



# Humidity levels drive reproductive modes and phylogenetic diversity of amphibians in the Brazilian Atlantic Forest

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## ABSTRACT

**Aim** The diversity of reproductive modes among amphibians constitutes a striking example of how differences in the biology of species provide important explanations for species distribution patterns on a broad scale. We hypothesize that sites with a higher humidity level will support more modes of reproduction than drier sites and will consequently exhibit a higher phylogenetic diversity. Furthermore, if there is a gradient in the tolerance of reproductive modes to desiccation, there will be a nested pattern in the composition of reproductive modes among sites.

**Location** Twenty-seven forest sites in the Brazilian Atlantic Forest.

**Methods** Through a path analysis approach, we evaluated the direct and indirect effects of the humidity level on the number of reproductive modes, as well as the relative importance of both variables on amphibian phylogenetic diversity. A nestedness analysis was used to quantify the extent to which the compositions of both species and reproductive modes in drier sites correspond to subsets of those in sites with higher annual precipitation.

**Results** We found that the reproductive modes present in drier sites are non-random subsets of those present in sites with higher humidity levels. Because reproductive modes are phylogenetically conserved among amphibians, sites with a greater number of reproductive modes supported greater phylogenetic diversity. Sites with high precipitation throughout the year provided suitable environmental conditions for a larger number of reproductive modes, whereas sites with low precipitation and typical seasonal climates supported only those reproductive modes specialized to resist desiccation.

**Main conclusions** Our results show that humidity-related variables are key environmental factors related to both the richness of reproductive modes and phylogenetic diversity. Our results support the hypothesis that the higher phylogenetic diversity found in moister sites reflects differences in the tolerance to desiccation among different reproductive modes. Given that reproductive modes are associated with susceptibility to desiccation, their incorporation into explanatory models may trigger a significant advance in the understanding of the mechanisms regulating the species richness and composition of amphibian communities.

## Keywords

Amphibian diversity, anurans, Atlantic Forest, biodiversity, biogeography, Brazil, conservation, humidity, precipitation.

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## INTRODUCTION

It is well known that the determinants of species richness and composition vary greatly among taxa, biomes and spatial scales (Ricklefs, 2004; Wolters *et al.*, 2006). Indeed, the relative importance of environmental factors in regulating species richness also differs among species groups of the same taxon from different regions (Ricklefs, 2004; Buckley & Jetz, 2007) and among different taxonomic groups from the same biome or geographical region (Similä *et al.*, 2006; Wolters *et al.*, 2006). A strong candidate explanation of these differences is that each taxonomic group has a unique set of morphological and physiological constraints and requirements, whereas different regions have distinct sets of environmental conditions (Kearney & Porter, 2009). For instance, it is not surprising that the relative importance of temperature-related variables for species richness differs between ectothermic vertebrates, which maintain their body temperatures by thermal exchange with the environment, and endothermic vertebrates, which control their temperature by shifts in their metabolic rate (Aragón *et al.*, 2010). Variations in the environmental factors regulating species richness even exist between amphibians and reptiles, both of which are groups of ectotherms (Qian *et al.*, 2007; Aragón *et al.*, 2010).

Amphibians are usually less tolerant of desiccation and require higher humidity levels than reptiles or other terrestrial vertebrates (Ludwig, 1945). The reason for these differences is that amphibians have highly permeable skin, complex life cycles that are typically dependent on both aquatic and terrestrial environments (Wilbur, 1980; Becker *et al.*, 2007; da Silva *et al.*, 2012), and low dispersal ability (Duellman & Trueb, 1986; Blaustein *et al.*, 1994; da Silva *et al.*, 2012). These characteristics explain why humidity-related variables, such as mean annual rainfall, tend to be more important for amphibian species richness (Rodríguez *et al.*, 2005; Vasconcelos *et al.*, 2010) than for the richness of other terrestrial vertebrates (Rahbek & Graves, 2001; Zhao *et al.*, 2006). Therefore, to understand and predict variation in amphibian species richness, it is necessary to consider how key climatic factors constrain or facilitate the occurrence of certain traits, such as the types of reproductive modes and the dependence of the organisms on aquatic environments.

In this study we examine the relative importance of the humidity level as a determinant of the variation in species richness among amphibians in the Brazilian Atlantic Forest, a global biodiversity hotspot (Mittermeier *et al.*, 2005). This Neotropical biome originally covered *c.* 1.3 million km<sup>2</sup>, of which between 84% and 89% has been converted to human use or degraded by human activities (Ribeiro *et al.*, 2009). It harbours as many as 460 amphibian species, of which *c.* 73% are endemic (AmphibiaWeb: <http://amphibiaweb.org/>). The great richness and endemism of amphibians in this region are usually attributed to the unusual extent of the ranges of topography (elevation varies from sea level to 2000 m a.s.l.) and latitude (of *c.* 25°), and the associated range of climatic conditions. In addition, the retraction of humid forests during

a cooler and drier period in the Quaternary may explain the regions of high endemism in the Brazilian Atlantic Forest, which served as a large climatic refugium for Neotropical species in the late Pleistocene, as well as local evolutionary differentiation and diversification (Carnaval *et al.*, 2009). The majority of reported declines of amphibian species in Brazil come from the Atlantic Forest (Eterovick *et al.*, 2005; Silvano & Segalla, 2005), and, according to IUCN (2009), 30% of the described amphibians in this biome are threatened with extinction (but for a different interpretation see Pimenta *et al.*, 2005; Haddad, 2008). Therefore, understanding the processes (e.g. extinction, colonization, speciation) regulating the distribution and species richness of amphibians in the Atlantic Forest is an important step towards the conservation and management of the threatened biodiversity of amphibians in this biome.

