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SHORT COMMUNICATION

Are reptile and amphibian species younger in the Northern Hemisphere than in the Southern Hemisphere?

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Abstract

A previous analysis of molecular phylogenies suggested that intraspecific diversification had occurred more recently in temperate-zone Northern Hemisphere reptiles and amphibians than in Southern Hemisphere taxa. Here, we test potential explanations for this pattern. We examined published phylogenetic analyses, derived from genetic sequence data, to generate two estimates of the age of species: (i) the oldest intraspecific diversification event within each taxon and (ii) the inferred timing of the split between two sister species. The timing of splits between species shows the same pattern as splits within species, and thus may be due to climatically driven cladogenic and extinction events or may be an artefact of differing levels of taxonomic knowledge about the fauna. Current rates of species descriptions suggest that many more taxa remain to be described in the Southern Hemisphere than the Northern Hemisphere; for that bias to fully explain our results on species age differences, the proportion of undescribed Southern taxa would need to be ≥ 12% in reptiles and ≥ 51% in anurans. For reptiles, taxonomic ignorance plausibly explains the apparent difference in mean age of species between the Southern and Northern Hemispheres; but this explanation can apply to amphibians only if a vast number of Southern taxa remain to be described.

Introduction

The growing availability of phylogenetic information about major lineages, based on standardized types of data (genetic sequence information) and analytical methods, provides an opportunity to explore broad geographic patterns in evolutionary history. Because rates of nucleotide substitution often are consistent within lineages, the phylogenies recovered by these analyses can be used to estimate the dates of major branching points. A recent analysis of reptile and amphibian species in this respect revealed a strong geographic pattern: the most recent common ancestor of all living populations within a species occurred much earlier, on average, in taxa from the temperate zone of the Southern Hemisphere than in taxa from equivalent latitudes in the Northern Hemisphere (Dubey & Shine, 2011). That is, Northern species seem to be 'younger' than Southern species.

Why might Northern species be younger? One possibility is a difference in climatic history: many areas of Europe and North America experienced severe glaciation in the Pliocene/Pleistocene, potentially eliminating much of the extant biodiversity (especially, of temperature-sensitive ectothermic taxa; Hewitt, 2000, 2003; Dubey *et al.*, 2006; Joger *et al.*, 2007). Modern populations of ectothermic vertebrates in this region might thus result from fairly recent re-invasion of the landscape, constraining intraspecific divergence between these lineages to recent (postglacial) timescales (Dubey & Shine, 2011). Similarly, in mammals and birds, the maximum coalescent dates for intraspecific diversification are shorter at high latitudes: that is, species are younger than in the tropics (Weir & Schluter, 2007). However, there are at least two other possible explanations for the geographic difference in species age. The first involves the way we measure the age of a species. Rather than looking for the most recent common ancestor of extant populations within a species, we could look at the dates of

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inferred speciation events – that is, how long since a species split from its most closely related sister taxon (as in Weir & Schluter, 2007). Geographic patterns in this type of 'species age' (i.e., how long since the species first appeared?) might differ from those based on intraspecific divergence (i.e., how long since extant populations diverged?). It is important to note that the processes influencing interspecific diversification differ from those influencing intraspecific diversification. For example, the rate and timing of speciation can be affected by ecological constraints, with rates of diversification dependent upon factors such as the availability of unfilled ecological niches (e.g., Raborsky & Alfaro, 2010), and climatic fluctuations and geological activities that fragment existing ranges and impose novel selective forces (e.g., Weir & Schluter, 2007). In contrast, the rate and timing of intraspecific differentiation are affected by a different suite of factors. For example, restriction to isolated refugia during unfavourable climate conditions can reduce effective population size, and the consequent decrease in genetic variation can influence subsequent rates of intraspecific diversification (e.g., Cruzan & Templeton, 2000).

Another possible explanation for the apparent geographic disparity in species age is that estimates (of either type above) may be biased by the level of taxonomic understanding of the organisms involved. If one region has a higher proportion of as-yet-undescribed taxa than another region, estimates of divergence times will be inflated in the former area. The reason for this artefact is that we will fail to detect some proportion of relatively recent cladogenic events, so that we end up by estimating the 'time since last recent common ancestor' of a species group rather than a single species. By definition, the former assemblage will be older than the latter.

In this study, we evaluate these potential explanations for the reported disparity in mean species ages between reptiles and amphibians from temperate-zone latitudes of the Northern vs. Southern Hemisphere.

