Available online at www.sciencedirect.com
SCIENCE DIRECT
Molecular Phylogenetics and Evolution xxx (2006) xxx–xxx
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Dispersal and vicariance: The complex evolutionary history of boid snakes

Brice P. Noonan [a,b,*], Paul T. Chippindale [b]

[a] *Department of Integrative Biology, Brigham Young University, Provo, UT 84602, USA*
[b] *Department of Biology, University of Texas at Arlington, Arlington, TX 76019, USA*

Received 18 November 2005; revised 2 March 2006; accepted 5 March 2006

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 (Ungaliophiidae, 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.

Keywords: Boidae; Biogeography; Gondwana; Vicariance

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 boids 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

* Corresponding author. Fax: +1 801 422 0090.
E-mail address: noonan@byu.edu (B.P. Noonan).

1055-7903/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2006.03.010

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 Burbrink (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 Burbrink (2005; Erycinae + Ungaliophiidae [sensu Zaher, 1994]). In fact, the study that produced the cytochrome *b* data used by Vences et al. (2001) and Burbrink (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; Burbrink, 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

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 (*Exiliboa* and *Ungaliophis*); A/I, Afro-Indian (*Eryx*); see text for details.

India/Madagascar and the Antarctica/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 *Exiliboa*, 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 erycine 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 erycine genera and multiple species of the only non-monotypic genus (*Eryx*) within this group. We rooted the tree with a typhlopid scolecophidian (*Ramphotyphlops*) and included representatives of the Aniliidae (*Anilius*), Cylindrophidae (*Cylindrophis*), Tropidophiidae (*Tropidophis*), Xenopeltidae (*Xenopeltis*), Caenophidia (*Acrochordus*), Loxocemidae (*Loxocemus*), Ungaliophiidae (*Exiliboa*), 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 new and previously existing sequences are listed in Table 1.

Genomic DNA was isolated using Qiagen DNeasy Tissue Kits according to the standard protocol, or by a high-salt precipitation method (Crandall et al., 1999). Portions of five nuclear loci were sequenced: brain-derived neurotrophic factor precursor (BDNF), neurotrophin-3 (NT3), oocyte maturation factor (c-*mos*), recombination activating gene 1 (RAG1), and ornithine decarboxylase (ODC). Primer sequences and amplification protocols for these loci are listed in Table 2. Cytochrome *b* sequences and some c-*mos* data were obtained from GenBank. Amplifications were performed in 13 µl reaction volumes using TaKaRa Hotstart *Taq* DNA polymerase and 10× reaction buffer (100 mM Tris–HCl [pH 8.3], 500 mM KCl, and 15 mM $MgCl_2$). PCR products were purified with Millipore MANU030 PCR cleanup plates. Purified double-stranded products were used directly in 1/32 dideoxy-termination sequencing reactions (10.0 µl total volume) using BigDye

Table 1
Institutional voucher numbers and GenBank accession numbers of data utilized in this study

Taxon	Museum #	*cyt b*	c-*mos*	NT-3	BDNF	RAG1	ODC
Corallus caninus	ZA A36702	U69763	AY987964	AY988044	AY988027	AY988061	DQ465528
Epicrates cenchria	UTA 50177	U69777	AY099966	AY988045	AY988028	AY988062	DQ465529
Epicrates striatus	Pet Trade	U69799	DQ465553	DQ465554	DQ465555	DQ465556	DQ465551
Eunectes notaeus	ZA 746701	U69810	AY099964	AY988046	AY988029	AY988063	DQ465530
Boa constrictor	Pending	U69740	AF471115	AY988047	AY988030	AY988064	DQ465531
Candoia carinata	YPM 12872	AY099984	AY099961	AY988048	AY988031	AY988065	DQ465532
Acrantophis dumerili	ZA956705	U69735	AY099963	AY988049	AY988032	AY988066	DQ465533
Sanzinia madagascariensis	FW 986704	U69866	AY099982	AY988050	AY988033	AY988067	DQ465534
Exiliboa placata	UTA 37871	AY099989	AY099973	AY988051	AY988034	AY988068	DQ465535
Morelia spilota	YPM 12876	U69851	AF544723	AY988052	AY988035	AY988069	DQ465537
Aspidites melanocephala	Pending	U69741	DQ465557	DQ465558	DQ465559	DQ465560	DQ465552
Loxocemus bicolor	ZA 46401	AY099993	AY099969	DQ465572	DQ465573	AY444061	DQ465545
Xenopeltis unicolor	Pending	AY121369	DQ465561	DQ465562	DQ465563	DQ465564	DQ465549
Acrochordus javanicus	YPM 13598	AF217841	AF471124	AY988053	AY988036	AY988070	DQ465538
Cylindrophis rufus	MVZ 170854	AF471032	AF471133	AY988054	AY988037	AY988071	DQ465539
Anilius scytale	YPM 10767	U69738	AY099965	AY988055	AY988038	AY988072	DQ465540
Tropidophis hateanus	BYU 48469	U69868	AY099962	AY988056	AY988039	AY988073	DQ465541
Eryx conicus	TP 28678	U69824	DQ469787	AY988057	AY988040	AY988074	DQ465547
Eryx jayakari	MVZ 236615	NA	DQ465565	NA	DQ465566	DQ465567	DQ465548
Eryx colubrinus	Pending	U69812	DQ465568	DQ465569	DQ465570	DQ465571	DQ465550
Eryx johni	TP 28719	U69823	AY099975	DQ465575	DQ465576	DQ465577	DQ465546
Calabaria reinhardtii	UTA 39598	AY099985	AY099978	AY988058	AY988041	AY988075	DQ465542
Charina bottae	BYU 48468	AY099986	AY099971	AY988059	AY988042	AY988076	DQ465543
Lichanura trivirgata	ASM 25201	U69844	AY099974	DQ465578	DQ465579	DQ465580	DQ465536
Ramphotyphlops	YPM 13663	AY099990	AY099980	AY988060	AY988043	AY988077	DQ465544