A great diversity in reproductive modes (hereafter termed RMs) is a distinctive feature of the class Amphibia, particularly of the order Anura. The concept of RMs (*sensu* Salthe & Duellman, 1973) incorporates the oviposition site, ovum and clutch characteristics, the rate and duration of development, the stage and size of hatchlings, and the type of parental care, if any. The most typical and generalized reproductive mode in amphibians is characterized by aquatic eggs that are deposited in lentic water and that develop into exotrophic aquatic tadpoles (Table 1). The development of RMs that are less dependent on aquatic environments is hypothesized to result from diverse selective pressures, such as predation on eggs and tadpoles and the desiccation of water bodies (Salthe & Duellman, 1973; Haddad & Prado, 2005). However, reproductive modes are not considered a fixed behaviour (Haddad & Prado, 2005), and natural variation in oviposition sites may reflect plasticity in the behavioural response of anurans to different environmental conditions (Touchon & Warkentin, 2010). For example, according to Haddad & Prado (2005), the nest-building gladiator frog of the Atlantic forest, *Hyla faber*, deposits eggs as a surface film on water accumulated in constructed clay nests (mode 4); however, if the water level rises so that muddy banks are not available for males to construct their nests, the eggs are deposited as a surface film in ponds (mode 1). Another phenotypically plastic aspect of reproduction with aquatic and terrestrial egg deposition was described by Touchon & Warkentin (2010) for the treefrog *Dendropsophus ebraccatus*, known to lay eggs on vegetation above water (mode 24). This treefrog also lays eggs in water, both at the surface and fully submerged (mode 1), and it chooses its oviposition sites in response to factors affecting the risk of egg desiccation.

Interestingly, the less RMs depend on non-temporary aquatic habitats, the more they tend to depend on predictable rainfall and/or high air humidity (Duellman & Trueb, 1986; Donnelly & Crump, 1998; Haddad & Prado, 2005). Indeed, few species with RMs whose eggs and tadpoles develop out of water in humid forested regions have been found in drier and/or highly seasonal habitats: see, for example, Duellman (1988), Haddad & Prado (2005) and Vieira *et al.* (2009). According to these authors, only species that are more resistant to

**Table 1** Reproductive modes (RMs) in anurans (adapted from Haddad & Prado, 2005) observed for the 27 Brazilian Atlantic forest sites used in the analysis.

RM	Egg deposition	Description of the RM	Number of species	Number of forest sites
1	Eggs deposited in water	Eggs and tadpoles in lentic water	107	27
2		Eggs and tadpoles in lotic water	21	23
3		Eggs and early larval stages in chambers; tadpoles in streams	10	11
4		Eggs and early larval stages in basins; tadpoles in ponds or streams	11	25
5		Eggs and early larval stages in subterranean nests; tadpoles in ponds or streams	7	17
6		Eggs and exotrophic tadpoles in water in tree holes or aerial plants	5	7
8		Eggs and endotrophic tadpoles in water in tree holes or aerial plants	2	8
10	Eggs in bubble nest	Bubble nest floating on pond; tadpoles in ponds	1	3
11	Eggs in foam nest	Foam nest on pond; tadpoles in ponds	19	26
12		Foam nest on pond; tadpoles in streams	1	1
13		Foam nest on water accumulated in constructed basins; tadpoles in ponds	3	11
14		Foam nest on water on the axils of terrestrial bromeliads; tadpoles in ponds	1	1
18	Eggs on ground	Eggs on ground or rock above water; upon hatching, tadpoles move to water	1	1
19		Eggs on rock or tree roots above water; semi-terrestrial tadpoles	5	9
20		Eggs hatch into tadpoles that are carried to water by adult	2	2
21		Eggs hatch into tadpoles that complete their development in the nest	4	5
23		Direct development of terrestrial eggs	27	20
24	Eggs arboreal	Eggs hatch into tadpoles that drop in lentic water	14	21
25		Eggs hatch into tadpoles that drop in lotic water	7	14
27		Eggs hatch into froglets	1	1
28	Eggs in foam nest outside	Foam nest on the humid forest floor; tadpoles in ponds	1	3
30		Foam nest with eggs and early larval stages in nests; tadpoles in ponds	12	22
32		Foam nest in subterranean nests; tadpoles complete development in nest	2	10
36	Eggs carried on dorsum	Eggs carried on dorsum or in dorsal pouch of female; tadpoles in bromeliads or bamboo	3	6
37		Eggs carried on dorsum or in dorsal pouch of female; direct development	1	1

desiccation and have more generalized RMs (modes 1 and 2) or modes adapted to insolation (e.g. modes 11, 13 and 30–32) can occupy habitats with unpredictable rainfall. Therefore, if air humidity and rainfall are important in limiting the occurrence of species with specialized RMs, we predict that drier sites in a given biome or region will harbour non-random subsets of the RMs found in moister sites; that is, a nested pattern in the composition of RMs among sites will be found. Nestedness in species composition has been widely reported (e.g. Patterson & Atmar, 1986; Ulrich *et al.*, 2009); it occurs if species-poor communities tend to harbour subsets of the species present in richer ones (Patterson & Atmar, 1986). The major hypotheses for the emergence of nestedness are related to differences in key habitat features (size, isolation, heterogeneity, and quality) and to certain species attributes (regional abundance, area requirement, niche breadth, and tolerance to abiotic conditions) (see Ulrich *et al.*, 2009 and references therein). For amphibians, differences in environmental and habitat factors can be important for determining nested patterns in species composition (Hecnar & M'Closkey, 1997; Hecnar *et al.*, 2002; Baber *et al.*, 2004).