Materials and Methods

Age of species

We analysed published phylogenetic hypotheses, derived from genetic sequence data, to generate two estimates of the age of species: (i) the oldest intraspecific diversification event within each taxon (as in Dubey & Shine, 2011) and (ii) the inferred timing of the split between two sister species. These measures estimate the amount of time since the most recent common ancestor of (i) existing populations within a species and (ii) sister species. Our data set is an expanded version of that used by Dubey & Shine (2011).

We reviewed published phylogenetic analyses (including species ages) based on 218 species of reptiles (Squamata) and amphibians (Anura) in temperate-zone (nontropical) areas, for a total of 183 intraspecific (vs. 152 in Dubey & Shine, 2011) and 113 interspecific splits, to test for differences in age between taxa from the Northern vs. Southern Hemispheres (see Appendix S1 for more details). Urodeles (salamanders and newts) were excluded from the analyses, as they do not occur in temperate-zone areas of the Southern Hemisphere. Testudines were excluded also, due to a lack of data on the timing of the splits between sister species in Southern Hemisphere taxa. Studies on nonmonophyletic species were also excluded, to avoid inaccurate species age estimations.

The statistical significance of the difference between Hemispheres in mean ages of species was tested with a three-way ANOVA with Hemisphere, measure of species age (intraspecific vs. interspecific diversification), Order (Anura vs.Squamata) or suborder (Ophidia [=Serpentes], Sauria [=Lacertilia], Amphisbaenia, Neobatrachia, Mesobatrachia and Archaeobatrachia) as factors and mean species age as the dependent variable, using JMP 7.0 (SAS Institute Inc., 2007). We also examined latitudinal subsets of the data, to check that the same patterns were present when we focused on taxa in comparable latitudinal zones. Thus, we repeated the above analysis for three categories within the broader data set: (i) species restricted to low latitudes only (between the tropics and 40°S or N), (ii) species distributed at higher latitudes only (above 40°S or N) and (iii) species distributed in both of the above latitudinal ranges.

Historical patterns in the number of described species

To quantify the extent of taxonomic knowledge of each area, we scored the number of new species of amphibians and reptiles described since 1970, using the databases (i) Amphibian Species of the World (http://research.amnh.org/vz/herpetology/amphibia/; Frost, 2011) and (ii) the Reptile database (http://www.reptile-database.org/; Uetz *et al.*, 2007), in the Northern Hemisphere (Europe, North America) and Southern Hemisphere (southern part of South America, South Africa, Australia, New Zealand). We only consider currently recognized species and the year of their first description as it appeared in the databases (past invalid species were excluded). Therefore, our analyses did not include lists of previously recognized species. To allow for geographic differences in total species richness, we expressed the cumulative number of taxa described by any given year as a proportion of the total number known at present (2011). If all extant species in an area have already been described, we expect a plateau in known species richness through time; but if many species remain to be discovered, the total number of described species will continue to increase to the present day.

Effects of omission of species on estimated divergence dates

We simulated 100 phylogenetic trees of 30 species with Mesquite (Maddison & Maddison, 2010) using birth–death models with three rates of extinction (1/3, 2/3 or equal to the speciation rate). We estimated the ages of all splits between sister species within each tree. Then, we randomly deleted 3, 5 and 10 species in each tree, before re-estimating the ages of speciation events. We used linear regression to compare the proportion of omitted species to the proportional change in estimated mean age of speciation events.

Results

Age of species

Including interspecific as well as intraspecific divergence events did not change the overall patterns in geographic disparities in mean age of species. Inevitably, the most recent common ancestor was later for intraspecific than interspecific divergence events (from the three-way ANOVA: intraspecific vs. interspecific diversification, $F_{1,295} = 49.61$, $P < 0.0001$; Fig. 1). As in the analysis of intraspecific divergences only (Dubey & Shine, 2011), present-day species of reptiles and amphibians from temperate-zone habitats of the Northern Hemisphere were on average younger than those from the Southern Hemisphere (Northern vs. Southern Hemisphere effect, $F_{1,295} = 12.63$, $P = 0.0004$). Reptiles and amphibians did not differ significantly in this respect (squamate vs. anuran, $F_{1,295} = 3.54$, $P = 0.061$), and no interaction terms were significant.

