Title*: The Origins and Drivers of Neotropical Diversity*

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Abstract

The origins and evolution of the outstanding Neotropical biodiversity are still debated. A comprehensive understanding is hindered by the lack of deep-time comparative data across wide phylogenetic and ecological contexts. Here, we evaluate four evolutionary scenarios assuming different diversification trajectories and drivers of Neotropical diversification, and assess their variation across Neotropical regions and taxa. Our analysis of 150 phylogenies (12,512 species) of seed plants and tetrapods reveals that Neotropical diversity has mostly expanded through time (70% of the clades), while scenarios of saturated and declining diversity also account for 21% and 9% of Neotropical diversity, respectively. We identify five biogeographic areas that represent distinctive units of long-term Neotropical evolution (Pan-Amazonia, Dry Diagonal, Bahama-Antilles, Galapagos, and an 'elsewhere' region) and find that diversity dynamics do not differ across these areas, suggesting no geographic structure in long-term Neotropical diversification. In contrast, diversification dynamics differ substantially across taxa: plant diversity mostly expanded through time (88%), while a substantial fraction (43%) of tetrapod diversity accumulated at a slower pace or declined toward the present. These opposite evolutionary patterns may reflect different capacities for plants and tetrapods to cope with climate change, with potential implications for future adaptation and ecosystem resilience.

Keywords: Diversity dynamics | Andes | climate | macroevolution | plants | tetrapods | speciation | extinction.

Introduction

Comprising most of South America, Central America, tropical Mexico and the Caribbean Islands, the Neotropics are arguably the most biodiverse region on Earth, being home to at least a third of global biodiversity (1). This region not only includes the largest tropical rainforest, Amazonia, but also eight of the world's 34 biodiversity hotspots (2). The tropical Andes, in particular, is considered to be the most species-rich region in the world for amphibians, birds, and plants (3), while Mesoamerica and the Caribbean Islands are the richest regions for squamates, and Amazonia has been identified as the primary biogeographic source of Neotropical biodiversity (4). The underlying drivers of the extraordinary biodiversity of the Neotropics are hotly debated in evolutionary ecology, and the mechanisms behind the origin and maintenance of diversity remain elusive (5–9).

Most attempts to explain Neotropical diversity have typically relied on two evolutionary models. In the first, tropical regions are described as the "*cradle of diversity*", the centre of origin from which species appeared, radiated, and colonized other areas (10–12). In the other, tropical regions are considered a "*museum of diversity*", where species suffered relatively fewer environmental disturbances over evolutionary time, allowing ancient lineages to be preserved for millennia (6, 13, 14). Although not mutually exclusive (15), the cradle *vs.* museum hypotheses primarily assume evolutionary scenarios in which diversity expands through time without limits (16). However, expanding diversity models may be limited in their ability to explain the entirety of the diversification phenomenon in the Neotropics; for example, expanding diversity models cannot explain the occurrence of ancient and species-poor lineages in the Neotropics (17–19) or the decline of diversity observed in the Neotropical fossil record (20–22).

A more comprehensive view of Neotropical diversification should consider four alternative evolutionary trajectories of species richness to explain the accumulation of Neotropical diversity (**Fig. 1**):

Gradual expansions (Scenario 1): This scenario proposes that species richness accumulated gradually through time in the Neotropics until the present, due, for example, to constant speciation and extinction rates. The gradual increase model received substantial support in the early and recent literature (14, 23–27), and is generally associated with the long-term environmental stability and large extension of the tropical biome across the South American continent (6, 13).

Exponential expansions (Scenario 2): An exponential increase in diversity model asserts that species richness accumulated through pulses. Such a pattern can result, for example, from constant extinction and increasing speciation rates. Support for this model generally comes from studies suggesting that geological and climatic perturbations, mostly associated with the elevation of the Andes, promoted pulses of diversification (8, 9, 28). This diversity scenario is probably the most supported across Neotropical studies, although never quantified, with models of increasing speciation (29–38) more often put forward than models of decreasing extinction (18).

Saturated or asymptotic expansions (Scenario 3): A saturated diversity model postulates that species richness accumulated more slowly towards the present than in the past, reaching a diversity plateau. This can result from constant extinction and decreasing speciation for example, such that speciation and extinction rates become equal towards the present. Diversification decreases could be due to ecological limits (39), damped increases (40, 41), or abiotic fluctuations (42). Some studies support this model for the Neotropics, and they generally associate it with an early burst of diversification under favourable climatic

conditions, followed by decelerations due to global cooling, and dispersal constraints (25, 43–46).

Declines in diversity (Scenario 4): Waxing and waning dynamics characterize clades that decline in diversity after periods of expansion. In a declining dynamic, diversification rates also decrease towards the present, but differ from saturated diversity in that extinction exceeds speciation, and diversity is lost. Waxing and waning dynamics may seem unlikely in a tropical context, but evidence for tropical diversity declines has been found at the global scale (47–49) and at the Neotropical scale in the fossil record (20–22, 50–53). Fossil studies additionally suggest a link between decreases in Neotropical diversity and global temperature. For example, plant diversity inferred from fossil morphotypes reached its maximum levels during hyperthermal periods in the Eocene, and decreased sharply with subsequent cooling (20, 21, 54).

Despite an increasing number of evolutionary studies on Neotropical groups, today the prevalence of these alternative modes of species accumulation and diversification (Sc. 1–4, **Fig. 1**) at a continental scale has been difficult to tease apart empirically (**Question 1**). But such an assessment is required to understand the origin of Neotropical diversity and why the Neotropics are more diverse than other regions in the world. Illuminating the historical causes of Neotropical diversity further requires a closer look at the regional determinants of diversification. Are species richness trends (Sc. 1–4) related to particular environmental drivers (**Question 2**), geographic settings (**Question 3**), or taxonomic groups (**Question 4**) in the Neotropics?

Previous studies indicate that diversification rates might be structured geographically in the Neotropics (55, 56), with geography and climate being strong predictors of evolutionary rate variation (57, 58). For example, speciation may be elevated in regions subjected to environmental perturbations, such as orogenic activity (33, 35, 59–61).

However, this view is not without controversy, as some studies found similar diversification patterns among Neotropical regions (27, 62, 63).

Still, little is known about the variation in diversity dynamics (Sc. 1–4, **Fig. 1**) across Neotropical regions at large temporal scales. Most studies investigating spatial patterns of Neotropical diversification focus on long-term diversification dynamics of particular clades, for example, diversification trends of orchids across Neotropical regions (36), or cross-taxonomic patterns in shallow evolutionary time, i.e., present-day speciation rates (27, 58, 63). However, present-day speciation rates might not represent long-term diversification dynamics, specially when rates vary through time. Diversification could be higher in one region than in other without providing information on the underlying diversification trend. Under time-variable rate scenarios, analyzing diversity trends is crucial, but requires changing the focus from species to clades as units of the analyses. Unfortunately, there is still a lack of large-scale comparative data across wide phylogenetic and ecological contexts (59, 64). Given the long history and vast heterogeneity of the Neotropics, general insights can only be provided if long-term patterns and drivers of diversification are shared among Neotropical lineages.

This lack of knowledge may be also due to the challenge of differentiating between evolutionary scenarios based on birth-death models and phylogenies of extant species alone (65, 66). Recent studies have raised concerns on difficulties in identifying parameter values when working with birth-death models under rate variation scenarios (67, 68) , showing that speciation (birth, λ) and extinction (death, μ) rates sometimes cannot be inferred from molecular phylogenies (69). This calls for *(i)* analysing 'congruent' models with potentially markedly different diversification dynamics but equal likelihood for any empirical tree (69), or *(ii)* implementing a solid hypothesis-driven approach, in which a small number of alternative hypotheses about the underlying mechanism are compared against data (70).

147 Based on an unparalleled comparative phylogenetic dataset containing 150 well-
148 sampled species-level molecular phylogenies and 12,512 extant species, we evaluate the
149 prevalence of macroevolutionary scenarios 1–4 (**Fig. 1**) as general explanations for
150 Neotropical diversification at a continental scale (**Q1**), their drivers **(Q2),** and their variation
151 across biogeographic units **(Q3)** and taxonomies **(Q4)**. To address Q3, we previously identify
152 evolutionary arenas of Neotropical diversification suitable for comparison. Depending on the
153 taxonomic source (1, 71), our dataset represents ~47–60% of all described Neotropical
154 tetrapods and ~5–7% of the known Neotropical plant diversity.
155

156 **Results**

157 ***Neotropical phylogenetic dataset***

158 We constructed a dataset of 150 time-calibrated clades of Neotropical tetrapods and plants
159 derived from densely-sampled molecular phylogenies (**Fig. 2** and *Appendix 1*). The dataset
160 includes a total of 12,512 species, consisting of 6,222 species of plants, including
161 gymnosperms and angiosperms (66 clades, representing 5–7% of the described Neotropical
162 seed plants; Supporting Information Table S1); 922 mammal species (12 clades, 51–77% of
163 the Neotropical mammals; Table S2); 2,216 bird species (32 clades, 47–59% of the
164 Neotropical birds; Table S3); 1,148 squamate species (24 clades, 30–33% of the Neotropical
165 squamates; Table S4); and 2,004 amphibian species (16 clades, 58–69% of the Neotropical
166 amphibian diversity; Table S5). Each clade in our dataset includes 7 to 789 species
167 (mean=83.4), with 53% of the phylogenies including more than 50% of the described
168 taxonomic diversity (sampling fraction mean=57%). Clade ages range from 0.5 to 88.5 Myr
169 (mean=29.9; **Fig. 2**, Fig. S1). Our dataset triples the data presented in previous meta-analyses
170 of the Neotropics in terms of number of species, for example, 214 clades and 4,450 species in
171 (4), and quadruples it in terms of sampling, with 20.8 species per tree in (4).

Estimating the tempo and mode of Neotropical diversification

a) Diversification trends based on traditional diversification rates

To understand the tempo (**Q1)** and drivers of Neotropical diversification (**Q2**), we compared the fit of birth-death models applied to 150 phylogenies, including models where diversification rates are constant, vary through time, vary as a function of past global temperatures, or vary according to past Andean elevation (see *Methods*). When only models with constant diversification and time-varying rates were considered, constant models best fit 67% of the phylogenies (101 clades), (Table S6). In the remaining 49 trees, we detected variation in diversification rates. Speciation decreased towards the present in 28 trees (57%), increased in 12, and remained constant (being extinction time-variable) in 9, although the proportions varied between lineages (**Fig. 3**). The proportion of clades that evolved at constant diversification decreased to 50.6% (76 clades) when the comparison included more complex environmental models (**Fig. 4;** *Appendix 2;* Table S7, S8). The proportion of time-variable models also increased to 74 trees.