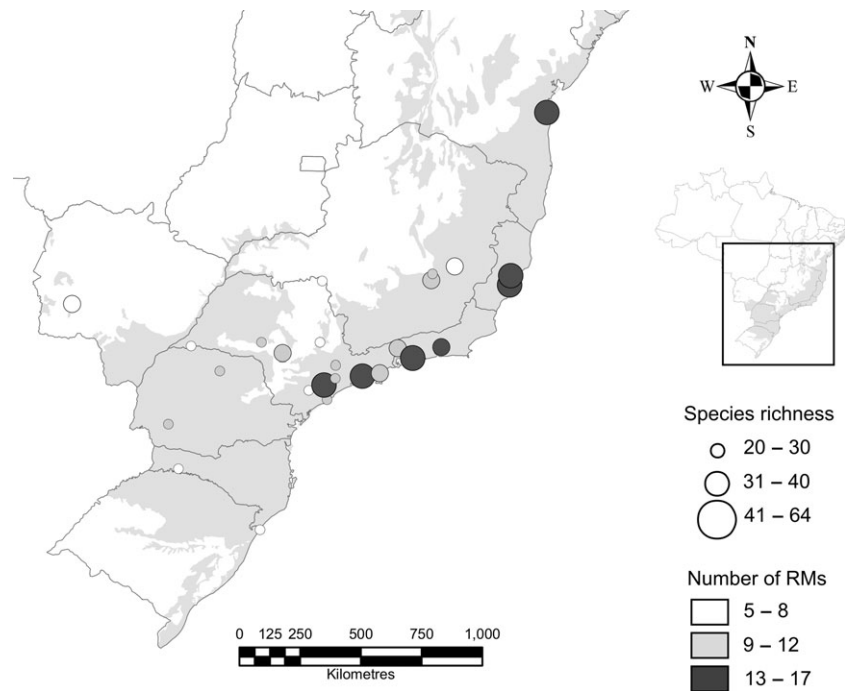
Here, we use checklists of local amphibian communities present in the Brazilian Atlantic Forest to investigate the influence of humidity level on three aspects of amphibian diversity: (1) species composition, (2) the number of RMs, and (3) phylogenetic diversity. Based on the assumption that RMs

differ in their susceptibility to desiccation, we hypothesized that sites with higher humidity levels support more types of RMs than drier sites. More specifically, drier sites will harbour subsets of the species composition and RMs present in sites with higher humidity levels. Furthermore, if RMs are phylogenetically conserved, we predict that sites with higher humidity levels will have higher levels of phylogenetic diversity than drier sites.

## MATERIALS AND METHODS

### Study system

We gathered data on amphibian records from the literature (see Appendix S1 in Supporting Information; data available from Dryad Repository at: <http://dx.doi.org/10.5061/dryad.6764jd6d>) for 27 forest sites (Fig. 1). Only forest communities within the Brazilian Atlantic Forest domain (*sensu* CONAMA, 1992) were included. Vegetation physiognomies ranged from rain forests to seasonal semi-deciduous forests. The sites were located from 19°54' S to 25°26' S and from 40°28' W to 56°48' W, and the highest elevation in each site ranged from 383 to 2000 m a.s.l. We chose survey data instead of range maps to evaluate the hypothesis that high-precipitation environments increase the number of RMs for the following reasons: (1) a species is not guaranteed to be present at every point within the range map delimited, and some RMs could be



**Figure 1** Distribution of the 27 Brazilian Atlantic Forest sites used in the analysis. Symbol size represents species richness. Symbol shade represents number of reproductive modes (RMs).

erroneously considered present in some areas; (2) climatic and physical geography predictors were available for all forest sites, fostering a stronger relationship between RMs and climatic predictors than would be obtained by the use of interpolated data on range maps; and (3) survey data allowed us to restrict the forest sites to the Atlantic Forest biome, whereas range maps would have included strong habitat differentiation and different historical biogeography from other biomes. Many studies have shown the relative merits of map-based versus survey-based data (e.g. Hurlbert & White, 2005; Hurlbert & Jetz, 2007; Hawkins *et al.*, 2008) in the identification of patterns of species richness and diversity hotspots. Hawkins *et al.* (2008) distinguished studies based on sampled data (i.e. richness measured over very local scales) from those based on range maps (i.e. richness estimated within large areas) and found no difference in the explanatory power of climate variables.

We used original checklist publications, local climate records (DNMET, 1992) and Google Earth to obtain the geographical and climatic data listed in Table 2. We compiled the following variables for each forest site: spatial coordinates (latitude and longitude; LAT and LONG, respectively), distance from the sea (DFS; km), elevational range (ELR; m), maximum elevation (MEL; m), annual mean temperature (ANNT; °C), and annual mean precipitation (PPT; mm year<sup>-1</sup>) (see Table 2). At least two of four different sampling methodologies (audio, active search, casual observations, and pitfall traps) were used to record the amphibian species at each site (Appendix S1). Although the number of sampling days varied greatly among the sites (from 12 to 260 days, Appendix S1), little information was available about the actual sampling effort per site (person-hours year<sup>-1</sup> or

**Table 2** Geographic and climatic variables used to assess whether the number of RMs is positively affected by explanatory variables in this study on Brazilian Atlantic Forest amphibians.

Abbreviation	Description	Mean (min, max)
LAT	Latitude	-22.57 (-13.87, -29.35)
LONG	Longitude	-46.89 (-39.13, -56.72)
ELR	Elevational range (m)	540 (160–1446)
MEL	Maximum elevation (m)	901 (383–2000)
PPT	Annual mean precipitation (mm year <sup>-1</sup> )	1775 (1200–3058)
ANNT	Annual mean temperature (°C)	20.8 (15.0–25.3)
DFS	Distance from the sea (km)	214 (1–1000)

season<sup>-1</sup>). Thus, to incorporate differences in sampling effort, we transformed the available information on sampling days according to 10 classes based on a log<sub>2</sub> scale (used as an exogenous explanatory variable; see data analysis).

The RMs of the amphibian species were determined using Haddad & Prado's (2005) classification criteria (Table 1). Among the 39 RMs described to date, 27 were reported for amphibian species occurring in the Brazilian Atlantic Forest, and six were restricted to this biome (Haddad & Prado, 2005). Certain amphibians exhibit multiple reproductive modes (see Haddad & Prado, 2005 for references). In these cases, we considered only the primary RM in the analysis.