We performed additional analyses including suborder (Ophidia, Sauria, Amphisbaenia, Neobatrachia, Mesobatrachia and Archaeobatrachia) as a factor. However, analyses of suborder effects were only performed within the Squamata (with suborders Sauria and Ophidia), due to a lack of data for Amphisbaenia in the Southern Hemisphere. In Anura, the only group for which data were available for both Hemispheres and intra–interspecific diversifications was the Neobatrachia, precluding analyses of suborder effects. Species of Lacertilia and Serpentes from the Northern Hemisphere were on average younger than those from the Southern Hemisphere (Northern vs. Southern Hemisphere effect, $F_{1,227} = 7.77$, $P = 0.0058$). Sauria and Ophidia did not differ significantly in this respect ($F_{1,227} = 0.08$ $P = 0.78$), and no interaction terms were significant. Consequently, the observed pattern is consistent across the tested groups. Similar patterns were present when the analysis was restricted to species of amphibians and reptiles that are found in low latitudes only (between the tropics and 40°N or S, including all orders: Northern vs. Southern Hemisphere effect, $F_{1,155} = 5.22$, $P = 0.024$; inter–intraspecific diversification, $F_{1,155} = 20.19$, $P < 0.0001$; squamate vs. anuran, $F_{1,155} = 3.46$, $P = 0.065$; Fig. 2), or when the analysis was restricted to species that are found in both moderate and high latitudes (Northern vs. Southern Hemisphere effect, $F_{1,118} = 5.14$, $P = 0.025$; interspecific vs. intraspecific diversification, $F_{1,118} = 25.92$, $P < 0.0001$; squamate vs. anuran, $F_{1,118} = 0.24$, $P = 0.62$). For the analysis of high-latitude species (above 40°N or S), the only taxa represented in our data set for the Southern Hemisphere were squamates, so we conducted a two-factor ANOVA with hemisphere as the factor and age of squamate taxa as the dependent variable. Species from this latitudinal zone were younger, on average, in the Northern Hemisphere than in the Southern Hemisphere ($F_{1,15} = 6.46$, $P = 0.025$; interspecific vs. intraspecific diversification, $F_{1,15} = 6.23$, $P = 0.027$).

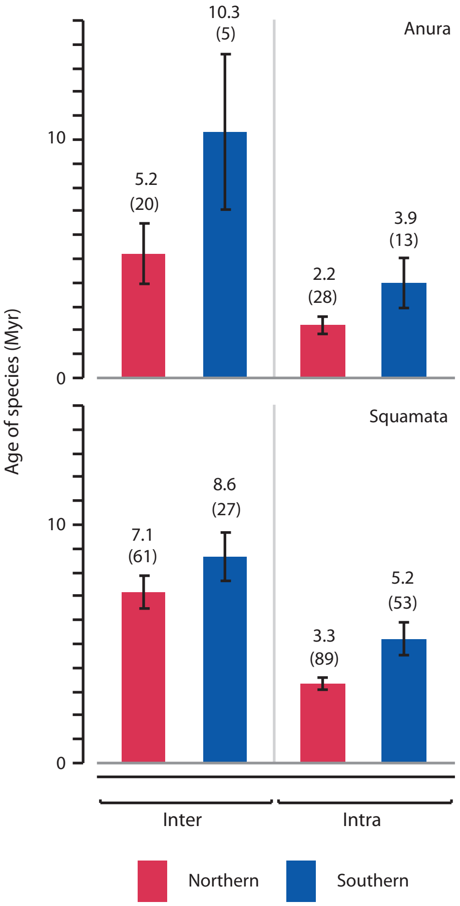

Fig. 1 Mean values and standard errors for estimated dates of the ages of anuran and squamate species from temperate-zone habitats of the Northern vs. Southern Hemispheres. Ages (expressed in millions of years ago: Ma) were calculated in two ways: as the most recent common ancestor of all living members of a species (intraspecific divergence age) and as the estimated date when two closely related species last shared a common ancestor (interspecific divergence age). Numbers show mean values and sample sizes.

