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Faculty of Tropical AgriSciences

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Impact of habitat loss on anuran diversity in different parts of Serra do Mar, the largest remnant of Mata Atlantica, SE Brazil

Master Thesis

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Assignment



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Methodology:	 To collect the data in the Serra do Mar area in Brasil. Data will be collected using the combination of transects and visual encounter survey methods. To carry out a high quality data treatment including taxonomic identification of anuran species, to provide protocols of the field work and to provide analysis of the data. Time schedule: The data for thesis were collected during February- March 2015. March - July – data preparation for statistical analyses; July-August 2015 – statistical treatment of data; September 2015 – March 2016 – results interpretation, writing the thesis. The study of relevant scientific literature is

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Declaration

"I hereby declare that this thesis entitled Impact of habitat loss on anuran diversity in different parts of Serra do Mar, the largest remnant of Mata Atlantica, SE Brazil, is my own work and all the sources have been quoted and acknowledged by means of complete references."

In Prague, 20.4.2016:

.....

Tomáš Holer

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Abstract

Mata Atlantica is one of the ancient forests. It is one of the five South American biodiversity hotspots. In the recent time, over 90% percent of this rich and unique biome has been lost. This loss and huge fragmentation of the forest, resulted in a decrease and extinction of many populations of endemic species of plants and animals. Over 450 amphibian species lives in the Atlantic forest of which 60% are endemic. My research area was localized inside one of the largest Atlantic forest fragments – Serra do Mar, in the buffer area of Serra da Bocaina National Park, Brazil. Aims of these thesis were to identify the anuran diversity in the abovementioned area, estimate species habitat preferences and compare the preferred habitat map of the three model species, with their distribution maps available on their IUCN Red List account. The methodology consisted of two parts. Three line transects were placed in three different forest parts and elevations. Three night and three day surveys were done on each transect by three people. Second part was non-invasive species identification on five selected breeding ponds in the research area. During my research, 31 species of frogs were recorded and 27 of them were identified, belonging to 8 families. Hylidae (16), Hylodidae (1), Brachycephalidae (3), Bufonidae (2), Odontophrynidae (3), Craugastoridae (1), Leptodactylidae (4), Centrolenidae (1). None of those frogs are endangered according to their IUCN Red List status (1 Data Deficient, rest Least Concern). After revision and re-estimation of three model species (Aplastodiscus callipygius, Cruz and Peixoto, 1985, Adenomera marmorata, Steindachner, 1867, Haddadus binotatus, Spix, 1824) distribution maps, according to their preferred habitat, using distribution maps available on IUCN Red List, It has been found, that the endanger status of many species of frogs is most likely underestimated. Results of this thesis suggest, that large-scale research should be conducted, to get more knowledge of Mata Atlantica amphibians, to understand their behavior and ecology and through the knowledge find the way how to protect them.

Keywords: Amphibians, anurans, Atlantic Forest, biodiversity, frogs

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1. Introduction and Literature Review

1.1. World biodiversity hotspots

There are 35 biodiversity hotspots in the world (Fig.1), with more than 1500 endemic species of plants (CEPF, 2016). All of them has in the recent time lost at least 2/3 of their original size (Brooks et. al., 2002). They now cover only 2.3 % of the land surface, since the original range was around 12 % (CEPF, 2016). Due to this loss, many endemic species of plants and animals became extinct or are threatened with extinction (Brooks et. al., 2002).



Figure 1: Map of world biodiversity hotspots. (Conservation International, 2012)

1.2. Atlantic forest

1.2.1. Historical and present distribution

The Atlantic forest has lost more than 90 % of its original range. Its range is now around 99 944 km² and it is highly fragmented as it can be seen in the map (Fig. 2). Protected areas cover the area of 50 370 km² of which 22 782 km² is protected in higher level of protection

(CEPF, 2016). The Atlantic forest has 14 terrestrial ecoregions: Ilha Grande mangroves, Rio Sao Francisco mangroves, Araucaria moist forests, Rio Piranhas mangroves, Bahia mangroves, Pernambuco coastal forest, Bahia coastal forest, Bahia interior forest, Caatinga Enclaaves moist forests, Parana-Paraíba interior forests, Pernambuco interior forests, Campos Rupestres montane savanna, Serra do Mar coastal forests and Atlantic Coast restingas (Galindo Leal and de Gusmao Camara, 2003).



Figure 2: Map of present and history extent of Atlantic forest. (IPCI-Canada, 2016)

1.2.2. Biodiversity

Despite the massive deforestation and degradation of the Atlantic forest, it still remains one of the most important biodiversity hotspots. There is more than 23 000 species of plants in the Atlantic forest. Approximately 40% of this species, are endemic, not to be found anywhere else. There could be approximately 450 species of trees in one hectare of the forest. Animals are also very diverse. For mammals, were recorded 264 species, nearly 1000 species of birds, 456 amphibians, more than 300 reptiles and species of freshwater fish. Around 31% of all vertebrates in the Atlantic forest are endemic. For amphibians is the endemism percentage even higher – 61%. Still new species are being discovered and probably many are not known yet (Galindo Leal and de Gusmao Camara, 2003). The high number of various amphibian species is caused probably by the variation of different vegetation types, created as a product of various altitudinal, longitudinal and latitudinal values (Haddad and Prado, 2005).

1.2.3. Threats and conservation

For the biome itself, the most threating factors are agricultural expansion, logging and growing of cities associated with road building. For the species, living in the Atlantic Forest it is mainly habitat loss and fragmentation of the forest and hunting and wildlife trade (WWF, 2016). Only 23 800 km² of the forest in Brazil is under high level of protection. There is 108 national and state parks, 85 federal and states biological reserves and 31 federal and state ecological stations and reserves (Galindo Leal and de Gusmao Camara, 2003).

1.3. Atlantic forest in comparison to other Neotropical biodiversity hotspots

1.3.1. Tropical Andes

Tropical Andes are the most diverse region of the earth. It is an area of 1 542 644 km², from western Venezuela through Columbia, Ecuador, Peru to northern Chile and Argentina,

where it's border is determined by the Tropic of Capricorn. The amazing diversity of this area has probably evolved due to great elevation differences. I can find there snow-covered peaks, steep slopes, deep canyons and other places, which creates microhabitats for many diverse species (CEPF, 2016). There are various types of vegetation, corresponding to the gradients in altitudes. Between 500 – 1500 m of altitude, there are mostly wet and moist forests. From 800 – 3500, various types of cloudy forest occur, which are especially rich in biodiversity. Higher altitudes, from 3000 to 4800, are usually grass, shrubs, lichens and mosses habitats, reaching the area of the permanent snow. Other habitats such as dry forest, woodlands, cactus stands, thorn scrub and matorral fund can be also found in the Tropical Andes, but covering smaller area (Kessler, 2000), (Young *et al.*, 2002).

There is around 30 000 species of vascular plants in the Tropical Andes and around 50% of them are endemic (Young *et al.*, 2002). Over 4200 vertebrate species, lives inside the Tropical Andes region. There are 1724 species of birds, including 579 endemic species and 66 endemic bird genera. Around 610 species of reptiles, including 275 endemic species. The highest proportion of endemic species among vertebrates in the Tropical Andes belongs to amphibians. Over 68% of the total number of 981 species, are endemic species. The last group of vertebrates, freshwater fishes, is represented by 380 species, including 131 endemics (CEPF, 2016).

This hotspot is for the amphibians the most important place on the world. The total number of amphibian species here is more than 980 species, including more than 670 endemic species and eight endemic genera. The amphibians of this region are unfortunately also the most threatened. There is around 450 species listed in IUCN Red List as threatened, including more than 360 endemic species. The major threat for local amphibians is habitat loss, but also diseases, especially chytridiomycosis (*B. dendrobatidis*) (Seimon *et al.*, 2007), (Navas, 2006). Most affected group by the chytridiomycosis is the genus *Atelopus*, when 56 species of this genus are considered as critically endangered (IUCN Red List, 2008). One of the most frequent and specific endemic genus occurring in the Tropical Andes is the genus *Telmatobius*, with around 45 species. Other non-endemic frequent genera, *Euletherodactylus*, has around 330

4

species. Some of the frogs of the family *Dendrobatidae*, are one of the most poisonous organisms and some others are also used in medical industry (Duellman, 1999) (Navas, 2006).

1.3.2. Cerrado

The Cerrado is a large region of Brazilian Plateu, occupying an area of 2 031 990 km², but only on 21% is remaining vegetation. Only 1.2% of the region is protected (Brasileiro *et al.*, 2005). The main habitats of Cerrado are savannas, woodland/savanna and dry forest. The savannas could be divided to grassland with scattered trees, trees and shrubs savanna and dry, closed canopy forest. Also gallery forests are present throughout the region. The rainfall in the region is around 1100 – 1600 mm per year and it is from October to April. The rest of the year is really dry, therefore most of the vegetation is adapted to fires. Plants on Cerrado has evolved various techniques how to survive the fires, such as thick bark, deep rood system and a rapid regeneration. The fires are really important to maintain the balance between the trees and grasses, especially in the case of invasive grasses. Some areas are on the other hand flooded and swampy in the It season (CEPF, 2016).

