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CAIO VINICIUS DE MIRA MENDES

PADRÕES DE DIVERSIDADE E COMPOSIÇÃO DOS ANFÍBIOS DE UMA FLORESTA
ATLÂNTICA DE TERRAS BAIXAS NA REGIÃO SUL DA BAHIA, NORDESTE DO
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BRASIL**

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Orientador: Dr. Julio Ernesto Baumgarten
Co-orientador: Dr. Mirco Solé

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INTRODUÇÃO GERAL

Ao longo da última década cerca de 13 milhões de hectares de florestas foram convertidas para outros usos ou perdidas naturalmente, a maior parte desta, concentrada na região tropical (FAO, 2010). Diversas causas têm sido associadas com a perda de florestas primárias ou maduras, sendo que a extração de madeira, agricultura e pecuária intensiva, continuam sendo os maiores responsáveis (FAO, 2010). No entanto, depois de exploradas, algumas áreas são posteriormente abandonadas, permitindo um processo de regeneração de florestas secundárias (Nepstad et al. 1991). As florestas secundárias compreendem cinco dos 11 milhões de km² dos remanescentes de floresta tropical ao redor do mundo (ITTO, 2002). Como resultado, florestas secundárias contribuem para o aumento da cobertura florestal, e diversos estudos vêm tentando compreender a importância destes ambientes para a conservação das espécies tanto de flora (e.g. Aide et al. 2000; Guaricata & Ostertag, 2001; Janzen, 2002; Liebsch et al. 2008) quanto da fauna (e.g. Dunn, 2004; Bowen et al. 2007; Gardner et al. 2007; Chazdon et al. 2009; Dent & Wright, 2009). Do mesmo modo, a relação entre a diversidade de espécies em diferentes tipos de plantações florestais (e.g. seringal) com florestas maduras também tem sido avaliada (e.g. Hartley, 2002; Lindenmayer & Hobbs, 2004; Gardner et al. 2007), uma vez que um aumento de 4,3 milhões de ha por ano destas áreas tem sido registrada em todo o mundo (FAO, 2010).

De maneira geral, os anfíbios são sensíveis a mudanças no ambiente e podem ser considerados bons modelos para compreensão da alteração de ambientes naturais (Young et al. 2004; Hopkins, 2007). Possuem pele permeável, o que os tornam mais vulneráveis a alterações microclimáticas (Lips, 1998; Blaustein et al. 2010); baixa mobilidade e área de vida pequena (Beebee, 1996), sendo mais suscetíveis a distúrbios ambientais (Todd et al. 2009). A alteração de ambientes naturais através do

desmatamento e degradação tem sido atualmente relacionada como a principal causa do declínio populacionais e extinções de espécies ao redor do mundo (Mace et al. 2005). Dentre os vertebrados, os anfíbios são considerados o grupo mais ameaçado no mundo, sendo que nas últimas décadas, um declínio acentuado de suas populações tem sido observado em todo o planeta (Hoffmann et al. 2010). Cerca de um terço de todas as espécies existentes estão ameaçadas (Stuart et al. 2008). A principal causa do seu declínio tem sido associada à perda e fragmentação do ambiente (Young et al. 2004; Becker et al. 2007; Stuart et al. 2004; Todd et al. 2009).

Apesar de a região neotropical abrigar um dos maiores níveis de biodiversidade no planeta (Myers et al. 2000), apenas uma pequena parcela tem sido descrita (Wilson, 2003). Estes fatores refletem os padrões de diversidade encontrados para os anfíbios, onde comunidades neotropicais abrigam os índices mais elevados de diversidade, com o Brasil representando o país com a maior riqueza conhecida atualmente (AmphibiaWeb, 2016). De acordo com a última lista divulgada pela Sociedade Brasileira de Herpetologia (SBH), 1026 espécies de anfíbios foram descritas até 2014 (Segalla et al. 2014). No entanto, a diversidade brasileira ainda é subestimada, sendo que de 2014 até hoje foram descritas cerca de 30 espécies pertencentes ao grupo (AmphibiaWeb, 2016). De acordo com Silvano & Segalla (2005), ainda há uma grande carência de estudos básicos sobre história natural, história de vida e distribuição geográfica dos anfíbios brasileiros. Este fato é comprovado por trabalhos de inventários de espécies, os quais tem revelado uma alta diversidade de anfíbios tanto em áreas pouco amostradas (e.g. Dias et al. 2014a; Dias et al. 2014b), como em áreas que vem sendo realizados esforços de coletas há mais de dez anos (e.g. Almeida et al. 2011; Trevine et al. 2014). Ainda nesse sentido, a freqüente descrição de novas espécies, mesmo em áreas muito amostradas reforça a carência de dados básicos (e.g. Motta et al. 2012; Lourenço-de-

Moraes et al. 2014; Ferreira et al. 2015). Estas informações servem de suporte para uma das questões mais importantes em ecologia, a compreensão de padrões de diversidade das espécies em diferentes ambientes (Begon et al. 2007). Além disso, auxiliam na diminuição de erros taxonômicos freqüentemente encontrados em estudos de ecologia (Bortulos, 2008), e servem como ferramentas importantes na implementação de ações conservacionistas (Haddad, 1998; Verdade et al. 2012).

A região sul da Bahia está inserida no corredor central da Mata Atlântica, e de acordo com Carnaval et al. (2009), foi climaticamente estável e serviu como um refúgio climático para os anfíbios neotropicais no Pleistoceno. Recentemente, a importância biológica da região tem sido reportada na literatura pelos altos níveis de riqueza e endemismos de anfíbios (Dias et al. 2014a; Dias et al. 2014b; Capítulo 1). Nas últimas décadas foi notado um avanço considerável de pesquisas na região, com descrições de novas espécies e ampliações de distribuição para o estado (e.g. Dias et al. 2010; Camurugi et al. 2010; Dias et al. 2011; Lourenço-de-Moraes et al. 2012; Teixeira-Jr et al. 2013; Caramaschi et al. 2013). No entanto, ainda há uma carência de estudos da anurofauna na região que visem tanto conhecer a diversidade de espécies, como compreender a relação das comunidades com os diferentes tipos de ambientes.

Neste sentido, o presente trabalho foi organizado em três capítulos, e busca expandir o conhecimento sobre a anurofauna de uma Floresta Atlântica de Terras Baixas localizada na região Sul da Bahia. No **Capítulo 1**, apresentamos um inventário conduzido diante de longo esforço amostral na Reserva Ecológica Michelin – REM, que resultou numa alta diversidade de anfíbios registrada em uma área de Floresta Atlântica de Terras Baixa. No **Capítulo 2**, buscamos compreender como as assembleias de anfíbios de serrapilheira estão organizadas nos diferentes ambientes da REM, como cultivo de seringueiras, florestas secundárias em diferentes estágios de sucessão e florestas

maduras. Neste capítulo, tentamos mostrar o tempo de recuperação das espécies de anfíbios em um gradiente de sucessão ecológica e identificar quais as principais variáveis ambientais responsáveis pela estruturação das assembleias. Por fim, no **Capítulo 3**, descrevemos uma nova espécie de anfíbio anuro miniaturizado do gênero *Adelophryne*, e ressaltamos a importância de estudos de história natural para a compreensão da diversidade de anuros na região Sul da Bahia.

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Capítulo I



**Amphibians of the Reserva Ecológica Michelin: A high diversity site in the lowland
Atlantic Forest of Southern Bahia, Brazil**

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Amphibians of the Reserva Ecológica Michelin Ecological: A high diversity site in the lowland Atlantic Forest of Southern Bahia, Brazil

Abstract. We present a complementary inventory checklist of the amphibians of the Reserva Ecológica Michelin - REM, southern Bahia, Brazil. Long sampling effort was carried out (about ten years), and as a result of our surveys, a total of 69 species were recorded from the MER, an increase of about 30 percent of previously recorded species. Amphibians were distributed in two orders (Gymnophyona and Anura), belonging to eleven families (Aromobatidae (1), Bufonidae (3), Centrolenidae (1), Craugastoridae (5), Eleutherodactylidae (3), Hemiphractidae (2), Hylidae (39), Leptodactylidae (7), Microhylidae (4), Odontophrynidae (3) and Caeciliidae (1)). Fifty per cent of the reproductive modes known for Atlantic forest anurans were recorded. No threatened species were found at MER, however, *Allobates olfersioides*, *Phasmahyla timbo*, *Phyllodytes* cf. *kautski*, *Scinax strigilatus*, *Leptodactylus cupreus*, *Chiasmocleis cordeiroi* and *C. crucis*, are classified as data deficient (DD) by IUCN and deserve special attention. Despite a higher diversity of amphibians in the Atlantic forest having been reported from mountainous regions, our results highlight an expressive richness of amphibians for lowlands forests as well.

Key words. Inventory, lowland Atlantic forest, species richness, amphibians.

Introduction

Over the past decades a marked decline in amphibian populations has been reported worldwide (YOUNG et al. 2001), and currently, amphibians are considered the most threatened vertebrate group on the planet (HOFFMANN et al. 2010). Approximately one-third of all extant species are threatened (STUART et al. 2008) which is a high rate compared to mammals (23%) and birds (12%) (BAILLIE et al. 2004). The major threats to the group are habitat alteration, fragmentation and destruction (YOUNG et al. 2004, BECKER et al. 2007, LOYOLA et al. 2007), exotic species (VREDENBURG 2004), infectious diseases (DASZK et al. 2003) and climate change (POUNDS et al. 2006, CAREY & ALEXANDER 2003).

The highest amphibian diversity is found in the neotropics, and Brazil shelters the largest number of described species worldwide (SEGALLA et al. 2014). According to the national assessment of the conservation status of Brazilian species (ICMBIO, 2014) only 4% of amphibians are threatened. However, approximately 17% of Brazilian species are classified as Data Deficient (DD) (ICMBio 2014), thus the real number of threatened species may be underestimated due to inadequate sampling as there are still many lacunas in geographical range assessments and few demographic studies (BROOKS et al. 2004, IUCN 2008).

The Amazon and Atlantic Forest harbor the highest values of species richness in Brazilian biomes (HADDAD et al. 2013, JENKINS et al. 2015). Particular attention should be paid to the Atlantic Forest, which is considered one of the five most important biodiversity hotspots of the world (MYERS et al. 2000) and has one of the highest levels of amphibian richness and endemism recorded in the country (MORELLATO & HADDAD 2000, SILVA & CASTELETI 2003, HADDAD et al. 2013). According to HADDAD et al. (2013) more than half of the country's species occur in Atlantic Forest, of which approximately 75% are considered endemics. However, this biome has been devastated by logging, urbanization and agricultural development, and currently there are only 11.4 - 16% of forest cover remaining (MORELLATO & HADDAD, 2000, RIBEIRO et al. 2009). Thus, given the high degree of threat and high values of richness and endemism, knowledge about the conservation status of the species is an essential step for planning future conservation actions.

The southern Bahia region is a critical part of the central Atlantic Forest corridor, and according to CARNAVAL et al. (2009), this area was the largest refugium in

the biome for amphibians through the last glacial maximum. Until recently the only report in the literature of amphibian diversity in southern Bahia was a rapid survey conducted by SILVANO & PIMENTA (2003). Although the sampling effort was limited (approximately four days/area), a representative richness was recorded in several areas. However, in the past decade there has been an increase in studies (i.e. DIAS et al. 2014a, DIAS et al. 2014b) which revealed high levels of amphibian richness and endemism, established the biological importance of this region.

Despite the increasing number of publications on amphibians in Bahia over the past decades, there is still lack of data on amphibian distribution patterns. The increasing number of publications reporting the geographic distribution of several species (e.g. CAMURUGI et al. 2010, DIAS et al. 2011, MATTEDI & PONTES 2014, DIAS et al. 2014a) and the description of new species corroborate this data (e.g. NAPOLI et al. 2011, LOURENÇO-DE-MORAES et al. 2012, TEIXEIRA-JR et al. 2013, CARAMASCHI et al. 2013; PONTES et al. 2014; JUNCÁ et al. 2015). Thus, inventories provide the baseline data that are essential for increasing our knowledge of amphibian community composition and allow a better understanding of species diversity patterns (HADDAD 1998). These studies also allow a better assessment of species conservation status that are necessary for developing future conservation plans (VERDADE et al. 2012).

The present study aims to update and expand the inventory of amphibians species from the Reserva Ecológica Michelin - REM, a lowland Atlantic Forest site in southern Bahia, northeastern Brazil. Our results highlight the importance of long sampling periods, showing an increase of 30% of species recorded compared with a previous study.

Materials and methods

Study area

The study was conducted in Michelin Ecological Reserve – MER (Figure 1), located in southern Bahia, northeastern Brazil. The private reserve is situated within the municipalities of Igrapiúna and Ituberá (13°50'S, 39°10'W). According to VELOSO et al. (1991), the region is characterized as Dense Lowland Rain Forest. The reserve comprises 3,096 ha, of which 1,800 ha are represented by lowland evergreen hill forest

distributed in three main fragments (Vila 5/Pancada Grande – 625 ha; Pacangê – 550 ha; and Luis Inácio – 140 ha). The Pacangê fragment is contiguous with a 13,000 ha forest located outside of the reserve. The reserve forests have a long history of human disturbance, mostly manioc farming and decades of intensive logging. Forest is predominantly secondary at different stages of regeneration and forests logged at varying intensities, with small patches of more intact forests on the steepest slopes and ridge tops. The remainder of reserve consists of wetlands, small forest fragments and areas with rubber plantations (*Hevea brasiliensis*) enriched with native forest trees (FLESHER 2015). The landscape to the east supports rubber, cocoa and banana groves, while to the south, southwest and north properties of small producers of mixed tree crops and small forest fragments. West of the REM lies the largest forest fragment in the region. The regional landscape (1,000 km²) retains 40% forest cover and a high diversity of agroforestry systems with more than 60 tree crops (FLESHER 2006). The average annual rainfall over the last 10 years was approximately 2,000 mm, the temperature varied between 21.7 and 30.8 ° C (data from Reserva Ecológica Michelin).

Data sampling

Eight years of sampling was carried out by researcher teams from Universidade Estadual de Santa Cruz (UESC) and Universidade Estadual de Feira de Santana (UEFS). Between March 2007 and December 2008 an inventory was conducted which revealed 48 species of anuran, distributed in 10 families (*see* CARAMUGI et al. 2010). Between 2010 and 2016 the two first authors regularly sampled amphibians and between March and December 2015 standard pitfall traps (CECHIN & MARTINS 2000, RIBEIRO-JÚNIOR et al. 2011) sampling all of the reserve habitats were used.

We used a total of 24 sets of pitfall traps with 40 cm tall drift fences that included five 30-liter buckets spaced at 8-m intervals, totaling 32 m length. Pitfall traps were kept active during six nights for fieldworks, totaling a sampling effort of 1,440 days during March and December 2015. Two to three scientists conducted active searches sampling amphibians by visual and acoustic search (RÖDEL & ERNST 2004). Individuals found during opportunistic encounters (i.e. along roads) were also recorded.

All animals were collected with a license conceded by the federal government upon and permission of the REM. Vouchers were deposited at the Museu da Universidade

Estadual de Santa Cruz (MZUESC), Ilhéus and Museu de Zoologia da Universidade Estadual de Feira de Santana (MZUEFS), both located in Bahia.

Results

We recorded sixty-nine species of amphibians in the REM: one species of Gymnophiona (*Siphonops annulatus* - Siphonopidae) and 68 species of anurans, belongs to ten families (Table 1; Figures 2-6). The following 21 species are new records to REM that were not recorded in the study of CAMURUGI et al. (2010): *Frostius erythropthalmus*, *Adelphophryne* cf. *pachydactyla*, *A. mucronatus*, *A. sp.*, *Gastrotheca* sp., *G. recava*, *Aparasphenodon brunoi*, *Dendropsophus decipiens*, *Hypsiboas exastis*, *Itapotihyla langsdorffii*, *Phyllodytes* cf. *kautskyi*, *P. sp1*, *P. sp2*, *P. sp3*, *P. wuchereri*, *Scinax argyreornatus*, *Leptodactylus cupreus*, *L. fuscus*, *L. vastus*, *Dermatonotus muelleri* and *Siphonops annulatus*.

According to the Brazilian list of threatened species (ICMBIO, 2014) most of the species are not threatened, (N = 55; 78.6%), about ten percent (N = 7) are listed as data deficient (DD) and the conservation status of seven species has not yet been defined. Special attention should be given to *Allobates olfersioides*. In a taxonomic review of *Allobates* from the Atlantic Forest, VERDADE & Rodrigues (2007) synonymized the four previously recognized species *Allobates olfersioides* (LUTZ, 1925), *A. capixaba* (BOKERMANN, 1967), *A. carioca* (BOKERMANN, 1967) and *A. alagoanus* (BOKERMANN, 1967) with *A. olfersioides*. However, in a recently assessment of threatened Brazilian amphibians, specialist recognized that only populations from Rio de Janeiro belong to *A. olfersioides*, (U. CARAMASCHI pers. comm.) with populations from Bahia placed with *A. capixaba* and classified as Data deficient (DD). However, due to the lack of a recent taxonomic analysis revalidating *A. capixaba*, we continue following VERDADE & RODRIGUES (2007) and assign the species from REM to *A. olfersioides*.

Considering the species identified at the species level or as “cf.”, the majority (n = 43; 61.4%) are endemic to the Atlantic Forest biome, and 18.6% (n = 13) are endemic to Bahia. *Phasmahyla timbo* is known only from the type locality and REM.

Almost half of the species were recorded in lotic habitats (n = 32; 45.7%), being that 17 species were restricted to temporary ponds, four to permanent ponds, and 11 to both habitats (Table 1). Eighteen species were found on leaf litter, of which four and

one, also occupied streams and temporary ponds, respectively. Eight species were found only in forest streams (*Vitreorana eurygnatha*, *Aplastodiscus cavicola*, *A. ibirapitanga*, *A. sibilatus*, *Bokermannohyla capra*, *Phasmahyla timbo*, *Scinax argyreornatus* and *S. strigilatus*). Epiphytes and bromeliads were used by species of the genus *Phyllodytes*. The canopy of forest was occupied by *Gastrotheca* sp. and *G. recava*, frequently found about five meters high. *Hypsiboas exastis* was mostly frequently earing calling in bromeliads at canopy, but was also found at streams. *Leptodactylus* cf. *latrans* was the species that demonstrated the highest habitat plasticity, occurring in streams, permanent and temporary ponds. Only *Siphonops annulatus* occurred in fossorial habitats. Regarding habitats, twenty-nine species were exclusively found inside the forest, ten only open areas and rubber plantations and 31 in both habitats (Table 1).

Taxonomic-remarks concerning CAMURUGI et al. (2010)

The previous checklist of anurans from the REM, (CAMURUGI et al. 2010) included some species with taxonomic uncertainties that are revised here. Most of the following species have cryptic patterns that hamper their taxonomic identification. Our analysis was based on larval (when possible) and adult morphological characteristics, and biocoustic parameters.

Ischnocnema aff. *ramagii* corresponds to *Pristimantis* sp. This species is widely distributed in the forests of southern Bahia and is currently being described (MARCIANO JR. et al. *in prep*). *Vitreorana* sp. was reported by Camurugi et al. (2010) only in larval form; in the present study adult males were collected and identified as *Vitreorana eurygnata* using morphological and bioacoustic parameters. *Scinax* aff. *alter* and *Chiasmocleis* sp. correspond to *Scinax juncae* and *Chiasmocleis crucis*, respectively (NUNES & POMBAL 2010, CARAMASCHI & PIMENTA 2003, FORLANI et al. 2013). *Phyllodytes luteolus* corresponds to *Phyllodytes* sp., and is in the process of being described (ORRICO AND MARCIANO JR. *in prep.*). Calls were recorded and voucher specimens collected in REM and identified by the authors cited above.

The species referred to as *Dendropsophus seniculus*, *Physalaemus signifer* and *Leptodactylus marmoratus* by CAMURUGI et al. (2010), correspond to *Dendropsophus novaisi*, *Physalaemus camacan* and *Adenomera thomei*, respectively. The tadpole of *Dendropsophus novaisi* differs from that of *D. seniculus* by the greater height of its fin

relative to the body, and a dorsal fin greater than the ventral fins. In *D. seniculus* dorsal and ventral fins are the same size. The advertisement call of *Dendropsophus novaisi* differs from that of *D. seniculus* by having a longer note duration, a larger number of pulses and a lower dominant frequency (RUAS et al. *in prep.*).

Tadpoles of *Physalaemus camacan* present “V” shaped lower jaw sheath and medial vent tube; two rows of marginal papillae on lower lip; whereas *P. signifer* has “U”-shaped lower jaw and dextral vent tube; a single row of marginal papillae. Furthermore, the advertisement call of *P. camacan* shows a pulsed structure and a lower dominant frequency (kHz) 0.66-1.69 compared with *P. signifer*, which has a harmonic call structure and a frequency of 0.7-2.5 kHz (PIMENTA et al. 2005).

As the tadpoles and the advertisement call of the populations found in the REM showed such characteristics, we conclude that they belong to the species *D. novaisi* and *P. camacan*.

In relation to *Adenomera*, tissues of three specimens from the REM were analyzed in a molecular review of the *Adenomera* genus (FOUQUET et al. 2013), and the results showed that *Adenomera* species from the REM form the sister clade to *A. thomei*. However, the same authors argued that there is insufficient data to confirm whether it is a new species, and therefore considered *Adenomera* species from MER as Unconfirmed Candidate Species (FOUQUET et al. 2013). Additionally, we consulted a specialist in the *Adenomera* species, who confirmed that species from REM correspond to *A. thomei* (C. S. CASSINI pers. comm.).

