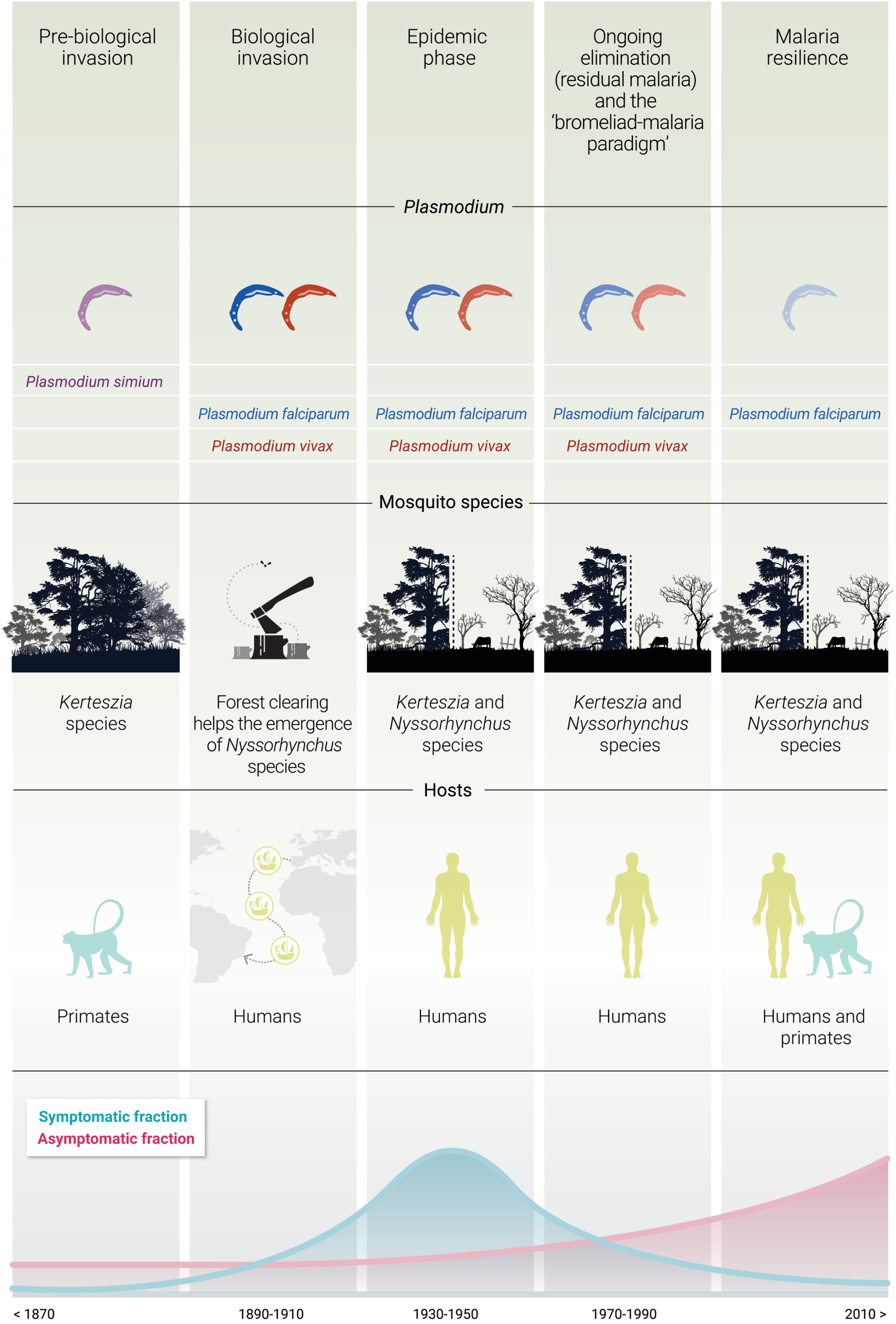


Figure 2. Hypothetical temporal framework that represents steps of malaria transmission system.

burden was so high that governments of endemic countries began to take serious control measures instigated by the Global Malaria Eradication Program (GMEP). In Brazil, transmission decreased after an intense eradication campaign that included chloroquine treatment of infected humans and control of *Kerteszia* mosquitoes by rapid bromeliad destruction (Griffing et al. 2015). During this elimination phase (1970-1990) in the Atlantic forest, chloroquine resistance plus vector persistence in forest fragments resulted in residual malaria, known as the bromeliad-malaria paradigm (Gadelha 1994). This paradigm is essentially the dynamics of vivax-malaria transmitted by local *Kerteszia* and *Nyssorhynchus* vectors. Finally, the finding of *P. falciparum* in humans who have never travelled to the Amazon or any other malaria endemic region, suggests an alternative cycle of transmission dynamics may have evolved, which is recognized herein as evolution of *P. falciparum* resilience (Figure 2).

It should be further suggested studies in such an intriguing scenario of *P. falciparum* resilience. This may be relevant because it may reveal important aspects of evolution of malaria transmission under environment disturbances (i.e., anti-malarial interventions). This should be therefore important to the implementation of malaria elimination programs in endemic countries.

Funding

São Paulo Research Foundation (FAPESP) and BIOTA-FAPESP Program (n. 2014/09774-1, 2015/09669-6).

Acknowledgements

Jan E. Conn and Maria A. M. Sallum for helping improvement of earlier versions. Anonymous reviewer for making insightful considerations.

Author Contributions

Gabriel Zorello Laporta: contribution in the concept and design of the study; contribution to manuscript preparation and critical revision.

Conflicts of interest

The author declares there are not conflicts of interest related to the publication of this manuscript.

References

BARBOSA, S., GOZZE, A. B., LIMA, N. F., BATISTA, C. L., BASTOS, M. S., NICOLETE, V. C., FONTOURA, P. S., GONÇALVES, R. M., VIANA, S. A. S., MENEZES, M. J., SCOPEL, K. K. G., CAVASINI, C. E., MALAFRONTE, R. S., SILVA-NUNES, M., VINETZ, J. M., CASTRO, M. C. & FERREIRA, M. U. 2014. Epidemiology of disappearing *Plasmodium vivax* malaria: a case study in rural Amazonia. PLoS Negl. Trop. Dis. 8(8):e3109.

BARROS, F.S.M & HONÓRIO, N.A. 2015. Deforestation and malaria on the Amazon Frontier: larval clustering of *Anopheles darlingi* (Diptera: Culicidae) determines focal distribution of malaria. Am. J. Trop. Med. Hyg. 93:939-953.

BRASIL, P., ZALIS, M.G., PINA-COSTA, A., SIQUEIRA, A.M., JUNIOR, C.B., SILVA, S., AREAS, A.L.L., PELAJO-MACHADO, M., ALVARENGA, D.A.M., SANTELLI, A.C.F.S., ALBUQUERQUE, H.G., CRAVO, P., ABREU, F.V.S., PETERKA, C.L., ZANINI, G.M. SUAREZ-MUTIS, M.C., PISSINATTI, A., LOURENCO-DE-OLIVEIRA, R., BRITO, C.F.A., FERREIRA-DA-CRUZ, M.F., CULLETON, R. & DANIEL-RIBEIRO, C.T. 2017. *Plasmodium simium* causing human malaria: a zoonosis with outbreak potential in the Rio de Janeiro Brazilian Atlantic forest. bioRxiv. doi: https://doi.org/10.1101/122127.

CARVALHO FILHO, I. & MONASTERIO, L. 2012. Immigration and the origins of regional inequality: Government-sponsored European migration to southern Brazil before World War I. Reg. Sci. Urban. Econ. 42:794–807.

CHANG, X., ZHONG, D., LO, E., FANG, Q., BONIZZONI, M., WANG, X., LEE, M.C., ZHOU, G., ZHU, G., QIN, Q., CHEN, X., CUI, L. & CHEN, X. 2016. Landscape genetic structure and evolutionary genetics of insecticide resistance gene mutations in *Anopheles sinensis*. Parasit.Vectors. 9:228.

COREY, V. C., LUKENS, A. K., ISTVAN, E. S., LEE, M. C., FRANCO, V., MAGISTRADO, P., COBURN-FLYNN, O., SAKATA-KATO, T., FUCHS, O., GNÄDIG, N.F., GOLDGOF, G., LINARES, M., GOMEZ-LORENZO, M.G., CÓZAR, C., LAFUENTE-MONASTERIO, M.J., PRATS, S., MEISTER, S., TANASEICHUK, O., WREE, M., ZHOU, Y., WILLIS, P.A., GAMO, F.J., GOLDBERG, D.E., FIDOCK, D.A., WIRTH, D.F. & WINZELER, E.A. 2016. A broad analysis of resistance development in the malaria parasite. Nature. Comm. 7:11901.

COTTER, C., STURROCK, H. J., HSIANG, M. S., LIU, J., PHILLIPS, A. A., HWANG, J., GUEYE, C. S., FULLMAN, N., GOSLING, R. D. & FEACHEM, R. G. 2013. The changing epidemiology of malaria elimination: new strategies for new challenges. Lancet. 382(9895): 900–911.

COUTO, R.D'A., LATORRE, M.R.D.O., DI SANTI, S.M. & NATAL, D. 2010. Autochthonous malaria notified in the State of São Paulo: clinical and epidemiological characteristics from 1980 to 2007. Rev. Soc. Bras. Med. Trop. 43:52–8.

DUARTE, A.M.R.C., MALAFRONTE, R.S., CERUTTI JR, C., CURADO, I., PAIVA, B.R., MAEDA, A.Y., YAMASAKI, T., SUMMA, M.E., NEVES, D.V.D.A., DE OLIVEIRA, S.G. & GOMES, A.C. 2008. Natural *Plasmodium* infections in Brazilian wild monkeys: Reservoirs for human infections? Acta Trop. 107:179–85.

ELBADRY, M.A., AL-KHEDERY, B., TAGLIAMONTE, M.S., YOWELL, C.A., RACCURT, C.P., EXISTE, A., BONCY, J., WEPPELMANN, T.A., BEAU DE ROCHARS, V.E.M., LEMOINE, J.F., OKECH, B. A. & DAME, J.B. 2015. High prevalence of asymptomatic malaria infections: a cross-sectional study in rural areas in six departments in Haiti. Malaria J. 14:510.

FERREIRA, M.U. & CASTRO, M.C. 2016. Challenges for malaria elimination in Brazil. Malaria J. 15: 284.

GADELHA, P. 1994. From "forest malaria" to "bromeliad malaria": a case-study of scientific controversy and malaria control. Parassitologia. 36(1-2):175–95.

GRIFFING, S.M., TAUIL, P.L., UDHAYAKUMAR, V. & SILVA-FLANNERY, L. 2015. A historical perspective on malaria control in Brazil. Mem. Inst. Oswaldo Cruz. 110(6):701-718.

GUERRA, C.A., SNOW, R.W. & HAY, S.I. 2006. A global assessment of closed forests, deforestation and malaria risk. Ann. Trop. Med. Parasitol. 100:189-204.

GUYANT, P, CORBEL, V., GUÉRIN, P. J., LAUTISSIER, A., NOSTEN, F., BOYER, S., COOSEMANS, M., DONDORP, A. M., SINOU, V., YEUNG, S. & WHITE, N. 2015. Past and new challenges for malaria control and elimination: the role of operational research for innovation in designing interventions. Malaria J. 14:279.

HAJI, K. A., KHATIB, B. O., SMITH, S., ALI, A. S., DEVINE, G. J., COETZEE, M. & MAJAMBERE, S. 2013. Challenges for malaria elimination in Zanzibar: pyrethroid resistance in malaria vectors and poor performance of long-lasting insecticide nets. Parasit. Vectors. 6:82.

HOLLING, C.S. 1973. Resilience and stability of ecological systems. Annu. Rev. Ecol. Syst. 4:1-23.

IMWONG, M., SUWANNASIN, K., KUNASOL, C., SUTAWONG, K., MAYXAY, M., REKOL, H., SMITHUIS, F.M., HLAING, T.M., TUN, K.M., VAN DER PLUIJM, R.W., TRIPURA, R., MIOTTO, O., MENARD, D., DHORDA, M., DAY, N.P.J., WHITE, N.J. & DONDORP, A.M. 2017. The spread of artemisinin-resistant *Plasmodium falciparum* in the Greater Mekong subregion: a molecular epidemiology observational study. Lancet Infect. Dis. 17:491-497.

KILLEEN, G.F. 2014. Characterizing, controlling and eliminating residual malaria transmission. Malaria J. 13:330.

LAPORTA, G.Z., BURATTINI, M.N., LEVY, D., FUKUYA, L.A., OLIVEIRA, T.M.P., MASELLI, L.M.F., CONN, J.E., MASSAD, E., BYDLOWSKI, S.P. & SALLUM, M.A.M. 2015. *Plasmodium falciparum* in the southeastern Atlantic forest: a challenge to the bromeliad-malaria paradigm? Malaria J. 14:181.

LIU, W., LI, Y., LEARN, G.H., RUDICELL, R.S., ROBERTSON, J.D., KEELE, B.F., NDJANGO, J.B., SANZ, C.M., MORGAN, D.B., LOCATELLI, S., GONDER, M.K., KRANZUSCH, P.J., WALSH, P.D., DELAPORTE, E.,

MPOUDI-NGOLE, E., GEORGIEV, A.V., MULLER, M.N., SHAW, G.M., PEETERS, M., SHARP, P.M., RAYNER, J.C. & HAHN, B.H. 2010. Origin of the human malaria parasite *Plasmodium falciparum* in gorillas. Nature. 467:420–5.

LOY, D.E., LIU, W., LI, Y., LEARN, G.H., PLENDERLEITH, L.J., SUNDARARAMAN, S.A., SHARP, P.M. & HAHN, B.H. 2016. Out of Africa: origins and evolution of the human malaria parasites *Plasmodium falciparum* and *Plasmodium vivax*. Int. J. Parasitol. 47:87-97.

MASELLI, L.M.F., LEVY, D., LAPORTA, G.Z., MONTEIRO, A.M., FUKUYA, L.A., FERREIRA-DA-CRUZ, M.F., DANIEL-RIBEIRO, C.T., DORLHIAC-LLACER, P.E., SALLUM, M.A.M. & BYDLOWSKI, S.P. 2014. Detection of *Plasmodium falciparum* and *Plasmodium vivax* subclinical infection in non-endemic region: implications for blood transfusion and malaria epidemiology. Malaria J. 13:224.

MASSEY, N.C., GARROD, G., WIEBE, A., HENRY, A.J., HUANG, Z., MOYES, C.L. & SINKA, M.E. 2016. A global bionomic database for the dominant vectors of human malaria. Sci. Data. 3:160014.

MENDRONE JR, A., CERUTTI, C., LEVI, J.E., BOULOS, M., ARROYO SANCHEZ, M.C., MALAFRONTE, R.S., DI SANTI, S.M. & ODONE, V. 2014. Unexpected detection of *Plasmodium vivax* and *Plasmodium falciparum* in asymptomatic blood donors: fact or artifact? Malaria J. 13:336.

MOLINA-CRUZ, A., ZILVERSMIT, M.M., NEAFSEY, D.E., HARTL, D.L. & BARILLAS-MURY, C. 2016. Mosquito vectors and the globalization of *Plasmodium falciparum* malaria. Annu. Rev. Genet. 50:447-465.

NAJERA, J.A., GONZÁLEZ-SILVA, M. & ALONSO, P.L. 2011. Some lessons for the future from the Global Malaria Eradication Programme (1955–1969). PLoS Med. 8: e1000412.

PINA-COSTA, A., BRASIL, P., DI SANTI, S.M., DE ARAUJO, M.P., SUÁREZ-MUTIS, M.C., SANTELLI, A.C.F.S., OLIVEIRA-FERREIRA, J., LOURENÇO-DE-OLIVEIRA, R. & DANIEL-RIBEIRO, C.T. 2014. Malaria in Brazil: what happens outside the Amazonian endemic region. Mem. Inst. Oswaldo Cruz 109:618-633.

POULIN, R. 2011. Evolutionary Ecology of Parasites, 2nd. Princeton University Press, New Jersey.

ROSS, R. 1910. The prevention of malaria. John Murray, London.

SALLUM, M.A.M., DANIEL-RIBEIRO, C.T., LAPORTA, G.Z., FERREIRA-DA-CRUZ, M.F., MASELLI, L.M.F., LEVY, D. & BYDLOWSKI, S.P. 2014. Finding connections in the unexpected detection of *Plasmodium vivax* and *Plasmodium falciparum* DNA in asymptomatic blood donors: a fact in the Atlantic forest. Malaria J. 13:337.

SANTOS, R.L.C. 2001. Medida da capacidade vetorial de *Anopheles albitarsis* e de *Anopheles (Kerteszia)* no Vale do Ribeira, São Paulo. Tese de doutorado, Universidade de São Paulo, São Paulo.

SÃO PAULO. http://www.saude.sp.gov.br/cve-centro-de-vigilancia-epidemiologica-prof.-alexandre-vranjac/areas-de-vigilancia/doencas-de-transmissao-por-vetores-e-zoonoses/agravos/malaria/dados-estatisticos (last access 17/May/2017).

SMITH, L.B. 1952. Bromeliad Malaria. Rep. Smithsonian Inst. 385–98.

TALMAN, A. M., DOMARLE, O., MCKENZIE, F. E., ARIEY, F. & ROBERT, V. 2004. Gametocytogenesis: the puberty of *Plasmodium falciparum*. Malaria J. 3(1):1.

THE LANCET INFECTIOUS DISEASES. 2017. Is malaria elimination within reach? Lancet Infect. Dis. 17:461.

TIEDJE, K.E., ODURO, A., AGONGO, G., ANYORIGIYA, T., AZONGO, D., AWINE, T., GHANSAH, A., PASCUAL, M., KORAM, K. & DAY, K. P. 2017. Seasonal variation in the epidemiology of asymptomatic *Plasmodium falciparum* infections across two catchment areas in Bongo District, Ghana. Ahead of print, doi: https://doi.org/10.4269/ajtmh.16-0959

WALTMANN, A., DARCY, A. W., HARRIS, I., KOEPFLI, C., LODO, J., VAHI, V., PIZIKI, D., SHANKS, G. D., BARRY, A. E., WHITTAKER, M., KAZURA JW & MUELLER I. 2015. High rates of asymptomatic, sub-microscopic *Plasmodium vivax* infection and disappearing *Plasmodium falciparum* malaria in an area of low transmission in Solomon Islands. PLoS Negl. Trop. Dis. 9(5): e0003758.

WHO. http://apps.who.int/iris/bitstream/10665/205565/1/WHO_HTM_GMP_2016.3_eng.pdf?ua=1 (last access 13/Dec/2016).

Received: 21/12/2016
Revised: 27/07/2017
Accepted: 28/07/2017

Biota Neotropica 17(3): e20170334, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
article

Composition of the ichthyofauna in Brazilian semiarid reservoirs

***Silvia Yasmin Lustosa Costa*[1]*, José Etham de Lucena Barbosa*[1]*, Leandro Gomes Viana*[1] *& Telton Pedro Anselmo Ramos*[2,3]*

[1]*Departamento de Ciências Biológicas e da Saúde, Universidade Estadual da Paraiba, Campina Grande, PB, Brazil*
[2]*Departamento de Botânica, Ecologia e Zoologia, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil*
[3]*Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba, João Pessoa, PB, Brazil*
**Corresponding author: Telton Pedro Anselmo Ramos, e-mail: telton@gmail.com*

COSTA, S.Y.L., VIANA, L.G., BARBOSA, J. E. L., RAMOS, T.P.A. **Composition of the ichthyofauna in Brazilian semiarid reservoirs**. Biota Neotropica. 17(3): e20170334. http://dx.doi.org/10.1590/1676-0611-BN-2017-0334

Abstract: The scale of impact that the São Francisco River transposition project will have on the drainage basins ichthyofauna is still unclear, however, changes in the fish community diversity and abundance is probable. Surveys and registries of the fish fauna within key systems of the catchment basins are priority actions for the conservation of the aquatic diversity. This study conducted a taxonomic survey of the Epitácio Pessoa (Boqueirão municipality) and Argemiro de Figueiredo (Itatuba municipality), reservoirs ichthyofauna, both belonging to the Paraíba do Norte River basin, important socioeconomic and environmental systems for the transposition project. Monthly sampling was carried out between October/2014 to September/2015 in the upstream and downstream zones of the reservoirs. The specimens were collected using dip nets, drag nets, and sieves, and placed on ice in the field. Later in the laboratory, they were transferred to 10% formaldehyde and then preserved in 70% alcohol. A total of 2,328 specimens were collected representing five orders, 14 families, and 31 species in both systems. At the upstream zones 2057 specimens were collected representing 17 species, and at the downstream zones 271 specimens were collected representing 24 species. Of the 31 species recorded, 13 species are endemic to watersheds that drain rivers from the Brazilian semi-arid region, and seven species (*Apareiodon davisi*, *Characidium bimaculatum*, *Hypostomus pusarum*, *Parotocinclus jumbo*, *P. spilosoma*, *Pimelodella enochi*, and *Prochilodus brevis*) are endemic to the Mid-Northeastern Caatinga Ecoregion (MNCE). Among the latter, *Parotocinclus spilosoma* and *Pimelodella enochi* are endemic to the Paraíba do Norte River. *Apareiodon davisi* is classified as Endangered according to the current published Brazil's official list of endangered species of fish and aquatic invertebrates. This pre-transposition ichthyofaunistic survey will serve as a basis for future post-transposition analyzes, considering this action will change the scope of the ecosystems diversity.

Keywords: *Endemism, Paraíba, Basins Transposition.*

Composição da ictiofauna em reservatórios do semiárido brasileiro

***Resumo*:** A dimensão do impacto que a transposição do rio São Francisco provocará na ictiofauna das bacias receptoras ainda é imprecisa, entretanto, é provável que haja alterações na riqueza e abundância da comunidade de peixes. Levantamentos e registros prévios da fauna íctica em sistemas chaves das bacias receptoras são ações prioritárias para a conservação da diversidade aquática. Este estudo realizou o levantamento taxonômico da ictiofauna dos reservatórios Epitácio Pessoa (município de Boqueirão), e Argemiro de Figueiredo (município Itatuba), ambos pertencentes a bacia hidrográfica do rio Paraíba do Norte, estado da Paraíba, importantes sistemas sócioeconômicos e ambientais para o projeto da transposição. Assim, foram realizadas amostragens mensais de Outubro/2014 a Setembro/2015 nas zonas lacustres e a jusante dos reservatórios. Os espécimes foram coletados com redes de espera, tarrafa, arrasto, peneiras e puçás, em seguida acondicionados em gelo no campo, posteriormente no laboratório, transferidos para o formol a 10% e conservados em álcool 70%. Foram coletados um total de 2328 espécimes distribuídas em 31 espécies, 14 famílias e cinco ordens nos dois sistemas. Nas zonas lacustres foram coletados 2057 espécimes distribuídos em 17 espécies e nas áreas a jusantes foram coletados 271 indivíduos, distribuídos em 24 espécies. Das 31 espécies registradas, 13 são endêmicas das bacias hidrográficas que drenam rios do semiárido brasileiro, destas, sete (*Apareiodon davisi*, *Characidium bimaculatum*, *Hypostomus pusarum*, *Parotocinclus jumbo*, *P. spilosoma, Pimelodella enochi* e *Prochilodus brevis*) são endêmicas do Nordeste Médio Oriental. Dentre estas últimas, *P. spilosoma e P. enochi* são endêmicas do rio Paraíba do Norte. *Apareiodon davisi* é classificada como Em Perigo de acordo com a atual lista oficial de espécies ameaçadas de peixes e invertebrados aquáticos no Brasil. Este levantamento ictiofaunístico pré-transposição servirá de base para análises futuras pós-transposição, considerando que esta ação acarretará mudanças no âmbito de diversidade dos ecossistemas em questão.

Palavras-chave: *Endemismo, Paraíba, Transposição de bacias.*

Introduction

Approximately 5,160 freshwater fish species are described from South America, representing 20 orders, 69 families, and 739 genera (Reis et al. 2016). Brazil covers majority of the Neotropical region, and its ichthyofauna consists of more than 3,000 freshwater species (Reis et al. 2016). According to Buckup et al. 2007, the number of freshwater fish species described in Brazil has increased significantly in recent years, with an annual growth greater than 20%, an average never yet recorded. However, the knowledge about the Brazilian ichthyofaunistic diversity is focused within certain regions, with the Northeast region displaying the lowest volume of published studies and a limited number of researchers (Rosa & Menezes 1996, Langeani et al. 2009, Ramos et al. 2014).

The lack of accurate knowledge related to systematics and distribution of fish taxa within Northeast Brazil is one of the main aspects limiting the ichthyofaunistic diversity evaluation and fish biogeographic determination of this region (Rosa et al. 2003, Ramos et al. 2014). However, in recent decades the number of research articles related to the ichthyofauna diversity of Brazilian semi-arid region has increased (Ramos et al. 2005, Novaes et al. 2013, Sánchez-Botero et al. 2013, Gurgel-Lourenço et al. 2013, Silva et al. 2014, Ramos et al. 2014, Silva et al. 2015, Gurgel-Lourenço et al. 2015).

In Brazil, approximately 12% of its territory is classified as being semi-arid with a population of about 23 million inhabitants (INSA 2015). Thus, the aquatic ecosystems are fundamental to the survival of local communities, taking into account that the construction of dams was primarily intended to increase water storage capacity for the urban network (Maltchik 1996, Rebouças et al. 2006, Medeiros et al. 2010).

Currently, Northeast region of Brazil is undergoing the process of channel construction that will transport water from the largest hydrographic basin in the region, the São Francisco River basin, to the basins of the Mid-Northeastern Caatinga Ecoregion - MNCE (ecoregion composed of hydrographic basins located between the São Francisco and Parnaíba basins - Rosa et al. 2003), among which is the Paraíba do Norte River basin. The Paraíba do Norte River watershed is the third largest of this ecoregion. In order to contribute to the knowledge of this systems ichthyofauna prior to the commencement of the transposition project, an ichthyofauna survey was undertaken focusing on two large reservoirs of the Paraíba do Norte River basin, the Epitácio Pessoa and the Argemiro de Figueiredo reservoirs, and their respective downstream areas.

Material and Methods

1. Study area

The study was carried out in the Epitácio Pessoa (Boqueirão) and Argemiro de Figueiredo (Acauã) reservoirs and their respective downstream areas. These reservoirs are situated in the Paraíba do Norte River basin, in Paraíba State (Figures 1 and 2). The Paraíba do Norte River is about 360 km in length, rising above a thousand meters elevation in the Serra de Jabitacá in Monteiro municipality, and flows into the Atlantic Ocean in Cabedelo

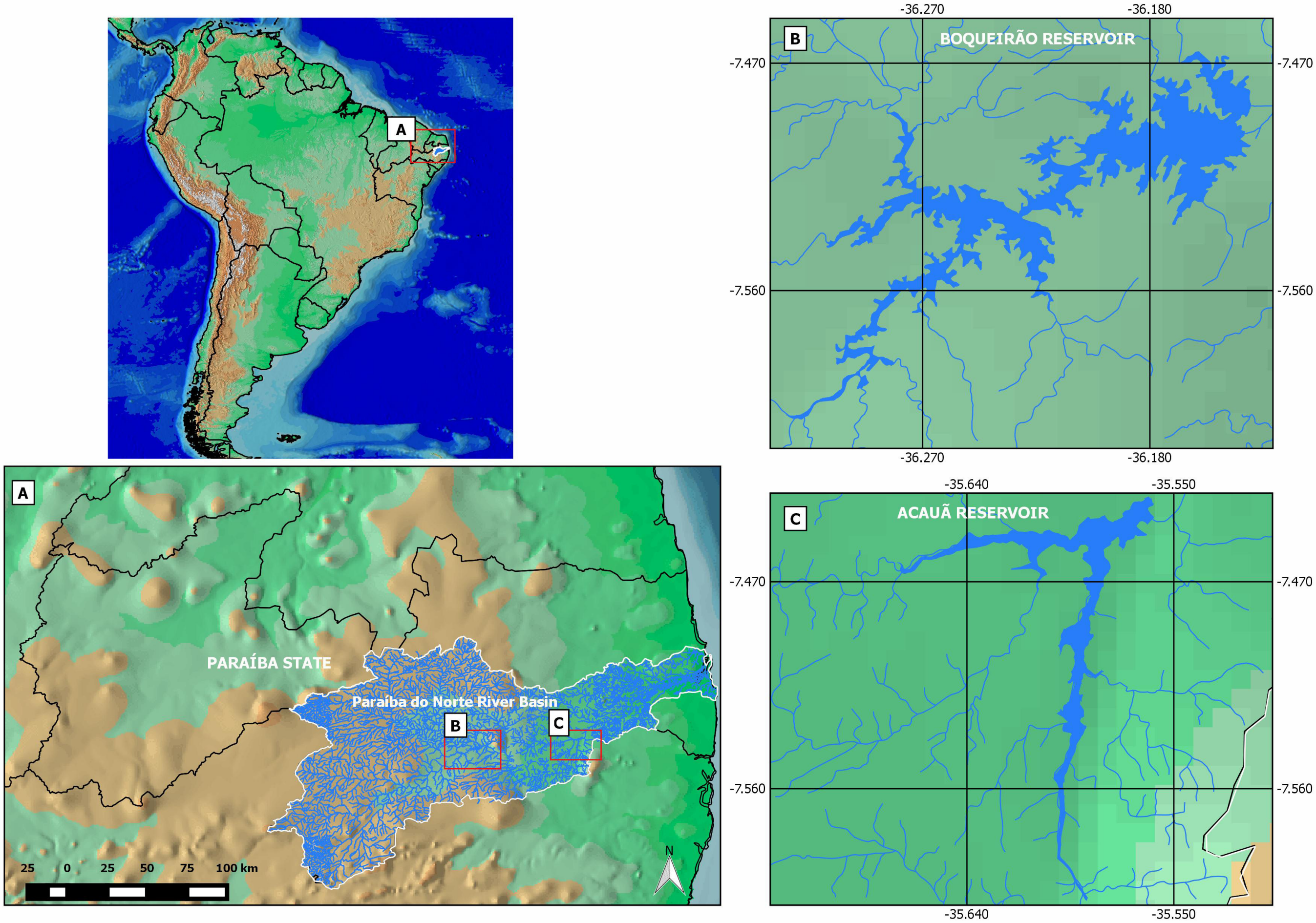


Figure 1. Map of Paraíba do Norte River basin (A): Boqueirão (B) and Acauã (C) reservoirs.

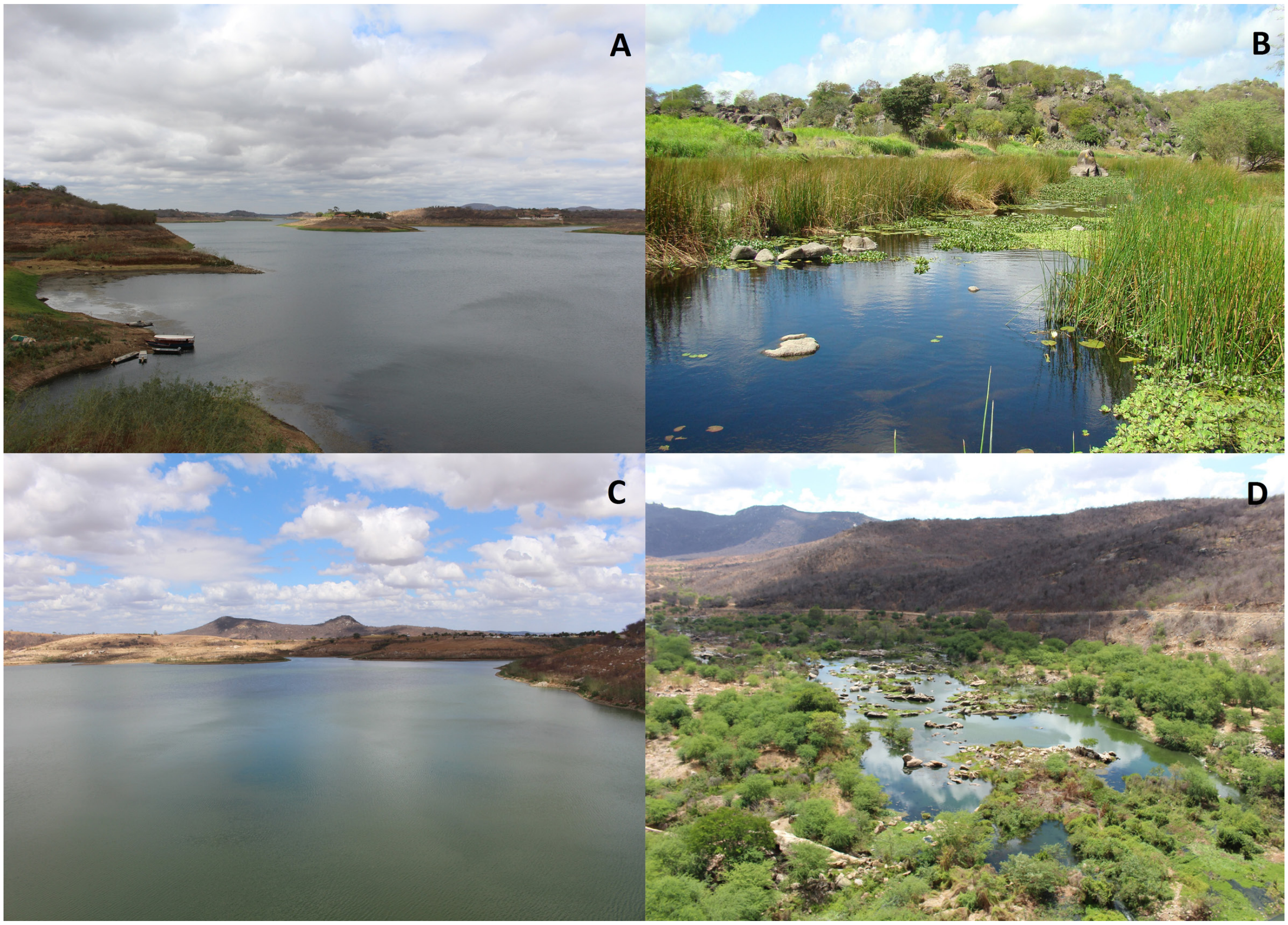


Figure 2. Epitácio Pessoa Reservoir (A) and its downstream area (B), Argemiro de Figueiredo Reservoir (C) and its downstream area (D).

municipality. The basin comprises an area of 20,071.83 km^2, making it the second largest in Paraíba State. It has five main reservoirs, among them are the Epitácio Pessoa and Argemiro de Figueiredo (Paraíba 2007).

The Epitácio Pessoa reservoir (7°29’20” S and 36º17’3” W), popularly known as Boqueirão, is located in the upper basin at Boqueirão municipality in the northeastern semi-arid region. It is the largest and main reservoir of the Paraíba do Norte River basin with a maximum capacity of 411,686.287m^3 and covering an area of about 14,000 km^2, supplying water to 26 municipalities in Paraíba State. Its main uses are human supply (approximately 600,000 people), animal husbandry and irrigation (Silva 1987, AESA 2015).

The Argemiro de Figueiredo reservoir, commonly known as Acauã, is located in the middle course of the Paraíba do Norte River between the geographical coordinates 7º36’51’’ S and 35º33’01’’ W in Itatuba municipality, supplying several cities as well as irrigation, water for livestock, and aquaculture. This reservoir has an area of 1,725 hectares with a 253,144,247 m^3 of accumulation capacity (SEMARH 2006).

2. Sampling

Sampling in the Epitácio Pessoa and Argemiro de Figueiredo Reservoirs was conducted monthly for one year, between October/2014 and September/2015. In addition, the downstream ebbing (environments that usually have a lotic regime) areas of the reservoirs were also sampled. In Argemiro de Figueiredo reservoir, the specimens were collected using a trawl net (20 m long, 2 m high and 10 mm mesh), cast net (2.4 m high, and 12 mm mesh) and gill nets (10 m long and 35, 45 and 55 mm meshes). Epitácio Pessoa specimens were collected by means of gill nets only. Gill nets were set at dusk and retrieved at dawn, having a soaking time of 12 hours in each of the sampled environments. Additional sampling methods were used when sampling downstream of the two reservoirs, including the use of sieves, grips and a trawl net (4 m long, 2 m high, and 5 mm mesh). Specimens collected in the reservoirs were placed on ice and taken to the Universidade Estadual da Paraíba (UEPB). Majority of the material was used for feeding studies, and the remaining specimens together with the material collected from the downstream areas were treated according to the scientific curatorial standards, by fixing the specimens in formaldehyde for two to eight days, then transferring them to a 70° GL ethyl alcohol solution, and labeling and sorting specimens into lots according to Malabarba & Reis (1987).

Specimen sampling and identifications were carried out at the Laboratório de Ecologia Aquática da Universidade Estadual da Paraíba (LEAQ/UEPB), Laboratório de Sistemática e Morfologia de Peixes da Universidade Federal da Paraíba (LASEP/UFPB) and Laboratório de Ictiologia Sistemática e Evolutiva da Universidade Federal do Rio Grande do Norte (LISE/UFRN). After identification, specimens were deposited in the Ichthyological collections at the UFPB (29 lots) and UFRN (two lots).

Meristic and morphometric data involved in the identification process were obtained according to Hubbs & Lagler (2006). Species identification was performed according to specialized literature (e.g., Kullander 1983, Britski et al. 1984, Vari 1989, Castro & Vari 2004, Ramos 2012). Taxonomic classification and endemism of species followed Eschmeyer et al. (2017) and Rosa et al. (2003), respectively.

3. Data analysis

Species occurrence was analyzed according to occurrence frequency (FO in percentage) of the species against evaluation criteria for values: Very common (FO > 70%); Common (FO between 50% and 70%); Uncommon (FO between 10% and 50%) and Rare (FO < 10%).

To evaluate abundance of each species, total number of species captured was considered and relative abundance of each species was obtained by dividing the number of individuals captured from each species by total number of specimens.

Results

A total of 2,057 specimens were collected in the Epitácio Pessoa and Argemiro de Figueiredo Reservoirs, representing freshwater fish from three orders, 10 families, 16 genera, and 17 species (Table 1). In the Argemiro de

Table 1. List of fish species recognized in Argemiro Figueiredo (AF) and Epitácio Pessoa (EP) reservoirs and their respective downstream areas (DAF) and (DEP), Paraíba do Norte River basin, Northeastern Brazil with their respective names. Endemic: endemism in Caatinga biome; Allochthonous: introduced from other regions; Autochthonous: native to the region; Exotic: introduced from other countries.

Taxon	Popular name	Origin	AF	EP	DAF	DEP	Voucher
CHARACIFORMES							
Crenuchidae							
Characidium bimaculatum Fowler, 1941	“Filhote de traíra”	Endemic			X		UFPB-10631
Erythrinidae							
Hoplias cf. *malabaricus* (Bloch, 1794)	“Traíra”	Autochthonous	X	X	X	X	UFPB-10604
Parodontidae							
Apareiodon davisi Fowler, 1941	“Piau”	Endemic	X			X	UFPB-10601
Anostomidae							
Leporinus piau Fowler, 1941	“Piau”	Endemic	X	X	X	X	UFPB -10606
Curimatidae							
Psectrogaster rhomboides Eigenmann & Eigenmann, 1889	“Cumatã”	Endemic					UFPB-10607
Steindachnerina notonota (Miranda Ribeiro, 1937)	“Piaba”	Endemic			X	X	UFPB-10616
Prochilodontidae							
Prochilodus brevis Steindachner, 1875	“Curimatã”	Endemic	X	X	X	X	UFPB - 10608
Triportheidae							
Triportheus signatus (Garman, 1890)	“Sardinha”	Endemic	X	X	X	X	UFPB-10600
Characidae							
Astyanax aff. *bimaculatus* (Linnaeus, 1758)	“Piaba”	Autochthonous	X	X	X	X	UFPB -10603
Astyanax aff. *fasciatus* (Cuvier, 1819)	“Piaba”	Autochthonous	X	X	X		UFPB-10635
Compsura heterura Eigenmann, 1915	“Piaba”	Autochthonous			X		UFPB-10627
Hemigrammus rodwayi Durbin, 1909	“Piaba”	Autochthonous			X		UFPB-10632
Hemigrammus marginatus Ellis, 1911	“Piaba”	Autochthonous			X		UFPB-10630
Serrapinnus heterodon (Eigenmann, 1915)	“Piaba”	Autochthonous			X		UFPB-10634
Hyphessobrycon parvellus Ellis, 1911	“Piaba”	Autochthonous			X		UFPB-10633
SILURIFORMES							
Heptapteridae							
Pimelodella enochi Fowler, 1941	“Cascudo”	Endemic			X	X	UFRN-0443
Rhamdia quelen (Quoy & Gaimard, 1824)	“Jundiá”	Autochthonous			X		UFPB-10625
Loricariidae							
Hypostomus pusarum (Starks, 1913)	“Cascudo”	Endemic	X	X		X	UFPB-10602
Parotocinclus jumbo Britski & Garavello, 2002	“Cascudinho”	Endemic			X		UFPB-10628
Parotocinclus spilosoma (Fowler, 1941)	“Cascudinho”	Endemic				X	UFRN-0731
CYPRINODONTIFORMES							
Poeciliidae							
Poecilia reticulata Peters, 1859	“Barrigudinho”	Allochthonous			X		UFPB-10629
Poecilia vivipara Bloch & Schneider, 1801	“Barrigudinho”	Autochthonous			X	X	UFPB-10626
SYNBRANCHIFORMES							
Synbranchidae							
Synbranchus aff. *marmoratus* Bloch, 1795	“Mussum”	Autochthonous			X		UFPB-10623
PERCIFORMES							
Sciaenidae							
Plagioscion squamosissimus (Heckel, 1840)	“Pescada”	Allochthonous	X	X			UFPB-9983
Cichlidae							
Astronotus ocellatus (Agassiz, 1831)	“Óscar”	Allochthonous		X			UFPB-10605
Cichla ocellaris Bloch & Schneider 1801	“Tucunaré”	Allochthonous		X			UFPB-10609
Cichlasoma orientale Kullander, 1983	“Corró preto”	Endemic			X	X	UFPB-9951
Crenicichla menezesi (Ploeg, 1991)	“Quatro-olho”	Endemic	X	X	X	X	UFPB-9980
Geophagus brasiliensis (Boulenger, 1897)	“Acará”	Autochthonous	X	X	X	X	UFPB-9952
Oreochromis niloticus (Linnaeus, 1758)	“Tilápia-do-Nilo”	Exotic	X	X			UFPB-9985
Coptodon rendalli (Boulenger, 1897)	“Tilápia”	Exotic	X	X			UFPB-2883

Figueiredo Reservoir a total of 1,482 specimens were collected, representing 13 species and in the Epitácio Pessoa reservoir a total of 575 specimens were collected, belonging to 16 species. Of the 17 species recorded, 12 species were common to both reservoirs, one species, *Apareiodon davisi*, was recorded only in the Argemiro Figueiredo Reservoir, and four species in the Epitácio Pessoa Reservoir: *Astronotus ocellatus*, *Cichlasoma orientale*, *Cichla ocellaris*, and *Psectrogaster rhomboides*.

Characiformes and Perciformes showed the highest species richness in the reservoir ichthyofauna, both with eight species (47.1%) of the 17 species registered. However, five species of the order Perciformes were introduced: *Astronotus ocellatus*, *Cichla ocellaris*, *Oreochromis niloticus*, *Coptodon rendalli*, and *Plagioscion squamosissimus*. Species from the order Characiformes were distributed within seven families and seven genera, and those species from the order Perciformes were distributed within two families and eight genera. The order Siluriformes, within the reservoirs, was represented by only one species (*Hypostomus pusarum*).

Cichlidae was family with the highest species richness in the Epitácio Pessoa and Argemiro Figueiredo Reservoirs, representing 41,1% with seven species. However, this family comprised only three species (17.7%) when allochthonous and exotic species are removed from this account, this is also the case of the Characidae family. *Oreochromis niloticus* (exotic species) was most abundant species, representing a total of with 473 (23%) specimens collected, and *Plagioscion squamosissimus* (allochthonous species) was second most abundant species, representing a total of 430 (21%) collected specimens. *Astyanax* aff. *bimaculatus* was the most abundant among the native species, representing a total of 189 (9%) collected specimens, followed by *Geophagus brasiliensis* with a total of 172 (8%) collected specimens. Cichlidae and Sciaenidae were the families with highest relative abundance values, comprising 45% and 21%, respectively, of all sampling of the two reservoirs. It is important to note that Sciaenidae family was represented by only one species, *Plagioscion squamosissimus*. Other families had a relative abundance percentage below 10%. Perciformes was the most abundant order in the Epitácio Pessoa and Argemiro de Figueiredo Reservoirs, representing a total of 1,372 (67%) collected specimens, followed by Characiformes with a total of 598 (29%) specimens and Siluriformes with a total of 87 (4%) specimens.

Analysis of frequency of occurrence (FO%) in the Argemiro de Figueiredo reservoir showed that six species were classified as “Very Common”, four species as “Common”, two species as “Uncommon” and one species as “Rare” (Table 2). For the Epitácio Pessoa Reservoir, five species were classified as “Very Common”, six species as “Common”, four species as “Uncommon” and a single species as “Rare” (Table 2).

In the lotic portion of the river downstream from Argemiro de Figueiredo and Epitácio Pessoa reservoirs, 271 specimens were collected and represented 24 species, 20 genera, 11 families, and five freshwater fish orders (Table 1). Additional species were recorded through downstream sampling but these were not recognized within the reservoirs. In the area downstream of Epitácio Pessoa Reservoir, 12 species were recorded in which five species were not collected in the reservoir: *Apareiodon davisi*, *Steindachnerina notonota*, *Parotocinclus spilosoma*, *Pimelodella enochi*, and *Poecilia vivipara*. In the area downstream of Argemiro de Figueiredo Reservoir, 20 species were recorded in which 11 species were not collected in the reservoir: *Characidium bimaculatum*, *Compsura heterura*, *Hyphessobrycon* cf. *parvellus*, *Hemigrammus marginatus*, *Hemigrammus rodwayi*, *Parotocinclus jumbo*, *Poecilia reticulata*, *Rhamdia quelen*, *Serrapinnus heterodon*, *Steindachnerina notonota*, and *Synbranchus* aff. *marmoratus*. In these two downstream areas, 12 families were recognized but seven of these families bear only one species.

A total of 31 species were collected in Argemiro Figueiredo and Epitácio Pessoa Reservoirs and their downstream areas, distributed within 27 genera, 14 families, and five orders (Table 1). Characiformes was the order with the largest species richness, comprising 15 species within 13 genera and eight families, and representing 48% of recorded species (Figure 3A). Perciformes was the second richest order with eight species recognized within eight genera and two families, and representing 26% of recorded species. Characidae and Cichlidae were families with the highest number of species, both with seven species representing 23% of species sampled, followed by Loricariidae with three (10%) species (Figure 3B).

Discussion

The dams built in the intermittent rivers of Brazilian Northeast region serve as a refuge for freshwater fishes during drought periods. The number of species (17) recorded in the two reservoirs in our study is in accordance to other reservoirs in the region. Gurgel-Lourenço et al. (2013) also listed 17 species from Paulo Sarasate and Edson Queiroz Reservoirs, both located

Table 2. Occurrence Frequency Classification (F.O.%) for species from Epitácio Pessoa (EP) and Argemiro de Figueiredo (AF) Reservoirs.

Species	EP			AF		
	N	FO (%)	Classification	N2	FO (%)	Classification
Hoplias cf. *malabaricus*	45	100	Very common	9	50	Common
Astronotus ocellatus	34	91,6	Very common	-	-	-
Cichla ocellaris	53	83,3	Very common	-	-	-
Prochilodus brevis	52	83,3	Very common	2	8,33	Rare
Geophagus brasiliensis	52	75	Very common	120	83,3	Very common
Leporinus piau	54	66,6	Common	6	41,6	Uncommon
Psectrogaster romboides	19	66,6	Common	-	-	-
Hypostomus pusarum	63	58,3	Common	24	50	Common
Triportheus signatus	58	58,3	Common	29	83,3	Very common
Cichlasoma orientale	19	50	Common	-	-	-
Crenicichla menezesi	11	50	Common	149	100	Very common
Oreochromis niloticus	67	41,6	Uncommon	406	100	Very common
Astyanax aff. *bimaculatus*	34	25	Uncommon	155	50	Common
Plagioscion squamosissimus	8	25	Uncommon	422	100	Very common
Coptodon rendalli	5	25	Uncommon	23	50	Common
Astyanax aff. *fasciatus*	1	8,3	Rare	30	16,6	Uncommon
Apareiodon davisi	-	-	-	104	75	Very common

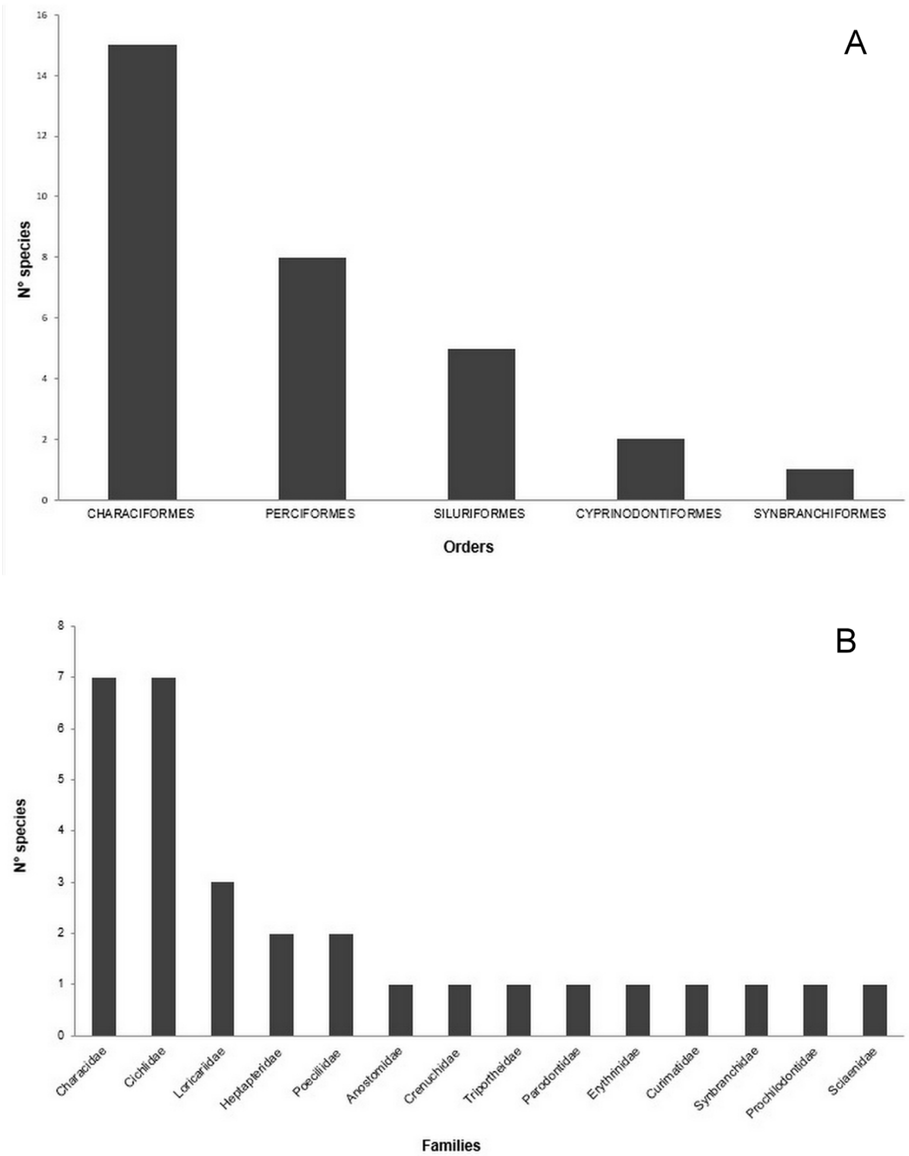


Figure 3. Fish species richness by Order (A) and Family (B) collected in Argemiro Figueiredo and Epitácio Pessoa reservoirs and their respective downstream areas.

in Ceará State. Sánchez-Botero et al. (2013) recognized 9 species by conducting monthly samplings for a period of six months in Santo Anastácio Reservoir at Ceará State. Silva-Filho et al. (2011) recorded 14 species in Duas Unas reservoir, review at Jaboatão River basin, Pernambuco State. Marinho et al. (2006) recognized seven species in Namorados (municipality of São João do Cariri) and Soledade (Soledade municipality) reservoirs at Paraíba do Norte River basin, which are under the hydrographic domain of Taperoá sub-basin. Montenegro et al. (2012) listed 11 species for Taperoá II reservoir, located in Taperoá municipality at Paraíba do Norte River basin. Our study supports a much higher number of species when compared to these previous studies in the same basins.

Characiformes and Siluriformes generally present the greatest species richness Neotropical regions (Lowe-McConnel 1987, Reis et al. 2003), as well as in Brazil (Buckup et al. 2007). The proportional richness of the order Characiformes has been a natural tendency of ichthyofauna basins under influence of the Caatinga biome (Ramos et al. 2005, Silva et al. 2014, Ramos et al. 2014, Silva et al. 2015). The Characidae family, one of the richest families in this study, generally has the greatest richness in fish diversity in Northeast Brazil (Ramos et al. 2005, Paiva et al. 2014, Silva et al. 2014, Ramos et al. 2014, Silva et al. 2015). Rosa et al. (2003) observed that Characidae was the most diversified family (50 species) when studied the diversity of Caatinga ichthyofauna. Most of its species are associated to inland waters of Brazil (Britski 1972).

However, order Perciformes displays higher species diversity than the order Siluriformes when it comes to reservoirs (Marinho et al. 2006, Montenegro et al. 2012, Sánchez-Botero et al. 2013, Gurgel-Lourenço et al. 2015). The higher species diversity of the order Perciformes in the semiarid reservoirs can be correlated to the lentic habit of Caatinga reservoirs, to which Cichlidae species, the most diverse family within the order, are well adapted (Kullander 2003, Langeani et al. 2007). Whereas species of the order Siluriformes are adapted to running water (lotic) environments (Nelson 2006). Epitácio Pessoa and Argemiro de Figueiredo Reservoirs as well as other Caatinga reservoirs are lentic environments that are more suitable for the predominance of cichlids. Most of the exotic species within the Caatinga rivers belong to the Cichlidae family, as pointed out in our results previously that noticed five out of eight Cichlidae species were introduced in these two reservoirs.

Oreochromis niloticus, popularly known as "Nile tilapia", is an exotic species that is well established in the Brazilian semiarid region and the most abundant species recorded in Epitácio Pessoa and Argemiro de Figueiredo Reservoirs. This species feeds mainly on phytoplankton and zooplankton, and it has great trophic plasticity and it is highly tolerant to environmental variations (Gurgel & Fernando 1994, Starling et al. 2002, Attayde et al. 2007). The abundance of *Oreochromis niloticus* is related to breeding in tanks from Argemiro Figueiredo Reservoir where 406 out of 473 specimens were collected. In addition to competition for food resources, mostly zooplankton, this species may affect other species through changes in habitat quality. Omnivorous filter-feeding fish such as tilapia tend to increase total phytoplankton biomass through predation on zooplankton and nutrient recycling (Drenner et al. 1996, Attayde et al. 2007).

Plagioscion squamosissimus, the second most abundant species, was introduced in reservoirs in Northeast Brazil during the early 1950s by Departamento Nacional de Obras Contra a Seca (DNOCS). However, fisheries were only productive from 1970s onwards (Fontenele & Peixoto 1978, Sato & Godinho 1999). Introduction of exotic species is considered to be one of the major reasons for biodiversity loss and the second major cause of animal extinction (Fontana et al. 2003, Ziller & Zalba 2007). Exotic fish may act as predators, competitors or even disseminate pathogenic organisms (Reis et al. 2003).

Astyanax aff. *bimaculatus* is a native species, is the third most abundant representative in the two reservoirs, along with other small Characidae species, popularly known as "piabas" in Northeastern Brazil, that is generally the most abundant group in the region (Ramos 2012, Paiva et al. 2014, Silva et al. 2014). These species are not valuable for fisheries purposes due to their relatively small size (Ramos 2012). However, these species are of importance as a food source for carnivorous species (Câmara et al. 1991). The relative low frequency of "piabas" in these reservoirs, and particularly in Epitácio Pessoa reservoir, when compared to *Oreochromis niloticus* and *Plagioscion squamosissimus* may be related to the abundance of *Cichla ocellaris* and *Hoplias* cf. *malabaricus*. The latter two species, such as *P. squamosissimus,* are considered top predators that have piscivorous habits and feed on small whole fishes such as "piabas" when adults (Goldstein 1973, Lowe-McConnell 1975, Peixoto 1982, Lowe-McConnell 1987, Resende et al. 1996, Almeida et al. 1997, Bennemann & Shibatta, 2002.).

Apareiodon davisi, one of the most representative species in our survey, was collected in Argemiro de Figueiredo Reservoir only, and registered during most of the sampling period. Species of the genus *Apareiodon* are generally found in habitats characterized by flowing water with high oxygen concentrations and rocky substrates, typical of lotic environments. Most of its species are found in rapids, and scrape rock-adhered algae off rocks as their food source (Pavanelli 2006). However, *A. davisi* was one of the six most abundant species from Argemiro de Figueiredo Reservoir, which leads us to infer that a population of this species is adapted to lentic conditions that are distinctive of this reservoir.

Apareiodon davisi is endemic to the Mid-Northeastern Caatinga ecoregion and it was described from specimens collected in drainages of Jaguaribe, Piranhas-Açu, and Paraíba do Norte rivers by Rodolfo Von Ihering during the decade of 1930 in Ceará and Paraíba States (Fowler, 1941). *Apareiodon davisi* is the only threatened species among those herein recognized, according to the criteria from the Brazil's official list of endangered species of fish and aquatic invertebrates (Brasil, 2014), due to the following aspects: 1) recent records detected reduction of population size. In the decades of 1940 and 1950, samples of *A. davisi* were abundant (around 100 specimens collected). However, recent expeditions have shown a decrease in the number of collected specimens (less than 10 specimens). Additionally, a single specimen has been recently collected in the type locality of *A. davisi* (b iv), in the North Paraíba River basin, with few additional specimens collected in recent years; 2) continuous decline of habitat quality (b iii). The locations in which *A. davisi* has been registered have suffered anthropogenic impacts such as deforestation due to expansions of sugar cane farming and extensive livestock, erosion, and river siltation. In addition, the main basins where this species is recognized are characterized by an intermittent regime and will receive water from the San Francisco River transposition. The consequences of changes in the hydrological regime (intermittent to perennial) are unknown to *A. davisi*. 3) severe fragmentation of its populations (a). Uninterrupted dams contribute to population fragmentation in the basins where *A. davisi* are registered. Population regressions were not yet quantified for this species, but occupation area (AOO) together with current records of this species were calculated as 93,9 Km^2 (B2), which categorize *A. davisi* as "endangered (EN)", according to B2 ab (iii,iv) criteria (Brasil, 2014).

Characiformes and Siluriformes, and the families Characidae, Loricariidae, and Cichlidae in the downstream area of Epitácio Pessoa and Argemiro de Figueiredo reservoirs were the most representative groups, which is in agreement with other studies from Northeast Brazil such as those by Ramos et al. (2005), Silva et al. (2014), Ramos et al. (2014), Silva et al. (2015). These results, however, differed regarding ichthyofauna within the reservoirs, as mentioned above.

Poecilia reticulata is the only species considered as introduced into the region that was not recorded within the two reservoirs among 24 species recognized in the downstream area of Argemiro de Figueiredo and Epitácio Pessoa Reservoirs. This is a small and well-disseminated species in Northeast Brazil, commonly used for aquarium trading and as pest control (Ramos 2012).

Two new occurrences were recorded from Paraíba do Norte River basin: *Hemigrammus rodwayi* Durbin, 1909, previously known from Guyana, Suriname, French Guiana Rivers and the Amazon basin; and *Hyphessobrycon* cf. *parvellus* Ellis, 1911 known from the coastal drainage of Bahia State, Brazil (Reis et al. 2003, Buckup et al. 2007). The occurrence of *Hemigrammus rodwayi* in the Brazilian Northeast is considered natural due to environmental similarities between the ichthyofauna of these regions as mentioned in Agassiz & Agassiz (1938) and Paiva (1978). *Hyphessobrycon* cf. *parvellus* was previously recorded from São Francisco River basin and its distribution is now extended to the MNCE. Both *Hemigrammus rodwayi* and *Hyphessobrycon* cf. *parvellus* were collected in several coastal rivers of Northeastern Brazil (Telton Ramos, personal communication).

Six species out of 31 species recorded in the present study were introduced, and 25 autochthonous species (Figures 4 and 5) in which 13 species (52%) are endemic to the basins that drain Caatinga biome. Six species (*Apareiodon davisi*, *Characidium bimaculatum*, *Hypostomus pusarum*, *Parotocinclus jumbo*, *P. spilosoma* and *Pimelodella enochi*) are endemic to the MNCE. *Parotocinclus spilosoma* and *Pimelodella enochi* are endemic to Paraíba do Norte River drainage, to which the water bodies of the studied regions belong (Reis et al. 2003, Rosa et al. 2003, Buckup et al. 2007). Studies on the biology of these endemic species that address adaptations in intermittent rivers such as Paraíba do Norte River are unknown, which makes it difficult to predict environmental impacts that changes in the hydrological regime may cause to them after the river transposition. However, special heterogeneity created from different hydrological phases and environmental variables (characteristics from the intermittent rivers) are important factors in the maintenance of a rich and mutable ichthyofauna as it decreases the comprehensive pattern of species dominance. Formation of pools of a variety of sizes and shapes in riverbeds increase fish resilience after flooding as many species use them as a habitat for reproduction (Medeiros & Maltchick 2001, Barbosa et al. 2012).

