

Evolutionary History of Amphibians in Biodiversity Hotspots

A Senior Honors Thesis

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By

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Abstract

Human activities are causing a global extinction event that rivals mass extinctions of the past. To counteract this crisis, conservationists have designated biodiversity hotspots, regions with exceptionally high species diversity that face imminent destruction. Setting aside these hotspots would be especially compelling if they contained not just enormous numbers of species but also excessive evolutionary history (i.e., older-than-typical lineages). A recent study seemed to provide evidence for this extra incentive for hotspot conservation. Sechrest *et al.* (2002) reported that hotspots contain more evolutionary history than expected based on the numbers of primate and carnivore species they contain. A recent study in our lab contested this claim, particularly for primates. We showed that the original analysis was driven by a single hotspot (Madagascar) that contains an ancient endemic clade. The remaining hotspots will not protect more evolutionary history than expected based on species numbers alone. In fact, for primates, these hotspots contain less evolutionary history than expected. Global conservation initiatives should not be developed under the false impression that hotspots generally contain excessive evolutionary history. In my study, we examined whether hotspots contain more (or less) evolutionary history of amphibians than expected. We used a phylogenetic tree of amphibian families combined with information on species within 34 hotspots. We compared the evolutionary history of species endemic to these hotspots with the amount represented by the same number of species chosen at random from the phylogeny. We found that hotspots do contain significantly more amphibian evolutionary history than expected, and that the vast majority of this extra evolutionary history is contained within just a few hotspots. Our findings provide new support for the global initiative to set aside biodiversity hotspots.

Introduction

The global extinction crisis demands immediate steps to conserve biodiversity. One step is setting aside biodiversity hotspots (1), originally defined as regions containing 1500 or more endemic plant species and with less than 30% of their original natural habitat remaining (2). These areas contain high endemic plant species richness and diversity. So, by conserving geographic regions of high plant endemism, we may succeed in conserving huge numbers of species in other taxa as well.

However, some conservationists have promoted an alternative approach. They have argued in favor of conserving areas with rich evolutionary history, or phylogenetic diversity (PD) (3-7). They argue that conserving the distinct lineages contained within such areas will maintain potential for evolutionary diversity. Under this optimistic view, maintaining the status quo (i.e., by continuing to identify hotspots based on endemic plant species) would be satisfactory if these areas also happen to contain excessive evolutionary history.

A recent study by Sechrest *et al.* (8) seemed to reinforce this conventional approach to identifying hotspots. By comparing observed values of PD in current biodiversity hotspots with expected PD values based on species richness, they reported that hotspots contain significantly excessive amounts of primate and carnivore PD. However, a reanalysis, conducted by Spathelf and Waite (9), suggests the opposite. Through an analysis where individual hotspots were systematically removed, a “leave-one-out” analysis, they claim that hotspots do not generally contain more PD than expected based on the number of species they contain. They concluded that the findings by Sechrest *et al.* had been driven by the enormous amount of primate PD found in Madagascar. Performing a detailed reanalysis, Spathelf and Waite found that, with the exception of Madagascar, hotspots do not contain more PD than expected. The remaining

hotspots appear to contain significantly *less* PD than expected (i.e., they contain younger-than-typical primate lineages).

Here, we apply the same methods that Spathelf and Waite used in order to quantify amphibian PD in hotspots. Worldwide, amphibians face serious threats. Like other taxa, they are sensitive to habitat destruction, but their physiological and developmental attributes make them especially sensitive to UV radiation, soil, water and air contaminants, and global warming (10-12). This sensitivity has led to their unofficial designation as the “miner’s canaries” in conservation biology (13). Knowing where species and phylogenetic diversity is concentrated should aid conservation planning (13).

Further motivation for this study was provided by the recent Amphibian Conservation Summit held in Washington, D.C., in September 2005. The summit’s declaration calls for: expanded understanding of the causes of declines and extinctions, ongoing documentation of amphibian diversity, development and implementation of conservation programs, and emergency responses to immediate crises. The goal of its action plan is to engage the global human community in fighting against the massive population declines documented throughout the world (14).

Our main objective here was to evaluate whether hotspots contain more PD in hotspots than expected based on the numbers of species they contain. We evaluate whether hotspots in general, and which in particular, will do a reasonable job of conserving amphibian evolutionary history. We also focus our attention on gymnophiona, an understudied order of amphibians (15), using the same techniques used for the whole tree of amphibians.

Methods

Designation of species

We designated species according to three criteria: 1) whether each species was strictly endemic to a single hotspot, 2) whether each species occurred in each hotspot, and 3) the identity of the hotspot for each endemic species. A slight majority of amphibians are endemic to hotspots (2515 endemic species of 5028 total; 2880 species occur in hotspots).

The same designations were also used for gymnophiona species, except the designation of data-deficient species (for definition see www.redlist.org), or species with a lack of population information, was used rather than the hotspot occurring designation. Like the two other orders of amphibians, many gymnophiona species are endemic to hotspots (77 of 168 species), while a high proportion of species are data deficient (111 of 168 species).

Our categorizations and hotspot information were obtained from the Global Amphibian Assessment website at www.globalamphibians.org (16) and the IUCN Red list of endangered species at www.redlist.org (17).

Tree of amphibians

We used the family tree of amphibians published by Hay *et al.* (Fig. 1), based on mitochondrial 12S and 16S RNA ribosomal genes (18). We modified the tree so that our analysis would reflect extant amphibians as accurately as possible. To do so, we eliminated 12 families (of 38 published) that no longer exist taxonomically (16). The tree was then saved as a Nexus file in order to edit and calculate evolutionary history contained in all hotspots.

For our analysis of gymnophiona, we used a tree generated by San Mauro *et al.* (Fig. 2), based on the mitochondrial genome and RAG1 (19). That tree was also saved as a Nexus file for editing and calculation of evolutionary history.

Phylogenetic diversity (PD)

For the main analysis, we used a measurement of evolutionary history called clade phylogenetic diversity. It measures the evolutionary history represented by a set of species and is calculated by summing all branch lengths originating from a common node (Fig. 3) (20). Shared branches are counted just once. Because the tips of the tree were families rather than species, we implicitly assigned identical branches lengths of zero to each individual species. Another measurement of evolutionary history, species phylogenetic diversity, was used by Sechrest *et al.* (8), but was not used in this analysis because we did not have evolutionary history recorded for individual species.

For a comparison of PD and endemism in gymnophiona, we used a modified version of the clade PD metric. As in the main analysis, PD for individual species was not recorded. Unfortunately, the small number of families (6) representing gymnophiona created a quantized pattern where PD took one of just seven possible values. This pattern arose from operationally assigning zero as the length of the terminal branch for each species. To adjust for this effect, we calculated the PD for each family and then multiplied those values by the number of species (endemic or data-deficient) in hotspots.

