Dispersal and vicariance: The complex evolutionary history of boid snakes

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Abstract

Since the early 1970s, boine snakes (Boidae: Boinae) have served as a prime example of a group whose current distribution was shaped by vicariant events associated with the fragmentation of the supercontinent Gondwana. Early phylogenetic treatments of this group, and what were thought to be closely related groups (Erycinae and Pythoninae) based on morphological features, produced a relatively stable view of relationships that has strongly influenced subsequent molecular-based work. We examined 4307 base pairs (bp) of nucleotide sequence data obtained from five nuclear loci (c-mos, NT3, BDNF, RAG1, and ODC) and one mitochondrial locus (cyt b) for all genera of erycines and boines, plus representatives of other groups, including those previously thought to be closely allied with boines (Ungaliophidae, Loxocemidae, Xenopeltidae, and Pythoninae). Our results suggest that the Boidae is not monophyletic, and its current division into three subfamilies (Erycinae, Boinae, and Pythoninae) does not accurately reflect evolutionary history. We find that the evolutionary relationships are better reflected by current geographic distributions and tectonic history than by the morphological characters that have long served as the foundation of boid phylogeny. Divergence time estimates suggest that this strong congruence between geography and phylogeny is the result of several vicariant and dispersal events in the Late Cretaceous and Paleocene associated with the fragmentation of the Gondwanan supercontinent. Our results demonstrate the importance of both vicariance and dispersal in shaping the global distributions of terrestrial organisms.

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1. Introduction

Despite widespread interest in boid snakes (family Boidae), and their use as a classic example of the influence of plate tectonics on organismal distributions (Bauer, 1993; Laurent, 1979; Rage, 1988, 2003), surprisingly few rigorous phylogenetic analyses of boines and relatives have been conducted. The most widely accepted hypotheses of boid relationships are based on analyses of morphology (Kluge, 1991, 1993a,b), although snakes may be especially prone to homoplasy in morphological characters (e.g., Burbrink, 2005). The few investigations of boid relationships employing molecular data have drawn their a priori concept of the ingroup from phylogenetic groupings based on these potentially homoplastic morphological data (e.g., Burbrink, 2005; Vences et al., 2001). Early morphological studies (Kluge, 1991, 1993a,b; Underwood, 1976; Underwood and Stimson, 1990) led to recognition of a Boidae consisting of three subfamilies: Boinae (Neotropics, Madagascar, Pacific Islands); Pythoninae (Australasia to Africa); and Erycinae (western North America, Africa, southeast Europe, southwest Asia, and India). While these authors acknowledged that the distributions of some morphological characters (e.g., Vidian canals, posterior hypapophyses, and paracotylar foramina; Underwood, 1976) conflicted with this arrangement, it appeared that most available evidence
supported recognition of these three subfamilies (Kluge, 1991). The most recent, comprehensive analysis of the relationships within the Boidae was undertaken by Kluge (1991, 1993a,b) in a three-part series addressing each subfamily separately and employing morphological data. Despite Kluge’s comprehensive analysis of relationships within the three boid subfamilies, little attention was given to the relationships among these groups, and their collective monophyly was assumed.

Subsequent to the work of Kluge (1993b), pythons (formerly Pythoninae) have received little attention with regard to relationships among the included taxa (but see Harvey et al., 2000; Rawlings et al., 2004). However, several studies of snake phylogeny have demonstrated that “true” pythons are quite distinct from the Boinae and Erycinae and are more closely related to Xenopeltis (Xenopeltidae; southeast Asia) and Loxocemus (Loxocemidae; Middle America) (Lee, 2004; Vidal and Hedges, 2002, 2004; Wilcox et al., 2002). The Old World pythons are now considered distinct from the Boidae and widely recognized as a separate family (Pythonidae; e.g., Vidal and Hedges, 2004), which we accept as the distinctiveness of these groups has been recovered by a number of recent phylogenetic analyses (Lawson et al., 2004; Lee, 2004; Slowinski and Lawson, 2002; Vidal and Hedges, 2002, 2004; Wilcox et al., 2002). Fine-scale relationships among members of Pythonidae are not considered here, although the relationships of Pythonidae to other taxa are examined.

