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Molecular systematics and historical biogeography of tree boas (*Corallus* spp.)

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ABSTRACT

Inferring the evolutionary and biogeographic history of taxa occurring in a particular region is one way to determine the processes by which the biodiversity of that region originated. Tree boas of the genus *Corallus* are an ancient clade and occur throughout Central and South America and the Lesser Antilles, making it an excellent group for investigating Neotropical biogeography. Using sequenced portions of two mitochondrial and three nuclear loci for individuals of all recognized species of *Corallus*, we infer phylogenetic relationships, present the first molecular analysis of the phylogenetic placement of the enigmatic *C. cropanii*, develop a time-calibrated phylogeny, and explore the biogeographic history of the genus. We found that *Corallus* diversified within mainland South America, via over-water dispersals to the Lesser Antilles and Central America, and via the traditionally recognized Panamanian land bridge. Divergence time estimates reject the South American Caribbean-Track as a general biogeographic model for *Corallus* and implicate a role for events during the Oligocene and Miocene in diversification such as marine incursions and the uplift of the Andes. Our findings also suggest that recognition of the island endemic species, *C. grenadensis and C. cookii*, is questionable as they are nested within the widely distributed species, *C. hortulanus*. Our results highlight the importance of using widespread taxa when forming and testing biogeographic hypotheses in complex regions and further illustrate the difficulty of forming broadly applicable hypotheses regarding patterns of diversification in the Neotropical region.

1. Introduction

Inferring the evolutionary history of a group in a particular region is one way to elucidate the processes by which the fauna of that region originated (Crisp et al., 2011; Riddle, 2009). In particular, comparing the evolutionary histories of multiple codistributed lineages allows one to formulate general biogeographic hypotheses that can be further tested and refined with additional taxa (Castoe et al., 2009; Daza et al., 2009). Extreme geological and environmental complexity as well as areas of high richness, high endemism, and multiple major biogeographic provinces (Udvardy, 1975) make the Neotropical region particularly interesting to biogeographers (Beheregaray, 2008; Graham, 1997). However, complex interactions among multiple factors have also precluded a consensus regarding the ecological and historical processes that have generated observed patterns of diversity. Although several broad biogeographic hypotheses have been proposed, the evolutionary history of most Neotropical taxa remains poorly understood and thus, the generality of these hypotheses remains untested in many clades (Beheregaray, 2008).

Multiple historical events have played a role in biological diversification in South America, but four major ones are of primary importance and have received the most attention (Rull, 2011).

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First, marine incursions from the Pacific and Caribbean covered large tracts of Amazonia with huge lakes and swamps during the Tertiary, isolating lineages in upland areas such as the Brazilian and Guianan shields (Haffer, 2008; Webb, 1995). Second, the Andean uplift during the Miocene created a major continental barrier to dispersal while also providing new montane habitat and opportunities for colonization and diversification (Haffer, 2008; Rull, 2011). Third, contraction of forests during Pleistocene climatic fluctuations is thought to have isolated populations of forest-adapted taxa in refugia, which subsequently led to speciation (Haffer, 1969, 2008; Vanzolini, 1970). Finally, closure of the Panamanian Isthmus provided a dispersal route between North and South America which has been coined the "Great American Biotic Interchange-GABI" (Lessa et al., 1997; Simpson, 1940). The GABI was first recognized by Wallace (1876) and has since been identified as important in structuring biogeographic patterns in multiple taxa including birds, mammals, and invertebrates (Johnson and Weckstein, 2011; Lessa et al., 1997; Zeh et al., 2003); however divergences in many squamate taxa that span both sides of the Panamanian Isthmus predate its formation (Castoe et al., 2009; Daza et al., 2009, 2010). Among these events, the formation of Pleistocene refugia has received the most attention and debate, whereas the closure of the Panamanian Isthmus is the only event for which the timing is precisely dated (Rull, 2011).