There is around 10 000 species of plant and nearly half (44%) of them are endemic. There is over 2000 species of vertebrates. The largest number of species belongs to freshwater fishes, which are very diverse in the rivers and streams of Cerrado, reaching the number of 800 species, including 200 endemic species. Second most diverse group of vertebrates this region are birds. There is 607 species, but in comparison to other South American hotspots, only 17 endemic species. More endemic species, 33, belong to reptiles, reaching the total number of 225 species. Pre-last group of vertebrates are mammals. I can find there 195 species including 14 endemics. The smallest group are amphibians, with their total number of 186 species, but 28 of them are endemic (Silva and Bates, 2002) (CEPF, 2016). There were not much studies on anuran fauna in the Cerrado (Brasileiro *et al.*, 2005).

1.3.3. Chilean Winter Rainfall – Valdivian Forest

Chilean Winter Rainfal-Valdivian Forest hotspots is often called as a continental island, meaning that it is surrounded by Pacific Ocean on one side and the Andes Mountains and Atacama Desert on the other sides. Its original range was 397 142 km², but less than one third of this remarkable habitat remains. The hotspot north border starts on central – northern Chile and Western Argentina and continue on the Pacific coast lined by the Andean mountains to the south, till the island Grande de Chiloé. Hotspot include also farther islands San Félix and San Ambrosio, and Juan Fernández Islands. Vegetation types in the area are winter-rainfall deserts, matorral and savannas, deciduous forests and high elevation alpine vegetation. Also a small strip of coastal rainforest is present (Arroyo *et al.*, 1996) (Arroyo *et al.*, 1999).

The hotspot isolation and different altitudes in this area lead to high endemism. Half of the plants, present in the Chilean Winter Rainfall – Valdivian Forest are endemic. The percentage of endemic species is even higher on the farther islands (over 60%). The total number of plants reaches 3 892 species. The most diverse group of vertebrates in this area are birds, with 226 species, of which 12 are endemic. But the highest percentage of endemism is present in local amphibians. Over 70% of 41 amphibian species living here, are endemic. Second highest number of endemic species is in reptiles, where 67% of 41 species cannot be found anywhere else out of the region. Even one whole family Rhinodermatidae and 5 genera are endemic to the region: Telmatobufo, Rhinoderma, Insuetophrynus, Caudiverbera and Hylorina, each including just one species. Freshwater fishes has also high number of endemic species (24 of 43). The number of mammals is relatively low, 68 species, but including 15 endemic (CEPF, 2016).

1.3.4. Tumbes – Chocó – Magdalena

The last and the originally smallest South American hotspot is the Tumbes – Chocó – Magdalena region, with its range 274 597 km². Only 65 903 km² of the region vegetation remains. The hotspot area is bordered by Panama Canal on the north, where the It and moist forests are present. On the north, one part of the hotspot is leading east, to inland to the

Magdalena valley. Than it continues south through the Columbian Chocó region and through the forests on the west coast of Ecuador to small part of northern Peru. Also the island of Malpelo and Galápagos Islands are included in the hotspot. The hotspot consist of many habitats: Mangroves, beaches, rocky shorelines, coastal dry forest and rainforests.

There is around 11 000 species of vascular plants, of which 25% cannot be found anywhere else. In vertebrates, the highest number of endemic species is in freshwater fishes. 115 species of total 251 are endemic. The most diverse group are the birds. I can find there 890 species, including 110 endemic. Mammals are in this area represented by 285 species, 11 endemic. Much more endemic species belongs to the reptiles. There is 327 species and 98 endemic. Just after fishes and reptiles, there are amphibians with their 30% of endemic species of the total 203 species. These endemic species usually live on very small part of some specific habitat and therefore they are vulnerable. There are also many species of amphibians, which are being discovered and not described yet. There are no native amphibians on Galápagos (Best and Kessler, 1995) (Parker and Carr, 1992) (CEPF, 2016).

1.3.5. Atlantic forest in context of other Neotropical biodiversity hotspots

As it can be seen from the table below (Tab. 1), the Atlantic Forest is the most disturbed hotspot of South America. It is on the second place in number of amphibian species and amphibian endemic species, just after Tropical Andes, which is but almost four times larger in its present extent. Percentage of amphibian endemic species is highest in the Chilean Winter Rainfall Valdivian Forest, where it is more than 70% of its 41 species.

	Original	Present	Habitat					
	extent	extent	Loss	Plant				Endemic
Hotspot	(Km²)	(Km²)	(%)	species	Endemic	Amphibians	Endemic	(%)
Atlantic	1 233							
Forest	875	99 944	92	23 000	8 000	456	282	61,8
Tropical	1 542	385						
Andes	644	661	75	30 000	15 000	981	673	68,8
	2 031	438						
Cerrado	990	910	78	10 000	4 400	186	28	15,1
Chilean								
Winter								
Rainfall -								
Valdivian		119						
Forest	397 142	143	70	3 892	1 957	41	29	70,7
Tumbes -								
Chocó -								
Magdalena	274 597	65 903	76	11 000	2 750	203	30	14,8

Table.1: Comparison of South American biodiversity hotspots. Data source: CEPF.net, 2016.

1.4. Serra da Bocaina national park and buffer area

The Serra da Bocaina national park is a protected area in one of the largest fragments of the Atlantic forest- Serra do Mar. Its position is on the border of the states of Rio de Janeiro and Sao Paulo. The size of the park is now around 1400 km² and its habitats are Atlantic rainforests and natural grasslands in the high elevation areas. The maximum altitude in the Serra da Bocaina national park reach up to 2088 m a.s.l. According to new studies, there is 68 species of amphibians in the park and its buffer area (Garey *et al.*, 2014), including eight endemics: *Brachycephalus vertebralis, Ischnocnema pusilla, Bokermannohyla ahenea, Bokermannohyla clepsydra, Scinax ariadne, Megalosia bocainensis, Physalaemus barriori and Paratelmatobius gigae* (Frost, 2013). They represent approximately 13% of Atlantic Forest anurans (Haddad *et al.*, 2013)

1.5. Amphibians in Atlantic forest

Approximately half of the global diversity of amphibians live in South America. There is over 450 amphibian species in the Atlantic forest, of which more than 60% are endemic. There is one whole endemic family, *Brachycephalidae* (Galindo Leal and de Gusmao Camara, 2003) (CEPF, 2016) and many endemic genera such as *Dendrophryniscus*, *Frostius*, *Hylomantis*, *Phrynomedusa*, *Crossodactylodes Cycloramphus*, *Euparkerella*, *Megaelosia*, and *paratelmabotius* (Haddad and Prado, 2005).

1.5.1. Amphibian world status

There is over 7 500 species of amphibians in the world (Amphibialb.org, 2016). Amphibians are currently the most threatened group of vertebrates. Over 1/3 of amphibian species is endangered and the number is still growing. Many species, 25% are also in data deficient status, which means, that there are no sufficient data to determine their threat status. Therefore more researches has to be done on this cold-blooded, ecologically important animals, to better understand their ecology and biology and use this knowledge for their conservation (IUCN RedList, 2015).

1.5.2. Biology of recorded amphibian species

This part is the summary of biological information about recorded species, their distribution range, preferred habitat, behavior, reproductive modes and status of thread.

1.5.2.1. Family Hylidae
Subfamily Hylinae:
Aplastodiscus callipygius (Cruz and Peixoto, 1985)
Common name: Bocaina TreeFrog
IUCN Status: Least concern
Trends: Decreasing

It is relatively large and robust treefrog with wide snout. Its basic coloration is green with some black and white dots on the dorsum and head. Also muscles and bones are green. The eyes are very prominent, red and yellow colored. High concentration of yellow spots in the area between hind legs. The tympanum is easy visible (Haddad *et al.*, 2008). Males have large vocal sacks, making short calls with mean duration of 0,3s. and intercall time interval of 2,8s (Abrunhosa *et al.*, 2005). Female lay eggs in narrow streams with clay bottoms (Gomes, 1996).

Bokermannohyla circumdata (Cope, 1871)

Common name: Espirito Santo Treefrog

IUCN Status: Least concern

Trends: Decreasing

The species used to belong to genus *Hyla* and was changed to genus *Bokermannohyla* recently (Faivovich *et al.*, 2005). It is relatively large tawny frog, with brown stripes on the hind legs and the side with blue and yellow background. The head is wide with slightly narrowed snout. This species can be found in the mountain areas in the states of Rio de Janeiro, Sao Paulo and Minas Gerais. The species inhabits edge of both primary and secondary forests. Female build nests in mud in forest streams, where she lay eggs (Napoli, 2005).

Dendropsophus elegans (Wied-Neuwied, 1824)

Common name: Elegant forest Treefrog

IUCN Status: Least concern

Trends: Stable

The species used to belong to genus *Hyla* and was changed to genus *Dendropsophus* recently (Faivovich *et al.*, 2005). This species occurs in large area of southeast Brazil in the elevations up to 1000 m. It inhabits both primary and secondary forest and also open areas near permanent and temporary ponds. The basic coloration is light, white or yellow with brown or red wide stripe going from behind the head through whole dorsum. Legs are orange. Female lays eggs in water (Abrunhosa *et al.*, 2006).

Dendropsophus minutus (Peters, 1872)

Common name: Lesser TreeFrog

IUCN Status: Least concern

Trends: Stable

The species used to belong to genus *Hyla* and was changed to genus *Dendropsophus* recently and it is probably a complex of more species (Faivovich *et al.*, 2005). Its basic coloration is yellow with square line ornaments on the head and dorsum. It is one of the most successful species with range covering almost whole east part of South America up to elevations of 1800 m. The species inhabits wet forests, forest edges and marshes. It can be found in almost every type of pond, both nature and artificial usually also near urban areas (Duellman, 1997). When not in reproduction period, it could be found on the branches of trees in the forest. Female lay eggs in the water ponds (Kwet, 1999). The males call from the water vegetation in the frequency of 3600 – 5800 Hz. Some males adapt the satellite strategy. Males are territorial, physical combat and different call types were observed by Haddad (1987).