Hereafter, we use the term Boidae for Erycinae + Boinae, and suggest that additional taxa be included in this family based on our molecular results.

Kluge’s phylogenetic work provided support for some previously assumed relationships within boines, erycines, and pythons, with several notable exceptions. Prior to Kluge’s studies, Erycinae generally was thought to include the widespread (Africa to India, hereafter Afro-India) “sand boas” (Eryx and Gongylophis, the latter genus now generally synonymized with Eryx), plus the monotypic North American genera Charina (“rubber boa”) and Lichanura (“rosy boa”) (Fig. 1). However, Kluge also included the monotypic, fossorial African genus Calabaria (widely thought to be closely allied with pythons) within Erycinae, considering Calabaria to be most closely related to Charina and Lichanura. Kluge (1993a) synonymized Lichanura and Calabaria under Charina for reasons of “taxonomic efficiency, and to emphasize the New–Old World geographic distribution of the three species in that assemblage.” Kluge’s most controversial conclusions concern relationships within the Boinae, and specifically the status of the genus Boa. Kluge’s (1991) morphological analysis shows the Neotropical boines to be polyphyletic, with Boa representing the sister group of the Malagasy Acrantophis + Sanzinia clade. This phylogenetic hypothesis was rejected in light of analysis of mitochondrial sequence data by Vences et al. (2001) and later by Burbbrink (2005) with a subset of the same molecular data and a reanalysis of Kluge’s morphological data. Rejection of a monophyletic [Boa (Acrantophis + Sanzinia)] has been borne out by analyses of nuclear markers (Noonan and Chippindale, in review). Despite the ongoing debates regarding the taxonomic rearrangements within the Boinae suggested by Kluge (1991) there has been no attempt to address the proposed relationships within the Erycinae, nor among Kluge’s three subfamilies within Boidae. The widespread acceptance of Kluge’s three subfamilies of Boidae is exemplified by the choice of outgroup taxa in recent analyses of relationships by Vences et al. (2001; a pythonid) and Burbbrink (2005; Erycinae + Ungaliophiidae [sensu Zaher, 1994]). In fact, the study that produced the cytochrome b data used by Vences et al. (2001) and Burbbrink (2005) showed the boines to be paraphyletic with respect to erycines (Campbell, 1997, p. 78). Thus, the only published tests of Kluge’s hypotheses of boid relationships have focused on relationships within a Boinae that the data employed had already suggested to be paraphyletic.

The biogeographic history of Boidae has received considerable attention due to their nearly global distribution, and seemingly unusual distributional patterns within subgroups. In particular, boines have elicited much interest because their distribution (Fig. 1, Neotropics + Pacific Islands + Madagascar) appears to support Gondwanan fragmentation and possibly oceanic dispersal (Austin, 2000; Bauer, 1993; Burbbrink, 2005; Underwood, 1976; Vences et al., 2001). Recent work has supported a Gondwanan, Late Cretaceous vicariant origin of the Malagasy boines Acrantophis and Sanzinia; (Noonan and Chippindale, in review) associated with a terrestrial connection linking

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**Fig. 1.** Geographic distribution of the Boidae (sensu this study). Abbreviations refer to well-supported clades; NT, Neotropical (Boa, Corallus, Epicrates, and Eunectes); A, African (Calabaria, Sanzinia, and Acrantophis); PI, Pacific Island (Candoia); N, North American (Lichanura and Charina); C, Central American (Exilbova and Ungaliophis); AI, Afro-Indian (Eryx); see text for details.
India/Madagascar and the Antarctic/South America/Australia landmass in the form of the Kerguelen Plateau (Hay et al., 1999) or Gunnerus Ridge (Case, 2002; Case and Krause, 2002). The pattern of relationships presented by (Noonan and Chippindale, in review) suggests that interpretation of the biogeographic history of the Boinae would ideally include both Erycinae and Ungaliophiidae (the latter comprising the Neotropical genera Ungaliophis and Exilloboa, thought by some early workers to belong within Boidae, but now generally considered a separate family).