The empirical support for the main species richness dynamics from the 150 phylogenies was as follows: gradual expansions (Sc. 1, constant diversification) were detected in 101 to 76 phylogenies if environmental models were considered; exponential expansions (Sc. 2, increases in diversification) were detected in 20–30 clades; and saturated expansions and declining dynamics (Sc. 3 and 4, diversification decreases) were supported in 24–31 and 5–9 clades, respectively (**Table 1** and **Fig. 4**). Diversification trends remained similar when small (<20 species) or poorly sampled (<20% of the species sampled) phylogenies were excluded from the analyses (99 and 137 trees remaining, respectively), although the proportion of constant diversification models decreased in all cases (55–35%; Fig. S2).

197 Rate variation was inferred from models that are able to capture the dependency of
198 speciation and/or extinction rates over time (time-dependent models) or over an
199 environmental variable (either temperature- or uplift-dependent models). Among them,
200 temperature-dependent models explained diversification in 40 phylogenies (26.7%). Time-
201 dependent models best fit 17 clades (11%). Uplift-dependent models explained another 11%
202 (**Fig. 5**, Table S7). The relative support for time-, temperature- and uplift-dependent models
203 remained similar regardless of whether we compared the fit of the best or second-best models
204 (defined based on ΔAIC values; Fig. S3), although overall support for constant-rate scenarios
205 decreased in the latter. Results also remained stable regardless of the paleo-temperature curve
206 (72–74) considered for the analyses (see SI; Fig. S4).
207
208 *b) Diversification trends based on pulled diversification rates*
209 To gain further insights in Neotropical diversification (**Q1**), we explored congruent
210 diversification models defined in terms of pulled diversification rates (PDR, r_p), (69, 75).
211 These analyses recovered consistent diversification trends with those found above: 63% of
212 the phylogenies (95 clades) better fit constant pulled models (**Fig. 3**; Table S9). Meanwhile in
213 37% of the phylogenies (55 clades) we found variation in PDR through time. Diversification
214 trends remained similar when small (<20 species) or poorly sampled (<20% of the species
215 sampled) phylogenies were excluded from the analyses (Fig. S5). We also detected negative
216 pulled present-day extinction rates $\mu_p(0)$ in most of the phylogenies (51 clades, 92%) in
217 which PDR varied through time, suggesting that speciation was decreasing. Based on PDR,
218 we could only detect constant diversification (Sc. 1) or decreases in speciation, and thus the
219 combined support for Sc. 2, 3 and 4 (**Table 1**).
220
221 ***Neotropical bioregionalization***

To examine the spatial variation of diversification dynamics within the Neotropics (**Q3**), we first had to identify geographic units of long-term Neotropical evolution suitable for comparison. We found that most clades in our study were distributed in most Neotropical WWF ecoregions (Table S10), suggesting that species presence-absence data might be of limited use for delimiting geographic units at the macroevolutionary scale of this study. In contrast, based on clades' abundance patterns, we identified 5 clusters of regional assemblages that represent long-term clade endemism (**Fig. 6a, b;** Fig. S6; Table S11): cluster 1 (including the Amazonia, Central Andes, Chocó, Guiana Shield, Mesoamerica, and Northern Andes), cluster 2 (Atlantic Forest, Caatinga, Cerrado, Chaco, and temperate South America), cluster 3 (Caribbean), cluster 4 ("elsewhere" region), and cluster 5 (Galapagos). An alternative clustering (Fig. S7) separating Mesoamerica from cluster 1, and the Chaco and temperate South America from cluster 2, received lower support (Fig. S6).

Variation of diversification dynamics across taxa, environmental drivers, and biogeographic units.

We evaluated the prevalence of macroevolutionary scenarios 1–4 (**Fig. 1**) across environmental drivers **(Q2),** biogeographic units **(Q3)** and taxonomies **(Q4)** (see *Methods*). **Table 2** summarize all the results. We found that species richness dynamics were related to particular environmental drivers (p=0.003; **Q2**). Pairwise comparisons indicated that temperature-dependent models tended to best fit clades experiencing saturating (p=0.049) and declining (p=0.05) diversity dynamics. Meanwhile, uplift- and time-dependent models tended to best fit clades with exponentially increasing diversity (p=0.03) (**Fig. 5c**).

In contrast, there is no evidence to suggest that species richness dynamics are related to particular geographic conditions when considering the whole dataset (**Fig. 6c–f; Q3**). Results of the Fisher's exact test show no significant differences in the proportion of clades

247 experiencing gradual expansions, exponential expansions, saturation or declining diversity
248 across biogeographic units (p=0.45) or elevation ranges (p=0.062). We obtained similar
249 results when the montane category was analyzed separately (p=0.5, Fig. S8). Analyses based
250 on PDR produce the same results, with no differences in the proportion of clades
251 experiencing constant (i.e., expanding diversity dynamics) or declining speciation trends
252 across biogeographic units (p=0.25), or elevation ranges (p=0.062), even when the montane
253 category was analyzed separately (p=0.839). Estimates of net diversification rates (rather than
254 diversity trajectories) derived from the constant diversification model did not differ across
255 biogeographic units (χ^2=5.05, p=0.17) or altitudinal ranges (χ^2=2.20; p=0.332) either.
256 Speciation rates did not differ between biogeographic units (χ^2=4.1, p=0.25), but did vary
257 across altitudinal ranges (χ^2=6.9, p=0.03). Speciation rates were significantly higher across
258 highland taxa (**Fig. 6**). In addition, PDR did not differ across biogeographic units (χ^2=6.7;
259 p=0.083) or elevations (χ^2=0.28; p=0.87).

260 Finally, diversity trajectories (Sc. 1–4) differed across taxonomic groups (p<0.0001,
261 Fisher's exact test; **Q4**). Pairwise comparisons indicated that plants differed significantly
262 from birds in the proportion of gradual (p<0.02), exponential (p<0.02) and saturated
263 (p<0.0001) increase models after correcting for multiple comparisons. Birds also differed
264 from amphibians in the proportion of saturated and exponential increases (p<0.02). Plants
265 differed from squamates in the proportion of exponential (p<0.0006) and saturated (p<0.008)
266 increases (**Fig. 4c**). Net diversification rates were also significantly lower for Neotropical
267 ectotherm tetrapods than for endotherms and plants (Kruskal-Wallis chi-squared: χ^2=36.7,
268 p<0.0001) (**Fig. 7**). We also found statistically significant differences in speciation rates
269 across groups (χ^2=60.8, p<0.0001): plants showed higher speciation rates than endotherms,
270 the latter, in turn, with higher speciation rates than ectotherms.

The number of species per phylogeny differed between model categories (phylogenetic ANOVA: F=10.9, p=0.002). Clades fitting gradual expansion models tended to have less species than clades fitting exponential (p=0.006) and declining (p=0.03) dynamics (Fig. S9). Taxon sampling, however, did not differ significantly (F=4.5, p=0.53). Crown age differed between model categories, being on average younger for gradual scenarios than for exponential (p=0.03) and declining (p=0.03) dynamics.

Discussion

Diversification dynamics

Neotropical biodiversity has long been considered as being in expansion through time due to high rates of speciation and/or low rates of extinction, either through a long-term (13, 27) or a recent (71) cradle and museum of diversity. Yet, the prevalence of the expanding dynamic was never quantified. The higher support for the expanding diversity trend found here align with these ideas. The majority of Neotropical clades (between 80% to 70%, if environmental models are considered) displayed expanding diversity dynamics through time (**Fig. 4**; **Table 1**). Most of these clades experienced a gradual accumulation of lineages (Sc. 1; between 67–50%), and a lower proportion (14–16%) expanded exponentially (Sc. 2), thus diversity accumulation accelerated recently. Results based on PDR support these conclusions, with the largest proportion of clades expanding diversity (63%) due to gradual increases (Sc. 1; **Fig. 3**).

Our results also provide evidence that cradle/museum models are not sufficient to explain Neotropical diversity. Based on traditional diversification rates, 16–21% of the Neotropical clades, mostly tetrapod clades, underwent a decay in diversification, hence a slower accumulation of diversity toward the present (Sc. 3). While a pervasive pattern of slowdowns in speciation has been described at various geographic and taxonomic scales, e.g.,

296 (41, 43, 76, 77), Neotropical tetrapod diversity levels have only rarely been perceived as
297 saturated (25, 27, 43, 46). Furthermore, waxing-and-waning dynamics (Sc. 4) also
298 characterize the evolution of 3–9% of the Neotropical diversity, consistent with
299 paleontological studies (20–22). We found that the species richness of five plant and eight
300 tetrapod clades decined toward the present (e.g., *Sideroxylon* [Sapotaceae], *Guatteria*
301 [Annonaceae], caviomorph rodents, Thraupidae birds, or Lophyohylinae [Hylidae] frogs).
302 This proportion might seem minor but is noteworthy when compared with the low support for
303 this model found in the Neotropical literature, which could be explained by the difficulties in
304 inferring negative diversification rates based on molecular phylogenies (66). Inferring
305 diversity declines is challenging, and often requires accounting for among-clade rate
306 heterogeneity (78). As shown here, incorporating environmental evidence could also help
307 identifying this pattern, increasing support for this scenario relative to the comparisons
308 without these models (**Fig. 4**).

309 Support for decreasing diversification through time was larger when PDR were
310 considered; 34% of the clades showed slowdowns in speciation (**Fig. 3**). Based on PDR,
311 however, we cannot infer if declines of speciation were accompanied by constant, declining
312 or increasing extinction (69), and thus determine the relative support for Sc. 2–4. If declines
313 in speciation were accompanied by larger extinction declines, it would be still possible to
314 recover expanding dynamics (Sc. 1, 2), but in most other cases, they would lead to declines in
315 diversification (Sc. 3, 4). The limited interpretability of PDR prevents the extraction of
316 further conclusions based on these rates (70).

317 Still, our study illustrates the robustness of the diversification trend in the Neotropics
318 to different modelling approaches. Despite parameter values varying substantially for some
319 trees between the traditional and PDR methods (Table S7, S9, **Fig. 6 d, e**), a pattern also

described in recent studies (70), our analyses support the generality of the expanding diversity trend for most clades in the Neotropics (**Fig. 4**).

