

# Ecological trait evolution in amphibian phylogenetic relationships

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Current biodiversity patterns of Neotropical amphibians are the result of their functional and phylogenetic relationships. Understanding the associations between ecological similarity and phylogenetic relatedness among species can provide a

convincing statement on the role of evolutionary history in the filling of the niche space. Here, we assessed the ancestral character states of amphibian ecological traits and their evolutionary history in the Atlantic Forest Hotspot. We used 12 genes (11,906 bp) to reconstruct a phylogeny for 207 amphibian species and related it to eight ecological traits regarding their morphology, life-history and behavioural features. We revealed that closely related species can have similar ecological traits, suggesting that these traits are driven by phylogenetic history. Despite the high endemism rate of Atlantic Forest amphibians, our findings heavily rely on good studies on complete amphibian phylogenetic lineages to overcome potential biogeographical constraints. Using mechanisms of adaptive evolution in the context of phylogenetic diversification, we suggest that closely related species have different phylogenetic signals and ecological traits can evolve without relatedness.

KEY WORDS: evolutionary changes, phylogenetic signal, functional niche, Atlantic Forest, Anura, Gymnophiona.

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## INTRODUCTION

Ecological trait evolution is the main result of the evolutionary history of functional interactions that addresses fundamental aspects on species' morphology, life-history and behaviour, according to their phylogenetic relationships (Wiens & Graham 2005; McGill et al. 2006; Kraft et al. 2007; Violle et al. 2007). As closely related species are often functionally similar (Losos 2008), many ecological traits show strong

phylogenetic signals (Freckleton et al. 2002; Webb et al. 2002; Moles et al. 2005; Donoghue 2008). In this context, a central question is how similar do traits need to be to qualify them as conserved (Pyron et al. 2015). The magnitude of phylogenetic signals can indicate the degree to which closely related species tend to have similar traits (Blomberg et al. 2003). Empirical evidence suggests that within a major clade, some lineages show strong phylogenetic signals in ecological traits, whereas others are likely to show predominantly divergent traits (Diniz-Filho et al. 2010). A single ecological trait can show different values of phylogenetic signal in relation to which node is considered to evaluate it (Swenson & Enquist 2009). Therefore, systematic measures that allow an evaluation how closely related species tend to retain more similar traits than distantly related may provide key insights into the evolutionary processes behind ecological patterns (Duarte et al. 2012).

One of the main challenges in evolutionary biology is to explain the relationships of ecological traits in functional niches, and how these traits can change multiple times (Gomez-Mestre et al. 2012). Another challenge which has yet to be addressed is to understand how evolutionary traits are related with the long-term maintenance of ancestral characters and the multiple origins of common ancestral states (e.g. Hansen & Houle 2004; Uyeda et al. 2011). The key strategy to address these challenges is assessing the relationships between ecological and phylogenetic traits (Cadotte et al. 2009; Gravel et al. 2011). Understanding the associations between ecological similarity and phylogenetic relatedness is an essential step to reveal biodiversity assembly mechanisms and ecosystem functioning (Hof et al. 2010; Bello et al. 2017).

Current patterns of diversity and distribution of tropical amphibians is a consequence of their ecological and phylogenetic traits (Jetz & Pyron 2018).

Evolutionary processes involving amphibians at a macroscale have been widely explored in previous studies (e.g. Wiens 2007; Hof et al. 2010; Ernst et al. 2012; Fritz & Rahbek 2012; Duarte et al. 2014; Trakimas et al. 2016). Some studies also highlight a tendency of amphibians to exhibit some degree of phylogenetic signal in their ecological traits (Wiens et al. 2006; Wollenberg et al. 2008; Moen et al. 2009). Incorporating phylogenetic information into trait-based approaches can be useful tools for predicting ecological processes (Cavender-Bares & Wilczek 2003), such as environmental filtering on amphibian community dynamics (Ernst et al. 2012).