ASM, Appalachain State University; UTA, University of Texas at Arlington; ZA, Zoo Atlanta; YPM, 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.

Table 2
Primer sequences, sources, aligned fragment lengths, and number of parsimony informative sites for the five nuclear loci used in this study

Primers	Primer sequence	Source	Fragment length (aligned)	Informative sites	Selected model
RAG1		Chiari et al. (2004)	854	119	K80 + G
Mart.FL1	AGCTGCAGYCARTAYCAYAARATGTA				
Amp.R1	AACTCAGCTGCATTKCCAATRTCA				
BDNF		Brandley pers. com.	671	53	HKY + I + G
BDNF-F	GACCATCCTTTTCCTKACTATGGTTATTTCATACTT				
BDNF-R	CTATCTTCCCCTTTTAATGGTCAGTGTACAAAC				
NT3		Noonan and Chippindale (in review)	498	102	TrN + G
NT3-F3	ATATTTCTGGCTTTTCTCTGTGGC				
NT3-R4	GCGTTTCATAAAAATATTGTTTGACCGG				
c-*mos*		Noonan and Chippindale (in review)	572	71	K80 + G
CMOS-Fsnk	GCTGTAAAACAGGTGAAGAGATGCAG				
CMOS-Rsnk	AGCACGATGGGTGTATGTTCCCCC				
ODC		Friesen et al. (1999)	613	118	HKY + G
OD-F	GACTCCAAAGCAGTTTGTCGTCTCAGTGT				
OD-R	TCTTCAGAGCCAGGGAAGCCACCACCAAT				

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 γ distribution of rate variation 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 $\geqslant$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

Taxon	Group	Period	Node	Reference
Coniophis	Aniliidae	Cretaceous	2	Rage (1984)
Dinilysia	Dinilysiidae	Cretaceous	3	Rage (1984); Albino (1996, 2000)
Nigerophis	Nigeropheidae	Paleocene	4	Rage (1984)
Indet.	Boidae	L. Cretaceous	6	Albino (1996)
Cheilophis	Boidae	Eocene	9	Rage (1984); Albino (1996, 1993)
Helagras	Boidae	Paleocene	12	Rage (1984)
Dunnophis	Boidae	Eocene (possibly Paleocene)	13	Rage (1984); Albino (1996)
Charina prebottae	Boidae	Mid Miocene	14	Rage (1984)
cf. Boa	Boidae	Early Eocene	15	Albino (1993, 1996)
Eunectes and *Pseudoepicrates*	Boidae	Miocene	23	Rage (1984)

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 ± 30 My. 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 MULTIDISTRIBUTE 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 substitiution 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 Ungaliophiidae) 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 *Acrochordus*) 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* (Aniliidae) as most closely related to the Tropidophiidae, as reported by Vidal and Hedges (2004). *Cylindrophis* (Cylindrophidae), which commonly has been linked to the Aniliidae, here is recovered as more closely related to the Xenopeltidae and the Acrochordidae. 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 monophly 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 *Acrantophis* + *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 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

