What drives the evolution of body size in ectotherms? A global analysis across the amphibian tree of life

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Abstract

Aim: The emergence of large-scale patterns of animal body size is the central expectation of a wide range of (macro)ecological and evolutionary hypotheses. The drivers shaping these patterns include climate (e.g. Bergmann's rule), resource availability (e.g. 'resource rule'), biogeographic settings and niche partitioning (e.g. adaptive radiation). However, these hypotheses often make opposing predictions about the trajectories of body size evolution. Therefore, whether underlying drivers of body size evolution can be identified remains an open question. Here, we employ the most comprehensive global dataset of body size in amphibians, to address multiple hypotheses that predict patterns of body size evolution based on climatic factors, ecology and biogeographic settings to identify underlying drivers and their generality across lineages.

Location: Global.

Time Period: Present.

Major Taxa Studied: Amphibians.

Methods: Using a global dataset spanning 7270 (>87% of) species of Anura, Caudata and Gymnophiona, we employed phylogenetic Bayesian modelling to test the roles of climate, resource availability, insularity, elevation, habitat use and diel activity on body size.

Results: Only climate and elevation drive body size patterns, and these processes are order-specific. Seasonality in precipitation and in temperature predict body size clines in anurans, whereas caecilian body size increases with aridity. However, neither of these drivers explained variation in salamander body size. In both anurans and caecilians, size increases with elevational range and with midpoint elevation in caecilians only. No effects of mean temperature, resource abundance, insularity, time of activity or habitat use were found.

Main Conclusions: Precipitation and temperature seasonality are the dominant climatic drivers of body size variation in amphibians overall. Bergmann's rule is consistently rejected, and so are other alternative hypotheses. We suggest that the rationale sustaining existing macroecological rules of body size is unrealistic in amphibians and
1 | INTRODUCTION

Body size influences most ecological, evolutionary and demographic processes underlying patterns of animal biodiversity from local to global scales (Calder, 1984; Peters, 1983; Smith & Lyons, 2013). On the one hand, the evolution of traits involved in ecological, sexual, reproductive and physiological performance is generally mediated by body size (Andersson, 1994; Brown et al., 2004). On the other hand, species predisposition to demographic collapses that lead to extinctions is often associated with their body sizes (Cardillo et al., 2005; Dirzo et al., 2014; Pincheira-Donoso, Harvey, Cotter, et al., 2021; Pincheira-Donoso & Hodgson, 2018; Ripple et al., 2017). Therefore, given the influence of body size on most components of an organism’s fitness (Andersson, 1994; Roff, 2002), elucidating the drivers behind the evolution of this complex trait has been a central focus of research for centuries (Peters, 1983; Smith & Lyons, 2013).

Most environmental factors that exert natural selection on body size, such as climate, length of breeding season and resource abundance, vary with geography (Ashmole, 1963; Pincheira-Donoso & Hunt, 2017). Therefore, body size diversity has traditionally been associated with spatial variation in environmental pressures (James, 1970; Meiri & Dayan, 2003; Smith & Lyons, 2013), deriving in multiple macroecological ‘rules’ that predict spatial patterns of body size. The foundational such rule, ‘Bergmann’s rule’, predicts that selection from temperature drives larger body sizes towards colder climates—higher latitudes and elevations—as adaptations for body heat conservation through adjustments of body-mass-to-surface-area ratio (Bergmann, 1847; Blackburn et al., 1999; James, 1970; Meiri & Dayan, 2003). Although Bergmann’s rule is more often supported in endotherms (Meiri, 2011; Olson et al., 2009; Pincheira-Donoso, 2010; Will et al., 2021), evidence is highly conflicting (Riemer et al., 2018), especially for ectotherms (Adams & Church, 2008; Ashton & Feldman, 2003; Feldman & Meiri, 2014; Pincheira-Donoso et al., 2007, 2008; Pincheira-Donoso & Meiri, 2013; Slavenko et al., 2019; Womack & Bell, 2020). Since ectotherms must gain body heat before they can preserve it, thermoregulation in these organisms is more likely to benefit from reductions in body size towards colder climates (Ashton & Feldman, 2003; Pincheira-Donoso, 2010; Pincheira-Donoso et al., 2008). This principle underlies an alternative rule that applies to ectotherms, the ‘heat balance hypothesis’, which predicts that thermoconformers enhance thermoregulation via smaller body size as climatic temperatures decline (Olalla-Tarraga, 2011; Olalla-Tarraga et al., 2006). Yet, a second alternative, the ‘water conservation hypothesis’ suggests that larger body sizes evolve to maximize the conservation of body water—rather than heat—thus predicting larger size towards arid environments (Ashton, 2002; Gouveia & Correia, 2016; Nevo, 1973; Olalla-Tarraga et al., 2009; Pincheira-Donoso et al., 2019). This mechanism is likely to apply in particular to organisms with permeable skins, such as amphibians (Pincheira-Donoso et al., 2019).