According to Maltchik (1999), fish diversity in rivers of the semi-arid region shows an inverse relationship with the hydrological stability, meaning that rivers of greater hydrological stability with minimal seasonal variability have lower rates of biological diversity than rivers with a more instable

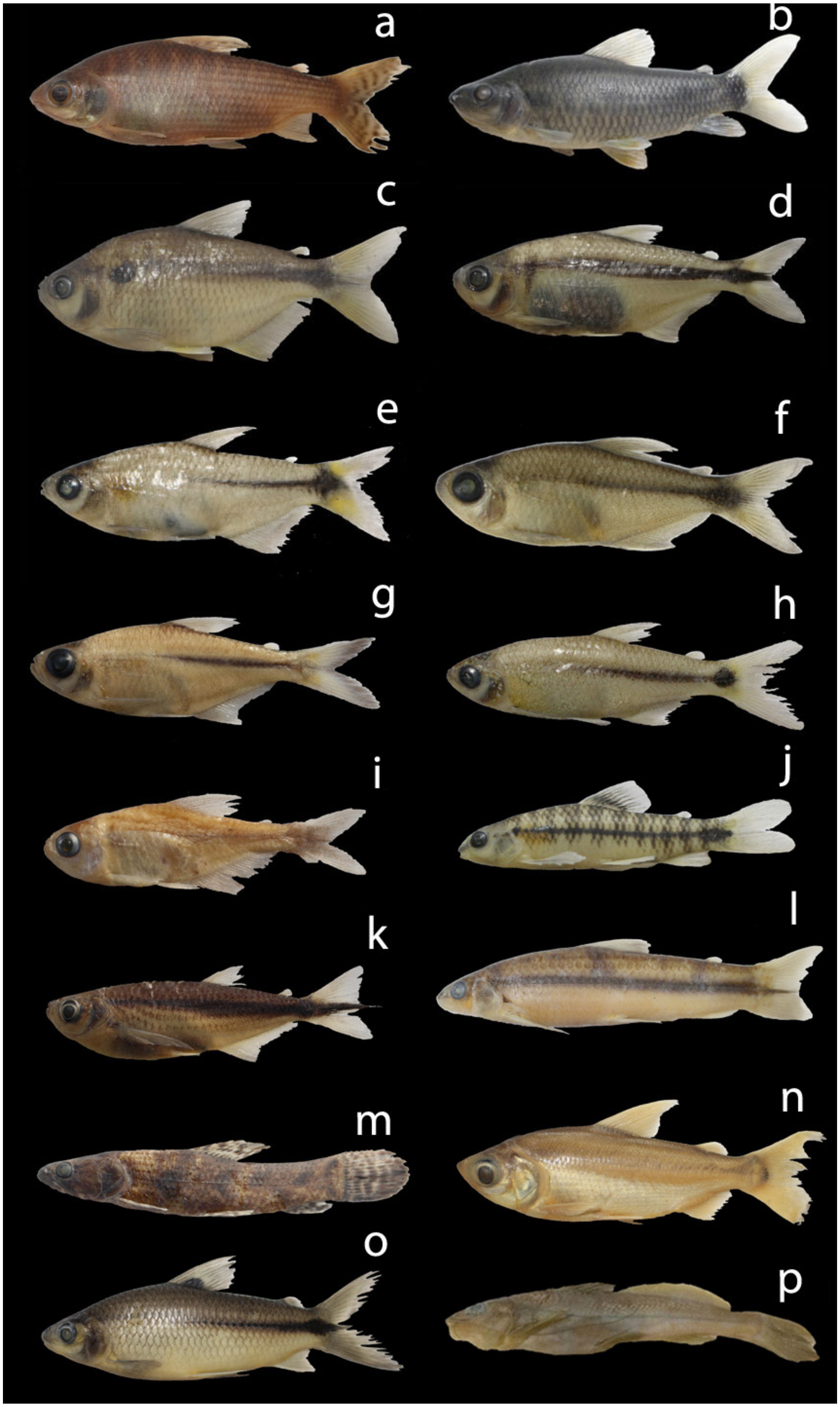


Figure 4. Fishes from the sampled areas of the Argemiro Figueiredo and Epitácio Pessoa reservoirs and their respective downstream areas. a) *Prochilodus brevis*, 120.5 mm SL; b) *Leporinus piau*, 101.2 mm SL; c) *Astyanax* aff. *bimaculatus*, 62.1 mm SL; d) *Astyanax* aff. *fasciatus*, 66.7 mm SL; e) *Compsura heterura*, 38.4 mm SL; f) *Hemigrammus rodwayi*, 26.2 mm SL; g) *Hemigrammus marginatus*, 34.7 mm SL; h) *Serrapinnus heterodon*, 35.2 mm SL; i) *Hyphessobrycon parvellus*, 21.4 mm SL; j) *Characidium bimaculatum*, 30.3 mm SL; k) *Triportheus signatus*, 84.5 mm SL; l) *Apareiodon davisi*, 75.4 mm SL; m) *Hoplias* cf. *malabaricus*, 92.7 mm SL; n) *Psectrogaster rhomboides*, 140.5 mm SL; o) *Steindachnerina notonota*, 96.5 mm SL; p) *Pimelodella enochi*, 175.3 mm SL.

http://dx.doi.org/10.1590/1676-0611-BN-2017-0334

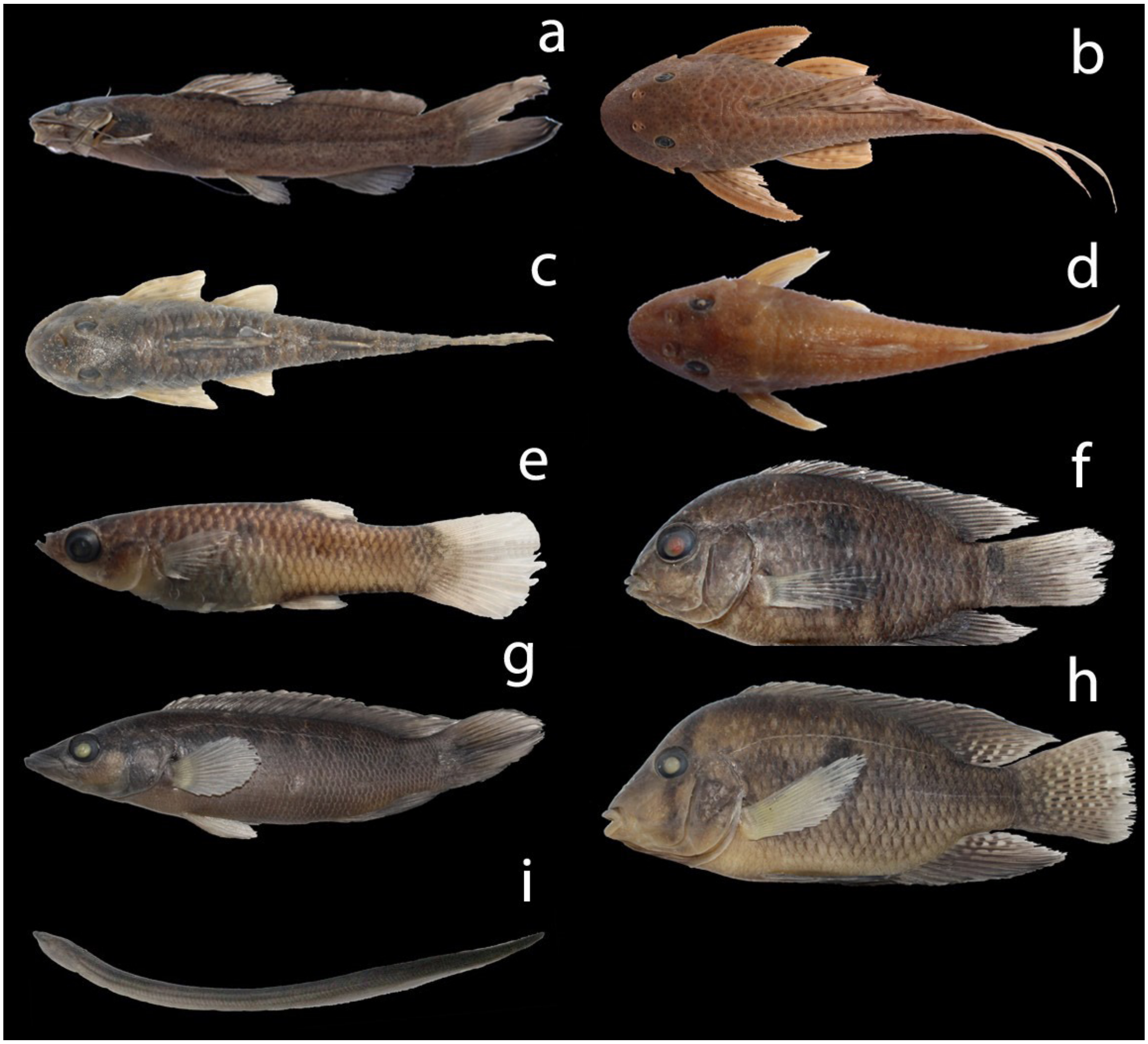


Figure 5. Fishes from the sampled areas of the Argemiro Figueiredo and Epitácio Pessoa reservoirs and their respective downstream areas. a) *Rhamdia quelen*, 129.1 mm SL; b) *Hypostomus pusarum*, 124.5 mm SL; c) *Parotocinclus jumbo*, 53.6 mm SL; d) *Parotocinclus spilosoma*, 38.1 mm SL; e) *Poecilia vivipara*, 27.1 mm SL; f) *Cichlasoma orientale*, 74.3 mm SL; g) *Crenicichla menezesi*, 89.7 mm SL; h) *Geophagus brasiliensis*, 118.4 mm SL; i) *Synbranchus* aff. *marmoratus*., 170.5 mm SL.

hydrology. The author further states that this pattern may be explained by the presence of dominant species in rivers of greatest hydrological stability.

The Northeast region of Brazil is currently undergoing a water transposition process, in which waters of São Francisco River basin, which is characterized by a perennial regime, will be artificially connected to the four largest basins of the MNCE that have an intermittent regime. In addition to Paraíba do Norte basin, the watersheds of the Apodi-Mossoró (Rio Grande do Norte), Jaguaribe (Ceará) and Piranhas-Açu Rivers (Paraíba and Rio Grande do Norte) will also receive water from São Francisco River basin through two main channels (Pittock et al. 2009). The transposition of water from the São Francisco basin to MNCE basins may cause changes in species composition, structure and dynamics of these ecosystem fish communities. Taxonomic studies such as the current study are crucial for comparative reevaluation post-transposition. The study of the ichthyofauna of Argemiro de Figueiredo and Epitácio Pessoa Reservoirs and their downstream areas improved the knowledge of fish diversity from Paraíba do Norte River basin, reinforcing subsidies for environmental protection and conservation of its watersheds. Understanding the local ichthyofauna and their ecosystems is essential for assessing future impacts caused by the transposition of São Francisco River as an example of direct anthropic action as well as by introducing exotic species.

Acknowledgements

We thank all members of Laboratório de Ecologia Aquática from Universidade Estadual da Paraíba (UEPB), especially Patricia Cruz for helping with field work.

Author Contributions

All authors contributed by collecting specimens, and writing this manuscript. SYLC and TPAR identified the species.

Conflicts of interest

The authors declare that have no conflict of interest related to the publication of this manuscript.

References

AESA. http://site2.aesa.pb.gov.br/aesa/volumesAcudesdomestodo=preparaUltimosVolumesPorMunicipio (the last access in 04/12/2015).

AGASSIZ, L. & AGASSIZ, E.C. 1938. Viagem ao Brasil: 1865-1866. São Paulo, Trad. de Edgar Siissekind de Mendonça.

ALMEIDA, V.L.L., RESENDE, E.K. & VAZZOLER, A.E. 1997. Feeding patterns in five predatory fishes of the hight Paraná River flooddplain (PR Brazil). Ecol. Freshw. Fish 6(3): 123-133.

ATTAYDE, J.L., OKUN, N., BRASIL, J., MENEZES, R., & MESQUITA, P. 2007. Impactos da introdução da tilápia do Nilo, *Oreochromis niloticus*, sobre a estrutura trófica dos ecossistemas aquáticos do bioma caatinga. Oecologia Brasiliensia. 11(3): 450-461.

BARBOSA, J.E.L., MEDEIROS, E.S.F., BRASIL, J., CORDEIRO, R.S., CRISPIM, M.C.B. & SILVA, G.H.G.S. 2012. Aquatic systems in semi-arid Brazil: limnology and management. Acta Limnol. Bras. 24(1): 103-118.

BENNEMANN, S.T. & SHIBATTA, O.A. 2002. Dinâmica de uma assembleia de peixes do rio Tibagi. A bacia do rio Tibagi. In (M.E. Medri, E, Bianchini, O.A. Shibatta & J. A. Pimenta eds). Londrina, M. E. Medri, p.433-442.

BRASIL. 2014. Lista Nacional Oficial de Espécies da Fauna Ameaçada de Extinção - Portaria N° 443, de 17 de dezembro de 2014. Ministério do Meio Ambiente. Diário Oficial da União, Brasília, Seção 1(245): 121-130.

BRITISKI, H.A. 1972. Peixes de água doce do Estado de São Paulo. In Poluição e Piscicultura. Comissão Interestadual da Bacia Paraná-Uruguai. Faculdade de Saúde Pública da USP, São Paulo, p.79-108.

BRITSKI, H.A., SATO, Y. & ROSA, A.B.S. 1984. Manual de identificação de peixes da região de Três Marias: com chave de identificação para os peixes da bacia do São Francisco. Brasília, Câmara dos Deputados, Codevasf, Divisão de Piscicultura e Pesca.

BUCKUP, P.A., MENEZES, N.A. & GHAZZI, M.S. 2007. Catálogo das espécies de peixes de água doce do Brasil. Museu Nacional, Rio de Janeiro.

CÂMARA, J.J.C., RODRIGUES A.M., CAMPOS, E.C., SANTOS, R.A. & MANDELLI J. 1991. Pesca seletiva do tambiú, *Astyanax bimaculatus* (Linnaeus, 1758) (Characiformes, Characidade), com a utilização de redes de emalhar, na represa de Ibitinga, rio Tietê, Estado de São Paulo, Brasil. Boletim do Instituto de Pesca. 18: 51-60.

CASTRO, R.M.C., & VARI R.P. 2004. Detritivores of the South American Fish Family Prochilodontidae (Teleostei: Ostariophysi: Characiformes): a phylogenetic and revisionary study. Smithsonian Contributions and Studies Series, p.622- 189.

DRENNER, R.W., SMITH, J.D. & THRELKELD, S.T. 1996. Lake trophic state and the limnological effects of omnivorous fish. Hydrobiologia. 319(3): 213-223.

ESCHMEYER, W.N., FRICKE, R. & VAN DER LAAN, R. (eds). 2017. Catalog of fishes: genera, species, references. http:// research.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (the last access in 06/02/2017).

FONTANA, C.S., BENCKE, G.A. & REIS, R.E. 2003. Livro vermelho da fauna ameaçada de extinção no Rio Grande do Sul. Edipucrs, Porto Alegre.

FONTENELE, O. & PEIXOTO, J.T. 1978. Análise dos resultados de introdução da pescada do Piauí, *Plagioscion squamosissimus* (Heckel,1840), nos açudes do Nordeste. Boletim Técnico DNOCS. 36(1):85-112.

FOWLER, H.W. 1941. A collection of freshwater fishes obtained in eastern Brazil by Dr. Rodolpho von Ihering. Proceedings of the Academy of Natural Sciences of Philadelphia. 93: 123–199.

GOLDSTEIN, R.J. 1973. Cichlids of the world. T.F.H. Publ. Inc. New Jersey.

GURGEL, J.J.S. & FERNANDO, C.H. 1994. Fisheries in semi-arid Northeast Brazil with special reference on the role of tilapias. International Review of Hydrobiology. 79(1): 77-94.

GURGEL-LOURENÇO, R.C., SOUSA, W.A., SÁNCHEZ-BOTERO, J.I. & GARCEZ, D.S. 2013. Ichthyofauna of two reservoirs in the middle Acaraú River basin, Ceará, Northeastern Brazil. Check List. 9(6): 1391–1395.

GURGEL-LOURENÇO, R.C., RODRIGUES-FILHO, C.A.S., ANGELINI, R., GARCEZ, D.S. & SÁNCHEZ-BOTERO, I.J. 2015. On the relation amongst limnological factors and fish abundance in reservoirs at semiarid region. Acta Limnol. Bras. 27(1): 24-38.

HUBBS, C.L. & LAGLER K.F. 2006. Fishes of the Great Lakes region. University of Michigan Press.

INSA. http://www.insa.gov.br/ (the last access in 04/12/2015).

KULLANDER, S.O. 1983. A revision of the South American cichlid genus *Cichlasoma* (Teleostei: Cichlidae). Swedish Museum of Natural History, Sweden.

KULLANDER, S.O. 2003. Family Cichlidae. In Check list of the freshwater fishes of South and Central America (R.E. Reis, S.O. Kullander & C.J. Ferraris Jr.). Edipucrs, Porto Alegre, p.605-654.

LANGEANI, F., CASTRO, R.M.C., OYAKAWA, O.T., SHIBATTA, O.A., PAVANELLI, C.S. & CASATTI, L. 2007. Diversidade da ictiofauna do Alto rio Paraná: composição atual e perspectivas futuras. Biota Neotrop. 7(3): 181-197.

LANGEANI, F., BUCKUP, P.A., MALABARBA, L.R., PY-DANIEL, L.H.R., LUCENA, C.A.S., ROSA, R.S., ZUANON, J.A.S., LUCENA, Z.M.S., DE BRITTO, M.R., OYAKAWA, O.T. & GOMES-FILHO, G. 2009. Peixes de Água Doce. In Estado da arte e perspectivas para a zoologia no Brasil (R.M. Rocha, & W.A.P. Boeger, orgs.). Ed. UFPR, Curitiba, p.211-230.

LOWE-MCCONNELL, R.L. 1975. Fish communities in tropical freshwaters. Longman London.

LOWE-MCCONNELL, R.H. 1987. Ecological studies in tropical fish communities, Cambridge University Press.

MALABARBA, L.R. & REIS, R.E. 1987. Manual de técnicas para a preparação de coleções zoológicas. Sociedade Brasileira de Zoologia, Campinas. 36: 1-14.

MALTCHIK, L. 1996. Nossos rios temporários, desconhecidos, mas essenciais. Ciências Hoje. 21(122): 64-65.

MALTCHIK, L. 1999. Ecologia de Rios Intermitentes Tropicais. In Perspectivas da Limnologia no Brasil (M.L.M. Pompêo, ed.). Gráfica e Editora União. São Luís.

MARINHO, R.S.A., TORELLI, J.E.R.S., SILVA, A.S. & RIBEIRO, L.L. 2006. Biodiversidade de peixes do semi-árido paraibano. Revista de Biologia e Ciências da Terra. (1): 112-121.

MONTENEGRO, A. K. A., TORELLI, J. E. R., CRISPIM, M. C., HERNÁNDEZ, M. I. M. & MEDEIROS, A.M.A. 2012. Ichthyofauna diversity of Taperoá II reservoir, semi-arid region of Paraíba, Brazil. Braz. J. Biol. 72(1): 113-120.

MEDEIROS, E.S. & MALTCHIK, L. 2001. Fish assemblage stability in an intermittently flowing stream from the Brazilian semiarid region. Austral Ecology. 26(2): 156–164

MEDEIROS, P.H.A., GÜNTNER, A., FRANCKE, T., MAMEDE, G.L. & ARAÚJO, J.C. 2010. Modelling spatio-temporal patterns of sediment yield and connectivity in a semi-arid catchment with the WASA-SED model. Hydrol. Sci. J. 55(4): 636-648.

NELSON, J.S. 2006. Fishes of the World. 4. ed. New Jersey: John Wiley & Sons.

NOVAES, J.L.C., MOREIRA, S.I.L., FREIRE, C.E.C., SOUSA, M.M.O. & COSTA, R.S. 2013. Fish assemblage in a semi-arid Neotropical reservoir: composition, structure and patterns of diversity and abundance. Braz. J. Biol. 74(2): 290-301.

PAIVA, M.P. 1978. A ictiofauna das Grandes Represas Brasileiras. Revista DAE. 38(116): 49-57.

PAIVA, R.E.C., LIMA, S.M.Q., RAMOS, T.P.A. & MENDES, L. 2014. Fish fauna of Pratagi River coastal microbasin, extreme north Atlantic Forest, Rio Grande do Norte State, northeastern Brazil. Check List. 10(5): 968-975.

PARAÍBA, SECRETARIA DE PLANEJAMENTO. 2007. Avaliação da infraestrutura hídrica e do suporte para o sistema de gerenciamento de recursos hídricos do Estado da Paraíba. João Pessoa, SEPLAN.

PAVANELLI, C.S. 2006. New species of *Apareiodon* (Teleostei: Characiformes: Parodontidae) from the Rio Piquiri, upper Rio Paraná basin, Brazil. Copeia. (2006)1: 89-95.

PEIXOTO, J.T. 1982. Alimento de tucunaré, *Cichla ocellaris* Bloch & Scheneider, 1801 no açude Lima Campos, Icó, Ceará, (Actinopterygii, Cichlidae). Colet. de Trab. Técn. DNOCS, Fortaleza, 2: 159-172.

PITTOCK, J., MENG, J. & CHAPAGAIN, A.K. 2009. Interbasin water transfers and water scarcity in a changing world -a solution or a pipedream? A discussion paper in a burning issue, 2nd, WWF Germany, Frankfurt.

RAMOS, R.T.C., RAMOS, T.P.A., ROSA, R.S., BELTRÃO, G.B.M. & GROTH, F. 2005. Diversidade de Peixes (Ictiofauna) da bacia do rio Curimataú, Paraíba. In Análise das variações da biodiversidade do bioma caatinga: suporte das estratégias regionais de conservação (F.S. Araujo, M.J.N. Rodal & M.R.V. Barbosa, eds.). Ministério do Meio Ambiente, Brasília, p. 291-318.

RAMOS, T.P.A. 2012. Ictiofauna de Água Doce da Bacia do Rio Parnaíba. Unpublished Ph.D. Universidade Federal da Paraíba, João Pessoa.

RAMOS, T.P.A. RAMOS, R.T.C. & RAMOS, S.A.Q.A. 2014. Ichthyofauna of the Parnaíba River Basin, Northeastern Brazil. Biota Neotrop. 14(3): 1-6. http://dx.doi.org/10.1590/S1676-06020140039.

REIS, R.E., ALBERT, J.S., DARIO, F. DI., MINCARONE, M.M., PETRY, P. & ROCHA, L.A. 2016. Fish biodiversity and conservation in South America. J. Fish Biol. 89(1): 12-47. doi:10.1111/jfb.13016.

REIS, R.E., KULLANDER, S.O. & FERRARIS JR, C.J. 2003. Check list of the freshwater fishes of South and Central America. Edipucrs, Porto Alegre.

REBOUÇAS, A.C., BRAGA, B.B., TUNDISI, J.G. 2006. Águas Doces no Brasil. 3° ed. São Paulo: Escrituras.

RESENDE, E.K., PEREIRA, R.A.C., ALMEIDA, V.L.L. & SILVA, A.G. 1996. Alimentação de peixes carnívoros da planície inundável do rio Miranda, Pantanal, Mato Grosso do Sul, Brasil. Embrapa Pantanal.

ROSA, R.S., MENEZES, N.A., BRITSKI, H.A., COSTA, W.J.E. & GROTH F. 2003. Diversidade, padrões de distribuição e conservação dos peixes da caatinga. In Ecologia e Conservação da Caatinga (I.R. Leal, M. Tabarelli & J.M.C. Silva eds.). Editora Universitária da Universidade Federal de Pernambuco, Recife, p.135–180.

ROSA, R.S. & MENEZES, N.A. 1996. Relação preliminar das espécies de peixes (Pisces: Elasmobranchii e Actinopterygii) ameaçadas no Brasil. Revista Brasileira de Zoologia 13 (3): 647-667.

SÁNCHEZ-BOTERO, I.J., REIS, V.C., CHAVES, F.D.N. & GARCEZ, D. 2013. Fish assemblage of the Santo Anastácio reservoir (Ceará state, Brazil). Bol. Inst. Pesca. 40(1): 1–15.

SATO, Y. & GODINHO, H. P. 1999. Peixes da Bacia do rio São Francisco. In Estudos ecológicos de comunidades de peixes tropicais (R.H. Lowe-Mcconnell ed.). Edusp, São Paulo, p.401-416.

SEMARH - Secretaria Extraordinária do Meio Ambiente, dos Recursos Hídricos. 2006. Plano Estadual de Recursos Hídricos da Paraíba-PERH-PB. João Pessoa, Resumo Executivo e Atlas.

SILVA, M.J., COSTA, B.G., RAMOS, T.P.A., AURICCHIO, P. & LIMA, S.M.Q. 2015. Ichthyofauna of the Gurgeia River, Parnaíba River basin, northeastern Brazil. Check List. 11(5): 1765.

SILVA, M.J., RAMOS, T.P.A., DINIZ, V.D., RAMOS, R.T.C. & MEDEIROS, E.S. F. 2014. Ichthyofauna of Seridó/Borborema: a semi-arid region of Brazil. Biota Neotrop. 14(3): 1-6. (the last access in 17/02/2016).

SILVA, M.A.V. 1987. Atlas climatológico do Estado da Paraíba. Universidade Federal da Paraíba, Núcleo de Meteorologia Aplicada, Paraíba.

SILVA-FILHO, E.G., SANTANA, F.M.S. & SEVERI, W. 2011. Ictiofauna do reservatório de Duas Unas, bacia do rio Jaboatão, Pernambuco: resultados preliminares da composição e estrutura da assembleia. Revista Brasileira de Ciências Agrárias. 6(2):351-361.

STARLING, F., LAZZARO, X., CAVALCANTI, C. & MOREIRA, R. 2002. Contribution of omnivorous tilapia to eutrophication of a shallow tropical reservoir: evidence from a fish kill. Freshwater Biol. 47(12): 2443-2452.

VARI, R.P. 1989. Systematics of the Neotropical Characiform genus *Psectrogaster* Eigenmann and Eigenmann (Pisces, Characiformes). Smithsonian Instituion Press. 481: 1-41.

ZILLER, S.R. & ZALBA, S. 2007. Propostas de ação para prevenção e controle de espécies exóticas invasoras. Nature and Conservation. 5(2):8-15.

Received: 07/02/2017
Revised: 17/07/2017
Accepted: 18/07/2017

Biota Neotropica 17(3): e20160251, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

short communication

Length-weight and length-length relationships for rockpool fishes on the Brazilian coast

***Fabiola Seabra Machado*[1*], ***Rory Romero de Sena Oliveira*[1], ***Arianderson Texeira Silva*[1] & ***Tommaso Giarrizzo*[1]

[1]Universidade Federal do Pará, Grupo de Ecologia Aquática, Avenue Perimetral, 2651, Terra Firme, Belém, PA, Brazil.

**Corresponding author: Fabiola Seabra Machado, e-mail: fabiola_seabra@hotmail.com*

MACHADO, F. S., OLIVEIRA, R. R. S., SILVA, A. T., GIARRIZZO, T. **Length-weight and length-length relationships for rockpool fishes on the Brazilian coast.** Biota Neotropica. 17(3): e20160251. http://dx.doi.org/10.1590/1676-0611-BN-2016-0251

Abstract: Fifty-nine length-weight (LWR) and length-length (LLR) relationships were estimated for 18 fish species, belonging to 10 families. The fish specimens were captured in rockpools of 21 sites along a stretch of 4,900 km of the Brazilian coast, between latitudes 00° and 22°S, in 2012. This study represents the first reference available for five fish species (*Gobiesox barbatulus, Bathygobius geminatus, Labrisomus nuchipinnis, Malacoctenus delalandii, Lutjanus alexandrei*) in LLR and six (*Gobiesox barbatulus*, *Bathygobius geminatus, Hypleurochilus fissicornis*, *Omobranchus punctatus*, *Entomacrodus vomerinus*, *Diplodus argenteus*) in LWR.

Keywords: *intertidal fish, tidepool, allometry.*

Relações comprimento-peso e comprimento-comprimento para os peixes de poças de maré na costa brasileira

Resumo: Cinquenta e nove relações peso-comprimento (RRC) e comprimento-comprimento (RCC) foram estimadas para 18 espécies de peixes, distribuídos em 10 famílias. Os espécimes de peixes foram capturados em poças de maré de 21 locais amostrados, ao longo de um trecho de 4.900 km da costa brasileira, entre as latitudes 00° e 22°S, durante o ano de 2012. Este estudo apresenta a primeira referência disponível para 5 espécies de peixes (*Gobiesox barbatulus, Bathygobius geminatus, Labrisomus nuchipinnis, Malacoctenus delalandii, Lutjanus alexandrei*) em RCC e 6 (*Gobiesox barbatulus*, *Bathygobius geminatus, Hypleurochilus fissicornis*, *Omobranchus punctatus*, *Entomacrodus vomerinus*, *Diplodus argenteus*) em RPC.

Palavras-Chave: *peixes de entremarés, poças de maré, alometria***.**

Introduction

In most fishery studies length–weight relationship (LWR) equations have been extensively used to estimate the fish weight from length given the technical difficulties (*e.g.,* bobbing motion of the boat) and the amount of time required to record weight in the field (Morato et al. 2001, Morey et al. 2003). However, the LWR of the same species could vary in space (*e.g.,* habitats and regional variation), so the use of a single equation throughout a large geographical area could introduce a bias in the estimates of weight (Morato et al. 2001, Mendes et al. 2004, Joyeux et al. 2008). Therefore, obtaining accurate local LWR parameter estimates is an important factor in the assessment of fish stocks and to subsidize the ecosystem modelling (Vaz-dos-Santos & Gris 2016). Additionally, length–length relationship (LLR) is very important for fisheries management and for comparative studies of population growth (*e.g.,* Moutopoulos & Stergiou 2002). In this study we report 59 LWR and LLR for 18 fish species caught in rockpools of 21 sites along a stretch of 4,900 km of the Brazilian coast, between latitudes 00° and 22°S.

Material and Methods

A Brazilian coastal sector between the Amazon estuary (0° of latitude) and Rio de Janeiro (22° S) was sampled between March and December 2012, in 21 rockpool sites spaced in average ~210 km apart (Figure 1).

The rockpool fish fauna was caught at low tide using anesthetic clove oil (40 ml from anesthetic dissolved in 1000 ml of ethanol) and small hand-nets (length 150 mm; mouth 101.6 mm and mesh 3 mm). The specimens were fixed in 10% formalin, preserved in 70% alcohol and identified at the lowest taxonomic level possible (Figueiredo & Menezes 1978, 1980a, 1980b, Menezes & Figueiredo 1985, Carvalho-Filho 1999, Carpenter 2002a, 2002b, Eschmeyer et al. 2016). Specimens were measured (nearest 0.01 cm standard length, SL and total length, TL) using digital slide calipers and weighed (nearest 0.01 g total weight, TW) using a digital balance.

The length–weight relationships (LWR) were calculated using the equation $TW = aTL^b$. Data were log transformed into: $\log TW = \log a + b\log TL$ (plots were performed for visual inspection of outliers, with extremes being excluded from the regression analyses) where TW is weight in

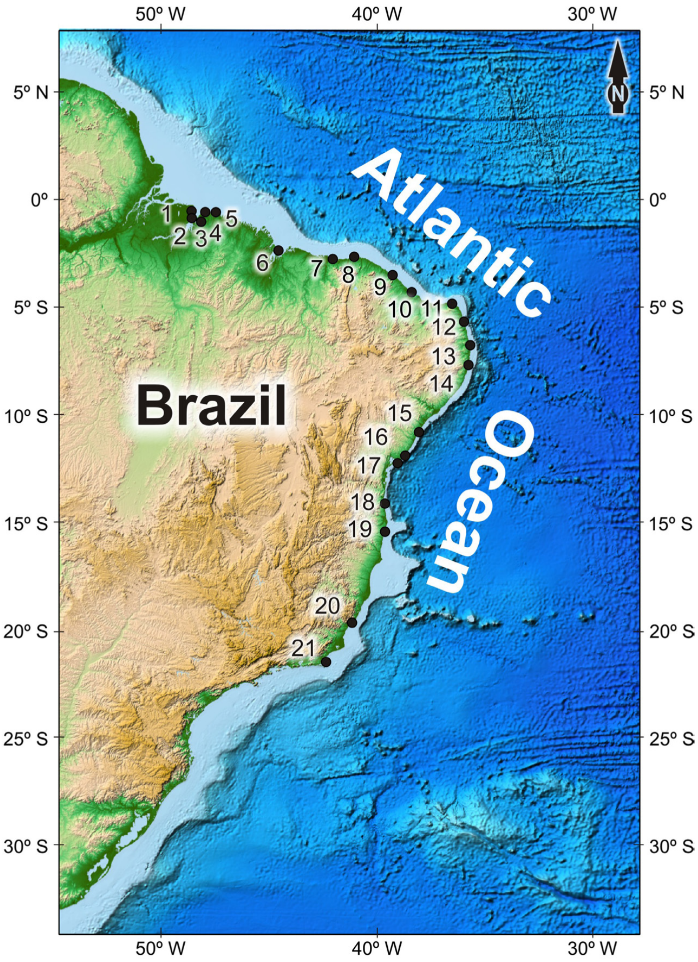


Figure 1. Geographic location of the 21 rockpools sites along the Brazilian coast. The names of locations are available in Table 1.

grams, TL is total length in centimeters, *a* is a constant, and *b* is the slope of the linear regression that sets the allometric growth (Froese 2006). Furthermore, length-length relationships (LLR) were estimated by the method of least squares to fit a simple linear regression model using the equation TL = *a* + *b*SL (Hossain et al. 2006). The association degree between variables (TW and TL for LWR and SL and TL for LLR), was measured by the coefficient of determination (r^2). In order to verify if *b* was significantly different from the isometric value (*i.e.*, *b* = 3 for LWR), the one-tailed Student's t-test (H0: *b* = 3) with a confidence level of ± 95% ($\alpha = 0.05$) was employed (Sokal & Rohlf 1987, Froese et al. 2011).

Results and Discussion

Overall, 4,299 specimens representing 18 different fish species belonging to 10 families were analyzed. The family Blenniidae were the most richness with four species, followed by Gobiidae and Pomacentridae (tree species each one), and Labrisomidae (two species). The five remaining families (Gobiesocidae, Carangidae, Gerreidae, Haemulidae, Sparidae and Lutjanidae) were represented by only one species. Sample size ranged from 9 specimens for *Labrisomus nuchipinnis* (Quoy & Gaimard, 1824) in Pirangi do Sul-RN (site 12), to 735 for *Scartella cristata* (Linnaeus, 1758) in Canoa Quebrada-CE (site 10). The inclusion of species with small sample size (< 20 individuals) is justified by using individuals with a spread in length broad enough to obtain the relationships (Froese 2006, Froese et al. 2011).

A total of 59 LLR and LWR were computed for all 18 fish species caught in one or more sampling sites (Table 1 and 2). All regressions were highly significant ($p < 0.001$), with the coefficient of determination r^2 ranging from 0.911 to 0.999.

The present study provides the first information on LLR and on LWR for five [*Gobiesox barbatulus* (Starks, 1913), *Bathygobius geminatus* (Tornabene, Baldwin & Pezold, 2010), *Labrisomus nuchipinnis* (Quoy & Gaimard, 1824), *Malacoctenus delalandii* (Valenciennes, 1836), *Lutjanus alexandrei* (Moura & Lindeman, 2007)] and five [*Gobiesox barbatulus*, *Bathygobius geminatus*, *Hypleurochilus fissicornis* (Quoy & Gaimard, 1824), *Entomacrodus vomerinus* (Valenciennes, 1836), *Diplodus argenteus* (Valenciennes, 1830)] native species (Table 1 and Table 2), respectively. It also presents the LWR

Table 1. Estimated parameters of length–length simple linear regression (LLR) to convert standard length (SL) to total length (TL) for 18 rockpool fish species in 21 sites along Brazilian coast.

Family/Species	Site/State	Site Code	n	Regression parameters				
				a	*b*	95% CL of *a*	95% CL of *b*	r²
Gobiesocidae								
Gobiesox barbatulus Starks, 1913	Areuá-PA	4	23	0.157	1.187	-0.009 to 0.325	1.131 to 1.243	0.98
	Farol Velho-PA	5	36	0.032	1.234	-0.173 to 0.238	1.162 to 1.278	0.98
Blenniidae								
Hypleurochilus fissicornis (Quoy & Gaimard, 1824)	Jericoacara-CE	8	82	0.131	1.139	0.0521 to 0.211	1.108 to 1.170	0.98
Omobranchus punctatus (Valenciennes, 1836)	Areuá-PA	4	11	0.007	1.176	-0.101 to 0.115	1.144 to 1.208	0.99
	Farol Veho-PA	5	50	-0.030	1.170	-0.145 to 0.084	1.140 to 1.200	0.99
Scartella cristata (Linnaeus, 1758)	Jericoacara-CE	8	264	0.106	1.169	0.034 to 0.178	1.155 to 1.184	0.98
	Canoa Quebrada-CE	10	735	0.060	1.182	0.018 to 0.102	1.171 to 1.192	0.98
	São M. do Gostoso-RN	11	39	0.038	1.166	-0.091 to 0.168	1.127 to 1.204	0.99
	Cabo Branco-PB	13	10	0.069	1.170	-0.128 to 0.267	1.117 to 1.222	0.99
	Boa Viagem-PE	14	65	0.121	1.134	0.059 to 0.1842	1.106 to 1.163	0.99
	Itapoã-BA	17	110	-0.022	1.203	-0.059 to 0.013	1.188 to 1.217	0.99
	Milagres-BA	18	97	0.117	1.145	0.051 to 0.183	1.118 to 1.172	0.98
Entomacrodus vomerinus Valenciennes, 1836	Itapoã-BA	17	90	0.037	1.201	-0.034 to 0.108	1.186 to 1.216	0.99
Carangidae								
Caranx latus Agassiz, 1831	Saco-SE	15	15	-0.476	1.296	-1.137 to 0.183	1.165 to 1.427	0.97

n, number of specimens; CL, confidence limits; Species in bold have length-length relationships estimates included in fishBase database (Froese & Pauly 2016) and literature.

Table 1. Continued...

Family/Species	Site/State	Site Code	n	Regression parameters				
				a	*b*	95% CL of *a*	95% CL of *b*	r²
Gerreidae								
Eucinostomus melanopterus (Bleeker, 1863)	Saco-SE	15	90	-0.129	1.305	-0.177 to -0.081	1.278 to 1.333	0.99
Gobiidae								
Ctenogobius boleosoma (Jordan & Gilbert, 1882)	Saco-SE	15	216	-0.096	1.300	-0.179 to -0.012	1.262 to 1.339	0.95
	Castelhanos-ES	20	24	-0.122	1.292	-0.300 to 0.059	1.202 to 1.381	0.97
Bathygobius soporator (Valenciennes, 1837)	Mata Fome-PA	1	95	-0.003	1.262	-0.168 to 0.161	1.223 to 1.301	0.97
	Joanes-PA	2	213	0.106	1.198	-0.014 to 0.228	1.166 to 1.231	0.96
	Paraiso-PA	3	55	0.079	1.219	-0.057 to 0.215	1.186 to 1.251	0.99
	Calhau-MA	6	34	-0.120	1.260	-0.200 to -0.040	1.244 to 1.277	0.99
	Barra Grande-PI	7	29	0.011	1.222	-0.109 to 0.131	1.187 to 1.256	0.99
	Jericoacara-CE	8	267	0.081	1.229	0.014 to 0.149	1.216 to 1.242	0.99
	Canoa Quebrada-CE	10	10	-0.096	1.254	-0.726 to 0.533	1.134 to 1.375	0.98
	Pirangi do Sul-RN	12	25	0.032	1.197	-0.130 to 0.194	1.153 to 1.241	0.99
	Cabo Branco-PB	13	141	-0.062	1.246	-0.114 to -0.010	1.231 to 1.262	0.99
	Saco-SE	15	122	0.010	1.234	-0.079 to 0.099	1.214 to 1.254	0.99
	Milagres-BA	18	17	0.078	1.164	-0.023 to 0.181	1.123 to 1.205	0.99
	Cabralia-BA	19	24	0.004	1.199	-0.063 to 0.072	1.165 to 1.233	0.99
	Castelhanos-ES	20	38	-0.080	1.253	-0.246 to 0.085	1.204 to 1.303	0.98
	Tartaruga-RJ	21	46	0.036	1.204	0.284 to -0.210	1.246 to 1.161	0.98
Bathygobius geminatus Tornabene, Baldwin & Pezold, 2010	Jericoacara-CE	8	96	0.085	1.227	0.169 to 0.169	1.253 to 1.253	0.98
	Canoa Quebrada-CE	10	13	0.108	1.193	-0.158 to 0.375	1.127 to 1.258	0.99
	Pirangi do Sul-RN	12	36	0.025	1.226	-0.091 to 0.143	1.185 to 1.268	0.99
	Cabo Branco-PB	13	132	0.108	1.182	0.010 to 0.207	1.145 to 1.220	0.96
	Saco-SE	15	24	0.240	1.198	-0.113 to 0.594	1.103 to 1.293	0.96
	Forte-BA	16	20	-0.003	1.222	-0.446 to 0.438	1.048 to 1.396	0.94
	Itapoã-BA	17	21	-0.044	1.219	-0.329 to 0.240	1.071 to 1.367	0.94
	Cabralia-BA	19	15	-0.082	1.277	-0.245 to 0.079	1.216 to 1.337	0.99
	Castelhanos-ES	20	67	0.190	1.125	0.074 to 0.306	1.072 to 1.178	0.96
	Tartaruga-RJ	21	17	-0.126	1.244	-0.264 to 0.012	1.212 to 1.276	0.99
Haemulidae								
Genyatremus luteus (Bloch, 1790)	Farol Velho-PA	5	14	0.266	1.137	-0.304 to 0.837	0.992 to 1.282	0.96
Sparidae								
Diplodus argenteus (Valenciennes, 1830)	Tartaruga-RJ	21	21	-0.508	1.416	-1.384 to 0.366	1.208 to 1.624	0.91
Labrisomidae								
Labrisomus nuchipinnis (Quoy & Gaimard, 1824)	Pirangi do Sul-RN	12	9	0.024	1.203	-0.398 to 0.447	1.132 to 1.274	0.99
	Forte-BA	16	14	-0.086	1.204	-0.504 to 0.331	1.153 to 1.255	0.99
	Itapoã-BA	17	18	0.116	1.160	0.0298 to 0.202	1.146 to 1.174	0.99
	Milagres-BA	18	28	0.149	1.157	0.005 to 0.292	1.102 to 1.212	0.98
Malacoctenus delalandii (Valenciennes, 1836)	Jericoacara-CE	8	85	0.075	1.194	-0.059 to 0.210	1.158 to 1.230	0.98
	São Miguel do Gostoso-RN	11	10	0.015	1.230	-0.154 to 0.186	1.168 to 1.291	0.99
	Pirangi do Sul-RN	12	15	-0.056	1.231	-0.277 to 0.164	1.138 to 1.323	0.98
Lutjanidae								
Lutjanus alexandrei Moura & Lindeman, 2007	Jericoacara-CE	8	11	0.170	1.226	-0.157 to 0.497	1.176 to 1.275	0.99
Pomacentridae								
Abudefduf saxatilis (Linnaeus, 1758)	Jericoacara-CE	8	180	-0.145	1.393	-0.249 to -0.041	1.366 to 1.420	0.98
	Canoa Quebrada-CE	10	41	-0.184	1.356	-0.369 to 0.001	1.273 to 1.439	0.96
	Saco-SE	15	136	0.030	1.286	-0.053 to 0.115	1.247 to 1.326	0.96
Stegastes fuscus (Cuvier, 1830)	Pirangi do Sul-RN	12	11	-0.388	1.378	-0.957 to 0.180	1.221 to 1.535	0.97
	Forte-BA	16	33	-0.063	1.320	-0.190 to 0.062	1.292 to 1.348	0.96
	Itapoã-BA	17	51	-0.052	1.304	-0.146 to 0.041	1.282 to 1.326	0.99
Stegastes variabilis (Castelnau, 1855)	Forte-BA	16	92	-0.021	1.324	-0.124 to 0.080	1.302 to 1.346	0.99
	Itapoã-BA	17	16	0.515	1.165	0.050 to 0.981	1.039 to 1.291	0.96

n, number of specimens; CL, confidence limits; Species in bold have length-length relationships estimates included in fishBase database (Froese & Pauly 2016) and literature.

Table 2. Estimated parameters of length-weight relationships (LWR) for 18 fish species in 21 rockpool sites along Brazilian coast.

Family/Species	Site code	n	TL (cm)		TW (g)		Regression parameters					T-test
			Min	Max	Min	Max	*a*	*b*	95% CL of *a*	95% CL of *b*	r^2	
Gobiesocidae												
Gobiesox barbatulus Starks, 1913	4	23	1.9	6.0	0.08	3.64	0.013	2.912	0.010 to 0.017	2.681 to 3.143	0.97	0.22
	5	36	1.6	5.9	0.06	2.80	0.012	2.994	0.011 to 0.012	2.870 to 3.118	0.99	0.46
Blenniidae												
Hypleurochilus fissicornis (Quoy & Gaimard, 1824)	8	82	1.7	5.6	0.04	2.05	0.010	3.116	0.008 to 0.011	2.969 to 3.261	0.96	0.06
Omobranchus punctatus (Valenciennes, 1836)	4	11	1.8	5.5	0.03	0.9	0.005	3.086	0.003 to 0.007	2.753 to 3.418	0.98	0.28
	5	50	1.8	6.8	0.02	1.61	0.005	3.064	0.004 to 0.006	2.949 to 3.178	0.98	0.13
Scartella cristata (Linnaeus, 1758)	8	264	2.3	10	0.15	13.19	0.012	3.002	0.011 to 0.013	2.935 to 3.069	0.97	0.47
	10	735	1.5	8.6	0.03	8.0	0.010	3.135	0.009 to 0.010	3.101 to 3.169	0.97	0.00
	11	39	1.2	6.5	0.02	3.42	0.009	3.099	0.007 to 0.011	2.960 to 3.238	0.98	0.07
	13	10	2.4	6.3	0.12	2.33	0.009	2.988	0.007 to 0.013	2.775 to 3.202	0.99	0.45
	14	65	1.0	8.3	0.01	5.85	0.009	3.037	0.008 to 0.010	2.890 to 3.185	0.96	0.30
	17	110	1.1	7.8	0.01	5.61	0.009	3.082	0.008 to 0.010	2.952 to 3.206	0.96	0.09
	18	97	1.1	6.4	0.02	2.53	0.009	3.019	0.008 to 0.010	2.911 to 3.127	0.97	0.35
Entomacrodus vomerinus Valenciennes, 1836	17	90	2.0	10	0.06	8.33	0.007	3.090	0.006 to 0.008	3.015 to 3.164	0.99	0.01
Carangidae												
Caranx latus Agassiz, 1831	15	15	4.8	8.9	1.53	9.52	0.014	2.962	0.009 to 0.021	2.745 to 3.179	0.99	0.35
Gerreidae												
Eucinostomus melanopterus (Bleeker, 1863)	15	90	1.1	4.4	0.01	0.79	0.009	2.812	0.009 to 0.010	2.693 to 2.931	0.96	0.01
Gobiidae												
Ctenogobius boleosoma (Jordan & Gilbert, 1882)	15	216	1.1	4.2	0.01	0.39	0.006	2.878	0.005 to 0.006	2.803 to 2.952	0.96	0.01
	20	24	1.1	4.0	0.01	0.35	0.006	2.901	0.006 to 0.008	2.724 to 3.077	0.98	0.13
Bathygobius soporator (Valenciennes, 1837)	1	95	1.4	9.3	0.02	9.46	0.010	3.046	0.008 to 0.012	2.956 to 3.135	0.98	0.15
	2	213	2.6	7.8	0.22	6.62	0.009	3.151	0.008 to 0.011	3.072 to 3.230	0.97	<0.001
	3	55	2	10.4	0.11	13.93	0.013	2.968	0.011 to 0.015	2.887 to 3.049	0.99	0.22
	6	34	0.8	9.9	0.01	13.79	0.009	3.129	0.007 to 0.012	2.960 to 3.298	0.98	0.06
	7	29	1.5	7.0	0.03	3.92	0.008	3.165	0.007 to 0.009	3.079 to 3.251	0.99	<0.001
	8	267	1.5	10.5	0.05	15.8	0.009	3.169	0.008 to 0.010	3.114 to 3.225	0.98	<0.001
	10	10	1.4	9.3	0.03	10.15	0.010	3.051	0.008 to 0.013	2.922 to 3.180	0.99	0.19
	12	25	0.8	8.5	0.01	7.06	0.008	3.137	0.006 to 0.010	2.930 to 3.345	0.98	0.09
	13	141	1.0	7.8	0.01	5.97	0.008	3.198	0.007 to 0.008	3.149 to 3.247	0.99	<0.001
	15	122	1.0	10.6	0.01	15.78	0.008	3.155	0.008 to 0.009	3.114 to 3.195	0.99	<0.001
	18	17	1.2	6.5	0.01	2.75	0.007	3.169	0.006 to 0.009	2.974 to 3.365	0.99	0.04
	19	24	1.0	4.5	0.01	0.7	0.007	3.042	0.006 to 0.009	2.820 to 3.263	0.97	0.34
	20	38	2.2	7.8	0.12	4.76	0.011	2.913	0.010 to 0.013	2.795 to 3.032	0.99	0.07
	21	46	1.0	10.9	0.01	12.08	0.011	2.973	0.009 to 0.012	2.900 to 3.046	0.99	0.23
Bathygobius geminatus Tornabene, Baldwin & Pezold, 2010	8	96	1.6	6.9	0.04	4	0.008	3.135	0.007 to 0.010	3.034 to 3.237	0.98	<0.001
	10	13	1	7.8	0.01	4.25	0.010	3.008	0.008 to 0.013	2.844 to 3.173	0.99	0.45
	12	36	1.5	6.1	0.03	1.64	0.008	3.002	0.007 to 0.011	2.829 to 3.175	0.97	0.49
	13	132	1.6	4.7	0.04	1.06	0.009	3.055	0.008 to 0.010	2.969 to 3.141	0.97	0.10
	15	24	1.8	8.6	0.06	7.69	0.008	3.142	0.007 to 0.009	3.040 to 3.244	0.99	0.49
	16	20	1.8	3.4	0.05	0.46	0.010	3.066	0.008 to 0.012	2.854 to 3.278	0.98	0.26
	17	21	1.2	3.8	0.01	0.47	0.007	3.096	0.006 to 0.009	2.846 to 3.346	0.97	0.21
	19	15	1.7	4.5	0.04	0.83	0.007	3.084	0.005 to 0.010	2.819 to 3.350	0.98	0.25
	20	67	1.1	4.2	0.01	0.66	0.009	3.008	0.008 to 0.010	2.878 to 3.138	0.97	0.45
	21	17	2.7	7.5	0.22	4.63	0.010	2.997	0.009 to 0.011	2.925 to 3.070	0.99	0.47
Haemulidae												
Genyatremus luteus (Bloch, 1790)	5	14	3.8	6.2	0.85	3.97	0.019	2.910	0.012 to 0.031	2.609 to 3.210	0.97	0.26
Sparidae												
Diplodus argenteus (Valenciennes, 1830)	21	21	3.6	6.4	0.51	2.99	0.010	3.045	0.008 to 0.014	2.871 to 3.218	0.99	0.30
Labrisomidae												
Labrisomus nuchipinnis (Quoy & Gaimard, 1824)	12	9	2.3	11.0	0.10	17.09	0.006	3.258	0.004 to 0.008	3.109 to 3.407	0.99	0.01
	16	14	6.2	11.9	2.95	20.66	0.007	3.184	0.005 to 0.012	2.990 to 3.379	0.99	0.01
	17	18	2.2	13	0.08	29.4	0.006	3.261	0.005 to 0.008	3.135 to 3.387	0.99	<0.001

n. number of specimens; TL. total length; TW. weight; CL. Confidence limits; Species in bold have length-weight relationships estimates included in FishBase database (Froese & Pauly 2016) and literature.

Table 2. Continued...

Family/Species	Site code	n	TL (cm)		TW (g)		Regression parameters					T-test
			Min	Max	Min	Max	*a*	*b*	95% CL of *a*	95% CL of *b*	r^2	
	18	28	2	7.3	0.04	2.41	0.004	3.196	0.003 to 0.005	2.935 to 3.457	0.96	0.06
Malacoctenus delalandii (Valenciennes, 1836)	8	85	3	6.5	0.31	3.16	0.012	2.987	0.010 to 0.013	2.898 to 3.076	0.98	0.39
	11	10	2	5.1	0.06	1.32	0.006	3.175	0.005 to 0.009	2.936 to 3.414	0.99	0.03
	12	15	1.7	4.2	0.04	0.65	0.008	3.028	0.006 to 0.010	2.792 to 3.263	0.98	0.40
Lutjanidae												
Lutjanus alexandrei Moura & Lindeman, 2007	8	11	4.4	11.3	1.25	19.76	0.015	2.955	0.007 to 0.030	2.625 to 3.285	0.98	0.38
Pomacentridae												
Abudefduf saxatilis (Linnaeus, 1758)	8	180	1.6	11.3	0.07	32.64	0.020	2.973	0.018 to 0.022	2.904 to 3.041	0.98	0.22
	10	41	1.7	5	0.08	1.96	0.015	3.016	0.014 to 0.018	2.892 to 3.139	0.98	0.39
	15	136	1.3	4.9	0.05	2.15	0.013	3.135	0.012 to 0.015	3.018 to 3.252	0.95	0.01
Stegastes fuscus (Cuvier, 1830)	12	11	2.3	7.2	0.02	7.16	0.017	3.040	0.011 to 0.025	2.769 to 3.310	0.99	0.37
	16	33	1.6	9.9	0.08	23.76	0.016	3.087	0.014 to 0.018	3.005 to 3.168	0.99	0.01
	17	51	1.4	12.0	0.07	41.63	0.014	3.135	0.012 to 0.017	3.039 to 3.230	0.99	<0.01
Stegastes variabilis (Castelnau, 1855)	16	92	1.5	10.4	0.08	26.18	0.016	3.083	0.014 to 0.018	3.0213 to 3.145	0.99	<0.01
	17	16	2.6	6.7	0.36	5.68	0.016	3.069	0.011 to 0.023	2.8287 to 3.310	0.99	0.27

n. number of specimens; TL. total length; TW. weight; CL. Confidence limits; Species in bold have length-weight relationships estimates included in FishBase database (Froese & Pauly 2016) and literature.

information of *Omobranchus punctatus* (Valenciennes, 1836), an exotic fish species from Indo-Pacific region widely introduced in the Western Atlantic Coast (Lasso-Alcalá et al. 2011).

Conversions among length measurements are given in Table 1. The parameter *b* of the LLR ranged from 1.125 for *Bathygobius geminatus* from Castelhanos beach (site 20, Espírito Santo state), to 1.416 for *Diplodus argenteus* from Tartaruga beach (site 21, Rio de Janeiro state). The slope (*b*) of LWR ranged from 2.81 for *Eucinostomus melanopterus* (Bleeker, 1863) from Saco beach (site 15, Sergipe state), to 3.26 for *Labrisomus nuchipinnis* from Itapuã beach (site 17, Bahia state), thus within the expected range of 2.50–3.50, as suggested by Froese (2006). The calculated mean value of all 59 estimations of *b* (± SE) was 3.06 (± 0.09), thus indicating a tendency towards positive allometry, which is in agreement with the majority of fish species (Froese 2006). Concerning the type of growth, 39 estimations (66.1% of the overall 59 estimations species × sites) evidenced isometric growth ($b = 3$), 17 estimations (28.8%) showed positive allometry ($b > 3$), and three estimations (5.1%) negative allometry (Figure 2). The most represented families in terms of species richness did not show a consistent tendency in type of growth among species.

Almost 90% of all fish collected were juveniles of larger species or adults of small species. Samples of three species [i.e. *Caranx latus* (Agassiz, 1831), *Diplodus argentus* and *Abdefduf saxatilis* (Linnaeus, 1758)] were represented by small length ranges due to the nursery function of the sampled tidalpools. Therefore, LWRs biased by inclusion of small specimens, should be used with caution (Giarrizzo et al. 2006). As pointed out by Petrakis and Stergiou (1995), the application of these LWRs should be limited to the size ranges used to estimate the parameters. Geographical differences in type of growth were observed for nine fish species. Seven species presented isometry or positive allometry, depending on the geographic site sampled [*Scartella cristata*, *Bathygobius geminatus*, *Labrisomus nuchipinnis*, *Malacoctenus delalandii* (Valenciennes, 1836), *Abudefduf saxatilis*, *Stegastes fuscus* (Cuvier, 1830) and *Stegaste variabilis* (Castelnau, 1855)]. One species presented isometry or negative allometry [*Ctenogobius boleosoma* (Jordan & Gilbert, 1882)] and the *Bathygobius soporator* species showed isometry and negative and positive allometry. The observed differences among localities could be explained by a number of factors including temperature, salinity, food availability, gonadal development, number of specimens, and the variations in the range of length of the sample populations (Pauly 1984, Weatherley & Gill 1987, Giarrizzo et al. 2006, Joyeux et al. 2008, Macieira & Joyeux 2008).

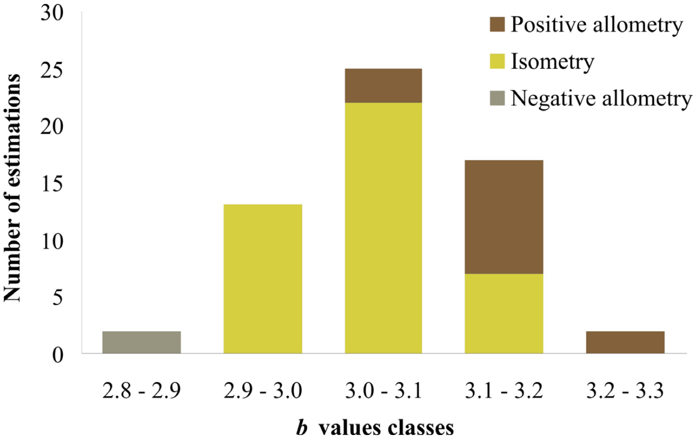


Figure 2. Frequency distribution of *b* values of 59 LWR estimations computed for 18 fish species caught in tidepool along the Brazilian coast.

In conclusion, this study has provided baseline information on the LWR and LLR of rockpool fish fauna sampled along a wide coastal sector of Brazil. The results obtained from this study will be useful to fishery biologists to employ adequate estimations for specific geographic areas.

Acknowledgements

The authors acknowledge Alfredo Carvalho Filho for assistance in identification of fish species and Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) for licensing n° 24917 for Biological material collected. First and second author acknowledge financial support by the CAPES and CNPQ-PPGEAP, respectively. The last author, Tommaso Giarrizzo, receives a productivity grant from the Conselho Nacional de Desenvolvimento Científico e Tecnológico [National Council for Scientific and Technological Development (CNPq; process CNPq # 310299/2016-0)].

Author Contributions

Fabiola Seabra Machado: Substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision, adding intelectual content.

Rory Romero de Sena Oliveira: Contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision, adding intelectual content.

Arianderson Texeira Silva: Contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation.

Tommaso Giarrizzo: Substantial contribution in the concept and design of the study; contribution to data collection; contribution to manuscript preparation; contribution to critical revision, adding intelectual content.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

CARPENTER, K.E. 2002a. The living marine resources of the western central Atlantic. FAO - Species Identification Guide for Fishery Purposes 2.

CARPENTER, K.E. 2002b. The living marine resources of the western central Atlantic. FAO - Species Identification Guide for Fishery Purposes 3.

CARVALHO FILHO, A. 1999. Peixes: costa brasileira. São Paulo: Melro 1.

ESCHMEYER, W.N., FRICKE, R. & VAN R. D. L. (EDS). 2016. Catalog of fishes: genera, species, references. World Wide Web electronic publication, www.calacademy.org, version accessed (11/2016).

FIGUEIREDO, J.L. & MENEZES, N. 1978. Manual dos peixes marinhos do sudeste do Brasil. Museu de Zoologia da USP, São Paulo, II Teleostei 1.

FIGUEIREDO, J.L. & MENEZES, N. 1980a Manual dos peixes marinhos do sudeste do Brasil. Museu de Zoologia da USP, São Paulo, III Teleostei 2.

FIGUEIREDO, J.L. & MENEZES, N. 1980b. Manual dos peixes marinhos do sudeste do Brasil. Museu de Zoologia da USP, São Paulo, IV Teleostei 3.

FROESE, R. 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. J. Appl. Ichthyol. 22:241-253.

FROESE, R, TSIKLIRAS, A.C. & STERGIOU, K.I. 2011. Editorial note on weight–length relations of fishes. Acta Ichtyol. Pisc. 41(4):261-263.

FROESE, R. & PAULY, D. 2016. FishBase. World Wide Web electronic publication, www.fishbase.org, version (01/2016).

GIARRIZZO, T., JESUS, A.J.S., LAMEIRA, E.C., ALMEIDA, J.B.A., ISSAC, V.J. & SAINT-PAUL, U. 2006. Weight-length relationships for intertidal fish fauna in a mangrove estuary in Northern Brazil. J. Appl. Ichthyol. 22:325-327.

HOSSAIN, M.Y., AHMED, Z.F., LEUNDA, P.M., JASMINE, S., OSCOZ, J., MIRANDA, R. & OHTOMI, J. 2006. Condition, length–weight and length–length relationships of the Asian striped catfish *Mystus vittatus* (Bloch, 1794) (Siluriformes: Bagridae) in the Mathabhanga River, southwestern Bangladesh. J. Appl. Ichthyol. 22: 304-307.

JOYEUX, J.C., GIARRIZZO, T., MACIEIRA, R.M., SPACH, H.L. & VASKE T., Jr. 2008. Length–weight relationships for Brazilian estuarine fishes along a latitudinal gradient. J. Appl. Ichthyol. 25(3):350-355.

LASSO-ALCALÁ, O NUNES, J.L.S., LASSO, C., POSADA, J., ROBERTSON, R., PIORSKI, N.M., TASSELL, J.V., GIARRIZZO, T. & GONDOLO, G. 2011. Invasion of the indo-pacific blenny *Omobranchus punctatus* (Perciformes: Blenniidae) on the Atlantic coast of central and south America. Neotrop. Ichthyol. 9(3):571-578.

MACIEIRA, R.M. & JOYEUX, J.C. 2008. Length–weight relationships for rockpool fishes in Brazil. J. Appl. Ichthyol. 25(3):358-359.

MENDES, B., FONSECA, P. & CAMPOS, A. 2004. Weight–length relationships for 46 fish species of the Portuguese west coast. J. Appl. Ichthyol. 20(5):355-361.

MENEZES, N.A. & FIGUEIREDO, J.L. 1985. Manual de peixes marinhos do sudeste do Brasil. Museu de Zoologia da USP, São Paulo, Teleostei 4.

MOUTOPOULOS, D.K. & STERGIOU, K.I. 2002. Length-weight and length-length relationships of fish species from the Aegean Sea (Greece). J. Appl. Ichthyol. 18(3):200-203.

MORATO. T., AFONSO, P., LOURINHO, P., BARREIROS, J.P., SANTOSA, R.S. & NASHC, R.D.M. 2001. Length-weight relationships for 21 coastal fish species of the Azores, north-eastern Atlantic. Fish. Res. 50:297-302.

MOREY, G., MORANTA, J., MASSUTÍ, E., GRAU, A., LINDE, M., RIERA, F. & MORALES-NIN, N. 2003. Weight-length relationships of littoral to lower slope fishes from the western Mediterranean. Fish. Res. 62(1):89-96.

PAULY, D. 1984. Fish population dynamics in tropical waters: a manual for use with programmable calculators. ICLARM Stud. Rev. 8.

SOKAL, R.R. & ROHLF, F.J. 1987. Introduction to Biostatistics. Freeman, New York, USA.

WEATHERLEY, A.H. & GILL, H.S. 1987. The biology of fish growth. Academic Press, London.

VAZ-DOS-SANTOS, A.M. & GRIS, B. 2016. Length-weight relationships of the ichthyofauna from a coastal subtropical system: a tool for biomass estimates and ecosystem modelling. Biota Neotrop. 16(3): e20160192.

Received: 17/08/2016
Revised: 19/07/2017
Accepted: 21/07/2017

Biota Neotropica 17(3): e20170326, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Short Communication

Common Vampire Bat (*Desmodus rotundus*) feeding on Lowland Tapir (*Tapirus terrestris*) in an Atlantic Forest remnant in southeastern Brazil

***Ariana Pignaton Gnocchi*[1] *& Ana Carolina Srbek-Araujo*[1,2,*]**

[1]*Universidade Vila Velha, Programa de Pós-Graduação em Ecologia de Ecossistemas, Laboratório de Ecologia e Conservação de Biodiversidade, Rua Comissário José Dantas de Melo, nº 21, Boa Vista, CEP 29102-920, Vila Velha, ES, Brazil*
[2]*Instituto SerraDiCal de Pesquisa e Conservação, Belo Horizonte, MG, Brazil*
**Corresponding Author: Dr. Ana Carolina Srbek-Araujo, email: srbekaraujo@hotmail.com*

GNOCCHI, A. P., SRBEK-ARAUJO, A. C. **Common Vampire Bat (*Desmodus rotundus*) feeding on Lowland Tapir (*Tapirus terrestris*) in an Atlantic Forest remnant in southeastern Brazil**. Biota Neotropica. 17(3): e20170326. http://dx.doi.org/10.1590/1676-0611-BN-2017-0326

***Abstract*:** There are few studies on the diet of Common Vampire Bat (*Desmodus rotundus*), despite its wide geographic distribution. The species is considered exclusively hematophagous, and medium and large-sized mammals are their main prey. In this study we report evidences of Common Vampire Bat feeding on Lowland Tapir (*Tapirus terrestris*) in a protected area located in the north of the state of Espírito Santo, southeastern Brazil, from camera trap records. The bat tried to access the Lowland Tapir by the posterior dorsolateral side of the body, and used the mean stratum of the vegetation as a point of support and observation between the consecutive offensives on the prey. In the same reserve, there were also two events of bat offensives on domesticated ox (*Bos* sp.). But in these cases the attacks occurred from the scapular region of the prey. The record here reported represents the first documented attack of *Desmodus rotundus* on *Tapirus terrestris* in the Brazilian Atlantic Forest and one of the first records in the South America as a whole. Previous records were in the Pantanal (Brazil) and in the Amazon rainforest (Ecuador). The feeding on wild and domestic prey by *Desmodus rotundus* in the same locality may favor the transmission of rabies to populations of wild mammals, as well as to domestic animals, and may represent an economic and public health issue with negative effects also for wildlife.