Hypsiboas albopunctatus (Spix, 1824)

Common name: Spotted Treefrog

IUCN Status: Least concern

Trends: Stable

The species used to belong to genus *Hyla* and was changed to genus *Hypsiboas* recently and it is a complex of more than one species (Faivovich *et al.*, 2005). The basic coloration of this species is tawny, with light belly and yellow spots on the sides and hind legs. The eyes are bright with horizontal pupil and blue circle around tuber. There is a brown stripe on the side of the head from snout over the eye, fading behind the tympanum. This species occur in large range of southeast Brazil, usually found on vegetation near to water ponds, where it breeds. It could be also seen in urban settlements (Cochran, 1955).

Hypsiboas faber (Wied-Neuwied, 1821)

Common name: The Blacksmith TreeFrog

IUCN Status: Least concern

Trends: Stable

The species used to belong to genus *Hyla* and was changed to genus *Hypsiboas* recently and it is a complex of more than one species (Faivovich *et al.*, 2005). It occurs on the large area of east Brazil and it is one of the most successful frog, probably thanks to its big size. This species inhabits humid forest and its edge. It is large tawny frog with yellow belly and stripes on the hind legs. Reproduction is done in permanent or temporary pools and also in slow flowing streams (Abrunhosa *et al.*, 2006). IUCN shows information about the maximum level of altitude for this species – 800 m a.s.l, which is not true according to my observations. These species was found in Plato pond and also Bruna pond which are both over 1000 m.a.s.l.

Hypsiboas pardalis (Spix, 1824)

Common name: Leopard Treefrog

IUCN Status: Least concern

Trends: Stable

The species used to belong to genus *Hyla* and was changed to genus *Hypsiboas* recently and it is a complex of more than one species (Faivovich *et al.*, 2005). It occurs in the states of Rio de Janeiro and Sao Paulo and also small part of Minas Gerais. Maximum sea level is up to 1000 m.a.s.l. It inhabits forest edge and gallery forests sitting on vegetation, often around small pools. Females build clay nests and the tadpoles developed in water (Bokermann, 1968).

Hypsiboas polytaenius (Cope, 1870)

Common name: Cope's Eastern Paraguay TreeFrog

IUCN Status: Least concern

Trends: Stable

The species used to belong to genus *Hyla* and was changed to genus *Hypsiboas* recently and it is a complex of more than one species (Faivovich *et al.*, 2005). This small tree frog is prominent due to the dark brown stripes on lighter background, leading from the snout through whole dorsum. This relatively small frog is known from the region of the states of Rio de Janeiro and Minas Gerais, usually above 900 m. a. s. l. It occurs on the leaves of low vegetation. It reproduce in small ponds or slow-flowing streams. Females lay eggs, which are in large groups floating on the water surface. It is considered as highly adaptable to disturbed habitat (Canelas, 2007).

Hypsiboas semilineatus (Spix, 1824)

Common name: n/a

IUCN Status: Least concern

Trends: Stable

The species used to belong to genus *Hyla* and was changed to genus *Hypsiboas* recently and it is a complex of more than one species (Faivovich *et al.*, 2005). It is a medium sized, brownish treefrog with prominent eyes. This species occur in the wide stripe on the east coast of Brazil, inhabiting both primary and secondary forest and also disturbed areas like pastures or gardens with water and shrubs. It is usually found on the vegetation near water. Their typical breeding spots are small ponds, but sometimes they lay eggs on the shore of lakes or water reservoirs. It is considered as a highly adaptable and common species, however, it was positively tested on the presence of *Batrachochytridium dendrobatidis* infection (Toledo *et al.*, 2006b).

Scinax flavoguttatus (A. Lutz and B. Lutz, 1939)

Common name: Yellowbelly snouted tree frog

IUCN Status: Least concern

Trends: Decreasing

This species of snouted tree frog occurs on the southeastern Brazil from the State of Espirito Santo to the State Santa Catarina, in the altitudes from 100 to 1300 m. a. s. l. This frog breeds in the streams and I can often found them on the vegetation in the primary and secondary forest. It is a medium sized dark frog with light belly and yellow or orange spots on the inner side of its hind legs (Duellman and Wiens, 1992).

Scinax hayii (Barbour, 1909)

Common name: Hay's snouted tree frog

IUCN Status: Least concern

Trends: Stable

This medium sized snouted tree frog distribution range is from the State of Espirito Santo through southeastern Minas Gerais to the State of Parana. It lives in the secondary and primary forest, on the forest edge and also in some disturbed areas like parks and gardens. It breeds in small ponds, even artificial, such as swimming pools. Its color is light with yellow spots on the inner sides of hind legs (Duellman and Wiens, 1992).

Sphaenorhynchus orophilus (A. Lutz a B. Lutz, 1938)

Common name: Lutz's Lime Treefrog

IUCN Status: Least concern

Trends: Unknown

This species is endemic to Brazil, occurring in the states of Rio de Janeiro, Sao Paulo and Minas Gerais up the elevations of 1000 m a.s.l. It is a small species with green coloration with white curves on the body. It has got typical arrow shape of the head. It inhabits vegetation in the marshes and large lakes. It can breed in deep ponds, both natural and artificial. Male vocalization are short "clicks" similar to the clicker used in dog training (Bertolutzi and Rodrigues, 2002).

Subfamily Phyllomedusinae:

Phyllomedusa burmeisteri (Boulenger, 1882)

Common name: Burmeister's Leaf Frog

IUCN Status: Least concern

Trends: Stable

This species occur in the eastern Brazil from the Sergipe to Sao Paulo up to 1000 m. a. s. l. It is usually found on the vegetation near water ponds, where it breeds. It is relatively large

frog of green basic color, with blue stripes and yellow spots on the sides. Belly is white with yellow spots. Head has strange square wide shape with large eyes. This species is usually not jumping, but more often it is climbing in the trees, thanks to its long limbs and toes. Female place the eggs in the leaf nests on the vegetation above the water surface. Tadpoles after hatch fall down into water, where they further develop (Pombal, 1992). The reproduce period is from December to February and sometimes also in November (Abrunhosa *et al.*, 2006).

1.5.2.2. Family Centrolenidae

Vitreorana uranoscopa (Müller, 1924)

Common name: Humboldt's Glass Frog

IUCN Status: Least Concern

Trends: Decreasing

This frog is widely spread on the east coast of Brazil, occurring in the forest remnants in the states of Espirito Santo, Rio de Janeiro, Minas Gerais, Sao Paulo, Parana, Santa Catarina and to the border of the state of Rio Grande do Sul. It has been found in the altitudes up to 1200 m a.s.l. (Heyer, 1985). Treefrog of the group of so-called "glass frogs" for their transparent skin. Basic coloration of this species is semi-transparent green with white and black spots on the dorsum and with transparent belly. Fingers are yellow. Its habitat are primary and secondary forests and forest edge, usually near running water and waterfalls. Female of this species lay eggs on the leaves above water and the tadpole continue the development, when it falls to the water. This species is not tolerant to disturbed and open areas. Male vocalization is really short, high and loud "tip" sound, but easily to be misheard in the noise of the waterfall (Stetson, 2001).

1.5.2.3. Family Craugastiridae

Haddadus binotatus (Spix, 1824)

Common name: Clay Robber Frog

IUCN Status: Least concern

Trends: Stable

This species is known from the east coast of Brazil and occur in elevations up to 1200 m a.s.l. and it is probably a complex of more species (Forlani *et al.*, 2010). Its habitat are dense primary and secondary forests. It usually avoid open forests and pastures. This frog is not a good climber and most often it is found in the leaf litter on the ground. This species is using direct development and therefore it do not need water for reproduction (Coco *et al.*, 2014).

1.5.2.4. Family Hylodidade

Hylodes asper (Spix, 1824)

Common name: Brazilian torrent frog

IUCN Status: Least concern

Trends: Stable

This species occur on the east coast of Brazil, in the states of Rio de Janeiro, Sao Paulo and Paraná, up to 1200 m a.s.l. It is a small frog of blackish and greenish color, with blue markings on their hind leg. It has very strong suckers at the end of their fingers, which help the frog to hold on slippery stones protuberant from the river. It lives on the rocks or vegetation near small rivers or streams, where it also breeds. Tadpoles of this frog live in the stream. It is usually found near some waterfalls. Probably due to the noise of the waterfall, males of this species use also visual signals together with vocalization. They stretch their hind leg above their body and show the blue markings on their thigh to the females (Haddad and Giaretta, 1999).

1.5.2.5. Family Brachycephalidae

Ischnocnema guentheri (Steindachner, 1864)

Common name: Steindachner's robber frog

IUCN Status: Least concern

Trends: Stable

In is a small frog of very various coloration from light brown to dark green and black, with various ornaments and sometimes a light dorsal strip. It can be identified by small black strip behind the eye. It lives mainly in the primary and secondary forests of the states of Rio de Janeiro and Sao Paulo. It is not tolerant to more open areas. This species breeds by direct development. Females lay eggs in small clutches in the leaf litter (Heyer, 1984).

