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Biodiversidade e conservação da herpetofauna do médio-Araguaia

Biodiversity and conservation of the middle-Araguaia herpetofauna

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Dedico este trabalho a todos os que sobrevivem na fronteira entre as sociedades humanas e a Natureza.

o júri

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palavras-chave

Amazónia, Araguaia, biodiversidade, Cerrado, conservação, herpetofauna.

resumo

A conservação da biodiversidade nunca foi uma assunto tão popular como nas últimas décadas, mas esta popularidade crescente é devida à pior das razões: o passo acelerado da extinção de espécies e habitats. Os ecossistemas tropicais são, ao mesmo tempo, os mais diversos e os mais ameaçados, em parte porque muitos países destas regiões emergem ainda de situações de instabilidade social, económica e política. O Brasil é o maior país Neotropical, onde se encontram alguns dos biomas com maior diversidade e mais ameaçados do planeta. Actualmente, é também um país líder ao nível da planificação e implementação de medidas de conservação da biodiversidade. Vários dos biomas tropicais mais diversos e ameaçados encontram-se em território brasileiro. Dois destes biomas, a Amazónia e o Cerrado, convergem numa região ecotonal sujeita a uma elevada pressão humana, conhecida como o arco do desmatamento. O Araguaia, um dos maiores rios do Brasil, corre ao longo desta paisagem e os efeitos do desmatamento são já evidentes em toda a sua bacia. Por causa do acelerado ritmo de degradação deste ecossistema, torna-se urgente obter uma imagem clara da biodiversidade regional e compreender como e se a estratégia de conservação para esta região é capaz de lidar com as correntes ameaças e alcançar o seu objectivo a longo prazo: conservar a biota regional. Tendo a herpetofauna como grupo-alvo, os nossos objectivos principais foram: aumentar o conhecimento das comunidades de anfíbios e répteis squamata da região do curso médio do Rio Araguaia; compreender a importância deste rio nos padrões intraespecíficos de estrutura e diversidade genética para diferentes espécies com diferentes características ecológicas; avaliar o potencial de diferentes metodologias para o estudo e monitorização da herpetofauna regional. Os nossos resultados revelam que a amostragem continuada e o uso de diferentes técnicas são essenciais para a obtenção de uma imagem precisa da diversidade da herpetofauna local. As comunidades locais de anfíbios e lagartos apresentaram maior riqueza específica na Área de Protecção Ambiental Bananal/Cantão (APABC), uma área tampão, do no Parque Estadual do Cantão (PEC), uma área de conservação estrita. A APABC é caracterizada por uma maior heterogeneidade de habitats e os nosso resultados corroboram a teoria da heterogeneidade espacial e resultados recentes que revelam uma maior diversidade de lagartos nas zonas interfluviais do Cerrado, do que nas matas de galeria. Os resultados aqui apresentados não corroboram a hipótese de que os ecótonos apresentam maior diversidade do que os biomas em redor. Os nossos resultados revelaram ainda que o Rio Araguaia afecta de forma diferente a estrutura genética de várias espécies de anfíbios e lagartos. Estas diferenças poderão estar relacionadas com a ecologia das espécies, nomeadamente com o uso de diferentes habitats, a vagilidade, ou a estratégia alimentar. Sugerimos que a gestão integração de diferentes unidades de conservação, com diferentes estatutos, podem ajudar a preservar melhor a biota regional.

keywords

Amazonia, Araguaia, biodiversity, Cerrado, conservation, herpetofauna.

abstract

Biodiversity conservation has never been such a popular matter as in the last decades, but this increasing popularity is due to the worst reason: the fast pace of extinction of species and habitats. Tropical ecosystems are both the most diverse and most threatened, in part because many countries from these regions are now emerging from decades of social, economic and political instability. Brazil is the largest country in the Neotropics and one of the world's megadiverse countries. Nowadays, it is also a leading country in terms of planning and implementing biodiversity conservation measures. Brazil harbors several of the most diverse and threatened tropical biomes. Two of them, Amazonia and Cerrado, converge in an ecotonal region subjected to high human pressure, known as the deforestation arc. Araguaia, one of the largest Brazilian rivers, runs along this landscape and the effects of deforestation are already evident throughout its basin. Because of the fast pace of degradation in this ecosystem, it becomes urgent to get a clear picture of the regional biodiversity and understand how and if the conservation strategy for the region is able to cope with the current threats and accomplish its long-term objectives of preserving the regional biota. Having herpetofauna as the target group, our main goals are: to improve the knowledge on the amphibian and lizard assemblages in the region of the middle Araguaia River; to understand the intra-specific patterns of genetic structure and diversity for different species with different ecological features; to assess the potential of different methodologies for the study and monitoring of the regional herpetofauna. Our results revealed that continued sampling and the use of several techniques are essential to achieve an accurate picture of the diversity of the local herpetofauna. The local lizard and amphibian assemblages presented higher species richness in the Área de Protecção Ambiental do Bananal/Cantão (APABC), a buffer area, than in Parque Estadual do Cantão (PEC), a strict conservation area. APABC is characterized by an higher habitat heterogeneity and our results corroborate the theory of spatial heterogeneity, as well as the conclusions of recent studies that reveal a higher diversity of lizards in the interfluvial áreas of Cerrado, than in gallery forest. The results here presented do not corroborate the hypothesis that ecotones present greater diversity than the surrounding areas. Our results also reveal that River Araguaia differently affects the genetic structure of several species of amphibians and lizards. These differences might be related with species ecology, namely with the use of different habitats, vagility or feeding strategy. We suggest that integrated management of different conservation units, with different conservation statuses might help to better preserve the regional biota.

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“Sempre pensara em ir
caminho do mar.
Para os bichos e rios
nascer já é caminhar.
Eu não sei o que os rios
têm de homem do mar;
sei que se sente o mesmo
e exigente chamar […]”

João Cabral de Melo Neto,
in Morte e Vida Severina

CHAPTER 1

General Introduction and Objectives

Chapter 1 cover photos (from left to right): Araguaia River after the rain season; Coco River at the peak of dry season; sunset at Javaés River.

1.1 – Introduction

One of the major obstacles to the conservation of the biodiversity, at the global scale, is that we live in a world in use. The destiny of millions of species is dependent on the will of a species, with thousands of millions, which inhabits the planet and uses its resources, but not like all the other species. This species, *Homo sapiens*, does not adapt itself to the environment; it adapts the environment to its needs, at a level without known parallel in the history of the planet.

Recent hypotheses about human dispersal place the Americas as the last region to be colonized by humans (Fagundes et al., 2007, 2008; Mulligan et al., 2008). Even though, this continent was colonized long before the modern paradigm of land-use and of the concept of natural world as a source of commodities. Contrarily to earlier believe, the peoples inhabiting Amazonia before European contact were also familiarized with plant domestication. However, the use and management of useful plants was not based on forest clearing and crop plantation, but on "landscape domestication" (Clement, 2006; Junqueira et al., 2010). In Cerrado, archeological evidences suggest that hunter-gatherer peoples were the first to colonize the biome, and were dependent on native plant and game species (Klink and Moreira, 2006). Common to both approaches was the sustainability of the coexistence between human populations and its environment.

At the present state of development, it is naïve to believe that such coexistence is still possible, or even desirable for the majority of human populations. Modern societies are dependent on domesticated plants and animals for almost everything and generally ignore the importance of wild species and habitats for the survival of peripheral human communities (Redford and Robinson, 1991a). The success of conservation efforts will depend on understanding and conciliating the needs of people living at the edge of these pristine areas with the need to preserve biodiversity (Fragoso et al., 2004; Schmink, 2004; Rudel, 2005). Meanwhile, biodiversity is being lost, sometimes even before being discovered (Fouquet et al., 2007). Species and ecosystems are not equally vulnerable and some adapt themselves to a rapidly changing world; others do not.

1.2 – Two Biomes: Amazonia and Cerrado

Forests used to be seen as pristine landscapes, while grasslands were traditionally considered as a transitional state in the succession to an equilibrium forest state (Bond and Parr, 2010). However, according to these authors, the tropical and subtropical long-

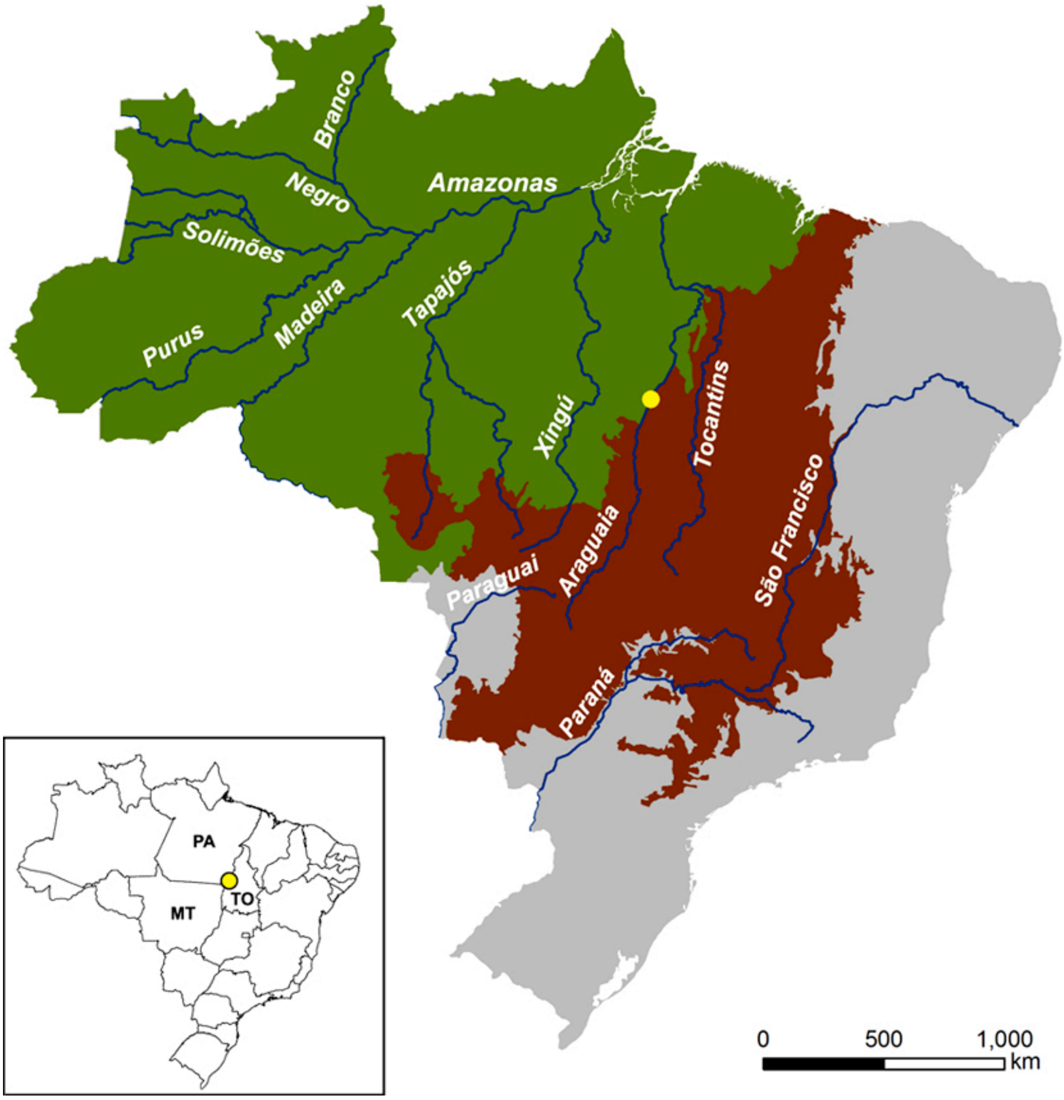

Figure 1.1 – Location of the study area (yellow dot) within the context of Amazonia (green) and Cerrado (brown) biomes, and Brazilian federal units: MT – sate of Mato Grosso; PA – state of Pará; TO – state of Tocantins. Major Brazilian rivers are also depicted.

standing grasslands are better explained in a framework of alternative ecosystem states. The main difference might be related with differences regarding shade tolerance, with shade-intolerant species dominating savannas and shade-tolerant species abounding in forests (Bond and Parr, 2010). A forest-dominated ecosystem, Amazonia, and a grassland/savanna-dominated ecosystem, Cerrado, merge along an ecotonal region that extends throughout several thousand kilometers, in central Brazil (Figure 1.1). In addition, several large savanna patches occur within the Hylean forests (Ratter et al., 2006), while gallery forests extend the Amazon influence along the watercourses in Cerrado (Oliveira-Filho and Ratter, 2002). In fact, a large portion of Cerrado biome is located within the limits of the legally defined Amazonia (Ratter et al., 2006).

Amazonian rainforest is the world's largest major wilderness area (Mittermeier et al., 1998) and about two thirds of this biome are located within the Brazilian borders (Silva et al., 2005). Amazonia is far from being a uniform tract of forest; rather it encompasses a wide variety of landforms such as the Andean foothills, the *tepuis* in the north, the *várzeas*, *terra firme* and *igapó* forests (Rudel, 2005). Different areas of endemism for terrestrial vertebrates, separated by the major Amazonian rivers, were recognized within the biome, of which Tapajós, Xingú, Belém and Roraima areas of endemism are exclusively or almost exclusively within Brazil (Silva et al., 2005). The climate varies along the Amazonian biome, with a marked seasonality in rainfall in the eastern regions that is absent in the western ones (Rudel, 2005). Amazonian habitats are largely dominated by trees and lianas (Ribeiro et al., 1994; Ivanauskas et al., 2004). Exception is made to the savanna enclaves that present higher proportions of grasses and shrubs, similarly to the open formations of the Cerrado biome (Ratter et al., 1997; Filgueiras, 2007). These enclaves of Amazonian savanna are however much less diverse than the core of Cerrado (Ratter et al., 2003), which is also true for faunal communities (Gainsbury and Colli, 2003).

The natural or anthropogenic origin of Cerrado has been a matter of debate (Ledru, 2002). There are strong evidences of a worldwide expansion of tropical and subtropical grasslands and savannas dominated by C4 grasses (such as Cerrado), starting around 8 million years ago, after a global decrease of atmospheric CO_2 (Cerling et al., 1997; Mayle et al., 2004; Simon et al., 2009; Bond and Parr, 2010). On the other hand, palynological records suggest that the earliest record of cerrado-type vegetation in Brazil dates back to 32,000 years before present, in the Central Brazilian Plateau (Ledru, 2002). Even though, according to this author, the fire-adapted vegetation of Cerrado probably existed before people arrived to South America. In either way, Cerrado is considered a global biodiversity hotspot for plants and vertebrates (Mittermeier et al., 1998; Myers et al., 2000). Species richness estimates for the Cerrado woody flora point to 950 to 1000 species, but the biome is widely dominated by only a small fraction of this assemblage (Ratter et al., 2003; Bridgewater et al., 2004). A strong geographical pattern in the distribution of the flora was found in Cerrado, which allowed the recognition of several distinct floristic regions within this biome (Oliveira-Filho and Ratter, 2002; Ratter et al., 2003). The Cerrado biome comprises several physiognomies ranging from grasslands with or without trees (*campo sujo* or *campo limpo*, respectively), to gradually more forested areas (*campo cerrado* and *cerrado sensu stricto*) until almost closed woodland (*cerradão*) areas (Oliveira-Filho and Ratter, 2002). The previous physiognomies are present in dry areas but Cerrado also includes gallery forests along the watercourses, and seasonally flooded forests and grasslands (Oliveira-Filho and Ratter, 2002).

1.3 – Deforestation, Land Use and Conservation Areas

Habitat fragmentation is one of the major causes of worldwide current biodiversity crisis (e.g., Skole and Tucker, 1993; Anderson and Jenkins, 2006), and the current main cause of habitat loss and fragmentation in tropical areas is deforestation. Recent studies showed that deforestation altered the patterns of species richness, diversity and abundance of the herpetofauna (Gardner et al., 2006; Bernarde and Macedo, 2008; Cano and Leynaud, 2010). On the other hand, some studies reveal that low-scale selective logging activities (Vallan et al., 2004) or silvicultural activities (Lopes, 2010) might present low impact on local herpetofauna.

Total deforested area, and sometimes the deforestation rates, is higher in Cerrado than in Amazonia (Klink and Machado, 2005). Estimates of total deforested area in Cerrado vary between 39% (Sano et al., 2010) and 80% (Myers et al., 2000), depending on the criterion: only clear cutted areas; clear cutted and edge areas; or all areas under human influence, such as natural pastures. Considering the most optimistic estimative, if secondary growth forests and exploited native pastures are included as areas under direct human intervention, then the lower threshold rises to 53%, approaching the estimates provided by Klink and Machado (2005). In the year of 1988, estimates of effectively deforested area in Amazonia corresponded to 6% of the total area of the biome, but the area affected by deforestation was already about 15% (Skole and Tucker, 1993). Cumulative deforested area in Legal Amazonia, which includes areas covered by Cerrado, reached 17% in the year of 2006 and most of the deforestation was concentrated in the border between Amazonia and Cerrado (Reid and Sousa, 2005; Foley et al., 2007). This area is known as the Deforestation Arc (Figure 1.2), which extends along the eastern and southern borders of Amazonia, cutting through the states of Maranhão, Tocantins, Pará, Mato Grosso, Rondônia, Acre and Amazonas (Vieira et al, 2008).

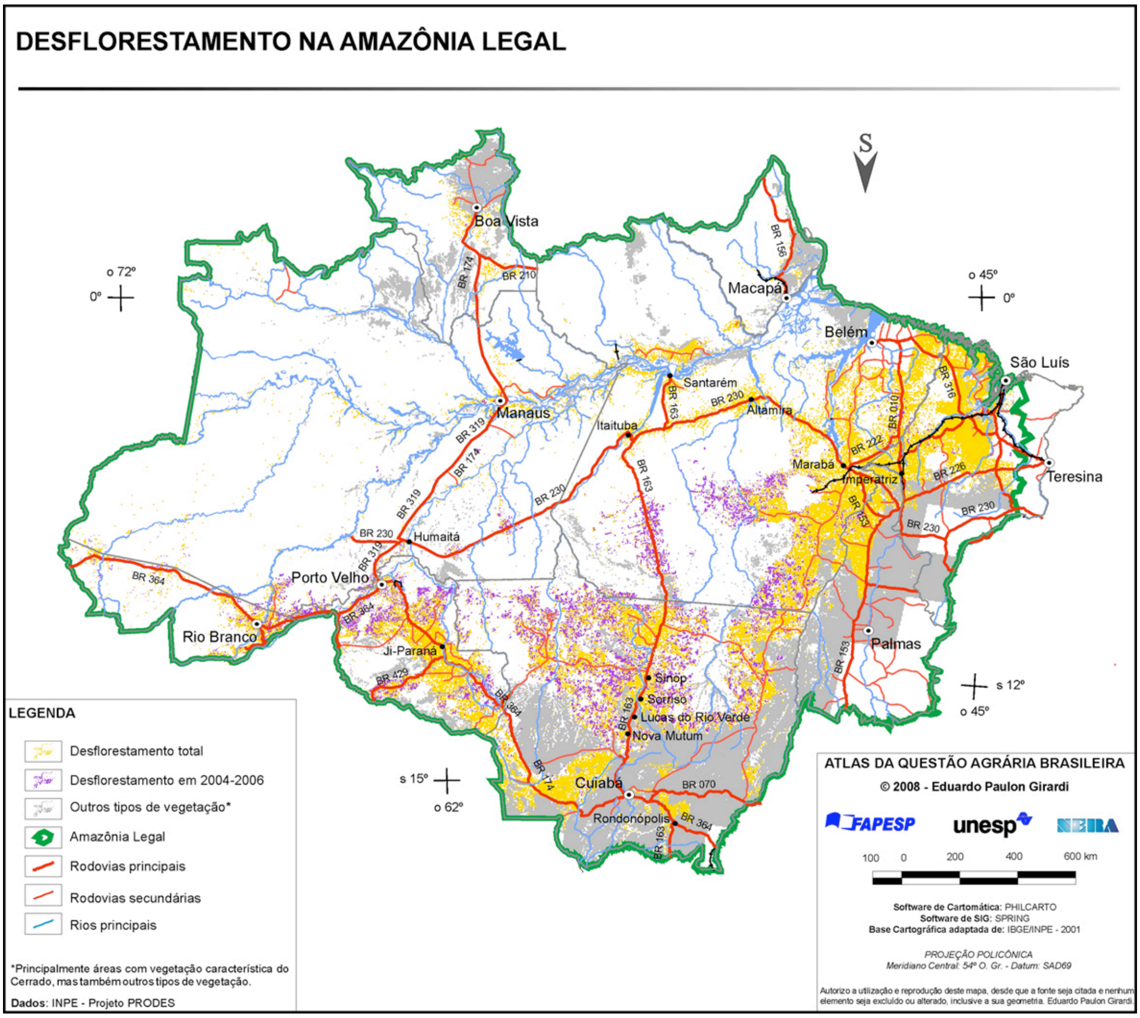

Figure 1.2 – Deforested areas within Legal Amazonia boundaries. The region known as deforestation arc corresponds to the continuous deforested area (yellow and purple) extending from São Luís (state of Maranhão) to Rio Branco (state of Acre). Reproduced with permission from Girardi (2008).

One of the reasons for the difference between the deforestation rates in the two biomes is that the Brazilian law (Klink and Machado, 2005). According to these authors, the latter requires that 80% of each holding in Amazonia should be preserved, but in Cerrado, this percentage is only 20%. In Cerrado, higher deforestation rates are reported for the southern states were only 13 to 32% of native areas still remain intact (Sano et al., 2010). According to the same authors, Tocantins is the northern state with the greatest percent of deforested areas (21%). In Amazonia, deforestation rates also vary among the major endemism areas (Silva et al., 2005). The Xingu area of endemism, at the core of the deforestation arc (Figure 1.2), is the most severely affected area, with almost 27% of its native area already converted for human use (Silva et al., 2005).

The most frequently appointed causes for deforestation are extensive agriculture and cattle ranching (Klink and Moreira, 2002; Fearnside, 2005; Vieira et al., 2008). In a recent study in the Cerrado biome, Carvalho and collaborators (2009) concluded that croplands are more damaging than pasturelands for the conservation of biodiversity. The soils under Cerrado and Amazonia are relatively infertile (Klink and Moreira, 2002; Stepan, 2006) and, in a context of large-scale crop and cattle production, cleared areas soon become exhausted and new large areas must be cleared. Other related causes for deforestation have also been suggested, and Fearnside (2005) pointed the role of clearing in the establishment of land claims as one of the root problems. In addition, government loans and subsidies were primarily oriented to large-scale agriculture and logging activities, and allocated based on the size of planted area, encouraging extensive and inefficient agriculture practices (Klink and Moreira, 2002; Rudel, 2005; Clement, 2006).

In this not very encouraging context, the great effort of Brazilian researchers, conservation professionals and governments in establishing a large and effective conservation program must be recognized (Rylands and Brandon, 2005). Brazil's present system of protected areas includes strictly protected areas, such as parks, biological reserves and ecological stations; and sustainable use areas, which allow different types and levels of human use, and include national forests, environmental protection areas and extractive reserves, among others (Rylands and Brandon, 2005). According to these authors, in 2005, there were 478 federal and state strictly protected areas and 436 sustainable-use areas, covering an area of about 37 million and 74.6 million hectares, respectively. Recent data from the Program of Amazonian Protected Areas (ARPA) reveal that 31 strict conservation areas and 30 conservation areas for sustainable use are presently included in this program, covering 32 million hectares of Amazonian forest (ARPA, 2011).

As suggested by Peres (2004), the long-term persistence of the Amazonian biome depends on a large and integrated system of strict conservation and sustainable use areas. Unfortunately, in the Amazonian area of endemism of Xingu, for example, only 0.29% is under strict protection and 2.72% are sustainable use areas (Silva et al., 2005). In the latter case, according to the authors, if indigenous areas are considered, 27.1% of this region is somehow preserved. The situation is not better in Cerrado, where only about 2.2% of its total extent is under any legal conservation statuses (Klink & Machado, 2005). Conservation efforts could benefit from the integration of indigenous and smallholder communities, and some successful cases where reported, resulting in the conservation of very large areas (Silvius, 2004; Campos and Nepstad, 2005). Large efforts for integrated protection of large extents of pristine areas are also being conducted by Brazilian conservation agencies, universities and international non-governmental organizations, by promoting the creation of ecological corridors. Some examples are: the “Corredor Jalapão – Oeste da Bahia”, in the transition between Cerrado and Caatinga biomes, in the border of the states of Tocantins, Piauí, Maranhão and Bahia; or the “Corredor Sul da Amazônia”, which includes all the forests located between the right margin of Madeira River and the central region of the state of Maranhão (CI-Brasil, 2011).

1.4 – The Araguaia River Basin

The Araguaia River (Figure 1.1) is the main fluvial system in Cerrado and, together with Tocantins River, constitutes the fourth largest drainage basin of South America (Aquino et al., 2008; Latrubesse et al., 2009). The basin extends through both Amazonia and Cerrado biomes (Aquino et al., 2008). Araguaia presents an anabranching pattern that is characteristic of many large rivers (Latrubesse, 2008). Anabranching rivers consist of multiple channels separated by vegetated semi-permanent alluvial islands excised from preexisting floodplain or formed by within-channel or deltaic accretion (Nanson and Knighton, 1996).

The Araguaia basin was recognized as a priority area for conservation (Azevedo-Ramos and Galatti, 2002; Cavalcanti and Joly, 2002), and a plant diversity hotspot that, despite the outlying location, is mainly composed of typical Cerrado species (Oliveira-Filho and Ratter, 2002). The area is now the core of political, scientific and social debates, because of the intensive deforestation in Cerrado and the expansion of agribusiness in the region (Aquino et al., 2008). One of the controversial issues is related with the damming projects for Araguaia, which is one of the last undammed large rivers in Brazil (Reid and Sousa, 2005; Latrubesse et al., 2009).

River damming can have direct impact on local fauna and habitats (Fearnside, 2001; Brandão and Araújo, 2008). Dams can degrade socially valued riverine and riparian ecosystems, and the services they provide (Esselman and Opperman, 2010). As an example of direct impacts on fauna, dam-induced changes such as siltation of ponds, increased water velocities and lowered water temperatures were shown to be pottentially deleterious to turtle populations (Reese, 1996). Flooding of dam reservoir also may lead to depletion of amphibian species in local amphibian communities in the islands formed from hill remnants, during the flooding of the reservoir (Brandão and Araújo, 2008). Habitat alteration caused by river damming was also shown to negatively affect native amphibian species, while promoting dominance by invasive species like *Rana catesbeiana* (Fuller et al, 2010).

However, direct human intervention in Araguaia is just one of the matters of concern. There are evidences that deforestation in Cerrado is triggering erosion all over the basin of Araguaia and other Brazilian rivers (Coe et al., 2009; Latrubesse et al., 2009; Coe et al., 2011) and this erosion has been affecting mostly large arboreal vegetation in the floodplain (Latrubesse and Stevaux, 2006). The middle Araguaia extends for 1160Km and corresponds to 79% of its total drainage area (Aquino et al., 2008). The floodplains in the middle river course retain about 30% of Araguaia's output during the flooding season, in part because of a complex lake system and because of the arboreal vegetation that slows down the flooding wave (Aquino et al., 2008). The question remains on how this dynamic is going to be affected by deforestation in the long term. Changes in land-cover are changing hydrology (Klink and Machado, 2005). The Araguaia basin still ensures the preservation of large Cerrado remnants (Latrubesse et al., 2009) but, in the long-term, this role might be compromised by the present deforestation rates.

1.5 – The Herpetofauna

Tropical regions present particularly rich amphibian and reptile faunas. Among amphibian taxa, the highest diversity of anurans and caecilians is found in the tropics, and only order Caudata is more diverse in temperate climates (Duellman, 1994; Wells, 2007). All reptilian taxa are represented in the tropics and some, such as crocodilians and several squamate families, are more diverse or even restricted to tropical and subtropical regions (Zug et al., 2001; Pough et al 2004). According to the Brazilian Society of Herpetology, there are currently 877 recognized species of amphibians (SBH, 2011a) and 721 recognized species of reptiles (SBH, 2011b), known to occur in Brazil, with several new species being described each year.

Several taxonomic rearrangements of squamate and amphibian taxa have been proposed in the last years, directly affecting the taxonomy of Neotropical taxa. The rearrangements included the establishment of new families, genera and species and the replacement and splitting of previously non-monophyletic taxa. Some examples are: Hylidae (Faivovich et al., 2005); Amphibia (Frost et al., 2006); Dendrobatidae and close relatives (Grant et al., 2006); Gekkota (Gamble et al., 2007); Terrarana (Hedges et al., 2008); or snakes (Adalsteinsson et al 2009; Zaher et al., 2009).

All the three groups of extant amphibians are present in Brazil, but anuran taxa are hegemonic in all biomes, both in the number of species, as in the number of families (Colli et al., 2002; Azevedo-Ramos & Gallati, 2002; Rodrigues, 2003; Avila-Pires et al., 2007; Cruz and Feio, 2007; Strüssman et al., 2007). The order Gymnophiona is represented by two families – Caeciliidae (26 species) and Rhinatrematidae (one species) – and the plethodontid *Bolitoglossa paraensis* is the only representative of order Caudata (SBH, 2010a). On the other hand, anurans constitute more than 96% of all amphibian species in Brazil, distributed into 19 families. The most diverse family of anurans in Brazil is by far the family Hylidae, with 339 recognized species, followed by: Leptodactylidae (74), Cycloramphidae (68), Bufonidae (67) and Leiuperidae (55) (SBH, 2010a). The same pattern also occurs in Cerrado and Amazonia, where hylids and leptodactylids are the most numerous families (Colli et al., 2002; Bastos, 2007; Ávila-Pires et al, 2007)

Most of world's non-avian reptilian species are squamates (Pough et al., 2004) and the same occurs for Brazilian biomes (Colli et al 2002; Ávila-Pires et al 2007; Rodrigues, 2003). Brazilian herpetofauna includes six crocodilian species, all from family Alligatoridae, and 36 chelonian species, distributed into eight families, including marine, terrestrial and fresh water representatives (SBH, 2010b). However, these two ancient taxa are outnumbered by squamates, which include 67 species of amphisbanids, 241 species of lizards and 371 species of snakes (SBH, 2010b). Autarchoglossa (133) is the most diverse lizard group, followed by Iguania (73) and Gekkota (34). At the family level, Gymnophtalmidae leads the ranking, with 82 valid species (SBH, 2010b). In Amazonia, this family also presents the highest number of species (Ávila-Pires, 2007), but the diversity of lizards in Cerrado is more evenly distributed, with Teiidae being the most speciose group (Colli et al., 2002).

Higher species richness does not warrant a higher perceived importance. Despite amphibian and squamate species clearly outnumber chelonians and crocodilians, the latter two groups are more familiar to common people in South America. In fact, they are the economically most important reptile taxa in the Neotropics (Redford and Robinson, 1991b; Vichers, 1991; Fachín-Teran et al., 2004; Seijas, 2004; Verdade and Piña, 2007). The same patern is verified locally, in the middle Araguaia basin, where turtles and their eggs are frequently consumed (Salera Jr., 2005; Salera Jr. et al., 2007; Ataídes 2009), as well as crocodilian meat, as reported by local people.

Large lizards, like iguanas or teiids, are important for some human communities in the Neotropics, as a protein source (meat and eggs) but also as a source of monetary income, because of skin trade (Fitzgerald et al., 1991; Werner, 1991; Fitzgerald et al. 1994). Some anuran species are also used for human consumption (Pough et al., 2004), which is the case of the large *Leptodactylus* species, in the Araguaia basin. Toxins from frog skins are traditionally used in rituals or hunting by indigenous people in the Amazon basin (Myers et al., 1978, Daly et al., 1992). Presently, amphibian toxins are also being used in medical and pharmaceutical research (Shwartz et al., 2007). Moreover, amphibians, lizards and snakes are much appreciated in the international pet market (Schlaepfer et al., 2005; Wells, 2007).

The first works about Cerrado herpetofauna (Duellman, 1979; Vanzolini, 1998; Sites, 1995) suggested its low differentiation, rate of endemism and lack of distinctiveness, but Colli and collaborators (2002) refuted these claims, stating that the horizontal habitat variability in Cerrado balanced the vertical variability typical of forested habitats, resulting in similar levels of local species richness. Recent studies reporting the highly habitat-structured nature of lizard assemblages in Cerrado supported this view (Nogueira et al., 2005; Costa et al., 2007; Nogueira et al., 2009). Some studies point to a greater diversity of anurans in the central and southeastern regions of Cerrado (Diniz-Filho et al., 2006, 2007) but Bini and collaborators (2006) suggest that a more intense sampling in the northern areas of the biome would probably expand the distribution of known anuran species and eventually contribute with the description of new species. In the case of Amazonia, Avila-Pires (1995) and Azevedo-Ramos and Galatti (2002) suggested the existence of distinctive amphibian and lizard assemblages across the biome. Vitt and collaborators (1999) found that Amazonian herpetofauna was also structured relatively to microhabitat and diet.

1.6 – The Riverine Barrier Hypothesis

The herps from the mid-Araguaia basin live in a river-dominated landscape. Besides the Araguaia itself, two major tributaries are present in this area: Coco and Javaés. The idea that these rivers could play an important role in the evolutionary history of herps is easily born in mind. However, this idea is not new. The role of rivers as barriers to animal populations and, thus, as a mechanism responsible for the diversification of the Amazonian fauna, was first formally proposed as the “Riverine Barrier Hypothesis”, in

1852, by Alfred Russell Wallace (Colwell, 2000). The idea that rivers could have shaped Amazonian biodiversity resulted from the observation that: (1) large rivers are common in the Neotropics since the Pleistocene; (2) they dissect the tropical forest in fragments of various sizes (Gascon et al., 1998); (3) and different species occurred in opposite banks of the same river. Several alternative hypotheses for the diversification of Amazonian diversity were proposed since then and reviews on this subject are available (Haffer, 1997; Moritz et al., 2000; Zeisset & Beebee, 2008; Haffer, 2008; Antonelli et al., 2010).

Some authors elaborated predictions that should be proven true, in the case of river-mediated diversification (Haffer, 1997; Moritz et al, 2000; Patton et al., 2000). For example, Patton et al (2000) suggested three possible scenarios of river mediated divergence: (1) reciprocally monophyletic and sister clades on opposite sides of the river would indicate primary diversification; (2) reciprocally monophyletic but not sister clades on opposite sides of the river would suggest secondary contact; (3) paraphyletic relationship from one bank relatively to the other would correspond to an event of dispersal across the river.

Patterns of species distribution or the phylogenetic patterns of vertebrate species sometimes appeared to fit the predictions of the Riverine Barrier Hypothesis. Some examples are: the distribution patterns of amphibians and other vertebrate groups in lowland Amazonia (Ron, 2000); and the phylogenetic patterns of amphibians (Noonan and Wray, 2006; Funk et al, 2007), lizards (Pellegrino et al., 2005; Rodriguez-Robles et al., 2008), birds (Bates et al., 2004), tamarins and rodents (Patton et al, 2000). However, several other (or even the same) studies reveal examples of distribution and phylogenetic patterns that contradict the predictions of this hypothesis (Gascon et al., 1998; Lougheed et al., 1999; Gascon et al., 2000; Patton et al., 2000; Symula et al., 2003). Several authors agree that no single model could adequately explain Amazonian diversity, and that different mechanisms should be relevant in speciation processes of different faunal groups (Noonan and Wray, 2006; Zeisset and Beebee, 2008; Haffer, 2008; Antonelli et al., 2010).

Alternative hypothesis for the origin of the Neotropical diversity have been tested in the last years. For example, the hypothesis of the Pleistocene refuge received support from the study by Carnaval and Moritz (2008), who found that paleoclimate modelling predicted the presence of historical refugia that matched current centres of endemism and biodiversity patterns. On a different study, Carnaval et al. (2009) found that areas of historical stability within Atlantic Forest presented higher genetic diversity than populations from unstable areas, providing support for the Pleistocene refuge hypothesis. Some support was also found for the role of Pleistocene climatic fluctuations in shaping phlylogeographic pattern and historical demography of the Neotropical rattlesnake, *Crotalus durissus* (Wüster et al., 2005; Quijada-Mascareñas et al., 2007). However, evidence from Amazonian lizard and amphibian taxa showed that, in the studied cases, interspecific, and even intraspecific diversification occurred well before the Pleistocene, dismissing the Pleistocene refugia as the mechanism for Amazonian diversification (Glor, 2001; Elmer et al. 2007; Gamble et al., 2008). There is also recent evidence contradicting the influence of historical refugia in the diversification of an Atlantic forest anuran (Thomé et al., 2010). An alternative hypothesis, the disturbance-vicariance hypothesis, received some support from the study of the phylogeographic patterns of the poison frog *Dendrobates tinctorius* (Noonan and Gaucher, 2006). Several authors agree that no single model could adequately explain Amazonian diversity, and that different mechanisms should be relevant in speciation processes of different faunal groups (Noonan and Wray, 2006; Zeisset and Beebee, 2008; Haffer, 2008; Antonelli et al., 2010).