The evolution of body size diversity is also influenced by selection from ecological resources. Central to macroecology, the ‘resource rule’ posits that constraints on upper body size limits relax as resource abundance increases, promoting larger size in more productive environments (Geist, 1987; Huston & Wulfferton, 2011; McNab, 2010; Rosenzweig, 1968; Yom-Tov & Geffen, 2006). The underlying mechanisms can involve increasing energy investment into body growth without the constraining effects of trade-offs, or selection for smaller body mass to reduce resource requirements as productivity declines (McNab, 2010). Similarly, the ‘seasonality hypothesis’ suggests that accumulation of nutritional reserves confers fitness advantages, promoting larger body mass towards more seasonal regions (Boyce, 1979; Calder, 1984; Lindsey, 1966). Finally, adaptive radiation theory predicts that adaptation of species to distinct niches can drive predictable patterns of body size diversity (Losos, 2009; Schluter, 2000). For example, use of similar microhabitats may drive convergent evolution in size across nonsister species (Butler et al., 2007; Losos, 1990, 2009; Pincheira-Donoso, Harvey, Grattarola, et al., 2021; Wainwright & Reilly, 1994; Womack & Bell, 2020). Likewise, given the different environmental demands imposed by adaptation to different parts of the 24-h cycle, body size optima are expected to differ between nocturnal and diurnal species (Huey & Slatkin, 1976; Meiri, 2008).

Insularity has also played a central role in elucidating the evolution of body size diversity (Losos, 2009; MacArthur & Wilson, 1967). The unique ecological features that prevail on islands (e.g. isolation, low predation and character release) have been hypothesized to drive species gigantism or dwarfism in size relative to their mainland close relatives (Benítez-López et al., 2021; Foster, 1964; Meiri, 2008; Van Valen, 1973). A stream of large-scale tests have both supported (Benítez-López et al., 2021; Clegg & Owens, 2002; Komoloni, 2005; Meiri, 2007; Raia et al., 2003) and challenged (Meiri et al., 2004, 2006, 2008) this prediction.

Collectively, theories and evidence have significantly advanced our understanding of the drivers and implications of body size diversity. However, (i) support for some predictions (e.g. Bergmann’s rule) is inherently weak to justify their validity; (ii) some hypotheses (e.g. resource rule vs water conservation hypothesis) make opposite
predictions based on the same sources of selection; (iii) most studies test one or a few of these hypotheses separately, without integrating them; and (iv) large-scale studies are available for some taxa such as lizards and birds (e.g., Benítez-López et al., 2021; Meiri, 2008; Olson et al., 2009; Slavenko et al., 2019) but not for others, for example amphibians, which lack comprehensive large-scale studies despite existing efforts (e.g. Adams & Church, 2008; Benítez-López et al., 2021; Slavenko & Meiri, 2015). Here, based on the most comprehensive global dataset of body size for amphibians compiled to date (spanning >87% of their known species diversity), we address the role that a range of key hypotheses (Table 1) attribute to climatic factors, ecology and biogeographic settings as the drivers shaping large-scale patterns of body size diversity in these organisms. Amphibians offer ideal model systems for testing these predictions at macroecological scale given their remarkable diversity in body size; their distribution across the full worldwide spectrum of environments (except the poles); the contrasting body plans across their three extant orders (Anura or frogs, Caudata or salamanders, and Gymnophiona or caecilians); and their unparalleled range of genome size diversity relative to tetrapods as a whole (Liedtke et al., 2018; Pincheira-Donoso et al., 2023). In addition, amphibians are nature’s most threatened animals (IUCN, 2021)—a phenomenon that has directly or indirectly been linked to their diversity in body size (Pincheira-Donoso, Harvey, Cotter, et al., 2021; Pincheira-Donoso & Hodgson, 2018; Ripple et al., 2017).