Here, we test whether closely related species are more similar in their ecological traits than expected if traits are independent of species phylogenetic relatedness, using the amphibians of the Atlantic Forest Hotspot as a case study. We calculated phylogenetic signals in ecological traits and provide evidence that these traits follow a Brownian evolution model. In this context, we determine whether the ecological traits of the Atlantic Forest amphibians are potentially driven by phylogenetic relationships. Our findings reveal that phylogenetically related species may have different ecological functions and the strength of the phylogenetic signals can vary across amphibian orders, families and subfamilies.

## MATERIALS AND METHODS

### *Study area*

The Atlantic Forest is one of the five most important biodiversity hotspots on Earth (Myers et al. 2000). Originally, it covered around 1,500,000 km<sup>2</sup> of which only about 12% (~ 195 km<sup>2</sup>) remains in Brazil, Paraguay and Argentina (Ribeiro et al. 2009), corresponding to around 100,000 km<sup>2</sup> of Brazilian forest remnants (Tabarelli et al. 2005). These forest remnants face a high rate of habitat loss (Teixeira et al. 2009),

which is one of the main factors driving amphibian populations to extinction (Stuart et al. 2004; Becker et al. 2007; Ferreira et al. 2016). Despite this, the Atlantic Forest is still considered the leading biome in terms of amphibian diversity in Brazil (Haddad et al. 2013), harbouring more than 50% of all amphibian species listed in this country (Haddad et al. 2013). Its geographical characteristics including a wide altitudinal range has favoured high species richness and levels of endemism, including more than 543 recognized amphibian species (Haddad et al. 2013). Here, we used the term Atlantic Forest according to the vegetation remnant map produced by the SOS Mata Atlântica/INPE (2015).

#### *Data acquisition*

We obtained spatial data on amphibian species following two approaches. First, we built a data set covering all currently recognized taxa known from the Atlantic Forest following Haddad et al. (2013); and second, we performed complementary fieldwork in seven protected areas across the Serra do Mar Coastal Forests and the Central Corridor of the Atlantic Forest, stretching from the South to the Northeast of the country (see Fig. S1) to compile additional data on species distribution and ecological traits. In all surveyed localities, we used acoustic and visual nocturnal/diurnal assessments (Crump & Scott 1994; Zimmerman 1994), through an active search for amphibians around water bodies, streams and along 2,000 m forest transects within each protected area. Following procedures approved by the American Society of Ichthyologist and Herpetologist (ASIH 2004), we euthanized the collected species with hydrochloride benzocaine ( $\geq 250$  mg/L), under the national biological sampling between 2015-2018 (ICMBio-SISBIO; license #30344/44755).

## *Ecological traits*

We characterized 207 amphibian species according to eight ecological traits that determine different dimensions of the amphibians' functional niches in relation to their morphology, life-history and behaviour. We used the following traits according to Haddad et al. (2013), with some additional complements obtained in the fieldwork: (1) Activity (nocturnal; diurnal; nocturnal and diurnal); (2) Body size (small < 3 cm; medium 3-10 cm; large > 10 cm); (3) Calling site (without calling; bamboo groove; swamp and pond; bromeliad; forest floor; tree crown; cave and burrow; stream and rivulet; river; low vegetation; leaf titter; swamp, pond, stream and rivulet); (4) Toxicity (unknown; toxic; unpalatable; non-toxic); (5) Habit (arboreal; terrestrial; cryptic; fossorial; rheophilic; semi-aquatic; aquatic; arboreal and cryptic; arboreal and aquatic; fossorial and aquatic; arboreal and terrestrial); (6) Habitat (forest; open area; forest and open area); (7) Developmental mode (direct; indirect); (8) Members (apod; tetrapod). Given the different modes of adaptive evolution at phylogenetic lineages both among "apods" and among "tetrapods", we used the category of "Members" in order to resolve the evolutionary origins between the Anura and Gymnophiona species. Considering the functional similarities among the ecological traits of the species evaluated, we used some non-exclusive character states, which can be represented by more than a single character. Such ecological traits contribute to ecosystem supporting services through direct and indirect changes on the ecosystem functions and processes (Hocking & Babbitt 2014). These functions can be structural (habitat and habit) and ecological (body size, members, activity, toxicity, calling site, and developmental mode). For further details, see the Supporting Information (Table S1), where we showed the specific functions and the ecosystem supporting services of each one of the ecological

traits assessed (Duellman & Trueb 1994; Toledo et al. 2007; Wells 2007; Haddad et al. 2013; Hocking & Babbitt 2014).