1.7 – Thesis Objectives

In face of the intensification of deforestation and agriculture pressure in Cerrado and Amazonia, most precisely in the deforestation arc and the transitional area between these two biomes, it becomes essential to have a clear picture of the regional biodiversity. There are plans for the establishment of a corridor of conservation areas in the middle Araguaia basin, involving several parks, sustainable use and indigenous areas, which also requires understanding how and if the conservation strategy for the region will be able to cope with the current threats and accomplish its long-term objectives of preserving the regional biodiversity.

Having this in mind, and choosing herpetofauna as the target faunal group, our main objectives are: to improve the knowledge on the amphibian and lizard assemblages in the region of the middle Araguaia River, namely how these are distributed across different conservation areas with different conservation statuses; to understand the intra-specific patterns of genetic structure and diversity for different species with different ecological features, within this region; and to to assess the potential of different methodologies for the study and monitoring of the regional herpetofauna.

I begin this thesis by this general introduction (Chapter I) with the aim of providing a better picture of the region of the mid-Araguaia basin. I tried to cover different aspects that I considered relevant in the context of this thesis: the two merging biomes; the economic and political context with focus on land use, current threats and conservation units; the characterization of the Araguaia hydrodynamics and the human impacts upon it; the characterization of the herpetofauna of Cerrado and Amazonia, within the Brazilian context, and finally the potential influence of rivers upon species diversification. Chapters II and III are presented with the objective of providing some information on the methods used during the collection of biological data. Fieldwork occupied more than two years of this study and is generally one of the most time-consuming tasks when studying biodiversity. We hope that the systematization of this kind of information might prove to be valuable for future researchers. Chapter IV focus on the distribution of amphibian and squamate species across the different conservation units, in an attempt to understand what is in fact being preserved and what is being left at the mercy of the fast-growing regional development. Chapters V and VI are focused on the role of the Araguaia on the diversification, respectively, of amphibian and lizard populations. In Chapter VII I try to summarize the main findings of the previous chapters and integrate them again in the larger context of the mid-Araguaia basin.

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CHAPTER 2

Biodiversity surveys in the Amazonia/Cerrado ecotone: the usefulness of different methods for capturing small mammals and herpetofauna

Ferreira E., Rocha R.G., Serafini A., Costa L.P., Nogueira A.J.A, Malvasio A., Martins I., Fonseca C. (2011). Biodiversity surveys in the Amazonia/Cerrado ecotone: the usefulness of different methods for capturing small mammals and herpetofauna. Manuscript submitted to *Studies on Neotropical Fauna and Environment.*

Chapter 2 cover photos (from left to right): artificial pipe refuge; detail of pitfall with drift fence; and Sherman trap.

2.1 – Abstract

Small mammals and herpetofauna were surveyed in the Amazonia/Cerrado ecotone and the capture efficiency of live traps and pitfalls was compared. We also evaluated if these methods alone accurately estimated local species richness, by using additional methods. Sixty-seven small vertebrate species were captured. Larger pitfalls were significantly more efficient than smaller ones. We also found significant differences among live traps and between live traps and pitfalls, at the family level. Pitfalls were the less selective method and might reveal very useful even in short-term biodiversity surveys. Additional sampling methods substantially increased the number of captured species. We suggest that it is important to diversify capture methodology and that variation within taxa must be considered when designing sampling strategy, or biodiversity will be underestimated.

Keywords: active search; Brazil; capture efficiency; live traps; pipe refuges; pitfalls.

2.2. – Resumo

A fauna de pequenos mamíferos e a herpetofauna do ecótono Amazônia/Cerrado foi amostrada e a eficiência de captura de armadilhas Sherman, Tomahawk e e armadilhas de queda foi comparada. Também avaliamos se estes métodos por si só estimariam de forma precisa a riqueza específica local, utilizando métodos adicionais. Sessenta e sete espécies de pequenos vertebrados foram capturadas. As armadilhas de queda maiores foram significativamente mais eficientes que as menores. Também encontramos diferenças significativas entre armadilhas Sherman, Tomahawk e armadilhas de queda, ao nível de família. As armadilhas de queda foram o método menos seletivo e podem revelar-se úteis mesmo em estudos de biodiversidade de curta duração. Os métodos de amostragem adicionais aumentaram substancialmente o número de espécies capturadas. Sugerimos que é importante diversificar a metodologia de captura e que a variação dentre taxa deve ser tida em conta durante o desenho da estratégia de amostragem, ou a biodiversidade será subestimada.

2.3 – Introduction

Brazilian Cerrado is the only tropical savanna included in the twenty-five biodiversity hotspots proposed by Myers et al. (2000), and Amazonian Rainforest is considered the largest and most diverse major tropical wilderness area (Mittermeier et al. 1998). These are the two largest Brazilian biomes (Klink & Machado 2005) and converge along a large ecotonal region in central Brazil, which is considered a conservation priority area (Azevedo-Ramos & Gallati 2002; Cavalcanti & Joli 2002). Despite its perceived importance, there is a generalized deficiency of sampling data in this area, common throughout the northern region of Cerrado (Marinho-Filho et al. 2002; Bini et al. 2006). Biodiversity surveys and complete species lists depend on efficient capture techniques. Careful evaluation of various techniques by experienced wildlife biologists is the key for successful capture programs (Schemnitz 1996). When available, published data about the efficiency of different methods could help to identify suitable techniques. Several studies about capture methodology were conducted in Amazonian Rainforest (Malcolm 1991; Voss & Emmons 1996; Woodman et al. 1996; Vieira 1998; Hice & Schmidly 2002; Lambert et al. 2005), but only a few exist for Cerrado, and only for the southern region of this biome (Cechin & Martins 2000; Vieira et al. 2004; Caceres et al. 2011).

Several factors can influence capture success: incidence and amount of precipitation (Gibbons & Bennett 1974); closed *versus* mesh trap (O'Farrell et al. 1994); size of the trap and mass of captured individuals (Slade et al. 1993); trap-habit and trap-avoidance (Sealander & James 1958); size of individuals and taxonomic group (Crosswhite et al. 1999) or ecological features across taxa (Malcolm 1991; Greenberg et al. 1994; Leite et al. 1996; Lambert et al. 2005). Generally, the simultaneous use of more than one method would help to increase the number of captured species (Mengak & Guyn 1987; Greenberg et al. 1994; Voss & Emmons 1996; Crosswhite et al. 1999).

Studies comparing different capture methods are normally developed within a particular region with a characteristic species assemblage. When extrapolating capture efficiency results from one area to another, wildlife professionals are confronted with different, but sometimes close, taxonomic assemblages. Therefore, comparisons made at higher taxonomical level, rather than species level, might be more useful. The aims of this study were: to survey small mammals and herpetofauna in the Amazonia/Cerrado ecotone; and to compare the capture efficiency of live traps (Sherman and Tomahawk traps) and pitfalls of two different sizes for different families of these vertebrate taxa.

Moreover, additional methods (active search and pipe traps) were also used to evaluate if live traps and pitfalls alone accurately estimate local species richness.

2.4 – Methods

2.4.1 – Study Area

This study was conducted in two conservation areas in the western region of the state of Tocantins, Central Brazil: Parque Estadual do Cantão (PEC) and the surrounding Área de Proteção Ambiental Bananal/Cantão (APABC). Fazenda Santa Fé (FSF) in the state of Pará – a nearby private ranch (65,000ha) without official conservation status – was also sampled. PEC (90,000ha) is a state natural reserve and APABC (1,700,000ha) is a conservation buffer area, where limited human activities (e.g. farming and forestry) are allowed. This buffer area surrounds PEC and the nearby Parque Nacional do Araguaia (PNA). A large river complex dominated by the Araguaia River, a natural border between the states of Tocantins and Pará, characterizes the area. The study area is located in the ecotonal region between the Cerrados from central Brazil and the Amazonian Rainforest, and is mainly composed of alluvial forests and, in a lesser extent, well-drained areas with more typical Cerrado *sensu lato* physiognomies (Oliveira-Filho & Ratter 2002). Seasonally flooded areas occupy most of PEC, and permanently dry areas are mostly located outside the park, in APABC and in the western margin of river Araguaia, state of Pará. Climate in this region of Brazil is tropical, with a rain season from October to April and a dry season from May to September (INMET 2010).

2.4.2 – Sampling

Two trapping methods were used: pitfalls (approximately 30L – diameter 32cm/height 38cm; and approximately 60L – diameter 38cm/height 54cm) with plastic drift fences (50cm height and 5m long) and live traps (Tomahawk – 45x21x21cm – and Sherman traps – 45x12.5x14.5cm). Four smaller sub-areas within the study area were sampled, each with five sampling points established at least 2 Km apart. Fourteen sampling points were established inside PEC and six in the surrounding areas. Sampling design at each point (Figure 2.1), consisted of: a line of sixteen 30L pitfalls with drift fences; a line of ten 60L pitfalls with drift fences; a mixed line with 22 Sherman traps and 10 Tomahawk traps. Lines were placed at least 150m apart. Sampling design for pitfall lines was based on a model proposed by A.P. Carmignotto (pers. comm.). Traps were checked daily, at early morning, to avoid desiccation of captured individuals. Water was added to pitfalls also to avoid desiccation, but without causing the drowning of small mammals, as reported earlier by Mengak & Guyn (1987). Excess water was removed after heavy rain. A small piece of wood or stone was placed inside the buckets to provide a dry surface for small mammals and lizards. Arthropods, mainly arachnids and ants, were removed from pitfalls, because they frequently preyed on vertebrates inside the buckets. Adding water also helped reduce vertebrate mortality caused by arthropods. Live traps were baited with peanut butter and pineapple and the bait was replaced every two days. At the end of each sampling period, buckets were closed, to prevent accidental death or injury of animals in the area, and live traps were removed.

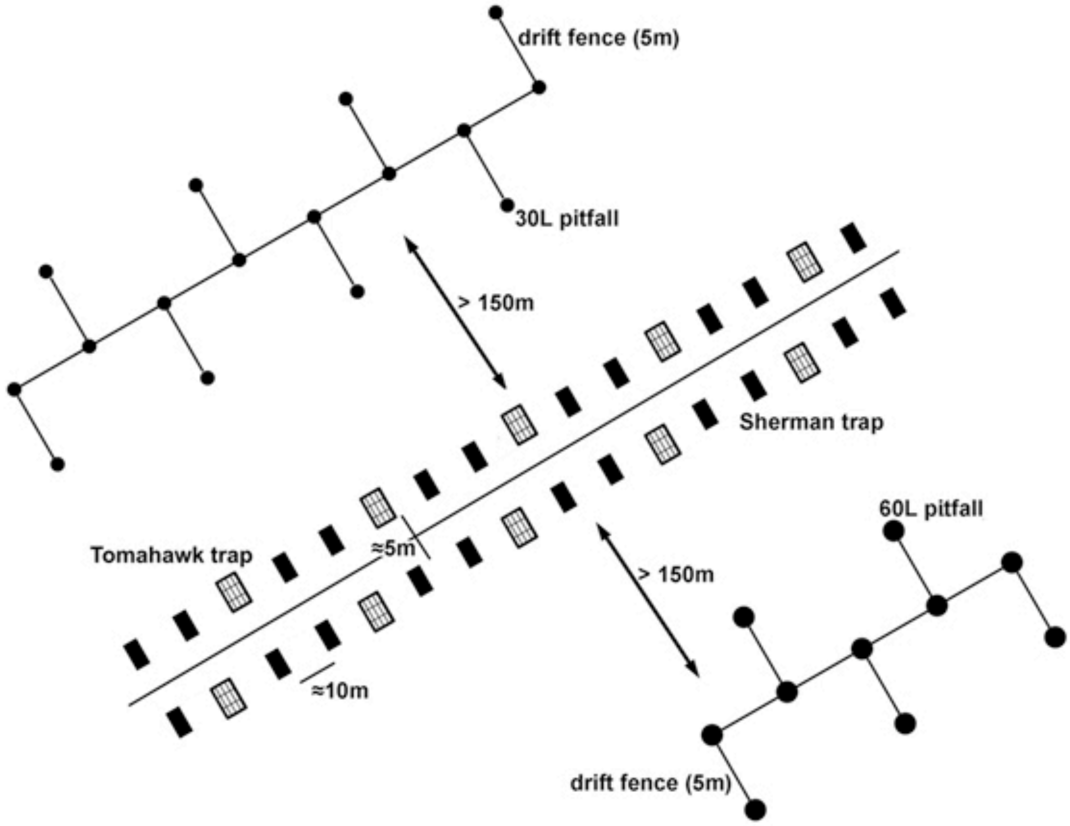

Figure 2.1 – Sampling design used in each one of the 20 sampling points. Lines of pitfalls and live traps were placed at least 150m apart

Sampling was carried out between June 2007 and November 2008. Three sampling periods averaging seven nights (range 5–9 nights) were performed for each area: 1) at the end of the rain season (June to July 2007; May to June 2008); 2) during the dry season (August to September 2007 and 2008); 3) at the beginning of the following rain season (October to November 2007 and 2008). Each sub area was sampled separately. Time intervals between consecutive samplings in the same area varied between one and a half and two months. We did not sample during the rain season since most of the area remains flooded. Small inconsistencies in trap-night numbers across methods were due to damaging of traps by wild animals or removal by local people. We also used active search and PVC pipe traps (tree pipe traps: 40cm long, adapted from Jonhson [2005]; lake pipe traps: 80cm long) as additional methods for surveying other species present in the area, but these methods were not used for statistical comparisons in this study.

2.4.3 – Collecting and Marking

All vertebrates captured in the traps (except snakes and some accidentally captured birds) were weighed with a digital scale (precision 0.1g) or with a spring scale (precision 20g), and identified to genus/species level, whenever possible. Individuals not identified in the field, as well as the first ones of each species caught, were collected as voucher specimens and deposited in "Coleção de Mamíferos da Universidade Federal do Espírito Santo" and "Coleção Herpetológica da Universidade de Brasília" (CHUNB). All other individuals were individually marked and released. Small mammals were marked with ear-tags and amphibians and lizards were marked with visible implant elastomer (VIE). All procedures were performed according to Brazilian national laws and guidelines. Fieldwork was carried out with permits from the federal (ICMBIO, permits: 200/2006; 036/2007; 13546-3 and 14307-1) and state (NATURATINS, permits: 019/2006; 009/2007 and 001/2008) conservancy agencies.

2.4.4 – Data Analysis

Capture data from all sampling points and periods were pooled by type of trap (30L or 60L pitfall and Sherman or Tomahawk), family and species. Only first captures of each individual, in each method, were included in odds-ratio analysis and graphical weight analysis. If an individual was captured more than once by the same method, it was considered a recapture. Capture and recapture rates (expressed as percentages) were calculated as the ratio of capture and recapture numbers over total trap-night numbers.

Captured individuals were classified by weight. Eight classes ranging from 0 to 2187g were defined according to a geometric series with base 3: $[0–3^0:3^6–3^7]$. Species capture rates (capture/1000 trap-nights) *per* weight class were used to visually compare capture efficiency by method, family and species.

Odds ratio meta-analysis was performed on capture data pooled by family using STATSDIRECT® statistical package, for each of the following pairs of methods: 60L pitfall *versus* 30L pitfall; Sherman traps *versus* Tomahawk trap; live traps *versus* pitfalls. For each comparison, only families captured in both methods under comparison were included in the analysis. We excluded from this analysis the accidental captures of: 1) Hylidae, in comparison among pitfalls, since these are not true captures individuals could easily escape from buckets; 2) Iguanidae, Polychrotidae and Scincidae, in comparison among live traps and pitfalls since only one individual of each family was captured in live traps.

Estimation of independent (family) and pooled odds-ratio weights the number of positive (capture) and negative cases (empty trap/pitfall), for both trap type. Thus, the calculation accounted for differences in trap-night numbers between different traps. Heterogeneity among independent odds-ratios was estimated in the analysis, using the inconsistency index I^2 (Higgins et al. 2003). A model accounting for random effects was chosen for estimating pooled odds-ratios (DerSimonian & Laird 1986). Confidence intervals (95%) for the independent and pooled estimates were also calculated in the analysis.

One-tailed *t*-test for unpaired samples was performed for statistical comparison between the average numbers of mammal recaptures, per night, in pitfalls, for: (1) one sampling period during dry season (9 nights, August 2007) and (2) total sampling time (84 nights). Our goal was to test the hypothesis that the average number of recaptures per night was higher during that particular dry season.

2.5 – Results

During this study, 2286 individuals were captured: 1507 amphibians (five families), 325 lizards (ten families) and 454 small mammals (three families). Total numbers of trap-nights (Table 2.1) were as follows: Sherman traps (8580 trap-nights); Tomahawk traps (3900); 30L pitfalls (6159) and 60L pitfalls (4079). Global capture rates were higher for 30L (15.12%) and 60L pitfalls (22.16%) than for Sherman (3.62%) and Tomahawk traps (4.10%) (Table I). Capture rates for amphibians in 30L and 60L pitfalls were very high compared with other vertebrate groups. Recaptures in pitfalls and live traps only occurred for mammals and teiid lizards. A negligible number of amphibian recaptures was observed in pipe traps and during active search.

Table 2.1 – Capture/recapture rates given by taxa and trap type, expressed as percentage of trap-nights number. When present, recapture rates are given after the dash.

Taxa	*Trap-nights*	**Sherman** *8580*	**Tomahawk** *3900*	**30L pitfall** *6159*	**60L pitfall** *4079*
Amphibia					
	Bufonidae	0.00	0.00	0.08	0.42
	Hylidae	0.00	0.00	0.06	0.02
	Leiuperidae	0.00	0.00	6.61	9.19
	Leptodactylidae	0.00	0.00	3.20	5.10
	Microhylidae	0.00	0.00	2.45	3.97
	Class totals	*0.00*	*0.00*	*12.40*	*18.71*
Reptilia					
	Amphisbaenidae	0.00	0.00	0.00	0.02
	Gekkonidae	0.00	0.00	0.10	0.07
	Gymnophtalmidae	0.00	0.00	0.42	0.25
	Iguanidae	0.01	0.00	0.02	0.00
	Polychrotidae	0.01	0.00	0.24	0.37
	Scincidae	0.01	0.00	0.06	0.07
	Teiidae	0.65/0.06	1.3/0.08	1.25/0.06	1.23/0.02
	Tropiduridae	0.00	0.00	0.05	0.05
	Class totals	*0.69/0.06*	*1.3/0.08*	*2.14/0.06*	*2.03/0.02*
Mammalia					
	Didelphidae	1.49/0.92	2.38/2.13	0.10	0.71/0.52
	Cricetidae	1.07/0.36	0.03	0.47	0.66/0.02
	Echimyidae	0.37/0.24	0.38/0.03	0.00	0.05/0.00
	Class totals	*2.94/1.53*	*2.79/2.15*	*0.57*	*1.42/0.54*
Totals		**3.62/1.59**	**4.10/2.23**	**15.12/0.06**	**22.16/0.56**

Sixty-seven vertebrate species (Table 2.2) were captured: 29 amphibian, 20 lizard and 18 small mammal species. Pitfalls captured 15 amphibian, 12 lizard and 14 small mammal species. From all these species, 7 were unique to pitfalls. Live traps captured 6 lizard and 12 small mammal species. Only 3 small mammal species were unique to live traps. Additional methods accounted for the highest number of amphibian (active search – 26 spp.; pipe traps – 8 spp.) and lizard species (active search – 15 spp.), but captured only 3 small mammal species (active search – 1 sp.; pipe traps – 2 spp.). Additional methods also accounted for the highest number of unique species: 8 amphibian and 6 lizard species by active search and 3 amphibian species in pipe traps.

Table 2.2 - Species captured by method, with reference to class/method totals. Unique species, defined as those captured by only one method, are presented between brackets.

Taxa			Method					
Class	Family	Species	Sherman trap	Tomahawk trap	30L pitfall	60L pitfall	Pipetrap	Active search
Amphibia								
	Bufonidae	*Rhaebo guttatus* (Schneider, 1799)			X	X		X
		Rhinella granulosa (Spix, 1824)						X
		Rhinella ocellata (Günther, 1859 “1858”)			X	X		X
		Rhinella schneideri (Werner, 1894)			X	X		X
	Craugastoridae	*Haddadus* sp.						X
	Hylidae	*Dendropsophus melanargyreus* (Cope, 1887)						X
		Dendropsophus minutus (Peters, 1872)					X	
		Dendropsophus nanus (Boulanger, 1889)					X	
		Hypsiboas fasciatus (Günther, 1859 "1858")					X	X
		Hypsiboas raniceps Cope, 1862					X	X
		Osteocephalus taurinus Steindachner, 1862					X	X
		Phyllomedusa azurea Cope, 1862						X
		Scinax fuscomarginatus (A. Lutz, 1925)					X	X
		Scinax fuscovarius (A. Lutz, 1925)			O	O		X
		Scinax gr. ruber (Laurenti, 1768)			O		X	X
		Trachycephalus venulosus (Laurenti, 1768)			O		X	X
	Leiuperidae	*Physalaemus centralis* Bokermann, 1962				X		X
		Physalaemus cuvieri Fitzinger, 1826			X	X		X
		Pseudopaludicola mystacalis (Cope, 1887)			X	X		X
	Leptodactylidae	*Leptodactylus bokermanni* Heyer, 1973			X	X		
		Leptodactylus fuscus (Schneider, 1799)						X
		Leptodactylus labyrinthicus (Spix, 1824)						X
		Leptodactylus leptodactyloides (Andersson, 1945)			X	X		X
		Leptodactylus mystaceus (Spix, 1824)			X	X		X
		Leptodactylus latrans (Steffen, 1815)			X	X		X
		Leptodactylus cf. *petersi* (Steindachner, 1864)						X
		Leptodactylus pustulatus (Peters, 1870)			X			X
	Microhylidae	*Chiasmocleis albopunctata* (Boettger, 1885)						X
		Elachistocleis ovalis (Schneider, 1799)			X	X		X
	Class totals	*29*	*0*	*0*	*14*	*12*	*8(2)*	*26(8)*
Reptilia								
	Amphisbaenidae	*Amphisbaena* sp.				X		
		Amphisbaena cf. *meringoera* Vanzolini, 1971						X
	Iguanidae	*Iguana iguana* (Linnaeus, 1758)	O					X
	Polychrotidae	*Anolis nitens (Wagler, 1830)*	O		X	X		X

	Anolis ortonii Cope, 1868				X		
	Polychrus acutirostris Spix, 1825						X
Tropiduridae	*Tropidurus torquatus* Wiegmann, 1834			X	X		X
	Tropidurus oreadicus Rodrigues, 1987			X	X		X
Gekkonidae	*Hemidactylus mabouia* (Moreau de Jonnès, 1818)						X
Phyllodactylidae	*Gymnodactylus amarali* Barbour,1825						X
Sphaerodactylidae	*Gonatodes humeralis* (Guichenot, 1855)			X	X		X
Teiidae	*Ameiva ameiva* (Linnaeus, 1758)	X	X	X	X		
	Cnemidophorus occellifer (Spix, 1825)						X
	Kentropix calcarata Spix, 1825	X		X	X		
	Tupinambis teguixin (Linnaeus, 1758)	X	X		X		
Gymnophtalmidae	*Colobossaura modesta* (Reinhardt & Luetken, 1862)			X	X		X
	Micrablepharus atticolus Rodrigues, 1996			X	X		X
	M. maximiliani (Reinhardt & Luetken, 1862)						X
Scincidae	*Mabuya frenata* (Cope, 1862)					X	X
	Mabuya nigropunctata (Spix, 1825)	O		X	X		X
Class totals	*20*	*6*	*2*	*9*	*12(2)*	*1*	*15(6)*
Mammalia							
Didelphidae	*Caluromys philander* (Linnaeus, 1758)		X	X		X	
	Didelphis albiventris Lund, 1840	X	X		X		
	Didelphis marsupialis Linnaeus, 1758	X	X	X	X		
	Gracilinanus agilis (Burmeister, 1854)	X		X			
	Marmosa murina (Linnaeus, 1758)	X	X	X	X		
	Metachirus nudicaudatus (É. Geoffroy, 1803)	X	X				
	Micoureus demerarae (Thomas, 1905)	X	X				
	Philander opossum (Linnaeus, 1758)	X	X		X		X
Cricetidae	*Calomys tocantinsi* Bonvicino, Lima & Almeida, 2003			X	X		
	Holochilus sciureus Wagner 1842			X			
	Hylaeamys megacephalus (Fischer, 1814)	X		X	X		
	Oecomys sp.				X		
	Oecomys roberti Thomas, 1904	X	X	X	X		
	Oligoryzomys sp.			X	X		
	Pseudoryzomys simplex Hershkovitz, 1962				X		
	Rhipidomys sp.*					X	
Echimyidae	*Makalata didelphoides* (Desmarest, 1817)	X	X		X		
	Proechimys roberti Thomas, 1901	X	X				
Class totals	*18*	*11*	*10*	*9(1)*	*11(2)*	*2(1)*	*1*
Total	**67**	**19**	**14**	**34(1)**	**37(4)**	**13(3)**	**44(14)**

Captured species – X; Accidental capture of a single individual – O; *a(b)*: a = total number of species; b = unique species. * - a single individual was captured in a preliminary sampling in a Sherman trap but was not captured again.

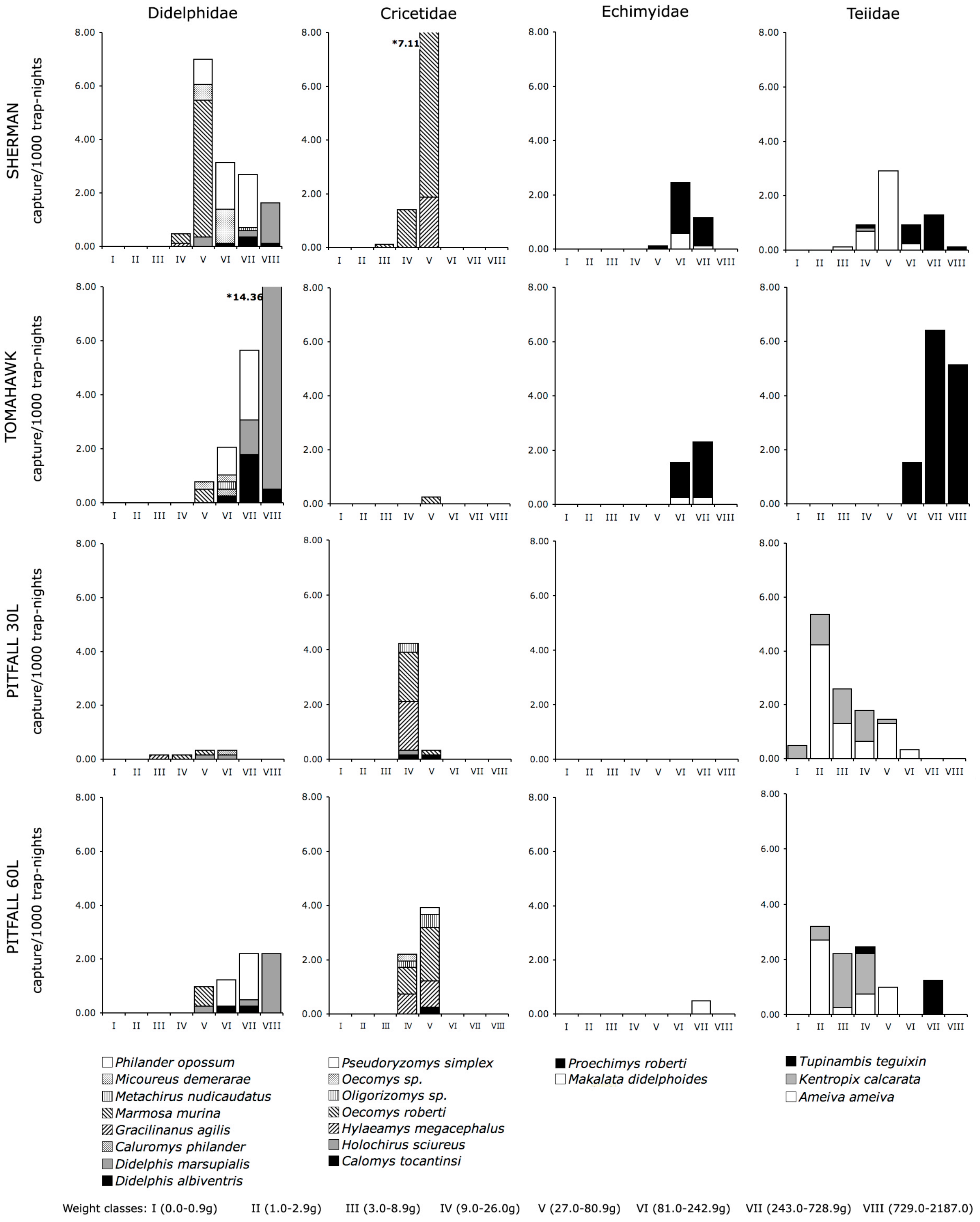

Figure 2.2 – Capture rates (captures/1000 trap-nights) for all families captured in all methods under comparison, presented by weight class. Species are identified by shades of grey and black and white patterns. Asterisks stand for off-scale values – actual capture rates for these two species are shown at the side of the bar

All small mammal families (Didelphidae, Cricetidae and Echimyidae) and one lizard family (Teiidae) were consistently captured in live traps and pitfalls (Figure 2.2). With exception to some accidental captures in live traps, other lizard or amphibian families were only captured in pitfalls (Figure 2.3).

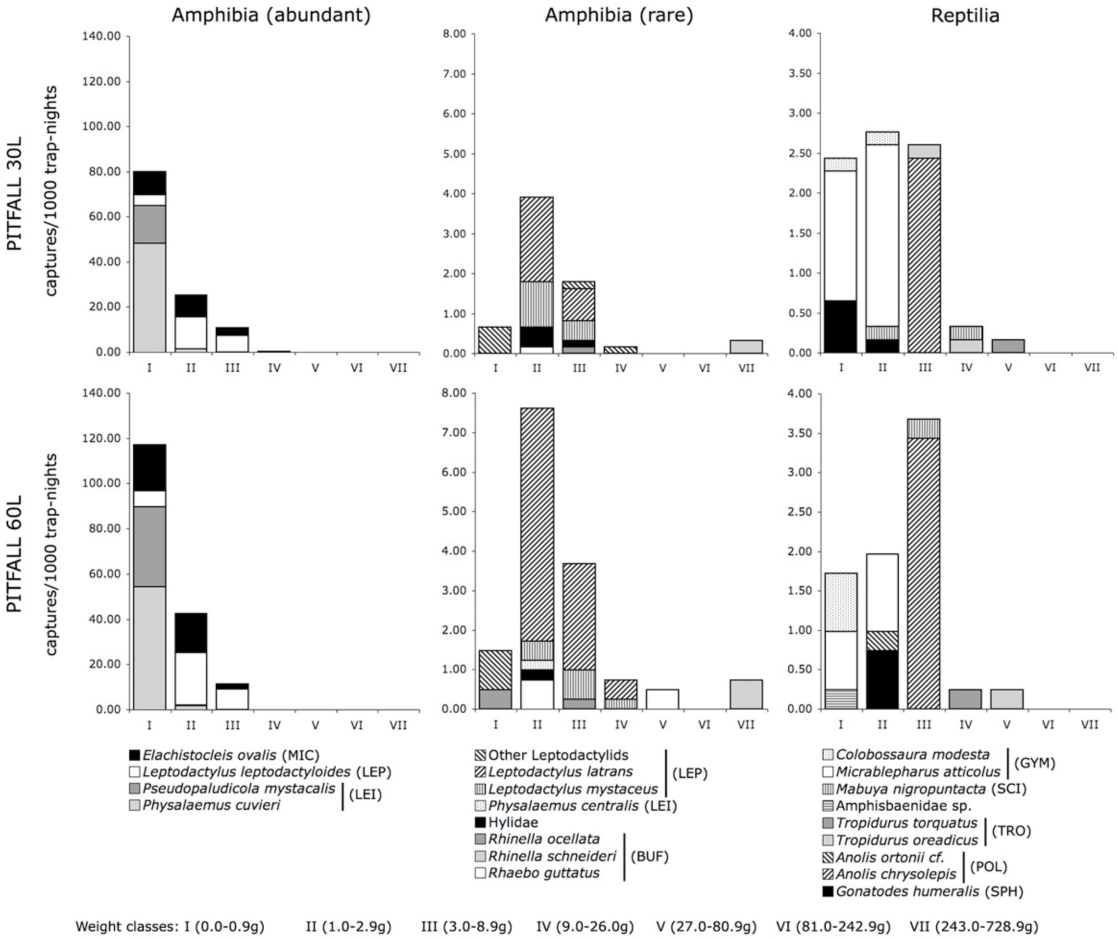

Figure 2.3 – Capture rates (captures/1000 trap-nights) for families captured only in pitfalls, presented by weight class: 3a) abundant amphibian species; 3b) rare amphibian species; 3c) lizard species. Species are identified by shades of grey and black and white patterns. BUF – Bufonidae; SPH – Sphaerodactylidae; GYM – Gymnophtalmidae; LEI – Leiuperidae; LEP – Leptodactylidae; MIC – Microhylidae; POL – Polychrotidae; SCI – Scincidae; TRO – Tropiduridae

Smaller teiid species were mainly captured in pitfalls. *Ameiva ameiva* (Linnaeus, 1758) was also frequently captured in Sherman traps. *Tupinambis teguixin* (Linnaeus, 1758)*,* the largest species of the family occurring in the area, was almost exclusively captured in Tomahawk traps. Larger didelphids were mainly captured in Tomahawk traps and, to a lesser extent, in Sherman traps and 60L pitfalls. Smaller didelphid species, such as *Marmosa murina* (Linnaeus, 1758), were mainly captured in Sherman traps. Larger cricetids were captured more frequently in Sherman traps and 60L pitfalls. Smaller cricetids were mainly captured in pitfalls, particularly in the larger ones. Echimyids were almost only captured in live traps.

Captures rates for amphibian taxa were consistently higher for 60L pitfalls, but both pitfalls captured individuals within the same weight-range. Families Leiuperidae, Leptodactylidae and Microhylidae were the major contributors to the observed capture rates. One or two species per family – *Pseudopaludicola mystacalis* (Cope, 1887, Leiuperidae) and *Physalaemus cuvieri* (Fitzinger, 1826, Leuiperidae), *Leptodactylus leptodactyloides* (Andersson, 1945, Leptodactylidae) and *Elachistocleis ovalis* (Schneider, 1799, Microhylidae), respectively – accounted for most of the captures, with a large proportion of juveniles in the latter three species. For lizard taxa, 30L and 60L also captured individuals within the same weight-range.

Heterogeneity estimates for odds-ratio analysis, using the inconsistency index (I^2) were: 67.0%, 90.0% and 97.0%, respectively. Heterogeneity among independent estimates (families) was globally high. Therefore, we used a random model to estimate the pooled odds-ratios. For overall comparisons (Figure 2.4), combined odds-ratio was only significantly different from 1 in the pair 60L/30L pitfalls (1.50, 95% CI = 1.19 to 1.88), where we can state that capture odds in favour of 60L pitfalls were about one and a half times greater than in 30L pitfalls.

In familial comparisons between pitfalls of different size: Bufonidae (5.15, 95% CI = 1.82 to 17.87), Leiuperidae (1.43, 95% CI = 1.23, 1.66, Leptodactylidae (1.62, 95% CI = 1.32 to 1.99), Microhylidae (1.65, 95% CI = 1.31 to 2.08) and Didelphidae capture odds (7.34, 95% CI = 2.99 to 21.65) were significantly greater in favour of 60L pitfalls. For the pair Sherman/Tomahawk traps, capture odds were significantly greater for: Cricetidae (42.27, 95% CI = 7.39 to 1687.94), in favour of Sherman traps; and for Teiidae (0.50, 95% CI = 0.33 to 0.74), in favour of Tomahawk traps. For the pair live traps/pitfalls, capture odds were significantly greater for: Didelphidae (4.43, 95% CI = 3.09 to 6.54) and Echimyidae (16.33, 95% CI = 4.27 to 138.85), in favour of live traps; and for Teiidae (0.59, 95% CI = 0.45 to 0.76), in favour of pitfall traps.