To clarify the status of the Boidae we examined ~4.3 kb of sequence data from five nuclear loci (RAG1, c-mos, NT3, ODC, and BDNF) and one mitochondrial (cyt b) gene for all boine and ericine genera and representatives of all major snake clades including key taxa whose affinities with Boidae have been controversial. Using these data we examine the support for the currently recognized subfamilies Erycinae and Boinae and relate the observed pattern of relationships to the historical biogeography of the family.

2. Materials and methods

2.1. Taxon sampling and laboratory methods

As our previous work (as well as that of Vidal and Hedges, 2004) suggested the paraphyly of the Boinae with respect to the Erycinae, we include all ericine genera and multiple species of the only non-monotypic genus (Eryx) within this group. We rooted the tree with a typhlolid scoloeophilid (Ramphotyphlops) and included representatives of the Aniliidae (Anilius), Cylindrophiidae (Cylindrophis), Tropidophiidae (Tropidophis), Xenopeltidae (Xenopeltis), Caenophidia (Acrochordus), Ungaliophiidae (Ungaliophis), and two pythonids (Morelia and Aspidites). Choice of taxa was based on results of recent analyses of snake phylogeny (Lee, 2004; Vidal and Hedges, 2002, 2004; Wilcox et al., 2002) and earlier hypotheses. Sources of tissue samples and GenBank accession numbers of data utilized in this study are listed in Table 2.

Table 2

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ASM, Appalachian State University; UTA, University of Texas at Arlington; ZA, Zoo Atlanta; UPM, Yale Peabody Museum; FW, Fort Worth Zoo; BYU, Monty L. Bean Museum, Brigham Young University; TP, Ted Papenfuss; MVZ, Museum of Vertebrate Zoology, University of California, Berkeley.
Terminator v3.1 (Applied Biosystems). Unincorporated dye terminators were removed by Sephadex cleanup.

2.2. Sequence analysis

Sequences were edited and aligned with Sequencher v. 4.1 (Gene Codes Corp) and checked by eye, particularly with respect to amino acid alignment. To optimize sequence alignment (when necessary) we employed Clustal X 1.83 (using default parameters). To determine optimal models of sequence evolution for each locus we employed the AIC criterion as implemented in ModelTest version 3.7 (Posada and Crandall, 1998). All phylogenetic analyses were performed on combined nuclear DNA (nDNA) and combined mitochondrial + nDNA sequences. Unweighted maximum parsimony (MP) and maximum likelihood (ML) analyses were conducted using PAUP* version 4.0b10 (Swofford, 2001). To determine node support we performed 2000 nonparametric bootstrap pseudoreplicates with the heuristic search option, tree-bisection-reconnection branch swapping (TBR) and 10 random-taxon-addition replicates for MP analyses, and 1000 nonparametric bootstrap pseudoreplicates for ML analyses. The substitution model of DNA evolution employed in ML analyses was obtained by AIC tests of the combined nDNA and mtDNA + nDNA data sets using ModelTest version 3.7 (Posada and Crandall, 1998). Bayesian analysis of the data employed MrBayes version 3.1 (Huelsenbeck and Ronquist, 2001) with a mixed model approach (Nylander et al., 2004). We also conducted Bayesian analyses of individual locus data sets to examine patterns of support across loci. For each analysis/partition we specified only the general structure of the substitution model (Table 2), the shape parameter of the $\gamma$ distribution and the proportion of invariant sites and a flat prior for both substitution rates and nucleotide frequencies. Bayesian searches were conducted using four chains (one cold) for 50 million generations, sampling every 1000 generations for a total of 50,001 trees of which the first 5000 were discarded as burnin. Adequacy of this burnin value was determined by examining uncorrected potential scale reduction values (Gelman and Rubin, 1992) as well as plots of parameter values of the cold chain for convergence on stationarity using TRACER v1.3 (Rambaut and Drummond, 2003). Results of simultaneous, independent Bayesian searches were compared to assess congruence of topologies and parameter estimates.