2 | MATERIALS AND METHODS

2.1 | Body size data

We created a global dataset on maximum body size (largest available record for a species regardless of sex) for 7270 amphibian species spanning all three living orders (Anura, Caudata and Gymnophiona). These data were collected from the primary literature, including articles and books, and from direct observation of specimens both in museums and in the field. Given that snout–vent length (SVL) is the most common proxy for body size in anurans and salamanders (Amado et al., 2021; Pincheira-Donoso, Harvey, Grattarola, et al., 2021; Wells, 2007), whereas total body length is used for caecilians (Pincheira-Donoso et al., 2019), we used species body mass as our measure for body size to make analyses comparable. Actual measures of body mass are considerably scarcer than body length measures both in the literature and in museum specimens. Therefore, we employed the approach presented by Pough (1980) for conversion of maximum SVL and maximum total length available for each species into body mass using order-specific allometric formulas (Pincheira-Donoso & Hodgson, 2018; Ripple et al., 2017). This dataset (Table S1) is part of the Global Amphibian Biodiversity Project (GABiP) initiative (www.amphibianbiodiversity.org).

|TABLE 1| Table of hypotheses, eco-evolutionary mechanism driving each hypothesis, and variables from the dataset used to test each hypothesis. |
|---|---|---|---|
|Hypothesis | Predictions | Eco-evolutionary mechanism | Variables used |
|Bergmann’s rule | Body size increases towards colder climate regions (higher latitudes and elevations) | Increased body mass relative to surface area is beneficial for thermoregulation in cold climates | Latitude, temperature, elevation midpoint |
|Heat balance hypothesis | Higher temperatures predict increased body size | Ectotherms which obtain heat from the surrounding environment have improved thermoregulation with smaller body sizes as temperatures decrease | Latitude, temperature, elevation midpoint |
|Water conservation hypothesis | Body size increases in more arid environments | Larger body sizes are required to maximize the conservation of body water; thus, larger organisms should exist in arid climates where water availability is scarce—especially in organisms with permeable skin | Precipitation |
|Resource rule | Body size should increase with higher net primary production | Increased availability of resources relax constraint on body size limits as excess energy availability can be invested into body size | Net primary production |
|Seasonality hypothesis | Seasonality (variations in temperature and precipitation) is related to increased body size | Accumulation from nutritional reserves confers fitness advantage to organisms, promoting larger body sizes in more seasonal environments | Temperature variation, precipitation variation |
|Habitat body size hypothesis | Different species in the same habitats will have the same body sizes | Adaptive radiation theory of species to distinct niches suggests different species within the same niche (habitat, diel activity) will have to evolve convergent body sizes | Microhabitat use, nesting site |
|Activity hypothesis | Different species with the same diel activity patterns will have the same body size | | Diel activity |
|Island Biogeography hypothesis | Island species will have smaller body sizes than continental species | Reduced resource availability on islands should select for smaller body sizes within insular environments | Insularity |
2.2 | Climatic, geographic and ecological predictors

To address the hypotheses that spatial gradients in climate drive the evolution of body size, we obtained extent of geographic occurrence range maps for all amphibian species from the International Union for Conservation of Nature (IUCN) archive (www.iucnredlist.org) for species for which body size data are available (Table S1). From the distributional data, we then created a dataset spanning a range of variables that have widely been associated with body size diversity in the literature (Olalla-Tárraga et al., 2009; Pincheira-Donoso et al., 2019; Riemer et al., 2018; Slavenko et al., 2019): mean annual temperature (averaged across 12 months of the year, in °C), temperature range (measured as the extent of variation in temperature between the warmest and the coldest month, in °C), mean annual precipitation (the amount of rainfall, measured in millimetres per year) and precipitation seasonality (coefficient of variation of monthly precipitation across the year) from the range of each species. These climatic predictors were obtained from the WorldClim 2 database (Fick & Hijmans, 2017; Hijmans et al., 2005), expressed at a spatial resolution of 2.5 arc-min (c. 5 km at the equator). We assigned each species a single value per predictor, calculated as the average of values obtained by dividing the geographical range polygon of each variable for each species into 2.5 arc-min grid cells using ArcGIS v.10.2. Finally, we added geographic data to the climatic database from the same sources described above. These data consist of elevational, latitudinal and insular distributions. For the elevational data, we obtained the minimum and maximum elevational records available for each species, from which we calculated both elevational midpoint (between maximum and minimum elevation) and elevational range (the range of metres above sea level contained between maximum and minimum known elevations). Latitude was extracted as the centroid for each species from the IUCN distributional maps described above. Insular data were obtained from the IUCN archive and from the literature (the full dataset is available in Table S1).