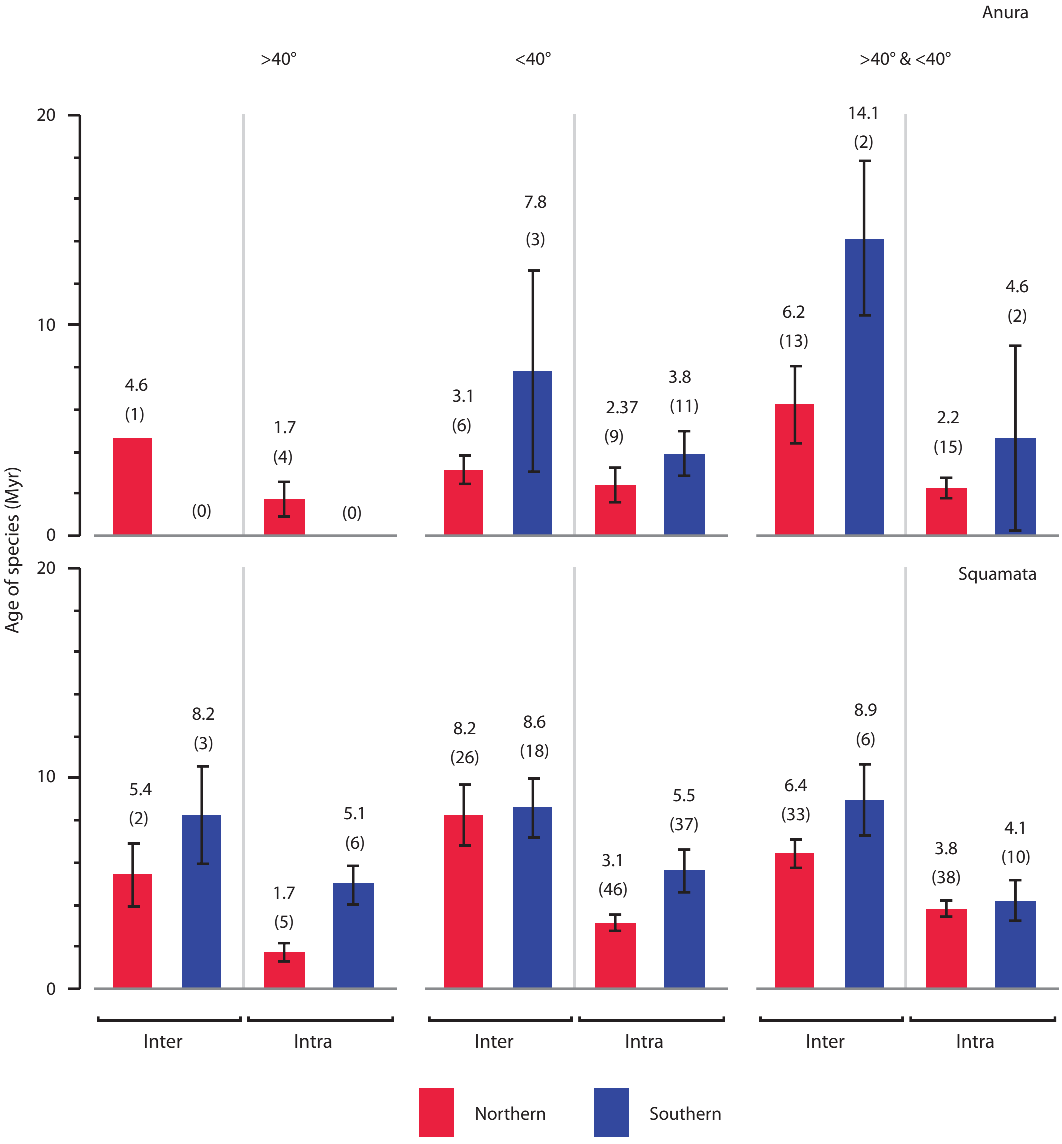

Fig. 2 Mean values and standard errors for the estimates of intraspecific and interspecific ages (millions of years ago: Ma) of Northern Hemisphere vs. Southern Hemisphere species in Anura and Squamata from comparable latitudinal range (with the number of comparisons included in our data set). The taxa were divided into (i) species distributed at high latitudes only (above 40°N or S), (ii) species restricted to lower latitudes only (between the tropics and 40°N or S) and (iii) species distributed in both of the above latitudinal ranges.

Historical patterns in the number of described species

Over the last 40 years, many more new species of reptiles and amphibians have been described from the Southern Hemisphere than from the Northern Hemisphere (Fig. 3). The known reptile fauna of the Southern Hemisphere in 1970 was only 64% of that currently recognized, whereas 93% of the taxa currently known for the Northern Hemisphere had already been described by that time (Fig. 3). For amphibians, the corresponding figures are 64% and 80% (Fig. 3). These geographic differences have decreased but not disappeared (Fig. 3), suggesting that coming years likely will see more new species described from the Southern than from the Northern Hemisphere.