Ischnocnema verrucosa (Reinhardt and Lütken, 1862)

Common name: n/a

IUCN Status: Data deficient

Trends: Unknown

This species was known only from three observations. One in Minas Gerais State and two from Espirito Santo State. All founds were in around 700 m a.s.l. It is a small frog with wrinkled skin and brown-orange-greenish coloration. It has got two specific orange spines on the head and red eyes. Not many thing about ecology and behavior of this species is known, but it is believed that it breeds by direct development, as its close relatives from the area (Canedo *et al.*, 2010).

1.5.2.6. Family Leptodactylidae

Leptodactylus fuscus (Schneider, 1799)

Common name: Rufous frog

IUCN Status: Least concern

Trends: Stable

This frog is widespread throughout the northern part of the South America in the states of Brazil, Venezuela, Guyana, Suriname, Trinidad and Tobago, French Guayana, Colombia, Panama, Bolivia, Paraguay and also small east parts of Peru and northern part of Argentina to the elevations of 1700 m a.s.l. This species is brown or grey color witch dark spots on the body and head and light snout-vent stripes on the dorsum. Auricular membranes are highly visible, as it is typical for the frogs of the genus *Leptodactylus*. It is a relatively common ground frog inhabiting open areas such as pastures, savannas, marshes, degraded forests and urban areas. Male vocalization are short whistle sounds in short intervals (Brasileiro and Oyamaguchi, 2008). Females lay eggs in the foam nests on the vegetation near water. When the nest is flooded, tadpoles escape to the water, where they further develop (Maragno and Cechin, 2009). It is highly adaptable species, which is tolerant to disturbed and modified habitats (Brasileiro and Oyamaguchi, 2008).

Leptodactylus labyrinthicus (Spix, 1824)

Common name: Labyrinth frog

IUCN Status: Least concern

Trends: Stable

This species occur in the Brazil, Paraguay, Bolivia and northern Argentina, inhabiting Cerrados, gardens, pastures, marshes and open forests up to 1000 m a.s.l. It is a very large species eating every animal that fits in its mouth. Its coloration is orange-brown with typical short black stripes around the mouth. It's a very adaptable species which lay eggs in foam nests. Tadpoles than live in ponds (Carvalho *et al.*, 2013).

Leptodactylus latrans (Steffen, 1815)

Common name: Criolla frog

IUCN Status: Least concern

Trends: Stable

This species is occurring on the large part of South America, with two synonym names *I. ocellatus* and *I. macrosternum.* It can be found in Argentina, Brazil, Bolivia, Colombia, French Guiana, Guyana, Paraguay, Suriname, Trinidad and Tobago, Uruguay and Venezuela up to the elevation of 1400 m a.s.l. It is a large frog with gray color with dark spots. This species is also very adaptable and inhabits different habitats such as pastures, savannas, marshes, gardens and open forests. The eggs are laid in foam nests and the female is guarding the eggs and the tadpoles from predators for few Weeks, using its masculine front legs and large mouth (Lavilla *et al.*, 2010).

Adenomera marmorata (Steindachner, 1867)

Common name: n/a

IUCN Status: Least concern

Trends: Stable

This species is endemic to Brazil, occurring in the states of Minas Gerais, Paraná, Rio de Janeiro and Sao Paulo up to elevations of 1200 m a.s.l. It is a small frog of black and brown color with light belly. It is a diurnal species inhabiting primary and secondary forests. It lay eggs in the leaf litter and tadpoles developed here. So it is vitally bounded to the forest and cannot therefore breed in disturbed areas (Almeida-Gomes *et al.*, 2007).

1.5.2.7. Family Odontophrynidae

Proceratophrys boiei (Wied-Neuwied, 1824)

Common name: Boie's frog

IUCN Status: Least concern

Trends: Stable

This species occurs only in Brazil, in the states of Alagoas, Bahia, Espirito Santo, Paraná, Pernambuco, Rio de Janeiro, Santa Catarina, Sao Paulo and Sergipe up to the elevations of 1200 m a.s.l. It can be found in the primary and secondary forests, in the leaf litter and reproduce in the slow-flowing forest streams. It has got rounded short snout, wide body and short legs. Its coloration is very variable with different ornaments. The basic color is brown with light belly. It has got dermal growths above eyes and usually not on the top of the snout, as it is in the other species. The size and shape is very variable both intraspecific and interspecific in the genus. Dorsum is covered with warts (Prado and Pombal, 2008). It is very similar to other two species occurring in the similar area and habitat – *P. apendiculata* and *P. melanopogon* and the identification is usually done by molecular biology or comparison of RTG of the cranium (Izecksohn *et al.* 2005)².

Proceratophrys apendiculata (Günther, 1873)

Common name: Gunther's Smooth Horned Frog

IUCN Status: Least concern

Trends: Decreasing

² Author's note: Identification was possible even without genetic material, because I had found all 3 species and I could compare the specimens.

This species occur in the Brazil states of Rio de Janeiro, Sao Paulo, Espirito Santo and Paraná between the elevations of 500-1700 m a.s.l. It can be found in primary and secondary forest. This species is not tolerant to open or disturbed areas. It breeds in the slow-flowing streams. It can be distinguished from *p. boiei* and *p. melanopogon* because of the presence of usually small sharp snout appendix and also eye dermal horns are smaller and sharper (Boquimpani-Freitas *et al.*, 2001). More significant identification can be done by molecular DNA analysis or cranial comparison (Izecksohn *et al.*, 2005).

Proceratophrys melanopogon (Günther, 1873)

Common name: Horned Frog

IUCN Status: Least concern

Trends: Decreasing

The third species of the genus *Proceratophrys* is found in the states of Rio de Janeiro, Sao Paulo and Minas Gerais in the elevations between 500-1300 m a.s.l. It breeds in the permanent streams in the primary and secondary forest. It has got large snout appendix and also larger eye horns. The coloration is brown with white belly but black ventral part of the head (Izecksohn and Peixoto, 1998).

1.5.2.8. Family Bufonidae

Rhinella icterica (Spix, 1824)

Common name: Yellow Cururu Toad

IUCN Status: Least concern

Trends: Stable

This large toad species is known from the Brazil, Argentina and Paraguay, where it occur on the elevations between 0-1200 m a.s.l. It has one of the largest diversity of habitats among amphibians, from dry Cerrado to It forest and agricultural area. It is highly adaptable to disturbed areas. It can breed both in flowing water and ponds, both natural and artificial, both temporary or permanent (Kwet and Di-Bernardo, 1999).

Rhinella ornata (Spix, 1824)

Common name: n/a

IUCN Status: Least concern

Trends: Unknown

This species of toad occurs in the states of Rio de Janeiro, Sao Paulo, Espirito Santo and Paraná up to the elevations of 1000 m a.s.l. It can be found near the temporary pools and in the forest and its edge. It breeds in the lentic water (Abrunhosa *et al.*, 2006).
2. Aims of the thesis

The main aim of my research was to identify anuran diversity in different elevations in selected parts in the buffer area of Serra da Bocaina NP, SE Brazil. Another objectives were to compare habitat preferences of recorded species and to compare 3 selected model species (*Aplastodiscus callipygius, Adenomera marmorata, Haddadus binotatus*) distribution maps, available on their IUCN Red List account, with the actual tree cover satellite maps (preferred habitat maps) in the study area, using Tree Cover Landsat maps. I have estimated hypothesis for this revision, that use of the Tree Cover Landsat maps (preferred habitat).

3. Materials and methods

3.1. Study area

The research was conducted in several private parts of Mata Atlantica forest, on the territory of the State of Sao Paulo, near Serra da Bocaina National Park, South-east Brazil (Viz. 3.3.1, 3.3.2., 3.3.3., 3.4.1., 3.4.2., 3.4.3., 3.4.4., 3.4.5.). The area has very diverse terrain and include different environments such as pastures, rain forests, highland forests and water areas. Stream density in the whole area is also very high and together with the frequent rains in the rain season, ensures a constant moisture in the forests. Compared to that, pastures between these forests were really dry, forming a big obstacle for amphibian movement.

3.2. Weather during data collection

I have started my research on 6th February 2015 till 6th March 2015, when the main rain season was in full swing. For first two weeks, almost every evening came storm with heavy rain, but with short duration, in approximately 2 hours. Rest of day was very warm with high temperatures above 35 °C. In the night temperature dropped down to 20-25 °C. On the high elevations, sometimes even bellow 20 °C. After first two weeks, there was a pause with no rain for few days and then it started to rain as usually, but mostly without storms.

3.3. Data collecting methods – Transects

Data were collected on three linear transects of equal length (around 1000m), each leading through different habitats and in different elevations. Each transect was searched three times in the day and three times in the night, always by three people. Search area was approximately 3 meters buffer area around transect. Day searching started in 7:00 am and ended in 10:00 am. Night searching started at 10:00 pm and ended in 1:00 am. Those habitats

were pastures or urban area, pasture/forest edge, forest. (Detail description of transects viz. 3.3.1, 3.3.2, 3.3.3.). Individuals were searched by vision (using head torches in the night) and also by listening to acoustic signals, produced by male vocalization. Individuals were catcher by hand, using wet gummy gloves to avoid spreading of diseases and parasites or by net. Captured individuals were placed separately in the plastic bags, for easier manipulation. The plastic bags were perforated to ensure air intake for the amphibian inside. After that I identified the species or take a photo for future identification and noted following data: Snoutvent length, using caliper with the accuracy of a tenth of a millimeter, GPS position, using mobile phone (HTC Desire Z) and free GPS map application (Locus Free), with offline vector maps, position of the individual (Ground, water, tree), activity (Vocalization, active, sleeping), age (juvenile, sub adult, adult), approximate distance from water, health of the animal and weather. Recapture can be identified using the lengths comparison.