Keywords: *camera trap, Chiroptera, hematophagy, micro predator, Sooretama Biological Reserve.*

Predação de antas (*Tapirus terrestris*) por morcegos vampiros (*Desmodus rotundus*) em área de Mata Atlântica no sudeste do Brasil

***Resumo*:** Apesar de sua ampla distribuição geográfica, pouco se conhece sobre a dieta do morcego vampiro (*Desmodus rotundus*). A espécie é considerada exclusivamente hematófaga, sendo os mamíferos de médio e grande porte suas principais presas. O presente trabalho apresenta evidências de predação de antas (*Tapirus terrestris*) por morcegos vampiros a partir de registros obtidos por armadilhas fotográficas em uma área protegida localizada no norte do estado do Espírito Santo, sudeste do Brasil. A partir dos registros fotográficos obtidos, foi possível observar que o morcego tentou acessar a anta pela parte dorsolateral posterior do corpo, utilizando o estrato médio da vegetação como ponto de apoio e observação entre as consecutivas investidas sobre a presa. Na mesma área, também foram registrados dois eventos de investida de morcegos sobre boi-doméstico (*Bos* sp.), mas, nestes casos, os ataques ocorreram a partir da região escapular da presa. O registro obtido representa o primeiro registro documentado de ataque de *Desmodus rotundus* sobre anta na Mata Atlântica brasileira e um dos primeiros na América do Sul de forma geral, havendo registros anteriores no Pantanal (Brasil) e na Floresta Amazônica (Equador). Ressalta-se que a utilização de presas silvestres e domésticas por *Desmodus rotundus* em uma mesma localidade pode favorecer a transmissão de raiva para populações de mamíferos silvestres, bem como para os rebanhos e outras espécies domésticas, podendo vir a representar um problema econômico e de saúde pública com efeitos negativos também sobre as espécies silvestres.

Palavras-chaves: *armadilha fotográfica, Chiroptera, hematofagia, micropredação, Reserva Biológica de Sooretama.*

Introduction

The Common Vampire Bat, *Desmodus rotundus* (É. Geoffroy, 1810) (Chiroptera, Phyllostomidae, Desmodontinae), is endemic to Latin America and is widely distributed, occurring from Mexico to central Argentina and Uruguay (Eisenberg & Redford 1999). It can be found in all Brazilian territory (Reis et al. 2013). Bats of this species roost in places of difficult access and form relatively small colonies, ranging from 10 to 200 specimens (Mayen 2003). Studies carried out in the Amazon region indicated *Desmodus rotundus* is not abundant, both in *terra firme* and in *várzea* forests (Bobrowiec et al. 2014), although the species is more frequently recorded in villages (Bobrowiec 2012). These areas would represent important foraging areas for Common Vampire Bats, where they would feed mainly on domestic animals, although human attacks may also occur (Bobrowiec 2012). Thus, when there are farms or villages near the areas where the Common Vampire Bats occur, the populations of *Desmodus rotundus* tend to increase due to the great availability of food (Bobrowiec et al. 2015).

There are few studies on the diet of *Desmodus rotundus*, despite its wide geographic distribution. Since the first report of *Desmodus rotundus* feeding, based on direct observations made by Charles Darwin in Chile (Darwin 1839), diverse techniques have been employed by researchers, including molecular (Bobrowiec et al. 2015) and isotopic analyzes (e.g. Voigt & Kelm 2006, Voigt et al. 2012, Streicker & Allgeier 2016), in addition to the camera traps (e.g. Castellanos & Banegas 2015, Galetti et al. 2016). Even with these methodological advances, the available records of diet of Common Vampire Bat are isolated and mainly from the western portion of South America (see discussion for details). It is known that this species is exclusively hematophagous and that its main prey are wild medium and large-sized mammals (Greenhall 1988). Common Vampire Bats use visual, acoustic and olfactory sensory abilities (Joermann et al. 1988) and infrared imaging pit organs (Campbell et al. 2002) to detect their potential prey, so they may also usually feed on livestock, horses, mules, goats, sheep, swine, and domestic birds (e.g. Delpietro et al. 1994, Bobrowiec 2012, Bobrowiec et al. 2015). These animals are frequently grouped in rural properties, being easily detected by Common Vampire Bats (Delpietro et al. 1994).

Here we report evidences of Common Vampire Bats feeding on Lowland Tapir, *Tapirus terrestris* (Linnaeus, 1758) (Perissodactyla, Tapiridae), in a protected area located in the north of the state of Espírito Santo, southeastern Brazil, from camera trap records.

Material and Methods

The records were obtained at Sooretama Biological Reserve (Reserva Biológica de Sooretama – RBS; 18º53' and 19º55' S, 39º55' and 40º15' W; 24,250 ha), located in the northern portion of the state of Espírito Santo, covering territories of Sooretama, Vila Valério, Jaguaré and Linhares municipalities. The RBS, together with the Vale Natural Reserve (Reserva Natural Vale – RNV; 22.711 ha), the Recanto das Antas Private Natural Heritage Reserve (Reserva Particular do Patrimônio Natural – RPPN Recanto das Antas) (221 ha) and the RPPN Mutum-Preto (379 ha), forms the largest continuous block of native vegetation in Espírito Santo, called Linhares-Sooretama Block (Srbek-Araujo et al. 2015). These reserves account for approximately 50,000 ha, representing more than 10% of the remaining forest area in the state (based on data available in FSOSMA & INPE 2014).

The Linhares-Sooretama Block is composed by a mosaic of habitats, in which the dense lowland forest (*Tabuleiro* forest) predominates (Jesus & Rolim 2005). This forest is interspersed with *várzea* forests, less dense forest on sandy soils (*Mussununga*) and occasional native grassland (*Campo nativo*) (Jesus & Rolim 2005, Kierulff et al. 2014). The climate in the region is tropical with dry winter, type Aw, according to Köppen classification (Alvares et al. 2014). The mean annual temperature in the region is 24.3°C, ranging between maximums around 30°C and minimum of 19°C (Kierulff et al. 2014). The average annual rainfall is around 1,214 mm, and the relative humidity of air is 84% (Kierulff et al. 2014). The Linhares-Sooretama Block is surrounded mainly by pastures, agricultural crops (especially fruit and coffee), and eucalyptus plantations.

The records were obtained by Bushnell Trophy Cam HD Aggressor digital camera traps (Bushnell Inc., Overland Park, USA). The camera traps were installed on 15 sampling points distributed along internal unpaved roads and in the forested interior of RBS and RNV. The camera traps were operated 24 hours a day. For each occasion of capture, a video (10 seconds duration) and two photographs were taken. The delay between consecutive records was set to 10 seconds. Camera traps were in place from October 2015 to August 2016, totaling 2,461 camera-days (for details on the calculation of the sampling effort, see Srbek-Araujo & Chiarello 2005).

Results and Discussion

Records evidencing the attempted predation of Lowland Tapir by Common Vampire Bats were obtained on November 17, 2015, at a sampling point located circa 30 m from Barra das Abóboras Road, close to the northern limit of RBS (18º59' S and 40º02' W). The feed attempts began at 02:04 h and followed for approximately three minutes, until 02:07 h. According to the sequence of videos and photographic records (six sets of records as a whole), the bat makes several attempts of attack, accessing the tapir by the posterior dorsolateral side of the body. Among the consecutive offensives on the prey, the bat rests on tree trunks with small diameter, in the middle layer of the vegetation, and wait for the best moment to make new attacks (Figure 1). During the record-keeping period, the Common Vampire Bat attempted to land on the posterior dorsolateral side of the tapir at least three times, all of them unsuccessful, and an aborted landing on the prey was also recorded. The tapir seems to notice the touch of the bat on all landing attempts and turns quickly to the side (Video 1).

On December 12, 2015, two other events of attempted predation by *Desmodus rotundus* were obtained at RBS, but on these occasions the attempts were on domesticated ox, *Bos* sp. (Linnaeus, 1758). The two events were obtained at the same sampling point, located at Barra das Abóboras Road, at the northern boundary of RBS (18º58' S e 40º06' W). Due to the proximity to pasture areas, which surrounding the reserve (neighboring properties), it is not uncommon to record domestic cattle on this road. Records were obtained between 21:30 h and 21:31 h (two consecutive videos and respective photos) and at 23:57 h (one set of records). The two events were on the same individual. On these occasions, at least two bats

Figure 1. Common Vampire Bat (*Desmodus rotundus*) perched on tree trunk after one of the attempted attacks on Lowland Tapir (*Tapirus terrestris*) at Sooretama Biological Reserve, Espírito Santo state, southeastern Brazil.

were observed simultaneously attacking the animal (Figure 2; Video 2). The attacks, with four offensives in the first event, occurred on the anterior part of the ox's body, near the scapular region, different from the attack observed on the tapir.

The record here reported represents the first documented record of *Desmodus rotundus* attack on *Tapirus terrestris* in the Brazilian Atlantic Forest. Previous records of Lowland Tapir predation by Common Vampire Bats in Brazil were obtained in the Pantanal (Galetti et al. 2016). There is also a record obtained in Ecuador (Amazon rainforest), in the region of the Yasuni National Park, where a bat was observed on the scapular region of a tapir (Castellanos & Banegas 2015). In these two studies, the records were also obtained from camera traps. Predation records of *Desmodus rotundus* on other wild mammals are still known, such as the South American Sea Lions, *Otaria flavescens* (Shaw, 1800), on islands along the coast of Peru (Catenazzi & Donelly 2008); the South American Red Brocket, *Mazama americana* (Erxleben, 1777), in the Atlantic Forest and in the Pantanal (Galetti et al. 2016); and the White-tailed Deer, *Odocoileus virginianus* (Zimmermann, 1780), in the El Platanal tropical forest, located in the Sierra Gorda Biosphere Reserve in Mexico (Sanchez-Cordero et al. 2011). Common Vampire Bats feed on invasive wild feral pigs (*Sus scrofa* Linnaeus, 1758) in the Atlantic Forest and in the Pantanal (Galetti et al. 2016). There are also reports of attacks on goats, cows and domestic pigs on farms in Argentina (Delpietro et al. 1994) and in different locations in the Amazon region, including attacks on domestic dogs (e.g. Bobrowiec 2012, Bobrowiec et al. 2015). Attacks on domestic pigs were also observed in the Atlantic Forest (Galetti et al. 2016). Records of predation of *Desmodus rotundus* on avian species are rare. There are records of attack on juvenile Humboldt Penguins (*Spheniscus humboldti* Meyen, 1834), in Pan de Azucar island in Chile (Luna-Jorquera & Culik 1995), and on chickens in Amazonia (e.g. Bobrowiec 2012, Bobrowiec et al. 2015).

To avoid predation, hematophagous bats exhibit cryptic foraging behavior, hunting their prey preferentially on darkest hours of the night (Flores-Crespo et al. 1974) and/or on darker nights (Delpietro et al. 1994). This behavior is corroborated by that recorded on the present study. According to the moon phase's calendar, the moon was in its New Moon phase on the days when our records were obtained.

The attacks on domesticated ox, in which at least two bats were observed, may constitute a typical hunting strategy of Common Vampire Bats (Greenhall 1971). According to Greenhall (1971), haematophagous bats usually hunt in groups of 2-6 individuals, and these groups live inside the colonies as a social structure. In our recorded cases, both the tapir and the domesticated ox, bats accessed their prey from the dorsal part of the body, although in distinct specific regions, similar to that observed in other records of attacks on medium and large sized mammals (e.g. Sanchez-Cordero et al. 2011, Castellanos & Banegas 2015, Galetti et al. 2016). It may characterize the typical attack strategy of *Desmodus rotundus* when the prey are moving. On the other hand, when feeding on South American Sea Lions, the prey were lying on the ground and the Common Vampire Bats were observed feeding on the neck and on the flipper of individuals (Catenazzi & Donelly 2008). In a resting domestic pig, the Common Vampire Bats feed on the ear of the prey (Galetti et al. 2016).

Figure 2. Two Common Vampire Bats (*Desmodus rotundus*) attacking a domesticated ox (*Bos* sp.) inside the Sooretama Biological Reserve, Espírito Santo state, southeastern Brazil.

Similarly to the behavior observed in the present study, the perception of the approach of the bats resulting in sudden movements to avoid predation was also registered in South American Red Brocket in the Atlantic Forest (Galetti et al. 2016). In both cases, bats tried to access the prey by the posterior dorsolateral side of the body resulting on unsuccessful attacks. It may suggest a lower attack efficiency performed by *Desmodus rotundus* attacking the posterior dorsal part of wild prey.

Only three independent records (more than 1 hour interval between records of the same species for each sampling point) of predation by Common Vampire Bats were obtained in the Linhares-Sooretama Block from a total of 1,087 independent nocturnal records of mammals. By analyzing only Lowland Tapir records, 191 independent nocturnal records of the species were obtained, resulting in a 0.5% predator-prey encounter rate recorded by camera traps (1 independent record of predation). For the domestic ox, only four independent nocturnal records were obtained, resulting in a 50% predator-prey encounter rate (2 independent records of predation). These values may indicate a preference in feeding on domestic prey by *Desmodus rotundus* even inside protected areas. The consumption of blood of wildlife species may decline in areas where the availability of livestock is greater (Streicker & Allgeier 2016). In these areas, cattle may become the main food source of *Desmodus rotundus* (Streicker & Allgeier 2016), with a high degree of preference for cows in some sites (Voigt & Kelm 2006). In the study developed by Galetti et al. (2016), the chances of a tapir being attacked by a Common Vampire Bat was estimated at 11% in the Pantanal. Although there is no abundance data available for *Desmodus rotundus* in the Linhares-Sooretama Block, the difference in rate of attack of tapirs between the two studies may indicate that the density of Common Vampire Bat in our study area is lower than in the region studied by Galetti et al. (2016).

The Common Vampire Bats can become infected with the rabies virus, which can lodge in the salivary glands of bats, allowing the transmission of the disease through its bite (Mayen 2003). Rabies can cause the death of wild mammals, including carnivores and ungulates (e.g. MacDonald 1993, Bengis et al. 2008), and domestic species, leading to heavy economic losses as result of herd infection and death of livestock (Almeida et al. 2008). We highlighted that diseases transmitted between wild and domestic species can be bidirectional, as in the case of rabies, and may be fatal for both groups and with potential for transmission to humans (Bengis et al. 2008). In this way, the potential transmission of rabies to humans and domestic animals, whether through the bite of infected bats or from the bite of sick domestic animals (especially domestic dog and domestic cat), can result in public health problems, mainly in rural areas and more isolated human villages. For this reason, it is important to maintain the vaccination of cattle and other domestic animals in areas subject to the presence of Common Vampire Bats, both for domestic animal welfare and for the protection and conservation of wildlife. In addition, it can be done the vaccinating of *Desmodus rotundus* against rabies applying a paste mixed with oral vaccine on the back of captured animals to indirectly protect other bats from the same colony (Almeida et al. 2008). The Lowland Tapir is classified as Vulnerable to extinction at global level (IUCN 2016) and in Brazilian territory (Ministério do Meio Ambiente 2014), and as Endangered in the state of Espírito Santo, where the species is restricted to a small number of protected areas (Chiarello et al. 2007, Flesher & Gatti 2010). It makes the contamination of the remaining populations of Lowland Tapir with rabies virus even more serious, particularly in Espírito Santo.

The records reported in the present study reinforce that camera traps may assist in obtaining sporadic records of predation by Common Vampire Bats, thus contributing to the investigation of their prey in natural habitat. The record here reported represents one of the first documented cases of *Desmodus rotundus* attack on *Tapirus terrestris* in Brazil and in South America as a whole, with previous records only in the Brazilian Pantanal and in the Ecuadorian Amazon. The feeding on wild and domestic prey by *Desmodus rotundus* in the same locality, as observed in the present study, may favor the transmission of rabies to populations of wild mammals, as well as to domestic species, and may represent an economic and public health issue with negative effects also for wildlife.

Supplementary material

The following online material is available for this article:

Video 1: Common Vampire Bat (*Desmodus rotundus*) attacking a Lowland Tapir (*Tapirus terrestris*) at Sooretama Biological Reserve, Espírito Santo state, southeastern Brazil.

Video 2: Two Common Vampire Bats (*Desmodus rotundus*) attacking a domesticated ox (*Bos* sp.) at Sooretama Biological Reserve, Espírito Santo state, southeastern Brazil.

Acknowledgments

APG is grateful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the postgraduate scholarship. ACSA tanks to Universidade Vila Velha and Fundação de Amparo à Pesquisa do Espírito Santo (FAPES 0607/2015), which sponsored the research of the Laboratório de Ecologia e Conservação de Biodiversidade (LECBio). The authors would like to thank anonymous reviewers for suggestions and comments.

Author Contributions

Ariana Pignaton Gnocchi: Analysis of camera trap records (photos and video) and preparation of the manuscript. Ana Carolina Srbek-Araujo: Camera trapping (data collection), analysis of camera trap records (photos and video), preparation of the manuscript and manuscript translation.

Conflicts of interest

The authors declare no conflict of interest.

References

ALMEIDA, M.F., MARTORELLI, L.F.A., AIRES, C.C., BARROS, R.F. & MASSAD, E. 2008. Vaccinating the vampire bat *Desmodus rotundus* against rabies. Virus Res. 137:275–277.

ALVAREZ, C.A., STAPE, J.L., SENTELHAS, P.C., GONÇALVES, J.L.M. & SPAROVEK, G. 2014. Köppen's climate classification map for Brazil. Meteorol. Z. 22(6):711–728.

BENGIS, R.G., KOCK, R.A. & FISCHER, J. 2002. Infectious animal diseases: the wildlife/livestock interface. Scientific and Technical Rev. - Off. Int. Epizoot. 21(1):53–65.

BOBROWIEC, P.E.D., LEMES, M.R. & GRIBEL, R. 2015. Prey preference of the common vampire bat (*Desmodus rotundus*, Chiroptera) using molecular analysis. J. Mammal. 96(1):54–63.

BOBROWIEC, P.E.D., ROSA, L.S., GAZARINI, J. & HAUGAASEN, T. 2014. Phyllostomid bat assemblage structure in Amazonian flooded and unflooded forests. Biotropica 46(3):312–321.

BOBROWIEC, P.E.D. 2012. A Chiroptera preliminary survey in the middle Madeira River region of Central Amazonia, Brazil. Mammalia 76:277–283.

CAMPBELL, A.L., NAIK, R.R., SOWARDS, L. & STONE, M.O. 2002. Biological infrared imaging and sensing. Micron 33:211–225.

CASTELLANOS, A.X. & BANEGAS, G.A. 2015. Vampire bats bite lowland tapirs in Yasuni National Park, Ecuador. Tapir Conservation 24(33):7.

CATENAZZI, A. & DONELLY, M.A. 2008. Sea lion *Otaria flavescens* as host of the common vampire bat *Desmodus rotundus*. Mar. Ecol-Prog. Ser. 360:285–289.

CHIARELLO, A.G., COSTA, L.P., LEITE, Y.L.R., PASSAMANI, M., SICILIANO, S. & ZORTÉA, M. 2007. Os Mamíferos Ameaçados de Extinção no Estado do Espírito Santo. In Espécies da fauna ameaçadas de extinção no estado do Espírito Santo (M. Passamani & S.L. Mendes, eds). Instituto de Pesquisas da Mata Atlântica, Vitória, p.29–45.

DARWIN, C.R. 1839. The zoology of the voyage of H.M.S. Beagle. Part 2: Mammalia. By George R. Waterhouse. Edited and superintended by Charles Darwin. Smith Elder and Co., London.

DELPIETRO, H., KONOLSAISEN, F., MARCHEVSKY, N. & RUSSO, G. 1994. Domestic cat predation on vampire bats (*Desmodus rotundus)* while foraging on goats, pigs, cows and human beings. Appl. Anim. Behav. Sci. 39:141–150.

EISENBERG, J.F. & REDFORD, K.H. 1999. Mammals of the Neotropics. Volume 3: Ecuador, Peru, Bolivia, Brazil. The University of Chicago Press, Chicago and London.

FLESHER, K.M. & GATTI, A. 2010. Tapirus terrestris in Espírito Santo, Brazil. Tapir Conservation 19/1(26):16–23.

FLORES CRESPO, R., LINHART, S.B., BURNS, R.J. & MITCHELL, G.C. 1972. Foraging behavior of the common vampire bat related to moon light. J. Mammal. 53:366–368.

FSOSMA & INPE. 2014. Atlas dos Remanescentes Florestais da Mata Atlântica - Período 2012–2013. São Paulo, Fundação SOS Mata Atlântica & Instituto Nacional de Pesquisas Espaciais. 61p. https://www.sosma.org.br/wp-content/uploads/2014/05/atlas_2012-2013_relatorio_tecnico_20141.pdf, (last access on 23/11/2014).

GALETTI, M., PEDROSA, F., KEUROGHLIAN, A. & SAZIMA, I. 2016. Liquid lunch - vampire bats feed on invasive feral pigs and other ungulates. Front. Ecol. Environ. 14:505–506.

GREENHALL, A.M .1971. Control of vampire bats. Study and program project for Latin. America. Bol. Oficina Sanit. Panam. 71:231–246.

GREENHALL, A.M .1988. Feeding behavior. In: GREENHALL A.M, SCHMIDT, U. (eds) Natural history of vampire bats, pp 111-132. CRC Press, Boca Raton, FL.

IUCN. 2016. The IUCN Red List of Threatened Species. Version 2016-1. International Union for Conservation of Nature and Natural Resources, Cambridge.

JESUS, R.M. & ROLIM, S.G. 2005. Fitossociologia da Mata Atlântica. Boletim Técnico da Sociedade de Investigações Florestais 19:1–149.

JOERMANN, G., SCHMIDT, U. & SCHMIDT, C. 1988. The mode of orientation during flight and approach to landing in two Phyllostomid bats. Ethology 78:332–340.

KIERULFF, M.C.M., AVELAR, L.H.S., FERREIRA, M.E.S., POVOA, K.F. & BÉRNILS, R.S. 2014. Reserva Natural Vale: história e aspectos físicos. Ciência & Ambiente 49:7-40.

LUNA-JORQUERA, G. & CULIK, B.M. 1995. Penguins bled by vampires. J Ornithol 136:471–472.

MACDONALD, D.W. 1993. Rabies and wildlife: a conservation problem? Onderstepoort Vet. Res. 60:351–355.

MAYEN, F. 2003. Haematophagous bats in Brazil, their role in rabies transmission, impact on public health, livestock industry and alternatives to an indiscriminate reduction of bat population. J. Vet. Med. B 50:469–472.

MIALHE, P.J. 2013. Preferential prey selection by *Desmodus rotundus* (E. Geoffroy, 1810, Chiroptera, Phyllostomidae) feeding on domestic herbivores in the municipality of São Pedro – SP. Braz. J. Biol. 74(3):579–584.

MINISTÉRIO DO MEIO AMBIENTE. 2014. Lista Nacional Oficial de Espécies da Fauna Ameaçada de Extinção. Portaria no 444, de 17 de dezembro de 2014. Diário Oficial da União 245, Brasília.

REIS, N.R., FREGONEZI, M.N., PERACCHI, A.L. & SHIBATTA, O.A. 2013. Morcegos do Brasil: Guia de Campo. Technical Books Editora, Rio de Janeiro.

SANCHÉS-CORDEIRO, V., BOTELLO, F., MANGAÑA-COTA, G. & IGLESIAS, J. 2011. Vampire bats, *Desmodus rotundus*, feeding on white-tailed deer, *Odocoileus virginianus*. Mammalia 75:91–92.

SRBEK-ARAUJO, A.C. & CHIARELLO, A.G. 2005. Is camera-trapping an efficient method for surveying mammals in Neotropical forests? A case study in south-eastern Brazil. J. Trop. Ecol. 21(1):121–125.

SRBEK-ARAUJO, A.C., MENDES, S.L. & CHIARELLO, A.G. 2015. Jaguar (*Panthera onca* Linnaeus, 1758) roadkill in Brazilian Atlantic Forest and implications for species conservation. Braz. J. Biol. 75(3):581–586.

STREICKER, D.G. & ALLGEIER, J.E. 2016. Foraging choices of vampire bats in diverse landscapes: potential implications for land-use change and disease transmission. J. Appl. Ecol. 53:1280–1288.

VOIGT, C.C., VOIGT-HEUCKE, S.L. & SCHNEEBERGER, K. 2012. Isotopic data do not support food sharing within large networks of female vampire bats (*Desmodus rotundus*). Ethology 118:260–268.

VOIGT, C.C. & KELM, D.H. 2006. Host preference of the common vampire bat (*Desmodus rotundus*; Chiroptera) assessed by stable isotopes. J. Mammal. 87:1–6.

Received: 24/01/2017
Revised: 04/04/2017
Accepted: 28/06/2017

Biota Neotropica 17(3): e20170328, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Occurrence of leucism in *Eira barbara* (Carnivora, Mustelidae) in Brazil

Sonia Talamoni[1*], Pedro Igor Macario Viana[1], Claudia Guimarães Costa[1], Lauro Palú[2], Raphaela Barcelos Oliveira[1] & Leila Maria Pessôa[3]

[1]Pontificia Universidade Católica de Minas Gerais, Belo Horizonte, MG, Brazil.
[2]Reserva Particular do Patrimônio Natural - Santuário do Caraça, Santa Bárbara, MG, Brazil.
[3]Universidade Federal do Rio de Janeiro, Departamento de Zoologia, Rio de Janeiro, RJ, Brazil.
**Corresponding author: Sonia Talamoni, e-mail: talamoni@pucminas.br*

TALAMONI, S., VIANA, P. I. M., COSTA, C. G., PALÚ, L., OLIVEIRA, R. B., PESSÔA, L. M. **Occurrence of leucism in *Eira barbara* (Carnivora, Mustelidae) in Brazil.** Biota Neotropica. 17(3): e20170328. http://dx.doi.org/10.1590/1676-0611-BN-2017-0328

Abstract: The occurrence of anomalous coloration (albinism, leucism and melanism) in mammals is a rare phenomenon in nature, but this phenomenon has been reported for several species of mammals. In this study, we report on the occurrence of leucism in *Eira barbara* by examining three road-killed individuals and two sightings of live animals in Reserva Particular do Patrimônio Natural Santuário do Caraça, southeastern Brazil. In addition, we examined tayra specimens housed in mammal collections from Brazil and USA. The animals found dead and those sighted had a whitish yellow fur on the body and head, resulting in lighter coloration than the coloring pattern commonly observed in tayras. Despite these lighter color pattern, the specimens showed parts of soft tissue, such as iris and the skin, with pigmentation very similar to that present in individuals with the typical color pattern. This set of factors indicates the specimens recorded were in fact leucistic and not albino. Among the specimens examined in the scientific collections, we found nine individuals from different localities that presented the whitish yellow color pattern. Some studies attribute the higher frequency of cases of leucism due to small populations and / or with some mechanism of reproductive isolation. Thus, analysis of the genetic variability of populations containing individuals with such characteristics should be considered. On the other hand, the occurrence of polymorphic color phenotype in tayras indicates that hypotheses related to the fixation of recessive characteristics, or on possible environmental adaptive advantages of these phenotypes can be tested.

Keywords***:*** *Leucism, fur coloration, tayra, Serra do Caraça*

Ocorrência de leucísmo em *Eira barbara* (Carnivora, Mustelidae) no Brasil

Resumo: A ocorrência de coloração anômala (albinismo, leucísmo e melanismo) em mamíferos é um fenômeno raro na natureza, mas ela tem sido relatada para diversas espécies. Neste trabalho nós relatamos a ocorrência de leucísmo em *Eira barbara* por meio do exame de três indivíduos encontrados mortos atropelados por veículos e pela visualização direta de indivíduos vivos na Reserva Particular do Patrimônio Natural Santuário do Caraça, sudeste brasileiro. Em adição, nós consultamos coleções de mamíferos em museus do Brasil e dos Estados Unidos da América. Os animais encontrados mortos e os avistados apresentavam pelagem amarela esbranquiçada no corpo e na cabeça, resultando em uma coloração muito mais clara que o padrão de coloração comumente observado em iraras. Apesar deste padrão de coloração mais claro, os espécimes apresentavam partes do tecido mole, tais como a íris e a pele, com pigmentação muito semelhante àquela presente em indivíduos com padrão de coloração típico da espécie. Este conjunto de fatores indicou que os espécimes registrados eram de fato leucísticos e não albinos. Dentre os espécimes examinados nas coleções científicas, nós encontramos nove indivíduos de diferentes localidades que apresentavam o padrão de coloração esbranquiçado. Alguns estudos atribuem a frequência elevada de casos de leucísmo a pequenas populações e / ou com algum mecanismo de isolamento reprodutivo. Dessa forma, análises da variabilidade genética de populações contendo indivíduos com essas características devem ser consideradas. Por outro lado, a ocorrência de fenótipos de coloração polimórficos em iraras indica que hipóteses podem ser testadas tanto com relação à fixação de caraterísticas recessivas, quanto sobre possíveis vantagens adaptativas ambientais desses fenótipos.

Palavras-chave*: Leucísmo, coloração de pelagem, irara, Serra do Caraça*

Introduction

Mammalian hair and skin coloration are related to concealment intra and interspecific communication, protection from ultraviolet (UV) radiation and to physiological functions (Caro 2005). The hair, skin and eye color are primarily determined by the quantity and distribution of eumelanin (black and brown coloration) and phaeomelanin (red and yellow coloration), which are produced by melanocytes present in the epithelium, iris, and hair (Prota 1980; Sanchez-Ferrer et al. 1995). The occurrence of phenotypic variations in the coat of animals is related to geographic distribution, seasonal climatic variations, age, sex, weight, and other factors (Ortolani 1999; Stoner et al. 2003; Kawanishi et al. 2010; Ancilloto & Mori 2017). Anomalous colors occur when integumentary pigments are present in excess or in deficient amounts in parts or in totality of the body; white is the complete lack of pigment (Prota 1980; Ortolani 1999; Caro 2005; Fertl & Rosel 2002). Such conditions have been categorized as melanism, leucism and albinism (Fertl & Rosel 2002).

Leucism is a condition involving the partial or total reduction of pigmentation without affecting soft tissues such as eyes and skin, which retain normal coloration (Miller 2005; Acevedo & Aguayo 2008). The causes of leucism are often attributed to the presence of recessive mutant alleles (Bensch et al. 2000), or to the lack of production of tyrosinase, the enzyme involved in melatonin biosynthesis, so that the body cannot synthesize melanin (Sanchez-Ferrer et al. 1995). Leucism differs from albinism, which is caused by a recessive disorder, causing the individual to have pink skin and eyes, and in mammals, a white fur (Cademartori & Pacheco 1999; Rodrigues et al. 1999; Oliveira 2009). Leucism is commonly confused with partial albinism or piebaldism (Fertl & Rosel 2002; Miller 2005), a type of disorder related to the lack of pigmentation in certain areas of the body of the animal, but maintaining normal eye color. On the other hand, melanism is an increase in the amount of pigment in an individual (Acevedo & Aguayo 2008).

In a recent study, 198 cases of abnormal coloration in 26 Neotropical mammal species was reported (Abreu et al. 2013), with cases being much more common in cetaceans, chiropterans and rodents (Pessôa & Reis 1995; Fertl et al. 2004; Abreu et al. 2013, Neves et al. 2014). Except for the report of the occurrence in pinniped species (Acevedo & Aguayo 2008; Abreu et al. 2013), anomalous colors in other Carnivora is known in *Eira barbara* (Trolle 2003; Reis et al. 2005; Tortato & Althoff 2007), in *Nasua narica* (Silva-Caballero et al. 2014), and in *Lontra longicaudis* (Arriaga-Flores et al. 2016).

Eira barbara (Linnaeus, 1758), from the Mustelidae family, is known as tayra and is a species unique in its genus. It is found from central Mexico to northern Argentina (Presley 2000). In Brazil, the tayra occurs in almost all of the territory, where it is more common in areas of dense vegetation (Cheida et al. 2006). The color of tayras may vary throughout its distribution area, but the dark brown coat of its body is predominant, with varying amounts of white or tan on the head and throat extending to the chest (Presley 2000). In Panama, specimens are usually completely black, but individuals presenting fur variations with a yellow-whitish body have been described in Guyana (Presley 2000) and in the Madidi National Park, Bolivia (Tarifa et al. 2001).

In Brazil, there are four reports of tayras with a yellow-whitish body; one individual was registered in the Jauaperi region, in the sub-basin of the Rio Negro, in the state of Amazonas (Trolle 2003); whitish tayras were sporadically sighted on the Monte Alegre farm, in the state of Paraná (Reis et al. 2005); six records of tayras were made in the State Biological Reserve of Sassafras in the state of Santa Catarina (Tortato & Althoff 2007). One recent record was made in the surroundings of the Itatiaia National Park, Rio de Janeiro state (Aximoff & Rosa 2016). In this study, we reported on the occurrence of individuals of *Eira barbara* with leucism characteristic at the Reserva Particular do Patrimônio Natural (R.P.P.N) Santuário do Caraça in Minas Gerais, southeastern Brazil. In order to expand the account of occurrence of leucism in tayras, we consulted some of the most representative mammal collections from Brazil, and also the mammal collection of the American Museum of Natural History (USA).

Material and Methods

The R.P.P.N. Santuário do Caraça is located (20°0’51”S, 43°29’28”W; 11,233 ha) between the cities of Catas Altas and Santa Bárbara, Minas Gerais, in southeastern Brazil. The “Santuário do Caraça” forms a mountainous setting, surrounding an interior plateau, whose altitude ranges from 750m to 2072 m above sea level. It is situated on the slopes of the mountain range known as the Serra do Espinhaço, and possesses a mosaic of different plant communities including semi deciduous forests, cerrado, and open areas such as high-altitude rocky fields (Talamoni et al. 2014).

We obtained the records of three tayras in 2009, all road-killed and found on the main road of the R.P.P.N. Two of them were taxidermized and their respective skulls and skins were deposited in the Mammalian Collection of the Museu de Ciências Naturais (MCNM PUC Minas) of the Pontifícia Universidade Católica de Minas Gerais. Two other records of tayras were made in the reserve, one occurred in 2012 and another in 2016.

We also examined the specimens of tayras deposited in the mammal collections of some Brazilian museums: Museu Nacional da Universidade Federal do Rio de Janeiro (MN-UFRJ), Museu de Zoologia da Universidade de São Paulo (MZUSP), Museu Paraense Emílio Goeldi (MPEG), Museu de Ciências Naturais da Pontifícia Universidade Católica de Minas Gerais (MCNM-PUC Minas), Museu João Moojen (MJM) and Mammal Collection of the Department of Zoology of Universidade Federal de Minas Gerais (UFMG collection), as well as the mammal collection of the American Museum of Natural History (AMNH).

Results

In this study, we report on 13 tayras with recorded characteristics of leucism, of which 11 are related to skins deposited in museums (Table 1). Some of the skins have biometric data available, while others do not (Table 1). Of the tayras found dead and deposited in MCNM-PUC Minas, one showed a pale cream color on the body and head (Figure 1A), while another (Figure 1B) had an intermediate color between a pale cream color and the common color pattern, typical for tayras in the state of Minas Gerais (Figure 1C). Another tayra found dead in 2009 (Figure 1D) also featured a pale cream color and the same was donated to a didactic collection in an educational institution, and therefore there was no collection number for this specimen. In 2012, a living tayra (Figure 1E) was sighted and photographed under an avocado pear tree, near the central area of the R.P.P.N., where there are several buildings and the constant presence of people. In 2016, a new sighting of a tayra showing the same characteristics of leucism occurred in the same place, and may have been the same individual sighted in 2012 (Figure 1F).

Regarding to museum specimens, we found nine individuals with pale cream color (Table 1, Figure 2 and 3). In the MPEG collection, we find four specimens deposited in the 1970s (Figure 2A - D, Table 1) from Oriximiná, state of Pará, in the Trombetas River basin, a tributary of the Amazonas River. In the UFMG collection a specimen from the same region was recently deposited (UFMG 4193, deposited in November 2009, Figure 2E, Table 1).

Four specimens have been recorded in the collection of MZUSP, being one from Boiuçu, state of Pará, two from the Public Garden of São Paulo, today known as Parque da Luz, and another from Iporanga in São Paulo (Figure 3A - D, Table 1). These specimens were compared with other species featuring a color similar to the pattern commonly known in tayras in Brazil and deposited in the last century: male (MZUSP 441, housed in 1901, Fig. 3E), Corupá, Santa Catarina. There is no biometric data available for these specimens (Table 1). The museums MN, João

Table 1. Collection site, sex and external measurements of leucistic tayras. Measurements are in millimeters, except weights, which are in grams.

Museum / Collection	Voucher number	Date	Locality	Geographic coordinate	Sex	Weight	Body length	Tail length	Hind foot length	Ear
MCNM	2981	Jan 11, 2009	R.P.P.N. Santuário do Caraça, Catas Altas, Minas Gerais	20°0'51" S 43°29'28" W	Female	2,450	550	283	98	32
MCNM	3012	Feb 22, 2009	R.P.P.N. Santuário do Caraça, Catas Altas, Minas Gerais	20°0'51" S 43°29'28" W	Male	9,150	691	394	114	39
MPEG	9210	Jul 15, 1978	Rio Trombetas, Oriximiná, Pará		Male	5,000	650	118	105-113	20-40
MPEG	10013	Apr 4, 1977	Cachoeira Porteira, Oriximiná, Pará	1°06'57.20" S 57°05'24.50" W	Female	4,100	630	450	95-105	25-35
MPEG	10014	Jul 26, 1978	Cachoeira Porteira, Oriximiná, Pará	1°06'57.20" S 57°05'24.50" W	Male	5,000	600	420	100-108	17-37
MPEG	10223	-	Porto Trombetas, Oriximiná, Pará	1°28'20.0" S 56°22'35.5" W						
UFMG	2981	Nov, 2007	Porto Trombetas, Oriximiná, Pará	1°28'20.0" S 56°22'35.5" W	Male	5,870	635	425	118	38
MZUSP	5186	Apr 26, 1965	Boiuçú, Pará	1°48'0.000" S 50°16'59.880" W	Female					
MZUSP	2061	Nov, 1905	Jardim Público de São Paulo (current Parque da Luz), São Paulo		Female					
MZUSP	2062	Dec, 1905	Jardim Público de São Paulo (current Parque da Luz), São Paulo		Male					
MZUSP	6295	Jan 28, 1944	Iporanga, São Paulo	24°58'41.67" S 48°59'27.00" W						

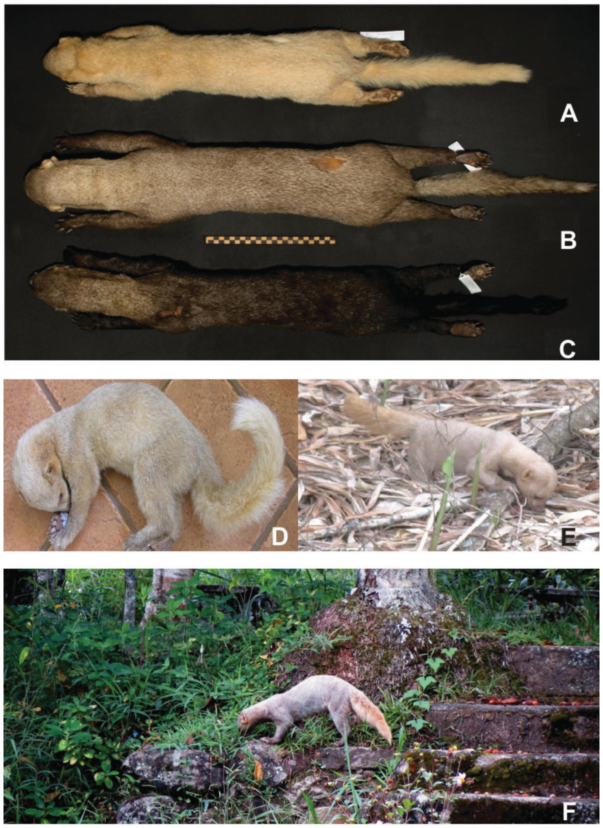


Figure 1. *Eira barbara* specimens with a yellow-whitish color recorded at R.P.P.N. Santuário do Caraça, Minas Gerais, Brazil. A. Female found dead in the road. Voucher number: MCNM 2981, registration date: 11/01/2009. B. Male found dead in the road. Voucher number: MCNM 3012, registration date: 02/22/2009. C. Male collected in the Municipality of Belo Vale, Minas Gerais, Brazil. Voucher number: MCNM 1915, registration date: 21/01/2011; this specimen was used in this study to represent the default color of the species *E. barbara* in the state of Minas Gerais. D. *Eira barbara* specimen found dead in the road. Registration date: 06/12/2009. E. Male recorded living at R.P.P.N., registration date: 07/17/2012. F. Individual recorded living at R.P.P.N., date of registration: 03/2016.

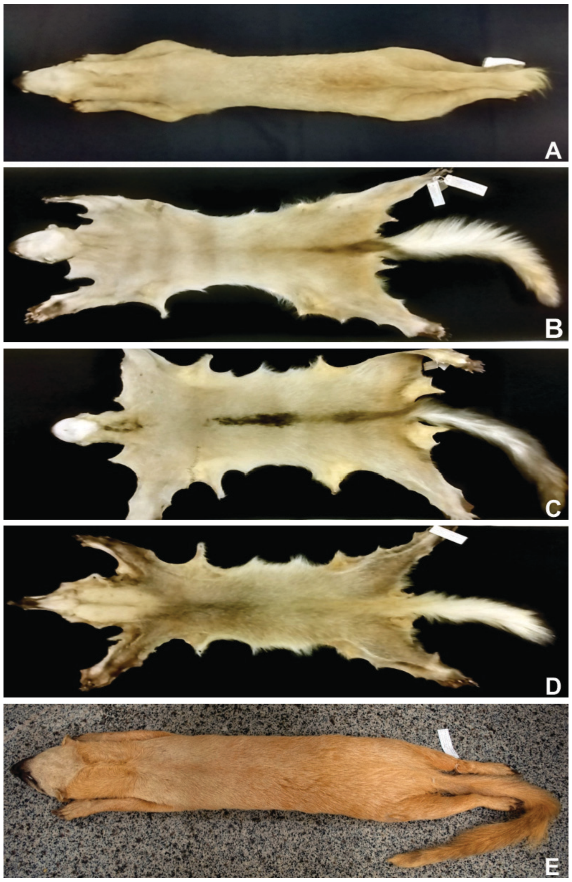


Figure 2. *Eira barbara* specimens with a yellow-whitish color recorded at Oriximiná, state of Pará, Brazil. A. Male collected in Rio Trombetas. Voucher number: MPEG: 9210, registration date: 15/07/1978. B. Female collected in Cachoeira Porteira. Voucher number: MPEG: 10013, registration date: 04/04/1977. C. Male collected in Cachoeira Porteira. Voucher number: MPEG: 10014. Registration date: 26/07/1978. D. no biological data, individual collected in Porto Trombetas, Voucher number: 10223, no date. E. Male collected in Porto Trombetas. Voucher number: UFMG: 2981, registration date: 11/2007.

Figure 3. *Eira barbara* specimens with a yellow-whitish color in Brazil. A. Female collected in Boiuçú, state of Pará. Voucher number: MZUSP: 5186, registration date: 26/04/1965. B. Female collected at Jardim Público of São Paulo (current known as Parque da Luz), but of unknown origin, state of São Paulo. Voucher number: MZUSP: 2061, registration date: 11/1905. C. Male collected at Jardim Público of São Paulo (current Parque da Luz), but of unknown origin, state of São Paulo. Voucher number: MZUSP: 2062, registration date: 12/1905. D. Individual collected in Iporanga, in the state of São Paulo. Voucher number: MZUSP: 6295, registration date: 28/01/1944. E. Male collected in Corupa, in the state of Santa Catarina. Voucher number: MZUSP: 441, registration date: 1901 year. This specimen was used in this study to represent the default of a very old skin presenting the common color pattern for *E. barbara*.

Moojen and the AMNH do not have any tayra with characteristics of leucism in their collections.

Discussion

Based on the available definitions about anomalous color (Fertl & Rosel 2002), and on the characteristics shown by individuals such as a lack of color in the guard hairs, maintenance of color in the under-hairs and body extremities (nose, ears, feet and tail) having dark pigmentation, we consider that the recorded individuals can be considered to have leucism. There is no information in the literature regarding the analysis of the consequences of the variation in coat color in tayras throughout its area of distribution, nor in relation to the consequences of leucism. Mammalian hair and skin color provides concealment for the mammals (Deblase & Martin 1980), while it is expected that leucism and/or albinism imposes on the individuals a greater difficulty in camouflage, possibly subjecting them to a greater risk of predation (Parsons & Bonderup-Nielsen 1995). Peles et al. (1995), however, in a rare study addressing this topic, it was shown that albinism in meadow voles (*Microtus pennsylvanicus*) proved not to be a disadvantageous trait in grassland habitats with high nutritional quality and heavy vegetative cover.

The MZUSP and MN are the natural history museums with the largest collections of mammals in the country. The rarity of older specimens and the absence of deposit of newly collected specimens in these two collections may indicate the rarity of leucism in tayras. Of the museum specimens analyzed, two of the MZUSP are dated to the beginning of the 20th century, both from Jardim Público, where today it houses the Parque da Luz within the city of São Paulo. Parque da Luz was created in 1825 as a Botanical Garden and in 1838 it was named the Jardim Público. Throughout the second half of the nineteenth century the Jardim Público underwent a series of reformulations, and the last major reform was the creation of a zoo, the first in the city of São Paulo, in the 1890s. This zoo housed species native to Brazil and some exotic (Ohtake & Dias 2011). In the middle of the nineteenth century and at the beginning of the twentieth century the area around Jardim da Luz was already quite urbanized (Ohtake & Dias 2011) and it was practically impossible that wild tayras could live freely in this central region of the city. Thus, the specimens of tayras from the Jardim Público were probably not native to the park and surrounding areas and may have come from other regions of Brazil.

Another specimen of the MZUSP collection comes from Boiuçu, in the state of Pará. Considering the records of Oriximiná, this region of the Amazon basin holds the largest number of cases of leucism in tayras. In addition, there is already the case reported in the region of the Jauaperi River, a sub-basin of Rio Negro (Toller 2003), a tributary of the Amazon River. It is notable that between the first and last record for the region of Oriximiná there is a time interval of 30 years. In Oriximiná and in the village of Porto Trombetas, in the mid-Amazonas region, several socio-environmental conflicts have been registered in the last five decades due to the establishment of a bauxite extraction plant in the region (Monteiro 2005; Wanderlei 2008). The local extraction of bauxite has led to numerous negative impacts such as deforestation, the contamination and silting of rivers and the displacement of riparian and "quilombola" peoples (Wanderlei 2008). The surroundings of the Caraça reserve is also characterized by intense mining activities (Marent et al. 2011), however, the reserve, with its more than 100 km^2, borders the Parque Nacional Serra do Gandarela (Marent et al. 2011), which has 312 km^2 of forested area (ICMBio 2017).

The occurrence of leucism is based on the expression of recessive alleles and individuals with leucism are more frequent in small and isolated populations because endogamy causes recessive alleles to be expressed (Bensch et al. 2000). Thus, if reproductive isolation occurs among local populations of tayras, it is necessary to understand it. Considering the number of records reported the analysis of the patterns of genetic diversity within and among local populations would be important. The occurrence of the leucistic phenotype observed in the Oriximiná and Caraça populations may represent an opportunity to investigate the possibility of establishing a recessive trait in these populations, such as the near fixation of melanism, another recessive color anomaly, observed in leopards of the Malay Peninsula (Kawanishi et al. 2010). On the other hand, the occurrence of different coloration phenotypes in tayras throughout its distribution area, provides an opportunity to investigate possible adaptive advantages of these phenotypes (Silva et al. 2016) in relation to the ecological conditions present in their areas of occurrence.

Acknowledgments

We are very grateful to Mario de Vivo (MZUSP), André Ravetta (MPEG), Silvia Pavan (AMNH), Gisele Lessa (Museu João Moojen), and Fernando Perini (UFMG) for kindly sending photos and for allowing the use of the records of the specimen housed in the respective collections. Our grateful thanks go to Mrs. Flavio Alves for allowing the use of her photographic record obtained in March 2016. Mario de Vivo and two anonymous reviewers contributed significantly to the improvement of the manuscript. Research in the RPPN Sanctuary of Caraça has been funded by the Research Incentive Fund (FIP) of PUC Minas and by FAPEMIG.

Author Contributions

Sonia Talamoni: substantial contribution in the concept and design of the study; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Claudia Guimarães Costa: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Pedro Igor Macário Viana: contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Lauro Palú: contribution to data collection; contribution to manuscript preparation and critical revision.

Raphaela Barcelos Oliveira: contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Leila Maria Pessôa: contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

ABREU, M.S.L, MACHADO, R., BARBIERI, F., FREITAS, N. S. & OLIVEIRA, L.R. 2013. Anomalous colour in Neotropical mammals: a review with new records for *Didelphis* sp. (Didelphidae, Didelphimorphia) and *Arctocephalus australis* (Otariidae, Carnivora). Braz. J. Biol. 73:185-194.

ACEVEDO, J. & AGUAYO, M. 2008. Leucistic South American sea lion in Chile, with a review of anomalously color in ottarids. Rev. Biol. Mar. Oceanogr. 43:413-417.

ANCILLOTO, L. & MORI, E. 2017. Adaptive significance of coat colouration and patterns of Sciuromorpha (Rodentia), Ethol. Ecol. Evol. 29 (3): 241-254.

ARRIAGA-FLORES, J.C., RODRÍGUEZ-RUÍZ, E R., GALLO-REYNOSO, J.P. & CASTRO-ARELLANO, I. 2016. Leucism in Neotropical otters (*Lontra longicaudis annectens*) from Mexico. Southwest. Nat. 61(1):63-68.

AXIMOFF, I.A. & ROSA, C.A. 2016. First records of albinism in greyheaded tayra (Carnivora, Mustelidae) and occurrence in high-altitude grassland in Brazil. Oecologia Australis 20(4): 526-531.

BENSCH, S., HANSSON, B., HASSELQUIST, D. & NIELSEN, B. 2000. Partial albinism in a semi-isolated population of Great Reed Warblers. Hereditas 133:167–170.

CADEMARTORI, C.V. & PACHECO, S.M. 1999. Registro de albinismo em Delomys dorsalis (Hensel, 1872) – Cricetidae, Sigmodontinae. Biociências 7(1):195-197.

CARO, T. 2005. The adaptive significance of coloration in mammals. Bioscience 55: 125-136.

CHEIDA, C.C., NAKANO-OLIVEIRA, E., FUSCO-COSTA, R., ROCHA-MENDES, F. & QUADROS J. 2006. Ordem Carnivora. In Mamíferos do Brasil (R. REIS, A.L. PERACCHI, W.A. PEDRO & I. LIMA, eds). EDUEL, Londrina, p.231-276.

DEBLASE, A.F. & MARTIN, R.E. 1980. A manual of Mammalogy, with keys to families of the world. William C. Brown Co. Publishers, Dubuque.

FERTL, D. & ROSEL, P. 2002. Albinism. In Encyclopaedia of Marine Mammals (W.F. PERRIN, B. WÜRSIG & J.G.M. THEWISSEN, eds). Academic Press, San Diego, p.16-18.

FERTL, D., BARROS, N.B., ROWLETT, R.A., ESTES, S. & RICHLEN, M. 2004. An update on anomalously white cetaceans, including the first account for the pantropical spotted dolphin (*Stenella attenuata graffmani*). LAJAM 3(2):163-166.

ICMBIO. 2017. Instituto Chico Mendes de Conservação da Biodiversidade. 2017. Parna da Serra do Gandarela. http://www.icmbio.gov.br/portal/unidadesdeconservacao/biomas-brasileiros/mata-atlantica/unidades-de-conservacao-mata-atlantica/5074-parna-da-serra-do-gandarela. Accessed 23 Jan 2017.

KAWANISHI, K., SUNQUIST, M.E., EIZIRIK, E., LYNAM, A.J., NGOPRASERT, D., WAN SHAHRUDDIN, W.N., RAYAN, D.M., SHARMA, D.S. K. & STEINMETZ, R. 2010. Near fixation of melanism in leopards of the Malay Peninsula. J. Zool. 282: 201-206.

MARENT, B.R., LAMOUNIER, W.L. & GONTIJO, B.M. 2011. Conflitos ambientais na Serra do Gandarela, Quadrilátero Ferrífero - MG: mineração x preservação. Geografias 7(1):99-113.

MILLER, J.D. 2005. All about albinism. Missouri Conservationist 66:5-7.

MONTEIRO, M.A. 2005. Meio século de mineração industrial na Amazônia e suas implicações para o desenvolvimento regional. Estudos Avançados 19(53):187 – 207.

NEVES, A.C.A., PESSÔA, L.M., COUTINHO, L.C., OLIVEIRA, M.B., NEVES, A.C.S.A., COUTINHO, L.C., OLIVEIRA, M.B., PESSÔA, L.M. 2014. First report of partial albinism in genus Thrichomys (Rodentia: Echimyidae). Pap. Avulsos Zool. 54:107-110-107.

OHTAKE, R. & DIAS, C. 2011. Jardim da Luz, um museu a céu aberto. Editora SENAC, São Paulo.

OLIVEIRA, S.V. 2009. Albinismo parcial em cutia *Dasyprocta azarae* (Lichtenstein, 1823) (Rodentia, Dasyproctidae), no sul do Brasil. Biotemas 22:243-246.

ORTOLANI, A. 1999. Spots, stripes, tail tips and dark eyes: Predicting the function of carnivore colour patterns in carnivores using the comparative method. Biol. J. Linnean Soc. 67:433-476.

PARSONS, G.J. & BONDERUP-NIELSEN, S. 1995. Partial albinism in an island population of Meadow Voles, *Microtus pennsylvanicus*, from Nova Scotia. Can. Field-Nat. 109:263-264.

PELES, J.D., LUCAS, M.F. & BARRETT, G.W. 1995. Population dynamics of agouti and albino meadow voles in high-quality, grassland habitats. J. Mammal. 76:1013-1019.

PRESLEY, S.J. 2000. *Eira barbara*. Mamm. Species 636:1-6.

PESSÔA, L.M.; REIS, S. F. 1995. Coat color variation in *Proechimys albispinus* (Geoffroy, 1838). Zoologia 361: 1-5.

PROTA, G. 1980. Recent advances in the chemistry melanogenesis in mammals. J. Investig. Dermatol. 75:122-127.

REIS, N.R., PERACCHI, A.L., FANDIÑO-MARINO, H. & ROCHA, V.J. 2005. Mamíferos da Fazenda Monte Alegre, Paraná. EDUEL, Londrina.

RODRIGUES, F.H.G., SILVEIRA, L., JÁCOMO, A.T. & MONTEIRO-FILHO, E.L.A. 1999. Um albino parcial de veado campeiro (*Ozotocerus bezoarticus*, Linnaeus) no Parque Nacional das Emas, Goiás. Rev. Brasil. Zool. 16:1229-1232.

SANCHEZ-FERRER, A., RODRÍGUES-LÓPEZ, J.N., GARCÍA-CÁNOVAS, F. & GARCÍA-CARMONA, F. 1995. Tyrosinase: a comprehensive review of its mechanism. Biochimica et Biophysica Acta 1247:1-11.

SILVA, L.G., OLIVEIRA, T.G., KASPER, C.B., CHEREM, J.J., MORAES jr, E.A., PAVIOLO, A. & EIZIRIK, E. 2016. Biogeography of polymorphic phenotypes: Mapping and ecological modelling of coat colour variants in an elusive Neotropical cat, the jaguarundi (Puma yagouaroundi). J. Zool. 299: 295-303.

SILVA-CABALLERO, A., MONTIEL-REYES, F., SÁNCHEZ-GARIBAY, E. & ORTEGA, J. 2014. Leucismo en el coatí de nariz blanca *Nasua narica* (Mammalia: Carnivora), en Quintana Roo, México. Therya 5(3):839-843.

STONER, C.J., BININDA-EMONDS, O.R.P. & CARO, T. 2003. The adaptive significance of coloration in lagomorphs. Biol. J. Linnean Soc. 79:309-328.

TALAMONI, S.A., AMARO, B.D., CORDEIRO-JÚNIOR, D.A. & MACIEL, C.E.M. A. 2014. Mammals of Reserva Particular do Patrimônio Natural Santuário do Caraça, state of Minas Gerais, Brazil. Check List 10:1005-1013.

TARIFA, T., ALIAGA, E., RÍOS, B. & HAGAMAN, D. 2001. Mamíferos del Parque Nacional Madidi. 1ed. Hisbol, La Paz, Bolivia.

TORTATO, R.F. & ALTHOFF, S.L. 2007. Variações na coloração de iraras (Eira barbara Linnaeus, 1758, Carnivora, Mustelidae) da Reserva Biológica Estadual do Sassafrás, Santa Catarina, sul do Brasil. Biota Neotrop. 7:365-367 http://www.biotaneotropica.org.br/v7n3/pt/abstract?article+bn02007032007 ISSN 1676-0603. Accessed 23 Jan 2017.

TROLLE, M. 2003. Mammal survey in the Rio Jauaperí region, Rio Negro Basin, the Amazon, Brazil. Mammalia 67:75-83.

WANDERLEY, L.J.M. 2008. Confitos e movimentos sociais populares em área de mineração na Amazônia brasileira. Dissertação de Mestrado, Universidade Federal do Rio de Janeiro, Rio de Janeiro.

Received: 26/01/2017
Revised: 19/05/2017
Accepted: 27/07/2017

Biota Neotropica 17(3): e20170363, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
inventory

Echinodermata associated to rhodoliths from Seixas Beach, State of Paraíba, Northeast Brazil

Jessica Prata[1*], *Dimitri Araujo Costa*[1,2], *Cynthia Lara de Castro Manso*[3], *Maria Cristina Crispim*[1] *&*

Martin Lindsey Christoffersen[1]

[1]*Universidade Federal da Paraíba, Departamento de Sistemática e Ecologia, Cidade Universitária, Campus I, s/n, 58051-900, João Pessoa, PB, Brazil*

[2]*Universidade Federal da Paraíba, Centro de Ciências Aplicadas e Educação, Campus IV, Rua da Mangueira s/n, 58297-000, Rio Tinto, PB, Brazil*

[3]*Universidade Federal de Sergipe, Laboratório de Invertebrados Marinhos, Departamento de Biociências, Itabaiana, SE, Brazil*

**Corresponding author: Jessica Prata, e-mail: jessie.prata@gmail.com*

PRATA, J., COSTA, D. A., MANSO, C. L. C., CRISPIM, M. C., CHRISTOFFERSEN, M. L. **Echinodermata associated to rhodoliths from Seixas Beach, State of Paraíba, Northeast Brazil.** Biota Neotropica. 17(3): e20170363. http://dx.doi.org/10.1590/1676-0611-BN-2017-0363

Abstract: This study presents the species of echinoderms found inside rhodolith branches from Seixas Beach, State of Paraíba, Brazil, during one year of rhodolith sampling. A total of 64 specimens were analyzed and identified into 12 species, belonging to 11 genera, eight families and three classes. Brief taxonomic descriptions, figures and ecological notes for recorded species are provided. Ophiuroidea and Holothuroidea were the most representative taxa. Biogenic structures are an important habitat for young specimens and some small species of Echinodermata. Some species complete their life cycle in these structures, while others spend part of their life in these substrates and may also migrate to other habitats. The work complements the knowledge of echinoderm biodiversity from Northeastern of Brazil and aims to support future projects of protection and sustainable management of this area.

Keywords: *Echinoderms, inventory, Brazilian coast.*

Echinodermata associados a rodolitos da Praia do Seixas, Estado da Paraíba, Nordeste do Brasil

Resumo: O presente estudo apresenta as espécies de equinodermos encontradas dentro de ramos de rodolitos provenientes da Praia do Seixas, Estado da Paraíba, Brasil. Um total de 64 espécimes foi analisado, sendo identificadas 12 espécies, pertencentes a 11 gêneros, oito famílias, e três classes. As amostras foram coletadas ao longo de um ano a partir de rodolitos. O artigo fornece uma breve descrição taxonômica, figuras e notas ecológicas para cada espécie. Ophiuroidea e Holothuroidea foram os grupos mais representativos. Os resultados mostram que essas estruturas biogênicas formam um habitat importante para espécies jovens e espécies pequenas de Echinodermata. Algumas espécies completam seu ciclo de vida dentro dos rodolitos, enquanto outras passam uma parte da vida nesses substratos para depois explorar em outros ambientes. O estudo complementa o conhecimento da biodiversidade de equinodermos para o nordeste do Brasil e fornece suporte para futuros projetos de proteção e uso sustentável da área.

Palavras-chave: *Equinodermos, inventário, costa brasileira.*

Introduction

Rhodoliths are aggregates of nodules of unarticulated encrusting coralline algae (Amado-Filho et al. 2012). These structures occur in shallow waters up to depths of 150 m, forming extensive rhodolith beds and hard bottom habitats. In Brazil, rhodolith beds are among the largest deposits of $CaCO_3$ in the world (Amado-Filho et al. 2012). These rhodoliths provide refuge, food and other resources, protecting organisms from competitive exclusion, predation, and physical disturbance (Riul et al. 2009, Horta et al. 2016). The Seixas Beach has a large bank of rhodoliths susceptible to degradation by anthropogenic impact, in the form of tourism and commercial exploitation.

Echinoderms can be found in various substrata. Small species and young specimens usually live associated with coral reefs, rhodolith beds, and algae. These animals are exclusively marine and belong to the benthic fauna, with many species inhabiting rocky shores, beaches, bays and subtidal zones, including the deep-sea (Pawson 2007). The phylum consists of approximately 7,000 living species and 13,000 extinct species, comprising the classes Crinoidea, Asteroidea, Ophiuroidea, Echinoidea and Holothuroidea (Pawson 2007). According to Ventura et al. (2012), very few studies on the biogeography of echinoderms have been done in Brazil. In part, this is due to the lack of reliable information on taxonomy and distribution ranges. About 340 species of Echinodermata are recorded along the Brazilian coast (Ventura et al. 2012). Here, we list the species of echinoderms found associated with rhodoliths from the Seixas Beach, followed by brief descriptions, ecological notes for species, and a discussion on the importance of this habitat for young and small species of echinoderms.

Material and Methods

1. Study site

The Seixas Beach is located about 700 m to the south of the municipality of João Pessoa, capital of the State of Paraíba, Brazil (Melo et al. 2008). The beach is located within the Tropical Northwesternn Marine Ecoregion (Spalding et al. 2007). The climate is tropical and humid, with a conspicuous seasonal pattern of rainfall. The rainy season goes from March to August, and the dry season extends from September to February. Temperature ranges are small, with annual averages varying from 20 to 37ºC. Mean yearly humidity is 80%, and yearly precipitation varies from 900 to 1800 mm (Lima & Heckendorff 1985).