Effects of omission of species on estimated divergence dates

Our simulations of taxonomic ignorance (i.e., omission of various proportions of taxa from phylogenetic trees) showed that underestimating species richness can inflate the estimated age of divergence events ($F_{1,399}$ = 120.60,

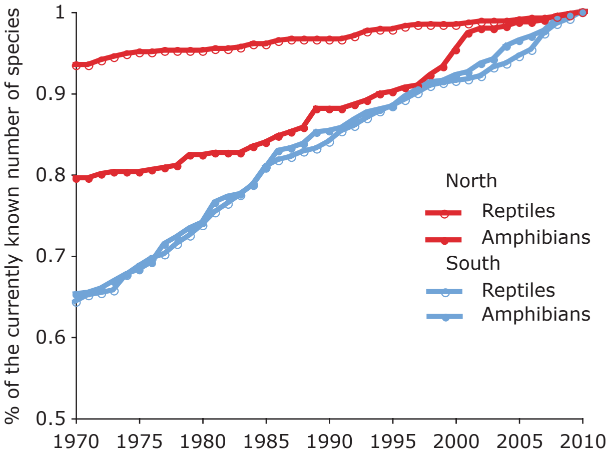

Fig. 3 Rate of taxonomic description of new species of amphibians and reptiles in Northern vs.Southern Hemisphere from 1970 to 2010, showing the cumulative number of known taxa at each date as a proportion of the total number of currently described species in each group in each Hemisphere (see Materials and Methods for more details).

$r^2 = 0.23$, $P < 0.0001$; $F_{1,399} = 51.33$, $r^2 = 0.11$, $P < 0.0001$; $F_{1,399} = 83.70$, $r^2 = 0.17$, $P < 0.0001$ for an extinction rate of respectively 1/3, 2/3 or equal to the speciation rate). For example, omitting 1/3 of species increases the estimated mean age for the split between sister species by 51% to 65% depending on the speciation rate (Fig. 4). Could this bias cause the calculated differences in mean interspecific age in amphibians and reptiles between Southern and Northern hemispheres (Fig. 1)? In order for those age differences to be entirely due to taxonomic ignorance, our simulations suggest that the proportion of as-yet-undescribed taxa in the Southern Hemisphere would need to be from 12% to 16% for reptiles, (realistic, based on Fig. 3), and from 50% to 64% for amphibians, depending on the extinction rate (unlikely: Fig. 3). The calculated divergence in ages of reptile species thus may well be a methodological artefact, but the same is unlikely to be true for amphibians unless current estimates of species richness in Southern Hemisphere anurans are massively in error.

Discussion

Our analyses clarify but do not entirely resolve the phenomenon of apparent differences in the mean age of reptile and amphibian species from temperate-zone habitats in the Northern vs. Southern Hemispheres. We can reject one plausible explanation that the geographic difference detected in our previous analysis (Dubey & Shine, 2011) was an artefact of using intraspecific rather than interspecific divergence times, because these measures can be influenced by very different factors. Contrary to this idea, we saw virtually identical geographic patterns in species age regardless of whether our analysis was based on intraspecific divergences (across orders and suborders) or on the cladogenic events responsible for species formation, suggesting that the same parameters influence these two measures. We are left with two other potential explanations: that the difference is real (possibly driven by climatic history) or that the difference is an artefact of geographic variation in the magnitude of taxonomic ignorance. The latter explanation is difficult to test empirically, because there is no way to measure the number of yet-to-be-described species in different parts of the world. However, our simulations suggest that a disparity in means species ages of the magnitude seen in reptiles could be explained by a plausibly low proportion of yet-to-be-described taxa (about 15%) in the temperate-zone habitats of Southern Hemisphere. The same explanation is less satisfying for amphibians, because it would require about half to two-thirds of Southern Hemisphere anuran taxa to be as-yet-undescribed. Nevertheless, that scenario is not impossible: a recent study suggested that about half of the amphibian species

