Slowing down: A macroevolutionary approach to the hypometabolic strategies of amphibians

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Abstract
The ability to survive harsh environmental conditions has probably been a key factor in the evolutionary success of organisms that cannot migrate long distances, such as amphibians. Therefore, we expect that the presence of a hypometabolic strategy (HS; like aestivation or hibernation) to deal with severe climatic conditions, would be a plesiomorphic trait. Here we 1) inferred the ancestral state of a HS in amphibians, 2) analyzed if amphibians with a HS have larger distributional ranges, and 3) explored how a HS may affect amphibian assemblage resilience using multiple models of climate change. We highlight that more research is necessary as information was available for many different lineages but < 10% of the total amphibian species. Ancestral state reconstruction showed a relatively high probability for the presences of a HS in the most recent common ancestors of Anura (73.09 ± 13.73), Caudata (78.66 ± 12.30), and Gymnophiona (77.26 ± 14.74). Our climate analyses predict that tropical zones will have the greatest degree of climate change, despite having the lowest proportion of species with HS. Thus, tropical amphibian assemblages at low elevations or in tropical

rainforests may be vulnerable to changes in climate. We found that species with a HS have significantly larger distribution ranges and most of those species are in the Least Concern IUCN category. It is likely that presence of HS as an ancestral state has been key for the evolutionary success in amphibians, and it will likely impact their future survival in the face of climate change.

Keywords (4-10): ancestral states**,** aestivation, conservation, hibernation, hypometabolism, [environmental] seasonality.

Introduction

Most animals seek shelter when environmental conditions turn harsh. For instance, amphibians use shelters to hide during their inactive hours on a daily basis (Forti *et al.*, 2022) in order to hide from predators and conserve water. Amphibians are required to keep their skin moist because it is crucial for breathing, and, therefore, their evaporative water loss (EWL) is normally high (Withers, 1998). Consequently, to survive long periods (weeks, months, or years) during adverse environmental conditions, amphibians resort to a type of dormancy or latency in a state of reduced or depressed metabolism (hereafter, hypometabolism) when they hide underground or underwater. The lengths of these hypometabolic periods vary considerably across species and populations because it seems to be driven by the local environmental conditions (Figure 1).

Hypometabolism is a general phenomenon that is not unique to amphibians and includes a wide range of metabolic depressions referred to with a variety of terms such as hibernation, estivation, dormancy, brumation and torpor. In general, dormancy is considered to be a term that encompasses all hypometabolic states, and the key aspect is that dormancy is preventive and anticipates stressful physiological conditions to avoid harmful changes in physiology (Navas & Carvalho, 2010; Storey & Storey, 2012; Reynolds, 2019). Torpor, from the Latin *torpōr* (Borror, 1960), means to be numb, inactive, or dull, and is referred to as a daily rest; it was commonly used as synonym of hibernation (i.e., winter torpor; Pinder *et al.*, 1992; Boutilier *et al.*, 1997; Withers & Cooper, 2010; Wilkinson *et al.*, 2017), yet it should not be considered a sustained hypometabolic state such as aestivation or hibernation (Castanho & De Luca, 2001; Navas & Carvalho, 2010). Aestivation or estivation, both derived from the Latin *aestās* that mean “pertaining to summer” (Borror, 1960), is defined as a state of reduced metabolism occurring in hot and dry conditions. Nevertheless, Pinder et al. (1992) consider that aestivation occurs in both hot and cold conditions and that its main driver is the lack of water. For example, toads of the genus *Scaphiopus* aestivate during winter (Seymour, 1973). Aestivation can occur when the amphibian burrows itself into the soil or seeks shelter in crevices or below logs and rocks, but it can also occur out in the open either on land or on

plants, although it is much less common (Navas & Carvalho, 2010). Finally, brumation and hibernation both come from Latin words meaning "related to winter" (Borror, 1960), the former from *bruma* and the latter from *hībern*. The use of the terms brumation and hibernation may be sometimes ambiguous, as they refer to the lethargic state that some animals assume during cold conditions. However, brumation was proposed to refer to obligate hibernators that cannot control their body temperature during winter dormancy rather than those that can (Mayhew, 1965).

Here we will use the most common terminology for the different states of dormancy: we will refer to summer dormancy as aestivation and winter dormancy as hibernation. We also make the assumption that both strategies imply a hypometabolic state. Here we will not include cryptobiosis from the Greek *crypto* and *bios* meaning 'hidden life', an extreme form of hypometabolic state, where metabolism is reduced to an undetectable but non-zero level (e.g., cryobiosis, when organism freeze in order to survive the winter), although it might be considered an extreme case of hypometabolism (Figure 1). Nonetheless, whether in the hottest and driest period of the year (i.e., aestivation) or in the coldest (i.e., hibernation), the end result is the same, some amphibians "slow their lives" (namely, lower their metabolism) in order to get through the unfavorable conditions that the environment imposes. In some cases, this pattern is so extreme that amphibians are active only a few weeks each year, like some desert amphibians (McClanahan, 1972; Cowan & Storey, 1999; Withers & Cooper, 2010). For example, the maximum aestivation period recorded in amphibians is five years in the Australian striped burrowing frog *Cyclorana platycephala* (Van Beurden, 1980). It is unknown if these extremely long periods of hypometabolism can be repeated several times throughout the animal's life or if there are limits. In some cases, aestivating amphibians produce a cocoon from several layers of shed skin (Withers, 1995); the cocoon provides extra insulation to prevent water loss and might increase the duration of aestivation periods in these species. For example, estimations show that it would take more than eight years for a *Cyclorana* frog with a cocoon and an empty bladder to lose 30% of its body mass by evaporation (Sadowski-Fugitt *et al.*, 2012).

According to Storey & Storey (2010), three requirements must be satisfied in order for a state to be considered hypometabolic: 1) an overall strong suppression of metabolic rate (at least a 70–80% reduction); 2) differential control over the rates of various metabolic processes so that energy use is reprioritized to favor core vital cell functions; and 3) implementation of actions that protect cells and preserve viability over long periods of time. There are several mechanisms that are activated during metabolic rate depression, and it is proposed that these mechanisms, their interactions, and their regulatory signals, form a common molecular basis for all states of metabolic depression including aestivation, hibernation, and some types of anoxybiosis (metabolic depression due to a lack of oxygen; Storey & Storey, 2004).

Many groups of frogs with aestivation evolved in arid or semiarid environments. For example, ancestors of family Ceratophryidae are thought to have evolved in a highly seasonal

115 and semiarid environment (Faivovich *et al.*, 2014), and all species in this group are able to
116 undergo prolonged aestivation and form cocoons, even the ones that no longer live in seasonal
117 environments (Zimicz *et al.*, 2024). Paleozoic amphibians appear to have burrowed in response
118 to seasonal droughts on the Permian midcontinental coastal plain in Kansas, USA (Hembree *et*
119 *al.*, 2004). Burrowing ability may be linked with aestivation in a response to climate variation.
120 For example, radiation of fossorial forms and increase in abundance and complexity of burrows
121 are contemporaneous with climatic crises such as the Cisuralian aridification and end-Permian
122 extinction events (Marchetti *et al.*, 2024). The ability of early Tertiary pelobatids to avoid
123 drought by estivating in burrows is thought to be a preadaptation to survive subfreezing
124 temperatures during the global cooling that began in the middle Eocene in Nevada, USA
125 (Henrici & Haynes, 2006). Therefore, we would expect to find a higher proportion of species
126 with a hypometabolic strategy in places with extreme environmental conditions, specifically
127 with aestivation occurring in semiarid conditions and hibernation in cold conditions (Hembree
128 *et al.*, 2004; Henrici & Haynes, 2006; Faivovich *et al.*, 2014; Marchetti *et al.*, 2024; Zimicz *et al.*,
129 2024).
130 The presence or absence of a hypometabolic strategy has possible implications for how
131 amphibians might respond to future changes in climate, and thus may be a concern for
132 conservation biologists. Indeed, climate change will impact amphibian distributions and ranges
133 (Ochoa-Ochoa *et al.*, 2012; Mokhatla *et al.*, 2015; Velasco *et al.*, 2021), will vary in speed across
134 regions (Ochoa-Ochoa & Velasco, 2024; García-Rodríguez et al., 2025), and produce climatic
135 debt—a disequilibrium between species communities' climatic tolerances and the magnitude of
136 climatic change (He *et al.*, 2023; Smith *et al.*, 2025). Here we propose that the climatic
137 resilience—the ability to cope with climatic stressors—of amphibian assemblages could be
138 related to the proportion of species with a hypometabolic state. Species that can enter
139 dormancy or a sustained hypometabolic state may be able to deal better with environmental
140 changes such as desertification, severe temperature/precipitation changes or extreme cold
141 conditions. Therefore, we expect that climatic resilience increases with increasing proportions
142 of species with hypometabolism (Figure 2). Moreover, having a hypometabolic strategy may
143 allow a species to survive over a broad range of environmental conditions. If true, we would
144 also expect that species with hypometabolism have larger distribution ranges on average.
145 In summary, the ability to tolerate unfavorable or extremely unfavorable environmental
146 conditions (such as very high or very low temperatures or droughts) has probably been a key
147 factor in the evolutionary success of organisms that cannot escape such harsh conditions. As a
148 group, amphibians have been on Earth for more than 300 mya and there have been many
149 periods of unfavorable conditions during that long time (Vitt & Caldwell, 2014; Jetz & Pyron,
150 2018; Judd *et al.*, 2024), including several mass extinctions. The ability to sustain a
151 hypometabolic state might have also been an important factor in the colonization of land, given
152 that lungfish also aestivate and produce cocoons (Jiang *et al.*, 2023). Thus, we would expect to