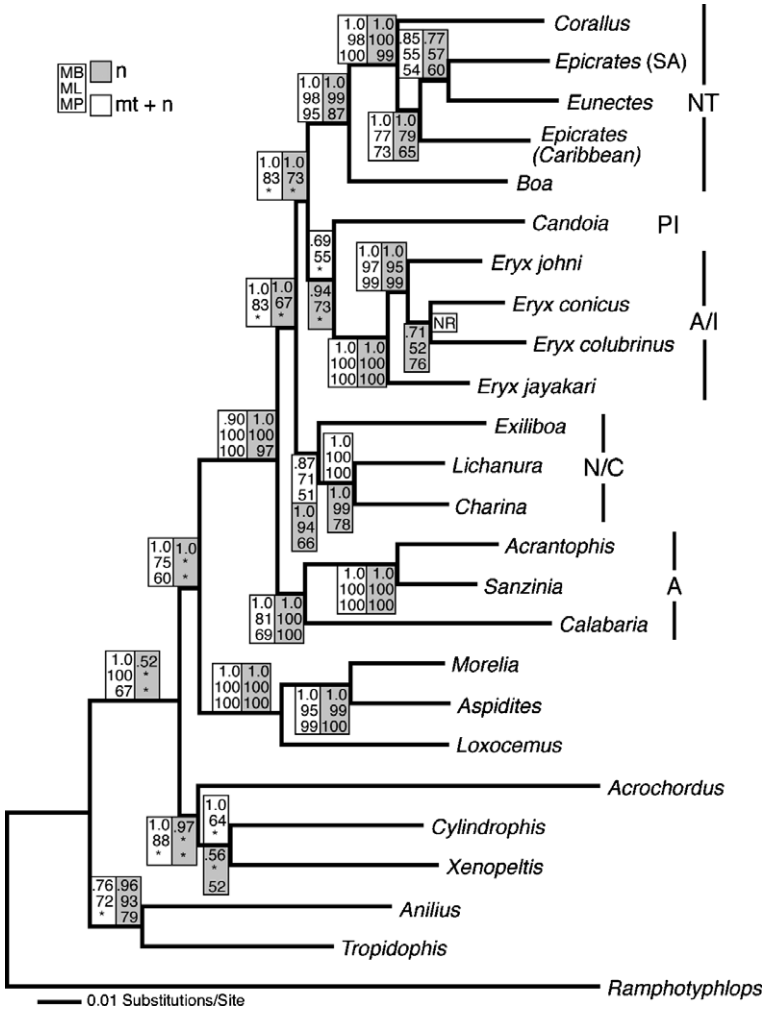

Fig. 2. Phylogeny obtained from Bayesian analysis of combined mitochondrial (mtDNA: *cyt b*) and nuclear (nDNA: c-*mos*, BDNF, RAG1, ODC, and NT3) data sets. Support values are reported in white (nDNA data set) and gray (mtDNA + nDNA) boxes in the order of Bayesian posterior probability, ML bootstrap, and MP bootstrap. Nodes not recovered by nDNA analysis are indicated by NR, support values below 50% are indicated by an ∗. Abbreviations to the right of taxon names indicate the geographic distribution of ingroup taxa as illustrated in Fig. 1.

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 + nDNA 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

Table 4
Divergence time estimates for nodes numbered in Fig. 5

Node	*cyt b*	NT3	c-*mos*	RAG1	BDNF	OD	nDNA	All
1	119 (101–129)	114 (94–128)	114 (93–129)	115 (94–129)	113 (93–128)	113 (94–127)	117 (100–129)	121 (106–129)
2	89 (75–116)	95 (76–119)	99 (77–122)	105 (82–124)	97 (77–121)	98 (78–119)	107 (88–122)	110 (93–123)
3	107 (89–119)	107 (88–119)	107 (87–119)	104 (84–119)	106 (86–119)	108 (90–119)	110 (95–119)	112 (99–119)
4	103 (85–117)	101 (80–116)	97 (75–115)	96 (75–115)	94 (70–114)	87 (67–109)	104 (88–117)	108 (94–118)
5	93 (74–110)	87 (60–110)	72 (31–105)	72 (33–104)	75 (38–106)	74 (53–97)	92 (74–108)	98 (82–111)
6	103 (86–117)	100 (81–116)	100 (80–116)	100 (81–116)	98 (78–115)	104 (86–117)	107 (92–117)	109 (96–118)
7	80 (61–100)	80 (47–107)	77 (48–104)	16 (2–45)	30 (6–69)	64 (39–91)	56 (40–73)	68 (53–82)
8	49 (31–70)	12 (8–39)	30 (5–63)	10 (0.3–36)	18 (0.7–52)	22 (5–47)	20 (8–37)	34 (22–48)
9	81 (69–96)	88 (70–108)	86 (69–106)	83 (68–101)	89 (71–109)	85 (69–104)	78 (68–91)	77 (68–89)
10	75 (60–92)	80 (58–102)	56 (24–89)	70 (47–92)	73 (43–100)	64 (40–89)	68 (53–83)	72 (61–85)
11	44 (30–61)	18 (0.1–44)	9 (0.3–31)	19 (2–45)	27 (2–64)	24 (4–50)	14 (4–25)	28 (19–39)
12	78 (67–93)	81 (66–102)	79 (66–99)	77 (66–95)	83 (67–103)	77 (66–95)	73 (65–85)	74 (65–85)
13	62 (55–76)	69 (56–90)	74 (56–92)	70 (57–89)	71 (56–94)	67 (56–86)	66 (56–79)	64 (56–77)
14	53 (42–68)	38 (18–65)	46 (22–73)	40 (19–65)	37 (17–69)	37 (18–62)	37 (24–51)	46 (36–58)
15	73 (61–88)	76 (60–96)	74 (59–94)	70 (57–89)	73 (67–95)	73 (60–91)	70 (61–82)	70 (61–81)
16	63 (48–79)	66 (41–88)	65 (45–87)	65 (50–85)	62 (39–87)	66 (50–86)	66 (56–79)	65 (55–78)
17	54 (35–73)	52 (26–79)	48 (24–74)	35 (15–60)	46 (19–75)	48 (27–72)	44 (30–60)	47 (33–62)
18	45 (25–64)	38 (17–63)	30 (9–56)	23 (7–46)	30 (8–59)	30 (12–53)	29 (18–42)	34 (24–46)
19	37 (23–53)	31 (10–55)	18 (2–43)	17 (2–38)	17 (1–44)	22 (5–44)	25 (14–37)	31 (21–42)
20	61 (48–77)	65 (45–87)	64 (45–86)	53 (36–74)	63 (42–86)	67 (51–85)	59 (47–72)	58 (48–70)
21	49 (36–64)	54 (34–76)	52 (34–75)	44 (30–65)	50 (32–74)	44 (30–65)	43 (32–56)	43 (34–55)
22	41 (29–55)	40 (27–60)	36 (25–53)	37 (57–89)	41 (27–63)	36 (26–53)	31 (25–40)	34 (26–43)
23	35 (25–49)	31 (24–78)	30 (24–44)	31 (24–47)	32 (24–52)	31 (24–46)	27 (24–35)	29 (24–38)