Sampling artefacts and reliability of these results

The pattern found in this study appears to be robust to sampling artefacts. Clade age and tree size can partially explain the better fit of the phylogenies to the constant diversification model, thus the gradually-expanding trend (Sc. 1), although these factors do not explain the relative support between time-varying increasing (Sc. 2) versus decreasing (Sc. 3, 4) scenarios (Fig. S9). Constant diversification prevails among recently-originated and species-poor clades, which may reflect that these clades had less time to experience changes in diversification. Alternatively, it has been suggested that the power of birth–death models to detect rate variation can decrease with the number of species in a phylogeny (79), suggesting that tree size could hinder the finding of rate-variable patterns. Still, the largest support for the expanding diversity trend persisted (72–60% of clades) after excluding small trees from the analyses (<20 species; Fig. S2). Then, the relative support for the exponentially expanding scenario (Sc. 2) increased at the expense of the gradually expanding scenario (Sc. 1), strengthening the conclusion of the generality of the expanding trend in the Neotropics.

It is also known that incomplete taxon sampling has the effect of flattening out lineages-through-time plots towards the present and artificially increasing the detection of diversification slowdowns (80). If this artefact affected our results, we would expect to see that weakly-sampled phylogenies tend to fit saturated diversity models (Sc. 3). Instead, we found that sampling fraction did not differ between lineages fitting saturated and expanding diversity models (Fig. S9), and the proportion of clades fitting saturated models increased (17–22%) after excluding poorly-sampled phylogenies (<20% of the species sampled; Fig. S2).

345

346 ***Taxon-specific patterns and drivers of diversification***

347 Our study revealed contrasting evolutionary patterns for plants and tetrapods in the
348 Neotropics (**Fig. 4**). Diversity expansions (Sc. 1, 2) were more frequent in plants (~88%, 59
349 clades) than in tetrapods (~57%, 48 clades). In contrast, asymptotic increases (Sc. 3) were
350 more frequently detected in tetrapods (33%, 28 clades) than in plants (4.5%, 3 clades;
351 *Tynanthus* [Bignoniaceae], Chamaedoreae [Arecaceae], and Protieae [Burseraceae]). The
352 study of PDR did not help to confirm/reject these conclusions. This contrasting evolutionary
353 pattern may result from differential responses of plants and tetrapods to environmental
354 changes (**Fig. 5**).

355 Global temperature change during the Cenozoic is found to be the main driver behind
356 diversification slowdowns (Sc. 3) and declines (Sc. 4), especially for endotherms (**Fig. 5**).
357 The positive correlation between diversification and past temperature in our temperature-
358 dependent models indicates these groups diversified more during warm periods, such as the
359 Eocene or the middle Miocene, and diversification decreased during cool periods. This result
360 is in agreement with a recent study showing a negative effect of climate cooling (and a
361 positive effect of Andean orogeny; see below) on Neotropical tetrapod diversification based
362 on spatially explicit simulation models of diversification (81). Only the New World monkeys
363 (Platyrrhini), diversified more as temperature dropped. This could reflect the role of
364 Quaternary events on primate speciation (9), and/or be an artefact of taxonomic over-splitting
365 in this clade (82). In contrast, for the few plant clades influenced by temperature changes,
366 diversification increased during Neogene cooling (i.e., a negative correlation between
367 diversification and temperature).

368 Diversification slowdowns and saturating dynamics have often been interpreted as the
369 signal of ecological limits on the number of species within a clade, implying that diversity is

370 bounded (39, 83). Among the tetrapod phylogenies supporting diversification slowdowns,
371 time-dependent models explain only 3% of them (4 phylogenies; **Fig. 5**, Table S7),
372 suggesting that ecological limits do not play an important role. Time-dependent models with
373 decreasing speciation have been suggested to be a good approximation of diversity-dependent
374 diversification, whereby speciation rates decline as species accumulate (84, 85). In fact,
375 recent studies show time- and diversity-dependent models are not distinguishable based on
376 extant phylogenies (86). Our results lend support to an alternative explanation for
377 diversification slowdowns: the idea that tetrapods fail to keep pace with a changing
378 environment (42, 87). According to the Metabolic Theory of Biodiversity, high temperatures
379 can increase enzymatic activity, generation times and mutation rates (88), which may in turn
380 affect diversification (89). Climate cooling could also decrease global productivity, resource
381 availability, population sizes (90) or even species interactions (91).

382 Diversification increases are attributed to different factors in our study, including the
383 uplift of the Andes and temperature cooling (**Fig. 5)**. The Andean orogeny mostly impacted
384 tetrapod diversification, especially ectotherms. Diversification of some lineages increased as
385 the Andes rose, including Andean-centred lineages such as Liolaemidae lizards, but also
386 others predominantly distributed outside the Andes, such as Leptodactylidae frogs. Sustained
387 diversification in the context of Andean orogeny, both into and out of the Andean region,
388 could be explained by increasing thermal and environmental gradients, from the equatorial
389 areas to Patagonia or from west-east (92, 93). Other possible correlates include changes in
390 elevational distributions of lineages (94, 95), or recurrent migrations (25, 33).

391 In contrast, plant diversity expansions were primarily associated with temperature
392 cooling and with time, where the latter represents a null hypothesis; the better fit of a time-
393 dependent model, in comparison to environmental models, is generally indicative of factors
394 not being investigated here (85). Many of the plant lineages fitting time-dependent models

395 represent textbook examples of ongoing radiations; e.g., centropogonids (35), *Lupinus* (34),
396 or *Inga* (96) whose diversification has been associated with biotic drivers, such as the
397 evolution of key adaptations or pollination syndromes. These factors are taxon-specific and
398 were not evaluated in this study, where we focused on global phenomena. Our results
399 therefore add support to the role of environmental and biotic factors as non-mutually
400 exclusive drivers of macroevolutionary changes on Neotropical plants.

401 Our plant dataset represents just a fraction (> 6,000 species, 66 clades; ~7% of the
402 total species) of the vast diversity described in the region. As such, future investigations
403 would be necessary to confirm the generality of the expanding trend for plants. Still, our
404 results suggest that Cenozoic environmental changes stimulated plant diversification, while
405 they drove diversification slowdowns for some tetrapods, with plants adapting to
406 deteriorating climatic conditions. Higher (mean) speciation rates in plants than in tetrapods
407 (**Fig. 7**) could have provided plant lineages more opportunities for adaptation to new
408 environments. Greater dispersal abilities in plants (4, 97) may also explain this pattern.

409 In our study, the relative support for time-, temperature- and uplift-dependent models
410 remained stable to AIC variations (Fig. S3). Model support also remained stable regardless of
411 the paleo-temperature curve considered for the analyses (Fig. S4). Still, the power of AIC
412 values to differentiate model support has been recently questioned (42, 69). As such, these
413 results should be interpreted with caution. The use of an hypothesis-driven framework has
414 been suggested as a potential solution to alleviate the problem of non-identifiability of
415 diversification parameters, by setting up explicit prior assumptions and delimiting the
416 potential parameter space (69, 70, 98). Here, we do not evaluate every possible factor that
417 could potentially explain Neotropical biodiversity, but only confront scenarios capturing
418 well-established hypotheses on Neotropical diversification. We focus on the role of the
419 Andes and climatic oscillations because they have previously been pinpointed as essential for

420 explaining Neotropical biodiversity (8, 57, 81). Thus, our interest is to explore which of these
421 factors best explains the data compiled, although other factors could have played a role.
422
423 ***The geographical structure of Neotropical diversification***
424 Understanding the spatial variation of biodiversity dynamics is key to understanding the
425 determinants of the exceptional diversity of the Neotropics. The first step towards this is the
426 identification of evolutionary arenas of Neotropical diversification.
427 Conventional bioregionalizations schemes, such as biomes (99), ecoregions (100) or
428 other pre-defined biogeographic units (4, 101, 102), could represent evolutionary arenas of
429 diversification suitable for comparison. Often, these schemes have been shown to be useful
430 for categorizing actual species ranges, but our study shows they are less appropriate for
431 examining lineage endemism at the macroevolutionary scale (i.e., above the species level);
432 most clades in our study are distributed in most Neotropical ecoregions (Table S10). The lack
433 of a clear geographical structure for taxa of higher rank could be explained by the fact that
434 conventional bioregionalizations generally represent categorizations based on data on the
435 contemporary distribution of species without explicitly considering ancestral distributions or
436 the relationships among species (103, 104).
437 We propose here an alternative bioregionalization scheme of the Neotropical region
438 that accounts for long-term regional assemblages at macroevolutionary scales (**Fig. 6**). We
439 identify five biogeographic units that represent macroregions of shared evolutionary history
440 for Neotropical clades – macroregions where independent Neotropical radiations occurred
441 over millions of years of biotic evolution. These regions are defined in terms of species-
442 richness patterns within clades (Table S10), showing that species-rich clades in the
443 Amazonia, also tend to be species rich in the Andes, Chocó, Guiana Shield, and Mesoamerica
444 (biogeographic cluster 1), without excluding that some species within these clades occur in

445 other regions. Meanwhile, clades that are species-rich in the Atlantic Forest tend to be rich in
446 the Caatinga, Cerrado, Chaco, and temperate South America (cluster 2). As such, these
447 clusters form distinctive units of Neotropical evolution and represent long-term clade
448 endemism.

449 The biogeographic cluster 1 roughly corresponds with a broad 'pan-Amazonian'
450 region that relied on the ancient Amazon Craton (8). Cluster 2 broadly groups different
451 formations of the area known as the 'Dry Diagonal' (105, 106), which are geologically
452 younger, dating from the Miocene (107–109), although lineage crown ages do not differ
453 between these regions (**Fig. 6**). Clusters 1 and 2 include regions identified as transition zones
454 in previous studies – Mesoamerica and temperate South America, respectively (110). Our
455 analyses merged these regions with the core area with which it showed the greatest affinity,
456 although other less supported classification schemes separate transition regions into
457 individual clusters (Fig. S6, S7).

458 When examining the connection between geography and long-term diversity
459 dynamics, we did not find evidence to reject the null hypothesis of equal diversification, with
460 similar diversity dynamics (Sc. 1–4) found across the biogeographic units of Neotropical
461 evolution identified here (**Fig. 6, Table 2**). We obtained the same result when Mesoamerica
462 and temperate South America transition zones were analysed separately (Fig. S7). In
463 addition, we did not find differences in diversification dynamics between elevational ranges.
464 These results were consistent whether we analysed net diversification rates or their derived
465 diversity trends (Sc. 1–4). In the former, Neotropical lineages distributed in different
466 elevations did differ in their speciation rates, as found in previous studies: speciation
467 increased with altitude (34, 46, 58, 59, 111). Elevated speciation rates might result from
468 ecological opportunities on newly formed high-altitude environments, or those newly
469 exposed after periods of cooling (112–114). However, elevated speciation rates were also

accompanied by elevated extinction in these habitats, hence net diversification remains comparable. The hypothesis of comparable diversification was also supported when comparing PDR (**Fig. 6**).

