



Molecular phylogeny and historical biogeography of West Indian boid snakes (*Chilabothrus*)



R. Graham Reynolds^{a,*}, Matthew L. Niemiller^b, S. Blair Hedges^c, Alex Dornburg^b, Alberto R. Puentes-Rolón^d, Liam J. Revell^a

^a Department of Biology, University of Massachusetts Boston, 100 Morrissey Blvd., Boston, MA 02125-3393, USA

^b Department of Ecology and Evolutionary Biology, Yale University, 21 Sachem St., New Haven, CT 06520, USA

^c Department of Biology, Pennsylvania State University, 208 Mueller Lab, University Park, PA 16802, USA

^d Departamento de Ciencias y Tecnología, Universidad Interamericana de Puerto Rico, Recinto de Arecibo, Arecibo, PR 00614, USA

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ABSTRACT

The evolutionary and biogeographic history of West Indian boid snakes (*Epicrates*), a group of nine species and 14 subspecies, was once thought to be well understood; however, new research has indicated that we are missing a clear understanding of the evolutionary relationships of this group. Here, we present the first multilocus, species-tree based analyses of the evolutionary relationships, divergence times, and historical biogeography of this clade with data from 10 genes and 6256 bp. We find evidence for a single colonization of the Caribbean from mainland South America in the Oligocene or early Miocene, followed by a radiation throughout the Greater Antilles and Bahamas. These findings support the previous suggestion that *Epicrates sensu lato* Wagler is paraphyletic with respect to the anacondas (*Eunectes* Wagler), and hence we restrict *Epicrates* to the mainland clade and use the available name *Chilabothrus* Duméril and Bibron for the West Indian clade. Our results suggest some diversification occurred within island banks, though most species divergence events seem to have occurred in allopatry. We also find evidence for a remarkable diversification within the Bahamian archipelago suggesting that the recognition of another Bahamian endemic species *C. strigilatus* is warranted.

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1. Introduction

More than one third (37%, 20 of 54 species) of all snakes in the family Boidae are island or archipelagic endemics, including the members of separate radiations in the archipelagos of Melanesia and the West Indies. West Indian boas (genus *Epicrates sensu lato*) provide an ideal case study to test whether the species diversity observed across the Caribbean is a consequence of multiple dispersal events from the mainland, or alternatively, one or a few colonization events followed by an among-island diversification of island endemics (e.g., Hedges et al., 1992; Hedges, 1996a). *Epicrates sensu lato* comprises 14 species, with 9 species and 14 subspecies distributed in the Greater Antilles (Jamaica, Hispaniola, Cuba, and Puerto Rico), the Turks and Caicos Islands, and the Bahama Islands, and an additional five species on the mainland of Central and South America (Sheplan and Schwartz, 1974; Tolson and Henderson,

1993; Passos and Fernandes, 2008; Rivera et al., 2011). West Indian boa species vary widely in body size (<1–4 m adult total length) and ecology, with members occupying many diverse habitats from the dense rainforests of the Greater Antilles to the xeric scrub of tiny (<0.1 km²) cays in the Bahamas. However, phylogenetic investigations of this group have been contradictory, hindering study of the biogeographic history underlying present day ecological and morphological diversity of these lineages. Furthermore, these species represent major conservation priorities, as they face a range of threats, including habitat encroachment and introduced predators or competitors (Hailey et al., 2011; Reynolds, 2011; Tolson and Henderson, 2011; Reynolds and Gerber, 2012; Reynolds et al., 2013). An understanding of the evolutionary relationships and biogeographic history of these lineages would be of high utility in identifying relevant conservation units (Frankham, 2006).

Systematics of West Indian *Epicrates* were formalized into the present classification by Sheplan and Schwartz (1974), who recognized a monophyletic group containing nine species in the West Indies, derived, they hypothesized, from the South American *Epicrates cenchria*. These authors proposed that the smaller species (*E. monensis*, *E. fordii*, *E. gracilis*, and *E. exsul*) represented a unique and independent lineage from the larger species (*E. angulifer*, *E. striatus*, *E. chrysogaster*, *E. subflavus*, and *E. inornatus*) (Fig. 1a).

* Corresponding author.

E-mail addresses: rgraham.reynolds@umb.edu (R.G. Reynolds), matthew.niemiller@yale.edu (M.L. Niemiller), sbh1@psu.edu (S.B. Hedges), alex.dornburg@yale.edu (A. Dornburg), albertonski@hotmail.com (A.R. Puentes-Rolón), liam.revell@umb.edu (L.J. Revell).

URL: <http://www.rgrahamreynolds.info> (R.G. Reynolds).

Although morphologically supported, these proposed relationships required multiple dispersal events to explain the movement of both ecotypes (small and large) from their centers of origin and diversification. Tolson (1987) tested the phylogenetic hypothesis of Sheplan and Schwartz (1974) using lipid electrophoresis and morphological data (Fig. 1a) and obtained a topologically similar estimate of the phylogenetic relationships of the group. Tolson (1987) further examined several biogeographic scenarios for the origins and diversification of West Indian *Epicrates*, including the vicariance model of Rosen (1975), by comparing area and geological cladograms. From these analyses he concluded that dispersal and not vicariance was the main force driving the origin and distribution of this genus (Tolson, 1987). Kluge (1989) obtained an identical phylogenetic hypothesis to that of Tolson (1987) in a combined analysis of osteological and lipid data. Kluge (1988a, Fig. 10) also proposed at least three vicariant events (and three dispersal events) to explain the present distribution of this genus in the West Indies. These vicariant (island separation) events are as follows: (1) vicariance between the mainland and the West Indies, (2) vicariance of the Cuban *E. angulifer*, and (3) one or more vicariant events in the remaining West Indies separating Jamaican, Hispaniolan, or Puerto Rican species.

Campbell (1997) was the first to apply molecular sequence data to the problem of West Indian *Epicrates* phylogenetics by sequencing the mitochondrial cytochrome *b* (*CYTB*) gene. Campbell combined these data with morphological characters and inferred an evolutionary tree that conflicted with relationships proposed by previous authors (Fig. 1b). Campbell's (1997) phylogeny provided the first evidence for the repeated evolution of small body size and strongly suggested that species occupying the same island, despite differing in ecology and morphology, might be more closely related. Since then, no study has explicitly examined the phylogenetic relationships and biogeography of the West Indian *Epicrates*. However, several broader molecular phylogenetic studies have suggested that our current understanding of the evolution and diversification of these boas requires further investigation. Burbrink (2004) examined *CYTB* variation across the Boidae and found evidence that *Epicrates sensu lato* might be paraphyletic with respect to anacondas (*Eunectes*). His analyses also suggested that

the Caribbean species *E. striatus sensu lato* may be paraphyletic with respect to *E. exsul* (Fig. 1b). Noonan and Chippindale (2006) extended this work using a concatenated analysis of five nuclear genes in a subset of taxa. These authors found weak support for paraphyly of *Epicrates sensu lato*. They also estimated divergence between mainland and West Indian *Epicrates* at 22 Mya. Tzika et al. (2008) examined the relationship of *E. subflavus* haplogroups with respect to other West Indian *Epicrates* using *CYTB*, and found support for a close relationship between *E. striatus* and *E. exsul*. Finally, Rivera et al. (2011) examined phylogenetic relationships among nine species of *Epicrates sensu lato* and close outgroups, including five species of mainland *Epicrates* (Passos and Fernandes, 2008), and found strong statistical support for the paraphyly of *Epicrates sensu lato* relative to *Eunectes*.

