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Article

Herpetofauna of Nísia Floresta National Forest, Rio Grande do Norte, Brazil: Richness and abundance differences among natural and secondary forests

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Abstract: In the Atlantic Forest, species distributions are not uniform. The biome has been divided into 8 biogeographic sub-regions, such as the Pernambuco Center of Endemism (PCE), in northeastern Brazil. Nísia Floresta National Forest (Flona) is a protected area situated in the municipality of Nísia Floresta, Rio Grande do Norte state, harboring native vegetation (Atlantic Forest and "Restingas or Tabuleiros") as well regenerating areas, with experimental plots of exotic plants for timber production. Herein, we present, for the first time, a species list of the herpetofauna surveyed at Flona over the past 10 years, using a standardized, long-term sampling design along with visual encounter surveys. We conducted monthly surveys across the area using 84 pitfall traps randomly distributed in 21 arrays throughout the forest's three phytogeographic zones (Restinga, Atlantic Forest, and Regeneration). In addition, we characterized ground-dwelling herpetofauna community diversity across these three zones over the course of an entire year. We collected a total of 39 species of reptiles (among lizards, snakes, chelonians, alligators, and amphisbaenians) and 24 species of frogs. The most frequent family found for reptiles was Dipsadidae, followed by Colubridae. In frogs, Leptodactylidae was the most common, followed by Hylidae. Herpetofaunal diversity in regeneration areas is smaller than forests, which in turn are marginally less diverse than the Restinga. Community descriptors such as equity, presence of exclusive species, and differences in abundances and composition indicate that distinct management strategies for each zone are needed for this protected area. At last, albeit smaller, diversity in regenerating areas is similar to natural areas, a reassuring result considering the significant deforestation the Atlantic Forest has suffered and the urgent need for restoration initiatives.
Keywords: *Conservation; Community Structure; Protected Areas; Atlantic Rain Forest.*

Herpetofauna da Floresta Nacional de Nísia Floresta, Rio Grande do Norte, Brasil: Diferenças na riqueza e abundância entre florestas naturais e secundarias

Resumo: Na Mata Atlântica, as distribuições de espécies não são uniformes. O bioma foi dividido em 8 sub-regiões biogeográficas, como o Centro de Endemismo de Pernambuco (CEP), no nordeste do Brasil. A Floresta Nacional de Nísia Floresta (Flona) é uma área protegida situada no município de Nísia Floresta, no Estado do Rio Grande do Norte, abrigando vegetação nativa (Mata Atlântica e "Restingas ou Tabuleiros"), bem como áreas em regeneração, com parcelas experimentais de plantas exóticas para produção de madeira. Aqui, apresentamos, pela primeira vez, uma lista de espécies da herpetofauna levantada na Flona ao longo dos últimos 10 anos, utilizando uma amostragem padronizada e de longo prazo, juntamente encontros visuais e ocasionais. Realizamos levantamentos mensais em toda a área utilizando 84 armadilhas de queda distribuídas aleatoriamente em 21 conjuntos ao longo das três zonas fitogeográficas da floresta (Restinga, Mata Atlântica e Regeneração). Além disso, caracterizamos a diversidade da herpetofauna terrestre nessas três zonas ao longo de um ano inteiro. Coletamos um total de 39 espécies de répteis (entre lagartos, serpentes, quelônios, crocodilianos e anfisbênios) e 24 espécies de anfíbios. A família mais frequente encontrada para os répteis foi Dipsadidae, seguida por Colubridae. Para anfíbios, Leptodactylidae foi a mais comum, seguida por Hylidae. A diversidade da herpetofauna em áreas de regeneração é menor do que em florestas, que por sua vez são marginalmente menos diversas do que a Restinga. Descritores da comunidade, como equidade, presença de espécies exclusivas e diferenças em abundâncias e composição indicam que estratégias de manejo distintas para cada área são necessárias para esta área protegida. Por fim, embora menor, a diversidade em áreas de regeneração é semelhante às áreas naturais, um resultado animador considerando o significativo desmatamento que a Mata Atlântica sofreu e a urgente necessidade de iniciativas de restauração.

Palavras-chave: *Conservação; Estrutura de Comunidades; Unidades de Conservação; Floresta Atlântica.*

Introduction

The Atlantic Forest is considered the second-largest forest in the Neotropical region, globally known as a biodiversity hotspot due to its high levels of threat and endemism (Mittermeier et al., 2004). Characteristics such as climate, heterogeneous phytogeographic patterns, and topography may explain the extensive diversity in the region (Moura et al., 2016). The combination of these factors bestows the Atlantic Forest with considerable temperature, precipitation, and humidity variation across its expanse, contributing to the diversification of the Neotropical biota (Alvares et al., 2013).

This biome once encompassed an area of approximately 1.5 million km², spanning Paraguay, Argentina, and with its largest portion situated in Brazil (Galindo-Leal & Camara, 2003). Intense anthropogenic pressure, driven by activities such as timber extraction, monoculture plantations (especially sugarcane and coffee), and land use for cattle ranching has significantly reduced the original extent of the Atlantic Forest. Today, estimates of the original coverage remaining range from 12% (Ribeiro et al., 2009) to 28% (Rezende et al., 2018). Most of these are comprised of small and isolated fragments, approximately 80% of which are less than 50 hectares in size (Ribeiro et al., 2009). The environment harbors around 2600 species of terrestrial vertebrates, with 954 of them being endemic (Figueiredo et al., 2021).

In the Atlantic Forest, species distributions are not uniform. The biome has been divided into eight biogeographic sub-regions based on primary areas of endemism and transition (Ribeiro et al., 2011). Among these, the Pernambuco Center of Endemism (PCE), located north of the São Francisco River, lays between northern of Rio Grande Norte state and Alagoas state. This region boasts high levels of biological diversity and consists of small forest fragments embedded in urban and agricultural matrices (Silva & Tabarelli, 2001; Filho et al., 2023). Consequently, it is considered the most threatened and least protected area within the Atlantic Forest hotspot (Porto et al., 2006; França et al., 2023). Furthermore, PCE's biodiversity was influenced by Pleistocene connections among the Atlantic and Amazon Forests, with species or closely-related species pairs occurring in both biomes, including mammals (Machado et al., 2024), birds (Batalha-Filho et al., 2013), amphibians (Coelho et al., 2022) and reptiles (Zamudio & Greene, 1997).