The lack of any clear geographic structure of diversification reveals that the evolutionary forces driving diversity in the Neotropics acted at a continental scale when evaluated over tens of millions of years. Despite the suggestion that present-day diversification rates are structured geographically in the Neotropics (55, 57), this variation might not represent long-term evolutionary dynamics. Evolutionary time and extinction could have eventually acted as levelling agents of diversification across the Neotropics over time. Geographic diversification may vary within taxonomic groups, though small sample sizes prevent us from drawing any firm conclusions on this. Future studies evaluating this question on a greater sample of Neotropical clades could help to assess the generality of this pattern.

Conclusion

The study of macroevolution using phylogenies has never been more exciting and promising than today (85, 115). The successful development of powerful analytical tools, in conjunction with the rapid increase in the availability of biological data, will allow us to disentangle complex diversification histories. We still face important limitations in data availability and methodological shortcomings, e.g., (68, 69, 86), but by acknowledging them we can better target our joint efforts as a scientific community (116). We hope that our approach will encourage movement in that direction, and that it will provide interesting perspectives for future investigations in the Neotropics and other regions.

This study represents a quantitative assessment of the prevailing macroevolutionary dynamics in the Neotropics, and their spatial variation, at a continental scale. Whether the pattern found here, i.e., a predominant expansion of diversity over time, can contribute to

495 explain why the Neotropics have more species than other regions in the world remains to be
496 evaluated based on comparative data from other regions (117). Such a comparison could
497 reveal contrasted diversity trajectories in different continents, and help to elucidate the
498 association between current diversity levels and long-term diversity dynamics. By identifying
499 distinct evolutionary arenas of long-term Neotropical diversification, we hope our study will
500 also stimulate research to examine hitherto investigated macroecological questions in shallow
501 evolutionary times, from a deep-time and clade-level macroevolutionary perspective.

502 Our results also have implications for discussing the future of biodiversity in the
503 context of current environmental changes and human-induced extinction (118). If half of the
504 lineages in our study expanded diversity through time following a constant diversification
505 mode, it may take tens of millions of years for biodiversity to reach its pre-extinction level.

506

507 **Methods**

508 ***Data compilation***

509 Neotropical clades, representing independent radiations in the Neotropics, were pulled from
510 large-scale time-calibrated phylogenies of frogs and toads (95), salamanders (119, 120),
511 lizards and snakes (121), birds (56) (including only species for which genetic data was
512 available), mammals (122, 123), and plants (124). To identify independent Neotropical
513 radiations, species in these large-scale phylogenies were coded as distributed in the
514 Neotropics – delimited by the World Wide Fund for Nature WWF (100) – or elsewhere using
515 the R package *speciesgeocodeR* 1.0-4 (125), and their geographical ranges extracted from the
516 Global Biodiversity Information Facility "GBIF" (https://www.gbif.org/), the PanTHERIA
517 database (https://omictools.com/pantheria-tool), BirdLife (http://www.birdlife.org) and eBird
518 (http://ebird.org/content/ebird), all accessed in 2018, in a procedure similar to (71). Next, we
519 pruned the trees to extract the most inclusive clades that contained at least 80% Neotropical

species, as previously defined. This procedure ensures that the diversification signal pertains to the Neotropics. In addition, phylogenies of particular lineages not represented in the global trees (or with improved taxon sampling) were obtained from published studies or reconstructed *de novo* in this study (for caviomorph rodents, including 199 species; see SI). In the case of plants and mammals, most phylogenies were obtained from individual studies, given the low taxon sampling of the plant and mammal large-scale trees. However, whenever possible, we extracted phylogenies from a single dated tree rather than performing a meta-analysis of individual trees from different sources (8, 126), such that divergence times would be comparable. The resulting independent Neotropical radiations could represent clades of different taxonomic ranks. We did not perform any specific selection on tree size, crown age, or sampling fraction, but tested the effect of these factors on our results.

Estimating the tempo and mode of Neotropical diversification

a) Diversification trends based on traditional diversification rates

We compared a series of birth-death diversification models estimating speciation (λ) and extinction (μ) rates for each of the 150 phylogenies with the R package *RPANDA* 1.9 (127). To make these results comparable with those derived from pulled diversification rates below, we followed a sequential approach by including models of increasing complexity. We first fitted a constant-rate birth–death model and compared it with a set of three models in which speciation and/or extinction vary according to time (78): $\lambda(t)$ and $\mu(t)$. For time-dependent models, we measured rate variation for speciation and extinction rates with the parameters α and β, respectively: α and $\beta > 0$ reflect decreasing speciation and extinction toward the present, respectively, while α and $\beta < 0$ indicate the opposite, increasing speciation and extinction toward the present.

We further compared constant and time-dependent models, described above, with a set of environment-dependent diversification models that quantify the effect of environmental variables on diversification (Q2) (128). Environmental models extend time-dependent models to account for potential dependencies between diversification and measured environmental variables, for example, speciation and extinction rates can vary through time and both can be influenced by environmental variables. We focus here on mean global temperatures and Andean uplift. Climate change is probably one of the most important abiotic factors affecting biodiversity, of which global fluctuation in temperatures is the main component (129). In addition, the orogenesis of the Andes caused dramatic modifications in Neotropical landscapes and has become paradigmatic for explaining Neotropical biodiversity (8). Temperature variations during the Cenozoic were obtained from global compilations of deep-sea oxygen isotope ratios ($\delta^{18}O$) (72, 129), but we also analysed other curves to assess the impact of paleotemperature uncertainty on our results (see SI). For Andean paleo-elevations we retrieved a generalized model of the paleo-elevation history of the tropical Andes, compiled from several studies ((35) and references therein). The elevation of the Andes could have indirectly impacted the diversification of non-Andean groups. We thus applied uplift models to all clades in our study.

We fitted three environmental models in which speciation and/or extinction vary continuously with temperature changes ($\lambda[T]$ and $\mu[T]$), and three others with the elevation of the Andes ($\lambda[A]$ and $\mu[A]$). In this case, λ_0 (μ_0) is the expected speciation (extinction) rate under a temperature of 0°C (or a paleo-elevation of 0 m for the uplift models). We also analysed whether the speciation (α) and extinction (β) dependency were positive or negative. For temperature models, $\alpha(\beta) > 0$ reflects increasing speciation (extinction) with increasing temperatures, and conversely. For the uplift models, $\alpha(\beta) > 0$ reflects increasing speciation (extinction) with increasing Andean elevations, and conversely. We accounted for missing

species for each clade in the form of sampling fraction *(ρ)* (78) and assessed the strength of support of the models by computing Akaike information criterion (AICc), ΔAICc, and Akaike weights (AICω) to select the best-fit model. We derived diversity dynamics (Sc.1–4) based on the inferred diversification trends according to **Fig. 1**.

b) Diversification trends based on pulled diversification rates

To gain further insights in Neotropical diversification (Q1), we explored congruent diversification models defined in terms of pulled diversification rates (PDR, r_p), and pulled extinction rates (PER, μ_p) (69, 75). Two models are congruent if they have the same r_p and the same product $\rho\lambda_0$, in which ρ is the sampling fraction and $\lambda_0 = \lambda(0)$. r_p is equal to the net diversification rate ($r = \lambda - \mu$) whenever λ is constant in time ($d\lambda/d\tau = 0$), but differs from r when λ varies with time. The PER μ_p is equal to the extinction rate μ if λ is time-independent, but differs from μ in most other cases. Pulled and canonical diversification parameters are thus not equivalent in most cases. Biological interpretation of pulled parameters is not obvious. However, some specific properties of PDR and PER allowed us to compare diversification dynamics estimated based on pulled and canonical diversification parameters. Specifically, changes in speciation and/or extinction rates usually lead to similarly strong changes in PDR, while constant PDR are strong indicators that both λ and μ were constant or varied only slowly over time (69, 75). PDR can also yield other valuable insights: if $\mu_p(0)$ is negative, this is evidence that speciation is currently decreasing over time (69, 75).

We estimated PDR values using the homogenous birth-death model on the R package *castor* 1.5.7 with the function *fit_hbd_pdr_on_grid* (130). We compared constant models (1 time interval) with models in which PDR values are allowed to vary independently on a grid of 3 time intervals. We set up the age grid non-uniformly, for example, age points were

594 placed closer together near the present (where information content is higher), and we selected
595 the model that best explained the lineage-through-time (LTT) of the Neotropical time trees
596 based on AIC. To avoid non-global local optima, we performed 20 independent fitting trials
597 starting from a random choice of model parameters. The *fit_hbd_pdr_on_grid* function
598 additionally provided estimates of $\rho\lambda o$ values. Knowing ρ, λ_0 could be derived as follows: λ_0
599 $= \lambda_0\rho/\rho$. Similarly, pulled extinction rates for each time interval could be derived as follows:
600 $\mu p := \lambda_0 - rp$. We limited the estimates to time periods with >10 species, using the *oldest_age*
601 function in *castor*, to avoid points in the tree close to the root, where estimation uncertainty is
602 generally higher.
603
604 ***Neotropical bioregionalization***
605 We used a quantitative approach to identify geographic units of long-term Neotropical
606 evolution. We divided the Neotropical region into 13 operational areas based on the WWF
607 biome classification (100) and similar to other studies, e.g., (4, 95) – Amazonia, Atlantic
608 Forest, Bahama-Antilles, Caatinga, Central Andes, Cerrado, Chaco, Chocó, Guiana Shield,
609 Mesoamerica, the Northern Andes, temperate South America, and an "elsewhere" region (see
610 SI for detailed definitions) – and assessed the distribution in these areas of the 12,512 species
611 included in our 150 phylogenies. Georeferenced records were downloaded for each species
612 through GBIF using the R package *rgbif* 0.9.9 (131). We removed points with precision
613 below 100 km, entries with mismatched georeference and country, duplicates, points
614 representing country capitals or centroids, using the R package *CoordinateCleaner* 1.0-7
615 (132). Then, we created 13 georeferenced polygons delimiting each operational area using
616 the WWF terrestrial ecoregions annotated shapefile in QGIS, and species were assigned to
617 each polygon according to coordinate observations using the R package *speciesgeocodeR*
618 1.0-4. GBIF records can result in an overestimation of widespread ranges (133), so species

distributions were manually inspected for completeness and accuracy with reference to databases (AmphibiaWeb 2018, Uetz et al. 2018, GBIF.org 2018, IUCN 2018). Based on the number of species belonging to each phylogenetic clade in the 13 ecoregions, we created a species abundance table (number of species per region per clade) that formed the basis for subsequent analyses.