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 Booidea (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) Macrostomata], 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 Booidea 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 + Ungaliophiidae) 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 Ungaliophiidae, *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 Ungaliophiidae (*Ungaliophis* + *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 Ungaliophiids [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 Booidea has been equivocal due to conflicting phylogenetic signal of morphological characters and the inadequacy of taxon and character sampling of molecular

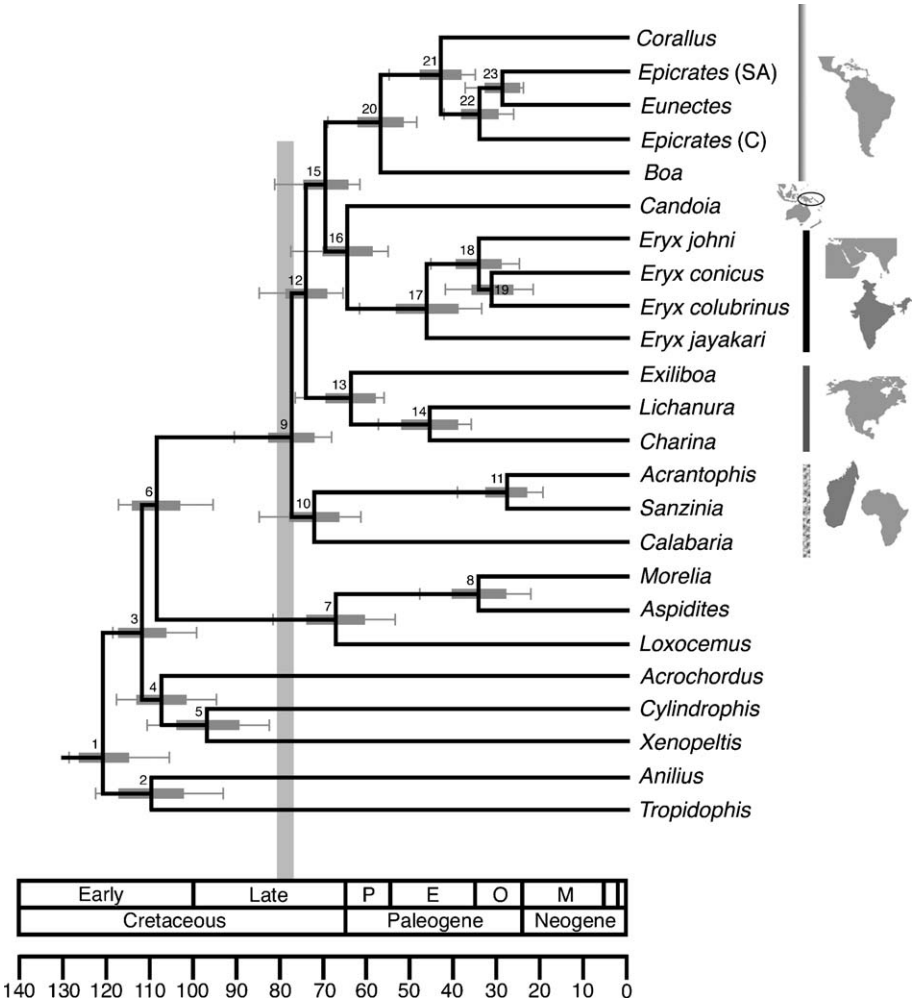

Fig. 3. Hypothesis of booid snake relationships with divergence times obtained using Bayesian MCMC methods. Maps to the right of taxon names illustrate geographic distribution of the specified clade. Solid gray bars illustrate divergence time ± one standard deviation and thin lines represent 95% credibility intervals. Node numbers refer to specific age estimates reported in Table 4 and fossil calibration points described in Table 3.

studies. 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 *Gongylophis* 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