Several herpetofauna surveys have been conducted in PCE (Oitaven et al., 2021; Roberto et al., 2017; Santana et al., 2008). A study in Parque Estadual de Dois Irmãos, Pernambuco state, for example, found 61 species of reptiles (Melo et al., 2018), while another study in Matas de Água Azul (also in Pernambuco state) recorded 83 species (Oliveira et al., 2021). Such studies have significantly improved our understanding of species richness and distribution along this region, uncovering richness up to 106 reptile species in a single locality (Mesquita et al., 2018). Likewise, in Rebio Serra Talhada, researchers identified 42 species of amphibians and 72 species of reptiles (Studer et al., 2015), while in Murici Ecological Station researchers recorded 89 species of reptiles (Dubeux et al., 2022). Nevertheless, considering the area of the Atlantic Forest in the PCE, works of this type remain scarce and relatively short-term in duration, hampering a proper ecological comparison of richness among areas. Even so, there are currently 97 species of amphibians and 143 species of reptiles known for this region (Filho et al., 2023).

Encompassing an area of approximately 174 hectares, Nísia Floresta National Forest is situated in the municipality of Nísia Floresta and was established in 2001 to preserve remnants of the Atlantic Forest in the state of Rio Grande do Norte. The forest's flora comprises native vegetation (Atlantic Forest and "Restingas or Tabuleiros") as well as abandoned areas where experimental plots with exotic plants for timber production, primarily eucalyptus. The area allocated for forest experimentation, which took place in Nísia Floresta National Forest until the late 1970s, features a well-advanced process of natural regeneration of native forest, where certain areas now exhibit floristic variations resembling those of Atlantic Forest areas (MMA, 2012). However, Nísia Floresta National Forest only possesses a preliminary list of reptile species and lacks a list for amphibians (MMA, 2012). Considering that this environment has undergone anthropogenic impacts resulting in vegetation conversion over the years (Lins-e-Silva et al., 2021), information on richness and abundances in the different physiognomies of the area is crucial for assessing the impact of forest modification on the diversity of these organisms in the Atlantic Forest.

Herein, we present a species list of the herpetofauna surveyed at Flona over the past 10 years, along with species abundances, using a standardized, long-term sampling design along with visual encounter surveys. To achieve this, we conducted monthly surveys across the area using 84 pitfall traps randomly distributed in 21 arrays throughout the forest's three phytogeographic zones (Restinga, Atlantic Forest, and Regeneration). In addition, we characterized ground-dwelling herpetofauna diversity across these three zones over the course of an entire year. We compared species richness among areas using rarefaction curves, diversity estimators, and exploratory statistical analyses (non-metric multidimensional scaling).

Material and Methods

1. Study area

We conducted field surveys in Nísia Floresta National Forest (Flona, from now on), located in the municipality of Nísia Floresta, Rio Grande do Norte State, Brazil (06º05'11" S; 35º11'03" W). This protected area is managed by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). Flona has three well-defined vegetation types: Atlantic Forest, Regeneration (Atlantic Forest secondary succession growth with exotic trees like *Pinus* and *Eucalyptus*), and Restinga (Figure 1). The biome is the Atlantic Forest which, originally, spanned more than 1.3 million km² in Brazil. However, currently the coverage area represents 29% of the original territory (Rezende et al., 2018), which leads to major impacts, with approximately 3000 species facing extinction in Brazil, most of them in the Atlantic Forest (IBGE, 2022). The Atlantic Forest is composed of native rainforests, which can be dense, open, or mixed rainforests, and associated with other ecosystems, such as mangroves, rocky outcrops ("*Campos Rupestres*"), and Restingas, which are costal sand forests from eastern Brazil and northern Uruguay, characterized by areas of open shrubby vegetation, formed by ridges of beaches and sandy dunes that have a strong marine influence (Marques & Grelle, 2021).

2. Data collection

Two different sampling methods were used, Visual encounter surveys and pitfalls traps (see Tables 1–3) (Heyer et al., 1994). Visual encounter surveys were conducted by a small group of researchers from 07:00 am to 12:00 pm, and from 05:00 pm to 10:00 pm in the survey