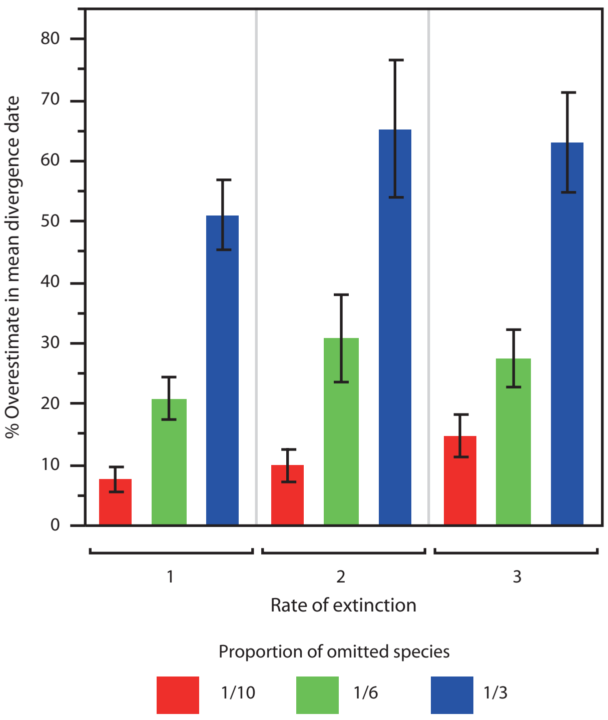

Fig. 4 The effects of taxonomic ignorance on estimated divergence times within phylogenies. The graph shows the relationship between the percentage of extant species in a hypothetical phylogeny that are omitted (as would occur if those species were as-yet undescribed) and the estimated mean ages of sister-species divergence events. The calculations are based on simulated trees containing 30 species and using birth–death models with three rates of extinction (1: 1/3, 2: 2/3, or 3: equal to the speciation rate).

of Madagascar are as yet undescribed (Vieites *et al.*, 2009).

The apparently greater geographic divergence in species ages in amphibians than reptiles thus may be attributable to biological factors rather than taxonomic or methodological artefacts. Amphibians tend to be more highly dependent on moisture than are reptiles and hence attain their highest species richness in cooler moister regions than do reptiles (Buckley & Jetz, 2007; McCain, 2010; Powney *et al.*, 2010). That divergence may have resulted in differential impacts of Pleistocene climatic fluctuations on the two groups. Extensive glaciation in the Northern Hemisphere may have left very few refuges for either amphibians or reptiles over very broad areas (Hewitt, 2004; Joger *et al.*, 2007), whereas for example the most significant Pleistocene climatic challenges in Australia may have been pulses of extreme aridity (Byrne *et al.*, 2008). Such pulses likely had less effect along the well-watered regions of Australia's east coast (where anuran species richness is high) than in the arid zone where reptiles dominate the vertebrate fauna (Williams, 2000; Powney *et al.*, 2010). Even during periods when much of central Australia was covered by sand dunes (Byrne *et al.*, 2008), extensive forest persisted along the eastern coast (Markgraf *et al.*, 1995). Hence, Pleistocene aridity may have eliminated a higher proportion of species-level lineages within Australia's reptiles than its anurans.

We are thus left with two plausible explanations for the strong trend for species of temperate-zone reptiles and amphibians from the Northern Hemisphere to be younger, on average, than are those from equivalent latitudes in the Southern Hemisphere. The difference may be due to evolutionary history (Pleistocene climatic extremes), perhaps differentially affecting reptiles and amphibians, or to the differing levels of taxonomic knowledge of the fauna in these two regions. Future work could usefully look for similar patterns in other lineages of vertebrates, invertebrates and plants. Broad climatic effects likely will be similar for sympatric taxa. To identify the role of ecological (habitat-specific) factors in the timing of cladogenic and extinction events, we will need detailed phylogenetic analyses of a much wider range of taxa than have been studied to date. However, knowledge in this field is accumulating rapidly, and at an ever-increasing speed as molecular phylogenetics methods become stronger, quicker and cheaper. We confidently expect that within a few years, the data will indeed be available to look for correlations between species age and specific habitat types (e.g., arid/mesic). The increasing availability of such data sets provides an exciting opportunity for researchers to ask questions about the timing of divergence events, and the reasons for taxonomic and geographic disparities in that timing, that have heretofore been inaccessible to empirical study.

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Supporting information

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

Appendix S1 Raw data on the age of species, based on (i) the oldest intraspecific diversification event (as based on molecular dating) within each taxon and (ii) the split between sister species.

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