2.3. Divergence time estimation

Divergence time estimates were obtained utilizing a relaxed Bayesian molecular clock method, which allows for variability in the evolutionary rate over time, using the MULTIDISTRIBUTE package (http://statgen.ncsu.edu/thorne/multidivtime.html, contents italicized below) developed by Thorne et al. (1998) and Thorne and Kishino (2002). This method was chosen as it allows for simultaneous estimates to be obtained of multiple locus data sets, rather than combining the information contained in these into a single data set (as in the penalized likelihood method of Sanderson, 1997, 2002). Fossil material (detailed in Table 3) and evidence from the literature were used to place constraints on the age of 10 nodes within the topology. Fossil material was used to infer minimum ages of nodes only, and no assumptions regarding the relationship of the first appearance in the fossil record and the upper limit were made (e.g., enforcing the time of first appearance in the fossil record plus some arbitrary number of years as the greatest possible age for a particular node). For this reason, the majority of calibration points used were open-ended minimum ages (e.g., node $\geq 60$ million years ago [Mya]).

A single upper constraint was placed on the deepest nodes of our tree specifying the upper limit of the divergence time among extant groups as less than the age of the oldest fossil snake (<130 My, Rage and Richter, 1994). This was done to
Table 3
Fossil material used to constrain the minimum age of nodes

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<th>Period</th>
<th>Node</th>
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<td>Nigerophiidae</td>
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<td>4</td>
<td>Rage (1984)</td>
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<td>Boidae</td>
<td>Eocene (possibly Paleocene)</td>
<td>13</td>
<td>Rage (1984); Albino (1996)</td>
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<td>Mid Miocene</td>
<td>14</td>
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<td>Boidae</td>
<td>Miocene</td>
<td>23</td>
<td>Rage (1984)</td>
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</table>

prevent the overlap of divergence time estimate credibility intervals with implausible estimates. Failure to include an upper bound for at least one node in the topology generally results in larger credibility intervals for node ages (BPN pers. obs.). Each of the six data sets was then analyzed using the BASEML program of the PAML package (Version 3.13; Yang, 1997) to determine appropriate nucleotide substitution parameters under the F84 model (Kishino and Hasegawa, 1989). The program estbranches was then used to estimate branch lengths and the variance–covariance structure of these parameter estimates for all loci. Finally, the application multidivtime was used to incorporate the results of estbranches and the fossil- and the literature-based calibrations to determine the posterior estimates of clade divergence times on each data set individually and for the combined data sets (nDNA and mtDNA + nDNA), while allowing for differences in the substitution parameters among genes. Estimates derived from analysis of multiple loci have been demonstrated to produce more accurate estimates with greater precision (Yoder and Yang, 2004) than those derived from analysis of single loci. For each analysis, one prior and two posterior distributions were obtained for all parameters. To verify that the MCMC had reached a stable approximation of the posterior distribution, analyses of combined data sets were conducted one additional time with ngen = 10^7. For all analyses, the topology specified was that shown in Fig. 2, with Ramphotyphlops designated as the outgroup. The prior distribution of the age of the root node (rttm), as required by multidivtime, was set to 110 Mya. The prior distribution for the rate at the root node (rtrate) was calculated individually for each data set as the median value of the sum of all branch lengths divided by rttm and the standard deviation of this value (rtratesd) as equal to rtrate, as suggested in the MULTIDISTRIBUTVE documentation. In each analysis the Markov chain was sampled every 100 generations for a total of 1 × 10^5 samples after a burnin of 1 × 10^5 generations.

3. Results

3.1. Phylogenetic analysis

Results of Modeltest analyses provided prior information on models of nucleotide substitution and parameter values that was incorporated into ML and Bayesian analyses (Table 2). In our phylogenetic analyses, relationships of taxa outside the focal group (Booidea: Pythonidae, Boidae, and Ungaliopiiidae) generally received less support from all analyses (partitions and methods) than those within this group, particularly based on MP analyses (Fig. 2). All nodes for these extra-booid taxa received greater support from the nDNA than the mtDNA + nDNA data sets. This appears to be the result of conflicting patterns supported by the mtDNA and nDNA (results of phylogenetic analysis of mtDNA not shown), primarily in the placement of a few taxa (Xenopeltis, Lichanura, and Acrachordus) and likely is due to substitutional saturation given the relatively ancient divergence of the taxa included and the relatively rapid rate of mitochondrial evolution.