Discussion

The present study increased amphibians species richness of the REM by 30%, bringing the total number of species for the reserve to 69. This richness is the second-highest recorded in Bahia, only lower than that of the RPPN Serra Bonita (80 ssp.), located along an altitudinal gradient from 200 to 950 m a.s.l. (DIAS et al. 2014a). Most other sites with high amphibian diversity, such as Santa Tereza municipality (92 ssp.), the Parque Natural Municipal Nascentes de Paranapiacaba (80 ssp.), Estação Ecológica de Boracéia (67 ssp.), Rio de Janeiro municipality (68 ssp.) and Parque Estadual Carlos Botelho (65 ssp.), are associated with mountainous Atlantic Forest areas (HEYER et al. 1990, IZECKSOHN & CARVALHO-E-SILVA 2001, FORLANI et al. 2010, ALMEIDA et al. 2011, TREVINE et al. 2014). Over the altitudinal gradients, changes in biotic and abiotic

features increases the availability of microenvironments which is believed to promote greater species diversity. VASCONCELOS et al. (2010) conducted a review study in various mountainous ranges in Brazil and found that the increasing amphibian richness is related to the degree of the altitude gradient. Unlike these areas, the REM is inserted in a region classified as Dense Atlantic Forest Lowlands (VELOSO 1998), with maximum altitudes reaching 393 m above sea level.

Despite the lack of distinct altitudinal gradients in the REM, the region has a high environmental heterogeneity, including agroforestry systems (rubber, cocoa and banana plantations), secondary forests at different regeneration stages, forests that were logged a varying intensities, and mature forests (FLESHER 2015). These diverse habitats provide a high diversity of breeding sites such as temporary and permanent ponds, streams, bromeliads, epiphytes and a dense leaf litter layer. This high habitat heterogeneity and elevated temperatures and rainfall throughout the year create an environment of a high air temperature and humidity which likely explains the expressive number of species recorded. Also, the high abundance of breeding habitats satisfies the reproductive requirements of a large number of species. According to DUELLMAN (1988) reproductive modes play an important role in understanding anuran species diversity. Currently, there are 27 reproductive modes recognized for Atlantic Forest amphibians (HADDAD et al. 2013). According to HADDAD & PRADO (2005), the high diversity of reproductive modes observed for the Atlantic Forest is the result of a successful utilization of the diverse humid microhabitats present in the biome. Fourteen out of the 27 reproductive modes from Atlantic Forest (~52%) were recorded at the REM.

Another important factor that likely affected the results was the sampling effort. With approximately 10 years of sampling this study had the highest sampling effort for northeastern Brazil to date. Long sampling periods are essential for understanding community structure and are necessary for reaching accurate values of diversity for an area. Although the present study shows an increase of 21 amphibians species compared to the previous study (CAMURUGI et al. 2010), further fieldwork may still reveal new species. The highest amphibian diversity areas in the Atlantic forest was also the result of long sampling periods. Despite the long sampling periods new species are still found in these regions, as the case of *Adelophryne glandulatta* and *Dendropsophus bromeliaceus*, recently described from Santa Tereza municipality (LOURENÇO-DE-MORAES et al. 2014, FERREIRA et al. 2015). Most long-term studies of amphibians are from southeastern Brazil, resulting in the better studied region in this biome (ROSSA-

FERES et al. 2011, CAMPOS et al. 2014), but the recent increase in studies from other regions of the country (e.g. southern Bahia) is expanding our knowledge to other regions (DIAS et al 2014a, DIAS et al. 2014b, present study).

The high diversity of species in southern Bahia has been associated with climate stability and forest conditions during glacial periods (CARNAVAL et al. 2009). According to the same authors, this region was a large pleistocenic climatic refuge for amphibians, and thus the high species diversity of the REM, as well as in other areas of southern Bahia, is also associated with these long-term biogeographical processes.

Typical of the Atlantic Forest the majority (n = 43, 61.4%) of the REM species identified at specific level or "cf"; are endemic to this biome (see HADDAD et al. 2013). Fourteen of the 69 species recorded (*Frostius erythropthalmus*, "*Eleutherodactylus*" *bilineatus*, *Pristimantis vinhai*, *Adeophryne* cf. *pachydactyla*, *A. mucronatus*, *Gatrotheca recava*, *Agalychnis aspera*, *Bokermannohyla capra*, *Phasmahyla timbo*, *Phyllodytes wuchereri*, *Scinax strigilatus*, *Physalaemus camacan*, *Chiasmocleis cordeiroi* and *C. crucis*) are also endemic to Bahia (ANGULO 2008, JUNCA & PIMENTA 2004, PEIXOTO & PIMENTA 2004, BORGES-NAJOSA & JUNCA 2004, LOURENÇO-DE-MORAES et al. 2012, Teixeira Jr. et al. 2012, Silvano & Pimenta 2010, Napoli & Pimenta 2009, ANGULO 2009, RODRIGUES 2006, JUNCA & SILVANO 2004, ANGULO 2008, FORLANI et al. 2013).

The results of this study expand the distribution of *Adelophryne mucronatus* 60 km to the north (LOURENÇO-DE-MORAES et al. 2012; DIAS et al. 2014a). Two species were recorded by collecting only a single individual: *Aparasphenodon brunoi*, a bromeliad species, which had its distribution increased from municipality of Una to the REM (RUAS et al. 2013), and *Dermatonotus muelleri* which is a species typically found in open areas (COLLI et al. 2004).

Eleven species (15%) are listed without a specific name or classified as similar. Some of them, like *Pristimantis* sp. and *Adelophryne* sp. are in final description processes (MARCIANO Jr. et al. in prep; CHAPTER 3). These results help compensate for the lack of basic data from Bahia, and corroborate the data presented by other recent inventory studies conducted in the state (DIAS et al. 2014a, DIAS et al. 2014b). Given the high levels of richness and endemism of amphibians already found for southern Bahia, we expect that future inventories in yet unsurveyed regions of southern Bahia may result in the discovery of additional new species and expand the ranges of already known species.

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TABLE 1. Amphibian species found in the Michelin Ecological Reserve, southern Bahia, Brazil. Caption. ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade) = Conservation status: DD = Deficient Data; LC = Least Concern. Microhabitat: LL = Leaf litter or understory; S = Streams; TP = Temporary ponds; PP = Permanent ponds; B = bromeliads or epiphytes; C = Canopy; F = Fossorial. Habitat: F = Forest; RP = Rubber plantation. Reproductive Modes (*sensu* Haddad et al. 2013). Source: PS = Present study. * = species only found in the inner forests; † = only acoustic record; # only recorded once or twice during the sampling.

Order/Family/Species	Conservation status	Microhabitat	Habitat	Reproductive Modes	Source
ANURA					
Aromobatidae					
<i>Allobates olfersioides</i> (Bokerman, 1967)	DD	LL	F	20	PS & Camurugi et al. 2010
Bufo					
<i>Frostius erythrophthalmus</i> Pimenta and Caramaschi, 2007*	LC	LL	F	?	Present study
<i>Rhinella hoogmoedi</i> (Caramaschi & Pombal, 2006)	LC	LL, S	F, RP	1	PS & Camurugi et al. 2010
<i>Rhinella crucifer</i> (Wied-Neuwied, 1821)	LC	LL, PP	F, RP	1,2	PS & Camurugi et al. 2010
Centrolenidae					
<i>Vitreorana eurygnatha</i> (A. Lutz, 1925)*	LC	S	F	25	PS & Camurugi et al. 2010
Craugastoridae					
<i>"Eleutherodactylus" bilineatus</i> (Bokermann, 1975)*	LC	LL	F	23	PS & Camurugi et al. 2010
<i>Haddadus binotatus</i> (Spix, 1824)	LC	LL	F	23	PS & Camurugi et al. 2010
<i>Pristimantis paulodutra</i> (Bokermann, 1975)	LC	LL	RP	23	PS & Camurugi et al. 2010
<i>Pristimantis</i> sp.*		LL	F	23	PS & Camurugi et al. 2010
<i>Pristimantis vinhai</i> (Bokermann, 1975)	LC	LL	F, RP	23	PS & Camurugi et al. 2010
Eleutherodactylidae					
<i>Adelophryne</i> cf. <i>pachydactyla</i> Hoogmoed, Borges & Cascon, 1994	LC	LL	F	23	Present study
<i>Adelophryne mucronatus</i> Lourenço-de-Morais et al., 2012*	LC	LL	F	23	Present study
<i>Adelophryne</i> sp. *	-	LL	F	?	Present study
Hemiphractidae					
<i>Gastrotheca</i> sp.	-	C	F	37	Present study
<i>Gastrotheca recava</i> Teixeira et al., 2012	-	C	F	37	Present study

Hylidae

<i>Agalychnis aspera</i> (Peters, 1873)*	LC	TP	F	18	PS & Camurugi et al. 2010
<i>Aparasphenodon bruno</i> i Miranda-Ribeiro, 1920*	LC	B	F	1	
<i>Aplastodiscus cavicola</i> (Cruz & Peixoto, 1984)*	LC	S	F	5	Camurugi et al. 2010
<i>Aplastodiscus ibirapitanga</i> (Cruz, Pimenta, & Silvano, 2003)*	LC	S	F	5	PS & Camurugi et al. 2010
<i>Aplastodiscus sibilatus</i> (Cruz, Pimenta, & Silvano, 2003)*	LC	S	F	5	PS & Camurugi et al. 2010
<i>Bokermannohyla capra</i> (Napoli & Pimenta, 2009)*	-	S	F	2	PS & Camurugi et al. 2010
<i>Dendropsophus anceps</i> (Lutz, 1929)	LC	TP	RB	1	Present study
<i>Dendropsophus branneri</i> (Cochran, 1948)	LC	PP, TP	F, RP	1	PS & Camurugi et al. 2010
<i>Dendropsophus decipiens</i> (Lutz, 1925)	LC	PP, TP	RB	24	Present study
<i>Dendropsophus elegans</i> (Wied-Neuwied, 1824)	LC	PP, TP	F, RP	1	PS & Camurugi et al. 2010
<i>Dendropsophus giesleri</i> (Mertens, 1950)	LC	TP	F, RP	1	PS & Camurugi et al. 2010
<i>Dendropsophus haddadi</i> (Bastos & Pombal, 1996)	LC	TP	F, RP	24	PS & Camurugi et al. 2010
<i>Dendropsophus minutus</i> (Peters, 1872)	LC	TP	F, RP	1	PS & Camurugi et al. 2010
<i>Dendropsophus novaisi</i> (Bokermann (1968)	LC	TP	RP	1	Present study
<i>Dendropsophus</i> aff. <i>oliveirai</i> (Bokermann, 1963)	LC	PP, TP	RB	1	PS & Camurugi et al. 2010
<i>Hypsiboas albomarginatus</i> (Spix, 1824)	LC	PP, TP	F, RP	1	PS & Camurugi et al. 2010
<i>Hypsiboas atlanticus</i> (Caramaschi & Velosa, 1996)	LC	PP, TP	F, RP	1,2	PS & Camurugi et al. 2010
<i>Hypsiboas crepitans</i> (Wied-Neuwied, 1824)	LC	PP	RB	4	PS & Camurugi et al. 2010
<i>Hypsiboas exastis</i> (Caramaschi & Rodrigues, 2003)*	LC	B, S	F	4	Present study
<i>Hypsiboas faber</i> (Wied-Neuwied, 1821)	LC	PP, TP	F, RP	1,4	PS & Camurugi et al. 2010
<i>Hypsiboas pombali</i> (Caramaschi, Pimenta & Feio, 2004)	LC	PP	F, RP	1,2	PS & Camurugi et al. 2010
<i>Hypsiboas semilineatus</i> (Spix, 1824)	LC	PP	F, RP	1,2	PS & Camurugi et al. 2010
<i>Itapotihyla langsdorffii</i> (Duméril and Bibron, 1841)	LC	TP	F, RP	1	Present study
<i>Phasmahyla timbo</i> Cruz, Napoli & Fonseca, 2008*	DD	S	F	25	PS & Camurugi et al. 2010
<i>Phyllodytes</i> cf. <i>maculosus</i> Cruz, Feio, and Cardoso, 2007†	DD	B	F	?	Present study
<i>Phyllodytes melanomystax</i> (Caramaschi, Silva & Britto-Pereira, 1992)	LC	B	F, RP	6	PS & Camurugi et al. 2010
<i>Phyllodytes</i> sp. 1	-	B	F, RP	?	Present study
<i>Phyllodytes</i> sp. 2	-	B	F, RP	?	Present study
<i>Phyllodytes</i> sp. 3†	-	B	F, RP	?	Present study

<i>Phyllodytes wuchereri</i> (Peters, 1873)†*	LC	B	F	6	Present study
<i>Phyllomedusa burmeisteri</i> (Boulenger, 1882)	LC	TP	F, RP	24	PS & Camurugi et al. 2010
<i>Phyllomedusa nordestina</i> (Caramaschi, 2006)	LC	PP, TP	F, RP	24	PS & Camurugi et al. 2010
<i>Phyllomedusa rohdei</i> (Mertens, 1926)	LC	PP, TP	F, RP	24	PS & Camurugi et al. 2010
<i>Scinax argyreornatus</i> (Miranda-Ribeiro, 1926)*	LC	S	F	1	Present study
<i>Scinax eurydice</i> (Bokermann, 1968)	LC	PP, TP	F, RP	1	PS & Camurugi et al. 2010
<i>Scinax juncae</i> Nunes and Pombal, 2010	LC	PP	F, RP	1	Present study
<i>Scinax strigilatus</i> Pimenta, Faivovich & Pombal Jr, 2007*	DD	S	F	1,2	PS & Camurugi et al. 2010
<i>Scinax x-signatus</i> (Spix, 1824)	LC	PP, TP	F, RP	1	PS & Camurugi et al. 2010
<i>Trachycephalus mesophaeus</i> (Hensel, 1867)	LC	TP	F, RP	1	PS & Camurugi et al. 2010
Leptodactylidae					
<i>Adenomera thomei</i> Almeida & Angulo, 2006	LC	LL	RP	32	PS & Camurugi et al. 2010
<i>Leptodactylus</i> cf. <i>latrans</i> (Steffen, 1815)	LC	S, PP, TP	F, RP	11	PS & Camurugi et al. 2010
<i>Leptodactylus cupreus</i> Caramaschi, Feio & São-Pedro, 2008*	DD	TP	F	30	Present study
<i>Leptodactylus fuscus</i> (Schneider, 1799)	LC	LL	RP	30	Present study
<i>Leptodactylus mystaceus</i> (Spix, 1824)	LC	LL	RP	30	PS & Camurugi et al. 2010
<i>Leptodactylus vastus</i> Lutz, 1930	LC	TP	RP	11	Present study
<i>Physalaemus camacran</i> Pimenta, Cruz, and Silvano, 2005	LC	TP	F, RP	11	PS & Camurugi et al. 2010
Microhylidae					
<i>Chiasmocleis cordeiroi</i> Caramaschi & Pimenta, 2003	DD	TP	F, RP	1	PS & Camurugi et al. 2010
<i>Chiasmocleis crucis</i> Caramaschi & Pimenta, 2003	DD	TP	F	1	PS & Camurugi et al. 2010
<i>Stereocyclops incrassatus</i> (Cope, 1870)	LC	TP	F, RP	1	PS & Camurugi et al. 2010
<i>Dermatonotus muelleri</i> (Boettger, 1885)	LC	TP	RP		Present study
Odontophrynidae					
<i>Macrogenioglottus alipioi</i> (Carvalho, 1946)*	LC	TP	F	1	PS & Camurugi et al. 2010
<i>Proceratophrys renalis</i> (Miranda-Ribeiro, 1920)	LC	LL, S	F	2	PS & Camurugi et al. 2010
<i>Proceratophrys schirchi</i> (Miranda-Ribeiro, 1937)*	LC	LL, S	F	2	PS & Camurugi et al. 2010
GYMNOPHYONA					
Caeciliidae					
<i>Siphonops annulatus</i> (Mikan, 1820)	LC	F	F, RP		Present study

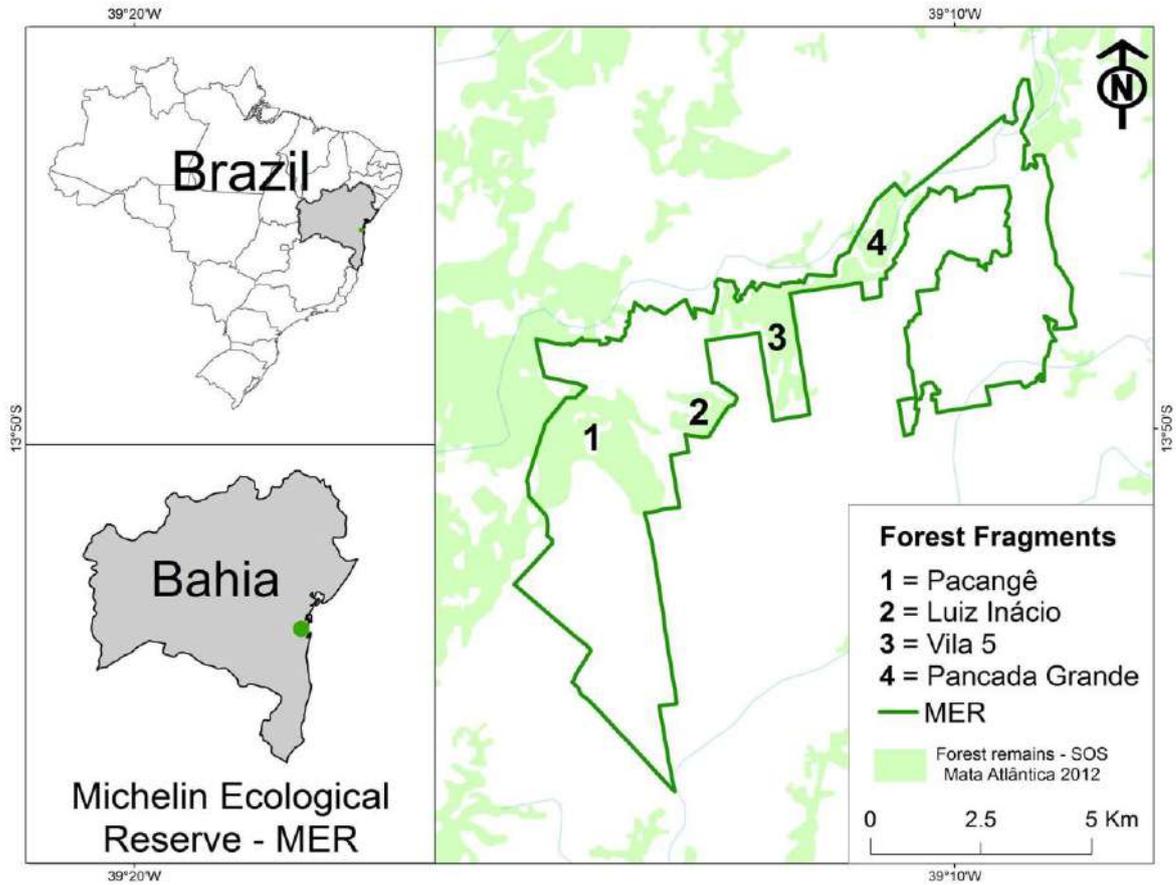


FIGURE 1. Map showing the perimeter of Michelin Ecological Reserve in the Atlantic forest of lowlands, Igrapiúna municipality, southern Bahia, Brazil.

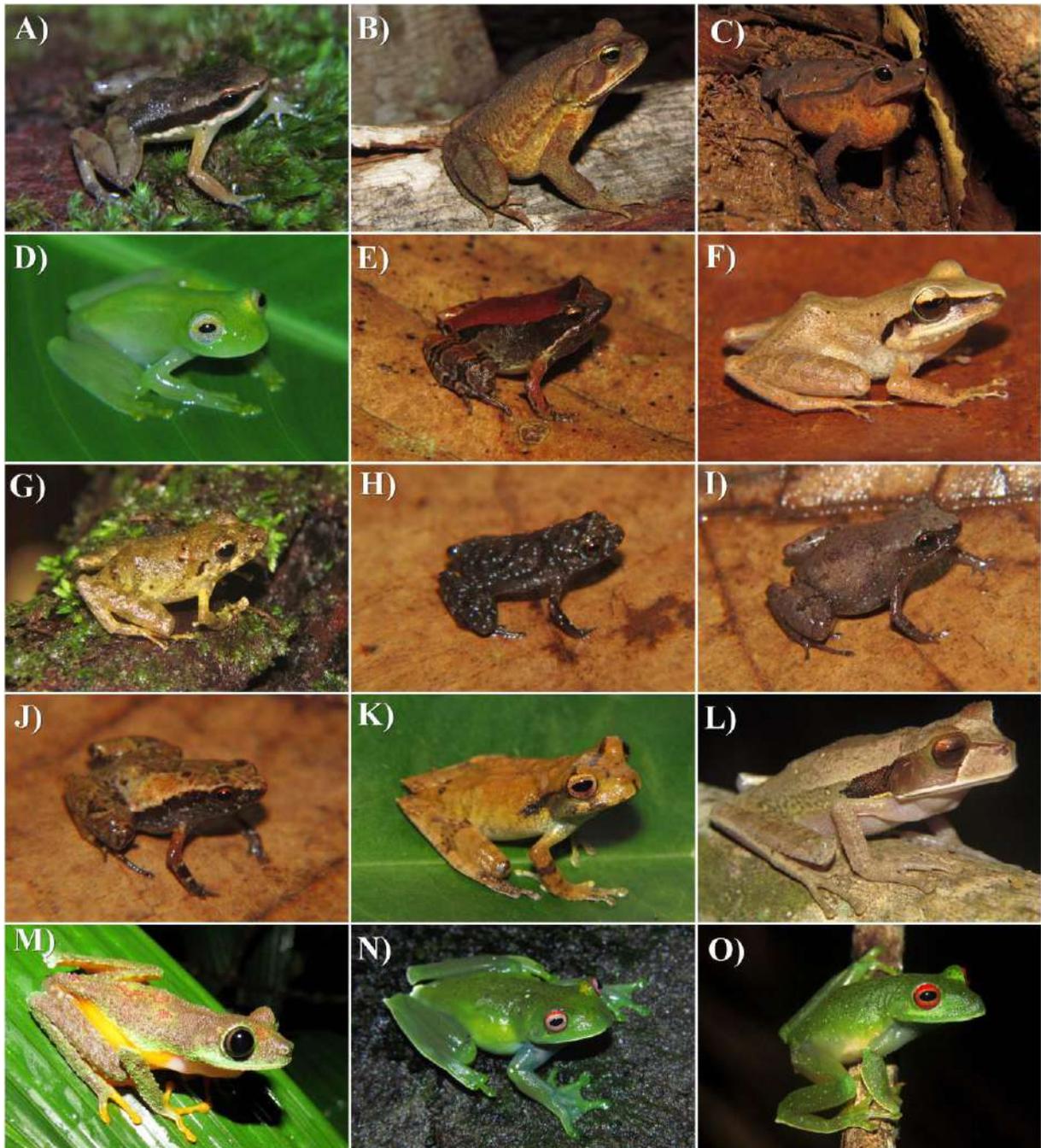


Figure 2. Some amphibian species from Michelin Ecological Reserve – MER, municipality of Igrapiúna, Bahia State, Northeastern Brazil. A) *Allobates olfersioides*; B) *Rhinella crucifer*; C) *Rhinella hoogmoedi*; D) *Vitreorana eurygnatha*; E) “*Eleutherodactylus*” *bilineatus*; F) *Pristimantis* sp.; G) *Pristimantis vinhai*; H) *Adelophryne* cf. *pachycadtyla*; I) *A. mucronatus*; J) *A.* sp. 2; K) *Gastrotheca* sp.; L) *Gastrotheca recava*; M) *Agalychnis aspera*; N) *Aplastodiscus ibirapitanga*; O) *A. sibilatus*.