The genus *Corallus* is a member of the subfamily Boinae, a group with clear Gondwanan origins (Noonan and Chippindale, 2006). Currently, eight species are recognized based on morphology or in some cases morphology and molecular data: *C. annulatus (sensu lato,* but see taxon sampling in Materials and Methods*), C. batesii, C. caninus, C. cookii, C. cropanii, C. grenadensis, C. hortulanus,* and *C. ruschenbergerii* (Henderson et al., 2009). Four species are endemic to South America, including the rare and enigmatic *C. cropanii,* which is only known from four specimens (three existing) and whose taxonomic placement has been of great interest (Kluge, 1991; Machado-Filho et al., 2011). Two species are distributed in both Central and South America (*C. annulatus* and *C. ruschenbergerii*), and two species are endemic to the Lesser Antilles (*C. cookii* and *C. grenadensis*) (Henderson, 2002; Henderson et al., 2009). Phylogenetic relationships have been explored using morphology (Kluge, 1991; Henderson, 1997, 2002; Henderson and Hedges, 1995; Henderson et al., 2009) and molecular phylogeographic analyses have been conducted on individual species (Vidal et al., 2005). However, a time-calibrated phylogeny for all members of the genus, which could be used to test both spatial and temporal components of biogeographic hypotheses, is not presently available.

Here, we use DNA sequence data from two mitochondrial and three nuclear genes of all species of *Corallus* to infer their phylogenetic relationships. We then reconstruct the biogeographic history of the group by integrating divergence times of species, as estimated by relaxed-clock divergence dating methods, with ancestral area analyses that account for dispersal and extinction to infer the biogeographic history of the group. Finally, we use our results to evaluate the potential roles of major Neotropical biogeographic events (e.g., marine incursions, Andean uplift, Pleistocene refugia, and Panamanian Isthmus) in the diversification of *Corallus*.

2. Materials and methods

2.1. Taxon sampling

We used 20 specimens of *Corallus* including all species from 18 localities across the distribution of the genus (Fig. 1, S1). We did not have access to tissue samples from specimens positively identified as *C. batesii* and available sequence data were restricted to cytochrome-*b* (*cyt-b*) from two individuals. Our Ecuadorian sample of *C. annulatus* could not be definitively classified as either *C. annulatus* or *C. blombergi*, and as there is no question whether these species are sister and we are not addressing the validity of *C. blombergi*, we treat *C. annulatus* herein as including both *C. annulatus* and *C. blombergi*. Tissue samples were collected by us, obtained through museum loans, or donated by private individuals from personal tissue collections. For phylogenetic analyses, we included sequences from GenBank for additional boid taxa and *Cylindrophis* for use as outgroups. These outgroup taxa were also included in divergence dating analysis in order to provide external nodes on which we could place prior age information based on the fossil record. A detailed list of specimens, including voucher numbers and GenBank accession numbers, is provided in Supplementary Information (S1).

2.2. Laboratory methods

We extracted total genomic DNA from liver tissue, muscle tissue, or shed skins either stored frozen or in ethanol using Qiagen DNeasy kits (tissue protocol). We amplified the complete