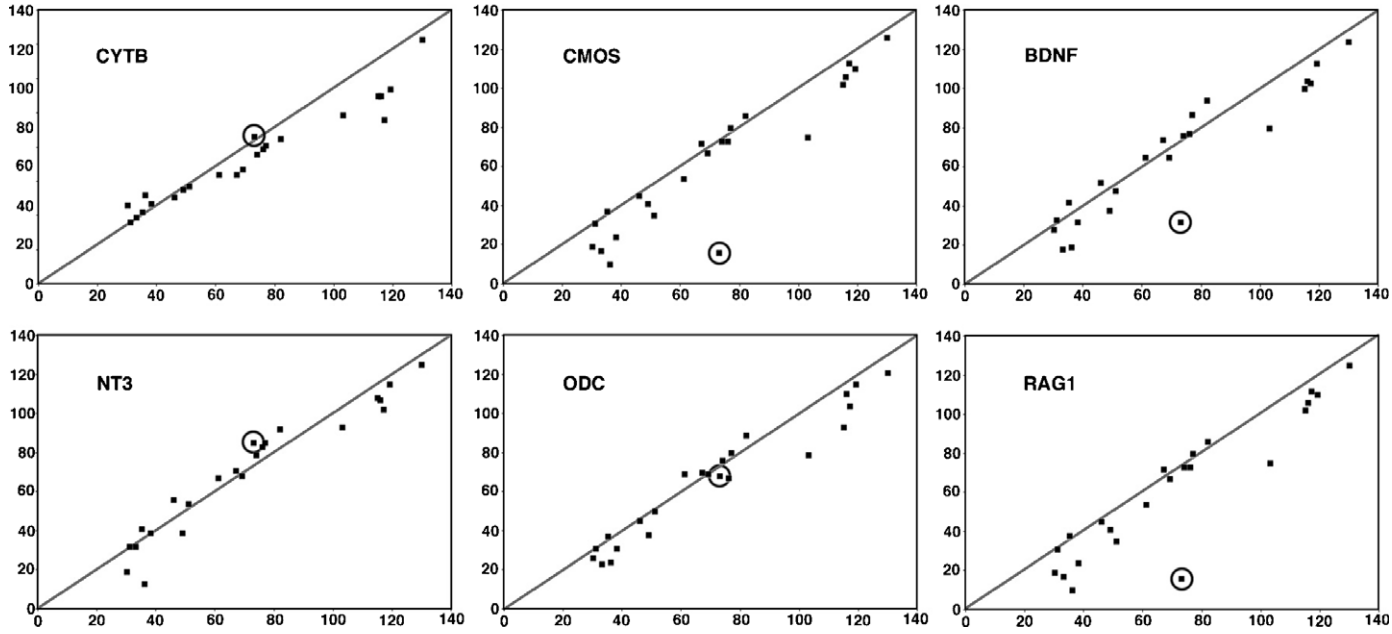

Fig. 4. Comparison of Bayesian posterior estimates of divergence times for individual loci (*Y* axis) and the combined mtDNA + nDNA analyses (*X* axis) for the 23 nodes of the phylogeny presented in this figure. The solid line represents perfect match between the two analyses with values above this line representing estimates of age for nodes based on analyses of individual loci that are greater than those of produced by the combined analysis. Data points below the line represent nodes for which individual locus estimates of age are less than that obtained in the combined analysis. Circled data points correspond to estimates of node 7 which are described in text.

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 Booidea, 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, (*Loxocemus* and *Xenopeltis*), the Mexican endemic *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 booid 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 leptodactylid 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-Effing, 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 Late 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

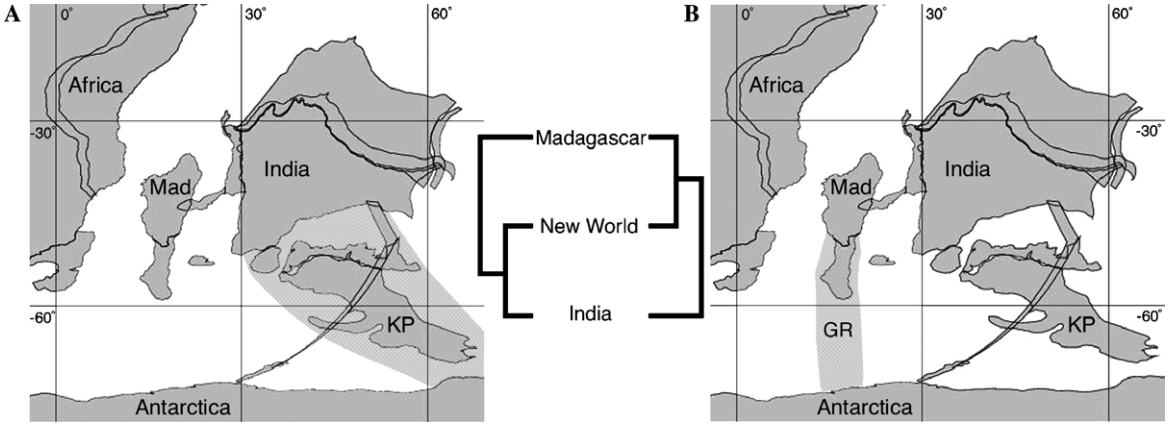

Fig. 5. Ninety Mya tectonic reconstruction illustrating alternative hypotheses of a Late Cretaceous terrestrial connection between India/Madagascar and Antarctica via either the Kerguelen Plateau (KP) (A: Hay et al., 1999) or the Gunnerus Ridge (GR) (B: Case, 2002; Case and Krause, 2002) and predicted biotic relationships based on each. The data presented here provide strong support for the early isolation of Madagascar and the continued biotic exchange across the Kerguelen Plateau after this separation.