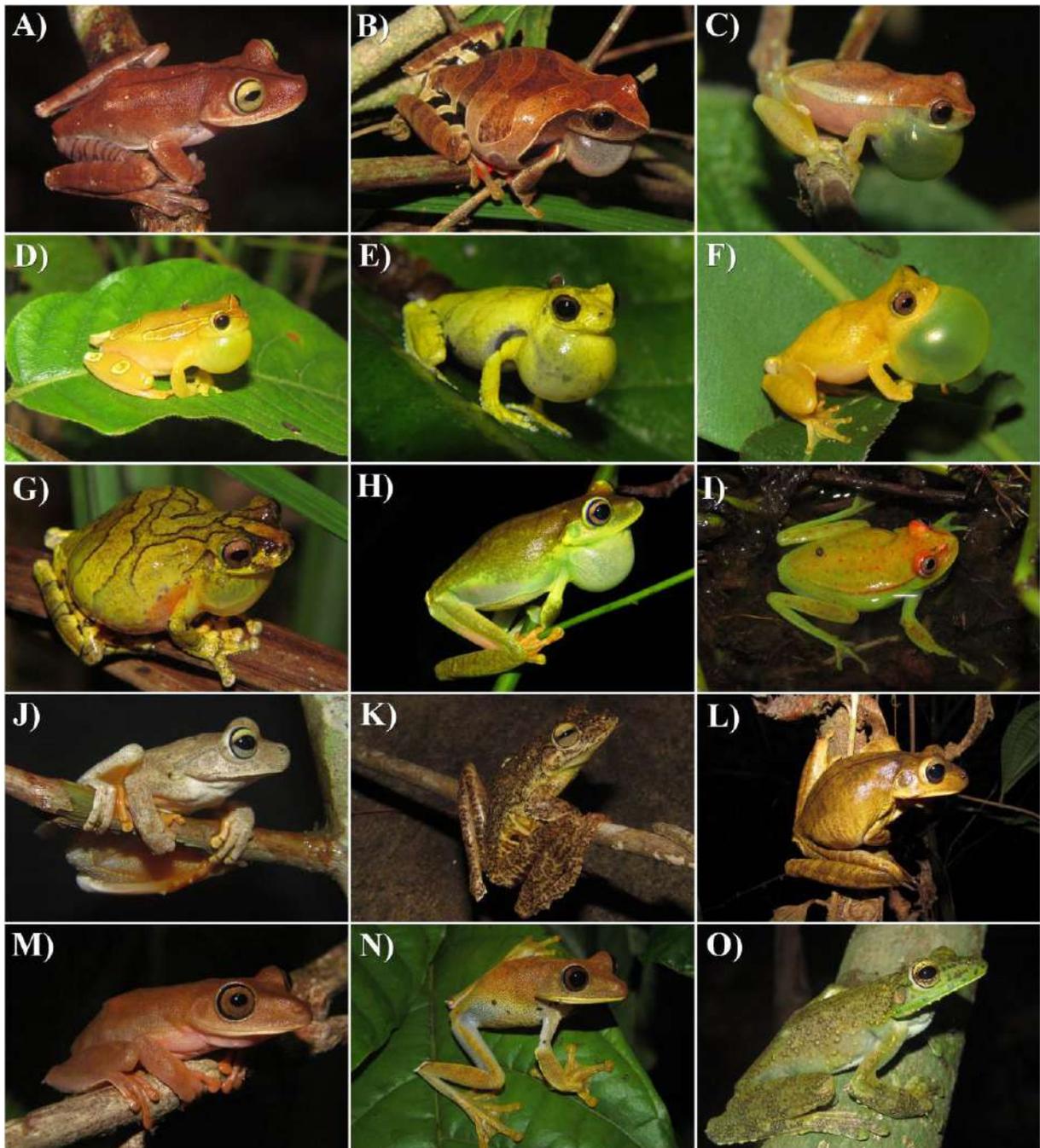


Figure 3. Some amphibian species from Michelin Ecological Reserve – MER, municipality of Igrapiúna, Bahia State, Northeastern Brazil. A) *Bokermannohyla capra*; B) *Dendropsophus anceps*; C) *D. branneri*; D) *D. elegans*; E) *D. giesleri*; F) *D. minutus*; G) *D. novaisi*; H) *Hypsiboas albomarginatus*; I) *H. atlanticus*; J) *H. creptans*; K) *H. exastis*; L) *H. faber*; M) *H. pombali*; N) *H. semilineaus*; O) *Itapotihyla langsdorffii*.

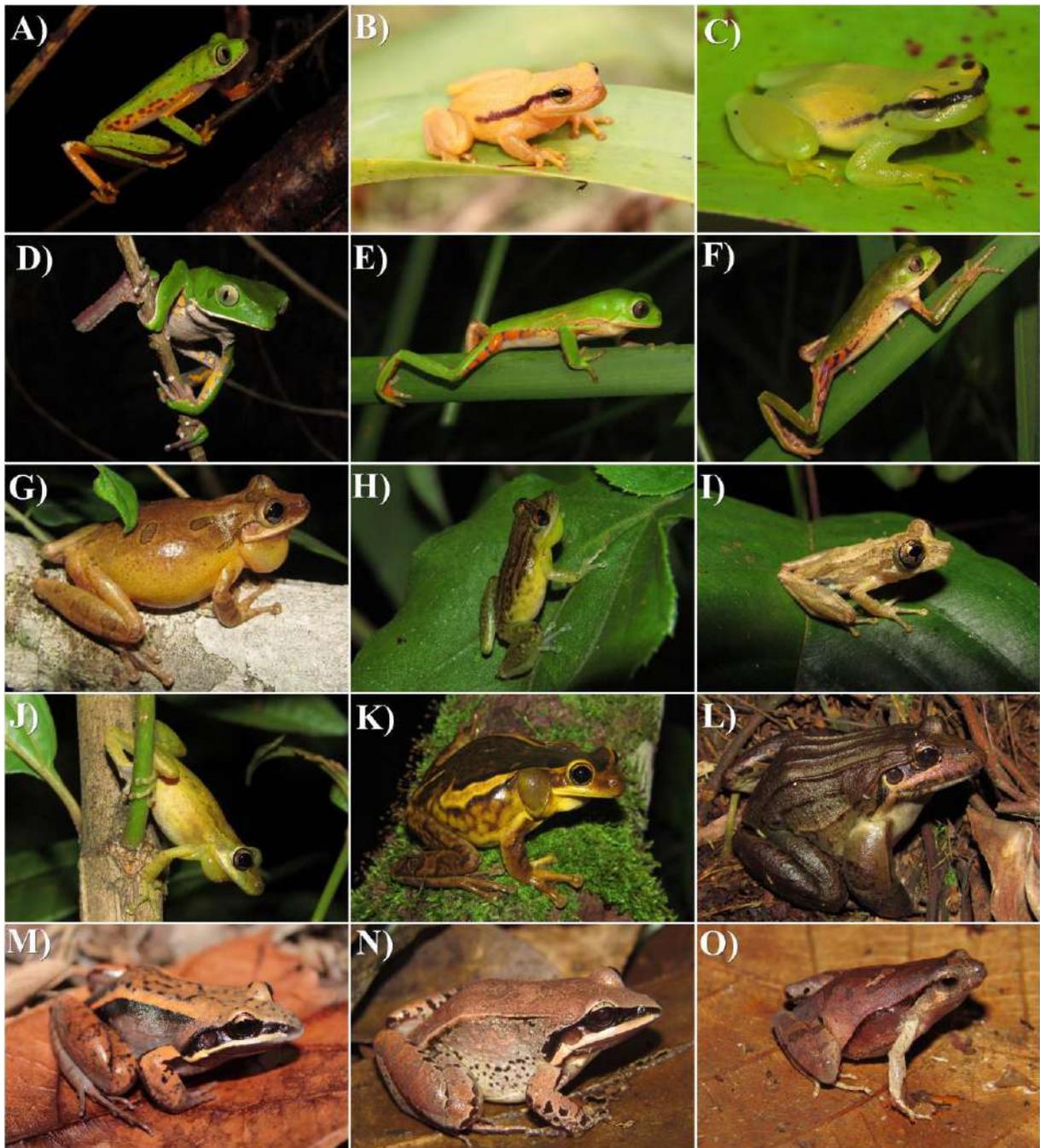


Figure 4. Some amphibian species from Michelin Ecological Reserve – MER, municipality of Igrapiúna, Bahia State, Northeastern Brazil. A) *Phasmahyla timbo*; B) *Phyllodytes* sp. 2; C) *P. melanomystax*; D) *Phyllomedusa burmeisteri*; E) *P. nordestina*; F) *P. rohdei*; G) *Scinax eurydice*; H) *S. juncae*; I) *S. strigilatus*; J) *S. x-signatus*; K) *Trachycephalus mesophaeus*; L) *Leptodactylus* cf. *latrans*; M) *L. cupreus*; N) *L. mystaceus*; O) *Physalaemus camacan*.

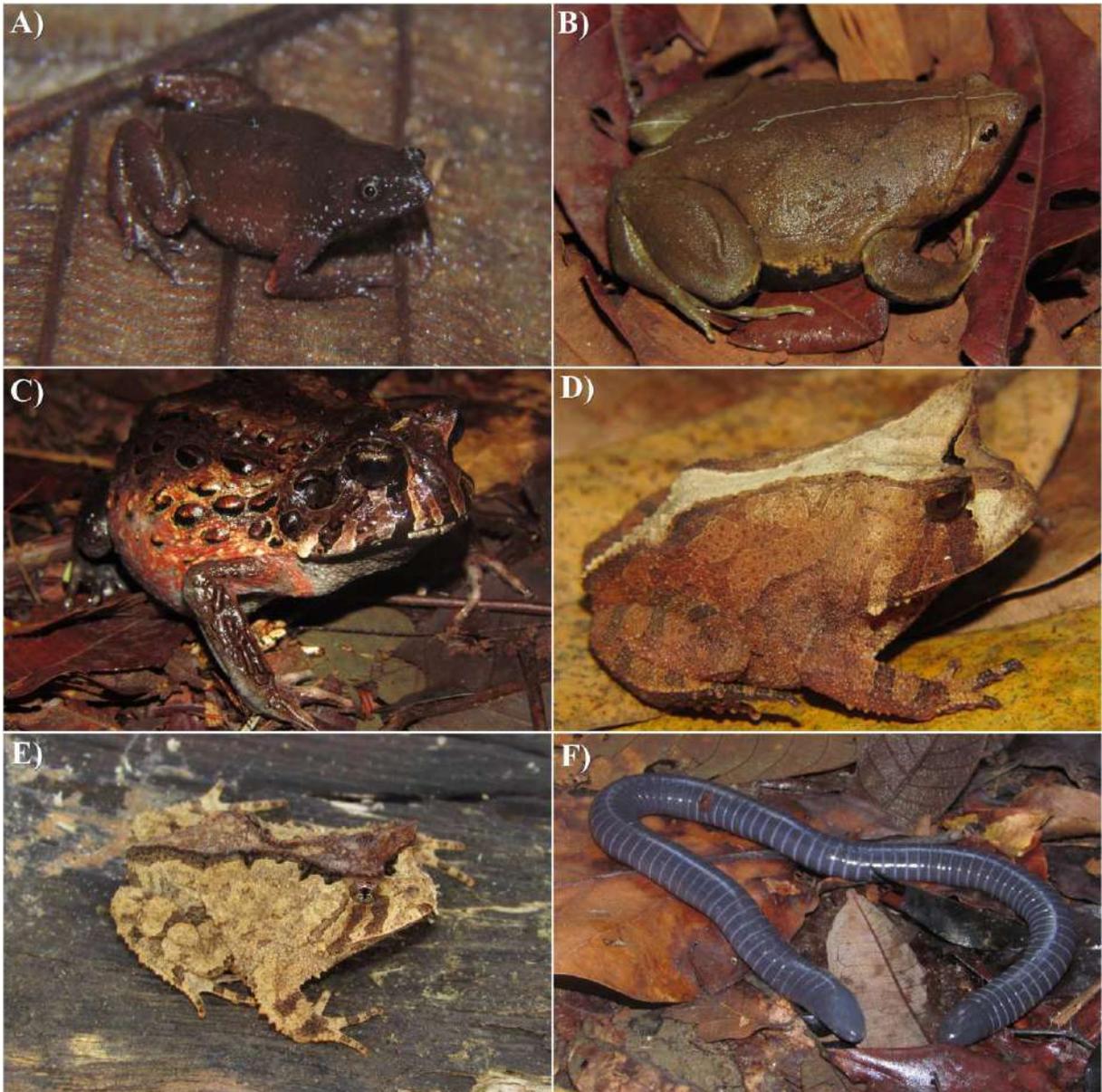


Figure 5. Some amphibian species from Michelin Ecological Reserve – MER, municipality of Igrapiúna, Bahia State, Northeastern Brazil. A) *Chiasmocleis cordeiroi*; B) *Stereocyclops incrassatus*. 2; C) *Macrogenioglottus alipioi*; D) *Proceratophrys renalis*; E) *P. schirchi*; F) *Siphonops annulatus*.

Capítulo II



**Changes in a leaf litter amphibian community along a tropical forest
chronosequence and rubber plantation in southern Bahia, Brazil**

(A ser submetido à revista *Biological Conservation*)

**Changes in a leaf litter amphibian community along a tropical forest
chronosequence and rubber plantation in southern Bahia, Brazil**

ABSTRACT: Over the past century human disturbance of tropical forests has led to widespread decline of pristine habitats and their replacement by forest plantations and secondary forests. However, information on how the native fauna is affected by these changes are still scarce. To investigate these effects we analyzed how leaf litter amphibian species richness, abundance and alpha diversity varies across several habitat categories (rubber plantation, secondary forest succession and old-growth forest) in an Atlantic Forest landscape of southern Bahia, Brazil. We compare the similarity and proportion of old-growth forest species with the amphibian communities found in rubber and secondary forest habitats and identified the principal environmental variables associated with changes in amphibian assemblages. We found no significant differences in the richness and diversity of leaf litter amphibians among the three habitats. However, when we analyzed each separately, forest specialists differed significantly among habitats, showing that diversity in RP and PF are significantly lower than in secondary forests. Our results suggest that richness and diversity of amphibians in secondary forests reach values similar to those found in old-growth forest within approximately 30 years after abandonment. Although the proportion and similarity of old-growth species increased with secondary forest succession, the recovery of the leaf litter amphibian assemblages occurs significantly more slowly, with mature secondary forest only reaching 56% of similarity with old-growth forest species after 30 years. Although rubber plantations harbor low richness, diversity and species compared to old-growth forest, if located closely to forest fragments, there is a increase in species diversity within 10 years of abandonment. Additionally, we found that leaf litter

density, canopy openness and temperature are significant explanatory variables for observed patterns of leaf litter amphibian assemblages in tropical secondary forest succession. Tropical secondary forests can play an important role in the conservation of amphibians, especially when the forest lies within a landscape context that provides favorable local environmental conditions that stimulate a the rapid recovery of the leaf litter amphibian species to approximate that of old-growth forests.

Keywords: Leaf-litter amphibians; species richness; rubber plantation; secondary forest succession; Atlantic Forest; Bahia.

1. Introduction

Recent studies indicated that forest area worldwide declined 3% in the last 25 years, which represents a loss of 129 million ha (FAO, 2015). Forest loss largely occurred in the tropics, especially in South America (Keenan et al., 2015) and the principal cause is agricultural expansion (FAO, 2010). However, increasing areas of agricultural land are being abandoned initiating a process of natural forest regeneration (Brown and Lugo, 1990; Nepstad et al., 1991; Chazdon et al., 2007). Tropical secondary forest comprises approximately five of the 11 million km² of forest cover (ITTO, 2002), and the importance of these forests for biodiversity conservation is increasingly recognized (e.g. Wright and Muller-Landau, 2006; Barlow et al. 2007; Chazdon et al., 2009).

While a number of studies investigate the recovery of plant species during secondary forest succession (e.g. Aide et al., 2000; Guaricata and Ostertag, 2001; Janzen, 2002; Liebsch et al., 2008; Chazdon et al., 2009), studies focused on animals are still scarce (Dunn, 2004; Bowen et al., 2007; Gardner et al., 2007; Chazdon et al., 2009). In general, these studies suggest that biodiversity increases in secondary forests over time,

and faunal species diversity reaches similar values to mature forests after 20 – 40 years (Dunn, 2004; Dent and Wright, 2009). However, the trajectory of faunal recovery during secondary succession depends on a set of factors, related both to landscape (e.g. land-use history and degree of isolation of secondary forest) as local variables (e.g. biotic and abiotic interactions).

Among vertebrates, amphibians are the most threatened group in the world, with around one-third of species globally threatened or extinct, mostly due to habitat loss and fragmentation (Stuart et al., 2004). Amphibians are particularly sensitive to habitat disturbance (Pechmann et al., 1991; Young et al., 2004; Todd et al., 2009), because, firstly, they have permeable skin which makes them vulnerable to changes in microclimate and microhabitats (Lips, 1998; Blaustein et al., 2010); and, secondly, because they have low mobility compared to other vertebrates group with resulting small home ranges (Beebee, 1996). This sensitivity makes amphibians useful models for understanding how the processes of secondary forest succession affects species diversity (Hopkins, 2007).

Studies designed to understand the recovery of amphibian assemblages during secondary forest succession, and also between forest areas and plantations have yielded contrasting results. In some cases amphibian assemblages recovered quickly, reaching similar compositions as old-growth forest in young (3 years in Ríos-López and Aide, 2007; 1 – 5 years in Herrera-Montes and Brokaw, 2010) and intermediate secondary forest (10 – 16 years in Hilje and Aide, 2012). On the other hand, some studies found that secondary forest 11 – 23 years only supported 55 and 60% of those found in mature forests (Hernandez et al., 2015; Gardner et al., 2006 respectively). Hilje and Aide (2002) suggested that this difference in recovery time is associated with landscape characteristics and a result of the spatial scale of the analysis.

The aim of the present study was to compare the richness, abundance, alpha diversity and composition of leaf litter amphibian assemblages in different habitats (rubber plantation, secondary forest, and old-growth forest) in southern Bahia, Brazil. The study was carried out at Reserva Ecológica Michelin, in a region composed predominantly of secondary forests, rubber groves, ~~plantation areas~~ and small patches of more intact forests (Flesher 2015). We assessed amphibian composition in six different habitat categories with the intent of answering the following questions: 1) Do richness, abundance, alpha diversity and composition change between rubber groves, secondary forests and old-growth forest? 2) Does the proportion and similarity of old-growth species in secondary forest increase with successional stages? 3) Which are the principal environmental variables associated with changes in amphibian assemblages in habitats with different land use histories and regeneration stages?

2. Methods

2.1. Study area

The study was conducted between March and December 2015 in the Reserv Ecológica Michelin – REM, located in southern Bahia, northeastern Brazil (Figure 1). The private reserve lies within the municipalities of Igrapiúna and Ituberá (13°50'S, 39°10'W) in a region of Dense Rain Forest of Lowlands (Veloso et al. 1991). The 3,096 ha reserve supports 1,800 ha of lowland evergreen hill forest distributed in three main fragments (Vila 5/Pancada Grande – 625 ha; Pacangê – 550 ha; and Luis Inácio – 140 ha). The Pacangê fragment is contiguous with a 13,000 ha forest located outside the reserve. The reserve has a long history of human disturbance, mostly manioc farming and decades of intensive logging. The forest today is predominantly secondary forest at different stages of succession, intensively logged forest, and-small patches of more intact forests on the

steepest slopes and ridge tops (Flesher 2015). The regional landscape is one of the most productive rubber areas in Brazil, and rubber plantations (*Hevea brasiliensis*) occupy about 15% of the reserve. The landscape to the east of the reserve is dominated by rubber, cocoa and bananas groves; to the south, southwest and north by properties of smallholder farmers with mixed tree crops and small forest fragments; while to the west lies the largest forest fragment in the region. The regional landscape (1,000 km²) retains 40% forest cover and a high diversity of agroforestry systems (Flesher, 2015). The average annual rainfall in MER during the last 10 years was approximately 2000 mm, and the temperature varied between 21.7 and 30.8 °C (Michelin Ecological Reserve data).

