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# Enhanced Phylogenetic Diversity of Anuran Communities: A Result of Species Loss in an Agricultural Environment

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Additional information is available at the end of the chapter

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## Abstract

The great environmental changes induced by anthropogenic activities reshaped many ecosystem processes. Thus, the previously natural landscapes have been turned into mosaics of natural and seminatural lands embedded into human-modified landscape. To understand effects of these landscape modifications on the anuran communities, we aimed to compare pond-breeding anuran communities in a well-preserved forest with communities in agricultural landscape. We tested the values of taxonomic, phylogenetic and functional diversity, by analyzing four data matrixes containing environmental, phylogenetic, morphological and abundance information from 15 anuran species along this environmental gradient. Our analysis showed that only the phylogenetic component of the diversity was linked to the changes on the gradient and that the loss of environmental quality generates enhanced phylogenetic diversity. We showed that the anthropogenic disturbance acts negatively on anuran communities, forcing the species to adapt and behave like generalist species in order to survive at the modified places now available, where there were well-preserved forests before the humans changed it.

**Keywords:** anuran diversity and conservation, landscape conversion, habitat loss, Atlantic rainforest, anthropogenic disturbance

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## 1. Introduction

The great environmental changes induced by anthropogenic activities are, in general, dated from before the industrial revolution (~1860 AD) [1]. These activities have reshaped many ecosystem processes across the globe since the human populations have become persistent

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in some places due the enhanced agricultural practices [2]. As a result of this growing phenomenon, the previously natural landscapes have been turned into mosaics of natural and seminatural lands embedded into human-modified landscapes [3]. Actually, these mosaic conditions of non-used land represent, almost 90% of the world's tropical forests which are inserted in reserves and parks within agricultural lands [4, 5]. Understanding the factors that affect the community assemblies has been the focus of many ecological studies [6–9]. Since the conversion of the previously undisturbed places is usually allied to unsustainable activities, which drives the ecosystem degradation throughout the loss of ecosystem services and the related cascade events [10] causing biodiversity loss across the globe and across spatial, temporal and organizational scales [11–13].

To understand these factors, some researchers have made use of a classical measure, the taxonomic unit (e.g., species) [10, 14], but it does not take into account ecological and evolutionary attributes of species. However, some modern approaches are combining functional attributes of species (individual characteristics that can be measured and that affect the fitness) with phylogenetic relationships. This approach, in addition to the taxonomic diversity, can bring different answers of a species community in relation to its habitat conditions, being these a combination of ecological and evolutionary answers [15, 16]. Environmental degradation process can be observed by studying diversity measures that are affected by disturbance conditions [17], and for this, the usage of functional, phylogenetic and taxonomic diversity is a growing tool that has been changing the focus of researchers from the use of species diversity or species composition that take no account of differences in species' life-history traits and ecological niches [17–19].

Although plenty of studies have shown strong relationships between community structure and environmental predictors and how the functional traits of species can match up with the environmental conditions [20], some adaptive processes remain unclear. This may be due the large number of traits presented by each species and/or the high species number existing in many habitats which generates an incomplete knowledge of which species traits can actually be an influence to the ecosystem processes [21]. Among all vertebrates, the amphibians are the group with highest proportion of species threatened with extinction [22], due to habitat loss, fragmentation [23], and other related environmental stressors like enhanced UV radiation incidence [24, 25] and canopy coverage loss [26]. Furthermore, the complexity on amphibian life cycle and the differences in life-history strategies between species and also their habitat associations generate a need for studies aiming to understand the true relationship between anthropogenic disturbance and the structure and organization of amphibian communities [7, 8].

In the present study, we aim to answer the following question: In relation to the anthropogenic disturbance in an agricultural-forest preserved gradient, would ponds in more preserved environment harbor higher taxonomic, functional and phylogenetic diversity patterns? So we tested the hypothesis that ponds located at most preserved and more heterogeneous environments would be taxonomically richest and would allow the coexistence of more functionally distinct species [27], expecting then a higher functional diversity and lower functional redundancy. We also expect an increase of the phylogenetic diversity (and thus a decrease in phylogenetic redundancy) at these sites, since more heterogeneous habitats can provide a wide range of microhabitat usage, diminishing the interspecific competition and allowing the coexistence of taxa with higher phylogenetic similarity [28, 29].