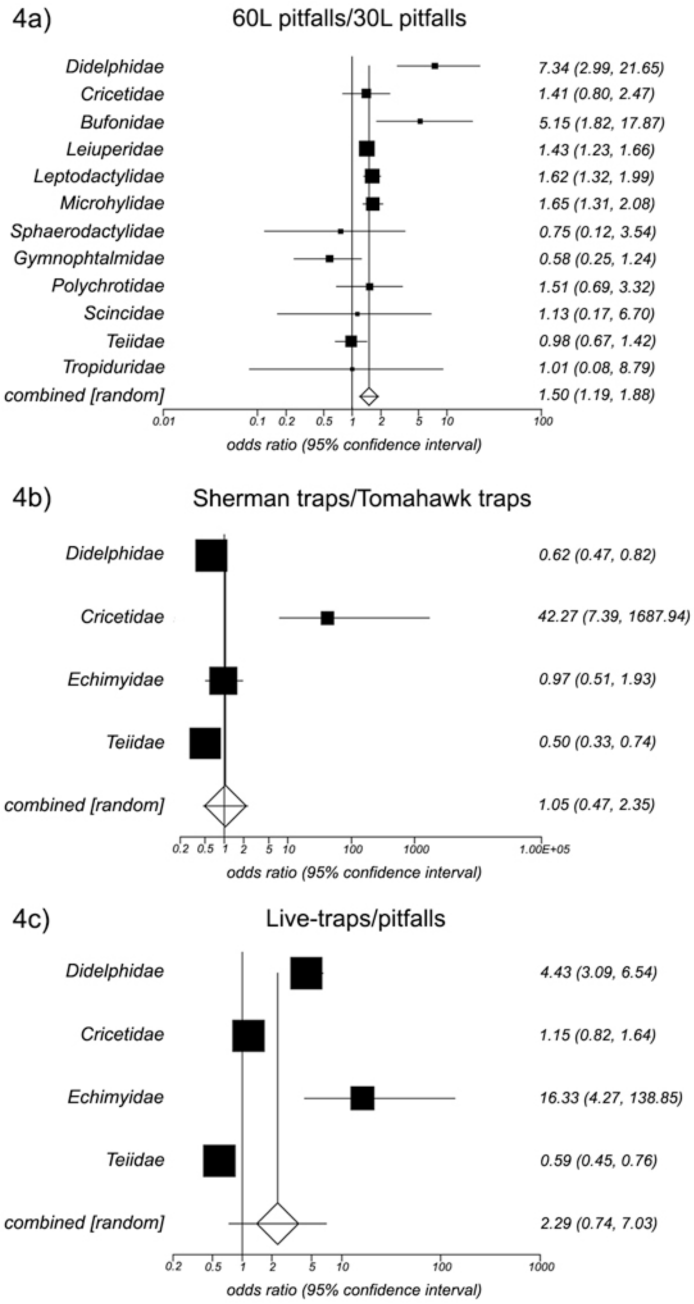

Figure 2.4 – Results from odds-ratio pairwise meta-analysis: 60L pitfall versus 30L pitfall (4a); Sherman traps versus Tomahawk trap (4b); live-trap versus pitfall trap (4c). Black solid squares represent ratios for each family; open rhombuses represent combined ratios and horizontal lines indicate 95% confidence intervals. Values are presented in the right side of the graphs. Size of squares is proportional to contribution of each family to combined ratios.

During the peak of the 2007 dry season, we detected that small mammals were being recaptured in pitfalls more frequently than average (for the total sampling period).

Thus, we compared the recapture rates between the dry season and the total sampling period. Mean number of mammal recaptures per night, in pitfalls, during the peak of 2007 dry season (1.00 ± 0.71, n = 9) was significantly greater ($t = 3.919$, $df = 91$, $p < 0.0001$) than mean number of mammal recaptures per night, in pitfalls, during total sampling period (0.26 ± 0.52, n = 84).

2.6 – Discussion

2.6.1 – Amphibians

Most amphibians present a typical sit-and-wait foraging strategy, using mainly visual cues for prey detection (Duellman & Trueb 1994). Baited live traps were primarily designed for capturing small mammals, which forage actively, using olfactory cues. This behavioural difference might be the reason for the absence of amphibian captures in live traps, as occurred in this study. There is no reason to believe that sit-and-wait predators would be more prone to fall inside pitfalls than to enter in live traps. However, animals might be captured simply because they move at some extent. Drift-fences enlarge the area of influence of pitfalls, by driving individuals into the buckets, and thus there is a higher probability of being intercepted by the drift-fences than by live traps. On the other hand, pitfalls allow multiple captures.

Several amphibians present explosive breeding behaviour and tend to be gregarious in early stages of life (Wells 2007). This behaviour was already reported for genus *Elachistocleis*, *Leptodactylus* and *Physalaemus* (Barreto & Andrade 1995; Rodrigues et al. 2003; Brasileiro et al. 2005), and is probably the reason for the observed high capture-rate for juveniles of some amphibian species. Despite the large capture rates, amphibians were never recaptured in pitfalls. Their low vagility (Blaustein et al. 1994), associated to the fact that they were released at some distance from capture location, probably contributed to this result.

Additional methods substantially increased the number of captured species. Several were unique species from families Hylidae (5 species) and Leptodactylidae (3 species), and one species was the single representative of the family Craugastoridae, in this study. Other authors had already suggested that the use of several methods is critical for adequately sampling herpetofauna (Greenberg et al. 1994; Crosswhite et al. 1999; Hutchens & DePerno 2009).

During fieldwork, several individuals from smaller weight classes were observed trying to climb pitfall walls. We suspect that even for smaller individuals, there is a chance of escape that is inversely proportional to the depth of the pitfall. Largest differences in capture rates between different pitfalls were for individuals from the lower weight classes, but also for medium-size leptodactylids. Leptodactylids are good jumpers and 30L pitfalls may be too shallow to prevent the escape of adult individuals of medium to large species. Bufonids, on the other hand, are not good jumpers, and even the larger ones were captured in both pitfalls. Larger pitfalls were more efficient than smaller ones for capturing amphibians.

2.6.22 – Lizards

Lizards can be broadly classified as widely (or active) foragers or as sit-and-wait (or ambush) predators, according to foraging mode (Miles et al. 2007). Sit-and-wait behaviour is frequently reported in families Polychrotidae, Tropiduridae (Iguania) and in Neotropical Gekkota (Gekkonidae, Sphaerodactylidae and Phyllodactylidae); while Teiidae, Gymnophtalmidae and Scincidae generally present active foraging behaviour (Colli et al. 2007; Vitt & Zani 1998a, 1998b; Miles et al. 2007). Teiids are active foragers that use mainly chemical signals for prey capture and discrimination (Cooper 2007), and it is likely that they were attracted to bait in live traps, like mammals. Capture rates, in live traps, were similar for teiids and small mammals. Recapture rates were however substantially lower for teiids.

Live traps failed to capture any other lizard family efficiently. Several causes can be suggested for this result. Iguania and Gekkota lizard families are mainly composed of ambush predators, as already discussed. Amphisbaenids are highly specialized fossorial squamates (Colli & Zamboni 1999). Family Scincidae also includes several ambush predators, such as genus *Mabuya* (Brown & Nagy 2007). The gymnophtalmid lizard *Micrablepharus atticolus* is an active predator, but specialized in ants (Rodrigues 1996), and is usually found near to ant and termite mounds. Live traps were significantly less efficient in capturing teiids, than pitfalls. Among live traps, Tomahwak traps appear to be the most suitable for capturing teiids. However, as already mentioned, there is a strong bias from small species towards Sherman traps, and from larger species towards Tomahawk traps.

Teiids and gymnophtalmids were the most captured lizards, in pitfalls. Besides feeding strategy, one probable cause is that they are more active on ground. The polychrotid *Anolis nitens* (Wagler, 1930, Polychrotidae) was the third most captured lizard, and also spends much time in leaf-litter (Vitt et al. 2008a). All other species that occur in the area have arboreal or fossorial habits (Vitt et al. 2008b).

2.6.3 – Small mammals

Endothermy demands a high energetic cost and mammals spend much of their time searching for food (Townsend et al. 2008). This physiological constraint is probably on of the reasons why live traps (baited) were globally more efficient for small mammals, than pitfalls (non-baited). If capture rates are pooled for pitfalls and live traps, didelphids and echimyids were captured significantly more often in live traps. Only cricetids were more frequently captured in pitfalls but this result was not significant. For this family, capture rate in Sherman traps was much higher than in Tomahawk traps. One possible explanation is that Tomahawk traps are not suitable for capturing cricetids, simply because most individuals do not have enough weight to trigger the trap. Alternatively, they could be able to escape through the trap mesh, as already suggested by Lyra-Jorge & Pivello (2001). Our study supports this hypothesis in that only individuals from weight class V were captured in Tomahawk traps but individuals from lower weight classes were captured both in Sherman traps and in pitfalls. Considering only Sherman traps, capture rate was almost twice the average rate for pitfalls. In general, recapture rates were also higher for live traps than for pitfalls, except for recapture rate of echimyids in Tomahawk traps, which was very low.

The overall number of small mammal species captured in pitfalls (14spp.) was greater than in live traps (12spp). This finding is in agreement with those from previous authors (Umetsu et al. 2006, Caceres et al. 2011). However, there were differences between the three mammal families. Pitfalls captured more species of cricetids, while live traps captured more echimyids and didelphids. Additional methods used in this study were mostly ineffective for capturing small mammals but two arboreal species, which were rarely (*Caluromys philander* Linaeus, 1758) or never (*Rhipidomys* sp. nov., Rocha et al. *in press*) captured in live traps or pitfalls, were found dead in pipe traps.

Capture odds for didelphids were significantly greater in favour of larger pitfalls. A possible explanation is that smaller pitfalls cannot prevent the escape of larger individuals, as already stated by several authors (Lyra-Jorge & Pivello 2001; Hice & Schmidly 2002; Umetsu 2006). Echimyids were rarely captured in pitfalls and only in the larger ones. Cricetids were not captured in significantly higher rates in any type of pitfall, possibly because both pitfalls were effective in preventing the escape of most of the captured individuals. Sherman and Tomahawk traps appeared to be equally suitable for capturing echimyids. Sherman traps, which in this study were larger than standard ones (Slade et al. 1993), clearly did not capture larger individuals from family Didelphidae as efficiently as Tomahawks.

2.6.4 – Trap-habit behaviour in pitfalls

Recapture rate for didelphids was higher than recapture rate for any other taxa, in larger pitfalls. This recapture rate was in fact comparable to those from live traps. In this study, didelphids generally presented the highest capture and recapture rates, suggesting the existence of trap-habit behaviour, which is frequently referred to in literature for live traps (Sealander & James 1958; Woodman et al. 1996; Umetsu et al. 2006). However, we found no references in literature that related trap-habit behaviour to pitfalls.

From 22 mammal recaptures in 60L pitfalls during the entire sampling, nine occurred in the sampling period of the 2007 dry season. Only three didelphids (two *Didelphis marsupialis* Linnaeus, 1758 and one *Philander opossum* Linnaeus, 1758) were recaptured in the referred sampling period. We hypothesized that these captured individuals were intentionally entering pitfalls. The number of recaptures during this period was significantly greater than recapture number for total period. We consider that there is enough evidence for trap-habit behaviour, eventually caused by the availability of a limited resource, inside the pitfalls. During the dry season, water was frequently dropped into the buckets. On the other hand, arthropods were frequently captured inside pitfalls. We cannot be sure if these individuals were being attracted by the water or by the arthropods. Both species commonly include arthropods in their diet (Emmons & Feer 1997). It is possible that, in response to the seasonal scarcity of fruits, seeds and arthropods in this region (Vieira 2003), these individuals found a suitable source of food inside the pitfalls.

2.6.5 – Final Considerations

Species within a family share several common features (e.g. ecological, morphological, behavioural), because of their shared ancestry. Based on our results, we suggest that the foraging strategy of different taxa can influence the efficiency of a given method. Taxonomically distinct taxa that present similar foraging strategy tend to be captured by the same techniques. One example is the unexpectedly capture efficiency of live traps revealed for teiids, despite the fact that these traps were primarily designed for mammals. The same way, vagility and size of individuals could affect capture rates for different methods. In this study, differences in size between two rodent families (Cricetidae and Echimyidae) or within the lizard family Teiidae reflected in significant differences in capture efficiency of distinct methods. This variation within vertebrate taxa must be considered when designing sampling strategy, or diversity might be underestimated.

Ambush predators, which include all anurans and most lizards in this study, were captured in pitfalls, but not in live traps. In addition, if pitfalls had not been used, eight species would not have been captured. This was the least selective method, capturing across all vertebrate taxa. Despite the initial effort of putting up pitfall lines, we suggest that these should be used together with other methods, particularly in short-term studies, when local biodiversity must be surveyed within a small period.

About one third of the species found in this study were unique to active search or pipe traps. These methods were not efficient for small mammals but, for herpetofauna, they represented a substantial increase in species number. This result stresses the importance of alternative methods, which are sometimes easier to implement and with lower cost (Hutchens & DePerno 2009). Our results show that diversifying capture methods increases the number of captured species, and thus species richness estimates, corroborating the findings of previous authors, in other regions (Mengak & Guyn 1987; Greenberg et al. 1994; Voss & Emmons 1996; Crosswhite et al. 1999; Umetsu 2006). We expect that our results and considerations will help researchers and other wildlife professionals to design sampling strategies that provide a more accurate picture of the state of biodiversity, in a region of fast-evolving human impact.

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CHAPTER 3

On the usefulness of pipe refuges for studying herpetofauna in the Amazonia/Cerrado ecotone

Ferreira E., Rocha R.G., Malvasio A., Fonseca C. (2011). On the usefulness of pipe refuges for studying herpetofauna in the Amazonia/Cerrado ecotone. Manuscript submitted to *Herpetological Journal*. (Short communication)

Chapter 3 cover photo (from left to right): *Mabuya nigropunctata* on tree trunk; set of artificial pipe refuges; and adult *Osteocephalus taurinus*.

3.1 – Abstract

We evaluated the usefulness of arboreal pipe refuges for studying Neotropical herpetofauna, by surveying the colonizing species and assessing if significant correlations between species and microhabitat variables could be found. Additionally, we assessed the influence of pipe colour on the colonization success. We used fifty-five sets of three refuges (white, grey and black). We registered 122 colonization events by four Hylidae and one Scincidae species. Refuge colour did not significantly affect colonization success. Environmental data explained a significant portion (10.6%) of total variance of species data. The variables vegetal type and insertion height presented the highest correlation with species data.

Keywords: Amazonia; Cerrado; colour; environmental variables; Hylidae; Scincidae

3.2 – Introduction

Tree frogs are easily observed in breeding sites, during the mating season but, due to their elusive behaviour, they are not easily detected outside these areas and periods (Pittman et al., 2008). For this reason, the knowledge about their biology outside of breeding habitats is limited (Lemckert, 2004). This lack of knowledge is critical, because terrestrial areas surrounding breeding habitats are crucial for the survival of amphibian populations (Marsh & Trenham, 2000; Semlitsch & Bodie, 2003). Even species with long reproductive season spend more time in terrestrial habitats (Wilbur, 1984). In the case of some Neotropical hylids, reproductive cycles are related to arboreal microhabitats such as tree holes and bromeliads (Haddad & Sawaya, 2000; Haddad & Prado, 2005).

Artificial pipe refuges have been suggested as an alternative sampling methodology for hylids (Boughton et al., 2000; Johnson, 2005; Myers et al., 2007). Pipes can be installed on trees or in the ground, allowing its use in forest habitats and reproductive areas. This methodology was successfully used in ecological (Mahan & Johnson, 2007; Liner et al, 2008; Pittman et al., 2008) and conservation studies (Schurbon & Fauth, 2003; Wyatt and Forys, 2004). Colonization success of pipe refuges can be influenced by: pipe diameter (Bartareau, 2004); placement of pipes (Myers et al., 2007); sex and size of tree frogs, and tree species (Johnson et al, 2008); and tree frog species (Hoffman et al (2009). The effect of refuge colour on the colonization success of pipe refuges was never studied, but Crawford and Kurta (2000) found that anurans were more efficiently captured in black pitfalls than in white ones. These authors suggested that anurans might perceive light-coloured traps as different from the substrate and avoid them, or dark traps as potential refuge and enter them. We hypothesize that colour might indirectly influence the colonization of artificial refuges, in the same way, because of differences in luminosity between lighter (white) and darker (black and grey) refuges.

Despite the high tree frog diversity in the Neotropics, pipe refuges are not commonly used in this region, judging from the lack of published references to its use (but see: Laurencio & Malone, 2009; Silva & Rossa-Feres, 2007). Our aim was to assess the usefulness of pipe refuges for biodiversity surveys and ecological studies in Neotropical habitats by: surveying which tree frog species colonize the refuges; and assessing if correlations between environmental and species data can be obtained. We also aimed to assess whether or not pipe colour influences colonization success.

3.3. – Methods

This study was conducted in two different areas at the ecotonal region between Cerrado and Amazonia, in Brazil: one at the mid-Araguaia River, including Parque Estadual do Cantão (PEC, east bank, state of Tocantins) and Fazenda Santa Fé (FSF, western bank, state of Pará); the other at Fazenda Lago Verde (FLV, state of Tocantins). PEC is a 90,000 ha state conservation unit mainly composed by seasonally flooded forests. FSF is a 65,000ha private ranch with 65% of its area covered by well-preserved semi-deciduous tropical forest. FLV is an 8,000ha private ranch with 70% of the area constituted by pristine Cerrado physiognomies. Natural forest fragments (*ipucas*) occur within the agricultural and Cerrado matrices.

Three sampling points were placed in PEC, two in FSF and three in FLV. We used 55 sets of three arboreal pipe refuges (one of each colour: white, grey and black). Five sets of pipes were installed in each sampling point in PEC and FSF, and ten in each sampling point in FLV. Pipes were installed at two heights: above head and waist level. Pipe refuges were adapted from Johnson (2005): length – 40cm; inner diameter – 4cm; maximum water level – 8cm.

Each area was sampled during three periods of seven days each: end of rain season (April to May 2008), dry season (June to September 2008) and beginning of rain season (October to November 2008). Refuges were visited every two days during the sampling periods, totalizing twelve visits *per* sampling point. Arboreal pipe refuges were only removed at the end of the study. Captured individuals were individually marked with visible implant elastomer and released about 30m away from capture location. The first three individuals of each species were collected as voucher specimens and deposited at Coleção Herpetológica da Universidade de Brasília (CHUNB). The snout-vent length (SVL) of all individuals was measured prior to collection or releasing.

For each arboreal pipe refuge, the following environmental variables were measured: height of opening; perimeter of insertion branch; perimeter of widest branch on insertion tree/shrub; distance to widest branch; number of branches; angle of insertion; percentage of canopy cover; vegetal type (tree or shrub) and bark rugosity [from smooth (0) to very rugose (3)]. Damages to pipe refuges were classified according to its intensity: no damages (0), and minor (1), moderate (2) or severe damages (3). Refuges were repaired or replaced whenever needed.

Individuals were always released relatively far from capture location, thus recaptures were considered independent colonization events and included in the analysis. We estimated the mean, minimum and maximum SVL of each captured species. We searched for significant differences in average number of captures and damage intensity among differently coloured refuges. Numbers of captured individuals *per* species were considered insufficient for independent statistical comparisons. Thus, statistical analysis was performed based on the total number of captured individuals. Our data did not conform the assumptions of homocedasticity and normality of distribution. Therefore, multiple comparisons were made using Kruskall-Wallis test. Pairwise comparisons were performed using Dwass-Steel-Chritchlow-Fligner test. Both tests were performed using STATSDIRECT®, considering a 0.05 significance level. Redundancy analysis (RDA) was performed using CANOCO® for Windows®, to search for correlation between environmental and species data. Sampling point and refuge colour were included as covariates in RDA. The null hypothesis of independence between species and environmental data sets was tested using a Monte Carlo test, with 1000 permutations. The significance test (p=0.05) was run for the first canonical axis and for all axes together.

3.4 – Results

We registered 114 colonization events by hylids (4 recaptures) and eight by the skink *Mabuya frenata*. A maximum of three individuals of *Trachycephalus venulosus* were found simultaneously colonizing the same pipe. Average SVL for captured species (Table 3.1) varied from 16.7 ± 1.71 mm (*Scinax fuscomarginatus*), to 53.2 ± 16.24 mm (*Osteocephalus taurinus*). The smallest individual colonizing the pipes was a *S. fuscomarginatus* (SVL = 14.2 mm), and the largest was an *O. taurinus* (SVL = 74.5 mm). We found no significant effect of colour on the average number of individuals per refuge (white, 0.84 ± 1.014; gray, 0.54 ± 0.741; black, 0.84 ± 1.102), for simultaneous comparison of all samples (groups = 3; *df* = 2, total observations = 165; T = 2.2403; *p* = 0.326). Several refuges were damaged during the study and sometimes were found lying at the base of insertion trees. Simultaneous comparison of all samples (groups = 3; *df* = 2, total observations = 165; T = 14.5037; *p* = 0.001) revealed a significant effect of colour on the average damage level of the arboreal refuges (white, 0.58 ± 0.956; gray, 0.11 ± 0.369; black, 0.13 ± 0.388).

Table 3.1 —Mean and SD, minimum and maximum snout-vent length (SVL) and number of colonizing individuals in arboreal refuges, given per species and refuge colour.

Species	N	Snout-vent length (mm)		Refuge colour		
		Mean ± SD	Min-max	White	Grey	Black
Hylidae						
Osteocephalus taurinus	5	53.2 ± 16.24	37.7–74.5	2	3	0
Scinax fuscumarginatus	39	16.7 ± 1.71	14.2–20.4	14	13	12
Scinax gr. *ruber*	39	30.6 ± 4.82	22.4–42.5	21	2	16
Trachycephalus venulosus	31	50.5 ± 7.49	37.0–69.3	8	10	13
Scincidae						
Mabuya frenata	8	58.8 ± 6.14	52.0–64.0	1	2	5
Totals	122			46	30	46

Pairwise comparisons (critical q = 3.3145; *df* = 162) revealed that average damage level in white refuges was significantly greater than in grey (*p* = 0.004) and black (*p* = 0.010) refuges, whereas there was no significant difference (*p* = 0.948) between the latter two.

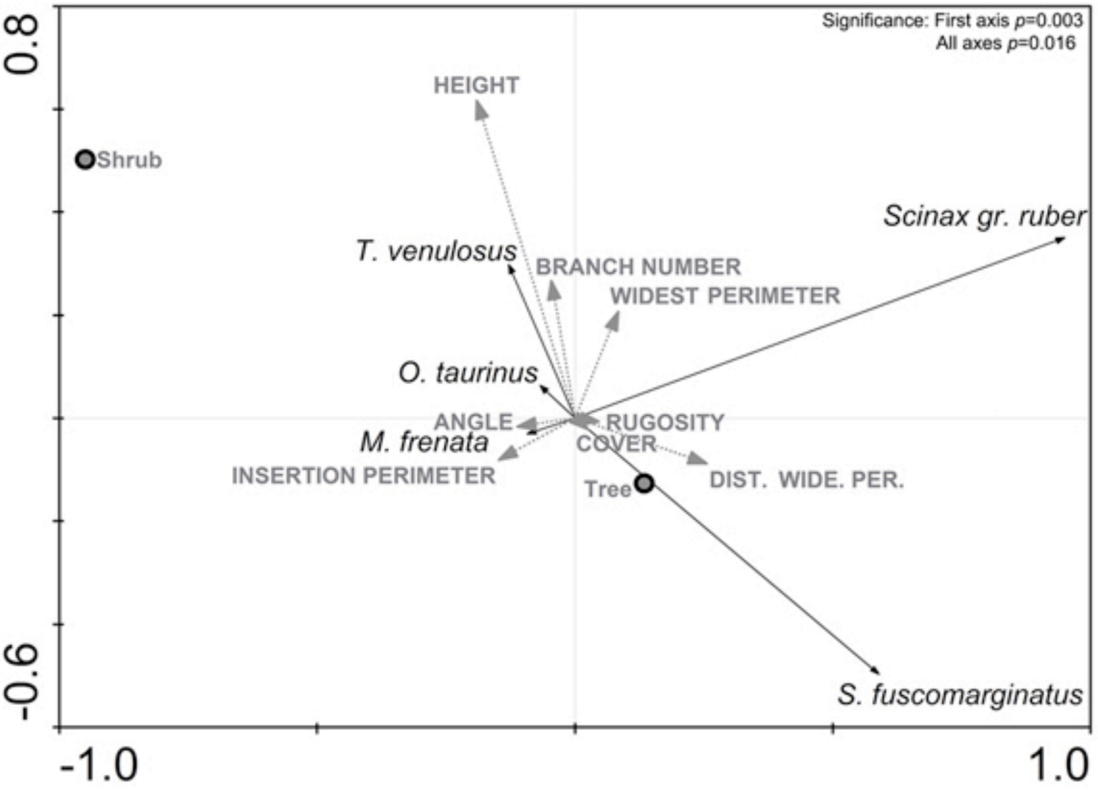

Figure 3.1 – RDA correlation biplot. Species – black solid lines; environmental variables (EV) – dotted grey lines and capitalized letters; Centroids from classes “tree” and “shrub” of nominal EV “vegetal type” – grey-filled circles.

The two first canonical axes of the RDA (Figure 3.1) explained 9.9% of cumulative percentage variance of species data. The sum of all canonical eigenvalues was 0.086, accounting for 10.6% of total variance of the species data. The portion of variance explained by the first and by all canonical axes was significantly greater than expected by chance alone (*p* = 0.003 and *p*=0.016, respectively). Vegetal type was the variable most correlated with the first environmental axis (tree – 0.380; and shrub – - 0.550) and the first species axis (tree – 0.178; and shrub – - 0.258). Insertion height was the variable most correlated with the second environmental (0.752) and the second species (0.201) axes. Relatively to the vegetal type, the score of the class “tree” was higher than for class “shrub”, for both *Scinax* species, and the inverse occurred for *T. venulosus* and *O. taurinus*. The latter two species presented strong positive correlation with the variable insertion height, while *S. fuscomarginatus* presented strong negative correlation and *Scinax* gr. *ruber* and *M. frenata* were not correlated with this variable.

3.5 – Discussion

Our results confirm the colonization of artificial arboreal refuges by four hylid species. A pilot survey, performed by us, revealed that four other – *Dendropsophus minutus*, *D. nanus*, *D. rubicundulus* and *Hypsiboas raniceps* – were able to colonize pipes placed around ponds. The presence of other six species in the region – *Dendropsophus melanargyreus*, *Hypsiboas fasciatus*, *H. punctatus*, *H. albopunctatus*, *Phyllomedusa azurea* and *Scinax nebulosus* – is documented by the voucher specimens (CHUNB44914–46212) collected by Guarino Colli and collaborators and deposited at Colecção Herpetológica da Universidade de Brasília. Hylid species that colonized the arboreal refuges are typical of forested or border areas (e.g. *O. taurinus*) and/or disturbed areas (e.g. *S.* gr. *ruber*) (Lutz, 1973; Lima et al., 2006). Species from genus *Trachycephalus*, including *T. venulosus*, are frequently found in hollow metal tubes containing water (Lutz 1973). All other species, that were found in ground pipes in the pilot study, or that did not colonized the arboreal pipes at all, are more frequent in open areas (Lutz 1973; Brasileiro et al. 2008). Some, like the small *Dendropsophus* species, are more frequently found in herbaceous vegetation at the margin of water bodies (Lutz 1973). Some of the smallest and largest species occurring in this region colonized the refuges. Therefore, the size and diameter of pipes do not appear to have limited the size of colonizing species. On the other hand, arboreal refuges were placed at the core or edge of forested patches and this might have restricted the range of colonizing species. We believe that if arboreal pipes are placed in more open habitats more species will be able to colonize the artificial refuges. On the other hand, if the objective is to maximize the number of captured species, than placing the refuges near reproduction areas might be more efficient.

The skink *Mabuya frenata* also colonized the arboreal refuges. Colonization of pipe refuges by lizards had already been reported by Johnson (2005). These findings suggest that artificial refuges might also be useful for studying other groups of herpetofauna.

Our results reveal that pipe refuges are more useful to survey Neotropical herpetofauna than suggested by previous studies (Silva & Rossa-Feres 2007; Laurencio & Malone, 2009), where none or only a few species and individuals colonized pipe refuges. In the study by Laurencio & Malone (2009), seven individuals from three species were captured in ground pipes, but none was captured in pipes placed in the canopy. However, almost fifty percent of the species sampled in the area were not captured in the refuges, and none of the captured species was only captured in the refuges. These results indicate that artificial refuges might be less usefull for species surveys, than for ecological studies aiming arboreal species.

Our results suggest that refuge colour did not affect colonization success, contrarily to our initial hypothesis based on the findings of Crawford & Kurta (2000). However, darker refuges were less prone to damage, and its use might be preferable because it would minimize the effort of fixing or substituting damaged refuges.

Despite the small number of colonization events, it was possible to explain a significant portion of the total variance of species data. The two environmental variables most correlated with species data were the height of insertion and the vegetal type. Boughton et al (2000) also found evidence for the influence of height of insertion on the colonization success by *Hyla* species. In their study, refuges placed higher in trees were more efficient than those at ground level. In our study, colonization by *T. venulosus* and *O. taurinus* was positively correlated with the insertion height, while colonization by *S. fuscomarginatus* was negatively correlated with this variable. Differences among species also occurred relatively to their frequency either on trees or shrubs.

More than helping to determine which factors influence colonization success, analyzing the microhabitat characteristics of artificial refuges can help us to understand the ecological preferences of hylids (Johnson & Semlitsch, 2003; Pittman et al., 2008). Considering the number of species and the ecological and reproductive diversity of tree frogs in the Neotropics, we believe that pipe refuges might become an important methodology in ecological studies in this region.

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CHAPTER 4

On the importance of spatial heterogeneity and buffer areas in biodiversity conservation: a story by the herps of the middle Araguaia River

Ferreira E., Rocha R.G., Colli G.R., Malvasio A., Fonseca C. On the importance of spatial heterogeneity and buffer areas in conservation: a story by the herps of the middle Araguaia River. Manuscript *in prep.*

Chapter 4 cover photos (from left to right): Cerrado *sensu strictu* and *varjão* with sprouting grass after small fire at the dry season; Coco River after rain season; and gallery forest during the dry season.

4.1 – Abstract

The Araguaia River basin lies along the ecotone between Amazonia and Cerrado, being one of the regions most threatened by deforestation and habitat degradation. Conservation units in the middle Araguaia present different levels of protection and habitat heterogeneity. According to the theory of spatial heterogeneity, more heterogeneous landscapes sustain greater diversity. Higher species richness are also frequently associated with ecotones. In addition, the middle Araguaia basin has also been suggested as a Pleistocene refuge for forest species. With the aim of clarifying these questions, we: (1) estimated anuran and lizard species richness in three conservation units; (2) assessed the contribution of Amazonia and Cerrado herpetofaunas for the regional assemblages; (3) and compared our species richness estimates with estimates from other Amazonian and Cerrado localities. We compiled data from two field campaigns performed between 2005 and 2008. Thirty-eight anuran, 20 lizard, 26 snake and two amphisbaenian species were found in the study area. Anuran and lizard species richness was significantly larger in the more heterogeneous buffer area, rather than in the strict conservation unit. Our results support the theory of spatial heterogeneity and previous findings that Cerrado lizard fauna is more diverse in the interfluvial areas than in gallery forests. Our results did not corroborate the role of the middle Araguaia basin as a refuge for forest species, nor the hypothesis that transitional areas bear higher diversity than surrounding biomes. This study provides a good example of the importance of environmental buffer areas, not only in reducing impacts on other conservation units, but also in the effective conservation of the regional biota.

Keywords: Amazonia; biodiversity; Cerrado; conservation status; herpetofauna.

4.2 – Introduction

The Brazilian Cerrado is the only tropical savanna among the twenty-five biodiversity hotspots proposed by Myers et al (2000), and Amazonian Rainforest is considered the largest and most diverse major tropical wilderness area (Mittermeier et al 1998). The Araguaia River runs between these two large and diverse biomes, and its basin is considered a biodiversity hotspot for plants as well (Oliveira-Filho & Ratter, 2002). Moreover, the middle Araguaia river, Coco river (a tributary), and Bananal Island were also listed as priority areas for conservation within Cerrado (Cavalcanti & Joly, 2002).

Parque Nacional do Araguaia (PNA), in Bananal Island, was the first Amazonian park; originally comprising an area of 20,000 Km^2, it was later reduced to a quarter of its original extent (Mittermeier et al, 2005). Recent conflicts between conservation authorities (ICMBio – Instituto Chico Mendes de Conservação da Biodiversidade) and indigenous people, motivated by the overlap between the park and the indigenous lands at its southern border, remain unsolved (MPF-TO, 2010). The establishment of Parque Estadual do Cantão (PEC), in 1998 (Tocantins state law nº996, 14/07/1998), granted protection to a large area of alluvial forests in the confluence of Araguaia and Coco Rivers. A large environmental buffer area, Área de Proteção Ambiental do Bananal/Cantão (APABC), was created one year before (Tocantins state law nº907 from 20/05/1997) to limit the human impacts in PEC and PNA. A later attempt to reduce the buffer area to about one tenth of its original size, by the state government of Tocantins, was stopped by the Federal Public Ministry (MPF-TO, 2005).

In addition to political and social tension regarding the establishment of conservation units, Amazonia and Cerrado biomes face other threats. Deforestation of Cerrado has been faster and more intense than in Amazonia, and it is estimated that 60% of this biome is now under direct human use (Klink & Moreira, 2002). Despite being lower, deforestation rates in Amazonia are not uniform and become higher at the agricultural frontier, in the contact zone with Cerrado (Foley et al 2007), affecting 27% of the original extent of Xingu's area of endemism (Silva et al, 2005). One of the reasons of the asymmetry between these two biomes is that Brazilian law requires that 80% of the forested area should be preserved in each holding in Amazonia, but this percentage is only 20% in Cerrado (Klink & Machado, 2005). Only 2.2% of Cerrado's original extent is under any legal protection (Klink & Machado, 2005). Fire (Fearnside 2005; Hoffman & Moreira 2002), habitat fragmentation (Carvalho et al 2009), and introduction of African grasses (Klink & Machado 2005, Pivello et al 1999) also contribute actively to the degradation of these biomes, which in turn threatens the persistence of many vertebrate populations (Paglia & Fonseca 2009). Cerrado deforestation promotes the erosion and alteration of flowing pattern of the Araguaia river basin (Latrubesse et al. 2009). Because of the fast pace of deforestation and habitat loss, the Amazonia/Cerrado frontier is considered a priority area for new studies (Azevedo-Ramos & Gallati, 2002).

Contrary to the early recognition of high plant diversity in Cerrado, first works about the Cerrado herpetofauna suggested low differentiation, low rate of endemism, and lack of distinctiveness (Duellman 1979; Vanzolini 1988; Silva & Sites 1995). Colli et al. (2002) refuted these claims, stating that the horizontal habitat variability in Cerrado balanced the vertical variability typical of forested habitats, resulting in similar levels of local species richness. Recent studies about the structure of Cerrado lizard fauna (Nogueira et al., 2005; Nogueira et al., 2009) revealed that it is mainly composed of habitat specialists, with little faunal overlap between gallery forests and open formations. This pattern was also found for small mammals (Alho, 2005). The studies by Nogueira et al. (2005, 2009) also revealed that lizard richness in Cerrado was greater in open formations than in gallery forests, revealing an opposite pattern to that observed for birds (Silva, 1997; Silva and Bates 2002) and mammals (Redford & Fonseca, 1986).

In the last decade, several attempts were made, based on macro-scale analyses, to identify patterns of diversity of squamates and anurans in Cerrado (Costa et al 2007; Diniz-Filho et al 2004, 2007), and to predict a network of conservation areas that maximizes the conservation of Cerrado anurans using the smallest possible area (Diniz-Filho et al 2004, 2006). In all these approaches, the middle Araguaia basin was estimated to have lower species richness than average. However, according to Bini et al. (2006), there is a generalized deficiency of sampling in the northern region of Cerrado. According to these authors, the middle Araguaia region presents a high potential to harbor amphibian species yet to be described. Recent studies in the middle Araguaia basin revealed high species richness of birds (Pinheiro & Dornas, 2009) and small mammals (Rocha et al, 2011), with the description of a new species, in the latter case.

The conservation units from the middle Araguaia present different conservation statuses and most importantly, different habitat characteristics. While PEC (strict conservation) is mostly composed of gallery and alluvial forests, highly subjected to seasonal flooding; APABC (buffer area) is more heterogeneous and less influenced by flooding regime, combining both closed, transitional and open formations. According to the theory of spatial heterogeneity, the more heterogeneous and complex the physical environment becomes, the more complex and diverse the plant and animal communities supported by that environment (Pianka et al, 1966, Richerson & Lum, 1980; Guégan et al, 2006). This greater diversity might be related to the presence of keystone structures, essential to particular species or faunal groups (Tews et al., 2004). One example comes from our study area, where the species *Gymnodactylus carvalhoi* was found to be restricted to termite mounds (Vitt et al., 2006), which are frequent in open Cerrado formations. Based on differences in habitat heterogeneity, we predict that APABC would present higher species diversity than PEC.