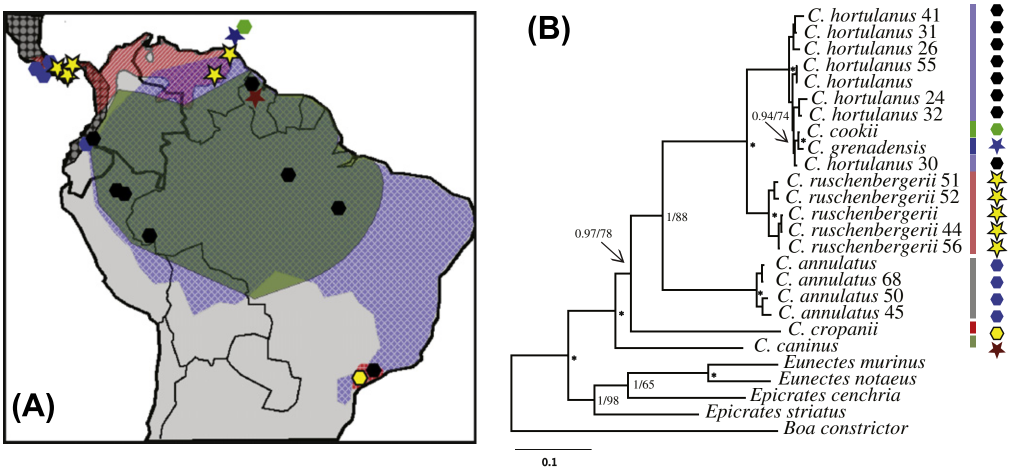

Fig. 1. (A) Map of Central and South America showing approximate distributions and sampling localities of *Corallus* species used in this study. (B) Phylogenetic estimate of relationships of the genus *Corallus* based on Bayesian Inference in the program Mr. Bayes. Tree topology was identical in both BI and ML inference. Node numbers indicate Bayesian posterior probability and ML bootstrap values (PP/ML). Nodes labeled with an asterisk (∗) had a posterior probability of 1 and a bootstrap value of 100%. Vertical colored bars correspond to colors in distribution map whereas symbols to the right of the bars correspond to sample localities on the map. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Primers, sources, and aligned fragment lengths (bp), number of parsimony informative sites for ingroup taxa, and chosen substitution model for loci used in this study.

Primers	Primer sequence	Source	Fragment length (aligned)	Informative sites	Selected model
BDNF		Noonan and Chippindale (2006)	713	30	HKY + GAMMA
BDNF-F	GACCATCCTTTTCCTKACTATGGTTATTTCATACTT				
BDNF-R	CTATCTTCCCCTTTTAATGGTCAGTGTACAAAC				
NT3		Noonan and Chippindale (2006)	542	11	TrN93 + GAMMA
NT3F3	ATATTTCTGGCTTTTCTCTGTGGC				
NT3R4	GCGTTTCATAAAAATATTGTTTGACCGG				
c-mos		Lawson et al. (2005)	596	28	HKY + GAMMA
CMOS-S77	CATGGACTGGGATCAGTTATG				
CMOS-S78	CCTTGGGTGTGATTTTCTCACCT				
cyt-b		Burbrink et al. (2000)	1117	190	HKY + GAMMA
L14910	GACCTGTGATMTGAAAACCAYCGTTGT (PCR)				
H16064	CTTTGGTTTACAAGAACAATGCTTTA (PCR)				
Corcyt-bF	TAACTCGATTCTTTGCACTACA (SEQ)	This study			
Corcyt-bR	TGGAATGGGATTTTRTCAAT (SEQ)				
12S		Palumbi et al. (1991)	439	45	GTR + GAMMA
12S-AL	AAACTCGGATTAGATACCCCACTAT				
12S-BH	GAGGGTGACGGGCGGTGTG				

cytochrome-*b* (*cyt-b*) mitochondrial gene and a portion of the 12S ribosomal RNA (rRNA) gene using Polymerase Chain Reaction (PCR) with GoTaq Green Master Mix (Promega Corp) following manufacturer's specifications. Additionally, we amplified and sequenced portions of three nuclear protein-coding loci: brain derived neurotrophic factor precursor (BDNF), neurotrophin-3 (NT3), and oocyte maturation factor (*c-mos*). Primer sequences and sources are listed in Table 1. We cleaned PCR products using ExoSap-IT (USB Corp.) and used purified double-stranded products in dideoxy-termination sequencing reactions (10.0 μl total volume) employing BigDye Terminator v3.1 (Applied Biosystems), DTCS (Beckman–Coulter), or DYEnamic ET Dye Terminator Cycle Sequencing Kit (GE Healthcare). We ran sequencing reaction products on an ABI 3730XL, Beckman–Coulter CEQ8000, or MegaBACE 1000 (GE Healthcare) automated sequencer following the manufacturer's protocols. We edited chromatograms by eye and aligned sequences using the MUSCLE algorithm (Edgar, 2004) in the program Geneious Pro 4.8.4 (Drummond et al., 2009). We verified an open reading frame for all protein-coding loci in all taxa. The final matrix for all five genes comprised 37 taxa and 3412 bp. Most ingroup samples were represented by at least three of the five loci.