### Data analysis

It is well documented that a number of ecological variables do not constitute spatially independent observations, owing to the

spatial structure of the abiotic factors operating at different scales (Legendre, 1993). Thus, we inspected all variables for spatial autocorrelation using Moran's *I* correlograms with an equal number of pairs in the distance class. Because we found no significant spatial autocorrelation for the explanatory (ELR, ANNT and PPT) or the response (amphibian species richness, number of RMs, and subfamilies and families as residuals from species) variables, no correction for the effects of spatial autocorrelation on degrees of freedom (i.e. for spatial dependence) was needed.

To assess the phylogenetic relatedness among all species in our data set, we organized the species according to taxonomic levels (genera, subfamily and family). A taxonomic topology at the genus, subfamily and family levels was compiled from the most recent study of amphibian phylogeny proposed by Pyron & Wiens (2011), which contains 2871 species (40% of the known extant species) from 432 genera (85% of the 500 currently recognized extant genera). Nine families (Alsodidae, Brachycephalidae, Bufonidae, Ceratophryidae, Cycloramphidae, Dendrobatidae, Hemiphractidae, Hylodidae, Odontophrynidae) contain genera that are not classified into any subfamily. For these genera, we implemented informal 'subfamily names', incorporating their phylogenetic relationships, which were assessed through cladograms from several sources (Appendix S1). Although this approach cannot reveal exact interfamily relationships, it can reveal distinct taxonomic levels based on phylogenetic studies. We did not include interspecies relationships in our analyses because phylogenetic studies that define species groups are available for only a minority of our taxa, for example Faivovich *et al.* (2005, 2010) for Hylines and Phyllomedusines, respectively. We constructed a cladogram based on the phylogenetic tree proposed by Pyron & Wiens (2011; Appendix S2) to illustrate the phylogenetic relationship of every species in our data set.

To test whether the RMs are phylogenetically conserved trait sets, we used the additive partitioning of species diversity as proposed by Crist *et al.* (2003). This approach was originally developed to measure beta diversity in a hierarchical sampling design, but it can also be used to examine the relative contribution of distinct taxonomic levels to the total diversity of one or a set of species traits, such as the RMs studied here. We used the unrestricted individual-based randomization algorithm, in which the probability of a given RM being found in a taxonomic level is equal to the proportion of RMs in that specific taxonomic level. This weighting procedure was used because the number of RMs varies greatly among amphibian taxa within a given taxonomic level. Thus, if a large and significant amount of the variation in RMs is explained at the family level, for instance, it means that the RMs are phylogenetically conserved at that level. The additive partitioning of species diversity was calculated using PARTITION 3.0 (Veech & Crist, 2009).

We used path analysis to evaluate (1) whether the number of RMs is positively affected by the humidity level and other potential explanatory variables, and (2) how the species richness and phylogenetic diversity of amphibians depend on

the number of RMs and on the humidity level. Path analysis dates back to Sewall Wright's method of path coefficients (Wright, 1921, 1934) and constitutes a suitable approach to investigate causal effects (i.e. direct and indirect pathways) in observational variables for linear relationships. This approach was well suited for our data because our aim was to disentangle the possible direct and indirect causal relationships of humidity level on diversity-related variables. For further details on path analysis, including applications in ecology, see Shipley (2000) and Grace (2006).

Because we did not have a single direct measure for the humidity level of each site, we used the scores of the first axis of a principal components analysis (PCA), with PPT and DFS (both log-transformed) as surrogates for the humidity level. The first axis explained 62% of the total variation in the PCA, and the surrogate measure of the humidity level showed higher correlations with the number of RMs compared with geographical and climatic data alone. The path analysis for amphibian species richness was based on the following rationale: (1) the humidity level was expected to be a key explanatory variable for amphibian occurrence and was included in all models; (2) species richness was expected to depend on the number of RMs rather than the converse, because the humidity level was assumed to be a major environmental filter for the occurrence of species with specialized RMs that are more susceptible to desiccation; (3) LONG, LAT, ANNT and ELR were potential explanatory variables for both species richness and the number of RMs, and their possible confounding effects had to be controlled; and (4) sampling effort was expected to affect both species richness and measures of phylogenetic diversity because longer sampling periods tend to include both more species and more phylogenetic groups. In addition, we incorporated both the direct and the indirect (through the number of RMs) effects of the humidity level and of other explanatory variables on amphibian species richness.

Because our sample size was relatively small ( $n = 27$  sites), we minimized the size and complexity of the path models by first evaluating whether potential explanatory (exogenous) variables were related to the response (endogenous) variables through paired linear correlations. As an objective criterion, we included only those explanatory variables that showed correlation coefficients (Pearson's  $r$ ) greater than 0.4 with the dependent variables in the models. We analysed adjustments to the models with a chi-square test and by inspecting the associated significance values. The fit of the models was also evaluated with three further fit statistics: the Tucker–Lewis coefficient, the comparative fit index, and the root mean square error of approximation (see these and other details in Appendix S3). Normality was checked with the Mardia test, and outliers were inspected using the Mahalanobis distance. We verified the normality assumption for the residuals of each variable by evaluating their kurtosis and asymmetries. To meet the normality requirements, we applied the logarithmic transformation to amphibian richness. The path analyses were constructed and analysed using AMOS 5.0 (Arbuckle, 2003).



To calculate the nestedness in the composition of both species and RMs, we employed the NODF metric, a nestedness index based on shared species and decreasing species richness (Almeida-Neto *et al.*, 2008). NODF ranges from 0 (no nestedness) to 1 (a perfectly nested matrix) or, alternatively, from 0 to 100 if a percentage scale is used. Almeida-Neto *et al.* (2008) showed that NODF is a robust and consistent nestedness index that is less susceptible to Type I error than other nestedness index (e.g. matrix temperature measure, standardized version of the NC metric and the discrepancy measure), even if liberal null models are used. NODF calculates the nestedness between all pairs of rows and columns independently by considering differences in their marginal totals and degree of overlap (Almeida-Neto *et al.*, 2008). Nestedness was determined using the NODF program (Almeida-Neto & Ulrich, 2011). We used two null models (EE, equiprobable–equiprobable; and FE, fixed richness and equiprobable incidences) to evaluate whether the observed nestedness degrees were significant. Null models are patterns produced by randomizations of incidence matrices in the absence of a particular ecological mechanism (Gotelli & Graves, 1996). If the patterns in incidence matrices were similar to those generated by null models, we considered that the observed nestedness degrees were randomly distributed. The null model FE is more conservative, because it constrains species richness, whereas the null model EE is more prone to passive sampling effects, because it does not incorporate differences in richness (strongly related to abundance and carrying capacity; see Ulrich *et al.*, 2009). Because our focus here was on species composition, we calculated nestedness only among sites.