find that the capacity for hypometabolism is a plesiomorphic trait in amphibians (e.g., Jiang *et al.*, 2023). In this paper we aim to collate data on hypometabolism in amphibians to: 1) infer the ancestral state and evolutionary trajectory of hypometabolic strategies in amphibians; 2) explore the association between the presence of hypometabolic strategies and distributional ranges; and 3) explore the vulnerability of amphibian assemblages due to climate change in relation to the presence of hypometabolic strategies.

Methods

Hypometabolism data collection. We compiled information about hypometabolic strategies, particularly aestivation and hibernation, through a systematic literature review (see *Systematic literature review*). A comparative dataset for hypometabolic states was assembled including aestivation/hibernation status (present/possible/absent), and range size in km^2 (Table S1 in the Supplementary Information). It is important to highlight that here we are not including cryobiosis for the analysis since it is a type of cryptobiosis and not a hypometabolic state *per se* (Navas & Carvalho, 2010).

For scoring aestivation/hibernation, we considered them as present in a species if it was reported in at least one population or subspecies in the literature. We recorded absences (tentatively) when published studies or researchers noted its absence from personal experiences in fieldwork. However, we note that these cases will likely require further validation, and therefore, we did not include them as part of the training dataset (see *State uncertainty*) for subsequent models. Rather, an estimated probabilistic value based on a model was used instead.

Systematic literature review. In order to compile the dataset of amphibians that present a hypometabolic state, an exhaustive bibliographic search was carried out. The systematic search was performed using the advanced search tool of each of the following search engines: Scopus, PubMed, and Web of Science, until December 2024. We used the words (aestivat* AND amphibian*) and (estivat* AND amphibian*) for aestivation, and (hibernat* AND amphibian*) for hibernation. Studies were selected according to the following criteria: 1) original and review manuscripts including not just research, but also theses; 2) original articles in which some biological, ecological, physiological, or genetic aspect of the aestivation process were mentioned; and 3) manuscripts where the focus was not related to dormancy processes, but an aestivating or hibernating species was mentioned. We also performed a search on AmphibiaWeb to review and complete our resulting list of species that aestivate and/or form cocoons while aestivating, hibernate, or have a cryptobiotic state. Scientific names follow AmphibiaWeb (2024) nomenclature.

Phylogenetic and geographic data. Because our subsequent analyses require a phylogenetic framework, we used the "fully-sampled" amphibian supertrees featured in Jetz and Pyron (2018). Given that these trees were generated with a mixed methodology incorporating a backbone for the species with available genetic information and the imputation of the species without data, the final consensus is highly polytomic. Therefore, in order to account for the inherent phylogenetic uncertainty (Rangel *et al.*, 2015), we subsampled 100 randomly resolved trees directly from VertLife (https://vertlife.org) that are from the same dataset. In addition, we downloaded the available distribution polygons for amphibians from the International Union for Conservation of Nature Red List (IUCN, 2024; https://www.iucnredlist.org) in its 2023-1 update. Because the trees and polygons may differ in the taxonomic treatment of some species, we harmonized the names between data sources to maximize the number of species in the final dataset. To do so, we manually validated the non-matching species against their synonym list available in the Amphibian Species of the World (https://amphibiansoftheworld.amnh.org) looking for alternative names. From the 857 species with hypometabolism data, 774 (approx. 90%) were shared between the phylogeny and geographic datasets. Although the non-matching species were not included in the phylogenetic comparative analyses, they were included to generate the hypometabolic probabilities (see *State uncertainty*) and to illustrate the summary information on the global status of hypometabolism in amphibians.

State uncertainty. Since we have literature information for only 857 species out of the 8,823 amphibians currently described, less than 10% (AmphibiaWeb, 2024), an important issue in this study is the large amount of missing data. Thus, we calculated the probabilities of the presence of a hypometabolic state in the tips with unknown states by assuming that hypometabolic states evolve in response to environmental conditions and that species are adapted to the environments they inhabit (Hembree *et al.*, 2004; Henrici & Haynes, 2006; Faivovich *et al.*, 2014; Zimicz *et al.*, 2024). Importantly, we highlight that this approach makes the assumption that species have tracked their niches over evolutionary time. To conduct this analysis, we first extracted for each species the maximum values for the 19 WorldClim bioclimatic variables (https://www.worldclim.org) at 10 arcmin resolution (approx. 18.5 km at the equator). To address multicollinearity, we thinned our dataset by excluding highly correlated variables using the Variance Inflation Factor (VIF) with the *vifstep* function as implemented in 'usdm' v.2.1 (Naimi *et al.*, 2014). We then fit a One-Class Support Vector Machine (OC-SVM) model using a subsampled training dataset that included only species with confirmed presence separately for aestivation and hibernation.

OC-SVM is suitable for presence-only data, allowing us to avoid model biasing given the uncertainty in the absence data (false positives). For this, we employed the *svm* function in the 'e1071' v.1.7-16 package (Meyer *et al.*, 2024), using a radial kernel with a custom gamma

228 (shape of the kernel) for each trait (aestivation = 0.25, hibernation = 0.15), nu = 0.1 (sensitivity
229 to outliers), and a 10 k-fold cross-validation strategy. We manually tuned the shape of the
230 kernel to optimize the model's performance by minimizing the number of false negatives (true
231 presences with low estimated probability) while increasing the model's accuracy (Figure S1).
232 Posteriorly, decision values were scaled between 0 and 1 and transformed into probabilities
233 using a sigmoid function. Subsequently, for species with confirmed presence we kept the
234 probability as 1, but replaced the other ones in our literature review (i.e., possible state of
235 aestivation or hibernation) with their inferred probability in order to account for the
236 uncertainty in defining true absences. We repeated this estimation twice, once for aestivation
237 and once for hibernation. For the species assigned by the literature review as "possible", two
238 outcomes were possible: 1) they were assigned a low probability, equivalent to an absence; 2)
239 they were assigned a high probability (> 80%), equivalent to a presence under our assumption
240 that climate is triggering such hypometabolic strategies (and our initial coding from the
241 literature is just a reflection of the absence of data).
242 Afterwards, in order to use these probabilities in the combined hypometabolism
243 analysis, we assigned the maximum probability of either aestivation or hibernation to each
244 species (i.e., if the species is predicted to estivate but not hibernate it would still be counted as
245 a "presence" for having a hypometabolic state). Although we calculated the missing data for all
246 the amphibians in the trees using probabilities, we performed subsequent ancestral state
247 reconstructions (see Reconstruction of ancestral states) in trees with twice the number of
248 species with hypometabolic data, by randomly subsampling another 774 species for each tree.
249 This means that we ran every reconstruction using the 774 species from the literature (fixed
250 species) and another 774 randomly selected for each tree, running the simulation 100 times.
251 Thus including 50% of species with literature hypometabolic state and 50% of species with
252 inferred hypometabolic state. The aim of this last is: 1) to increase the taxonomic
253 representativeness of the different amphibian taxa; 2) to reduce the bias in the results by
254 accounting only for species with presence or potential presence data; and, finally, 3) to balance
255 the effect of using the entire estimated dataset.
256
257 **Reconstruction of ancestral states.** Here, we modeled the evolutionary transitions between the
258 presence and inferred absence of aestivation, hibernation, and hypometabolism (either
259 aestivation or hibernation), the last following the assumption that all states of metabolic
260 depression have a common molecular basis (Storey & Storey, 2004). Subsequent analyses were
261 performed using 'phytools' v.2.3 (Revell, 2024), and a script can be found as part of the
262 Supplementary Files. First, we employed the Markov k model (hereafter Mk model) for discrete
263 character evolution of characters with k unordered states (Lewis, 2001) to find the best state
264 transition model, as implemented in the function *fitMk*. As a Markovian model, it assumes that
265 transitions between states depend only on the current state and not on previous ones. We

tested two different models of transition rates: equal (ER) and all different (ARD), allowing the π parameter to estimate the prior distribution of the root state jointly with the other model parameters rather than defining it *a priori* (Fitzjohn *et al.*, 2009). Since we are using a collection of trees rather than a single one, we ran the analyses 100 times and chose the best model based on its likelihood and Akaike information criterion (AIC) values to plot the ancestral state reconstruction.