Notably, among the extra-booid taxa, we recover strong support from the nDNA analysis for Anilius (Anilidae) as most closely related to the Tropidophiidae, as reported by Vidal and Hedges (2004). Cylindrophis (Cylindrophiidae), which commonly has been linked to the Anilidae, is recovered as more closely related to the Xenopeltidae and the Acrachordidae. Vidal and Hedges (2004) found Xenopeltis to be the sister taxon of the Loxocemus + Pythonidae clade of the Booidea, but our evidence strongly indicates that it is outside of the Booidea (Fig. 2). The monophyly of the Booidea (Pythonidae [here including Loxocemus] + Boidae + Ungaliophiidae) is strongly supported by Bayesian (MB) and ML analysis of the combined nDNA and by MB analysis of the mtDNA + nDNA datasets.

Within the Boidae, the monophyly of an “African” (= continental African Calabaria + the Malagasy Acranotphis + Sanzinia) group is strongly supported in all analyses of all data sets, a relationship recovered but without strong support by Vidal and Hedges (2004). Monophyly of the boid group exclusive of this African clade is well supported by both MB and ML analyses of the nDNA and mtDNA + nDNA datasets, as is the monophyly of both the Neotropical and North/Central American boids (Fig. 1). Support for the grouping of Candoia + Eryx, which was first suggested (with weak support) by Vidal and Hedges (2004), is high only in MB and ML analyses of the mtDNA + nDNA data sets. Within the Neotropical Boidae, in all analyses, the widespread genus Epicrates is recovered as polyphyletic with respect to Eunectes, suggesting that the
Caribbean Epicrates are the sister group to an Eunectes + South American Epicrates clade, although support for this pattern is generally weak.

3.2. Divergence time estimation

Estimates of divergence times based on the inferred topology (Fig. 2) and specified calibration points (Table 3) of individual loci and combined nDNA and mtDNA datasets provided similar results (Table 4). The timing of the divergence between Loxocemus and the Pythonidae (node 7, Fig. 3), a relationship strongly supported in all phylogenetic analyses (Table 4), represented the only instance of major discordance in clade-age estimates (Fig. 4). Specifically, the estimates based on the analysis of RAG 1, c-mos, and BDNF alone produced estimates of divergence time that were comparatively low with 95% credibility intervals that fell either entirely or largely outside those of all other analyses (Fig. 4, Table 4). Despite the general concordance between combined and individual locus analyses, estimates of nodal age for the combined (nDNA and mtDNA + nDNA) datasets for the earliest divergence events (nodes 1–6, Fig. 3) were slightly higher (average = 9.3%) than estimates resulting from analyses of individual genes (Fig. 4). As expected (Yoder and Yang, 2004), a general increase in the precision of divergence time estimates (~35%, as measured by the 95% credibility interval [CI]) was observed in the combined data analyses (nDNA and mtDNA + nDNA average CI = 25 and 24 My, respectively) relative to the analysis of individual loci (overall average CI = 38 My, range of averages for six loci = 30–45 My).

4. Discussion

4.1. Phylogenetic relationships

Although the focus of this study is the diversification of the Booidea and specifically patterns within the Boidae, some interesting phylogenetic relationships among the
other taxa included merit discussion. Most notable is the placement of the Xenopeltidae (Xenopeltis). This monotypic southeast Asian family has long been allied with the Neotropical genus *Loxocemus* and/or the Pythonidae (see Lee, 2004; Vidal and Hedges, 2002, 2004; Wilcox et al., 2002), yet these groupings receive no support based on our results. All analyses support the traditional placement of *Loxocemus* within the Booidae (sister taxon to the Pythonidae), and the placement of *Xenopeltis* outside this group, more closely allied with *Cylindrophis* (see also Lawson et al., 2004) which is also from southeast Asia. Our analyses also support the distant relationship between the Uropeltidae (*Cylindrophis*) and the Aniliidae (*Anilius*), formerly grouped together as the sister group to the Macrostomata, as suggested by Wilcox et al. (2002) (see also Lawson et al., 2004; Vidal and Hedges, 2004). It appears that the Uropeltidae are indeed within the Macrostomata, in contrast to early hypotheses placing them at the base of the Alethinophidia, which traditionally includes [(Uropeltidae + Aniliidae) Macrostromata], primarily based on several morphological features of the skull associated with gape size. The comparatively small gape (and associated osteological features) of the Uropeltidae thus appear to be the result of adaptation for fossorial habits (see Vidal and Hedges, 2002 for further discussion).