## RESULTS

Overall, 25 distinct RMs were found among the 268 amphibian species listed for the 27 Atlantic Forest sites (Table 1; Appendix S1). These species involved 52 genera from 14 families. The number of species was highly unequal among families and subfamilies (Appendix S1). The subfamily Hyliinae was represented by 111 species (41.4% of total), whereas Caugrastorinae, for instance, was represented by only one species. The number of species was also highly unequal among RMs. The RM characterized by the deposition of eggs and exotrophic tadpoles in lentic water (RM1) was the most common; it was identified in 107 species (39.9% of the species), whereas seven other RMs were found in only one species each (Table 1).

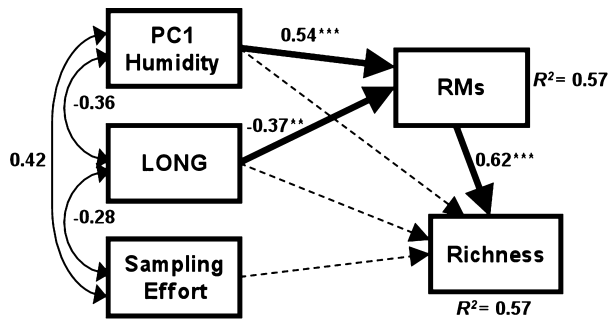
By partitioning the total number of RMs among the taxonomic levels (from species to family level), we found that differences in RMs among species from the same genus and among genera from the same subfamily explained only 9.5% and 8.9%, respectively, of the total number of RMs recorded. Both values were significantly lower than those expected by randomly allocating the RMs among the amphibian species [95% confidence intervals (CIs): 23.3–30.5 for species from the same genera and 13.2–29.8 for genera from the same subfam-

ily]. The differences in RMs among subfamilies from the same family explained only 2.9% of the total number of RMs and did not differ from the value expected by chance (95% CI: 1.6–6.2). In contrast, differences in RMs among the recorded amphibian families explained 78.7% of the total number of RMs, with an expected 95% CI of 39.7–56.4. These results support the assumption that RMs tend to be phylogenetically conserved, because they vary much less among closer taxonomic groups (within genera or subfamily) than among distant groups (e.g. families).

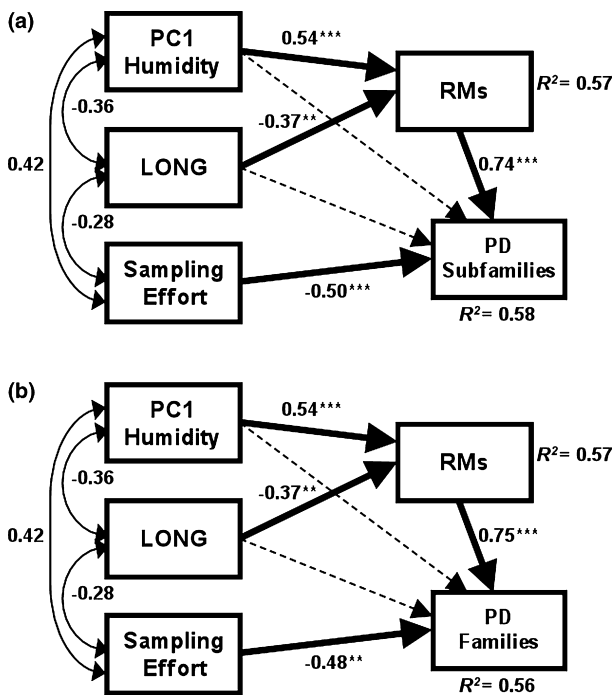
The numbers of amphibian species and RMs per site ranged from 20 to 64 and from 5 to 17, respectively (Appendix S1). We found a strong positive correlation between amphibian species richness and the number of RMs ( $r = 0.712$ ,  $P < 0.001$ ). Because the maximum number of RMs is constrained by species richness (i.e. a single RM for each species) and the minimum species richness is constrained by the number of RMs at each site (i.e. if a site has  $n$  distinct RMs, the minimum species richness at that site will be  $n$ ), a monotonic positive relationship is necessarily expected between the two variables, but the occurrence of this relationship does not mean that the number of RMs per site and the species richness per site will be strongly linked. The humidity level had a moderate to strong significant positive correlation with amphibian species richness ( $r = 0.465$ ,  $P = 0.0139$ ) and with the number of RMs ( $r = 0.667$ ,  $P < 0.0001$ ).

The positive correlation between the number of RMs and the humidity level indicates that the compositions of both species and RMs are non-randomly distributed among sites. We found a significant weak to moderate nested pattern of species composition if the sites were sorted by species richness (NODF = 31.9;  $P < 0.0001$  for both null models). If the sites were sorted by humidity level, the nestedness in species composition decreased to a lower but still significant degree (NODF = 20.24;  $P < 0.001$  for both null models). In contrast, the level of nestedness was high for the composition of RMs if the sites were sorted by the number of RMs (NODF = 75.1;  $P < 0.0001$  for both null models) and was moderate if the sites were sorted by humidity level (NODF = 55.3;  $P < 0.0001$  for both null models). These findings showed that in the drier forests, the compositions of amphibian species and of amphibian RMs tend to be non-random subsets of the compositions found in the wetter forests.