### *Phylogenetic tree*

According to the time-calibrated phylogenetic framework proposed by Jetz & Pyron (2018), we used an existing molecular supermatrix to produce a novel phylogeny estimate for the Atlantic Forest amphibians (i.e. 11,906 bp for each species), through three mitochondrial (i.e. Cyt-b, 12s and 16s) and nine nuclear genes (i.e. CXCR4, H3A, NCX1, POMC, RAG1, ROHD, SIA, SLC8A3 and TYR). For the length-variable regions, we performed multiple pairwise comparisons by the online version of MAFFT v.6.8 and the G-INS-i algorithm (Katoh & Toh 2008). After, we put together alignments of all genes in the same alignment using the software SequenceMatrix 1.7.7 (Vaidya et al. 2011) to concatenate the supermatrix previously produced. We obtained the molecular data of 207 species in terms of nucleotide sequences obtained from GenBank (Benson et al. 2013; Table S2), provided by the National Center for Biotechnology Information (NCBI).

We inferred the phylogenetic relationships performing Bayesian analyses in software BEAST 1.7 (Drummond et al. 2012), which was based on the combined data matrix, a HKY model of sequence evolution for all genes, using a Yule speciation process as the tree prior and an uncorrelated relaxed clock. We run the Yule process for 100 million generations, ensuring that the number of generations convergence were sufficient assessed with Tracer 1.6 (Rambaut et al. 2014), removing a conservative 10% burn-in fraction for the final tree. We combined these results with the use of LogCombiner 1.8 (Rambaut & Drummond 2013a). We conducted additional phylogenetic estimations based on 100 stochastic trees to account for the phylogenetic

uncertainty of the single reconstructed phylogenetic tree for our 207 species assessed. Given these 100 random simulations, we built the maximum clade credibility tree (summary tree) with the use of TreeAnnotator 1.8 (Rambaut & Drummond 2013b). To account node support, we used posterior probability of each node in accordance with the 100 trees generated automatically. We considered the nodes strongly supported if they received posterior probability  $\geq 0.95$ .

The reconstructed phylogenetic tree provides a revised taxonomic classification that incorporates the newly obtained phylogenetic information slightly changing in an existing classification (Frost 2019). Thus, we did not take the low supported nodes as a reason of changes in the current classification. We only used species from one geographic region, which in case of low node support (not shown), could be a result of an incomplete lineage sorting and thus the lack of information in the sequence data.

We estimated the phylogenetic relationships of the ecological traits by using the most recent maximum-likelihood topology for 7,238 extant amphibian species, according to the time-calibrated reconstruction proposed by Jetz and Pyron (2018). To edit our phylogenetic tree based on this recent time-calibrated reconstruction, we used the package ‘ape’ (Paradis 2004), in the R software (R Development Core Team 2017). Finally, we revealed a wide-ranging phylogenetic tree across two orders, 17 families and 15 subfamilies (Fig. 1).

#### *Reconstruction of ancestral character states*

We reconstructed the ancestral character states using maximum-likelihood estimations under stochastic character mapping analysis (SIMMAP, Bollback 2006), using 1,000 simulations for discrete characters based on the ecological-trait matrix (Table S3). We compared the likelihood estimations with a value of  $\lambda = 1$  for



providing a standardized method to draw the reconstructed phylogenies on the distribution of ecological traits, accounting the phylogenetic uncertainty for the ancestral character states. We also used null simulations across the tips of the reconstructed trees indicating the expected variances within the ancestral characters under Brownian motion. We performed the stochastic character mapping analysis and the null simulations in the R software (R Development Core Team 2017), using the “make.simmap” and “fastBM” functions of the package “phytools” (Revell 2012).