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.

Acknowledgments

We thank Jack Sites and Jennifer Pramuk for reviewing drafts of the manuscript. We also thank Zoo Atlanta, Fort Worth Zoo, St. Louis Zoo, Ryan Hoyer, R. Wayne Van Devender, Jonathan Campbell (University of Texas at Arlington Amphibian and Reptile Biodiversity Research Center), Troy Hibbits, Wally Schargel, and Jim McGuire (Museum of Vertebrate Zoology, University of California at Berkeley) for providing tissue samples. We thank Matthew Brandley (U. California, Berkeley) for sharing BDNF primers. NT-3 primers were developed in the labs of T. Reeder and J. Sites with support from NSF Assembling the Tree of Life award NSF EF-0334966. Tissues obtained in the field were collected during research supported by Grants from NSF (DEB-0206562) to PTC and BPN and the National Geographic Society (#7509-03) to BPN.

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

Albino, A.M., 1993. Snakes from the Paleocene and Eocene of Patagonia (Argentina): paleoecology and coevolution with mammals. Hist. Biol. 7, 51–69.

Albino, A.M., 1996. The South American fossil Squamata (Reptilia: Lepidosauria). Münch. Geo. Abh. 30, 185–202.

Albino, A.M., 2000. New record of snakes from Cretaceous of Patagonia (Argentina). Geodiversitas 22, 247–253.

Austin, C.C., 2000. Molecular phylogeny and historical biogeography of Pacific island boas. Copeia 2, 341–352.

Bauer, A.M., 1993. African–South American relationships: a perspective from the Reptilia. In: Goldblatt, P. (Ed.), Biological Relationships between Africa and South America. Yale Univ. Press, New Haven, CT, pp. 244–288.

Beck, R.A., Burbank, D.W., Sercombe, W.J., Riley, G.W., Barndt, J.K., Berry, J.R., Afzal, R., Kahn, A.M., Jurgen, H., Metje, J., Cheema, J., Safique, N.A., Lawrence, R.D., Kahn, M.A., 1995. Stratigraphic evidence for an early collision between northwest India and Asia. Nature 272, 55–58.

Bonaparte, J.F., 1984a. La Formacion Los Alamitos (Campaniano-Maastrichtiano) del Sudeste de Rio Negro, con descripcíon de *Kritosaurus australis* n. sp. (Hadrosauridae). Significado paleogeográfico de los Vertebrados. Assoc. Geol. Argentina. Rev. 39, 284–299.

Bonaparte, J.F., 1984b. Late Cretaceous faunal interchanges of terrestrial vertebrates between the Americas. In: Reif, W.-E., Westphal, F. (Eds.), Third Symposium on Mesozoic Terrestrial Ecosystems, Short Papers. ATTEMPTO Verlag, Tübingen, pp. 19–24.

Bossuyt, F., Milinkovitch, M.C., 2001. Amphibians as indicators of early Tertiary "Out-of-India" dispersal of vertebrates. Science 292, 93–95.

Briggs, J.C., 2003. The biogeographic and tectonic history of India. J. Biogeogr. 30, 381–388.

Burbrink, F.T., 2005. Inferring the phylogenetic position of *Boa constrictor* among the Boinae. Mol. Phylogenet. Evol. 34, 167–180.

Campbell, B.N. 1997. Hic Sunt Serpentes—Molecular phylogenetics and the Boidae (Serpentes: Booidea). Unpublished Ph.D. dissertation. Queen's University, Kingston Ontario. p. 167.

Case, J.A., 2002. A new biogeographic model for dispersal of Late Cretaceous vertebrates into Madagascar and India. J. Vert. Paleo. 22 (suppl. 3), 42A.

Case, J.A., Krause, D.W., 2002. A tribosphenic lower molar from the Maastrichtian of Madagascar: Phyletic affinities, biogeography and a new dispersal model. International Palaeo Congress, Sydney, Australia. p. 31.

Chiari, Y., Vences, M., Vietes, D.R., Rabemananjara, F., Bora, P., Ramilijaona Ravoahangimalala, O., Meyer, A., 2004. New evidence for parallel evolution of color patterns in Malagasy poison frogs (*Mantella*). Mol. Ecol. 13, 3763–3774.

Conti, E., Eriksson, T., Schönenberger, J., Sytsma, K.J., Baum, D.A., 2002. Early Tertiary out-of-India dispersal of Crypteroniaceae: evidence from phylogeny and molecular dating. Evolution 56, 1931–1942.

Crandall, K.A., Fetzner Jr., J.W., Lawler, S.H., Kinnersley, M., Austin, C.M., 1999. Phylogenetic relationships among the Australian and New Zealand genera of freshwater crayfishes (Decapoda: parastacidae). Aust. J. Zool. 47, 199–214.

Crawford, A.J., Smith, E.N., 2005. Cenozoic biogeography and evolution in direct-developing frogs of Central America (Leptodactylidae: *Eleutherodactylus*) as inferred from a phylogenetic analysis of nuclear and mitochondrial genes. Mol. Phylogenet. Evol. 35, 536–555.

de Queiroz, A., 2005. The resurrection of oceanic dispersal in historical biogeography. Trends Ecol. Evol. 20, 68–73.