The number of species distributed in each region within each clade were transformed using Hellinger transformations to account for differences in species richness between clades, and the *Morisita-horn* distance metric was selected to quantify pairwise dissimilarities of regional assemblages using the R package *vegan* 2.5-7 (134). We used K-means cluster analyses to form groups of similar regional assemblages. We determined the optimum number of groups by the elbow method. We use the function *fviz_cluster* in the R package *factoextra* 1.0.7 (135) to visualize K-means clustering results using principal component analysis.

Variation of diversification dynamics across taxa, environmental drivers, and biogeographic units.

We classified each clade in our study according to their main taxonomic group (plant [n=66], mammal [n=12], bird [n=32], squamate [n=24], amphibian [n=16]), environmental correlate (as estimated above: time [n=17], temperature [n=40] or uplift [n=17]), species richness dynamic based on canonical diversification rates (as estimated above: Sc. 1 [n=76], Sc. 2 [n=30], Sc. 3 [n=31], Sc. 4 [n=13]), and species richness dynamic based on pulled diversification rates (constant speciation [n=83], and decreasing speciation [n=51]).

We also classified each clade into the biogeographic units identified above (see results): cluster 1 (including the Amazonia, Central Andes, Chocó, Guiana Shield, Mesoamerica, and Northern Andes, [n=97]), cluster 2 (Atlantic Forest, Caatinga, Cerrado,

644 Chaco, and temperate South America, [n=10]), cluster 3 (Bahama-Antilles, [n=4]), cluster 4
645 ("elsewhere" region, [n=0]), or cluster 5 (Galapagos, [n=0]). Clades were assigned to a given
646 cluster only if > 60% of the species appeared in the cluster, otherwise clades were classified
647 as 'mixed' (n=39). We additionally classified clades according to the main elevational range
648 of their constituent species (lowland [< 1000 m; n=42], montane [1000–3500 m; n=8],
649 highland [> 3500 m; n=6], mixed [n=94]; see SI for detailed definitions). Note that for the
650 elevation, in our dataset most clades fell into the mixed category, with montane species most
651 often occurring within clades of lowland species, and rarely forming a clade of their own. To
652 account for this pattern (and minimize the number of clades classified as "mixed"), we
653 performed additional analyses pooling "lowland" and "montane" categories and considered a
654 clade "mixed" only if contained species in lowlands, montane and highlands (lowland-
655 montane [n=124], highland [n=6], mixed [n=20]).

656 We assessed the phylogenetic signal of each multi-categorical trait (i.e.,
657 biogeographic units, elevation, diversity dynamics, and environmental drivers) using the δ
658 statistics (136) over a phylogeny including one tip for each of the 150 clades represented in
659 this study. This tree was constructed using TimeTree (137). High δ-value indicates strong
660 phylogenetic signal. δ can be arbitrarily large, and thus significance was evaluated by
661 comparing inferred δ-values to the distribution of values when the trait was randomised along
662 the phylogeny. We evaluated the phylogenetic signal of continuous traits (i.e., diversification
663 $[r]$, speciation $[\lambda]$, and pulled diversification $[r_p]$ rates) using Blomberg's K (138) in the R
664 package *phytools* 0.7-80 (139). Since time-varying diversification curves are hardly
665 summarized in a single value, comparisons of net diversification values are based on
666 estimates derived from the constant-rate model.

667 As any continuous (K_r=0.06, p=0.6; K_λ=0.07, p=0.4; K_{rp}=0.07, p=0.6) or multi-
668 categorical trait displays phylogenetic signal (Fig. S10), suggesting that the distribution of

trait values is not explained by the phylogeny itself, statistical tests were conducted without applying phylogenetic corrections to account for the non-independence of data points. Fisher's exact test was used in the analysis of contingency tables, performing pairwise-comparison with corrections for multiple testing (140), and Kruskal-Wallis Tests for comparing means between groups.

We also tested the effect of clade age, size and sampling fraction on the preferred species-richness dynamic (Sc. 1–4) using a phylogenetic ANOVA in *phytools* with posthoc comparisons, checking if the residual error controlling for the main effects in the model and the tree were normally distributed. We applied phylogenetic corrections in this case because phylogenetic signal was detected for sampling fraction ($K_{sampling}$=0.12, p=0.001) and crown age (K_{age}=0.22, p=0.001), not for tree size (K_{size}=0.49, p=0.9).

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Tables

Table 1. Alternative species richness dynamics (sc. 1–4) and the corresponding diversification models (a–c) able to explain Neotropical diversity. Species richness dynamics represent scenarios of expanding (sc. 1–2), saturating (sc. 3) and contracting (sc. 4) diversity, in which speciation *(λ)* and/or extinction *(μ)* remain constant or vary through time.

The number of phylogenies supporting each model is provided for all lineages pooled together, and for plants and tetrapods separately. Empirical support for each evolutionary model is based on canonical diversification rates (CDR), and pulled diversification rates (PDR), by comparing the constant model against different sets of time-variable models. For CDR, we provide as well the results (in italic) based on model comparisons including constant, time-variable, and paleoenvironmental dependent (temperature and uplift) models.

Diversity dynamics	CDR all(plant/tetra)	PDR all(plant/tetra)	Diversification mode	Model parameters	CDR all(plant/tetra)	PDR all(plant/tetra)
Sc 1. Gradual increase	101 (47/54) *76 (40/37)*	95 (39/56)	**(b)** Cradle & Museum	$\lambda(t) = \lambda_0$, $\mu(t) = \mu_0$	101 (47/54) *77 (40/37)*	95 (39/56)
			(c) Recent cradle & ancient museum	$\lambda(t) = \lambda_0 e^{\alpha t}$, $\mu(t) = \mu_0 e^{\beta t}$, $\lambda_0 = \mu_0$, $\alpha = \beta$	0 (0/0) *0 (0/0)*	
			(d) Both		0 (0/0) *0 (0/0)*	
Sc 2. Exponential increase	20 (17/3) *30 (19/11)*	51 (25/26) *	**(b)** Recent cradle	$\lambda(t) = \lambda_0 e^{\alpha t}$, $\alpha < 0$, $\mu(t) = \mu_0$	9 (7/2) *9 (8/1)*	51 (25/26) *
			(c) Recent museum	$\lambda(t) = \lambda_0$, $\mu(t) = \mu_0 e^{\beta t}$, $\beta > 0$	10 (10/0) *13 (11/2)*	
			(d) Both		1 (0/1) *8 (0/8)*	
Sc 3. Saturated increase	24 (1/23) *31 (3/28)*		**(b)** Ancient cradle	$\lambda(t) = \lambda_0 e^{\alpha t}$, $\alpha > 0$, $\mu(t) = \mu_0$	24 (1/23) *29 (3/27)*	
			(c) Ancient Museum	$\lambda(t) = \lambda_0$, $\mu(t) = \mu_0 e^{\beta t}$, $\beta < 0$	0 (0/0) *0 (0/0)*	
			(d) Both		0 (0/0) *1 (0/1)*	
Sc 4. Declining diversity	5 (1/4) *13 (5/8)*		**(b)** Ancient cradle & recent decline	$\lambda(t) = \lambda_0 e^{\alpha t}$, $\alpha > 0$, $\mu(t) = \mu_0$	1 (1/0) *(1/5)*	
			(c) Ancient Museum & recent decline	$\lambda(t) = \lambda_0$, $\mu(t) = \mu_0 e^{\beta t}$, $\beta < 0$	1 (0/0) *(1/1)*	
			(d) Both		3 (0/4) *8 (3/2)*	

*Pulled extinction rates (μ_p) can be useful for inferring speciation trends, for example, a negative present-day pulled extinction rate ($\mu_{p(0)} < 0$) is indicative that λ decreases through time. But the opposite is not necessarily true, i.e., a positive present-day pulled extinction rate ($\mu_{p(0)} > 0$) does not necessarily indicate that λ increases through time (Louca & Pennell 2020). Based on pulled extinction, we cannot infer either if diversification dropped below 0, and thus differentiate between the two scenarios in which λ decreases through time (damped increase and waxing and waning dynamics). Similarly, based on pulled diversification rates, we cannot identify increases in speciation or time changes in extinction rates (scenarios 1b, 2a, 2b, 3c, 4c).

1059 **Table 2**. Summary *p* value results derived from the analysis of canonical diversification (*r*)
1060 and pulled diversification (r_p) rates. Significant differences in the proportion of clades
1061 experiencing different diversity trajectories (based on canonical diversification rates: gradual
1062 expansions, exponential expansions, saturation or declining diversity; based on pulled
1063 diversification rates: expanding vs. declining speciation) across biogeographic units,
1064 elevations, taxonomic groups, and environmental drivers as derived from Fisher's exact tests.
1065 Significant differences in net diversification, pulled diversification, and speciation rates
1066 across biogeographic units, elevations and taxonomic groups derive from Kruskal-Wallis chi-
1067 squared analyses. Significant results are highlighted in bold.

1068

	Diversity trajectories		**Diversification rates**		**Speciation rates**
	r	r_p	*r*	r_p	*r*
Biogeographic units (5 clusters)	0.459	0.252	0.168	0.083	0.248
Biogeographic units (7 clusters)	0.503	0.947	0.198	0.424	0.277
Elevation	0.504	0.839	0.672	0.277	**0.034**
Elevation (lowland-montane combined)	0.062	0.062	0.332	0.869	**0.031**
Taxonomic groups	**0.000**	0.126	**0.000**	**0.000**	**0.000**
Environmental drivers	**0.003**	–	–	–	–

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1074 ## Figure legends

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1076 **Figure 1.** Alternative hypotheses to explain current Neotropical diversity. **(a)** Main species
1077 richness dynamics through time, and **(b, c)** the alternative evolutionary processes that could
1078 generate the corresponding patterns. **(Sc. 1a)** A gradual increase of species richness could
1079 result from constant speciation and extinction rates (1b; cradle & museum model), or through

1080 a comparable increase in speciation and extinction rates (1c; recent cradle & ancient museum
1081 model). **(Sc. 2a)** An exponential increase in species numbers could be attained through
1082 constant extinction and increasing speciation (2b; recent cradle model), or constant speciation
1083 and decreasing extinction rates (2c; recent museum). **(Sc. 3a)** Saturated increase scenarios,
1084 with species accumulation rates slowing down towards the present, could result from constant
1085 extinction and decreasing speciation (3b; ancient cradle model), or through constant
1086 speciation and increasing extinction rates (3c; ancient museum model). **(Sc. 4a)**. Waxing and
1087 waning dynamics could result from constant extinction and decreasing speciation (4b; ancient
1088 cradle & recent decline model), or constant speciation and increasing extinction (4c; ancient
1089 museum & recent decline model). Waxing and waning scenarios differ from saturated
1090 increases in that extinction exceeds speciation towards the present, such that diversification
1091 goes below 0. Scenarios (b-c) represent the simplest and most general models to explain
1092 species richness patterns in (a), but other combinations of speciation and extinction rates
1093 could potentially generate these patterns; for example, an exponential increase of species (2a)
1094 could also result from increasing speciation and punctual increases in extinction, or through
1095 increasing speciation and decreasing extinction.
1096
1097 **Figure 2.** Time of origin for Neotropical tetrapods and plants. Horizontal bars represent
1098 crown ages of 150 phylogenies analyzed in this study. Shaded boxes represent the
1099 approximate duration of some geological events suggested to have fostered dispersal and
1100 diversification of Neotropical organisms. Inset histograms represent summary statistics for
1101 crown age (mean = 29.9 Myrs), sampling fraction (mean = 57%) and tree size (mean = 83.4
1102 species/tree). Mean global temperature curve from (Zachos et al. 2008). Abbreviations: K,
1103 Cretaceous; P, Paleocene; E, Eocene; O, Oligocene; M, Miocene; P, Pliocene (Pleistocene

follows but is not shown); GAARlandia, Greater Antilles and Aves Ridge. Animal and plant silhouettes from PhyloPic (http://-phylopic.org/).