Using MeSA to analyze PD

We used MeSA to calculate PD, run simulations, and edit trees loaded into a matrix. MeSA produces a Nexus file as the output file. We loaded the modified family tree of amphibians (18), as well as the gymnophiona tree (19), into a program queue that randomly removed a specified number of target species. The PD of the remaining species was then calculated and recorded. MeSA was used to perform these actions 1000 times for each global endemic, hotspot-occurring, data-deficient, and leave-one-out analysis (see below). This allowed us to average the values of PD to produce “expected” values and to use the distribution of random values to calculate a *P*-value for the observed amount of PD. Specifically, we generated a distribution of 1000 random PD values against which to compare the calculated PD value for species in hotspots. We computed the *P*-value as the proportion of random values more extreme than the observed PD. We performed the same protocol for the gymnophiona species. The endemic, occurring, gymnophiona endemic, data-deficient and hotspot trees were edited by Tree Edit and Tree Thief and loaded into MeSA to calculate PD (observed values) (21).

Results

Randomization tests

Our main analysis shows that biodiversity hotspots contain significantly more clade PD than expected based on species numbers alone (Table 1). The observed PD for amphibian species globally endemic to hotspots is 2.1% greater than the expected value ($P < 0.001$), and observed PD for amphibian species that occur in one or more hotspots is 1.5% greater than the expected value ($P < 0.001$). Hotspots contain approximately 5.1 billion years of evolutionary history more than expected summed across the globally endemic species and approximately 4.2 billion years more than expected summed across the hotspot-occurring species (Table 1).

As with the above analysis for all amphibians, our analysis restricted to gymnophiona (Table 2) provided evidence for a concentration of clade PD within some hotspots. Overall, hotspots contained significantly more evolutionary history than expected based on the numbers of gymnophiona species they contained. They contained 40.5% more than expected ($P = 0.01$; Table 2) for this clade, which contains an unusually large proportion of data-deficient species (see below).

Leave-one-out analysis

Our results show that the original finding of excessive clade PD within hotspots (Table 1) did not arise as a spurious byproduct of some extreme concentration of PD within any particular hotspot. No matter which individual hotspot was temporarily removed from the analysis, the original finding was supported. The remaining hotspots always contained significantly more PD than expected (all P s < 0.001 ; Table 3) and the difference between observed and expected PD showed only minor variation (Fig. 4).

By contrast, the analysis restricted to gymnophiona produced a more complex pattern. In all cases, the initial evidence that hotspots contain excessive evolutionary history for gymnophiona was lost (Table 4), regardless of which hotspot had been temporarily removed. In most cases, removal of a hotspot seemed to suggest that the initial evidence for excessive PD in hotspots was largely due to that particular hotspot (Fig. 5). The variable effect of removing individual hotspots reflects the very small number of species (globally endemic [77] minus data deficient [53] = 24) included in the analysis (Table 2).

Data deficiency within gymnophiona

Data-deficient species represent over half (54%) of the total PD of endemic species within gymnophiona. These data-deficient taxa also represent 22% more PD than expected, a nearly significant excess ($P = 0.06$; Table 2). The proportion of PD represented by these species varies widely among hotspots (Fig. 6). The greatest proportion is found in the Tumbes-Chocó-Magdalena hotspot, where all (both) of the species are data deficient. Lower proportions but greater numbers of species are data deficient in other hotspots: 15 of 17 species are data deficient in the Tropical Andes and 10 of 12 species are data deficient in Sundaland.

Endemicity and PD

A comparison of PD and endemicity revealed a strong positive relationship between numbers of endemic species and amounts of PD contained within hotspots. This relationship was similarly strong for hotspot endemic species throughout the amphibian phylogeny ($r^2 = 0.94$) and for those in gymnophiona ($r^2 = 0.91$) (Fig. 7a) and b), respectively). These tight, positive relationships

suggest that it might be reasonable to use endemic species richness as a surrogate for evolutionary history.

Distribution of endemic species and PD among hotspots

The biodiversity hotspots vary widely in endemic species richness. Tropical Andes ranks highest with 673 globally endemic species and Succulent Karoo is at the bottom with just a single species (Fig. 8a). (Only 13 of the 34 hotspots contain gymnophiona species [Fig. 8b]. Tropical Andes again ranks highest with its 17 globally endemic species within gymnomphiona.)

Considering the tight relationship between endemism and PD, it makes sense that the vast majority of amphibian PD is contained within just a few of the biodiversity hotspots (Fig. 9).

Discussion

Amphibian PD and Biodiversity Hotspots

Our analysis revealed that hotspots do contain more evolutionary history than expected based on species richness alone. In other words, hotspots as a group do contain older-than-typical amphibian lineages. The leave-one-out analysis showed that no single hotspot was responsible for producing this result. This reinforces our claim that hotspots contain “extra” phylogenetic diversity for amphibians.

Why hotspots contain more evolutionary history than expected for amphibians, but not primates (9), is a compelling question that needs further study. For now, we emphasize that the findings in this paper provide a new incentive for setting aside biodiversity hotspots. We have shown, for at least amphibians, that more evolutionary history than expected could be conserved by setting aside hotspots. Whether the same can be said for other taxa awaits study.

Our findings, as possible support for hotspot conservation initiative, should be interpreted cautiously. Our results show an uneven distribution of endemic amphibian species across hotspots, implying that most hotspots will do little to conserve amphibian evolutionary history. Only a few hotspots contain most of the global evolutionary history of amphibians. It will be interesting to see whether a similar pattern emerges for various other taxa. If so, the design of the global network of hotspots could be optimized accordingly. It may be possible to reduce the set-aside costs of a global system of hotspots while conserving an increased amount of evolutionary history.

PD and Endemicity

Our analysis revealed a tight positive correlation between endemic species richness and PD. This result prompts the question, “Why even worry about evolutionary history?” This is a legitimate question in light of the need for emergency measures to save declining populations of amphibians and other taxa. If the need to save species is urgent, why not just save as many species as possible and not worry about phylogenetic diversity per se?