Estes, R., Báez, A., 1985. Herpetofaunas of North and South America during the Late Cretaceous and Cenozoic: evidence for interchange. In: Stehil, G.M., Webb, S.D. (Eds.), The Great American Biotic Interchange. Plenum Publishing Corporation, New York, New York, pp. 139–197.

Friesen, F.L., Congdon, B.C., Kidd, M.G., Birt, T.P., 1999. Polymerase chain reaction (PCR) primers for the amplification of five nuclear introns in vertebrates. Mol. Ecol. 8, 2141–2152.

Gelman, A., Rubin, D.B., 1992. Inference from iterative simulation using multiple sequences. Stat. Sci. 7, 457–511.

Gower, D.J., Kupfer, A., Oommen, O.V., Himstedt, W., Nussbaum, R.A., Loader, S.P., Presswell, B., Müller, H., Krishna, S.B., Boistel, R., Wilkinson, M., 2002. A molecular phylogeny of ichthyophiid caecilians (Amphibia: Gymnophiona: Ichthyophiidae): out of India or out of south east Asia? Proc. R. Soc. Lond. B 269, 1563–1569.

Harvey, M.B., Barker, D.G., Ammerman, L.K., Chippindale, P.T., 2000. Systematics of pythons of the *Morelia amethistina* complex (Serpentes: Boidae) with the description of three new species. Herptol. Monogr. 14, 139–185.

Hay, W.W., DeConto, R.M., Wold, C.N., Wilson, K.M., Voigt, S., Schulz, M., Wold, A.R., Dullo, W., Ronov, A.B., Balukhovsky, A.N., Söding, E., 1999. Alternative global Cretaceous paleogeography. In: Barrera, E., Johnson, C. (Eds.), Evolution of the Cretaceous Ocean-Climate System. Geological Society America Spec Pap 332, Boulder, pp. 1–47.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. J. Mol. Evol. 29, 170–179.

Kluge, A.G., 1991. Boine snake phylogeny and research cycles. Misc. Pub. Mus. Zool. U. Mich. 178, 1–58.

Kluge, A.G., 1993a. *Calabaria* and the phylogeny of erycine snakes. Zool. J. Linn. Soc. 107, 293–351.

Kluge, A.G., 1993b. *Aspidites* and the phylogeny of pythonine snakes. Rec. Aus. Mus.(Suppl. 19), 77.

Knapp, M., Stöckler, K., Havell, D., Delsuc, F., Sebastiani, F., Lockhart, P.J., 2005. Relaxed molecular clock provides evidence for long-distance dispersal of *Nothofagus* (southern beech). PLos Biol. 3, e14.

Laurent, R.F., 1979. Herpetofaunal relationships between Africa and South America. In: Duellman, W.E. (Ed.), The South American Herpetofauna: Its Origin, Evolution and Dispersal, 7. Mus Nat Hist Univ Kansas, Monogr, pp. 55–71.

Lawson, R., Slowinski, J.B., Burbrink, F.T., 2004. A molecular approach to discerning the phylogenetic placement of the enigmatic snake *Xenophidion schaeferi* among the Alethinophidia. J. Zool. Lond. 263, 285–294.

Lee, M.S.Y., 2004. Molecular evidence and marine snake origins. Biol. Lett. 1, 227–230.

McGlone, M.S., 2005. Goodbye Gondwana. J. Biogeogr. 32, 739–740.

Noonan, B.P., Chippindale, P.T., in review. Vicariant origin of Malagasy reptiles supports Late Cretaceous Antarctic landbridge. Am. Nat.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J.L., 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53, 47–67.

Posada, D., Crandall, K.A., 1998. Modeltest; testing the model of DNA substitution. Bioinformatics 14, 817–818.

Poux, C., Madsen, O., Marquard, E., Vietes, D.R., de Jong, W.W., Vences, M., 2005. Asynchronous colonization of Madagascar by the four endemic clades of primates, tenrecs, carnivores, and rodents as inferred from nuclear genes. Syst. Biol. 54, 719–730.

Rage, J.-C., 1972. *Eryx* Daudin et *Gongylophis* Wagler (Serpentes, Boidae): Etude osteologique. Bull. Mus. Nat. Hist. Paris 57, 893–898.

Rage, J.-C., 1981. Les Continents Péri-atlantiques au Crétacé Supérieur: migrations des Faunes Continentales et Problémes Paléogéographiques. Cret. Res. 2, 65–84.

Rage, J.-C., 1984. Serpentes. In: Wellnhofer, P. (Ed.), Handbuch der Paläoherpetologie.. Gustav Fischer Verlag, Stuttgart, New York, pp. 1–80.

Rage, J.-C., 1986. South American/North American terrestrial interchanges in the latest Cretaceous: short comments on Brett-Sturman and Paul (1985), with additional data. J. Vert. Paleo. 6, 382–383.