The Araguaia River basin was also proposed as a subspecies-endemicity center, highly correlated with the presence of paleoecological forest refuges for Neotropical forest butterflies (Brown 1982). Later, this region was also proposed as a refuge for forest bird species during the climate fluctuations of the Quarternary (Silva, 1997; Silva & Bates, 2002). If the middle Araguaia River was a historical refuge for lizard and amphibian forest species, in the same manner as for butterfly and bird species, we would predict an higher proportion of species and endemics associated to forest habitats, and thus to the alluvial and gallery forests of PEC (Silva, 1995). In addition, ecotones are sometimes considered a source of higher species diversity and evolutionary novelty (Yahner, 1988; Risser, 1995; Smith et al., 1997). Therefore, because of the confluence of the Cerrado and Amazonian biome in this area, we would also predict higher species richness than the average for Cerrado or Amazonian localities.

In order to clarify these issues, and using data collected by us and other workers, we: (1) estimated the species richness of amphibians and lizards in each conservation unit; (2) assessed the contribution of Amazonia and Cerrado herpetofaunas in the regional species assemblage (3) compared estimates of anuran and squamate richness of this region with estimates for other Amazonian and Cerrado localities, and with estimates for the entire Cerrado biome, based on macro-scale approaches. The understanding of the partitioning of faunal richness across the different conservation units in the middle Araguaia is important because of the different conservation statuses and also because of the recent attempts to reduce the size of APABC. Increasingly more complete species lists for the whole region will also help to understand the contribution of Amazonian and Cerrado faunas to the regional herpetofaunal assemblages.

4.3 – Methods

4.3.1 – Study Area

This study was conducted in three different areas (Fig. 4.1): Parque Estadual do Cantão (PEC), a strictly protected area; Área de Proteção Ambiental do Bananal/Cantão (APABC), a sustainable use protected area; and Fazenda Santa Fé (FSF), a private area. Climate in the region is tropical, with a rainy season from October to April and a dry season from May to September (INMET 2009).

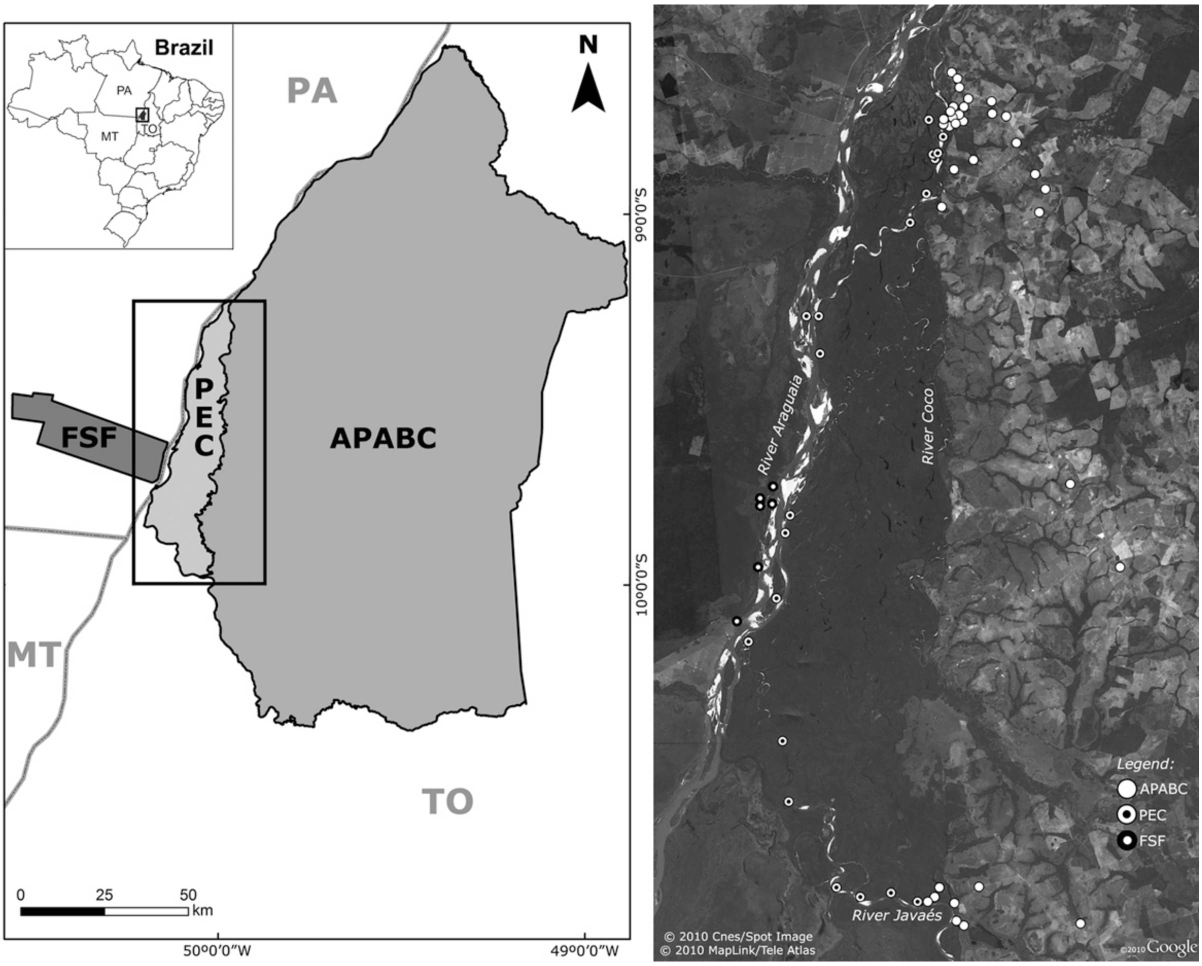

Figure 4.1 – Map of the study area (right); its location within Brazil (top corner); and detail of sampling area (left), showing sampled locations (by any sampling methods) and major rivers. Sampled locations depicted by black and white circles. Federal units: MT – Mato Grosso; PA – Pará; TO – Tocantins. Conservation areas: APABC – Área de Protecção Ambiental da Ilha do Bananal/Cantão; FSF – Fazenda Santa Fé; PEC – Parque Estadual do Cantão.

PEC is a state park with 90,000 ha, located within the municipality of Pium, in west Tocantins state, at the border with Pará state. It is a strict conservation unit corresponding to World Conservation Unit (IUCN) category II (Rylands & Brandon, 2005). The park is located in the alluvial basin of the Araguaia River and two of its tributaries (Coco and Javaés). It is bordered by the Araguaia River in the east, by the Coco River in the west, and by the Javaés River in the south. The park is primarily covered by seasonally flooded alluvial forests, and to a lesser extent by non-flooded forests. PEC also includes some islands along the Araguaia River that can be more than six kilometers long.

APABC is a 1,678,000 ha buffer area, at the eastern border of PEC, created to buffer the impacts over PEC and PNA. This conservation area comprises nine municipalities from the state of Tocantins. Our work was developed within the area of the three municipalities closer to PEC: Caseara, Marianópolis, and Pium. APABC is a sustainable use protected area, corresponding to IUCN category V (Rylands & Brandon 2005). APABC presents greater habitat heterogeneity, including seasonally flooded and non-flooded grasslands with or without sparse trees, dry savannas, and deciduous gallery forests across the waterways. Because human activities are allowed within APABC, there are also roads, pastures, croplands, settlements, and villages. The buffer area is divided in zones for conservation, economical development, and special use. Because our interest was the area closer to PEC and the Araguaia River basin, we did not sample across all the extent of this area, but focused on conservation zones bordering PEC, and Coco and Javaés rivers.

FSF is a private ranch, with 65,000 ha, located in the municipality of Santana do Araguaia, state of Pará, at the western margin of Araguaia River. The main activity is cattle ranching, but about 65% of the ranch is still covered by deciduous forest. The forested area is concentrated near the margin of the Araguaia River, in the continuity of APABC and PEC, following a longitudinal axis. These forests are drier than PEC and less influenced by seasonal flooding.

Conservation areas were not sampled throughout its entire extent. Therefore, effectively sampled areas were determined by the method of the convex hull, including all sampling points in each sampling area. The effectively sampled areas were as follows: PEC – 77,270 ha; APABC – 87,304 ha; and FSF – 1,090 ha.

4.3.2 – Data collection

Field-data included in this study come from two different sources, separated by a temporal gap of one and a half year. The first dataset was the result of a sampling campaign performed by Guarino Colli and collaborators, during 2005. These authors sampled amphibians and squamates continuously during 47 days, from September 5 to October 22. Sampling was done in the following habitats within PEC and APABC: alluvial forests, dry deciduous forests, savannas, and grasslands. Sampling methods included arrays of pitfalls with drift-fences, funnel-traps, and active search. All collected individuals were deposited at Coleção Herpetológica da Universidade de Brasília (CHUNB). Voucher specimens constitute a continuous series (CHUNB44914-46211) including 754 anurans and 532 squamates. Sampling date and geographical coordinates are available for all collected individuals.

The second data set was the result of a sampling campaign done by E.F. and R.R., from June 2007 to November 2008, with a total number of 97 sampling days. This sampling included PEC, APABC, and FSF, widening the sampling area in the two first areas. We sampled amphibians and lizards in the several different habitats mentioned above. No directed effort was made for capturing snakes, contrary to the 2005 campaign. Different areas were sampled in the two years. Each area was sampled consecutively during the end of the rainy season, dry season, and beginning of the rainy season, for equal periods.

Sampling methods included arrays of pitfall traps with drift-fences, Sherman and Tomahawk traps, pipe-traps and active search. All methods were used in the three conservation areas at comparable proportions. However, sampling effort was greater in PEC and APABC than in FSF, and the number of captured individuals provides a fair idea of the differences of sampling effort across the three areas. Data on seven individuals (four lizards and three snakes) found dead on road in APABC were also included.

The first captured individuals of each species, as well as individuals not identified in the field, were collected. The remaining individuals were marked with visible implant elastomer (VIE) and released. During this campaign, we captured 1844 anurans and 406 lizards, including 158 anuran and 72 lizard voucher specimens, deposited at CHUNB (CHUNB58031–58260). All individuals were handled and/or killed ethically.

All species considered in this study are documented by voucher specimen (CHUNB58103 – 58260) or, when not possible, by tissue samples (CHUNB58036 – 58208; 59185 – 59239; 59580 – 59613). This was the case of one *Tupinambis merianae* and three *T. quadrilineatus* specimens, found dead on road, but not suitable for collection as voucher specimens. Exception was made for three snake species

(*Anilius scytale, Caudisona durissa* and *Boa constrictor*), for which we have no voucher or tissue, but only photographic record. Nomenclature for species and families follows lists available at the Brazilian Herpetology Society site, for amphibians (SBH, 2011a) and reptiles (SBH, 2011b).

4.3.3 – Data Analysis

Comparisons among the three areas were made using only amphibian and lizard taxa, because no special effort was made for capturing snakes or amphisbaenians in the 2007-08 campaign. Including these taxa in the analysis would surely constitute a source of bias. However, snakes and amphisbaenians were included in the total number of squamate species known from the middle Araguaia region.

The two data sets were merged and individuals were pooled by area (PEC, APABC and FSF). Only the first capture of each individual released during the 2007/08 campaigns was included in this analysis. Information on the number of captured individuals, microhabitat, conservation areas, and habitat characteristics of capture locations was compiled for each species. Characteristics of capture locations (within an aproximate 100m radius) were classified according to: (1) cover: closed (alluvial and gallery forests), mosaic (cerrado *sensu strictu* and cerradão), and open areas (grasslands and more open Cerrado formations); (2) flooding regime: seasonally flooded and non-flooded; (3) signs of anthropogenic disturbance: dirt roads, asphalt roads, and buildings; and (4) presence of permanent water bodies: rivers, lakes, and ponds. For each species, habitat characteristic classes were only considered when the number of individuals captured in that given class was greater than 5% of total number of captured individuals of that species. Information about the typical biomes of occurrence (Amazonia or Cerrado) was collected from the literature (Table 1).

Information about sampling date and site of capture was available for all individuals, and it was possible to sort them by chronological order of capture, for each of the conservation areas and for the total set of individuals captured in the region. Amphibians and lizards were then divided in groups of 40 and 20 individuals, respectively, and subsequent analyses were preformed based on the chronologically arranged groups of individuals. Individual-based rarefaction curves (*sensu* Gotelli and Colwell, 2001), or Coleman curves, were generated separately for anurans and lizards, using the software EstimateS 8.2.0 for Mac (Colwell, 2006). Abundance based richness estimatives – Chao1 (Chao, 1984) and ACE (Chazdon et al., 1998; Chao, 2000) –and Shannon's H diversity index (Magurran, 2004) were estimated using the same software, for each point of the rarefaction curves. Buzas and Gibson's E eveness index (Buzas and Gibson's, 1969), was estimated according to Hayek & Buzas (1997) using the following equation:

$$E = \frac{e^H}{S}$$

where H is the Shannon's information index and S is observed species richness, estimated for each point of the rarefaction curves:

4.4 – Results

Considering the two data sets, 3539 individuals were captured in the study area: 2598 anurans, 857 lizards, 81 snakes, and 3 amphisbaenians. Anurans were represented by seven families and 38 species, and lizards by nine families and 20 species (Table 4.1). Snakes comprised seven families and 25 species, and amphisbaenians one family and two species (Table 4.2). Among anurans, 22 species occur in Cerrado, three in Amazonia and nine in both biomes. Among lizards, 12 occur in Cerrado, one in Amazonia and seven in both biomes. Ten anuran species and ten lizard species only occurred in habitats not subjected to seasonal floods. On the other hand, no lizard species and only five anurans were only found in or near seasonally flooded habitats. Relatively to vegetation cover, five lizards, but no anuran species, were found only in closed forest formations. On the other hand, 15 anuran and six lizards were only found in open or mosaic formations. A higher number of anuran species was found most frequently on open/mosaic formations (26) than in close formations (12). However, roughly the same number of lizard species was found more frequently in open/mosaic formations (9) and in closed formations (11).

Table 4.1 – Captured anuran and lizard species (and number of individuals) and conservation units where they occurred. Information about habitat characteristics of capture locations is presented in decreasing order of frequency. Biomes converging in this ecotonal region, where the listed species are typically found are presented with references.

Family	**Species (n)**	**Conservation Unit**	**Habitat Characteristics**				**Biomes**	**Micro-habitat**
			Cover	Flooding regime	Anthropogenic disturbance	Water bodies		
Anuran								
Bufonidae	*Rhaebo guttatus* (70)	APABC/FSF/PEC	M/C	N	Dr/Bu/–	Po/–	Ce[4,13]	Te
	Rhinella granulosa (40)	APABC	M/O	N/F	Dr/–	Ri/La/–	Am/Ce[4,7,13]	Te
	Rhinella ocellata (23)	APABC	M/O	N/F	Dr/–	–	Ce[4,13]	Te
	Rhinella schneideri (46)	APABC/FSF/PEC	C/O/M/B	N/F	–/Dr/Bu	Ri/La/–	Ce[4,13]	Te
Craugastoridae	*Haddadus* sp. (14)	APABC	M/C/O	N/F	Dr/–	–/La/Ri	?	Lt
Hylidae	*Dendropsophus melanargyreus* (12)	APABC	M/C	N	Dr/Bu/–	Po/Ri/–	Ce[4,8]	Ar
	Dendropsophus minutus (2)	APABC	M/O	F	–	–	Am/Ce[4,7,8]	Ar
	Dendropsophus nanus (57)	APABC/FSF/PEC	M/C	F/N	–/Dr	–/Po/La/Ri	Ce[4,8]	Ar
	Dendropsophus sp. (2)	APABC	M	F	–	–	?	Ar
	Hypsiboas albopunctatus (9)	APABC	M/C	N	Dr/Bu	Po/Ri/–	Ce[4,8,13]	Ar
	Hypsiboas gr. *albopunctatus* (17)	APABC	M/O	N/F	Dr/–	–/La/Ri/Po	?	Ar
	Hypsiboas punctatus (3)	APABC	M	N	Dr	Po	Am/Ce[4,8,13]	Ar
	Hypsiboas raniceps (78)	APABC/FSF/PEC	O/M/B/C	N/F	–/Bu/Dr	Ri/Po/La/–	Am/Ce[4,8,13]	Ar
	Osteocephalus taurinus (9)	APABC/PEC	C/M/O	F/N	–/Dr	–/Ri	Am[7]	Ar
	Phyllomedusa azurea (19)	APABC	M/O	N/F	Dr/–	Po/–	Ce[2]	Ar
	Pseudis caraya (27)	APABC	M	N	Dr	Po	Ce[4]	Aq
	Scinax fuscomarginatus (34)	APABC/FSF	M/C	F	–/Dr	–/Ri	Ce[4,8,13]	Ar
	Scinax fuscovarius (7)	APABC/FSF/PEC	M/O/C	N/F	Bu/Dr/–	Ri/–/La	Ce[4,8,13]	Ar
	Scinax nebulosus (10)	APABC	M	F	–	–	Ce[4]	Ar
	Scinax gr. *ruber* (134)	APABC/FSF/PEC	C/M	F/N	–	Po/Ri/La/–	Am/Ce[7,13]	Ar
	Scinax sp. (4)	APABC	M/O	N/F	Dr/Bu/–	–	?	Ar
	Trachycephalus venulosus (63)	APABC/FSF/PEC	C/M/O	N/F	–/Bu/Dr	Ri/–/Po	Am/Ce[4,8]	Ar
Leiuperidae	*Physalaemus centralis* (69)	APABC	M/O	N	Dr/–	–	Ce[4]	Lt
	Physalaemus cuvieri (524)	APABC/FSF/PEC	C/M	F/N	–/Dr	Ri/La/–	Ce[4,13]	Lt
	Pseudopaludicola mystacalis (258)	APABC/FSF/PEC	C/M	F/N	–/Dr	Ri/La/–	Ce[4,13]	Lt
Leptodactylidae	*Leptodactylus bokermanni* (49)	APABC/FSF/PEC	M/C	N	Dr/–	–/Ri	Ce[4]	Lt
	Leptodactylus fuscus (34)	APABC	M/O	F/N	Dr/–	–/Po/La	Am/Ce[4,7]	Te
	Leptodactylus labyrinthicus (12)	APABC/PEC	M/C/B/O	F/N	Dr/–/Bu	Ri/–/La	Ce[4,6]	Te
	Leptodactylus latrans (119)	APABC/FSF/PEC	C/M/B/O	N/F	–/Dr/Bu	Ri/–	Ce[4,8]	Te
	Leptodactylus leptodactyloides (362)	APABC/FSF/PEC	C/M	F/N	–	Ri/La	Am[13]	Te
	Leptodactylus martinezi (3)	APABC	M/O	N	Dr	–	Ce[4,13]	Lt
	Leptodactylus mystaceus (37)	APABC/FSF/PEC	C/M	N/F	–/Dr	Ri/–	Am/Ce[4,7]	Te
	Leptodactylus petersii (66)	APABC/PEC	C/M/B	F/N	–/Dr	Ri/–	Am/Ce[4,5,7]	Te
	Leptodactylus pustulatus (44)	APABC/PEC	M/C/B	N/F	Dr/–	Po/Ri/–	Ce[4]	Te
	Leptodactylus syphax (1)	APABC	M	N	Dr	–	Ce[4]	Te

Microhylidae	*Chiasmocleis albopunctata* (3)	APABC	C/M	N	Dr	–	Ce[4]	Lt
	Elachistocleis ovalis (335)	APABC/FSF/PEC	C/M	N/F	–/Dr	Ri/La/–	Ce[4,13]	Lt
Pipidae	*Pipa pipa* (2)	APABC	M	F	–	–	Am[7]	Aq
Lizards								
Gekkonidae	*Hemidactylus mabouia* (18)	APABC/FSF	M	N	Bu	–	Am/Ce[4,14]	Te/Ar
Phyllodactylidae	*Gymnodactylus carvalhoi* (107)	APABC	M/O	N	–/Dr/Bu	–	Ce[4,12]	Te
Sphaerodactylidae	*Gonatodes humeralis* (36)	APABC/FSF/PEC	C	F/N	–	Ri/La/–	Am/Ce[1,4,14]	Ar
Gymnophtalmidae	*Colobosaura modesta* (38)	APABC/PEC	C/M/O	N	–/Dr/Bu	–/Ri	Ce[1,4]	Lt
	Micrablepharus atticolus (33)	APABC/FSF/PEC	C/M	N/F	–/Dr	Ri/La/–	Ce[4,11]	Lt
	Micrablepharus maximiliani (5)	APABC	O/M/C	N	Dr/–	–	Ce[1,4,11]	Lt
Iguanidae	*Iguana iguana* (13)	APABC/FSF/PEC	C/M/B	F/N	–/Dr	Ri/La/–/Po	Am/Ce[1,4,14]	Ar
Polychrotidae	*Anolis nitens brasiliensis* (138)	APABC/FSF/PEC	C	N/F	–	Ri/–/La	Ce[1,4]	Te/Ar
	Anolis ortonii (1)	FSF	C	N	Dr	La	Am[9,14]	Ar
	Polychrus acutirostris (4)	APABC	M	N	Dr/Ar	Po/–	Ce[1,4,13]	Ar
Scincidae	*Mabuya frenata* (30)	APABC/PEC	C	F/N	–	Ri/–	Ce[4]	Te/Lt/Ar
	Mabuya nigropunctata (69)	APABC/FSF/PEC	M/C/O	N	–/Dr	–/Ri	Am/Ce[1,4,14]	Te/Lt/Ar
Teiidae	*Ameiva ameiva* (154)	APABC/FSF/PEC	C/M	N/F	–/Bu	Ri/La/–	Am/Ce[1,4,14]	Te
	Cnemidophorus occellifer (4)	APABC	M/O	N	Bu/–	–	Ce[4]	Te
	Kentropyx calcarata (53)	APABC/PEC	C	N/F	–	Ri/La/–	Am/Ce[1,4,14]	Te/Ar
	Tupinambis merianae (1)	APABC	O	N	Ar	–	Ce[4,1]	Te
	Tupinambis quadrilineatus (3)	APABC	M	N	Ar	–	Ce[3,4,13]	Te
	Tupinambis teguixin (75)	APABC/FSF/PEC	C/M	N/F	–	Ri/La	Am/Ce[1,4,14]	Te
Tropiduridae	*Tropidurus oreadicus* (48)	APABC/PEC	M/C/O	N/F	Bu/Dr/–	–/Ri	Ce[1,4,10]	Te
	Tropidurus torquatus (27)	APABC/PEC	C/M	N/F	–/Bu	Ri/–/La	Ce[4,10]	Te/Ar

Conservation Unit: APABC - Área de Proteção Ambiental do Bananal/Cantão; PEC - Parque Estadual do Cantão; FSF - Fazenda Santa Fé. **Habitat Characteristics**: Cover: closed (C), mosaic (M), open (O) and beach (B); Flooding regime: flooded (F) and non-flooded (N); Anthropogenic disturbance: asphalt road (Ar), building (Bu), dirt-road (Dr), none (–); Permanent water bodies: lake (La), pond (Po), river (Ri), none (–). Biome: refers to the biomes converging in the ecotone, where the species regularly occurs - Amazonia (Am); Cerrado (Ce). **Micro-habitat**: aquatic (A), ground (Te), leaf-litter (Lt), tree (Ar). **References for biome information**: [1] Avila-Pires (1995); [2] Caramaschi (2006); [3] Colli et al (1998); [4] Colli et al (2002); [5] Heyer (1994); [6] Heyer (2005); [7] Lima et al (2005); [8] Lutz (1973); [9] Peters & Donoso-Barros (1986); [10] Rodrigues (1987); [11] Rodrigues (1996); [12] Vanzolini (2004); [13] Vitt et al (2002); [14] Vitt et al (2008).

Table 4.2 – List of snakes and amphisbaenians recorded in the study areas (APABC/PEC), with reference to type and year of first record.

Family	Species	Type of record	Year
Amphisbaenians			
Amphisbaenidae	*Amphisbaena alba*	CHUNB	2008
	Amphisbaena cf. miringoera	CHUNB	2008
Snakes			
Boidae	*Boa constrictor*	DOR/VE	2008
	Corallus hortulanus	CHUNB	2005
	Epicrates cenchria	CHUNB	2005
Colubridae	*Chironius exoletus*	CHUNB	2005
	Spilotes pullatus	CHUNB	2005
	Tantilla melanocephala	CHUNB	2005
Dipsadidae	*Helicops angulatus*	CHUNB	2005
	Helicops polylepis	CHUNB	2005
	Helicops trivittatus	CHUNB	2005
	Leptodeira annulata	CHUNB	2005
	Liophis poecilogyrus	CHUNB	2005
	Oxyrhopus guibei	CHUNB	2005
	Oxyrhopus trigeminus	CHUNB	2005
	Philodryas olfersii	CHUNB	2005
	Psomophis joberti	CHUNB	2005
	Pseudoboa nigra	CHUNB	2005
	Sibynomorphus mikanii	CHUNB	2005
	Taenophallus occipitalis	CHUNB	2005
	Thamnodynastes sp.	CHUNB	2005
	Xenodon merremii	CHUNB	2005
Elapidae	*Micrurus frontalis*	CHUNB	2005
Leptotyphlopidae	*Epictia albifrons*	CHUNB	2005
	Rena cf. *dimidiata*	CHUNB	2005
Viperidae	*Bothrops moojeni*	CHUNB	2005
	Caudisona durissa	DOR/VE	2008
Aniliidae	*Anilius scytale*	DOR	2008
Total species	**28**		

Type of record: CHUNB – specimens collected with vouchers deposited in CHUNB. DOR/VE – Individuals fond dead on road (DOR) or visual encounters (VE) during the 2007/2008 campaign, with no voucher but with photographic record.

Individual-based rarefaction curves appear to approach an asymptote in all cases, except for lizard in FSF (Figure 4.2). The rarefaction curves stabilized at higher values of species richness in APABC than in PECC and FSF, both for anurans and lizards. This result holds true even considering the same numbers of individuals. Rarefaction curves based on the total number of individuals in the study area also present asymptotic behavior.

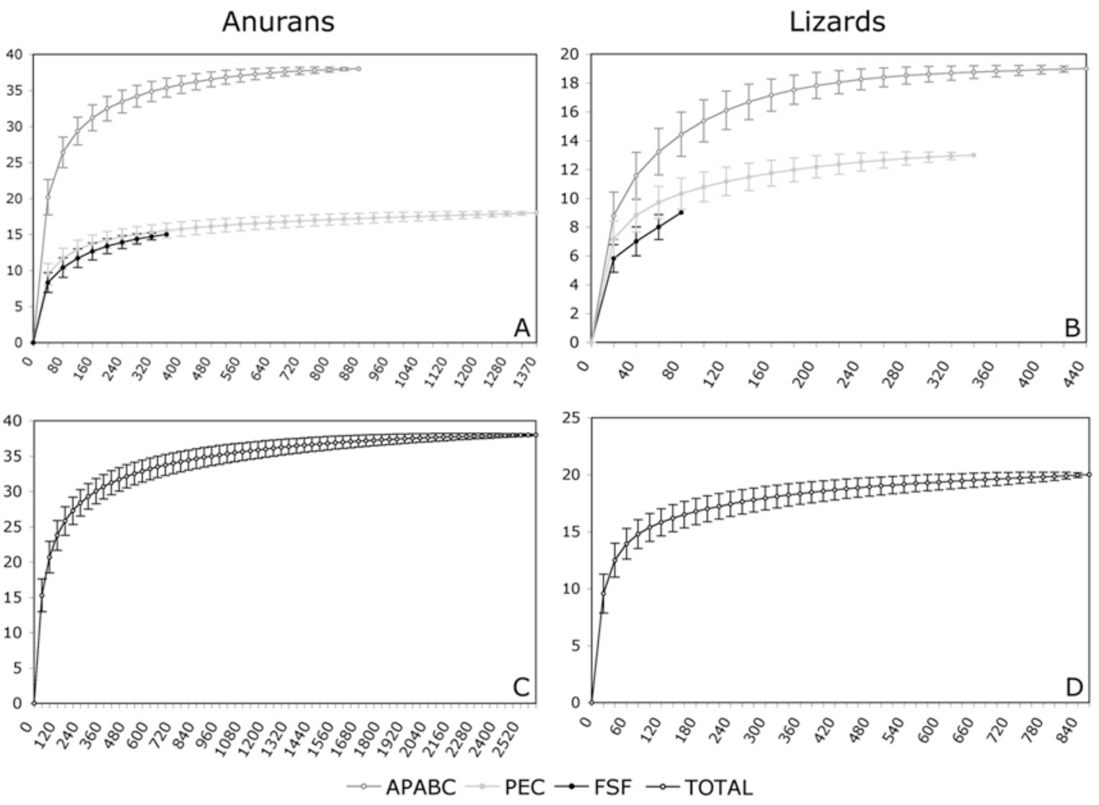

Figure 4.2 – Individual-based rarefaction curves for anurans and lizards. Separate curves are presented for each of the three sampled areas (A and C) and for the middle Araguaia region (B and D). Bars represent standard deviation of estimates.

Species richness estimates were also higher in APABC than in PEC (Figure 4.3), both for lizards and anurans, and for the two estimators (ACE and Chao1). Due to the low number of captured individuals in FSF, the evolution of the richness estimators for this area was always very erratic, preventing comparisons with the other two areas. Anurans species richness estimators appear to converge to a stable estimate, with standard deviation (in the case of ACE) and 95% confidence intervals (in the case of Chao 1) steadily decreasing towards the end of the curves. On the other hand, for lizards, standard deviation (ACE) and confidence intervals remain broad even after adding all the individuals. It is important to mention that for ACE, standard deviation is based on the variation in sample order among randomizations and, without sample replacement, it must reach zero after adding the last set of individuals.

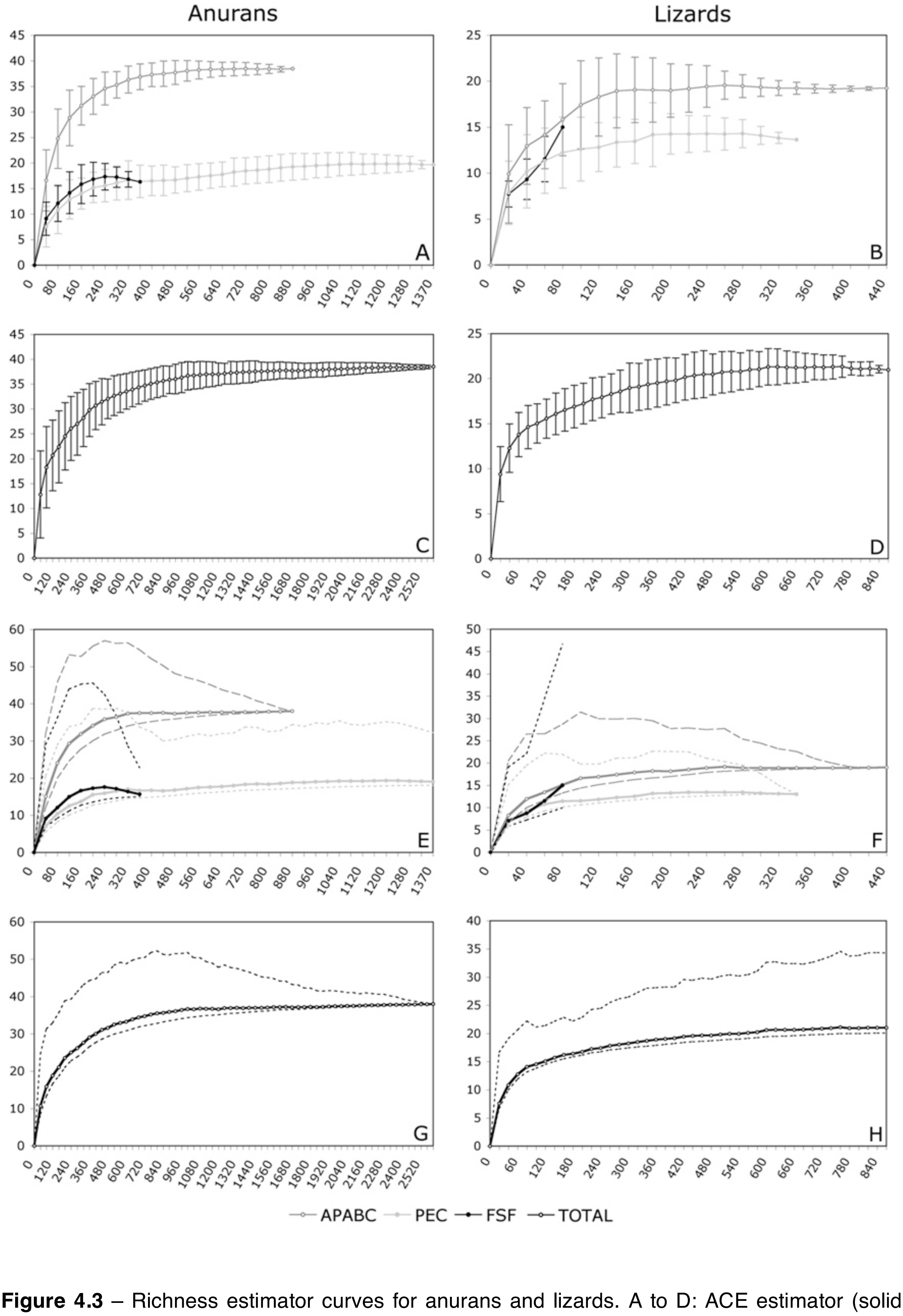

Figure 4.3 – Richness estimator curves for anurans and lizards. A to D: ACE estimator (solid lines) and standard deviation (bars). E to H: Chao 1 estimator (solid lines) and 95% confidence intervals (dashed and dotted lines).

Eveness of the anuran assemblage was higher in APABC than in PEC and FSF (Figure 4.4). On the other hand, the lizard assemblage in PEC was more even than in APABC. Once again, the low number of captured individuals in FSF did not allow comparisons with this area. Evenness of the anuran assemblage considering all individuals was lower than in PEC and APABC considered separately, but the opposite result was obtained for lizards.

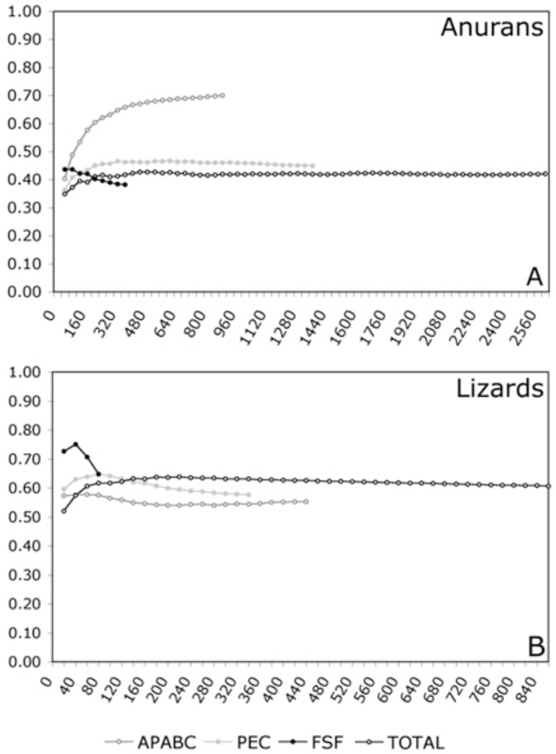

Figure 4.4 – Buzas and Gibson's evenness estimator curves for anurans and lizards. Separate curves are presented for each one of the three sampled areas and for the middle Araguaia region.

Species richness estimates based on ACE and Chao 1 estimators were in agreement, in all cases (Table 4.3). These estimates were higher than the observed number of species in: PEC (ACE 19.7; Chao1 19.0) and FSF (ACE 16.3; Chao 1 15.7), in the case of anurans; and in FSF (ACE 15.0; Chao 1 15.0) and total study area (ACE 21.0; Chao 1 21.0), in the case of lizards.

Table 4.3 – Numbers of individuals, observed number of anuran and lizard species for each conservation unit (CU) and for the entire study area. Presented species richness and evenness estimates are based on the total numbers of captured individuals.

	CU	N	S	Species Richness Estimators		E
				ACE	**Chao 1 (95% CI)**	
Anurans	APABC	868	38	38.4	38.0 (38.0 – 38.0)	0.700
	PEC	1369	18	19.7	19.0 (18.07 – 32.2)	0.450
	FSF	361	15	16.3	15.7 (15.1 – 22.8)	0.383
	Total	*2598*	*38*	*38.5*	*38.0 (38.0 – 38.0)*	*0.420*
Lizards	APABC	443	19	19.2	19.0 (19.0 – 19.0)	0.553
	PEC	341	13	13.6	13.0 (13.0 – 13.0)	0.576
	FSF	73	9	15.0	15.0 (9.95 – 46.7)	0.648
	Total	*857*	*20*	*21.0*	*21.0 (20.1 – 34.3)*	*0.606*

N – number of individuals; S – observed species; ACE – abundance-based coverage estimator; Chao 1 – Abundance-based estimator of Chao. E – Buzas & Gibson's E evenness index.