## 2. Methods

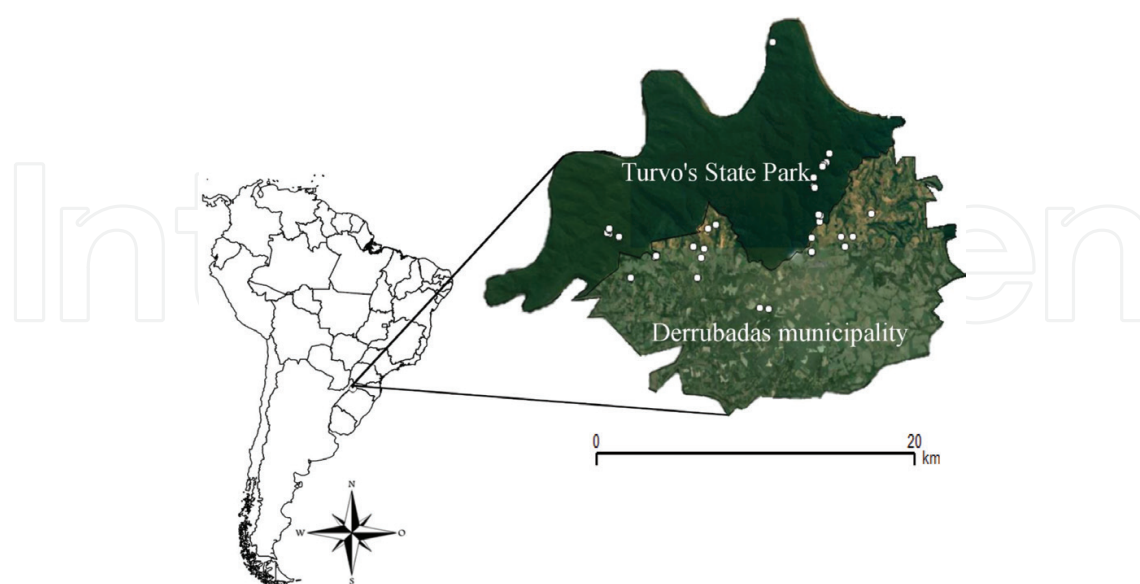
### 2.1. Description of the study site

The sampling areas are located at the Parque Estadual do Turvo (PET) and its adjacencies, and both belong to the Atlantic Rainforest biome. The PET is located at the Rio Grande do Sul State ( $27^{\circ} 07' - 27^{\circ} 16' \text{ S}$ ,  $53^{\circ} 48' - 54^{\circ} 04' \text{ W}$ ; 100–400 a.s.l), at the municipality of Derrubadas, covering an area of 17,491 ha with about 90 km of perimeter of semi-deciduous forest. The study site differs from the wet evergreen forests since it is dryer and presents more open areas, sharing this same vegetational classification with the Republic of Argentina by the Moconá Provincial Park (about 1000 ha) and the Yabotí International Biosphere Reserve (236,613 ha), as well the Brazilian state of Santa Catarina by the Uruguay River [30] (**Figure 1**).

The vast majority of the surrounding areas of PET were converted into intensively agricultural landscapes dominated by crops of soybeans (~22.000 t/year), maize (7.560 t/year), wheat (6.840 t/year) and cattle (~8700 animals) pasture and where the legal buffer zones are not implemented or respected [31]. The climate is characterized as subtropical highly humid with average rainfall between 1.700 and 1.900 mm with reduction of precipitation at the winter season, and the average of temperature ranges from 20 to 23°C [32].

### 2.2. Data collection

The fieldwork was conducted during two anuran breeding seasons at southern Brazil, the first one from September 2013 to March 2014 and the second from September 2015 to March 2016, which comprises the spring and summer seasons at the southern hemisphere. The field



**Figure 1.** Map representing the sampling area on the extreme north-western of the Rio Grande do Sul State, Brazil. The white points on the map show location of the sampled ponds along the agricultural-preserved forest gradient at Parque Estadual do Turvo and surrounds. The study was performed at two consecutive breeding seasons of anurans between 2014 and 2016.

campaigns were made monthly, for approximately 10 days, when we sampled 38 ponds following an environmental gradient. The gradient ranged from the agricultural landscape where 19 ponds were located (outside of the park borders) to a preserved undisturbed forest, the inner portion of the gradient, with 19 ponds too (**Figure 1**). We collected adult anurans by using the method of “survey at breeding sites” [33], recording the number of calling males along all perimeter of ponds. Then, the maximum abundance data from each species in each pond was used to construct the composition matrix to be used at the subsequent analysis.