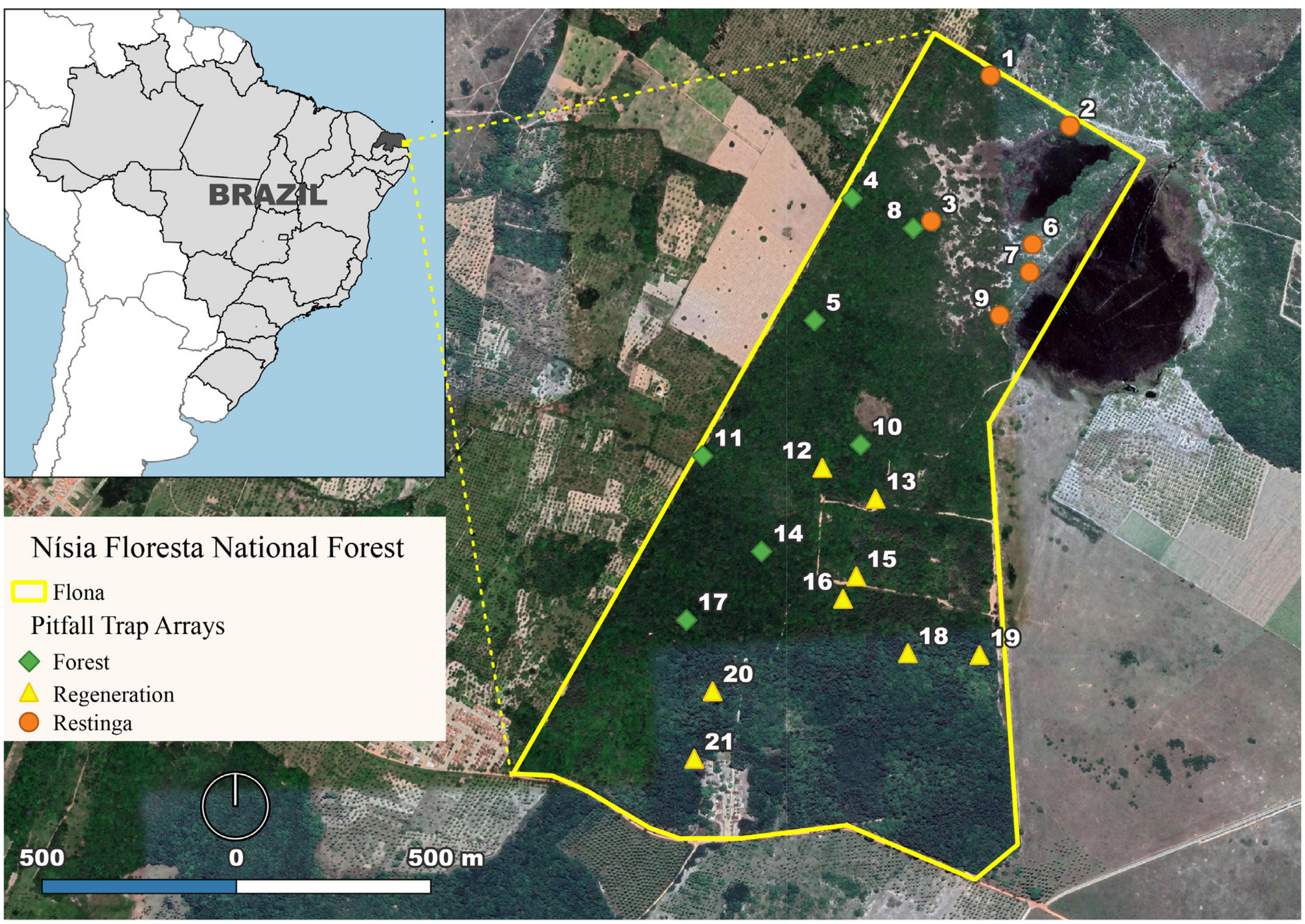

Figure 1. Location of the study area — Floresta Nacional de Nísia Floresta, in South America, Brazil, Rio Grande do Norte State. Symbols represent our pitfalls traps arrays, which are colored according to vegetation type.

Figure 2. Collecting Sites at Flona: (A) Lagoa Seca, (B) Lagoa da Coruja, (C) Pitfall trap array 11 in the Forest, (D) Pitfall trap array 6 in Restinga, (E) Pitfall trap array 18 in Regeneration. Photo credits: Photo E by Maria Beatriz Sousa, all others by Adrian Garda.

of 2012-2013 when traps were open. For the 2022-2024 survey, visual encounters were conducted while walking the trails to check pitfall traps. We conducted pitfall trap field surveys during two periods, ten years apart. Firstly in 2012 and 2013, and secondly between June 2022 and August 2023.We used twenty-one pitfall trap arrays, distributed across three different physiognomies: Atlantic Forest, Regenerating forest, and Restinga (Figures 1, 2). We selected sites to install pitfall trap arrays through a random search using ArcGIS, with the following conditions: arrays should be at least 50 meters apart and 100 meters or less to one of the access roads of the protected area. Each array contained four 30 liters buckets distributed in a Y-shape with one central bucket linked to three peripheral ones by six meters plastic drift fences (Cechin & Martins, 2000). In each bucket we left a piece of styrofoam to help reduce mortality rates in case of flooding and covered exposed buckets with their lids 30cm above ground to protect animals against sun exposure.

Each pitfall array was sampled for a total of seven consecutive days each month from June 2022 to August 2023, and for varying periods of time during the period of 2012 to 2013. Traps were checked every morning by four observers. Each amphibian and reptile captured was identified and measured. The measurements (snout-vent length, tail length, tail base) were taken with a ruler and the weight was taken using a dynamometer scale, after all measures they were released back into the environment at least 100m away from pitfall trap arrays. We conducted this sampling in the protected area with permits from ICMBio (#82300-1) and the ethics committee at UFRN (#283.013/2022 and # 017/2011).

3. Specimens

We collected up to three voucher specimens per species, especially during the 2012–2013 survey. Specimens were given a unique field number (AAGARDA Field Series) and latter deposited in the Coleção Herpetológica da Universidade Federal da Paraíba (CHUFPB). Specimens were killed by rubbing benzocaine cream onto frogs' bellies or by injection of a lethal dose of barbiturate into the body cavity of lizards and snakes. We removed tissue samples and preserved specimens in formalin 10%, later storing them in ethanol 70%. We followed the taxonomic arrangements of Uetz et al. (2024) and Burbrink et al. (2020) for reptiles and Frost (2024) for amphibians.

4. Data analyses

Species data obtained exclusively through visual encounter surveys were used solely to compile a complete species list. Consequently, these data were not utilized for comparing diversity across different Flona vegetation types. Instead, only data from the pitfall sampling survey were used for this comparison. We generated rarefaction curves for the three areas of the Flona: Restinga, forest and regeneration, to compare diversity within and among the environments using *"iNext"* package (Gotelli & Colwell, 2001; Hsieh et al., 2016). To quantify species diversity across different vegetation types, we used observed species richness, rarefied species richness, and rarefied effective number of species (Hill numbers order q=1; Jost, 2006). The effective number of species for q=1 is a transformation of the Shannon diversity index which weighs all species by their frequencies, without favoring either common or rare species (Chao et al., 2014). To observe the proportion of abundances among the areas, we created species rank curves using the *"BiodiversityR"* package (Kindt & Kindt, 2019). Multivariate patterns were inspected with a non-metric multidimensional scaling (nMDS) ordination to check for similarity in species composition

Table 1. Amphibians recorded at Floresta Nacional de Nísia Floresta grouped by family, followed by genus and then species, the area where it was found and sampling method. Rs: Restinga; Rg: Regeneration; F: Forest; AS: Active Survey; and PT: Pitfall Trap. Vouchers with AAGARDA tag numbers are in the process of being incorporated into CHUFPB collection.