4.5 – Discussion

4.5.1 – Species richness in the conservation areas: spatial heterogeneity and refuge theories

Our results revealed that anuran and lizard species richness are significantly higher in APABC than in PEC. As previously mentioned, APABC is a more heterogeneous area, that includes several open and mosaic Cerrado formations, besides the gallery and alluvial forests that dominate PEC. Most anuran species found in the study area were only or mostly found in open areas (both non-flooded and seasonally flooded) and mosaic cerrado formations. In this regard, lizard species were more evenly distributed between closed and open habitats. However, because APABC included all habitats, species richness was still higher there. These results are in agreement with the findings of Nogueira et al. (2005, 2009), in that lizard fauna from Cerrado was more diverse in the interfluvial areas than in gallery forests, contrarily to earlier findings for bird and mammal assemblages (Redford & Fonseca, 1986; Silva, 1997; Silva and Bates 2002).

The higher herpetofauna species richness found in the more heterogeneous APABC corroborates the theory of spatial heterogeneity (Pianka, 1966). The diversity of habitats in this area, in opposition to the dominance of alluvial and gallery forests in

PEC, must have contributed to the higher species richness in APABC. The abundance of key structures (Tews et al., 2004) in the latter area, such as termite mounds, enabled the presence of specialist species like *Gymnodactylus carvalhoi* (Vitt et al., 2006). Other species, like *Amphisbaena* cf. *meringoera* and *M. maximiliani*, were also only or most frequently found in termite mounds, in APABC.

Recent studies with medium and large mammals also reported higher species richness in non-flooded areas (Haugaseen & Peres, 2007; Negrões et al., 2011), which suggests that flooding regime, which is stronger in PEC, could also be limiting species richness inside the park. Differences in species richness between PEC and APABC were higher in the case of amphibians. Curiously, the five species restricted to flooded areas occurred in APABC but not in PEC. On the other hand, from the 19 species restricted to APABC, 12 occurred in flooded areas or present aquatic microhabitat. In the case of lizards, five species are restricted to APABC and to non-flooded habitats. However, all five species are typical of Cerrado biome and characteristic of more open formations. We therefore conclude that spatial heterogeneity must play a more important role in the partitioning of the herpetofauna assemblage, than flooding regime.

No support was found for the role of the middle Araguaia basin as a refuge for forest lizard or amphibian species. This theory was proposed by Silva (1997) for birds. In the case of herpetofauna, only five species were confined to forested areas, and 23 species were more frequent in forested areas. On the other hand, 21 species were restricted to the mosaic and open formations and 35 species were more frequent there. Species richness was also much lower in the alluvial and gallery forests of PEC, where only 18 anuran and 12 lizard species were found, against 38 anuran and 19 lizard species in APABC. If the Araguaia basin had been a refuge for forest species, we would expect a higher proportion of species in the gallery and alluvial forests of PEC. Also, it would be expected that endemic species associated with forest areas would be present. According to Silva (1997), in putative pleistocene refuges for forest species, we would predict the existence of recently formed endemic species associated with forest habitats. Two lizard (*Tupinambis quadrileneatus* and *Micrablepharus atticolus*) and four anuran species (*Elachistocleis ovalis*, *Leptodactylus martinezi*, *L. syphax* and *Rhinella ocellata*) found in the study area are endemic of Cerrado (Colli et al., 2002; Bastos, 2007). From these species, only *M. aticollus* was more frequent in forest habitat, but also occurred in more open formations. We therefore conclude that, because higher proportions of species richness and endemism are related to more open formations, it is unlikely that this region has been a refuge for forest anuran and lizard species during the Pleistocene.

4.5.2 – Species richness in the middle Araguaia: Amazonia and Cerrado contributions

Most of the species (22 anurans and 12 lizards) found in this study, in the middle Araguaia basin, are strictly associated with the Cerrado biome. Only one lizard (*Anolis ortonii)*, and three anurans (*Leptodactylus leptodactyloides, Osteocephalus taurinus* and *Pipa pipa*) are strictly associated with the Amazonian biome. One of this species, *A. ortonii*, was only found once, in FSF, at the western border of the Araguaia River. Despite the much larger sampling effort in APABC and PEC, this species was not found on the eastern bank of the Araguaia River. Eventually, a larger sampling effort in FSF might reveal the presence of more Amazonian species on the western bank of the river. The presence of more species in FSF is suggested by the mean values and dispersion measures of the estimates of species richness obtained with both estimators (ACE and Chao1), which are above the observed species richness.

Species richness estimates for the middle Araguaia River were within the range of the values estimated for other Amazonian and Cerrado localities. Anuran richness estimates for the middle Araguaia River (38) were within the values reported for Cerrado localities (Vitt et al., 2002 – 24 spp.; Bastos, 2007 – 27-43 spp.) but lower than the values reported for Amazonian localities (Lima et al., 2006 – 50spp.; Bastos, 2007 – 39-125 spp.; Bernarde, 2007 – 47spp.). Lizard richness estimates for this area (21) were also within the range of values reported for Cerrado (Colli et al., 2002 – 14-25 spp.; Vitt et al., 2002 – 18spp.; Nogueira et al., 2005 – 17spp.; Nogueira et al., 2009 – 13-28 spp.) and Amazonian localities (Colli et al., 2002 – 16-30 spp.; Vitt et al., 2008 – 32 spp.).

These results suggest that the ecotonal confluence between Amazonia and Cerrado, in this region, is not characterized by an increase in species richness, relatively to the merging biomes. Thus, these findings do not corroborate the idea that transitional areas bear greater diversity (Yahnen 1988; Risser, 1995). Instead, our results agree with the results of recent macro-scale approaches, which estimate lower values of squamate species richness at the edges of the Cerrado biome (Costa et al., 2007). However, more sampling is needed in the western margin of the Araguaia River (FSF), where more species characteristic of the Amazonian biome might still be found, as suggested by the rarefaction curves and species richness estimators, as well as by the presence of *Anolis ortonii* in the area.

Species richness estimates based on macro-scale approaches are available both for anurans and squamates. Based on available data sampled in several Cerrado localities, Diniz-Filho et al. (2006, 2007) extrapolated anuran species richness for the entire biome. These estimates predicted a maximum of 42 species for the region of the Bananal Island, and of 32 species for region where PEC and APABC lie. Our results, based on field data, confirmed the presence of at least 38 anuran species. The species richness of this area exceeded by six species the previous estimates, which corresponds to an increase of about 19% relatively to the 32 species previously estimated. Based on the asymptotic behavior of the rarefaction curves and on the species richness estimates for the total study area, the observed number of anuran species already matched the theoretical maximum to the area. On the other hand, we could not assign three anuran taxa to the species level, which might suggest that, as predicted by Bini et al. (2006), there are still some species to be described in this region. The number of endemic anuran species found in PEC and APABC reached (3 sp.) or even slightly exceeded (4 sp.) the one estimated by Diniz-Filho (2007), depending on which species are considered to be Cerrado endemics (Colli et al., 2002; Bastos, 2007).

A recent study regarding squamate richness in Cerrado, the area of the middle Araguaia, including Bananal Island, PEC, and APABC estimated a maximum of 30 squamate species (Costa et al 2007). If we count all lizard, snake, and amphisbaenian species known to occur in this area, the observed squamate richness reaches 48 species. However, contrary to the anuran assemblage, at least one new lizard species is predicted by both species richness estimators, and the upper bound of the 95% confidence interval of Chao 1 estimate reaches 34 species. In fact, during the second field campaign, new squamate species were still being recorded. Some of these species, such as *Micrablepharus atticolus* (small-size and habitat specialist) and *Amphisbaena* cf *meringoera* (small-size and fossorial) are more difficult to sample. However, large species such as *Tupinambis quadrilineatus*, *T. merianae*, *Boa constrictor* and *Amphisbaena alba* were only recorded during the second half of the second campaign, found dead in a road within APABC. These results suggest that it is possible that more lizard species can still be recorded in the region and that, even after intense sampling, a complete species list might not yet be a reality.

4.5.3 – Conservation implications

As mentioned at the beginning of this manuscript, Cerrado and Amazonia biomes face severe threats to their integrity (Azevedo-Ramos & Galatti 2002; Klink & Moreira 2002; Silva & Rylands 2005; Fearnside 2005; Carvalho et al 2009). Agriculture and pastures have supplanted Cerrado and, most recently, the transitional areas between Cerrado and Amazonian forests (Sousa & Reid 2005). Deforestation can have a direct negative effect on major rivers (Latrubesse et al. 2009) and Brazilian government has development projects for two of them, Araguaia and Xingu (Sousa & Reid 2005). A large effort has been made in Brazil for the establishment of protected areas (Rylands & Brandon 2005), but still a very small part of both biomes is under legal protection (Silva 2005; Klink & Machado 2005). The long-term persistence of Amazonian and Cerrado biotas depends not only on strictly protected areas but also on sustainable use reserves (Peres 2004). Successful cases of integration of sustainable use reserves and indigenous lands in large extents of pristine areas exist (Campos & Nepstad 2006). Efforts towards such an integration of conservation areas with different statuses are being conducted for the middle Araguaia basin. However, the area is under strong human pressure and tension frequently arises. During the writing of this manuscript, 10% of the area of PNA burned, threatening once again the integrity of regional conservation areas.

Our data suggest that a significant part of herpetofauna diversity of the middle Araguaia exists outside strictly protected areas (PEC) and that surrounding areas, such as buffer areas (APABC) and private reserves (FSF) play an important role in the preservation of the herpetofauna of this region. This study focused only on three conservation areas but, nevertheless, we consider that the data here presented corroborate the importance of long-term collection of field data as the basis for efficient conservation strategies. Moreover, this is a good example of the major importance of environmental buffer areas, not only in reducing impacts on other conservation units, but also in the effective conservation of the regional biota.

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CHAPTER 5

Is Araguaia River a genetic barrier to amphibian populations?

Ferreira E., Rocha R.G., Tonini J.F., Costa L.P., Carlos Fonseca. Riverine barriers and amphibians: A local-scale analysis in the middle Araguaia basin. Manuscript *in prep.*

Chapter 5 cover photos (from left to right): adult *Elachistocleis ovalis*; adult *Leptodactylus latrans*; and adult *Trachycephalus venulosus*.

5.1 – Abstract

Several hypotheses have been raised, to explain the mechanisms that shaped Amazonian biodiversity. The oldest is the Riverine Barrier Hypothesis, postulated by Alfred Russel Wallace, in 1852. This hypothesis resulted from the observation that large rivers were very frequent in the region and they dissect the Amazonian Rainforest in fragments of variable size. Recently, several studies using amphibian species distribution or molecular data have contributed to corroborate or refute this hypothesis. However, none of these studies focused on the clear-water rivers that flow from the Brazilian Shield and run towards the Amazonian biome. Here we tested the Riverine Barrier Hypothesis in the Araguaia River, the largest basin draining the Cerrado biome. For this purpose, we collected molecular data from three amphibian species (*Elachistocleis ovalis*, *Leptodactylus latrans* and *Trachycephalus venulosus*), using the gene coding for the cytochrome c oxidase subunit 1 (*cox1*). Our results do not support the Riverine Barrier Hypothesis, since we did not found reciprocally monophyletic (or even paraphyletic) clades on both banks of the river. Rather, we found that haplotypes were shared between both banks of the Araguaia River, for the three species. *Elachistocleis ovalis* presented greater genetic diversity and structure than *L. latrans* and *T. venulosus*, and two strongly divergent lineages were found for the former species. The geographic dispersal of these two clades could support the role of Araguaia as of a semi-permeable barrier. We found no correlation between genetic structure and colour pattern variation, which might suggest the existence of two sympatric cryptic species. The existence of these two sympatric clades is discussed in the light of recent taxonomic changes within genus *Elachistocleis*. Additionally, the existence of two colour morphs in *T. venulosus*, consistent with the *zonata* and *bufonia* patterns described by Lutz in 1973, is reported for individuals sharing the same *cox1* haplotype.

Keywords: Amazonia; Cerrado; cytochrome oxidase subunit-1 gene; COI; *Elachistocleis ovalis*; *Leptodactylus latrans*; Riverine Barrier Hypothesis; *Trachycephalus venulosus*.

5.2 – Introduction

Amazonian biodiversity has been puzzling naturalists for centuries and several hypotheses were raised in order to identify the mechanisms that shaped this amazing diversity (reviews by Haffer, 1997; Zeisset & Beebee, 2008; Haffer, 2008; Antonelli et al., 2010). The first of these hypotheses was the “Riverine Barrier Hypothesis”, proposed in 1852 by Alfred Russell Wallace (Colwell, 2000). The idea that rivers could have shaped Amazonian biodiversity resulted from the observation that: large rivers are common in the Neotropics, since the Pleistocene; and they dissect the tropical forest in fragments of various sizes and different species occur on opposite banks (Gascon et al., 1998).

Support for this theory came for example from the study by Ron (2000), using distribution data of amphibians and other vertebrate groups in Neotropical lowlands. Funk and collaborators (2007) tested two biogeographic hypothesis using *Engystomops* (formerly *Physalaemus*) *petersi*, and their results provided some additional support for the Riverine Barrier Hypothesis. Noonan and Wray (2006) also suggested that phylogenetic relationships within populations of *Dendrobates ventrimaculatus* in Napo region might be in agreement this hypothesis. Further support came from studies with amphibians in Chagres River, in Central America (Lampert et al., 2003), and Yalong and Dadu Rivers, in China (Li et al., 2009). However, evidence against the role of rivers as barriers to amphibian species came from several studies performed in Amazonian rivers (Gascon et al., 1998; Lougheed et al., 1999; Gascon et al., 2000; Symula et al, 2003) and, curiously, also in rivers Yalong and Dadu (Zhao et al., 2009).

According to some authors (Haffer, 1997; Noonan and Wray, 2006; Zeisset & Beebee, 2008; Antonelli et al., 2010), no single model can adequately explain Amazonian diversity. In fact, whether or not rivers can act as barriers seems to depend both on the river and on the species being study. The Riverine Barrier Hypothesis has been tested for amphibians in several Amazonian rivers, but not for the older clear-water rivers that flow from the Brazilian Shield (Gascon et al, 2000). One of these rivers, Tapajós, was studied by Bates and collaborators (2004), which found evidence of river-mediated genetic structure in populations of several bird species in Tapajós headwaters.

Here we tested whether or not the Araguaia River, which borders the southeastern limits of Amazonia, acts as a barrier to gene flow in amphibian species. The Araguaia River is the main fluvial system in Cerrado and, together with Tocantins River, constitutes the fourth largest drainage basin of South America (Aquino et al., 2008; Latrubesse et al., 2009). Despite draining mostly within the Cerrado biome, Araguaia borders Amazonian rainforest throughout most of its lower course. Therefore, we considered Araguaia to be a suitable candidate for testing the Riverine Barrier Hypothesis.

Genealogical data indicate that, in addition to historical biogeographical events, behaviour and natural history of species can also impact phylogeographic patterns (Avise, 2009). Comparative phylogeographic studies enable the distinction between historical events, which may have influenced whole communities, from ecological or demographic forces acting on single lineages or species (Carnaval, 2002; Crawford et al., 2007). For this reason, we propose to test the Riverine Barrier Hypothesis using three amphibian species with wide distribution in the Neotropical region, and with different life-history and ecology: the microhylid *Elachistocleis ovalis*, the leptodactylid *Leptodactylus latrans* and the hylid *Trachycephalus venulosus*.

Elachistocleis ovalis is a leaf-litter species, presenting the borrowing habits shared by many microhylid species (Wells, 2007). This species reproduces after heavy rains, during the rain season in more seasonal areas such as Cerrado (Rodrigues et al., 2003; Prado et al, 2005; Bernarde, 2007), or throughout the whole year, in the less seasonal central Amazonia (Lima et al., 2006*). Leptodactylus latrans* is the current valid name for the species formarly known as *L. ocellatus* (Lavilla et al., 2010a). This species is considered to be an early stage in the trend towards terrestrial habit observed in *Leptodactylus* (Heyer, 1969). This species is an explosive breeder that reproduces occasionally, during the dry season (Prado et al, 2005; Wells, 2007), placing the eggs in foam-nests above water surface (Heyer, 1969). *Trachycephalus venulosus* is a canopy-dwelling species (Wells, 2007) that is able to glide for several meters in the horizontal (Duellman & Trueb, 1994). This species is considered an explosive breeder, which reproduces after heavy rains (Prado et al., 2005; Rodrigues et al., 2005; Bernarde, 2007). We expect to find greater genetic structure and diversity, and a stronger influence of Araguaia on this structure in the case of *E. ovalis*, rather than in the more vagile *L. latrans* and *T. venulosus*.

By analyzing the genetic structure of these three species around the mid-course of the Araguaia River, we hope to understand if this river constitutes a barrier to gene flow across different groups of amphibians, at a local scale. In the end, we aim to understand if our results help to corroborate or refute the Riverine Barrier Hypothesis of the diversification of Amazonian fauna.

5.3 – Methods

5.3.1 – Study area

This study was conducted in the region of the mid-Araguaia River, in an area comprising four different conservation units (Figure 5.1), with different conservation statuses: Parque Estadual do Cantão (PEC); Área de Protecção Ambiental do Bananal/Cantão (APABC); Fazenda Santa Fé (FSF) and Fazenda Lago Verde (FLV). The regional climate is tropical and markedly seasnal, with a rain season from October to April and a dry season from May to September (INMET 2010).

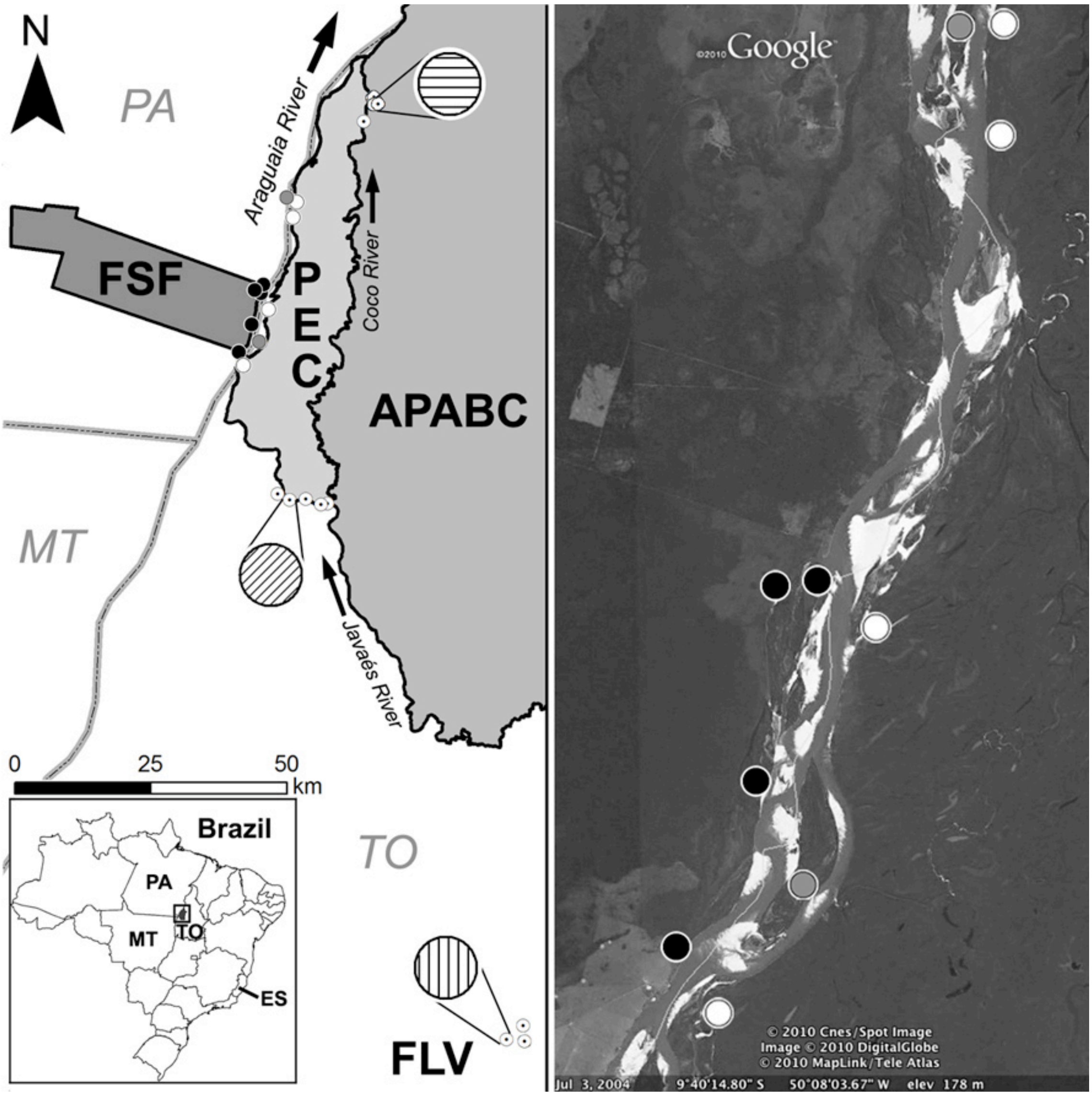

Figure 5.1 – Study area in the mid-Araguaia River basin. Location of study area in Brazil (bottom left corner); Map including all the sampled conservation units (left) and detail of sampling points near River Araguaia (right). Grayscale and hatched patterns correspond to those in figures 5.2, 5.3 and 5.4. Federal Units: ES – Espírito Santo; MT – Mato Grosso; PA – Pará; TO – Tocantins. Major rivers and direction of flow are also depicted. Sample areas: PEC – Parque Estadual do Cantão; APABC – Área de Protecção Ambiental do Bananal/Cantão; FSF – Fazenda Santa Fé; FLV – Fazenda Lago Verde.

PEC is a state park with 90,000ha, in the west of the state of Tocantins, at the border with the state of Pará. It is a strict conservation unit corresponding to World Conservation Unit (IUCN) category II (Rylands & Brandon, 2005). The park is located in the alluvial basin of the Araguaia River and two of its tributaries, Coco and Javaés. The park is mostly composed of seasonally flooded alluvial forests, and non-flooded forests at a lesser extent. PEC also includes some islands along the Araguaia River that can be more than six kilometres long. APABC is a 1,700,000ha buffer area, at the eastern border of PEC, created to buffer the impacts over PEC and Parque Nacional do Araguaia (PNA), at the south. APABC is a sustainable use protected area, corresponding to IUCN category V (Rylands & Brandon 2005). FSF is a private ranch with 65,000ha, located in the municipality of Santana do Araguaia, state of Pará, at the western margin of Araguaia River. The main activity is cattle ranching but about 65% of the ranch is still covered by deciduous forest. The forested area is concentrated near the margin of the Araguaia River, in the continuity of APABC and PEC. These forests are drier than PEC and less influenced by seasonal flooding. FLV is an 8,000ha private ranch managed for irrigated crop production (rice, maize, bean and soybean), with almost 70% of the area constituted by pristine Cerrado sensu lato physiognomies. Natural forest fragments, locally named *ipucas*, occur within agricultural and Cerrado matrices.

5.3.2 – Sample collection

Sampling was carried out between June 2007 and November 2008, using pitfalls, PVC pipetraps and active search. Detailed descriptions of sampling strategy can be found in Ferreira et al. (submitted, Chapter II and III of this thesis). The first individuals of each species were collected, prepared as voucher specimens and are deposited at “Coleção Herpetológica da Universidade de Brasília” (CHUNB). All other individuals were individually marked with visible implant elastomer (VE) and released. Blood samples were collected directly from adult individuals into FTA® cards (Whatman™). Blood was collected after punching the forearm with a small lancet. Individuals were kept under observation and released in the following morning. We observed no mortality resulting from blood collection procedure. Occasionally, tissue was collected from the thigh muscle of individuals found dead inside pitfalls, but that were no longer suitable for voucher preparation.

5.3.3 – Laboratory protocols

DNA was extracted from tissue samples preserved in FTA cards or ethanol using the salt-extraction method (Bruford et al. 1992) and the concentration of DNA isolates was quantified using spectophotometer (model NanoDrop® ND-1000 UV/Vis, Thermo Fisher Scientific). A 663-bp fragment of the gene coding for the subunit I of the cytochrome oxidade (*cox1*) was amplified by polymerase chain reaction using the primers dgLCO1490 and dgHCO2198 (Meyers, 2003). Amplification reactions were performed using the following PCR profile: initial denaturation at 95°C for 1 min; 37 cycles with denaturation at 95 °C for 40 s, annealing at 48 °C for 40 s and polymerization at 72°C for 60 s; final extension at 72°C for 5 min. In order to amplify particular samples, small adjustments to this profile were sometimes needed. PCR reactions were performed in a 25*μ*l total volume, using 1.0 unit of Taq-polymerase and final concentration of 0.12 *μ*M of each primer and 0.25*μ*g/*μ*l of bovine serum albumin. Other reagents were added according to manufacturer's instructions. Quantity of DNA template was aimed at 50-100ng, but successful amplification occurred with as little as 10ng. The 663-bp fragment was sequenced once for each primer, in an automated sequencer ABI 310 using the Big Dye Terminator Cycle Polymerase (Perkin Elmer, Applied BiosystemsTM, Foster City, California).

5.3.4 – Data Analysis

Sequences[1] were aligned using CLUSTALW algorithm implemented in MEGA version 4.1 (Kumar et al. 2008), and then checked and edited by eye. Alignments were performed on the consensus sequences generated for each individual, using the raw sequences from forward and reverse sequencing.

Numbers of haplotypes, haplotype (h) and nucleotide (π) diversity indices were estimated for each species, using DNASP version 5.10.01 (Librado & Rozas, 2009)), using the alignments of all sequences from the Araguaia basin. Lists of haplotypes were generated with the same software and subsequent analyses were conducted based in haplotypes rather than in individual sequences. To investigate whether or not river Araguaia acts as a barrier to gene flow in these three amphibian species, we generated phylogenetic trees using both distance-based and criterion-based algorithms, as well as haplotype median-joining networks. For each species, the same taxa were used as

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[1] The sequences generated during this study were not yet submitted to GenBank, but will be submitted prior to manuscript submission.

outgroups (Table 5.1) in all analyses except MJ networks, which were calculated without outgroups.

Neighbour-joining (NJ) trees (Saitou & Nei, 1987) were inferred based on the genetic distances estimated by the Kimura 2-parameter model (Kimura, 1980), using MEGA software. Bootstrap-consensus tree was inferred based on 1000 replicates (Felsestein, 1985).

Most-parsimonious (MP) trees were inferred using PAUP* 4.0b10 (Swofford 1998) by heuristic search. Starting trees were generated by stepwise addition (Farris, 1970), using 1000 replicates and a tree-bisection-reconnection (TBR) branch-swapping algorithm (Swofford and Sullivan, 2009). Bootstrap 50% majority-rule consensus-tree was inferred based on 500 replicates.

Bayesian inference (BI) of phylogenetic relationships among haplotypes was conducted using MrBayes version 3.1.2 (Ronquist and Hueselbeck, 2003). Prior best model of nucleotide substitution was chosen based on the scores of Akaike information criteria, estimated using MrModelTest version 2.3 (Nylander, 2004). The settings for the chosen model, generated by MrModelTest, were then incorporated into MrBayes' input file. Settings for the Metropolis coupling behavior were kept at default values. Markov chains ran for 1 million generations and were sampled each 100th generation. We confirmed that chains had reach stationarity by inspecting the value of the standard deviation of split frequencies. A 50% majority-rule consensus tree was generated after discarding 25% of the simulated generations as *burn-in*. NJ, MP and BI phylogenetic trees were visualized and edited using FigTree version 1.3.1 (Rambaut, 2009).

Median-joining networks (MJ) were generated using the software NETWORK (Bandelt et al, 1999). Only polymorphic nucleotide sites were included in MJ analyses. Geographical location of haplotypes across the mid-Araguaia basin was superimposed to the MJ networks *a posteriori*, following the grayscale and hatched patterns from figure 1, for better visualization of geographical distribution of haplotypes.

Average pairwise genetic divergence between and within groups of haplotypes was estimated using the Kimura 2-parameter method (Kimura, 1980) implemented in MEGA. Standard error estimates were obtained by bootstrap procedure (1000 replicates). Confidence intervals (95% CI) of the average pairwise distances were estimated according to Hayek & Buzas (1997).

5.4 – Results

We obtained DNA sequences corresponding to fragments (663bp) of the *cox1* gene from 72 *Elachistocleis ovalis*, 52 *Leptodactylus latrans* and 39 *Trachycephalus venulosus* individuals. Additional sequences were included in the phylogenetic analysis: *Elachistocleis ovalis* from Panamá – KRL1136 and KRL1137, with the following GenBank accession numbers: FJ766754 and FJ766753; *Trachycephalus venulosus* from the Atlantic Forest, Espírito Santo, Brazil – JFT473, JFT757, JFT955, provided by João F. Tonini. Sequences for outgroup taxa were generated in this study or downloaded from Genbank (Table 5.1).

Table 5.1 – Outgroup taxa used in the phylogenetic analyses (Genbank accession numbers).

Species	Outgroup taxa
Elachistocleis ovalis	*Nelsonophryne aterrima* (FJ766759) – Microhylidae *Chiasmocleis shudikarensis* (EF396044) – Microhylidae
Leptodactylus latrans	*Leptodactylus fuscus* (E0272, this study) – Leptodactylidae *L. labyrinthycus* (E0731, this study) – Leptodactylidae
Trachycephalus venulosus	*Hemiphractus fasciatus* (FJ766707) – Hylidae *Hypsiboas raniceps* (E0641, this study) – Hylidae

The alignment was unambiguous and the inferred sequence contained no stop codons. The estimated best prior evolution models for Bayesian inference were: GTR (Rodriguez et al. 1990) with gamma-distributed substitution rates for *E. ovalis* and *T. venulosus*; and HKY (Hasegawa et al, 1985) with gamma-distributed substitution rates for *L. latrans.* Numbers of haplotypes, and polymorphic and parsimony informative sites were higher in *E. ovalis*, and lower in *T. venulosus* (Table 5.2). The latter also presented the lowest levels of haplotype and nucleotide diversity. *Elachistocleis ovalis* presented the highest nucleotide diversity but haplotype diversity was slightly lower than in *L. latrans*.

According to the results of phylogenetic analysis, *E. ovalis* population presents genetic structure, but this structure appears not to be related with Araguaia River (Figure 5.2). Two different clades (named EAraguaia and WAraguaia) were evident in the area of the mid-Araguaia. Only the monophyly of WAraguaia was supported by BI, but both clades were supported by NJ and MP analysis, and were also evident in the MJ network. Samples from the western bank (FSF, state of Pará) were all clustered in only one of these clades (WAraguaia), but they were not monophyletic or even paraphyletic relatively

Table 5.2 – Molecular diversity indices for *cox1*, for the sets of samples of the three amphibian species used in this study. Numbers of individual sequences (N), polymorphic sites/parsimony informative (Np) and haplotypes (H); Haplotype (h) and nucleotide (π) diversity (± SD).

Species	N	Np	H	h	π
Elachistocleis ovalis	73	48/40	27	0.928 ± 0.018	0.01916 ± 0.00052
Leptodactylus latrans	52	33/23	23	0.946 ± 0.014	0.00786 ± 0.00056
Trachycephalus venulosus	39	24/14	13	0.870 ± 0.030	0.00642 ± 0.00106

to the samples from the eastern bank (state of Tocantins), which were present in both clades. There was also haplotype sharing between the two banks of the Araguaia River, but once again only in one clade (WAraguaia). According to the results of MJ analysis, a minimum of 14 mutations separated the ancestral haplotypes from both clades, which were not sampled. The monophyly of the mid-Araguaia haplotypes relative to two haplotypes collected in Panamá was only supported by NJ analysis. The number of mutation steps separating these haplotypes (25) from the unsampled common ancestor of the three clades was almost two times the number of mutation steps separating both mid-Araguaia clades (14).

In *Elachistocleis ovalis*, the estimated evolutionary divergence (Table 5.3) was lower between the clades from the river Araguaia (3.5%) than among these and the clade from Panama (5.9 and 7.0%). However, only the Western Araguaia clade was significantly more divergent from the Panama clade. Indeed, the confidence intervals of the divergence estimate between both Araguaia clades overlapped with the confidence intervals of the divergence estimate for the pair Panama/Eastern Araguaia. The average distances between clades were always higher than the average distances within clades, which varied from 0.6% (WAraguaia and Panama clades) to 0.7% (EAraguaia clade).

Leptodactylus latrans haplotypes presented no structure at all, in the region of the mid-Araguaia (Figure 5.3). Only external nodes received some support from NJ and MP phylogenetic analyses. BI only supported the monophyly of the ingroup relatively to other sympatric species of the same genus. Extensive polytomy was observed throughout the whole tree, and only some external clades presented associated moderately high levels of posterior probability that were, however, not significant. These findings were posteriorly corroborated by MP analysis, which retained three best trees (not shown) and also presented extensive polytomy. Extensive haplotype sharing occurred among all

Table 5.3 – Average pairwise divergence estimates among *E. ovalis* clades (lower diagonal) and 95% confidence intervals (upper diagonal), given as percentages.

	Panama	WAraguaia	EAraguaia
Panama		±2.2%	±2.0%
WAraguaia	7.0%		±1.2%
EAraguaia	5.9%	3.5%	

locations, including FSF and FLV, in opposite banks of the Araguaia River and about 150 Km apart (Figure 5.1).

Trachycephalus venulosus also did not present genetic structure relatively to the Araguaia River (Figure 4). Besides the support for the monophyly of the ingroup, three other clades were supported by MP and NJ analyses. One clade (Atl. Forest), also supported by BI analysis, correspond to haplotypes sampled in the Atlantic forest, state of Espírito Santo (Southeastern Brazil), which were monophyletic relatively to the mid-Araguaia haplotypes. However, the haplotypes from the mid-Araguaia were not reciprocally monophyletic relatively the Atlantic forest haplotypes. NJ and MP analyses supported the monophyly of a clade that comprises the majority of the haplotypes from our study area (Araguaia I). The posterior probability (BI) associated with this clade was also high, but not significant (<0.95). Another clade (Araguaia II), composed by two haplotypes, was supported by NJ and MP analysis. However, the relationships among the three clades and between these and the ungrouped haplotypes were inconclusive. MP analysis retained ten best trees (not shown) and corroborated these results.

Average pairwise divergence estimates for *T.venulosus* were lower than the estimates for *E. ovalis*, even for the divergence between the clades from River Araguaia (Table 5.4). One of the two clades from the River Araguaia appears to be genetically closer to the Atlantic forest clade. However, for this species, divergence estimates among clades were not significantly different from each other.

Table 5.4 – Average pairwise divergence estimates among *T. venulosus* clades (lower diagonal) and 95% confidence intervals (upper diagonal), given as percentages.