Around this rhodolith formation, water depths vary from 0.50 m to 1.50 m at low tides, except for some areas that become exposed during the lowest tides. The deeper portions vary from 3.0 to 6.0 m (Melo et al. 2014). The region is located between the coordinates of 07º08'45"S to 07º09'20"S and 34º47'45"W to 34'47'35"W. Figure 1 indicates collecting locations at the Seixas Beach (PB).

2. Sampling

Expeditions were conducted in the infralittoral of the Seixas Beach. Small boats were used to access to the reef environment. Collecting location were positioned at depths of 1.5 and 4.0 m. Geographical coordinates of each collecting site were recorded with a GPS. The depth of 1.5 m has coordinates 07º09'13"S and 34º47'21"W, while the depth of 4.0 m has the coordinates 07º09'13"S and 34º47'10"W. Collections were made in January, March, May, July, September and November, during the lowest tides along the year of 2015. Samples were replicated each month, totaling 12 samples in the year. Specimens were collected manually, with the help of a square of 15 X 15 cm, within which 4-5 rhodoliths were collected. The samples was conditioned in labelled plastic bags and sorted in the Laboratory of Aquatic Ecology (LABEA), Federal University of Paraíba (UFPB).

In the lab, samples were maintained separate in trays with sea water, with salinity at 35 ppm and continuously aerated, up to the time of processing of the material with a hammer and stylets. Echinoderms were separated and subsequently anesthethized with menthol, fixed in formalin at 4%, diluted with sea water, and finally preserved in alcohol at 70%. Additional material present in the Collection of Invertebrates Paulo Young (CIPY) was also considered.

Taxonomic identifications were based on Tommasi (1970), Hendler et al. (1995), Borges et al. (2002), Borges & Amaral (2005), Manso (2008), Benavides-Serrato et al. (2011), Borrero-Pérez et al. (2012), Tommasi (1969), Pawson et al. (2010), and Martins et al. (2012). Specimens were photographed with a Canon Powershot A2000IS digital camera, and a Leica MZ12.5 stereomicroscope. Measurements were obtained from fixed specimens. All specimens were deposited at CIPY, Department of Systematics and Ecology (DSE), Federal University of Paraíba (UFPB).

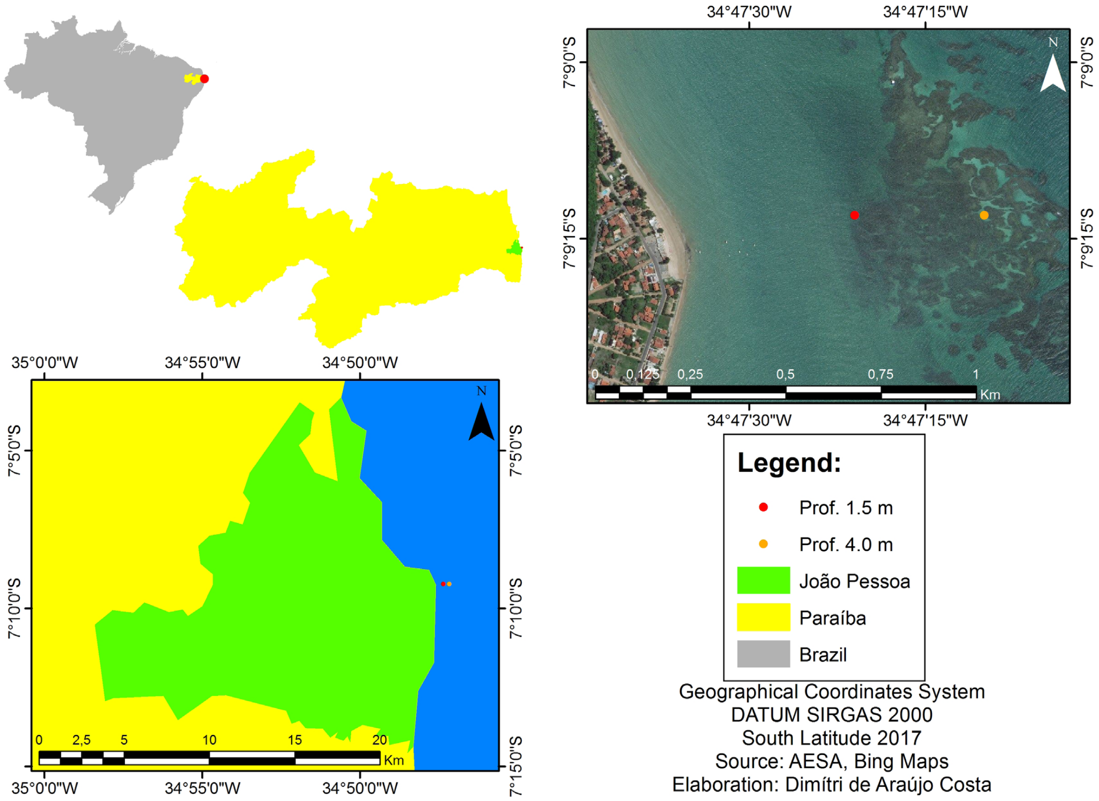


Figure 1. Study area with location of collecting points at Seixas Beach, João Pessoa, Paraíba, Brazil.

Results

A total of 62 individuals were recorded from inside rhodoliths, belonging to three classes, four orders, eight families, 11 genera and 12 species of echinoderms, as listed below.

Class Ophiuroidea Gray, 1840
Order Ophiurida Müller & Troschel, 1840
Family Amphiuridae Ljungman, 1867
Genus *Amphipholis* Ljungman, 1866
Amphipholis squamata (Delle Chiaje, 1828)
(Figure 2)

Axiognathus squamatus—Lima-Verde, 1969: 11.

Amphipholis squamata—Albuquerque, 1986: 103, fig. 18a–c, est. V, fig. 1a–c; Tommasi, 1999; Borges, 2006: 34; Magalhães et al., 2005: 63; Gondim et al. 2008; Manso et al. 2008: 191, fig. 18 e–g; Oliveira et al., 2010: 6; Barboza & Borges, 2012: 11; Paim et al., 2015: 5; Alitto et al. 2016: 4.

Material examined: 5 specimens (UFPB.ECH–2260), Seixas Beach, Paraíba State, Brazil, depth 1.5 m; 1 specimen (UFPB.ECH–2275), Seixas Beach, Paraíba State, Brazil, depth 4.0 m.

Description: Disk rounded, covered by medium size scales, with circular to semicircular imbricated scales (Figure 2a, 2b). Primary scales large, easily observed (Figure 2c). Radial shields slightly long than wider, separated by a thin scale up to the distal region of the shields. Ventral surface of the disk covered by scales similar to dorsal scales (Figure 2d). Bursal slits narrow, near the first plate of the arms. Diamond-shaped oral shield, adoral shields longer than wide, touching the proximal edge. Two oral papillae in each side of jaw, the more distal bigger and trapezoidal, other rounded and smaller. A pair of elongated infradental papillae (Figure 2e). Five arms, about five times the disk diameter. Dorsal arm plate slightly pentagonal, with distal edge rounded (Figure 2f). Ventral arm plate pentagonal, two tentacular scales (Figure 2g). Lateral arm plates meeting in the longitudinal mid-line, with four arm spines (Figure 2h). Color white.

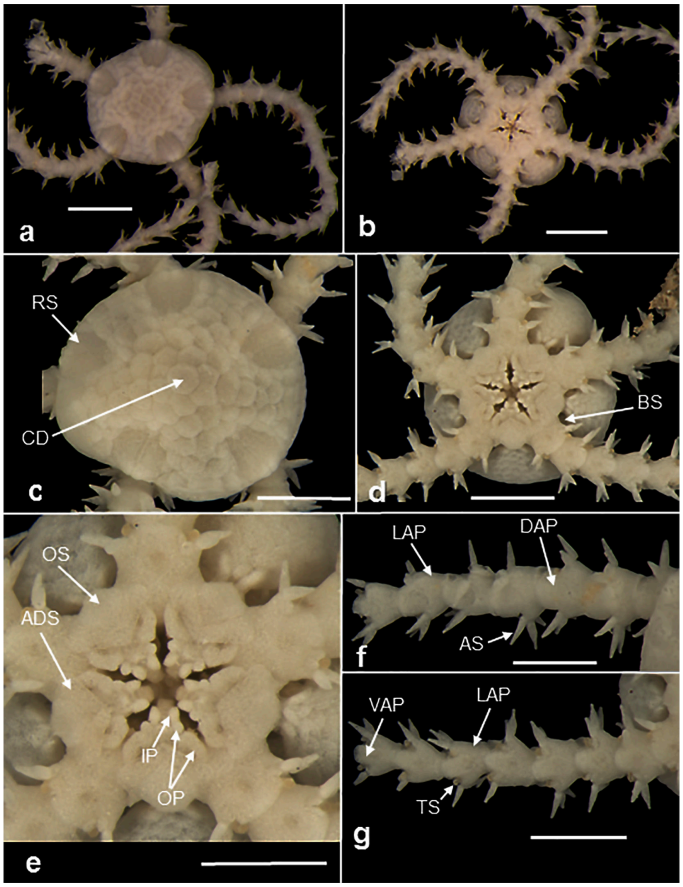


Figure 2. *Amphipholis squamata*. a, dorsal view of the animal; b, ventral view of the animal; c, dorsal view of the disk; d, ventral view of the disk; e, detail of the jaws; f, dorsal view of the arm; g, ventral view of the arm. ADS, adoral shield; AS, arm spine; BS, bursal slit; CD, central dorsal scale; DAP, dorsal arm plate; IP, infradental papillae; LAP, lateral arm plate; OP, oral papillae; OS, oral shield; RS, radial shield; TS, tentacle scale; VAP, ventral arm plate. Scales: a-d, f, 1 mm; e, 0.5 mm. The arms are oriented from the distal to proximal region.

Distribution: In the Western Atlantic, the species is found from Canada to Argentina (Hendler et al. 1995, Benavides-Serrato et al. 2011). In Brazilian coast, it was reported in Pará, Maranhão, Ceará, Paraíba (Gondim et al. 2013), Alagoas (Lima et al. 2011), Bahia (Magalhães et al. 2005), Rio de Janeiro (H.L. Clark 1915) and, São Paulo (Borges et al. 2002). The species occurs from shallow waters to 1962 m depth (Pawson et al. 2009).

Ecological notes: The specimens were found inside rhodoliths but can be buried in crevices, sand and coral rocks. The species presents bioluminescence (Bernasconi & Agostino 1975) and incubates the juveniles (Borges & Amaral, 2005).

Amphipholis januarii Ljungman, 1866
(Figure 3)

Amphipholis januarii—Tommasi, 1967: 1, fig. 1; Lima-Verde, 1969: 11; Alves & Cerqueira, 2000: 545; Magalhães et al. 2005: 63; Gondim et al., 2008: 154; Manso et al., 2008: 190, fig. 18a–d; Oliveira et al., 2010: 6; Barboza & Borges, 2012: 11; Paim et al., 2015: 3-4; Alitto et al. 2016: 4.

Material examined: 16 specimens (UFPB.ECH–2248, 2249, 2252, 2265, 2276), Seixas Beach, Paraíba State, Brazil, depth 1.5 m; 19 specimens (UFPB.ECH–2253, 2256, 2257, 2258), Seixas Beach, Paraíba State, Brazil, depth 4 m.

Description: Disk circular to pentagonal, with reentrances in inter-radial areas (Figure 3a). Disk covered by small and imbricated scales. Radial shields narrow, longer than wide, usually separated by one or two scales, the internal more elongated (Figure 3c). Ventral side of the disk covered by smaller scales, imbricated (Figure 3d). Bursal slit long, near the first arm plate. Oral shield diamond-shaped, adoral shield triangular. Two oral papillae in each side of jaw, the distal triangular and robust, a pair of elongated and robust infradental papillae (Figure 3e). Five elongated arms, about seven to ten times the diameter of the disk (Figure 3b). Arms tapering distally. Dorsal arm plate wider than long (Figure 3f), ventral arm plate pentagonal (Figure 3g), with rounded edges. Two tentacle scales, small and flattened. Lateral arm plate with three to four elongated spines, similar in size (Figure 3h). The arm spine of middle with one or two teeth at the apex. Color white to yellow.

Distribution: South Carolina, Florida, Texas, Mexico, Antilles, Belize, Panama and Brazil (Hendler et al. 1995, Alvarado et al. 2008). In Brazilian coast, the species was reported in Pará (Albuquerque 1986), Ceará (Lima-Verde 1969), Paraíba (Gondim et al. 2008), Alagoas (Lima et al 2011), Bahia (Magalhães et al. 2005), Rio de Janeiro (Ljungman 1867), and São Paulo (Tommasi 1970, Alitto et al. 2016). From 1 to 311 m depth (Laguarda-Figueras et al. 2009).

Ecological notes: This species usually is found in sand bottom and gravel (Tommasi 1970), algae, under rocks (Hendler et al. 1995).

Genus *Microphiopholis* Turner, 1985
Microphiopholis gracillima (Stimpson, 1854)
(Figure 4)

Micropholis gracillima—Tommasi, 1970: 39, figs. 39–40; Alves & Cerqueira, 2000: 545.

Amphipholis gracillima—Guille & Albuquerque, 1987: 147; Albuquerque & Guille, 1991: 6; Oliveira et al., 2010: 6.

Microphiopholis gracillima—Tommasi, 1999; Magalhães et al., 2005: 63; Barboza & Borges, 2012: 13.

Material examined: 3 specimens (UFPB.ECH–2259), Seixas Beach, Paraíba State, Brazil, depth 4 m.

Description: Disk rounded with indentations in the radial region (Figure 4a). Disk covered by numerous small and imbricated scales. Radial shields narrow and elongated, joined at half of length, and then separated by three scales on the proximal edge (Figure 4c). Ventral surface of disk covered by small and imbricated scales. Bursal slit large, near the first to fourth ventral arm plate (Figure 4d). Oral shield diamond-shaped. Adoral shield elongated and slightly wide distally. Jaws with three oral papillae, the more distal rectangular, bigger than proximal papilla. Arms long, about six to eight times the diameter of the disk (Figure 4b). Dorsal arm plate trapezoidal (Figure 4f). Ventral arm plate square-shaped, with distal edge rounded (Figure 4g). Two tentacle scales, the most internal robust. Lateral arm plate with four arm spines (Figure 4h). Second arm spine near the ventral arm plate, with small teeth. Color light brown to yellowish.

Remarks: Young specimens with only three arm spines, the first smaller than others.

Distribution: South Carolina, Florida, Antilles, Puerto Rico, Tobago, Curaçao, Belize and Brazil. In Brazilian coast, the species was reported in Paraíba (Gondim et al. 2008), Bahia (Alves & Cerqueira 2000), and Rio de Janeiro (Tommasi 1970). From intertidal zone to 26 m depth (Hendler et al. 1995).

Ecological notes: This species is usually found in soft bottom (Tommasi 1970), with intermediate granulometry and high organic content (Zimmerman 1988).

Family Ophiactidae Matsumoto, 1915

Genus *Ophiactis* Lütken, 1856

Ophiactis savignyi (Müller & Troschel, 1842)

(Figure 5)

Ophiactis krebsii—Verril, 1868: 366.

Ophiactis savignyi—Brito, 1960a: 4, fig.3; 1962: 2; Lima–Verde, 1969: 12; Tommasi, 1970: 24, figs.16–17; 1999; Nunes, 1975: 181; Alves & Cerqueira, 2000: 545; Magalhães et al., 2005: 63; Gondim et al., 2008: 154; Manso et al., 2008: 188, fig. 14f–h; Lima & Fernandes, 2009: 61; Oliveira et al., 2010: 6; Barboza & Borges, 2012: 9; Paim et al., 2015: 10-11; Alitto et al., 2016: 7.

Material examined: 1 specimen (UFPB.ECH–2277), Seixas Beach, Paraíba State, Brazil, depth 1.5 m; 9 specimens (UFPB.ECH–2250, 2251), Seixas Beach, Paraíba State, Brazil, depth 4 m.

Description: Disk rounded to pentagonal, covered by medium size scales, imbricated, more numerous in the center and in the interradial surface (Figure 5a). Small rough-tipped spines scattered over the disk, more

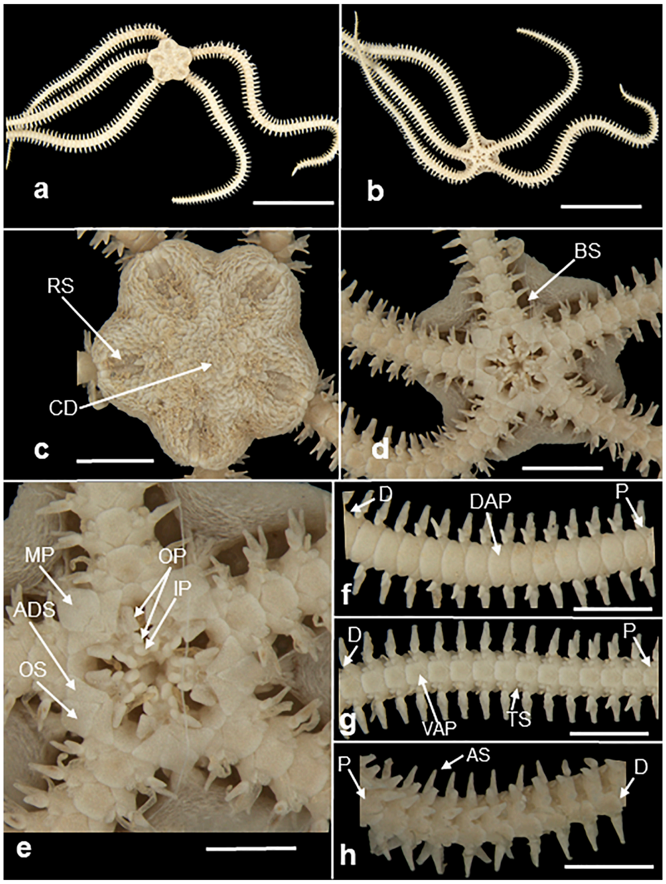


Figure 3. *Amphipholis januarii*. a, dorsal view of the animal; b, ventral view of the animal; c, dorsal view of the disk; d, ventral view of the disk; e, detail of the jaws; f, dorsal view of the arm; g, ventral view of the arm; h, lateral view of the arm. ADS, adoral shield; AS, arm spine; BS, bursal slit; CD, central dorsal scale; D, distal region; DAP, dorsal arm plate; IP, infradental papillae; MP, madreporite; OP, oral papillae; OS, oral shield; P, proximal region; RS, radial shield; TS, tentacle scale; VAP, ventral arm plate. Scales: a-b, 10 mm; c-d, f-h, 2 mm; e, 1 mm.

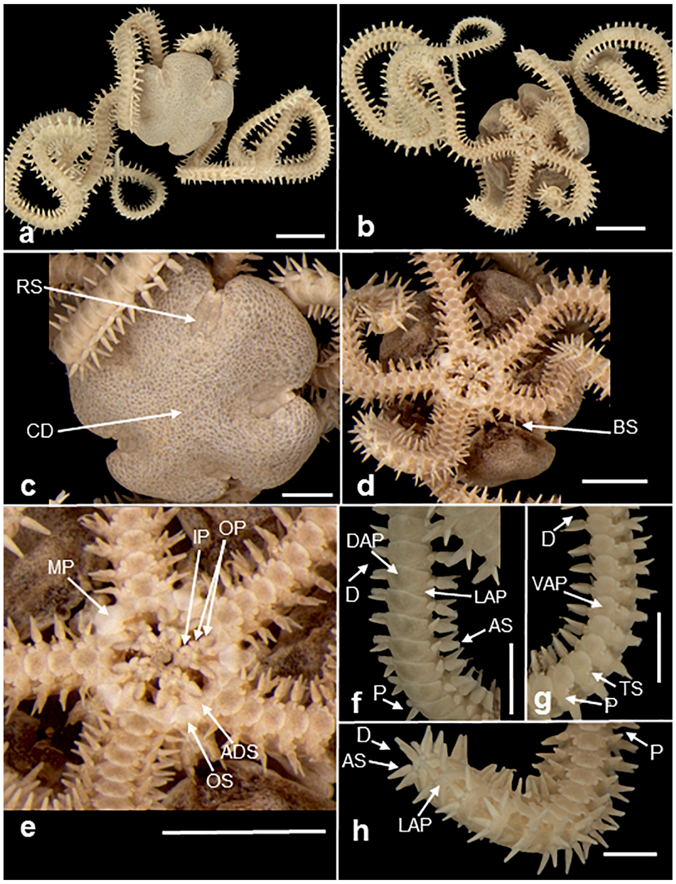


Figure 4. *Microphiopholis gracilimna*. a, dorsal view of the animal; b, ventral view of the animal; c, dorsal view of the disk; d, ventral view of the disk; e, detail of the jaws; f, dorsal view of the arm; g, ventral view of the arm; h, lateral view of the arm. ADS, adoral shield; AS, arm spine; BS, bursal slit; CD, central dorsal scale; D, dorsal region; DAP, dorsal arm plate; IP, infradental papillae; LAP, lateral arm plate; MP, madreporite; OP, oral papillae; OS, oral shield; P, proximal region; RS, radial shield; TS, tentacle scale; VAP, ventral arm plate. Scales: a-b, 5 mm; c, 1 mm; d-h, 2 mm.

numerous at the edges. Radial shield large and triangular, occupying more than half the disk. They are united distally and separated by two scales proximally, the most internal more elongated (Figure 5c). Ventral surface of the disk covered by small and imbricated scales (Figure 5d). Bursal slits large. Oral shield sub-diamond shaped. Adoral shield longer than wide, more wide distally, separated proximally. Two oral papillae flattened and robust, similar in size. An apical papillae large and triangular (Figure 5e). Six arms, about five times the diameter of the disk, tapering distally (Figure 5b). Dorsal arm plate trapezoidal (Figure 5f). Ventral arm plate octagonal (Figure 5g). A flattened and large tentacle scale. Lateral arm plate with five arm spines, short and flattened. They are more robust and elongated near the dorsal arm plate. The arm spines with marginal teeth. Color olive green in the disk, with some white spots. Arms with olive green plates interspersed with white plates. Radial shields with a white spot on the distal edge.

Distribution: This species occurs in the Indo-West-Pacific, eastern Pacific, and Atlantic Ocean. In the western Atlantic it can be found in South Carolina, Bermuda, Puerto Rico, Gulf of Mexico, Colombia and Brazil (Hendler et al. 1995, Benavides-Serratto et al. 2011). In Brazilian coast, it was reported in Amapá, Pará, Maranhão (Albuquerque 1986), Ceará (Lima-Verde 1969), Paraíba (Gondim et al. 2008), Pernambuco (Tommasi 1970), Alagoas (Lima et al. 2011), Bahia, Rio de Janeiro (Rathbun 1879), and São Paulo (Tommasi 1970). From intertidal zone to 550 m depth (Pawson et al. 2009).

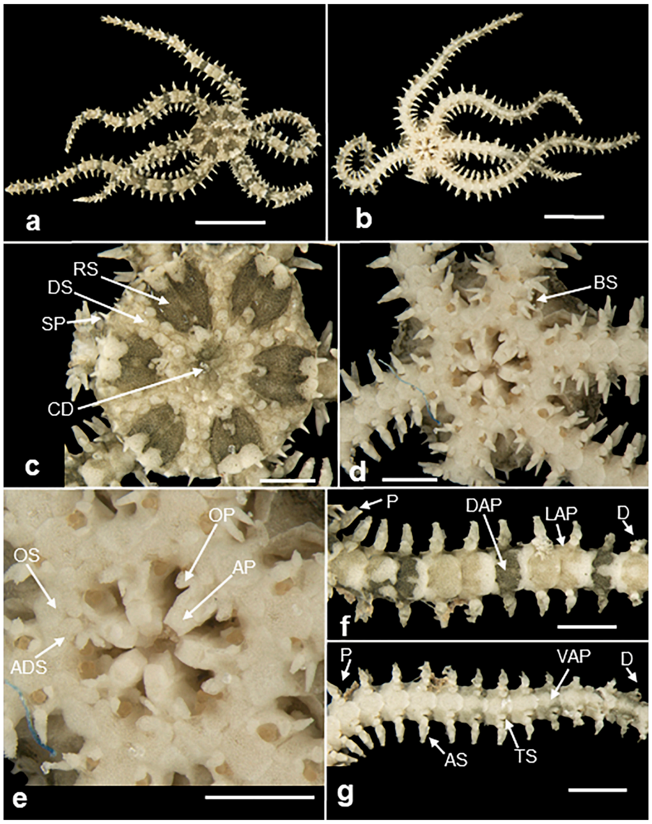


Figure 5. *Ophiactis savignyi*. a, dorsal view of the animal; b, ventral view of the animal; c, dorsal view of the disk; d, ventral view of the disk; e, detail of the jaws; f, dorsal view of the arm; g, ventral view of the arm. ADS, adoral shield; AP, apical papillae; AS, arm spine; BS, bursal slit; CD, central dorsal scale; D, dorsal region; DAP, dorsal arm plate; DS, dorsal scales; LAP, lateral arm plate; OP, oral papillae; OS, oral shield; P, proximal region; RS, radial shield; SP, spines of the disk; TS, tentacle scale; VAP, ventral arm plate. Scales: a-b, 5 mm; c-g, 1 mm.

Ecological notes: This species occurs in hard bottoms, coral reefs, algae, and mangroves. Young specimens are found in sponges or algae (Tommasi 1970, Pawson et al. 2009).

Family Ophiocomidae Ljungman, 1867

Genus *Ophiocomella* A.H. Clark, 1939

Ophiocomella ophiactoides (H.L. Clark, 1900)

(Figure 6)

Ophiocomella ophiactoides—Magalhães et al. 2005: 63; Oliveira et al. 2010: 7.

Material examined: 2 specimens (UFPB.ECH–2255), Seixas Beach, Paraíba State, Brazil, depth 1.5 m; 1 specimen **(**UFPB.ECH–2254), Seixas Beach, Paraíba State, Brazil, depth 4 m.

Description: Disk pentagonal, covered by imbricated scales with spine-like granules (Figure 6a). Radial shields small, a little wider than long, separated by some scales and spine-like granules (Figure 6c). Ventral surface of disk covered by imbricated scales (Figure 6d). Bursal slits slender, long, from first to second or third ventral arm plate. Oral shield sub-elliptical. Adoral shield longer than wide, sub-triangular. Four flattened oral papillae and a pair of apical papillae (Figure 6e). Six arms, about three times the diameter of the disk, tapering distally (Figure 6b). Dorsal arm plate sub-pentagonal, proximal edge rounded (Figure 6f). Ventral arm plate slightly as wide as long (Figure 6g). Tentacle scale diamond-shaped. Lateral arm plate with four arm spines. That near the ventral plate longer and robust. Arm spines with small teeth. Color orange or whitish to white.

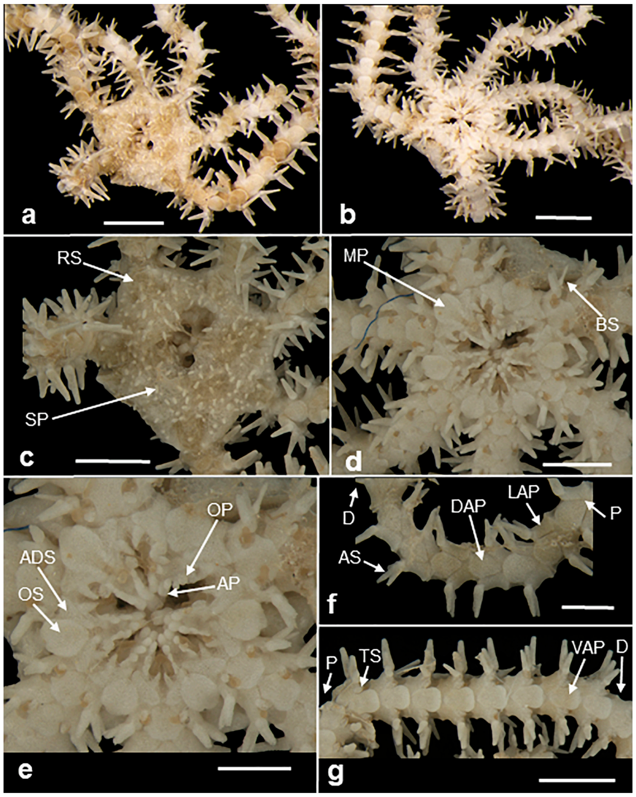


Figure 6. *Ophiocomella ophiactoides*. a, dorsal view of the animal; b, ventral view of the animal; c, dorsal view of the disk; d, ventral view of the disk; e, detail of the jaws; f, dorsal view of the arm, oriented from the distal to proximal region; g, ventral view of the arm, oriented from the proximal to the distal region. ADS, adoral shield; AP, apical papillae; AS, arm spine; BS, bursal slit; D, distal region; DAP, dorsal arm plate; LAP, lateral arm plate; MP, madreporite; OP, oral papillae; OS, oral shield; P, proximal region; RS, radial shield; SP, spines of the disk; TS, tentacle scale; VAP, ventral arm plate. Scales: a-b, d-f, 1 mm; c, g, 2 mm.

Distribution: From Florida, Mexico, Bermuda, Puerto Rico, Jamaica, Virgin islands, Curaçao, Trinidad and Tobago, Belize, Panama, Colombia and Brazil (Hendler et al. 1995, Pawson et al. 2009). In Brazilian coast, the species was reported in Paraíba (Oliveira et al. 2010), Alagoas (Lima et al. 2011) and Bahia (Albuquerque & Guille 1991). From one to 18 m depth (Hendler et al. 1995).

Ecological notes: This species is found in reef systems (Hendler et al. 1995), usually associated with algae, inside rhodoliths.

Family Ophiodermatidae Ljungman, 1867

Genus *Ophioderma* Müller & Troschel, 1840

Ophioderma appressa (Say, 1825)

(Figure 7)

Ophioderma appressum—Lima–Verde, 1969: 12; Tommasi, 1999; Gondim et al., 2008: 154; Lima & Fernandes, 2009: 60.

Ophioderma apressum—Brito, 1962: 1, fig. 8; Alves & Cerqueira, 2000: 546; Magalhães et al., 2005: 63; Oliveira et al., 2010: 7.

Ophioderma appressa—Tommasi, 1970: 65, figs. 62–63. Barboza & Borges, 2012: 7; Paim et al., 2015: 12-13.

Material examined: 1 specimen (UFPB.ECH–2261), Seixas Beach, Paraíba State, Brazil, depth 1.5 m.

Description: Disk pentagonal, covered by small granules (Figure 7a). Radial shields small, covered by granules. Ventral surface of the disk covered by a thin granulation. Bursal slits short and slender, with two openings (Figure 7c). That the most proximal appear before the first ventral arm plate and the more distal occur from third to fourth ventral arm plates. Oral shield diamond-shaped, rounded on the edge. Adoral shield flattened, triangular, not touching the proximal edge. Eight oral papillae, the most proximal slender, the second more proximal robust and elongated. Others gradually decrease along the length. The most distal papillae larger and flattened. Small apical papillae (Figure 7e). Five arms, about five times the diameter of the disk. Dorsal arm plate trapezoidal, proximal edge rounded (Figure 7b). Ventral arm plate squared to trapezoidal (Figure 7d). Two tentacle scales, the most internal elongated, and the external scale flattened with a slight projection at the apex. Lateral arm plate with eight arm spines, the most internal more robust (Figure 7f). Color gray to dark brown, arms with light and dark brown bands.

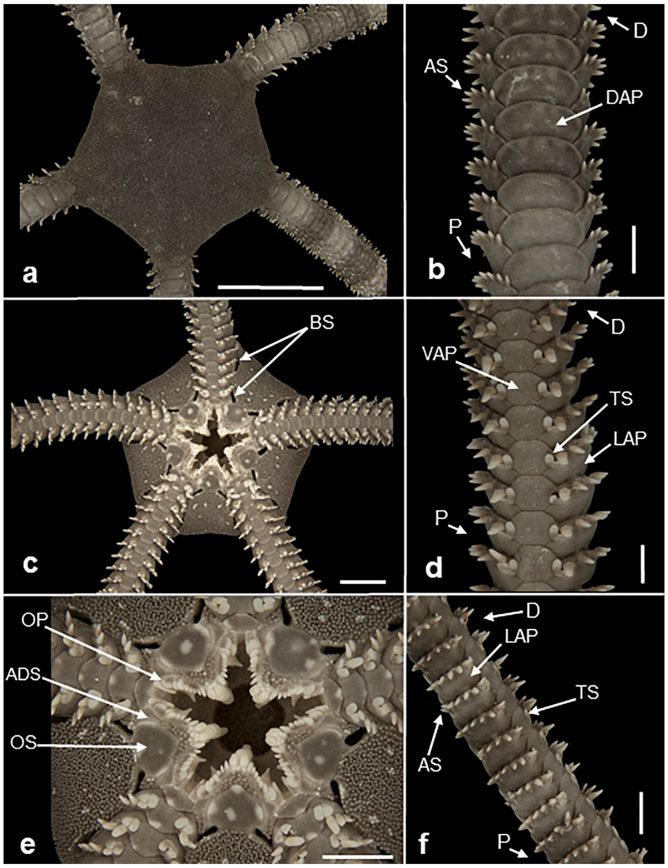


Figure 7. *Ophioderma apressa*. a, dorsal view of the disk; b, dorsal view of the arm; c, ventral view of the disk; d, ventral view of the arm; e, detail of the jaws; f, lateral view of the arm. ADS, adoral shield; AS, arm spine; BS, bursal slit; D, dorsal region; DAP, dorsal arm plate; LAP, lateral arm plate; OP, oral papillae; OS, oral shield; P, proximal region; TS, tentacle scale; VAP, ventral arm plate. Scales: a, 10 mm; b, d, f, 2 mm; c, 5 mm; e, 1 mm.

Distribution: From South Carolina, Florida, Mexico, Bermuda, Bahama Islands, Tortugas, Gulf of Mexico, Antilles, Belize, Panama, Colombia, Venezuela, and Brazil (Hendler et al. 1995, Laguarda-Figueras et al. 2009, Pawson et al. 2009). In Brazilian coast, the species was reported in Paraíba to Bahia (Rathbun 1879), Rio de Janeiro, São Paulo (Tommasi 1970). From intertidal zone to 364 m depth (Tommasi 1970).

Ecological notes: *Ophioderma appressa* usually occurs under rocks or in association with algae in reef ecosystems (Hendler et al. 1995).

Class Echinoidea Leske, 1778

Order Echinoida Claus, 1876

Family Echinometridae Gray, 1825

Genus *Echinometra* Gray, 1825

Echinometra lucunter (Linnaeus, 1758)

(Figure 8a-b)

Echinus lucunter—Linnaeus, 1758: 665.

Echinometra subangularis—Rathbun, 1879: 143.

Echinometra lucunter—Bernasconi, 1955: 62-63; Tommasi, 1957: 29, pr. 1, fig. 1-2; Brito, 1960b: 4; 1968: 21-22; 1971: 263; Lima-Verde, 1969: 10; Alves & Cerqueira, 2000: 547; Fernandes et al., 2002: 422; Magalhães et al., 2005: 63.

Material examined: 1 specimen (UFPB.ECH–2262), Seixas Beach, Paraíba State, Brazil, depth 1.5 m.

Diagnosis: Elongate oval test with two rows of large tubercules along the ambulacra and interambulacra, pairs of pores arranged in arcs of six, and a large peristome. Spines long and slender, thickened at the base, and sharply pointed at the tips. On aboral side, primary and secondary spines dark olive green, with greenish violet to purple tips. In general, the color is blackish (Hendler et al. 1995).

Remarks. Specimen young, 3 cm in diameter (Figure 8a, b).

Distribution: Western Atlantic, from North Carolina to South Brazil (Pawson et al. 2009). In Brazilian coast, the species was reported in Ceará (Martins & Martins de Queiroz 2006), Paraíba (Gondim et al. 2008), Pernambuco to Santa Catarina (Rathbun, 1879). It is found until 45 m depth (Hendler et al. 1995).

Ecological notes: The species is usually found in hard bottoms (Tommasi 1966). It prefers exposed areas, where there are large quantities of macroalgae and food available.

Class Holothuroidea

Order Dendrochirotida Grube, 1840

Family Phyllophoridae Oestergren, 1907

Genus *Euthyonidiella* Heding & Panning, 1954

Euthyonidiella occidentalis (Ludwig, 1875)

(Figure 8c-i)

Thyonidium occidentale—Ludwig, 1875: 119.

Phyllophorus occidentalis—Deichmann, 1930: 148.

Euthyonidium occidentalis—Deichmann, 1938: 380; 1941:124.

Thyonidium constituta—Sluiter, 1910: 340; Deichmann, 1926: 124.

Trachythyonidium occidentale—Deichmann, 1954: 402.

Phyllophorus (*Urodemella*) *occidentalis*— Heding & Panning, 1954: 164; Domantay, 1959: 191; Tommasi, 1969: 10.

Euthyonidiella occidentalis—Martins & Souto, 2015: 362-374.

Material examined: 1 specimen (UFPB.ECH-2264), Seixas Beach, Paraíba State, Brazil, depth 1.5 m.

Description: Rounded body, slightly narrow in the ends (Figure 8c). Tegument thin, smooth. Mouth and anus terminal. Tube feet numerous, small, arranged in the radii and some in interradii. Calcareous ring complex, with high radial pieces and posterior processes divided in two small pieces. Color brown. Body wall with tables of dentate margins, four large central holes and some lateral, about three in each side or two more proximal bigger than other distal tables (Figure 8g). Spire of two pillars ending in three teeth at the apex. Disk of tables from ventral region with eight or more holes and three or more spines in each side of the margin. Tube feet with supporting plates, large endplate (Figure 8h) and supporting rods (Figure 8i). Introvert with rosettes, rods and tables. Tentacles with rosettes (Figure 8d), rods (Figure 8f) and tables (Figure 8e).

Geographical distribution: Florida, Puerto Rico, Antilles, Aruba, Trinidad, Suriname and Brazil (Hendler et al. 1995). In Brazilian coast, the species was reported in (Gondim et al. 2008), Alagoas (Miranda et al. 2012), Bahia (Deichmann 1930), Espírito Santo, Rio de Janeiro (Mondin 1973), São Paulo (Ancona Lopez 1962). It found until 45 m deep (Hendler et al. 1995). The species can be found until 99 m depth (Miller & Pawson 1984).

Ecological notes: The specimen was found in rhodoliths, but can also be found in rocks, gravel, sand and coral stones.

Genus *Stolus* Selenka, 1867

Stolus cognatus (Lampert, 1885)

(Figure 9a-f)

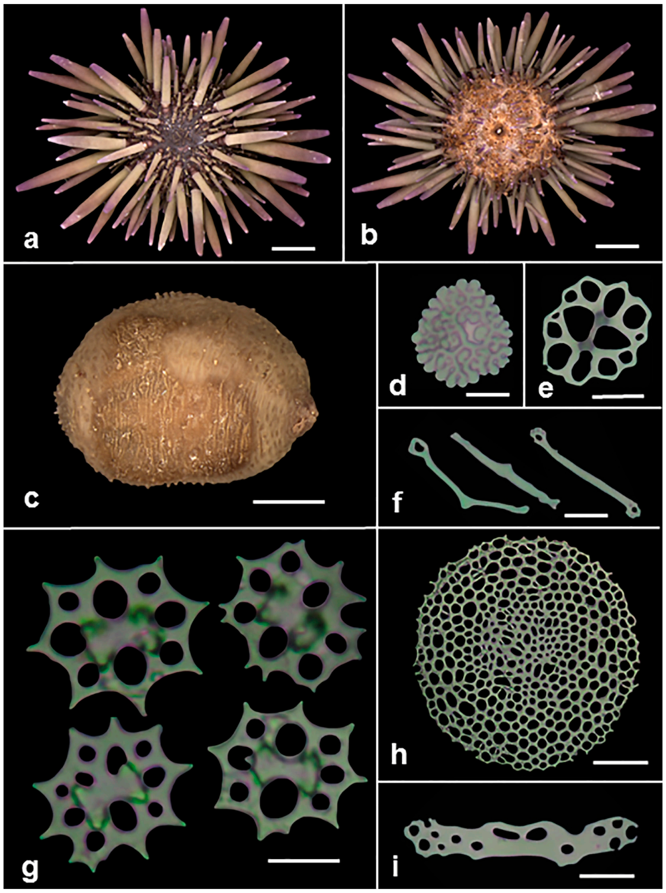


Figure 8. *Echinometra lucunter*. a, dorsal view of the animal; b, ventral view of the animal; *Euthyonidiella occidentalis*. c, dorsal view of the animal; d, rosette from tentacle; e, table from tentacles; f, rods from tentacle; g, tables from body wall; h, endplate from tube feet; i, rod from tube feet. Scales: a-b, 5 mm; c, 2 mm; d, f, 10 µm; e, g, i, 20 µm; h, 50 µm.

Thyone cognita—Deichmann, 1930: 169; Tommasi, 1969: 14.

Thyone cognate—Clark, 1933: 115; Mondin, 1973: 9.

Stolus cognatus—Caycedo, 1978: 165; Hendler et al., 1995: 275; Laguarda-Figueras et al., 2001: 33–34.

Material examined: 1 specimen (UFPB.ECH-1954) Seixas Beach, Paraíba State, Brazil.

Description: Young specimen. Body curved, with tapered ends, posterior end narrower (Figure 9a). Tegument slightly thick, rough. Mouth and anus terminal. Ten small dendritic tentacles, similar size. Tube feet arranged in double rows in the radii, larger ventrally. Calcareous ring complex, formed by small distinct pieces, posterior processes long. Color white, tentacles light brown. Body wall with numerous elongated plates, with two rows of holes (Figure 9d), sometimes perforations alternate; small buttons plate-shape with four subequal holes. Tube feet with small endplate, and supporting rods (Figure 9e), some with a third arm, and supporting plates (Figure 9f). Introvert with irregular rosettes, and large thick plates. Tentacles with rods (Figure 9c), some with holes in the ends, and rosettes (Figure 9b).

Distribution: Florida, Antilles, Venezuela and Brazil (Hendler et al. 1995). In Brazilian coast, the species was reported in Paraíba (Gondim et al. 2008), Alagoas (Miranda et al. 2012), Bahia (Deichmann 1930), Espírito Santo, Rio de Janeiro (Mondin 1973), São Paulo (Tommasi 1969). The species is found until 5 m depth (Laguarda-Figueras et al. 2001).

Ecological notes: The specimen was found inside rhodoliths. This species can be found also in coral reefs, and under rocks.

Genus *Thyone* Oken, 1815

Thyone pawsoni Tommasi, 1972

(Figure 9g-i)

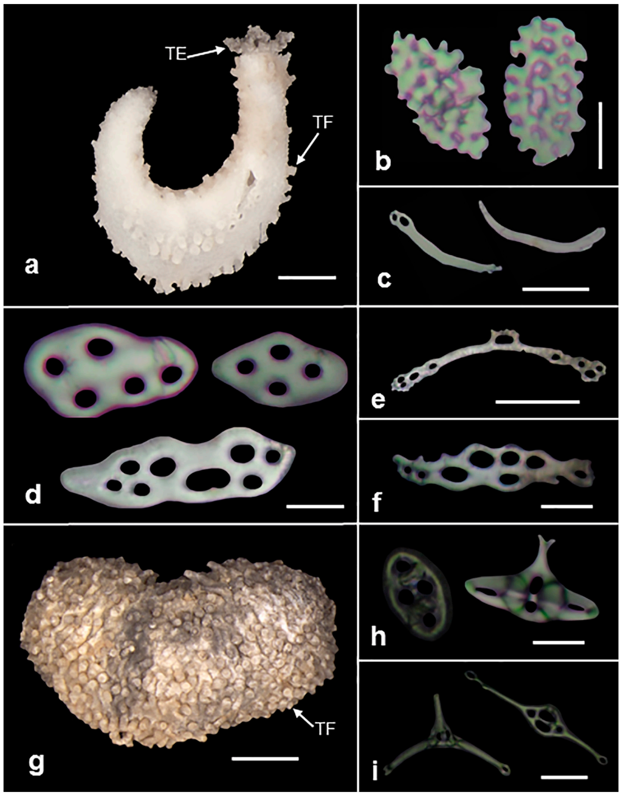


Figure 9. *Stolus cognatus*. a, dorsal view of the animal; b, rosettes from tentacle; c, rods from tentacles; d, plates from body wall; e, supporting rod from tube feet; f, supporting plate from tube feet. *Thyone pawsoni*. g, dorsal view of the animal; h, tables from body wall; I, tables from tube feet. TE, tentacles; TF, tube feet. Scales: a, 2mm; b-c, 10 µm; d-e, 50 µm; f, h-i, 20 µm; g, 1 mm.

Thyone pawsoni—Tommasi, 1972: 19; Pawson & Miller, 1981: 397-398; Miller & Pawson, 1984: 42; Pawson et al., 2010: 30; Martins et al., 2012: 3-5.

Material examined: 1 specimen (UFPB.ECH-2266), Seixas Beach, Paraíba State, Brazil, depth 1.5 m.

Description: Short body, slightly curved (Figure 9g). Mouth and anus terminal. Tube feet elongated, numerous, scattered over the body, arranged in double rows in each end on the radii, and several tube feet in interradii, arranged into two to three rows, more abundant and bigger ventrally. Calcareous ring complex, with long posterior processes. Color light brown, with brown and white spots. Body wall with tables of disk oval, four holes and high spire of two pillars, ending in two to four teeth (Figure 9h). Tube feet with elongated supporting tables (Figure 9i), with four large central holes and one or more small holes in the ends, endplate and supporting plates with irregular holes. Introvert with tables, rosettes and plates. Tentacles with rosettes and small rods, with some perforations and sometimes prolongations.

Distribution: From North Carolina, Gulf of Mexico, Venezuela, and Brazil (Bahia) (Pawson et al. 2010, Martins et al. 2012). The species is found until 5 m deep (Laguarda-Figueras et al. 2001). It is found from 6 to 51 m depth (Pawson et al. 2010, Martins et al. 2012).

Ecological notes: The specimen was found in rhodoliths. The species also can be found buried in crevices and sand (Martins et al. 2012).

Family Psolidae Burmeister, 1837

Genus *Lissothuria* Verrill, 1867

Lissothuria braziliensis (Théel, 1886)

(Figure 10)

Psolus braziliensis—Thèel, 1886: 15.

Thyonepsolus braziliensis—Deichmann, 1930: 192; Clark, 1933: 117; Deichmann, 1954: 401.

Lissothuria braziliensis—Pawson, 1967: 8–10; Tommasi, 1969: 8–9.

Material examined: 1 specimen (UFPB.ECH-2274), Seixas Beach, Paraíba State, Brazil, depth 1.5 m.

Description. Body elongate, ventrally flattened, forming a sole (Figure 10a, b, c). Tegument covered by imbricate scales, thick. Ten dendritic tentacles, with two ventral ones smaller. Mouth antero-dorsal, surrounded by numerous pointed scales. Anus postero-dorsal, surrounded by scales, small plates alternating with large plates. Five anal teeth present. Introvert short, well delimited. Tube feet from dorsal side small, covering the whole surface uniformly. Tube feet from ventral side arranged into set of four to five rows in each radius. Color light pink to white. Body wall from dorsal side with large scales (Figure 10g), with small holes and more than one network. Numerous high tables hourglass-shaped (Figure 10d, e), small tables of four pillars of reticulate structure and small perforated plates. Sole with plane plates, with irregular margins (Figure 10i). Ventral tube feet with endplate (Figure 10f), several elongated and curved supporting rods (Figure 10h, j), and irregular smooth buttons with five central holes. Tentacles with rosettes, large, flattened, perforated plates, and small plates with irregular holes and rods.

Distribution. From Antilles to Brazil (Tommasi 1969). In Brazilian coast, the species was reported in Paraíba, Alagoas (Miranda et al. 2012), Bahia (Théel, 1886), Espírito Santo (Mondin 1973). The species can be found until 5 m depth (Laguarda-Figueras et al. 2001).

Ecological notes. The species usually is found in rhodoliths and other algae.

Order Apodida Brandt, 1835

Family Chiridotidae Östergren, 1898

Genus *Chiridota* Eschscholtz, 1829

Chiridota rotifera (Pourtalès, 1851)

(Figure 11)

Synapta rotifera—Pourtalès, 1851: 15.

Chiridota rotifera—Ludwig, 1881: 41; Clark, 1907: 115; Sluiter, 1910: 341; Heding, 1928: 293; Deichmann, 1930: 212; 1963: 112; Pawson, 1976: 381; Hendler et al., 1995: 313; Laguarda-Figueras et al., 2001: 36.

Material examined: 1 specimen (UFPB.ECH–2263), Seixas Beach, Paraíba State, Brazil, depth 1.5 m.

Description: Body cylindrical, elongated. Tegument thin, with some papillae or warts formed by agglomeration of ossicles (Figure 11a). Mouth and anus terminal. Color light pink to translucent. Body wall with

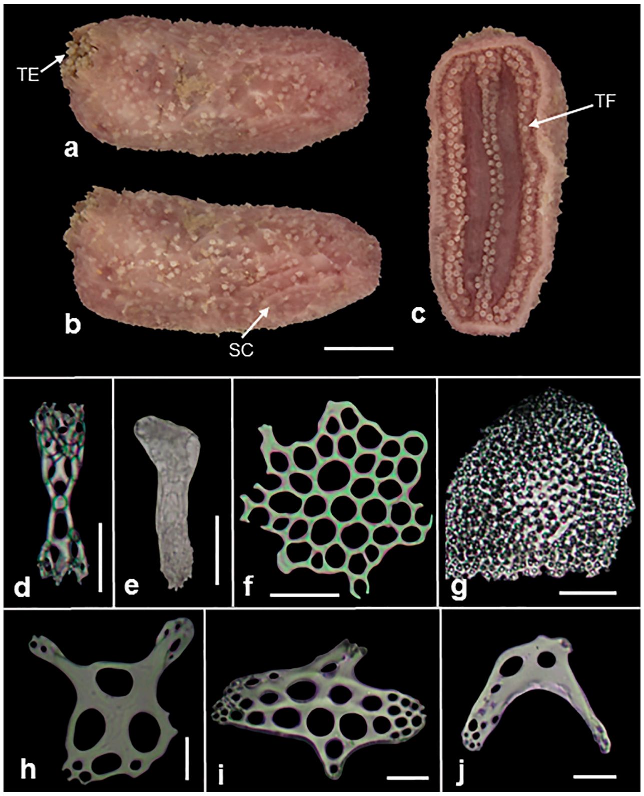


Figure 10. *Lissothuria braziliensis*. a, dorsal view of the animal; b, lateral view of the animal, c, ventral view of the animal; d-e tables hourglass-shaped; f, endplate; g, scales from body wall; h, j, curved supporting rods from tube feet; i, with plane plates from tube feet. SC, large scales from dorsal side; TE, tentacles; TF, tube feet. Scales: a-c, 6 mm; d-e, h-j, 20 µm; f, 40 µm; g, 100 µm.

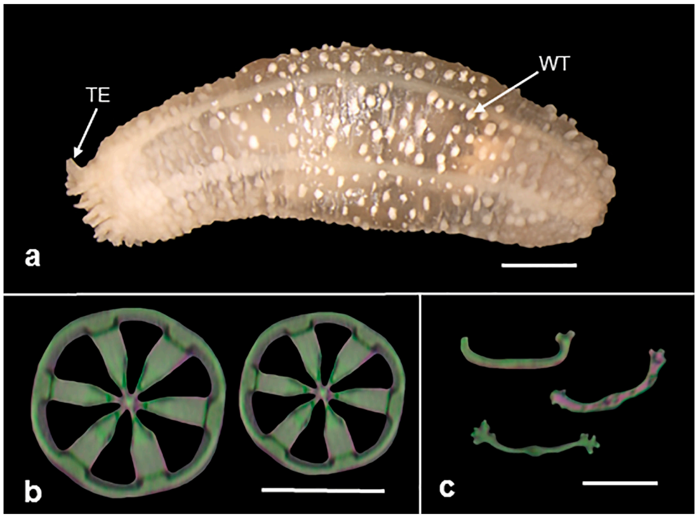


Figure 11. *Chiridota rotifera*. a, dorsal view of the animal; b, wheel ossicles from body wall; c, rods from body wall. TE, tentacles; WT, warts or papillae formed by agglomeration of ossicles. Scales: 2 mm; b, 50 µm; c, 20 µm.

wheels with six holes (Figure 11b). Small, straight to curved (C-shaped) rods in radial zones (Figure 11c). Tentacles with rods similar to those of body.

Distribution: From Florida, Mexico, Belize, Antilles, Puerto Rico, Bonaire and Brazil (Hendler et al. 1995). In Brazilian coast, the species was reported in Ceará (Martins & Martins de Queiroz 2006), Paraíba (Gondim et al. 2008), Pernambuco (Fernandes et al. 2002), Alagoas (Miranda et al. 2012), Bahia (Rathbun 1879), Rio de Janeiro (Brito 1960), São Paulo (Ancona Lopez 1957). The species occurs up to about 15 m depth (Hendler et al. 1995).

Ecological notes: The species can be found buried in the sand, under rocks, in coral reefs, rhodoliths and sponges (Hendler et al. 1995).

Discussion

The Seixas Beach has a diverse echinoderm fauna, with species of Ophiuroidea and Holothuroidea being most abundant. The species recorded here were similar to those recorded from the Cabo Branco beach (Gondim et al. 2008), an area adjacent to the Seixas Beach. We found eight of the species previously recorded from Cabo Branco Beach. Another area from the State of Paraíba, Areia Vermelha, has also been inventoried for its echinoderm fauna (Gondim et al. 2011), but only four species were recorded additionally at Seixas. The species *Ophiocomella ophiactoides* was found only at the Seixas Beach. *Amphipholis januarii* Ljungman was the most abundant species, with 34 specimens.

The species inventoried from rhodoliths of the Seixas Beach were recorded in other areas of Northeastern coast: in Ceará, by Martins & Martins de Queiroz (2006); in Pernambuco, by Lima & Fernandes (2009); in Alagoas, by Lima et al. 2011 and Miranda et al. (2012); and in Bahia, by Alves & Cerqueira (2000), Magalhães et al. (2005), Manso et al. (2008) and Paim et al. (2015). These species also occur in the Caribbean Sea. Thus, the echinoderm fauna of the Seixas Beach presents tropical species, usually found in shallow waters and with a large distribution in the Tropical Atlantic Marine Province.

Rhodolith beds provide refuge for very small species of marine invertebrates (Scherner et al. 2010). The results show that these biogenic structures are an important habitat for young specimens and some small species of Echinodermata. *Lissothuria braziliensis* has been found only rarely in other substrata. The small species *Pentamera paraibanensis* Prata & Christoffersen, 2016 was also found inside rhodoliths (Prata & Christoffersen 2016). The fauna associated with rhodoliths needs more research in order to know what species live there, and if these species complete their life cycle in these structures or if the species spend only part of their life in rhodoliths and then explore other environments.

According to the analyzed material, *Amphipholis januarii* and *Microphiopholis gracillima* are represented by young and mature species. This suggests that these species have part of their life cycle in rhodoliths in this environment. Usually, both species are found buried in mud or sand (Hendler et al. 1995). *Ophioderma appressa* usually lives under rocks, and the specimen found is a young form. The small species *Amphipholis squamata*, *Ophiactis savignyi* and *Ophiocomella ophiactoides* seem to spend their life cycle in the rhodoliths. Usually these species are common in association with algae, among its leaves (Hendler et al. 1995, Benavides-Serrato et al. 2011). The found specimen of Echinoidea represents a young individual; these animals usually build holes in hard substrata.

Small dendrochirotids holothurians *Lissothuria braziliensis* and *Thyone pawsoni* seem to prefer to live in rhodoliths and can pass the whole life cycle in these structures. The specimens of *Euthyonidiella occidentalis* and *Stolus cognatus* are young; this can suggest that these species have a part of their life cycle inside rhodoliths. This substrate can provide protection and easy access to food, ensuring species survival in the initial ages. The species *Chiridota rotifera* presents viviparity and inhabit several kinds of substrates (Hendler et al. 1995, Laguarda-Figueras et al. 2001). Probably this species also passes the whole life cycle inside these rodoliths and may reproduce there.

Most species were found at depths of 1.5 m. The species *Amphipholis squamata*, *Amphipholis januarii*, *Ophiactis savignyi* and *Ophiocomella ophiactoides* were observed at both depths, 1.5 m and 4m. *Microphiopholis gracillima* (Stimpson, 1854) was found only while sampling at 4 m. In general, these species have a wide bathymetric distribution; however, the species recorded at 1.5 m seems to prefer shallower waters.

Shallow reef ecosystems are threatened environments due to various interferences resulting from human activities (Leão et al. 2003). The area of Cabo Branco and Seixas beaches is utilized for fisheries and tourism. Tourists walk over the fauna and collect souveniers. Residents modify the natural scenery by buildings on the waterfront, mainly for commercial activities and in an attempt to control coastal erosion (Costa et al. 2007). Sustainable exploitation and management of resources in marine and coastal areas depend heavily on adequate scientific knowledge of the physical, chemical and biological processes in the oceans and their interactions with coastal areas (Awozika & Marone 2000). Studies should thus be encouraged towards inventorying the entire fauna of echinoderms and other marine invertebrates of the Seixas Beach. Surveys of species should be conducted in other substrata as well as the rhodoliths. This study shows the need for further research in the area and the importance of rhodoliths for the survival of some species of Echinodermata.

Acknowledgments

We thank the facilities provided by Federal University of Paraíba, Brazil (Laboratory of Aquatic Ecology-LABEA and Laboratory of Invertebrates Paulo Young-LIPY). J.P. acknowledges the doctoral in Sciences Scholarship received from Coordenação de Aperfeiçoamento do Ensino Superior (CAPES). D.A.C. acknowledges the Ph. D. Scholarship received from Fundação de Apoio à Pesquisa do Estado da Paraíba (FAPESQ) in partnership with CAPES. M.L.C. was supported by a productivity grant from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Author Contributions

J.P. contributed substantially in the conception and organization of the work, in the acquisition, analysis, and interpretation of the data and in the writing of the manuscript. D.A.C. contributed in data acquisition, collection of specimens and drafting of the work. C.L.C.M. contributed to the analysis, and interpretation of data and in critical reviewing the paper, adding intellectual content. M.L.C. contributed in critically reviewing and adding intellectual content, being responsible for the English text; finally, M.C.C. contributed to critical review and the adding of intellectual content.

Conflicts of interest

The authors declares that they have no conflict of interest related to the publication of this manuscript.

References

ALBUQUERQUE, M.N. 1986. Ophiuroidea Gray, 1840 (Echinodermata) da Plataforma Continental do Norte e Nordeste do Brasil. Tese de doutorado, Universidade de São Paulo, Instituto Oceanográfico, São Paulo.

ALBUQUERQUE, M.N. & GUILLE, A. 1991. Ophiuroidea (Echinodermata) ao largo do Brasil: Banco dos Abrolhos, cadeia submarina Vitória-Trindade e plataforma continental adjacente. Bol. Mus. Nac. 353: 1-30.

ALVARADO, J.J., SOLÍS-MARÍN, F.A., & AHEARN, C. 2008. Equinodermos (Echinodermata) del Caribe Centroamericano. Rev. Biol. Tropical 56(3): 37-55.

ALVES, O.F.S. & CERQUEIRA, W.R.P. 2000. Echinodermata das praias de Salvador (Bahia, Brasil). Rev. Bras. Zool., 17(2): 543-553.

AMADO-FILHO, G.M., MOURA, R.L., BASTOS, A.C., SALGADO, L.T., SUMIDA, P.Y., GUTH, A.Z., FRANCINI-FILHO, R.B., PEREIRA-FILHO, G.H., ABRANTES, D.P., BRASILEIRO, P.S., BAHIA, R.G., LEAL, R.N., KAUFMAN, L., KLEYPAS, J.A., FARINA, M. & THOMPSON, F.L. 2012. Rhodolith beds are major CaCO3 bio-factories in the Tropical South West Atlantic. PLoS ONE 7(4): 1-6.

AWOZIKA, L. & MARONE, E. 2000. Scientific needs to assess the health of the oceans in coastal areas: a perspective of developing countries. Ocean Coast Manag. 43:781-791.

BARBOZA, C.A.M. & BORGES, M. 2012. A checklist of the extant species of ophiuroids (Echinodermata: Ophiuroidea) from Brazilian waters. Zootaxa 3447: 1-21.

BENAVIDES-SERRATO, M., BORRERO-PÉREZ, G.H. & DIAZ-SANCHEZ, C.M. 2011. Equinodermos del Caribe colombiano I: Crinoidea, Asteroidea y Ophiuoridea. Serie de Publicaciones Especiales de Invemar, 22, Santa Marta.

BERNASCONI, I. 1955. Equinoideos y Asteroideos de la Colección del Instituto Oceanografico de la Univerdidad de San Pablo. Primera contribución. Bol. Inst. Oceanogr. 6: 51-57.

BERNASCONI, I. & D'AGOSTINO, M.M. 1975. Equinodermos Antarticos. III. Ofiuroideos. 2) Ofiuroideos de Georgias del Sur. Rev. Mus. Argent. Cienc. Nat. 5: 3-23.

BORGES, M. 2006. Taxonomia, distribuição e biologia reprodutiva de Ophiuroidea (Echinodermata) das Regiões Sudeste e Sul do Brasil. Tese de doutorado. Universidade Estadual Paulista, Instituto de Biociências-Zoologia, São Paulo.

BORGES, M. & AMARAL, A.C.Z. 2005. Filo Echinodermata. In Manual de Identificação dos Invertebrados Marinhos da Região Sudeste-Sul do Brasil (A.C.Z. Amaral, A.E. Rizzo & E.P. Arruda eds). Editora da Universidade de São Paulo, São Paulo, p.235-283.

BORGES, M., MONTEIRO, A.M.G. & AMARAL, A.C.Z. 2002. Taxonomy of Ophiuroidea (Echinodermata) from the continental shelf and slope of the southern and southeastern Brazilian coast. Biota Neotrop. 2(2):1-69.

BORRERO-PÉREZ, G.H., BENAVIDES-SERRATO, M. & DIAZ-SANCHEZ, C.M. 2012. Equinodermos del Caribe colombiano II: Echinoidea y Holothuroidea. Serie de Publicaciones Especiales de Invemar, 30, Santa Marta.

BRASIL, MMA-Ministério do Meio Ambiente, dos Recursos Naturais Hídricos e da Amazônia Legal. 1996. Macrodiagnóstico da zona costeira do Brasil. Brasília: Programa Nacional de Gerenciamento Costeiro.

BRITO, I.M. 1960a. Os ofiuróides do Rio de Janeiro. Parte I – Ophiothrichidae, Ophiochitonide e Ophiactidae. Contrib. Avuls. Univ. Bras. Fac. Nac. Filos. Cent. Est. Zool. 6:1-4.

BRITO, I.M. 1960b. Os Equinoideos regulares do litoral do Rio de Janeiro. Contrib. Avuls. Univ. Bras. Fac. Nac. Filos. Cent. Est. Zool. 4:1-8.

BRITO, I.M. 1962. Ensaio de catálogo dos equinodermas do Brasil. Contrib. Avuls. Univ. Bras. Fac. Nac. Filos. Cent. Est. Zool., 13:1-13.

BRITO, I.M. 1968. Asteróides e equinóides do Estado da Guanabara e adjacências. Bol. Mus. Nac. 260:1-51.

BRITO, I.M. 1971. Contribuição ao conhecimento dos Equinodermos da Ilha da Trindade, Brasil – Arq. Mus. Nac. 54:261-265.

CAYCEDO, I. E. 1978. Holothuroidea (Echinodermata) de águas someras em la costa norte de Colombia. An. Inst. Invest. Mar. 10:149-198.

CLARK, H.L. 1907. The apodous holothurians: a monograph of the Synaptidae and Molpadidae, including a report on the representatives of these families in the collections of the United States National Museum. Smithson. Contrib. Knowl., 35(1723):1-231.