The direct and indirect effects of the humidity level on the species richness and phylogenetic diversity of amphibians were assessed through three path models that controlled for the effects of longitude and sampling effort. Three other potential explanatory variables, namely LAT, ANN and ELR, were not included in the models because they had weak and non-significant correlations (Pearson's  $r < 0.36$ ) with the endogenous variables. The three path models fit the data well ( $\chi^2 < 0.142$ ; d.f. = 1;  $P > 0.706$ ; see Appendix S3). Together, the humidity level and longitude explained 57% of the variation in the number of RMs, but the former had a stronger effect (Figs 2 & 3; Table 3). The path model also explained 57% of the variation in amphibian species richness



**Figure 2** Path analysis for the number of reproductive modes (RMs) and amphibian species richness based on scores of the first axis of a principal components analysis (PCA) with annual mean precipitation and distance from sea (PC1 Humidity, see text), longitude (LONG), and sampling effort in 27 Brazilian Atlantic Forest sites. Numbers associated with paths between variables are path coefficients presented as standardized values (scaled by the standard deviations of the variables). Solid arrows represent significant effects. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . For further details see Table 3 and Appendix S3.



**Figure 3** Path analyses for the number of reproductive modes (RMs) and (a) subfamilies, and (b) families as residuals from amphibian species based on scores of the first axis of a principal components analysis (PCA) with mean annual precipitation and distance from sea (PC1 Humidity, see text), longitude (LONG) and sampling effort in 27 Brazilian Atlantic Forest sites. For further details see Fig. 2, Table 4 and Appendix S3.

(log-transformed). Because only the number of RMs had a direct significant effect on amphibian species richness, both the humidity level and longitude affected species richness indirectly through variation in the number of RMs (Fig. 2).

The path models for subfamilies and families as residuals from species explained almost 60% of the variation in both variables (Fig. 3a,b). These models showed that the number of RMs is also a key explanatory variable for the richness of higher taxa (subfamilies and families) and, consequently, for the phylogenetic diversity of amphibians in forest sites of the Brazilian Atlantic Forest.

## DISCUSSION

### The importance of the humidity level for amphibian species richness

Rainfall and other humidity-related variables have been shown to be key environmental determinants of the richness and composition of amphibian communities (Duellman & Trueb, 1986; Duellman, 1988). In this study we showed an indirect but significant effect of humidity level (measured by PPT and distance from the sea) on amphibian species richness via the number of RMs. Recently, Vasconcelos *et al.* (2010) found similar results related to amphibian species richness and the number of RMs in several Brazilian ecoregions. These results are consistent with the proposition of Hawkins *et al.* (2003) that water variables tend to be better predictors of species richness gradients than energy variables if the geographical scope of the data is restricted to tropical and subtropical areas. Studies on species richness gradients encompassing broader spatial scales are expected to fit the water–energy conjecture especially well because they use data from regions with contrasting moisture conditions, such as rain forests and arid biomes (see Hawkins *et al.*, 2003; Rodríguez *et al.*, 2005; Buckley & Jetz, 2007). However, the spatial scale at which environmental conditions constrain species richness will differ across clades with different home ranges and dispersal abilities (Belmaker & Jetz, 2011). According to Belmaker & Jetz (2011), both biotic and abiotic filters may regulate the species from the regional pool that will appear in fine-grained assemblages through community assembly processes. Our findings show that the humidity level can be a major predictor of amphibian richness even for sites within the same biome and within a relatively small geographical range. To our knowledge, this study provides the first empirical evidence of the importance of the humidity level for amphibian phylogenetic diversity.

Both elevation- and temperature-related variables constitute important predictors of amphibian richness (Williams & Hero, 2001; Buckley & Jetz, 2007). These variables operate at different spatial scales: elevational range is related to climatic effects at mesoscales, whereas annual mean temperature is a measure of climate variation at broader scales (Rodríguez *et al.*, 2008). Interestingly, we did not detect a significant effect of annual mean temperature or elevational range on species richness or on the number of RMs. The small difference in annual mean temperature detected among sites (range: 18–22 °C) is a potential explanation for the observation that this variable had no effect on amphibian richness. The sites cover a small latitudinal range and have a high overlap in their

**Table 3** Explanatory model for the number of reproductive modes (RMs) and species richness (log-transformed) of amphibians in 27 Brazilian Atlantic Forest sites according to the path model in Fig. 2. Direct and indirect coefficients and their relative contributions are included to explain the variation ( $R^2$ ). Non-significant paths are depicted only if their indirect effect is significant.

Dependent variable	Explanatory variable	Coefficient				Determination	
		Correlation ( $r$ )	Direct ( $d$ )	Indirect ( $i$ )	Effect ( $e = d + i$ )	Effect ( $e \times d$ )	Total ( $r \times d$ )
No. of RMs	PC1 (humidity level)	0.670	0.537	–	0.537	0.288	0.360
	LONG	–0.563	–0.375	–	–0.375	0.141	0.211
Log of anuran species richness	PC1 (humidity level)	0.467	–0.096	0.333	0.237	–0.023	–0.045
	LONG	–0.496	–0.112	–0.230	–0.342	0.038	0.056
	No. of RMs	0.712	0.621	–	0.621	0.386	0.442
	Sampling effort	0.482	0.265	–	0.265	0.070	0.128

elevational ranges, thus minimizing the influence of both annual mean temperature and elevational range on amphibian richness.

It is generally accepted that the deposition of eggs and exotrophic tadpoles in lentic water is the most generalized and ancestral RM among amphibians, and that other RMs represent grades of specialization associated with adaptive radiation into various environments (Duellman & Trueb, 1986). Although the phylogenetic relationships used in this study are different from those used by Duellman (1985) and Duellman & Trueb (1986), and we are aware that previous conclusions and comparisons among studies should be approached with caution, the findings were similar. According to Duellman (1985), there appear to be phylogenetic trends in RMs from families to lower taxonomic levels (e.g. subfamilies and genera). At higher taxonomic levels (e.g. family), however, amphibian RMs appear to be convergent adaptations related to local environmental conditions in independent clades (Duellman & Trueb, 1986). Although our finding that most species within amphibian clades exhibit the same RM did not discriminate between higher and lower taxonomic levels, it supports our assumption that RMs are phylogenetically conserved among amphibians. Haddad & Prado (2005) showed that the more specialized reproductive modes in the Atlantic Forest are generally found in species-poor genera

of forest specialists (e.g. *Brachycephalus*, *Dendrophryniscus*, *Flectonotus*, *Gastrotheca*, *Crossodactylodes* and *Myersiella*).