	Atlantic Forest	Araguaia I	Araguaia II
Atlantic Forest		±1.0%	±0.8%
Araguaia I	1.6%		±0.8%
Araguaia II	1.0%	1.6%	

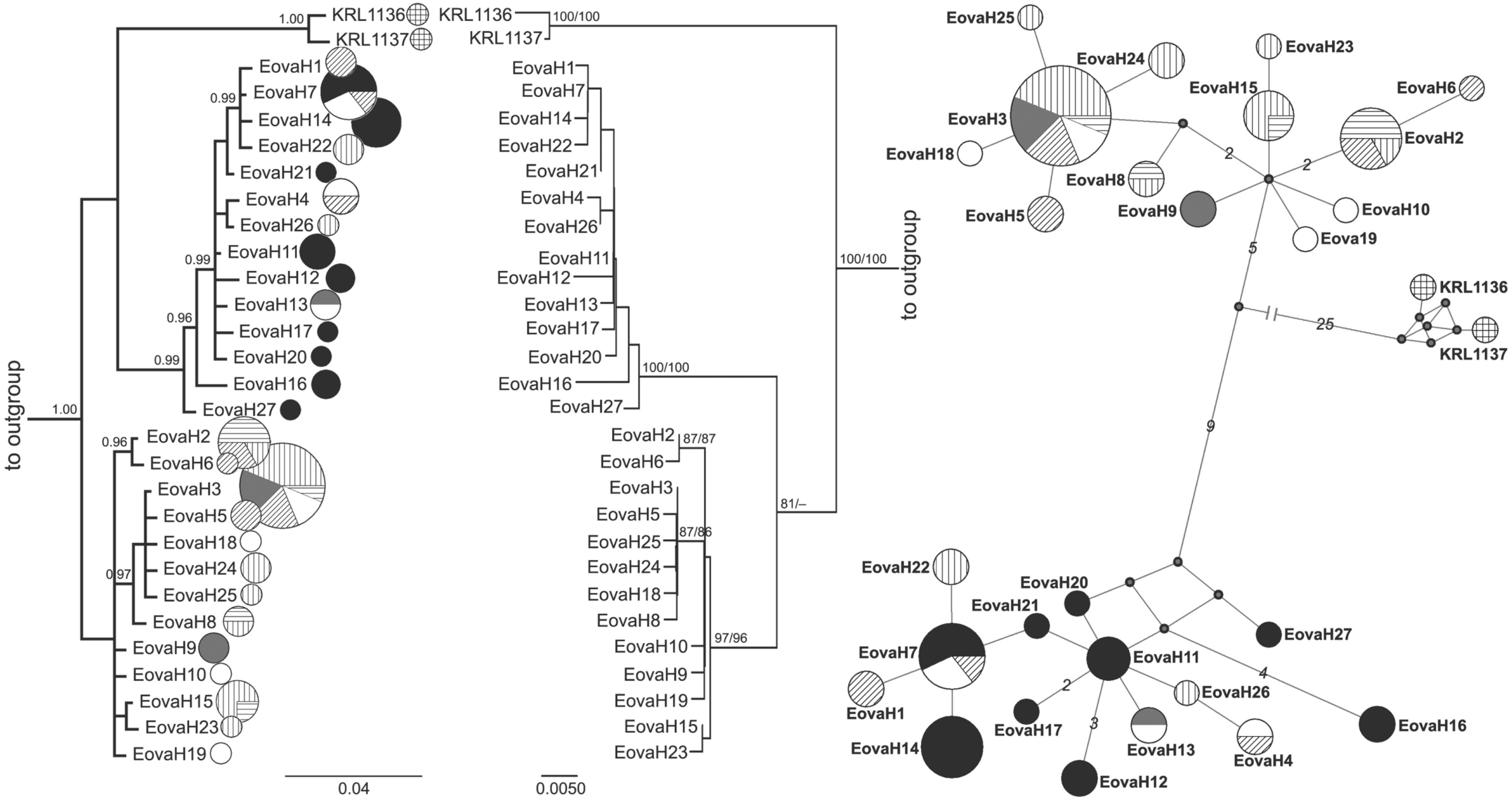

Figure 5.2 – *Elachistocleis ovalis*. **Left**: Bayesian inference (BI) majority-rule 50% consensus tree. **Centre**: Neighbour-joining (NJ) bootstrap consensus tree. Posterior probability (BI) or bootstrap support (NJ/MP) is presented above each node. Only nodes with significant support are labelled. **Right**: Median-Joining (MJ) networks – haplotype pies correspond to those in BI trees. Grayscale/hatched patterns correspond to those in figure 5.1. Clade names (gray) correspond to those in Table III. Numbers of mutations between haplotipes in the MJ network are indicated except for links corresponding to one mutation.

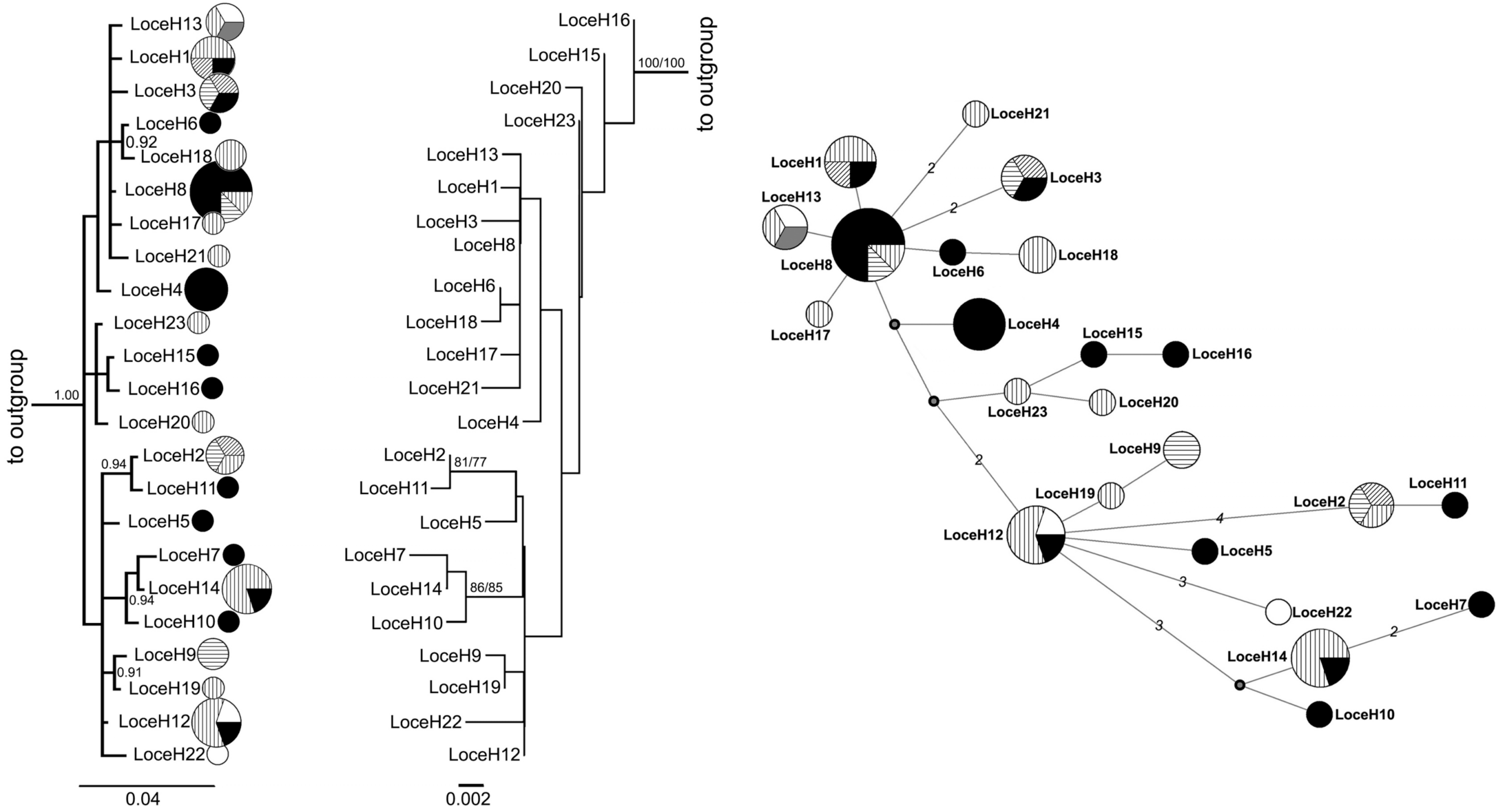

Figure 5.3 – *Leptodactylus latrans*. **Left**: Bayesian inference (BI) majority-rule 50% consensus tree. **Centre**: Neighbour-joining (NJ) bootstrap consensus tree. Posterior probability (BI) or bootstrap support (NJ/MP) is presented above each node. Only nodes with significant support are labelled. **Right**: Median-Joining (MJ) networks – haplotype pies correspond to those in BI trees. Grayscale/hatched patterns correspond to those in figure 5.1. Numbers of mutations between haplotipes in the MJ network are indicated except for links corresponding to one mutation.

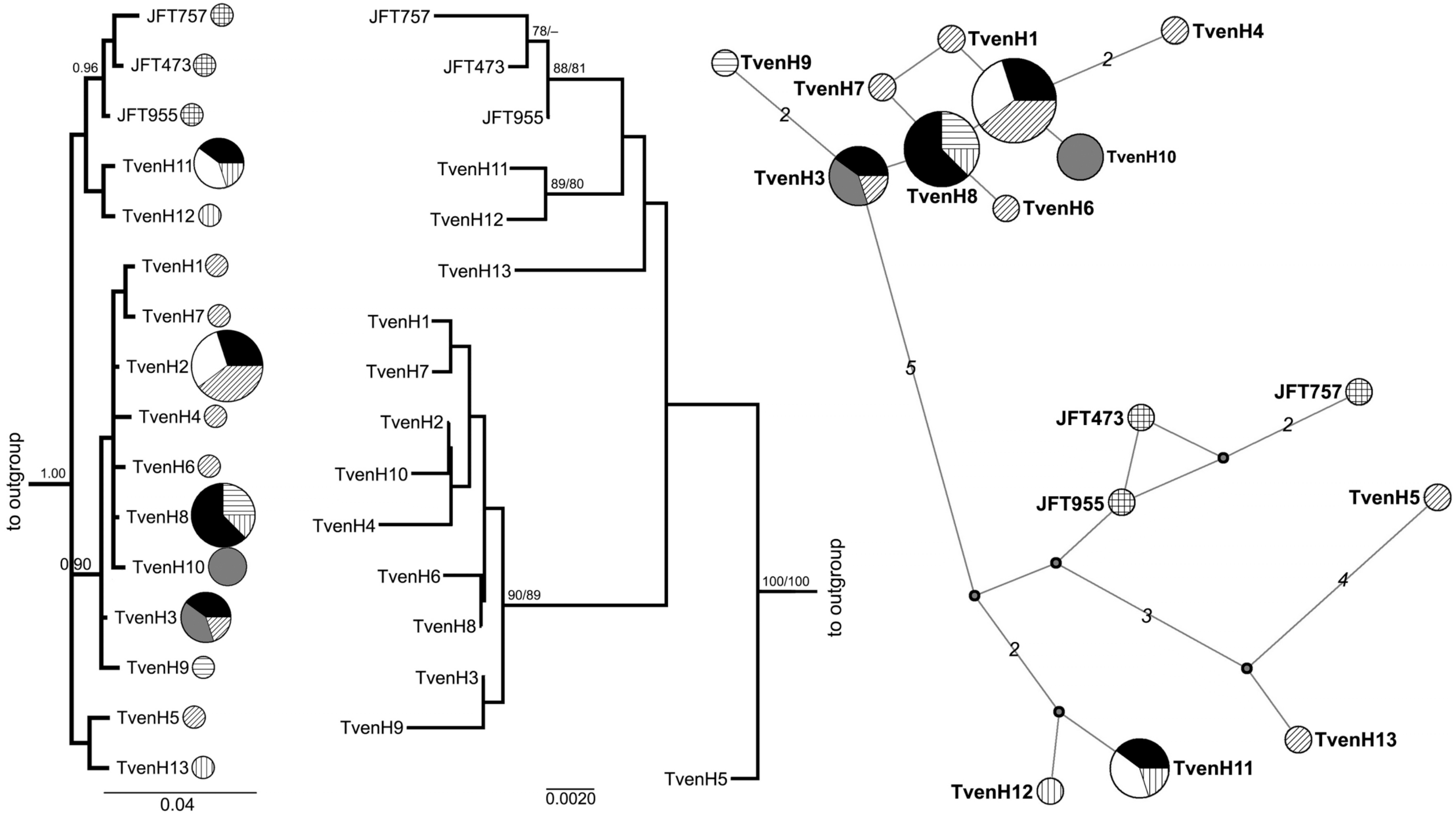

Figure 5.4 – *Trachycephalus venulosus*. **Left**: Bayesian inference (BI) majority-rule 50% consensus tree. **Centre**: Neighbour-joining (NJ) bootstrap consensus tree. Posterior probability (BI) or bootstrap support (NJ/MP) is presented above each node. Only nodes with significant support are labelled. **Right**: Median-Joining (MJ) networks – haplotype pies correspond to those in BI trees. Grayscale/hatched patterns correspond to those in figure 5.1. Clade names (in gray) correspond to those in Table IV. Numbers of mutations between haplotipes in the MJ network are indicated except for links corresponding to one mutation.

5.5 – Discussion

Cox1 gene is considered to perform well in discriminating amphibian species (Vences et al., 2005; Smith et al., 2008). It also presents low amino acid divergence but high rates of nucleotide substitution and appears not to recover incorrect branches (Mueller et al, 2006). However, some disagreement exists regarding the overlap of intraspecific and interspecific variation in *cox1* (Vences et al., 2005; Rubinoff et al., 2006; Smith et al., 2008). The fast evolution rate of the *cox1* and its apparent reliability to recover phylogenetic relationships were the reasons for choosing this genetic marker to test the Araguaia River as a barrier to gene-flow at the intraspecific level of three amphibian species.

Haplotype diversity was high for the three species, considering estimates for other amphibian taxa, using mtDNA genes (Lougheed et al, 1998; Vences et al., 2004; Makowsky et al., 2009; Zainudin et al, 2010). Nucleotide diversity estimates obtained in this study were located within the range of estimates for other amphibian species (Lougheed et al, 1998; Austin et al., 2002; Vences et al., 2004; Funk et al., 2007; Makowsky et al., 2009; Zainudin et al., 2010). However, nucleotide diversity was much higher for *E. ovalis* than for the other two species. In the study on *Pseudacris crucifer*, by Austin and collaborators (2002), the highest levels of nucleotide diversity occurred in areas of secondary contact among two different lineages. In our study, *E. ovalis* was indeed the only species that appears to include two distinct lineages, supported by all analyses except by bayesian inference.

5.5.1 – Is Araguaia River a barrier to gene flow?

The general pattern revealed by phylogenetic trees and haplotype networks, generated for the three species studied here, shows no congruence between geography and genetic relationships. For all three species, several haplotypes (generally the most frequent ones) occur on both banks of the Araguaia River. As expected, diversity was higher in *E. ovalis*, even though haplotype diversity was slightly higher in *L. latrans*. In the latter species, haplotypes were more evenly distributed and the ratio between haplotypes/individuals (23/52) was higher than in *E. ovalis* (27/73). However, haplotypes in *L. latrans* were less divergent than in *E. ovalis*, which presented a greater proportion of parsimony informative sites and much higher nucleotide diversity. The level of structure was also higher in *E. ovalis*. Two distinct clades were detected, with haplotypes from the western bank restricted to one clade, and haplotypes sampled at the northern portion of the eastern bank restricted to the other clade. But even for *E. ovalis*, it appears that Araguaia River does not constitute an effective barrier to gene flow, since haplotypes from the two riverbanks are present, and even shared, in one of the clades.

Expectations from the riverine barrier hypothesis include: 1) occurrence of two reciprocally monophyletic clades on opposite banks of the river (Haffer, 1997; Lougheed et al., 1998; Patton et al., 2000; Funk et al, 2007); 2) occurrence of a paraphyletic clade on one bank of the river, resulting from the dispersal of a population established on the other bank of the river (Haffer, 1997; Patton et al., 2000); 3) or strong genetic structure between populations on opposite banks of the river and little structure between populations on the same bank (Gascon et al., 2000; Funk et al., 2007; Zhao et al, 2009). The phylogeographic relationships revealed by our analysis do not corroborate any of these expectations and thus, do not support the Riverine Barrier hypothesis. Our results corroborate previous findings for Amazonian rivers (Gascon et al., 1998; Lougheed et al., 1999; Gascon et al., 2000). All three species were captured near the river margins or even in the large islands that occur throughout the river. Frequently, haplotypes found in the islands also occurred in mainland, at one or both banks of the Araguaia River.

Previous authors hypothesized that gene-flow can occur when extensive portions of land are passively transferred from one bank to another in a river, each time a meander loop is cut off or a new river course is carved out within the floodplain (Haffer, 1997; Gascon et al, 1998). In this study, all species were found in the islands. It is known that separation and accretion of islands to the mainland occurs in the Araguaia, and that larger islands are stable in a decadal scale (Latrubesse, 2009). Therefore, we consider that these islands can provide a mechanism of passive transfer of individuals from one bank of the river to another.

5.5.2 – Two lineages of E. ovalis in the Araguaia River

Our results support two distinct lineages of *E.ovalis* in the region of the mid-Araguaia: one only at the eastern bank of the river and other mainly on the western bank. These lineages presented high divergence (3.5%) and their monophyly relatively to haplotypes of *E. ovalis* from Panama was not supported by MP and BI analyses. This result might be related to the fact that, despite the Western Araguaia Clade being significantly more divergent from the Panama Clade than from the Eastern Araguaia Clade, the magnitude of the divergence rates from the latter clade to the two former clades did not differ significantly.

The taxonomy of genus *Elachistocleis* is still unresolved (Toledo et al., 2010), despite recent attempts to clarify old controversies (Lavilla et al., 2003). Several species have been recently described or revalidated (*Elachistocleis skotogaster* – Lavilla et al., 2003; *E. cesarii* – Toledo et al., 2010; *E. magnus* – Toledo, 2010; *E. surumu*, *E. matogrosso*, *E. helianneae*, *E. carvalhoi* and *E. bumbameuboi* – Caramaschi, 2010), adding to five previously known species. Species in this genus are mainly diagnosed by their colour patterns and the presence of postcommisural glands (Lavilla et al., 2003). In the description of *E.ovalis* presented by Parker (1934), two colour varieties occur in *Elachistocleis ovalis,* which ultimately might prove to be different species.

The individuals collected in the mid-Araguaia basin are in general agreement with the description provided by Parker (1934) and presented great colour pattern variation (Supporting information – Figures 5.S1a to 5.S1c): presence/absence of discrete light dorsal strip; broad/thin femoral stripe, always with inguinal spots; more or less profusely spotted belly (sometimes not spotted at all). We did not found any correlation among coloration patterns or between these patterns and the identified clades, or even haplotypes. We could not safely assign the individuals from the Araguaia to any of the recently described species, based on morphological characters. However, because the individuals from this area fitted the description by Parker (1934), we considered that assigning them to *E.* ovalis would be a conservative approach. In addition, the specimens we collected (CHUNB58168, CHUNB58177, CHUNB58165, CHUNB58180, CHUNB58185, CHUNB58150) were very similar to other specimens previously collected in the same area (CHUNB45831-45846), by Janalee Caldwell and collaborators, and deposited as *Elachistocleis ovalis* in the Colecção Herpetológica da Universidade de Brasília. We do not exclude that ultimately they might be assigned to one of the recently described *Elachistocleis* species, eventually when molecular data becomes available for comparison.

According to Bickford and co-authors (2006), morphological change might not be correlated with species boundaries because cryptic species are differentiated by nonvisual mating signals and/or could be under selection that promotes morphological stasis. Amphibians do tend to exhibit conservative morphological evolution and the application of molecular and bioacustic tools are helping to reveal morphologically cryptic species (Bickford et al., 2006; Stuart, 2006). Many cryptic complexes are sympatric (Bickford et al, 2006) but are not usually each other's closest relatives (Stuart, 2006). The existence of two considerably divergent lineages in the mid-Araguaia basin, that might not be each other's sister group (not supported as sister clades by MP and BI analyses), suggests that these two lineages might be part of a cryptic species complex. Unravelling cryptic species complexes is very important since actual biological species in those complexes have more limited distributions than the complex itself, making each more prone to extinction (Köller et al., 2005; Bickford et al, 2006).

The average pairwise divergence rate between both Araguaia clades (3.5%) is above the threshold for intraspecific variation (2%) and bellow the threshold for interspecific variation (4%) estimated by Smith and collaborators (2008) for Holoarctic amphibian species. Both average pairwise divergence rates between the Araguaia clades and the Panama clade are above the latter threshold (5.9% and 7.0%). However, taking into account the values of intraspecific variation (mean 5.4%, regularly 10-14%) estimated by Vences and collaborators (2005) for Mantellid frogs, all the divergence rates estimated for *E. ovalis* might be considered intraspecific variation.

If the variation that we encountered in the mid-Araguaia basin prove to be intraspecific, it might be possible that in fact the Araguaia River might pose some resistance to gene flow, acting as a semi-permeable barrier that reduces but does not eliminate the probability of dispersal between geographic areas. Such kind of barrier (filter barrier, in the sense of Remington, 1968) was found by Crawford and collaborators (2007) in the region of Bocas del Toro, Costa Rica. This way, two different lineages could have evolved at both banks of Araguaia River, with the posterior occurrence of dispersal across the river, eventually by a mechanism of passive transfer mediated by the islands.

5.5.3 – Considerations about the dorsal patterns in T. venulosus

The taxonomy of the genus *Trachycephalus* has also received recent contributions. Faivovich et al., (2005) included genus *Phrynoyas* (and thus *P. venulosa*) into genus *Trachycephalus*. Three new species were described recently: *Trachycephalus lepida* (Pombal et al, 2003), *Trachycephalus dibernardoi* (Kwet & Solé, 2008) in the Atlantic Forest, and *Trachycephalus mambaiensis* (Cintra et al., 2009) in Cerrado. All the species in the genus are easily distinguishable from *T. venulosus*. However, Lavilla et al. (2010b) suggested that this species might in fact constitute a species complex. In the account by Lutz (1973), *T. venulosus* is described as presenting two distinct dorsal patterns: the uniform *bufonia* pattern, and the ornate *zonata* pattern, that vaguely resembles the dorsal pattern of *Trachycephalus mesopheus*, from the Atlantic Rainforest.

According to Lutz (1973), it was unlikely that both patterns were in fact two different species.

These two patterns occur in sympatry in the mid-Araguaia basin. Our results indicate no genetic structure relative to dorsal pattern (Supporting information, Figure 5.S2). In fact, the same *cox1* haplotype is shared by individuals presenting both patterns (eg. Figure 5.S2, TvenH8). These findings corroborate the hypothesis that both patterns correspond to the same species. Most of the haplotypes from the mid-Araguaia form a monophyletic clade. However, the relationship between this clade with the other haplotypes from this region and those from the Atlantic Forest (which also present the *zonata* pattern) is unresolved. In the case of *T. venulosus*, it also appears that several different lineages occur in the Araguaia basin. However, in this case, the divergence rates are rather consistent with a scenario of intraspecific variation.

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Supporting Information

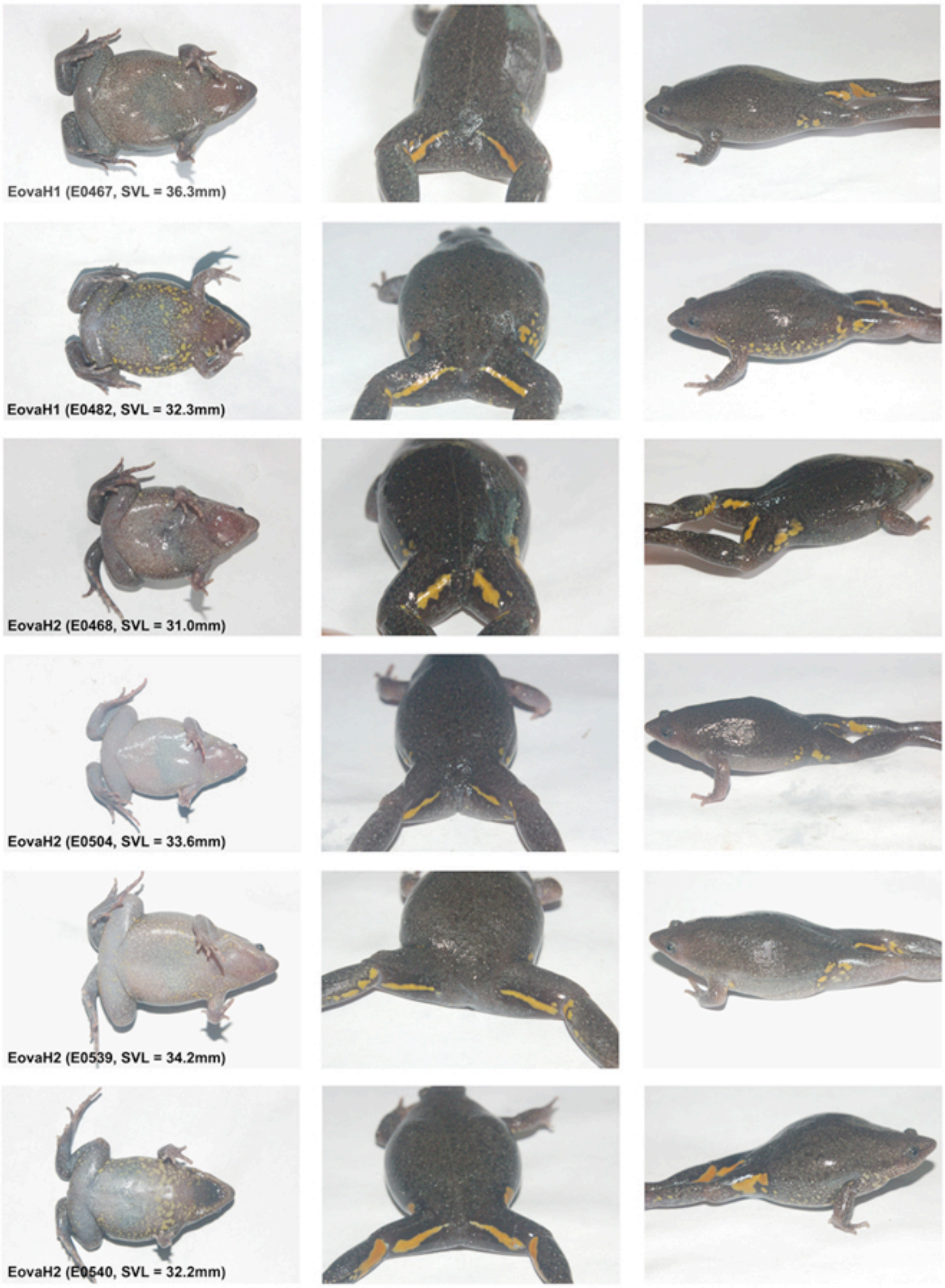

Figure 5.S1a – Ventral, posterior and lateral view of several *E. ovalis* individuals captured at the study area, around the Araguaia River. The haplotipe and snout-vent length of each individual is presented rightmost picture.

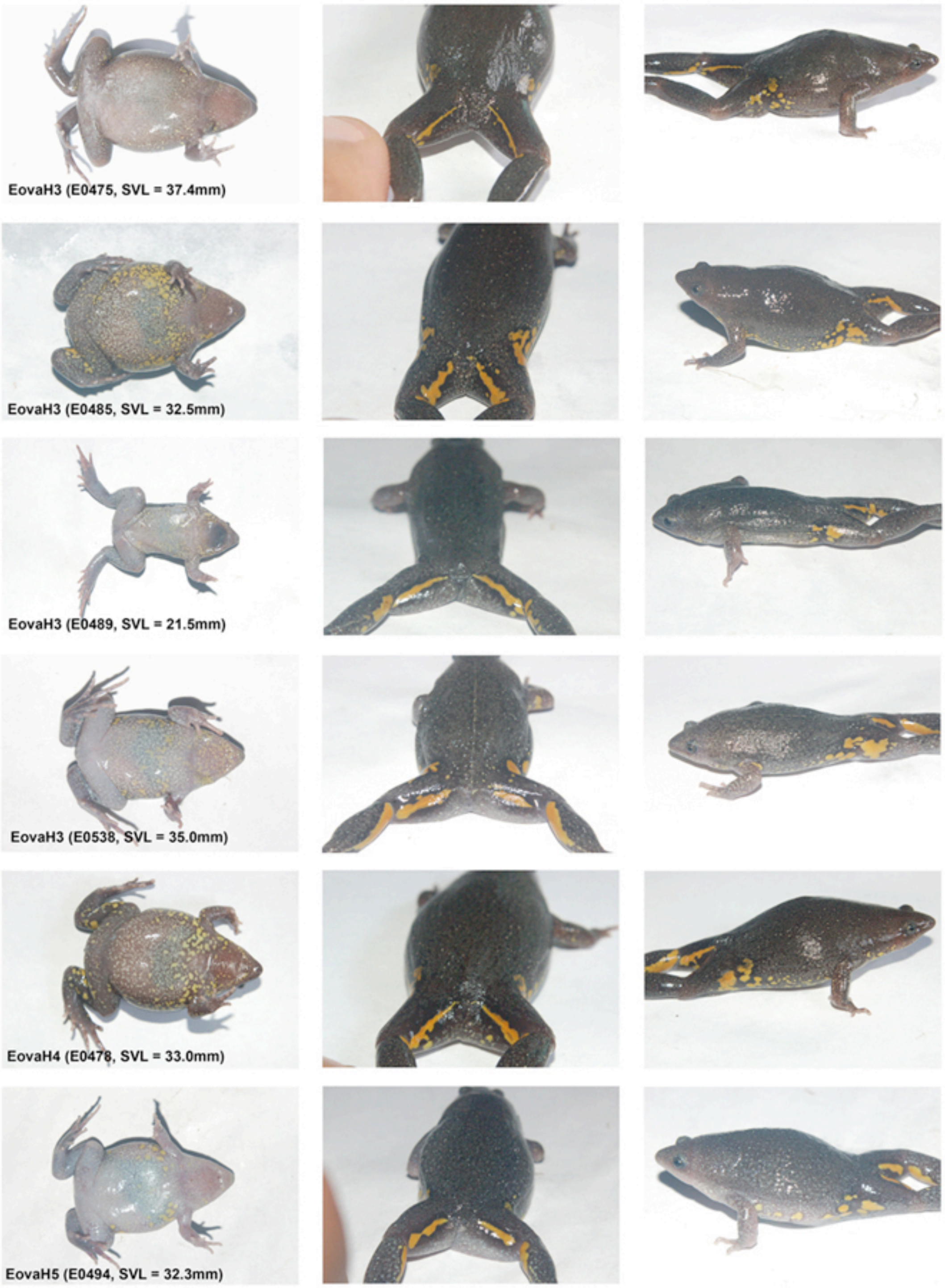

Figure 5.S1b – Ventral, posterior and lateral view of several *E. ovalis* individuals captured at the study area, around the Araguaia River. The haplotipe and snout-vent length of each individual is presented rightmost picture

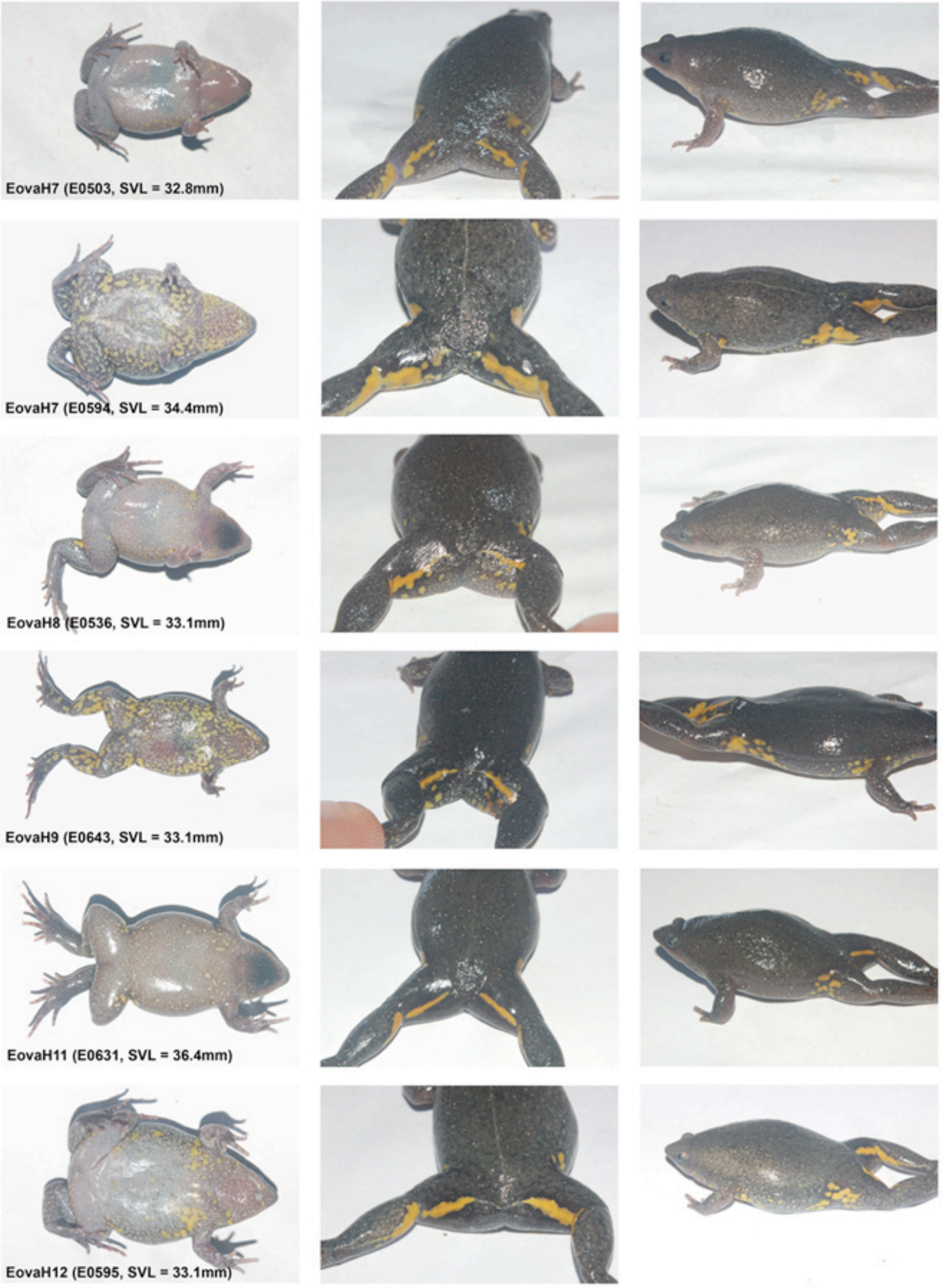

Figure 5.S1c – Ventral, posterior and lateral view of several *E. ovalis* individuals captured at the study area, around the Araguaia River. The haplotipe and snout-vent length of each individual is presented rightmost picture.

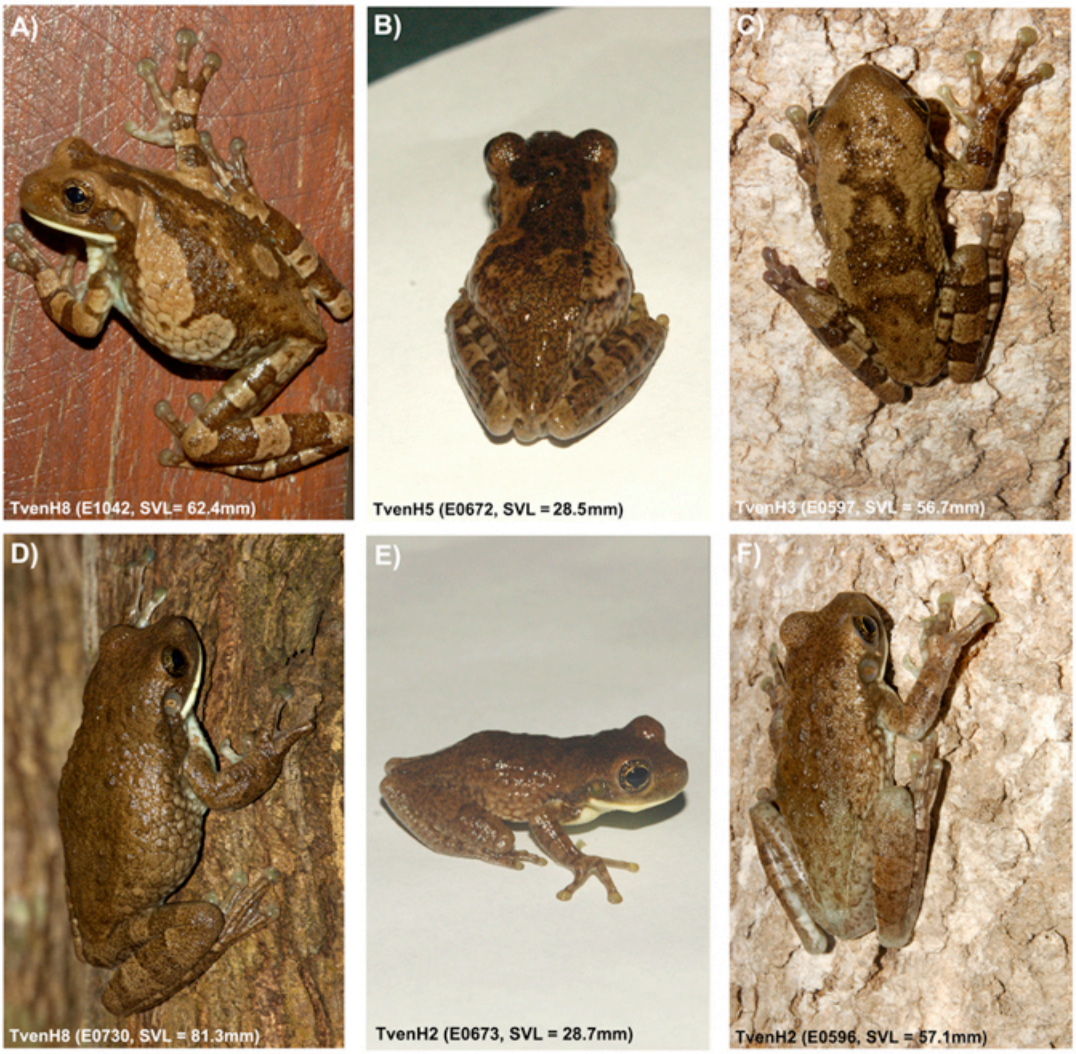

Figure 5.S2 – View of the ventral pattern of several *T. venulosus* individuals, captured in the study area, around Araguaia River. The haplotipe and snout-vent length of each individual is presented in each picture. Pictures A, B and C correspond to pattern "*zonata*", and pictures D, E and F correspond to pattern "*bufonia*" in Lutz (1973).