Given that the presence of a hypometabolic state for many species may be ambiguous given the paucity of data, we accounted for uncertainty in trait states as probabilities. Then, we mapped possible scenarios of trait evolution using stochastic character mapping over the 100 trees for aestivation, hibernation, and hypometabolism separately. We employed the *make.simmap* function with 100 simulations per tree using the ARD transition model, π as in the fitMk, a non-fixed transition matrix (Q) estimated with a Markov Chain Monte Carlo (MCMC) approach, empirical priors from observed data, a variance of 0.1 (vQ), and a sampling frequency of 10. Thus, we ran a total 1000 MCMC iterations, sampling every 10 for a total of 100 independent histories per tree. Subsequently, we calculated the average number of transitions across 100 simulations for each of the 100 trees using the countSimmap function. Since the Q matrices were estimated independently for each simulation and tree, the MCMC algorithm may get stuck in suboptimal peaks, yielding extremely low or high numbers of transitions in some cases and increasing the uncertainty in the transition counts. Therefore, the average number of gains and losses was calculated excluding outlier values (those above or below 1.5 times the interquartile range).

Because each tree differs in topology, it is not possible to summarize the nodes across the entire set of reconstructions (10,000 different sampled histories). However, given the method used for inferring the base topology we used (Pyron & Jetz, 2018), such differences are expected to be more pervasive at lower taxonomic levels (e.g., species or genus). Therefore, we identified the most recent common ancestor (MRCA) for superfamilies and orders using the fixed species on each tree (i.e., those non-randomly selected), and averaged the posterior probabilities of those nodes to account for phylogenetic uncertainty. It is important to note that, those MRCA are not necessarily the same in all the trees but the most inclusive monophyletic groups encompassing our fixed species. Because this kind of reconstruction requires discrete traits at tips, we considered values equal or greater than 80% as strong evidence for presence, given that the probability for all true presences from the literature was estimated with the same threshold (see Figure S1). Moreover, in order to explore the transition of the states in the nodes along time, we also performed a joint ancestral state reconstruction over the 100 random trees using the function *ancr*, to quantify the proportion of nodes with aestivation or hibernation on 5-my bins and its possible association with the mean Earth surface temperature (Judd *et al.*, 2024).

Geographic distribution of species with hypometabolism. To explore geographic patterns in our data, we generated maps of species richness with aestivation, hibernation, and hypometabolism by overlapping the IUCN species polygons (IUCN, 2024; 2023-1 update) with a grid of 1° × 1° (ca. 111 km at the equator); we also generated maps using the proportion of species with a hypometabolic state compared to the assemblage. We first generated maps using only the species with data from the literature review, including any species with presence or possibly presence of a hypometabolic state as presence. Then, we repeated the calculation of species richness with all the species in a grid cell to calculate the proportions of the total species in each cell with a HS.

Distributional range analyses. In order to explore whether the species with a hypometabolic strategy had larger geographic ranges, we first created two datasets, one with the dataset reduced to include only the 857 species with data from literature ("reduced dataset") and one with all species included ("complete dataset") to explore if there were differences due to literature bias. In both cases, we included data from the literature as well as the inferred probabilities for the species with "possible" states, for the complete dataset we also included species without literature data. We then divided the species into three probability groups using equal quantiles (100/3): high, medium, and low, respectively. We then graphed the $\log_{10}$ of the areas from IUCN data (2023-1 update) and tested for differences between categories using Wilcoxon tests with Bonferroni correction. Finally, we quantified the threatened category of the species in the dataset and discussed the implications for conservation, particularly regarding the upcoming environmental changes.

Amphibian assemblage climatic resilience. According to our hypothesis, the greater the proportion of species with hypometabolism, the higher climatic resilience the assemblage would have. To test this idea, we quantified dissimilarities between the baseline (1990–2020) and future climate scenarios (2070–2100) by calculating the standardized local anomalies (i.e., how atypic is a value compared with the historical variability) per raster pixel (~1 km^2), using the standardized Euclidean distance (SED) (Williams *et al.*, 2007). In other words, the temporal differences for each climate parameter are standardized using the local inter-annual standard deviation for that parameter. This method is able to provide information on the extent of the change in each location in absolute terms, therefore it gives direct information on the magnitude of climate change to which the assemblage might be exposed in a particular grid cell (Williams *et al.*, 2007; Taheri *et al.*, 2024a).

We focused on the monthly values of the multi-model mean of precipitation, and maximum and minimum of temperature from the experiments from KNMI 2023 standard set of CMIP6 General Circulation Models (GCMs). Historical and future change scenarios were obtained from the Climate Explorer (https://climexp.knmi.nl). We choose a middle shared

342 socioeconomic pathway: the regional rivalry climate change scenario ssp370 or the "rocky
343 road", where countries prioritize national interests over global cooperation thus leading to an
344 increase in greenhouse gases. We use the mean of all 24 models for historical and future
345 scenarios of climate change (CCESS-CM2, ACCESS-ESM1-5, AWI-CM-1-1-MR, BCC-CSM2-MR,
346 CMCC-CM2-SR5, CNRM-CM6-1-HR-f2, CNRM-CM6-1-f2, CNRM-ESM2-1-f2, CanESM5-CanOE-p2,
347 CanESM5, EC-Earth3-Veg, EC-Earth3, FGOALS-g3, GFDL-ESM4, GISS-E2-1-G-p3, INM-CM4-8,
348 INM-CM5-0, IPSL-CM6A-LR, MIROC-ES2L-f2, MIROC6, MPI-ESM1-2-HR, MPI-ESM1-2-LR, MRI-
349 ESM2-0, and UKESM1-0-LL-f2). In order to estimate the standardized Euclidean distance (SED),
350 we employed the *sed* function in the 'climetrics' package v.1.0-15 (Taheri *et al.*, 2024b),
351 analyses were performed using the platform R version 4.4.2 (R Core Team, 2024).
352 To explore the relation between the changes in climate with the proportion of species
353 with hypometabolic data (including "possible" hypometabolic states), we first averaged climatic
354 standardized distance per grid (1° × 1°) to match the scale of the species richness maps. We
355 generated a heat map of the climate change magnitude (SED) against the proportion of species
356 with a HS under the assumption that the higher the proportion of species with a HS, the higher
357 the expected resilience to climatic change. In order to localize the most vulnerable assemblages
358 we selected the sites with highest increase in climate variance (> 2 of SED) and low proportion
359 of species with hypometabolism (≤ 0.5). Afterwards, we plotted the selected assemblages on a
360 map, we also included grid cells with the 10th highest percentile of the anomaly scores which
361 corresponds to large changes in temperature and precipitation.
362
363 **Results**
364 The systematic search rendered 280 articles for aestivation and 650 for hibernation. We found
365 information about a hypometabolic strategy for 857 amphibian species (Table S1). From these
366 species, 648 had some information about aestivation, 342 with presence, 235 with the
367 possibility of presenting that strategy, and 52 as non-aestivating species. In the case of
368 hibernation, we found information for 479 species, 308 with presence, 62 species with possible
369 hibernation, and 48 that showed no hibernating strategy. The representativity of this data set is
370 as follow: Gymnophiona one of nine families (11%), Caudata ten of ten (100%) and Anura 28 of
371 59 (48%). Most of the species with hypometabolic strategies fall in the Least Concern IUCN
372 category: 388 of the aestivating species, including possible aestivation (> 67 % of species with
373 information), and 242 of the species with hibernation including possible hibernation (> 65 % of
374 species with information) (Figure S2).
375
376 **Ancestral states.** The accuracy of the traits inferred using OC-SVM compared to data from the
377 literature was high, with 73 % for aestivation and 72 % for hibernation matching known
378 observations and all confirmed presences in both categories with >80 % probability (Figure S1).
379 The best transition model for aestivation and hypometabolism was inferred to be the ARD,