Amphibians are very susceptible to water loss. Their requirement for moist habitats is a significant ecological factor limiting the occurrence of most species because it affects their reproduction, egg development, growth rates, adult activity, and mortality (Duellman & Trueb, 1986; Duellman, 1988; Donnelly & Crump, 1998; Haddad & Prado, 2005). According to Haddad & Prado (2005), those amphibian species that depend on humidity and moist forest microhabitats to reproduce in the Atlantic Forest are disappearing from deforested areas and degraded forests because these sites have become drier and more seasonal. Touchon & Warkentin (2010) reported that even short-term variation in precipitation patterns (e.g. an increase in the number of dry days in the rainy season) in a Neotropical rain forest affects the survival of a tropical amphibian's arboreal eggs, chiefly as a result of desiccation and predation. Pearman (1997) showed that the species richness of the genus *Pristimantis* (treated as *Eleutherodactylus*), in which all of the species exhibit direct development of terrestrial eggs, decreased owing to microclimate changes in forest humidity levels within deforested areas in equatorial Amazonia. If moist localities become drier and more seasonal as a result of anthropogenic habitat degradation (e.g. an increase in air temperature and light intensity, a

**Table 4** Explanatory models for subfamilies and families as residuals from amphibian species richness in 27 Brazilian Atlantic Forest sites according to the path model in Fig. 3. Direct and indirect coefficients and their relative contributions are included to explain variation ( $R^2$ ). Non-significant paths are depicted only if their indirect effect is significant.

Dependent variable	Explanatory variable	Coefficient				Determination	
		Correlation ( $r$ )	Direct ( $d$ )	Indirect ( $i$ )	Effect ( $e = d + i$ )	Effect ( $e \times d$ )	Total ( $r \times d$ )
Subfamilies as residuals from species	PC1 (humidity level)	0.365	0.186	0.395	0.581	0.108	0.068
	LONG	–0.055	0.288	–0.273	0.015	0.004	–0.016
	No. of RMs	0.535	0.736	–	0.736	0.542	0.394
	Sampling effort	–0.260	–0.501	–	–0.501	0.251	0.130
Families as residuals from species	PC1 (humidity level)	0.357	0.160	0.402	0.562	0.090	0.057
	LONG	–0.057	0.291	–0.278	0.013	0.004	–0.017
	No. of RMs	0.537	0.749	–	0.749	0.561	0.402
	Sampling effort	–0.243	–0.475	–	–0.475	0.226	0.115



decrease in air and soil moisture) a homogenization of RMs can occur in such habitats. As a consequence, degraded habitats would harbour only generalized RMs and RMs resistant to insulation/desiccation (the same subset of RMs as reported for arid regions or with high seasonality) and a low phylogenetic diversity of amphibians.

It has long been known that amphibians have a wide array of adaptive mechanisms to withstand desiccation. According to Navas *et al.* (2004), many of the amphibian species in the Caatinga biome, a semi-arid region in north-eastern Brazil, have developed physiological (e.g. a tolerance of temperatures that would impair or kill typical tropical anurans), behavioural (e.g. the ability to aestivate), and/or morphological (e.g. a ventral skin morphology that affects water uptake, such as thickness, sculpturing, and/or the presence of hydrophilic verrucae) adaptations to survive dry periods. More recently, Vieira *et al.* (2009) reported that amphibian species inhabiting the Caatinga biome use several behavioural and reproductive strategies to avoid or minimize the negative effects of high temperatures and water scarcity on egg development. Interestingly, Olalla-Tárraga *et al.* (2009) showed that mean amphibian body size tended to increase towards areas with high water deficits in the Brazilian Cerrado. They suggested that the smaller surface-to-volume ratios associated with larger body sizes reduce evaporative water loss, an ability that may be critical for surviving in dry environments. Therefore, our results confirm those of previous studies, specifically that independent traits of amphibians (e.g. body size, reproductive modes, physiological tolerance) are associated with water availability, furnishing adaptive mechanisms for withstanding desiccation.

### Nested composition of amphibian reproductive modes

Several studies have shown that differences in environmental and habitat factors can be important biological explanations for nested patterns in amphibian species distribution (Hecnar & M'Closkey, 1997; Hecnar *et al.*, 2002; Baber *et al.*, 2004). Our results highlight the importance of the humidity level in the compositions of both species and RMs of amphibians at different sites in the Brazilian Atlantic Forest. The RMs exhibited a strong nestedness distribution, in which the RMs in the drier forest sites were a subset of the RMs displayed by the species in sites with higher humidity levels. We hypothesize that the diversity of humid microhabitats necessary for the reproduction of species with specialized RMs is lower in sites with lower humidity levels. In addition, it is probable that the humidity level has a constraining effect on species with a higher susceptibility to desiccation. Hence, the drier sites tend to harbour species with RMs that are completely dependent on water bodies or that are more resistant to desiccation and adapted to regular dry periods (see Haddad & Prado, 2005).

A weakly significant nested pattern in amphibian species composition was found if the sites were sorted by humidity level. A probable explanation for this finding is the high degree

of speciation and endemism in coastal Atlantic regions (Duellman, 1988; Haddad, 1998; Santos *et al.*, 2009). Indeed, 62% of the amphibians in the Atlantic Forest are endemic species restricted to a single region in the biome (Amphibia-Web: <http://amphibiaweb.org>). Because endemism reduces nestedness, this result is as expected for amphibians in the Atlantic Forest. Furthermore, the distribution of amphibian species in the Brazilian Atlantic Forest appears to be strongly related to the geomorphological characteristics of the study sites (Santos *et al.*, 2009). The coastal Atlantic Forest sites in the mountain complex of Serra do Mar and Serra da Mantiqueira, which experience an annual precipitation of 3600 mm and no dry season, promote the geographical isolation of populations and favour speciation and endemism (Haddad, 1998). However, the inland Atlantic Forest (1300–1600 mm precipitation and typical seasonal climate) does not have endemic species (Santos *et al.*, 2009) and has a lower species richness than the coastal Atlantic Forest (see Heyer *et al.*, 1990; Santos *et al.*, 2009).