Phylogenetic patterns within the Booidae suggest a radical rearrangement of accepted relationships. The Pythonidae are strongly supported as the sister group of the monotypic Loxocemidae. Support for (Pythonidae + *Loxocemus*) + (Boidae + Ungalliophiidae) is strong in analyses of nDNA, but the addition of mtDNA reduces support in both MP and ML analyses to below 50%. Within the Boidae, our data provide strong support for five geographically distinct clades that can be generally characterized as: (1) African boids including the mainland Calabaria and the Malagasy Acrantophis and Sanzinia; (2) North/Central American boids including the Ungalliophiidae, Lichanura, and Charina; (3) Neotropical boids; (4) the Afro-Indian Eryx; and (5) the Pacific island *Candoia*.

Within the African clade, the two Malagasy genera represent a monophyletic group, whose sister taxon is the mainland Calabaria. We are aware of only one previous study to report this pattern (Vidal and Hedges, 2004), although its support in their study was negligible (MP bootstrap <50; Bayesian posterior probability = 0.57). The morphological differences between Calabaria and the Malagasy taxa are so great that the mainland and Malagasy taxa have each been associated with different groups of New World boids rather than one another throughout their respective taxonomic histories. Nonetheless, support for this pattern is extremely strong and demonstrates the high levels of morphological homoplasy within boids that presumably results from habitat specialization, particularly the fossorial lifestyles of taxa traditionally referred to Erycinae.

Previous studies have suggested that the Ungalliophiidae (Ungalliophis + Exiliboa) are closely related to the North American Lichanura and Charina based on fossil material (e.g., Dunnophis, which is most similar to Charina and the ungalliophiids [Rage, 1984]), morphological characteristics of extant taxa (e.g., the naso-frontal joint; Rieppel, 1978; Zaher, 1994), and molecular data (Lawson et al., 2004; Lee, 2004; Vidal and Hedges, 2002, 2004). However, placement within the Booidae has been equivocal due to conflicting phylogenetic signal of morphological characters and the inadequacy of taxon and character sampling of molecular
The relationships suggested by our data strongly support this North/Central American clade as the sister group to a monophyletic [Neotropical, (Afro/Indian, Pacific Island)] group. This North/Central American clade was also recovered by Burbrink (2005) based on analysis of cyt b sequence data.

The Neotropical boid clade has been examined repeatedly (Burbrink, 2005; Vences et al., 2001) to test the controversial phylogeny and taxonomy proposed by Kluge (1991). The hypotheses of relationships of Neotropical boines from these mitochondrially based studies are entirely congruent with the results of our analysis of nDNA. Like Burbrink (2005), we find strong support for an Epicrates + Eunectes clade, with moderate support for the paraphyly of Epicrates (as did Lawson et al., 2004). While support for paraphyly of Epicrates is relatively low based on analyses of mtDNA only (Burbrink, 2005) and our combined nDNA data set, we interpret the independent corroboration of the nDNA and mtDNA data sets as being consistent with Caribbean dispersal prior to the divergence between South American Epicrates and Eunectes.

Within the Afro-Indian genus Eryx, there has been considerable controversy regarding phylogeny and taxonomy based on morphology, including whether these taxa should be placed in a single genus (see Kluge, 1993a; Rage, 1972; and Tokar, 1989 for a review). The morphological disparity within this genus is so great that Rage (1972) reported the variation in cranial morphology as being greater than that observed between the genera Python and Boa, which are now considered members of different families. The erycine genus Gongyllophis has been proposed to include the morphologically ‘primitive’ taxon E. conicus (see Rage, 1972). Our results suggest that Eryx represents a monophyletic group with the morphologically divergent E. conicus nested within, rather than as the sister taxon to, the other members of the genus.