Given the discordance between these prior studies of evolutionary relationships, as well as between phylogenetic hypotheses and historical biogeography, we have undertaken the first multilocus phylogenetic analysis to focus specifically on West Indian *Epicrates* and their close relatives. Here, we infer a phylogeny from 10 genes (two mitochondrial and eight nuclear loci) comprising 6256 base pairs among eight species and four subspecies of West Indian *Epicrates*, two species of mainland *Epicrates*, two species of *Eunectes* (anacondas), and outgroups using a Bayesian framework allowing for the possibility of incongruence between gene trees and the species tree. In addition, we estimated the historical timing of island colonization by conducting fossil-calibrated divergence time analyses, which we then used as the evolutionary framework for reconstructing the history of island colonization in the West Indies in a likelihood-based analysis of historical biogeography.

2. Materials and methods

2.1. Sample collection and DNA sequencing

We obtained samples of West Indian *Epicrates*, mainland *Epicrates*, and *Eunectes* species, along with several outgroup species, from field collection, museum tissue collections, public and private reptile collections (zoos), and U.S. private reptile breeders

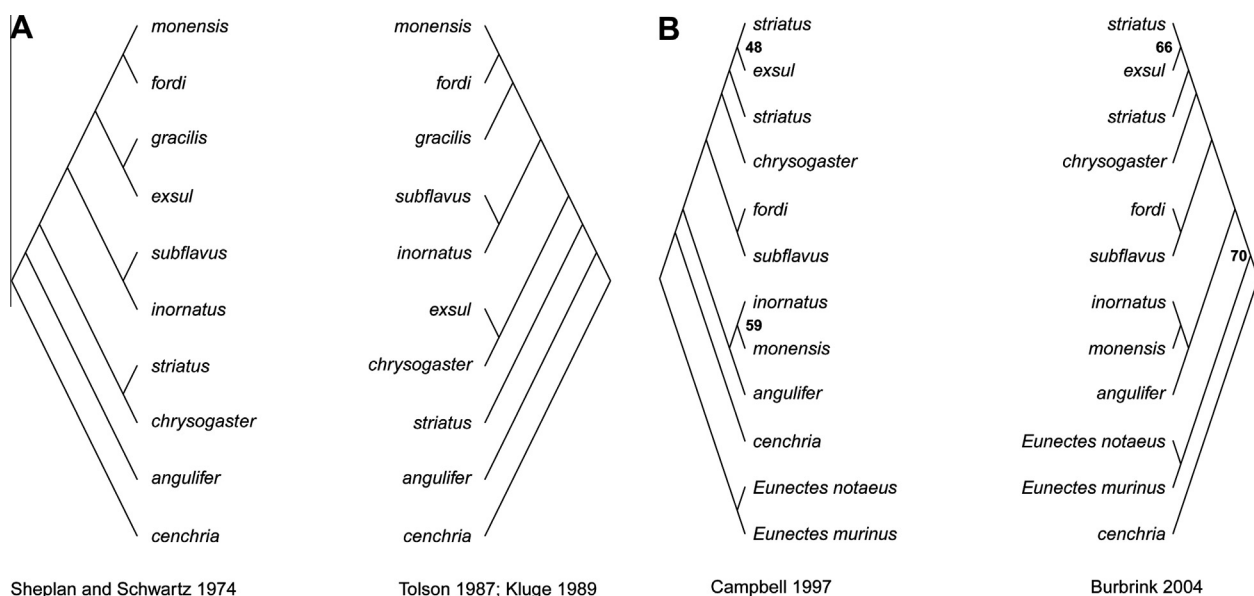


Fig. 1. Previous phylogenetic hypotheses for *Epicrates* boid snakes. (A) Morphological cladogram of Sheplan and Schwartz (1974) is of similar topology to the lipid cladogram of Tolson (1987) and the "total evidence" approach of Kluge (1989). (B) Molecular cladogram based on the mitochondrial cytochrome *b* gene. Numbers at nodes correspond to maximum likelihood support values less than 90% (Campbell, 1997) and Bayesian posterior probabilities less than 0.90 (Burbrink, 2004). Note that both authors find weak support for paraphyly of *Epicrates striatus*, and Burbrink (2004) finds support for the paraphyly of *Epicrates* with respect to anacondas (*Eunectes*).

(Appendix S1). Samples consisted of tissue biopsies preserved in 95% ethanol or frozen freshly-shed skins. We attempted to include at least two samples from every described species of West Indian *Epicrates*; however, we were unable to obtain a sample from the Hispaniolan species *E. gracilis*, and no other published molecular study has included a sample from this species. In addition, some authors treat the species *E. monensis* as actually being composed of two species: *E. monensis* on Isla Mona and *E. granti* on Puerto Rico and the eastern Puerto Rican Bank (e.g., Platenberg and Harvey, 2010). While we included individuals from Puerto Rico and the U.S. Virgin Islands, we were unable to obtain a sample from Isla Mona. We included four subspecies of the Hispaniolan/Bahamian species *E. striatus sensu lato* (*E. s. fosteri* [$n = 1$], *E. s. fowleri* [$n = 3$], *E. s. striatus* [$n = 6$], and *E. s. strigilatus* [$n = 5$]). Though four other subspecies are recognized, they were not included in our analysis due to scarcity of samples or samples of questionable origin. We also included two of five species of continental *Epicrates* (*E. cenchria* [South America] and *E. maurus* [South and Central America]) and two of four species of South American anacondas (*Eunectes murinus* and *Eu. notaeus*). Outgroups were chosen based on previously established relationships with the West Indian boa clade (e.g., Burbrink, 2004; Wiens et al., 2008; Rivera et al., 2011; Colston et al., 2013) and availability of tissue or sequences. Outgroup taxa included one species of *Boa constrictor* (South America), two species of *Corallus* (*Cor. batesii* and *Cor. annulatus*) [South America], one species of North American boid (*Lichanura trivirgata*), and one species of burrowing python (*Calabaria reinhardtii*) [Africa], the latter two of which are represented by sequences available on Genbank (Appendix S2).