Each day on each transect, there was randomly chosen one square of 5 meters, lying on transect, which was twice (day and night) invasively explored in detail. Process of the capture and measuring of the amphibians was the same. Each square was labeled with first letter of the transect name and the number of the square: x1, x2, x3, p1, p2, p3, b1, b2, b3.

3.3.1. Transect I - Xandoca

First transect (Fig. 3) started at the private lonely estate, leading through garden (Fig. 4) to the forest (Fig. 5). Altitude of transect was around 800 meters above the sea level and the terrain is very dynamic. First part copy the driveway to the house. This way is surrounded by fruit trees such as mangos and limes and also by some bushes, weeds and bromeliads on the trees. There is a stream on the right side of transect and behind that stream rises hillside covered with long grasses. After first crossing of the stream, on the edge of the forest, there is also some bamboo around the trail. When entering the forest, trail continue down to the stream, leading through rocky path plummeting to the stream. This stream is very rocky, fast flowing and its creek bed is approximately 5 meters wide. After crossing the stream near the small waterfall, there is rocky path rising. Transect continues on the pre-cut old trail, leading

above the stream on the hillside and cross two more small streams. The trees around all the way through the forest were different size. There were trees about more than 20 meters high, medium high trees up to 10 meters with connected canopy and small trees or bushes up to 5 meters. Epiphytes were not much abundant on the trees. The lowest vegetation zone consist mostly of small green individual plants, often with large leafs and also many creepers. The ground is covered with leaf litter of about 5 cm deep on average. Also lot of rotten wood is lying on the ground and some rocks were present occasionally. A lot of invertebrates is present in the forest. Mainly arachnids, crickets, cockroaches and millipedes and also some small flying insects. GPS: 22°44'5.65"S, 44°23'35.83"W.



Figure 3: Scheme map of the first transect. Created using google.com satellite maps and ArcGis 10.2.2.



Figure 4. Photo of the garden part, transect I.



Figure 5: Walking in the forest part of the first transect.

3.3.2. Transect II - Plato

Second transect (Fig. 6) is placed on plateau, 1000 meters above the sea level and the terrain is almost flat. It starts on pasture (Fig. 7) with two natural water ponds. Grass on the pasture is sometimes grazed by cows and horses and wild animals. This pasture is from all sides surrounded by forest. It was used by the buffalos in the past. The buffalos completely destroy the forest edge. The trail enters the forest on straight path. First 200 meters is the transect leading through very open area almost without low and middle vegetation. On this part of transect, there were usually only high trees with connected canopy or some very high trees with individual canopy and just very few shrubs, small trees or green plants (Fig. 8). More epiphytic plants can be found there, compared to the previous transect. The leaf litter on this part is up to 5 cm deep. Ground is also often covered with rotten wood and only few small rocks can be found around the stream. Transect continues following the stream at the opposite direction of its flow. Vegetation here is now really different. High trees remain, but more vegetation appear as transect is going further. Approximately from the half of the forest part of transect, the trail must have been newly cut, because of very dense vegetation including creepers, shrubs and large-leaved plants. Also the leaf litter here is deeper, up to 10 cm. On this part transect several times cross the stream, due to easier movement and less invasion in the habitat. GPS: 22°44'54.41"S, 44°24'6.70"W.



Figure 6: Second transect scheme map. Created using google.com satellite maps and ArcGis 10.2.2.



Figure 7: Photo of the pasture part of the second transect.



Figure 8. Forest part of the second transect.

3.3.3. Transect III - Bruna

The third transect (Fig. 9) is in the highest altitude – around 1200 meters above the sea level. The terrain is almost flat. It starts on the edge of the small ranch copying the road and the riverside, on the left of the road. Cattle was present on the road (Fig. 10). On the right there is forest all the way. Second half leads through the forest, which is completely different from both previous transects (Fig. 11). The forest is open, so the movement is easy, almost without need of cutting. The trees were high and medium sized, mainly with connected canopy. Almost all the trees were wrapped by epiphytic plants, mainly bromeliads. Low vegetation zone is not as dense as on previous transects and shrubs appears only occasionally. Leaf litter depth range from 5 to 10 cm. Lot of rotten wood cover the ground, but almost no rock can be find in this area. Except the river, there were no streams in the area, but one small forest pond. At the end, transect again leave the forest and continue on the road. GPS: 22°49'59.77"S, 44°27'22.27"W.



Figure 9. Scheme map of the third transect. Created using google.com satellite maps and ArcGis 10.2.2.



Figure 10: Cattle presence on the road part of third transect.



Figure 11: Forest part of the third transect.

3.4. Data collecting method - Breeding sites

Research also include detailed, but non-invasive examination of water ponds, which have been found used by some species of amphibians, as their breeding sites. Species occupying the pond were determined by visual and acoustic contact. I also estimate quantity percentage of present species (males only), according to number of voices. Surrounding area, position, type and vegetation cover were described in each pond.

3.4.1. Xandoca pond 1

The first pond is artificial and it is located directly next to the non-frequent road and it is surrounded by urban buildings and pastures in the altitude of 500 m above sea level (Fig. 12). It is fed by small stream and its size is about 50 square meters. Cattle is present here, so it could be used by it. Closest continuous forest edge is about 200 m distance from the pond. Shores of the pond were covered with reeds and shrubs. Some reeds were also directly in the water. About one half of the pond is covered with water vegetation, the other is clear water surface. The maximum depth of the pond is about 1 m and the bottom is covered with deep mud. GPS: 22°43'53.2"S, 44°22'33.5"W.



Figure 12: Scheme of the Xandoca 1 breeding pond and surrounding area. Created using google.com satellite maps and ArcGis 10.2.2.

3.4.2. Xandoca pond 2

The second pond is located on the pasture between two parts of continuous forest in the altitude of 850 m above the sea level (Fig. 13). Its size is about 15 square meters. The distance of the forest edge is about 50 m. Cattle and horses were present. One third of the pond is covered by water vegetation and there were few small trees on the shore of the pond. The maximum depth is about 50 cm with shallow mud cover on the bottom. The pond is not fed by any stream, so it could maybe dry out in the dry season. GPS: 22°44'16.9"S, 44°24'03.5"W.



Figure 13: Scheme of the Xandoca 2 breeding pond and surrounding area. Created using google.com satellite maps and ArcGis 10.2.2.

3.4.3. Plato pond

The third breeding site is located on the pasture, which is surrounded by continuous forest in the altitude of 1000 meters above the sea level (Fig. 14). The pond consist of two parts one with open water surface and the other is in half covered by water vegetation. The two ponds could by connected in the rain season. Maximum depth in both ponds is about half

meter and the bottom is covered with shallow mud. The cattle and buffalos were present in the past, now the pond could be used by some wild animals such as peccaries or tapirs. The pond is not fed by any stream, so it could maybe dry out in the dry season. GPS: 22°'44"47.7S, 44°24'01.8"W.



Figure 14: Scheme of the Plato breeding pond and surrounding area. Created using google.com satellite maps and ArcGis 10.2.2.

3.4.4. Bruna pond

The third breeding side is a swamp located in the agricultural area in the altitude of 1200 meters above the sea level (Fig. 15). The swamp is fenced, so wild animals has no access. It is highly used by cattle and horses, but only on the shores. There is almost no clear water surface. The water surface is mostly covered with floating carpet of vegetation. The maximum depth under the carpet is about one meter. The bottom is in some parts covered with deep mud, but mostly there is only shallow mud. The swamp is fed by small stream. GPS: 22°49'59.78"S, 44°27'21.09"W.



Figure 15: Scheme of the Bruna breeding pond and surrounding area. Created using google.com satellite maps and ArcGis 10.2.2.

3.4.5. Lucia pond

The last breeding is located in the urban area, near to frequent road, directly in the fenced surroundings of one populated building in the altitude of 500 meters about the sea level. Horses, cattle and dogs were present. The breeding sites consist of two ponds, which were separated by the driveway to the house (Fig. 16). Shores were covered with reeds, which cover also approximately one half of the water surface. Ponds were fed by small stream. The maximum depth is about one meter. The bottom is covered by deep mud. GPS: 22°39'56.3"S, 44°16'19.0"W.



Figure 16: Scheme of the Lucia breeding pond and surrounding area. Created using google.com satellite maps and ArcGis 10.2.2.

3.5. IUCN Species distribution maps revision

3.5.1. Data source

Geographic data of species distribution were downloaded from IUCN RedList web pages, where it is available for free, for non-commercial use. The surface reflectance maps including the background satellite maps, were downloaded through the web pages of GLFC – Global Land Cover Facility. As data source, was chosen the NASA Landsat Imagery, which is monitoring the Earth surface with six satellites, since 1972. The datasets were selected using map-search. Six nearest squares to the study area were selected. Landsat Tree Cover dataset were selected and downloaded with geographic data of each square. HydroSHEDS (RIV) – South American River Network data were downloaded using data search tool in the ESRI ArcMap Software, version 10.2.2.

3.5.2. Creating maps

The data were processed in ESRI ArcMap Software, version 10.2.2. Both datasets, species distribution and NASA Landsat Imagery Tree Cover Were connected in one map. The species data were already in vector version, but the Landsat Tree Cover data were manually vectorised, creating new shapefile. Symbology was selected, legend created and the map was exported.

3.6. Diversity indices

I have calculate Shannon-Wiener diversity index and also Simpson's diversity index to compare amphibian diversity between three selected transects in different elevations.