To test the hypotheses on the role of habitat structures as drivers of body size evolution, from the same sources described above, we created a dataset consisting of two variables that capture habitat use by species: ‘perching’ sites (‘microhabitats’) and microhabitat structures for egg deposition (‘nesting sites’). Each species was assigned to one of four perching microhabitat structures: aquatic (species that depend on direct contact with water bodies, including strictly aquatic species and species whose subsistence depends on permanent contact with water bodies), terrestrial (ground-dwellers that do not depend on permanent contact with water), vegetation (bush dwellers and arboreal species) and fossorial (species that, except for the breeding seasons, have underground lifestyles). For nesting site data, we assigned each species to one of five categories depending on whether parents lay their eggs in water (e.g. streams, lakes and seasonal pools, but not on vegetation, e.g. pitcher plants), the ground (terrestrial sites separated from water), burrows (enclosed nests in the ground, or in caves), on vegetation (bushes and trees, including those that use small accumulations of water within flowers and pitchers, or between leaves) or in the body of the parents in gastric brooder or skin-brooder species (Table S1). To test the role of diel activity for influencing body size, we also collated data from the same sources described above, categorizing species as either diurnal, nocturnal, cathemeral or crepuscular. Species for which different literature sources provided conflicting data on the use of habitat structures were removed from the analyses.

2.3 | Statistical analyses

Phylogenetic MCMC generalized linear mixed models, MCMCglmm hereafter (Hadfield, 2021), run through R 4.1.0 (R Development Core Team, 2021) were used to predict body mass from environmental variables using a cross-species approach. Further, the simplicity of Bayesian regressions considering phylogenetic nonindependence compared with phylogenetic path analyses is better suited to highlight the patterns and hypotheses we are testing (Table 1). Comparatively, an alternative method such as an assembly-based approach would not be as insightful for our study, given we are testing >7000 species of amphibians across the entire globe which is ideally suited to cross-species phylogenetic methods (Olalla-Tárraga et al., 2010; Pennell et al., 2014). Assemblage-based approaches are ideal for looking within specific clades over smaller spatial scales, rather than identifying global-scale patterns across an entire taxonomic class (Olalla-Tárraga et al., 2010). Phylogenetic analyses were performed based on Jetz and Pyron’s (2018) amphibian phylogenetic tree that covers ~90% of the world’s extant species. In analyses, this phylogeny was implemented as a random effect where the inverse of the sum matrix of phylogenetic correlation was calculated (Gamszegi, 2014). Environmental predictors were assessed for collinearity using a conservative threshold of 0.65 to exclude variables from multivariate models owing to collinearity. None of the environmental predictors were collinear, so were all included in our models. A multiple regression model of the four climatic predictors (temperature, precipitation, temperature seasonality and precipitation seasonality) and net primary production (NPP) was performed to predict body mass. We then performed separate models with latitude as a predictor of body size variation. The reason behind the use of latitude separately is that, although it is widely used as a key predictor of geographic biodiversity clines in macroecology (Womack & Bell, 2020), latitude is in fact a ‘catch-all’ proxy for most environmental factors that vary geographically, rather than an environmental source of selection that could be implicated as the mechanism driving the evolution of those gradients (Pincheira-Donoso, Harvey, Cotter, et al., 2021). Therefore, latitude was not incorporated into the same models with the environmental predictors that covary strongly with it. Measures of elevation (elevation range and elevation midpoint) were co-linear, so multiple univariate models were implemented to predict body mass with these elevation measures as predictors. All predictors were
standardized to a mean of zero and variance of 1 to aid convergence to deal with data of varying orders of magnitude. The log-transformed body mass values were used for all models, specified with a ‘Gaussian’ family distribution. Given that anurans account for >88% of the world’s diversity of amphibians, models were run for each of the three amphibian orders separately (global models with all amphibians were qualitatively identical to anuran-only models), while still considering phylogeny as a random effect. For all models, default flat normal priors were used for the fixed effects which assume a normal posterior distribution with large variance. The random effect prior was specified to an inverse-Gamma distribution with shape and scale parameters equal to 0.01, canonical for phylogenetic regressions in a Bayesian framework (Garamszegi, 2014). All models were run for a minimum of 20,000 iterations and 5000 discarded as burn-in. Iterations and burns were increased if the effective sample size of any model was below 1000 (Hadfield, 2021). Trace plots of the fixed and random effects were visually assessed for convergence (Hadfield, 2021).