Phylogenetic placement of the Pacific Island endemic genus Candoia as the sister taxon to the Afro-Indian Eryx radiation, though recovered, received little support from nDNA alone. Inclusion of mtDNA provided additional support for this hypothesis of relationships in MB and ML analyses. Several studies that have included Candoia in larger examinations of squamate relationships have...
recovered this genus as most closely related to the Malagasy boids (Austin, 2000; Burbrink, 2005; Lawson et al., 2004; Vidal and Hedges, 2002). However, these results were poorly supported (Austin, 2000; Lawson et al., 2004; Vidal and Hedges, 2002) or hampered by the constraints placed on the composition of the ingroup (Burbrink, 2005).

4.2. Divergence times and biogeography

Estimates of divergence times suggest a rapid radiation of the snake families in the Early Cretaceous. Consistent with this hypothesis, the six deepest nodes of our phylogeny (nodes 1–6, Fig. 3) all occur within the span of 25 My and the 95% credibility intervals of these nodes are all overlapping. Given this early, rapid diversification of extant snake clades, it is not surprising that relationships among these taxa have proven difficult to resolve using mtDNA alone. This may explain the reduction in support values for the deepest nodes that resulted from addition of mitochondrial cyt b sequences to our nDNA dataset (Fig. 2).

The deepest divergence within the Booidae, that of the Pythonidae–Boidae (node 6, Fig. 3), appears to have occurred in the late Early Cretaceous (Albian) with the diversification of extant clades within both families not taking place for another 30–40 My in the Late Cretaceous. Further discussion of the temporal framework of pythonid diversification is not possible with the limited sampling presented here. However, of the two taxa often considered closely related to the Pythonidae, \( \text{Loxocemus and Xenopeltis} \), the Mexican endemic \( \text{Loxocemus} \) appears, based on our data, to be the sister lineage of the otherwise exclusively Old World radiation of pythons. The timing of their divergence at the very end of the Cretaceous (potentially into the early Paleocene) seems unusual given that the only likely terrestrial connection between these clades at this time was the South America/Antarctica/Australia land block, which is not known to have undergone any physical separation for approximately another 30 My. This timing does coincide with the Late Cretaceous North America/South America biotic exchange (Bonaparte, 1984a,b; Estes and Báez, 1985; Rage, 1988) and suggests a pythonid presence in South America with subsequent extinction. A Gondwanan distribution of pythons is not supported by the fossil record of South America (Albino, 1996), although the morphological homoplasy that has plagued boid phylogenetics is also problematic in the assignment of fossil forms, especially considering that most remains are limited to vertebral elements which are themselves not particularly rich in characters.

The results of these analyses augment the growing amount of information that supports a Late Cretaceous isolation of the African (Malagasy) taxa, and thus the role of an Indo/Malagasy/Antarctic connection in the origin of the Malagasy fauna (Case, 2002; Case and Krause, 2002; Noonan and Chippindale, in review). The taxon sampling in this study provides further insight into the timing of Indo/Malagasy fragmentation and the submersion of an Antarctic landbridge. The monophyly of the non-African Boidae strongly suggests that, given a contiguous, Late Cretaceous, anti-African Gondwana, the initial event driving boid diversification was vicariance associated with the
isolation of Madagascar from India (biological separation here dated at ~77 Mya). Thus, after this separation, the physical connection between India and South America via Antarctica may have remained intact for a short period of time. This biogeographic pattern thus supports the Kerguelen Plateau rather than Gunnerus Ridge as the landbridge connecting Indo-Madagascar to Antarctica (Fig. 5). Subsequent differentiation between remaining boid clades appears to have occurred in a short period of time (all within 9 My; Fig. 3).