2.3. Phylogenetic inference

We conducted phylogenetic analyses using Bayesian Inference (BI) and Maximum Likelihood (ML) methods with different substitution models applied to each gene and codon position (Brandley et al., 2005; Castoe and Parkinson, 2006). The best substitution model for each partition was determined using the Akaike Information Criterion (AIC) in the program FindModel (http://www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html), a web implementation of ModelTest (Posada and Crandall, 1998).

For Bayesian Inference, we used MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) with two independent runs of four chains (3 hot and 1 cold) for 20×10^6 generations sampling every 1000. We used default priors for all parameters and assessed convergence of the MCMC chains and independent runs by viewing trace plots of the tree –lnL values in the program Tracer v1.5 (Rambaut and Drummond, 2007). Additionally we used the program AWTY (Nylander et al., 2008) to view changes in the posterior probability of splits over the course of each MCMC run. We discarded trees sampled prior to stationarity as burn in and combined post-burn in trees from the two runs.

We conducted maximum likelihood analysis using the program RAxML 7.2.5 (Stamatakis et al., 2008) via the CIPRES portal v2.2 (Miller et al., 2010). The tree was inferred under the GTRGAMMA (GTR + Γ) model, partitioned by gene and codon position, and node support was obtained from 1000 non-parametric bootstrap replicates under the partitioned rapid bootstrapping GTRCAT model.

We compared trees from BI and ML analyses to infer the most credible relationships and considered nodes well supported when both the Bayesian posterior probability was $\geqslant 0.95$ and the nonparametric bootstrap value $\geqslant 70\%$. Including the *cyt-b* sequences from the two individuals identified as *C. batesii* in phylogenetic analyses produced questionable topologies therefore we ran comparative analyses both with and without these data.

2.4. Divergence time estimates

To test temporal aspects of biogeographic hypotheses, a time-calibrated phylogeny is needed. To this end, we simultaneously inferred divergence times and phylogeny using a relaxed-clock method in the program BEAST v1.6.2 (Drummond and Rambaut, 2007). We used fossil material to place priors on the ages of three nodes (Table 2). In each case, we used an offset with a lognormal prior to place a hard minimum, and soft maximum bounds on node ages (Yang and Rannala, 2006). Failure to include a hard upper bound on at least one node can result in large credibility intervals for other node ages (Noonan and Chippindale, 2006; Yang and Rannala, 2006). Therefore, we placed a maximum age on the root of the tree encompassing the age of the oldest known snake fossil (145 million years; Hofstetter, 1960), which predates all possible

Table 2
Fossil material and lognormal prior settings used to constrain minimum ages of nodes in Fig. 2.

Taxon	Group	Period (Age)	Mean (SD)	Offset	Node	Reference
Corallus	Boinae	Middle Paleocene (61-58MY)	1	58	C	(Pascual and Ortiz-Jaureguizar (1991); Medeiros and Bergqvist (1999); Rage (2001)
Titanoboa	Boinae	Middle Paleocene (60 MY)	1	60	B	Head et al. (2009)
Morelia	Boidae	Miocene (25-22 MY)	1	22	A	Scanlon (2001)