2.2. Sample design

Amphibians were sampled in the following habitats: (1) *Rubber plantations - RP*: Actively exploited rubber groves with periodic maintenance of tapping trails with pesticides (Glyphosate-based herbicide) and the inter-rows left in low pioneer vegetation and cut every 6-12 months; (2) *Pioneer forest - PF*: Tall natural early succession vegetation between rows of rubber trees has not been cut in the last 10 years; (3) *Young secondary forest - YSF*: Areas that were clear-cut, burned and used for manioc farming, but were last planted 30-50 years ago, and are now regenerating with natural vegetation; (4) *Mature secondary forests - MSF*: Sites that were clear-cut, burned and last planted with manioc >50 years ago and are now regenerating with natural vegetation; (5) *Intensively selectively logged - ISL*: Most medium and large trees were felled 50-70 years ago; (6) *Lightly selectively logged*: Few trees removed 50-70 years ago. Although, large and medium sized trees have been removed, individual mature trees (most *Eriotheca spp.*) and/or clusters of large and medium sized trees were

left standing. In the present study we consider the category Lightly selective logged as the most pristine in the reserve, and it will be treated as old-growth forest – OGF. The classification was based on history of land use data collected through interviews, an analysis of aerial photos 1964 and 1974, SPOT satellite data (1989), Landsat 1998, and by sampling the flora (Flesher 2015).

We selected 4 sampling units in each habitat category, except in MSF, where one sample unit was excluded due to logistic problems—for a total of 23 sampling units in the six categories sampled. Sites of the same habitat category were located at least 1 km away from each other to guarantee independence, and all the sites were located with a minimum of 100 m from the forest edge. All sampling points were at least 300 m from water bodies such as streams and pools.

2.3. Amphibian sampling

Sampling was carried out over six field expeditions between March and December 2015, with a duration of seven day each, for a total of 42 days. Amphibians were sampled by visual and acoustic active searching (Rödel and Ernst, 2004) and by using pitfall traps with drift fences (Cechin and Martins, 2000; Ribeiro-Júnior et al., 2011).

At each sample unit we installed one set of pitfall traps with five 30 litter buckets spaced at 8-m intervals and 40 cm tall drift fences with 32 m lines. Pitfall traps remained open during three consecutive nights, totaling a sampling effort of 90 traps/day in each sample unit, and 340 traps/day in each category.

Active searching was mostly conducted by two researchers (but occasionally by three) in a radius of approximately 30 meters from the center point of the pitfall traps. We employed a sampling effort of 30 minutes of active search at each site, totaling 3 hours/man in each sample unit, and 12 hours/man in each category.

2.4. Forest characteristics

To measure the vegetational structure, three plots of 5m x 5m were delimited in each sample unit. The plots were placed parallel to the 32 meters line of the pitfall traps as follows: First plot (five meter length); interval of 8.5 meters; Second plot (five meter length); interval of 8.5 meters; and Third plot (five meter length). In each plot the following environmental variables were sampled: canopy cover was evaluated through three photographs, one in each plot and distanced 13,5 meters. The pictures were made using a fisheye lens on a digital camera (Canon Powershot, 14 megapixels). The camera was set up on a 1.70 m tripod and pointed directly towards the sky. Pictures were analyzed using the ImageJ software (Rasband, 2008). To measure leaf-litter density, we delimited 50 cm x 50 cm plots on the forest floor in each sample unit, removed all leaf litter and placed it into a plastic bag. Afterwards, the leaf litter was dried and its weight was measured with a digital scale. Diameter at breast height (DBH) of all the trees in the plots was measured, and later divided in categories for analysis: 0 – 40 cm; 40 – 60 cm; 60 – 100 cm and > 100 cm DBH. Up to 1m tall shrubs and tree-trunks (DBH > 20 cm and with more than 1 m length) were counted in each plot. Temperature and light were recorded with data loggers (Onset HOBO® Pendant Temp/Light Data Logger) at each sample unit. Data loggers were scheduled to record temp/light at each 30 minutes during the entire study period.

2.5. Data analysis

To estimate efficiency and completeness of amphibian inventories were calculated the nonparametric species estimators ACE, Chao-1 and the first-order jackknife using EstimateS v.8.0 (Colwell, 2006). According to Colwell and Coddington (1994) these estimators show the best performance for samples based on abundance data. Rank-

abundance curves were performed to assess the evenness of species compositions (Magurran 1998). To assess species diversity in each habitat category we used the effective number of species, or “true diversity” as suggested by Jost (2006). This method uses orders of diversity ($q = 0$, $q = 1$ and $q = 2$), indicating the sensitivity to common and rare species.

The Sorensen similarity index (S) was used to assess the similarity between habitat categories. We also calculated the proportion of old-growth species present in rubber plantation and in secondary forest sites. To identify the species associated with or indicative of habitat categories we perform an Indicator Species Analysis (ISA; Cáceres et al., 2010). Diversity analysis; Indicator Species Analysis (ISA) and Sorensen similarity index (S) were performed using the R statistical software (v. 2.15.2) R Development Core Team, Vienna, Austria.

To measure the structure of amphibian assemblages between the habitat categories, we conducted a non-metric multidimensional scaling analysis (NMDS) with PC-ORD v. 4 (McCune and Mefford, 1999). We constructed a presence/absence matrix of amphibians per habitat categories, with sample units in rows and species in columns. The Bray-Curtis distance was used as a metric of similarity. For this analysis we followed the procedures currently used in herpetological studies (Rios-López and Aide, 2007; Hernandez et al., 2015). Firstly, ordination was performed using the routine settings of PC-ORD software. Afterwards, we selected the best solution and performed a new ordination with only one axis. As recommended by McCune and Grace (2002), in order to avoid the local minimum problem, the ordination was performed with a stability criterion < 0.0005 , 500 iterations, and 50 runs for random and real data. The Monte Carlo test was used to assess if the extracted axis was stronger than expected by chance. The Mantel's correlation between the original data matrix (Bray-curtis distance) and the

NMDS ordination axis (Euclidian distance) was performed to evaluate the percentage that the original distances represented in the ordination space. The probability between this association was calculated through the Monte Carlo test with 1000 randomizations. Cluster analysis (ANOSIM) was conducted as complementary to NMDS, in order to statistically check the differences of structure of the amphibian assemblages between habitat categories. Bray-Curtis distance measure was used (Clarke, 1993).

To reduce the set of environmental variables we conducted a Principal Components Analysis (PCA) (Manly, 1994). This analysis allows to order variables correlated in a few axes. The principal components are formed by one or more original variables and explain a percentage of the variation in the multidimensional space, allowing the simplification of environmental variables correlated.

Finally, to verify the relationship between the environmental variables (PCA axes), with the amphibian attributes, richness, abundance and the first axis of NMDS we conducted a Multiple Regression Analysis (MRA). PCA analysis and regressions were performed with the software PAST (Paleontological Statistic), v. 3.11 (Hammer et al., 2001).

3. Results

3.1 Richness, Abundance and Alpha Diversity

We recorded a total of 1025 individuals belonging to 18 species of leaf litter frogs in the six different habitat categories (Table 1). The richness (S_{obs}) between categories did not differ significantly ($H= 6.74$, $p = 0.24$), although showed a rising order, with the RP comprising the lowest number of species ($N=7$) and the OGF the highest number ($N=12$). According to results of estimators, the completeness was between 75 to 100 percent, meaning that a representative proportion of species were sampled (Tabela 2).

Abundance differed significantly between habitat categories ($H= 12.02$, $p = 0.03$), with RP showing the greatest values compared to the other habitats. In relation to evenness, all categories showed a similar pattern, with two dominant species, representing over 50 percent of the total individuals. In some cases, as in RP and PF, dominant species had a high proportion with 81 and 76 percent of total individuals in those categories, respectively. Rare species (doubletons and singletons), were highest in OGF and PF, represented by five species in each category. The rank-abundance curve showed that *Pristimantis* sp. was the dominant species in all categories, except in RP, where, *Pristimantis paulodutra* was the dominant species, followed by *Adenomera thomei* (Figure 2). In general, most species were distributed over more than three habitat habitats, with only few species restricted to one or two habitats. The results of indicator species analysis (ISA) showed that *A. thomei*, *P. paulodutra* and *L. mystaceus* characterized RP, being that the two first had high fidelity and the latter high specificity, occurring only in this habitat. The ISA indicated that *Leptodactylus cupreus* and *Proceratophrys schirchi* were characteristic of the ISL and OGF, respectively.

The true diversity (orders = 0, 1 and 2) at all community did not differ significantly among habitat categories (Table 3), being that habitats ISL and YSF had relatively higher values of diversity followed by MSF and OGF (Figure 3A). The categories RP and PF showed the lowest level of α -diversity. However, when analyzing, forest specialist and disturbance-tolerance species separately, the α -diversity differs significantly among habitat categories (Table 3; Figure 3B). The results for forest specialist species, showed that α -diversity from RP and PF was significantly lower than for other categories. Considering $q = 1$, α -diversity values from OGF ($> \alpha$ -diversity) was 3.1 and 2.0 times higher than from RB and PF, respectively. The opposite happened for disturbance-tolerance species, where the highest values of α -diversity are

represented by RP, PF and YSF. The remaining categories (MSF, ISL and OGF) had no values of α -diversity (Figure 3C).

3.2. Similarity in species composition among habitat categories

Both mean proportion and similarity (S) with OGF species, increased significantly with the habitat categories ($F= 41.65$, $p < 0.01$; $F = 52.55$, $p < 0.01$, respectively). Turkey pairwise comparison shows that RP and PF, differ significantly from others categories, representing a mean proportion of $15(\pm 0.13)$ and $42(\pm 0.11)$ percent of OGF species, respectively. The mean proportion of OGF species in YSF, MSF and ISL did not differ significantly, and corresponded to $55(\pm 0.15)$, $54(\pm 0.19)$ and $61(\pm 0.14)$ percent of OGF species, respectively (Figure 4A). The same pattern was found for similarity (S), with mean values of $0.17(\pm 0.14)$ of the RP differing significantly from others categories. The similarity value $0.48(\pm 0.10)$ of the PF differed significantly only from ISL. The similarity with OGF species from others categories was $0.45(\pm 0.11)$ in YSF, $0.56(\pm 0.14)$ in MSF, and $0.58(\pm 0.12)$ in ISL (Figure 4B).

3.3. Environmental variables

The results of the Principal component analysis (PCA) for all habitat categories revealed three main axis, which explained 74.6% of the total environmental variation. The first principal components (PC1) explained 48.6% of total variation, followed by PC2 with 14.4% and PC3 with 11.5%. The environmental variables DBH 0 – 40 (0.89), DBH > 100 (0.72), fallen tree-trunks (0.82) and leaf litter mass (0.78) were positively related to the first axis (PC1), while canopy cover (-0.77) and maximum temperature (-0.92) were negatively related to this axis. The minimum temperature (0.83) and DBH 60 – 100

(0.85) were positively related to second (PC2) and third axis (PC3), respectively (Table 4).

The plot between the two first axis of PCA showed that RP , PF, YSF were clearly separated from other habitat categories by the PC1, which was influenced by the density of small and large trees, fallen tree trunks, leaf litter density; highest canopy cover and temperatures (Figure 5). In relation to the PC2 there was an overlap of most habitat categories, except by OGF, which was separated from YSF and PF. PC2 was influenced only by minimum temperature.

3.4. Structure of anuran communities

The results of the NMDS analysis showed a good stress value (15.88) for the amphibian community ordination, considering that original data were reduced to a single axis. Mantel's test showed high correlation ($r^2 = 0.98$, $p < 0.001$) between dissimilarities matrix, representing community structure. The Monte Carlo's test indicated that the NMDS analysis extracted a stronger axis than expected by chance ($p = 0.002$). ANOSIM shows that composition between habitat categories differs significantly ($r = 0.52$, $p = 0.0001$). The results of pair-wise comparisons, shows that RP differs significantly from all other habitat categories; PF differs from MSF, ISL and OGF; and YSF differs from ISL.

The results of multiple regression analysis between the NMDS axis and the three first axes of PCA revealed a significant correlation of the leaf litter amphibian assemblage structure with environmental variables ($F = 35.44$, $r^2 = 0.84$, $p < 0.001$; Figure 6). However, the ANOVA test to partial regressions showed significant association only between NMDS axis and PC1 ($r^2 = 0.34$, $p < 0.01$).

The gradient constituted by the higher values of canopy openness and maximum temperature; the increased of small and large-sized trees, fallen tree trunks and leaf litter density, had an influence on the anuran community structure along of the forest successional categories. Thus, the Rubber plantation and initial categories (e.g. Pioneer forest) tended to present low environmental complexity and high temperatures levels, while advanced categories (e.g. MSF, ISL and OGF) showed an increase in environmental complexity and milder temperatures.

4. Discussion

4.1. Richness, Abundance and Alpha Diversity

Our results do not suggest significant changes in species richness and alpha diversity ($q = 1$ and 2) across the habitat categories, although there was a slight tendency of increase in from more disturbed to less disturbed forest. Some studies report the same pattern (e.g. Herrera-Montes and Brokaw, 2010; Hilje and Aide, 2012; Cruz-Elizalde et al., 2016), while others report an abrupt increased in both species richness and diversity from plantation areas towards pristine forest as along forest succession (e.g. Gardner et al., 2007; Bernie et al., 2013). A compilation of the data from studies on the replacement along a chronosequence shows that species richness in secondary forest can be exactly the same as in an old-growth forest <40 years after abandonment (Hernandez et al., 2015). Our results corroborate this statement, as an intermediate stage of succession forest (YSF \pm 30 years abandonment) at our site had a similar species richness and diversity as in MSF, ISL and OGF. However, according to Cortés-Gomes et al. (2013), the species richness is not an efficient attribute to assess the replacement of amphibians in forest chronosequence studies. These authors suggest that in fragmented landscapes, ecotone areas can favor the occurrence of disturbance-tolerance

species in some secondary forests, increasing the values of species richness. However, some species require the structural complexity only found in old-growth forest (Dunn 2004). As expected, our results show that forest specialist species increased significantly toward later successional and more intact forest. Undisturbed forest contains greater environmental complexity, and consequently greater availability of resources that tend to be more efficiently used by forest specialists (Vallan, 2002; Ríos-Lopes and Aide, 2007). The opposite is recorded for disturbance-tolerant species, where high values were reported in degraded categories (RP and PF) and with for the other forest categories.

In relation to assemblage size, our result shows that amphibian abundances were significantly higher in rubber plantations than all other successional habitat categories. Comparisons of leaf litter herpetofauna between a chornosequence of abandoned cacao plantations to pristine forest showed that the abundance values were greater in more recently disturbed sites (Heinen, 1992). However, this pattern can be influenced by the disproportional high abundance of common species, as is shown in our results, where *Pristimantis paulodutra* and *Adenomera thomei* represented about 80 percent of total abundance in RP. According to Hernandez et al. (2015), after the initial successional stages, the abundance of amphibians in ≤ 40 year old secondary forest, can reach similar values of abundance as those in old-growth forest. Our data shows that amphibian abundances did not increase significantly beyond the Pioneer Forest (± 20 years abandonment) stage.

4.2. Similarity in species composition

Unlike of the relatively rapid recovery time of leaf litter amphibian richness and diversity as forest succession progresses (both observed in our results as well as in

previous studies e.g. Gardner et al., 2007; Bernie et al., 2013), the recovery of composition of amphibians assemblages is a slower process and will likely take decades before it resembles the toward old-growth forest assemblage. Species compositional similarity between tropical mature secondary forest and old-growth forest ranges from 42 – 75 percent (Hernandez et al., 2015). A quantitative review of 65 studies found that regenerating secondary forests have a mean similarity of 0.58 (\pm 0.006) of faunal composition as old-growth forest (Dent and Wright, 2009). Our results corroborate these findings, with values for Mature Secondary Forest (\pm 50 years abandonment) representing 54 and 56 percent of the proportion and similarity of species to old-growth forest, respectively.

The recovery of composition of the amphibian assemblages during secondary forest succession can be affected by different variables. Studies have found that tropical secondary forest succession can occur at an accelerated rate, with a rapid increase in forest complexity (Guarigata and Ortertag 2001; Siminski et al., 2011). Thus, species that depend on environmental features that recover rapidly will benefit and have a faster recovery (Dent and Wright, 2009). In general, amphibians are sensitive to environmental changes, and in tropical forests; these changes can result in complex responses (Vitt et al., 1990; Tocher et al., 2002; Rödel and Ernst, 2005). Our results show a clear replacement of amphibian species along the continuum of successional trajectories. For example, *Pristimantis paulodutraei*, *Adenomera thomei*, *Leptodactylus* cf. *latrans* and *L. mystaceus* tolerate a wide range of environmental conditions, and thus are considered disturbance-tolerant species (Pimenta and Junca, 2004; Angulo, 2008; Heyer et al., 2010; Heyer and Rodrigues, 2010). The abundance of these species decreased rapidly from disturbed categories toward old-growth forests, being reported only in RP, PF and rarely in YSF. On the other hand, some species are forest specialists

and are highly sensitive to disturbance and only found in old-growth or mature secondary forest. This is the case of *Adelophryne mucronatus*, *Leptodactylus cupreus*, *Macrogenioglottus alipioi* and *Proceratophrys schirchi*, that were reported only for OGF, ISL and MSF. The two first species (*A. mucronatus* and *L. cupreus*) have been reported especially from fragments of pristine or undisturbed forest (Lourenço-de-Moraes et al., 2012; Cassini et al. 2013) which corroborates our results. Although *P. schirchi* and *M. alipioi* are considered species tolerant to a certain degree of habitat modification (Pimenta et al., 2004; Carvalho-e-Silva et al., 2010), they were reported only in old-growth forest categories in the present study. Furthermore, the record of these species may be related to abundance, because, although they are tolerant, they should have a greater abundance in most preserved sites, which would facilitate the record. Additionally, fieldwork carried out in southern Bahia, found *P. schirchi* only in undisturbed areas (Mira-Mendes pers.comm).

Another factor that plays an important role in the recovery of amphibian assemblages in secondary tropical forest are specific characteristics of the landscape (Chazdon, 2003; Gardner et al., 2007; Hilje and Aide 2012). The degree of isolation of the secondary forest is an important factor and has been related with the recovery time of species composition. Gardner et al. (2006) shows that species composition in secondary forest reaches approximately two-third of the similarity of that of old-growth forest, after only 14 – 19 years of abandonment. In that study, secondary forest sites were located at a mean distance of 1,316m (650 – 2800m) from continuous primary forest (Gardner et al., 2006). Amphibians from tropical secondary forest in Mexico (23 years of abandonment), showed 55% of similarity to old-growth forest, however, the sites were located approximately 200m distance from pristine forest (Hernandez et al., 2015). By contrast, some studies carried out in secondary forest in Puerto Rico (1 – 5 years of

abandonment) and Costa Rica (10 – 16 years after abandonment) reached similar values of composition as that of old-growth forest (Ríos-López and Aide 2007; Herrera-Montes and Brokaw 2010; Hilje and Aide 2012). In Puerto Rico all sites of secondary forest were located less than 100m from pristine forest, while in Costa Rica they were approximately at 500m. In our study, the distance of secondary forest to pristine forest was a mean of 400m (200 – 1000m). Most of the landscape of REM shelters 40% of forest cover and a high diversity of agroforestry systems, linked by riparian vegetation (Flesher, 2015). These connections should facilitate the dispersal of amphibian species between sites and consequently reduce the recovery time of amphibians in secondary forest (Ficetola et al., 2008; Hilje and Aide, 2002). However, our results showed that even in Mature Secondary Forest (± 50 years of abandonment) the similarity was a mean of 54% (range 33 – 77%) as that of the old-growth forest. Thus, although the degree of isolation and the connectivity of secondary forest play an important role in the recovery of amphibian species compositions, other environmental factors may also be at play along of successional trajectories.

4.3. Environmental variables and community structure

The process of forest succession forms a gradient of vegetation structure, which may reflect changes in forest complexity and habitat heterogeneity (Tews et al., 2004). Consequently, these changes in the forest structure may influence the pattern of amphibian compositions at different scales (Bastazini et al., 2007; Vasconcelos et al., 2009). Our result shows that vegetation characteristics, along with abiotic features are directly related to the structure of the leaf litter amphibian community along forest succession gradients.

For amphibians, microclimatic factors, especially moisture and temperature, are essential for the maintenance of physiological and metabolic functions (Duellman and Trub, 1994; Wells, 2007). Canopy openness and temperature were the environmental variables that most contributed negatively to the first component. These variables are closely related, and the way that they are arranged may determine vegetation structure and environmental conditions. For example, in RP and PF the more open canopies may influence the environmental temperature by stimulating microclimatic changes, which tends to be negative for forest specialist species, while it favors disturbance-tolerant species (Ernest et al., 2007). Current studies have shown that tropical forest species exhibit low thermal tolerance and are live close to their physiological limits (Duarte et al., 2012; Tejedo et al., 2012; Gutiérrez et al., 2016). This is the case of the species of the genus *Adelophryne*, miniature frogs that inhabit forest leaf litter (Hedges et al., 2008). Critical thermal limits (CT_{max}) of the species from REM are approximately 34°C (D.S. Ruas unpub. data). In our study, we found three species of the genus, especially in YSF, MST, ISL and OGL. Only three individuals of one of the species were found in a Pioneer forest, and none in Rubber plantations, which reaches mean values of maximum temperature of 34.4° and 42°C, respectively. Thus, our results show that at least in the Rubber groves, high temperatures associated with other environmental variables (e.g. low density leaf-litter and greater canopy openness) may create conditions that make these habitats inhospitable to some species. Other studies have reported the importance of canopy openness and temperature in structuring amphibian communities in the tropics (Urbina-Cardona and Londoño 2003; Cortés et al., 2008; Cortéz-Gomés et al., 2013).

The density of small and large trees, leaf litter, and fallen tree-trunks were the variables that mostly contributed positively to the first component. According to Hillers et al.