CHAPTER 6

Genetic structure of three lizard populations along the middle Araguaia River: the influence of size and ecology

Ferreira E., Rocha R.G., Fonseca C. Genetic structure of three lizard populations along the mid-Araguaia River: the influence of size and ecology. Manuscript *in prep.*

Chapter 6 cover photos (from left to right): adult *Anolis nitens brasiliensis*, adult *Ameiva ameiva*; and young *Tupinambis teguixin*.

6.1 – Abstract

Since the formulation of the Riverine Barrier Hypothesis for explaining the diversification of the Amazonian fauna, several alternative models have been proposed. Evidence for and against this and alternative hypotheses have been generated based on the work of several authors. Here, we propose to assess the genetic structure of three lizard species along the mid-Araguaia River, the largest drainage basin of the Cerrado biome. These species, *Anolis nitens brasiliensis*, *Ameiva ameiva* and *Tupinambis teguixin*, differ in ecological requirements and size and we expect them to respond differently to the presence of Araguaia River. Ultimately, we aim to understand if the genetic structure of these three species agrees with the predictions of the Riverine Barrier Hypothesis. We collected tissue samples of these species along the mid-course of the Araguaia. We analyzed two fragments of mitochondrial DNA, corresponding to the genes coding for the cytochrome oxidase subunit 1 (*cox1)* and to the NADH dehydrogenase subunit 4 (*ND4*). As expected, our results revealed a different scenario for each different species. *T. teguixin* revealed the lowest levels of diversity and genetic structure, followed by *A. ameiva* and *A. n. brasiliensis*. Haplotype sharing between the eastern and western bank occurred in *T. teguixin* and *A. ameiva*, but not in *A. n. brasiliensis*. For the latter species, haplotypes from the western bank formed a monophyletic clade that rendered the eastern bank paraphyletic. These results suggest that Araguaia is not a barrier for both teiid species, but might constitute a barrier for gene flow in *A. n. brasiliensis*. We suggest that these differences might be a result of the combined effect of habitat preferences, ecology and body size. Our results provide new information on how the genetic structure and diversity of sympatric but ecologically distinct species can respond differently to the same environmental constraints. Phylogeograhic relations within *Tupinambis* were also discussed, by combining our data with previously published ones. The populations of *T. teguixin* from the mid-Araguaia and Roraima formed a monophyletic clade, but the monophyly of *T. teguixin* (when including a population from Equador) relatively to *T. quadrilineatus* and *T. longilineus* was not supported by our analysis.

Keywords: Amazonia; *Ameiva ameiva*; *Anolis nitens brasiliensis*; Cerrado; Riverine Barrier Hypothesis; *Tupinambis teguixin*;

6.2 – Introduction

The role of rivers as barriers to animal populations and, thus, as one of the mechanisms responsible for the diversification of the Amazonian fauna, was first suggested in a formal framework as the “Riverine Barrier Hypothesis”, in 1852, by Alfred Russell Wallace (Colwell, 2000). Some support for this hypothesis came from studies on the distribution of Neotropical vertebrate taxa (Ron, 2000) and on the phylogenetic structure of amphibians (Noonan and Wray, 2006; Funk et al, 2007), lizards (Pellegrino et al., 2005; Rodriguez-Robles et al., 2008), birds (Bates et al., 2004) and mamals (Patton et al, 2000; Rocha et al., 2011). However, the phylogenetic patterns revealed for several other species refuted the predictions of the Riverine Barrier Hypothesis. Evidence against the dominant role of rivers in the diversification of Amazonian fauna came, sometimes, from the same studies (Patton el al, 2000; Noonan and Wray, 2006), but also from studies with other species (Gascon et al., 1998; Lougheed et al., 1999; Gascon et al., 2000; Symula et al., 2003). Several authors agree that no single model could adequately explain Amazonian diversity, and that different mechanisms should be relevant in speciation processes of different faunal groups (Noonan and Wray, 2006; Zeisset and Beebee, 2008; Haffer, 2008; Antonelli et al., 2010).

Insights about the role of rivers in the genetic structure of lizard species came from a study by Glor and collaborators (2001), which found evidence of a very deep split between the subspecies of *A. nitens* at the north and at the south of the Amazon River. Evidence came also from studies in the Brazilian Atlantic rainforest (Pellegrino et al., 2005) and in the Caribbean island of Puerto Rico (Rodriguez-Robles et al., 2008). However, there are no published data, at least to our knowledge, on the genetic structure or phylogeography of lizard species along the several large rivers flowing from the Brazilian Shield.

Here we propose to assess the potential role of one of these rivers, the Araguaia, as a barrier to the gene flow of lizard species. The Araguaia River is the main drainage basin of the Cerrado biome and, together with the Tocantins River, constitutes the fourth largest drainage basin of South America (Aquino et al., 2008; Latrubesse et al., 2009). The Araguaia basin has been recognized as a priority area for conservation (Azevedo-Ramos and Galatti, 2002; Cavalcanti and Joly, 2002).

The natural history of species can impact phylogeographic patterns (Avise, 2009), but comparative phylogeography might help to discriminate community-level historical events from ecological or demographic forces acting on single lineages or species

(Carnaval, 2002; Crawford et al., 2007). For this reason, we chose to study three species that diverge in their evolutionary history, ecology and size: *Anolis nitens brasiliensis*, *Ameiva ameiva* and *Tupinambis teguixin*.

Anolis nitens is a polychrotid lizard with typical sit-and-wait predator behaviour (Colli et al., 1997; Vitt and Zani, 1998; Miles et al., 2007). This species is divided in several recognized subspecies, which were proven to be genetically divergent (Ávila-Pires, 1995; Glor et al., 2001). *Anolis n. brasiliensis* is widely distributed in the Cerrado biome (Colli et al., 2002; Vitt et al., 2008a), occurring also periferically in Amazonia (Ávila-Pires, 1995), namely in the mid-Araguaia basin (Vitt et al., 2008a). Similarly to its Amazonian relatives (Ávila-Pires, 1995; Vitt and Zani, 1998; Vitt et al, 2001; Vitt et al., 2008b), this subspecies is a forest specialist, non-heliothermic, being predominantly found in the leaf litter and in the basal portion of trunks within forested habitats (Mesquita et al, 2006; Vitt et al., 2008a). This species is very rarely observed in open areas (Colli et al., 2002; Vitt et al., 2008a).

The teiids *Ameiva ameiva* and *Tupinambis teguixin* are ground-dwelling active foragers (Colli et al., 1997; Vitt and Zani, 1998; Miles et al., 2007). Both species are heliotherm (Vitt and Colli, 1994; Sartorius et al., 1999; Vitt et al, 2008b), and occur both on forested and open areas, preferentially in ecotones and naturally or antropogenically disturbed areas such as tree-falls, clearings or river bluffs (Vitt and Colli, 1994; Ávila-Pires, 1995; Sartorius et al., 1999; Vitt et al. 2008a). At least *T. teguixin* is known to be a good swimmer, despite its terrestrial habit (Ávila-Pires, 1995; Vitt et al., 2008b). *Ameiva ameiva* is a medium-sized lizard with a wide-distribution that extends from Panama to the tropical South America (Peters and Donoso-Barros, 1986), reaching the north of Argentina, east of the Andes (Colli, 1991). *Tupinambis teguixin* is a large lizard, distributed throughout Amazonia and in the northern part of South-America east of Andes. Its distribution limits are extended southwards, in gallery forests, to the state of São Paulo (Ávila-Pires, 1995).

With this study, we aim to assess the genetic structure of these ecologically distinct lizards, in the region of the mid-Araguaia river. We wish to understand if and how the Araguaia River interferes with the gene flow in these species and if, therefore, these findings would provide evidence for or against the Riverine Barrier Hypothesis. We expect levels of structure and diversity to be higher in the sit-and-wait predator and forest specialist, *A. n. brasiliensis*, than in both the more active and less habitat-restricted teiid species. At last, we expect that the much larger *Tupinambis teguixin* will reveal the lowest levels of genetic structure and diversity.

6.3 – Methods

6.3.1 – Study area

This study was conducted in the region of the mid-Araguaia River, in an area comprising four different conservation units (Figure 6.1), with different conservation statuses: Parque Estadual do Cantão (PEC); Área de Protecção Ambiental do Bananal/Cantão (APABC); Fazenda Santa Fé (FSF) and Fazenda Lago Verde (FLV). The regional climate is tropical and markedly seasonal, with a rain season from October to April and a dry season from May to September (INMET 2010).

PEC is a state park with 90,000ha, in the west of the state of Tocantins, at the border with the state of Pará. It is a strict conservation unit corresponding to World Conservation Unit (IUCN) category II (Rylands & Brandon, 2005). The park is located in the alluvial basin of the Araguaia River and two of its tributaries, Coco and Javaés. The park is mostly composed of seasonally flooded alluvial forests, and non-flooded forests at a lesser extent. PEC also includes some islands along the Araguaia River that can be more than six kilometres long. APABC is a 1,700,000ha buffer area, at the eastern border of PEC, created to buffer the impacts over PEC and Parque Nacional do Araguaia (PNA), at the south. APABC is a sustainable use protected area, corresponding to IUCN category V (Rylands & Brandon 2005). FSF is a private ranch with 65,000ha, located in the municipality of Santana do Araguaia, state of Pará, at the western bank of Araguaia River. The main activity is cattle ranching but about 65% of the ranch is still covered by deciduous forest. The forested area is concentrated near the Araguaia River, in the continuity of APABC and PEC. These forests are drier than PEC and less influenced by seasonal flooding. FLV is an 8,000ha private ranch managed for artificially irrigated crop production (rice, maize, bean and soybean), with almost 70% of the area constituted by pristine Cerrado sensu lato physiognomies. Natural forest fragments, locally named *ipucas*, occur within agricultural and Cerrado matrices.

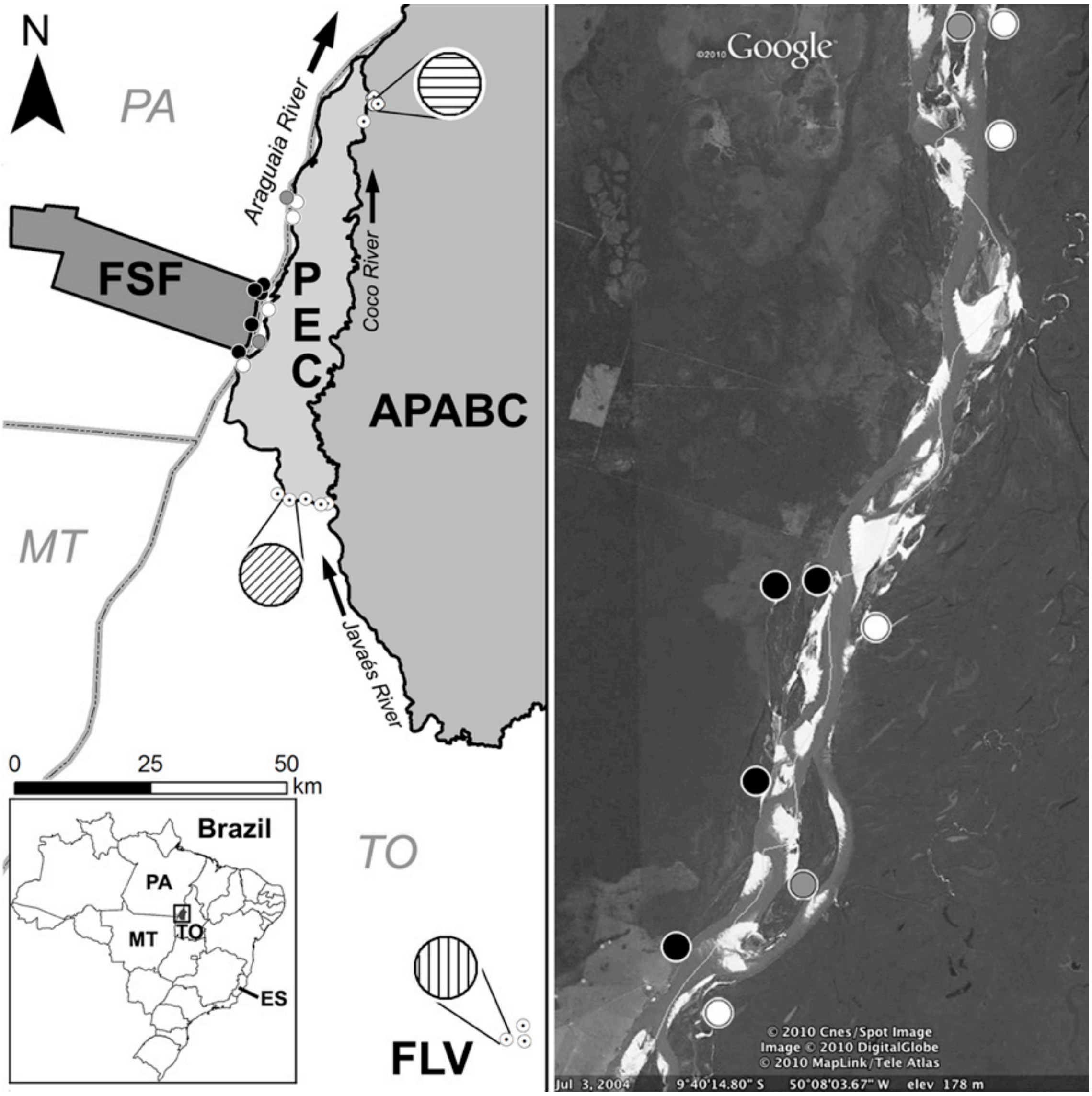

Figure 6.1 – Study area in the mid-Araguaia River basin. Location of study area in Brazil (bottom left corner); Map including all the sampled conservation units (left) and detail of sampling points near River Araguaia (right). Grayscale and hatched patterns correspond to those in figures 6.2, 6.3 and 6.4. Federal Units: ES – Espírito Santo; MT – Mato Grosso; PA – Pará; TO – Tocantins. Major rivers and direction of flow are also depicted. Major rivers and direction of flow are also depicted. Sample areas: PEC – Parque Estadual do Cantão; APABC – Área de Protecção Ambiental do Bananal/Cantão; FSF – Fazenda Santa Fé; FLV – Fazenda Lago Verde.

6.3.2 –Sample collection

Sampling was carried out between June 2007 and November 2008, using pitfalls, Sherman and Tomahawk traps and active search. Detailed descriptions of sampling strategy can be found in Ferreira et al. (submitted, Chapter II of this thesis) and Rocha et al. (2011). The first individuals of each species were collected, prepared as voucher specimens and are deposited at “Coleção Herpetológica da Universidade de Brasília” (CHUNB). All other individuals were individually marked with visible implant elastomer (VE) and released. Blood samples were collected directly from adult individuals into FTA® cards (Whatman™). Blood was collected after punching between the lizard fingers with a small lancet. Individuals were kept under observation and released in the following morning. We observed no mortality resulting from blood collection procedure. Alternatively, tail tissue samples were collected whenever lizards released the tail-tip, during handling. Occasionally, tissue was collected from the thigh muscle of individuals found dead inside pitfalls, but that were no longer suitable for voucher preparation.

6.3.3 – Laboratory protocols

DNA was extracted from tissue samples preserved in FTA cards or ethanol using the salt-extraction method (Bruford et al. 1992) and the concentration of DNA isolates was quantified using spectophotometer (model NanoDrop® ND-1000 UV/Vis, Thermo Fisher Scientific). A 663-bp fragment of the gene coding for the subunit I of the cytochrome oxidade (*cox1*) was amplified by polymerase chain reaction using the primers dgLCO1490 and dgHCO2198 (Meyers, 2003). An initial 840 to 860-bp fragment of the gene coding for the NADH dehydrogenase subunit 4 (*ND4*) was amplified using the primers ND4 and LEU (Arévalo et al. 1994).

PCR reactions were performed in a 25μl total volume, using 1.0 unit of Taq-polymerase and final concentration of 0.12μM (*cox 1*) or 0.16μM (*ND4*) of each primer and 0.25μg/μl of bovine serum albumin. Other reagents were added according to manufacturer’s instructions. Quantity of DNA template was aimed at 50-100ng, but successful amplification occurred with as little as 10ng.

Amplification of *cox1* fragment was performed using the following PCR profile: initial denaturation at 95°C for 1 min; 37 cycles with denaturation at 95°C for 40 s, annealing at 48°C for 40 s and polymerization at 72°C for 60 s; final extension at 72°C for 5 min. In order to amplify particular samples, small adjustments to this profile were sometimes needed. Amplification of *ND4* fragment was performed using the following PCR profile: initial denaturation at 95°C for 5 min; 32 cycles with denaturation at 95 °C for 30s, annealing at 54°C for 45s and polymerization at 72°C for 60 s; final extension at 72°C for 5 min. The fragments were sequenced once for each primer, in an automated sequencer ABI 310 using the Big Dye Terminator Cycle Polymerase (Perkin Elmer, Applied BiosystemsTM, Foster City, California).

6.3.4 – Data Analysis

Sequences[2] were aligned using CLUSTALW algorithm implemented in MEGA version 4.1 (Kumar et al. 2008), and then checked and edited by eye. Alignments were performed on the consensus sequences generated for each individual, using the raw sequences from forward and reverse sequencing.

Numbers of haplotypes, haplotype (h) and nucleotide (π) diversity indices were estimated for each species, for each gene in separate and for concatenated data, using DNASP version 5.10.01 (Librado & Rozas, 2009), using the alignments of all sequences from the Araguaia basin. Lists of haplotypes were generated with the same software and subsequent analyses were conducted based in haplotypes rather than in individual sequences. To investigate whether or not river Araguaia acts as a barrier to gene flow in these three amphibian species, we generated phylogenetic trees using both distance-based and criterion-based algorithms, as well as haplotype median-joining networks. For each species, the same taxa were used as outgroups (Table 6.1) in all analyses except MJ networks, which were calculated without outgroups.

Neighbour-joining (NJ) trees (Saitou & Nei, 1987) were inferred based on the genetic distances estimated by the Kimura 2-parameter method (Kimura, 1980), using MEGA software. Bootstrap-consensus tree was inferred based on 1000 replicates (Felsestein, 1985).

Most-parsimonious (MP) trees were inferred using PAUP* 4.0b10 (Swofford 1998) by heuristic search. Starting trees were generated by stepwise addition (Farris, 1970), using 1000 replicates and a tree-bisection-reconnection (TBR) branch-swapping algorithm (Swofford and Sullivan, 2009). Bootstrap 50% majority-rule consensus-tree was inferred based on 500 replicates.

Bayesian inference (BI) of phylogenetic relationships among haplotypes was conducted using MrBayes version 3.1.2 (Ronquist and Hueselbeck, 2003). Prior best model of nucleotide substitution was chosen based on the scores of Akaike information criteria, estimated using MrModelTest version 2.3 (Nylander, 2004). The settings for the chosen model, generated by MrModelTest, were then incorporated into MrBayes' input

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[2] The sequences generated during this study were not yet submitted to GenBank, but will be submitted prior to manuscript submission.

file. Settings for the Metropolis coupling behavior were kept at default values. Markov chains ran for 1 million generations and sampled each 100th generation. We confirmed that chains had reach stationarity by inspecting the value of the standard deviation of split frequencies. A 50% majority-rule consensus tree was generated after discarding 25% of the simulated generations as *burn-in*. NJ, MP and BI phylogenetic trees were visualized and edited using FigTree version 1.3.1 (Rambaut, 2009). Phylogenetic analysis was conducted for each gene in separate and for the the concatenated data.

Median-joining networks (MJ) were generated using the software NETWORK (Bandelt et al, 1999). Only polymorphic nucleotide sites were included in MJ analyses. Geographical location of haplotypes across the mid-Araguaia basin was superimposed to the MJ networks *a posteriori*, following the grayscale and hatched patterns from figure 1, for better visualization of geographical distribution of haplotypes.

Average pairwise genetic divergence between groups of haplotypes was estimated using the Kimura 2-parameter (K-2p) method (Kimura, 1980) implemented in MEGA. Divergence was estimated considering pairwise deletion of nucleotide sites and gamma distributed evolutionary rates. Standard error estimates were obtained by bootstrap procedure (1000 replicates). Confidence intervals (95% CI) of the average pairwise distances were estimated according to Hayek & Buzas (1997).

6.4 – Results

We obtained DNA sequences corresponding to fragments (663bp) of the *cox1* gene from 27 *Anolis nitens brasiliensis* and 69 *Ameiva ameiva*. Sequences from *T. teguixin* consistently presented too much ambiguity, despite the overall good quality of the signal, and were discarded from the analyses. We also obtained DNA sequences corresponding to fragments of the *ND4* gene from 27 *A. n. brasiliensis* (860bp), 72 *A. ameiva* (840bp) and 67 *T. teguixin* (840bp). Several stop codons and gaps were found in the last portion of these sequences, which should correspond to the genes coding for a set of three t-RNA molecules (Arévalo, 1994). We choose to eliminate this segment and only the initial portion of the sequence (until the first stop codon) was used in the analysis. This resulted in a *ND4* fragment of 696-bp for *A. n. brasiliensis*, and 684-bp for *A. ameiva* and *T. teguixin*. For the latter species, additional sequences were included in the phylogenetic analysis (supporting information, Table 6.S1). Sequences used as outgroup taxa were all generated in this study (Table 6.1).

Table 6.1 – Outgroup taxa used in the phylogenetic analyses.

Species	Outgroup taxa
Anolis nitens brasiliensis	*Anolis ortonii* (E0353, this study) – Polychrotidae *Polychrus acutirostris* (E1115, this study) – Polychrotidae
Ameiva ameiva	*Kentropyx calcarata* (E0575, this study) – Teiidae *Tupinambis quadrilineatus* (E0746, this study) – Teiidae
Tupinambis teguixin	*Ameiva ameiva* (E0586, this study) – Teiidae *Kentropyx calcarata* (E0575, this study) – Teiidae

The estimated best prior evolution model for Bayesian inference was the general time reversible (GTR – Rodriguez et al. 1990), for all species/gene combinations. Additionaly, shape parameter of gamma-distribution of substitution rates (GTR+G) was selected for: *ND4* and for concatenate data, in *A. ameiva* and *Tupinambis*; and *cox1* in *A. n. brasiliensis*. Proportion of invariable sites (GTR+I) was selected for: *ND4* and concatenated data in *A. n. brasiliensis*; and *cox1* in *A. ameiva.*

Anolis n. brasiliensis presented higher diversity in *cox1* than in *ND4*, but the opposite occurred in *A. ameiva* (Table 6.2). *Anolis n. brasiliensis* presented the highest nucleotide diversity, in all cases. The values for this species were about two (*ND4*) to almost five times higher (*cox1*) than in *A. ameiva*, and almost nine times higher (*ND4*) than in *T. teguixin*. The number of *cox1* haplotypes, in *A. n. brasiliensis,* was two times greater than in *A. ameiva*. However, the number of haplotypes in *A. ameiva* was higher both for *ND4* gene and for concatenated data. All diversity indices were fairly lower in *T. teguixin* than in the other two species.

Globally, the results from phylogenetic analysis based on the two separated genes and on the concatenated data revealed very similar phylogenetic relationships. For this reason, only the results obtained from the concatenated data will be presented. When appropriate, disagreement among the results from the different data sets will be referred.

Anolis nitens brasiliensis appears to be structured along the Araguia River, with no haplotypes shared between riverbanks or among different areas in the eastern bank (Figure 6.2). However, few haplotypes were shared by more than one individual. Maximum parsimony and Neighbour-joining analyses supported the monophyly of the haplotypes from the western bank. The Bayesian support was also high but not significant. These results were corroborated by the three methods in *cox1* (0.98/97/97), but received low support in *ND4* (0.87/68/72)*.* A deeper divergence appears to exist between the haplotypes from the Araguaia basin and those from FLV. The monophyly of the haplotypes from FLV received maximum support from all the analysis using the three data sets. The monophyly of the Araguaia haplotypes was supported by MP and NJ analyses, but not by BI. The exact same support values were obtained using all data sets. In the MJ network, is possible to observe the very high number (106) of mutation that separates the hypothetic common ancestor from FLV haplotypes (not sampled) from the closest haplotype sampled in the Araguaia. The divergence estimate between the two detected clades was 10.1% ± 2.0%.

Table 6.2 — Molecular diversity indices for *cox1*, *nd4* and concatenated data, for the sets of samples of the three lizard species used in this study: numbers of individual sequences (N), polymorphic sites/parsimony informative (Np) and haplotypes (H); Haplotype (h) and nucleotide (π) diversity (± SD).

	N	Length	Np/Pa	H	h	Π
A. nitens brasiliensis						
Cox 1	27	663	77/61	18	0.960±0.021	0.01927±0.00729
ND4	27	696	71/60	15	0.932±0.031	0.01686±0.00648
Concatenated	27	1359	148/121	20	0.969±0.021	0.01803±0.00704
Ameiva ameiva						
Cox 1	69	663	27/23	9	0.543±0.069	0.00404±0.00126
ND4	72	684	49/39	25	0.822±0.027	0.00800±0.00177
Concatenated	68	1347	75/60	28	0.924±0.020	0.00537±0.00145
Tupinambis teguixin						
ND4	67	684	20/12	8	0.407±0.071	0.00190±0.00055

A different situation occurs in *A. ameiva*, where haplotypes are shared between riverbanks and among different areas from the eastern Araguaia bank (Figure 6.3). The most frequent haplotype appears to be restricted to the eastern bank, but the second most frequent one is shared by both banks and by the river islands. The NJ tree presents extensive politomy, with only one deep divergence between two clades: one (FLV) comprising only haplotypes sampled in FLV and another (Araguaia) including all the haplotypes from the Araguaia River and one haplotype found in FLV. The monophyly of the clade FLV received high support in all analyses, except in BI (0.89) using the *ND4* data set. The monophyly of the Araguaia clade also received high bootstrap support from MP and NJ, but BI was only obtained in the case of *cox1.* The estimated divergence (K-2p) between the two clades was 3.3%±1.0%. Haplotypes sampled in the mid-Araguaia basin appear to radiate from a central and most frequent haplotype (AameH2), as depicted in MJ network. Clade FLV does not connect to the network through this central haplotype, but through a close one (AameH1), sampled at the southern area or PEC. The number of mutations (38) separating the haplotypes from clade FLV is several-fold larger than the number of mutations separating any other two haplotypes in the MJ network.

In the case of *T. teguixin* the analysis was performed including sequences from this and other species of the genus *Tupinambis*, used in previous studies (Supporting Information, Table 6.S1), available at Genebank. Several of these sequences corresponded to smaller fragments (375-bp) of the *ND4* gene. In order to use as much data as possible, analyses were performed using pairwise deletion of nucleotide sites when necessary.

Phylogenetic analyses (Figure 6.4) confirmed the occurrence of three *Tupinambis* species in the study area: *T. teguixin* (TtegH1-H8), *T. quadrilineatus* (TquaH1) and *T. merianae* (TmerH1). The original data set from Fitzgerald et al (1999) did not include *T.quadrilineatus*. Here, the monophyly of this species is highly supported by the three methods, appearing as the sister species to *T. longilineus*. The monophyly of the haplotypes of *T. teguixin* from the Araguaia is supported by MP and NJ analyses,but not by BI. This clade is presented as the sister clade to *T. teguixin* from Roraima, Brazil, with high support from MP and NJ analyses. The monophyly of the three *T. teguixin* populations was only supported by MP analysis (83), but the monophyly of *T. teguixin* + *T. longilineus* + *T. quadrilineatus* is highly supported by the three methods of phylogenetic analysis. Divergence estimates (Table 6.3) were significantly lower than others involving the same clades, in the following cases: *T. teguixin* from the Araguaia and from Roraima (5.8%±2.5%); *T. longilineus* and *T. quadrilineatus* (6.6%±2.7%); *T. merianae* and *T. rufescens*/*T. duseni* (11.1%±3.7%). *Tupinambis teguixin* from Cuyabeno (Equador) was not significantly more divergent from *T. longilineus* (14.6%±4.7%) or *T. quadrilineatus*

(12.6%±4.1%) than from the other two *T. teguixin* populations (Araguaia, 11.1%±3.7%; Roraima, 10.4%±3.9%).

Table 6.3 – Average pairwise divergence estimates (K-2P) between *Tupinambis* clades. Divergence estimates are presented below diagonal and confidence intervals (95%) are presented above the diagonal. Clade numbers correspond to those in figure 4. BR – Brazil; EQ – Equador.

	1	**2**	**3**	**4**	**5**	**6**	**7**
1 *T. teguixin* Araguaia BR		2.5%	3.7%	3.9%	3.3%	6.7%	7.3%
2 *T. teguixin* Roraima BR	5.8%		3.9%	4.7%	5.5%	8.2%	7.8%
3 *T. teguixin* Cuyabeno EQ	11.1%	10.4%		4.7%	4.1%	6.7%	6.7%
4 *T. longilineus*	11.0%	13.7%	14.6%		2.7%	7.1%	6.9%
5 *T. quadrilineatus*	14.5%	15.0%	12.6%	6.6%		6.3%	7.1%
6 *T. merianae*	24.6%	27.9%	23.1%	23.8%	23.6%		3.7%
7 *T. rufescens* + *T.duseni*	25.2%	26.2%	24.0%	23.0%	22.7%	11.1%	

We found very little variation in the *T. teguixin* haplotypes from the Araguaia. Fifty-one individuals shared the same haplotype (TtegH1), which corresponded to more than 75% of the sampled individuals. This haplotype occurred with high frequency in both banks and in the Araguaia islands, suggesting lack of structure in the study area. The lack of structure is also patent in the NJ tree, where most of the nodes have no support from any of the analysis. All other haplotypes sampled more than once (TtegH2 and TtegH4) occurred in more than one area. All haplotypes appear to radiate from the most abundant one. One haplotype (TtegH2) is separated from the nearest one by 9 mutations, being supported as sister to the remaining haplotypes by MP and NJ analyses.

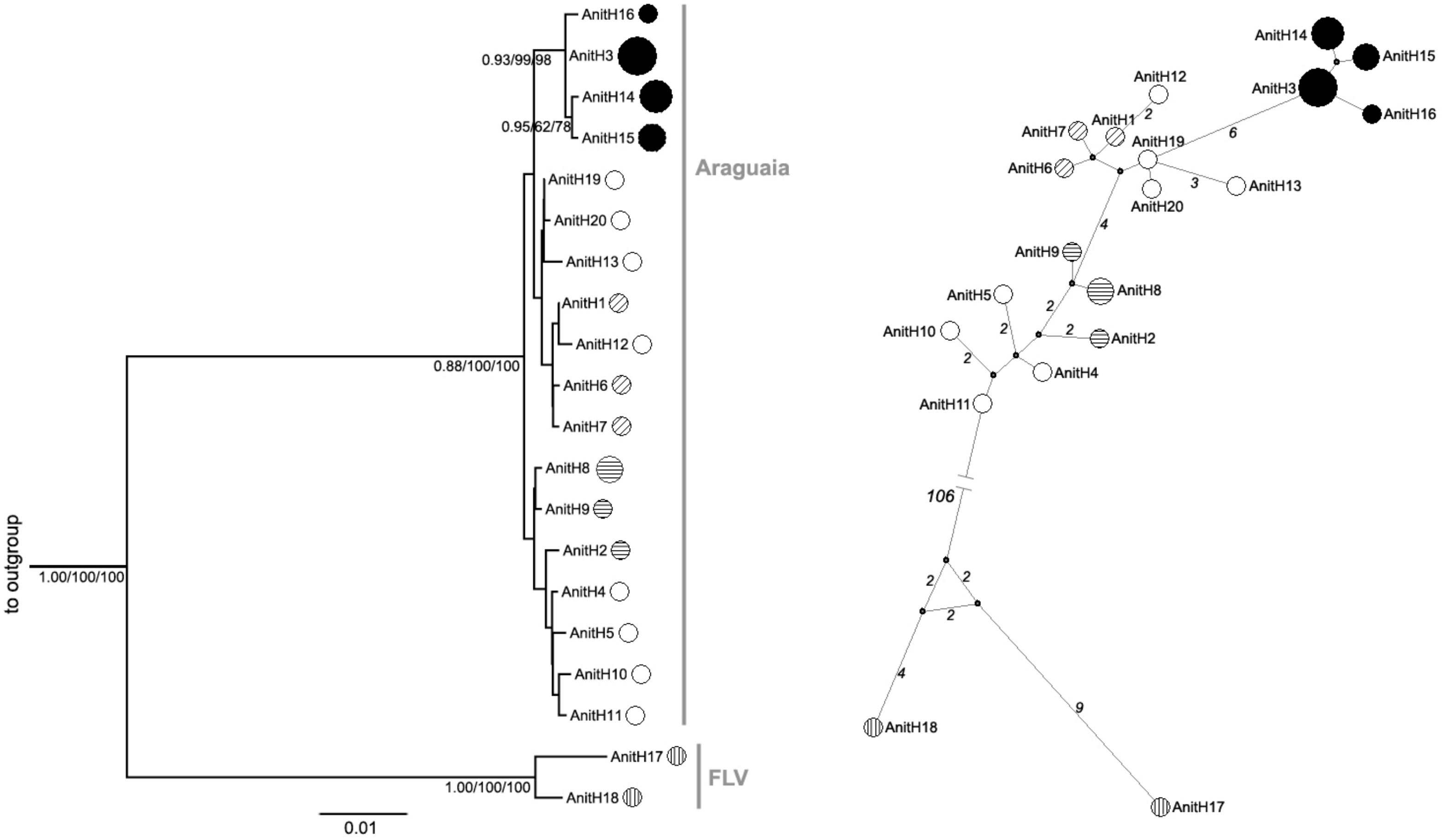

Figure 6.2 – *Anolis nitens brasiliensis*. **Left**: Neighbour-joining (NJ) bootstrap consensus tree. Posterior probability (BI) and bootstrap support (MP/NJ) for nodes are presented bellow each node; only nodes supported by at least to of the methods are labelled. **Right**: Median-joining (MJ) network – haplotype pies correspond to those in NJ trees. Grayscale/hatched patterns correspond to those in figure 1. Numbers of mutations between haplotipes in the MJ network are indicated except for links corresponding to a single mutation.

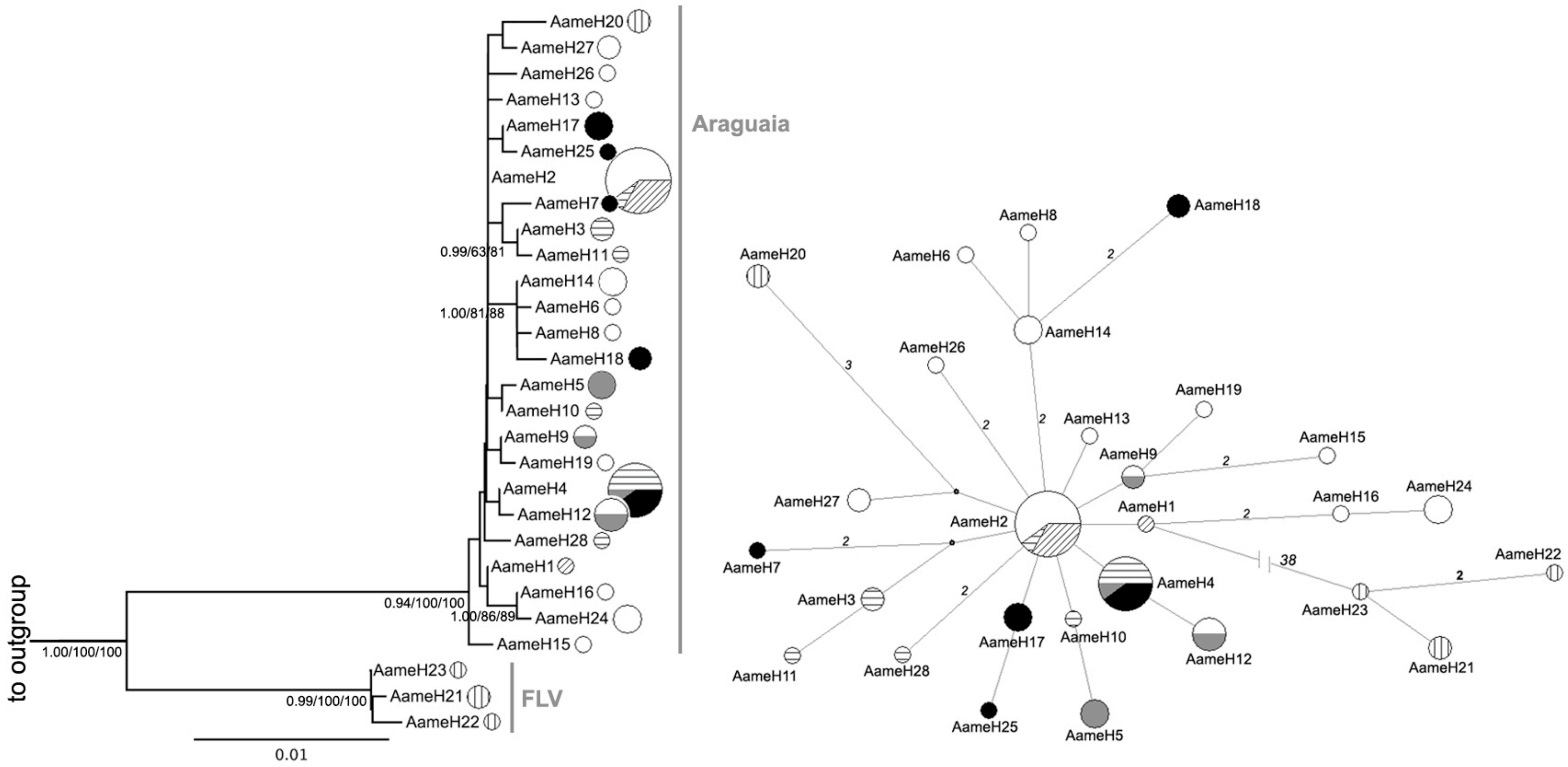

Figure 6.3 – *Ameiva ameiva*. **Left**: Neighbour-joining (NJ) bootstrap consensus tree. Posterior probability (BI) and bootstrap support (MP/NJ) for nodes are presented bellow each node; only nodes supported by at least to of the methods are labelled. **Right**: Median-joining (MJ) network – haplotype pies correspond to those in NJ trees. Grayscale/hatched patterns correspond to those in figure 1. Numbers of mutations between haplotipes in the MJ network are indicated except for links corresponding to a single mutation.