CLARK, H.L. 1915. Catalogue of recent ophiurans: based on the collection of the Museum of Comparative Zoology. Mem. of the Mus. of Comp. Zool. 25(4):165-376.

CLARK, H.L. 1933. Scientific Survey of Porto Rico and the Virgin Island. A Handbook of the Littoral Echinoderms of Porto Rico and the other West Indian Island. New York Acad. Sci. 16(1):3-147.

COSTA, C.F.; SASSI, R.; COSTA, M.A.J. & BRITO, A.C.L. 2007. Recifes costeiros da Paraíba, Brasil: usos, impactos e necessidades de manejo no contexto da sustentabilidade. Gaia Scientia. 1(1):37-45.

DEICHMANN, E. 1926. Report on the Holothurians collected by the Barbados-Antigua Expedition of the University of Iowa in 1918. Univ. Iowa Stud. 1:102.

DEICHMANN, E. 1930. The Holothurians of the western part of the Atlantic Ocean. Bull. Mus. Comparat. Zool. Harv. Coll. 71(3):42-276.

DEICHMANN, E. 1938. Eastern Pacific Expeditions of the New York Zoological Society. XVI. Holothurians from the western coasts of Lower California and Central America, and from Galápagos Islands. Zool. Soc. 23(18):361-387.

DEICHMANN, E. 1954. The holothurians of the Gulf of México. Bull. U.S. Fish Comm. 55:381-410.

DEICHMANN, E. 1963. Shallow water holothurians known from the Caribbean waters. Studies Fauna Curaçao, Caribbean Islands, v.63, p.100-118.

DOMANTAY, J.S. 1959. Some holothurians from Florida in the collections of the Allan Hancock Foundation. Agra Univ. J. Res. Sci. 7(2):181-202.

FERNANDES, M.L.B., TOMMASI, L.R. & LIMA, E.J.B. 2002. Filo Echinodermata de Pernambuco. In Diagnóstico da Biodiversidade de Pernambuco (M. Tabarelli & J.M.C. Silva, orgs.), v.2, p.405-427.

GONDIM, A.I., LACOUTH, P., ALONSO, C. & MANSO, C.L.C. 2008. Echinodermata da Praia do Cabo Branco, João Pessoa, Paraíba, Brasil. Biota Neotrop. 8(2):151-159.

GONDIM, A.I., DIAS, T.L.P., CAMPOS, F.F., ALONSO, C. & CHRISTOFFERSEN, M.L. 2011. Benthic macrofauna from Areia Vermelha Marine State Park, Cabedelo, Paraíba, Brazil. Biota Neotrop. 11(2): http://www.biotaneotropica.org.br/v11n2/en/abstract?article+bn01811022011

GONDIM, A.I., ALONSO, C., DIAS, T.L.P., MANSO, C.L.C., CHRISTOFFERSEN, M.L. 2013. A taxonomic guide to the brittlestars (Echinodermata, Ophiuroidea) from the State of Paraíba continental shelf, Northeastern Brazil. ZooKeys. 307:45-96. doi: 10.3897/zookeys.307.4673

GUILLE, A. & ALBUQUERQUE, M.N. 1987. Les echinoderms duplateau continental et des seamounts MD 55 / Brésil à borddu "Marion Dufresne". Technical Report, Instaprint, La Riche, p.146-150.

HEDING, S.G. 1928. Papers from Dr. Th. Mortensen's Pacific Expedition 1914-1916. XLVI. Synaptidae. Vidensk. Medd. Dan. Naturhist. Foren. Kbh. 85:105-323.

HEDING, S.G. & PANNING, A. 1954. Phyllophoridae. Eine bearbeitung der polytentaculaten dendrochiroten holothurien des Zoologischen Museums in Kopenhagen. Spolia Zoologica Musei Hauniensis XIII. Skrifter udgivet af Universitetets Zoologiske Museum, Kobenhavn XIII, p.1-209.

HENDLER, G., MILLER, J.E., PAWSON, D.L. & KIER, P.M. 1995. Sea stars, sea urchins and allies: echinoderms of Florida and the Caribbean. Smithsonian Institution Press, Washington.

HORTA, P.A., RIUL, P., AMADO FILHO, G. A., GURGEL, C.F.D., BERCHEZ, F., NUNES, J.M.C., SCHERNER, F., PEREIRA, S., LOTUFO, T., LETÍCIA, L., SISSINI, M., BASTOS, E.O., ROSA, J., MUNOZ, P., MARTINS, C., GOUVÊA, L., CARVALHO, V., BERGSTROM, E., SCHUBERT, N., BAHIA, R.G., RODRIGUES, A.C., RÖRIG, L., BARUFI, J.B., FIGUEIREDO, M. 2016. Rhodoliths in Brazil: Current knowledge and potential impacts of climate change. Braz. J of Oceanography. 64(2): 117-136.

LAGUARDA-FIGUERAS, A., SOLÍS-MARÍN, F.A., DURÁN-GONZALEZ, A., PLIEGO, P.H. & LEÃO, Z.M.A.N., KIKUCHI, R.K.P. & TESTA, V. 2003. Corals and Coral Reefs of Brazil. In Latin America Coral Reefs (J. Cortês ed.). Elsevier Publisher, Amsterdam, p.9-52.

LAGUARDA-FIGUERAS, A., HERNÁNDEZ-HERREJÓN, A., SOLÍS-MARÍN F. A. Y A. DURÁN-GONZÁLEZ. 2009. Los ofiuroideos del Caribe Mexicano y Golfo de México. 249 pp. CONABIO-UNAM.

LIMA, E.J.B. & FERNANDES, M.L.B. 2009. Diversidade de equinodermos (Echinodermata) no Estado de Pernambuco (Brasil). Rev. Bras. Zooc. 11(1):55-63.

LIMA, M.L.F., CORREIA, M.D., SOVIERZOSKI, H.H. & MANSO, C.L.C. 2011 New records of Ophiuroidea (Echinodermata) from shallow waters off Maceió, Alagoas, Brazil. Mar. Biodivers. Rec. 4:1-10.

LIMA, P.J. & HECKENDORFF, W.D. 1985. Climatologia. In Atlas Geográfico do Estado da Paraíba (Governo do Estado da Paraíba, Secretaria da Educação, eds.). Grafset, João Pessoa, p.34-43.

LIMA-VERDE, J.S. 1969. Primeira contribuição ao inventário dos equinodermas do nordeste brasileiro. Arq. Ciênc. Mar. 9(1): 9-13.

LINNAEUS, C. 1758. Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio decima, reformata. Laurentius Salvius, Holmiae. ii, pp.824.

LUDWIG, H.L. 1875. Beitrage zur kenntniss der holothurien. Arb. Zool. Zootom. Inst. Würz. 2(2):77-120.

LUDWIG, H.L. 1881. Über eine lebendiggebarende Synaptide und zwei andere holothurienarten des Brasilianischen kuste. Arch. Ital. Biol. 2:41-58.

MAGALHÃES, W.F., MARTINS, L.R. & ALVES, O.F.S. 2005. Inventário dos Echinodermata do Estado da Bahia. Braz. J. Aquat. Sci. Tech. 9(1):61-65.

MANSO, C.L.C., ALVES, O.F.S. & MARTINS, L.R. 2008. Echinodermata da Baía de Todos os Santos e da Baía de Aratú (Bahia, Brasil). Biota Neotrop. 8(3):179-196.

MARTINS, I. X. & MARTINS DE QUEIROZ, A. C. 2006. Equinodermos do litoral do estado do Ceará, pp.199-220. In: MATTHEWS-CASCON, H. & LOTUFO, T. M. C. (ed.). Biota Marinha da Costa Oeste do Ceará. Brasília, Ministério do Meio Ambiente. 248pp.

MARTINS, L. & SOUTO, C. 2015. Taxonomic review of four western Atlantic dendrochirotids (Holothuroidea) with the description of a new Brazilian cucumariid species and designation of neotypes. Zootaxa 3919(2):362-374.

MARTINS, L., SOUTO, C. & MENEGOLA, C. 2012. First record of *Holothuria (Theelothuria) princeps* and *Thyone pawsoni* (Echinodermata: Holothuroidea) in the South Atlantic Ocean. Mar. Biodiv. Rec., 5.

MELO, R.S., CRISPIM, M.C., VIANA, E.R. & LINS, R.P. 2008. Planejamento turístico e zoneamento ambiental: um estudo de caso nos ambientes recifais das praias do Seixas, Penha e Arraial – PB. Cad. Virt. Tur. 8(2):23-33.

MELO, R.S., LINS, R.P.M. & ELOY, C.C. 2014. O impacto do turismo em ambientes recifais: caso praia Seixas-Penha, Paraíba, Brasil. Rev. Elet. Prod. 8(1):67-83.

MILLER, J.E. & PAWSON, D.L. 1984. Holothurians (Echinodermata: Holothuroidea). Mem. Hourg. Crui. 7(1) 1-79.

MIRANDA, A.L.S., LIMA, M.L.F., SOVIERZOSKI, H.H. & CORREIA, M.D. 2012. Inventory of the Echinodermata collection from the Universidade Federal de Alagoas. Biota Neotropica 12(2):135-146.

MONDIN, M. A. 1973. Estudo sobre os Holothuroidea do Brasil. Holothuroidea de Santa Cruz, Estado do Espírito Santo. Atas Soc. Biol. Rio Jan. 17(1):5-11.

NUNES, T.B. 1975. Echinoderma da Baía de Aratu (Bahia, Brasil). Memories of the second Latin American Symposium of Oceanographic Biology, Universidad de Oriente, Cumana, Venezuela.

OLIVEIRA, J.P., OLIVEIRA, J. & MANSO, C.L.C. 2010. Inventário da coleção de equinodermos do LABIMAR, Campus Prof. Alberto Carvalho, Universidade Federal de Sergipe. Sci. Plena 6(12): 1-14.

PAIM, F.B., GUERRAZZI, M.C., BORGES, M. 2015. Descriptive and illustrated diagnosis of Ophiuroidea fauna (Echinodermata) in the shallow waters of North-eastern Brazil. Marine Biodiversity Records 8: 1-17. doi:10.1017/S1755267215000652.

PAWSON, D. L. 1967. The Psolid Holothurian Genus *Lissothuria*. Proc. U. S. Nat. Mus. 122(3592):1-17.

PAWSON, D.L. 1976. Shallow-Water Sea Cucumbers (Echinodermata: Holothuroidea) from Carrie Bow Cay, Belize. P. Biol. Soc. Wash. 89:369-382.

PAWSON, D.L. 2007. Phylum Echinodermata. Zootaxa 1668:749-764

PAWSON, D.L. & MILLER, J.E. 1981. Western Atlantic sea cucumbers of the genus *Thyone*, with description of two new species (Echinodermata: Holothuroidea). Proc. Biol. Soc. Wash. 94(2):391-403.

PAWSON, D.L., VANCE, C. M., SOLIS-MARIN, F. A., CHRISTOPHER, L. M. 2009. Echinodermata of the Gulf of Mexico. Volume 1. Austin, TX: Texas A & M University Press, pp.1177-1204.

PAWSON, D.L., PAWSON, D.J. & KING, R.A. 2010. A taxonomic guide to the Echinodermata of the South Atlantic Bight, USA: 1. Sea cucumbers (Echinodermata: Holothuroidea). Zootaxa 2449:1-48.

POURTALÈS, L.F. 1851. On the Holothuriae of the Atlantic Coast of the United States. Am. Assoc. Adv. Sci., Washington, pp.8-16.

PRATA J. & CHRISTOFFERSEN M.L. 2016. A new species of *Pentamera* Ayres, 1852 from the Brazilian coast (Holothuroidea, Dendrochirotida, Phyllophoridae). ZooKeys, 634:1-14.

RATHBUN, R. 1879. A List of the Brazilian echinoderms, with notes on their distribution. T. Connecticut Academy of Arts and Sciences. 5:139-151.

RIUL, P.; LACOUTH, P.; PAGLIOSA, P. R.; CHRISTOFFERSEN, M. L.; HORTA, P. A. 2009. Rhodolith beds at the easternmost extreme of South America: Community structure of an endangered environment. Aquat. Bot. 90:315-320.

SLUITER, C. P. 1910. Westindische holothurien. Zool. Jahrb. Supp. 11(2):331-342.

SPALDING, M.D., FOX, H.E., ALLEN, G.R., DAVIDSON, N., FERDAÑA, Z.A., FINLAYSON, M., HALPERN, B.S., JORGE, M.A., LOMBANA, A., LOURIE, S.A., MARTIN, K.D., MCMANUS, E., MOLNAR, J., RECCHIA, C.A., ROBERTSON, J. 2007. Marine Ecoregions of the World: a bioregionalization of coast and shelf areas. BioScience. 57:573-583.

SCHERNER, F., RIUL, P., BASTOS, E., BOUZON, Z. L., PAGLIOSA, P. R., BLANKENSTEYN, A., OLIVEIRA, E. C. & HORTA, P. A. 2010. Herbivory in a rhodolith bed: a structuring factor? Pan-Am. J. Aquat. Sci. 5:358-366.

THÉEL, H. 1886. Report on Holothurioidea. Reports on the results of dredging, under the Supervision of Alexander Agassiz, in the Gulf of Mexico (1877-78), in the Caribbean Sea (1879-80), and along the Eastern Coast of the United States during the Summer of 1880, by the U.S. Coast Survey Steamer "Blake". Bull. Mus. Comp. Zool. 13:1-21.

TOMMASI LR. 1957. Os Echinodermata do litoral de São Paulo - I. Echinoidesa, Crinoidea e Holothurioidea do Bentos Costeiro. Pap. Depart. Zool. São Paulo, 13(12):19-44.

TOMMASI, L.R. 1966. Lista dos Equinoides recentes do Brasil. Contrib. Inst. Oceanogr. Univ. 11:1-50.

TOMMASI, L.R. 1967. Sobre dois Amphiuridae da fauna marinha do Sul do Brasil. Contrib. Avuls. Inst. Oceanogr. Univ. São Paulo 12:1-5.

TOMMASI, L.R. 1969. Lista dos Holothuroidea recentes do Brasil. Contrib. Avuls. Inst. Oceanogr. Univ. São Paulo 15:1-29.

TOMMASI, L.R. 1970. Os ofiuróides recentes do Brasil e de regiões vizinhas. Contrib. Avuls. Inst. Oceanogr. Univ. São Paulo 20:1-146.

TOMMASI, L.R. 1972. Equinodermes da região entre o Amapá (Brasil) e a Flórida (E.U.A.). II. Echinozoa. Bol. Inst. Oceanogr. Univ. São Paulo 21:15-67.

TOMMASI, L.R. 1999. Echinodermata Recentes e Fósseis do Brasil. Base de Dados Tropical, Campinas.

UNDERWOOD, A.J. & CHAPMAN, M.G. 2013. Design and analysis in benthic surveys in environmental sampling. In Methods for the Study of Marine Benthos (A. Eleftheriou, ed). 4 ed. John Wiley & Sons, Chichester, p.1-45.

VENTURA, C.R.R., BORGES, M., CAMPOS, L.S., COSTA-LOTUFO, V., FREIRE, C.A., HADEL, V.F., MANSO, C.L.C., SILVA, J.R.M.C., TAVARES, Y. & TIAGO, C.G. 2012. Echinoderm from Brazil: historical, research and the current state of biodiversity knowledge. In Echinoderm research and diversity in Latin America (J.J. Alvarado & F.A. Solís-Marín, ed.), v. 1. Springer, Verlag Berlin Heidelberg, p.301–344.

VERRIL, A.E. 1868. Notes of the radiate on the Museum of Yale College, with descriptions of genera and species. 4. Notice on the corals and echinoderms collected by Prof. C.F. Hartt at the Abrolhos Reefs, Province of Bahia, Brazil, 1867. Trans.-Connect. Acad. Arts Sci. 1(2):351-371.

ZIMMERMAN, K.M., STANCYK, S.E. & CLEMENTS, L.A.J. 1988. Substrate selection by the burrowing brittlestar *Microphiopholis gracillima* (Stimpson) (Echinodermata: Ophiuroidea). Mar. Behav. Physiol. 13:239-255.

Received: 18/04/2017
Revised: 21/08/2017
Accepted: 22/08/2017

Biota Neotropica 17(3): e20170338, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Selective advantage conferred by resemblance of aposematic mimics to venomous model

Frederico Gustavo Rodrigues França[1*], Vívian da Silva Braz[2] & Alexandre Fernandes Bamberg de Araújo[3]

[1]Universidade Federal da Paraíba, Centro de Ciências Aplicadas e Educação, Departamento de Engenharia e Meio Ambiente, CEP 58297-000, Rio Tinto, PB, Brazil

[2]Centro Universitário de Anápolis, Programa de Pós-Graduação em Sociedade, Tecnologia e Meio Ambiente, Anápolis, GO, Brazil

[3]Universidade Federal Rural do Rio de Janeiro, Instituto de Biologia, Departamento de Biologia Animal, 23851-970, Seropédica, RJ, Brazil

**Corresponding author: Frederico Gustavo Rodrigues França, e-mail: fredericogrf@gmail.com*

FRANÇA, F. G. R., BRAZ, V. S., ARAÚJO, A. F. B. **Selective advantage conferred by resemblance of aposematic mimics to venomous model.** Biota Neotropica. 17(3): e20170338. http://dx.doi.org/10.1590/1676-0611-BN-2017-0338

Abstract: Mimicry is an excellent example of how natural selection can act on color, morphology, and behavior of species. Herein we assess predation rates on coral snake mimics in Central Brazil, a region with many mimics but only a single model, to answer the following questions: (i) Do predators avoid attacking coral snake mimics? (ii) Does the degree to which mimics resemble their venomous model affect the frequency of predator attacks? (iii) Do predators attack different body regions in mimics with different color patterns? Our experiment was conducted in the Chapada dos Veadeiros National Park, in the municipality of Alto Paraíso de Goiás, state of Goiás, Brazil. To evaluate predation rates on the different mimic patterns, we made 2,400 clay snake replicas using pre-colored nontoxic plasticine and distributed them in open savanna landscapes within the park. A total of 164 (6.83%) replicas were attacked by predators of snakes. Among these attacks, 121 were attacks by birds, and 43 were attacks by carnivorous mammals. Logistic regression and Fisher's exact test indicated that replicas with red, white, and black coloration are less likely to be attacked than were grey replicas, and coral snake replicas were attacked more often at the "head" end. Also, the greater the similarity to the pattern of venomous coral snakes, the rarer the attack on the replica. Our study underscores the strong selective force that protects coral snake mimics from predators. Our findings reinforce resemblance to the model as an extremely effective strategy in a complex natural system with only one model and numerous mimics.

Keywords*:* *mimicry, coral snake, Central Brazil, predation.*

Vantagem seletiva atribuída à semelhança de mímicos aposemáticos ao modelo venenoso

Resumo: O mimetismo é um ótimo exemplo de como a seleção natural pode agir sobre a coloração, morfologia e comportamento das espécies. Neste trabalho nós utilizamos as taxas de predação em mímicos de cobras corais no Brasil Central, uma região com muitos mímicos mas apenas um modelo, para responder as seguintes questões: (i) Os predadores evitam atacar os mímicos das cobras-corais? (ii) O grau de semelhança de cada mímico em relação ao modelo afeta a frequência de ataque dos predadores? (iii) A região do corpo na qual os predadores atacam varia entre os diferentes mímicos? O estudo foi realizado no Parque Nacional da Chapada dos Veadeiros, município de Alto Paraíso de Goiás, estado de Goiás, Brasil. Para avaliar as taxas de predação nos diferentes padrões, nós utilizamos 2400 réplicas construídas com massa de modelar pré-colorida e não tóxica e distribuídas em fitofisionomias abertas de Cerrado do Parque. Um total de 164 (6.83%) réplicas foram atacadas por predadores de serpentes, sendo 121 réplicas atacadas por aves e 43 por mamíferos. A análise de regressão logística e o teste exato de Fisher indicaram que as réplicas com cores vermelha, preto e branco são menos propensas a serem atacadas do que as réplicas cinzas e que essas réplicas com cores de cobra-coral são mais atacadas na extremidade da "cabeça". Além disso, quanto maior a semelhança do padrão da cobra-coral verdadeira, menor a probabilidade da réplica ser atacada. Este estudo evidencia a grande força seletiva de serpentes corais sobre a proteção de serpentes miméticas e reforça a semelhança do modelo como uma estratégia extremamente eficiente em um sistema complexo com um modelo e vários mímicos.

Palavras-chave*:* *mimetismo, cobra coral, Brasil Central, predação.*

Introduction

Mimicry is an excellent example of how natural selection can act on color, morphology, and behavior of species. In order to avoid predation, plants, invertebrates, and vertebrates have evolved a myriad of mimicry strategies worldwide (Ruxton et al. 2004). Coral snake mimicry systems enable the study of the selective pressures driving the evolution of mimicry in vertebrates and involve the following understandings. (i) Brightly banded neotropical snakes are mimics of elapid coral species. In spite of a previous debate, coral snake mimicry is widely supported by studies of geographic concordance (Greene & McDiarmid 1981, Marques & Puorto 1991, Harper & Pfennig 2007), allopatric mimicry (Pfennig et al. 2001, Pfennig et al. 2007, Pfennig & Mullen 2010, Akcali & Pfennig 2014), and genetics and evolution (Harper & Pfennig 2008, Pyron & Burbrink 2009, Kikuchi & Pfennig 2010, Pfennig et al. 2015). (ii) A wide range of mimics of poisonous species are protected from predation. This assumption was confirmed by various experiments with clay models (Brodie 1993, Buasso et al. 2006, Kikuchi & Pfennig 2010). (iii) The success of avoiding predation increases with resemblance to the model. Brodie (1993) suggested some mimics would be less predated than others. Furthermore, he proposed that predation rate on mimics would decline as resemblance to the model increased. However, he stated that testing this hypothesis would be difficult because there are multiple models in his study area and suggested further experimentation on assemblages with multiple mimics and a single model pattern to address this issue. Pfennig & Kikuchi (2012) demonstrated differences in predation rates among mimics of *Lampropeltis elapsoides* (Holbrook, 1838) (Colubridae), in which poor mimics are more susceptible to attacks than perfect or good mimics.

Herein we assess predation rates on coral snake mimics in Central Brazil, a region with many mimics but only a single model, to answer the following questions: (i) Do predators avoid attacking coral snake mimics? The hypothesis is that snakes with bright red, white, and black coloration are attacked less often than brown/grey snakes, as these colors work as an aposematic signal for natural predators of snakes, and predators avoid these colors even when the patterns presented are imperfect (Smith 1975, Greene & McDiarmid 2005, Kikuchi & Pfennig 2010). (ii) Does the degree to which mimics resemble their venomous model affect the frequency of predator attacks? The hypothesis is that mimics that closely resemble the pattern of venomous coral snakes (tricolor triads in red, white, and black along the entire body) will be less attacked than false-coral snakes with dissimilar patterns (imperfect patterns). The prediction is that predators, even if they generally avoid aposematic color snakes, can distinguish poor mimics from good ones in an aposematic system (Brodie 1993, Pfennig & Kikuchi 2012). (iii) Does the body region that predators attack vary among mimics with different patterns? The hypothesis is that predators principally attack coral snakes and mimics in the head, to avoid possible injuries from a bite, whereas they attack harmless snakes in the body (Brugger 1989, Greene & McDiarmid 2005).

Material and Methods

Our experiment was conducted in the Chapada dos Veadeiros National Park (CVNP) (13º 51'S to 14º 10'S and 47º 25'W to 47º42'W), a 65,512-ha protected area at the core of the Cerrado biome, in the municipality of Alto Paraíso de Goiás, northern Goiás state, central Brazil. Approximately 50 species of snakes live in the park (França & Braz 2013), of which one species is venomous, *Micrurus frontalis* (Duméril, Bibron & Duméril, 1854) (Elapidae). There are also nine coral snake mimics: *Tantilla melanocephala* (Linnaeus, 1758) (Colubridae), *Apostolepis ammodites* Ferrarezzi, Barbo & Albuquerque, 2005 (Elapomorphini: Xenodontinae: Dipsadidae), *Erythrolamprus aesculapii* (Linnaeus 1758) (Xenodontini: Xenodontinae: Dipsadidae), *Oxyrhopus guibei* Hoge & Romano 1977 (Pseudoboini: Xenodontinae: Dipsadidae), *Oxyrhopus rhombifer* Duméril, Bibron & Duméril, 1854 (Pseudoboini: Xenodontinae: Dipsadidae), *Oxyrhopus trigeminus* Duméril, Bibron & Duméril, 1854 (Pseudoboini: Xenodontinae: Dipsadidae), *Oxyrhopus petolarius digitalis* (Reuss, 1834) (Pseudoboini: Xenodontinae: Dipsadidae), juvenile *Pseudoboa nigra* (Duméril, Bibron & Duméril, 1854) (Pseudoboini: Xenodontinae: Dipsadidae), and juvenile *Boiruna maculata* (Boulenger 1896) (Pseudoboini: Xenodontinae: Dipsadidae).

Snake replicas were made using pre-colored nontoxic plasticine (Acrilex) threaded onto a plastic tube and a wire to create an S-shape (following Brodie 1993). All snake models looked very similar to juvenile or adult snake species found in the CNPV. Replicas were 1.5 cm in diameter and 20 cm in length, and each replica had a "head" representing the anterior end of the snake, while the posterior end was anchored into the ground by wire. Six different color patterns were constructed following the classification proposed by Savage & Slowinski (1992): (A) TT (the model/perfect mimic, a tricolor triad characteristic of the venomous coral snake *M. frontalis* and mimics such as *Simophis rhinostoma*, *O. trigeminus*, and *O. guibei*), (B) TD (tricolor dyad characteristic of *E. aesculapii*), (C) TSB (black diamond blotches (outlined in yellow) on red, characteristic of the imperfect mimic *O. rhombifer*), (D) BIR (red and black bicolor, characteristic of *O. petola*), (E) UCD (unicolor in red with black nuchal collar characteristic of non- or mildly venomous rear-fanged colubrid snakes of the genera *Apostolepis* and *Phalotris* and juveniles of *Clelia*) and (F) CTR (uniformly gray control, similar to non-venomous colubrid snakes of the genera *Chironius* and *Erythrolamprus*) (Figure 1).

Figure 1. Clay model replicas of six snake patterns. The patterns are: A- TT (tricolor triad), B- TD (tricolor diad), C- TSB (black diamond blotches (outlined in yellow) on red), D- BIR (red and black bicolour), E- UCD (unicolor in red with black nuchal collar), and F- CTR (uniformly gray control).

To evaluate predation rates on the different mimic patterns, we used 2,400 clay replicas. We distributed 600 clay replicas (100 of each pattern) during each of four months (May, June, September, and November 2007). These 600 clay replicas were distributed randomly in four transects, in commonly open landscapes within the CVNP, such as grasslands and montane savannas. The transects were at least 30 km apart from one another, and replicas inside each transect were placed at least 10 m apart. Transects were not located exactly in the same places each month but were allocated in the same region of the CVNP, with the following geographical coordinates (SAD69): (1) 14º09'S and 47º46'W; (2)14º08'S and 47º45'W; (3) 14º06'S and 47º38'W; (4) 14º05'S and 47º37'W. Each replica remained in the field for seven consecutive days, after which any attack marks were registered and the replica was then removed. Replicas were not reused in subsequent months.

We did not attempt to compensate for the relative conspicuousness of the models in the environment because previous studies on aposematic snakes demonstrated that there were no differences in attack rates when snakes had different backgrounds (Brodie 1993, Wüster et al. 2004, Buasso et al. 2006). All replicas in each transect were in place for 10 consecutive days, after which we checked replicas for evidence of predator attacks. We counted only marks made by birds (V- and U-shaped) and carnivorous mammals (Canidae, Didelphidae, Mustelidae) (teeth-shaped) as predator attacks. Marks made by rodents and arthropods were ignored. Replicas with evidence of more than one predator attack were scored for only a single predator attack based on Brodie (1993). All marked replicas were preserved and housed in the Laboratório de Ecologia Animal of Universidade Federal da Paraíba (UFPB).

We employed logistic regression to model differences in predation rates among coloration patterns and estimate the odds of a predator attack for each pattern (Tabachnick & Fidell 2007). The binary logistic model was used to estimate the probability of a binary response based on one or more predictor (or independent) variables (Tabachnick & Fidell 2007). To perform logistic regression, we considered predation a binary dependent variable (0 = non attack, 1 = attack) tested against the different color patterns of replicas of snakes (TT, TD, TSB, BIR, UCD, CTR). Each attacked replica was computed as 1, regardless of the type of predator or number of attacks. We tested the null hypothesis that the color patterns did not influence predation on snake replicas. Also, we used Fisher's exact test to determine whether the body region attacked by predators differed by pattern. We carried out statistical analyses using R (R Development Core Team 2010), with a significance level of 5% to reject the null hypothesis. Logistic regression was done using the lmr function of the rms package (Harrell 2011).

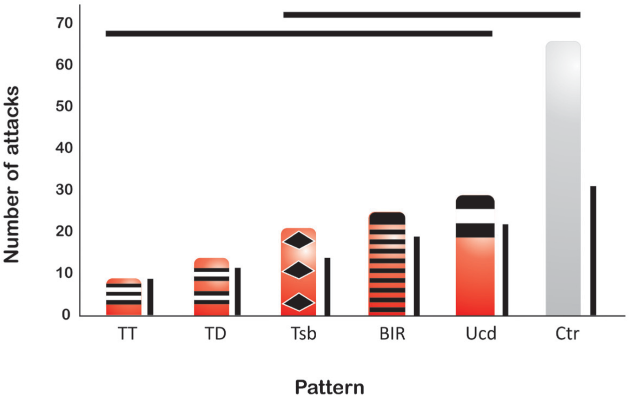


Figure 2. The number of attacks by natural predators on each of six patterns of snakes. Solid lines above bars indicate groups that differ significantly from each other by Kruskal-Wallis analysis. Solid lines on the side of the bars denote the number of attacks on the head of clay models.

Results

Of the 2,400 clay models, 164 (6.83%) were attacked by predators of snakes. Among these attacks, 121 (74%) were inflicted by birds and 43 (26%) were attacks by carnivorous mammals. In addition to marks on the models, there was other evidence of predators near some models, such as foot imprints, feathers, or fur. We recorded attacks on nine models of pattern TT, 14 of TD, 21 of TSB, 25 of BIR, 29 of UCD, and 66 of CTR. Logistic regression indicated that pattern significantly affected the probability of being predated (likelihood ratio $X^2 = 71.18$, df = 5, $p < 0.0001$) and estimated the odds of being attacked for each pattern (Table 1). We observed that CTR pattern was more attacked than any other red, black and white patterns and also an increase in predation odds as resemblance to the model decreased (Figure 2). There were more attacks on the head than on the body for patterns TT (Fisher's exact test, $p = 0.004$), BIR (Fisher's exact test, $p = 0.013$), and UCD (Fisher's exact test, $p = 0.002$), but the number of attacks did not significantly differ between the head and body ($p < 0.05$) for TD (Fisher's exact test, $p = 0.055$), TSB (Fisher's exact test, $p = 0.183$), and CTR (Fisher's exact test, $p = 0.7$).

Discussion

We observed that models with patterns that mimicked coral snakes were less predated than were models that did not mimic coral snakes; there was a direct relationship between predation and resemblance to the model, and predators appeared to target the heads of mimics more often. The single venomous model in the CVNP, the coral snake *M. frontalis*, conferred some degree of protection from predators to an ample range of imperfect mimics. Coral snakes (TT) combine dangerous venom with difficulty of capture, secretive habits, and defensive behavior, making them excellent models for mimicry (Greene & McDiarmid 1981). Imperfect mimics receive some protection even in allopatric regions (Pfennig & Mullin 2010). Moreover, frequency-dependent selection predicts that if harmless and edible perfect mimics become too common, predators will learn to ignore the warning coloration. Very similar morphs should receive the best protection, leading to the possibility of stable polymorphisms (Mallet & Joron 1999).

Table 1. Summary of the Logistic regression results of predation in intensity in snake models. The intercept includes the pattern TT.

	Parameter Estimate	Standard Error	Wald Chi-Square	Pr >Chi-Sqr	Odds Ratio
Intercept	-3.7715	0.3371	-11.19	<0.0001	0.02
TD	0.4547	0.4332	1.05	0.2939	1.58
TSB	0.8785	0.4049	2.17	0.0300	2.41
BIR	1.0634	0.3954	2.69	0.0072	2.90
UCD	1.2226	0.3884	3.15	0.0016	3.40
CTR	2.1500	0.3631	5.92	<0.0001	8.58

The small number of attacks on TT, TD, and TSB patterns agrees with the results of a previous study in the Argentinian Chaco (Buasso et al. 2006). Furthermore, the UCD result was similar to findings in Costa Rica, in which the collar pattern was the most attacked coral snake phenotype (Brodie 1993). Our findings support Brodie's (1993) prediction and suggest that the most similar phenotypes enjoy a selection advantage for predator deterrence over imperfect mimics. Kikuchi & Pfennig (2010) changed the proportion and the order of the colors and demonstrated no difference in predation between a perfect mimic and a good one (with a different order of colors), but both were less predated than a poor mimic (with both a different proportion and order of colors). These results confirm Brodie's prediction that predation on mimics will increase as difference to the model increase. Kikuchi & Pfennig (2013) also predicted loose selection (i.e., an increase in predation rate) on the proportions of dorsal colors, a generalization of the deadly model scenario. Although our data cannot confirm any specific assumptions regarding colors, our results match the prediction of an increase in predation rate as resemblance with the model decreased, using the natural assemblage of species as a proxy for a resemblance gradient. Finally, regarding the higher predation on CTR, most snakes in neotropical assemblages are plain brown or grey, and all previous studies have found the highest rates of attacks on control patterns (Brodie 1993, Buasso et al. 2006).

The attacks directed to the head end instead the body portion in aposematic (red, white, and black) replicas are evidence that the predators recognize them as potentially dangerous snakes. Advantages for head-first attack include a decrease in the probability of incurring injury when handling dangerous prey, such as deadly coral snakes, and a reduction in swallowing time (Greene 1976).

Our study underscores the strong selective force protecting coral snake mimics from predators. Although all mimic phenotypes appear to have an advantage over gray-colored snakes, selection favors the best mimics. Our findings reinforce resemblance to the model as an extremely effective strategy in a complex natural system with only one model and a variety of mimics. Even imperfect mimics were less predated than non-coral snakes, revealing that even imperfect mimicry carries an advantage. This result confirmed previous assumptions regarding the avoidance of good mimics (Greene & McDiarmid 2005, Kikuchi & Pfennig 2013) and suggests that this evolutionary strategy may be widespread in dissimilar neotropical environments.

Acknowledgments

The authors are grateful to Daniel Rios de Magalhães Borges and ICMbio for work permits in the Chapada dos Veadeiros National Park. We thank Santos Balbino, Davi Pantoja, and Iubatã Farias for their generous help during fieldwork. We thank Ailton Maciel for help with the figures. We also thank the National Counsel of Technological and Scientific Development (CNPq) for a graduate scholarship.

Author Contributions

Frederico Gustavo Rodrigues França: Substantial contribution in the concept and design of the study; Contribution to the acquisition of data; analysis and interpretation of data; manuscript preparation and critical review adding intellectual content.

Vívian da Silva Braz: Substantial contribution in the concept and design of the study; Contribution to the acquisition of data; analysis and interpretation of data; manuscript preparation and critical review adding intellectual content.

Alexandre Fernandes Bamberg de Araújo: Substantial contribution in the concept and design of the study; Contribution to the analysis and interpretation of data and critical review adding intellectual content.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

AKCALI, C.K. & PFENNIG, D.W. 2014. Rapid evolution of mimicry following local model extinction. Biol. letters 10(6):20140304. http://dx.doi.org/10.1098/rsbl.2014.0304

BRODIE, E.D., iii. 1993. Differential Avoidance of Coral Snake Banded Patterns by Free-Ranging Avian Predators in Costa Rica. Evolution 47(1):227-235. http://dx.doi.org/10.1111/j.1558-5646.1993.tb01212.x.

BRUGGER, K.E. 1989. Red-tailed hawk dies with coral snake in talons. Copeia 1989(2):508-510. http://dx.doi.org/10.2307/1445456

BUASSO, C.M., LEYNAUD G.C. & CRUZ, F.B. 2006. Predation on snakes of Argentina: Effects of coloration and ring pattern on coral and false coral snakes. Stud. Neotrop. Fauna E. 41(3):183-188. http://dx.doi.org/10.1080/01650520600630725

FRANÇA, F.G.R. & BRAZ, V.S. 2013. Diversity, activity patterns, and habitat use of the snake fauna of Chapada dos Veadeiros National Park in Central Brazil. Biota Neotropica 13(1):74-84 http://www.biotaneotropica.org.br/v13n1/pt/abstract?article+bn01313012013 (last access in 24/02/2017). http://dx.doi.org/10.1590/S1676-06032013000100008

GREENE, H.W. 1976. Scale overlap, a directional sign stimulus for prey ingestion by ophiophagous snakes. Z. Tierpsychol. 41(2):113-120. http://dx.doi.org/10.1111/j.1439-0310.1976.tb00473.x

GREENE, H.W. & MCDIARMID, R.W. 1981. Coral snake mimicry: does it occur? Science 213(4513):1207-1212. http://dx.doi.org/10.1126/science.213.4513.1207

GREENE, H.W. & MCDIARMID, R.W. 2005. Wallace and Savage: heroes, theories and venomous snake mimicry. In Ecology and evolution in the tropics: a herpetological perspective (M. Donnelly, B. Crother, C. Guyer, M.H. Wake, & M. E. White, eds.). University of Chicago Press. Chicago, p.190-208.

HARPER, G. R., jr & PFENNIG, D. W. 2007. Mimicry on the edge: why do mimics vary in resemblance to their model in different parts of their geographical range? P. Roy. Soc. B-Biol. Sci. 274:1955-1961. http://dx.doi.org/dx.doi.org/10.1098/rspb.2007.0558

HARPER, G. R., jr & PFENNIG, D. W. 2008. Selection overrides gene flow to break down maladaptive mimicry. Nature 451:1103-1106. http://dx.doi.org/10.1038/nature06532

HARRELL, F.E., Jr. 2011. rms: Regression Modeling Strategies. R package version 3.3-0. http://CRAN.R-project.org/package=rms (last access in 24/02/2017)

KIKUCHI, D.W. & PFENNIG, D.W. 2010. Predator cognition permits imperfect coral snake mimicry. Am. Nat. 176(6):830-834. http://dx.doi.org/10.1086/657041

KIKUCHI, D.W. & PFENNIG, D.W. 2013. Imperfect mimicry and the limits of natural selection. Q. Rev. Biol. 88(4):297-315. http://dx.doi.org/10.1086/673758

MALLET, J. & JORON, M.1999. Evolution of Diversity in Warning Color and Mimicry: Polymorphisms, Shifting Balance, and Speciation. Annu. Rev. Ecol. Syst. 30:201-233. http://dx.doi.org/10.1146/annurev.ecolsys.30.1.201

MARQUES, O.A.V. & PUORTO, G. 1991. Padrões cromáticos, distribuição e possível mimetismo em *Erythrolamprus aesculapii* (Serpentes, Colubridae). Mem. Inst. Butantan 53(1): 127-134.

PFENNIG, D.W., HARCOMBE, W.R. & PFENNIG, K. S. 2001. Frequency-dependent Batesian mimicry. Nature 410(6826): 323. http://dx.doi.org/10.1038/35066628

PFENNIG, D. W., jr, G.R.H., BRUMO, A.F., HARCOMBE, W.R. & PFENNIG, K.S. 2007. Population differences in predation on Batesian mimics in allopatry with their model: selection against mimics is strongest when they are common. Behav. Ecol. Sociobiol. 61: 505-511. http://dx.doi.org/10.1007/s00265-006-0278-x

PFENNIG, D.W. & MULLEN, S.P. 2010. Mimics without models: causes and consequences of allopatry in Batesian mimicry complexes. P. Roy. Soc. B-Biol. Sci. 277:2577-2585. http://dx.doi.org/10.1098/rspb.2010.0586

PFENNIG, D. W., & KIKUCHI, D. W. 2012. Competition and the evolution of imperfect mimicry. Curr. Zool. 58(4): 608-619. http://dx.doi.org/10.1093/czoolo/58.4.608

PFENNIG, D.W., AKCALI, C.K. & KIKUCHI, D.W. 2015. Batesian mimicry promotes pre- and postmating isolation in a snake mimicry complex. Evolution 69(4): 1085-1090. http://dx.doi.org/10.1111/evo.12624

PYRON, A.R. & BURBRINK, F.T. 2009. Lineage diversification in a widespread species: roles for niche divergence and conservatism in the common kingsnake, Lampropeltis getula. Mol. Ecol. 18(16): 3443-3457. http://dx.doi.org/10.1111/j.1365-294X.2009.04292.x

R DEVELOPMENT CORE TEAM. 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

RUXTON, G.D., SHERRATT, T.N. & SPEED, M.P. 2004. Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals and Mimicry. Oxford University Press, Oxford.

SAVAGE, J.M. & SLOWINSKI, J.B. 1992 The coloration of the venomous coral snakes (family Elapidae) and their mimics (families Aniliidae and Colubridae). Biol. J. Linn. Soc. 45:235–254. http://dx.doi.org/10.1111/j.1095-8312.1992.tb00642.x

SMITH, S.M. 1975. Innate recognition of coral snake pattern by a possible avian predator. Science 187:759-760. http://dx.doi.org/10.1126/science.187.4178.759

TABACHNICK, B.G. & FIDELL, L.S. 2007. Using Multivariate Statistics (5th ed.). Allyn and Bacon, New York.

WÜSTER, W., ALLUM, C.S.E., BJARGARDÓTTIR, I.B., BAILEY, K.L., DAWSON, K.J., GUENIOUI, J., LEWIS, J., MCGURK, J., MOORE, A.G., NISKANEN, M. & POLLARD C.P. 2004. Do aposematism and Batesian mimicry require bright colours? A test, using European viper markings. P. Roy. Soc. B-Biol. Sci. 271:2495-2499. http://dx.doi.org/10.1098/rspb.2004.2894

Received: 07/03/2017
Revised: 04/08/2017
Accepted: 09/08/2017

Biota Neotropica 17(3): e20170342, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Inventory

Freshwater mollusks and environmental assessment of Guandu River, Rio de Janeiro, Brazil

Igor Christo Miyahira[*1], *Jéssica Beck Carneiro*[2], *Isabela Cristina Brito Gonçalves*[2], *Luiz Eduardo Macedo de Lacerda*[2], *Jaqueline Lopes de Oliveira*[2], *Mariana Castro de Vasconcelos*[2] & *Sonia Barbosa dos Santos*[2,3].

[1]*Universidade Federal do Estado do Rio de Janeiro, Departamento de Zoologia, Rio de Janeiro, RJ, Brazil.*
[2]*Universidade do Estado do Rio de Janeiro, Programa de Pós-Graduação em Ecologia e Evolução, Rio de Janeiro, RJ, Brazil.*
[3]*Universidade do Estado do Rio de Janeiro, Departamento de Zoologia, Rio de Janeiro, RJ, Brazil.*
**Corresponding author: Igor Christo Miyahira, e-mail: icmiyahira@yahoo.com.br*

MIYAHIRA, I. C., CARNEIRO, J. B., GONÇALVES, I. C. B., LACERDA, L. E. M. de, OLIVEIRA, J. L. de, VASCONCELOS, M. C. de, SANTOS, S. B. **Freshwater mollusks and environmental assessment of Guandu River, Rio de Janeiro, Brazil**. Biota Neotropica. 17(3): e20170342. http://dx.doi.org/10.1590/1676-0611-BN-2017-0342

Abstract: The Guandu River Basin is extremely important to state of Rio de Janeiro, as a water supplier of several municipalities. However, the malacological knowledge and environmental status is not well known to this basin. The aim of this paper is to present an inventory of freshwater mollusks, as well as an environmental assessment through a Rapid Assessment Protocol, of ten sampling sites at Guandu River basin in six municipalities (Piraí, Paracambi, Japeri, Seropédica, Queimados and Nova Iguaçu). Thirteen species of molusks were found, eight native (*Pomacea maculata*, *Biomphalaria tenagophila*, *Gundlachia ticaga*, *Gundlachia radiata*, *Omalonyx matheroni*, *Diplodon ellipticus*, *Anodontites trapesialis* and *Eupera bahiensis*) and five exotics (*Melanoides tuberculata, Ferrissia fragilis*, *Physa acuta, Corbicula fluminea* and *Corbicula largillierti*). The environmental analysis ranked most sites as "modified". The expansion of exotic species with their associated negative effects, as well the gap in knowledge of native species calls attention to the need of future studies of biology and ecology of the species found.

Keywords: *Gastropoda, Bivalvia, Mollusca, exotic species, RAP.*

Moluscos de água doce e avaliação ambiental do rio Guandu, Rio de Janeiro, Brasil

Resumo: A bacia do rio Guandu é de extrema importância para o estado do Rio de Janeiro, pois abastece grande parte da região metropolitana. Contudo, aspectos malacológicos e ambientais desta bacia são pouco conhecidos. O presente trabalho teve por objetivo realizar um inventário das espécies de moluscos de água doce ocorrentes na região, assim como uma avaliação ambiental através de um Protocolo de Avaliação Rápida, de dez estações de coleta na bacia do rio Guandu em seis municípios (Piraí, Paracambi, Japeri, Seropédica, Queimados e Nova Iguaçu). Foram encontradas 13 espécies, sendo oito nativas (*Pomacea maculata*, *Biomphalaria tenagophila*, *Gundlachia ticaga*, *Gundlachia radiata*, *Omalonyx matheroni*, *Diplodon ellipticus*, *Anodontites trapesialis* e *Eupera bahiensis*) e cinco exóticas (*Melanoides tuberculata, Ferrissia fragilis*, *Physa acuta*, *Corbicula fluminea* e *Corbicula largillierti*). A avaliação ambiental caracterizou a maior parte das estações de coleta como "modificado". A dispersão das espécies exóticas e os efeitos negativos a elas associados, além da nossa falta de conhecimento sobre as espécies nativas chama a atenção para a necessidade de futuros estudos biológicos e ecológicos das espécies encontradas.

Palavras-chave: *Gastropoda, Bivalvia, Mollusca, espécies exóticas, RAP.*

Introduction

Few molluscan studies were conducted in Guandu River Basin. Tubbs-Filho & Vettorazzi (2012) recorded the occurrence of the exotic bivalve *Corbicula fluminea* (Müller, 1774) in the tributaries of Ribeirão das Lajes which is an affluent of Guandu River. Lacerda et al. (2013) reported the first occurrence of *Gundlachia radiata* (Guilding, 1828) from southeast Brazil in this area. Thiengo et al. (2001) reported sixteen species of limnic gastropods in the municipalities of Itaguaí, Seropédica, Queimados, Japeri, Paracambi and Nova Iguaçu, but without the information of the water body surveyed.

The concern with the conservation of natural resources is growing due to constant alteration of habitats, overuse of natural resources and introduction of exotic species, which causes loss or reduction of the diversity at all scales (Lydeard et al. 2004, Amaral et al. 2008, Santos et al. 2012, Miyahira et al. 2012). An important step for conservation is making the information of occurrence and distribution of different taxa available (Metzger & Casatti 2006). Therefore it is necessary to inventory native flora and fauna, in order to support conservation plans (Moulton et al. 2000). Molluscan fauna were not considered in most surveys, although it is the most diverse group after arthropods, occurring in different freshwater habitats such as streams, rivers, lakes, wetlands, swamps, dams, ponds and drainage ditches, with different degrees of eutrophication. Moreover, freshwater molluscs are among the most threatened faunal group in the world (Lydeard et al. 2004, Bogan 2008, Strong et al. 2008, Pereira et al. 2014).

Thus, the goals of this work were to: 1) study the freshwater mollusks diversity and distribution at the Guandu River Basin and, 2) evaluate the degree of preservation of the studied area through the application of a Rapid Assessment Protocol (RAP).

Material and methods

1. Study area and sampling

The Guandu River is formed mainly by the Ribeirão das Lajes River and the waters transposed of the Paraiba do Sul River (Tubbs-Filho & Vettorazzi 2012, Costa et al. 2013). The total length of the Guandu River is 108.5 km (Costa et al. 2013), flowing through the municipalities of Piraí, Paracambi, Japeri, Seropédica, Queimados, Nova Iguaçu and Rio de Janeiro, all in the state of Rio de Janeiro. In Guandu River Basin there are crops, pastures, sand extraction areas, cities, industries, hydroelectric plants and a reservoir at the responsibility of CEDAE (Companhia Estadual de Águas e Esgotos), that provide water to approximately eight million people in the metropolitan area of Rio de Janeiro (Tubbs-Filho & Vettorazzi 2012).

It were performed 10 sampling sites in six municipalities: Piraí (PR1; -43.81638, -22.68165 and PR2; -43.81779, -22.68307), Paracambi (PA1; -43.80007, -22.69054 and PA2; -43.71366, -22.64109), Itaguaí (ITA; -43.78791, -22.69493), Japeri (JAP; -43.66498, -22.65561), Seropédica (SE1; -43.64508, -22.73790 and SE2; -43.63964, -22.71110), and Nova Iguaçu (NI1; -43.62473, -22.80675 and NI2; -43.62626, -22.80764) (Figure 1). The sampling sites were done at confluence of Floresta River and Ribeirão das Lajes River (PR1), Ribeirão das Lajes River (PR2 and PA1), Guandu River (PA2, ITA, JAP, SE1 and SE2) and at Guandu Reservoir (NI1 and NI2). The sampling sites have different degrees of anthropic impacts (Figure 2). PR1 and PR2, as also NI1 and NI2 are sites close together (Figure 1).

1.1. Collection and analysis

The mollusks were searched in all suitable habitats, including marginal and floating vegetation, using a handled metallic scoop as described by Fernandez et al. (2008). Large freshwater mussels (Unionida) were searched in sand and muddy substrates using bare hands and feet. The search was made by three collectors during 20 minutes, totalizing one hour per sampling site. The abundance values presented are the sum of the specimens obtained in each site. The field work was done at May/2013 and January/2014.

After field work the gastropods were submitted to a parasitological test, light/dark cycle exposures test (Fernandez et al. 2008). The cercariae were identified following Schell (1970) and preserved in AFA. Thereafter, the mollusks were anesthetized with menthol ($C_{10}H_{20}O$), sacrificed in hot water (only gastropods), soft parts were preserved in 70% ethanol and shells were preserved dry.

Mollusks were identified under a stereomicroscope according to specialized literature (e.g., Santos 2003, 2012, Simone 2006, Arruda et al. 2006, Lacerda et al. 2011, 2013, Miyahira et al. 2013) and with the assistance of specialists. The classification above genus level followed Bouchet & Rocroi (2005), Simone (2006) and Albrecht et al. (2007) for the gastropods and Graf & Cummings (2006) and Bieler et al. (2010) for bivalves. The mollusks collected were housed at Mollusk Collection of Universidade do Estado do Rio de Janeiro (UERJ).

We used the RAP developed by Callisto et al. (2002) to evaluate the conservation status in each sampling site at the Guandu River Basin. This protocol evaluates 22 characteristics of the water body, margins and surroundings. The final score reflects the conservation level of the site:

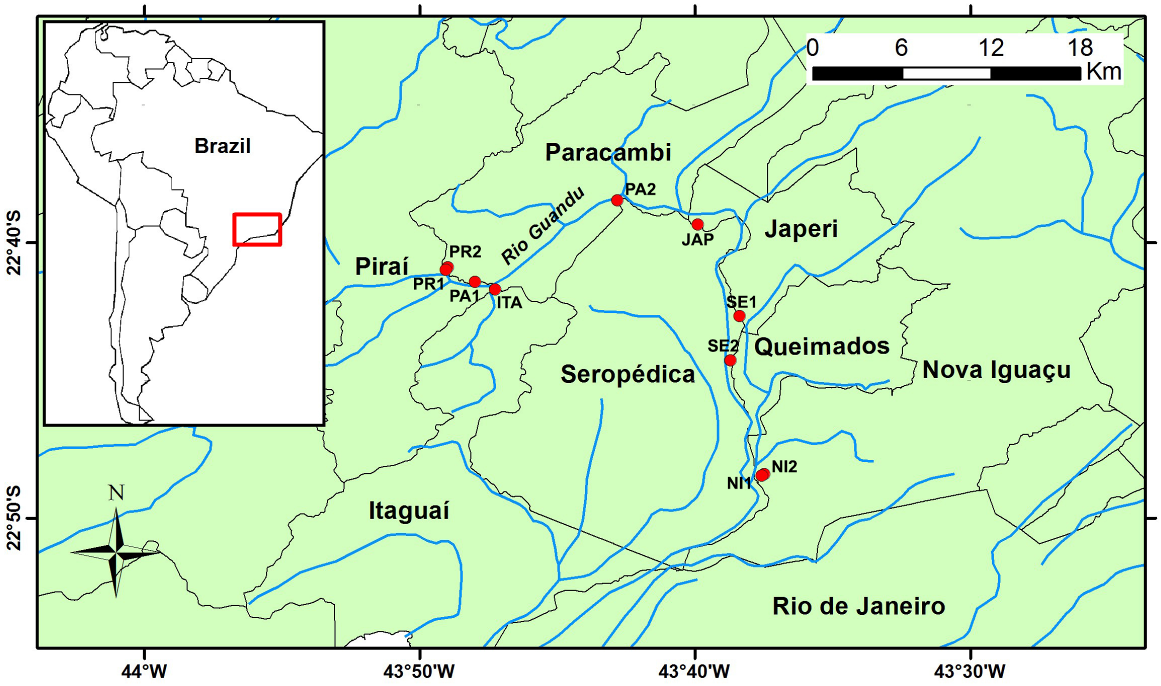


Figure 1. Studied area and the ten sampling sites at Guandu River Basin in six municipalities of the state of Rio de Janeiro.

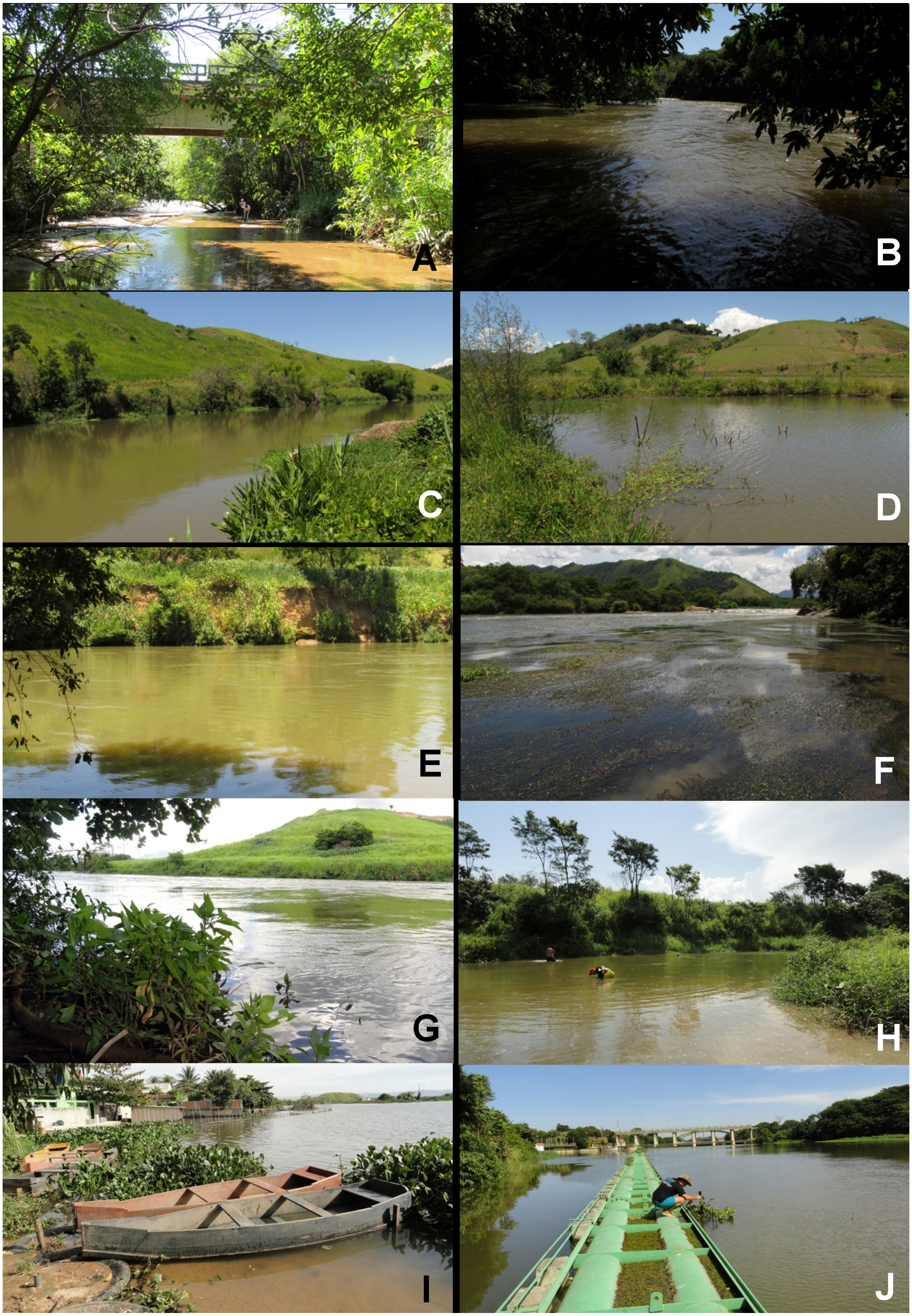


Figure 2. Overview of the sampled sites at Guandu River Basin. A - Piraí (PR1), B - Piraí (PR2), C – Paracambi (PA1), D - Paracambi (PA2), E - Itaguaí (ITA), F - Japeri (JAP), G - Seropédica (SE1), H - Seropédica (SE2), I - Nova Iguaçu (NI1) and J - Nova Iguaçu (NI2).

zero to 40 points is “impacted”, 41 to 60 points is “modified” and above 61 points is “natural”.

Results

1. Species diversity, distribution and parasitological test

Thirteen species of freshwater mollusks were found: eight gastropods and five bivalves (Table 1, Figure 3). Only two genera were represented by more than one species: *Gundlachia* Pfeiffer, 1849 and *Corbicula* Megerle von Mühlfeld, 1811. Five species were exotic: *C. fluminea, Corbicula largillierti* (Philippi, 1844), *Melanoides tuberculata* (Müller, 1774), *Physa acuta* Draparnaud, 1805 and *Ferrissia fragilis* (Tyron, 1863).

Only the exotic species *C. fluminea* was found in all sampling sites. Another widespread exotic species in Guandu River Basin was *M. tuberculata*, missing in only one sampling site. The native species with widest distribution was *Pomacea maculata* Perry, 1810 found in seven sites. However, most species presented smaller distributions, restricted to three, *Diplodon ellipticus* Spix in Wagner, 1827*, G. radiata*, *Gundlachia ticaga* (Marcus & Marcus, 1962) and *Omalonyx matheroni* (Potiez & Michaud, 1835); two, *F. fragilis* and *Anodontites trapesialis* (Lamarck, 1819), or even only one sampling site, *Eupera bahiensis* (Spix in Wagner, 1827) (Table 2).

Gundlachia ticaga is found at upstream-intermediate sites (PR1, PA1 and ITA); *F. fragilis* is found at intermediate sites (ITA and SE2); and *G. radiata*, *D. ellipticus*, *A. trapesialis* and *E. bahiensis* at intermediate-downstream

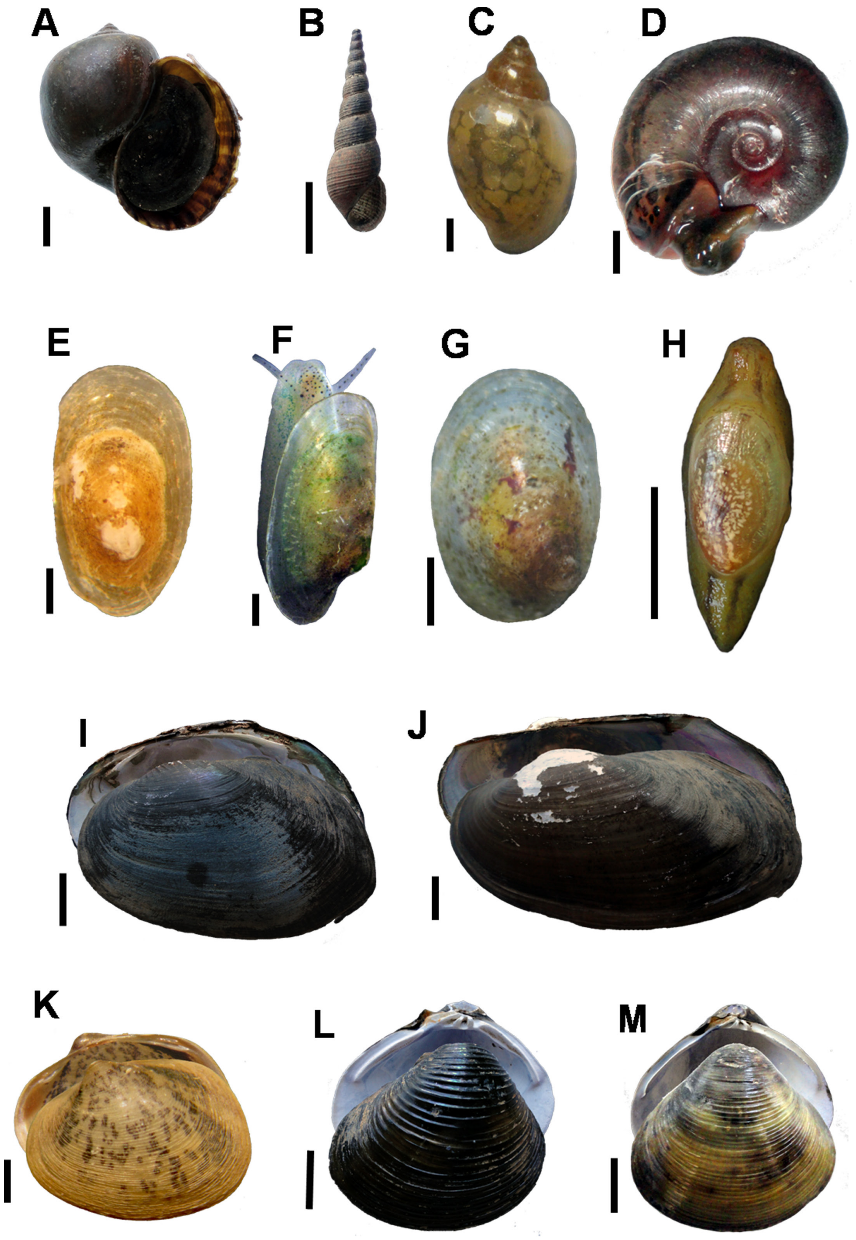


Figure 3. Freshwater molluscs from Guandu River Basin. A- *Pomacea maculata* Perry, 1810. B – *Melanoides tuberculata* (Müller, 1774). C – *Physa acuta* Draparnaud, 1805. D – *Biomphalaria tenagophila* (d’Orbigny, 1835). E – *Ferrissia fragilis* (Tryon, 1863). F – *Gundlachia radiata* (Guilding, 1828). G – *Gundlachia ticaga* (Marcus & Marcus, 1962). H – *Omalonyx matheroni* (Potiez & Michaud, 1835). I – *Diplodon ellipticus* Spix in Wagner, 1827. J – *Anodontites trapesialis* (Lamarck, 1819). K – *Eupera bahiensis* (Spix in Wagner, 1827). L – *Corbicula fluminea* (Müller, 1774). M – *Corbicula largillierti* (Philippi, 1844). Scale bar: A, B, H, I, J, L and M = 1 cm; C, D, E, F, G and K = 1 mm.