380 while for hibernation, there were no differences between the ARD and the ER (Figure S3). Thus,
381 we will present only the results of the reconstructions using the ARD model.
382 The ancestral state reconstruction analyses for the 100 trees showed a high uncertainty
383 regarding the state for the most recent common ancestor of all amphibians, with an estimate
384 close to 50/50 presence/absence for each dataset: aestivation 48.33/51.67 (SD ± 9.07),
385 hibernation 51.51/48.49 (SD ± 14.70), and hypometabolism 48.27/51.73 (SD ± 9.83) (Figure 3;
386 Table 1). It is worth mentioning that the standard deviation is the same for presence and
387 absence since the values for each tree node are complementary. The probability of the
388 presence of a hypometabolic state for either aestivation or hypometabolism increases rapidly
389 towards the present, being the most probable state for the most recent common ancestor of
390 each of the major amphibian clades: probabilities for Anura (frogs) of aestivation 62.79 (SD ±
391 11.23) and hypometabolism 73.09 (SD ± 13.73), for Caudata (salamanders) 68.34 (SD ± 11.97)
392 and 78.66 (SD ± 12.30), and for Gymnophiona (caecilians) 71.72 (SD ± 16.22) and 77.26 (SD ±
393 14.74) (Table 1). But this was not the case for hibernation where Anura (53.97 ± 14.47) and
394 Caudata (67.23 ± 18.49) tended for a presence while in Gymnophiona hibernation seems more
395 likely to be absent (64.74 ± 15.44) than present (35.26 ± 15.44). Nonetheless, the internal nodes
396 tended to be predicted to have presence of hibernation even in the superfamilies (Table 1). On
397 average, 362 (SD ± 86) gains were inferred for aestivation and 262 (SD ± 62) for hibernation;
398 327 (SD ± 80) and 248 (SD ± 84) losses were inferred for aestivation and hibernation,
399 respectively. In the combined hypometabolism dataset, 283 (SD ± 76) gains and 250 (SD ± 69)
400 losses were inferred. In all three datasets, there were more gains than losses (Figure S4).
401 For aestivation it is possible to observe a complex pattern of gains and losses of the trait
402 (Figure 4, exemplification with the best tree), where most of the clades have representative
403 lineages of both although there are a few groups exhibiting only presence such as the
404 Pelodryadinae subfamily (i.e., *Cyclorana* genus), and some genera within the subfamilies
405 Hyloxalinae and Mantellinae, and the Ceratophryidae family. The transitional evolution of
406 aestivation is likely to be complex, for example being the most likely ancestral state in groups
407 like Myobatrachoidea, but for Ranoidea although probabilities are above 50% there seems to
408 be multiple independent losses in groups such as *Odorrana* and some species of *Rana.* For
409 hibernation there are only a few groups that show a high probability for the presence of
410 hibernation as an ancestral state, such as Caudata, as well as in some anurans, including
411 Myobatrachoidea and Paleobatoidea, while Hylodea seems to have a very low probability of
412 presence of hibernation as well as in Gymnophiona (Table 1, Figure 4). In the ancestral state
413 reconstruction analysis for hypometabolism, there are few groups that show high probability
414 for the absence of hypometabolism as the ancestral state, such as Hyloidea, although it is
415 important to note that there are some species in this clade that showed hypometabolism such
416 as *Eleutherodactylus* and *Craugastor*, probably as a subsequent gain. Something similar occurs
417 in the Bufonidae family, with a most likely ancestral state of absence of hypometabolism, a

418 state that is maintained in genera such as *Atelopus* but subsequently gained in *Incilius*,
419 *Anaxyrus* and *Bufo*, just to mention a few. Meanwhile groups such as Sirenoidea,
420 Salamandroidea, Myobatrachoidea, and Bombinatoroidea showed remarkably high
421 probabilities of presence of hypometabolism (Table 1).
422 At a broad scale, based on the best tree only, the proportion of ancestral nodes with
423 aestivation decreased during the Jurassic when the Earth's surface temperature was
424 transitioning to a cool-house (ca. 175–160 mya), to posteriorly recover again in the transition to
425 a warm-house (ca. 160–150 mya). Unexpectedly, aestivation's greatest depression was inferred
426 during a warmer period between 150–125 mya, with a posterior recovery and fluctuating but
427 steady trend to the present. On the other hand, the hibernation trend was stable during the
428 cooled period in the Jurassic, with a drop during the transitional period to warmer
429 temperatures 160 mya, with a posterior recovery but reduction again during the remaining
430 Mesozoic, and a recovery during the transition to a cooler Earth in the past 50 mya (Figure 5).
431
432 **Geographic distribution of species with hypometabolism.** As mentioned in the methods, in this
433 section when we mention a hypometabolic state we are also including the species with the
434 "possibility" of presenting that state but haven't been confirmed. The pattern of aestivating
435 species richness (Figure S5) is really striking in some parts of the world such as in the east coast
436 of the United States where there is a high number of species that present aestivation. In other
437 regions such as Australia, South Africa, the arid zones of North America and the Arid Diagonal in
438 South America, the high number of species that present aestivation is unsurprising due the high
439 precipitation seasonality in those regions. The pattern of the proportion of species that present
440 aestivation (Figure S6) follows very closely the arid zones of the world (Zomer *et al.*, 2022), with
441 obvious exceptions where no amphibians are found such as the Sahara, Arabian and Gobi
442 Deserts which are deemed as hyper-arid zones (Yang & Williams, 2015).
443 The pattern of hibernating species richness is also interesting in some parts of the world
444 such as in the east coast of the United States and southeast China, where there is a high
445 number of species that present hibernation (Figure S7). In other parts such as the rest of North
446 America, Europe, and northern Asia, the high number of species that present hibernation is
447 expected due the high seasonality in temperature in those regions as they coincide with
448 Köppen cold climates map (Beck *et al.*, 2023). The areas that show a high proportion of species
449 that present hibernation are mainly in the Global North (Figure S8).
450
451 **Distributional range analyses.** The results of the Wilcoxon tests for the reduced dataset
452 revealed that species with the highest probability of aestivation (> 66%) do not tend to have
453 larger areas. In fact, the low (< 33%) probabilities (p) group showed the largest areas, with a
454 decreasing tendency for intermediate (33% > p < 66%). There were significant differences
455 between low-mid (p = 0.013), but not between low-high (p = 0.200), nor mid-high (p = 0.103;

Figure 6a). For hibernation and hypometabolism, the group with the high probabilities of HS presented the larger distribution ranges. There were no significant differences between the range sizes of species with high and medium probability of hibernation ($p = 0.5669$), however, the low probability showed smaller range sizes than the intermediate probability, with significant differences: low-mid ($p = 0.00045$), and low-high ($p > 0.0001$; Figure 6b). For hypometabolism, there were significant differences between low-high ($p = 0.0047$), but not in low-mid ($p = 1.00$) and mid-high ($p = 0.7433$; Figure 6c).

However, the results of the Wilcoxon tests for the complete dataset revealed in all cases, aestivation, hibernation and hypometabolism ($n = 6130$), that species with the highest probability of presenting HS tend to have larger areas, with a decreasing tendency for intermediate and low probabilities. Between the categories there were significant differences in almost all cases. In aestivation there were significant differences in low-high ($p > 0.0001$), and mid-high ($p = 0.00033$) but not in low-mid ($p = 0.79$; Figure 6d). Hibernation showed significant differences in all pairs (with $p > 0.0001$; Figure 6e). In hypometabolism, there were also significant differences: low-mid ($p = 0.0011$), low-high ($p > 0.0001$), and mid-high ($p > 0.0001$; Figure 6f).

Amphibian assemblage climatic resilience. The results of the standardized climate anomalies showed higher magnitude in climate change are actually in the tropics (Figure 7). Results show that most of the sites will experience low climate change and those have a wide range of proportions of species with a HS (Figure 7a), it can also be observed that there are few sites that will be faced with great climate change but that have a high proportion of species with a hypometabolic state therefore, a climatically resilient assemblage (Figure 7a). We consider that most vulnerable assemblages are sites with high climate variance (> 2 of SED). If we take the subset of these that contain a low proportion of species with hypometabolism (≤ 0.5), there are a total of 238 assemblages, all of which are located in the tropics (Figure 7b). These sites are going to experience the greatest change in climate, according to the models, and therefore amphibian populations living there are going to experience a substantial pressure to adapt, possibly using hypometabolic states to help them to survive.