Given that the characters in common for several lineages can originate from the common ancestor of those lineages and may converge to the tips of the tree (Pavoine et al. 2008), we assessed the relationships among the diversity of ancestral states at different taxonomic levels. Resolving the evolutionary origins of the ecological traits, we used an additive partitioning of diversity based on the ancestral states observed and expected for the taxonomic levels “species”, “subfamilies”, “families”, and “orders”. For each taxonomic level, a value of alpha diversity was calculated (i.e. the number of character states). Therefore,  $\alpha_1$  represented the alpha diversity in the lower taxonomic level (species), so that  $\alpha_2$ ,  $\alpha_3$  and  $\alpha_4$  corresponded to alpha diversity in the three subsequent levels (subfamilies, families and orders). According to the protocol proposed by Crist et al. (2003), we tested whether the distribution of diversity across levels differs from the expected by chance using a null model under a 95% confidence interval with 999 randomizations. We used expected diversity partitions through the null model implemented in the “r2dtable” function, which is based on the Patefield algorithm for generating random matrices of diversity across levels (see Blüthgen et al. 2008). We performed all analyses for the additive partitioning of diversity in the R software (R Development Core Team 2017), using the algorithms “boot” and “mass” through the “adipart” function of the package “vegan” (Oksanen et al. 2013).

We evaluated the ancestral traits for the following "lineages" from the original tree: Orders – Gymnophiona and Anura; Families – Typhlonectidae, Siphonopidae, Microhylidae, Leptodactylidae, Pipidae, Ranidae, Aromobatidae, Hylodidae, Cycloramphidae, Bufonidae, Craugastoridae, Brachycephalidae, Eleutherodactylidae, Odontophrynidae, Hemiphractidae, Phyllomedusidae and Hylidae; and Subfamilies – Gastrophryninae, Paratelmatobiinae, Leiuperinae, Leptodactylinae, Allobatinae, Craugastorinae, Holoadeninae, Ceuthomantinae, Phyzelaphryninae, Hemiphractinae, Scinaxinae, Pseudinae, Lophyohylineae, Dendropsophinae and Cophomantinae. We followed Frost (2019) for the taxonomic nomenclature of families, subfamilies and species.

#### *Phylogenetic signal*

We assessed the phylogenetic signal of each ecological trait using a robust test proposed by Abouheif (1999). The Abouheif's  $C_{\text{mean}}$  test uses the Geary's C and Moran's I indices, providing a phylogenetic proximity matrix that does not relate to branch length but focuses on topology of the tree and has a non-zero diagonal values (see Pavoine et al. 2008). We estimated Abouheif's  $C_{\text{mean}}$  with 999 randomizations using the package "adephylo" (Jombart et al. 2010), in the R software (R Development Core Team 2017).

In order to test what ecological traits follow a stochastic Brownian evolution model, we used a measure based on the maximum likelihood of phylogenetic signals, called lambda ( $\lambda$ ), developed by Pagel (1999). This metric is a scaling parameter that measures the phylogenetic dependence of observed trait data as a multiplier applied to the internal branches of the tree. The lambda parameter ranges from 1 to 0, where  $\lambda$  equal to 1 correspond to the Brownian evolution model, whereas  $\lambda$  equal to 0

correspond to a completely stochastic distribution within the tree branches and an absence of phylogenetic signal (Münkemüller et al. 2012). The strongest argument for using Pagel's  $\lambda$  is that it provides a reliable effect size measure besides testing for phylogenetic signal for both continuous and discrete traits (e.g. Gumm & Mendelson 2011; Münkemüller et al. 2012; Best & Stachowicz 2013). For this analysis, we used the "phylosig" function of the package "phytools" (Revell 2012), in the R software (R Development Core Team 2017).