(2008) changes in environmental variables, such as leaf litter characteristics and tree size, can cause significant changes in the composition of amphibian assemblages. These findings agree with the results of others studies (e.g. Van Sluys et al., 2007; Córtez-Gómez et al., 2013). Leaf litter density plays an important role especially for terrestrial amphibians, influencing prey availability, shelter areas and providing suitable microclimatic environments (Fauth et al., 1989; Van Sluys et al.; 2007). Our results showed a slight decrease in both density of leaf litter and fallen tree-trunks from old-growth forest disturbed areas. However, the change in these variables was more pronounced in the Rubber groves. Compared with forest areas, RP produce few leaves, and is a deciduous tree resulting in a thin layer of leaf litter, creating environmental conditions that only few species can tolerate. When considering only chronosequence of secondary forest habitats, density of leaf litter and fallen tree-trunks do not present a relevant weight. Although other studies have shown the same pattern (e.g. Allmon, 1991; Siqueira et al., 2014), the density of leaf litter seems to play an important role for amphibians that inhabit the forest floor.

5. Conclusions

The results found in the REM suggest that secondary forests of the region with 30+ years of abandonment reach values of richness and alpha diversity of leaf litter amphibians similar to those found in old-growth forests. As shown in other studies, the proportion and similarity of old-growth species increased with secondary forest succession and degree of forest intactness. However, the recovery of richness and diversity in the composition of amphibian assemblages takes place significantly more slowly.

Rubber groves had values of richness and diversity similar to the other habitat categories, however when analyzed for the adequacy of this habitat for forest specialists RP proved a poor habitat with the lowest values of richness and diversity and the lowest in the proportion and similarity to old-growth species. However, when abandoned rubber groves are associated with forest fragments, there is a relative increase in species diversity after 10 years.

Finally, our study has shown that both vegetation structure and microclimatic variables contribute to explain patterns of leaf liter amphibian assemblage structure in tropical secondary forest succession.

Thus, our results shown that tropical secondary forests play an important role in the conservation of leaf-liter amphibians, and linked to landscape features and local environmental conditions, may provide the rapid recovery of amphibian species in relation to old-growth forests. According to Chazdon et al. (2009) all secondary forest, especially more species-rich secondary forest near protected areas should be highest protection priorities. The REM is a private area that comprises 3,096 ha, of which 1,800 ha are represented by tropical forest lowlands with different regeneration stages. Although, the landscape surrounding (1,000 km²) shelters 40% of forest cover, including a contiguous forest fragment with 13,000 ha, most is comprised by unprotected areas. Regarding the high diversity of amphibians found in the region (Chapter 1) and the rapid potential of recovery of amphibians in secondary forests succession, we encourage the planning of actions for the implementation of new protected areas.

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Table 1 List and abundance of 18 species of amphibians recorded in six habitat categories in southern Bahia, Brazil (Rubber plantation = RP, Pioneer forests = PF, Young secondary forest = YSF, Mature secondary forest = MSF, Intensive selectively logged = ISL, Old-growth forest = OGF).

Order/Family/Species	RP	PF	YSF	MSF	ISL	OGF
Bufonidae						
<i>Rhinella hoogmoedi</i>	13	1	5	1	0	1
Craugastoridae						
<i>"Eleutherodactylus" bilineatus</i>	0	0	13	5	1	3
<i>Haddadus binotatus</i>	0	13	17	17	38	39
<i>Pristimantis paulodutraei</i>	124	9	5	0	0	0
<i>Pristimantis</i> sp.	2	74	50	56	44	65
<i>Pristimantis vinhai</i>	18	28	6	26	16	18
Eleutherodactylidae						
<i>Adelophryne</i> cf. <i>pachydactyla</i>	0	3	5	4	2	1
<i>Adelophryne mucronatus</i>	0	0	0	18	17	7
<i>Adelophryne</i> sp.	0	0	25	37	23	13
Leptodactylidae						
<i>Adenomera thomei</i>	107	2	0	0	0	0
<i>Leptodactylus</i> cf. <i>latrans</i>	7	0	0	0	0	0
<i>Leptodactylus cupreus</i>	0	0	0	0	3	1
<i>Leptodactylus mystaceus</i>	13	0	0	0	0	0
<i>Physalaemus camacan</i>						
Microhylidae						
<i>Chiasmocleis cordeiroi</i>	0	1	1	1	2	0
Odontophrynidae						
<i>Macrogenioglottus alipioi</i>	0	0	0	0	4	2
<i>Proceratophrys renalis</i>	0	1	3	4	3	4
<i>Proceratophrys schirchi</i>	0	0	0	0	0	3

Table 2 Observed amphibian species richness (S_{obs}), species predicted by estimators ACE, Cha 1, Cha2 and Jackknife 1, and proportion of completeness according to such estimator by habitat categories (Rubber plantation = RP, Pionner forets = PF, Young secondary forest = YSF, Mature secondary forest = MSF, ISL = Intensive selectively logged ,Old-growth forest = OGF).

Habitat Category	Abundance	S_{obs}	Species predicted ACE	%	Species predicted Chao 1	%	Species predicted Chao 2	%	Species predicted Jackknife 1	%
OGF	156	13	17	75,8	16	81,5	14	94	16	79,6
ISL	156	12	12	97	12	100	12	100	13	93,5
MSF	168	11	12	90,6	12	95,7	11	97,5	13	86,8
YSF	134	11	11	97,9	11	100	11	100	12	93
PF	134	10	13	79,6	12	81,8	13	75	13	75
RP	285	7	7	100	7	100	7	100	7	100

Table 3 Values of true diversity of each habitat category (Rubber plantation = RP, Pionner forets = PF, Young secondary forest = YSF, Mature secondary forest = MSF, Intensive selectively logged = ISL, Old-growth forest = OGF), and results of Kruskal Wallis post-hoc test (Mann-Whitney) from each comparison.

	Orders	RP	PF	YSF	MSF	ISL	OGF	<i>H</i>	<i>P value</i>
Total Community	q = 0	5,0	5,3	7,3	6,3	7,3	7,0	<i>6.08</i>	<i>0.29</i>
	q = 1	3,3	3,2	5,1	4,6	5,4	4,3	<i>8.26</i>	<i>0.14</i>
	q = 2	2,8	2,5	4,0	3,8	4,4	3,3	<i>8.88</i>	<i>0.11</i>
Forest specialist	q = 0	2,0	4,0	6,5	6,3	7,8	7,0	<i>14.38</i>	<i>0.01</i>
	q = 1	1,7	2,6	4,7	4,6	5,4	4,3	<i>14.98</i>	<i>0.01</i>
	q = 2	1,6	2,2	3,8	3,8	4,4	3,3	<i>14.59</i>	<i>0.01</i>
Disturbance tolerance	q = 0	3,0	1,3	0,8	0,0	0,0	0,0	<i>17.27</i>	<i>> 0.01</i>
	q = 1	2,5	1,2	0,8	0,0	0,0	0,0	<i>17.37</i>	<i>> 0.01</i>
	q = 2	2,3	1,2	0,8	0,0	0,0	0,0	<i>17.37</i>	<i>> 0.01</i>

Table 4 Loadings from Principal Components Analysis (PCA) from 10 environmental variables of 23 combined sample units from the Michelin Ecological Reserve, southern Bahia, Brazil.

Environmental variables	PC1	PC2	PC3
DBH 0 - 40	0,8885	0,07032	0,00149
DBH 40 - 60	-0,6941	0,2664	-0,43
DBH 60 - 100	-0,3408	-0,2831	0,8578
DBH > 100	0,7184	0,4792	-0,0359
Tree-trunk fallen	0,8489	-0,1717	-0,0177
Leaf litter	0,8033	0,1228	-0,0906
Shrub	0,3844	-0,5414	-0,2559
Canopy openness	-0,7804	0,2187	-0,2019
Maximum temperature	-0,9199	0,06613	0,0249
Minimum temperature	0,2026	0,8306	0,3449

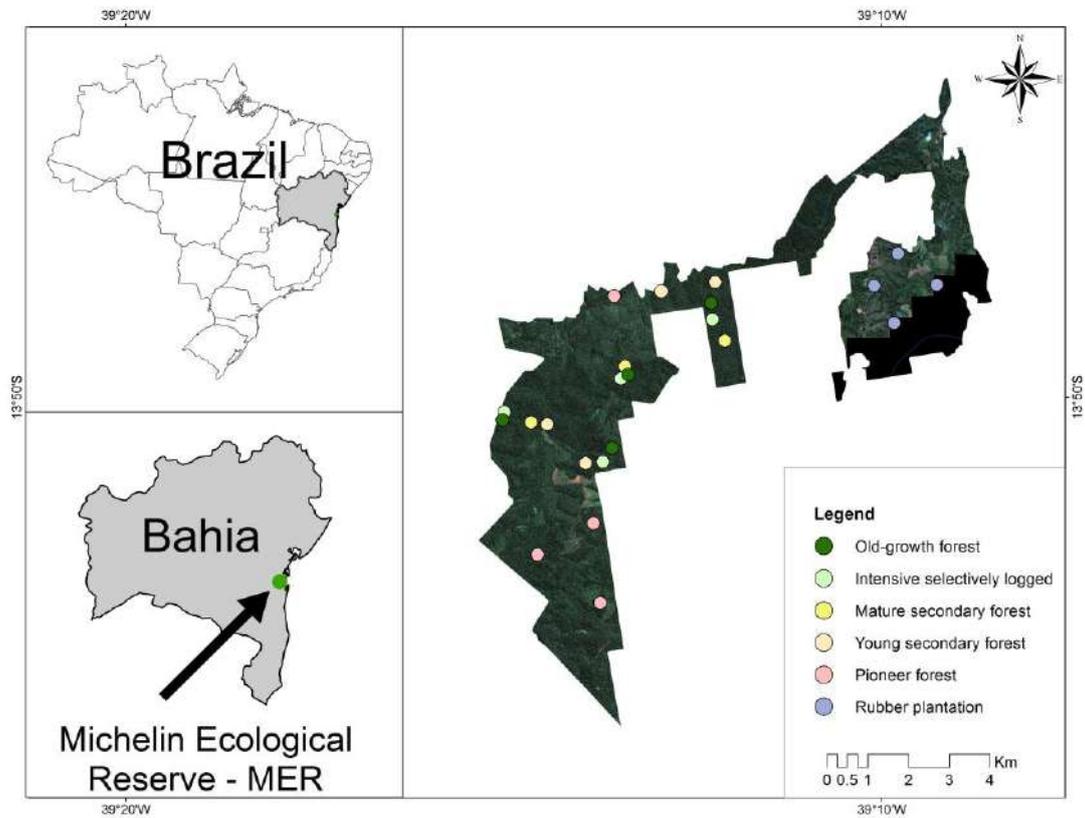


Fig. 1. Location of the 23 sample units from six habitat categories at Michelin Ecological Reserve, southern Bahia, Brazil.

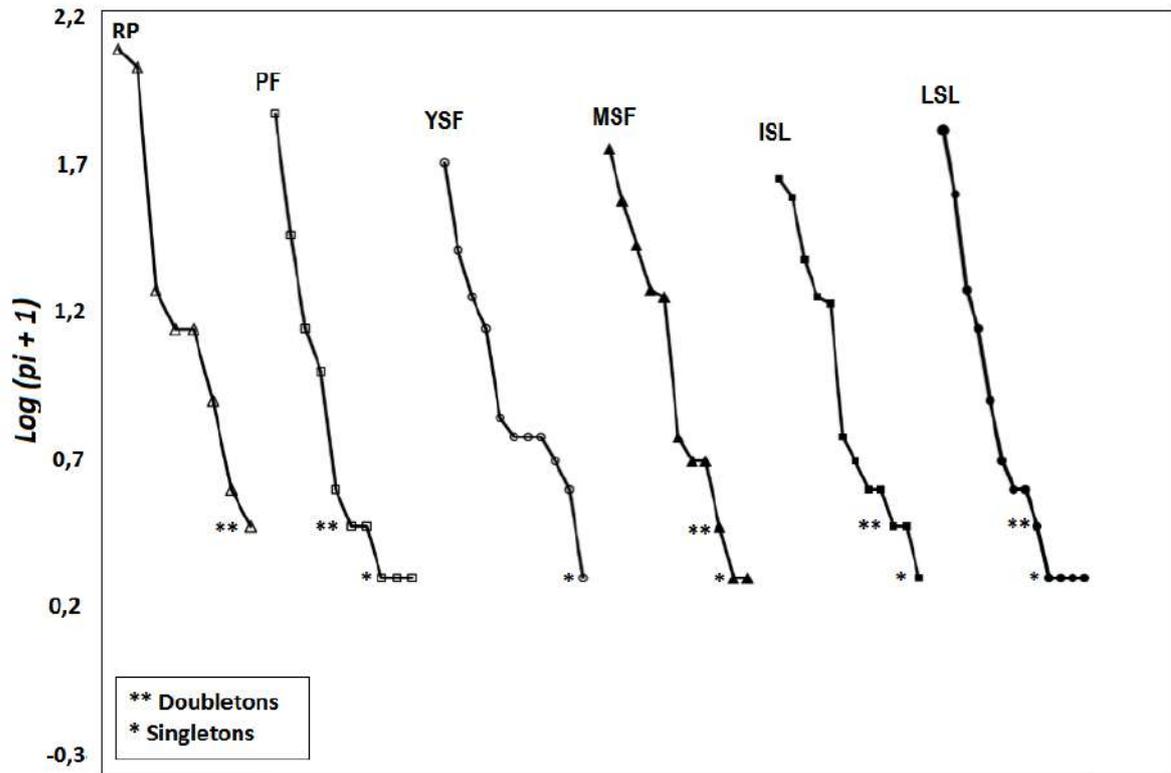


Fig. 2. Rank-abundance curves of amphibians species of the six habitat categories (Rubber plantation = RP, Pioneer forest = PF, Young secondary forest = YSF, Mature secondary forest = MSF, Intensive selectively logged = ISL, Old-growth forest = OGF) from Michelin Ecological Reserve, southern Bahia, Brazil.

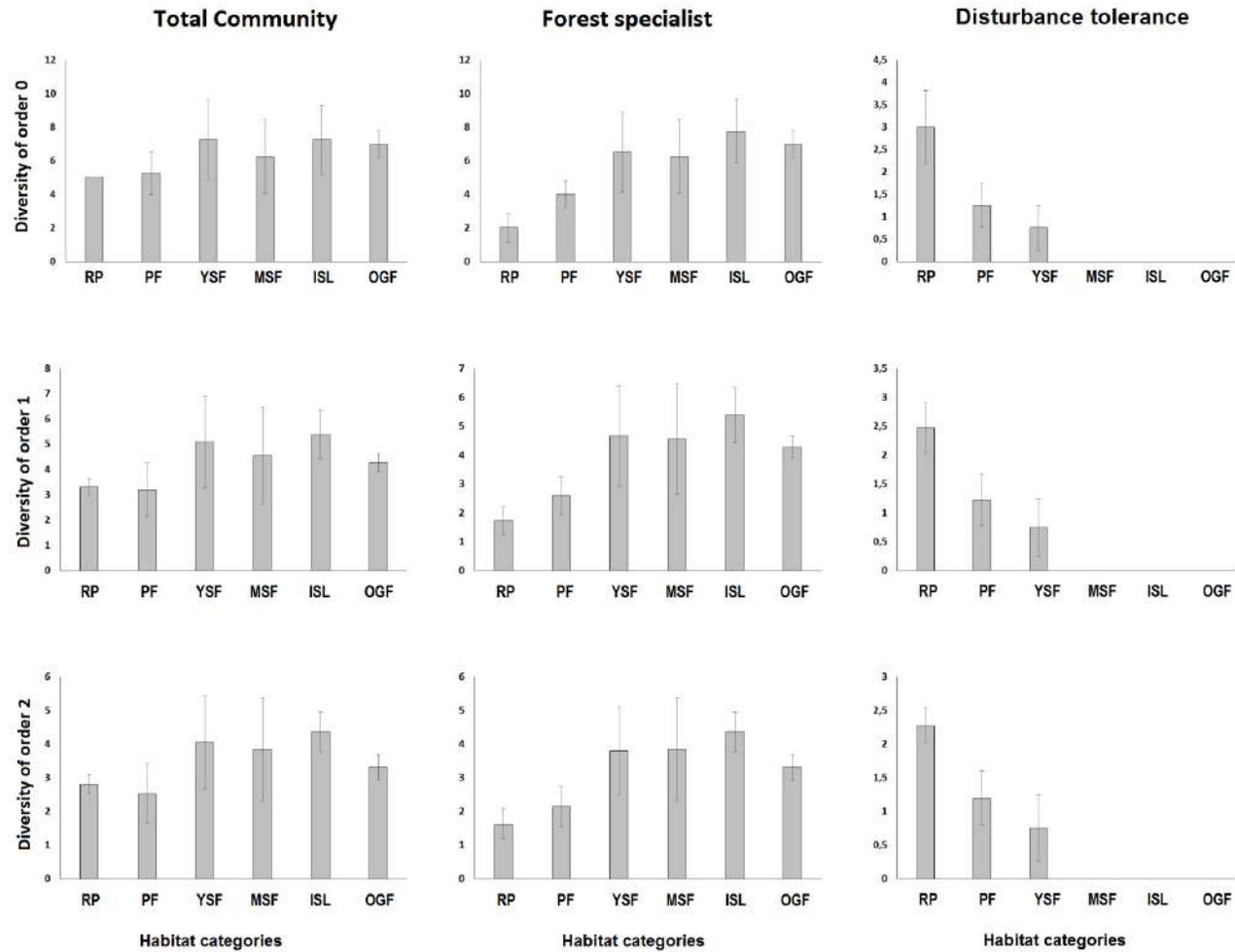


Fig. 3. Diversity of amphibian species of the six habitat categories (Rubber plantation = RP, Pioneer forest = PF, Young secondary forest = YSF, Mature secondary forest = MSF, Intensive selectively logged = ISL, Old-growth forest = OGF) resulting of three comparisons: Total community, Forest specialist and Disturbance tolerance species.

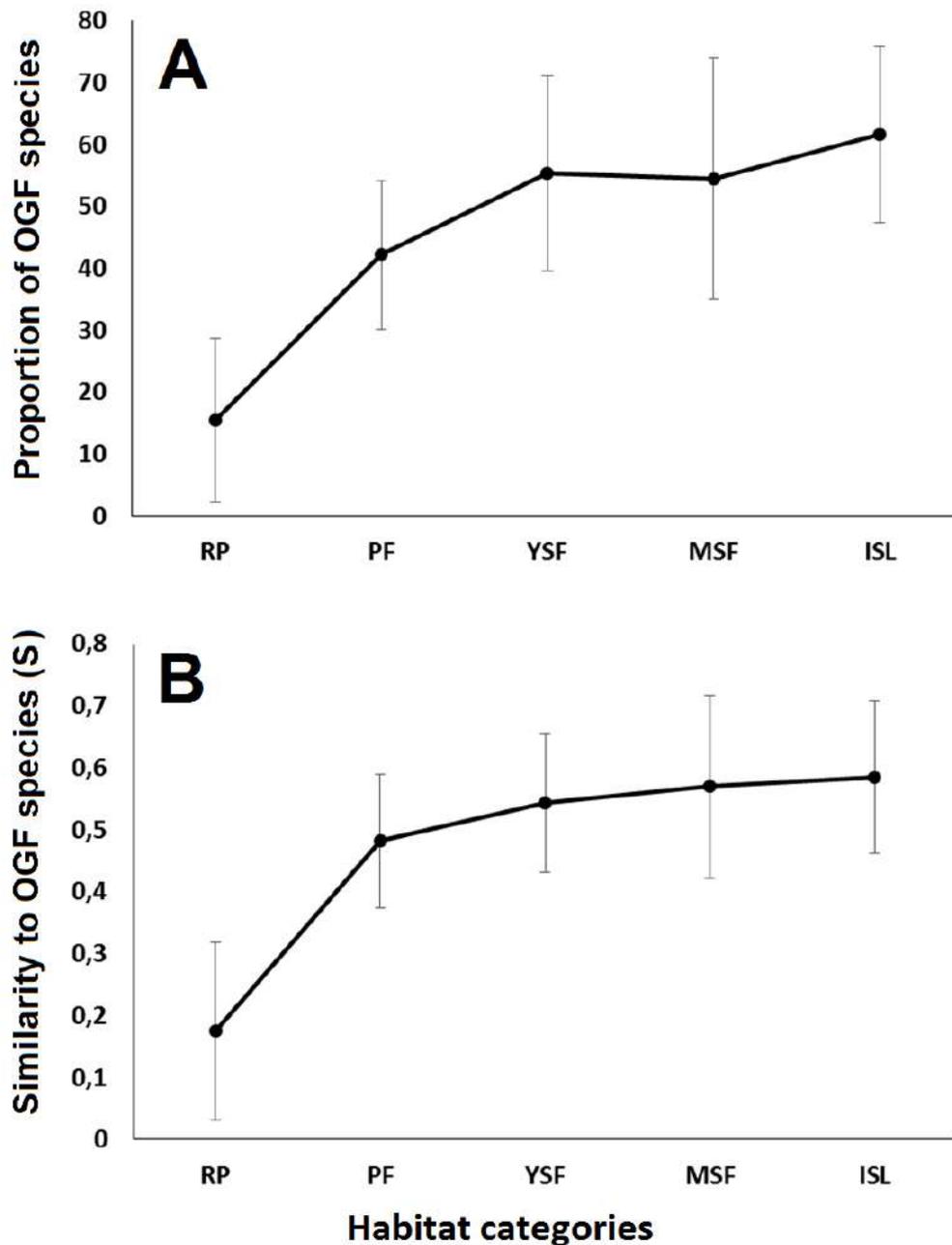


Fig. 4. Proportion of OGF species (A) and Sorensen similarity (*S*) indices (B) comparisons between the habitat categories (Rubber plantation = RP, Pioneer forest = PF, Young secondary forest = YSF, Mature secondary forest = MSF, Intensive selectively logged = ISL, Old-growth forest = OGF) from Michelin Ecological Reserve, southern Bahia, Brazil.