Discussion

With more than 8,800 described amphibian species, we only recovered information for less than 10%, clearly indicating that more information about the ecology of the amphibian species is needed. This is troublesome to say the least, not only because it increases the uncertainty in the analyses (see below) but also because it means that for the majority of the species, we do not have the minimum information to establish effective conservation plans and we will need to rely on assumptions derived from < 10% of each group or class. Nevertheless, we note that these 857 species include a variety of distinct lineages from caecilians to salamandrids, pipids,

494 and hylids to name just a few. Another aspect to take into account is the inherent geographical
495 bias of the data, where most of the data is from the USA, Australia, New Zealand, and the
496 European region. Although, it is worth mentioning that other regions also stand out, such as
497 South America and South Africa, probably due to the extreme seasonality and the number of
498 species that are known to have estivation in these regions.
499
500 **Reconstruction of ancestral states.** Since ancestral state reconstruction is a phylogenetic
501 comparative method that involves estimating the unknown trait values of hypothetical
502 ancestral taxa at internal nodes of a phylogenetic tree, it involves quite a bit of uncertainty.
503 Revell (2025) recommends '*considerable caution and circumspection in the use and*
504 *interpretation of ancestral reconstruction in empirical evolutionary research*'. The results of our
505 analyses showed that it is probable that a hypometabolic strategy has a single origin in the
506 ancestor of amphibians, not because of the probabilities at the root node itself but when
507 considering the probabilities of the ancestors of the three orders. For instance, at the order
508 level and superfamily level, some hypometabolic states were inferred with high probability
509 (above 70% for hypometabolism and aestivation). For hibernation the case is different since the
510 probabilities are lower for Anura and Caudata (< 70%), and for Gymnophiona the ancestral
511 state points to an absence of hibernation which is probably related to the geographic history of
512 the clade.
513 The hypometabolic strategies (HS) seem to have been secondarily lost multiple times
514 within the amphibian tree; the complex pattern presented in the different reconstruction
515 analyses prevents us from drawing a general inference for the whole group. The loss of HS is
516 most evident for clades that are largely distributed in the tropics, compared to clades that are
517 distributed toward the poles; however, we cannot make a generalization due to the gain of HS
518 in some cases. For example, the loss of aestivation is most evident for clades that are largely
519 aquatic or restricted to areas with accessible water such as *Amolops* or *Odorrana*. If aestivation
520 is triggered by drought, having water availability all year round might permit its loss. The
521 aestivation loss in the case of *Pristimantis* might be associated with the type of habitat where
522 they are found such as cloud montane forest or tropical forest, where the humidity is constant
523 around the year since they are distributed near the equator (AmphibiaWeb, 2024). The loss of
524 hibernation is most evident for clades that are largely in the tropics, such as Dendrobatidae,
525 *Hyalinobatrachium*, *Oreophryne, Pristimantis*, *Eleutherodactylus*, and *Craugastor* just to
526 mention some. As we acknowledged, there is very limited data regarding the absence of either
527 aestivation or hibernation, so additional data and analyses will be necessary to confidently
528 assign losses of the trait. For example, our OC-SVM model yielded several "false positive"
529 species. In this regard, if the climate is indeed highly influencing the presence of a
530 hypometabolic strategy, and it is somehow predictable, those "false positive" could be "true

positive" reflecting a bias in the lack of knowledge about the species' ecological traits, also known as Raunkiæran shortfall (Hortal *et al.*, 2015).

If the discrepancy in the reconstruction of ancestral states among estivation and hibernation is a reflection of the evolutionary history of both states, it is possible to consider that estivation and hibernation are both cases of an adaptive dormancy (Navas & Carvalho, 2010), since the mechanisms that trigger each of the different states of hypometabolism are completely different. On the one hand, aestivation seems to be triggered by lack of water and excessive heat, on the other, hibernation seems to be triggered by low temperatures, and it is commonly associated with accessible water. Nonetheless, it is possible that the regulatory mechanisms responsible for depressing metabolisms are the same between aestivation and hibernation (e.g., methylation of DNA; Hawkins & Storey, 2020; Bell & Hellmann, 2025) but the triggering event is different in each case. So, our results seem to agree partially with the proposal of Storey & Storey (1990) in that if having an hypometabolic state is an ancestral state, therefore the mechanisms and regulatory signals that are activated during metabolic depression have a common molecular basis for all states of metabolic depressions, including aestivation, hibernation, and some types of anoxybiosis, probably even cryobiosis. It might be possible to go even further and predict that torpor also relies on the same mechanisms; however, little is known in this aspect for amphibians since most of the literature about torpor is actually referring to winter torpor, here classified as hibernation (Boutilier *et al.*, 1997; Wilkinson *et al.*, 2017) but see Castanho and De Luca (2001).

Distributional range analyses. When using the complete dataset, we found that species with a hypometabolic strategy have significantly larger distribution ranges. We suspect that this pattern arises because having a hypometabolic strategy allows individuals and populations to overcome harsh environments since they do not have to 'suffer' those conditions. Therefore, this ability could allow them to disperse to stressful or unfavorable environments because they are not limited by narrow environmental constraints. Given that one of the criteria for IUCN criteria for assessing threatened species is small range size, it is not surprising that most of the species with estivation (and large range sizes) are within the Least Concern category (IUCN, 2024). What do these data imply for conservation programs? While we do not want to lure readers into a false sense of "these species are going to be fine", because there are other threats besides climate change, such as deforestation (Tan *et al.*, 2023), pollution (Egea-Serrano *et al.*, 2012; Martin *et al.*, 2024), and invasive species (Falaschi *et al.*, 2020), just to mention some (see Catenazzi, 2015 for a deeper assessment), species that aestivate or hibernate might be relatively resilient in the face of climate change (but see Rollins-Smith, 2017).

Amphibian assemblage climatic resilience. It is interesting that when accounting for the variability along the periods tested (i.e., using the SED method), the results showed that the

largest differences between current and future climate is going to occur in the tropics. It does make sense because much of the tropics have little variability in temperature and precipitation compared to the higher latitudes where the inter-annual variation is huge. In addition, most of the species with a hypometabolic strategy occur outside the tropics (Figs. S3-S6). Consequently, the most vulnerable assemblages are located in the tropics, but not just anywhere in the tropics, they are mostly concentrated in the lowlands. For example, soil temperature variation in tropical mountains like the Andes may reach almost 50 °C in one day, ranging from -7 °C at 3,500 m a.s.l. or -14° at 4,300 m a.s.l. during the night to more than 40 °C during the day (Navas, 1996), and we expect that these species are likely to demonstrate some form of hypometabolism. For example, *Atelopus carrikeri* lives at 4,500 m a.s.l. where temperatures can be high during the day but it can be trapped occasionally in ice sheets and survives (Navas, 1997). It is important to stress that high-elevation Neotropical amphibians do not belong to a single taxonomic group. Species from several divergent families such as Bufonidae, Leptodactylidae, Dendrobatidae, and Hylidae are represented at elevations over 3,000 m a.s.l. in the tropical Andes, and these high-elevation species are apparently derived from low elevation stocks (Lynch & Suárez-Mayorga, 2002). Thus, given the results of the ancestral state reconstruction, it might be possible to assume that these species were able to persist in those conditions because they have converged on the mechanisms to survive harsh conditions.

Another aspect that is worth highlighting is the temperature spectrum of activity. Navas (1996) showed that high-elevation anurans are capable of performing activities across a wide range of temperatures, however low-elevation species can be active in narrower ranges of temperature. This supports our results of assemblage climatic resilience in the sense that most vulnerable assemblages were found in tropical lowlands. Plastic responses to past environments shape adaptation to novel selection pressures (Coates *et al.*, 2025), therefore it is logical to assume that if there is geographical variation in the response to climate across population, that is, not necessarily all populations aestivate or hibernate, this plasticity may give said species a chance to adapt to novel climate.

Uncertainty. Even though we have tried to account for the limited number of species used for inferences, it is important to stress that the results presented here have to be taken with caution as the information used in the analyses represents less than 10% of the total amphibian species described (Frost, 2024; AmphibiaWeb, 2024). Two aspects emerge from this lack of data, the first is the high uncertainty that was generated by inferring the probabilities of a hypometabolic strategy based only on climate without taking into account the intrinsic variation of the phylogenetic signal of each group. Thus, it led, in part, to uncertainty in the reconstruction of the ancestral state, although it is important to stress that the inferences did correctly predict all of the species known to have hypometabolic strategies. Nevertheless, it is also necessary to highlight that the possibility of the hypometabolic state as an ancestral state

was around 50%, which is high given the complexity of the tree and the amount of data. The second aspect regarding uncertainty in our analyses is the assumption that species without information either do not or cannot present a hypometabolic strategy. For example, *Rhinella marina* does not normally aestivate under natural conditions but when subjected to experimental conditions of water stress and temperature it does aestivate (Boutilier *et al.*, 1979 but see Hilje & Arévalo-Huezo, 2012). Solving this dilemma will be crucial in the years to come when planning effective conservation strategies, either by collecting more information in the field or by performing experiments. It would be ideal to have information for all the genera described.

Future research directions. Aside from a call to ameliorate the general paucity of data on hypometabolism in amphibians, we propose a few research themes that would be fruitful to pursue in the future. First, how long can species aestivate or hibernate? A few accounts suggest that amphibians can survive five or more years while estivating (Van Beurden, 1980) and estimations suggest even more (Sadowski-Fugitt *et al.*, 2012), which conflicts with common narratives that amphibians are sensitive and fragile organisms. Persisting in a hypometabolic state for years would provide impressive resiliency to sudden or sustained environmental change and may start to explain how amphibians have already survived several mass extinction events. Aestivation may also help amphibians survive wildfires. Second, could resorting to aestivation or hibernation affect longevity in populations? Third, do amphibians have different levels of hypometabolism, for example, very shallow (days), shallow (weeks) or deep (months to years)? In this sense should we classify torpor as an actual hypometabolic state? Are the triggers and the molecular mechanisms the same? Fourth, what are the physiological mechanisms of the species that live in mountains, such as the Andes, where they can experience an oscillation of temperature of more that 40 °C in one day? Finally, in the era of huge environmental changes, will the limits of hypometabolic ability be reached in the near future (Macip-Ríos *et al.*, 2023)? Would having a hypometabolic strategy give populations resilience to face coming changes in climate?