Shannon-Wiener diversity index (Shannon and Weaver, 1948):

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

P_i= Number of individuals of species/total number of individuals

In= Natural logarithm

Simpson's index (Simpson, 1949):

$$l = \frac{\sum_{i=1}^{R} n_i (n_i - 1)}{N(N - 1)}$$

n_i= Number of individuals of particular species

N= Total number of species

4. Results

4.1. Species

Table 2: Table of recorded species, their IUCN Status and trend

Family	Subfamily	Genus	Species	IUCN Status	Trend	
		Aplastodiscus	callipygius	LC	Decreasing	
		Bokermannohyla	circumdata	LC	Decreasing	
		Dendropsophus	elegans	LC	Stable	
		Dendropsophus	minutus	LC	Stable	
		Hypsiboas	albopunctatus	LC	Stable	
		Hypsiboas	faber	LC	Stable	
		Hypsiboas	pardalis	LC	Stable	
Hulidaa	Hylinae	Hypsiboas	polytaenius LC		Stable	
пушае		Hypsiboas	semilineatus	LC	Stable	
		Hypsiboas	-	-	-	
		Scinax	spp.1	-	-	
		Scinax	flavoguttatus	LC	Decreasing	
		Scinax	hayii	LC	Stable	
		Scinax	spp.2	-	-	
		Sphaenorhynchus	orophilus	LC	Unknown	
	Phyllomedusinae	Phyllomedusa	burmeisteri	LC	Stable	
Centrolenidae	Centroleninae	Vitreorana	uranoscopa	LC	Decreasing	
		Ischnocnema	guentheri	LC	Stable	
Brachycephalidae		Ischnocnema	verrucosa	DD	Unknown	
		Ischnocnema	spp.	- - LC LC - LC LC LC LC LC LC LC LC LC LC		
Craugastoridae		Haddadus	binotatus	LC	Stable	
Hylodidae		Hylodes	asper	LC	Stable	
		Leptodactylus	fuscus	LC	Stable	
Lantodactulidao		Leptodactylus	labyrinthicus	LC	Stable	
Leptodactyndae		Leptodactylus	latrans	LC	Stable	
		Adenomera	marmorata	LC	Stable	
		Proceratophrys	boiei	LC	Stable	
Odontophrynidae		Proceratophrys	apendiculata	LC	Decreasing	
		Proceratophrys	melanopogon	LC	Decreasing	
Dufanidaa		Rhinella	icterica	LC	Stable	
Buronidae		Rhinella	ornata	LC	Unknown	

I have found 31 species in total and identificated 27 of them. Thirteen species Were found only on transects, fourteen only around and in the breeding ponds, two was both on transect and on breeding site and two outside the methodology, found by random encounter. Found species belong to 8 families (Tab. 2). The majority of the species (16) belongs to family Hylidae, others to *Hylodidae* (1), *Centrolenidae* (1) (Fig. 17), *Bufonidae* (2), *Odontophrynidae* (3), *Craugastoridae* (1), *Brachycephalidae* (3), *Leptodactylidae* (4) (Fig. 18). In three found frogs I Were able to classify them only to genus.



Figure 17: Photos of recorded species of the families Bufonidae (a-b), Leptodactylidae (c-e), Craugastoridae (f), Brachycephalidae (g-h) and Odontophrynidae (i-k). a) *Rhinella ornata*, b) *R.icterica*, c) *Adenomera marmorata*, d) *Leptodactylus fuscus*, e) *L.latrans*, f) *Haddadus binotatus*, g) *Ischnocnema guentheri*, h) *I.verrucosa*, i) *Proceratophrys boiei*, j) *P.melanopogon*, k) *P.appendiculata*. I)³

³ I lack the photo of *Leptodactylus labyrinthicus*, because it was not taken, before I lost only specimen I saw.



Figure 18: Photos of recorded species of the families Hylidae (a-m), Centrolenidae (n) and Hylodidae (o). a) *Hypsiboas polytaenius*, b) *Phyllomedusa burmeisteri*, c) *Hypsiboas faber*, d) *H.albopunctatus*, e) *H.semilineatus*, f) *Dendropsophus elegans*, g) *Hypsiboas pardalis* (juvenile), h) *Sphaenorhynchus orophilus*, i) *Aplastodiscus callipygius*, j) *Scinax flavoguttatus*, k) *Dendropsophus minutus*, I) *Scinax hayii*, m) *Bokermannohyla circumdata*, n) *Vitreorana uranoscopa*, o) *Hylodes asper*

4.2. Results on transects

During all 18 surveys on transects, 6 on each, 3 days, 3 nights, I have recorded 57 individuals, belonging to fifteen species. Only 12 species were identified, 3 species remains unrevealed. In total, most individuals were found during the night surveys (52) and only 5 individuals during the day surveys. Only one species, *Proceratophrys apendiculata*, was only found during the day surveys, all other species were found in the night or both in day and night. Only one species, *Ischnocnema guentheri*, was found on all three transects. It has got very variable coloring and the change for other species of *Ischnocnema* genus is likely possible, when the identification is not confirmed by genetic data.

SPECIES	Transect I Xandoca	Transect II Plato	Transect III Bruna	Sum
Bokermanohyla circumdata	3	0	0	3
Hypsiboas polytaenius	0	0	1	1
Scinax flavoguttatus	2	0	7	9
Scinax spp.1	14	0	0	14
Scinax spp.2	0	1	0	1
Ischnocnema spp.	0	0	1	1
Ischnocnema guentheri	1	4	1	6
Ischnocnema verrucosa	1	0	0	1
Haddadus binotatus	5	0	0	5
Rhinella icterica	1	0	5	6
Rhinella ornata	2	0	0	2
Proceratophrys boiei	1	1	0	2
Proceratophrys appendiculata	0	1	0	1
Proceratophrys melanopogon	1	1	0	2
Vitraorana uranoscopa	3	0	0	3
Sum	34	8	15	57
Species	11	5	5	<u>15</u>
Transect-unique species	6	2	2	10

Table 3: Table of species recorded on transects, number of individuals and transect-unique species.

Other 4 species, were found on two transects. *Scinax flavoguttatus* – Xandoca and Bruna transects, *Rhinella icterica* – Xandoca and Bruna transects and *Proceratophrys melanopogon* – Xandoca and Plato transects. *Proceratophrys boiei* – Xandoca and Plato. The rest of the species is unique to one transect. I have not record any recapture, except in *Scinax spp*.₁, where the snout went lengths were very close, so there was a possibility of recapture. In one species, *Ischnocnema verrucosa*, I have update the range for around 100 km, from its IUCN distribution map.

4.2.1. Transect I – Xandoca results

Most species (11) and individuals (34) were found on the first transect, Xandoca (Fig. 20). The habitat preference of the species is visible in the graph (Fig. 19). The forest here was very moist and It and dense. Also the terrain was more dynamic than on other two transects. Six species were ground species, easy to find. Other species were usually found in the trees. Some were hidden high in the trees, especially Vitreorana uranoscopa, which was recorded just by acoustic male vocalization during the last night survey. After few days it was also found by visual contact and finally identified. The most numerous species was the unidentified *Scinax spp.*₁, which was found in large numbers only around one small waterfall. Only two individuals, one Ischnocnema quentheri and one Rhinella ornata were found during the day survey. For I. quentheri it was the only record on this transect. Only one individual of Rhinella icterica was found on the garden part of transect. Largest species in the forest was Bokermannohyla circumdata, found in 2 individuals, but each on different side of the forest transect. Also two individuals of Scinax flavoguttatus were found in different parts. Two individuals, one Proceratophrys boiei and one Haddadus binotatus were found on the first selected square – x1 and one another specimen of *Haddadus binotatus* on the second square -x2. No frog was recorded on the third square. Outside the transect survey, I have found in the forest two more species, which I later also find on the Plato transect – Adenomera marmorata and Scinax spp.2.



Figure 19: Species habitat preference according to my records on Xandoca transect. Created using ArcGis 10.2.2.



Figure 20: Graph comparing the number of individuals and the number of new species found each survey on the Xandoca transect.

4.2.2. Transect II – Plato results

Eight individuals in five species were found on the second transect (Fig. 22). The habitat preference of the species is visible in the graph (Fig. 21). One species was not identified yet (*Scinax spp.*₂), belonging likely to the genus *Scinax*. The most common species was Ischnocnema guentheri. All 4 individuals were freshly metamorphosed juveniles. All 3 species of the genus *Proceratophrys* (*P. Apendiculata, P. Boiei, P. Melanopogon*), occurring in the area of Serra da Bocaina national park, were found on this transect, each in just one individual. More individuals were found later, not during the survey. This group is very diverse in coloration and eye and nose appendices. Identification of these frogs is not easy and the individuals found during my survey were also very diverse and there is a possibility, they belong to more than 3 species. Genetic material will be needed for study of those frogs. All of the specimens were found on the open forest part of transect. I have not found any frog on the freshly cut trail in the dense vegetation. Only one frog, *Ischnocnema guentheri*, was found on one square – p1. Nothing was recorded on other two squares.



Figure 21: Species habitat preference according to my records on Plato transect. Created using ArcGis 10.2.2.



Figure 22: Graph comparing the number of individuals and the number of new species found each survey on the Plato transect.