Family	Species	Voucher	Area	Sampling methods
Bufonidae	1. *Rhinella granulosa* (Spix, 1824)	CHUFPB0023287	Rs	AS/PT
	2. *Rhinella diptycha* (Cope, 1862)	AAGARDA6210	F/Rs	AS/PT
Hylidae	3. *Boana albomarginata* (Spix, 1824)	AAGARDA13654	Rs	AS
	4. *Boana raniceps* (Cope, 1862)	AAGARDA13656	Rs	AS
	5. *Dendropsophus minutus* (Peters, 1872)	AAGARDA13657	Rs	AS
	6. *Dendropsophus nanus* (Boulenger, 1889)	CHUFPB0026593	Rs	AS
	7. *Pithecopus gonzagai* Andrade, Haga, Ferreira, Recco-Pimentel, Toledo, and Bruschi, 2020	AAGARDA13666	F	AS
	8. *Scinax fuscomarginatus* (Lutz, 1925)	CHUFPB0020398	Rs	AS
	9. *Scinax nebulosus* (Spix, 1824)	AAGARDA6251	Rs	AS
	10. *Scinax pachycrus* (Miranda-Ribeiro, 1937)	AAGARDA13616	Rs	AS
	11. *Scinax x-signatus* (Spix, 1824)	AAGARDA13655	Rs	AS
Leptodactylidae	12. *Adenomera hylaedactyla*	AAGARDA13571	F/Rs	AS/PT
	13. *Leptodactylus macrosternum* Miranda-Ribeiro, 1926	AAGARDA13659	Rs/F	AS/PT
	14. *Leptodactylus natalensis* Lutz, 1930	AAGARDA13614	Rs	AS/PT
	15. *Leptodactylus troglodytes* Lutz, 1926	CHUFPB0020873	Rs/Rg/F	AS/PT
	16. *Leptodactylus vastus* Lutz, 1930	Unvouchered	Rs/Rg/F	AS/PT
	17. *Physalaemus albifrons* (Spix, 1824)	CHUFPB0021089	F/Rs	AS/PT
	18. *Physalaemus cuvieri* Fitzinger, 1826	CHUFPB0023793	Rs/F/Rg	AS/PT
	19. *Pleurodema diplolister* (Peters, 1870)	AAGARDA13605	Rs/F/Rg	AS/PT
	20. *Pseudopaludicola mystacalis* (Cope, 1887)	CHUFPB0020049	Rs/F	AS/PT
	21. *Pseudopaludicola pocoto* Magalhães, Loebmann, Kokubum, Haddad & Garda, 2014	Unvouchered	Pond	AS/PT
	22. *Pseudopaludicola jaredi* Andrade, Magalhães, Nunes-de-Almeida, Veiga-Menoncello, Santana, Garda, Loebmann, Recco-Pimentel, Giaretta, and Toledo, 2016	AAGARDA9197	Rs	AS
Microhylidae	23. *Elachistocleis cesarii* (Miranda-Ribeiro, 1920)	CHUFPB0023477	F/Rs	AS/PT
Strabomantidae	24. *Pristimantis ramagii* (Boulenger, 1888)	AAGARDA13404	F	AS/PT

within and between groups in the *"vegan"* package (Dixon, 2003). We conducted all statistical analysis using R 4.3.1 (R Core Team, 2023).

Results

We collected a total of 17 species of lizards (11 families and 17 genera), 15 species of snakes (4 families and 12 genera), three species of chelonians (3 families and 3 genera), two species of amphisbaenians (1 family and 1 genera), one species of alligator, and 24 species of frogs (5 families and 11 genera) (Tables 1–3; Figures 3–6).

Amphibians – the family Leptodactylidae was the richest with 11 species, followed by Hylidae (9 spp.), Bufonidae (2 spp.) and Strabomantidae and Microhylidae one species each.

Reptiles – the richest lizard family was Teiidae with 4 species followed by Gymnophthalmidae (3 spp.), Mabuyidae (2 spp.) and Anolidae, Gekkonidae, Iguanidae, Leiosauridae, Phyllodactylidae, Polychrotidae, Sphaerodactylidae and Tropiduridae with a singles species each. For snakes, Dipsadidae family was the richest with 9 species, followed by Colubridae (4 spp.), Elapidae (2 spp.) and Typhlopidae with a single species.

Considering solely pitfall trapped animals, we sampled 31 species, 12 amphibians, and 19 reptiles. The Venn diagram illustrates species compositions across the three environments within Flona, revealing that 12 species occur in all areas, three species exclusively inhabit the forest, four species are exclusive to the Restinga, and the regeneration area harbors no species unique to it (Figure 7A). Among these species, we identified *Ameivula ocellifera*, *Pleurodema diplolister*, and *Pseudopaludicola mystacalis* as dominant species in the Restinga environment, while *Kentropyx calcarata* and *Physalaemus cuvieri* were noted as the most abundant species in the forest and regeneration areas.

On the other hand, we observed species restricted to only one environment within the Flona. Specifically, *Oxyrhopus trigeminus*, *Gymnodactylus geckoides*, *Vanzosaura multiscutata*, and *Leptodactylus*

Table 2. Squamates (lizards) recorded at Floresta Nacional de Nísia Floresta grouped by family, followed by genus and then species, the area where it was found and the sampling method. Rs: Restinga; Rg: Regeneration; F: Forest; AS: Active Survey; and PT: Pitfall Trap. Vouchers with AAGARDA tag numbers are in the process of being incorporated into CHUFPB collection.

Family	Species	Voucher	Area	Sampling methods
Anolidae	1. *Norops fuscoauratus* (D'Orbigny, 1837)	AAGARDA13611	F	AS/PT
Gekkonidae	2. *Hemidactylus mabouia* (Moreau de Jonnès, 1818)	AAGARDA6232	Rg	
Gymnophthalmidae	3. *Dryadosaura nordestina* Rodrigues et al., 2005	AAGARDA8787	F/Rg	PT
	4. *Micrablepharus maximiliani* (Reinhardt & Lütken, 1862)	AAGARDA7318	Rs	PT
	5. *Vanzosaura multiscutata* (Amaral, 1933)	AAGARDA13602	Rs	PT
Iguanidae	6. *Iguana iguana* (Linnaeus, 1758)	AAGARDA8887	Rs	AS
Leiosauridae	7. *Enyalius bibronii* Boulenger, 1885	AAGARDA13610	F/Rg	PT
Phyllodactylidae	8. *Gymnodactylus geckoides* Spix, 1825	AAGARDA9442	Rs	PT
Polychrotidae	9. *Polychrus acutirostris* Spix, 1825	AAGARDA10415	Rs/Rg	AS
Scincidae	10. *Brasiliscincus heathi* (Schmidt & Inger, 1951)	AAGARDA8748	Rg/Rs	PT
	11. *Psychosaura macrorhyncha* (Hoge, 1946)	AAGARDA9805	F/Rg	PT
Sphaerodactylidae	12. *Coleodactylus meridionalis* (Boulenger, 1888)	AAGARDA8768	F/Rg/Rs	PT
Teiidae	13. *Ameiva ameiva* Linnaeus 1758	AAGARDA9823	Rg/Rs	PT
	14. *Ameivula ocellifera* (Spix, 1825)	AAGARDA13604	Rs/Rg	PT
	15. *Kentropyx calcarata* Spix, 1825	AAGARDA13606	F/Rg	PT
	16. *Salvator merianae* Duméril & Bibron, 1839	AAGARDA7313	Rg/Rs	PT
Tropiduridae	17. *Tropidurus hispidus* (Spix, 1825)	AAGARDA7362	Rg/Rs/F	PT
Amphisbaenidae	18. *Amphisbaena alba* Linnaeus, 1758	AAGARDA8788	F	AS
	19. *Amphisbaena vermicularis* Wagler, 1824	AAGARDA9814	Rg	AS