The main reason behind our choice by the Abouheif's  $C_{\text{mean}}$  and the Pagel's  $\lambda$  tests is their high power to reduce uncertainty besides testing for phylogenetic signal in large phylogenies, providing a level of performance substantially better than other methods (i.e. Fritz & Purvis' D or Blomberg's K; Münkemüller et al. 2012). Fritz & Purvis' D test is a statistic approach to measure phylogenetic signal strength only in binary traits, which are not the encountered in our case study. In this context, we followed the guidelines proposed by Münkemüller et al. (2012) to better assess phylogenetic signal and distinguish it from random trait distributions. Given that Pagel's  $\lambda$  is most valuable for discrete traits that follow Brownian motion (Best & Stachowicz 2013), we fitted the reconstructed phylogenetic trees to a model selection approach based on the delta Akaike Information Criterion ( $\Delta\text{AIC}$ ) weights for three evolutionary models (i.e. BM = Brownian-motion model; EB = Early-burst model; White = White-noise model). We performed these models of character trait correlated evolution using the "fitDiscrete" function of the "geiger" package (Harmon et al. 2007), in the R software (R Development Core Team 2017).

In addition to the measures described above, we created a traitgram to visualize the evolutionary diversification for the ecological traits across the reconstructed phylogeny under ancestral character estimation by likelihood, using the "phenogram"

function of the package "phytools" (Revell 2012), in the R software (R Development Core Team 2017). Time scales analysis of the multigene dataset are congruent with previous studies (San Mauro et al. 2005; Roelants et al. 2007; Wiens 2007; Blackburn et al. 2010; Roelants et al. 2011) and support anuran radiation episodes in the Triassic/Early Jurassic (basal anuran radiation).

## RESULTS

Maximum-likelihood ancestral state reconstructions suggested different evolutionary patterns on the distribution of ecological traits, fitting the Brownian-motion evolution model (Fig. 2). Activity trait indicated the “nocturnal” character as an ancestral state shared by the orders Anura and Gymnophiona, and the “diurnal” character as a derived state for the families Aromobatidae, Hylodidae and Brachycephalidae (exclusive for the genus *Brachycephalus*) (Fig. 2a). Body size indicated the “large (> 10 cm)” character as an ancestral state shared by the orders Anura and Gymnophiona, and the “medium (3-10 cm)” and “small (< 3 cm)” characters as derived states for the order Anura (Fig. 2b). Calling site and Toxicity showed derived states among anuran families due to emerged independently through different ancestors (Fig. 2c-d). Habit trait indicated the “aquatic” character as an ancestral state for the subfamily Pseudinae, and the “arboreal” character as a derived state for the families Hylidae, Hemiphractidae and Phyllomedusidae (Fig. 2e). Habitat trait indicated all characters as derived states ancestrally from forest environments (Fig. 2f). Developmental mode indicated the “direct” and “indirect” characters as ancestral states (Fig. 2g). Members indicated the “apodal” character as a derived state for the order Gymnophiona, and the “tetrapod” character as an ancestral state for the order Anura (Fig. 2h).

Additive partitioning of diversity showed the greatest number of ancestral states at the lower taxonomic level (i.e. species), indicating multiple evolutionary origins and adaptive outcomes (i.e. 97% of total observed ancestral states; Fig. 3). Comparing these results with the expected diversity provided by the null model, only the levels “species” and “families” showed diversity values significantly higher than random expectations ( $P < 0.05$ ). However, the levels “subfamilies”, “families” and “orders” did not show relevant diversity values, together accounting for less than 3% of the total diversity of ancestral states evaluated.