Phylogenetic ANOVAs using the ‘aov.phylo’ function from the ‘geiger’ package (Harmon et al., 2008) were performed to statistically analyse differences in body mass between groups (i.e. insularity, microhabitat, activity and nesting site). The phylogenetic signal, estimated based on Pagel’s lambda (Pagel, 1999), of group effects on body size was calculated using the ‘phylosig’ function from the ‘phytools’ package (Revell, 2012). The phylogenetic tree shown in Figure 2 was plotted using the ‘ggtree’ and ‘ggtreeextra’ packages implemented in R (Xu et al., 2021; Yu, 2020; Yu et al., 2017, 2018).

3 | RESULTS

Body size diversity spans several orders of magnitude across extant amphibians (Figures 1 and 2), ranging from 8 mm (0.03 g) of total body length in the frog *Pseudophryne amauensis* to >1600 mm (1593 g) in the caecilian *Caecilia guntheri*, and 1500 mm (10,793 g) in the giant salamander *Andrias japonicus* (Table S1). Among orders, anurans have the smallest body sizes overall (median body mass: 4.54 g, range: 0.03–5497.2; median SVL: 38 mm, range: 8–340, n=6440). Salamanders follow, although their maximum body mass is the largest among amphibians (median mass: 3.67 g, range: 0.09–10,792.7; median SVL: 61 mm, range: 17.2–923.1, n=617). Caecilians are the largest amphibians overall (median mass: 12.1 g, range: 0.49–1592.8; median body length: 318 mm, range: 110–1600, n=213). The frequency distribution of body size is strongly right-skewed in all three orders (Kolmogorov-Smirnov tests for anurans: D=0.42, df=6440, p<0.0001, for salamanders: D=0.47, df=617, p<0.0001, for caecilians: D=0.37, df=213, p<0.0001; Figure 1).

3.1 | Climatic drivers of body size

Climatic analyses consistently reject Bergmann’s rule. Geographic gradients in environmental temperature failed to predict variations of body size in any direction for each order separately (Table 2). In contrast, our analyses reveal that body size in anurans decreases predictably towards environments with increasing seasonality in precipitation and with decreasing thermal seasonality (Table 2; Figure 2). In caecilians, body size decreases towards environments with increasing levels of annual precipitation, but seasonality plays no role (Table 2; Figure 2). All our climatic predictors failed to significantly explain body size variation in salamanders, with seasonality in precipitation having a borderline effect in the opposite direction observed for anurans (Table 2; Figure 2). The resource rule was consistently rejected by all our analyses as NPP failed to predict body size variation in any direction for each group separately (Table 2; Figure 2). All analyses incorporating amphibians of all three orders combined yielded results that consistently matched the findings observed in anurans (Figure 2).

3.2 | Geographic and ecological drivers of body size

Our models consistently failed to identify a role for latitude as a predictor of spatial variation in body size among each of the three amphibian orders separately (Table 2), revealing the lack of a latitudinal gradient of body size (Figure 3). Our analyses addressing the influence of elevation on body size variation revealed a significant increase in caecilian body mass towards higher elevations (Table 2). No such relationship was observed among anurans or salamanders, although a weak, nonsignificant tendency for anuran body size to decrease with increasing elevation was observed (Table 2). Elevational range, in contrast, showed a strong positive relationship with body size in anurans and caecilians, but not in salamanders (Table 2).