We initially assayed eight taxa for primer amplification using the polymerase chain reaction (PCR) across 23 loci obtained from the literature. We also tested five internal primers designed from a template of *E. striatus* available on Genbank using Primer-Blast (NCBI). Of these 28 primer-pairs, we selected ten loci that amplified across taxa and have been previously used in phylogenetic studies of boid snakes (Table 1), including cytochrome b (*CYTB*), oocyte maturation factor (*c-mos*), brain-derived neurotrophic factor (*bdnf*), neurotrophin-3 (*ntf3*), bone morphogenetic protein 2 (*bmp2*), recombination activating protein-1 (*rag1*), NADH dehydrogenase subunit 4 (*ND4*), prostaglandin E receptor 4 (*ptger4*), protein tyrosine phosphatase non-receptor type 12 (*ptpn12*), and ornithine decarboxylase (*odc*). We visualized PCR products by gel electrophoresis and purified and sequenced products (both strands) on an automated sequencer (ABI 3730XL) at Massachusetts General Hospital DNA Core Facility, Cambridge, MA. We assembled each gene region and manually verified ambiguous base calls using SEQUENCHER 5.1 (Gene Codes). We then aligned sequences using the CLUSTALW 2.1 algorithm (Larkin et al., 2007) implemented in MESQUITE 2.75 (Maddison and Maddison, 2011) using reference sequences. Because all loci except *odc* are exons from protein-coding

genes, alignment was straightforward and no indels were found in in-group taxa; however, we could not amplify the ND4 region for Bahamian *E. striatus* owing to a likely mutation in the priming region. Additional primer pairs designed from other boid templates were also unsuccessful. The intron locus *odc* contains indels and hence was aligned in a step-wise fashion using reference sequences from other boas and pythons. We deposited all 367 newly generated sequences into GenBank (Appendix S2) and accessioned the alignment in TreeBase (<http://purl.org/phylo/treebase/phylogenetics/study/TB2:S14064>).

2.2. Species tree and divergence time

A number of methods are available for estimating species trees from multiple gene trees, though some require *a priori* species assignments or restrictive assumptions, such as constant population size along a branch (Carstens and Knowles, 2007; Edwards et al., 2007; Liu and Pearl, 2007; Niemiller et al., 2012). We chose to use the flexible MCMC method *BEAST (Heled and Drummond, 2010) implemented in the program BEAST v1.7.4 (Drummond et al., 2006; Drummond and Rambaut, 2007). This method jointly estimates species tree topology, divergence times, and population sizes from multiple embedded gene trees under the multispecies coalescent model, which assumes that incongruence among gene trees owes to incomplete lineage sorting in lieu of gene flow between species. We feel that this is a reasonable assumption for lineages that are primarily restricted to islands. We conducted three independent MCMC runs for 300 million generations for each analysis, sampling every 10,000 generations. The two mitochondrial genes (*CYTB* and *ND4*) were concatenated and treated as a single locus. In all analyses, sequence data were partitioned by locus and assigned a locus-specific model of nucleotide substitution chosen using Akaike's Information Criterion implemented in Model-Test Server (Posada, 2006; Table 1). Nucleotide substitution models, clock models, and gene trees were unlinked in all analyses. We employed an uncorrelated lognormal (UCLN) relaxed molecular clock model of rate variation for each locus with a Yule process speciation prior for the branching rates. We assured adequate mixing of the MCMC by calculating the effective sample size (ESS) values for each model parameter, with ESS values greater than 200 indicating adequate sampling of the posterior distribution. We assessed convergence of the independent runs by a comparison of likelihood scores and model parameter estimates in TRACER v1.5. We discarded the first 150 million generations as burn-in.

We incorporated four fossil priors on the species tree following McCormack et al. (2010), using an offset with a lognormal prior to set a hard minimum and a soft maximum bound on node ages (Yang and Rannala, 2006) [Table 2]. The divergence of henophidian and caenophidian snakes likely occurred 100–94 Mya during the Cenomanian (Rage and Werner, 1999; Noonan and Sites, 2010;

Table 1

Genes, primers, and selected best-fit models of evolution. Internal primers were designed on an *Epicrates striatus* template. See Appendix S2 for GenBank accession numbers.

Gene	Abbreviation	Length (bp)	Ploidy	Primers	Selected Model
Cytochrome b	<i>CYTB</i>	1077	N	Burbrink et al. (2000)	HKY+I+G
Oocyte maturation factor	<i>c-mos</i>	465	2n	Noonan and Chippindale (2006)	K81uf
Brain-derived neurotrophic factor	<i>bdnf</i>	711	2n	Wiens et al. (2008)	TrN
Neurotrophin-3	<i>ntf3</i>	519	2n	Wiens et al. (2008)	TrN+I
Bone morphogenetic protein 2	<i>bmp2</i>	660	2n	Wiens et al. (2008)	TrNef+G
Recombination activating protein-1	<i>rag1</i>	678	2n	F-5'-GCAGCTTTGGTGGCTGCCCT R-5'-ACAGTGCAGTGCATCTATTGAAGGC	HKY+I
NADH dehydrogenase subunit 4	<i>ND4</i>	636	N	Janzen et al. (2002)	TVM+I+G
Prostaglandin E receptor 4	<i>ptger4</i>	507	2n	Wiens et al. (2008)	HKY+I
Protein tyrosine phosphatase non-receptor type 12	<i>ptpn12</i>	387	2n	F-5'-GCCCTTCAGGAAGGACCACG R-5'-TGCCATCTGGACTGGCACC	HKY
Ornithine decarboxylase	<i>odc</i>	610	2n	Friesen et al. (1999)	HKY+G

Table 2

Fossils and calibrations used in divergence time analysis, with minimum and maximum priors for specified nodes. See text for additional information.

Taxon	Group	Node	Hard minimum (Mya)	Soft maximum (Mya)	Reference(s)	
Boidae	Boidae	Late Cretaceous	A	75	94	Rage (1984) and Albino (1996, 2000)
† <i>Titanoboa</i>	Boinae	Middle-Late Paleocene	C	58	65.5	Jaramillo et al. (2007) and Head et al. (2009)
<i>cerrejonensis</i>						
<i>Corallus</i>	Boinae	Middle-Late Paleocene	D	58	61	Pascual and Ortiz-Jaureguizar (1991), Medeiros and Bergqvist (1999) and Rage (2001)
† <i>Eunectes stirtoni</i>	Boinae	Middle Miocene	H	12	13.8	Hoffstetter and Rage (1977)