Overall, Abouheif’s  $C_{\text{mean}}$  and Pagel’s  $\lambda$  tests indicated that all ecological traits showed significant phylogenetic signals against random expectations. Results of the model selection approach based on the delta Akaike Information Criterion ( $\Delta\text{AIC}$ ) weights support the Brownian-motion as the best fitted model for our trait evolution approach (Table 1). The second best fitted model was the Early-burst (EB), due to some characters’ change tends to be concentrated toward the base of the tree. According to Abouheif’s  $C_{\text{mean}}$ , the traits that had the highest values were developmental mode ( $C_{\text{mean}} = 0.919$ ,  $P < 0.001$ ) and members ( $C_{\text{mean}} = 0.872$ ,  $P < 0.001$ ). Abouheif’s simulations represented the distribution of the statistical  $C_{\text{mean}}$  calculated from each ecological trait along the phylogeny evaluated (Fig. 4). On the other hand, Pagel’s  $\lambda$  also indicated the highest values of the maximum likelihood for the traits developmental mode and members ( $\lambda > 0.999$ ,  $P < 0.001$ ), totally supporting a Brownian evolution model. The traits body size, toxicity and activity also had high  $\lambda$  values ( $\lambda > 0.900$ ,  $P < 0.001$ ), showing close relations to the Brownian evolution model as well. However, the traits habit and habitat showed moderate phylogenetic signals under this same model ( $\lambda > 0.600$ ,  $P < 0.001$ ).

The traitgram visualised the evolution of traits through time, using a projection for the evolutionary diversification of all ecological traits assessed under ancestral character estimation by likelihood (Fig. 5). In addition, this estimate accounts for the uncertainty about ancestral states along branches and at nodes. Potential evolutionary shifts over longer time scales was based on the basal anuran radiation in the Triassic and Early Jurassic periods (i.e. about 200 million years ago). This projection illustrated a horizontal dimension of evolutionary divergence time, showing a quantitative trait evolution for the multiple ancestral characters of the Atlantic Forest amphibians.

## DISCUSSION

Our results showed a strong tendency to retain ancestral ecological traits in Atlantic Forest amphibians. Given the partitioning diversity of reconstructed ancestral states at taxonomic levels, we suggest the additive partitioning of ancestral states as an additional approach for the stochastic character mapping, in an attempt to reduce the uncertainty behind the evolutionary origin of the ecological traits among taxonomic groups. Despite this uncertainty, phylogenetic characteristics of some species can be influenced or directly affected by other not phylogenetically related species. Under similar ecological pressures, some species are reinforced to exhibit the same ecological traits, due to the high phenotypic plasticity of amphibians (Relyea 2001; Urban et al. 2014; Delia et al. 2019), which might in turn imply a lower functional adaptability of species to current and future climate change (Urban et al. 2014).

We highlighted Developmental mode (i.e. direct and indirect) and Members (i.e. apod and tetrapod) as the most ancestral traits across the long sequence of changes in the basal amphibian radiation at the Atlantic Forest. In many cases, indirect development characters seem to represent terminal stages that are retained for tens of

millions of years without proceeding to direct development (e.g. Phyllomedusidae and Hylidae families). Although we have shown high levels of homoplasy, some lineages do not seem to have been hampered in their ecological diversification. Many specialization modes can be evolving with variations in different ecological traits related to behaviour, habitat and developmental modes (Roelants et al. 2011). The development of the same ecological traits using different evolutionary mechanisms cannot be distinguished by our results. However, most of specialization modes behind the ecological traits assessed can have been widespread with homoplasy across parallel evolutions (see Bossuyt & Milinkovitch 2000).