We undertook a series of 14 measures (averages from the continuous values, chosen given their environmental and/or reproductive values) on morphological and ecological traits from eight individuals of each recorded species, to access data on functional diversity and redundancy (**Table 1**). Since the sampling method is based on the calling males, the morphometric measures were taken only from adult males. The data acquisition was performed from anurans collected during the field campaigns and also on specimens already deposited at the Universidade Federal de Santa Maria collection (ZUFMSM Appendix A) between the years of 2010 and 2012 from the same area, to enlarge the database.

We also constructed a phylogenetic matrix based on the phylogenetic information of the species to access data on phylogenetic diversity and redundancy. We manually inserted six

Trait type	Variable	Trait
Snout vent length	Continuous	Total size of the body, from the tip of the nose to the cloaca
Mouth ratio	Continuous	Distance between the rear edge of the jaw joint and the tip of the snout, divided by the snout vent length
Forelimb ratio	Continuous	Greater distance from the “shoulder” to the tip of the “hand,” divided by the snout vent length
Hind limb ratio	Continuous	Distance between the cloaca and the tip of the “foot,” divided by the snout vent length
Perched	Binary	Place of activity/or vocalization
Ground	Binary	Place of activity/or vocalization
Water	Binary	Place of activity/or vocalization
Prolonged breeding	Binary	Time of breeding season
Explosive breeding	Binary	Time of breeding season
Reproductive Mode 1*	Binary	Reproductive mode
Reproductive Mode 2*	Binary	Reproductive mode
Reproductive Mode 11*	Binary	Reproductive mode
Reproductive Mode 24*	Binary	Reproductive mode
Reproductive Mode 30*	Binary	Reproductive mode
*Reproductive modes based on [43].		

**Table 1.** Description of the ecomorphological traits of the anurans recorded in ponds monitored at Parque Estadual do Turvo and adjacencies between 2014 and 2016.



species (*Elachistocleis bicolor*, *Ololygon aromothyella*, *Physalaemus* aff. *gracilis*, *Rhinella ornata*, *Scinax granulatus* and *S. perereca*) not present at Pyron and Wiens' work [34]. The position of these insertions (missing species) was defined according to the position of the closest species or closest species group [35–38].

### 2.3. Statistical analysis

We calculated the functional and phylogenetic patterns by using Rao's quadratic entropy and the taxonomic diversity by using the Gini-Simpson's index [39, 40]. These analyses were based on [15, 16] by constructing a dataset composed by four matrices. The first one (matrix B) contains the species functional traits, the second one (matrix W) contains the abundance of species in each sampled pond, the third one (matrix E) with the environmental filter (distance from the nearest border of PET, negative values for outside and positive values for inside) and the fourth (matrix F) with the phylogenetic information (transformed then into a matrix of phylogenetic distance) of the recorded species. To perform these analyses, we used the software Phylocom [41] and SYNCSEA (available at <http://ecoqua.ecologia.ufrgs.br/SYNCSEA.html>).

As a way to explore our database and better understand the effects of richness and equability of the species distributed along the measured gradient of distance, we constructed a Whittaker diagram (or dominance diagram). After these procedures, we tested the relation of the obtained values of functional diversity and redundancy, taxonomic diversity and redundancy and phylogenetic diversity and redundancy of each pond with its distance from PET's nearest border (positive values represented the ponds inside PET's area and negative values represented ponds outside PET's boundaries). In addition, we also tested the relation of the components of the community weighted means matrix (CWM matrix containing the weighted functional traits) with the distance from the PET's nearest borders (*Vegan* Package, *lm* function, [42]).

## 3. Results

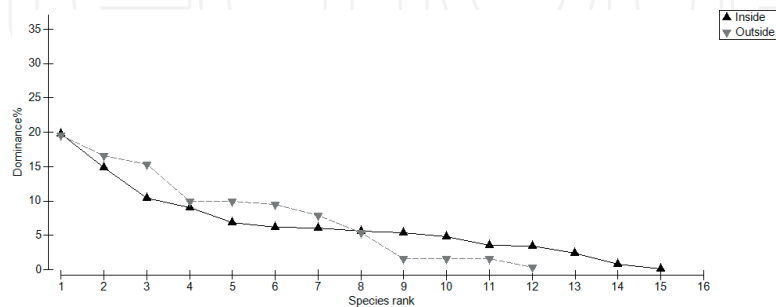
We found 15 anuran species from five families: Hylidae (four species), Leptodactylidae (four species), Bufonidae (two species), Phyllomedusidae, Microhylidae and Ranidae, both with one species each. We registered all the 15 species in the inner portion of gradient (the portion inside the PET) and only 10 species in the outside portion. The most conspicuous species were *Dendropsophus minutus* and *Scinax granulatus* both occurring at 31 of the 38 sampled ponds, respectively (**Table 2**). We found, based on the abundance distribution curve, that the ponds located at inner portion of the gradient have the species abundance more equally distributed (equability) than the ponds located at the outside portion (**Figure 2**).