One counterargument is that phylogenetic analysis can provide clues about underlying causes of extinction and population decline. For example, if we know that a certain species is at risk, it may be reasonable to assume that many of its close evolutionary relatives are also at risk (22). Corey and Waite (23) have recently discovered extreme clumping of specific kinds of extinction threat within the amphibian phylogeny. In addition, phylogenies can be used not only to identify which groups of species might be extinction-prone due to shared evolutionary history, but can also serve as a model for the evolutionary processes that have generated those species (24). Evolutionary relationships of at-risk taxa can be used to identify the common environment that favored radiation of those species (25). Finally, knowledge about at-risk species strongly motivates research regarding their phylogenetic relationships (26). Knowing as much as we can about a species now, may help us to recognize indicators of future risk.

But how should we meet the immediate needs? If endemicity is tightly correlated to PD, we should be able to conserve endemic species and conserve PD at the same time. In general, areas of high endemicity do not necessarily overlap with areas rich in phylogenetic diversity. This lack of spatial congruence reflects the fact that older species tend to be more widespread than younger species (26, 27). If we simply target centers of endemicity, we may do so at the cost of discarding older taxa. On the other hand, habitat-restricted species, young and old, tend

to occupy similar regions (26). These conclusions suggest that the value of phylogenetic analysis for emergency conservation policy may depend on the taxa in question. For amphibians, this dilemma depends on their ability to distribute themselves and the restrictiveness of their ranges. Rodrigues and Gaston (28) suggest that it is safe to use species richness as a predictor of PD as long as the phylogenetic tree of the taxa in question is relatively balanced, with even distribution of long and short branches, and that these two types of branches are not regionally separated. Our data suggest that endemism is a good indicator of PD, although tree balance could not be evaluated because we lacked branch lengths to the species level. Together, these considerations suggest that value of phylogenetic information should be ascertained on a taxon-by-taxon basis for urgent conservation needs. For amphibians, we have reason to believe that saving many endemic species will translate into saving much evolutionary history.

Data deficiency and gymnophiona

Hotspots contain more PD in gymnophiona than expected, but the amount of PD represented by data-deficient species is a concern. Over half of the gymnophiona species, and 68% of the endemic species, are data deficient. This is nontrivial considering the massive losses and declines of amphibians throughout the tree and across the globe. The rates at which other species of amphibians have been declining calls for action in understanding evolutionary processes for this understudied order. We may be losing clusters of PD that we will never get a chance to study (19). The level of ignorance about gymnophiona combined with the fact that so few of the current hotspots contain gymnophiona species suggests that we may be losing PD before we even discover the species that represent it.

Identifying regions to maximize amphibian PD

Acknowledging the possibility that current hotspots are less than ideal for amphibian conservation, how should we identify those regions that are good refuges for amphibian evolution? The first, most basic approach is to target areas with high amphibian endemism that face imminent threat by human activity. In the original criteria, hotspots were identified in part by the number of endemic plant species they contained. Later additions to the list of hotspots considered birds, amphibians, mammals, and reptiles, as well as plants (7). To identify amphibian hotspots per se, it would be necessary to focus on centers of amphibian endemism. However, because the balance in the phylogenetic tree of amphibians is poorly understood, it may be necessary to establish additional criteria for amphibian hotspots such as the distribution of old and young species, the ecological importance of a species to other endemic species, or the level of knowledge about a species. Phylogenetic analyses could be used to identify regions with high PD to ensure targeting of both young and old clades (28).

Conclusion

We found that hotspots contain significantly more amphibian phylogenetic diversity than expected. The bulk of this wealth of evolutionary history is concentrated in just a few hotspots, suggesting that the current system of hotspots may be inadequate for amphibian conservation. We would like to continue studying the conservation of hotspots and amphibian phylogenetic diversity by using a new, updated phylogenetic tree of amphibians. We would also like to develop and perform new analyses to study the relationships among endemism, phylogenetic diversity, and hotspot distribution. Future studies will create a rank-ordered list of politically defined regions by species richness and PD, quantify the amount of PD potentially lost with the

loss of the best hotspots for amphibians, and quantify PD in hotspots for other taxa. These and other studies will contribute to the increased role of phylogenetic analysis in efforts to conserve biodiversity worldwide (29).

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Table 1. Results of randomization tests for species globally endemic to hotspots and species occurring in hotspots. The amount of clade PD (my) represented in these species was compared to the amount from random samples of the same numbers of species from throughout the phylogeny.

	No. species	Phylogenetic diversity (my)		<i>P</i> -value	Difference	
		Observed	Random		Observed-Random	%
Global endemic	2515	248161.17	243072.50	<0.001	5088.66	2.1
Hotspot occurring	2880	282511.02	278260.66	<0.001	4250.35	1.5

Table 2. Results of randomization tests for endemic and data-deficient gymnophiona species in hotspots.

	No. species	Modified Clade PD (my)		<i>P</i> -value	Difference	
		Observed	Random		Observed-Random	%
Globally endemic	77	1846.26	1313.85	0.01	532.41	40.5
Data deficient	53	1846.26	1506.16	0.06	340.10	22.6

Table 3. Summary of the leave-one-out analysis for all amphibian species endemic to hotspots. Remaining hotspots contained significantly more PD than expected, regardless of which hotspot was temporarily removed from the analysis (all $P_s < 0.001$).

Hotspot left out	No. endemic hotspot species	Phylogenetic diversity (my)		Difference	
		Observed	Random	Observed-Random	%
Atlantic Forest	275	223345.34	217425.19	5920.15	2.7
California Floristic Province	24	245254.54	240500.62	4753.92	2.0
Cape Floristic Region	8	247335.62	242265.77	5069.84	2.1
Caribbean Islands	170	230208.08	225950.00	4258.07	1.9
Caucasus	2	247990.21	242930.25	5059.96	2.1
Cerrado	28	245954.31	240805.15	5149.16	2.1
Chilean Winter Rainfall - Valdivian Forests	27	245289.77	240396.50	4893.27	2.0
Coastal Forests of Eastern Africa	5	247565.32	242529.00	5036.32	2.1
East Melanesian Islands	38	244508.70	239041.84	5466.86	2.3
Eastern Afromontane	41	244097.50	239478.00	4619.51	1.9
Guinean Forests of West Africa	51	242818.21	237958.03	4860.18	2.0
Himalaya	23	245861.01	240846.68	5014.33	2.1
Horn of Africa	6	247632.23	242563.11	5069.12	2.1
Indo – Burma	90	239107.35	234201.29	4906.06	2.1
Irano – Anatolian	2	247947.51	242919.52	5027.99	2.1
Japan	22	246113.09	241003.88	5109.21	2.1
Madagascar and Indian Ocean Islands	229	224299.60	220307.78	3991.82	1.8
Madrean Pine - Oak Woodlands	50	242642.99	237959.18	4683.80	2.0
Maputaland - Pondoland - Albany	6	247537.99	242522.26	5015.73	2.1
Mediterranean Basin	25	245691.75	240695.63	4996.12	2.1
Mesoamerica	357	212167.55	207926.38	4241.17	2.0
Mountains of Central Asia	2	247968.59	242891.69	5076.90	2.1
Mountains of Southwest China	5	247744.05	242718.11	5025.94	2.1
New Zealand	4	247782.16	242705.66	5076.50	2.1
Philippines	65	241713.24	236760.37	4952.87	2.1
Polynesia – Micronesia	3	247872.30	242820.57	5051.74	2.1
Southwest Australia	5	247501.59	242416.97	5084.61	2.1
Succulent Karoo	1	248049.36	243008.10	5041.26	2.1
Sundaland	122	236190.38	231254.64	4935.74	2.1
Tropical Andes	673	185216.57	179049.42	6167.15	3.4
Tumbes - Chocó - Magdalena	18	246304.36	241361.02	4943.34	2.0
Wallacea	42	244215.51	239265.48	4950.03	2.1
Western Ghats and Sri Lanka	70	241094.99	236208.93	4886.06	2.1