Despite an overall and robust observed trend of detection of phylogenetic signals in ecological traits, we found large variation in ancestral character histories among taxonomic groups. Both measures used to determine the strength of the phylogenetic signals were highly significant, suggesting that they are following a Brownian evolution model. This variation in ancestral character histories was supported by the length of the branches (a surrogate of time), corresponding to the characters' rhythm of evolution expected under a random walk model (Gingerich 2009; Hunt 2012; Hunt & Rabosky 2014). However, low phylogenetic signals in ecological traits (i.e.  $C_{\text{mean}} < 0.6$ ) also showed exceptions from the random walk model, especially in the terminal branches. These exceptions may indicate that ecological condition changes could be fast in these taxonomic bifurcation points and not in the overall reconstructed tree. On one hand, mapping trait evolution on reconstructed phylogenies allows estimating where the phylogenetic nodes connect the species trait values to the trait values of their potential ancestors (Ackerly 2009; Kembel et al. 2010; Revell 2012). On the other hand, the phylogenetic relationships of species showed by the traitgram can be difficult to understand, mainly when species in different parts of the phylogeny have similar

ecological traits (Revell 2013). In this context, Campos et al. (2017) revealed a high congruence of functional and phylogenetic patterns of amphibian biodiversity, providing potential trade-offs for ecological and evolutionary processes in the Brazilian Atlantic Forest.

Some other studies stated a doubt if the establishing of the existence of a phylogenetic signal is a useful approach for integrating evolution, ecology, and conservation biology (Wiens & Graham 2005; Wiens 2008). However, there is an ongoing demand for further evidence to identify phylogenetic signals in different communities (Losos 2008). The attempts to assess the existence of a phylogenetic signal are very relevant to researches of biodiversity gradients and species distribution models for climate change predictions (Hof et al. 2010), while the fact of its generality is still under discussion.

We observed that many ecological traits are conserved enough to show strong phylogenetic signals when closely related species have similar traits, whereas convergence has occurred when species from different lineages have similar ecological characters. Conserved traits have been observed in different amphibian lineages, at least within lower clades such as families or genera (Wiens et al. 2006; Wollemborg et al. 2008; Algar et al. 2009; Moen et al. 2009). Among these clades, the Hylidae family showed to be generally conserved across the evolutionary history of the group in relation to climatic factors (Wiens et al. 2006). This association with speciation rates can be explained by latitudinal diversity gradient (Wiens et al. 2011). Some salamanders of the genera *Plethodon* and *Desmognathus* also showed relationships between speciation and endemism rates with conserved traits across the phylogeny (Kozak & Wiens 2006). The widespread conservatism hypothesis is supported by phylogenetic clusters based on ecological niche constraints (Pianka et al. 2017) and



their geographic location (Wiens et al. 2006). However, we need to take into account that most associations between phylogenetic and ecological traits are scarcely based on an entire phylogenetic tree (Diniz-Filho et al. 2010). Therefore, some phylogenetically conserved traits apparently attributed to regional amphibian clades can be underestimated due to history-related biogeographical constraints on different phylogenetic lineages (Hof et al. 2010; Ernst et al. 2012).

We showed a straightforward and promising approach in how amphibian ecological traits can recover significant phylogenetic signals in the Atlantic Forest. However, our findings heavily rest on good studies on complete amphibian phylogenetic lineages to overcome potential biogeographical constraints. Our study includes basically all Atlantic Forest amphibian species with available data on both phylogenetic and ecological features, highlighting how badly more basic research is needed to provide empirical data for testing evolutionary and ecological questions.

Although we found that closely related species can show different ancestral states, we revealed that most amphibians of the Atlantic Forest have ecological traits driven by phylogenetic history. However, the strength of their phylogenetic signals varied considerably across amphibian orders, families and subfamilies. In summary, we used a novel approach for investigating reconstructed ancestral states under maximum likelihood and phylogenetic signals across an entire class of organisms in the Atlantic Forest, accounting differences from lower (species) to higher (orders) taxonomic levels.

Despite the potential biogeographical constraints of our assumptions, our results address how the ecological trait evolution of amphibians can be informative to describe regional phylogenetic patterns based on multiple and discrete characters. A starting point to address questions related to conservatism hypothesis and biogeographical constraints of different phylogenetic lineages may help to describe the evolutionary

patterns that may be important for environmental filtering. This work has sought to move forward the use phylogenetic signals as a proxy for ecological similarities, supporting conservation studies that explore ecological drivers of phylogenetic loss in biodiversity hotspots.