Table 1. Taxonomic list of freshwater molluscs found at Guandu River basin.

Gastropoda				
	Caenogastropoda		Ampullariidae	***Pomacea maculata* Perry, 1810** (Figure 3A)
			Thiaridae	***Melanoides tuberculata* (Müller, 1774)** (Figure 3B)
	Hygrophila		Physidae	***Physa acuta* Draparnaud, 1805** (Figure 3C)
			Planorbidae	***Biomphalaria tenagophila* (d'Orbigny, 1835)**
				***Ferrissia fragilis* (Tryon, 1863)** (Figure 3E)
				***Gundlachia radiata* (Guilding, 1828)** (Figure 3F)
				***Gundlachia ticaga* (Marcus & Marcus, 1962)** (Figure 3G)
	Eupulmonata		Succineidae	***Omalonyx matheroni* (Potiez & Michaud, 1835)** (Figure 3H)
Bivalvia				
	Paleoheterodonta	Unionida	Hyriidae	***Diplodon ellipticus* Spix in Wagner, 1827** (Figure 3I)
			Mycetopodidae	***Anodontites trapesialis* (Lamarck, 1819)** (Figure 3J)
	Heterodonta	Venerida	Sphaeriidae	***Eupera bahiensis* (Spix in Wagner, 1827)** (Figure 3K)
			Cyrenidae	***Corbicula fluminea* (Müller, 1774)** (Figure 3L)
				***Corbicula largillierti* (Philippi, 1844)** (Figure 3M)

Table 2. Distribution and abundance of freshwater molluscs in Guandu River basin. PR – Piraí, PA – Paracambi, ITA – Itaguaí, JAP – Japeri, SE – Seropédica, NI – Nova Iguaçu. RAP - Rapid Assessment Protocol. I – Impacted, M – Modified, N – Natural.

Species	Sampling sites										Material studied (UERJ numbers)
	PR1	PR2	PA1	PA2	ITA	JAP	SE1	SE2	NI1	NI2	
Pomacea maculata	1	-	3	-	22	1	-	6	9	54	10557-58; 10577; 10588; 10593; 10601; 10610;
Melanoides tuberculata	1	1	3	1	2	22	37	20	-	8	10561; 10582; 10584; 10591; 10597; 10599; 10603; 10606; 10612
Physa acuta	1	-	17	-	-	3	2	2	-	1	10564; 10579; 10585; 10600; 10604; 10608;
Omalonyx matheroni	1	-	3	-	-	-	-	-	-	1	10567; 10576; 10587
Ferrissia fragilis	-	-	-	-	1	-	-	2	-	-	10595; 10554
Gundlachia radiata	-	-	-	-	-	-	-	41	28	91	10409; 10611; 10560
Gundlachia ticaga	6	-	2	-	9	-	-	-	-	-	10578; 10589; 10594
Biomphalaria tenagophila	-	-	12	-	3	-	-	2	-	1	10586;10562; 10592; 10609
Diplodon ellipticus	-	-	-	-	-	-	-	21	12	72	10555; 10566; 10607
Anodontites trapesialis	-	-	-	-	-	-	-	-	3	3	10556; 10570
Eupera bahiensis	-	-	-	-	-	-	-	-	-	96	10559
Corbicula fluminea	17	66	11	55	16	57	37	150	5	17	10565; 10571-72; 10580; 10583; 10590; 10596; 10598; 10602; 10605
Corbicula largillierti	-	14	-	-	-	-	-	-	5	2	10568-69; 10581
Richness	6	3	7	2	6	4	3	8	6	11	-
RAP Score	76	80	48	59	57	55	59	58	38	43	-
RAP Category	N	N	M	M	M	M	M	M	I	M	-

sites (SE2, NI1 and NI2). The other species not presented a distribution limited to any specific section of the basin.

The most abundant species at Guandu River Basin was *C. fluminea* (431 specimens), and the less abundant was *F. fragilis* (3 specimens) and *O. matheroni* (5 specimens) (Table 2). *Gundlachia radiata*, *D. ellipticus* and *E. bahiensis* were restricted to few sampling sites; however these species were abundant locally.

The parasitological survey revealed only the interaction of *G. ticaga* with Longifurcate pharyngeate monostome cercaria (Vivax cercaria) (Trematoda: Digenea) in PR1. The exposition of the other gastropods to light/dark cycles returned negative results.

2. Habitat and environmental evaluation

All bivalves were found buried in soft sediment with some organic matter, except by *E. bahiensis* found attached to the roots of *Eichornia* sp. *Ferrissia fragilis*, *G. radiata*, *G. ticaga* and *P. acuta* were found attached to aquatic plants, decayed debris and on artificial substrates such as plastic bottles. *Melanoides tuberculata* and *P. maculata* were found over soft and hard substrates. *Omalonyx matheroni* was found on plants at interface water-air.

Only PR1 and PR2 were considered as "natural" on RAP; NI1 was characterized as "impacted" and the remaining sites as "modified" (Table 2). The downstream stations received the lowest scores on the RAP.

Discussion

Five exotic species were found: three freshwater snails (*M. tuberculata, P. acuta* and *F. fragilis*) and two freshwater bivalves (*C. fluminea* and *C. largillierti*); except by *F. fragilis*, a cryptic species that were recently reported to Brazil (Lacerda et al. 2015), the other species can also be considered as invasive. Among these species, the most widespread and harmful are *C. fluminea* and *M. tuberculata*. Both species can cause modification of community (Santos et al. 2012). *Melanoides tuberculata* is widespread in Brazil (Fernandez et al. 2003, Thiengo et al. 2007, Santos et al. 2012), reaching high population densities that affect native species (Freitas et al. 1987, Giovanelli et al. 2003, Braga et al. 2014). Furthermore, it is the first host of different parasites that causes human diseases (Vaz et al. 1986, Guimarães et al. 2001, Pinto & Melo, 2010). However, no parasitological interaction was found for this species in Guandu River Basin.

Corbicula fluminea is a widespread species in Brazil compared to *C. largillierti*, that has a restrict distribution (Santos et al. 2012). These observations are consistent with the situation that we found in the Guandu River Basin, where *C. largillierti* had a restricted distribution whereas *C. fluminea* was found everywhere. Previous record of *C. fluminea* on Guandu River Basin stated only tributaries of Ribeirão das Lajes River (Tubbs-Filho & Vettorazzi 2012), but it is clear that is widespread at the basin. This last species also has caused serious economic damage to industries and power plants, due to obstruction of water pipes and competition with native bivalves (like *D. ellipticus* and *A. trapesialis* that occurs at Guandu River Basin), reducing their populations (Mansur et al. 2004, Santos et al. 2012). *Corbicula fluminea* is also the most abundant species at Guandu River Basin, as already observed by other authors in other sites (e.g., Mansur et al. 2004, Santos et al. 2012, Pereira et al. 2014, Meyer et al. 2017).

The Unionida species needs a minimum amount of nutrients in the water to survive and usually occurs in lower sections of the rivers, where there is large amount of nutrients (Pereira et. al. 2011, 2014), as observed for *D. ellipticus* and *A. trapesialis* at Guandu River Basin. *Gundlachia radiata* also prefer the large section of the river, at least at Guandu River Basin (Table 2), and it is possible that have higher nutrients requirements compared to other ancylids species. The first record of this species was in this section of the river, also occurring downstream of Guandu Reservoir (Lacerda et al. 2011). It is not possible to found a reason to the restricted distributions of *G. ticaga* and *F. fragilis* based in our data.

Eupera bahiensis is recorded for the first time to the state of Rio de Janeiro and it is also the southernmost record of this species. Until now, it was found in the states of Bahia (Mansur & Meier-Brook 2000), Pernambuco (Haas 1939), Ceará (Haas 1939), Pará (Haas 1949 a,b; Haas 1952) and Amazonas (Haas 1949b).

All other species were previously recorded to state of Rio de Janeiro. However some of them, like *A. trapesialis* was rarely reported. This species was recorded only one time to state of Rio de Janeiro at Juturnaíba Reservoir, municipality of Silva Jardim (Alvarenga et al. 1979). *Diplodon ellipticus* is only mentioned to Paraíba do Sul River in the state of Rio de Janeiro (Morretes, 1949). These new records evidence the gaps on the distribution of freshwater mollusks species as a result of the low sampling effort.

We noticed some new municipalities occurrences compared to Thiengo et al (2001): *F. fragilis* to Seropédica; *G. radiata* to Seropédica and Nova Iguaçu; and *M. tuberculata, O. matheroni* and *P. maculata* to Paracambi and Nova Iguaçu.

We noticed only the presence of Longifurcate pharyngeate monostome cercaria (Vivax cercaria) in *G. ticaga.* Previous studies in the state of Rio de Janeiro have pointed out other cercaria types in this species: Gymnocephalous cercaria to the municipality of Angra dos Reis (Lopes et al. 2011), Echinostome cercaria to Itaguaí and Vassouras (Thiengo et al. 2001) and Nova Friburgo (Thiengo et al. 2002). The final host of longifurcate pharyngeate monostome cercaria are birds and mammals (Pinto & Melo 2013).

The RAP classification identified the most upstream collecting sites (PR1 and PR2) as natural environments. In PR1 were found more species than in PR2 (Table 2) due the greater environmental heterogeneity (more habitats available). In PR1 there are more macrophytes and some side pools, habitats for *O. matheroni* and *G. ticaga*. As the Guandu River flows across the cities of Paracambi, Itaguaí, Japeri, Seropédica and Nova Iguaçu, receives domestic and industrial sewage and margins are sharply modified. NI2 was the richest in freshwater molluscs species, and also one of the most clearly anthropized. However, it was on a small bay near the main channel of the river, thus with a good water renovation. In NI1, near to NI2, with same habitats available (soft and hard substrates, as also rooted and floating macrophytes) but with more organic input and less water circulation sustained fewer species (Table 2).

In a dense populated area, in the metropolitan area of Rio de Janeiro, we found new municipalities records, and even a new record to the state of Rio de Janeiro. The dominance and wide distribution of *M. tuberculata* and *C. fluminea* in almost all sampling sites, emphasizes the need for a continuous monitoring and control of the spread of exotic species. Also, biological and ecological aspects of native species must be studied to support conservation strategies and reduce the effects of exotic species.

Acknowledgements

We would like to thank to J. Arruda (FZB-RS), S. Thiengo (Fiocruz-RJ) and M.C.D. Mansur (UFRGS) for species identification; Maria Regiana Salgado de Mello for the help with curatorial work; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support to SBS (CNPq/PROTAX 562291/2010-5) and Doctoral scholarships to LEML and ICM ; to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for Master scholarships to ICBG, JBC, MCV and JLO; Instituto Chico Mendes de Conservação da Biodiversidade for the licenses (63407-4 to ICM and 10812-1 to SBS); and to the two anonymous reviewers that improved this manuscript.

Authors Contributions

Igor Christo Miyahira: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Jéssica Beck Carneiro: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Isabela Cristina Brito Gonçalves: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Luiz Eduardo Macedo de Lacerda: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Jaqueline Lopes de Oliveira: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Mariana Castro de Vasconcellos: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Sonia Barbosa dos Santos: contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

ALBRECHT, C., KUHN, K. & STREIT, B. 2007. A molecular phylogeny of Planorboidea (Gastropoda, Pulmonata). Zool. scr. 36: 27-39.

ALVARENGA, L.C.F., COELHO, A.C.S., RICCI, C.N., GOMES, L.A.L. & BARROS, H.M. 1979. Resultados preliminares dos trabalhos ecológicos realizados na lagoa de Juturnaíba, município de Araruama, Estado do Rio de Janeiro, criadouro natural dos bivalves *Diplodon besckeanus* (Dunker, 1849) (Unionoidea; Hyriidae) e *Anodontites trapesialis* (Lamarck, 1819) (Muteloidea; Mycetopodidae). Anais do V Encontro de Malacologistas Brasileiros. 1:73-89.

AMARAL, A.C.Z., RIBEIRO, C.V., MANSUR, M.C.D., SANTOS, S.B., AVELAR, W.E.P., MATTHEWS-CASCON, H., LEITE, F.P.P., MELO, G.A.S., COELHO, P.A., BUCKUP, G.B., BUCKUP, L., VENTURA, C.R.R. & TIAGO, C.G. A situação de ameaça dos invertebrados aquáticos no Brasil. In Livro vermelho da fauna brasileira ameaçada de extinção Série Biodiversidade vol. 19 – I (A.B.M. Machado, G.M. Drummond & A.P. Paglia, orgs). Ministério do Meio Ambiente, Brasília, p. 156-301.

ARRUDA, J.O., GOMES, S.R., RAMIREZ, R. & THOMÉ, J.W. 2006. Morfoanatomia de duas espécies do gênero *Omalonyx* (Mollusca, Gastropoda, Succineidae) com novo registro para Minas Gerais, Brasil. Biociências. 14(1):61-70.

BIELER, R., CARTER, J.G. & COAN, E.V. 2010. Classification of bivalve families. Malacologia. 52(2):113-184.

BOUCHET, P. & ROCROI, J.P. 2005. Classification and nomenclator of gastropods families. Malacologia. 47(1-2):1-396.

BRAGA, R.M.R., MIYAHIRA, I.C., LACERDA, L.E.M., GONÇALVES, I.C.B. & SANTOS, S.B. 2014. The influence of an invasive gastropod on the population dynamics of *Pisidium punctiferum* (Sphaeriidae) in an insular impacted stream in Rio de Janeiro, Brazil. In Medical and Applied Malacology Crossing Boundaries: Integrative Approaches to Malacology (A.R.T. Piza, L.F. Tallarico, G.O. Introíni & S.B. Santos, orgs.). Cambridge Scholars Publishing, Newcastle, p. 97-114.

BOGAN, A.E. 2008. Global diversity of freshwater mussels (Mollusca, Bivalvia) in freshwater. Hydrobiologia 595:139-147.

CALLISTO, M., FERREIRA, W.R., MORENO, P., GOULART, M. & PETRUCIO, M. 2002. Aplicação de um protocolo de avaliação rápida da diversidade de habitats em atividades de ensino e pesquisa (MG-RJ). Acta limnol. bras. 14(1):91-98.

COSTA, M.R., MATTOS, T.M., BORGES, J.L. & ARAÚJO, F.G. 2013. Habitat preferences of common native fishes in a tropical river in Southeastern Brazil. Neotrop. ichthyol. 11(4):871-880.

FERNANDEZ, M.A., THIENGO, S.C. & SIMONE, L.R.L. 2003. Distribution of the introduced freshwater snail *Melanoides tuberculatus* (Gastropoda: Thiaridae) in Brazil. The Nautilus. 117:78-82.

FERNANDEZ, M.A., THIENGO, S.C. & AMARAL, R.S. 2008. Técnicas Malacológicas. In Vigilância e controle de moluscos de importância epidemiológica. (Ministério da Saúde. Departamento de vigilância epidemiológica, ed.). Editora do Ministério da Saúde, Brasília, p. 43-70.

FREITAS, J.R., BEDÊ, L.C., MARCO J.R., ROCHA, L.A. & SANTOS, M.B.L. 1987. Population dynamics of aquatic snails in Pampulha reservoir. Mem. Inst. Oswaldo Cruz. 82 (Suppl. IV):299-305.

GIOVANELLI, A., VIEIRA, M.V. & SILVA, C.L.P.A.C. 2003. Apparent competition through facilitation between *Melanoides tuberculatus* and *Biomphalaria tenagophila* and the control of schistosomiasis. Mem. Inst. Oswaldo Cruz. 98(3):429-431.

GUIMARÃES, C.T., SOUZA, C.P. & SOARES, D.M. 2001. Possible competitive displacement os Planorbids by *Melanoides tuberculata* in Minas Gerais, Brazil. Mem. Inst. Oswaldo Cruz. 96:173-176.

GRAF, D.L. & CUMMINGS, K.S. 2006. Palaeoheterodont diversity (Mollusca: Trigonioida + Unionoida): what we know and what we wish we knew about freshwater mussel evolution. Zool. j. Linn. Soc. 148:343-394.

HAAS, F. 1939. Zur Kenntnis der Binnen-Mollusken NO-Brasiliens. Senckenbergiana. 21(3-4):254-278.

HAAS, F. 1949a. Land- und Süßwassermollusken aus dem Amazonas-Gebiete. Arch. Molluskenkd. 78(4-6):149-156.

HAAS, F. 1949b. On fresh water mollusk from the Amazonian region. An. Inst. Biol. 20:301-314.

HAAS, F. 1952. South American non-marine shells: further remarks and descriptions. Fieldiana Zool. 34(9):107-132.

LACERDA, L.E.M., MIYAHIRA, I.C. & SANTOS, S.B. 2011. Shell morphology of the freshwater snail *Gundlachia ticaga* (Gastropoda, Ancylidae) from four sites in Ilha Grande, southeastern Brazil. Zoologia. 28(3):334-342.

LACERDA, L.E.M., MIYAHIRA, I.C. & SANTOS, S.B. 2013. First record and range extension of the freshwater limpet *Gundlachia radiata* (Guilding, 1828) (Mollusca: Gastropoda: Planorbidae) from southeast Brazil. Checklist. 9(1):125-128.

LACERDA, L.E.M., RICHAU, C.S., AMARAL, C.R.L.; SILVA, D.A.; CARVALHO, E.F. & SANTOS, S.B. 2015. *Ferrissia fragilis* (Tryon, 1863): a freshwater snail cryptic invader in Brazil revealed by morphological and molecular data. Aquat. Invasions. 10(2):157-168.

LOPES, B.G., MATTOS, A.C., FERNANDEZ, M.A., LACERDA, L.E.M., MIYAHIRA I.C., SANTOS, S.B. & THIENGO, S.C. 2011. Primeiro registro de "Gymnocephalous cercaria" (TREMATODA; FASCIOLIDAE) em *Gundlachia ticaga* (Marcus & Marcus, 1962) (GASTROPODA; ANCYLIDAE) NO BRASIL. In XXII Encontro Brasileiro de Malacologia (C.A.O. Meirelles, org.). Sociedade Brasileira de Malacologia, Fortaleza, p.465-468.

LYDEARD, C., COWIE, R.H., PONDER, W.F., BOGAN, A.E., BOUCHET, P., CLARK, S., CUMMINGS, K.S., FREST, T.J., GARGOMINY, O., HERBERT, D.G., HERSHLER, R., PEREZ, K., ROTH, B., SEDDON, M., STRONG, E.E. & THOMPSON, F.G. 2004. The global decline of nonmarine mollusks. BioScience. 54(4):321-330.

MANSUR, M.C.D. & MEIER-BROOK, C. 2000. Morphology of *Eupera* Bourguignat, 1854, and *Byssanodonta* Orbigny, 1846 with contributions to the phylogenetic systematics of Sphaeriidae and Corbiculidae (Bivalvia: Veneroida). Arch. Molluskenkd. 128(1-2):1-59.

MANSUR, M.C.D., CALLIL, C.T., CARODOSO, F.R. & IBARRA, J.A.A. 2004. Uma retrospectiva e mapeamento da invasão de espécies de *Corbicula* (Mollusca, Bivalvia, Veneroida, Corbiculidae) oriundas do sudeste asiático, na América do Sul. In Água de Lastro e Bioinvasão (J.S.V. Silva & R.C.C.L. Souza, orgs.) Editora Interciência, Rio de Janeiro, p. 39-58.

METZGER, J.P. & CASATTI, L. 2006. Do diagnóstico à conservação da biodiversidade: o estado da arte do programa BIOTA/FAPESP. Biota Neotrop. 6(2): 1-23. http://www.biotaneotropica.org.br/v6n2/pt/abstract?point-view+bn00106022006 (último acesso em 12/10/2016).

MEYER, A.A.N., OLIVEIRA, E., SILVA, I.S. & PRESTES, J.G. 2017. Evaluation of density of freshwater bivalves from the Environmental Protection Area (APA) of Verde River, Campo Magro, Paraná, Brazil. Pan-American Journal of Aquatic Sciences 12(1): 21-30.

MIYAHIRA, I.C., SANTOS, S.B., MANSUR, M.C.D. & CARNEIRO, J.B. 2012. Freshwater mussels in Brazil: past, presente and future, at least, we hope they have one. American Conchologist 40(3): 16-18.

MIYAHIRA, I.C., MANSUR, M.C.D. & SANTOS, S.B. 2013. Revision of the type specimens of *Diplodon ellipticus* and *Diplodon expansus* (Bivalvia, Unionoida, Hyriidae). Spixiana. 36(2):173-182.

MORRETES, F.L. 1949. Ensaio do catálogo dos moluscos do Brasil. Arq. mus. Paranaense 7: 5-216.

MOULTON, T.P., MELO, G.A.S., NESSIMIAN, J., SALGADO, N.C., PAIVA, P.C., ABSALÃO, R.S., SANTOS, S.B., VELOSO, V.G. & COSTA, J.M. 2000. Invertebrados aquáticos. In A fauna ameaçada de extinção do estado do Rio de Janeiro (H.G. Bergallo, C.F.D. Rocha, M.A.S. Alves & M. van Sluys, orgs.) Editora UERJ, Rio de Janeiro, p.45-51.

PEREIRA, D., ARRUDA, J.O., MENEGAT, R., PORTO, M.L., SCHWARZBOLD, A. & HARTZ, S.M. 2011. Guildas tróficas, composição e distribuição de espécies de moluscos límnicos no gradiente fluvial de um riacho subtropical brasileiro. Biotemas 24(1): 21-36.

PEREIRA, D., MANSUR, M.C.D., DUARTE, L.D.S., OLIVEIRA, A.S., PIMPÃO, D.M., CALLIL, C.T., ITUARTE, C., PARADA, E., PEREDO, S., DARRIGRAN, G., SCARABINO, F., CLAVIJO, C., LARA, G., MIYAHIRA, I.C., RODRIGUEZ, M.T.R. & LASSO, C. 2014. Bivalve distribution in hydrographic regions in South America: historical overview and conservation. Hydrobiologia 735(1): 15-44.

PINTO, H.A. & MELO, A.L. 2010. *Melanoides tuberculata* (Mollusca: Thiaridae) as an intermediate host of *Centrocestus formosanus* (Trematoda: Heterophyidae) in Brazil. Rev. Inst. Med. Trop. São Paulo. 52(4):207-210.

PINTO, H.A. & MELO, A.L. 2013. Larvas de trematódeos em moluscos do Brasil: panorama e perspectivas após um século de estudos. Rev. Patol. Trop. 42(4): 369-386.

SANTOS, S.B. 2003. Estado atual do conhecimento dos ancilídeos na América do Sul. (Mollusca, Gastropoda, Pulmonata, Basommatophora). Rev. Biol. Trop. 50(3):191-224.

SANTOS, S.B., THIENGO, S.C., FERNANDEZ, M.A., MIYAHIRA, I.C., GONÇALVES, I.C.B., XIMENES, R.F., MANSUR, M.C.D. & PEREIRA, D. 2012. Espécies de moluscos límnicos invasores no Brasil. In Moluscos Límnicos invasores no Brasil - Biologia, prevenção, controle (M.C.D. Mansur, C.P. Santos, D. Pereira, I.C.P. Paz, M.L.L. Zurita, M.T.R. Rodriguez, M.V. Nehrke & P.E.A. Bergonci, eds.). Editora Redes, Porto Alegre, p. 25-49.

SCHELL, S. 1970. How to know the trematodes. Dubuque, WMC Brown Co. Publisher, 355pp.

SIMONE, L.R.L. 2006. Land and freshwater mollusks of Brazil. EGB/FAPESP, São Paulo.

STRONG, E.E., GARGOMINY, O.G., PONDER, W.F. & BOUCHET, P. 2008. Global diversity of gastropods (Gastropoda; Mollusca) in freshwater. Hydrobiologia 595:149-166.

THIENGO, S.C., FERNANDEZ, M.A., BOAVENTURA, M.F., GRAULT, C.E., SILVA, H.F.R., MATTOS, A.C. & SANTOS, S.B. 2001. Freshwater Snails and *Schistosomiasis Mansoni* in the State of Rio de Janeiro, Brazil: I - Metropolitan Mesoregion. Mem. Inst. Oswaldo Cruz. 96 (Suppl.):177-184.

THIENGO, S.C., FERNANDEZ, M.A., BOAVENTURA, M.F., SANTOS, S.B. & MATTOS, A.C. 2002. Freshwater Snails and *Schistosomiasis Mansoni* in the State of Rio de Janeiro, Brazil: II – Centro Fluminense Mesoregion. Mem. Inst. Oswaldo Cruz. 96 (Suppl.):177-184.

THIENGO, S.C., FERNANDEZ, M.A., MATTOS, A.C. & BARBOSA, A.F. 2007. Dispersão do molusco introduzido *Melanoides tuberculatus* (Müller, 1774) (Gastropoda; Thiaridae) no Brasil. In Tópicos em Malacologia - ECOS do XVIII EBRAM (S.B. Santos, A.D. Pimenta, S.C. Thiengo, M.A. Fernandez, & R.S. Absalão, orgs.) SBMa, Rio de Janeiro, p.101-106.

TUBBS-FILHO, D. & VETTORAZZI, J.S. 2012. Presença do molusco bivalve *Corbicula fluminea* em afluentes da bacia hidrográfica do Rio Guandu. In Bacia Hidrográfica dos Rios Guandu, da Guarda e Guandu-Mirim/Comitê da Bacia Hidrográfica Guandu (D. Tubbs-Filho, J.C.O. Antunes & J.S. Vettorazzi, orgs.). INEA, Rio de Janeiro, p. 257-272.

VAZ, J.F., TELES, H.M.S., CORREA, M.A. & LEITE, S.P.S. 1986. Ocorrência no Brasil de *Thiara* (*Melanoides*) *tuberculata* (Muller, 1774) (Gastropoda, Prosobranchia), primeiro hospedeiro intermediário de *Clonorchis sinensis* (Cobbold, 1875) (Trematoda, Plathyhelmintes). Rev. Saúde Pública. 20(4):318-322.

Received: 14/03/2017
Revised: 07/08/2017
Accepted: 09/08/2017

Biota Neotropica 17(3): e20160310, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Inventory

Odonates from Bodoquena Plateau: checklist and information about endangered species

Ricardo Koroiva[1], Marciel Elio Rodrigues[2], Francisco Valente-Neto[1] & Fábio de Oliveira Roque[1]*

[1]Universidade Federal do Mato Grosso do Sul, Instituto de Biociências, Cidade Universitária,
79070-900, Campo Grande, MS, Brazil
[2] Universidade Estadual de Santa Cruz, Departamento de Ciências Biológicas, Rod. Jorge Amado,
km 16, 45662-900, Ilhéus, BA, Brazil
**Corresponding author: Ricardo Koroiva, e-mail: ricardo.koroiva@gmail.com*

KOROIVA, R., RODRIGUES, M.E., VALENTE-NETO, F., ROQUE, F.O. **Odonates from Bodoquena Plateau: checklist and information about endangered species.** Biota Neotropica. 17(3): e20160310. http://dx.doi.org/10.1590/1676-0611-BN-2016-0310

Abstract: Here we provide an updated checklist of the odonates from Bodoquena Plateau, Mato Grosso do Sul state, Brazil. We registered 111 species from the region. The families with the highest number of species were Libellulidae (50 species), Coenagrionidae (43 species) and Gomphidae (12 species). 35 species are registered in the IUCN Red List species, four being Data Deficient, 29 of Least Concern and two species being in the threatened category. *Phyllogomphoides suspectus* Belle, 1994 (Odonata: Gomphidae) was registered for the first time in the state.

Keywords*: Dragonfly, Damselfly, inventory, Cerrado, Brazil*

Libélulas da Serra da Bodoquena: lista de espécies e informações sobre espécies ameaçadas

Resumo: Nós apresentamos um inventário atualizado das espécies de libélulas ocorrentes na Serra da Bodoquena, Estado de Mato Grosso do Sul, Brasil. Nós registramos 111 espécies para a região. As famílias com o maior número de espécies foram Libellulidae (50 espécies), Coenagrionidae (43 espécies) e Gomphidae (12 espécies). 35 espécies são registradas na Lista Vermelha de espécies da IUCN e duas estão em categorias de ameaçadas. *Phyllogomphoides suspectus* Belle, 1994 (Odonata: Gomphidae) foi registrado pela primeira vez no estado.

Palavras-chave*: Libélulas, donzelinhas, Inventário, Cerrado, Brasil*

Introduction

Insects are arthropod group with largely unknown distribution patterns (Diniz-Filho et al. 2010), due to their high species richness, difficulties related to sampling and lack of taxonomic experts. Applying all of these conditions, odonates (Insecta: Odonata) constitutes an important group that play a major role as predators, with several insects making up a large portion of their diets. This group is also a good indicators of water quality, with their nymphs being an important link in food chains for fish and other aquatic vertebrates and serve as an inspired subject for artists, naturalists and collectors (Abbott 2015).

Even with important taxonomists in the recent Brazilian history, odonates are still poorly described and its distribution is deficiently known in the Neotropical region (Kalkman et al. 2008). Local inventories are recorded to several parts of Brazil like São Paulo (Costa et al. 2000), Espirito Santo (Costa & Oldrini 2005), Rio de Janeiro (Anjos-Santos & Costa 2006), Goiás and Distrito Federal (Nóbrega & De Marco 2011), Minas Gerais (Souza et al. 2013), and Rio Grande do Sul (Renner et al. 2016). In Mato Grosso do Sul, state originally covered by Cerrado, Pantanal, Atlantic Forest and Chaco vegetation, 198 odonatan species were registered (Rodrigues & Roque 2017). Despite this number, information gaps are recognized from many areas in this state (Vianna & De Marco 2012, Rodrigues & Roque 2017).

Bodoquena Plateau is a threatened area in the Mato Grosso do Sul state because of the broad conversion of natural areas to pastures and croplands (Roque et al. 2016). In the last five years, several research projects about biodiversity and the ecology of aquatic insects have been developed in Bodoquena Plateau, (e.g. LTER Planalto da Bodoquena, Dragonflies of Serra da Bodoquena Project and Observagua Project). These projects have promoted a great opportunity for extending sampling effort of aquatic insects, especially for odonates (e.g. Koroiva et al. 2016, Rodrigues et al. 2016a, Rodrigues et al. 2016b, Valente-Neto et al. 2016). Until 2011, about 68 Odonata species were registered to Bodoquena Plateau; a number that was considered underestimated because of the broad unsampled area (Dalzochio et al. 2011b). Therefore, even with the publications of checklists (Dalzochio et al. 2011a, Dalzochio et al. 2011b, Rodrigues & Roque 2017), the amount of information generated by these new sampling efforts and visits to museums demand an update.

Here, we provide an updated checklist of the odonates from Bodoquena Plateau, including species records from recent sampling efforts, from the literature, and from specimens deposited in the Reference Collection of the Federal University of Mato Grosso do Sul (CEUFMS). We also provide information about endangered species from the IUCN Red List (IUCN 2016), which is fundamental for updating the status of Brazilian species, as recently emphasized in the 1st Meeting of the Latin American Odonatological Society - SOL.

Material and Methods

1. Sources of data

We used different sources of information to produce the checklist, including samplings (from 2011 to 2016), previous available records on literature and information collected in zoological reference collections of the Mato Grosso do Sul state. For each source, we searched for names and geographic coordinates of the locations. For records that did not have latitude and longitude values, geographic coordinates of the centroid of the place where the site is located.

2. Sampling in study region

All the sampling sites were located within Bodoquena Plateau region (21º08'02" to 20º38'26"S and 56º48'31" to 56º44'28"W). The Bodoquena Plateau is located in the mid-southern portion of Mato Grosso do Sul State, including the cities of Bodoquena, Bonito and Jardim. The region is characterized by a mountain chain, with altitudes varying between 450 and 800 m. The climate is temperate humid with hot summers and two well-defined seasons. The average annual temperature varies between 20 and 22ºC and annual rainfall varies between 1300 and 1700 mm. The hydrography of the region is characterized by limestone, rivers with high alkalinity and low turbidity. Its tributaries are part of the Paraguay River watershed (PCBAP 1997).

Bodoquena Plateau has both areas of the Cerrado (Brazilian savanna) and Atlantic Forest biomes. This region is an important ecological corridor for biodiversity and one of the most important areas of Ecotourism in Brazil (Klein et al. 2011). Considered a priority area for conservation by the Brazilian Ministry of Environment (MMA) (Brasil 2016), the region is currently threatened by inadequate exploitation of its resources and the progressive agricultural expansion.

The dataset used in this checklist included 85 lotic environments (Figure 1) sampled during the period between 2011 and 2016. These samples were concentrated on adults from Odonata and were performed during Ph.D studies (Valente-Neto 2015, Rodrigues 2016), Long term ecological research in the Bodoquena Plateau (LTER-Serra da Bodoquena), Dragonflies of Serra da Bodoquena Project and Observagua Project. The sampling method used handhold insect nets, in sunny days, during the peak time of Odonata activities (between 09:00 h to 16:00 h). The collection authorization process was issued by IBAMA, through SISBio system under the number 41313-3 and 54386-1. All specimens collected were deposited in the Reference Collection of the Universidade Federal de Mato Grosso do Sul (CEUFMS), afterwards determined in laboratory according to Garrison et al. (2006, 2010), Heckman (2006, 2008) and Lencioni (2005, 2006). For the systematics classification, we followed Dijkstra et al. (2014) and for scientific names and followed the synonymic list of Garrisson & von Ellenrieder (2016). In the laboratory, dragonflies were analyzed under Zeiss Discovery V20 stereoscope and recorded with an AxioCam Icc 5 Microscope Camera. For capturing and editing images, we used the software Zen 2-2011.

3. Data analysis

We estimated the sampling accuracy of sampling sites with incidence-based non-parametric estimators: Chao 2, Jacknife 1 and Jacknife 2. Non-parametric methods have better performance compared to others estimators for aquatic insects (Melo & Froelich 2001). These analyses were performed with EstimateS version 9.1.0 software (Colwell 2013). We set 1000 repetitions to calculated mean values with confidence intervals of 95%.

4. Literature and reference collections information

Aiming to improve our list of species, we used online available databases to find registers of Odonata species from Bodoquena Plateau, specifically Web of Knowledge (which includes the main journals of world), Scielo (which include the main journals of South America) and Google Scholar.

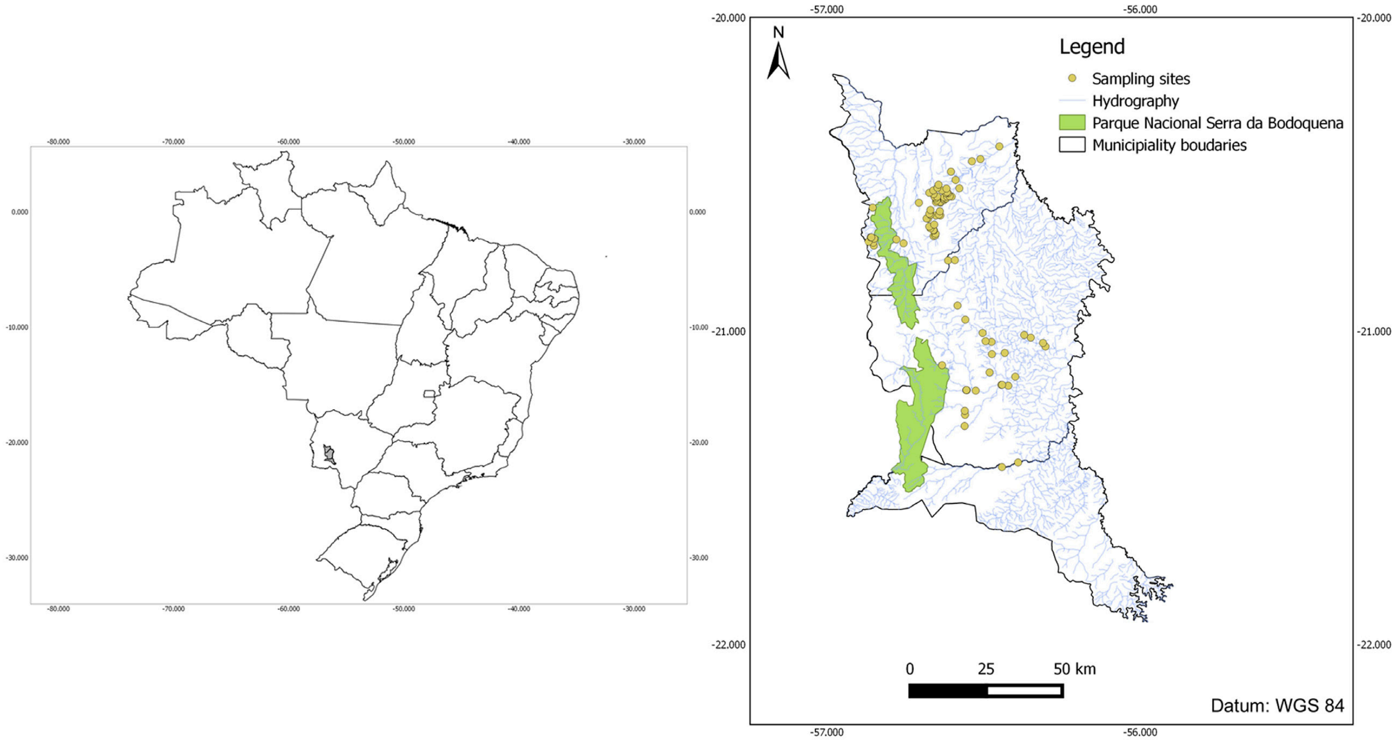


Figure 1. Sampling locations in the Bodoquena Plateau region. On the north is the municipality of Bodoquena; on the middle is the municipality of Bonito and on the south is the municipality of Jardim.

We did the search on general keywords like “Odonata”, “dragonfly” or “libélula” (“Dragonfly” in Portuguese) in combination with “Mato Grosso do Sul”, “Bodoquena Plateau” or “Serra da Bodoquena” (“Bodoquena Plateau” in Portuguese). Souza (2003), Dalzochio et al. (2011a) and Dalzochio et al. (2011b) provided additional information about registers of Odonata species (“Reference” in Table 1).

In addition, we analyzed the deposited specimens from two important Zoological collections of Mato Grosso do Sul state: Reference Collection Universidade Federal de Mato Grosso do Sul (CEUFMS) and Biodiversity Museum of Universidade Federal da Grande Dourados (UFGD). CEUFMS provided additional register besides that found in the project samples or in literature (“CEUFMS” in Table 1).

Table 1. Inventory list of Odonata species from Bodoquena Plateau with information about endangered species (IUCN). CR = Critically Endangered, VU = Vulnerable, LC = Least Concern, DD = Data Deficient; *, first record to Bodoquena Plateau.

Suborder	Family	Species	Source	IUCN Red list status
Anisoptera	Aeshnidae	*Castoraeschna* sp Calvert, 1952 *	This study (BOMS 0052/UFMS BOMS 0053/UFMS)	-
		Staurophlebia reticulata Burmeister, 1839 *	This study (CANI 414/UFMS)	-
	Gomphidae	*Agriogomphus ericae* Belle, 1966	Reference (Dalzochio et al. 2011b)	-
		Archaeogomphus sp Williamson, 1919	Reference (Dalzochio et al. 2011b)	-
		Cyanogomphus sp Selys, 1873	Reference (Dalzochio et al. 2011b)	-
		Gomphoides infumata Rambur, 1842	Reference (Dalzochio et al. 2011a)	-
		Phyllogomphoides suspectus Belle, 1994 *	This study (6-R15-04/UFMS	DD
		Phyllocycla sp Calvert, 1948	This study (CANI336/UFMS)	-
		Progomphus amazonicus Belle, 1973	Reference (Dalzochio et al. 2011a)	DD
		Progomphus intricatus Hagen in Selys, 1858	Reference (Dalzochio et al. 2011b)	LC
		Progomphus montanus Belle, 1973	Reference (Dalzochio et al. 2011a)	LC
		Progomphus sp1 Selys, 1854	This study (CANI 411/UFMS)	-
		Progomphus sp2 Selys, 1854	This study (CANI 334/UFMS)	-
		Remartinia sp Navás, 1911 *	This study (CANI 017/UFMS)	-
	Libellulidae	*Brechmorhoga* sp Kirby, 1894	Reference (Dalzochio et al. 2011b)	-
		Dythemis sterilis Hagen, 1861	This study (6-R15-21/UFMS)	-
		Elasmothemis cannacrioides Calvert, 1906 *	This study (6-R15-28/UFMS, 6-R15-14/UFMS, 6-R15-8/UFMS)	-
		Elasmothemis williamsoni Ris, 1919	Reference (Dalzochio et al. 2011a)	-
		Elga newtonsantosi Machado, 1992 *	This study (CANI 833/UFMS, CANI 413/UFMS)	CR
		Erythemis vesiculosa Fabricius, 1775	This study (1-R19-3/UFMS)	LC
		Erythemis mithroides Brauer in Therese, 1900 *	CEUFMS (CEUFMS 6048)	LC
		Erythemis peruviana Rambur, 1842 *	CEUFMS (CEUFMS 6044, CEUFMS 6046, CEUFMS 6047)	-
		Erythemis plebeja Burmeister, 1839	CEUFMS (CEUFMS 6043)	LC
		Erythrodiplax basalis Kirby, 1897 *	This study (CANI 011/UFMS, CANI 015/UFMS, CANI 09/UFMS, CANI 222/UFMS)	-
		Erythrodiplax branconensis Sjöstedt, 1929 *	This study (CANI 217/UFMS)	-
		Erythrodiplax famula Erichson in Schomburgk, 1848	Reference (Dalzochio et al. 2011a)	-
		Erythrodiplax fusca Rambur, 1842	This study (3-R14-11/UFMS, 3-R14-10/UFMS, 3-R14-24/UFMS, 6-R15-3/UFMS)	-
		Erythrodiplax kimminsi Borror, 1942 *	This study (CANI 088/UFMS)	-
		Erythrodiplax lativittata Borror, 1942 *	This study (CANI 405/UFMS, CANI 499/UFMS, CANI 505/UFMS, CANI 502/UFMS)	-
		Erythrodiplax ochracea Burmeister, 1839	This study (8-R22-1/UFMS, 8-R22-4/UFMS, 8-R22-5/UFMS)	LC
		Erythrodiplax paraguayensis Förster, 1905	This study (BNBTO-0005/UFMS)	LC
		Erythrodiplax umbrata Linnaeus, 1758	This study (7-R21-1/UFMS, 7-R21-2/UFMS, 7-R21-4)	-
		Macrothemis flavescens Kirby, 1897 *	This study (CANS 480/UFMS)	LC
		Macrothemis hemichlora Burmeister, 1839	This study (BOMS-0328/UFMS)	LC
		Macrothemis heteronycha Calvert, 1909	Reference (Dalzochio et al. 2011b)	LC
		Macrothemis imitans imitans Karsch, 1890	Reference (Dalzochio et al. 2011a)	-
		Macrothemis sp Hagen, 1868 *	This study (BOMS0379/UFMS)	-
		Miathyria marcella Selys in Sagra, 1857	This study (2-R16-3/UFMS, 7-R21-6/UFMS)	LC
		Micrathyria catenata Calvert, 1909	Reference (Souza 2003)	LC
		Micrathyria hesperis Ris, 1911	CEUFMS (CEUFMS 6019, CEUFMS 6020, CEUFMS 6021)	-
		Micrathyria laevigata Calvert, 1909	This study (CANI 019/UFMS)	-
		Micrathyria longifasciata Calvert, 1909	Reference (Dalzochio et al. 2011b)	LC

Table 1. Continued...

Suborder	Family	Species	Source	IUCN Red list status
		Micrathyria ocellata Martin, 1897	CEUFMS (CEUFMS 5998, CEUFMS 5999, CEUFM 6008)	-
		Micrathyria pseudeximia Westfall, 1992	Reference (Souza 2003)	-
		Micrathyria pseudhypodidyma Costa, Lourenço & Viera, 2002 *	This study (CANI 433/UFMS, CANI 432/UFMS)	VU
		Micrathyria stawiarskii Santos, 1953 *	This study (BOMS0076/UFMS)	LC
		Micrathyria tibialis Kirby, 1897	Reference (Souza 2003)	LC
		Micrathyria ungulata Förster, 1907 *	This study (CANI 013/UFMS)	-
		Oligoclada sp. Karsch, 1890	Reference (Souza 2003)	-
		Orthemis cultriformis Calvert, 1899 *	This study (BNBTO202-12/UFMS)	-
		Orthemis discolor Burmeister, 1839	This study (1-R19-7/UFMS)	-
		Orthemis ferruginea Fabricius, 1775 *	CEUFMS (CEUFMS 5886, CEUFMS 5888, CEUFMS 5889)	LC
		Orthemis schmidti Buchholz, 1950	CEUFMS (CEUFMS 59891)	-
		Pantala flavescens Fabricius, 1798	This study (BOMS0088/UFMS)	LC
		Pantala hymenae Say, 1839 *	This study (CANI 041/UFMS)	-
		Perithemis electra Ris, 1930 *	This study (CANI 588/UFMS, CANI 514/UFMS, CANI 517/UFMS, CANI 501/UFMS)	LC
		Perithemis icteroptera Selys in Sagra, 1857 *	This study (13-R31-2/UFMS)	LC
		Perithemis lais Perty, 1834	This study (CANI 301/UFMS, CANI 459/UFMS)	LC
		Perithemis mooma Kirby, 1889	This study (CANI 460/UFMS)	-
		Perithemis thais Kirby, 1889	This study (CANI 481/UFMS, CANI 468/UFMS, CANI 464/UFMS, CANI 463/UFMS)	-
		Tauriphila australis Hagen, 1867	Reference (Souza 2003)	-
		Tramea cophysa Hagen, 1867 *	CEUFMS (CEUFMS 6082, CEUFMS 6093, CEUFMS 6084)	-
		Tramea minuta De Marmels & Rácenis, 1982	Reference (Dalzochio et al. 2011a)	-
		Tramea rustica De Marmels & Rácenis, 1982 *	CEUFMS (CEUFMS 6074, CEUFMS 6075, CEUFMS 6076)	LC
Zygoptera	Calopterygidae	*Hetaerina mortua* Hagen in Selys, 1853 *	This study (CZYG 693/UFMS)	-
		Hetaerina rosea Selys, 1853	This study (6-R15-23/UFMS, 02-R16-01/UFMS, 4-R17-1/UFMS, 4-R17-2/UFMS)	-
	Coenagrionidae	*Acanthagrion aepiolum* Tennessen, 2004	This study (12-R25-18/UFMS, 9-R27-5/UFMS, 10-R28-8/UFMS, 13-R30-16/UFMS)	-
		Acanthagrion apicale Selys, 1876	Reference (Dalzochio et al. 2011a)	-
		Acanthagrion ascendens Calvert, 1909	Reference (Dalzochio et al. 2011a)	-
		Acanthagrion chararum Calvert, 1909	Reference (Dalzochio et al. 2011b)	-
		Acanthagrion cuyabae Calvert, 1909	This study (10-R28-2/UFMS, 10-R28-6/UFMS, 10-R28-7/UFMS)	LC
		Acanthagrion gracile Rambur, 1842	This study (8-R22-2/UFMS, 12-R25-20/UFMS, 12-R25-17/UFMS)	-
		Acanthagrion temporale De Marmels, 1985	Reference (Souza 2003)	-
		Acanthagrion truncatum Selys, 1876	Reference (Souza 2003)	LC
		Aeolagrion dorsale Burmeister, 1839 *	This study (CZYG 1011/UFMS, CZYG 1551/UFMS)	-
		Argentagrion ambiguum Ris, 1904 *	CEUFMS (CZYGO 5001/UFMS)	-
		Argia chapadae Calvert, 1909 *	This study (5-R13-1/UFMS, 3-R14-16/UFMS, 6-R15-1/UFMS, 1-R19-10/UFMS)	-
		Argia croceipennis Selys, 1865	This study (CZYG 1504/UFMS, CZYG 1837/UFMS, CZYG 1328/UFMS, CZYG 1329/UFMS)	LC
		Argia cupraurea Calvert, 1902	Reference (Souza 2003)	-
		Argia hasemani Calvert, 1909 *	This study (CZYG 1779/UFMS, CZYG 1791/UFMS, CZYG 501/UFMS)	-
		Argia indocilis Navás, 1934	This study (CZYG 873/UFMS)	-
		Argia lilacina Selys, 1865 *	This study (CZYG 412/UFMS)	-
		Argia modesta Selys, 1865 *	This study (BNBTO-0288/UFMS)	-
		Argia mollis Hagen in Selys, 1865 *	This study (3-R14-17/UFMS)	-
		Argia reclusa Selys, 1865 *	This study (6-R15-16/UFMS, 1-R19-11/UFMS, 9-R27-1/UFMS, 13-R30-2/UFMS)	-
		Argia smithiana Calvert, 1909 *	This study (BNBTO-0093)	-

Table 1. Continued...

Suborder	Family	Species	Source	IUCN Red list status
		Argia subapicalis Calvert, 1909 *	This study (CZYG 509/UFMS)	DD
		Argia tamoyo Calvert, 1909	This study (BNBTO-0289/UFMS)	-
		Enallagma novaehispaniae Calvert, 1907	This study (CZYG 742/UFMS, CZYG 1128/UFMS, CZYG 1137/UFMS)	-
		Epipleoneura metallica Rácenis, 1955 *	This study (BNBTO244-12/UFMS)	LC
		Epipleoneura venezuelensis Rácenis, 1955	This study (CZYG 958/UFMS, CZYG 1112/UFMS, CZYG 1116/UFMS)	-
		Forcepsioneura sp. Lencioni, 1999	Reference (Souza 2003)	-
		Homeoura chelifera Selys, 1876	Reference (Dalzochio et al. 2011a)	-
		Homeoura nepos Selys, 1876 *	This study (CZYG 433/UFMS, CZYG 437/UFMS)	-
		Ischnura capreolus Hagen, 1861	Reference (Souza 2003)	-
		Ischnura fluviatilis Selys, 1876	Reference (Souza 2003)	LC
		Metaleptobasis lillianae Daigle, 2004 *	This study (CZYG 1358/UFMS, CZYG 1359/UFMS)	DD
		Neoneura bilinearis Selys, 1860 *	This study (CZYG 2091/UFMS, CZYG 2101/UFMS, CZYG 2099/UFMS)	LC
		Neoneura ethela Williamson, 1917	This study (CZYG 1815/UFMS, CZYG 1828/UFMS, CZYG 1397/UFMS)	-
		Neoneura rubriventris Selys, 1860	This study (CZYG 8908/UFMS, CZYG 8907/UFMS, CZYG 8906/UFMS	-
		Neoneura sylvatica Hagen in Selys, 1886	This study (05-R13-09/UFMS, 06-R15-31/UFMS, 13-R30-22/UFMS)	-
		Oxyagrion chapadense Costa, 1978	Reference (Dalzochio et al. 2011b)	-
		Oxyagrion sulmatogrossense Costa, Souza & Santos, 2000 *	This study (9-R27-3/UFMS)	-
		Oxyagrion terminale Selys, 1876 *	This study (CZYG 189/UFMS)	-
		Peristicta aeneoviridis Calvert, 1909	This study (CEUFMS 8925, CEUFMS 8926, CEUFMS 8924)	-
		Peristicta muzoni Pessacq & Costa, 2007	Reference (Dalzochio et al. 2011b)	-
		Telebasis sanguinalis Calvert, 1909	Reference (Souza 2003)	-
		Telebasis willinki Fraser, 1948	Reference (Dalzochio et al. 2011a)	LC
		Tigriagrion aurantinigrum Calvert, 1909 *	This study (13-R30-24/UFMS)	-
	Lestidae	*Lestes forficula* Rambur, 1842	This study (CEUFMS 8903, CEUFMS 8902, CEUFMS 8901)	-
		Lestes curvatus Belle, 1997	Reference (Souza 2003)	LC

Results

1. Species list

Sampling projects data recorded 67 species, belonging to 42 genera in six families, totaling 1902 collected specimens. The estimators showed that this richness corresponds to 79.99, 76.35 and 69.99% of the medium estimated richness by, respectively, Chao 2 (Sest = 83.76, standard deviation. = ±9.59), Jackknife 1 (Sest = 87.75, standard deviation = ±4.85) and Jackknife 2 (Sest = 95.72). The accumulation curves obtained are non-asymptotic (Figure 2), thus indicating that total species richness would probably increase with additional sampling effort.

The Odonata list records from the Bodoquena Plateau are presented in Table 1. We added another fourty-four species that were registered in the literature (34 species) or deposited in the Reference Collection of the Federal University of Mato Grosso do Sul (10 species). Thus, we have considered the presence of 111 Odonata species. We recorded for the first time the presence of 42 species from Bodoquena Plateau (24 anisopterans and 18 zygopterans). In this inventory list, 35 species are registered in IUCN red List (IUCN 2016), with four being in Data Deficient (DD), 29 in Least Concern (LC) and two species are in threatened categories, *Elga newtonsantosi* Machado, 1992 like Critically Endangered (CR)

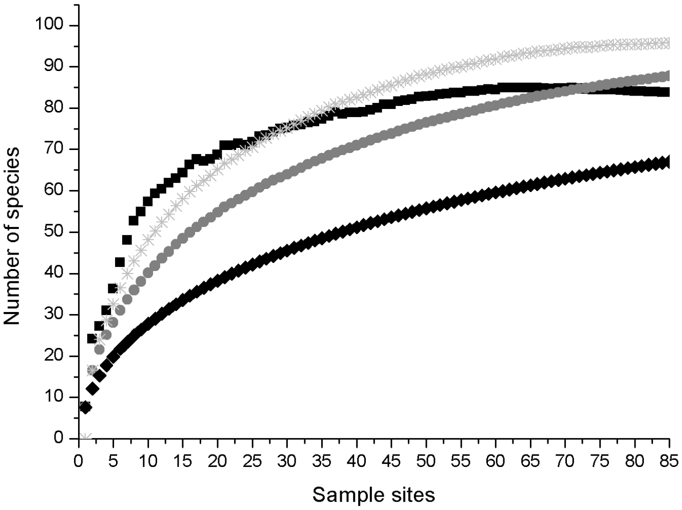


Figure 2. Accumulation curves for observed and estimated number of species collected at 85 sample points. The estimates were calculated with the estimator Chao 2, Jackknife 1 and Jackknife 2. Black diamonds = number of species observed (est); Black squares = Chao 2 richness estimator; Grey circles = Jackknife 1 richness estimator; Grey stars = Jackknife 2 richness estimator . All curves are plotted form the means of 1000 randomizations of sample accumulation order.

and *Micrathyria pseudhypodidyma* Costa, Lourenço & Viera, 2002 like Vulnerable (VU).

The families with the higher number of species were Libellulidae (45.05%), Coenagrionidae (38.74%) and Gomphidae (10.81%) (Table 2). The most speciose genus were *Argia, Micrathyria* and *Erythrodiplax*, represented by twelve, ten and nine species, respectively. The endangered species classified in the IUCN Red List, *Elga newtonsantosi* and *Micrathyria pseudhypodidyma*, were registered only in two and one sampled sites, respectively.

2. New record

We found that *Phyllogomphoides suspectus* Belle, 1994 represents a new records of odonate species for Mato Grosso do Sul state, compared with the list published by Rodrigues & Roque (2017). The genus *Phyllogomphoides* Belle, 1970, is composed of 45 species in the Neotropical Region, 29 of these in South America (Belle 1994). *Phyllogomphoides suspectus* Belle, 1994 has a distribution recorded by Belle (1994) and von Ellenrieder (2009a) and is considered exclusive to Brazil. The species distribution ranges from Rondonia to the Mato Grosso with specimens found in Rio Ouro Preto and Sinop, respectively (Belle 1994), around 800 km northwards of our sampling site.

We collected one male adult of *Phyllogomphoides* in the rural area of Bonito, Mato Grosso do Sul state, Brazil. This specimen was identified as *P. suspectus* (Figure 3a), according to the characteristics indicated by Belle (1994) and Heckman (2008), like spines along ventral tergal margins of abdominal segment 7 small and largely black (Figure 3b), the cerci, in dorsal view, with forcipate shape (Figure 3c) and with a hatchet-shaped inferior protuberance near the base (Figure 3d) and anterior genital hamules without a posterior black hook (Figure 3e).

Table 2. Number of Odonata species collected per family in the Bodoquena Plateau.

SUBORDER FAMILY	Number	%
ANISOPTERA		
Aeshnidae	2	1.80
Gomphidae	12	10.81
Libellulidae	50	45.05
ZYGOPTERA		
Calopterygidae	2	1.80
Coenagrionidae	43	38.74
Lestidae	2	1.80
TOTAL	111	100

Discussion

Inventories promote skeletal support in many fields, including biogeography, population and community ecology. Such data establish a baseline of biodiversity and ecological information, which allow their application in conservation strategies. The list of species shown here represents 55.77% of all species recorded for Mato Grosso do Sul (199 species) (Rodrigues & Roque 2017, Rodrigues et al. 2016a), and is a significant advance in comparison with previous accounts (Souza 2013, Dalzochio et al. 2011a, Dalzochio et al. 2011b), increasing by 42 odonate species list of Bodoquena Plateau region.

Considering that insect inventories, especially those of very species-rich taxa in tropical region, are often incomplete (Gotelli & Colwell 2001), our results show that accumulation curves have not tended to stabilize. The reference source emphasize this result, because there are 44 species registered by Souza (2003) and Dalzochio et al. (2011a, 2011b) and not sampled by the projects. Other reasons for the high number of non-sampled species are the large territory of the Bodoquena Plateau and many of these species had been collected in the southern part, region not sampled by the projects. Furthermore, the projects sample sites, like the other previous studies (Souza 2003, Dalzochio et al. 2011a, Dalzochio et al. 2011b), focused on lotic system rather lentic ones, such as lakes and reservoirs. Thus, future inventories in lentic environments and with other sampling methods (e.g. visual record, Malaise trap, light trap) may still increase the species records for Bodoquena Plateau, highlighting the importance of this region, which lies into two highly threatened biomes.

Our new records extend the register of distribution to many species, information considered critical for the definition of their threatened status (IUCN 2016). When considering the IUCN red list, the Odonata species of the Neotropical region have a high number of species listed as Data Deficient (Clausnitzer et al. 2009) or without status definition. In this study, 2/3 of the species do not have classification, which reinforces this information gap related to conservation for the group. Regarding the species classified as threatened, *Elga newtonsantosi* and *Micrathyria pseudhypodidyma* have limited information available about their distribution (number of locations), population size, and ability to survive in reduced habitat (von Ellenrieder

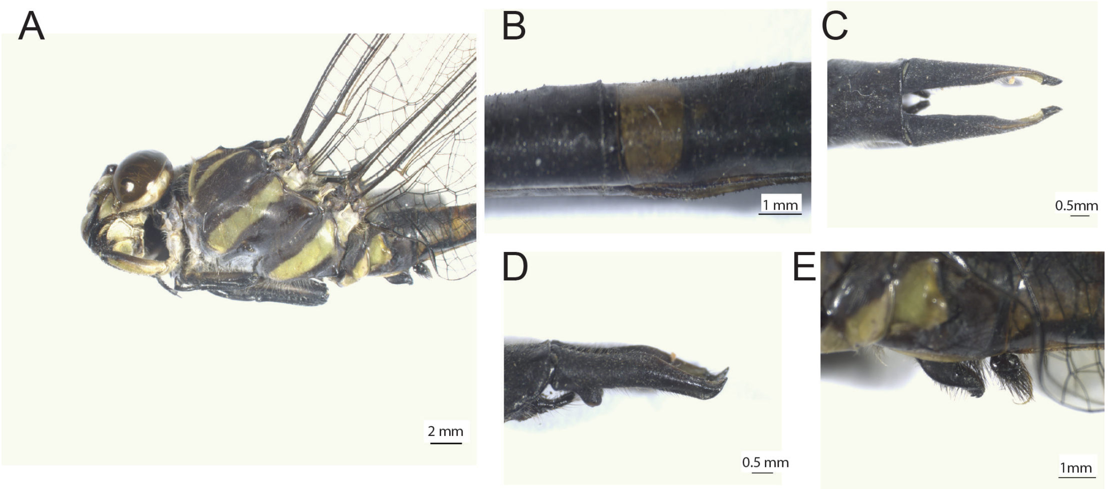


Figure 3. *Phyllogomphoides suspectus* (Belle, 1994): (A) adult male specimen; (B) lateral overview of abdomen (segment 7); (C) male caudal appendices: dorsal view; (D) male caudal appendices: lateral view; (E) male genital hamule: lateral view.

2009b, von Ellenrieder 2009c). From a conservation perspective, the knowledge about threatened species distributions is crucial for biodiversity management, especially for the development and implementation of conservancy plans. Thus, both of them shall be targets of governmental protection plan, as required in the Brazilian legislation on endangered species. In summary, this study highlighted the Bodoquena Plateau as an important habitat for odonates, including species classified as threatened.

Acknowledgements

We are grateful to several colleagues for their help: Janira Martins Costa, Cesar Carriço, Danielle Anjos-Santos, National Museum (MNRJ, Rio de Janeiro, Brazil), Mr. Javier Muzon, Alexandro Del Placio, Federico Lozano and Lia Ramos, National University of Avellaneda (Avellaneda, Argentina) helped us with the identifications. We also thank the projects Long Term Ecological Research in the Bodoquena Plateau (LTER-Serra da Bodoquena), NGO Neotropica do Brasil, Observagua Project and the hotel Cabanas staff. RK receives a scholarship of CAPES and grants from Rufford Foundation (Dragonflies of Serra da Bodoquena Project, RSGF 19689-1). FOR received a productivity grant, no. 09908/2013-2, from CNPq. This study was partially funded by the Fundect.

Authors' Contributions

KOROIVA, R., RODRIGUES, M., VALENTE-NETO: contribution to data collection.

KOROIVA, R., RODRIGUES, M., VALENTE-NETO, F. and ROQUE, F.O: contribution to data analysis and interpretation.

KOROIVA, R., RODRIGUES, M., VALENTE-NETO, F. and ROQUE, F.O: contribution to manuscript preparation.

KOROIVA, R., RODRIGUES, M., VALENTE-NETO, F. and ROQUE, F.O: contribution to critical revision, adding intelectual content.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

ABBOTT, J.C. 2015. Dragonflies of Texas: A field guide. University of Texas Press, Austin.

ANJOS-SANTOS, D. & COSTA, J.M. 2006. A revised checklist of Odonata (Insecta) from Marambaia, Rio de Janeiro, Brazil with eight new records. Zootaxa 1300: 37-50.

BELLE, J. 1994. On five species of Phyllogomphoides Belle, 1970, from Brazil with the descriptions of three new taxa (Odonata: Gomphidae). Zool. meded. 68: 73-85.

BRASIL. 2016. Áreas prioritárias para conservação, uso sustentável e repartição dos benefícios da biodiversidade. Ministério do Meio ambiente. World Wide Web publication. http://www.mma.gov.br/images/arquivo/80049/Areas%20Prioritarias/Cerrado%20e%20Pantanal/FICHAS%20Areas%20Prioritarias%20Cerrado%20e%20Pantanal_2%20atualizacao%2022ago16.pdf (last access at 23/November/2016).

CLAUSNITZER, V., KALKMAN, V.J., RAM, M., COLLEN, B., BAILLIE, J.E.M., BEDJANIC, M., DARWALL, W.R.T., DIJKSTRA, K.B., DOW, R., HAWKING, J., KARUBE, H., MALIKOVA, E., PAULSON, D., SCHÜTTE, K., SUHLING, F., VILLANUEVAM, R.J., VON ELLENRIEDER, N. & WILSON, K. 2009. Odonata enter the biodiversity crisis debate: the first global assessment of an insect group. Biol. Conserv. 142: 1864-1869.

COLWELL, R.K. 2013. EstimateS 9.1.0. World Wide Web publication. http://viceroy.eeb.uconn.edu/estimates (last access at 23/November/2016).