Table 4. Results of leave-one-out analysis for gymnophiona species endemic to hotspots. The results show a more complicated pattern of percent difference than the analysis for global amphibians.

Hotspot left out	Expected PD (my)	Observed PD (my)	% difference	<i>P</i>-value
Atlantic Forest (5:3)	510.13	350.51	-31.29	0.03
Coastal Forests of Eastern Africa (1:0)	333.66	350.51	5.05	0.38
Eastern Afromontane (3:2)	436.98	465.61	6.55	0.43
Guinean Forests of West Africa (6:3)	541.55	465.61	-14.02	0.24
Himalaya (1:0)	330.82	336.05	1.58	0.37
Indo - Burma (4:2)	472.92	465.61	-1.54	0.44
Madagascar and Indian Ocean Islands (6:0)	543.71	350.51	-35.53	0.01
Mesoamerica (9:6)	617.80	350.51	-43.26	0.00
Philippines (3:2)	433.59	465.61	7.39	0.42
Sundaland (12:10)	684.26	350.51	-48.78	0.00
Tropical Andes (17:15)	769.10	465.61	-39.46	0.02
Tumbes - Chocó - Magdalena (2:2)	386.43	350.51	-9.29	0.19
Western Ghats and Sri Lanka (8:6)	600.89	465.61	-22.51	0.13

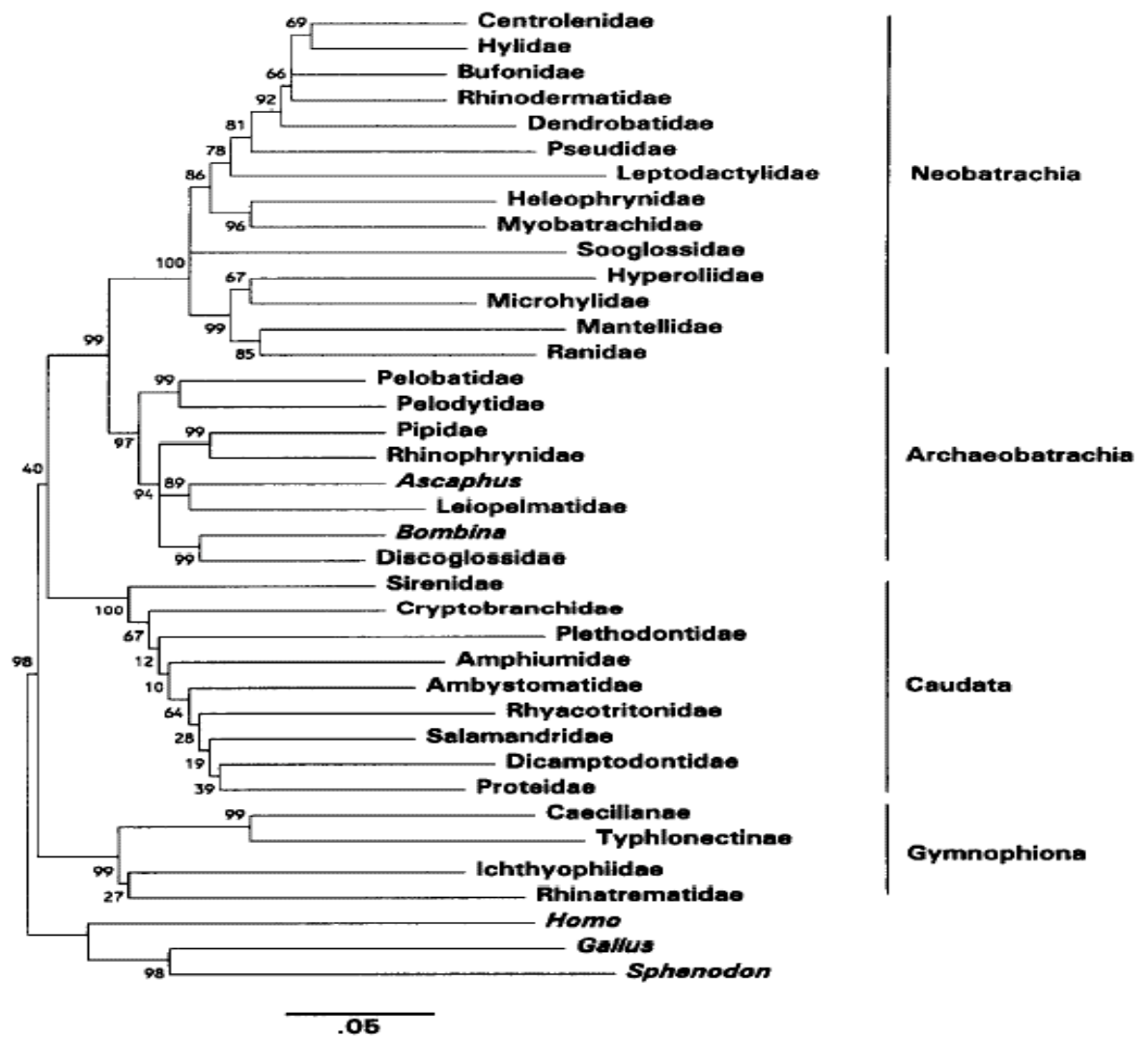


Figure 1. Amphibian phylogeny used in our analysis (redrawn from Hay *et al.* 1995) based on mitochondrial 12S and 16S ribosomal RNA genes.