Regarding the taxonomic, functional and phylogenetic patterns of diversity that we analysed, only the phylogenetic diversity and phylogenetic redundancy were related to the studied gradient ( $r^2 = 0.14$ ,  $p > 0.05$  and  $r^2 = 0.20$ ,  $p < 0.05$ , respectively). The phylogenetic diversity (opposed to what we assumed) decreased at the inner portion of the gradient, while the phylogenetic redundancy increased (see **Figure 3A** and **B**).

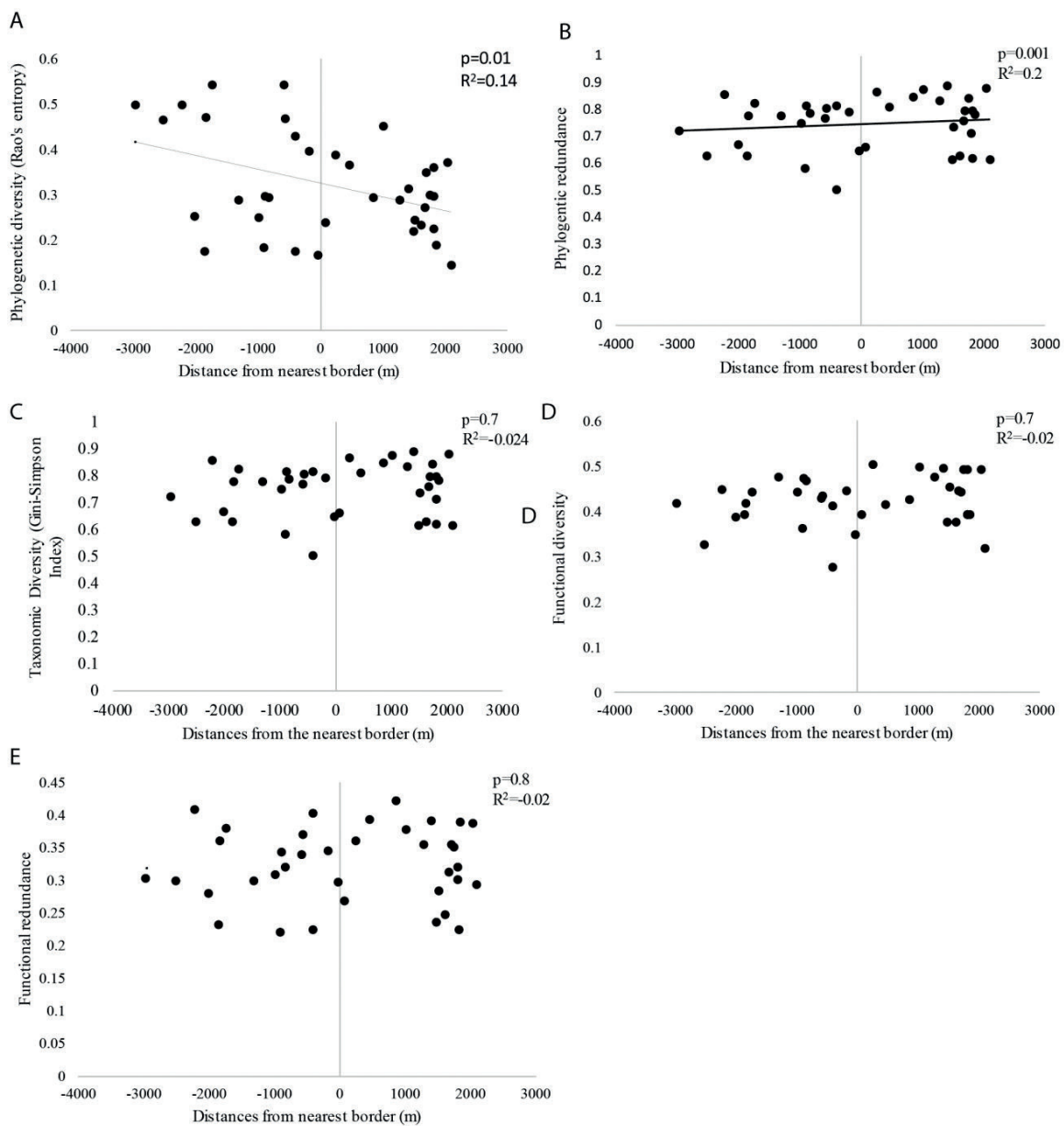
Family/species	Inside	Outside	Number of ponds (total 38)
<b>Bufonidae</b>			
<i>Rhinella icterica</i>	01	04	04
<i>Rhinella ornata</i>	34	00	07
<b>Hylidae</b>			
<i>Dendropsophus minutus</i>	74	40	31
<i>Hypsiboas faber</i>	30	24	18
<i>Ollolygon aromothyella</i>	17	00	03
<i>Scinax fuscovarius</i>	04	00	01
<i>Scinax granulatus</i>	58	23	31
<i>Scinax perereca</i>	24	00	10
<b>Leptodactylidae</b>			
<i>Leptodactylus latrans</i>	27	13	12
<i>Leptodactylus mystacinus</i>	28	19	21
<i>Physalaemus cuvieri</i>	45	37	25
<i>Physalaemus</i> aff. <i>gracilis</i>	31	04	15
<b>Microhylidae</b>			
<i>Elachistocleis bicolor</i>	12	24	15
<b>Phyllomedusidae</b>			
<i>Phyllomedusa tetraploidea</i>	18	00	08
<b>Ranidae</b>			
<i>Lithobates catesbeianus</i> *	50	28	23