4.2.3. Transect III – Bruna results

I have recorded five species on the third transect (Fig. 24). Fifteen individuals was found in total. The habitat preference of the species is visible in the graph (Fig. 23). All frogs were found during the night surveys. I have not recorded any frog in the day. The reason for that is most likely the high humidity difference between day and night. Days were very hot and dry, but in the night the temperature fall down by 10° C and the humidity was also significantly higher, after evening rains. Five individuals of the species *Rhinella icterica* were found on the side of the road, first part of transect, together with one individual of *Hypsiboas polytaenius*, which was a juvenile in froglet stage. The most numerous species, 7 individuals, was *Scinax flavoguttatus* species. The coloration of these frogs was slightly different than in the same species on the first transect. The spots on the inner side of their legs seemed to be rather yellow than orange, as it was in the Xandoca population. One individual of the lschnocnema belongs to the genus *Ischnocnema* which is complicated and in many species, there is no any photo or characterization of the species. Only one individual was found on one square – b1. It was the unidentified *Ischnocnema spp*.



Figure 23: Species habitat preference according to my records on Bruna transect. Created using ArcGis 10.2.2.



Figure 24: Graph comparing the number of individuals and the number of new species found each survey on the Bruna transect.

4.3. Diversity indices

For the diversity I have calculated two biodiversity indices: Shannon-Wiener index and Simpson's index. For Shannon-Wiener index calculation I used natural logarithm (In). As you can see in the table (4), Shannon-Wiener index shows the first transect as one with the highest diversity of amphibians. Second is Plato and third Bruna transect. Surprisingly, the diversity is decreasing with increasing of the altitude. On the other hand Simpson's index shows that Plato transect has highest diversity, Xandoca on second and Bruna again on third place (Tab. 4).

Table 4: Table comparing Shannon-Wiener index and Simpson's index of biodiversity, for each transect. When the number of Shannon-Wiener index increases, biodiversity also increases, while when the Simpson's index increases the biodiversity decreases.

Transect	Xandoca	Plato	Bruna
Shannon- Wiener index	1.82	I.43	I.26
Simpson´s index	0.19	0.11	0.30

4.4. Breeding sites

Sixteen species in total were recorded in the area around five selected breeding sites, in different elevations. Individuals were not counted, because such counting would meant invasive searching in the breeding ponds, which would lead to disturbance of reproducing frogs and cause damage to their eggs and tadpoles. Three species, *Dendropsophus minutus*, *Hypsiboas faber* and *Rhinella icterica* were the only species found on all five breeding pond areas. *Dendropsophus elegans*, was recorded on all breeding ponds except Xandoca 1. Nine species, *Aplastodiscus callipygius*, *Hypsiboas pardalis*, *Hypsiboas semilineatus*, *Leptodactylus fuscus*, *Leptodactylus labyrinthicus*, *Leptodactylus latrans*, *Adenomera marmorata*, *Hypsiboas* *spp.* and *Sphaenorhynchus orophilus* were unique only for one breeding pond. Last three species, *Hypsiboas polytaenius*, *Phyllomedusa burmeisteri* and *Hypsiboas albopunctatus* were found on two of five breeding ponds. One species was not identified yet, belonging to the genus *Hypsiboas*, probably relative to *Hypsiboas pardalis* species, but with different coloration and body shape (Fig. 25, Tab. 5).



Figure 25: Graph illustrating the number of selected breeding ponds occupied by particular species.

Table 5: Table of species presence on selected breeding ponds

Species	Xandoca A	Xandoca B	Plato	Bruna	Lucia
Aplastodiscus callipygius				•	
Dendropsophus elegans		•	•	•	•
Dendropsophus minutus	•	•	•	•	•
Hypsiboas albopunctatus			•		•
Hypsiboas faber	•	•	•	•	•
Hypsiboas pardalis				•	
Hypsiboas polytaenius	•			•	
Hypsiboas semilineatus					•
Leptodactylus fuscus					•
Leptodactylus labyrinthicus					•
Leptodactylus latrans					•
Adenomera marmorata			•		
Phyllomedusa burmeisteri	•	•			
Hypsiboas spp.					•
Rhinella icterica	•	•	•	•	•
Sphaenorhynchus orophilus				•	

4.5. IUCN Maps Update, revision

I have chosen 3 model species for IUCN maps revision. I was comparing their habitat, forest cover, with their distribution map on IUCN Red List pages. On the following maps (Fig. 26-28) you can see, that the hypothesis estimated for this revision was confirmed. Actual distribution of those 3 selected model species (*Aplastodiscus callipygius, Adenomera marmorata, Haddadus binotatus*) were after permeation with the Tree Cover Landsat maps

significantly reduced and the habitat is also fragmented, which suggest, that the populations of those species are also severely fragmented.



Figure 26: Revised IUCN map of Aplastodiscus callipygius distribution. Created using IUCN Aplastodiscus



Figure 27: Revised IUCN map of *Adenomera marmorata* distribution. Created using IUCN *Adenomera marmorata* species distribution map dataset and GLFC Tree Cover satellite maps and ArcGis 10.2.2.



Figure 28: Revised IUCN map of *Haddadus binotatus* distribution. Created using IUCN *Haddadus binotatus* species distribution map dataset and GLFC Tree Cover satellite maps and ArcGis 10.2.2.

4.6. New record of Ischnocnema verrucosa species

I have found two specimens of *Ischnocnema verrucosa*. One during the night survey on transect I and the other one on the same transect, but not during survey. Both individuals were found on leaf of low vegetation. The border of the distribution map of this species is 140 km far away from my record (Fig. 29). Both frogs were of green-brown-orange coloration, with two specific spines on the head. Both had red eyes. When disturbed, they pressed down to the substrate. I also observe changes in coloration and structure of their skin.



Figure 29: Distribution map of *Ischnocnema verrucosa* with my record of this species. Created using IUCN *Ischnocnema verrucosa* species distribution map dataset and GLFC Tree Cover satellite maps and ArcGis 10.2.2.

5. Discussion

The main purpose of this study was to identify anuran biodiversity in the selected area of Atlantic Forest – Serra do Mar fragment, buffer area of Serra da Bocaina National Park. The line transect methodology was found effective on particular species, which I have recorded. Only five frogs of total 57 individuals (2.85%), recorded on transects, were found during the invasive search on 9 selected squares, which were invasively searched (Ch. 3.3.). Such a small number can confirm us, that the error of line transect methodology – missing, overlooking some individuals, is minimal. I could have probably missed species, which live only in very high altitudes or specific microhabitat or which are active in different time over year, so I could not record them by acoustic identification. In the next study, one or two transects in the higher altitudes should be included, because many anuran species occur in minimal altitude of 1300 m a.s.l and higher (Garey *et al.*, 2014). More time will be needed for the high-altitude transects, because those locations in the study area are very hard to access, because of the difficult terrain (Steep slopes, rocks, waterfalls) and dense vegetation.

Fifty-seven individuals belonging to 15 species were recorded on transects. The number of individuals, recorded on transect, could be influenced by the weather. I have recorded, that anurans of this area were mostly active at the night, few hour after rain. They were less active, when it was not raining before and also during the rainfall. For more quantitative and qualitative result, I should repeat the study in the same time period after one or more years.

Second methodology was non-invasive species identification on the selected breeding sites. Mostly different species were recorded on these breeding sites, than those on transect. This methodology cover species, which were not present in the forest, because they migrate in the purpose of reproduction or they permanently live near water on grasslands. I have recorded 16 species in total on the breeding sites, 14 of them was not found in the forest. Without the breeding site methodology, I would have missed those species. This point out,

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that conservation of grasslands and especially small temporary and permanent water bodies which are present in those habitats is also important, same as the conservation of the forest biome. For example in the Xandoca valley (Transect I, II), I have recorded only 3 breeding ponds for the whole area and one of them was artificially created. Larger study in the scale of time over year and range should be conducted, to get more qualitative and quantitative data. I will get also more data by sampling tadpoles (Silva, 2010), although I did not find many of them during my research, probably because the breeding season of most species was just at beginning during my research (Haddad *et al.*, 2013).

According to the new and historical studies, there was 68 species of anurans present in the Serra da Bocaina National Park and its buffer area (Garey *et al.*, 2014), which is approximately 13% of Mata Atlantica anurofauna (Haddad *et al.*, 2013). I have recorded 31 species and identified 27 of them. Twenty species recorded during my research were recorded also in history in Serra da Bocaina National Park and its buffer area and 6 species should represent new records for the area. *Leptodactylus fuscus, Ischnocnema verrucosa, Hypsiboas albopunctatus, Hypsiboas semilineatus, Adenomera marmorata, Phyllomedusa burmeisteri* (Serafim *et al.* 2008, Heyer 1985, Cruz and Peixoto 1984, Luzt and Lutz 1939, Lutz and Lutz 1938, USNM⁴, Zaher *et al.*, 2005). *Leptodactylus fuscus, Hypsiboas albopunctatus, Hypsiboas polytaenius and Hypsiboas semilineatus* were found in the close municipality of Sao Jose do Barreiro (Serafim *et al.*, 2008). Serafim *et al.* (2008) wrote, that those 4 species and also *Phyllomedusa burmeisteri* were known from preliminary research in Bananal municipality, close to Serra da Bocaina National Park and they have cited this information from the list of anuran species occurring in the Bananal municipality, provided by Zaher *et al.* (2005). However, only *Hypsiboas polytaenius* is actually mentioned in this list (Zaher *et al.*, 2005).

Species habitat preference was estimated according to their presence or absence on particular parts of transects or breeding sites. Two species, *Hypsiboas polytaenius* and *Rhinella*

⁴ USNM = Smithsonian National Museum of Natural History

icterica, were found to have very large habitat range, as they were found both on transect and breeding sites. However neither of this species were found directly inside the forest, only on its edge.