COSTA, J.M., MACHADO, A.B.M., LENCIONI, F.A.A. & SANTOS, T.C. 2000. Diversidade e distribuição dos Odonata (Insecta) no estado de São Paulo, Brasil. Pub. Avul. Mus. Nac. 80(1): 1-27.

COSTA, J.M. & OLDRINI, B.B. 2005. Diversidade e distribuição dos Odonata (Insecta) no Estado do Espírito Santo, Brasil. Publ. Avulsas Mus. Nac. 107:1-15.

DALZOCHIO, M.S., COSTA, J.M. & UCHÔA, M.A. 2011a. Diversity of Odonata (Insecta) in lotic systems from Serra da Bodoquena, Mato Grosso do Sul State, Brazil. Rev. Bras. Entomol. 5(1): 88-94.

DALZOCHIO, M.S., SOUZA, L.O.I., UCHÔA, M.A. & COSTA, J.M. 2011b. First records of Odonata (insecta) From the Bodoquena Mountains, Mato Grosso do Sul, Brazil. EntomoBrasilis 4(3): 135-138.

DINIZ-FILHO, J.A.F., DE-MARCO, P. & HAWKINS, B.A. 2010. Defying the curse of ignorance: perspectives in insect macroecology and conservation biogeography. Insect Conserv. Diver. 3: 172-179.

GARRISON, R. & VON ELLENRIEDER, N. 2016. A synonymic list of the New World Odonata. World Wide Web publication. http://www.odonatacentral.org/docs/NWOL.pdf (last access at 23/November/2016).

GARRISON, R.W., VON ELLENRIEDER, N. & LOUTON, J.A. 2006. Dragonfly genera of the new world: an illustrated and annotated key to the Anisoptera. The John Hopkins University Press, Baltimore.

GARRISON, R.W., VON ELLENRIEDER, N. & LOUTON, J.A. 2010. Damselfly genera of the new world: an illustrated and annotated key to the Zygoptera. The John Hopkins University Press, Baltimore.

GOTELLI, N.J. & COLWELL, R.K. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecol. Lett. 4: 379-391.

HECKMAN, C.W. 2006. Encyclopedia of South American aquatic insects: Odonata - Anisoptera. Springer, Dordrecht.

HECKMAN, C.W. 2010. Encyclopedia of South American aquatic insects: Odonata - Zygoptera. Springer, Dordrecht.

IUCN (International Union for Conservation of Nature). 2016. The IUCN Red List of Threatened Species, Version 2016-2. World Wide Web publication. http://www.iucnredlist.org. (last access at 23/November/2016).

KALKMAN, V.J., CLAUSNITZER, V., DIJKSTRA, K.D.B., ORR, A.G., PAULSON, D.R. & VAN TOL, J. 2008. Global diversity of dragonflies (Odonata) in freshwater. Hydrobiologia 595: 351-363.

KLEIN, F.M., ESCANDOLHERO, J.P.O., LUCCHESE, N.R., MERCANTE, M.A., FÁVERO, S. & RODRIGUES, S.C. 2011. Educação ambiental e o ecoturismo na Serra da Bodoquena em Mato Grosso do Sul. Soc. Nat. 23(2): 311-321.

KOROIVA, R., VALENTE-NETO, F., RODRIGUES, M.E. & ROQUE, F.O. 2016. As reveladoras libélulas da Bodoquena. Ciência Pantanal 2: 26-27.

LENCIONI, F.A.A. 2005. Damselflies of Brazil: An Illustrated Identification Guide – 1 – Non-Coenagrionidae Families. All Print Editora, São Paulo.

LENCIONI, F.A.A. 2006. The Damselflies of Brazil: An Illustrated Identification Guide 2 - Coenagrionidae. All Print Editora, São Paulo.

MELO, A.S. & FROELICH, C.G. 2001. Evaluation of methods for estimating macroinvertebrate species richness using individual stones in tropical streams. – Freshwat. Biol. 46: 711–721.

NÓBREGA, C.C. & DE MARCO, P. jr. 2011. Unprotecting the rare species: a niche-based gap analysis for odonates in a core Cerrado área. Divers. Distrib. 17(3): 491-505.

PCBAP (Plano de Conservação da Bacia do Alto Paraguai - Pantanal). 1997. Análise integrada e prognóstica da Bacia do Alto Paraguai. MMA/SEMAM/PNMA, Brasília.

RENNER, S., PERICO, E, & SAHLEN, G. 2016. List of Odonates from the Floresta Nacional de São Francisco de Paula (FLONA - SFP), with two new distribution records for Rio Grande do Sul, Brazil. Biota Neotrop. 16(3): e20150132. http://www.biotaneotropica.org.br/v16n3/pt/download?inventory+bn00616032016+item. (last access at 23/November/2016).

RODRIGUES, M.E. 2016. Odonates as indicators of landscape change in a region of the Cerrado. 2016. Tese de doutorado, Universidade Federal de Mato Grosso do Sul, Campo Grande.

RODRIGUES, M.E., KOROIVA, R., RAGALZI-DA-SILVA, E. & MOURA, E.B. 2016a. Mecistogaster linearis (Fabricius) (Odonata: Coenagrionidae): First Record from Mato Grosso do Sul State, Brazil. EntomoBrasilis. 9: 212-215.

RODRIGUES, M.E., ROQUE, F.O., OCHOA-QUINTERO, J.M., PENA, J.C.C., SOUSA, D.C. & DE-MARCO, P. Jr. 2016b. Nonlinear responses in damselfly community along a gradient of habitat loss in a savanna landscape. Biol. Conserv. 194: 113-120.

RODRIGUES, M.E. & ROQUE, F.O. 2017. Checklist de Odonata do estado de Mato Grosso do Sul, Brasil. Iheringia Ser. Zool. 107(suppl.): e2017117.

ROQUE, F.O., OCHOA-QUINTERO, J.M., RIBEIRO, D.B., SUGAI, L.S.M, COSTA-PEREIRA, R., LOURIVAL, R. & BINO, G. 2016. Upland habitat loss as a threat to Pantanal wetlands. Conserv. Biol. 30 (5): 1131–1134.

SOUZA, L.O.I. 2003. A Influência dos Fatores Ambientais na Distribuição da Fauna de Odonata (Insecta) em Riachos da Serra da Bodoquena, MS. Dissertação de Mestrado, Universidade Federal de Mato Grosso do Sul, Campo Grande.

SOUZA, M.M., SOUZA, B., PEREIRA, M.C.S.A. & MACHADO, A.B.M. 2013. List of Odonates from Mata do Baú, Barroso, Minas Gerais, Brazil. Check List 9: 1367-1370.

VALENTE-NETO, F. 2015. Ecological drivers of aquatic metacommunities in a riverine network. 2015. Tese de doutorado, Universidade Federal de Mato Grosso do Sul, Campo Grande.

VALENTE-NETO, F., ROQUE, F.O., RODRIGUES, M.E., JUEN, L. & SWAN, C.M. 2016. Toward a practical use of Neotropical odonates as bioindicators: Testing congruence across taxonomic resolution and life stages. Ecol. Indic. 61: 952-959.

VIANNA, D.M. & DE-MARCO, P. Jr. 2012. Higher-taxon and cross-taxon surrogates for odonate biodiversity in Brazil. Nat. Conservacao 10: 34–39.

VON ELLENRIEDER, N. 2009a. Phyllogomphoides suspectus. The IUCN Red List of Threatened Species. World Wide Web publication. http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T159007A5305086.en (last access at 23/November/2016).

VON ELLENRIEDER, N. 2009b. Elga newtonsantosi. The IUCN Red List of Threatened Species. World Wide Web publication. http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T159064A5318685.en (last access at 23/November/2016).

VON ELLENRIEDER, N. 2009c. Micrathyria pseudhypodidyma. The IUCN Red List of Threatened Species. World Wide Web publication. http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T159112A5315873.en (last access at 23/November/2016).

Received: 05/12/2016
Revised: 02/06/2017
Accepted: 10/07/2017
Published online: 31/07/2017

Biota Neotropica 17(3): e20170344, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Inventory

Survey of fish species from plateau streams of the Miranda River Basin in the Upper Paraguay River Region, Brazil

Fabiane Silva Ferreira[1], *Gabriela Serra do Vale Duarte*[1], *Francisco Severo-Neto*[2], *Otávio Froehlich*[3] *&*

Yzel Rondon Súarez[4]*

[1]*Universidade Estadual de Mato Grosso do Sul, Centro de Estudos em Recursos Naturais, Dourados, MS, Brazil*
[2]*Universidade Federal de Mato Grosso do Sul, Laboratório de Zoologia, Campo Grande, CG, Brazil*
[3]*Universidade Federal de Mato Grosso do Sul, Departamento de Zoologia, Campo Grande, CG, Brazil*
[4]*Universidade Estadual de Mato Grosso do Sul, Centro de Estudos em Recursos Naturais, Lab. Ecologia, Dourados, MS, Brazil*
**Corresponding author: Yzel Rondon Súarez, e-mail: yzel@uems.br*

FERREIRA, F. S., DUARTE, G. S. V., SEVERO-NETO, F., FROEHLICH O., SÚAREZ, Y. R. **Survey of fish species from plateau streams of the Miranda River Basin in the Upper Paraguay River Region, Brazil.** Biota Neotropica. 17(3): e20170344. http://dx.doi.org/10.1590/1676-0611-BN-2017-0344

Abstract: The objective of this study was to provide a comprehensive list of the fish fauna of headwater streams of the Miranda River in the Upper Paraguay River Basin. Our primary data set was constructed from sampling of fish using a rectangular sieve, trawl, and gill nets from 2004 to 2015. Our secondary data were derived from published reports conducted in the Miranda River Basin, in addition to taxonomic and distribution data from other studies conducted in the basin. All data were compiled, which in the end encompassed a period from 1999 to 2015. The datasets yielded a total of 143 species, 104 from the primary data (72.7%) and 39 from the secondary data (27.3%). Species were distributed among seven orders and 30 families were found in the Miranda River Basin. Characiformes and Siluriformes were the predominant orders, and the families Characidae and Loricariidae had the greatest number of species. Our results indicate a greater number of species compared to other studies of the Upper Paraguay Basin headwaters, likely due to the longer time frame covered by our primary and secondary datasets.

Keywords: *Species List, Pantanal, streams ichthyofauna, plateau, Paraguay basin.*

Levantamento das espécies de peixes da bacia do Rio Miranda em riachos do planalto, Alto Rio Paraguai, Brasil

Resumo: O objetivo deste trabalho é o de prover uma listagem da ictiofauna de riachos de cabeceira da bacia do Rio Miranda, Alto Rio Paraguai. Os peixes foram amostrados utilizando uma peneira retangular, rede de arrasto e rede de espera no período de 2004 a 2015, além disso, outros trabalhos realizados na bacia do Rio Miranda foram compilados e adicionados na listagem total, assim como, artigos de descrição e distribuição de espécies amostradas na bacia compreendendo o período de 1999 a 2015. A bacia do Rio Miranda apresentou um total de 143 espécies, 104 espécies oriundas de dados primários (72,7%) e 39 de dados secundários (27,3%). As espécies estão distribuídas em sete ordens e 30 famílias. Characiformes e Siluriformes foram as ordens predominantes e as famílias Characidae e Loricariidae apresentaram maior número de espécies. Os resultados obtidos neste estudo demonstram um maior número de espécies quando comparados com outros estudos realizados em cabeceiras na bacia do Alto Rio Paraguai decorrente ao longo período de estudo.

Palavras-chave: *Lista de espécies, Pantanal, ictiofauna de riachos, planalto, bacia do Paraguai.*

Introduction

The Paraguay River Basin is an important drainage of the La Plata River Basin (Lowe-McConnell 1999), and occupies a total area of about 496,000 km^2 (Junk et al. 2006). The Brazilian Pantanal has a total area of approximately 361,700 km^2, with 72.7% of the total territory lying in Brazil and the remainder in Bolivia and Paraguay. The upper Paraguay basin in Brazilian divided into two distinct regions: a floodplain known as the 'Pantanal', which at approximately 138,200 km^2 constitutes one of the largest wetland areas in the world; and a plateau comprised of areas with altitudes greater than 200 m encompassing approximately 223,500 km^2 (Silva & Abdon 1998). These two regions are distinguished by geological and geomorphological characteristics, however they are also interdependent since the headwaters of the most important rivers draining into the Pantanal are in the plateau region, resulting in strong hydrologic connectivity (Willink et al. 2000, Teresa et al. 2010).

Approximately 270 fish species have been identified in the Paraguay River Basin (Britski et al. 2007). However, the total number of species would likely be higher if the plateau streams had been included (Alho & Sabino 2012). Numerous studies have been carried out in the basin over the past two decades, including the floodplain region (Catella & Petrere-Jr 1996, Willink et al. 2000, Junk et al. 2006, Baginski et al. 2007, Pacheco & Silva 2009, Pains-Silva et al. 2010, Tondato et al. 2013, Súarez et al. 2013, Pains-Silva et al. 2014, Polaz et al. 2014, Botini et al. 2015 and Severo-Neto et al. 2015) and plateau waters (Súarez et al. 2007, Terra & Sabino 2007, Teresa et al. 2010, Casatti et al. 2010, Teresa & Romero 2010, Castro & Vizzotto 2013, Krinski et al. 2015 and Oliveira et al. 2015). In studies of the plateau portion of the basin, analyses at small spatial scales were the predominant.

Compared to Brazilian hydrographic basins (e.g. the Upper Paraná River system), the fish fauna of central Brazil is poorly described (Casatti et al. 2010). Despite being a hotspot for endemic fauna (Willink et al. 2000) the main Pantanal headwater streams are understudied. The goal of this study was thus to describe the ichthyofauna of plateau region streams of the Miranda River Basin by combining primary and secondary data compiled from previous studies on the basin.

Materials and Methods

1. Study site

The Miranda River Basin is entirely within the limits of the Brazilian region, in Mato Grosso do Sul State. The total basin area is 42,993.83 km^2, with Serra da Bodoquena as the western boundary and the Paraguay River as the northwestern boundary (Plano de Recursos Hídricos da Bacia Hidrográfica do Rio Miranda 2014). The main tributaries are the Miranda and Aquidauana Rivers. The rivers and streams of the Aquidauana and Miranda Rivers flow into the Brazilian Cerrado (savanna), which is composed of patches of deciduous, semi-deciduous, and riparian forest that vary in density depending on the ecological drainage system in which they lie (Jesus 2003, Junk et al. 2006).

Surveys were conducted at 42 sites in the Miranda River Basin from January 2004 to January 2015 (primary data). Additional secondary data were compiled from 16 collection sites from published work on stream fish assemblages in the Miranda River Basin (Willink et al. 2000, Casatti et al. 2010, Teresa et al. 2010, Teresa & Romero 2010). These 58 sampling sites are distributed among streams from first to third order in 18 municipalities in Mato Grosso do Sul, Brazil (Figure 1). Taxonomic and distribution data from additional studies were also added to the final species list (Sabino & Trajano 1997, Willink et al. 2000, Ribeiro et al. 2007, Terra & Sabino 2007, Zawadzki et al. 2014, Shibatta 2016).

2. Sampling

For the primary data set, fish were sampled predominantly using a 0.8 x 1.2 m rectangular sieve and 5 x 1.5 m trawl nets, both with 2 mm mesh. In streams with a higher volume, sampling was supplemented with 10 x 1.5 m gill nets with mesh sizes of 15, 25, 30, 40, and 50 mm between adjacent knots. Sampled fish were anesthetized with Eugenol solution and subsequently fixed by immersion in 4% formaldehyde solution for at least 48 hours. Specimens were then washed and transferred to 70% ethanol.

Fish identification was performed mainly using the Pantanal fish identification key of Britski et al. (2007), and specific keys were used when necessary. Voucher specimens were deposited in the fish collection at the Universidade Federal de Mato Grosso do Sul (ZUFMS/UFMS) and the Núcleo de Pesquisa em Limnologia, Ictiologia e Aquicultura at the Universidade Estadual de Maringá (Nupelia/UEM). For the secondary data set, obtained by published articles and fish collection, voucher specimens were deposited in the fish collection of the Departamento de Zoologia e Botânica at the Universidade Estadual Paulista, São José do Rio Preto (DZSJRP/UNESP), Fish collection of Laboratório de Ictiologia de Ribeirão Preto (LIRP/USP) and Fish collection of Museu de Zoologia of University of Londrina (MZUEL/UEL). Collections were authorized by IBAMA (collection permit number SISBIO; process number 13458-1). Fish classification follows Eschmeyer et al. (2017).

3. Data analysis

To evaluate sampling efficiency, the total richness in the basin was estimated using a species accumulation curve with the bootstrap method (Smith & Van Belle 1984), as well as standard error using the function 'specaccum' in the 'vegan' package (Oksanen et al. 2011) in R (R Development Core Team 2013).

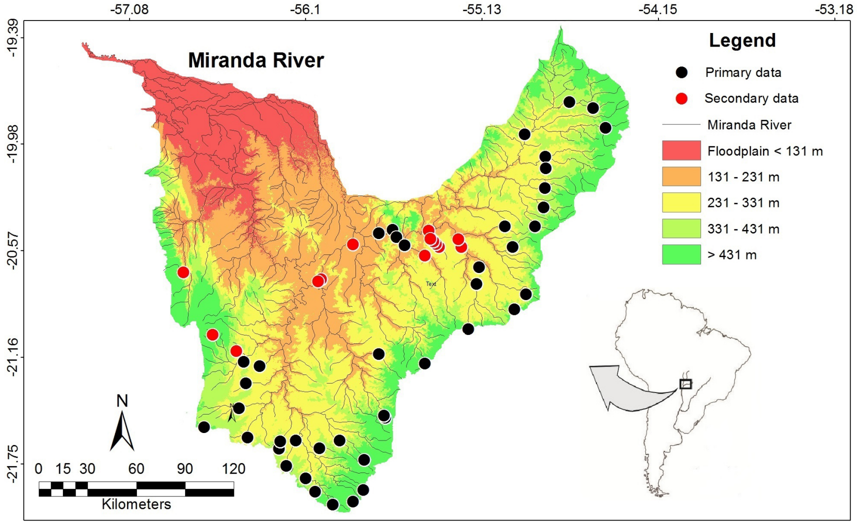


Figure 1: Locations of the sampling points in the Miranda River Basin of the Upper Paraguay River, Mato Grosso do Sul, Brazil. Black dots: Primary data; Red dots: Secondary data.

Results

The datasets yielded a total of 143 species, 104 from the primary data (72.7%) and 39 from the secondary data (27.3%) data. Species were distributed among seven orders and 30 families (Table 1). Characiformes (75 species) and Siluriformes (48 species) were the predominant orders, representing together 86% of the recorded species. Among the families of these orders, the largest numbers of species were from Characidae and Loricariidae, representing 32.2% (46 species) and 15.4% (22 species) of the total sample, respectively (Figure 2).

The species accumulation curve shows that richness does not reach the asymptotic threshold (Figure 3), indicating the need for further collection efforts (i.e., increasing the number of sampling sites should increase estimates of species richness). The bootstrap method estimated approximately 169 with confidence interval (α=0.05) from 150 to 188 species, represent approximately 84.6% of the estimated by datasets (primary and secondary) the total number of species in the Miranda River basin.

In relation the species sampled *Piaractus mesopotamicus* (Holmberg 1887), *Pseudoplatystoma corruscans* (Linnaeus 1766) are on the National List

Table 1: Final list of species from sampled and compiled data from the Miranda River Basin, Upper Paraguay River. Location of voucher deposition: DZSJRP (Departamento de Zoologia e Botânica da Universidade Estadual Paulista, São José do Rio Preto – UNESP); ZUFMS (Coleção Zoológica da Universidade Federal do Mato Grosso do Sul – UFMS); LIRP (Coleção de Peixes do Laboratório de Ictiologia de Ribeirão Preto, Universidade de São Paulo - USP); MZUEL (Museu de Zoologia da Universidade Estadual de Londrina - UEL); NUP (Núcleo de Pesquisa em Limnologia, Ictiologia e Aquicultura da Universidade Estadual de Maringá – Nupelia/UEM).

Taxon	Voucher
BELONIFORMES	
Belonidae	
Potamorrhaphis eigenmanni Miranda-Ribeiro 1915	DZSJRP-12880
CHARACIFORMES	
Acestrorhynchidae	
Acestrorhynchus pantaneiro Menezes 1992	ZUFMS-PIS01657
Anostomidae	
Leporinus friderici (Bloch 1794)	ZUFMS-PIS01063
Leporinus lacustris Campos 1945	DZSJRP-12439
Leporinus striatus Kner 1858	DZSJRP-12438
Leporellus vittatus (Valenciennes 1850)	[5]LIRP-5334
Megaleporinus obtusidens (Valenciennes 1836)	DZSJRP-12858
Bryconidae	
Brycon hilarii (Valenciennes 1849)	DZSJRP-11992
Salminus brasiliensis (Cuvier 1816)	DZSJRP-000534
Characidae	
Galeocharax humeralis (Valenciennes 1834)	ZUFMS-PIS03418
Roeboides descalvadensis Fowler 1932	DZSJRP-12871
Phenacogaster tegatus (Eigenmann 1911)	ZUFMS-PIS03802
Odontostilbe pequira (Steindachner 1882)	ZUFMS-PIS03908
Odontostilbe paraguayensis Eigenmann & Kennedy 1903	ZUFMS-PIS03815
Serrapinnus calliurus (Boulenger 1900)	ZUFMS-PIS03847
Serrapinnus kriegi (Schindler 1937)	ZUFMS-PIS01399
Serrapinnus microdon (Eigenmann 1915)	[5]MZUEL-12591
Serrapinnus notomelas (Eigenmann 1915)	ZUFMS-PIS04040
Characidae Incertae Sedis	
Aphyocharax anisitsi Eigenmann &Kennedy 1903	ZUFMS-PIS01275
Aphyocharax dentatus Eigenmann & Kennedy 1903	ZUFMS-PIS03791
Prionobrama paraguayensis (Eigenmann 1914)	DZSJRP-12861
Astyanax abramis (Jenyns, 1842)	DZSJRP12873
Astyanax lacustris (Lütken 1875) (syn. *Astyanax asuncionensis*)	ZUFMS-PIS03848
Astyanax aff. *fasciatus* (Cuvier 1819)	NUP-17163
Astyanax lineatus (Perugia 1891)	NUP-17166
Astyanax marionae Eigenmann 1911	NUP17197
Astyanax sp. 1	NUP-17162
Astyanax sp. 2	NUP-17178
Astyanax sp. 3	NUP-17161
Hyphessobrycon cf. *arianae* Uj & Géry 1989	DZSJRP-12898
Hyphessobrycon eques (Steindachner 1882)	DZSJRP-12863
Hyphessobrycon elachys Weitzman 1984	DZSJRP-12866
Hyphessobrycon luetkenii (Boulenger 1887)	DZSJRP-11969
Psellogrammus kennedyi (Eigenmann 1903)	ZUFMS-PIS01427
Brachychalcinus retrospina Boulenger 1892	NUP-17213

[1]Sabino & Trajano (1997); [2]Ribeiro et al. (2007); [3]Zawadzki et al. (2014); [4]Terra & Sabino (2007); [5]Willink et al. (2000); 6Rizzato, Costa, Trajano & Bichuette (2011); [7]Shibatta (2016). * Species occurrence information, but not sampled.

Table 1: Continued...

Taxon	Voucher
Bryconops melanurus (Bloch 1794)	DZSJRP-12917
Hemigrammus lunatus Durbin 1918	DZSJRP-12882
Hemigrammus tridens Eigenmann 1907	DZSJRP-12885
Moenkhausia bonita Benine, Castro & Sabino 2004	NUP-17165
Moenkhausia forestii Benine, Mariguela & Oliveira 2009	ZUFMS-PIS04042
Moenkhausia intermedia Eigenmann 1908	ZUFMS-PIS03832
Moenkhausia oligolepis (Günter 1864)	ZUFMS-PIS03863
Moenkhausia sanctafilomenae (Steindachner 1907)	ZUFMS-PIS00173
Jupiaba acanthogaster (Eigenmann 1911)	NUP-17168
Gymnocorymbus ternetzi (Boulenger 1895)	ZUFMS-PIS01449
Poptella paraguayensis (Eigenmann 1907)	ZUFMS-PIS01450
Bryconamericus exodon (Eigenmann 1907)	NUP-17196
Bryconamericus stramineus Eigenmann 1908	NUP-17205
Creagrutus meridionalis Vari & Harold 2001	NUP-17195
Piabarchus analis (Eigenmann 1914)	NUP-17190
Piabarchus torrenticola Mahnert & Géry 1985	NUP-17214
Xenurobrycon macropus Myers & Miranda Ribeiro 1945	DZSJRP-12881
Oligosarcus perdido Ribeiro, Cavallaro & Froehlich 2007	[2]LIRP-5893
Oligosarcus pintoi Campos 1945	ZUFMS-PIS04038
Tetragonopterus argenteus (Cuvier 1816)	ZUFMS-PIS01541
Crenuchidae	
Characidium borellii (Boulenger 1895)	ZUFMS-PIS02505
Characidium aff. *fasciatum* Reinhardt 1866	DZSJRP-13743
Characidium laterale (Boulenger 1895)	DZSJRP-12855
Characidium aff. *zebra* Eigenmann 1909	NUP-17172
Characidium cf. *gomesi* Travassos 1956	DZSJRP-12876
Characidium sp. 1	NUP-17559
Characidium sp.	NUP-17181
Curimatidae	
Cyphocharax gillii (Eigenmann & Kennedy 1903)	ZUFMS-PIS03850
Steindachnerina brevipinna (Eigenmann & Eigenmann 1889)	ZUFMS-PIS03794
Erythrinidae	
Hoplias sp. 1	ZUFMS-PIS03877
Erythrinus erythrinus (Bloch & Schneider 1801)	DZSJRP-12900
Hoplerythrinus unitaeniatus (Agassiz 1829)	DZSJRP-12902
Iguanodectidae	
Piabucus melanostoma Holmberg 1891	DZSJRP-000620
Lebiasinidae	
Pyrrhulina australis Eigenmann & Kennedy 1903	DZSJRP-12864
Parodontidae	
Apareiodon affinis (Steindachner 1879)	DZSJRP-12910
Parodon nasus Kner 1859	ZUFMS-PIS04057
Prochilodontidae	
Prochilodus lineatus (Valenciennes 1836)	LIRP-5849
Serrasalmidae	
Metynnis maculatus (Kner 1858)	MZUEL-5235
Piaractus mesopotamicus (Holmberg 1887)	[5]DZSJRP-007798
Serrasalmus maculatus Kner 1858	[5]MZUEL-14023
Serrasalmus marginatus Valenciennes 1837	ZUFMS-PIS03836
GYMNOTIFORMES	
Apteronotidae	
Apteronotus albifrons (Linnaeus 1766)	DZSJRP-12407
Gymnotidae	
Gymnotus inaequilabiatus (Valenciennes 1839)	DZSJRP-12856
Gymnotus sp.	ZUFMS-PIS04043
Hipopomidae	

[1]Sabino & Trajano (1997); [2]Ribeiro et al. (2007); [3]Zawadzki et al. (2014); [4]Terra & Sabino (2007); [5]Willink et al. (2000); 6Rizzato, Costa, Trajano & Bichuette (2011); [7]Shibatta (2016). * Species occurrence information, but not sampled.

Table 1: Continued...

Taxon	Voucher
Brachyhypopomus sp. B	MZUSP-59444.0
Brachyhypopomus sp. C	ZUFMS-PIS3820
Brachyhypopomus sp.	DZSJRP-12862
Rhamphichthyidae	
Gymnorhamphichtys britskii Carvalho, Ramos & Albert, 2011	ZUFMS-PIS01487
Sternopygidae	
Eigenmannia trilineata López & Castello 1966	DZSJRP-12865
Sternopygus macrurus (Bloch & Schneider 1801)	ZUFMS-PIS03397
SILURIFORMES	
Auchenipteridae	
Tatia neivai (Ihering 1930)	ZUFMS-PIS01419
Callichthyidae	
Callichthys callichthys (Linnaeus 1758)	ZUFMS-PIS04046
Hoplosternum littorale (Hancock 1828)	ZUFMS-PIS01132
Lepthoplosternum pectorale (Boulenger 1895)	DZSJRP-12901
Corydoras aeneus (Gill 1858)	DZSJRP-12886
Corydoras areio Knaack 2000	ZUFMS-PIS01105
Corydoras hastatus Eigenmann & Eigenmann 1888	DZSJRP-12904
Corydoras sp.	ZUFMS-PIS05366
Cetopsidae	
Cetopsis gobioides (Kner 1858)	ZUFMS-PIS04039
Heptapteridae	
Imparfinis mirini Haseman 1911	ZUFMS-PIS02493
Imparfinis cf. *schubarti* (Gomes 1956)	DZSJRP-12911
Imparfinis stictonotus (Fowler 1840)	NUP-17194
Imparfinis sp. 1	DZSJRP-12913
Imparfinis sp. 2	DZSJRP-12887
Imparfinis sp.	NUP-17180
Pimelodella gracilis (Valenciennes in d'Orbigny 1835)	DZSJRP-12914
Pimelodella taenioptera Miranda-Ribeiro 1914	ZUFMS-PIS01052
Phenacorhamdia hoehnei (Miranda-Ribeiro 1914)	DZSJRP-12410
Rhamdia quelen (Quoy & Gaimard 1824)	ZUFMS-PIS01046
Loricariidae	
Otocinclus bororo Schaefer 1997	[4]NUP-13347
Otocinclus vittatus Regan 1904	DZSJRP-12874
*Hypostomus basilisko*Tencatt, Zawadzk i& Froehlich 2014	ZUFMS-PIS01294
Hypostomus boulengeri (Eigenmann & Kennedy 1903)	DZSJRP-12908
Hypostomus cochliodon Kner 1854	DZSJRP-12437
*Hypostomus khimaera*Tencatt, Zawadzki & Froehlich 2014	NUP-17207
Hypostomus aff. *latirostris* (Regan 1904)	NUP-17170
Hypostomus piratatu Weber 1986	ZUFMS-PIS02539
Hypostomus perdido Zawadzki, Tencatt & Froehlich 2014	[3]ZUFMS-PIS1468
Hypostomus sp.	NUP-17537
Ancistrus formoso Sabino & Trajano 1997	[1]MZUSP-51836
Ancistrus sp.	NUP-17209
Farlowella cf. *isbrueckeri* Retzer & Page 1997	ZUFMS-PIS01291
Farlowella paraguayensis Retzer & Page 1997	NUP-17202
Loricaria cf. *coximensis* Rodriguez, Cavallaro & Thomas 2012	NUP-17204
Loricaria luciae Thomas, Rodriguez, Cavallaro, Froehlich & Macedo Corrêa e Castro 2013	NUP-17171
Loricaria sp.	DZSJRP-12920
Proloricaria prolixa Isbrücker & Nijssen 1978	MZUEL-10006
Pyxiloricaria menezesi Isbrücker & Nijssen 1984	NUP-17203
Rineloricaria cacerensis (Miranda-Ribeiro 1912)	ZUFMS-PIS01344
Rineloricaria lanceolata (Günther 1868)	NUP-17192
Rineloricaria parva (Boulenger 1895)	ZUFMS-PIS03839
Pimelodidae	

[1]Sabino & Trajano (1997); [2]Ribeiro et al. (2007); [3]Zawadzki et al. (2014); [4]Terra & Sabino (2007); [5]Willink et al. (2000); 6Rizzato, Costa, Trajano & Bichuette (2011); [7]Shibatta (2016). * Species occurrence information, but not sampled.

Table 1: Continued...

Taxon	Voucher
Pseudoplatystoma corruscans (Spix & Agassiz 1829)	MZUSP-51269.0
Pseudoplatystoma reticulatum Eigenmann & Eigenmann 1889	*[5]
Pseudopimelodidae	
Microglanis leniceae Shibatta 2016	ZUFMS-PIS01657[7]
Pseudopimelodus mangurus (Valenciennes 1835)	ZUFMS-PIS03397
Rhyacoglanis pulcher (Boulenger 1887)	DZSJRP-12141
Trichomycteridae	
Paravandellia oxyptera Miranda-Ribeiro 1912	DZSJRP-12877
Trichomycterus dali Rizzato, Costa, Trajano & Bichuette 2011	MZUSP 166630[6]
Trichomycterus sp.	NUP-9351
CYPRINODONTIFORMES	
Poeciliidae	
Poecillia reticulata Peters 1859	ZUFMS-PIS03799
Cynolebiidae	
Melanorivulus punctatus (Boulenger 1895)	DZSJRP-12905
SYNBRANCHIFORMES	
Synbranchidae	
Synbranchus marmoratus Bloch 1795	ZUFMS-PIS03797
CICHLIFORMES	
Cichlidae	
Aequidens plagiozonatus Kullander 1984	DZSJRP-12906
Bujurquina vittata (Heckel 1840)	ZUFMS-PIS03843
Cichlassoma dimerus (Heckel 1840)	NUP-17187
Crenicichla lepidota Heckel 1840	DZSJRP-12889
Crenicichla vittata Heckel 1840	[5]ZUFMS-PIS01307
Crenicichla sp.	ZUFMS-PIS03800

[1]Sabino & Trajano (1997); [2]Ribeiro et al. (2007); [3]Zawadzki et al. (2014); [4]Terra & Sabino (2007); [5]Willink et al. (2000); 6Rizzato, Costa, Trajano & Bichuette (2011); [7]Shibatta (2016). * Species occurrence information, but not sampled.

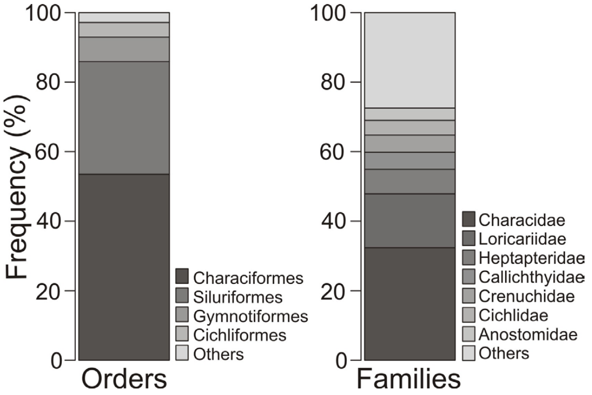


Figure 2: Frequency of orders and families from to primary and secondary data from the Miranda River Basin of the Upper Paraguay River, Mato Grosso do Sul, Brazil.

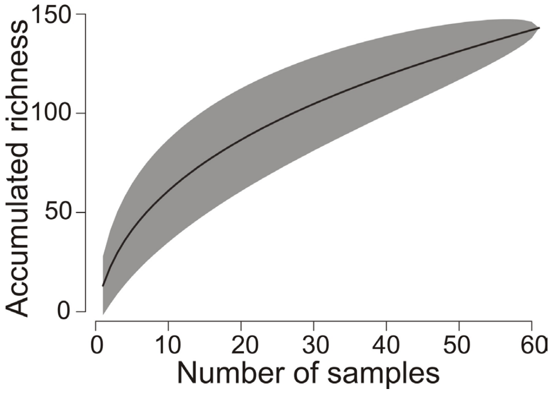


Figure 3: Species accumulated curve generated from to primary and secondary data from the Miranda River Basin of the Upper Paraguay River, Mato Grosso do Sul, Brazil.

of Near-threatened Species. Furthermore *Characidium* aff. *fasciatum* Reinhardt 1866, *Farlowella* cf. *isbrueckeri* Retzer & Page 1997 are listed as data deficient. The cave fishes *Ancistrus formoso* Sabino & Trajano 1997 and *Trichomycterus dali* Rizzato, Costa, Trajano & Bichuette 2011 are listed as vulnerable. We encountered six possible new species, including *Astyanax* sp. 1, *Astyanax* sp. 2, *Astyanax* sp. 3, *Characidium* sp. 1, *Imparfinis* sp. 1, *Imparfinis* sp. 2. Six species from our data sets were reported as only being found in the Paraná River basin, such as *Serrapinnus notomelas* (Eigenmann 1915), *Astyanax* aff. f*asciatus* (Cuvier 1819), *Imparfinis mirini* Haseman 1911, *Imparfinis* cf. *schubarti* (Gomes 1956), *Proloricaria prolixa* Isbrücker & Nijssen 1978 and *Hoplias* sp. 1 from our primary data set, and *Oligosarcus pintoi* Campos 1945, *Rineloricaria lanceolata* (Günter 1868) from our secondary data set.

Discussion

Studies of fishes from the Paraguay River basin usually concentrate on the main rivers of the Pantanal region (Britski et al. 2007, Terra & Sabino 2007, Polaz et al. 2014), due mainly to the easy access into these areas, when compared to the headwaters, this makes the knowledge of the fauna of streams almost non-existent (Krinski et al. 2015). However, to understand the environmental structural dynamics, the accomplishment of ichthyofauna inventories and systematic studies are extremely important. Besides, they constitute the basis for better understanding the dynamics of species distribution (Vilar et al. 2011), providing data on the species composition, management and conservation (Pains-Silva et al. 2014).

Our results showed predominance of Characiformes and Siluriformes, and the families Characidae and Loricariidae. Other studies conducted in streams of the Paraguay River Basin reported similar results (Oliveira et al. 2015, Vizzotto & Castro 2015), which reflect typical species patterns for Neotropical icthyofauna (Reis et al. 2016).

The combination of primary and secondary data for the Miranda River Basin produced a data set from a greater number of sample sites, over longer time periods, and at larger spatial scales, and the end product is a more complete assessment of regional fish fauna. Other studies of fish fauna in the Paraguay River Basin at smaller spatial scales report fewer numbers of species, including studies from streams of the headwaters of the Rio Vermelho (Oliveira et al. 2015, Vizzotto & Castro 2015) and Rio Sepotuba (Krinski et al. 2015) in northern Pantanal. In other words, by increasing spatial scale and the number of sample sites, we also increase the probability of greater among-site variation in species numbers by virtue of including a greater diversity of mesohabitats with different substrate types and riparian vegetation. These differences in habitat structural complexity (Teresa et al. 2010, Teresa Romero & 2010, Krinski et al. 2015, Vizzotto & Castro 2015) result in a gradient of heterogeneity that, through niche differentiation, favors a greater number of species.

Some species in the current study should be highlighted due to conservation status. Serrasalmidae and Pimelodidae, *Piaractus mesopotamicus* and *Pseudoplatystoma corruscans* are on the National List of Near-threatened Species (NT) in Brazil (data published by ICMBio 2014), despite this, these species have broad distribution in the Paraguay River Basin, in addition, they are important species for artisanal and sports fishing in the region, being *P. corruscans* represent approximately 32% and *P. mesopotamicus* 17.3% of artisanal fisheries in 2015 (Catella et al. 2016). So, this results suggest a need of effort to better definition of conservation status at upper Paraguay basin scale.

Among the species sampled were *Characidium* aff. *fasciatum* and *Farlowella* cf. *isbrueckeri* from the families Crenuchidae and Loricariidae, respectively, both of which are listed at Brazilian law as Data Deficient (DD) (data published by ICMBio 2014) due to low numbers of recorded occurrences or absence in study samples. *Ancistrus formoso* and *Trichomycterus dali* are the only encountered species listed as vulnerable, likely a result of its limited distribution due to restriction to cave habitats in the karst environments of the Serra da Bodoquena (Cordeiro et al. 2014); the restricted range and the frequent change of the landscape to harbor crop fields in the region represent a great menace to these species. The Miranda River Basin seems to offers sufficient resources to maintain numerous species, in particular species of concern with respect to conservation. The collective data thus suggest that preservation of this basin ecosystem is critical for perpetuation of natural resources and biodiversity.

There are roughly 270 described species in the Pantanal floodplain (Britski et al. 2007), and our list presents 39 additional species not included in that estimate. Several species from our data sets were reported as only being found in the Paraná River basin, this suggests dispersion during the formation of the Pantanal, and serves as evidence of connectivity between the headwaters of the Paraná and Paraguay River Basins. This finding illustrates the importance of expanding sampling effort for interface regions of the basins, and reviewing the material deposited in biological collections.

In addition to future studies of species richness in the Paraguay River Basin, more detailed information on species distributions is needed to better gauge conservation status of fish in the area. There have been a limited number of studies in the area, particularly in headwater streams and their tributaries (Oyakawa & Menezes 2011, Krinski et al. 2015). Many species of headwater streams are potentially geographically isolated (Lowe-McConnell 1999) that are specific to these areas (Krinski et al. 2015), whereby headwaters may host very different fauna than exists in major rivers (Pedroza et al. 2012, Volcan et al. 2012). Based on previous experience and distribution maps from SpeciesLink, we can point out in this whor *Astyanax lineatus*, *A. marionae*, *Creagrutus meridionalis*, *Oligosarcus perdido*, *Hypostomus basilisko*, *H. perdido* and the cave fish *Ancistrus formoso* and *Trichomycterus dali* as exclusive species that inhabit headwaters. This understanding of fish fauna in headwater streams and associated tributaries is essential for maintaining fish biodiversity in the Paraguay River Basin. Inventory studies in the basin would also be useful for evaluation of potential environmental impacts on ichthyofauna, as these environments are subject to anthropogenic modification (e.g., due to the construction of hydroelectric dams, which has increased in last years). Such anthropogenic impacts have potential to modify species distribution (Mariano et al. 2012), by limiting species dispersion and altering resource availability in the entire plateau region of the Upper Paraguay Basin. In the Miranda River Basin, at least eleven small hydroelectric projects have recently approved for construction, two in Miranda sub-basin and nine in Aquidauana sub-basin. More attention should be given to the actual and potential negative impacts of these and other anthropogenic activities upon fish diversity and distributions in the basin area.

Acknowledgments

We thank the Biota-MS program in association with the Superintendência de Ciência e Tecnologia de MatoGrosso do Sul (SUCITEC), CAPES, CNPq and FUNDECT for financial and logistical support. We are grateful to Walmir B. F. Mundim Jr., Wagner Vicentin, Marcelo S. Maldonado and Gabriel Nakamura for help with field work.

Author Contributions

Fabiane S. Ferreira, Gabriela S. V. Duarte, Francisco Severo Neto, Otávio Froehlich and Yzel R. Súarez contributed to the field work and in secondary data collection. FSF and YRS also realized a statistical analysis and all authors contributed to manuscript preparation and critical revision.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

ALHO, C.J.R. & SABINO, J. 2012. Seasonal Pantanal flood pulse: implications for biodiversity conservation – A review. Oecol. Aust. 16(4): 958-978. doi: http://dx.doi.org/10.4257/oeco.2012.1604.17.

BAGINSKI, L.J., FLORENTINO, A.C., FERNANDES, I.M., PENHA, J.M.P. & MATEUS, L.A.F. 2007 The spatial and temporal dimension of fish diversity of the vegetated littoral zone of marginal lagoons of the Cuiabá river floodplain, Pantanal, Brazil. Biota Neotrop 7(3): 233-238. http://www.biotaneotropica.org.br/v7n3/pt/abstract?article+bn04007032007 (Accessed 24 Jun 2016).

BOTINI, A.F., BARROS, C.A., SOUZA, T.H., BOTINI, N., MOURA, N.A. 2015. Diversidade de peixes no Rio Mutum e baía marginal no Pantanal – Matogrossense através da coleta ativa. Enciclop Biosf 11(21): 2183-2197.

BRITSKI, H.A., SILIMON, K.Z.S. & LOPES, B.S. 2007. Peixes do Pantanal: manual de identificação. 2 ed. Embrapa, Brasília, 227p.

CASATTI, L., ROMERO, R.M., TERESA, F.B., SABINO, J. & LANGEANI, F. 2010. Fish community structure along a conservation gradient in Bodoquena Plateau streams, central West of Brazil. Acta Limnol Brasil 22(1): 50-59. doi: http://dx.doi.org/10.4322/actalb.02201007.

CASTRO, R.J. & VIZZOTTO, P.C. 2013. Fishes of the Vermelho River, São Lourenço River basin, MatoGrosso state, Brazil. Check List 9(1): 001-003.

CATELLA, A.C., CAMPOS, F.L.R., ALBUQUERQUE, S.P. 2016. Sistema de controle de pesca de Mato Grosso do Sul SCPESCA/MS 22- 2015. Corumbá: Boletim de pesquisa e desenvolvimento – Embrapa Pantanal. p. 55.

CATELLA, A.C. & PETRERE-JR, M. 1996. Feeding patterns in a fish community of Baia da Onça, a foodplain lake of the Aquidauana River, Pantanal, Brazil. Fisheries Manag Ecol 3: 229-237.

CORDEIRO, L.M.; BORGHEZAN, R. & TRAJANO, E. 2014. Subterranean biodiversity in the Serra da Bodoquena karst area, Paraguay river basin, Mato Grosso do Sul, Southwestern Brazil. Biota Neotropica14: 1-28. doi: http://dx.doi.org/10.1590/1676-06032014011414.

ESCHMEYER, W.N., FRICKE, R. & VAN DER LAAN, R. (eds.). 2017. Catalog of Fishes: Genera, Species, References.http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp (Accessed 20 May 2017).

JESUS, F. 2003. Classificação dos ecossistemas aquáticos do Pantanal e da bacia do Alto Paraguai. Brasília: The Nature Conservancy. p. 136.

JUNK, W.J., CUNHA, C.N., WANTZEN, K.M., PETERMANN, P., STRÜSSMANN, C., MARQUES, M.I. & ADIS, J. 2006. Biodiversity and its conservation in the Pantanal of Mato Grosso, Brazil. Aquat Sci 68: 278-309. doi: http://dx.doi.org/10.1007/s00027-006-0851-4.

KRINSKI, D., CARMO, E.J. & MIYAZAWA, C.S. 2015. Ichthyofauna of headwaters from Sepotuba River Basin, Upper Paraguay River Basin, Tangará da Serra, Mato Grosso state, Brazil. Panamjas 10(4): 283-292.

MARIANO, J.R., MAKRAKIS, M.C., KASHIWAQUI, E.A.L., CELESTINO, E.F. & MAKRAKIS, S. 2012. Longitudinal habitat disruption in Neotropical streams: fish assemblages under the influence of culverts. Neotrop Ichthyol 10(4):771-784. doi: http://dx.doi.org/10.1590/S1679-62252012000400010.

OKSANEN, J., BLANCHET, F.G., KINDT, R., LEGENDRE, P., O'HARA, R.B., SIMPSON, G.L., STEVENS, M.H.H. & WAGNER, H. 2011. Vegan: community ecology package. Version 1.17-11. Available from: http://vegan.r-forge.r-project.org/

OLIVEIRA, V.A., MATEUS, L.A., LOVERDE-OLIVEIRA, S. & PIETRO-SOUZA, W. 2015. Fish from urban tributaries to the Vermelho River, upper Paraguay River Basin, Mato Grosso, Brazil. Check List 11(1): 1-6. doi: http://dx.doi.org/10.15560/11.1.1516.

OYAKAWA, O.T. & MENEZES, N.A. 2011. Checklist of fresh water fishes from São Paulo State, Brazil. Biota Neotrop 11(1a): 19-32. doi: http://dx.doi.org/10.1590/S1676-06032011000500002.

PACHECO, E.B. & SILVA, C.J. 2009. Fish associated with aquatic macrophytes in the Chacororé-Sinhá Mariana lake system and Mutum River, Pantanal of Mato Grosso Brazil. Braz J Biol 69(1): 101-108. doi: http://dx.doi.org/10.1590/S1519-69842009000100012.

PAINS-SILVA, H., SOUZA, F. & CORRÊA, F. 2014. Icththyofauna of the Bento Gomes River in the Pantanal Matogrossense, Brazil. Panamjas 9(1): 48-53.

PAINS-SILVA, H., PETRY, A.C. & DA SILVA, C.J. 2010. Fish communities of the Pantanal wetland in Brazil: evaluating the effects of the Upper Paraguay River flood pulse on baía Caiçara fish fauna. Aquat Ecol 44: 275-288. doi: http://dx.doi.org/10.1007/s10452-009-9289-9.

PEDROZA, W.S., RIBEIRO, F.R.V., TEIXEIRA, T.F., OHARA, W.M. & PY-DANIEL, L.H.R. 2012. Icththyofauna survey of stretches of the Guariba and Roosevelt Rivers, in Guariba State Park and Guariba Extractive Reserve, Madeira River basin, Amazonas, Brazil. Check List 8(1): 8-15.

PLANO DE RECURSOS HÍDRICOS DA BACIA HIDROGRÁFICA DO RIO MIRANDA. 2014. http://www.imasul.ms.gov.br/wp-content/uploads/sites/74/2015/06/Diagn%C3%B3stico-Final-Plano-de-Bacia-Miranda.pdf (Acessed 20 May 2017).

POLAZ, C.N.M., MELO, B.F., BRITZKE, R., RESENDE, E.K., MACHDO, F.A., LIMA, J.A.F. & PETRERE jr, M. 2014. Fishes from the Parque Nacional do Pantanal Matogrossense, upper Paraguai River basin, Brazil. Check List 10(1): 122-130. doi: http://dx.doi.org/10.15560/10.1.122.

R DEVELOPMENT CORE TEAM. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

REIS, R.E., ALBERT, J.S., DI DARIO, F., MINCARONE, M.M., PETRY, P. & ROCHA, L.A. 2016. Fish biodiversity and conservation in South America. J. Fish Biol.89(1):12–47. doi: http://dx.doi.org/10.1111/jfb.13016

RIBEIRO, A.C., CAVALLARO, M.R. & FROEHLICH, O. 2007. *Oligosarcus perdido* (Characiformes, Characidae), a new species of fresh water fish from Serra da Bodoquena, upper Rio Paraguai basin, Brazil. Zootaxa 1560: 43-53.

SABINO, J. & TRAJANO, E. 1997. A new species of blind armoured catfish, genus *Ancistrus*, from caves of Bodoquena region, Mato Grosso do Sul, southwestern Brazil (Siluriformes, Loricariidae, Ancistrinae). Revue Fr Aquariol 23: 73-78.

SEVERO-NETO, F., TENCATT, L.F.C., COSTA-PEREIRA, R. & TAVARES, L.E.R. 2015. Fishes from Baía da Medalha, southern Pantanal, Brazil: A 20 years review. Biota Neotrop 15(2): 1-9. doi: http://dx.doi.org/10.1590/1676-06032015011614.

SILVA, J.S.V. & ABDON, M.M. 1998. Delimitação do Pantanal Brasileiro e suas sub-regiões. Pesq. Agropec. Bras. 33: 1703-1711.

SHIBATTA, O.A. 2016. A new species of bumblebee catfish of the genus *Microglanis* (Siluriformes: Pseudopimelodidae) from the upper rio Paraguay basin, Brazil. Neotrop Ichthyol 14(3): e160031. http://dx.doi.org/10.1590/1982-0224-20160031.

SMITH, E.P. & VAN BELLE, G. 1984. Nonparametric estimation of species richness. Biometrics 40(1):119-129. doi: http://dx.doi.org/10.2307/2530750.

SÚAREZ, Y.R., FERREIRA, F.S. & TONDATO, K.K. 2013. Assemblage of fish species associated with aquatic macrophytes in Porto Murtinho Pantanal, Mato Grosso do Sul, Brazil. Biota Neotrop 13(2): 182-189. http://www.biotaneotropica.org.br/v13n2/en/abstract?inventory+bn02313022013 (Accessed 24 Jun 2016)

SÚAREZ, Y.R., VALÉRIO, S.B., TONDATO, K.K., FLORENTINO, A.C., FELIPE, T.R.A., XIMENES, L.Q.L. & LOURENÇO, L.S. 2007. Fish species diversity in headwaters streams of Paraguay and Paraná basins. Braz Arch Biol Techn 50(6): 1033-1042. doi: http://dx.doi.org/10.1590-89132007000700014.

TERESA, F.B. & ROMERO, R.M. 2010. Influence of the riparian zone phytophysiognomies on the longitudinal distribution of fishes: evidence from a Brazilian savanna stream. Neotrop Ichthyol 8(1): 163-170. doi: http://dx.doi.org/10.1590/S1679-62252010000100019.

TERESA, F.B., ROMERO, R.M. & LANGEANI, F. 2010. Pisces, Aquidauana and Miranda drainages, upper Paraguay River basin, Mato Grosso do Sul, Brazil. Check List 6(4): 596-601.

TERRA, L.C.C. & SABINO, J. 2007. Composição da ictiofauna de dois riachos, com diferentes graus de conservação, na bacia do Rio Formoso, município de Bonito, Mato Grosso do Sul, Brasil. Redalyc 11(1): 49-58. http://www.redalyc.org/articulo.oa?id=26012838005 (Accessed 24 Jun 2016).

TONDATO, K.K., FANTIN-CRUZ, I., PEDROLLO, O.C., SÚAREZ, Y.R. 2013. Spatial distribution of fish assemblages along environmental gradients in the temporary ponds of Northern Pantanal, Brazil. J Limnol 71(1): 95-102. doi: http://dx.doi.org/10.4081/jlimnol.2013.e8.

VILAR, C.C., SPACH, H.L. & SOUZA-CONCEIÇÃO, J.M. 2011. Fish assemblage in shallow areas of Baía da Babitonga, southern Brazil: structure, spatial and temporal patterns. Panamjas 6(4): 303-319.

VIZZOTO, P.C. & CASTRO, R.J. 2015. Ichthyofauna of tributaries of the Vermelho River, upper Paraguay River basin, region of Rondonópolis, state of Mato Grosso, Brazil. Check List 11(5): 1-5. doi: http://dx.doi.org/10.15560/11.5.1750.

VOLCAN, M.V., LANÉS, L.E.K., GONÇALVES, A.C., FONSECA, A.P. & CIRNE, M.P. 2012. The fish fauna of the Corrientes stream basin, Patos lagoon system, state of Rio Grande do Sul, southern Brazil. Check List 8(1): 77-82. doi: http://dx.doi.org/10.15560/8.1.077.

WILLINK, P.W., FROEHLICH, O., MACHADO-ALISSON, A., MENEZES, N., OYAKAWA, O., CATELLA, A., CHERNOFF, B., LIMA, F.C.T., TOLEDO-PIZA, M., HORTEGA, H., ZANATA, A.M. & BARRIGA, R. 2000. Diversidade, distribuição e habitats críticos dos peixes dos rios: Negro, Negrinho, Taboco, Taquari e Miranda, e sua importância para conservação e desenvolvimento sustentável do Pantanal, Mato Grosso do Sul, Brasil. Pp. 63-81. In: WILLINK, P. W., CHERNOFF, B., ALONSO, L., MONTAMBAULT, J.R. & LOURIVAL, R. (eds). RAP Bulletin of Biological Assesment, 18: Uma Avaliação Biológica dos Ecossistemas Aquáticos do Pantanal, Mato Grosso do Sul, Brasil. Conservation International, Washington.

ZAWADZKI, C.H., L.F.C. TENCATT, and O. FROEHLICH. 2014. A new unicuspid-toothed species of *Hypostomus* Lacépède, 1803 (Siluriformes: Loricariidae) from the rio Paraguai basin. Neotrop Ichthyol 12(1): 97-104. doi: http://dx.doi.org/10.1590/S1679-62252014000100010.

Received: 15/03/2017
Revised: 20/07/2017
Accepted: 21/07/2017
Published online: 14/08/2017

Biota Neotropica 17(3): e20170381, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
article

Blattaria from Museu de La Plata, Argentina: New records for *Blaberus affinis* Jurberg, Albuquerque, Rebordões, Gonçalves & Felippe, 1977 and *Blaberus scutatus* Saussure & Zehntner, 1894 (Blaberidae, Blaberinae)

***Sonia Maria Lopes*[1*] *& Mariana Assumpção*[1]**

[1]*Universidade Federal do Rio de Janeiro, Museu Nacional - Entomologia, Quinta da Boa Vista,*
Rio de Janeiro, RJ, Brazil
**Corresponding author: Sonia Maria Lopes, e-mail: sonialfraga@gmail.com*

LOPES S. M., ASSUMPÇÃO, M. **Blattaria from Museu de La Plata, Argentina: New records for *Blaberus affinis* Jurberg, Albuquerque, Rebordões, Gonçalves & Felippe, 1977 and *Blaberus scutatus* Saussure & Zehntner, 1894 (Blaberidae, Blaberinae)**. Biota Neotropica. 17(3): e20170381. http://dx.doi.org/10.1590/1676-0611-BN-2017-0381

Abstract: The habitus of the males of seven species of *Blaberus* that belong to the Blattaria collection of the La Plata Museum from Argentina (of which two are new occurrences for the country) are illustrated and are classified into the appropriate groups. Information on their collection sites, synonyms and updated geographic distribution is also provided. In total, 27 *Blaberus* specimens were studied and will be returned to the collection of the Entomology from the Museu de La Plata, Buenos Aires, Argentina.

Keywords*: Collection, La Plata Museum, Blattaria, new records.*

Blattaria do Museu de La Plata, Argentina: Novos Registros para *Blaberus affinis* Jurberg, Albuquerque, Rebordões, Gonçalves & Felippe, 1977 and *Blaberus scutatus* Saussure & Zehntner, 1894 (Blaberidae, Blaberinae)

Resumo: Os habitus dos machos de sete espécies de *Blaberus* que pertencem ao Museu de La Plata na Argentina (dos quais duas são novas ocorrências) são ilustrados e assinalados em grupos apropriados. Informação sobre seus locais de coleta, sinonímias e sua distribuição geográfica atual são também citadas. No total, 27 espécimens de *Blaberus* foram estudados e serão acondicionados à Divisão do Museu de La Plata, Buenos Aires, Argentina.

Palavras chave*: Coleção, Museu de La Plata, Blattaria, nova ocorrência.*

Introduction

The genus *Blaberus* was described by Serville (1831), who designated *Blatta giganteus* Linnaeus, 1758 as type-species. The males of the genus have the following characteristics: large individuals, 45-88 mm; usually with small cerci, not reaching the posterior margin of the supranal plate; subgenital plates with two similar styles, simple, and small, genital hook on the right side. Females with atrium enlarged, where the eggs are incubated. In a literature review of the genus, Lopes & Oliveira (2013) listed 20 species, including them into three groups that were based on the male internal genitalia: the atropos, brasilianus and giganteus groups. They also described one new group based on the female (macurus group). Intra and interspecific variations among the species were defined with respect to habitus, morphology of the head and the pronotum, and genital characters. The following species were included in the atropos group: *B. amazonensis* Lopes & Oliveira, 2013, *B. atropos* (Stoll, 1813), *B. colombianus* Lopes & Oliveira, 2013, *B. duckey* Jurberg et all, 1977, *B. parabolicus* Walker, 1868, *B. paulistanus* Lopes & Oliveira, 2000, *B. matogrossensis* Rocha e Silva & Aguiar, 1977, *B. neomatogrossensis* Lopes & Oliveira, 2013, *B. peruvibolicus* Lopes & Oliveira, 2013 and *B. yuracianus* Lopes & Oliveira, 2013. In the brasilianus group, the following species were included: *B. affinis* Jurberg, R. S. Albuquerque, Rebordões, Gonçalves & Felippe, 1977, *B. chacoensis* Lopes & Oliveira, 2013, *B. neofusiformis* Lopes & Oliveira, 2013, *B. parafusiformis* Lopes & Oliveira, 2013, *B. nigrocephalicus* Lopes & Oliveira, 2013, *B. scutatus* Saussure & Zehntner, 1894, *B. valleyanus* Lopes & Oliveira, 2013. No grupo giganteus are included *B. giganteus* (Linnaeus, 1758), *B. nigromaculatus* Lopes & Oliveira, 2013. Roth (1969) also included *B. minor* Saussure, 1864 in brasilianus group.

Beccaloni (2014), in his “on line” catalog, listed only 19 species in the genus.

In this contribution, the habitus of the males of seven species of *Blaberus* are illustrated and the species are assigned to one of the following groups 1) the *atropos* group (*B. atropos, B. neomatogrossensis*) and 2) the *brasilianus* group (*B. affinis*, *B. scutatus, B. neofusiformis, B. parafusiformis* and *B. minor*). Two new records for *B. affinis* and *B. scutatus* are given. The collection sites, synonyms and updated geographic distribution are also provided.

Material and Methods

The specimens were analyzed morphologically following routine techniques developed in the laboratory and described by Lopes & Oliveira (2000). The terminology for the genital parts, literature and taxonomic classification were based on Roth (1970, 1976 and 2003). The phylogenetic position of the genus follows Khambhampati, 1995 and Klass & Meier, 2006. After analysis, the genital parts were stored in microvials containing glycerin and were stored next to the specimen on the same entomological pin, following Gurney et al (1964). In total, 27 *Blaberus* specimens were studied and identified and will be returned to the collection of the Entomology of the Museu de La Plata, Buenos Aires, Argentina (MLP).

Results

1) **The atropus group-** species in this group lack a hook shaped or tumor-like growth at the apex of the median sclerite (Roth 1976). Truncated or rounded projections or crests are present only of the left side of the prepuce, which are much larger than the spines of the right side. Preputial spines are often arranged in a single series, but may also be in a double or partially double series on the right side, in which case they are more numerous than those on the left side.

Blaberus atropus (Stoll, 1813)

(Figure 1a)

B. atropos Stoll, 1813:4 (*Blatta*); Saussure, 1864: 233-234; Brunner von Wattenwyl, 1865 : 22, 23, 40, 44, 372, 375, 376, 377, 410, 415; Walker, 1868: 2, 4, 5, 6, 7; Saussure & Zehntner, 1894: 118; Rehn, 1903:131,288; Kirby, 1904:164; Hebard, 1921:217; Rehn & Hebard, 1927:261-162; Hebard, 1931; Princis, 1946:145-146; Princis & Kevan, 1955:159, 167; Princis, 1958:75; Bruijning, 1959: 3,6-7; Princis. 1963:131-132; Rocha e Silva-Albuquerque, 1964:5; Vanschuytbroeck, 1969:5; Roth, 1969:217-250; Rocha e Silva, 1982:3; Beccaloni, 2014 http://blattodea.speciesfile.org; Grandcolas, 2008:43; Lopes & Oliveira, 2013:82

Material examined: 1♂ e 1♀ Venezuela, Delta Amacuro, Araguaimujo, 12/VIII/1964, without collector.

Distribution: São Domingos, Jamaica, Guyana, Suriname, Trinidad, Tobago bay, Cuba, Mexico, Venezuela, Colombia, Chile, Brazil (Rio de Janeiro, Rondônia, Amazonas, Pará, São Paulo).

Blaberus neomatogrossensis Lopes & Oliveira, 2013

(Figure 1b)

Blaberus neomatogrossensis Lopes & Oliveira, 2013:86

Material examined: Paratypes 2♂, Venezuela, Guárico, S. Juan de los Morros, Cuevas de Los Morritos, 3/IX/1961, Bordon col.

Distribution: Brazil (Mato Grosso); Venezuela (Guárico).

2) **The brasilianus Group** – This group includes species whose male genitalia lack a hook-shaped projection or a tumor-like growth. The prepucial spines are numerous on the left and right sides and are generally arranged in a row.

Blaberus affinis Jurberg, R.S. Albuquerque, Rebordões, Gonçalves & Felippe, 1977

(Figure 1c)

B. affinis Jurberg, R.S. Albuquerque, Rebordões, Gonçalves & Felippe, 1977: 539-540, figs. 5-15; Lopes & Oliveira, 2000:3; Beccaloni, 2014. http://blattodea.speciesfile.org>; Pellens & Grandcolas, 2008:49; Lopes & Oliveira, 2013:88

Material examined: 1♂ Argentina, Salta, Dto. Victoria, 17/VII/1933, P. Denier col.

Distribution: Brazil (Rio de Janeiro, Mato Grosso, Goiás, São Paulo).

New record: Argentina, Brazil (Ceará, Sergipe).

Blaberus parafusiformis Lopes & Oliveira 2013

(Figure 1d)

Blaberus parafusiformis Lopes & Oliveira, 2013:90

Material examined: 1♂, Paratype. Brazil. Mato Grosso, without data, Museu de La Plata, Scardinsky col.

Distribution: Brazil (Mato Grosso, Rio de Janeiro, São Paulo, Rondônia, Goiás) Argentina.