natalensis occur exclusively in the Restinga, while *Norops fuscoauratus*, *Adelphostigma occipitalis*, *Pithecopus gonzagai* and *Micrurus corallinus* are found solely in the forest. Regarding species evenness, we observe a subtle difference among the areas when examining the rank curve, with Restinga exhibiting the highest number of species and greater species evenness (Figure 7B).

Pitfall trap species richness was highest in the Restinga (518 individuals; richness = 27; 95% CI [23.99, 30.01]), followed by Atlantic Forest (240 individuals; richness = 22; 95% CI [19.02, 24.98]), and Regeneration areas (203 individuals; richness = 18; 95% CI [15.37, 20.63]). These results indicate that the overall richness of amphibians and reptiles do not differ between Restinga and the Atlantic Forest, as their confidence intervals overlap. Still, Regeneration areas exhibit significantly lower richness than Restinga, but do not differ from the Atlantic Forest.

To compare richness among the three areas, we used rarefaction curves for trap-sampled species based on the smallest sample size of 203 individuals, which was observed in the regeneration area (Figure 7D). This analysis indicates that richness is lowest in Regeneration areas (observed richness = 18; 95% CI [15.37, 20.63]), followed by Atlantic Forest areas (rarefied richness = 21.28; 95% CI [18.52, 24.04]) and Restinga (rarefied richness = 22.18; 95% CI [20.36, 24.00]). However, since these confidence intervals overlap, the rarefied richness cannot be considered significantly different between the vegetation types, particularly between Restinga and Forest areas.

When comparting richness among areas based on Hill numbers (q = 1) we recover similar results. Diversity was highest in Restinga (rarefied diversity = 11.38; 95% CI [10.40, 12.37]), followed by the Atlantic Forest (rarefied diversity = 10.20; 95% CI [8.91, 11.50]), and Regeneration areas (diversity = 8.33; 95% CI [6.94, 9.71]). The rarefaction curve for amphibians showed that the Forest and the Restinga are not significantly different, but both present significant differences from the Regeneration sites (Figure 7E). Meanwhile, for reptiles, results are similar to total richness, with no significant differences between the areas (Figure 7F).

Discussion

Nísia Floresta National Forest presents a moderate diversity of amphibians and reptiles compared to other areas of Atlantic Forest within the PCE, but significant considering is small size and insertion in a human disturbed matrix. In fact, there are important herpetofauna surveys in PCE, as well as studies that assessed taxonomy, ecology, and conservation status of individual species and communities (Dubeux et al., 2020; Filho et al., 2021; Oliveira et al., 2021). However, when it comes to comparing richness between areas, the lack of standardization in studies and the absence of details regarding sampling effort hinder the possible comparisons, limiting our ability to assess the true parameters responsible for the differences in richness between areas (França et al., 2023).

Table 3. Squamates (Snakes) and Chelonians recorded at Floresta Nacional de Nísia Floresta grouped by family, followed by genus and then species, the area where it was found and the sampling method. Rs: Restinga; Rg: Regeneration; F: Forest; AS: Active Survey; and PT: Pitfall Trap. Vouchers with AAGARDA tag numbers are in the process of being incorporated into CHUFPB collection.

Family	Species	Voucher	Area	Sampling methods
	Squamata (snakes)			
Colubridae	1. *Chironius flavolineatus* (Boettger, 1885)	AAGARDA8888	Rs	AS
	2. *Leptophis dibernardoi* Albuquerque, Santos, Borges-Nojosa & Ávila, 2022	AAGARDA9813	RS	AS
	3. *Oxybelis aeneus* (Wagler, 1824)	AAGARDA5866	RS	AS
	4. *Tantilla melanocephala* (Linnaeus, 1758)	AAGARDA10418	Rs/Rg	PT
Dipsadidae	5. *Adelphostigma occipitalis* (Jan, 1863)	AAGARDA13573	F	AS
	6. *Apostolepis cearensis* Gomes, 1915	Unvouchered	Rs	PT
	7. *Apostolepis longicaudata* Gomes, 1921	AAGARDA13505	Rs/Rg	PT
	8. *Dipsas mikanii* Schlegel, 1837	AAGARDA9441	F	PT
	9. *Hydrodynastes gigas* (Duméril, Bibron & Duméril, 1854)	AAGARDA8745	Rs	AS
	10. *Oxyrhopus trigeminus* Duméril, Bibron & Duméril, 1854	AAGARDA10417	Rs	PT
	11. *Philodryas olfersii* (Lichtenstein, 1823)	AAGARDA10436	Rs	AS
	12. *Philodryas nattereri* (Steindachner, 1870)	AAGARDA9372	Rs	AS
	13. *Xenodon merremii* (Wagler, 1824)	AAGARDA8826	F	AS
Elapidae	14. *Micrurus corallinus* (Merrem, 1820)	Unvouchered	F	PT
	15. *Micrurus potyguara* Pires, Silva Junior, Feitosa, Prudente, Pereira-Filho & Zaher, 2014	AAGARDA8890	Rs	PT
Typhlopidae	16. *Amerotyphlops paucisquamus* (Dixon & Hendricks, 1979)	AAGARDA13603	Rs/Rg	PT
	Testudines			
Kinosternidae	17. *Kinosternon scorpioides* (Linnaeus, 1766)	Unvouchered	Rs	AS
Chelidae	18. *Mesoclemmys tuberculata* (Luederwaldt, 1926)	Unvouchered	Rs	AS
Testudinidae	19. *Chelonoides carbonarius* (Spix, 1824)	Unvouchered	Rs	AS
	Crocodylia			
Alligatoridae	20. *Caiman latirostris* (Daudin, 1801)	Unvouchered	Rs	AS