Vidal et al., 2010; Mulcahy et al., 2012; Pyron and Burbrink, 2012), while the age of the split between Boidae and Calabaridae has been estimated between 86–55 Mya (Rage, 1984; Albino, 1996, 2000; Vidal et al., 2009; Noonan and Sites, 2010; Pyron and Burbrink, 2012). We therefore calibrated the root node to capture crown Boidae, using a hard minimum of 75 Mya and a soft maximum of 94 Mya to encompass the appearance of the Boidae. We calibrated the most recent common ancestor of *Boa*, *Corallus*, *Eunectes*, and *Epicrates* with the age of the fossil †*Titanoboa cerrejonensis*, from the Cerrejón Formation (middle-late Paleocene), La Puente Pit, Guajira Peninsula, Colombia, which is ancestral to modern boids (Jaramillo et al., 2007; Head et al., 2009). In the absence of prior information for a maximum age for this split, the soft maximum age was set corresponding to the start of the Paleocene. We used a fossil calibration identified as †*Eunectes stirtoni* (Hoffstetter and Rage, 1977) for the *Eunectes* lineage, though the distinction from *Eu. murinus* has been questioned (Hecht and LaDuke, 1997; Hsiou and Albino, 2009) and additional material associated with the holotype might have been misidentified due to the exclusion of diagnostic vertebral characteristics in the reconstructed illustration of the material (Hecht and LaDuke, 1997). However, *Eunectes* certainly appear to have evolved by the middle Miocene (Hoffstetter and Rage, 1977; Hsiou and Albino, 2009). The fossil record for *Epicrates* is largely unknown, as the fossils *Paraepicrates* and *Pseudoepicrates* are now thought to have belonged to the North American *Lichanura* boids (Kluge, 1988b). A set of Miocene fossils tentatively attributed to aff. *Epicrates* (Hsiou and Albino, 2010) shares vertebral characteristics with *Corallus hortulanus*, in that these mid-trunk vertebrae fossils have a large protruding median lobe, a characteristic that is shared between *Epicrates* and *Corallus* (Hsiou and Albino, 2010). The authors suggest that because the prezygapophyses in the three series are inclined (*Epicrates*) and not horizontal (*Corallus*) relative to the horizontal plane of the vertebrae, they tentatively assign these fossils to aff. *Epicrates*. Another study has used this fossil, though the authors acknowledged that the identification is tentative (Head et al., 2012). Based on this phylogenetic uncertainty, we have taken a cautious approach and excluded this particular fossil from our analysis.

We calibrated the split between *Corallus* and other neotropical boids to have occurred in the middle-late Paleocene (Pascual and Ortiz-Jaureguizar, 1991; Medeiros and Bergqvist, 1999; Rage, 2001), which is consistent with other studies. Previous work (e.g., Colston et al., 2013) using this calibration has additionally recovered older than expected age estimates for deeper nodes in the South American boid tree relative to other studies (e.g. Noonan and Chippindale, 2006). In order to compare node ages inferred with and without this prior age calibration, we conducted two separate divergence time analyses in *BEAST. The first analysis included all four prior age calibrations mentioned above, while in the second we excluded the *Corallus* fossil as a calibration.

To compare our topological results to prior studies of concatenated datasets, we also conducted a concatenated Bayesian phylogenetic analysis using MrBayes 3.2 (Ronquist et al., 2012). We

partitioned the analysis by locus (eight nuclear loci plus concatenated mtDNA), choosing models of nucleotide substitution for each locus using Akaike's Information Criterion implemented in Model-Test Server (Posada, 2006). We conducted two independent runs using six Markov chains and default prior settings for 10 million generations, sampling every 100th generation. We used random trees to begin each Markov chain and did not enforce a molecular clock. We assessed convergence of runs by visual examination of the state likelihoods, calculation of potential scale reduction factors, and examination of the average deviation between the clade splits of the two independent runs, with a value below 0.01 indicating that the runs had converged. We used our sample of trees from the stationary distribution to generate a 50% majority-rule consensus tree.

2.3. Ancestral area reconstructions

All ancestral area reconstructions were based on the assignment of contemporary lineages to eight geographic areas: (1) Great Bahama Bank; (2) Little Bahama Bank, (3) Southern Bahamas; (4) Puerto Rico Bank; (5) Hispaniola; (6) Cuba; (7) Jamaica; and (8) Mainland South America (Fig. 2A), encompassing the extent of the historical range of *Epicrates sensu lato* boas (*E. maurus* of Panama and Costa Rica is also found in northern S.A.). As *Calabaria reinhardtii* (Africa) and *Lichanura trivirgata* (SW United States and NW Mexico) were included only as a distantly related outgroups, these lineages were pruned from the tree prior to all biogeographic analyses. To infer historical range shifts based on our ultrametric phylogeny, we used a likelihood dispersal–extinction–cladogenesis (DEC) model implemented in the software package Lagrange (Ree et al., 2005; Ree and Smith, 2008). One advantage of this approach for ancestral area reconstruction is the incorporation of temporal information contained in branch lengths, thereby allowing for changes in area connectivity through time. We partitioned the dated tree into two time slices and tested models with a range of dispersal probabilities between geographic areas. We tested four models of area reconstruction: (1) restricted dispersal (Model 1) between all areas from the present day until 15 Mya based on reconstructions of historical connectivity owing to ocean surface currents and hurricane tracks (Hedges, 1996b, 2001; Fig. 2A and B); (2) restricted long distance dispersal ($p = 0$) against prevailing surface currents and long distance dispersal events (Model 2; Fig. 2C); (3) reduced probabilities ($p = 0.1$) against prevailing surface currents and long distance dispersal events (Model 3; Fig. 2D); and (4) equal probabilities of dispersal between all areas throughout the history of the radiation (Model 4; Fig. 2E). For the first three models, the geologically earlier time slice included an unconstrained probability of dispersal between islands, as the Proto-Antilles likely consisted of a complex of fragmented islands and the geologic and oceanographic record is sufficiently vague as to prevent refined modeling prior to the mid-Miocene (Robinson, 1994; Iturralde-Vinent and MacPhee, 1999; Hedges, 2001; Iturralde-Vinent, 2006; Pindell and Kennan, 2009). We compared the fit of these four models using an information theoretic framework