\*Exotic species.

**Table 2.** Anuran species, place of occurrence, frequency of occurrence and maximum abundance recorded in ponds monitored at Parque Estadual do Turvo and adjacencies between 2014 and 2016.



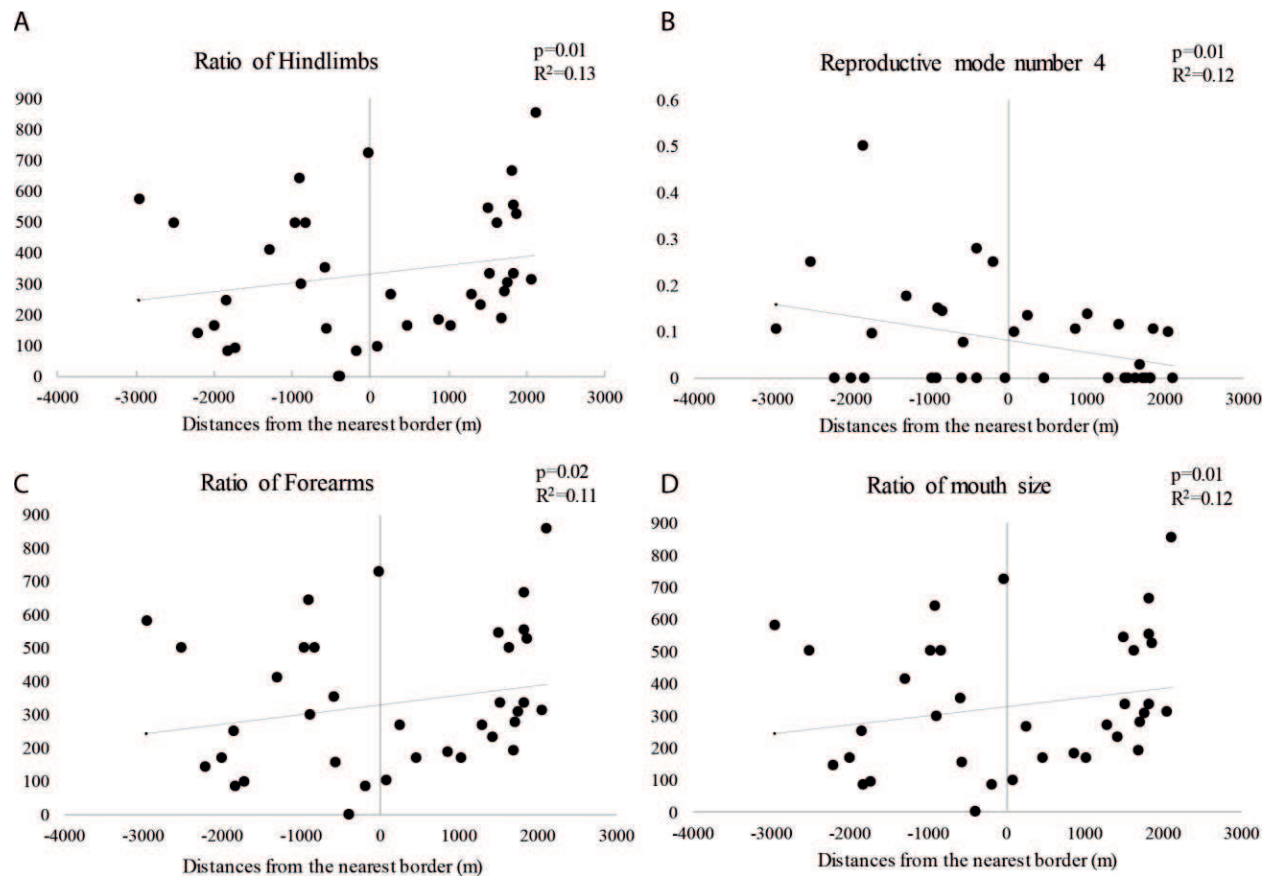
**Figure 2.** Whittaker diagram, showing the distribution of abundance of 15 anuran species recorded along an agricultural-preserved forest gradient at the Parque Estadual do Turvo, Rio Grande do Sul, southern Brazil. Black triangles represent the inner portion of the gradient, and the gray triangles represent the outside portion.