Nevertheless, we know that richness can vary depending on the size of the region, position in relation to ecotones, and diversity of microhabitats (Garda et al., 2013; MacArthur & Wilson, 2001; Ramanamanjato et al., 2002). In Guaribas Biological Reserve, Paraíba State, for example, 106 of reptiles and amphibians have been reported, a large number likely related to the protected area's size (4,000 hectares, or 23 times larger than Flona), and the presence of Tabuleiros (Vegetation type related to Restingas but geologically older), which enable richer communities associated to such environments (Mesquita et al., 2018). Additionally, there is a decrease in humidity and extension of the Atlantic Forest in the east-west direction as we enter Rio Grande do Norte; due to these characteristics, the state is sometimes excluded from studies involving the northeastern portion of the Atlantic Forest in Brazil (Ribeiro et al., 2011; Tabarelli et al., 2005). Despite the smaller area of the National Forest, proximity to the Caatinga may be contributing to the increase in richness. Here, Flona lacks flowing water, which limits or prevents the presence of some amphibian species such as *Proceratophrys renalis*, typical of the Atlantic Forest, and *P. cristiceps*, typical of the Caatinga. But ecotones may harbor species absent from adjacent environments. A study conducted in a transition zone between Caatinga and Atlantic Forest in Rio Grande do Norte revealed a remarkable diversity of amphibians (Magalhães et al., 2013). Similarly, we were able to observe species typical of the Caatinga at Flona, such as the lizard *Vanzosaura multiscutata* and the frog *Pseudopaludicola pocoto* (Magalhães et al., 2014; Recoder et al., 2014).

Some notable absences from our list of amphibians for Flona include Atlantic Forest species such as *Dendropsophus oliveirai*, *Lithobates palmipes*, and *Scinax cretatus*, which have been recorded north of this site in areas at the transition with the Caatinga Biome (Escola Agrícola de Jundiaí – EAJ, Magalhães et al., 2013). Likewise, species usually more associated to the Caatinga, like *Dermatonotus muelleri* and *Proceratophrys cristiceps*, were found at EAJ and in fragments south of Flona.

Such absences are likely linked to the ecological idiosyncrasies of these species. Ecologically, species like *P. cristiceps* and *L. palmipes* are associated to flowing streams, in which they call and where their tadpoles develop (in small ponds associated with flowing streams) (Nunes et al., 2015; Volpe & Harvey, 1958). The temporary and permanent water bodies at Flona are large and rest on sandy soils, possibly precluding the