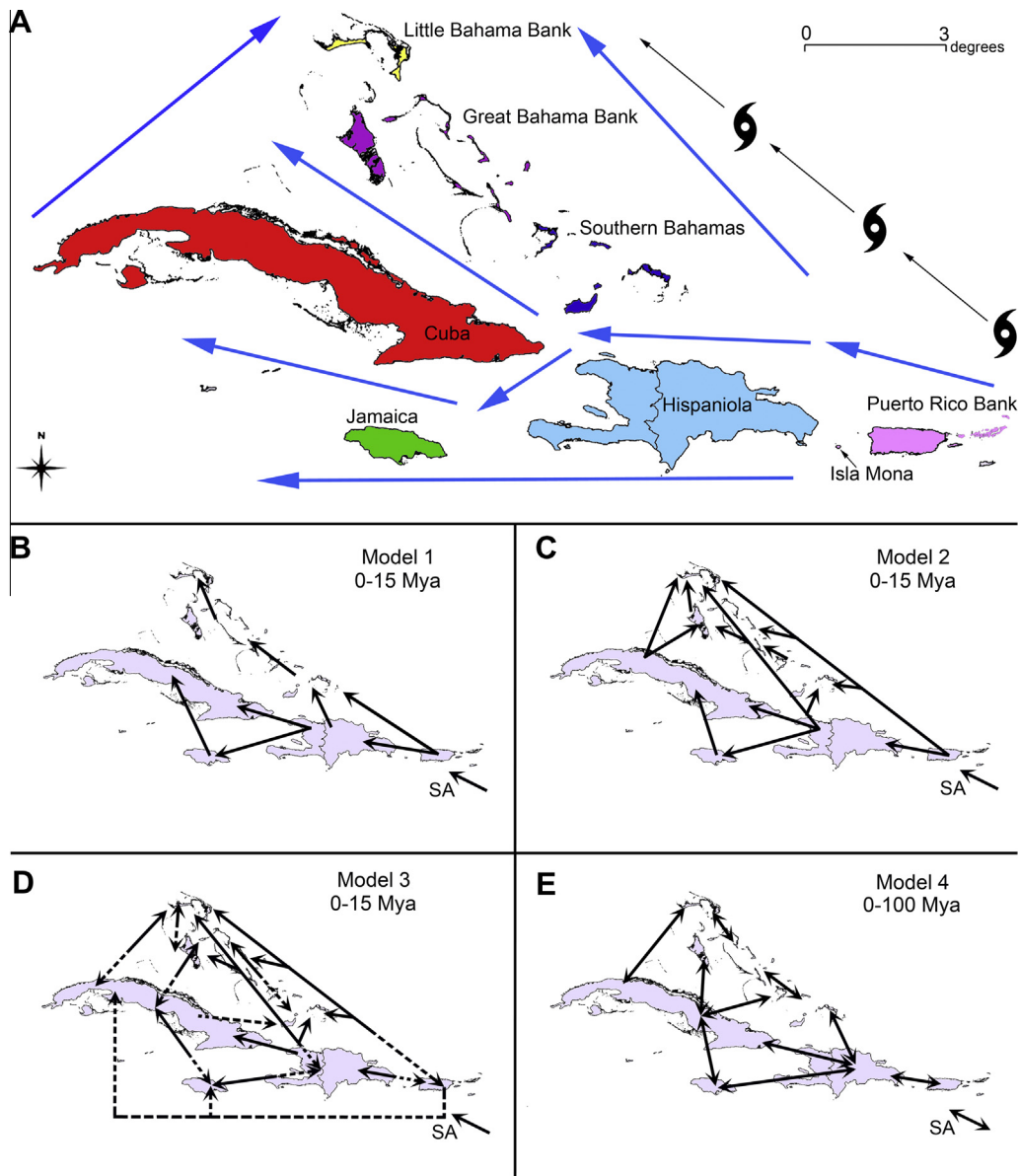


Fig. 2. Map of the Greater Antilles and Bahamas Banks, with each island or island group color-coded and panels illustrating ocean currents and dispersal models. (A) The predominant directions of surface ocean currents (blue arrows) and hurricane tracks (black arrows) [after Hedges, 1996b]; (B through E) models used in DEC analyses. Solid black arrows indicate high probability of dispersal between islands; dashed arrows indicate marginal probability of dispersal. Dispersal to or from South America is denoted by black arrows and the label SA. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that accounts for small sample size (AICc, Burnham and Anderson, 2004).

3. Results

3.1. Species tree and divergence times

Our species tree analysis (Fig. 3) resulted in an identical topology to our concatenated analysis (Fig. 4). This topology is similar to previous studies of West Indian boas (i.e., Burbrink, 2004; Rivera et al., 2011), with a few notable exceptions (Fig. 3). We found strong support (posterior probability [PP] = 1.0) for the paraphyly of *Epicrates sensu lato* with respect to the anacondas (*Eunectes*), recovering a closer relationship between the mainland *Epicrates* and *Eunectes* that diverged approximately 25.3 Mya (95% highest posterior density interval [95% HPD]: 19.2–32.5 Mya; Table 3). This

is similar to previous estimates of 24 Mya (Rage, 1994) and 29 Mya (Noonan and Chippindale, 2006). We estimated a mean divergence of 30.2 Mya (95% HPD 24.5–35.8 Mya) for the most recent common ancestor of the mainland clades (*Epicrates* and *Eunectes*) and the West Indian *Epicrates* clade, henceforward *Chilabothrus*, slightly older than a previous study (22 Mya; Noonan and Chippindale, 2006). This divergence is also younger than (and hence consistent with) a frequently used maximum upper age of 37.2 Mya for the earliest date after which there has been a continuous presence of emergent land masses in the West Indies (Iturralde-Vinent and MacPhee, 1999; Hedges and Conn, 2012). In the West Indies, the Cuban boa (*Chilabothrus angulifer*) represents the most basal extant lineage of the radiation, splitting off from the Caribbean taxa approximately 21.7 Mya (95% HPD 16.9–26.0 Mya). A Puerto Rican bank clade consisting of the large *C. inornatus* and small arboreal *C. monensis* diverged soon after (19.2 Mya; 95% HPD 15.3–23.2 Mya). Jamaican boas (*C. subflavus*) diverged 17.3 Mya (95% HPD

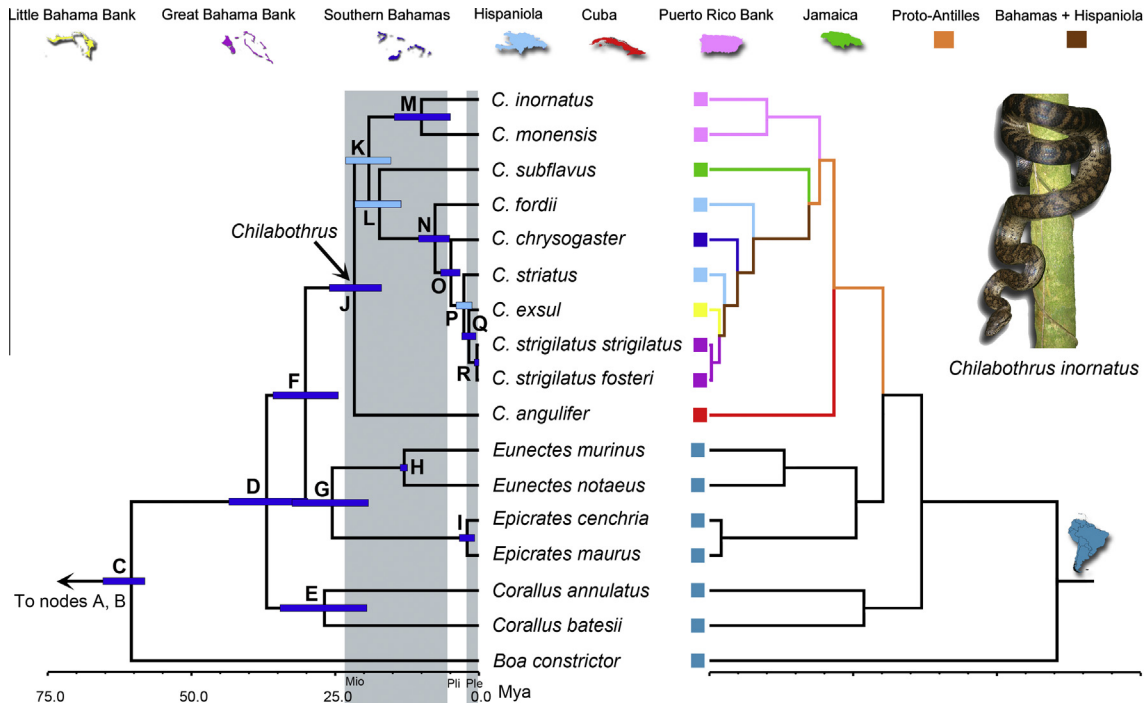


Fig. 3. Fossil-calibrated species tree (left) for the ingroups *Boa*, *Chilabothrus*, *Corallus*, *Epicrates* and *Eunectes*. Nodes are labeled with letters (Table 3) and 95% HPD intervals are shown, with shading corresponding to the posterior probabilities for each node (dark blue ≥ 0.95 ; light blue < 0.95). The species tree is reflected across the median of the figure, and on the right the results from the biogeographic DEC analysis are shown. Colored branches above and below each node represent the inferred splits between lineages (Table 3), which are color-coded by island or island group as shown above the figure. Each tip is labeled with a colored square corresponding to the present range of that species. A representative West Indian boa (*C. inornatus* from Arecibo, Puerto Rico) is shown on the far right. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