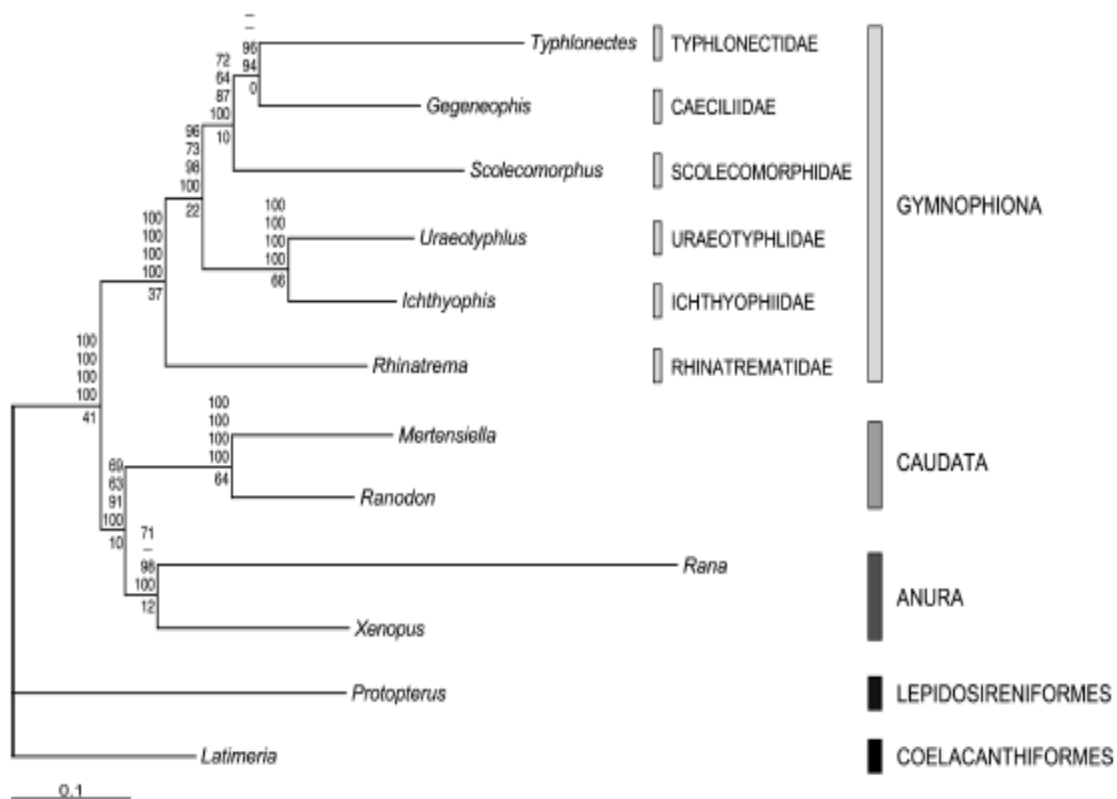


Figure 2. Phylogeny of gymnophiona used in our analysis (redrawn from San Mauro *et al.* 2004) based on the mitochondrial genome of gymnophiona and RAG1 gene.

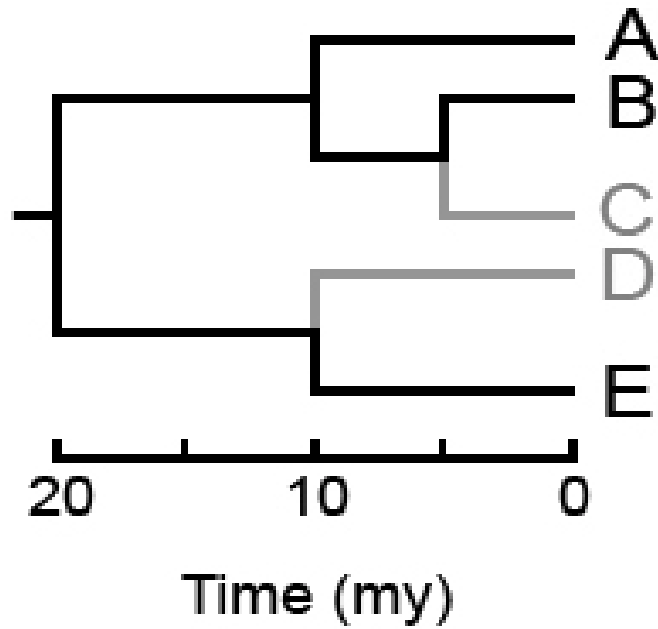


Figure 3. Hypothetical tree to illustrate the calculation of clade PD, which is the sum of all branch lengths for a set of taxa, with shared ancestral branches counted once. For example, clade PD for taxa A, B and E is 50 million years. (Note that a modified method was used for gymnophiona [Figs. 6-7].)