Analyses testing the role of ecological predictors consistently failed to identify any effect of diet activity, microhabitat use or nesting site choice as drivers of body size variation across all three amphibian orders (Table 3; Figure S1).
DISCUSSION

Our study provides a global-scale empirical overview addressing a range of classic hypotheses on the drivers behind the evolution of animal body size, using amphibians—the vertebrate class for which evidence remains more scattered—as model systems. By using amphibians as a model system, our study covers these classic questions across a range of evolutionary traits, habitat types and genome sizes unique to the amphibian tree of life. Consistent with recent global-scale phylogenetic studies spanning all three other tetrapod classes (Riemer et al., 2018; Slavenko et al., 2019), our analyses failed to identify a role for mean climatic temperature as a source of selection on amphibian body size. These findings add to the widespread evidence that rejects ‘Bergmann’s rule’, as well as the alternative ‘heat-balance hypothesis’. Similarly, all analyses consistently rejected the ‘resource rule’, as no role for NPP was identified. In contrast, as shown by other phylogenetic studies (Olalla-Tarraga et al., 2009; Pincheira-Donoso et al., 2019), our analyses revealed a more predominant role for seasonality as a driver of large-scale patterns of body size evolution. Anurans evolve larger body sizes towards climates where seasonality in rainfall decreases but the extremes in temperature increase—this may indicate that selection from extreme thermal ranges may be a stronger driver of reserve accumulation (i.e. the ‘seasonality hypothesis’) than precipitation. In caecilians, our results are fully aligned with a recent study (Pincheira-Donoso et al., 2019) where species were found to evolve larger body sizes towards more arid environments. These authors suggested that aridity may favour larger body sizes to reduce the risk of evaporative dehydration. Yet, an alternative explanation could be associated with the progressively hypoxic conditions in wet and swampy soils (Silver et al., 1999; Wells, 2007). Given that ~45% of oxygen has been recorded to be taken through the skin in some caecilians (Smits & Flanagin, 1994), a reduction in size (and thus, a corresponding increase in surface area relative to mass) is likely to increase overall efficiency of O₂ consumption in hypoxic environments (Wells, 2007). Elevation was found to be an equally strong predictor of body size gradients. Whereas increasing body mass is associated with increasing tolerance to a wider elevational range in both anurans and caecilians, body mass in caecilians increases in species occurring at higher elevations. Remarkably, none of these climatic or elevational factors showed any relationship with body size diversity in salamanders.

Finally, our analyses failed to identify an effect of ecological factors (diel activity and use of habitat structures) and insularity on the predictable evolution of body size patterns. Combined, these findings suggest that climatic gradients are the dominant drivers of body size patterns at large scales. However, even these dominant effects are inconsistent, with different factors driving body size clines in different clades, or not driving them at all. It remains possible that studies at smaller spatial scales may identify factors that operate at regional scale or within specific clades. Our study, however, has
JOHNSON et al. aimed to identify the underlying factors that drive body size evolution more ‘universally’.

4.1 The climatic macroecology of body size: Why are body size rules often exceptions?

The study of macroecological patterns of body size across the animal tree of life has been guided for over 150 years by Bergmann's rule (Bergmann, 1847; James, 1970), which simultaneously combines the statuses of being both a landmark and one of the most discredited ecogeographic predictions (Adams & Church, 2008; Olalla-Tarraga, 2011; Pincheira-Donoso, 2010; Pincheira-Donoso et al., 2008; Riemer et al., 2018; Slavenko et al., 2019; Slavenko & Meiri, 2015; Watt et al., 2010; Watt & Salewski, 2011). Among amphibians in particular, a range of comparative studies varying in geographic and taxonomic scale have provided evidence that rejects (Adams & Church, 2008; Ashton, 2002; Olalla-Tarraga et al., 2009; Table 2 Results of phylogenetic analyses of body mass variation as a function of multiple climatic, environmental and geographic (latitude and elevation) predictors. Amphibian orders are tested separately (results of models combining all amphibian orders were qualitatively identical to results from anuran models). An effect is considered to exist if the 95% credible intervals (CI) do not cross zero. Significant results in boldface.