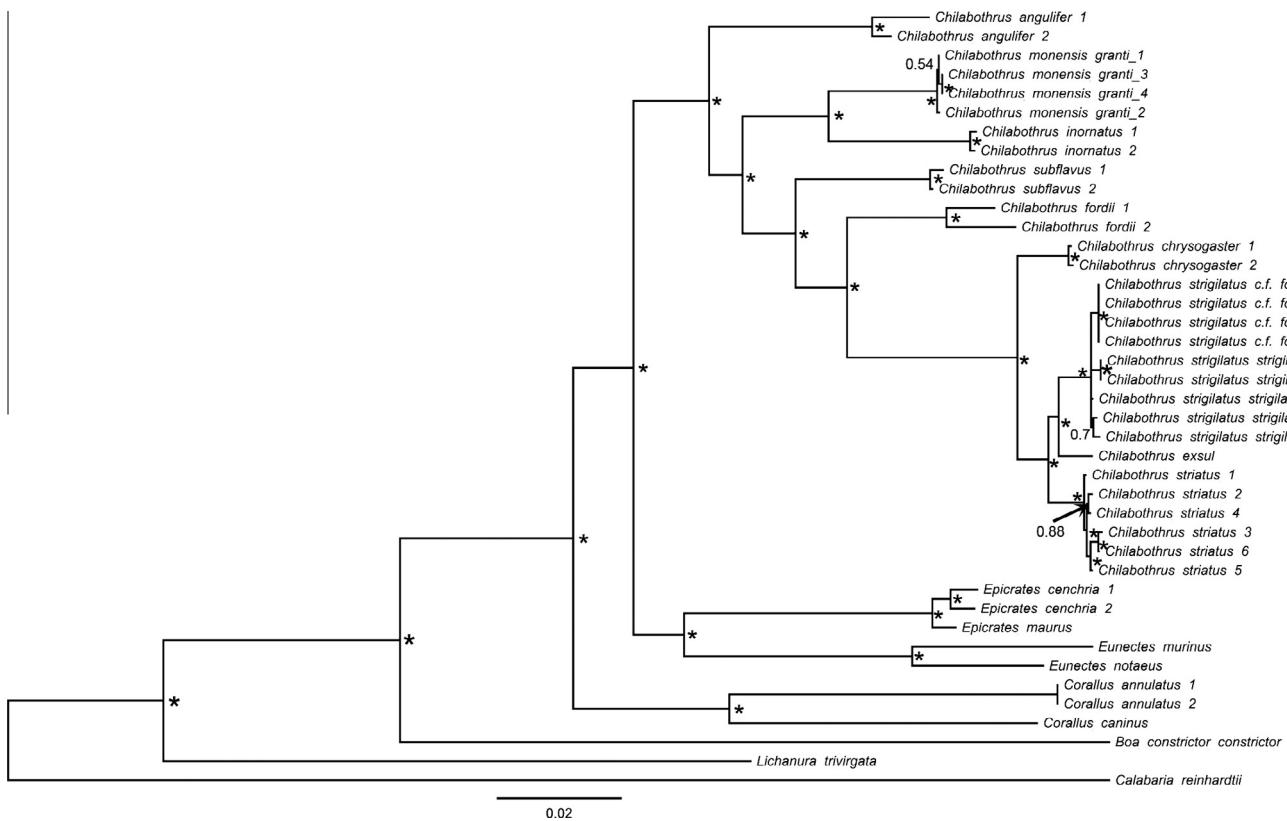


Fig. 4. Bayesian MCMC consensus tree from the concatenated and partitioned 10-gene dataset. Nodes with posterior probabilities > 0.95 are indicated by an asterisk (*), while numbers indicate posterior probabilities at nodes with lower support. Refer to Supplementary Data S1 for more information on tip labels.

Table 3

Estimated divergence times (in millions of years) from *BEAST and ancestral area splits from Model 2 in LAGRANGE for nodes in Fig. 2.

Node	Posterior probability	Divergence times mean, [95% HPD]	LAGRANGE (LG) splits	LG likelihood
A	1.0	106.2, [75.0, 126.5]	–	–
B	1.0	85.0, [68.4, 103.3]	–	–
C	1.0	60.5, [58.1, 65.4]	ML_HS_PR_GB_LB_SB_JA_CB ML	0.28
D	1.0	36.9, [29.8, 43.5]	ML ML_HS_PR_GB_LB_SB_JA_CB	0.52
E	1.0	26.9, [19.5, 34.6]	ML ML	0.99
F	1.0	30.2, [24.5, 35.8]	ML HS_PR_GB_LB_SB_JA_CB	0.62
G	0.96	25.5, [19.2, 32.5]	ML ML	0.99
H	1.0	13.0, [12.4, 13.7]	ML ML	1.0
I	1.0	2.1, [0.7, 3.4]	ML ML	0.99
J	1.0	21.7, [16.9, 26.0]	HS_PR_GB_LB_SB_JA CB	0.75
K	0.90	19.2, [15.3, 23.2]	HS_GB_LB_SB_JA PR	0.78
L	0.70	17.3, [13.6, 21.6]	JA HS_GB_LB_SB	0.80
M	1.0	10.0, [5.0, 14.7]	PR PR	0.99
N	1.0	7.7, [5.1, 10.5]	HS_GB_LB_SB HS	0.93
O	1.0	4.9, [3.3, 6.7]	SB HS_GB_LB	0.97
P	1.0	2.6, [1.2, 4.0]	GB_LB HS	0.99
Q	0.51	1.8, [0.5, 3.0]	GB LB	0.99
R	0.98	0.4, [0, 0.8]	GB GB	0.99

13.6–21.6 Mya), while the Bahamian and Hispaniolan boas show a more complicated pattern of divergence. The small, fossorial, Hispaniolan *C. fordii* splits prior to the origin of a clade primarily restricted to the Southern Bahamas (mean 7.7 Mya; 95% HPD 5.1–10.5 Mya), with the Hispaniolan *C. striatus* diverging from the clade containing *C. exsul* from the Little Bahama Bank and *C. striatus sensu lato* from the Great Bahama Bank approximately 2.6 Mya (95% HPD 1.2–4.0 Mya). This raises the possibility of a paraphyletic relationship in *C. striatus*, whereby *C. exsul* would be the closest relative of the Bahamian *C. striatus* with a divergence time of 1.8 Mya corresponding to the Pre-Illinoian mid-Pleistocene, though support for divergence between *C. exsul* and Bahamian *C. striatus* is low (PP = 0.51) in the species-tree analysis but not in the concatenated analysis (Fig. 4). The western Great Bahama Bank subspecies *C. striatus fosteri* (Bimini) and *C. striatus fowleri* (Berry Is./Andros Is.) are found to be a separate lineage (PP = 0.98) from eastern Great Bahama Bank *C. striatus strigilatus* (Eleuthera/Long Island) on the eastern side of the Great Bahama Bank, with an estimated mean divergence time of 0.35 Mya (95% HPD 0.0–0.83 Mya). All Bahamian species and populations appear to have diverged after the Miocene, which is consistent with diversification of other squamates in the region (Hedges and Conn, 2012).