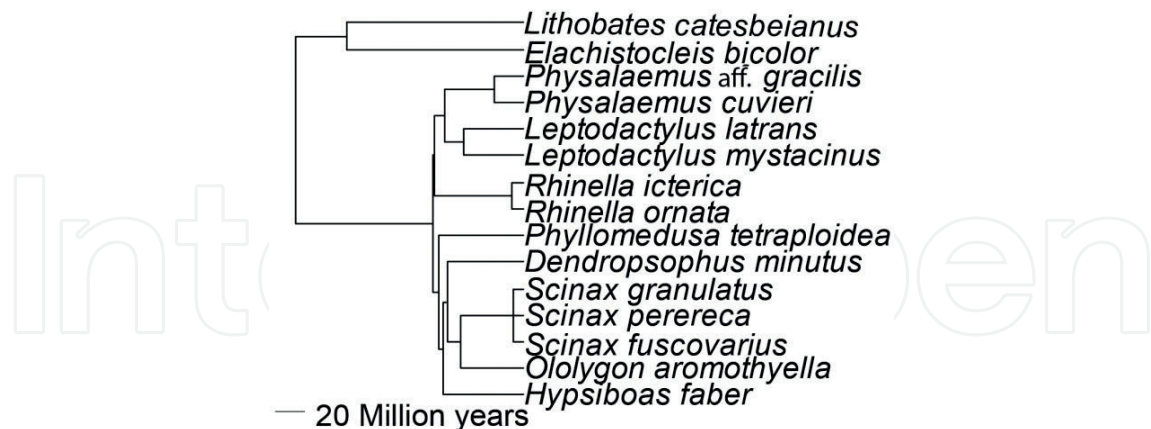
**Figure 3.** Regression results between anuran functional, phylogenetic ( $p < 0.05$ ) and taxonomic diversities and redundancy along the agricultural-preserved forest gradient (environmental filter) at Parque Estadual do Turvo and adjacencies, southern Brazil between 2014 and 2016.

Despite the total functional diversity not showing statistical significance, when evaluated alone with the distance gradient, the attributes (CWM matrix) showed that the individuals from the inner portion of the gradient presented higher values of the forelimb ratios (hind limbs  $r^2 = 0.13$ ,  $p < 0.05$ , forearms  $r^2 = 0.11$ ,  $p < 0.05$ ) and mouth size ratio ( $r^2 = 0.12$ ,  $p < 0.05$ ) than the individuals found at the outside portion of the gradient. The results also showed that the reproductive mode number 4 (eggs laid on small ponds constructed by the males) [43], exhibited by *Hypsiboas faber*, is more commonly found at the outside than the inner portion ( $r^2 = 0.12$ ,  $p < 0.05$ ; see **Figure 4A–D**).





**Figure 4.** Regression results between the ratios of the morphological characteristics of anurans and their reproductive modes along the agricultural-preserved forest gradient (environmental filter) at Parque Estadual do Turvo and adjacencies, southern Brazil between 2014 and 2016.