Clearly the major drawback of the analyses and results presented here are the number of species used for the analyses. Therefore, we call on our colleagues to report whether or not a species goes into a hypometabolic state, and if possible, to test them.

Supplementary material

File S1. Additional methods and results. This file contains additional results.

File S2. R **scripts used for the analyses performed**.

Table S1. Dataset of species with hypometabolic strategy information. Compiled dataset containing the species found in literature regarding hypometabolic strategy (HS): aestivation or hibernation. HS was classified as **present** = confirmed hypometabolic state, **possible** = author mention the possibility but it was not confirmed, **absent** = the reference clearly mention that the species/population does not present the hypometabolic state**, unknown** = when the authors clearly mention that is unknown. In this table is also listed the threatened category from IUCN (2024), the name in the tree (Jetz & Pyron, 2018), and the calculated probabilities for the states.

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Figure legends

Figure 1. Representation of the different levels of metabolic depressions currently reported in amphibians. The ovals represent the range in which each state would be denominated as such. For example, there would be a wide range of aestivation and hibernation levels. Illustrations were generated using the Adobe Illustrator generative AI.

Figure 2. Resilience of the amphibian assemblage due to climate change. Highly resilient assemblages are expected to be either located in a cell with severe climate change but would be composed by a high proportion of species with presence of hypometabolism. In contrast, highly vulnerable assemblages would be where very few species have hypometabolism regardless of the amount of change in climate that is going to occur. Hypothetical lines are drawn to picture thresholds of vulnerability.

Figure 3. Probabilities from the ancestral state reconstruction and age node estimation for A) aestivation, B) hibernation and C) hypometabolism**.** Result from the MCMC approach to sample character histories from their posterior probability distribution (multi simmap analyses) for 100 trees.

Figure 4. Stochastic character mapping for the hypometabolic strategies in amphibians based on the best tree. The internal colored ring depicts the current state of each tip (red = presence; blue absence), while the external, major groups as featured in Jetz and Pyron (2018): 1. Gymnophiona, 2. Cryptobranchoidea, 3. Sirenoidea, 4. Salamandroidea, 5. Leiopelmatoidea, 6. Bombinatoroidea, 7. Pipoidea, 8. Paleobatoidea, 9. Myobatrachoidea, 10. Hyloidea, and 11. Ranoidea.

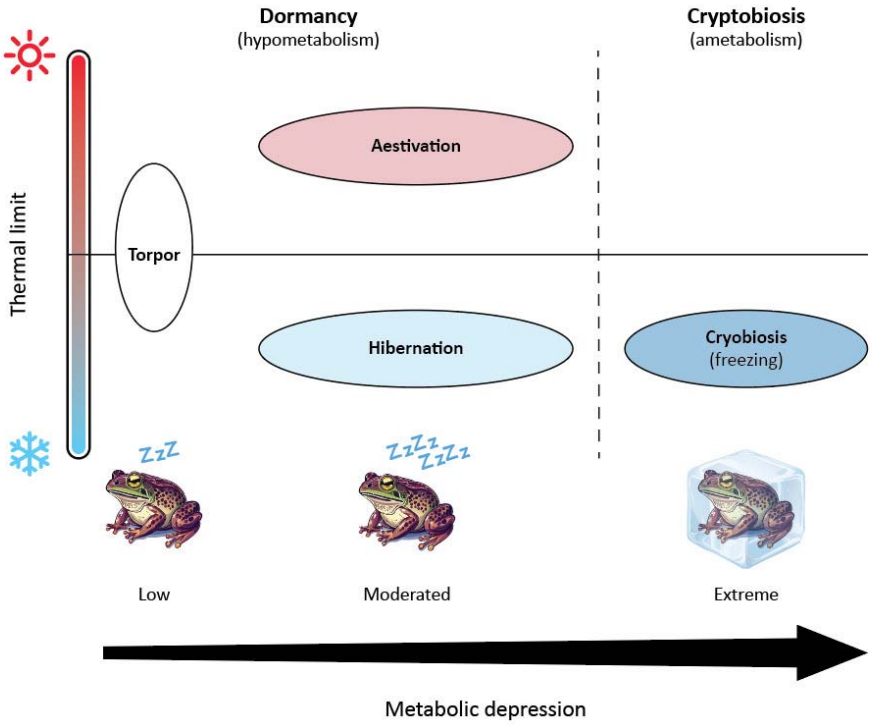
Figure 5. Average proportion of nodes that were inferred as presence as the most likely state for one of the two hypometabolic strategies, according to the joint ancestral state reconstruction for best tree. The temperature bar is the same as featured in Judd et al. (2024). Curves were smoothed using the smoothing splines method and shaded areas are derived from the confidence interval (95%). Dotted lines with the fish skeleton indicate major extinction events, the Triassic–Jurassic (Tr-J) 200 mya, and the Cretaceous–Paleogene (K–Pg) 66 mya.

Figure 6. Distribution range size comparison between species with probabilities of having a hypometabolic strategy calculated with the OC-SVM model. All amphibians (a, b, c) and only the species with data from the literature (d, e, f), with probabilities Wilcoxon test with Bonferroni correction. Significance codes for p-value are as follows (ns: > 0.05 / * ≤ 0.05 / ** ≤ 0.01 / ***: ≤ 0.001 / ****: ≤ 0.0001*).

Figure 7. Magnitude of climate change based on temporal differences for each climate parameter which are standardized using the local inter-annual standard deviation for that parameter in both periods of time, in this case t1 (1990-2020) and t2 (2070-2100). The higher the standardized local anomaly scores the larger the predicted changes in temperature and precipitation. The most vulnerable amphibian assemblages are highlighted with a red star.

854 **Figures**

855 **Figure 1**



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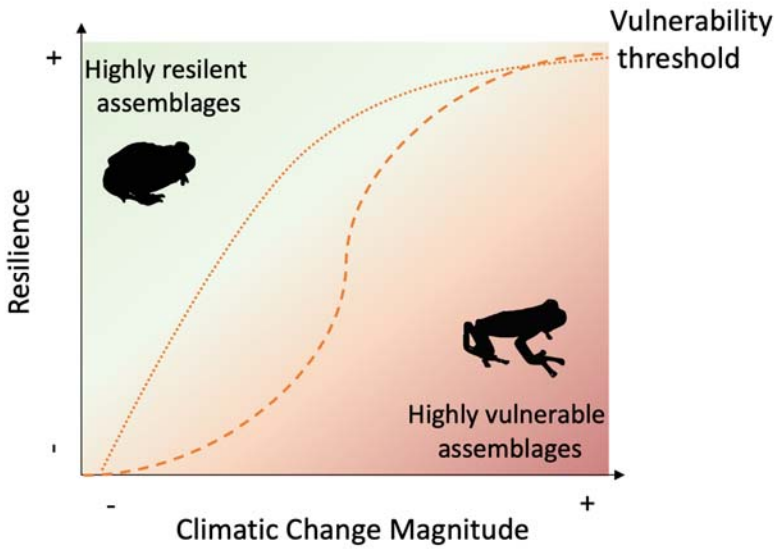
858 **Figure 2**