<table>
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<th>Model</th>
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<td>Caudata</td>
<td>476</td>
<td>0.96</td>
<td>0.105</td>
<td>−0.0476, 0.2689</td>
<td>1500</td>
<td>0.199</td>
</tr>
<tr>
<td>Gymnophiona</td>
<td>154</td>
<td>0.60</td>
<td>0.046</td>
<td>−0.2395, 0.3085</td>
<td>1500</td>
<td>0.739</td>
</tr>
<tr>
<td>3. Elevation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anura</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Elevational midpoint</td>
<td>3181</td>
<td>0.86</td>
<td>−0.034</td>
<td>−0.0728, 0.0085</td>
<td>1500</td>
<td>0.091</td>
</tr>
<tr>
<td>Elevational range</td>
<td>3181</td>
<td>0.86</td>
<td>0.204</td>
<td>0.1732, 0.2355</td>
<td>1500</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Caudata</td>
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<tr>
<td>Elevational midpoint</td>
<td>431</td>
<td>0.94</td>
<td>−0.018</td>
<td>−0.0939, 0.0529</td>
<td>1666.7</td>
<td>0.628</td>
</tr>
<tr>
<td>Elevational range</td>
<td>431</td>
<td>0.94</td>
<td>0.049</td>
<td>−0.0078, 0.1016</td>
<td>1500</td>
<td>0.087</td>
</tr>
<tr>
<td>Gymnophiona</td>
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<td></td>
<td></td>
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<tr>
<td>Elevational midpoint</td>
<td>82</td>
<td>0.69</td>
<td>0.547</td>
<td>0.1293, 0.9528</td>
<td>1500</td>
<td>0.011</td>
</tr>
<tr>
<td>Elevational range</td>
<td>82</td>
<td>0.65</td>
<td>0.468</td>
<td>0.2013, 0.7264</td>
<td>1236.3</td>
<td>&lt;0.001</td>
</tr>
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</table>
FIGURE 3  Global distribution of median log(body mass) for amphibians combined (top), and for anurans, salamanders and caecilians (as shown by the representative diagram depicting each order) separately. Map uses Behrmann equal area projection and 1 x 1° grid cells.
Pincheira-Donoso et al., 2019; Slavenko & Meiri, 2015) or only partially supports (Olalla-Tarraga & Rodriguez, 2007) Bergmann’s rule. Our study, the largest in both geographic and taxonomic scale conducted in these organisms, rejects the role of mean environmental temperature predicted by both Bergmann’s rule and its alternative, the ‘heat balance’ hypothesis. Therefore, the findings we present add to a history of conflicting evidence, where both the underlying cause and the predicted outcome of Bergmann’s rule are regularly discredited. In fact, a similar rationale extends to most ecogeographic rules on body size, for which support is conflicting and conditioned by multiple exceptions, such that they may apply depending on geographic region, lineage or size itself (Krizmanic et al., 2005; Olalla-Tarraga & Rodriguez, 2007; Pincheira-Donoso et al., 2008).

Why do predictions from ecogeographic rules about body size fail to find empirical support so often? A straightforward answer is the unrealistic logic of such predictions. Body size, like other complex phenotypes, is shaped by the additive effects of a wide array of selection pressures operating simultaneously on ecological (e.g. predation avoidance, climatic resistance and habitat use), sexual (e.g. mate competition) and reproductive (e.g. fecundity performance) (Andersson, 1994; Calder, 1984; Losos, 2009; Lynch & Walsh, 1998; Peters, 1983; Pincheira-Donoso & Hunt, 2017; Roff, 2002; Rosenthal, 2017; Smith & Lyons, 2013), in addition to the effects of shared ancestry (as demonstrated by the consistently high phylogenetic signal in all our models; see Table 2). Consequently, the optimal body size (i.e. the phenotypic point at which fitness is maximized) favoured by these different selection pressures may not necessarily be the same across the tree of life. For example, in ectotherms at high latitudes, fecundity selection may favour larger body size to compensate with larger clutch sizes for the limited reproductive opportunities caused by shorter breeding seasons (Cox et al., 2003; Pincheira-Donoso, Harvey, Cotter, et al., 2021), whereas lower temperatures may favour smaller size to enhance thermoregulation (Ashton & Feldman, 2003; Olalla-Tarraga et al., 2006; Pincheira-Donoso et al., 2008). Therefore, body size is often the outcome of trade-offs between the demands of multiple selection pressures, thus making the expectation that large-scale patterns of body size variation across organisms (e.g. endotherms and ectotherms) will be neatly explained by one single factor unrealistic.