#### ACKNOWLEDGMENTS

We especially thank the Asociación Española de Ecología Terrestre (AEET) for the research award granted to conduct this research. We are grateful to L. Rincón, P. Pintanel, A. Beckerman, and two anonymous reviewers for the constructive comments and great suggestions on the paper. We also thank the Institut de Biologia Evolutiva (CSIC-UPF) for making the use of lab computers available. All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supporting Information (Appendix I). Additional data related to this paper may be requested from the authors.

#### DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors

#### FUNDING

This research work was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES: 99999.001180/2013-04), and the National Council for Scientific and Technological Development (CNPq: 140710/2013-2).

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#### AUTHOR CONTRIBUTION

Felipe S. Campos conceived the study and wrote the manuscript with contributions from all co-authors. Felipe S. Campos, Ricardo Lorenço-de-Moraes and Andrey Rudoy designed the analyses, collected the data, and created the figures. All co-authors discussed the results and edited the manuscript.

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## Figure Captions

Fig. 1. — Reconstructed phylogenetic tree for 207 amphibian species of the Atlantic Forest. Numbers indicate nodes of Families, and circles indicate nodes of Subfamilies. Gymnophiona: 1. Typhlonectidae; 2. Siphonopidae; Anura: 3. Pipidae; 4. Microhylidae (Gastrophryninae); 5. Ranidae; 6. Aromobatidae (Allobatinae); 7. Hemiphractidae (Hemiphractinae); 8. Craugastoridae (Holoadeninae, Craugastorinae, Ceuthomantinae); 9. Eleutherodactylidae (Phyzelaphryninae); 10. Brachycephalidae; 11. Cycloramphidae; 12. Hylodidae; 13. Phyllomedusidae; 14. Hylidae (Scinaxinae, Pseudinae, Lophyohylineae, Dendropsophinae, Cophomantinae); 15. Leptodactylidae (Paratelmatobiinae, Leiuperinae, Leptodactylinae); 16. Odontophrynidae; 17. Bufonidae.

Fig. 2. — Maximum-likelihood ancestral state reconstruction for the amphibian ecological traits of the Atlantic Forest (N = 207 species). (a) Activity; (b) Body size; (c) Calling site; (d) Toxicity; (e) Habit; (f) Habitat; (g) Developmental mode; (h) Members. Reconstructed phylogenetic trees show discrete traits through stochastic character mapping (SIMMAP), based on based on 1,000 simulations. Vertical bars across the tips of the trees indicate the expected variances on ancestral states among species under the Brownian-motion evolution model.

Fig. 3. — Partitioning diversity of the reconstruction of ancestral states observed and expected at different taxonomic levels (species, subfamilies, families, and orders), according to the reconstructed phylogenetic tree for 207 amphibians of the Atlantic Forest. Random expectations are based on a null model under a 95% confidence interval



772 with 999 randomizations. Bars are organized from lower (species) to higher (orders)  
773 taxonomic levels.

774

775 Fig. 4. — Phylogenetic signal simulations according to the Abouheif's test calculated  
776 for each ecological trait across the reconstructed phylogeny of amphibians of the  
777 Atlantic Forest (N = 207 species). Black diamonds indicate the position of the observed  
778 mean distributions in relation to the  $C_{\text{mean}}$  randomizations. All  $P$ -values < 0.001.

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780 Fig. 5. — Traitgram showing a projection for the evolutionary diversification of  
781 ecological traits across the reconstructed phylogeny of amphibians of the Atlantic Forest  
782 (N = 207 species). Y-axis represents the ecological trait values under ancestral character  
783 estimation by likelihood (log10-transformed). X-axis represents the relative time  
784 (millions of years) and the length of the branches represents a surrogate of time.  
785 Transparent blue stain represents 95% of confidence interval. All  $P$ -values < 0.001.

**Commented [U1]:** The Figure 5 was updated