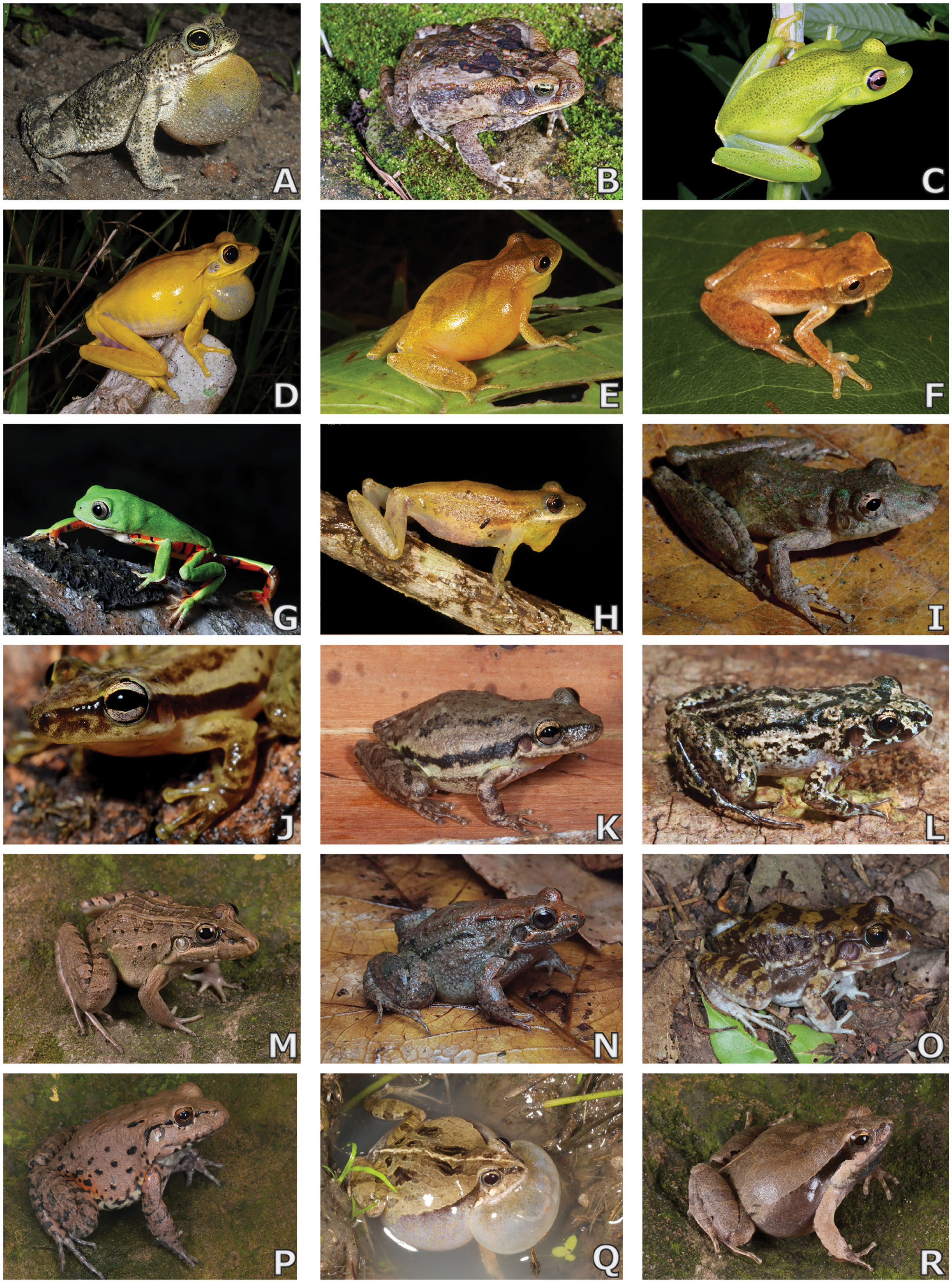

Figure 3. Reptiles and Amphibians of Floresta Nacional de Nísia Floresta, Rio Grande do Norte State, Brazil. Amphibians: **Bufonidae**: (A) *Rhinella granulosa*, (B) *R. diptycha*. **Hylidae**: (C) *Boana albomarginata*, (D) *B. raniceps*, (E) *Dendropsophus minutus*, (F) *D. nanus*, (G) *Pithecopus gonzagai*, (H) *Scinax fuscomarginatus*, (I) *S. rostratus*, (J) *S. pachycrus*, (K) *S. x-signatus*. **Leptodactylidae**: (L) *Adenomera hylaedactyla*, (M) *Leptodactylus macrosternum*, (N) *L. natalensis*, (O) *L. troglodytes*, (P) *L. vastus*, (Q) *Physalaemus albifrons*, (R) *P. cuvieri*. Photo credits: I, J, and N by Diego J. Santana; all other pictures by Adrian A. Garda.

Figure 4. Reptiles and Amphibians of Floresta Nacional de Nísia Floresta, Rio Grande do Norte State, Brazil. Amphibians: **Leptodactylidae (cont.)**: (A) *Pleurodema diplolister*, (B) *Pseudopaludicola mystacalis,* (C) *P. pocoto*, (D) *P. jaredi*. **Microhylidae**: (E) *Elachistocleis cesarii*. **Strabomantidae**: (F) *Pristimantis ramagii*. Squamata (Lizards): **Anolidae**: (G) *Norops fuscoauratus*. **Gymnophthalmidae**: (H) *Dryadosaura nordestina*, (I) *Micrablepharus maximiliani*, (J) *Vanzosaura multiscutata*. **Iguanidae**: (K) *Iguana iguana*. **Leiosauridae**: (L) *Enyalius bibronii*. **Phyllodactylidae**: (M) *Gymnodactylus geckoides*. **Polychrotidae**: (N) *Polychrus acutirostris*. **Scincidae**: (O) *Brasiliscincus heathi*, (P) *Psychosaura macrorhyncha*. **Sphaerodactylidae**: (Q) *Coleodactylus meridionalis*. **Teiidae**: (R) *Ameiva ameiva*. Photo credits: K and P by Willianilson Pessoa; all other pictures by Adrian A. Garda.

Figure 5. Reptiles and Amphibians of Floresta Nacional de Nísia Floresta, Rio Grande do Norte State, Brazil. SQUAMATA (LIZARDS): **Teiidae**: (A) *Ameivula ocellifera* (B) *Kentropyx calcarata*, (C) *Salvator merianae*. **Tropiduridae**: (D) *Tropidurus hispidus*. **Amphisbaenidae**: (E) *Amphisbaena alba*, (F) *A. vermicularis*. SQUAMATA (SNAKES): **Colubridae:** (G) *Chironius flavolineatus*, (H) *Leptophis dibernardoi*, (I) *Oxybelis aeneus*, (J) *Tantilla melanocephala*. **Dipsadidae:** (K) *Adelphostigma occipitalis*, (L) *Apostolepis cearensis*, (M) *A. longicaudata*, (N) *Dipsas mikanii*, (O) *Hydrodynastes gigas*, (P) *Oxyrhopus trigeminus*, (Q) *Philodryas olfersii*, (R) *P. nattereri*. Photo credits: C, E, F, I, O, Q, R by Willianilson Pessoa; L by Maria Beatriz Sousa; all other pictures by Adrian A. Garda.

Figure 6. Reptiles and Amphibians of Floresta Nacional de Nísia Floresta, Rio Grande do Norte State, Brazil. Squamata (Snakes): **Dipsadidae:** (A) *Xenodon merremii* **Elapidae** (B) *Micrurus corallinus*, (C) *M. potyguara*. **Typhlopidae**: (D) *Amerotyphlops pauscisquamus*. **Chelidae**: (E) *Mesoclemmys tuberculata*. **Testudinoidea**: (F) *Chelonoidis carbonarius*. **Alligatoridae:** (G) *Caiman latirostris.* Photo credits: A by Willianilson Pessoa; B by Maria Beatriz Sousa; G by FLONA employees; other pictures by Adrian A. Garda.

use of such areas by Atlantic Forest species that use smaller and more permanent ponds like *D. oliveirai* and *S. cretatus*. These water bodies at Flona are associated with Restinga areas with no nearby streams, which likely makes them less stable, exposed to high temperatures and very unpredictable, making such environments hostile for species where tadpoles take time to develop like *P. gonzagai* (Brasileiro et al., 2022). Indeed, we recorded *P. gonzagai* for the first time in July 2024, after three consecutive years of significant rain that filled a temporary pond that had been dry for almost a decade. Diversity of reproductive sites has been shown to significantly determine amphibian species richness (Bickford et al., 2010).

For squamates, species recorded correspond to the diversity reported to other Atlantic Forest sites in the Atlantic Forest of Rio Grande do Norte (Freire, 1996; Lion et al., 2016). Compared to coastal sites near the Capital of the state – Natal, where similar habitats are found and long-term work has been conducted, the absence of Caatinga gecko *Hemidactylus brasilianus*, recorded at Parque das Dunas (Freire, 1996) and Centro de Lançamento da Barreira do Inferno (Adrian Garda, unpub. data), indicates a stochastic occurrence of Caatinga species within Atlantic Forest and Restinga sites in this northernmost portion of the Atlantic Forest. This is expected given climatic oscillations during the Pleistocene, with expansion and retraction of Forests and Caatinga areas, possibly leaving populations of caatinga species trapped within Atlantic Forest matrices (Guillory et al., 2024). Other examples of Caatinga species include *Lygodactylus klugei*, found in a forest fragment south of Flona, and *Vanzosaura multiscutata*, first recorded at Flona after 3 years of intense sampling.

Species diversity was only marginally higher in Restinga compared to forest areas, but both were richer than regeneration sites. These results may be related to the fact that the Flona has been the subject of forest experiments with exotic species for many years ago (MMA, 2012). Lezzi et al. (2018) observed that mammal and bird diversity was higher in natural environments than in plantations. Just like for these groups, reptiles and amphibians can also be affected and, depending on the dynamics of the environment, may exhibit different patterns of species richness, abundance, and composition, whether in natural areas, secondary forests, or plantations (Gardner et al., 2007). This indicates that anthropogenic factors (such as deforestation, timber harvesting, monoculture plantations) may be linked to the distribution of these species in this fragment, as habitat modification is a crucial factor in the changes of biological diversity. The alteration of natural landscapes due to human action can affect the diversity of microhabitats, resource availability, hiding places, and cause changes in plant physiognomy (Cordier et al., 2021).