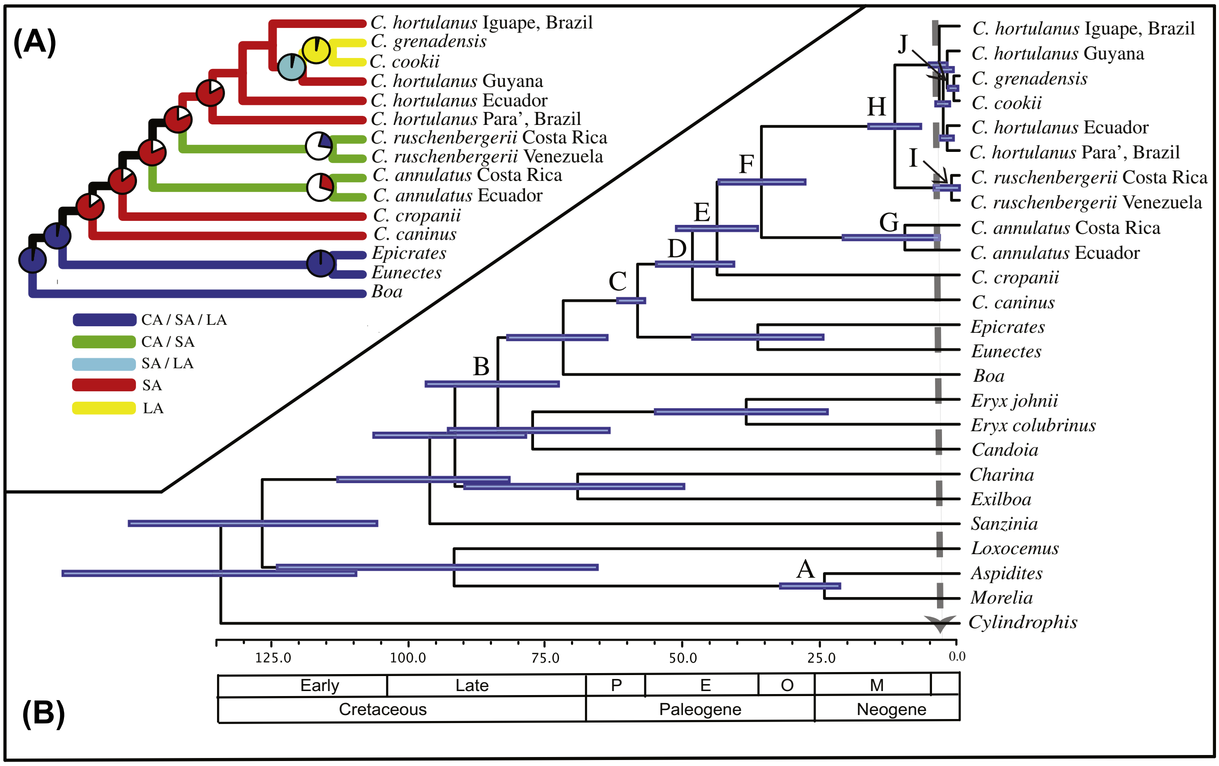

Fig. 2. (A) Chronogram of *Corallus* with outgroup taxa pruned for ancestral area reconstruction analyses. Branch colors reflect biogeographic ranges for terminal tips and pie charts reflect the area with highest probability from Lagrange and DIVA analyses. Biogeographic regions are SA = South America, C = Central America, and LA = Lesser Antilles. (B) Chronogram for *Corallus* and outgroup taxa based on relaxed-clock Bayesian MCMC methods in BEAST. Error bars on nodes indicate 95% highest posterior densities around the mean dates and vertical dashed grey line indicates the time of closure of the Panamanian Isthmus (~3 Ma). All nodes had a posterior probability >0.95. Node letters correspond to fossil calibration points described in Table 2 and divergence estimates reported in Table 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

divergences within the clade of interest. We conducted two independent searches of 30×10^6 generations on the five-gene dataset sampling every 1000 generations under the same models and partitioning strategy used in BI. We combined output log files from the two runs using the program LogCombiner v1.6.2 (Drummond and Rambaut, 2007) and discarded the first 7.5 million generations as burn in. We ensured that all ESS values based on post-burnin samples were >200 for all estimated parameters in the program Tracer v1.5 (Rambaut and Drummond, 2007). We used Tree Annotator (Drummond and Rambaut, 2007) to combine post-burn in trees from the two runs and present the mean and 95% highest posterior density (HPD) of node ages.