Figure 3. Speciation trends in 150 phylogenies of Neotropical plants and tetrapods. The histograms show the proportion of phylogenies for which constant vs. time-variable diversification models were the best fit, as derived from pulled diversification rates **(a)**, and canonical diversification rates **(b)**, when comparing time-dependent models against constant models. In subfigure b, the proportion of time-variable models is subdivided by the proportion of phylogenies in which speciation rates increase through time, decrease through time, or speciation remains constant (being extinction time-variable). In subfigure a, speciation trends are derived from present-day pulled extinction rates $\mu p(0)$: negative present-day pulled extinction rates values ($\mu p(0) < 0$) indicate decreasing speciation trends through time (Louca & Pennell, 2020). Positive $\mu p(0) > 0$ values are possible under both increasing or decreasing speciation rates, in which case speciation trends are designed as "unknown".

Figure 4. Diversity dynamics in 150 phylogenies of Neotropical plants and tetrapods. The histograms show the proportion of phylogenies for which gradual increase (Sc. 1), exponential increase (Sc. 2), saturated increase (Sc. 3) and waxing & waning (Sc. 4) scenarios were the best fit, as derived from net diversification trends when comparing **(a)** time-dependent models against constant models and **(b)** environmental (temperature- and uplift-dependent models) against time-dependent and constant models. **(c)** Correspondence analysis showing the association between species richness dynamics (represented by blue arrows) and major taxonomic groups (red arrows). If the angle between two arrows is acute, then there is a strong association between the corresponding variables.

1129 **Figure 5.** Drivers of Neotropical diversification in 150 phylogenies of Neotropical plants and
1130 tetrapods. The histograms report the proportion of **(a)** phylogenies whose diversification rates
1131 are best explained by a model with constant, time-dependent, temperature-dependent, or
1132 uplift-dependent diversification. The number of phylogenies (and species) per group is shown
1133 in parentheses. **(b)** The histograms report the number of phylogenies whose diversification
1134 rates are best explained by a model with constant, time-, temperature-, or uplift-dependent
1135 diversification according to different species richness scenarios (Exp = Exponential increase
1136 [Sc.2], Sat = Saturated increase [Sc.3] and Dec = Declining diversity [Sc.4]), for plants,
1137 endotherm tetrapods, ectotherms and all clades pooled together. **(c)** Correspondence analysis
1138 for the pooled dataset showing the association between species richness dynamics
1139 (represented by red arrows) and the environmental drivers (blue arrows). If the angle between
1140 two arrows is acute, then there is a strong association between the corresponding variables.
1141
1142 **Figure 6.** Geographical component of long-term Neotropical diversification. **(a)** PCA
1143 representation of the 5 biogeographic clusters identified based on K-means clustering of 13
1144 areas (WWF ecoregions) and 150 clades. **(b)** Resulting clusters (1–5) in geographic space.
1145 Colors correspond with the biogeographic clusters in (a). Thick lines delineate the original 13
1146 ecoregions used in the analyses. **(c)** Box plot showing differences in crown age of the
1147 phylogenies distributed in each of the biogeographic clusters. **(d)** Variation in diversification
1148 and **(e)** pulled diversification rates (derived from the constant-rate model) across geographic
1149 clusters. **(f)** Number of phylogenies for which species richness scenarios Sc. 1–4 (Grad =
1150 Gradual increase [Sc.1], Exp = Exponential increase [Sc.2], Sat = Saturated increase [Sc.3]
1151 and Dec = Declining diversity [Sc.4]) were the best fit, across geographic clusters as derived
1152 from canonical diversification rates. **(g)** Number of phylogenies for which constant vs.

declining speciation rates were the best fit, across geographic clusters as derived from pulled diversification rates.

Figure 7. Diversification rates compared across plants and tetrapods (endotherms and ectotherms). Diversification and speciation rates are derived from the constant-rate model (Table S6). Letters are used to denote statistically differences between groups, with groups showing significant differences in mean values denoted with different letters. The y-axis was cut off at 1.0 to increase the visibility of the differences between groups. Upper values for plants are therefore not shown, but the quartiles and median are not affected. Units are in events per million years.

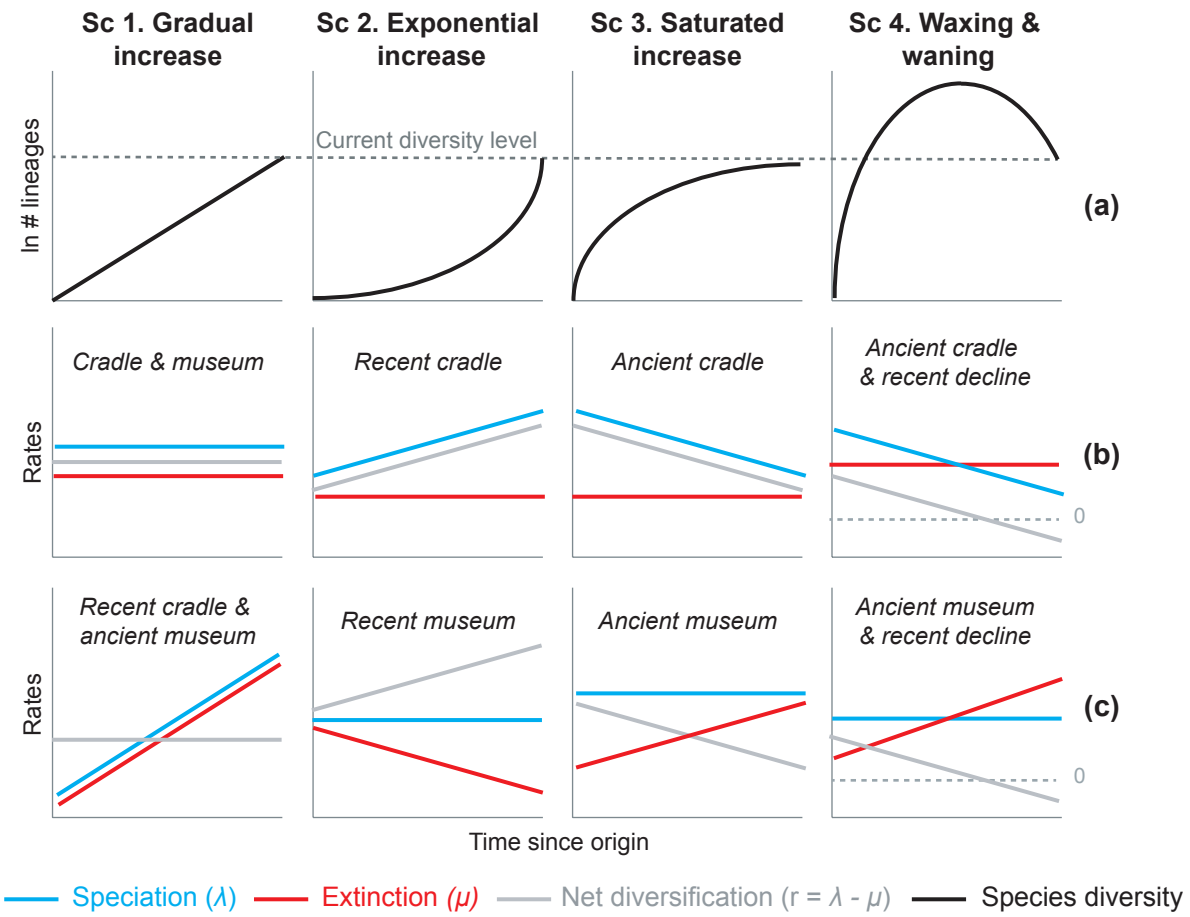


Figure 1. Alternative hypotheses to explain current Neotropical diversity. (a) Main species richness dynamics through time, and **(b,c)** the alternative evolutionary processes that could generate the corresponding patterns. **(Sc. 1a)** A gradual increase of species richness could result from constant speciation and extinction rates (1b; cradle & museum model), or through a comparable increase in speciation and extinction rates (1c; recent cradle & ancient museum model). **(Sc. 2a)** An exponential increase in species numbers could be attained through constant extinction and increasing speciation (2b; recent cradle model), or constant speciation and decreasing extinction rates (2c; recent museum). **(Sc. 3a)** Saturated increase scenarios, with species accumulation rates slowing down towards the present, could result from constant extinction and decreasing speciation (3b; ancient cradle model), or through constant speciation and increasing extinction rates (3c; ancient museum model). **(Sc. 4a)** Waxing and waning dynamics could result from constant extinction and decreasing speciation (4b; ancient cradle & recent decline model), or constant speciation and increasing extinction (4c; ancient museum & recent decline model). Waxing and waning scenarios differ from saturated increases in that extinction exceeds speciation towards the present, such that diversification goes below 0. Scenarios (b-c) represent the simplest and most general models to explain species richness patterns in (a), but other combinations of speciation and extinction rates could potentially generate these patterns; for example, an exponential increase of species (2a) could also result from increasing speciation and punctual increases in extinction, or through increasing speciation and decreasing extinction.