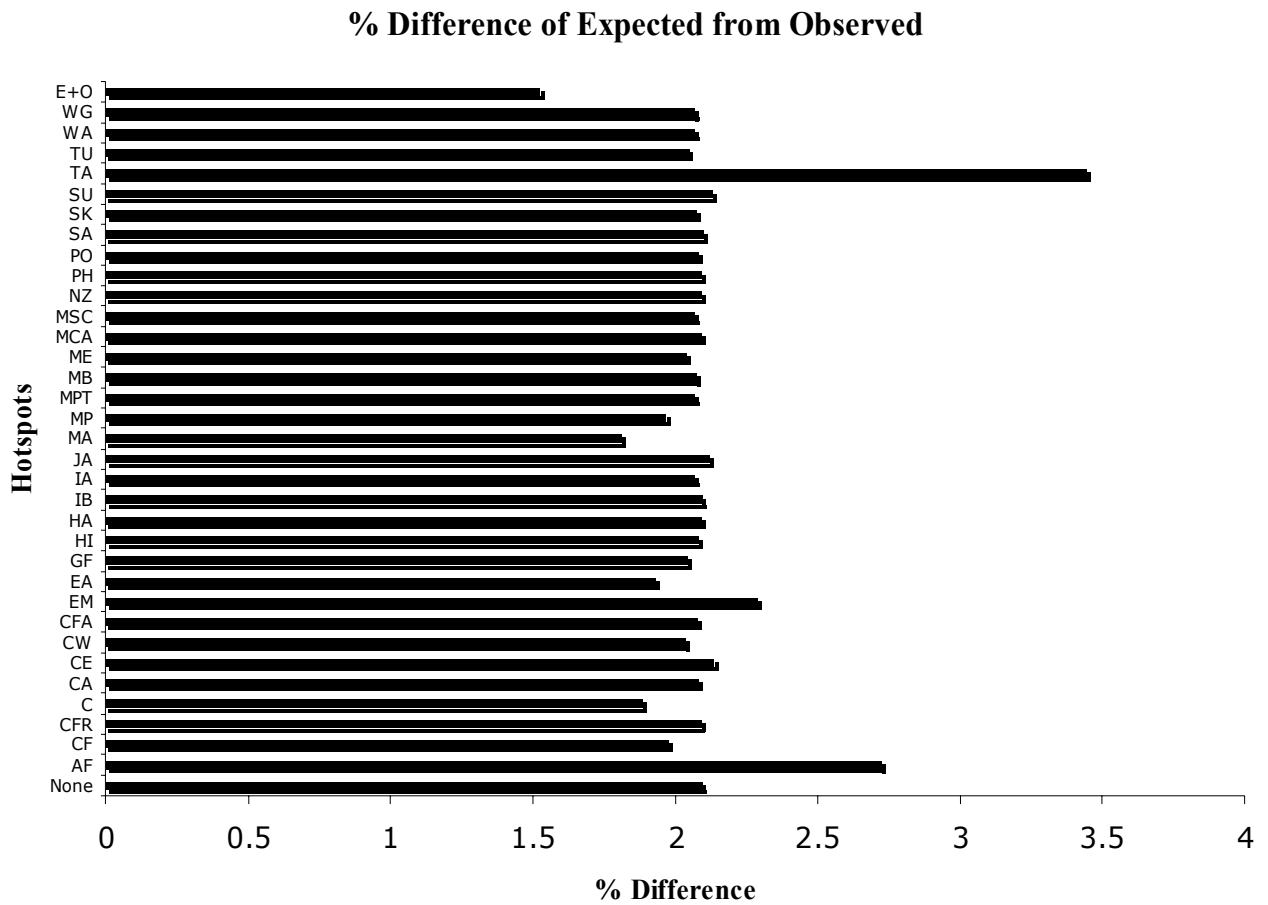


Figure 4. Difference between observed and random clade PD in the leave-one-out analysis for amphibian species endemic to hotspots.

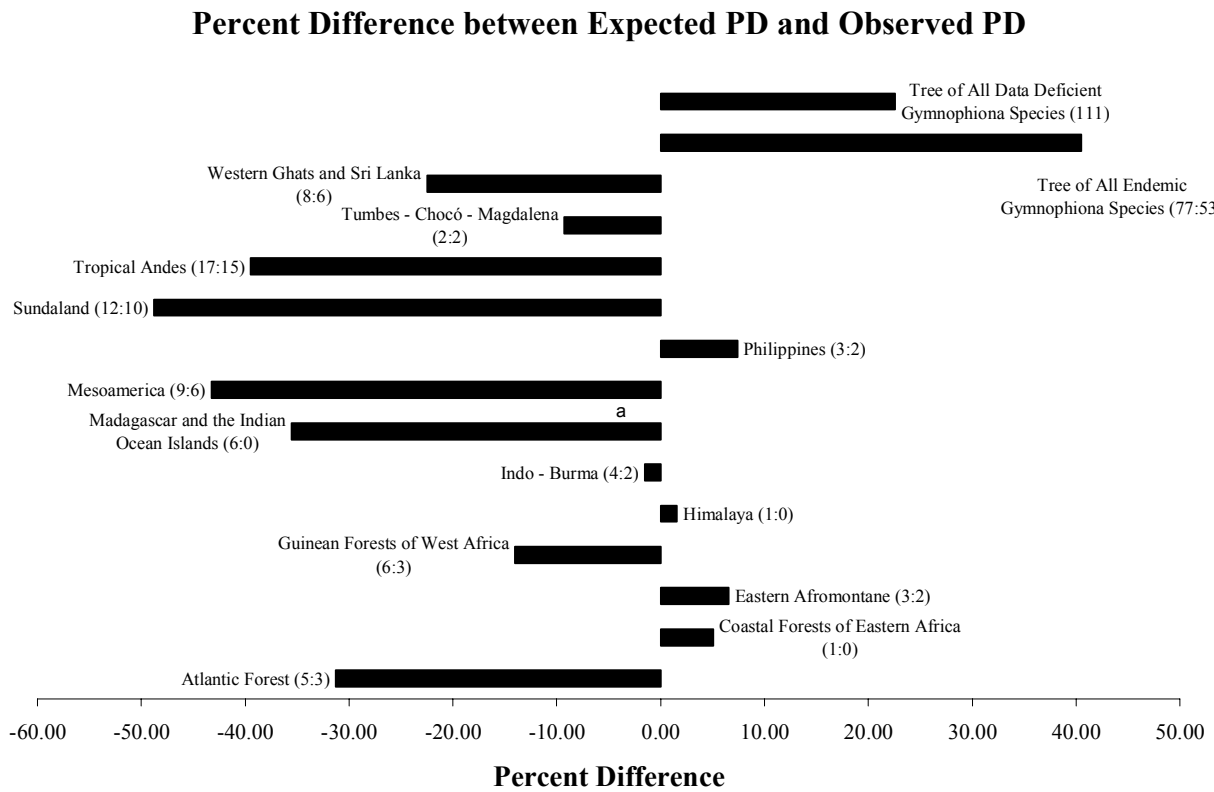


Figure 5. Difference between observed and random clade PD for the leave-one-out analysis of gymnophiona species endemic to hotspots.

Comparison of Proportion of PD represented by Gymnophiona and Data Deficient Species