2.5. Biogeographic analyses

We inferred the historical biogeography of *Corallus* using the ingroup topology and where appropriate the node ages, of the BEAST chronogram (Fig. 2). We categorized species as having one of four distributions: (1) South American (SA) only – *C. caninus, C. cropanii, C. hortulanus*; (2) Central American (CA) and SA – *C. annulatus, C. ruschenbergerii*; (3) Lesser Antilles only – *C. cookii, C. grenadensis*, or (4) CA, SA, and Lesser Antilles (for outgroup ranges).

We reconstructed ancestral areas for each node in the phylogeny of *Corallus* using the program DIVA 1.2 (Ronquist, 2001). DIVA is a parsimony method that places ancestral areas onto internal nodes of a phylogeny using a three-dimensional step matrix. Ancestral areas are optimized by minimizing the allowed number of duplication and extinction events required to explain the current distributions of descendant lineages. Because optimizations can become less reliable as the root node is approached (Ronquist, 2003, 1997) we rooted the tree with the outgroup *Boa constrictor*, included the sister group of *Corallus* (*Eunectes* + *Epricrates*), and assumed the ancestral area of *Boa constrictor* encompassed all possible ancestral areas for *Corallus* (as does *Eunectes* + *Epicrates*). Additionally, we used a likelihood method of geographic range reconstruction implemented in the program Lagrange v2.0.1 (Ree and Smith, 2008). This maximum likelihood approach is based on a stochastic model of range evolution that incorporates rates of dispersal, extinction and cladogenesis. Instantaneous transition rates between ranges along phylogenetic branches were specified and these rates were used to estimate the likelihood of ancestral range inheritance at branching events using the program's default settings.

3. Results

3.1. Phylogenetic relationships

FindModel identified the best model for each locus (Table 1). Results from the BI and ML analyses were congruent and support was high for the monophyly of *Corallus*. The *cyt-b* sequences from the two individuals identified as *C. batesii* produced questionable topologies with low support (e.g., placing *C. batesii* sister to *Eunectes* rather than *C. caninus*). A NCBI blast-n search of one sequence identified the individual as *Eunectes notaeus* with a 85.9% match (e-score 2.0e−177). Because neither specimen was available for examination by the primary author and the sister relationship of *C. batesii* and *C. caninus* is not in question we chose to exclude these two sequences from further analyses. We note however that the species status of *C. batesii* and divergence times from *C. caninus* warrant further investigation.

In both BI and ML analyses, we found strong support for the sister relationship of *C. caninus* and all other species of *Corallus*. The enigmatic *C. cropanii* was strongly supported as sister to all

Table 3
Divergence estimates for nodes indicated in Fig. 2.

Node	Mean	95% HPD
C	59.5	58.1–63.1
D	49.3	41.6–56.0
E	44.7	37.3–52.3
F	36.5	28.5–44.5
G	10.1	3.7–21.4
H	11.9	7.1–16.7
I	1.5	0.1–4.5
J	1.0	0.3–1.2

species of *Corallus* exclusive of *C. caninus*. *Corallus grenadensis* and *C. cookii* were recovered as sister taxa, but nested within *C. hortulanus* (Fig. 1). *Corallus ruschenbergerii* was the well-supported sister taxon to the clade comprised of *C. hortulanus*, *C. grenadensis* and *C. cookii* with *C. annulatus* being sister to that clade (Fig. 1).

3.2. Divergence times

Estimates of divergence times for deeper nodes within Boidae were concordant with ages found in other recent studies (Noonan and Chippindale, 2006; Pyron and Burbrink, 2010). The MRCA of *Corallus* is estimated to be 49 Ma (95% HPD 41.6 – 56.0 Ma; Fig. 2, Table 3), and with the exception of *C. caninus* and *C. cropanii*, all extant species originated between 0.2 and 37 Ma (95% HPD 0.1 – 44.5 Ma; Fig. 2 and Table 3).

3.3. Historical biogeography

Ancestral area reconstructions from Lagrange and DIVA analyses inferred identical ancestral ranges for internal nodes and are summarized in Fig. 2. Lagrange incorporates both branch lengths and topology, and thus makes more effective use of phylogeny than DIVA. Therefore, we present the Lagrange results and choose the scenario with the highest relative probability for each node.