It is probable that seasonality is one of the most important factors contributing to the nested pattern of RMs because it reflects environmental conditions that change with time and are directly related to the humidity level. Interspecific differences in the tolerance to environmental conditions provide a possible explanation for nestedness (Patterson & Brown, 1991; Hecnar *et al.*, 2002). According to Hylander *et al.* (2005), nested habitat quality represents a situation in which all species in an assemblage are favoured by the same habitat or environmental factor but differ in specialization or tolerance to that factor. This pattern appears to explain the nested distribution we found for RMs in the Atlantic Forest sites because humidity level is a variable that favours all RMs. However, various RMs also differ in their specialization or tolerance to the decreased humidity levels observed at certain study sites. The explanation of the nested RM pattern might be that sites with adequate environmental conditions and high humidity levels throughout the year support the largest number of RMs, whereas sites with low humidity levels and typical seasonal climates harbour only RMs that are generalized or that are specialized in their resistance to desiccation. A similar pattern was found by Worthen *et al.* (1998) for mycophagous flies, whereby all species were favoured by a moist substrate, but desiccation stress – which was tolerated to different degrees among species – was responsible for creating the nested subset structure in those communities.

Other processes that can produce nested patterns are differential extinction, differential colonization, and sampling effects (see Ulrich *et al.*, 2009). The inland Atlantic Forest is considered a remnant of the Pleistocene Arc, which extended over South America during a cooler and drier period that coincided with the retraction of humid forests (Pennington *et al.*, 2000). Here, we present two possible hypotheses, which are not mutually exclusive, to explain the nested patterns of RMs. First, assuming that the RM distribution in the Atlantic Forest was the same in the past as it is now, the nested patterns of RMs found in Atlantic Forest sites may have resulted from

the extinctions of certain RMs that required habitats with high humidity at inland Atlantic Forest sites after the retraction of the humid forests. This hypothesis is consistent with the results of a recent phylogeographical study on climatic stability across the Brazilian Atlantic Forest by Carnaval *et al.* (2009). According to these authors, the São Paulo refugium in the coastal Atlantic Forest harbours a substantial genetic endemism and species richness of amphibians because this area served as a climatic refugium for Neotropical species in the late Pleistocene. This hypothesis is not easily proved because we do not know which RMs were present at each site in the past. The alternative is that the high degree of speciation and endemism in the coastal Atlantic Forest (Haddad, 1998) might be responsible for the high number of RMs at these sites. It is probable that the low humidity levels and/or other barriers to displacement would have prevented the colonization of new sites far from coastal forests by species with these new RMs. In contrast, RMs specialized to be more resistant to desiccation could colonize sites with both high and low humidity levels. Carnaval *et al.* (2009) report that amphibian species with broader ranges and generalized RMs colonized unstable areas from adjacent refugial populations following climate changes, in contrast to the long-term persistence of populations in unstable areas. Therefore, the high degree of endemism and speciation and the different requirements for colonization among sites with different humidity levels could explain the nested pattern found for RMs.

## CONCLUSIONS

The direct and indirect effects described above imply that humidity level is a key environmental variable related to both species richness and phylogenetic diversity. We hypothesized that the higher phylogenetic diversity found in moister sites reflects differences in tolerance to desiccation among different RMs. Given that RMs are associated with desiccation susceptibility (Pearman, 1997; Haddad & Prado, 2005), their incorporation into explanatory models may trigger a great advance in the understanding of the mechanisms regulating the species richness and composition of amphibian communities.

With regard to the nested pattern of RMs found in the Atlantic Forest sites, the protection and management of sites with high humidity levels would benefit most of the amphibians recognized as inhabitants of the Atlantic Forest and, consequently, contribute to preserving the phylogenetic diversity of amphibians in this biome. This factor is rendered even more important when considering that the greatest number of RMs is found in the Neotropics, where amphibians have evolved 31 RMs, 27 of which are present in the Atlantic Forest (Haddad & Prado, 2005). Our results show that the geographical distribution pattern of amphibian species differs from that of their RMs, and, because the majority of species identified as threatened with extinction (IUCN, 2009) are closely related, conservation specifically focused on these species will affect only some of the RMs recognized as occurring within the

Atlantic Forest. Loyola *et al.* (2008) were the first to include amphibian life history traits in the selection of priority areas for conservation, in particular those essential for species that require an aquatic habitat for reproduction. Here, we emphasize that future conservation assessments for Atlantic Forest amphibians should include RM distributions in their analyses in order to incorporate these important aspects of amphibian life history into conservation practices.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Description of Brazilian Atlantic Forest sites used in the analysis, taxonomic classification and reproductive modes of amphibians, and presence–absence matrix showing the distribution of 25 anuran reproductive modes.

**Appendix S2** Cladogram of amphibians demonstrating the phylogenetic relationships of our data set.

**Appendix S3** Analysis summary for the path models shown in Figs 2 and 3.

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## BIOSKETCH

**Fernando Rodrigues da Silva** has a background in natural history, community ecology, landscape ecology and macroecology. His main interest lies in understanding the factors and processes underlying amphibian species distribution.

Author contributions: F.R.dS. gathered and analysed the data and supervised the writing; V.H.M.dP. conceived the original idea for the study and helped with gathering the data and writing; M.A.-N. conceived the present study version and helped with analyses and writing; D.C.R.-F. and C.F.B.H. helped with the writing and through their knowledge of the natural history and biology of the amphibian species, from dry and wet Atlantic forest, respectively.

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