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863 **Figure 3**

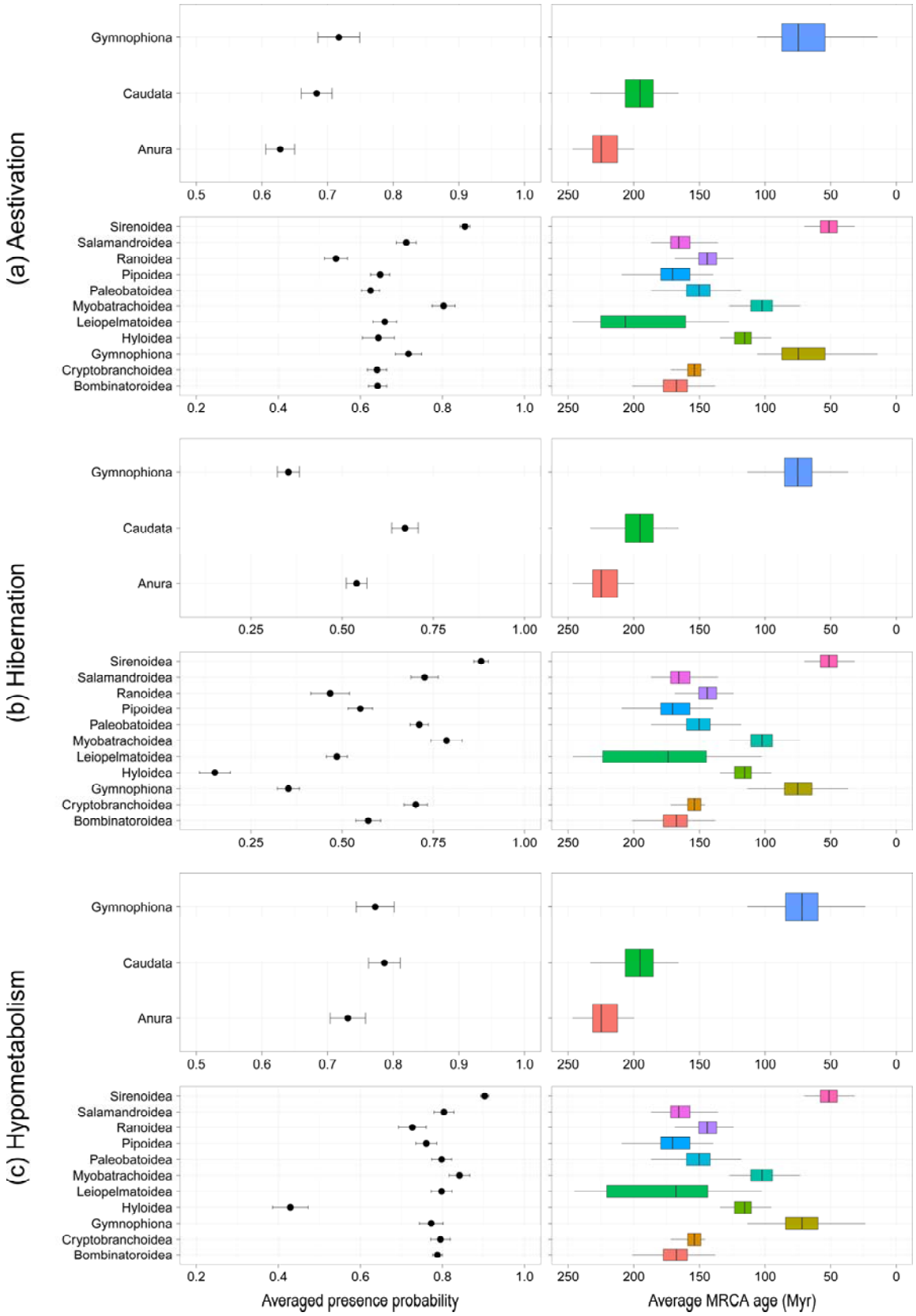

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Figure 4

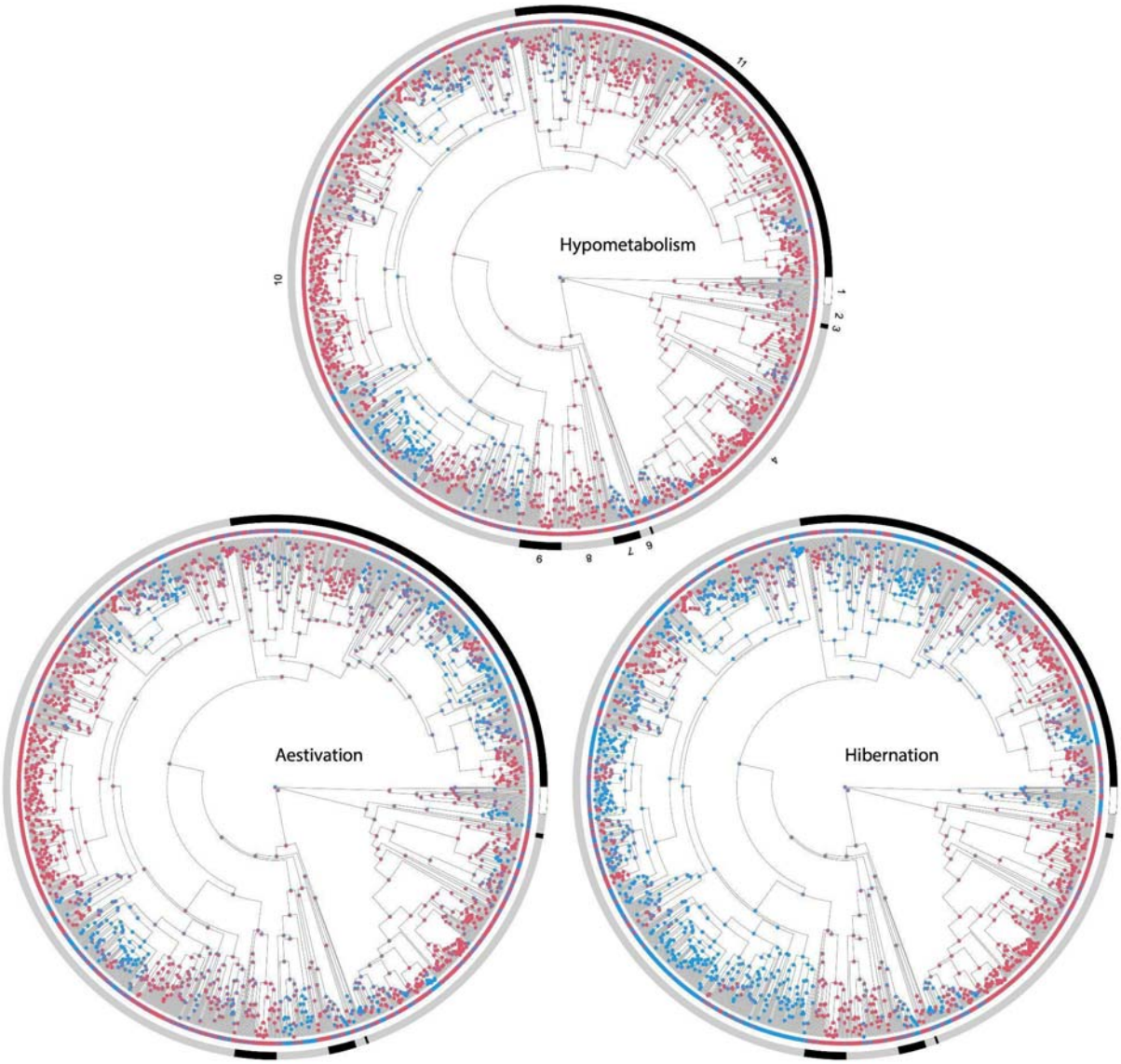


Figure 5

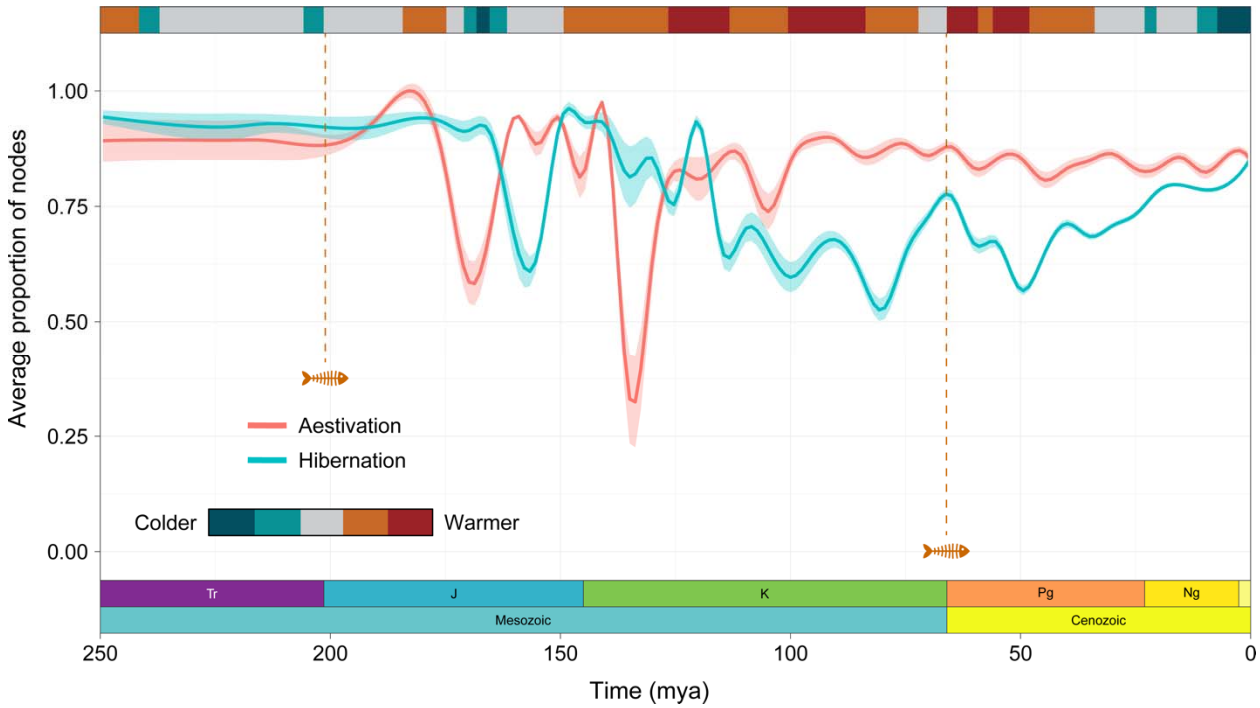

Average proportion of nodes
1.00
0.75
0.50
0.25
0.00
Aestivation
Hibernation
Colder
Warmer
Tr
J
K
Pg
Ng
Mesozoic
Cenozoic
250
200
150
100
50
0
Time (mya)