The phylogenetic placement of *Calabaria* contradicts traditional (morphology-based) hypotheses of relationships. The well-supported relationship between this African burrowing taxon and the Malagasy boids suggests that phenotypic similarities among distantly related taxa with similar life history strategies (e.g., *Calabaria*–*Eryx* and *Boa*–*Acrantophis*) has concealed an African–Malagasy dispersal event. This dispersal event appears to have occurred in the latest Late Cretaceous, with divergence between the terrestrial and arboreal Malagasy genera *Acrantophis* and *Sanzinia* not occurring until the Oligocene. Such dispersal across the Mozimbaque channel has been documented in numerous other vertebrate groups (Poux et al., 2005; Raxworthy et al., 2002; Yoder et al., 1996, 2003).

Among the non-African boids, our data indicate that the North/Central American group was isolated from a contiguous South America–Antarctica–India group roughly 74 Mya. The geographic distribution of extant taxa of the North/Central American group and the timing of this divergence closely mirrors the Late Cretaceous biotic interchange between North America and South America supported by the distributional and temporal patterns of numerous fossil forms (reviewed by Rage, 1988). This biotic interchange, and particularly a northward dispersal, has been supported phylogenetically and temporally in recent analyses of leptodactyloid frogs (Crawford and Smith, 2005). This pattern is temporally congruent with the proposed proto-Antillean landbridge/island-arc model that would have allowed dispersal of terrestrial South American organisms into North America (Rage, 1981, 1986; Schmidt-Effling, 1979).

The origin of the Pacific Island boids has long been problematic. Their close relationship to the Afro-Indian *Eryx* and a Cretaceous/Tertiary origin does little to alleviate this situation. The origins of this clade remain a mystery, and our data neither confirm nor reject previous hypotheses (e.g., oceanic dispersal from South America or vicariance associated with the South America/Antarctica/Australia [including New Guinea] landmass). Our results also suggest a third possibility, oceanic dispersal from the migrating Indian subcontinent.

An Indian origin of the widespread *Eryx* has been suggested previously (see Tokar, 1996) and is supported by both the phylogenetic placement and timing of divergence within the genus. The collision of the Indian subcontinent with Eurasia was complete ~60 Mya (Beck et al., 1995) with final suturing occurring ~42–55 Mya (Briggs, 2003). The earliest divergence recovered within this genus (47 Mya) is consistent with dispersal from the previously isolated landmass into Afro-Arabia, corroborating the “Out-of-India” hypothesis suggested for numerous other groups (Bossuyt and Milinkovitch, 2001; Conti et al., 2002; Gower et al., 2002).

Subsequent to their isolation in the latest Cretaceous (~70 Mya), the Neotropical Boidae underwent little diversification until the invasion of the Caribbean at the beginning of the Oligocene, at which time the connectivity to what now represents the Greater Antilles appears to have reached its peak (see Fig. 5 of Crawford and Smith, 2005).

5. Conclusions

Studies of boid phylogeny and biogeographic history long have been complicated by potentially homoplastic morphological characters. Our findings suggest the need for a radical rearrangement of the traditional subfamilial hypotheses. Boid snakes are truly “Gondwanan” in origin.
with a presence on every major fragment of this former landmass and an evolutionary history that closely mirrors geological evidence of the timing and sequence of fragmentation of this supercontinent. Although recent inferences of dispersal origins for numerous groups have highlighted the importance of this phenomenon (de Queiroz, 2005; Knapp et al., 2005; McGlone, 2005; Vences et al., 2003; Yoder et al., 1996, 2003), the history of the Boidae supports the roles of both Gondwanan vicariance and dispersal in shaping their current distribution.

Our results corroborate a number of dispersal and vicariant events that have been proposed such as the Out-of-India hypothesis, the proto-Antillean land bridge/island arc hypothesis, oceanic dispersal between Africa and Madagascar, and the existence of a Late Cretaceous terrestrial connection between Antarctica and India/Madagascar. While studies of numerous groups have shown that one or two of these phenomena have influenced evolutionary history, we are aware of no other group whose evolutionary history simultaneously supports so many of these biogeographic hypotheses.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2006.03.010.

References