Rage, J.-C., 1988. Gondwana, Tethys, and terrestrial vertebrates during the Mesozoic and Cainozooic. In: Audley-Charles, M.G., Hallam, A. (Eds.), Gondwana and Tethys Geological Society Special Publication No. 37, pp. 255–273.

Rage, J.-C., 2003. Relationships of the Malagasy fauna during the Late Cretaceous: Northern or Southern routes. Acta Pal. Pol. 48, 661–662.

Rage, J.-C., Richter, A., 1994. A snake from the Lower Cretaceous (Barremian) of Spain: the oldest known snake. N. Jb. Geol. Paläont. Mh. 9, 561–565.

Rambaut, A., Drummond, A.J., 2003. Tracer v1.3, Available from: <http://evolve.zoo.ox.ac.uk>.

Rawlings, L.H., Barker, D., Donnellan, S.C., 2004. Phylogenetic relationships of the Australo-Papuan region pythons, *Liasis* (Reptilia: Macrostomata) based on mitochondrial DNA. Aust. J. Zool. 52, 215–227.

Raxworthy, C.J., Forstner, M.R.J., Nussbaum, R.A., 2002. Chameleon radiation by oceanic dispersal. Nature 415, 784–787.

Rieppel, O., 1978. The evolution of the naso-frontal joint in snakes and its bearing on snake origins. Zeit. Zool. Syst. Evol. 16, 14–27.

Sanderson, M.J., 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. 14, 1218–1231.

Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol. Biol. Evol. 19, 101–109.

Schmidt-Effing, R., 1979. Alter und Genese des Nicoya-Komplexes, einer ozeanischen Paläokruste (Oberjura bis Eozän) im südlichen Zentralamerika. Geo. Rundsch. 68, 457–494.

Slowinski, J.B., Lawson, R., 2002. Snake phylogeny: evidence from nuclear and mitochondrial genes. Mol. Phyl. Evol. 24, 194–202.

Swofford, D.L., 2001. PAUP* Phylogenetic analysis using parsimony (* and other methods), version 4b10 Sinauer, Sunderland, MA.

Thorne, J.L., Kishino, H., 2002. Divergence time and evolutionary rate estimation with multilocus data. Syst. Biol. 51, 689–702.

Thorne, J.L., Kishino, H., Painter, I.S., 1998. Estimating the rate of evolution of the rate of molecular evolution. Mol. Biol. Evol. 15, 1647–1657.

Tokar, A.A., 1989. A revision of the genus *Eryx* using osteological data. Vestinik Zoologii, No. 4, 46–55.

Tokar, A.A., 1996. Taxonomic revision of the genus *Gongylophis* Wagler 1830: *G. colubrinus* (L. 1758) (Serpentes Boidae). Trop. Zool. 9, 1–17.

Underwood, G., 1976. A systematic analysis of boid snakes. In: d'ABeallairs, A., Cox, C.B. (Eds.), Morphology and Biology of Reptiles, Linn. Soc. Symp. Ser., vol. 3. Academic Press, London, pp. 151–175.

Underwood, G., Stimson, A.F., 1990. A classification of pythons (Serpentes, Pythoninae). J. Zool. Lond. 221, 565–603.

Vences, M., Glaw, F., Kosuch, J., Böhme, W., Veith, M., 2001. Phylogeny of South American and Malagasy Boine snakes: molecular evidence for the validity of *Sanzinia* and *Acrantophis* and biogeographic implications. Copeia, 1151–1154.

Vences, M., Vieites, D.R., Glaw, F., Brinkmann, H., Kosuch, J., Veith, M., Meyer, A., 2003. Multiple overseas dispersal in amphibians. Proc. R. Soc. Lond. B 270, 2435–2442.

Vidal, N., Hedges, S.B., 2002. Higher-level relationships of snakes inferred from four nuclear and mitochondrial genes. C. R. Biol. 325, 977–985.

Vidal, N., Hedges, S.B., 2004. Molecular evidence for a terrestrial origin of snakes. Proc. R. Soc. Lond. B Suppl. 271, S226–S229.

Wilcox, T.P., Zwickl, D.J., Heath, T.A., Hillis, D.M., 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Mol. Phylogenet. Evol. 25, 361–371.

Yang, Z., 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. CABIOS 13, 555–556.

Yoder, A.D., Yang, Z., 2004. Divergence dates for Malagasy lemurs estimated from multiple gene loci: geological and evolutionary context. Mol. Ecol. 13, 757–773.

Yoder, A.D., Cartmill, M., Ruvolo, M., Smith, K., Vilgalys, R., 1996. Ancient single origin of Malagasy primates. Proc. Natl. Acad. Sci. USA 93, 5122–5126.

Yoder, A.D., Burns, M.M., Zehr, S., Delefosse, T., Vernon, G., Goodman, S.M., Flynn, J.J., 2003. Single origin of Malagasy Carnivora from an African ancestor. Nature 421, 734–737.

Zaher, H., 1994. Les Tropidopheoidea (Serpentes; Alethinophidia) sont-ils réellement monophylétiques? Arguments en faveur de leur polyphylétisme. Comptes Rendus de l'Academie des Sciences, Paris. 317, 471–478.