Blaberus minor Saussure, 1864

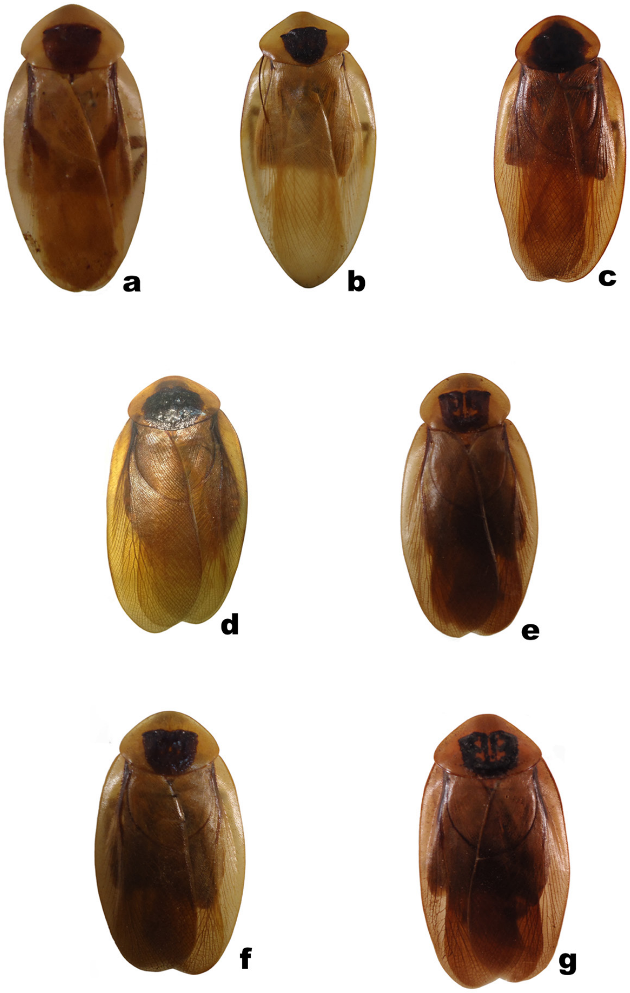
(Figure 1e)

Blaberus minor Saussure, 1864:347; Beccaloni, 2014.http://blattodea.speciesfile.org>

B. fraterna Saussure, 1864-65: 238-242; Walker, 1868:2-7; Kirby, 1904: 165; Hebard, 1931:261; Princis, 1963:130; Roth, 1970:312-313, 319; Jurberg; R.S. Albuquerque; Rebordões, Gonçalves & Felippe, 1977:545-547 Princis, 1963:130; Pellens & Grandcolas, 2008:50; Beccaloni, 2014.http://blattodea.speciesfile.org>

Material examined: 1♂ Argentina (Formosa), 31/10/1940, P. Denier col.

Distribution: Brazil, Paraguay, Argentina and Bolivia



Figures 1 Habitus. a. *Blaberus atropos* (Stoll, 1813); b. *Blaberus neomatogrossensis* Lopes & Oliveira, 2013; c. *Blaberus affinis* Jurberg, R.S.Albuquerque, Rebordões, Gonçalves & Felippe, 1977; d. *Blaberus parafusiformis* Lopes & Oliveira, 2013; e. *Blaberus minor* Saussure, 1864; f. *Blaberus neofusiformis* Lopes & Oliveira, 2013; g. *Blaberus scutatus* Saussure & Zehntner, 1894.

Blaberus neofusiformis Lopes & Oliveira, 2013
(Figure 1f)

Blaberus neofusiformis Lopes & Oliveira, 2013:89

Material examined: 1♂ Paratype. Argentina, Jujuy, 23-28/II/1945, Biraben col.; 1♂ Paratype, Argentina, Salta, Juramento, Dept. Robles, 02/XI/1939; 1♂ Paratype, Argentina, Salta, Orán, 5/VI/1947, Denner col.; 1♂ e 1♀ Paratype, Argentina, Jujuí, Santa Bárbara Palmar, V/1947, Biraben col.

Distribution: Brazil (Ceará, Pernambuco, São Paulo, Goiás), Argentina

Blaberus scutatus Saussure & Zehntner, 1894
(Figure 1g)

B. scutatus Saussure & Zenhtner, 1894:119 (*Blabera*); Princis, 1963:132; Rocha e Silva-Albuquerque, 1964:5; Roth, 1969:220-221,228; Vanschuyetbroecker, 1969: 6; Roth, 1970:312, 319; Beccalloni, 2014: http://blattodea.speciesfile.org; Pellens & Grandcolas, 2008:44; Lopes & Oliveira, 2013:92

Material examined: 1♂ Argentina, Salta, Juramento, 02/XI/1939, without collector; 2♂ Argentina, Corrientes, 15-30/II/1959, Biraben col.; 1♂ Argentina, Formosa, Mojon de Fierrro, 11/XII/1939. P. Denier col.; 1♀ Argentina, Formosa, 15/XI/1942, M. Birabén col.; 2♂ Argentina, Estancia de La Amalia, Resistencia Chaco, 13/IX/1955, E. Martin M. Calvan col.; 1♂ Argentina, Chaco, XII/1913. M. Currens Leg; 2♂ and 2♀ Argentina, Chaco, Estancia Anello, Cam a. Zapallar 10-15/III/1959, Birabén col.; 2♂ e 2♀. Argentina, Córdoba, Los Cocos “El Piquillin”, 17/II/1944, Sta Pritchard; 1♂. Argentina, Museu de La Plata.

Distribution: BRAZIL (Piauí, Ceará, Minas Gerais, Rio Grande do Norte, Pernambuco, Sergipe, Bahia, Rio de Janeiro), Peru, Paraguay.

New record: Argentina

Author Contributions

Sonia Maria Lopes: substantial contribution in the concept and design of the study; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Mariana Assumpção: substantial contribution in the concept and design of the study.

Conflicts of interest

The authors declares that they have no conflict of interest related to the publication of this manuscript.

References

BECCALONI, G.W. 2014. Cockroaches Species File Online. Version 5.0/5.0. World Wide Web electronic publication. <http://blattodea.speciesfile.org> [accessed 17/01/17].

GURNEY, A.B., KRAMER, J. P. & STEYSKAL, G.C. 1964. Some Techniques for the Preparation, Study and Storage in Microvials of Insect Genitalia. Annals of the Entomological Society of America, Washington 57(2):240-242.

KHAMBHAMPATI, S. 1995. A phylogeny of cockroaches and related insects based on DNA sequence of mitochondrial ribosomal RNA genes. Proceedings of the Academy of Natural Sciences of Philadelphia 92:2017-2020.

KLASS, K.D. & MEIER, R. 2006. A phylogenetic analysis of Dictyoptera (Insecta) based on morphological characters. Entomologische Abhandlungen 63:3–50. Konowia 10: 257-285.

LOPES, S.M. & OLIVEIRA, E.H. 2000. Espécie nova de *Eublaberus* Hebard, 1919 do Estado de Goiás, BRASIL e notas sobre *E. marajoara* Rocha e Silva-Albuquerque, 1972 (Blaberidae, Blaberinae). Boletim Museu Nacional Rio de Janeiro, Nova Série, Zool. 433:1-5.

LOPES, S.M. & OLIVEIRA, E.H. 2013. New group, new species and new records from Brazil of the atropos, brasilianus and giganteus groups (Blattaria, Blaberidae, Blaberinae). Biota Neotropica, vol. 13, no. 2 (on line)

ROTH, L.M. 1969. The male genitalia of Blattaria I. *Blaberus* spp. (Blaberidae Blaberinae. Psyche 76 (3): 217-250

ROTH, L.M. 1970. The male genitalia of Blattaria IV. Blaberidae: Blaberinae. Psyche *77*(2):217-236.

ROTH, L.M. 1976. The male genitalia of Blattaria. I. *Blaberus* spp. (Blaberidae: Blaberinae). Psyche 76: 217-250

ROTH, L.M. 2003. Systematics and Phylogeny of Cockroaches (Dictyoptera, Blattaria). Oriental Insects 37: 1-186.

SERVILLE, J. G.A. 1831. Revue méthodique des insectes de l’ordre des Orthoptères. Annales des Sciences Naturelles, Zoologie et Biologie Animale 22: 166–167.

Received: 21/05/2017
Revised: 28/08/2017
Accepted: 31/08/2017

Biota Neotropica 17(3): e20170335, 2017
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Inventory

Butterflies of Amazon and Cerrado remnants of Maranhão, Northeast Brazil

Lucas Pereira Martins[*1,2], *Elias da Costa Araujo Junior*[1,3], *Ananda Regina Pereira Martins*[1,4],
Mairla Santos Colins[1], *Gabriela Cristina Fonseca Almeida*[1] & *Gisele Garcia Azevedo*[1]

[1]*Universidade Federal do Maranhão, Departamento de Biologia, São Luís, Maranhão, Brazil.*
[2]*Universidade Federal de Goiás - Programa de Pós-Graduação em Ecologia e Evolução, Goiânia, Goiás, Brazil.*
[3]*Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil.*
[4]*McGill University, Department of Biology, Montreal, Quebec, Canada.*
**Corresponding author: Lucas Pereira Martins, e-mail: martinslucas.p@gmail.com*

MARTINS, L. P., ARAUJO JUNIOR, E. C., MARTINS, A. R. P., COLINS, M. S., ALMEIDA, G. C. F., AZEVEDO, G. G. **Butterflies of Amazon and Cerrado remnants of Maranhão, Northeast Brazil**. Biota Neotropica. 17(3): e20170335. http://dx.doi.org/10.1590/1676-0611-BN-2017-0335

Abstract*:* Species inventories are important tools to evaluate biodiversity losses and contribute to the conservation of endangered areas. The Amazon and Cerrado are the largest Brazilian biomes and represent some of the most threatened regions of the country. Due to its location between these biomes, the state of Maranhão, Northeast Brazil, possesses a great variety of habitats and a high local diversity. Nonetheless, few faunistic inventories of diversified groups have been performed in the state. In the specific case of butterflies, a well-known biological indicator, no inventories have been published in the past years. This study aimed to expand the knowledge on the composition of butterflies in Amazon and Cerrado remnants of Maranhão. Butterflies were sampled between 2011 and 2015 across eight municipalities of the state. Captures were made through entomological nets and baited traps. In total, 189 species were sampled, of which 165 were captured in the Amazon, 65 in the Cerrado and 41 in both biomes. We sampled 167 species through entomological nets and 43 through baited traps, representing 12% of similarity in species composition between sampling methods. We estimate that the recorded species represent a small subset of the butterflies from Maranhão. Therefore, long-term researches in poorly studied areas of the state are recommended to identify novel and/or endemic taxa.

Keywords: *tropical forest, diversity, Lepidoptera, Neotropical region, savanna.*

Borboletas de remanescentes de Amazônia e Cerrado do Maranhão, nordeste do Brasil

Resumo: Inventários de espécies são ferramentas importantes para avaliar perdas de biodiversidade e contribuir para a conservação de áreas ameaçadas. A Amazônia e o Cerrado são os maiores biomas brasileiros e representam algumas das regiões mais ameaçadas do país. Devido à sua localização entre estes biomas, o estado do Maranhão, nordeste do Brasil, possui uma grande variedade de habitats e uma alta diversidade local. No entanto, poucos inventários faunísticos de grupos diversificados foram realizados no estado. No caso específico de borboletas, um indicador biológico bem conhecido, nenhum inventário foi publicado nos últimos anos. Este estudo objetivou expandir o conhecimento sobre a composição de borboletas de remanescentes de Amazônia e Cerrado do Maranhão. As borboletas foram amostradas entre 2011 e 2015 em oito municípios do estado. As capturas foram realizadas através de redes entomológicas e armadilhas com iscas. No total, 189 espécies foram amostradas, das quais 165 foram capturadas na Amazônia, 65 no Cerrado e 41 em ambos os biomas. Nós amostramos 167 espécies através de redes entomológicas e 43 através de armadilhas com iscas, representando 12% de similaridade na composição de espécies entre métodos de amostragem. Nós estimamos que as espécies registradas representam um pequeno subconjunto das borboletas do Maranhão. Deste modo, pesquisas de longa duração em áreas pouco estudadas do estado são recomendadas para identificar taxa novos e/ou endêmicos.

Palavras-chave: *floresta tropical, diversidade, Lepidoptera, região Neotropical, savana.*

Introduction

Species inventories contribute to the conservation of endangered areas by providing relevant data for conservation plans, such as occurrence, richness and diversity (Kremen et al. 1993, Santos et al. 2008, Santos et al. 2016). Unfortunately, conservation plans are usually restricted to studies focusing vertebrates and higher plants (Santos et al. 2008). The scarcity of basic knowledge studies on megadiverse groups, such as insects, increases the difficulty of cataloging all species present in a region (Santos et al. 2008). Despite that, insects are suggested as suitable biological indicators in studies of environmental monitoring and evaluations of natural landscape diversity and integrity. Thus, insect inventories are important tools to biological evaluations in regions threatened by anthropic disturbances (Kim 1993, Brown Jr 1997, Uehara-Prado et al. 2007, Santos et al. 2016).

Among the insects, butterflies are considered excellent organisms to indicate the "health state" of ecosystems and an effective "umbrella group" for biodiversity conservation (Uehara-Prado et al. 2007, Bonebrake et al. 2010, Santos et al. 2016). There are about 3,300 species of butterflies in Brazil (Brown Jr 1996, Lewinsohn et al. 2005, Francini et al. 2011), but few inventories have been performed in large biomes of the country (Santos et al. 2008). The low number of researchers and difficult access to some sites partially explain the lack of information for many areas of Brazil, especially within the North and Northeast regions (Santos et al. 2008). As a consequence, relatively little is still known about the biodiversity of butterflies in the country considering its vast area, hindering the development of management and conservation strategies (Santos et al. 2008).

The Amazon is the largest biome in Brazil and possesses the world highest absolute rate of forest reduction (Laurance et al. 2000, Silva et al. 2005). Anthropogenic effects caused by the insertion of enterprises and the implementation of monocultures and livestock are the main causes of deforestation, especially in the Eastern portion, which is part of the "deforestation arch" (Silva et al. 2005, Vieira et al. 2008, Martins & Oliveira 2011). Considering the Amazonian high biodiversity, information on insect composition and distribution remains scarce (Santos et al. 2008). Butterfly inventories have been performed in the Amazon since the 19th century (Bates 1867, Santos et al. 2008), but many of these do not present a delimitation of the sampled area, reducing their scientific reliability (Santos et al. 2008, Casagrande et al. 2012).

The Cerrado is the second largest biome in Brazil and consists of a mosaic of vegetation types, varying from savannas to dense forests (Klink & Machado 2005). Human occupation has caused extensive habitat loss and transformed large areas of this domain into crops and pastures (Klink & Machado 2005, Diniz-Filho et al. 2009). Due to the increasing land-use and the high degree of endemism, the Cerrado is considered a biodiversity hotspot (Myers et al. 2000). However, despite the existence of previous butterfly lists performed in this biome, butterfly fauna is poorly understood especially in regions with insufficient number of research centers, such as the state of Maranhão, one of the poorest known areas in Brazil regarding butterflies (Santos et al. 2008).

The state of Maranhão, Northeast Brazil, contains portions of Amazon, Cerrado and Caatinga vegetation, contributing to the maintenance of a high local diversity. Patterns of habitat reduction are evident within the Amazon and Cerrado areas of Maranhão, and major deforestations are already assumed for the next years in these areas (Vieira et al. 2008, Diniz-Filho et al. 2009, Martins & Oliveira 2011, Barreto et al. 2012). For this reason, long-term butterfly inventories are crucial for the development and application of efficient conservation measures in the state. The present study aims to broaden the knowledge of the butterfly fauna in the state of Maranhão and provide perspectives for future researches.

Material and Methods

1. Study areas

Field work was performed in forest remnants of Amazon (six municipalities) and Cerrado (two municipalities) (Figure 1). The studied remnants of Amazon forest presented different vegetation types and degrees of human impact (Figure 2): (1) Maracanã, located in the municipality of São Luís (2º 36' S and 44º 17' W), possesses an area of 1,813 ha and vegetation characterized as open ombrophilous forest with high anthropic impacts in several points; (2) Sítio Aguahy, located in the municipality of São José de Ribamar (2° 38' S and 44° 08' W), possesses an area of 635 ha composed by portions of stacional semidecidous forest, being

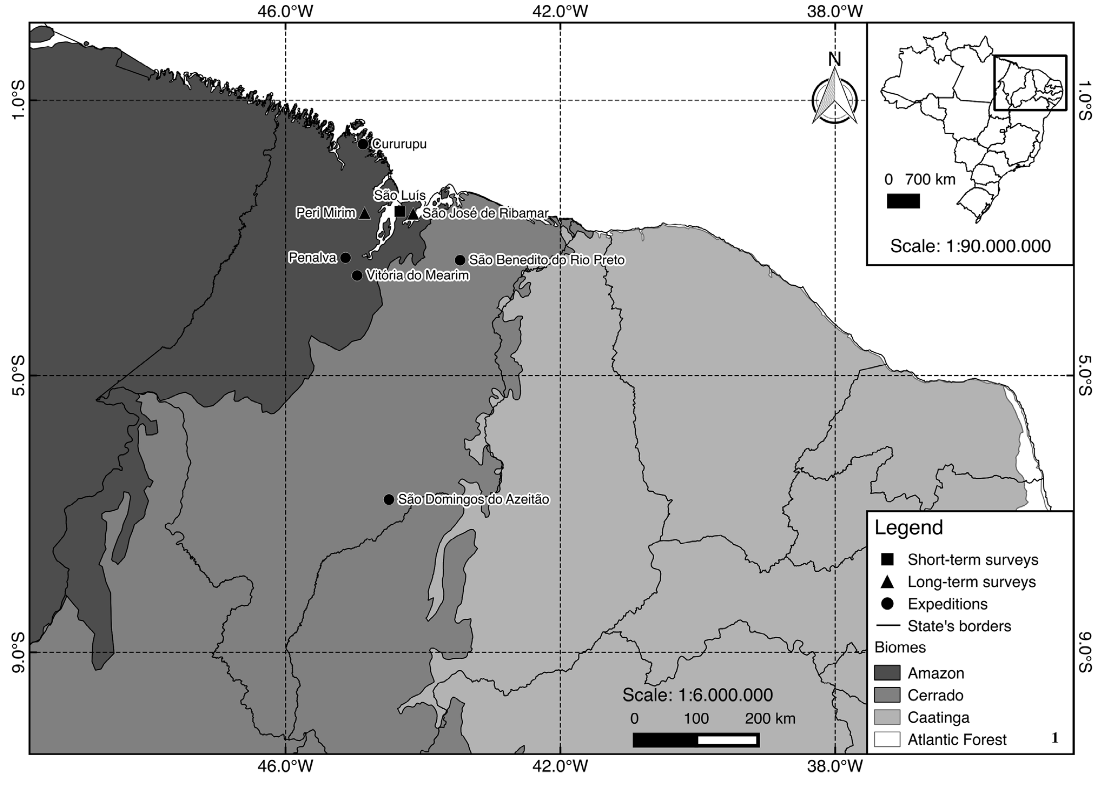


Figure 1. Map of the state of Maranhão, Northeast Brazil. Six study areas were selected in the Amazon and two study areas were selected in the Cerrado. Projection system: UTM. Datum: WGS 84.

Figure 2. Studied landscapes of the Amazon biome in the state of Maranhão, Northeast Brazil. A. Stacional semideciduous forest in São José de Ribamar. B. Transition zone between Amazon forest and coastal vegetation in Cururupu. C. Natural open fields in Peri Mirim. D. Amazon forest remnant in Vitória do Mearim.

surrounded by crops of *Manihot* sp. (Euphorbiaceae) and *Zea* sp. (Poaceae); (3) Cururupu (1° 49'S and 44° 51' W), located in a coastal region inside the Environmental Protection Area of "Reentrâncias Maranhenses", is characterized by the predominance of transition zones between Amazon forest and coastal vegetation, with anthropic impacts of the surrounding villages; (4) Parque Agroecológico de Buritirana, located in Peri Mirim (2° 38'S and 44° 50'W); (5) Fazenda Coque, located in Vitória do Mearim (3° 32'S and 44° 57'W); and (6) Fazenda Canadá & Boa Esperança, located in Penalva (3° 17'S and 45° 07'W), are situated in the region of "Baixada Maranhense" - 1,775,035 ha of Amazon forest remnants characterized by flooded areas and natural open fields inserted in a matrix of urban settlements and secondary forests.

The study areas of Cerrado were selected in two municipalities (Figure 3): (7) São Benedito do Rio Preto (3° 19' S and 43° 31' W), located in the Northeast of Maranhão, is characterized by tablelands and gallery forests following the Preto river; and (8) São Domingos do Azeitão (6º 47' S and 44º 29' W), located between the headwaters of Itapecuru and Alpercatas rivers, is characterized by open areas with sparse trees and gallery forests, being surrounded by soy crops.

2. Butterfly sampling and identification

Butterflies were sampled between 2011 and 2015 by two to four collectors in each locality. Field trips were classified into three categories according to the duration and sampling effort: (1) Short-term survey: two sampling days; (2) Long-term surveys: two sampling days per month during 24 months; and (3) Expeditions: one sampling week.

Using entomological nets, we surveyed for butterflies every sampling day. Butterflies were sampled along transects that varied in extension from 1 - 5 km. At least two collectors walked along transects collecting with entomological nets in a given amount of time (from 8 – 12 a.m. and from 2 – 5 p.m., totalling 7 hours*person/day). Cylindrical traps were also used to capture fruit-feeding butterflies (family Nymphalidae) (Uehara-Prado et al. 2007, Freitas et al. 2014). In each transect, at least five traps were disposed with minimum distances of 100 meters between traps. Five traps were used in our short-term survey and 10 traps were used in expeditions. For the long-term surveys, 10 traps were used monthly in Peri Mirim and 18 traps were used monthly in São José de Ribamar. In total, 707 traps were disposed in the Amazon biome and 20 in the Cerrado. Traps were placed 1.5 meters from the ground and baited with cat feces, rotting fish or fruits. The proportion of four traps baited with bananas or mango with sugarcane juice to one trap baited with cat feces or rotting fish was maintained for all transects in expeditions. On the other hand, only bananas with sugarcane juice were used as baits in short and long-term surveys. We inspected traps twice a day. For each collected specimen, date, time and habitat type was recorded.

Captured butterflies were taken to the Laboratório de Ecologia e Sistemática de Insetos Polinizadores e Predadores (LESPP/UFMA) and identified through comparison with specimens from the Museu de Zoologia da Universidade de São Paulo (MZUSP), Museu de Zoologia da Universidade Estadual de Campinas (ZUEC), Coleção Entomológica Pe. Jesus Santiago Moure (DZUP) and specialized catalogues (D'abrera 1995, Garwood et al. 2009). Taxonomy follows mostly Lamas (2004), Mielke

Figure 3. Studied landscapes of the Cerrado biome in the state of Maranhão, Northeast Brazil. A. Tablelands of Cerrado in the Northeast of the state. B. The Preto river and its gallery forest. C. Typical Cerrado vegetation in São Benedito do Rio Preto. D. Gallery forest following the Alpercatas river in São Domingos do Azeitão.

(2005) and Duarte & Robbins (2010). Vouchers were deposited in four collections: LESPP/UFMA, MZUSP, ZUEC and DZUP.

3. Data analysis

We used the Jaccard index (J) to evaluate quantitatively the similarity in species composition between sampling methods (entomological nets and baited traps). This index is based on presence-absence data and has been widely used in ecological studies (Magurran 2004, Jost et al. 2011). In order to compare our list to other lists performed in the state, we searched for papers in the Web of Science, Scopus and Google Scholar databases. Only papers that provided a list of butterfly species and a clear indication that the field work was performed in the state of Maranhão (e.g.: coordinates, region or municipality) were included in the analysis.

Results

We recorded 189 butterfly species, of which 165 were captured in the Amazon, 65 in the Cerrado and 41 in both biomes. Nymphalidae was the best represented family, with 41.3% of the total richness (n = 78), followed by Hesperiidae (n = 53), Riodinidae (n = 29), Lycaenidae (n = 16), Pieridae (n = 10) and Papilionidae (n = 3) (Table 1). The richest subfamilies were, respectively, Riodininae with 29 species (15.3% of the species collected), Satyrinae with 24 species (12.7%) and Hesperiinae with 23 species (12.2%). *Hamadryas februa* (Hübner, [1823]), an indicator of disturbed habitats (Brown Jr 1992), was the most widespread species, occurring in all municipalities. Regarding sampling effort, 146 species were captured in long-term surveys, 112 in expeditions and 15 in our short-term survey. Specifically, the municipality of Peri Mirim presented the greatest number of species (n = 101), followed by São José de Ribamar (n = 99). A total of 167 species were sampled through entomological nets and 43 through baited traps. Similarity in species composition between sampling methods was recorded in 12% (J = 0.12).

Four lists performed in the state of Maranhão that meet the established criteria were included in the analyzes (Table 2). Of these studies, three used exclusively entomological nets to collect butterflies (Bates 1867, Garcia et al. 1990, Garcia & Bergman 1994), and one used cylindrical traps baited with bananas and sugarcane juice (Ramos 2000). Additionally, all these studies were performed in the Amazon biome, being two of these at the state capital, São Luís (Garcia et al. 1990, Garcia & Bergman 1994).

Discussion

The butterfly fauna captured in this study represents approximately 6% of the species recorded for Brazil (Brown Jr 1996, Lewinsohn et al. 2005, Francini et al. 2011). It is known that species richness is strongly dependent on sampling effort, partially explaining the lowest richness in our short-term survey when compared to our expeditions and long-term surveys. The influence of sampling effort on species richness is also observed when we compare our total richness (189 species) to intensively sampled areas in the Amazon, such as Rondônia (843) (Emmel & Austin 1990), Parque Nacional del Manu, in Peru (1,300) (Robbins et al. 1996) and Parque Estadual do Chandless, Acre (482) (Mielke et al. 2010), and Cerrado sites, such as Distrito Federal (504) (Emery et al. 2006). Comparing our results with those from other biomes, such as the Atlantic forest, one of the most

http://dx.doi.org/10.1590/1676-0611-BN-2017-0335

Table 1. List of butterflies of Amazon and Cerrado remnants of the state of Maranhão, Northeast Brazil.

Taxa (Family, Subfamily, Species, Subspecies)	**Amazon**						**Cerrado**		**Sampling method**	
	CP	**PV**	**PM**	**SJR**	**SL**	**VT**	**SBR**	**SDA**	**EN**	**BT**
Nymphalidae (S=78)										
Biblidinae (S=14)										
Biblis hyperia (Cramer, 1779)				x						x
Callicore astarte astarte (Cramer 1779)				x						x
Catonephele acontius (Linnaeus 1771)				x						x
Dynamine agacles (Dalman, 1823)	x								x	
Dynamine myrson myrson (Doubleday, 1849)	x								x	
Dynamine paulina (Bates, 1865)				x					x	
Dynamine postverta postverta (Cramer, 1779)			x	x					x	
Ectima iona E. Doubleday, [1848]	x									x
Eunica maja (Fabricius, 1775)				x						x
Hamadryas amphinome (Linnaeus, 1767)	x		x	x		x	x		x	x
Hamadryas chloe (Stoll, 1787)	x		x	x			x		x	x
Hamadryas februa (Hübner, [1823])	x	x	x	x	x	x	x	x	x	x
Hamadryas feronia (Linnaeus, 1758)			x	x	x	x	x	x	x	x
Hamadryas laodamia (Cramer, 1777)				x						x
Charaxinae (S=10)										
Archaeoprepona demophon (Linnaeus, 1758)			x	x	x	x				x
Archaeoprepona demophoon (Hübner, [1814])			x							x
Fountainea ryphea (Cramer, 1775)			x	x	x	x			x	x
Hypna clytemnestra (Cramer, 1777)			x	x					x	x
Memphis acidalia (Hübner, [1819])				x						x
Memphis leonida (Stoll, 1782)				x						x
Prepona laertes (Hübner, [1811])			x	x		x				x
Prepona pheridamas (Cramer, 1777)			x	x					x	x
Prepona pseudomphale Le Moult, 1932			x							x
Zaretis isidora (Cramer, 1779)		x	x	x		x	x		x	x
Cyrestinae (S=2)										
Marpesia chiron (Fabricius, 1775)			x	x					x	
Marpesia petreus (Cramer, 1776)			x	x		x			x	
Danainae (S=5)										
Danaus eresimus (Cramer, 1777)			x						x	
Danaus gilippus (Cramer, 1775)			x					x	x	
Danaus sp Kluk, 1780			x						x	
Lycorea halia (Hübner, 1816)			x	x		x			x	
Methona sp Doubleday, 1847			x	x	x				x	
Heliconiinae (S=13)										
Agraulis vanillae (Linnaeus, 1758)			x						x	
Dryas iulia (Fabricius, 1775)	x		x	x	x			x	x	
Dryadula phaetusa (Linnaeus, 1758)			x	x		x			x	
Euptoieta hegesia (Cramer, 1779)			x	x		x	x		x	
Heliconius antiochus (Linnaeus, 1767)	x	x	x	x			x		x	
Heliconius erato phyllis (Fabricius, 1775)				x					x	
Heliconius melpomene melpomene (Linnaeus, 1758)	x	x	x	x		x			x	
Heliconius melpomene nanna Stichel, 1899			x	x	x			x	x	
Heliconius ricini (Linnaeus, 1758)			x	x		x			x	
Heliconius numata (Cramer, 1780)			x	x					x	
Heliconius sara sara (Fabricius, 1793)				x				x	x	
Neruda mentis Moreira & Mielke, 2010								x	x	
Philaethria dido (Linnaeus, 1763)	x		x	x		x			x	
Limenitidinae (S=2)										
Adelpha cytherea cytherea (Linnaeus, 1758)	x			x					x	
Adelpha iphiclus iphiclus (Linnaeus, 1758)	x		x	x		x			x	
Nymphalinae (S=8)										
Anartia amathea (Linnaeus, 1758)			x					x	x	
Anartia jatrophae (Linnaeus, 1763)	x		x	x		x		x	x	

CP = Cururupu; PV = Penalva; PM = Peri Mirim; SJR = São José de Ribamar; SL = São Luís; VT = Vitória do Mearim; SBR = São Benedito do Rio Preto; SDA = São Domingos do Azeitão; EN = Entomological net; BT = Baited trap.

Table 1. Continued...

Taxa (Family, Subfamily, Species, Subspecies)	Amazon						Cerrado		Sampling method	
	CP	**PV**	**PM**	**SJR**	**SL**	**VT**	**SBR**	**SDA**	**EN**	**BT**
Colobura dirce (Linnaeus, 1758)	x		x	x		x			x	x
Historis acheronta (Fabricius, 1775)			x	x						x
Historis odius (Fabricius, 1775)			x	x						x
Junonia evarete (Cramer, 1779)	x	x	x	x		x			x	x
Siproeta stelenes (Linnaeus, 1758)			x	x					x	
Tigridia acesta (Linnaeus, 1758)	x								x	
Satyrinae (S=24)										
Brassolis sophorae (Linnaeus, 1758)			x					x	x	
Catoblepia berecynthia (Cramer, 1777)	x		x	x			x	x	x	x
Cissia penelope (Fabricius, 1775)	x	x		x		x		x	x	x
Caligo teucer (Linnaeus, 1758)			x	x						x
Caligo illioneus (Cramer, 1775)			x		x	x			x	x
Hermeuptychia hermes (Fabricius, 1775)				x		x		x	x	x
Magneuptychia libye (Linnaeus, 1767)			x	x		x			x	x
Magneuptychia ocypete (Fabricius, 1776)				x						x
Magneuptychia pallema (Schaus, 1902)				x						x
Morpho helenor (Cramer, 1776)	x		x	x		x			x	x
Morpho menelaus terrestris (Butler, 1866)				x	x				x	x
Morpho rhetenor Butler, 1866				x					x	x
Narope panniculus Stichel, 1904							x			x
Opsiphanes invirae (Hübner, [1808])			x	x					x	x
Opsiphanes quiteria (Stoll, 1780)				x		x			x	x
Pierella hyalinus (Gmelin, [1790])	x			x					x	
Pierella lamia (Sulzer, 1776)				x				x	x	
Pharneuptychia innocentia (C. Felder & R. Felder, 1867)							x		x	
Selenophanes cassiope (Cramer, 1775)								x	x	
Taygetis echo (Cramer, 1775)			x							x
Taygetis laches Fabricius, 1793			x	x					x	x
Taeygetis sosis Hopffer, 1874			x							x
Yphthimoides renata (Stoll,1780)				x						x
Yphthimoides affinis (Butler, 1867)						x				x
Lycaenidae (S=16)										
Polyommatinae (S=2)										
Hemiargus hanno hanno (Stoll, 1790)			x	x			x	x	x	
Leptotes cassius (Cramer, 1775)							x	x	x	
Theclinae (S=14)										
Arawacus aetolus (Sulzer, 1776)	x			x				x	x	
Calycopis demonassa (Hewitson, 1868)				x					x	
Chlorostrymon telea (Hewitson, 1868)							x		x	
Evenus satyroides (Hewitson,1865)				x					x	
Iaspis castitas (H. H. Druce, 1907)			x						x	
Rekoa palegon (Cramer, 1780)							x		x	
Ministrymon megacles (Stoll, 1780)			x				x		x	
Ministrymon zilda (Hewitson, 1873)			x				x		x	
Nicolaea socia (Hewitson, 1868)							x		x	
Panthiades phaleros (Linnaeus, 1767)			x	x					x	
Pseudolycaena marsyas (Linnaeus, 1758)	x		x	x			x		x	
Strymon mulucha (Hewitson, 1867)			x				x		x	
Tmolus echion (Linnaeus, 1767)							x		x	
Ziegleria hesperitis (Butler & H. Druce, 1872)			x						x	
Pieridae (S=10)										
Coliadinae (S=8)										
Anteos menippe (Hübner, [1818])	x								x	
Aphrissa statira statira (Cramer, 1777)			x	x			x		x	
Eurema albula (Cramer, 1775)			x	x			x		x	

CP = Cururupu; PV = Penalva; PM = Peri Mirim; SJR = São José de Ribamar; SL = São Luís; VT = Vitória do Mearim; SBR = São Benedito do Rio Preto; SDA = São Domingos do Azeitão; EN = Entomological net; BT = Baited trap.

Table 1. Continued...

Taxa (Family, Subfamily, Species, Subspecies)	Amazon						Cerrado		Sampling method	
	CP	PV	PM	SJR	SL	VT	SBR	SDA	EN	BT
Eurema elathea (Cramer, 1777)				x		x	x	x	x	
Phoebis argante argante (Fabricius, 1775)			x			x			x	
Phoebis philea philea (Linnaeus, 1763)			x	x					x	
Phoebis sennae marcellina (Cramer, 1777)			x	x	x	x	x	x	x	
Pyrisitia nise (Cramer, 1775)				x			x	x	x	
Pierinae (S=2)										
Ascia monuste (Linnaeus, 1764)		x	x	x		x	x		x	
Itaballia demophile (Linnaeus, 1763)			x			x			x	
Papilionidae (S=3)										
Papilioninae (S=3)										
Battus polydamas (Linnaeus, 1758)	x	x	x	x		x	x	x	x	
Heraclides thoas (Linnaeus, 1771)		x		x				x	x	
Heraclides anchisiades (Esper, 1788)			x						x	
Riodinidae (S=29)										
Riodininae (S=29)										
Aricoris campestris (Bates, 1868)			x					x	x	
Aricoris propitia (Stichel, 1910)			x					x	x	
Baeotis euprepes (Bates, 1868)							x		x	
Calospila lucianus (Fabricius, 1793)							x		x	
Calospila sp Geyer, 1832			x	x				x	x	
Detritivora zama (Bates, 1868)	x								x	
Emesis diogenia Prittwitz, 1865							x		x	
Eurybia elvina Stichel,1910				x					x	
Eurybia patrona Weymer, 1875				x					x	
Helicopis cupido (Linnaeus, 1758)						x	x		x	
Isapis agyrtus (Cramer, 1777)							x		x	
Juditha odites odites (Cramer, 1775)							x		x	
Lemonias zygia Hübner, 1807			x	x			x		x	
Melanis smithiae (Westwood, 1851)								x	x	
Mesosemia steli Hewitson 1858			x						x	
Mesosemia sp Hübner, [1819]				x					x	
Napaea beltiana beltiana (Bates, 1867)			x	x					x	
Napaea eucharila (Bates, 1867)			x						x	
Mesene phareus (Cramer, 1777)	x								x	
Phaenochitonia cingulus (Stoll, 1790)			x	x					x	
Stalachtis phlegia (Cramer, 1779)			x	x	x		x		x	
Synargis axenus axenus (Hewitson, 1876)							x		x	
Synargis agle (Hewitson, [1853])			x						x	
Synargis calyce (C. Felder & R. Felder, 1862)			x				x		x	
Synargis galena (Bates, 1868)	x								x	
Synargis gela (Hewitson, [1853])	x								x	
Thisbe irenea (Stoll, 1780)	x								x	
Thisbe molela (Hewitson, 1865)	x								x	
Theope foliorum Bates, 1868							x		x	
Hesperiidae (S=53)										
Eudaminae (S=16)										
Astraptes fulgerator fulgerator (Walch, 1775)			x						x	
Aguna asander asander (Hewitson, 1867)								x	x	
Aguna metophis (Latreille, [1824])				x					x	
Autochton neis (Geyer, 1832)				x	x				x	
Chioides catillus catillus (Cramer, 1779)			x						x	
Epargyreus clavicornis clavicornis (Herrich-Schäffer, 1869)			x						x	
Euriphellus euribates (Stoll, 1782)				x					x	
Hyalothyrus leucomelas (Geyer, 1832)			x						x	
Phocides pigmalion hewitsonius (Mabille, 1883)				x					x	

CP = Cururupu; PV = Penalva; PM = Peri Mirim; SJR = São José de Ribamar; SL = São Luís; VT = Vitória do Mearim; SBR = São Benedito do Rio Preto; SDA = São Domingos do Azeitão; EN = Entomological net; BT = Baited trap.

Table 1. Continued...

Taxa (Family, Subfamily, Species, Subspecies)	Amazon						Cerrado		Sampling method	
	CP	PV	PM	SJR	SL	VT	SBR	SDA	EN	BT
Typhedanus crameri McHenry, 1960	x								x	
Udranomia orcinus (C. Felder & R. Felder, 1867)				x					x	
Urbanus chalco (Hübner, 1823)			x						x	
Urbanus dorantes dorantes (Stoll, 1790)		x	x	x		x	x		x	
Urbanus procne (Plötz, 1881)		x	x						x	
Urbanus proteus (Linnaeus, 1758)	x			x			x		x	
Urbanus simplicius (Stoll, 1790)		x	x	x					x	
Hesperiinae (S=23)										
Augiades crinisus (Cramer, 1780)			x						x	
Aides duma duma Evans, 1955			x						x	
Aides duma argyrina Cowan, 1970		x					x		x	
Aides aegita (Hewitson, 1866)					x				x	
Calpodes ethlius (Stoll, 1782)			x	x					x	
Carystoides basoches (Latreille, [1824])			x						x	
Carystoides maroma (Möschler, 1877)			x	x					x	
Carystus phorcus phorcus (Cramer, 1777)			x						x	
Cobalus calvina (Hewitson, 1866)			x						x	
Cymaenes tripunctus theogenis (Capronnier, 1874)			x						x	
Cynea irma (Möschler, 1879)		x							x	
Cynea robba robba Evans, 1955				x					x	
Enosis uza uza (Hewitson, 1877)			x						x	
Mnasicles hicetaon Godman, 1901			x						x	
Morys valerius (Möschler, 1879)								x	x	
Nyctelius nyctelius nyctelius (Latreille, [1824])			x						x	
Panoquina fusina fusina (Hewitson, 1868)				x					x	
Phanes aletes (Geyer, 1832)								x	x	
Panoquina ocola ocola (Edwards, 1863)			x						x	
Polites vibex (Geyer, 1832)					x				x	
Pompeius pompeius (Latreille, [1824])			x						x	
Pyrrhopygopsis socrates (Ménétriés, 1855)	x						x		x	
Saliana saladin Evans, 1955				x	x				x	
Synale hylaspes (Stoll, 1781)								x	x	
Pyrginae (S=14)										
Elbella sp Evans, 1951				x					x	
Grais stigmaticus stigmaticus (Mabille, 1883)			x						x	
Heliopetes arsalte (Linnaeus, 1758)	x	x	x	x				x	x	
Heliopetes omrina (Butler, 1870)	x					x			x	
Jemadia fallax fida Evans, 1951				x					x	
Mysoria barcastus antila Evans, 1951		x	x						x	
Pyrgus orcus (Stoll, 1780)			x	x		x	x	x	x	
Pyrgus veturius Plötz, 1884				x					x	
Pyrrhopyge phidias (Linnaeus, 1758)						x			x	
Pyrrhopyge sp Hübner, [1819]			x						x	
Timochares trifasciata (Hewitson, 1868)				x					x	
Timochreon satyrus (C. Felder & R. Felder, 1867)								x	x	
Viola violella (Mabille, 1898)							x		x	
Zopyrion evenor evenor Godman,1901								x	x	

CP = Cururupu; PV = Penalva; PM = Peri Mirim; SJR = São José de Ribamar; SL = São Luís; VT = Vitória do Mearim; SBR = São Benedito do Rio Preto; SDA = São Domingos do Azeitão; EN = Entomological net; BT = Baited trap.

Table 2. Butterfly inventories previously published in the state of Maranhão, Northeast Brazil.

Authors and year	Municipality	Coordinates	Sampling method	Species richness
Bates 1867	Maracaçumé	02º 02’ S 45º57’ W	Entomological net	364 *
Garcia et al. 1990	São Luís	02 º39’ S 44 º15’ W	Entomological net	39
Garcia & Bergman 1994	São Luís	02 º39’ S 44 º15’ W	Entomological net	19
Ramos 2000	Açailândia	5°01´S, 47°32´W	Baited traps	90

*There are 23 species mentioned in the paper, but the author states that 364 species were sampled and deposited in collections.

well inventoried biomes regarding butterflies in Brazil (Santos et al. 2008), we observe higher richness in several sites of the later, which shelter from 218 to 914 species (Brown Jr & Freitas 2000, Francini et al. 2011). On the other hand, the richness of 189 species that was recorded in the present study was greater than in three of the four butterfly lists previously performed in the state of Maranhão (Garcia et al. 1990, Garcia & Bergman 1994, Ramos 2000), highlighting the need for greater sampling effort towards butterflies in the state. Although not directly comparable, these data indicate that new records should be expected in our less sampled study areas with the inclusion of rare and inconspicuous taxa, which are usually only captured after extensive samplings (Magurran 2004).

Patterns of endemism to a specific biome have been proposed for different groups of butterflies in Brazil (Pinheiro et al. 2010), suggesting that some of the collected species could be endemic. However, the task of determining endemism has been hampered by the scarcity of available data, especially for transition zones (Silva et al. 2005, Pinheiro et al. 2010). From the collected species, we highlight *Nicolaea socia* (Hewitson, 1868), which has been proposed to be endemic of Cerrado (Pinheiro et al. 2010). Considering that *Nicolaea socia* (Hewitson, 1868) was only captured in São Benedito do Rio Preto, we reinforce its distribution as an endemic species of this biome. The high number of species that only occurred in the Amazon (124) is probably related to the greater sampling effort applied in our Amazonian sites. Since Bates (1867), Garcia et al. (1990), Garcia & Bergmann (1994) and Ramos (2000) field works were performed exclusively in the Amazon, we believe that new inventories in Maranhão's Cerrado are necessary to evaluate the distribution and endemism of butterflies from Maranhão.

The low similarity between species captured through entomological nets and cylindrical traps was expected. Entomological nets usually capture larger number of species, but its dependence on good weather and collector's abilities may limit the capture of inconspicuous groups (Sparrow et al. 1994). On the other hand, baited traps sample fewer species, but consistently yields species rarely observed by researchers (Sparrow et al. 1994, Freitas et al. 2014). Consequently, the low value of similarity obtained in this study reinforces that entomological nets and baited traps contribute with complementary data for butterfly inventories in the Neotropics (Sparrow et al. 1994, Caldas & Robbins 2003).

1. *Taxonomic composition*

The richest family in our list was Nymphalidae, following the same pattern of other butterfly lists performed in Brazil (Zacca et al. 2011, Morais et al. 2012). Most recorded species of this family are widespread throughout the country, such as *Hamadryas februa* (Hübner, [1823]), *Hamadryas feronia* (Linnaeus, 1758), *Hamadryas amphinome* (Linnaeus, 1767), *Junonia evarete* (Cramer, 1779) and *Agraulis vanillae* (Linnaeus, 1758). The fact that *Hamadryas februa* (Hübner, [1823]) was our most widespread species is concerning but expected, since this species is characteristic of modified habitats and secondary forests (Brown Jr 1992). Individuals of *Hamadryas februa* (Hübner, [1823]) were also captured by Garcia et al. (1990), Garcia & Bergmann (1994) and Ramos (2000), suggesting that this species is common in different regions of the state. Six species of the genus *Heliconius* Kluk, 1780, one of the most studied groups of butterflies regarding evolution and diversity patterns (Mallet 1993, Mavárez et al. 2006, Merrill et al. 2015), were sampled, including two races of *Heliconius melpomene* (Linnaeus, 1758): *Heliconius melpomene melpomene* (Linnaeus, 1758) and *Heliconius melpomene nanna* Stichel, 1899. Bates (1867) described different variations of *Heliconius* species captured in Maracaçumé, including intermediate varieties between *Heliconius melpomene melpomene* (Linnaeus, 1758) and *Heliconius melpomene thelxiope* Hübner [1806]. Considering the distribution of *Heliconius* butterflies and the suggested hybridization zones for this group in the Amazon (Mallet 1993, Merrill et al. 2015), new species and races of *Heliconius* are expected to be found in Maranhão.

The second richest family was Hesperiidae, including common species in the Neotropics, such as *Astraptes fulgerator fulgerator* (Walch, 1775), *Autochton neis* (Geyer, 1832) and *Heliopetes arsalte* (Linnaeus, 1758). Hesperiidae is considered, along with Nymphalidae, the richest families in the Neotropical region (Lamas 2004). Moreover, Francini et al. (2011) stated that Hesperiidae is expected to be the richest family in relatively complete inventories of Brazil, while Nymphalidae is the best represented butterfly group in short-term studies, probably because nymphalids are easily visualized and identified in the field. In this study, Nymphalidae was considerably richer than Hesperiidae, implying that additional records of hesperids should be expected with an increased sampling effort.

Riodinidae was the third family in species richness, followed by Lycaenidae. This result agrees with those from lists performed in other Amazonian sites (Emmel & Austin 1990, Brown Jr & Freitas 2002), although lists from other biomes frequently demonstrate Lycaenidae being richer than Riodinidae (Francini et al. 2011). Riodinidae richness has been correlated with the mean temperature of the study areas, explaining its greater number in tropical regions (Brown Jr 2005, Francini et al. 2011), such as Maranhão. From the captured riodinids, we highlight *Napaea beltiana beltiana* (Bates, 1867) and *Napaea eucharila* (Bates, 1867), species also captured and described by Bates (1867). Lycaenidae was the fourth family in number of species, but we expect the richness of Lycaenidae to increase, since this family include small and inconspicuous species, requiring longer temporal surveys for adequate samplings (Francini et al. 2011).

Pieridae and Papilionidae are the families with lowest species richness in Brazil (Emery et al. 2006, Francini et al. 2011), and our findings corroborate this pattern. Most captured species of Pieridae and Papilionidae are considered widely distributed throughout the country and characteristic of modified habitats (Emery et al. 2006, Zacca et al. 2011, Morais et al. 2012), suggesting that the study areas have been harmed by anthropic influences.

2. *Perspectives for future research*

Considering our sampling effort and the difficulties to sample a large Brazilian state, we estimate that the recorded species represent a small subset of the butterflies from Maranhão. Broader and longer samplings could result in much greater richness, especially for the families Hesperiidae, Riodinidae and Lycaenidae. Centuries of butterfly records from Maranhão in museum collections must also be analyzed in the future to achieve better understanding of butterflies' diversity and distribution in the state.

Two of the four lists previously performed in Maranhão were conducted at the state capital, São Luís. The proximity to the major research centers of the state and the easy access to study areas may be the main reason for this geographic bias. Consequently, little or no effort is applied for distant and complicated access areas, resulting in the current lack of information. Among the priority areas for butterfly inventories, the western region of Maranhão should be focused by researchers, since this portion of Amazon is still poorly studied and under human pressures (Silva et al. 2005, Martins & Oliveira 2011). Moreover, this region is part of the most threatened endemism center in Brazil, the Belém Center of Endemism (Silva et al. 2005). Only about one-third of its forest remains, and the increasing deforestations may represent the extinction of several species, including endemic butterflies (Hall & Harvey 2002, Silva et al. 2005). Therefore, inventories in this area are recommended to identify novel and/or endemic taxa. Long-term researches are also requested for Maranhão's Cerrado, since different butterfly species have been considered endemic of this endangered biome (Brown Jr & Gifford 2002, Pinheiro et al. 2010) and there are no previous butterfly lists published for this region.

Species inventories are important tools for a better understanding of the biodiversity. In this context, it is urgent to perform studies that generate lists of species in poorly studied areas to evaluate the distribution and endemism of different taxa, such as megadiverse groups of insects. As previously mentioned, the richness of 189 butterfly species collected in the present study can be considered low when compared to intensively sampled areas in the Amazon and Cerrado. On the other hand, we presented the greatest number of species in butterfly inventories from Maranhão since Bates (1867). Furthermore, some of our study areas were sampled for the first time and may shelter a greater butterfly diversity, including species not yet known. Thus, we call attention to the urgent measures that should be taken to preserve Amazon and Cerrado remnants of Maranhão and their local biodiversity, since these areas have been increasingly threatened by anthropic disturbances.

Acknowledgments

We would like to thank the researchers of the Laboratório de Ecologia e Sistemática de Insetos Polinizadores e Predadores (LESPP/UFMA), especially David Barros Muniz for helping with the confection of the map. Marcelo Duarte helped in various phases of this project, including technical support. Renato de Oliveira, André Victor Lucci Freitas, Diego Rodrigo Dolibaina, Ricardo Russo Siewert and Jessie Pereira dos Santos helped greatly with identifications. Our sincere gratitude to Quercegen S.A and Instituto Formação for allowing us to develop part of this research in Sítio Aguahy and Parque Agroecológico de Buritirana, respectively. We are grateful to two anonymous reviewers for helpful comments on the original version of the manuscript. This study was supported by the Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão (FAPEMA/APP-UNIVERSAL-00404/11 and FAPEMA/CBIOMA 02986/12).

Author Contributions

Lucas Pereira Martins: substantial contribution in the concept and design of the study; contribution to manuscript preparation, data collection, interpretation and analysis.

Elias da Costa Araujo Junior: substantial contribution in the concept and design of the study; contribution to manuscript preparation, data collection and critical revision.

Ananda Regina Pereira Martins: substantial contribution in the concept and design of the study; contribution to manuscript preparation and critical revision.

Mairla Santos Colins: substantial contribution to manuscript preparation, data collection and critical revision.

Gabriela Cristina Fonseca Almeida: substantial contribution to manuscript preparation, data collection and critical revision.

Gisele Garcia Azevedo: substantial contribution in the concept and design of the study; contribution to manuscript preparation and critical revision.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

BARRETO, L., VAN EUPEN, M., KOK, K., JONGMAN, R.H.G., RIBEIRO, M.C., VELDKAMP, A., HASS, A. & OLIVEIRA, T.G. 2012. The impact of soybean expansion on mammal and bird, in the Balsas region, north Brasilian Cerrado. J Nat Conserv. 20(6):374-383.

BATES, H.W. 1867. On a collection of butterflies formed by Thomas Belt, Esq., in the interior of the province of Maranhão, Brazil. T Roy Ent Soc London. 5(7):535-546.

BONEBRAKE, T.C., PONISIO, L.C., BOGGS, C.L & EHRLICH, P.R. 2010. More than just indicators: a review of tropical butterfly ecology and conservation. Biol. Conserv. 143(8):1831–41.

BROWN JR, K.S. 1992. Borboletas da Serra do Japi: diversidade, habitats, recursos alimentares e variação temporal. In Historia Natural da Serra do Japi: ecologia e preservação de uma área florestal no sudeste do Brasil (L. Morellato, ed). Unicamp, Campinas, p.142–187.

BROWN JR, K.S. 1996. Diversity of Brazilian Lepidoptera: History of study, methods for measurements, and use as indicators for genetic, specific and system richness. In Biodiversity in Brazil: a first approach (C.E.M. Bicudo & N.A. Menezes, eds). CNPq/Instituto de Botânica, São Paulo, p.221–253.

BROWN JR, K.S. 1997. Diversity, disturbance, and sustainable use of Neotropical forests: insects as indicators for conservation monitoring. J Insect Conserv. 1(1):25–42.

BROWN JR, K.S. & FREITAS, A.V.L. 2000. Atlantic forest butterflies: Indicators for Landscape Conservation. Biotropica 32(4b):934–956.

BROWN JR, K.S. & FREITAS, A.V.L. 2002. Diversidade biológica no Alto Juruá: avaliação, causas e manutenção. In O Alto Juruá: Práticas e Conhecimentos das Populações (M.M.C. Cunha & M.B. Almeida, eds). Enciclopédia da floresta, São Paulo, p.33–42.

BROWN JR, K.S. & GIFFORD, D.R. 2002. Lepidoptera in the Cerrado landscape and the conservation of vegetation, soil, and topographical mosaics. In The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna (P.S Oliveira & R.J. Marquis, eds). Columbia University Press, New York, p.201–222.

BROWN JR, K.S. 2005. Geological, evolutionary and ecological bases of the diversification of Neotropical butterflies: implications for conservation. In Tropical rainforests: Past, Present and Future (E. Bermingham, C.W. Dick & C., Moritz, eds). University of Chicago Press, Chicago, p.166–201.

CALDAS, A. & ROBBINS, R.K. 2003. Modified Pollard transects for assessing tropical butterfly abundance and diversity. Biol. Conserv. 110:211–219.

CASAGRANDE, M.M., MIELKE, O.H.H., CARNEIRO, E., RAFAEL, J.A. & R.W. HUTCHINGS. 2012. Hesperioidea e Papilionoidea (Lepidoptera) coligidos em expedição aos Rios Nhamundá e Abacaxis, Amazonas, Brasil: novos subsídios para o conhecimento da biodiversidade da Amazônia Brasileira. Rev Brasil Entomol 56(1):23-28.

DINIZ-FILHO, J.A.F., OLIVEIRA, G., LOBO, F., FERREIRA JR, L.G., BINI, L.M. & RANGEL, T.F.V.L.B. 2009. Agriculture, habitat loss and spatial patterns of human occupation in a biodiversity hotspot. Sci Agric 66(6):764–771.

DUARTE, M., ROBBINS, R. K. 2010. Description and phylogenetic analysis of the Calycopidina (Lycaenidae, Theclinae, Eumaeini): a subtribe of detritivores. Rev Brasil Entomol. 54(1):45–65.

D'ABRERA, B. 1995. Butterflies of the Neotropical region: Nymphalidae. Hill House, Victoria.

EMERY, E.O., BROWN JR., K.S. & PINHEIRO, C.E.G. 2006. As borboletas (Lepidoptera, Papilionoidea) do Distrito Federal, Brasil. Rev Brasil Entomol. 50(1):85-92.

EMMEL, T.C. & AUSTIN, G.T. 1990. The tropical rain forest butterfly fauna of Rondônia, Brazil: species diversity and conservation. Trop Lepid. 1(1):1–12.

FRANCINI, R.B., DUARTE, M., MIELKE, O.H.H., CALDAS, A. & FREITAS, A.V.L. 2011. Butterflies (Lepidoptera, Papilionoidea and Hesperioidea) of the "Baixada Santista" region, coastal São Paulo, southeastern Brazil. Rev Bras Entomol. 55(1):55–68.

FREITAS, A.V., ISERHARD, C.A, SANTOS, J.P., CARREIRA, J.Y.O., RIBEIRO, D.B, MELO, D.H.A, ROSA, A.H.B, MARINI-FILHO, O.J., ACCACIO, G.M. & UEHARA-PRADO, M. 2014. Studies with butterfly bait traps: an overview. Rev Colomb Entomol. 40(2):209-218.

GARCIA, I.P., BERGMANN, E.C. & NETTO, S.M.R. 1990. Diversidade mensal de borboletas da ilha de São Luís (MA). Arq. Inst. Biol. 57(1/2):39-44.

GARCIA, I.P. & BERGMANN, E.C. 1994. Borboletas da Ilha de São Luís (MA). Arq. Inst. Biol. 56(1/2):37-38.

GARWOOD, K., LEHMAN, R., CARTER, W. & CARTER, G. 2009. Butterflies of Southern Amazonia. A photographic checklist of common species. RiCalé Publishing, Texas.

HALL, J.P.W. & HARVEY, D. 2002. The phylogeography of Amazonia revisited: new evidence from riodinid butterflies. Evolution 56(7):1489-1497.

JOST, L., CHAO, A. & CHAZDON R.L. 2011. Compositional similarity and beta diversity. In Biological Diversity: Frontiers in Measurement and Assessment (A.E. Magurran & B.J. McGill, eds). Oxford University Press, Oxford, p.66-84.

KIM, K.C. 1993. Biodiversity, conservation, and inventory: why insects matter. Biodivers. Conser. 2(3):191- 214.

KLINK, C.A. & MACHADO, R.B. 2005. Conservation of the Brazilian Cerrado. Conserv Biol. 19(3):707–713.

KREMEN, A.C., COLWELL, R.K., ERWIN, T.L., MURPHY, D.D., NOSS, R.F. & SANJAYAN, M.A. 1993. Terrestrial Arthropod Assemblages: Their Use in Conservation Planning. Conserv Biol. 7(4):796–808.

LAMAS, G. 2004. Checklist: Part 4A. Hesperioidea – Papilionoidea. In Atlas of Neotropical Lepidoptera. Association for Tropical Lepidoptera (J. B. Heppner, ed). Scientific Publishers, Gainesville, p.1- 439.

LAURANCE, W.F., VASCONCELOS, H. L. & LOVEJOY, T. E. 2000. Forest loss and fragmentation in the Amazon: implications for wildlife conservation. Oryx. 34(1):39–45.

LEWINSOHN, T.M., FREITAS, A.V.L. & PRADO, P.I. 2005. Conservation of Terrestrial Invertebrates and Their Habitats in Brazil. Conserv Biol. 19(3):640–645.

MAGURRAN, A.E. 2004. Measuring biological diversity, 1 ed. Blackwell, Oxford.

MALLET, J. 1993. Speciation, raciation and color pattern evolution in *Heliconius* butterflies: evidence from hybrid zones. In Hybrid Zones and the Evolutionary Process (R.G. Harrison, ed.) Oxford University Press, New York, p.226-260.

MARTINS, M.B & OLIVEIRA, T.G. 2011. Amazônia Maranhense: Diversidade e Conservação. 1 ed. MPEG, Belém.

MAVÁREZ, J., SALAZAR, C.A., BERMINGHAM, E., SALCEDO, C., JIGGINS, C.D. & LINARES, M. 2006. Speciation by hybridization in Heliconius butterflies. Nature. 441:868-871.

MERRILL, R.M., DASMAHAPATRA, K.K., DAVEY, J.W., DELL'AGLIO, D.D., HANLY, J.J., HUBER, B., JIGGINS, C.D., JORON, M., KOZAK, K.M., LLAURENS, V., MARTIN, S.H., MONTGOMERY, S.H., MORRIS, J., NADEAU, N.J., PINHARANDA, A.L., ROSSER, N., THOMPSON, M.J., VANJARI, S., WALLBANK, R.W.R. & YU, Q. 2015. The diversification of Heliconius butterflies: what have we learned in 150 years? J. Evol. Biol. 28:1417-1438.

MIELKE, O.H.H. 2005. Catalogue of the American Hesperioidea: Hesperiidae (Lepidoptera). 6 ed. Sociedade Brasileira de Zoologia, Curitiba.

MIELKE, O.H.H., CARNEIRO, E. & CASAGRANDE, M.M. 2010. Lepidopterofauna (Papilionoidea e Hesperioidea) do Parque Estadual do Chandless e arredores, Acre. Biot Neotrop. 10(4): http://www.biotaneotropica.org.br/v10n4/pt/fullpaper?bn03210042010 (last access on 27/05/2017)

MORAIS, A.B.B., LEMES, R. & RITTER, C.D. 2012. Borboletas (Lepidoptera: Hesperioidea e Papilionoidea) de Val de Serra, região central do Rio Grande do Sul, Brasil. Biot Neotrop. 12(2): http://www.biotaneotropica.org.br/v12n2/en/fullpaper?bn01412022012 (last access on 01/02/2017)

MYERS, N., MITTERMEIER, R.A., MITTERMEIER, C.G., DA FONSECA, G.A.B. & KENT, J. 2000. Biodiversity hotspots for conservation priorities. Nature. 403:853-858.

PINHEIRO, C.E.G., MALINOV, I.K., EMERY, E.O. & SCHMIDT, K. 2010. Endemismos e conservação de borboletas (Lepidoptera: Papilionoidea e Hesperioidea) no bioma Cerrado. In Cerrado: Conhecimento científico quantitativo como subsídio para ações de conservação (I.R. Diniz, J. Marinho-Filho, R.B. Machado & R.B. Cavalcanti, eds). UNB, Brasília, p.225–238.

RAMOS, F.A. 2000. Nymphalid butterfly communities in an Amazonian forest fragment. J Res Lepid. 35:29-41.

ROBBINS R.K., LAMAS, G., MIELKE, O.H., HARVEY, D.J., CASAGRANDE, M.M. 1996. Taxonomic composition and ecological structure of the species-rich butterfly community at Pakitza, Parque Nacional del Manu, Peru. In La Biodiversidad del Sureste del Peru. Lima, Peru (D.E. Wilson & A. Sandoval, eds.). Smithsonian Institution Press, Washington D.C., p.201–236.

SANTOS, E.C, MIELKE, O.H.H. & CASAGRANDE, M.M. 2008. Butterfly inventories in Brazil: the state of the art and the priority-areas model for research aiming at conservation. Nat Conservação. 6(2):178–200.

SANTOS, J.P., MARINI-FILHO, O.J., FREITAS, A.V.L. & UEHARA-PRADO, M. 2016. Monitoramento de Borboletas: o Papel de um Indicador Biológico na Gestão de Unidades de Conservação. Biodiversidade Brasileira. 6(1):87-99.

SILVA, J. M. C., RYLANDS, A. B. & FONSECA, G. A. B. 2005. O destino das áreas de endemismo da Amazônia. Megadiversidade. 1(1):124-131.

SPARROW, H.R., SISK, T.D., EHRLICH, P.R. & MURPHY D.D. 1994. Techniques and guidelines for monitoring neotropical butterflies. Conserv Biol. 8(3):800-809.

VIEIRA, I.C.G., TOLEDO, P.M., SILVA, J.M.C. & HIGUCHI, H. 2008. Deforestations and threats to the biodiversity of Amazonia. Braz J Biol. 68(4):949-956.

UEHARA-PRADO, M., BROWN JR., K.S. & FREITAS, A.V.L. 2007. Species richness, composition and abundance of fruit-feeding butterflies in the Brazilian Atlantic Forest: comparison between a fragmented and a continuous landscape. Glob Ecol Biogeog. 16(1):43–54.

ZACCA, T., BRAVO, F., ARAÚJO, M.X. 2011. Butterflies (Lepidoptera: Papilionoidea and Hesperioidea) from Serra da Jibóia, Bahia State, Brazil. Entomobr. 4(3):139–143.

Received: 20/02/2017
Revised: 28/05/2017
Accepted: 13/08/2017