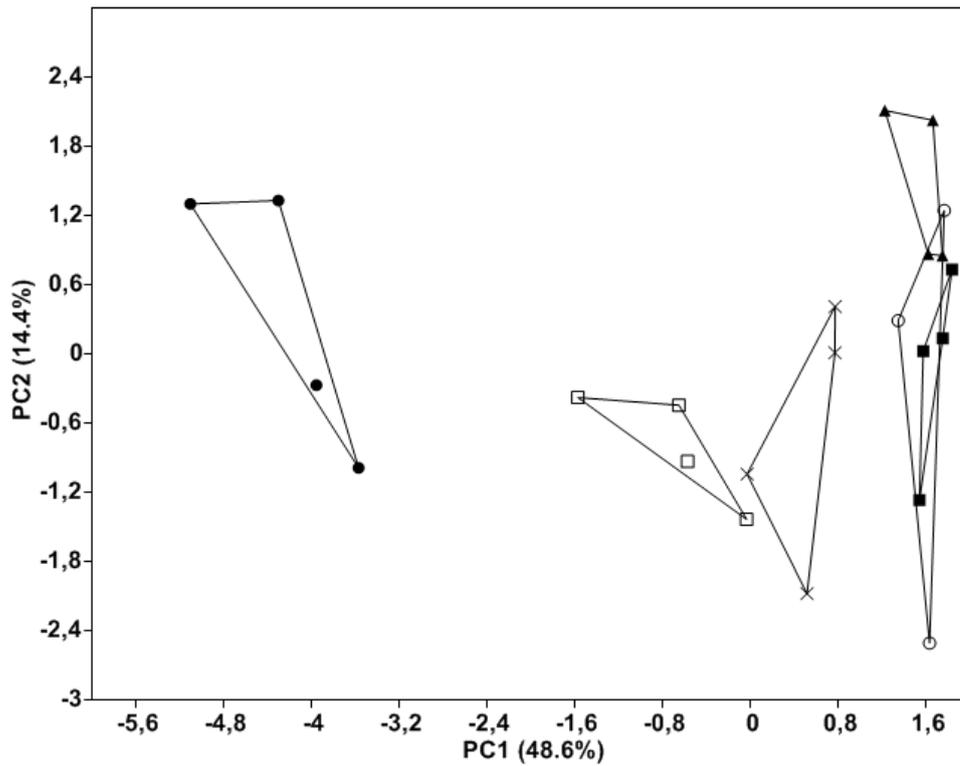


Fig. 5. Projection of individuals scores (PC1 and PC2) from Principal Component Analysis (PCA) for the ten environmental variables. Legend: ● = Rubber plantation, □ = Pioneer forest, × = Young secondary forest, ○ = Mature secondary forest, ■ Intensive selectively logged ▲ Old-growth forest.

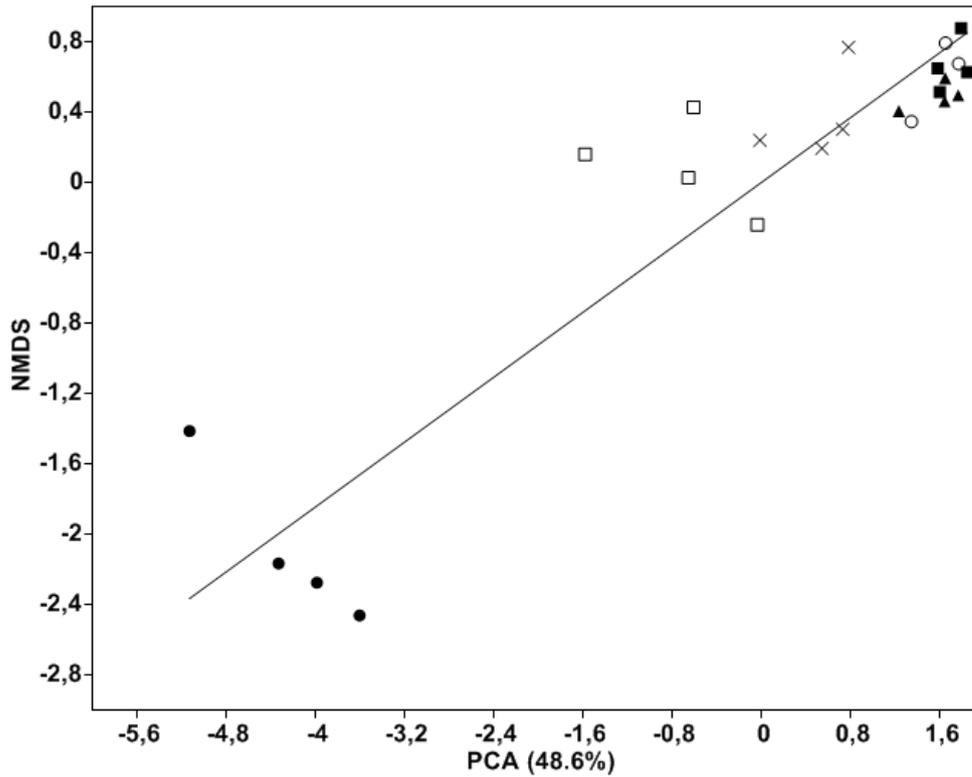


Fig. 6. Partial regression ($r^2 = 0.83$, $p = 0.0001$) of the non-metric multidimensional scaling (NMDS) on the first principal component (PC1). Legend: ● = Rubber plantation, □ = Pioneer forest, × = Young secondary forest, ○ = Mature secondary forest, ■ Intensive selectively logged forest, ▲ Old-growth forest.

Capítulo III



The miniature genus *Adelophryne* (Anura, Eleutherodactylidae): a new species from the Atlantic forest of northeastern Brazil.

(A ser submetido para a revista *Herpetologica*)

**The Miniature Genus *Adelophryne* (Anura: Eleutherodactylidae): a New Species
from the Atlantic Forest of Northeastern Brazil.**

ABSTRACT: The number of species of the genus *Adelophryne* has significantly increased in recent years. Herein we describe a new species of *Adelophryne* from the municipality of Igrapiúna, southern Bahia, northeastern Brazil. The new species is characterized by its small body size, indistinct tympanum, and two phalanges in the finger I and IV with reduction in the first phalange. This new species lives in pristine forest, is probably sensitive to degraded areas and presumably has a small distribution. Thus, we suggest that *Adelophryne "igrapiuna"* sp nov. may be listed as Endangered (EN) under B1ab (iii) criteria of the IUCN Red List.

Key Words: *Adelophryne* sp. nov.; Amphibia; Taxonomy; Southern Bahia.

THE GENUS *Adelophryne* Hoogmoed and Lescure 1984 is represented by poorly known diminutive frogs inhabiting leaf litter of forest, with discontinuous distribution in eastern Brazil, the Guiana Shield, and the upper Amazon Basin (Hedges et al. 2008; Frost et al. 2016). In the article describing the genus, two species were reported: *Adelophryne adiastrata* and *Adelophryne gutturosa* Hoogmoed and Lescure 1984. Ten years after, three additional new species had been described: *Adelophryne baturitensis*, *Adelophryne maranguapensis* and *Adelophryne pachydactyla* Hoogmoed, Borges and Cascon 1994. Until recently, only these five species were recognized, however, from 2008-2016, four new species have been described: *Adelophryne patamona* MaCculloch, Lathrop, Kok, Minter, Khan and Barrio-Amorós 2008; *Adelophryne mucronatus*

Lourenço-de-Moraes, Solé and Toledo, 2012; *Adelophryne meridionalis* Santana, Fonseca, Neves and Carvalho 2012 and *Adelophryne glandulata* Lourenço-de-Moraes, Ferreira, Fouquet and Bastos 2014, currently totaling nine species belonging to the genus.

Molecular phylogeny of Phyzelaphryninae conducted by Fouquet et al. (2012) confirmed the monophyly of the group and revealed the existence of seven more candidate species, of which one was recently described (*see* Lourenço-de-Moraes et al. 2014). According to Fouquet et al. (2012), the genus is represented by three deeply divergent and well-sustained clades that are geographically circumscribed to North Amazonia (NAMC), North Atlantic Forest (NAFC) (from Ceará to northern Bahia) and South Atlantic Forest (SAFC) (from southern Bahia, Minas Gerais and Espírito Santo).

In this paper, we describe a new species of the genus *Adelophryne* from the Atlantic Rain Forest of southern Bahia, northeastern Brazil. We also use a molecular analysis to present new data on the distribution of *A. mucronatus* and *A. glandulata*, and provide new information on *A. sp. 5* and *A. sp. 2* (*sensu* Fouquet et al. 2012).

MATERIALS AND METHODS

Study Area

The new species was found in the forest fragments of the Reserva Ecológica Michelin – REM, located at the municipality of Igrapiúna (13°49'15" S, 39°11'52" W, 343 m above sea level; Fig. 1), southern Bahia, northeastern Brazil. According to Veloso et al. (1991) the region is characterized as Dense Lowland Rain Forest. The MER comprises a total area of 3,096 ha, of which 1,800 ha are represented by three tropical rainforest fragments. Most of the forest is secondary at different stages of succession, and intensively logged forest, with small patches of intact forest areas of

hillsides and hilltops (Flesher 2015). According to Köppen-Geiger's climate classification (Peel et al. 2007), the region has the AF type (Landau and Resende 2003). All specimens were collected at the type locality. Voucher specimens were deposited in the Zoological Collections of Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil (MZUESC). CVMM correspond to Caio Vinícius de Mira Mendes field numbers respectively.

Morphological analysis and specimens

Measurements were taken under a stereomicroscope with digital calipers. The measurements follow Lourenço-de-Moraes et al. (2012, 2014): snout-vent length (SVL); head length (HL); head width (HW); eye diameter (ED); upper eyelid width (UEW); interorbital distance (IOD); internarial distance (IND); eye-nostril distance (END); nostril to tip of snout distance (NSD); eye to tip of snout distance (ETSD); foot length (FL); thigh length (THL); and tibia length (TL). The description of snout shape in lateral view follows Cei (1980) and terminology of ventral and dorsal view follow Heyer et al. (1990); terminology of the tympanum, fingers, toes and pads follow Hoogmoed et al. (1994) and Lourenço-de-Moraes et al. (2012) for terminal tips; terminology of skin texture follow Kok and Kalamandeen (2008). Skeletal characters were determined from two cleared and stained individuals.

Comparisons between species were made from original species descriptions (Hoogmoed and Lescure 1984; Hoogmoed et al. 1994; MacCulloch et al. 2008; Lourenço-de-Moraes et al. 2012; Santana et al. 2012; Lourenço-de-Moraes et al. 2014) and direct examination of specimens (Célio F.B. Haddad Collection, Universidade Estadual Paulista - CFBH, División de Herpetología, Museo Ecuatoriano de Ciencias Naturales, Quito, Ecuador - DHMECN, Museu Nacional do Rio de Janeiro - MNRJ,

Museu de Zoologia João Moojen, Universidade Federal de Viçosa - MZUFV, Museu de Zoologia da Universidade Estadual de Campinas “Adão José Cardoso” - ZUEC, Museu de Zoologia da Universidade Estadual de Santa Cruz - MZUESC, Museu de Biologia Mello Leitão - MBML, Coleção Zoológica da Universidade Federal de Goiás - ZUFG and NUROF-UFC, Núcleo Regional de Ofiologia, Laboratório de Herpetologia da Universidade Federal do Ceará; see Appendix for details)

Molecular procedures

We conducted a phylogenetic analysis using the sequences generated from five specimens of *Adelophryne* collected in MER, Igrapiúna, Bahia. Additionally, we conducted molecular sequencing of a paratype of *A. glandulata* (MZUESC 12180) from Santa Tereza, Espírito Santo. The analysis was conducted together with the data generated by Fouquet et al. (2012) for various species of Phyzelaphryninae. Available sequences in Genbank of *Diasporus diastema* (EU186682) and *Eleutherodactylus coqui* (EF493550), species belonging to Eleutherodactylinae closely related to Phyzelaphryninae (Fouquet et al. 2012), were used as outgroups.

We extracted genomic DNA from the leg muscle of individuals using a standard salt extraction protocol (Bruford et al. 1992). Sequences from partial mitochondrial 16S rDNA were amplified using published primer sets AC_16S_AR (F: 5'-CGC CTG TTT ATC AAA AAC AT-3') and 16SBH (R: 5'- CCG GTC TGA ACT CAG ATC ACG T-3') (Palumbi et al. 2002). PCR Reactions were performed in a final volume of 11.5 µl using the following concentration: 0.3 µl of each primer, 0.25 µl of dNTP, 2.5 µl PCR buffer, and 0.1 µl of GoTaq DNA polymerase (Promega, Mannheim, Germany) and 1 µl of DNA. Amplification conditions consisted of a pre-desnaturation step of 3 min at 92 °C, followed by 38 cycles of a desnaturation step of 3 min at 92 °C, annealing at 48 °C

for 50 sec and extension at 72 °C of 3min and storage at 8 °C. PCR products were purified with enzymatic process: 0.15 units of Shrimp Alkaline Phosphatase (SAP) and 1 unit of Exonuclease I (New England Biolabs, Frankfurt am Main, Germany) incubated for 15 min at 37°C followed by 15 min at 80°C. Purified PCR products were sequenced on an automated DNA sequencer (Applied Biosystems ABI 3130XL). Sequences were checked and edited using CodonCode Aligner 3.7.1(CodonCode Corporation, Dedham, MA, USA).

We generated static alignments in MAFFT (Kato and Toh 2008) with L-INS-i strategy. Sequences were submitted to Genbank (to be added upon manuscript acceptance). We performed Bayesian analyses using MrBayes 3.2 (Huelsenbeck and Ronquist 2011) as implemented in the CIPRES web based platform (Miller et al. 2010) The models of molecular evolution were determined using Partition Finder 1.1.0 (Lanfear et al. 2012). The GTR + I + G substitution model was selected as the optimal nucleotide substitution model for the 16S rRNA. Bayesian analyses included two independent runs, each with four chains and sampling every 1000 generations for 60 million generations. We examined trace plots and effective sample size (ESS) in Tracer v1.6 to determine MCMC mixing and convergence. We removed trees from the first 20% of the samples as burn-in. A consensus of the post-burning trees was visualized in Fig Tree v1.4.2. Genetic distances (p-distance) were also computed for mitochondrial loci using MEGA 5.1 (Tamura et al. 2011).

RESULTS

Adelophryne "**igrapiuna**" sp. nov.

(Figs. 2, Table 1)

Holotype.— Adult female (CVMM 001 ; Fig. 2) collected on March 2012, in forest areas at REM, municipality of Igrapiúna, State of Bahia, northeastern Brazil (13°49'15" S, 39°11'52" W, 95 m above sea level). Collected by MS, CVMM and DSR.

Paratopotypes.— Seven adult males (CVMM 002 – 008), and twelve adult females (CVMM 009 – 020). These specimens were collected between March 2012 to October 2014 in forest fragments of REM, municipality of Igrapiúna, State of Bahia, northeastern Brazil (13°49'15" S, 39°11'52" W, 95 m above sea level). Collected by CVMM, DSR and RMO.

Diagnosis.— The new species is included in the subfamily Phyzelaphryninae based on the molecular evidence and due to the apically pointed digits. It inhabits the leaf litter of forest floor; the terminal digits are either barely or not expanded; none exceeds 20 mm in SVL (Hedges et al. 2008). In addition to the results of molecular analysis, the generic assignment of *Adelophryne "igrapiuna"* sp. nov. is based on the possession of a head narrower than body, cranial crests absent, small size, finger IV with single subdigital pad and mucronate tip, toes III and IV with discs and mucronate tips, and terminal phalanges of toes and fingers T-shaped.

Adelophryne "igrapiuna" sp. nov. can be distinguished from species of the genus *Phyzelaphryne* by not presenting subarticular tubercles, by presenting an indistinct tympanum, and reduction of the phalanges in finger IV. *Phyzelaphryne* has subarticular tubercles, distinct tympanum and no reduction of the phalanges (*see* Hoogmoed and Lescure, 1984; Hogmoed et al. 1994; Hedges et al. 2008).

The new taxon is diagnosed by the following combination of character states:

(1) snout-vent length smaller than 11.4 mm (in males 7.6–9.1, in females 10.0–11.4); (2) tympanum indistinct without visible membrane; (3) tympanic annulus absent; (4) dentigerous processes of vomers present; (5) fingers without terminal discs, fingers with mucronate tips, terminal phalanges T-shaped; (6) toes with terminal discs or circumferential grooves and mucronate tips; (7) terminal phalanges of toes T-shaped and sharply reduced; (8) finger I shorter than finger II; (9) finger I and IV with two phalanges; (10) toe III longer than toe V; (11) belly skin smooth; (12) dorsum skin smooth; (13) anal flap absent.

Comparison with other species.— *Adelophryne "igrapiuna"* sp. nov. is distinguished from all other congeners by having reduction of pads formula (2–3–4–2 in *A. pachydactyla*; 1–1–2–2 in *A. maranguapensis*; 1–1–2–1 in *A. adiaastola*, *A. gutturosa*, *A. meridionalis* and *A. patamona*; 1–2–3–1 in *A. mucronatus*; 1–2–2–1 in *A. glandulata*; 1–1–1–1 in *Adelophryne "igrapiuna"* sp. nov.). It also can be distinguished from all other congeners (except *A. glandulata* and *A. meridionalis*) by having an indistinct tympanum (distinct in *A. baturitensis*, *A. adiaastola*, *A. gutturosa*, *A. maranguapensis*, *A. mucronatus*, *A. pachydactyla*, and *A. patamona*). The new species is distinguished from *Adelophryne baturitensis*, *A. gutturosa*, *A. maranguapensis*, *A. mucronatus*, and *A. patamona* by its smaller size with maximum SVL of 11.4 mm in *Adelophryne "igrapiuna"* sp. nov (maximum SVL - 16.3 mm in *A. baturitensis*, 16.0 mm in *A. gutturosa*, 17.4 mm in *A. maranguapensis*, 14.9 mm in *A. mucronatus*, and 23.0 mm in *A. patamona*) and by having two phalanges in finger IV (three phalanges in finger IV in species mentioned above). *Adelophryne "igrapiuna"* sp. nov is distinguished from *A. adiaastola*, *A. glandulata*, *A. mucronatus*, and *A. patamona* by skin texture of dorsum

(shagreened with small and rounded granules in *A. glandulata*, smooth with scattered small granules in *A. mucronatus*, shagreened to granular in *A. adiastrata*, tuberculated in *A. patamona* and smooth in *Adelophryne "igrapiuna"* sp. nov). It can also be distinguished from *A. glandulata* and *A. gutturosa* by the absence of a distinct glandular ridge line that runs from the posterior part of eye to the insertion of the forelimb. It is distinguished from *A. meridionalis* by having toe I, II, III, and IV with circumferential grooves or disc (only toe IV with circumferential grooves in *A. meridionalis*), and from *A. baturitensis* and *A. maranguapensis* by lacking discs or circumferential grooves on fingers. *Adelophryne "igrapiuna"* sp. nov. is distinguished from *A. mucronatus* and *A. maranguapensis* by the absence of an anal flap (anal flap present in *A. mucronatus* and *A. maranguapensis*).

Description of the holotype.— Adult, SVL 10.5 mm (Fig. 2). Snout rounded slightly triangular in ventral and dorsal view (Fig. 2B, C) and truncate, slightly rounded in lateral views (Fig. 2A). ETSD larger than the ED. END smaller than the IND. Nostrils not protruding and round. IND slightly larger than the IOD. Canthus rostralis indistinct; loreal region slightly concave. Choanae small, round, located very laterally. Dentigerous processes with two rows of four; widely separated below choanae, centrally localized. Tongue ovoid, free except its anterior margin. Pupil horizontally oval. Upper eyelid slightly convex. Temporal region vertical, tympanum indistinct. Skin texture of venter, dorsum and limbs smooth; flanks and ventral region of thighs areolate. Anal flap absent, cloacal opening horizontally positioned at slightly below the level of the dorsal surface of the thigh. Fingers without disks; fingers I, II and III with mucronate tips, finger IV with rounded tip. Fingers thin, depressed and short, without webbing. Finger formula: IV<I<II<III (Fig. 2C). Phalangeal formula 2–2–3–2 (Fig. 3A). Fingers and

palm appear to be surrounded by a narrow strip of transparent skin. Subarticular tubercles absent with round subdigital pads, formula 1–1–1–1; no pads under ultimate phalanges and no supernumerary tubercles. Inner metacarpal tubercle ovoid, outer metacarpal tubercle round, slightly larger than inner. Toes without webbing, cylindrical, slightly flattened. Toes formula: I<V<II<III<IV; toes II, III and IV with discs and mucronate tips, toe V with circumferential grooves and mucronate tips; toe I with circumferential groove (Fig. 2D). Phalangeal formula 2–2–3–4–3 (Fig. 3B). Skin transparent only on the distal portion of toes II, III, and IV. Subarticular tubercles absent with round subdigital pads (0–0–2–1–0); no pads under ultimate phalanges and no supernumerary tubercles. Inner metatarsal tubercle oval; outer metatarsal tubercle smaller and rounded. For measurements see Table 1.

Color.— Venter dark with numerous small white dots. Throat and underside of thighs and shanks beige. Dorsum beige with two stripes lines dark brown of various widths in the middle region of dorsum in “Λ”. Loreal region dark brown, with a dark brown stripe extending along the flanks and reaching the groin, with numerous small white dots. A dark brown stripe interorbital, of various widths in “V”. Thigh and tibia with two or one dark brown band line. Eyes red-orange with black pupil (Fig. 4A). The color pattern does not change in preservative, shades of colors become darker than in life.

Variation.— The specimens can vary the pattern of coloration, with some species showing a gray-bluish dorsal coloration (Fig. 4B). The forelimbs can be brown-reddish. The coloration of venter can be scattered with dark spots or uniform. The

formula of pads and discs in the toes vary in number; males are small, toes IV and V are more depressed than females. For morphometric variation see Table 1.

Etymology.— The specific name will be presented when we proceed to the submission for publication. For this paper we apply the temporary name “igrapiuna” not latinized, in honor to the municipality of Igrapiúna, Bahia.

Common name.— Igrapiuna Flea-frog or Rãzinha-pulga igrapiuna (in Portuguese).