The IUCN map revision confirm the estimated hypothesis. First species was the Bocaina tree frog, *Aplastodiscus callipygius*, occurring in the forest in and around Serra da Bocaina National Park. This species is living in the trees and it is therefore vitally dependent on the forest, as it's natural habitat (Gomes, 1996). As it can be seen on the map (Ch. 4.5., Fig. 26), the tree cover map, blending with the species distribution area, shows two main forest fragments, which are not connected by any continuous stripe of forest. Those two habitats are therefore completely isolated and animals from each fragment cannot sustain optimal gene flow. Besides those two large fragments, few significantly smaller forest fragments are present on the rest of the species distribution area. Those fragments are but again isolated from each other and from the two large fragments. Although ICUN Red List states, that populations of this species are not severely fragmented, this map shows the opposite (IUCN, 2015).

The second species, which I choose for this revision is Adenomera marmorata, which is vitally bounded to the forest, because it lay eggs in the deep leaf litter and her tadpoles also developed here. It is more tolerant to the open areas, but still it cannot reproduce without the forest and its ground covered with leaf litter (Almeida-Gomes *et al.*, 2007). On the map (Ch. 4.5., Fig. 27) I can see, that there is one large, main forest fragment and smaller, unconnected fragments. It is obvious that the population is severely fragmented, although the IUCN Red List account for this species says, that it is not fragmented (IUCN, 2015). It is a ground species and can migrate between forests, but only at night or during the rainfall and also not for large distances. Some fragments are very distant from each other and the animals from those populations cannot sustain optimal gene flow.

Third and the last model species is the *Haddadus binotatus* as one of the typical inhabitants of the Atlantic forest. It is not tolerant to open or disturbed areas and live only in the primary and secondary forest and on the forest edge. It is bounded to the forest even

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because it has got a direct development reproduction type (Haddad and Giaretta, 1999). On the map (Ch. 4.5., Fig. 28) there are again some main unconnected fragments and smaller, tree-covered areas.

5.1. Unidentified species

The species were identified using photos and descriptions from scientific articles and other publications, in some cases Brazilian scientists were asked to help us identify some species. Four of the 31 recorded species were not identified. They could represent a brand new species, or just have not been identified due to lack of available photos or description. Genetic data should be collected for better and more precise identification. Permission from Brazilian government is needed for that collection and transport of the samples. I will try to get that permission in further years.

5.1.1. *Scinax spp*¹

Genus: Scinax

Location: Xandoca Transect

Number of specimens: 20+

Position: Vegetation near stream waterfall

This unknown frog is small greenish snouted tree frog, which I have found near small stream waterfall in the forest. At least 20 specimens were observed during few days, including those found during transect survey. There was a small pond near the streams, where tadpoles were found, probably belonging to this *Scinax* species.


Figure 30: Photo of *Scinax spp*₁

5.1.2. Scinax spp₂

Genus: Scinax or fritziana

Location: Xandoca Transect, Plato Transect

Number of specimens: 7

Position: Trees

This unidentified species belong probably to the genus *Scinax* or *Fritziana* and could represent a brand new species. I found one specimen on the Plato transect and 6 individuals on the Xandoca transect, outside the survey time. They were always sitting on the tree branch

or leaf, in the high of person's eyes. It is brown/grey color with blue marking on the inside of the hind legs.



Figure 31: Photo of Scinax spp.2

5.1.3. Ischnocnema spp.

Genus: Ischnocnema

Location: Bruna transect

Number of specimens: 1

Position: Trees

I have found just one specimen of this unidentified species, which most likely belongs to the genus *lschnocnema*. This genus is very variable and also specimens were interspecifically variable, therefore the identification is not easy. It is not probably a new species, but only some species, of which the photo is not available for us to see.



Figure 32: Photo of Ischnocnema spp.

5.1.4. Hypsiboas spp.

Genus: *Hypsiboas*

Location: Lucia Pond

Number of specimens: 3

Position: Water vegetation

This unidentified frogs belongs to the genus *Hypsiboas*. It is very similar to *h. pardalis* species, but it has got different coloration, slightly different body and snout shape and significantly different vocalization. I have seen only 1 individuals, 2 others were identified by

acoustic signals. Research should be conducted on this species for more information about its morphology and behavior. The frog had some special apparat on its front legs, which is being used when the frog is in danger. I felt short strong pain on the hand when holding the frog and when she perform weird movement with her front legs. I was not allowed to collect any biologic material, therefore further I will need to get permission for this, to get more information about this frog.



Figure 33: Photo of *Hypsiboas spp*.

6. Conclusion

This study recorded 31 species of anurans in the Serra da Bocaina National Park buffer area. Only 27 species were identified due to lack of photos or description to compare or they may represent a brand new species. Six species represent first records for the area. Habitat preference of the recorded species was illustrated on the transect graphs (Fig. 19, Fig. 21, Fig. 23), those found of the breeding sites were found to prefer grassland habitat. I have found, that IUCN species distribution maps are not accurate in the case of habitat preference of the particular model species, which I have chosen. Populations of those model species were found as fragmented, in opposite of what is estimated on their IUCN account. Further revision on more species should be conducted, which may lead to change of the thread status of those species. Larger scale study should be also conducted to more quality data for identification of the biodiversity in the area and to understand the species habitat preferences and behavior and use this knowledge for conservation activities.

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Appendices

Appendix 1:

Anuran species list of Serra da Bocaina with my records

Bold letters represent species, I have recorded in my research:

Brachycephalus ephippium (Spix, 1824) Brachycephalus vertebralis (Pombal, 2001) Ischnocnema parva (Girard, 1853) Ischnocnema pusilla (Bokermann, 1967) Ischnocnema vizottoi (Martins & Haddad, 2010) Ischnocnema sp. (aff. guentheri) Ischnocnema sp. (aff. melanopygia) Ischnocnema sp. (gr. lactea) Rhinella icterica (Spix, 1824) Rhinella ornata (Spix, 1824) Vitreorana eurygnatha (Lutz, 1925) Vitreorana uranoscopa (Müller, 1924) Ceratophrys aurita (Raddi, 1823) Haddadus binotatus (Spix, 1824) Holoaden suarezi (Martins & Zaher, 2013) Cycloramphus eleutherodactylus (Miranda-Ribeiro, 1920) Cycloramphus granulosus (Lutz, 1929) Thoropa taophora (Miranda-Ribeiro, 1923) Fritziana sp. Aplastodiscus albosignatus (Lutz & Lutz, 1938) Aplastodiscus arildae (Cruz & Peixoto, 1987) Aplastodiscus callipygius (Cruz & Peixoto, 1985) Aplastodiscus leucopygius (Cruz & Peixoto, 1985) Aplastodiscus perviridis (Lutz, 1950) Bokermannohyla ahenea (Napoli & Caramaschi, 2004) Bokermannohyla circumdata (Cope, 1871) Bokermannohyla clepsydra (Lutz, 1925) Dendropsophus elegans (Wied-Neuwied, 1824) Dendropsophus microps (Peters, 1872) Dendropsophus minutus (Peters, 1872) Hypsiboas bandeirantes (Caramaschi & Cruz, 2013) Hypsiboas pardalis (Spix, 1824) Hypsiboas faber (Wied-Neuwied, 1821) Phasmahyla cochranae (Bokermann, 1966) Scinax ariadne (Bokermann, 1967) Scinax atratus (Peixoto, 1989) Scinax crospedospilus (Lutz, 1925)

Scinax eurydice (Bokermann, 1968) Scinax flavoguttatus (Lutz & Lutz, 1939) Scinax fuscomarginatus (Lutz, 1925) Scinax fuscovarius (Lutz, 1925) Scinax hayii (Barbour, 1909) Scinax squalirostris (Lutz, 1925) Scinax sp. (aff. duartei) Scinax sp. (aff. obtriangulatus) Sphaenorhynchus orophilus (Lutz & Lutz, 1938) Trachycephalus imitatrix (Miranda-Ribeiro, 1926) Crossodactvlus dispar (Lutz, 1925) Hylodes asper (Müller, 1924) Hylodes phyllodes (Heyer & Cocroft, 1986) Hylodes sp. Megaelosia bocainensis (Giaretta, Bokermann & Haddad, 1993) Megaelosia goeldii (Baumann, 1912) Adenomera bokermanni (Heyer, 1973) Leptodactylus furnarius (Sazima & Bokermann, 1978) Leptodactylus labyrinthicus (Spix, 1824) Leptodactylus latrans (Steffen, 1815) Leptodactylus mystacinus (Burmeister, 1861) Paratelmatobius gaigeae (Cochran, 1938) Physalaemus barrioi (Bokermann, 1967) Physalaemus cuvieri (Fitzinger, 1826) Physalaemus maculiventris (Lutz, 1925) Physalaemus olfersii (Lichtenstein & Martens, 1856) Chiasmocleis mantiqueira (Cruz, Feio & Cassini, 2007) Myersiella microps (Duméril & Bibron, 1841) Proceratophrys appendiculata (Günther, 1873) Proceratophrys boiei (Wied-Neuwied, 1824) Proceratophrys melanopogon (Miranda-Ribeiro, 1926)

(Garey et al., 2014)

New records:

Ischnocnema verrucosa Leptodactylus fuscus Adenomera marmorata Hypsiboas albopunctatus Hypsiboas semilineatus Phyllomedusa burmeisteri