As expected, inclusion of the *Corallus* fossil calibration point significantly increased node age estimates for all taxa, pushing the crown boid node back to the mid-Jurassic (168.4 Mya, [95% HPD 144.2–197.5 Mya] [Appendix S3 and S5] predating all other estimates of the origin of the entire Alethinophidian clade (e.g., Vidal et al., 2010; Pyron and Burbrink, 2012). The oldest known snake fossils date from 145 to 112 Mya (Hoffstetter, 1960; Vidal et al., 2009) and our deeper node HPD intervals from the analysis without the *Corallus* calibration are consistent with previous hypotheses of boid divergence (e.g., Noonan and Chippindale, 2006). Additionally, many internal node age estimates obtained with this calibration are inconsistent with previous studies, as well as expected divergences based on the geological record. For example, the divergence of Jamaican boa (*C. subflavus*) predates the estimated emergence of the island of Jamaica in the Miocene (Draper, 1987). Further study is warranted to determine the accuracy of this fossil calibration and the influence of additional calibrations on age estimates for deeper nodes in the boid tree.

3.2. Historical biogeography

Comparison of historical biogeographic models supported Model 2, in which we restricted dispersal pathways based on prevailing

Table 4

Historical biogeographic models and model selection for the 0–15 Mya time slice. See text for details.

Model	Parameters	DEC ln(L)	AICc	AICc weights
Model 1	49	–29.4748	8.46	0.119
Model 2	43	–29.3271	4.51	0.859
Model 3	63	–29.4935	13.10	0.119
Model 4	64	–29.6076	13.32	0.010

ocean surface currents and hurricane tracks, over the other models of dispersal (Table 4). However, likelihoods were very similar suggesting that model selection is likely dominated by parameterization, and inferences of ancestral ranges under all models were identical (Appendix S4). Our results corroborated previous suggestions (e.g., Tolson, 1987; Kluge, 1988a) that West Indian *Chilabothrus* form a monophyletic group derived from a single dispersal from Mainland Central/South America into the Proto-Greater Antilles (Fig. 2). Two species, *C. monensis* and *C. inornatus*, are found to be closest relatives, suggesting the potential for within-bank divergence from an ancestral isolation on the Puerto Rican Bank. From the Proto Greater Antilles, we inferred the isolation of *C. subflavus* on Jamaica subsequent to node L (Fig. 3; Table 3) approximately 17.3 Mya. The *C. fordii* and *C. chrysogaster* lineages were subsequently isolated in Hispaniola and the Southern Bahamas, respectively. We inferred the isolation of the Hispaniolan *C. striatus* lineage from the Great and Little Bahama banks subsequent to node P. Finally, we inferred an isolation of the Great and Little Bahama banks lineages subsequent to node Q.

4. Discussion

Earlier studies indicated that the West Indian boas form a monophyletic clade resulting from a single ancestral dispersal into the West Indies (Cuba) from mainland South America (Sheplan and Schwartz, 1974; Rosen, 1975). Tolson (1987) suggested that the actual ancestral origin was North or Central America based on two now outdated lines of evidence: *Paraepicrates* and *Pseudoepicrates* fossils (almost exclusively from North America that are now not considered to be ancestral to *Epicrates*; Kluge, 1988b) and an inferred lack of differentiation in the Central and South American species *E. cenchria sensu lato* (now five species, only one in Central America; Passos and Fernandes, 2008; Rivera et al., 2011). Given the diversity of *Epicrates* in South America, the close relationship

to the exclusively South American anacondas, and our biogeographic reconstruction, we suggest that the ultimate origin of the genus *Chilabothrus* is South America. However, we cannot completely exclude the possibility that the dispersal to the West Indies occurred from Nuclear Central America, though this would be against historical currents, and no evidence has been found for passive dispersal in this direction in other vertebrate groups (Hedges, 2001). Our divergence time analysis suggests that this dispersal event occurred between nodes F and J in Fig. 3, and therefore in the Oligocene or early Miocene (30–22 Mya); a time after which several components of the Proto Greater Antilles were emergent (Perfit and Heezen, 1978; Pregill, 1981; Hedges, 2001; Pindell and Kennan, 2009). *Chilabothrus* in the West Indies subsequently underwent a radiation of ecomorphological diversity consisting of nine (now 10, see Section 4.2) species and 14 subspecies. The timing of this radiation is consistent with the diversification of alsophiine snakes across the Greater Antilles in the mid-Miocene to the Pleistocene (Burbrink et al., 2012). Our historical biogeographic reconstruction suggested an initial arrival into the Proto Greater Antilles followed by isolation and subsequent speciation of the Cuban population, which is consistent with previous hypotheses (e.g., Tolson, 1987; Kluge, 1988a). The early separation of boas on the Puerto Rico Bank resulted in a speciation event around the end of the Miocene, giving rise to the contemporaneous arboreal *C. monensis* and generalist *C. inornatus*. This speciation event could have occurred parapatrically, as both *C. inornatus* and *C. monensis* occur on Puerto Rico; or *C. monensis* could have speciated allopatrically on Isla Mona (which is isolated in the Mona Passage and has never been connected to Puerto Rico) and then colonized the Puerto Rico Bank. Though dispersal in this direction (due east) would be against the prevailing ocean currents, an occasional hurricane track does push winds in this direction (Hedges, 2001). Alternatively, fluctuating sea levels could have led to allopatric speciation across the Puerto Rican Bank, as *C. monensis*, but not *C. inornatus*, is found on emergent islands on the submerged eastern Puerto Rican Bank (specifically, the Spanish, US, and British Virgin Islands, excluding St. Croix), which have been periodically connected and separated from the main island of Puerto Rico through geological time. Additional sampling and explicit tests of species boundaries from Isla Mona boas might shed more light on this speciation event as well as whether *C. monensis* is in fact composed of two allopatric species (e.g., *C. monensis* and *C. granti*). Diversification of extant lineages in Hispaniola and the Bahamas originated at the end of the Miocene, leading to small body size in *C. fordii* (Hispaniola) and *C. exsul* (Little Bahama Bank), a characteristic that apparently evolved independently on the Puerto Rico Bank and Isla Mona (*C. monensis*). As the other small Hispaniolan species (*C. gracilis*) is not included in our analysis, we are unable to determine whether this species evolved in Hispaniola and whether it is the closest relative of the other Hispaniolan *Chilabothrus* species (*C. fordii* and *C. striatus*).