Molecular analysis.— Our phylogenetic results recovered *Adelophryne "igrapiuna"* sp. nov. in the North Atlantic Forest Clade - NAFC, formed by *A. baturitensis*, *A. maranguapensis*, *A. sp.1*, *A. sp. 2* and *A. sp. 3* (Fig. 5). The phylogenetic relationships among the species of this clade were not established, probably only one molecular marker was used. Based on the phylogenetic position and high molecular divergence, *Adelophryne "igrapiuna"* sp. nov. does not represent any of the candidate species listed by Fouquet et al. (2012). Values of genetic divergence of *A. "igrapiuna"* sp. nov. with other species of the genus range between 10.3 to 20.7% (Table 2). The remaining samples recovered two other species that occur sympatrically with *A. "igrapiuna"* sp. nov. in MER. One corresponds to *Adelophryne mucronatus* (two specimens) and another one to *A. sp. 2* (one specimen). The results of molecular sequencing recovered the paratype of *Adelophryne glandulata* as the *A. sp 5* (*sensu* Fouquet et al. 2012).

Geographic Distribution.— *Adelophryne "igrapiuna"* sp. nov. is known only from the type locality, at the Michelin Ecological Reserve – MER, municipality of Igrapiúna, southern Bahia, northeastern Brazil.

Natural History and Ecology. — *Adelophryne "igrapiuna"* sp. nov. occurs in the leaf litter of forest areas in the Michelin Ecological Reserve, Igrapiúna, Bahia, Brazil. Advertisement calls of *Adelophryne "igrapiuna"* sp. nov. could not be recorded, especially due to the occurrence of three species of the genus in the REM. Lourenço-de-Moraes et al. (2012) comments on the difficulty of locating calling males. Two large ovarian eggs (2.0 mm) were found in one female of *Adelophryne "igrapiuna"* sp. nov. Data on eggs and clutch characteristics have been reported in the literature for several species of the genus: *Adelophryne baturitensis* and *A. maranguapensis* (Hoogmoed et al. 1994; Cassiano-Lima et al. 2011), *A. gutturosa* (Ayarzaguena and Diego-Aransay 1985; MacCulloch et al. 2008), *A. mucronatus* (Lourenço-de-Moraes et al. 2012) and *A. glandulata* (Lourenço-de-Moraes et al. 2014). As well as most Terrarana, the pattern of direct development of eggs should be characteristic for all species of the genus *Adelophryne*, especially due to the large size of the eggs and the low number per clutch that range from 1 to 8 eggs (Hedges et al. 2008; Cassiano-Lima et al. 2011). We dissected four specimens of *Adelophryne "igrapiuna"* sp. nov. for stomach content analysis. One specimen contained one beetle larva and three specimens revealed ants. Beetles were found in five specimens of *A. glandulata* and ants also were found in specimens of *A. glandulata* (Lourenço-de-Moraes et al. 2014), in *A. gutturosa* (MacCulloch et al. 2008) and in *A. mucronatus* (Lourenço-de-Moraes et al. 2012).

DISCUSSION

The genus *Adelophryne* is among the smallest sized anuran groups of the world and its diversity is still underestimated. Although Fouquet et al. (2012) revealed a high number of candidate species of *Adelophryne*, the same authors highlighted that these results represent a tiny portion of the real potential distribution of the genus, especially due to the low number of sampled localities. The reduced number of digits and phalangeal elements and the loss or reduction of some cranial elements shows the miniaturization within this genus (Estrada and Hedges 1996; Yeh 2002). Some studies have shown high patterns of diversity in miniaturized species (Kraus 2011; Wollenberg et al. 2011; Fusinato et al. 2013). For example, in *Brachycephalus* species, a minute toad genus, the tolerance to certain environmental conditions seems to play an important role in the high diversity and levels of microendemism (Pie et al. 2013; Ribeiro et al. 2015). We believe that miniaturization seems to have been an important evolutionary step for diversity of *Adelophryne* species, especially in the wet-forests of Atlantic Forest. However, miniature species have a high surface to volume ratio, and could be more susceptible to desiccation (MacLean 1985), and this could be an important factor regarding the pattern of distribution of these species. A recent study carried out in MER, comparing the critical thermal maximum (CT_{max}) of *Adelophryne* with maximum temperature (T_{max}) of the environmental, showed that these species are close to their thermal limits (D. S. Ruas, personal observation).

The species of the genus *Adelophryne* are sensitivity to anthropogenic changes and live exclusively in forest areas (Kok and Kalamandeen 2008; Lourenço-de-Moraes et al. 2012, 2014). *Adelophryne "igrapiuna"* sp. nov was collected in a private protected area (Michelin Ecological Reserve) in pristine forest and is probably sensitive to

degraded areas, having presumably a small distribution. Although the landscape of REM comprises a great area of forest cover, which is represented mostly by small fragments, the surrounding of reserve has been affected with increasing areas rubber, cacao and banana plantations, besides highly diverse agroforestry systems. Thus, we suggest that *Adelophryne "igrapiuna"* sp. nov. should be listed as Endangered (EN) under B1ab (iii) criteria of the IUCN Red List (IUCN 2001).

Our molecular results showed the presence of three species of *Adelophryne* in the MER. Besides *Adelophryne "igrapiuna"* sp. nov., we recorded a new population of *A. mucronatus* living in sympatry and syntopy with *A. "igrapiuna"* sp. nov. This record increases the known geographic distribution of *A. mucronatus* 60 km north from its nearest locality (airline), municipality of Itacaré (Lourenço et al. 2012). The same is reported for *Adelophryne* sp. 2 (*sensu* Fouquet et al. 2012), known previously only from municipality of Wenceslau Guimarães, approximately 60 km from MER. Santana et al. (2012) suggested that *A. sp 5* (*sensu* Fouquet et al. 2012) could represent *A. meridionalis*. However, the molecular sequencing results from the paratype of *Adelophryne glandulata*, assign it to *A. sp 5* (*sensu* Fouquet et al. 2012), and the current phylogenetic position of *Adelophryne meridionalis* remains uncertain. Thus, we extend the geographic distribution of *A. glandulata* approximately 180 km west from its type locality, Santa Tereza – Espírito Santo State, to Mariana and Marliéria municipalities, and Serra do Cipó, Minas Gerais.

Although our results of molecular analysis based on gene 16S rRNA shows that *Adelophryne "igrapiuna"* sp. nov. does not belong to any of the candidate species suggested by Fouquet et al. (2012), we cannot affirm the same in relation to *A. sp. 3*. This is because some molecular data of *Adelophryne sp. 3* was missing, and the

mitochondrial gene 16S rRNA was not sequenced, which prevents the use of this species in phylogenetic analyses.

According to our molecular analysis *Adelophryne "igrapiuna"* sp. nov. was included in the North Atlantic Forest Clade – NAFC species (*sensu* Fouquet et al. 2012). This group is formed currently by *Adelophryne baturitensis*, *A. maranguapensis*, *A. sp. 1* (identified as *A. baturitensis* see Loebman et al. 2011), *A. sp. 2* and *A. sp. 3*. Except for *A. sp. 3*, for which we did not have voucher access, the remaining species of the NAFC show similar morphological features, such as SVL greater than 12 mm, three phalanges in finger IV (not confirm to *A. sp. 2*), distinct tympanum and disks in the fingers. On the other hand, these morphological features of the NAFC species are different in *Adelophryne "igrapiuna"* sp. nov.

Herein, we described a new miniature species of *Adelophryne*, highlighting once again that the diversity within the genus is still underestimated. Furthermore, we report for the first time the occurrence of at least 3 species of the genus living sympatrically at the same locality. Thus, we encourage the collection and description of candidates species listed by Fouquet et al. (2012) to enable a better understanding of both the diversity and the evolution of morphological characters within the genus (e.g. reduction in phalanges in finger IV and indistinct tympanum).

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APPENDIX

Additional specimens examined

Adelophryne glandulata BRAZIL: ESPÍRITO SANTO, Santa Teresa, Reserva Biológica Santa Lúcia (MZUESC 12178–81). *Adelophryne adiastrata*: ECUADOR: PASTAZA, Kurintza (DHMECN 4378). *Adelophryne baturitensis*: BRAZIL: CEARÁ, Guaramiranga (CFBH 20469–76); Tiangua (CFBH 24554–67); Viçosa do Ceará (CFBH 24579–85). *Adelophryne maranguapensis*: BRAZIL: CEARÁ, Maranguape (CFBH 24515–27). *Adelophryne mucronatus*: Brazil: BAHIA, Una (CFBH 23672, ZUEC 16169,12139,16626). *Adelophryne pachydactyla*: BRAZIL: BAHIA, Itacaré, RPPN Capitão (ZUEC 18212–13); Una, RPPN Nova Angélica (ZUEC 17825). *Adelophryne meridionalis*: BRAZIL: MINAS GERAIS, Juiz de Fora, Parque Municipal de Lajinha (MZUFV 12625).

TABLE 1. — Measurements of the type series of *Adelophryne "igrapiuna"* sp. nov.
 Values presented in millimeters as mean \pm standard deviation (range).

Measurement	Holotype	Paratopotypes	
	Female	Males (N = 7)	Females (N = 12)
SVL	10.5	8.5 \pm 0.5 (7.6-9.1)	10.7 \pm 0.4 (10.0-11.4)
HL	3.3	3.2 \pm 0.4 (2.5-3.6)	3.7 \pm 0.4 (3.1-4.1)
HW	3.8	3.1 \pm 0.2 (2.8-3.4)	3.7 \pm 0.1 (3.5-3.9)
ED	0.9	1.0 \pm 0.2 (0.9-1.3)	1.2 \pm 0.3 (0.9-1.8)
UEW	0.9	0.8 \pm 0.1 (0.6-1.0)	1.0 \pm 0.1 (0.8-1.2)
IOD	1.7	1.3 \pm 0.1 (1.1-1.5)	1.6 \pm 0.1 (1.4-1.8)
IND	1.2	1.0 \pm 0.1 (0.9-1.2)	1.2 \pm 0.1 (1.0-1.4)
END	1.2	0.7 \pm 0.1 (0.5-0.9)	1.1 \pm 0.3 (0.7-1.5)
NSD	0.7	0.6 \pm 0.1 (0.5-0.7)	0.7 \pm 0.1 (0.6-0.9)
ETSD	1.9	1.3 \pm 0.2 (1.0-1.5)	1.8 \pm 0.3 (1.3-2.4)
FL	7.2	6.7 \pm 1.1 (4.9-8.4)	7.6 \pm 0.5 (6.7-8.2)
THL	4.9	4.5 \pm 0.5 (3.8-5.1)	5.1 \pm 0.2 (4.8-5.5)
TL	4.8	4.1 \pm 0.5 (3.0-4.6)	4.9 \pm 0.2 (4.6-5.2)

TABLE 2. — Genetic divergence (%) between samples of the *Adelophryne*. Values are estimated from a dataset constructed from 364 bp of the 16S rRNA gene.

Species	GenBank	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
<i>A. adiastrata</i>	JX298299																		
<i>A. baturitensis</i>	JX298282	20.7																	
<i>A. glandulata</i>	MZUESC 12180	19.5	22.5																
<i>A. glandulata</i>	JX298287	19.5	22.3	6.2															
<i>A. gutturosa</i>	JX298300	10.7	21.9	20.1	19.8														
<i>A. maranguapensis</i>	JX298285	23.3	14.8	21.6	20.7	23.1													
<i>A. "igrapiuna"</i>	CVMM 004	19.6	11.9	20.7	19.8	20.6	14.2												
<i>A. "igrapiuna"</i>	CVMM 005	19.6	11.9	20.7	19.8	20.3	14.2	0.3											
<i>A. mucronatus</i>	CVMM 030	17.2	21.1	12.3	14.0	17.8	21.0	18.7	18.4										
<i>A. mucronatus</i>	JX298291	19.2	21.1	12.6	14.0	19.8	20.7	19.5	19.3	4.8									
<i>A. pachydactyla</i>	JX298294	20.4	23.5	14.6	16.9	19.0	21.9	22.2	21.9	13.5	14.3								
<i>A. patamona</i>	JX298297	12.5	19.5	17.4	16.9	12.8	20.5	18.8	18.8	16.0	16.6	17.5							
<i>A. sp. 1</i>	JX298284	20.5	13.6	19.7	19.4	21.1	15.7	10.3	10.3	19.7	19.7	22.9	20.2						
<i>A. sp. 2</i>	JX298283	22.1	16.2	25.0	24.4	20.4	19.0	15.0	14.7	23.9	24.4	24.8	21.2	16.5					
<i>A. sp. 2</i>	ID_935*	22.4	16.2	25.6	25.0	21.0	18.8	15.3	15.0	24.2	24.7	24.8	21.5	16.8	0.8				
<i>A. sp. 4</i>	JX298290	19.2	20.5	12.3	14.6	18.7	22.4	18.1	17.8	10.9	13.2	13.8	16.3	19.4	21.1	21.6			
<i>A. sp. 7</i>	JX298295	18.9	21.3	17.7	17.7	19.1	21.3	21.4	21.4	17.2	17.7	18.4	15.7	22.5	25.6	25.9	14.5	-	

* number of fieldwork

CAPTIONS FOR FIGURES

FIG. 1. — Map showing the known distribution of Atlantic Forest species of the genus *Adelophryne* and candidate species (*sensu* Fouquet et al. 2012).

FIG. 2. — Adult holotype of *Adelophryne "igrapiuna"* sp. nov.. Dorsal (A), ventral (B), and lateral (C) views of head, ventral views of hand (D) and foot (E).

FIG. 3. — Views of hand (A) and foot (B) of a cleared and stained paratype *Adelophryne "igrapiuna"* sp. nov.

FIG. 4. — Adult individuals of *Adelophryne "igrapiuna"* sp. nov. in life. (specimens not identified). Individual (A) showed common coloration, individual (B) showed coloration gray-bluish not usual.

FIG. 5. — Bayesian phylogenetic inference among analyzed species based on 16s rRNA. Posterior probabilities are indicated to the left of nodes. Asterisks represents posteriors equal or higher than 0.95 and numbers below 0.7 are not show. GenBank access code after the species name.

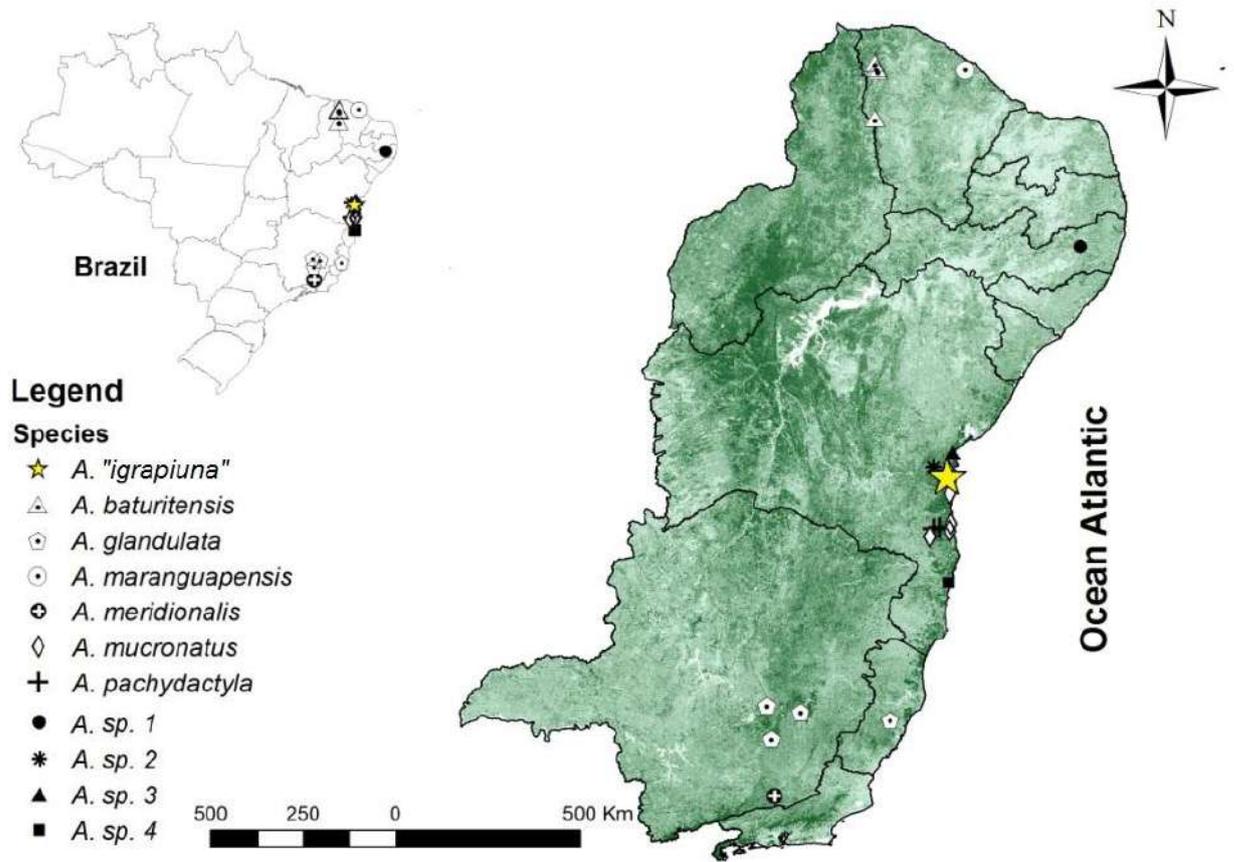


Figure 1.

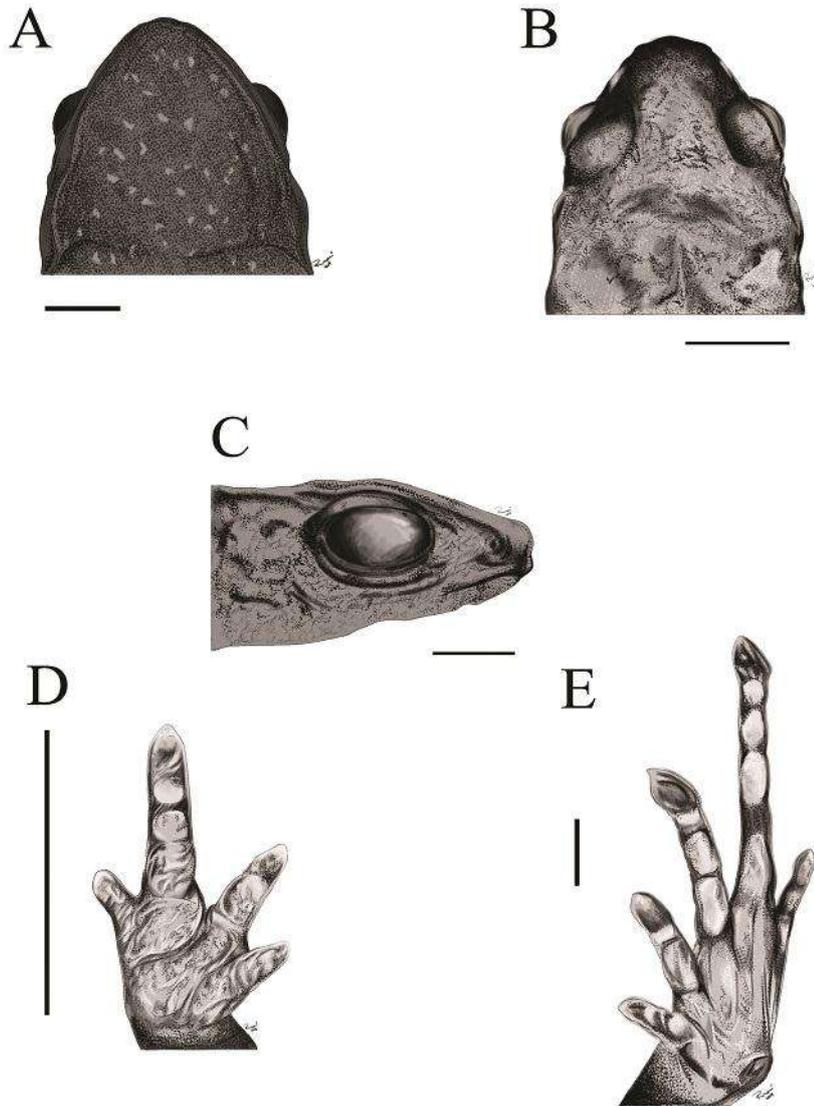


Figure 2.



Figure 3.

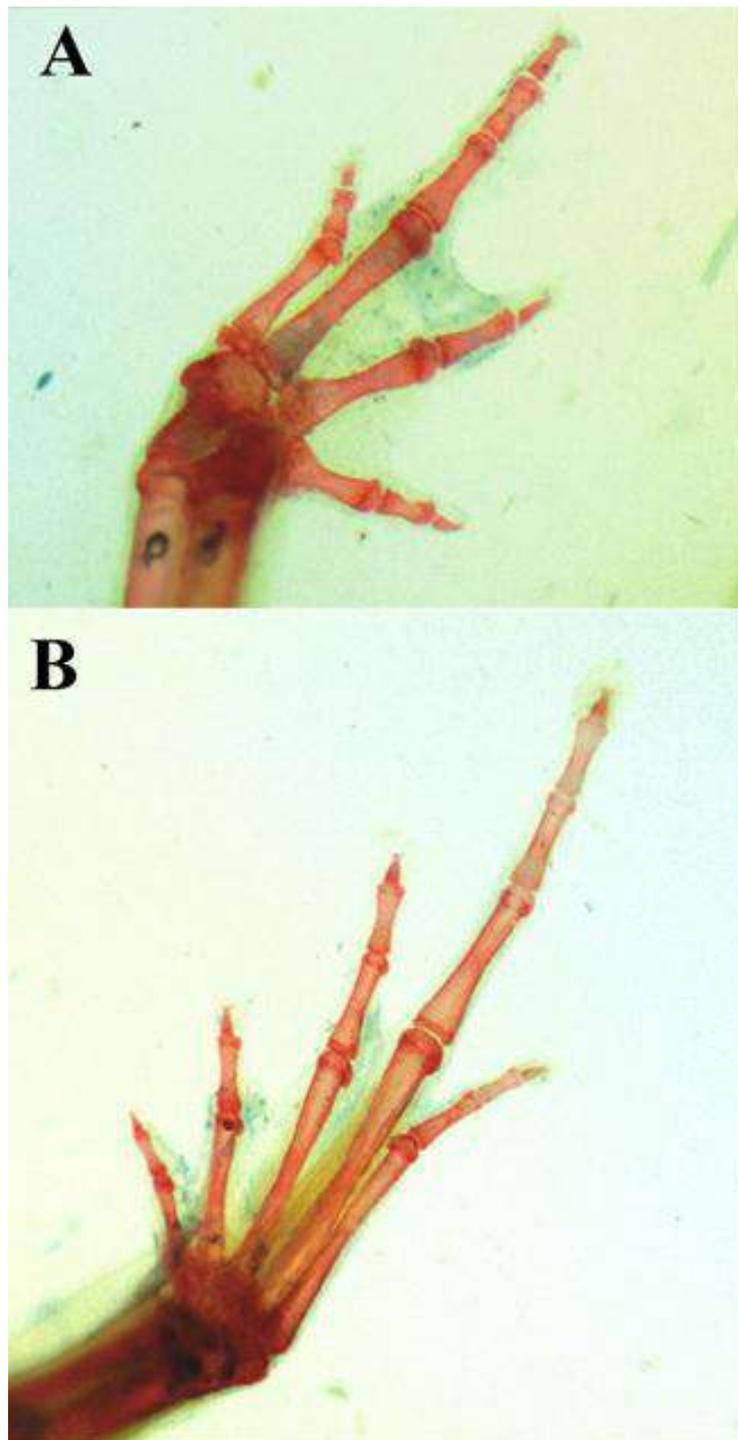


Figure 4.