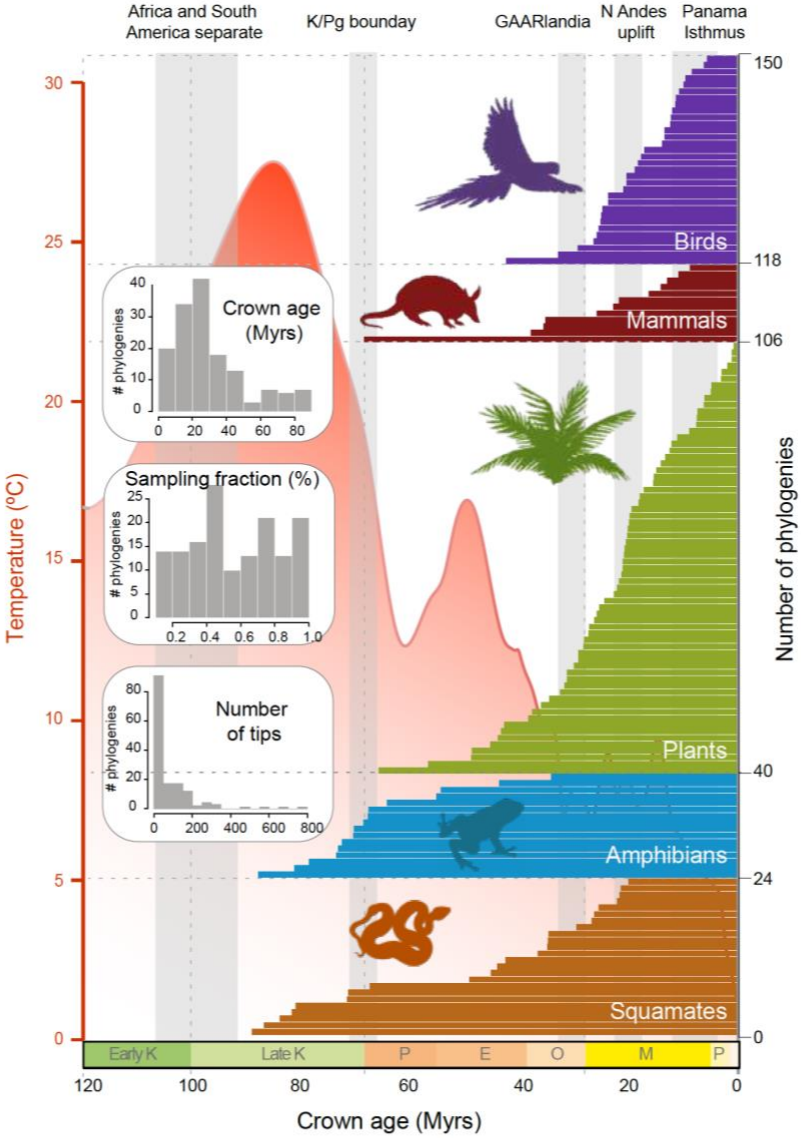


Figure 2. Time of origin for Neotropical tetrapods and plants. Horizontal bars represent crown ages of 150 phylogenies analysed in this study. Shaded boxes represent the approximate duration of some geological events suggested to have fostered dispersal and diversification of Neotropical organisms. Inset histograms represent summary statistics for crown age (mean = 29.9 Myrs), sampling fraction (mean = 57%) and tree size (mean = 83.4 species/tree). Mean global temperature curve from (Zachos et al. 2008). Abbreviations: K, Cretaceous; P, Paleocene; E, Eocene; O, Oligocene; M, Miocene; P, Pliocene (Pleistocene follows but is not shown); GAARlandia, Greater Antilles and Aves Ridge. Animal and plant silhouettes from PhyloPic (http://-phylopic.org/).

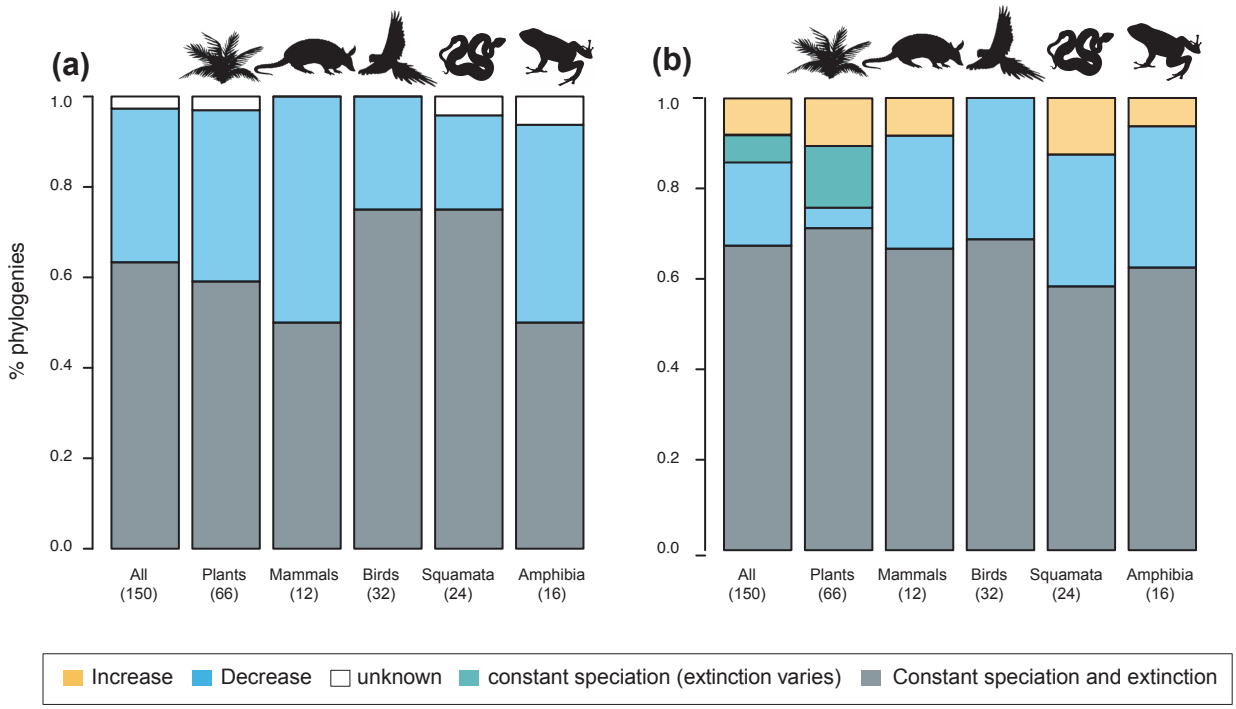


Figure 3. Speciation trends in 150 phylogenies of Neotropical plants and tetrapods. The histograms show the proportion of phylogenies for which constant vs. time-variable diversification models were the best fit, as derived from pulled diversification rates **(a)**, and canonical diversification rates **(b)**, when comparing time-dependent models against constant models. In subfigure b, the proportion of time-variable models is subdivided by the proportion of phylogenies in which speciation rates increase through time, decrease through time, or speciation remains constant (being extinction time-variable). In subfigure a, speciation trends are derived from present-day pulled extinction rates *μp(0):* negative present-day pulled extinction rates values (*μp(0)* < 0) indicate decreasing speciation trends through time (Louca & Pennell, 2020). Positive *μp(0)* > 0 values are possible under both increasing or decreasing speciation rates, in which case speciation trends are designed as “unknown”.

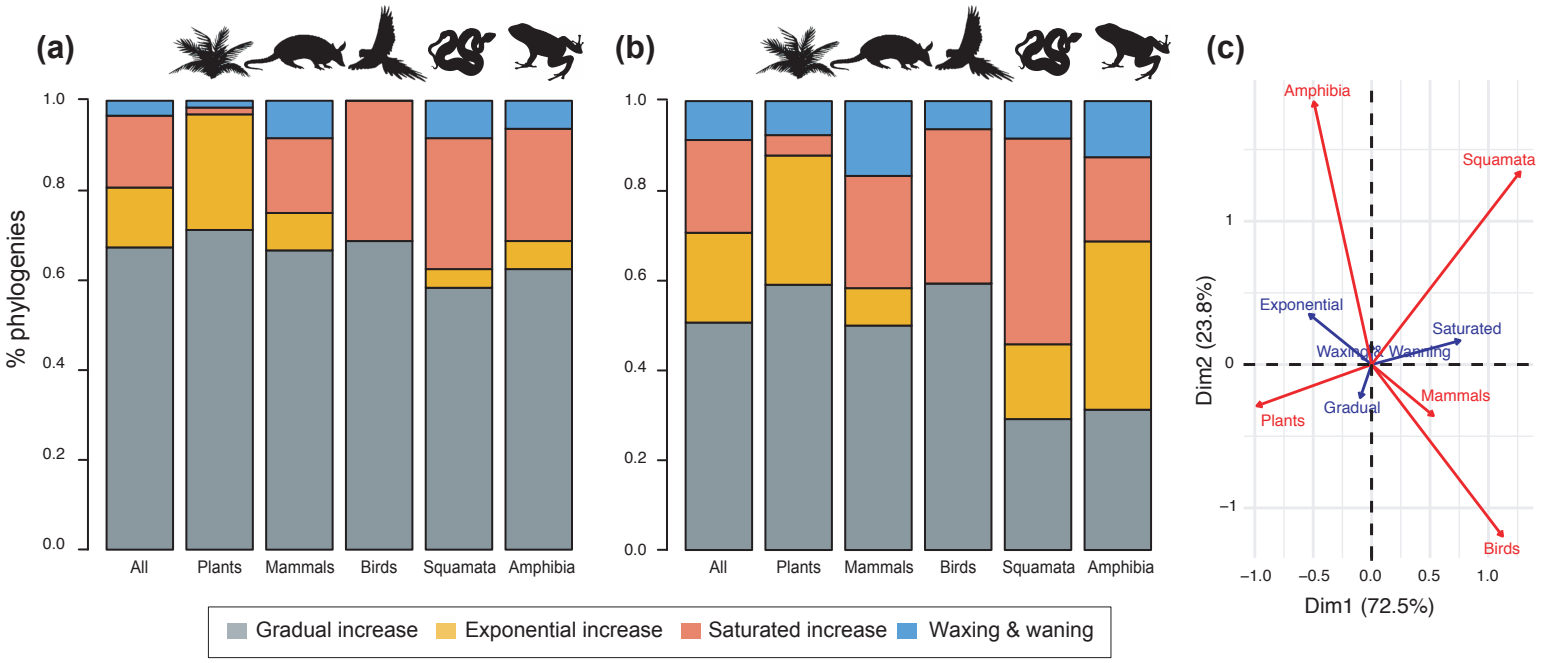


Figure 4. Diversity dynamics in 150 phylogenies of Neotropical plants and tetrapods. The histograms show the proportion of phylogenies for which gradual increase (Sc. 1), exponential increase (Sc. 2), saturated increase (Sc. 3) and waxing & waning (Sc. 4) scenarios were the best fit, as derived from net diversification trends when comparing **(a)** time-dependent models against constant models and **(b)** environmental (temperature- and uplift-dependent models) against time-dependent and constant models. **(c)** Correspondence analysis showing the association between species richness dynamics (represented by blue arrows) and major taxonomic groups (red arrows). If the angle between two arrows is acute, then there is a strong association between the corresponding variables.