Our results show that regenerating areas contain a significant and diverse but depauperate community of amphibians and reptiles compared to natural areas. The regions near water bodies and in the Restinga areas have the highest diversity, yet they are the smallest in extent within Flona. The natural substitution of exotic plants and the subsequent regeneration of the Atlantic Forest is expected to contribute

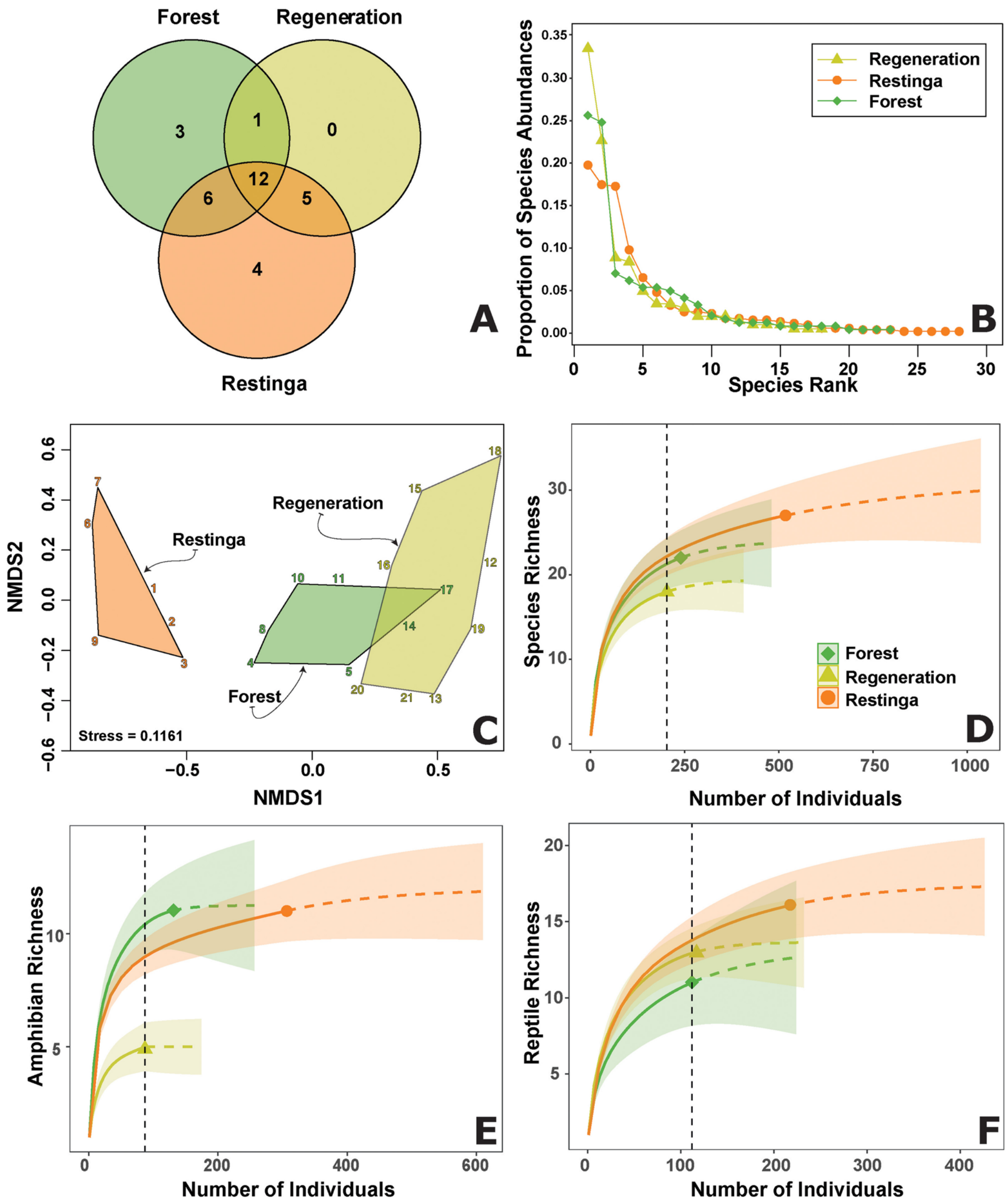

Figure 7. Descriptive statistics of the herpetofauna found in the Floresta Nacional de Nísia Floresta. (A) Veen Diagram showing the distribution of the number of species among different vegetation types. (B) Rank Curve presenting species proportions in a decrescent order of species abundance, within each vegetation type of the present study; (C) NMDS graphic presenting similarity species composition in and among different vegetation types; (D) Rarefaction curves with standard deviations presenting diversity of species in each vegetation type; y axis shows species richness and x axis number of individuals; (E) Rarefaction curves with standard deviations presenting amphibian species diversity in each vegetation type; (F) Rarefaction curves with standard deviations presenting species reptile diversity in each vegetation type.

to an increase in the occurrence area for endemic species of the Atlantic Forest biome. The disparity in species richness between disturbed and natural sites within the studied fragment underscores the need for future investigations into the underlying ecological mechanisms. Further research could explore the specific factors driving these differences, assess long-term effects of disturbance on populations, consider landscape connectivity, and collaborate with conservation efforts to translate findings into effective management strategies, ultimately enhancing our understanding of reptile and amphibian ecology in fragmented landscapes.

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Nicolas Vinicius A. A. Leite: Conducted sampling; contributed to discussing and editing the manuscript.

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Felipe de Medeiros Magalhães: Conceived the research; obtained funding for sampling and analysis; supervised the study; contributed to discussing and editing the manuscript.

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Ricardo Rodrigues da Silveira-Filho: Conducted sampling; analyzed the data; contributed to discussing and editing the manuscript.

Matheus Lucas Azevedo Soares: Conducted sampling; contributed to discussing and editing the manuscript.

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Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Data Availability

The data and scripts used in this study are available at https://data.scielo.org/dataset.xhtml?persistentId = doi:10.48331/scielodata.HGMNQ5.

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