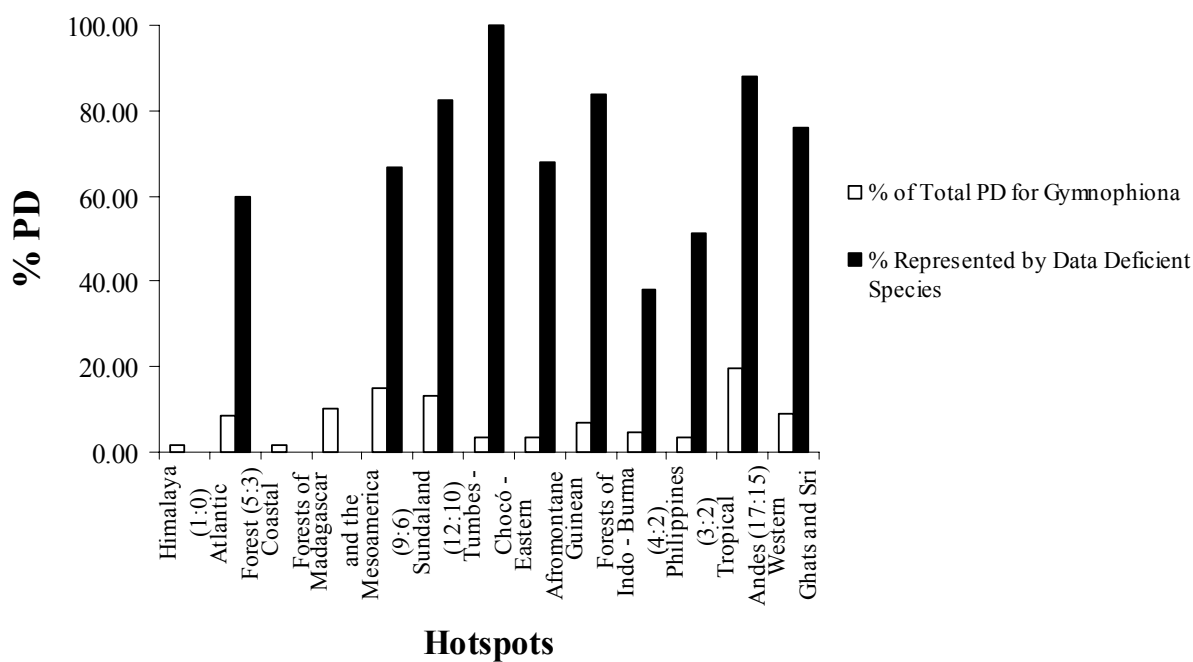
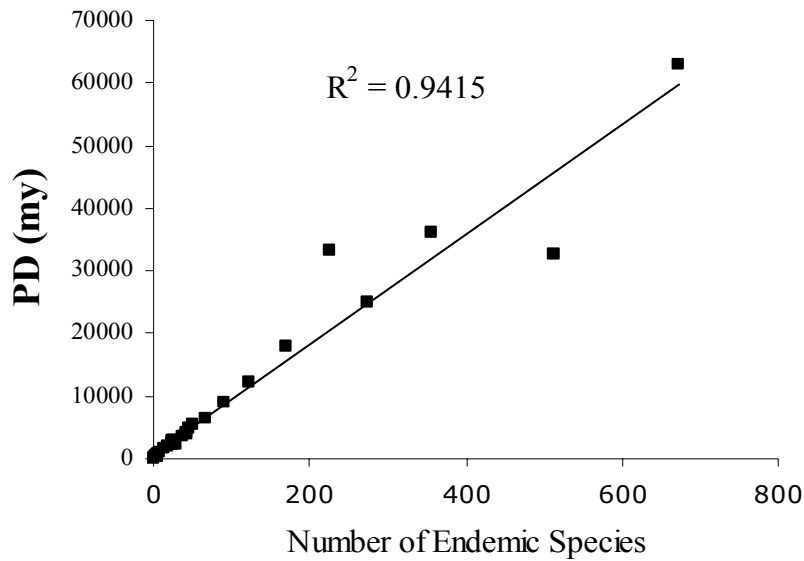


Figure 6. Percent of gymnophiona modified clade PD (methods) within each hotspot represented by data-deficient species and the percent of gymnophiona PD represented by endemic species in each hotspot.

Endemicity vs PD



Endemicity vs PD for Gymnophiona

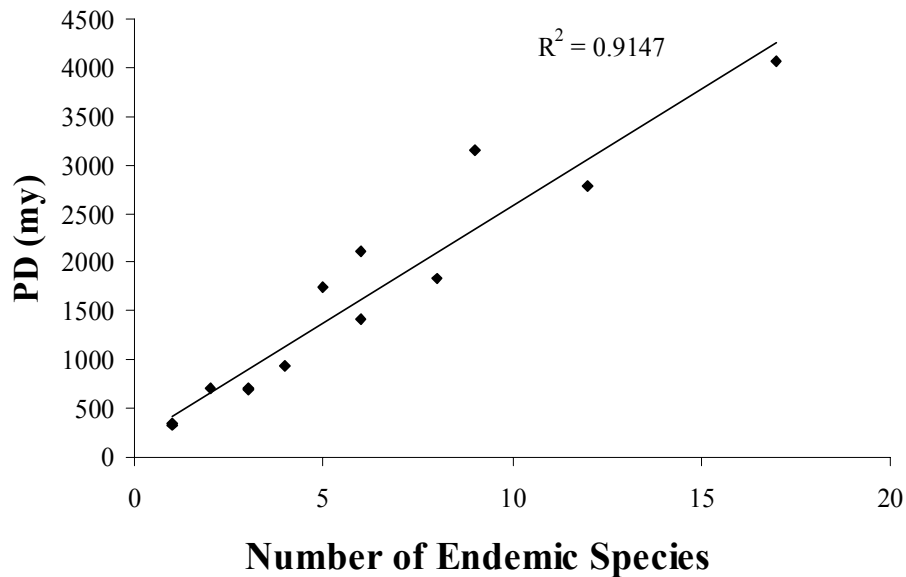
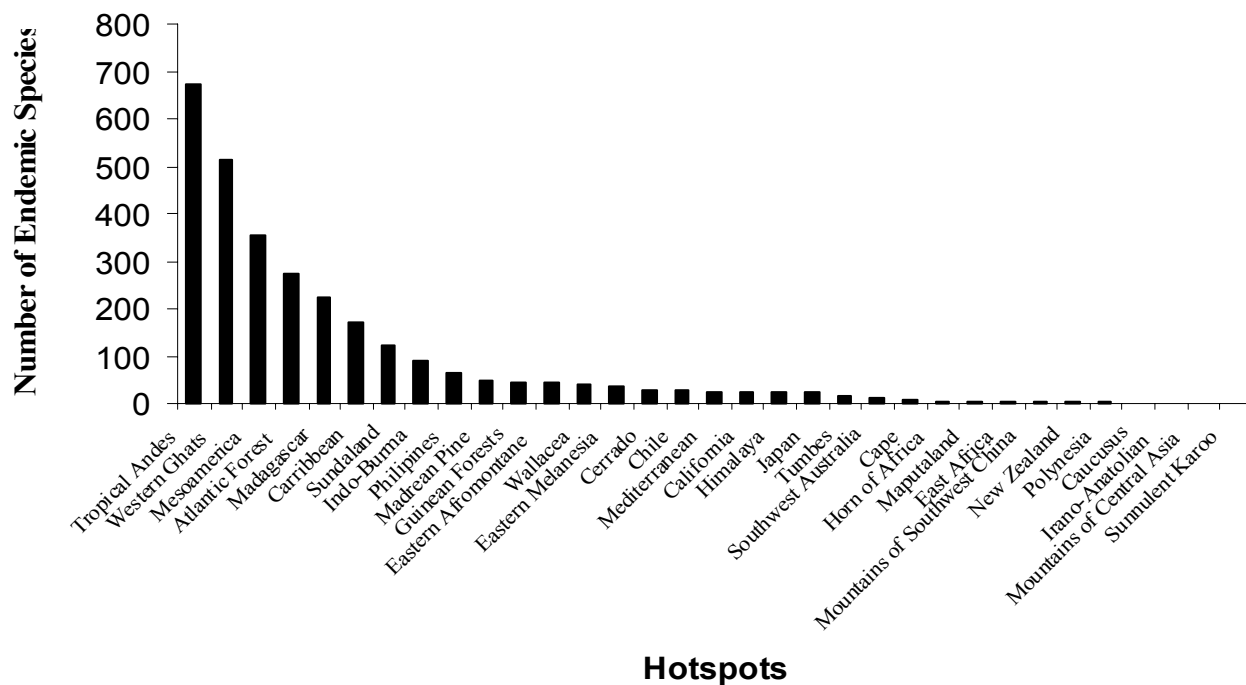