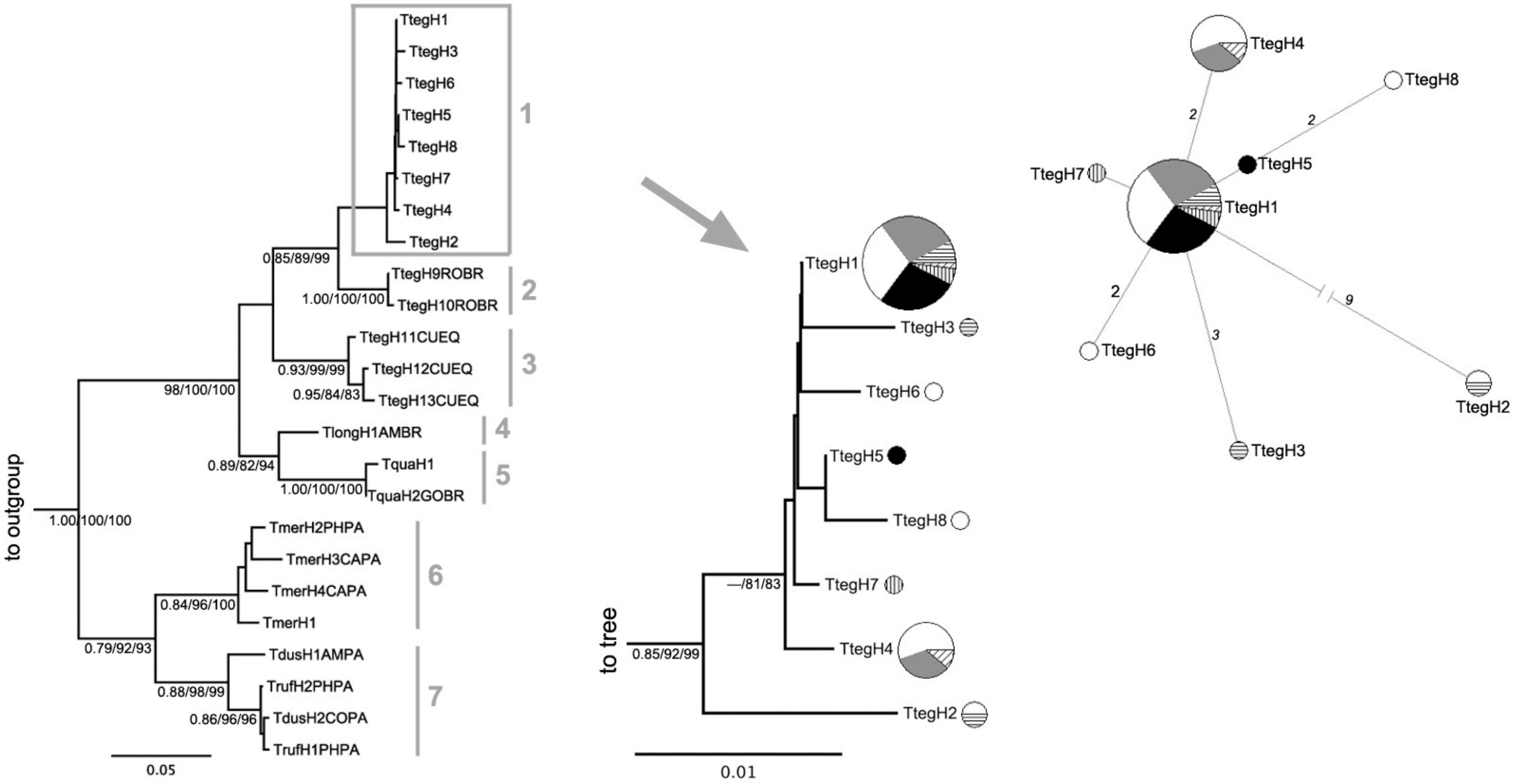

Figure 6.4 – *Tupinambis*. **Left:** Neighbour-joining (NJ) bootstrap consensus tree from genus *Tupinambis*, including samples from the studies by Fitzgerald et al (1999) and Pellegrino et al (2005) – see Table S2. **Centre**: detail from clade 1, corresponding to *T. teguixin* from the middle Araguaia basin. Posterior probability (BI) and bootstrap support (MP/NJ) for nodes are presented bellow each node; only nodes supported by at least to of the methods are labelled. **Righ**t: Median-joining (MJ) network – haplotype pies correspond to those in clade 1. Grayscale/hatched patterns correspond to those in figure 1. Numbers of mutations between haplotipes in the MJ network are indicated except for links corresponding to a single mutation.

6.5 – Discussion

6.5.1 – Patterns of genetic structure

Anolis nitens is a polychrotid lizard with typical sit-and-wait predator behaviour (Colli et al., 1997; Vitt and Zani, 1998; Miles et al., 2007) and the teiids *Ameiva ameiva* and *Tupinambis teguixin* are ground dwelling and active foragers (Colli et al., 1997; Vitt and Zani, 1998; Miles et al., 2007). Besides these ecological differences, the three species also differ greatly in size, which was confirmed by the average size of individuals captured during this study (supporting information, Table S2). Average size for *A. n. brasiliensis* reported here is slightly lower than in the study by Vitt and collaborators (2008a), for the same area. Our smaller sample size, and the inclusion of sub-adult individuals in our case, was probably the main causes for this difference. Here, we chose to exclude all juveniles from the morphometric analysis but, because we did not evaluate the reproductive condition, some non-reproductive individuals were certainly included. Nevertheless, there is a large difference in size among the three species. Based on these differences, we would expect that *A. n. brasiliensis* presented a greater level of genetic structure and diversity.

The lack of shared haplotypes between riverbanks and among different areas in *A. n. brasiliensis*, contrasting with the extensive haplotype sharing in both teiid species confirms our suspects. The fact that all *T. teguixin* haplotypes sampled in more than one individual were present in different geographic areas and the hegemony of a single and widely dispersed haplotype also suggests an absence of structure in this species. On the other hand, the support for two distinct and, at some extent, geographically separate clades in *Ameiva ameiva*, as well as the existence of some geographic segregation of less frequent (and not shared) haplotypes, indicates an higher level of structure in this species, comparatively to *T. teguixin*. Nucleotide and haplotype diversity were always higher in *A. n. brasiliensis* than in *A. ameiva* and *T. teguixin*. In this regard, genetic diversity was also higher in *A. ameiva* than in *T. teguixin*. As would be expected, there was a decreased level of structure and diversity with the increased vagility, which is a result of the combined effects of feeding ecology, habitat preferences and body size.

Two strongly supported and widely divergent lineages were found in *A. ameiva* and *A. n. brasiliensis*, corresponding to a distinction between the haplotypes from the Araguaia and the haplotypes from FLV. These two areas are approximately 100 km apart, which is more than the 70 km that separate the northern and southern sampling locations along the Araguaia. In the case of A. ameiva, haplotypes from both clades were sampled in FLV. Even though, the haplotype sampled in FLV that belongs to the Araguaia clade (AameH20) was not sampled along the Araguaia and was four mutations apart from the closest sampled haplotypes (AameH2 and AameH27). This contrasted with the maximum divergence of two mutations between any two closest haplotypes sampled in the Araguaia. Because of the very high divergence (K-2p = 3.3% ± 1%; 38 mutations) between both clades, and because one of the clades was only composed by haplotypes sampled in FLV, we believe that the two clades correspond to distinct and long diverged populations of A. ameiva and the presence of haplotype AameH20 in FLV is an evidence of recent migration from the Araguaia to Cerrado. FLV is located in an area dominated by Cerrado sensu latu physiognomies, with remnants of forested patches dominated by plant species belonging to the families Favaceae, Arecaceae, Chrysobalanaceae and Vochysiaceae (Martins et al. 2008). The mid-Araguaia basin is more densely vegetated, dominated by gallery and alluvial forests. *Ameiva ameiva* occurs both on forested and open areas, preferentially in ecotones and naturally or antropogenically disturbed areas such as tree-falls, clearings or river bluffs (Vitt and Colli, 1994; Ávila-Pires, 1995; Sartorius et al., 1999; Vitt et al. 2008a). For this reason, we did not found evidence for the existence of barriers to the dispersal of this species that could justify the divergence here found. Because individuals appear to be able to cross from one area to the other but clearly two different lineages are present in this region, one might suspect that this area can actually be a secondary contact area between two different lineages. We hypothesize that the population from the Araguaia might be closer related to Amazonian populations and the FLV to Cerrado populations. However, molecular samples from a broader area are needed to confirm or refute this hypothesis.

Two very distinct lineages (10.1% ± 2.0%) were also found in the case of *Anolis nitens brasiliensis*. We were not able to collect vouchers from the two specimens captured in FLV. Both specimens were caught in pitfalls and had been attacked and partially eaten by ants. The only reliable sources of information were the measurements taken and the tissue sample that was collected and deposited at CHUNB. We could suspect that the two individuals belonged to another closely related species, *Anolis meridionalis*, which is typical from Cerrado biome (Colli et al., 2002; Nogueira et al, 2005; Langstroth, 2006), being most commonly found in grasslands, but never in densely forested areas (Nogueira et al., 2009).

However, several lines of evidence help us to refute this hypothesis. First, the measured snout-vent length of the two individuals (58.6 and 64.0mm) is consistent with *A. n. brasiliensis* (Ávila-Pires, 1995; Mesquita et al., 2006; Vitt et al., 2008) but not consistent with *A. meridionalis* (Gainsbury and Colli, 2003; Colli and Oliveira, 2011). Second, both individuals were captured inside the natural forested fragments and not in the Cerrado matrix and, as referred above, *A. n. brasiliensis* is a forest species and *A. meridionalis* is characteristic of open areas. Third, the two individuals were more divergent among each other (15 mutations), despite being captured about 2 Km apart from each other, than the two most divergent haplotypes from the eastern bank of the Araguaia (AnitH11 and AnitH13, 13 mutations), sampled about 28 km apart. The difference is that the two haplotypes from FLV were sampled in two forested remnants within a matrix of Cerrado and agricultural fields, while the Araguaia haplotypes were sampled in a continuous forested area. Forth, the average pairwise divergence between the FLV and the Araguaia clade (10.1% ± 2.0%) is perfectly consistent with intraspecific divergence values found between the Amazonian subspecies of *A. nitens*, or even within populations of the subspecies *A. nitens tandai* (Glor et al., 2001). Therefore, we have reason to suspect that the two clades might correspond to different subspecies of *Anolis nitens*, or two highly divergent populations within *A. nitens*. The subspecies *A. n. brasiliensis*, which occurs in Cerrado, was previously identified in our study area along Araguaia and Coco rivers (Vitt et al., 2008). The other known subspecies of *A. nitens* occur in the Amazonia. Whether the individuals from FLV are a different subspecies or are related to Amazonian subspecies is dependent on including molecular data from the other subspecies in these phylogenetic analyses. If individuals captured in FLV are more related with Amazonian A. nitens, it will provide evidence for an ancient relationship between the forested remnants, *ipucas*, and Amazonian rainforest.

6.5.2 – Is Araguaia River a barrier for lizard species?

The frequent occurrence of the same haplotypes on both banks of the Araguaia river, in *A. ameiva* and *T. teguixin* is enough to refute the hypothesis that this river might have a strogn effect in limiting the gene flow in this species. Both species are abundant in the Araguaia islands and haplotypes shared between banks are often also found in the islands. On this regard, it is important to refer that previous authors hypothesized that gene-flow could occur when extensive portions of land are passively transferred from one bank to another in a river, each time a meander loop is cut off or a new river course is carved out within the floodplain (Haffer, 1997; Gascon et al, 1998). This is known to occur in the Araguaia, where some large islands are stable for as long as a decade (Latrubesse, 2009).

Another indirect source of evidence comes from the support for the monophyly of the populations of T. teguixin from the Araguaia basin and from Roraima, relatively to the populations of Cuyabeno (Equador). The two Brazilian populations are separated by the lower course of several large rivers flowing from the Brazilian Shield (Araguaia, Tocantins, Xingú and Tapajós), and of the Amazonas itself. On the other hand, several rivers like Negro, Japurá and Napo exist between the populations of Roraima and Cuyabeno. However, in the latter case, the areas are separated by the headwaters of these rivers. These findings appear not to be consistent with the predictions of the Riverine Barrier Hypothesis (Haffer, 1997; Lougheed et al, 1999), since it would be expected that the river barrier effect would be greater in the lower course of the river.

A very different situation occurs with *A. n. brasiliensis*. Despite being no reciprocal monophyly of the haplotypes from both banks, the monophyly of the western bank is strongly supported. The eastern bank is, however, paraphyletic relatively to the western bank, which might suggest dispersal from one bank to the other (Patton et al., 2000), most probably from the eastern (paraphyletic) to the western (monophyletic) bank. The haplotypes from FLV are located eastern to Araguaia (and Javaés), and are much more divergent from the Araguaia haplotypes, than both banks are from each other. However, as previously referred, the FLV clade might represent a different subspecies. Thus, we consider that river Araguaia limitates, at least partially, the genetic flow in *A. n. brasiliensis*. Glor and collaborators (2001) detected a very deep split between the subspecies of *A. nitens* north and south of the Amazonas river.

Tupinambis teguixin and *Ameiva ameiva* are heliothermic lizards (Vitt and Colli, 1994; Sartorius et al., 1999; Vitt et al, 2008b) and at least *T. teguixin* is known to be a good swimmer (Ávila-Pires, 1995; Vitt et al., 2008b). We had the opportunity, for a couple of times, to observe *A. ameiva* swimming in flooded pitfalls and submerging for several times when we tried to capture them. At the time, they also seem to have no problem dealing with water. Vitt and Zani (1998) found this species several times in wet ground. We also frequently observed both species in beaches and river bluffs along Araguaia and its tributaries. On the contrary, *A. n. brasiliensis* was never found in these habitats, probably because of thermal restrictions (Vitt et al., 2008a), since beaches and bluffs were frequently exposed to direct sunlight. *Anolis n. brasiliensis* is a diurnal and non-heliothermic lizard, occurring almost only in shaded places or exposed to filtered sunlight (Mesquita et al., 2006; Vitt et al., 2008a). For a forest-specialist species, a river such as Araguaia, with exposed bluffs and large sand beaches, might be an even greater barrier than just the flowing water, much more than for heliothermic species that are frequently active on transitional or open areas.

6.5.3 – Some considerations on genus Tupinambis

The taxonomy of the genus *Tupinambis* was involved in substantial confusion until the 1990 decade. Ávila-Pires (1995) clarified part of this confusion by assigning the Amazonian form, previously known as *T. nigropunctatus*, to *T. teguixin* (which is now the valid name for that species) and the Central Brazilian form, previously known as *T. teguixin*, to *T. merianae*. A few years later, a new species, *T. quadrilineatus*, was described for the Cerrado biome, almost simultaneously by different authors (Manzani and Abe, 1997; as *T. cerradensis* by Colli et al. 1998). A molecular phylogeny by Fitzgerald et al. (1999) clarified the phylogenetic relationships inside *Tupinambis*, but draw attention to the non-monophyletic relation between *T. duseni* and *T. rufescens*. However, these authors considered them to be valid species based on morphological data. These findings were later corroborated by Peres and Colli (2004). A new species *T. palustris* was described by Manzani and Abe (2002) but was considered a junior synonim of *T. teguixin* by Peres (2003). Fitzgerald et al. (1999) found substantial genetic divergence between population of *T. teguixin* from Roraima (Brazil) and Cuyabeno (Equador). The divergence between these two populations was comparable to the divergence of valid species within genus *Tupinambis*. Peres (2003) found no morphological support for geographical structure within *T. teguixin*.

The analysis of our data together with the data by Fitzgerald et al. (1999) and an additional sample of T. quadrilineatus generated in the study by Pellegrino et al. (2001), allowed us to clarify some issues. First, we were able to confirm the sympatry of three Tupinambis species in the region of the mid-Araguaia. Despite the large size of this species, the sympatry of *Tupinambis* species was reported several times (Ávila-Pires 1995; Colli et al, 1998; Fitzgerald et al, 1999; Peres and Colli, 2003). There is a report on the sympatry of *T. duseni*, *T. merianae* and *T. teguixin* in the state of Goiás (Abe et al., 1992), and Ávila-Pires (1995) suggests the possibility of sympatry of the two latter species and *T. longilineus*. To our knowledge, this is however the first report on the sympatry of *T. teguixin*, *T. quadrilineatus* and *T. merianae*. Voucher specimens were only collected for *T.*

teguixin, since *T. quadrilineatus* (3 individuals) and *T. merianae* (1 individual) were only found dead on road, not suitable for voucher preparation. Even though, coloration patterns were consistent with the patterns of the latter two species, and their identification was corroborated by molecular data analysis. The three species were detected within a short range from each other (less than 10 Km), but in different habitats. *T. teguixin* was allways captured inside or near gallery forests and seasonally flooded forests around Araguaia, Coco and Javaés rivers. *T. quadrilineatus* was found in a road crossing a Cerrado sensu strictu area, near forested patches, while *T. merianae* in a more open area. These findings are consistent with the ecology of these tree species (Ávila-Pires, 1995; Colli et al, 1998; Colli et al, 2002). Niche segregation might justify the frequent sympatry between such large lizards.

Second, by adding samples from *T. quadrilineatus* to phylogenetic analyses we found some evidence that this species would be sister to *T. longilineus*. When describing *T. quadrilineatus* (in the original reference: *T. cerradensis*), Colli and collaborators (1998) analyzed allozyme data and found greater genetic distances between *T. quadrilineatus* and *T. merianae* than between the former and *T. teguixin*. Based on morphological data, Ávila-Pires (1995) concluded that *T. longilineus* was closer to *T. teguixin* than to the other known species. These findings were supported by the morphological data analysed by Colli and collaborators (1998) and later by molecular analysis (Fitzgerald et al., 1999). It is important to notice that tissue samples were not available for *T. longilineus* in the study from Colli et al. (1998) and that *T. quadrilineatus* was also not included in the molecular analysis by Fitzgerald et al. (1999). Thus, our findings do not contradict any of the previous findings, but suggest that *T. quadrilineatus* and *T. longilineus* might in fact be sister taxa. These two species would then by closer to *T. teguixin* than to the species from the southern clade detected by Fitzgerald et al. (1999).

Third, our re-analysis of the data from Fitzgerald and collaborators, provides very little support for the monophyly of *T. teguixin*. In the original analysis, the authors found that the divergence between *T. teguixin* from Roraima and Cuyabeno was comparable to some interspecific relationships within the genus *Tupinambis*. Here, the monophyly of the Roraima and the Araguaia populations is corroborated by MP and NJ analysis, but receives little support from BI analysis. However, even less support, in all phylogenetic analysis, is provided to the monophyly of the three intraspecific populations of *T. teguixin*. Average pairwise divergence between the clades corroborates that *T. teguixin* populations from Roraima and from the Araguaia are significantly less divergent than other taxa. The divergence between any of these populations and the population from Cuyabeno is however not significantly lower the divergence between the latter and the closest relatives to *T. teguixin*, which appear to be *T. longilineus* and *T. quadrilineatus*. Thus, and despite the lack of support from morphological data (Peres, 2003), we reinforce the suggestion by Fitzgerald and collaborators, that significant variation within *T. teguixin* might be found in an expanded analysis.

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Supporting Information

Table 6.S1 – Information about the external molecular data (*ND4* gene) used in this study, with reference to original source and to Genebank accession number. Haplotype denomination correspond to the one in figure 4.

Haplotype	**Species**	**Location**	**Original ref.**[a]	**Genebank**
TtegH9ROBR	*T. teguixin*	Faz. Nova Esperança, Roraima, Brazil	LSUMNS H-12450	AF151213.1
			LSUMNS H-12431	AF151212.1
TtegH10ROBR	*T. teguixin*	Faz. Nova esperança, Roraima, Brazil	LSUMNS H-12405	AF151211.1
TtegH11CUEQ	*T. teguixin*	Cuyabeno, Sucumbios, Equador	LSUMNS H-12678	AF151200.1
TtegH12CUEQ	*T. teguixin*	Cuyabeno, Sucumbios, Equador	LSUMNS H-12715	AF151202.1
TtegH13CUEQ	*T. teguixin*	Cuyabeno, Sucumbios, Equador	LSUMNS H-12703	AF151201.1
TlongH1AMBR	*T. longilineus*	Rio Ituxi, Amazonas, Brazil	LSUMNS H-14135	AF151203.1
			LSUMNS H-14136	AF151204.1
TquaH2GOBR	*T. quadrilineatus*	Niquelândia, Goiás, Brazil	LG1132	AF420912.1
TmerH2PHPA	*T. merianae*	Presidente Hayes, Paraguay	USNMField 166649	AF151194.1
		Concepcíon, Paraguay	USNMField 166756	AF151197.1
TmerH3CAPA	*T. merianae*	Caazapa, Paraguay	USNMField 166779	AF151209.1
TmerH4CAPA	*T. merianae*	Caazapa Paraguay	USNMField 166780	AF151210.1
TdusH1AMPA	*T. duseni*	Amambay, Paraguay	USNMField 166778	AF151198.1
TdusH2COPA	*T. duseni*	Concepcíon, Paraguay	USNMField 166766	AF151199.1
		Paraguay	USNMField 166777	AF151208.1
TrufH1PHPA	*T. rufescens*	Presidente Hayes, Paraguay	USNMField 166740	AF151195.1
TrufH2PHPA	*T. rufescens*	Presidente Hayes, Paraguay	USNMField 166743	AF151196.1

a) All sequences from Fitzgerald et al. (1999), except *T. quadrilineatus* (LG1132), obtained from Pellegrino et al. (2005).

Table 6.S2 – Average and maximum snout-vent length (SVL) and weight for each species. Data are based in measurements made during this study and are presented separately for males, females and for all individuals, including non-sexed individuals, but excluding juveniles.

	N	SVL (mm)	Max. SVL	Weight (g)	Max. Weight
A. nitens brasiliensis					
Male	11	65.3 ± 3.9	70	5.34 ± 0.74	6.3
Female	6	63.9 ± 6.6	72	5.00 ± 1.17	6.9
All	36	62.1 ± 6.1	72	4.80 ± 0.99	6.9
Ameiva ameiva					
Male	21	136.1 ± 19.4	175	63.65 ± 26.62	139.0
Female	15	118.3 ± 16.1	145	38.46 ± 14.78	69.6
All	49	128.4 ± 18.5	175	52.37 ± 23.52	139.0
Tupinambis teguixin					
Male	28	296.0 ± 39.4	356	780.0 ± 275.4	1240
Female	27	250.9 ± 40.4	323	465.5 ± 195.9	710
All	67	268.6 ± 47.8	356	580.5 ± 296.9	1240

CHAPTER 7

Final Discussion

Chapter 7 cover photos (form left to right): adult *Phyllomedusa azurea*; juvenile *Gymnodactylus carvalhoi*; and fishermen colony at Barreira de Campos, state of Pará.

7.1 – On the study and monitoring of herpetofauna

One of the most important steps in research is to clearly identify the questions to be answered and to devise an approach based on these questions (Heyer et al., 1994; Ratti and Garton, 1996). However, if different questions are to be addressed at the same time, demanding different methodological approaches, a level of compromise must be achieved. To update the species check-list for a given taxonomic group and region, at the same time that one tries to obtain data that allow the comparison of different areas, and to obtain a sufficiently large and widespread sample of some target species can become conflicting objectives.

As reported in this thesis (Chapter II), different techniques capture different sets of species, and individual species are more or less prone to be captured by each capture technique. If the objective is to try to sample as much species as possible in a particular region, it is a common opinion that it is advisable to apply a set of different capture techniques (e.g.: Mengak and Guyn 1987; Greenberg et al. 1994; Voss and Emmons 1996; Crosswhite et al. 1999; Hutchens and DePerno, 2009). An alternative approach to capture, which can be applied in the study of some particular taxa, is the use of artificial habitats. Artificial pools (Gascon, 1994), artificial covers (Fellers and Drost, 1994), or artificial pipe refuges (as reported in Chapter III) can be used, with different levels of success, in the study of amphibian and reptile taxa. The use of artificial habitats allows some level of standardization, which can be important for long-term studies and population monitoring. The analysis of different characteristics of artificial refuges can provide us an insight into the ecological preferences of colonizing species, as already suggested by Johnson and Semlitsch (2003) and Pittman et al. (2008). It is important to stress that some species would certainly be more prone to use these artificial habitats, than others, and that inter-specific comparisons might become compromised.

However, this is a problem I found to be transversal to several capture methods. Excluding methods that result in total counts of individuals, the reliability of estimation methods depend on several variables, such as: *a*) the accuracy of the observer (Hayek, 1994); *b)* the size, color, vagility or any other factor that influences the detectability of a species (Lancia et al, 1996); *c*) the different efficiency of the method for different taxa (Corn, 1994); *d*) or the social behavior or any kind of territoriality or gregarious behavior (Scott and Woodward, 1994). I believe that these limitations should not be considered an impediment to the collection and analysis of field data, rather should work as cautionary notes to be accounted when choosing methods and analyzing and comparing data from different taxa or locations.

Field data are still the primary and most important kind of data for informed decision-making regarding conservation strategies. Estimation of species richness, complementarity, irreplaceability (Diniz-Filho et al., 2004, 2006, 2007; Costa et al 2007) or any other dimension of biodiversity based on meta-data is a very useful approach when field-data are sparse, unevenly distributed or even inexistent. These macro-scale are subjected to bias (Bini et al., 2006) but might provide important insights to biodiversity patterns and point regional and local biodiversity hotspots. An example is the corroboration of the results of Costa et al. (2007), which point lower species richness at the periphery of Cerrado biome, by the data presented in Chapter IV. For the middle Araguaia basin, both the anuran and squamate species diversity proved to be greater than previously estimated but, even though, not higher than in other Cerrado or Amazonian localities. Our results also corroborated previous field data (Nogueira et al, 2005; Nogueira et al., 2009), which point for higher diversity in the interfluvial areas than in gallery forests, in the Cerrado biome. These findings support the idea that long-term biodiversity assessments and monitoring are still one of the keystones of efficient wildlife conservation and management planning.

7.2 – The dynamic relation between river and herps

Large rivers, just as mountains and canyons, are frequently considered as potential barriers to the dispersal of species and the gene flow between populations. Here, we tested the hypothesis that the Araguaia could be a barrier to a different set of species (Chapters V and VI). In most cases, in turned out that species were able to cross the river and exchange genes. This was the case for the three studied amphibian species (Chapter V). However, in the case of the least vagile species, *Elachistocleis ovalis*, two divergent lineages were found. The single fact that some haplotypes were shared between riverbanks should not constitute evidence that Araguaia River was not the cause of this divergence. The fact that individuals could migrate from on bank to the other does not invalidate that it was sufficiently low to allow diversification. However, in such a situation, it would be expected that the populations on the two sides of the river were sister to each other (Patton et al, 2000; Haffer, 2008). However, our phylogenetic analyses failed to support the monophyly of the two Araguaia clades relatively to a very distant population from Panama. This result better suggests that the Araguaia is probably an area of secondary contact between two non-sister populations originated elsewhere (Patton et al., 2000), and that these are able to cross Araguaia at some extent.

The results obtained with lizards were not much different (Chapter VI). The Araguaia seems to constitute no barrier for the two teiid lizards, which are larger, more active and less habitat-restricted than *Anolis nitens brasiliensis*. The former, *Ameiva ameiva* and *Tupinambis teguixin* are not restricted to forested areas (Vitt and Colli, 1994; Ávila-Pires, 1995; Sartorius et al., 1999) and spend much time actively searching for food (Colli et al., 1997; Vitt and Zani, 1998). *Anolis n. brasiliensis*, on the other hand, spends most of its time within forested areas, in fixed positions, ambushing preys (Vitt and Zani, 1998; Vitt et al., 2008). This species appears not to be able to cross the Araguaia River, at least frequently enough to leave a genetic signature. In the case of this polychrotid lizard, we found no evidence of haplotype share across the river and the western bank was monophyletic relatively to the eastern bank. This result is consistent with a scenario of dispersal of a original population from one side to the other of Araguaia. The hypothesis that Araguaia might be a barrier to this species is supported in two ways. First, at the subspecies level, the phylogeny of *Anolis nitens* appears to be influenced by the Amazon River (Glor et al., 2001). Second, despite the much larger sampling effort in the eastern side of the Araguaia, only one *Anolis* species was found on the eastern bank. However, a second species, *Anolis ortonii* was once captured in the western bank, very near to the river (Chapter IV). *Anolis ortonii* is a species widely distributed in Amazonia and in the northern portion of the Atlantic Forest (Peres and Donoso-Barros, 1986; Ávila-Pires, 1995). Because, despite all sampling effort, we were never able to find this species on the eastern bank, we believe that the Araguaia River might constitute a distribution limit for this species.

In resume, the Araguaia River appears to play different roles with different species (see also Rocha et al., submitted), most often not limiting their dispersal. The dynamics of this river, with the accretion and isolation of large islands (Latrubesse, 2009), might provide the appropriate vehicle for transferring the individuals, and their genes, from one bank to the other, as already suggested by other authors (Haffer, 1997; Gascon et al, 1998). For some Amazonian species, like the large *Tupinambis teguixin*, the alluvial and gallery forest of the Araguaia and its tributaries are better explained as corridors, extending their distribution into the Cerrado biome (Ávila-Pires,1995).

7.3 – Conservation areas and species assemblages

A project for the establishment of an ecological corridor, with an area of 10 million hectares, in the region of the middle Araguaia has been in the paper for a long time (Arruda, 2003; MMA, 2005). According to these authors, this corridor would comprise: four indigenous areas, three sustainable use areas, and one federal and two state strict conservation areas. It would comprise 36 municipalities from the states of Goiás, Mato Grosso, Tocantins and Pará. However, the implementation depends on the participative management of all the intervenient parts, which has not yet been achieved. In some cases, the borders of the conservation areas included in the project still are, or recently were, matter of debate (MPF-TO, 2005; MPF-TO, 2010). The area of implementation of the ecological corridor Bananal-Araguaia lies within the deforestation arc between Amazonia and Cerrado, a region under strong deforestation pressure (Azevedo-Ramos and Galatti, 2002; Vieira et al., 2008).

The human pressure in the five municipalities that comprise our study area goes with the general trend (Table 7.1). It becomes clear from the data in the table that human pressure is much higher in the western border of Araguaia River, in Santana do Araguaia, state of Pará, where no state or federal conservation units exist. On the eastern side, the municipality of Pium encompasses both the Parque Estadual do Cantão (PEC) and Parque Nacional do Araguaia (PNA). The remaining area of Pium municipality and from the municipalities of Caseara and Marianópolis are located within the Área de Protecção Ambiental do Bananal/Cantão (APABC). The latter is a sustainable use area and human pressure is much higher in APABC than in PEC, where only a handful of *ribeirinhos* is allowed to reside.

Table 7.1 – Anthropogenic pressure in the study area. Figures are relative to annual values for 2008, according to the IBGE census (IBGE, 2010).

Municipality	Conservation Area	Area (Km^2)	People/Km^2	Per./temp. crops (ha)	Bovine herd (heads)	Timber (m^3)
Caseara TO	APABC	1,692	2.9	34/1,835	47,000	2,950
L. Confusão TO	FLV	10,565	0.82	45/53,299	109,580	25,450
Marianópolis TO	APABC	2,091	2.26	20/1,010	99,000	4,700
Pium TO	APABC/PEC	10,013	0.67	600/6,471	139,330	10,130
S. Araguaia PA	FSF	11,591	4.75	392/10,095	485,859	244,420

Key: L. Confusão – Lagoa da Confusão; S. Araguaia – Santana do Araguaia; TO – State of Tocantins; PA – State of Pará; Perm./temp. crops – permanent and temporary crops.

However, according to our results, APABC harbors a significant portion of the regional biodiversity that is not present within the strict conservation area of PEC. Despite being less intensively sampled, Fazenda Santa Fé (FSF), in the state of Pará, also appears to have the potential to harbor some species that do not occur in the eastern border of the Araguaia River. In a recent study by Negrões et al. (2011), FSF presented a higher species richness for medium and large mammals, than PEC. In our study, only one species captured in FSF was not present in PEC or APABC. The highly seasonal nature of the Araguaia alluvial plains, and its low heterogeneity, are surely an impediment for the establishment of several vertebrate species, which are able to colonize the surrounding areas, which are drier and more heterogeneous. In resume, it becomes clear that successful conservation will result not only from the creation of strict conservation areas, but also from integration with surrounding areas. Indigenous lands, sustainable use areas and private forests might play an important role in the preservation of regional fauna and ecosystems, an opinion shared by several other researchers (Peres, 2004; Campos and Nepstad, 2005; Rylands and Brandon, 2005; Azevedo-Ramos et al., 2006; Gardner et al, 2006; Negrões et al., 2011).

7.4 – Is there room for everyone?

Besides deforestation rates, the region of the arc of deforestation in the Southeastern Amazonia is in the top list for several other reasons: number and size of bovine herds; violence against rural workers; slave work; land concentration and, recently, land redistribution (Girardi et al., 2008). Data from South America and other tropical areas indicate that environmental degradation is intimately connected with social instability and poverty (Fragoso et al., 2004; Rudel, 2005; Sanchez et al., 2005). However, and in the particular case of Brazil, environmental degradation is also the result of decades of inefficient large-scale agricultural and logging activities, many times with the political and economical support of national governments (Klink and Moreira, 2002; Fearnside, 2005; Rudel et al., 2005; Silva et al., 2005; Clement, 2006; Ratter et al., 2006). A recent study by Rangel and co-authors (2007) found that patterns of modern agriculture and cattle ranching were better predictors of conflict with conservation planning, than human population density.

Brazilian Ministry of Environment recently released a list of the top 100 illegal deforesters in Amazonia (MMA, 2008), which included nine settlements of rural landless people, with the remaining positions belonging to large private corporations or individual owners related with logging, livestock and agricultural activity. Almost all the 100 top deforesters come from the states of Mato Grosso, Pará and Rondônia, with the few remaining scattered by other Amazonian states. Judicial measures were taken against the transgressors. Unfortunately, deforested areas can no longer be turned into pristine areas.

The solution might pass by the enforcement of the law in the deforestation border. However, there are other possible and complementary measures. There have been some examples of successful integration of smallholders, indigenous and local communities in the preservation of very large tracts of forest (McGrath et al., 2004; Sears and Pinedo-Vasquez, 2004; Silvius, 2004; Campos and Nepstad, 2006). All these cases imply a level of use of forest resources, such as small-scale selective logging or subsistence hunting, but prevent deforestation and leave untouched large nuclear areas. Thus, measures such as the mediation of conflicts between the different stakeholders, the enforcement of Brazilian law and the integration of local communities in conservation policies, providing alternative sources of income, might turn to be as important as surveying and understanding biodiversity. The question is if we should try to preserve natural habitats despite the existence of local human populations or should we preserve it for and with the help of these communities?

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