Rather than aiming to make predictions about specific spatial clines of body size evolution as a function of single selection pressures (e.g. larger size with decreasing temperature), the elucidation of mechanisms underlying macroecological processes may benefit from a focus on how patterns of body size are shaped by the constraints that environmental pressures impose to set (maximum) thresholds beyond which the trait becomes inviable. For example, as climates get progressively colder, larger ectotherm body sizes are likely to be less viable (e.g. Ashton & Feldman, 2003; Pincheira-Donoso et al., 2008). As expected, no truly large ectotherms exist towards temperate regions (e.g. Pough et al., 2015; Sindaco & Jeremcenko, 2008; Wells, 2007). However, these constraints are likely to be relaxed, rather than reversed, towards lower latitudes as temperatures become warmer. As a result, tropical latitudes are more likely to accommodate a higher variance in body sizes across species. Therefore, while colder climates are expected to set a ‘maximum viable body size’ at any given latitudinal point (regardless of whether sexual or fecundity selection favours body masses larger than the maximum viable), the relaxation of this threshold towards tropical latitudes is more likely to allow the evolution of a wider variation of body sizes, from very small to very large. This is, again, the case—tropical climates host the smallest as well as the largest sized amphibians.

### 4.2 | A ‘third universal response’ to climate change?

The influence of the temperature–body size ‘rules’ led to the emergence of the hypothesis that anthropogenic global warming should
drive widespread changes (mostly reductions) in animal body size (Gardner et al., 2011; Sheridan & Bickford, 2011). This phenomenon has been deemed a potential ‘third universal response’ to global warming alongside phenological and geographic range shifts (Daufresne et al., 2009; Gardner et al., 2011). The lack of consistent evidence for the temperature–body size relationship (e.g. Riemer et al., 2018; Slavenko et al., 2019) has led to questioning the significance of this warming hypothesis (Riemer et al., 2018). However, rising temperatures are associated with severe alterations in other climatic components, particularly changes in precipitation (Fischer & Knutti, 2016; Krauss, 2021; Masson-Delmotte et al., 2021), and in its seasonality (Easterling et al., 2000; Feng et al., 2013; Ning et al., 1999). In concordance with our analyses, previous studies reveal a widespread role for precipitation as a driver of body size variation in amphibians, where body mass in both anurans and caecilians decreases towards environments with increasing rainfall (Olalla-Tarragà et al., 2009; Pincheira-Donoso et al., 2019). Therefore, based on the rationale of the warming hypothesis (Gardner et al., 2011), strong natural selection on body mass may arise from rapid alterations in precipitation regimes, leading to rapid size shifts. The directions in body size shifts are expected to be spatially heterogeneous given the geographic heterogeneity in the predicted changes in rainfall regimes (Chadwick et al., 2016; Feng et al., 2013; Marvel & Bonfils, 2013; Masson-Delmotte et al., 2021). Yet, the tendencies for extreme climatic events to aggravate under climate change, with intensification of rainfall, lead to the expectation that average body size in amphibians is likely to decrease. In addition to the wider impacts predicted to result from widespread reductions in animal body sizes (Daufresne et al., 2009; Gardner et al., 2011; Sheridan & Bickford, 2011), emerging evidence has shown how smaller size in amphibians is linked to higher extinction risk (Pincheira-Donoso & Hodgson, 2018) given the detrimental demographic effects of lower reproductive output (Pincheira-Donoso, Harvey, Cotter, et al., 2021). Collectively, we argue that this ‘third universal response’ should remain as a next frontier for empirical interrogation.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflict.

DATA AVAILABILITY STATEMENT
All the datasets used in this study are available as supplementary material to the main article, and will be made open-access at the GABiP initiative’s repository at http://www.amphibianbiodiversity.org upon publication.

REFERENCES


**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.