Contrary to what might be expected based on geographic proximity (but not necessarily contemporary ocean current patterns) we do not find evidence for dispersal of boas directly from Cuba to the Bahamas. Instead, the Southern Bahamas lineage became separated from ancestral populations on Hispaniola at the end of the Pliocene. Previous authors (Tolson, 1987) hypothesized that multiple dispersal events from Hispaniola to the Bahamas may have occurred as recently as the Pleistocene from well-differentiated species (*C. exsul* and *C. chrysogaster*). In addition to finding an older origin for the Bahamian *Chilabothrus*, our analysis suggests that an additional vicariance of ancestral *Chilabothrus* populations on Hispaniola and the Great Bahama Bank in the mid-Pleistocene explains the diversification of the species *C. striatus sensu lato*, followed by separation of the Great and Little Bahama banks (*C. exsul*) and incipient separation across the Great Bahamas Bank (*C. strigilatus* and *C. strigilatus cf. fosteri*).

4.1. Generic taxonomy

Given the deep divergence of the West Indian boa clade and paraphyly within the genus *Epicrates sensu lato*, taxonomic revision of this group is warranted. We use the available name *Chilabothrus* (Duméril and Bibron, 1844; type species, *Chilabothrus inornatus*) for species in the West Indian clade. As given in the original etymology, the generic name is from the Greek *cheilos* (lip), *a* (without), and *bothros* (pits), referring to the absence of labial pits in the clade (pits present only in *Chilabothrus angulifer*). We recognize the following 10 species of *Chilabothrus*: *C. angulifer*, *C. chrysogaster*, *C. exsul*, *C. fordii*, *C. gracilis*, *C. inornatus*, *C. monensis*, *C. striatus*, *C. strigilatus*, and *C. subflavus*.

Eunectes appears to have diverged from South American *Epicrates* in the Oligocene (95% HPD 19.2–32.5 Mya), followed by subsequent diversification into the four extant species. We restrict the genus *Epicrates* (Wagler, 1830) to include the five recognized continental species (*E. alvarezzi*, *E. assisi*, *E. cenchria*, *E. crassus*, and *E. maurus*) and the genus *Eunectes* (Wagler, 1830) remains unchanged and comprises four South American species (*Eu. beniensis*, *Eu. deschauenseei*, *Eu. murinus*, and *Eu. notaeus*).

4.2. Origin of Bahamian boas

West Indian boas in the Bahamas were previously hypothesized to be Pleistocene arrivals derived from a Hispaniolan stock dating to at least the Miocene or Pliocene (Tolson, 1987). Studies of other squamates in the region found that diversification in the Bahamas and Turks and Caicos occurred after the Miocene, likely due to the influence of sea level changes on the low-lying Bahamian islands throughout the Pliocene and Quaternary (Hedges and Conn, 2012). Our study suggests that Bahamian boas were derived from two independent range contractions that separated ancestral populations of *Chilabothrus* from Hispaniola. We suggest that the first of these occurred 4.9 Mya to the southern Bahamas and led to the isolation of *Chilabothrus chrysogaster*, whereas the second occurred 2.6 Mya to the Great Bahamas bank and led to the origin of the two species presently found in the central and northern Bahamas. Taxonomic distinction between the Hispaniolan and Bahamian boas (*C. striatus sensu lato*) has been previously suggested (e.g., Campbell, 1997; Burbrink, 2004; Henderson and Powell, 2007; O'Shea, 2007), although never explicitly demonstrated. The two groups are morphologically similar but consistent differences in squamation, body size, and coloration are known (Sheplan and Schwartz, 1974; Tolson and Henderson, 1993). From our analysis, it appears that the Hispaniolan and Bahamian species *C. striatus sensu lato* is paraphyletic; whereby the Great Bahama Bank *C. striatus* are closest relatives of the Little Bahama Bank species *C. exsul*. Our species tree further suggests some distinction between the subspecies of *C. striatus sensu lato*, corresponding to a western Great Bahama Bank lineage composed of *C. striatus fosteri* (Bimini) and *C. striatus fowleri* (Berry Islands), as well as the eastern bank *C. striatus strigilatus* (Eleuthera/Long Island). This distinction is supported by morphological and coloration differences (Sheplan and Schwartz, 1974; Tolson and Henderson, 1993). We therefore recognize the distinction of Hispaniolan *C. striatus* (Fischer, 1856), which retains the binomial and is composed of the Hispaniolan subspecies *C. striatus striatus*, *C. striatus exagistus* (Sheplan and Schwartz, 1974), and *C. striatus warreni* (Sheplan and Schwartz, 1974). Furthermore, we elevate *C. strigilatus* (Cope, 1863) to include boas from the eastern Great Bahama Bank islands of Eleuthera and Long Island. Finally, we suggest the epithet *C. strigilatus cf. fosteri* (Barbour, 1941) to include the subspecies *C. strigilatus fosteri* and *C. strigilatus fowleri* (Sheplan and Schwartz, 1974) on the Bimini and Berry islands, respectively, of the western Great Bahama Bank. Though we did not include the Bahamian subspecies *C. strigilatus ailurus* (Cat

Island) and *C. strigilatus mcraniei* (Ragged Islands) in our analyses, previous studies using mtDNA (Campbell, 1997; Burbrink, 2004) have shown that these subspecies are closely related to other Great Bahama Bank subspecies. Verifying this relationship, plus a more detailed analysis of Great Bahama bank boas in general, would be an important next step for future research.

4.3. Conclusions

Although much work had been previously done on the systematics and biogeography of West Indian boas through the 1970s and 1980s (e.g. Sheplan and Schwartz, 1974; Tolson, 1987; Kluge, 1988a, 1989), more recent molecular analyses contradicted these earlier hypotheses (e.g., Campbell, 1997; Burbrink, 2004; Zika et al., 2008). Our study has helped to clarify evolutionary relationships and times of divergence within West Indian boas. In particular, we find support for the hypothesis that West Indian boas represent a single colonization of the Caribbean followed by diversification in the Greater Antilles, and that diversification involved serial range contraction events throughout the Greater Antilles and potential, though limited, within-island (or island bank) evolution of differing ecological morphologies. In addition to the Greater Antilles, the Bahamas appear to have been a source of diversification in this group, as we found evidence that at least three species evolved in this region following two independent range contractions from Hispaniola. Although we were unable to include one small, arboreal species from Hispaniola, our results suggest repeated evolution of small, possibly neotenic (Kluge, 1989; Campbell, 1997) body forms in this genus. Finally, our results have important implications for the conservation of this group, given the imperiled status of most species of West Indian boas (e.g., Hailey et al., 2011; Tolson and Henderson, 2011). By demonstrating the presence of cryptic diversity in *C. striatus* and relatives (Bahamian *C. strigilatus*) we have identified an additional species that should likely be prioritized for conservation. While *C. striatus* remains common to abundant on Hispaniola, *C. strigilatus* is under increasing threat from encroachment, malicious killing, collection, and invasive predators in its relatively small effective range (Knapp et al., 2011). We anticipate that future research will establish the extent and distribution of genetic diversity in these species and hope this study highlights the urgent need for this work.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2013.04.029>.

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