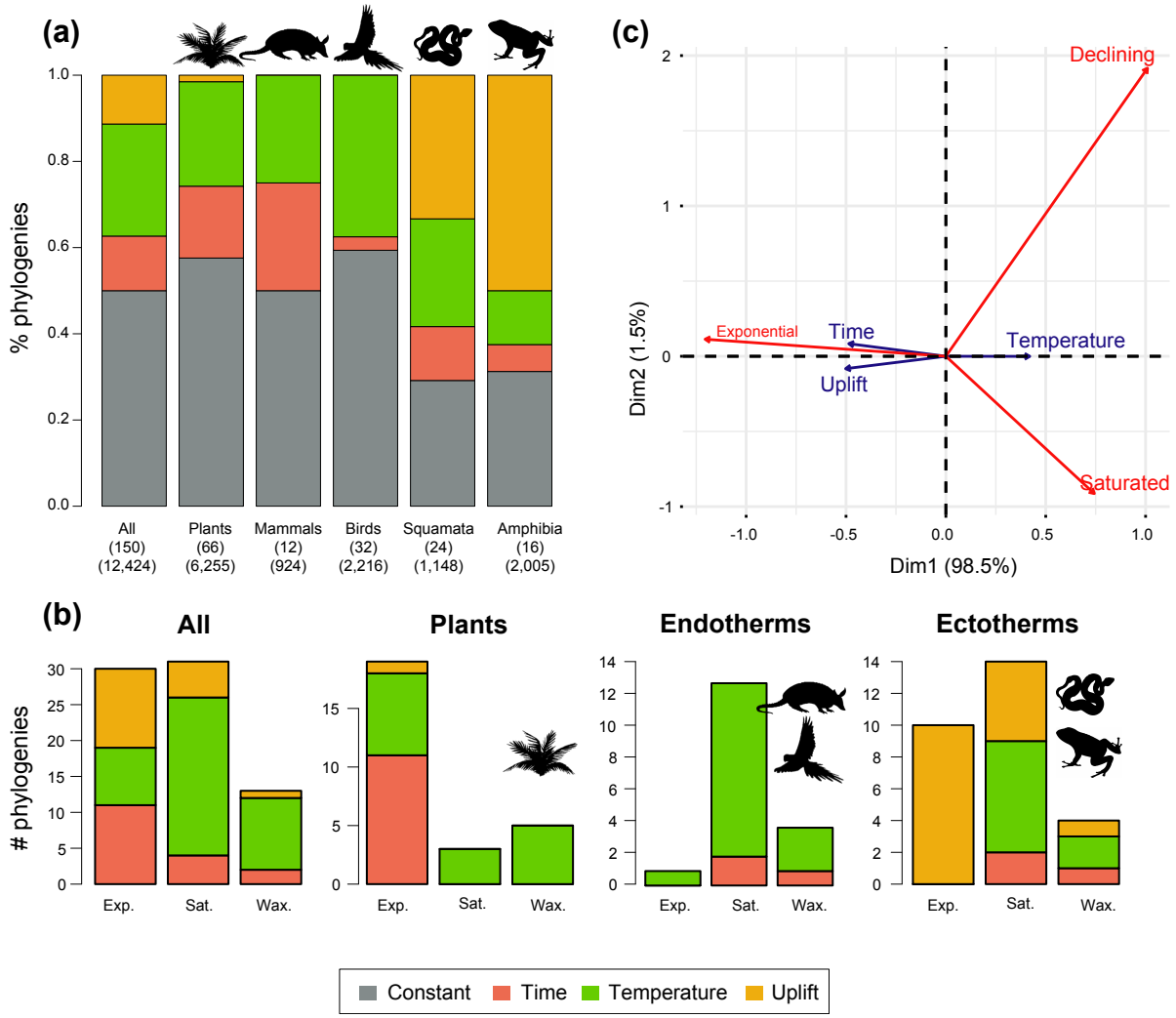


Figure 5. Drivers of Neotropical diversification in 150 phylogenies of Neotropical plants and tetrapods. The histograms report the proportion of ***(a)*** phylogenies whose diversification rates are best explained by a model with constant, time-dependent, temperature-dependent, or uplift-dependent diversification. The number of phylogenies (and species) per group is shown in parentheses. ***(b)*** The histograms report the number of phylogenies whose diversification rates are best explained by a model with constant, time-, temperature-, or uplift-dependent diversification according to different species richness scenarios (Exp = Exponential increase [Sc.2], Sat = Saturated increase [Sc.3] and Dec = Declining diversity [Sc.4]), for plants, endotherm tetrapods, ectotherms and all clades pooled together. ***(c)*** Correspondence analysis for the pooled dataset showing the association between species richness dynamics (represented by red arrows) and the environmental drivers (blue arrows). If the angle between two arrows is acute, then there is a strong association between the corresponding variables.

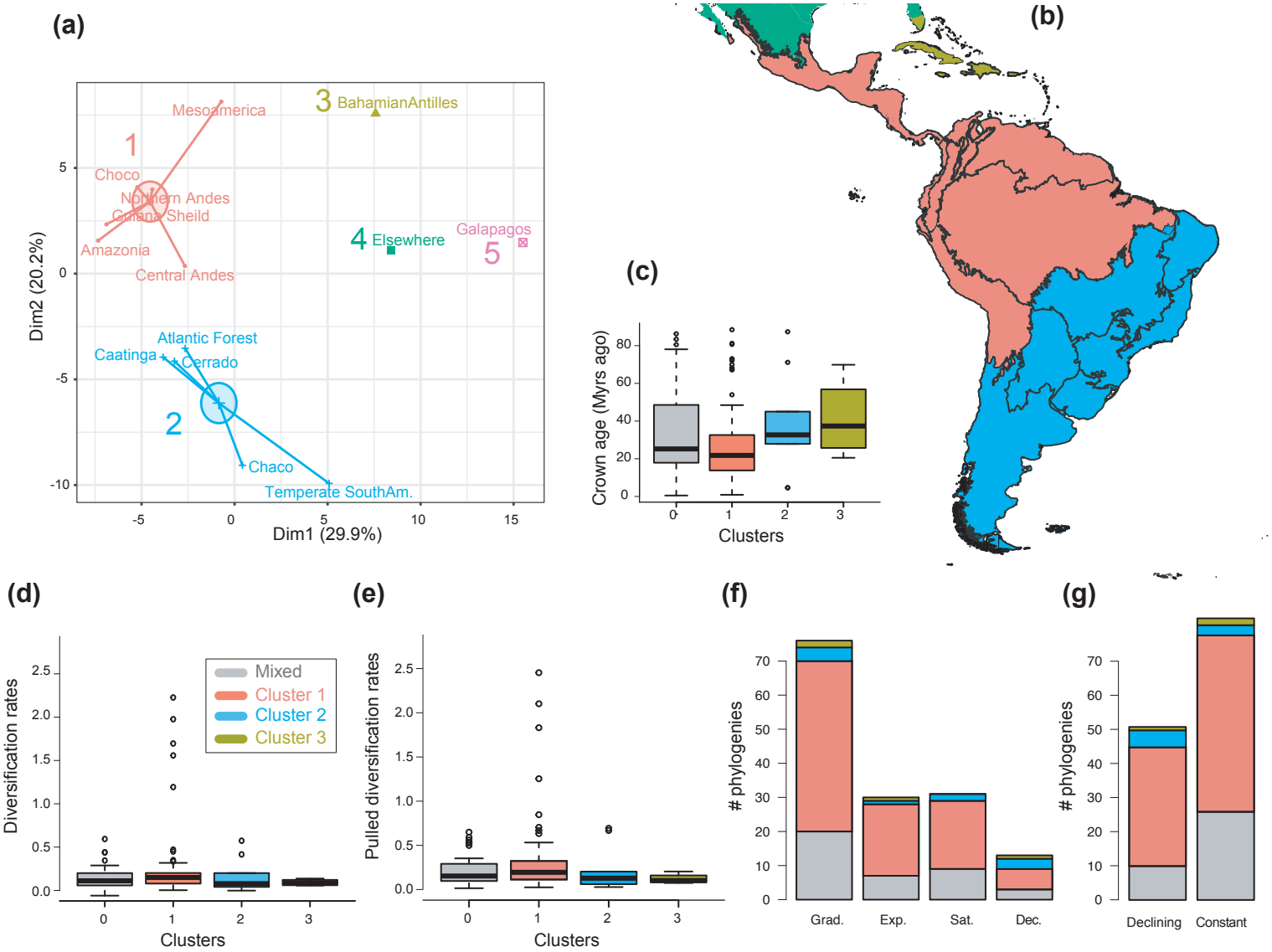
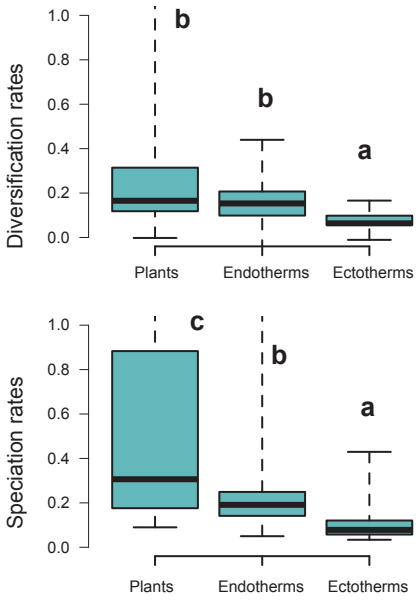


Figure 6. The geographical structure of Neotropical diversification. (a) PCA representation of the 5 biogeographic clusters identified based on K-means clustering of 13 areas (WWF ecoregions) and 150 clades. **(b)** Resulting clusters (1–5) in the geographic space. Colors correspond with the biogeographic clusters in (a). Thick lines delineate the original 13 ecoregions used in the analyses. **(c)** Box plot showing differences in crown age of the phylogenies distributed in each of the biogeographic clusters. **(d)** Variation in diversification and **(e)** pulled diversification rates (derived from the constant-rate model) across geographic clusters. **(f)** Number of phylogenies for which species richness scenarios Sc. 1–4 (Grad = Gradual increase [Sc.1], Exp = Exponential increase [Sc.2], Sat = Saturated increase [Sc.3] and Dec = Declining diversity [Sc.4]) were the best fit, across geographic clusters as derived from cannonical diversification rates. **(g)** Number of phylogenies for which constant *vs.* declining speciation rates were the best fit, across geographic clusters as derived from pulled diversification rates.



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