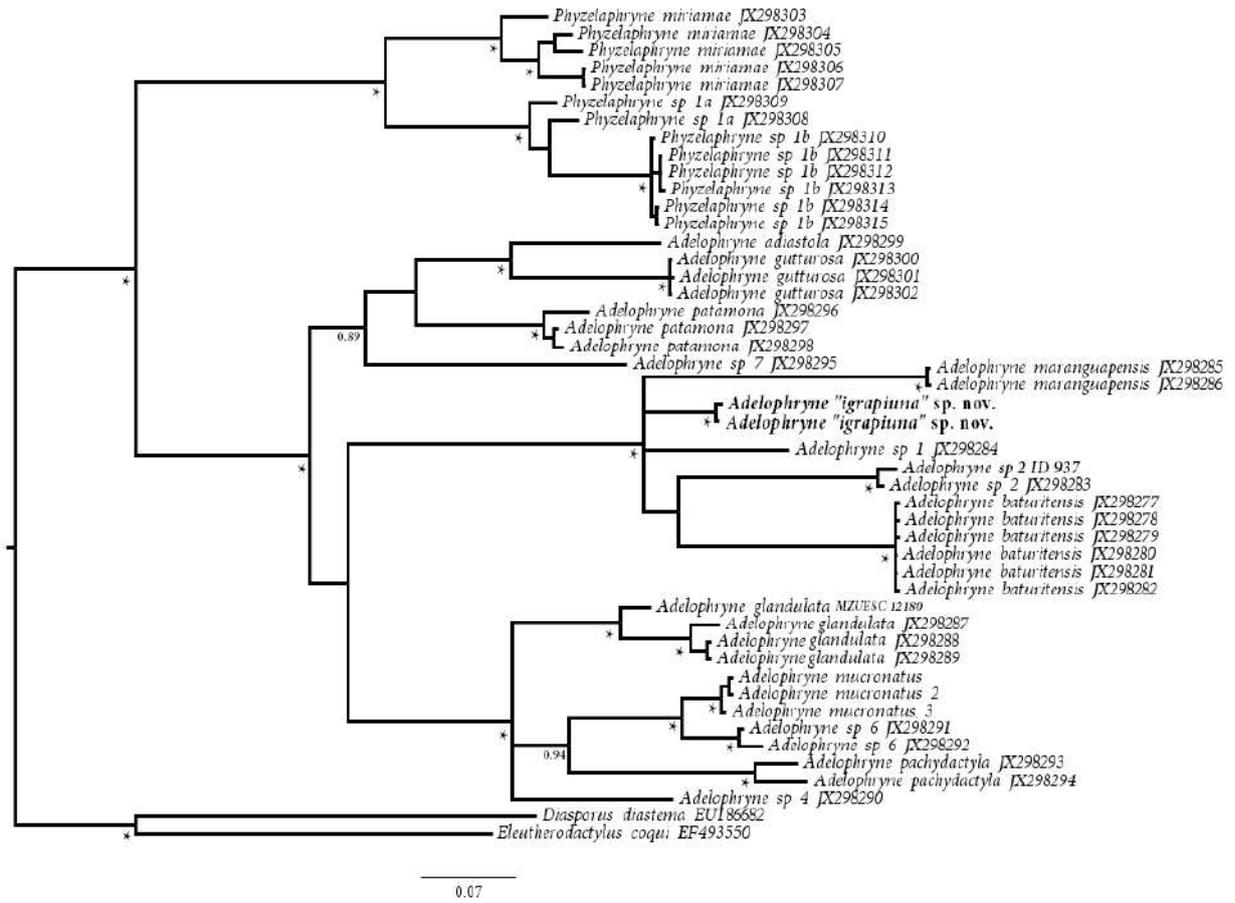


Figure 5.

CONSIDERAÇÕES FINAIS

- O número de espécies de anfíbios registrados na Reserva Ecológica da Michelin – REM (69 ssp.) constitui a segunda maior riqueza do estado da Bahia, além de representar uma das áreas com maior diversidade de anfíbios no bioma Mata Atlântica.

- Importante ressaltar, que as áreas com maior diversidade no bioma estão localizadas em ambientes montanhosos, nos quais alterações de fatores bióticos e abióticos tendem a aumentar a disponibilidade de microambientes e conseqüentemente promover uma maior diversidade de espécies. Por outro lado, apesar da REM está localizada em uma região de Floresta Atlântica de Terras Baixas a alta heterogeneidade ambiental e disponibilidade de ambientes reprodutivos deve desempenhar um papel fundamental na diversidade da anurofauna da reserva.

- Mesmo em áreas já inventariadas, o aumento do esforço amostral pode acrescentar novos registros para determinadas áreas, além de possibilitar o descobrimento de novas espécies para a ciência. No presente trabalho aumentamos em 30% a anurofauna da REM, além de registrar cinco novas espécies confirmadas: uma espécie do gênero *Adelophyrne* (Capítulo 3), uma do gênero *Gastrotheca*, e três do gênero *Phyllodytes*.

- A riqueza e diversidade de anuros de serrapilheira não apresentaram diferenças significativas entre as áreas de Plantação de seringueira, Florestas secundárias e Floresta madura. Contudo, quando avaliadas separadamente (espécies especialistas em florestas e espécies tolerantes a distúrbios), verificou-se uma clara substituição das espécies entre as diferentes categorias de habitats. Espécies especialistas em florestas ocorreram predominantemente em Florestas maduras e Florestas secundárias jovens e tardias, apresentando uma diminuição significativa em Florestas pioneiras e Plantações de

seringueira; por outro lado, espécies tolerantes a distúrbios ocorreram predominantemente em Plantações de seringueira e Florestas pioneiras, raramente em Florestas secundárias jovens, e não sendo registradas nas demais categorias.

- A regeneração da riqueza de espécies de anfíbios de serrapilheira em Florestas secundárias da REM atinge valores similares a Florestas maduras em cerca de ± 30 anos abandono (Floresta secundária jovem). Por outro lado, a composição de espécie acontece em um processo mais lento, com Florestas secundárias tardias (± 50 anos de abandono) possuindo cerca de 54% de similaridade em relação a Florestas maduras. Apesar da Plantação de seringueira apresentar baixa riqueza, diversidade e composição de espécies em relação aos ambientes florestais, um aumento significativo nestes atributos pode ser observado em um período de tempo relativamente curto (± 10 anos de abandono), quando associadas a fragmentos florestais preservados.

- A estruturação das assembleias de anfíbios de serrapilheira da REM depende tanto de variáveis microclimáticas (temperatura) quanto da estrutura da vegetação (abertura dossel e densidade de serrapilheira). A distância entre áreas de plantações e Florestas secundárias em relação a Florestas maduras desempenham fator fundamental no tempo de regeneração das espécies de anfíbios anuros de serrapilheira.

APÊNDICE I: Manuscritos aceitos e publicados a partir de dados coletados ao longo do doutorado

Advertisement call of *Scinax strigilatus* (Spix, 1824) (Anura: Hylidae) from southern Bahia, Brazil

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Scinax strigilatus (Spix, 1824) is a member of the *Scinax catharinae* species group that inhabits forested streams in the Atlantic Rainforest of southern Bahia, northeastern Brazil. The loss of the holotype resulted in years of nomenclatural confusion solved with the designation of a neotype and the redescription of the species by Pimenta *et al.* (2007). Aiming to contribute to the taxonomic status and natural history of the species, we describe herein the advertisement call of *Scinax strigilatus* (Fig. 1A).

Calls of one male (SLV = 25.4 mm; n = 7; air temperature = 23° C; MZUESC 10510) were record at Serra Grande Mountain, Itamaraju (16°59'35" S, 39°35'40" W) on 27 April 2012, and calls from another male (SLV = 27 mm; n = 40; air temperature = 21.2° C; MZUESC 10889) were record at Reserva Ecológica Michelin, Igrapiúna (13°49'15" S, 39°11'52" W) on 18 September 2012. Both areas are in southern Bahia, northeastern Brazil. The first recorded individual was found calling while perched on the vegetation of a stream bank, with two more calling males located at a distance of about two meters. The second male was calling alone, also from the vegetation of a stream bank. We used a Marantz PMD 660 digital audio recorder with a unidirectional Yoga HT-320A microphone. Calls were analyzed at a resolution of 16 bits and 48 kHz sampling rate, mono channel. Waveform and spectrogram (Fig. 1B and C) were made using Raven Pro 3 and analyzed with a Fast Fourier Transformation of 256 points, 50% overlap for an entire call and Window Hamming. Terminology follows Duellman and Trueb (1994). Data are presented as mean ± SD (range).

The advertisement call of *S. strigilatus* is pulsed, with one note per call, with mean 6.51 ± 0.41 pulses per call (5-8 pulses). The mean call duration was 0.014 ± 0.0013 (0.011-0.017 seconds). The interval between calls was 11.47 ± 10.68 (1.89 - 55.39 seconds). Pulse per second rate was 455 ± 56 (294.11-545.45 second). The mean dominant frequency was 3,279 ± 150.7 kHz (2,625-3,379 kHz). There is an increasing modulation of the amplitude until the middle of the call, followed by decreasing modulation. There are not noticeable harmonics (Fig. 1C).

The call of *S. strigilatus* has a pulsed structure, common to the calls of the genus *Scinax* (Pombal *et al.* 1995). Within the *S. catharinae* clade (sensu Faivovich 2002), the call of *S. strigilatus* is more similar to that of *S. heyeri* and *S. luizotavioi* considering that the variation in the number of pulses per call in the three species overlap (Peixoto & Weygoldt 1987; Lourenço *et al.* 2009). As in *S. luizotavioi*, *S. strigilatus* has one note per call, while all other species of the group have more than one note per call.

Nonetheless, the call of *S. strigilatus* differs from the call of all other species in the group by having the shortest call duration (0.011-0.017 seconds). The call duration of other species ranges from 0.08 s in *S. luizotavioi* (0.08-0.54 seconds) (Lourenço *et al.* 2009) to 52.04 s in *S. berthae* (3.2–52.04 seconds) (Pereyra *et al.* 2012).

Its pulsed, but not harmonic structure, distinguishes the call of *S. strigilatus* from that of *S. albicans*, *S. catharinae* (Heyer 1980) and *S. machadoi* (Bokermann & Sazima 1973), which are pulsed and harmonic.

The dominant frequency of the call of *S. strigilatus* (2,625-3,379 kHz) is higher than that of *S. skaios* (2.20-2.24 kHz) (Pombal *et al.* 2010) and lower than that of *S. agilis* (5.6-7.92 kHz) (Nunes *et al.* 2007), *S. argyreornatus* (5.0-6.5 kHz) (Pombal *et al.* 1995), *S. aromothyella* (4.76-5.53 kHz) (Pereyra *et al.* 2012), *S. berthae* (4.41-5.36 kHz) (Pereyra *et al.* 2012), *S. centralis* (3.20-4.89 kHz) (Pombal & Bastos, 1996; Bastos *et al.* 2011), and *S. machadoi* (3.5 kHz) (Bokermann & Sazima 1973).

Advertisement call of *Rhinella crucifer* (Wied-Neuwied, 1821) (Anura: Bufonidae) from southern Bahia, Brazil

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The *Rhinella crucifer* species group is currently composed by six species: *Rhinella crucifer* (Wied-Neuwied, 1821); *R. ornata* (Spix, 1824); *R. henseli* (A. Lutz, 1924); *R. abei* (Baldissera, Caramaschi & Haddad, 2004); *R. pombali* (Baldissera, Caramaschi & Haddad, 2004) and *R. inopina* Vaz-Silva, Valdujo & Pombal, 2012. Until 2012, the group was known to occur only in the Brazilian Atlantic Rain Forest from Rio Grande do Sul to Ceará, and adjacent areas in Minas Gerais and São Paulo (Baldissera *et al.* 2004). The recently described *R. inopina* revealed an allopatric distribution, occurring in forest vegetation types in eastern Cerrado (Vaz-Silva *et al.* 2012). *Rhinella crucifer* (Wied-Neuwied, 1821), occurs throughout the Atlantic Rain Forest from Rio de Janeiro to Ceará including the northeast of Minas Gerais (Baldissera *et al.* 2004; Frost, 2014). Only two species in the group have described calls: *R. ornata* as *Bufo crucifer* in Heyer *et al.* (1990) and *R. pombali* (Lourenço *et al.* 2010). The type locality of *R. crucifer* was informed as being between São Pedro de Alcântara in Santa Catarina and Barra da Vereda in Bahia, but as stated by Bokermann (1966) the type locality can be narrowed down to an area between the rivers Piabanda and Issara, both affluents of the Ilhéus river, approximately 40 km above the municipality of Itabuna. Herein we describe the advertisement call of *R. crucifer* from a population in the municipality of Igrapiúna, Bahia, Brazil, located less than 100 km from the city of Itabuna.

Between August and October 2012 we recorded 33 calls from several males, calling in a temporary pond located in the Atlantic Rain Forest domain in the Michelin Ecological Reserve, Igrapiúna, southern Bahia (13°49'15" S, 39°11'52" W). *Rhinella crucifer* is an explosive breeder showing opportunistic behavior during mate selection and males alternate between calling activity, territorial behavior and quite periods. Due to the difficulty of recording single individuals, we cannot state how many individual males were recorded, since records were made in six different occasions in the same population, thus the same males may have been recorded more than one time. Males were found calling at night, above partly submerged leaves, trunks and also at the edge of the pond (Fig. 1A). *Rhinella crucifer* was identified combining distribution data (Thomé *et al.*, 2012) and morphological characters like absence of vertebral line and absence of yellow marks on flanks (Baldissera *et al.*, 2004; Vaz-Silva *et al.*, 2012). We used a Marantz PMD 660 digital audio recorder with a unidirectional Yoga HT-320A microphone. Calls from several males were pooled and analyzed at a resolution of 16 bits and 48 kHz sampling rate, mono channel. Waveform and spectrogram (Fig. 1B and C) were made using Raven Pro 1.3 (Bioacoustics Research Program, 2008) and analyzed with a Fast Fourier Transformation of 256 points, 50% overlap for an entire call and Window Hamming. Terminology of temporal and acoustic parameters follows Heyer *et al.* (1990). Data are presented as mean ± standard deviation (range). One individual of the recorded population was collected as voucher specimen (MZUESC-12167, SVL = 99.7 mm). Measures of note duration and interval between notes were made using five notes from the middle of the call and five notes from the beginning. Mean air temperature during recordings was 22°C (21°C–24°C) and mean air humidity was 95% (92%–99%).

The advertisement call of *R. crucifer* has no harmonic structure and is composed by a series of pulsed notes (Fig. 1B, 1C). The call begins quietly, with the first notes shorter than the following, quickly becoming intense and staying intense. The mean call duration was 3.54 ± 0.85 s (1.47–5.53 s; N = 33) with 72 ± 16 notes per call (31–104; N = 33). The first notes were shorter than the following ones. First notes duration was 0.016 ± 0.004 s (0.008–0.029 s; N = 145) and the center call notes duration was 0.027 ± 0.003 s (0.017–0.035 s; N = 130). The mean interval of first notes was 0.031 ± 0.006 s (0.015–0.044 s; N = 116) and central notes last 0.023 ± 0.013 s (0.011–0.15 s; N = 104). The mean dominant

Defensive behaviours in the Bahia forest frog *Macrogenioglottus alipioi* Carvalho, 1946 (Anura: Odontophrynidae), with a review of the stiff-legged posture

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Macrogenioglottus alipioi Carvalho, 1946 is a large odontophrynid toad that occurs in the Atlantic Forest domain from the state of São Paulo to Alagoas (Frost 2015; Bourgeois 2010). It inhabits the leaf litter of preserved forests and can be found calling in temporary ponds formed on the forest floor after extremely heavy rains during explosive breeding events (Abravaya and Jackson 1978).

Amphibians serve as prey for a great variety of animals including vertebrates, as well as some arthropods (Wells 2007). To avoid being preyed upon, they have evolved several defensive strategies, which provide protection from potential predators (Duellman and Trueb 1986). Among anurans, 31 defensive behaviours were recognized by Toledo et al. (2011), mostly associated to remaining motionless or fleeing (Jared et al. 2011). We report a sequence of defensive behaviours performed by *Macrogenioglottus alipioi* in an Atlantic forest fragment in Northeastern Brazil.

On June 06, 2015, at 22.30 h, during fieldwork in the Michelin Ecological Reserve (13°49'35" S, 39°08'32"

W, approximately 90–400 m a.s.l.), located in the municipality of Igrapiúna, southern Bahia, Brazil, we found an adult male of *M. alipioi* calling in a temporary pond inside a forest fragment. We captured the individual to take some photographs and the toad immediately reacted by inflating its body (Figure 1A). After placing it back on the forest floor it kept this posture (Figure 1B), and when touched, directed its back towards the hand, performing a body-tilting posture (Figure 1C). Then the toad tried to escape and reacted to our attempt to recapture it by flattening its body on the floor and stretching out its legs (Figure 1D), adopting the posture called stiff-legged behaviour (*sensu* Toledo et al. 2011). The toad remained in this position for about 10 minutes, and then tried to flee again.

Puffing-up-the-body is a common behaviour performed by anurans. It consists of enlarging the body size by filling the lungs with air (Toledo et al. 2011). This increase in size may discourage the predator to capture it (Williams et al. 2000; Toledo et al. 2011). In some cases, puffing-up-the-body may be accompanied by other defensive postures, such as body-tilting. This defensive behaviour consists of directing the dorsal part of the body towards the predator (Toledo et al. 2011). In toads with dorsal macroglands, such as *M. alipioi*, by adopting a body-tilting posture the glands would most likely be the first part of the toad's body that the predator would bite. Thus, noxious secretions could come into contact with the mouth of the predator, allowing the escape of the toad (Toledo et al. 2011).

Stiff-legged behaviour has been reported for forest-floor toads that have cryptic colouration (Toledo et al. 2011). According to Sazima (1978), this behaviour may protect the toad against visually oriented predators,

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Escalated antipredator mechanisms of two neotropical marsupial treefrogs

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The sequence and intensity of antipredator mechanisms may be displayed according to the risk of predation. We tested this hypothesis using two species of marsupial treefrogs from Brazil's Atlantic Forest. We observed *Gastrotheca recava* and *G. megacephala* displaying nine antipredator mechanisms and three types of defensive calls. These behaviours were displayed in an escalated sequence from motionless (passive behaviour) to biting (the most aggressive behaviour). This diversified set of antipredator mechanisms may be related to the interaction between predator and prey at the local scale. The escalated sequence of defensive behaviours should be considered in future studies on anuran-predator interaction.

Key words: Amphibia, Atlantic forest, defensive behaviour, defensive call, *Gastrotheca*, Hemiphractidae

INTRODUCTION

Studies of predator-prey interactions continue to be one of the most fascinating and important aspects of ecological research (Mukherjee & Heithaus, 2013). Due to selective pressures from a variety of predators, anurans display a wide diversity of antipredator mechanisms (at least 31 types) that range from immobility to aposematic behaviours and defensive calls (Toledo et al., 2010, 2011; Jared et al., 2011; Haddad et al., 2013). Anuran antipredator mechanisms are diverse not only in their variety but also how and when they are displayed.

The diversity of antipredator mechanisms of amphibians may depend primarily on the characteristics of their skin secretions (Brodie, 1977, 1983), which may be noxious and toxic (Daly et al., 1987; Bevins & Zasloff, 1990; Erspamer, 1994) or adhesive (Arnold, 1982; Evans & Brodie, 1994). Skin secretions often act synergistically with defensive behaviour and aposematic colour patterns

(Johnson & Brodie, 1975; Brodie, 1977, 1983; Williams et al., 2000; Toledo et al., 2011).

Studies have shown that an individual frog may display several behaviours, which are presumably exhibited according to the degree of stress imposed by the predator (Williams et al., 2000). Some behaviours are displayed exclusively during the approach and others exclusively during handling of the potential predator (Toledo et al., 2005; Ferreira et al., 2013; Lourenço-de-Moraes et al., 2014). In addition, studies have shown that there are differences of antipredator mechanisms across species, populations, and sexes (Williams et al., 2000; Toledo et al., 2005; Heyer & Giaretta, 2009).

Predation involves several phases such as locate, identify, approach, subjugate, ingest, and digest prey (Edmunds, 1974). Therefore, one can hypothesise that the more types of antipredator mechanisms a species displays, the more likely it will escape from different predators. However the sequence of the behaviours may be more

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