Figure 7. Relationship between number of endemic species and phylogenetic diversity, measured as clade PD for all amphibian species (top panel) and as modified clade PD (methods) for gymnophiona species (bottom panel).

Rank Ordering of Hotspots Based on Endemic Species



Rank Order of Gymnophiona Containing Hotspots

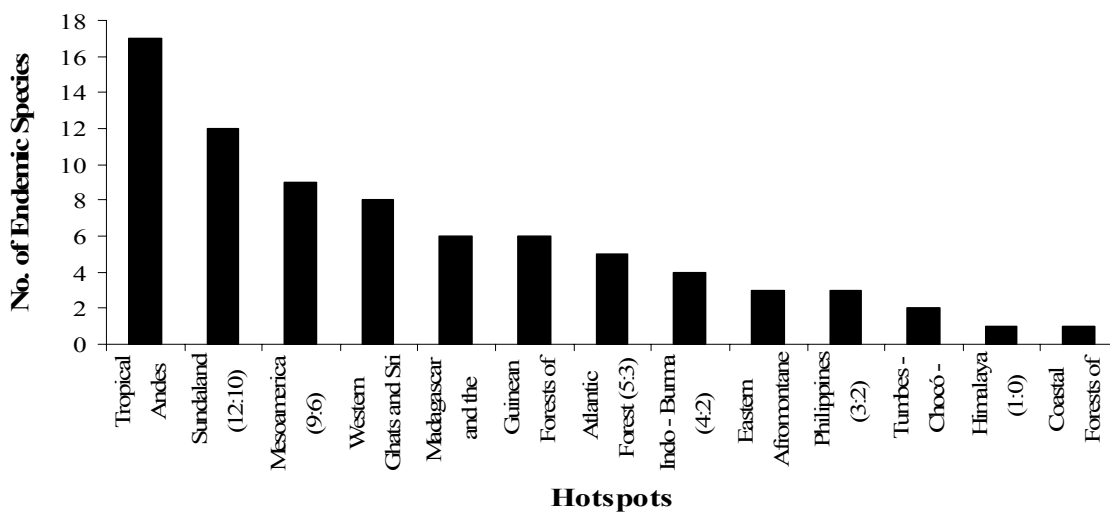


Figure 8. Hotspots ranked by number of endemic species for all amphibians (top panel) and gymnophiona (bottom panel).

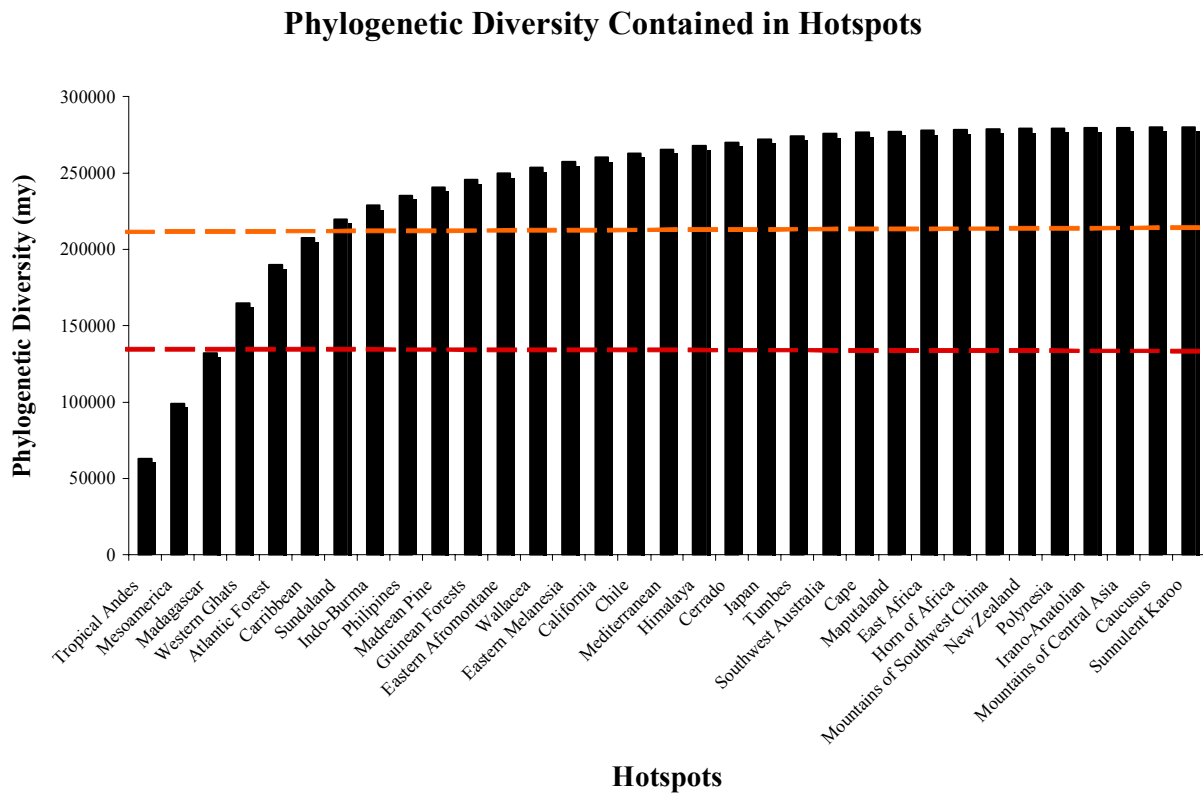


Figure 9. Cumulative clade PD contained within hotspots listed in descending order of PD.

Much of the evolutionary history of amphibians is represented within just a few hotspots.

Dashed lines indicate the 50th (red) and 75th (orange) percentiles.