**Figure 5.** Phylogeny of the anurans recorded at waterbodies monitored along an environmental gradient ranging from an agricultural landscape to a well-preserved forest at the southern Brazil. Generated based in Pyron and Wiens [34], Narvaes and Rodrigues [37], Faivovich (2002), Nascimento et al. [36], de Sá et al. (2012), [38] and Vieira (2010). We defined the branch length based on the estimative of the age of the clades, given by the TimeTree (Hedges et al., 2006).

The phylogeny generated concerning the anurans showed the formation of two distinct clades (Figure 5). The first, the oldest clade (about 90 million years), is formed by two genera with one species each (*Lithobates catesbeianus* and *Elachistocleis bicolor*); the second clade is formed

by eight genera (*Physalaemus* [two species], *Leptodactylus* [two species], *Rhinella* [two species], *Phyllomedusa* [one species], *Dendropsophus* [one species], *Scinax* [three species], *Ololygon* [one species] and *Hypsiboas* [one species]).

## 4. Discussion

The anuran community of the PET is characterized as a mixture of species (from several families and genera) [44] given their distribution patterns. From the 15 species found at our study, five occurred only at the inner portion of the gradient (*Ololygon aromothyella*, *Phyllomedusa tetraploidea*, *Rhinella ornata*, *S. fuscovarius* and *S. perereca*), and four of them are quite dependent of the arboreal strata or the different types of vegetation at water surface (*O. aromothyella*, *P. tetraploidea*, *S. fuscovarius* and *S. perereca*) [43, 45, 46].

The pattern observed at the Whittaker's diagram (**Figure 2**) showed the dominance of a low number of species at the inside and outside portions of the gradient. This kind of pattern is considerably recurrent; other studies already showed the decrease of richness and enhancing on dominance at places affected by anthropogenic disturbance [47–49]. In the present case, at both portions of the studied gradient, the native species *Dendropsophus minutus* and *S. granulatus* and the exotic species *Lithobates catesbeianus* presented higher abundances when compared to other species. These two native and abundant species share not just the reproductive mode but are also highly tolerant to human induced disturbances, being found close to human dwelling (or inside of them, like *S. granulatus*) and man-made water bodies. However, *L. catesbeianus* presents a high invasive potential, and as explained by Madalozzo et al. [50], its distribution is facilitated by the influence of the edge effect and the man-made water bodies along the borders of PET.

Our results show that despite the initial assumption of a higher taxonomic and functional diversity at the inner portions of the gradient, there is no significant difference between the two sampling sites. The great number of man-made water bodies available outside of PET area may explain the similarity on the taxonomic and functional diversity given the high number of generalist species that inhabit both agriculture and forest environments (e.g. *D. minutus*, *S. granulatus*, *L. catesbeianus*, and *Physalaemus* spp.) and their reproductive modes, associated to both permanent and temporary ponds. This pattern of occurrence is commonly found at studies on Atlantic rainforest *lato sensu* (with exception of the wet evergreen forest), mainly at locations that present ecotonal characteristics (given the recent anthropogenic modifications). This landscape feature may exert influence on anuran reproductive behavior and physiology, given the unpredictability of variables like temperature and evaporation at these places, enhancing the establishment of more plastic species which can respond differently and maybe more efficiently to disturbed environmental conditions [51, 52]. In this way, it is expected to find similar species (with similar functional traits) when thinking only on the pond-dwelling anurans, both, in and outside of the gradient, since they have to deal with the diversity of microhabitat of both places, diminishing the difference of this diversity patterns. However, when adding the stream (e.g. *Vitreorana uranoscopa*, *Hypsiboas curupi*, *Crossodactylus schmidtii*) the marsh-dwelling anurans (e.g. *Odontophrynus americanus*, *Proceratophrys avelinoi* and *P. bigibosa*) and the extremely ephemeral pond-dwelling anurans (e.g. *Melanophryniscus*), we can expect to see greater differences.

Despite the lack of difference on taxonomic and functional diversities (and also functional redundancy), we found evidence of a decrease on the phylogenetic component of diversity toward the interior of the park. This result suggests that the environmental filter (distance) has influence on the phylogenetic structure of the assemblages and also suggests low phylogenetic competition, opposing to what can be seen outside of the park [53, 54]. However, another possibility may arise (concerning the decrease of phylogenetic diversity) with the presence of strong competitors (clades); in this case the competition would be also a biotic influence on these assemblages [55, 56]. These two non-excluding possibilities agree with the hypothesis of the niche conservatism [56, 57], so the similar ecological traits shared by the phylogenetically close species would allow them to coexist and the conservative similarity on niche usage by these species would have shaped the actual clustering or over dispersion (e.g. outside portion of the gradient).