Figure 6

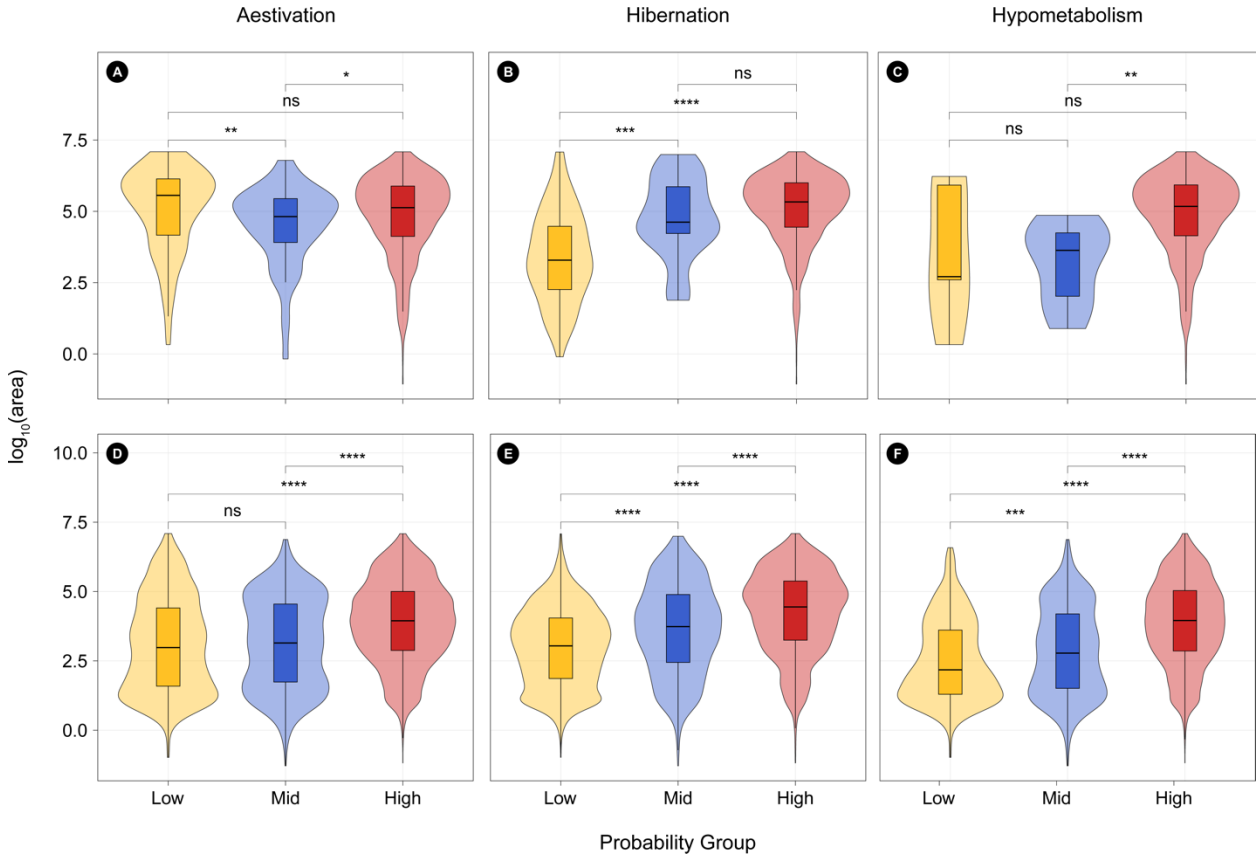
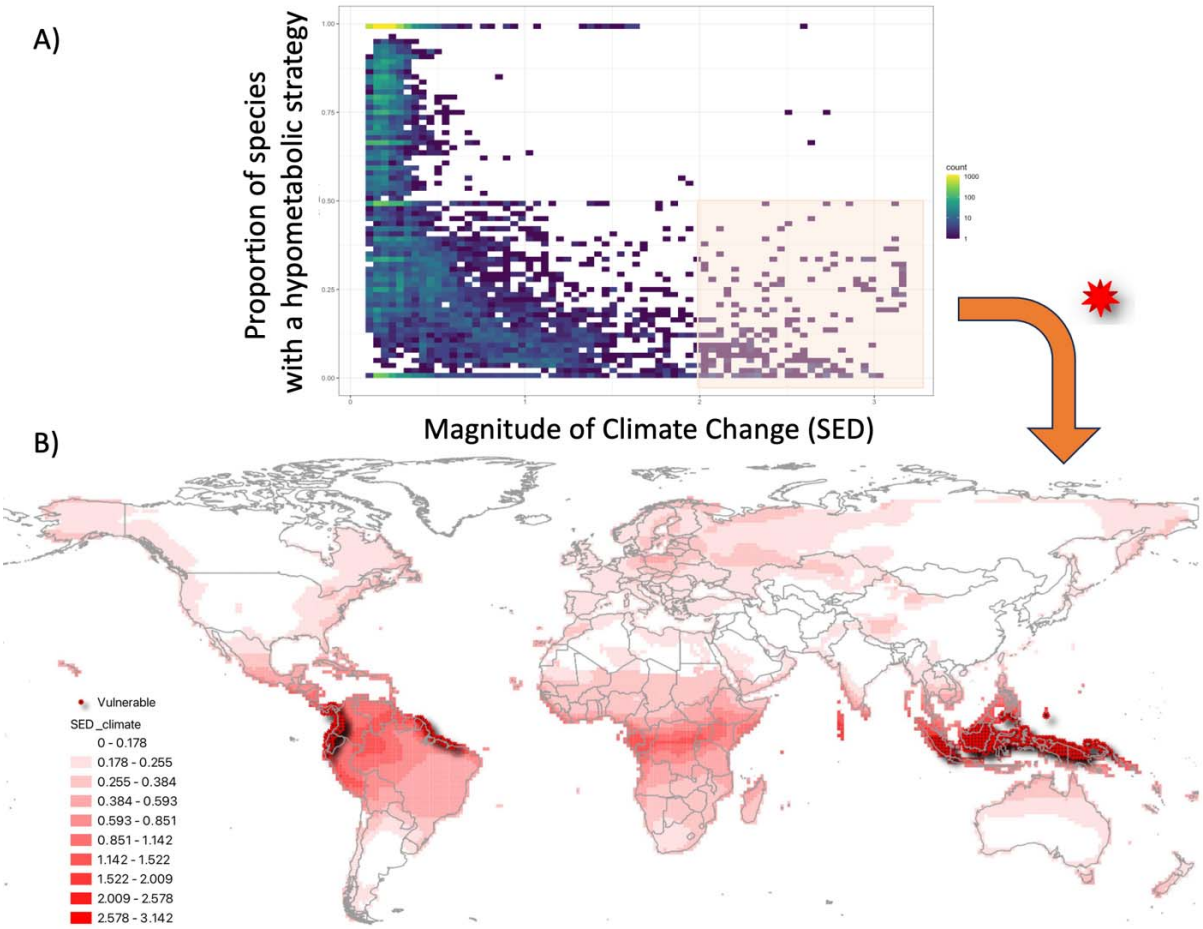

Aestivation
Hibernation
Hypometabolism
A
B
C
D
E
F
ns
*
**
***
****
log10(area)
7.5
5.0
2.5
0.0
10.0
Low
Mid
High
Probability Group

Figure 7


A)
Proportion of species with a hypometabolic strategy
Magnitude of Climate Change (SED)
count
1000
100
10
1
B)
Vulnerable
SED_climate
0 - 0.178
0.178 - 0.255
0.255 - 0.384
0.384 - 0.593
0.593 - 0.851
0.851 - 1.142
1.142 - 1.522
1.522 - 2.009
2.009 - 2.578
2.578 - 3.142