In this way, it is expected that species occupying the same habitat (e.g. inner portion or outside portion of the gradient) will show similar morphological traits in response to the environment [58]. However, under a more competitive scenario, it would be expected that they show differences on morphology and, then, show the existence of some degree of niche specialization [59]. Despite the assumption of a similar response in morphology trait from closely related species (evolutionary), the difference found on the size of some morphological traits (e.g. hind limbs, forearms and mouth), greater from individuals from inside and smaller to individuals from outside, here, this pattern occurs following the premises of adaptive radiation, showing that when the species (or lineages) adapt themselves to explore new or different niches, the changes can be rapid [60–62]. The individuals present at the inner portion of the gradient are adapted to cope with some barriers of dispersion (e.g. fallen trees, streams) and/or make use of a larger number of habitats than the individuals present on the outside portion that are susceptible to predation, to desiccation and to pesticides (given the anthropic nature of the landscape). This is also corroborated when we see that species that construct nests that can hold water (reproductive mode 4) could be better distributed or more frequently found at places with hydrological deficit.

It is widely known that land-use intensification is one of the major threats to biodiversity in local and global perspectives. Several studies have shown that anthropogenic influence can cause a decline in several aspects of diversity in natural assemblages [22]. In this way, these modifications would not allow the species to track their optimum environment, forcing them to adapt in situ to avoid extinction [63]. These adaptations can be seen when the functional traits (functional diversity) from individuals of a highly preserved area, show similarity from individuals of a highly converted area; it is the phenotypical plasticity of these individuals that seems to be needed at these places. In the present study, we found evidence of a strong influence of the environmental conditions shaping the assemblages, given the phylogenetic clustering and the lack of difference on functional diversity.

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## Appendix I. Examined anuran specimens from the Herpetological Collection of Universidade Federal de Santa Maria (ZUFSM). Missing numbers are individuals measured in field and from didactic collection

*Dendropsophus minutus*: ZUFSM4540, ZUFSM4558, ZUFSM4621, ZUFSM4622, ZUFSM4630 and ZUFSM4632; *Elachistocleis bicolor*: ZUFSM4575; *Hypsiboas faber*: ZUFSM 4476, ZUFSM4619 and ZUFSM4661; *Leptodactylus fuscus*: ZUFSM4585, ZUFSM4628 and ZUFSM 4660; *Leptodactylus latrans*: ZUFSM4557 and ZUFSM4604; *Leptodactylus mystacinus*: ZUFSM4473, ZUFSM4526 and ZUFSM4551; *Ololygon aromothyella*: ZUFSM4547, ZUFSM4566, ZUFSM4596, ZUFSM4598, ZUFSM4616, ZUFSM4623, ZUFSM4633, ZUFSM4634 and ZUFSM4635; *Phyllomedusa tetraploidea*: ZUFSM4533, ZUFSM4580 and ZUFSM4581; *Physalaemus cuvieri*: ZUFSM4555, ZUFSM4563, ZUFSM4578 and ZUFSM4579; *Physalaemus aff. gracilis*: ZUFSM4356, ZUFSM4358, ZUFSM4359, ZUFSM4368, ZUFSM4553, ZUFSM4572 and ZUFSM4609; *Rhinella icterica*: ZUFSM4529, ZUFSM4518, ZUFSM4516, ZUFSM4515, ZUFSM10000, ZUFSM10009, ZUFSM10010 and ZUFSM10011; *Rhinella ornata*: ZUFSM4477, ZUFSM4496, ZUFSM4497, ZUFSM4498, ZUFSM4499, ZUFSM4527, ZUFSM4659, ZUFSM10005, ZUFSM10006 and ZUFSM10007; *Scinax fuscovarius*: ZUFSM4549, ZUFSM4556, ZUFSM4576 and ZUFSM4610; *Scinax granulatus*: ZUFSM4550, ZUFSM4559, ZUFSM4594 and ZUFSM4607; *Scinax perereca*: ZUFSM2810, ZUFSM2956, ZUFSM4513, ZUFSM4597, ZUFSM4599, ZUFSM4606, ZUFSM4613, ZUFSM4617, ZUFSM4627 and ZUFSM4808.

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