Combining the results from Lagrange with divergence times above, *Corallus* most likely originated in South America (SA) during the Middle Eocene (∼49 Ma; Fig. 2, Table 3). *Corallus caninus* and *C. cropanii* both originated within SA ∼35 – 49 Ma in the Eocene. *Corallus annulatus* dispersed from SA to Central America (CA) in the Late Miocene ∼10 Ma. The ancestral area of *C. ruschenbergerii* was inferred to be SA, thus indicating a second dispersal to CA during the Pleistocene (∼1.5 Ma). The widespread *C. hortulanus* arose within South America during the Middle Miocene (∼11.9 Ma). Notably, the common ancestor of *C. grenadensis* and *C. cookii* was inferred to been distributed in both SA and the Lesser Antilles (relative probability = 1) thus requiring an over-water dispersal to LA by the MRCA of *C. grenadensis* and *C. cookii* during the Pleistocene.

4. Discussion

4.1. Phylogenetic relationships within Corallus

Our molecular-based phylogeny generally supports the traditional systematic relationships within *Corallus* based on morphology (Kluge, 1991; Henderson, 1997, 2002; Henderson et al., 2001, 2009; Henderson and Hedges, 1995) and confirms the inclusion of the enigmatic species *C. cropanii* within the genus (Kluge, 1991). The agreement between molecular- and morphological-based phylogenies suggests that our inference of relationships is not being misled by gene-tree discordance, which is a concern when using multiple loci because they may have different genealogies. Using species tree methods to infer phylogenetic relationships would be the next logical step, but we currently lack sequence data for all loci for multiple individuals of each species. The one notable exception between molecular and morphological phylogenies is the nesting of *C. grenadensis* and *C. cookii* within *C. hortulanus* in our molecular phylogeny. This relationship was recovered using mitochondrial, nuclear, and the combined datasets, indicating that either *C. grenadensis* and *C. cookii* are not valid species or that *C. hortulanus* is composed of several cryptic species, some of which are more closely related to *C. grenadensis* and *C. cookii* than they are to each other. Mitochondrial DNA sequence divergence among these three taxa is minimal (<2% uncorrected sequence divergence), questioning the validity of the taxonomic status of *C. grenadensis* and *C. cookii*. However, additional studies of these taxa that include better sampling and additional loci, and explicit tests of alternative species hypotheses using coalescent methods for species delimitation (e.g. Yang and Rannala, 2010; Fujita et al., 2012) are needed to properly address their status.

4.2. Historical biogeography

Species distributions in *Corallus* largely follow major Neotropical biogeographic provinces. The Amazon Basin, Guiana Shield, Brazilian Atlantic Forest, and Lesser Antilles all contain endemic *Corallus* lineages, and a few widespread taxa span multiple regions. Interestingly, the two Central American species are co-distributed in northern South America as well. These patterns reveal that multiple dispersal and vicariant events have shaped diversification in *Corallus*, highlighting the complexity of processes driving species diversification in the biodiverse Neotropical region.

The South American–Caribbean track (SAC) hypothesis, which describes the biogeography of faunal exchange between North and South America, has been posited for multiple squamate clades (Savage, 2002), although this hypothesis has yet to be explicitly tested. The SAC predicts that *Corallus* initially diversified within mainland South America during the Eocene, dispersed to the Lesser Antilles via the Aves Ridge and subsequent stepping stone islands in the Late Tertiary (and should thus be relatively older than the lower Central American clades of South American origin), and then dispersed to Central America in the Middle to Late Pliocene (∼3 Ma) with the closure of the Panamanian Isthmus (Savage, 1982; see Savage, 2002 for further review). Consistent with this, we found that *Corallus* initially diversified within South America with speciation events giving rise to *C. annulatus*, *C. caninus*, *C. cropanii*, *C. hortulanus, and C. ruschenbergerii* dating to the Oligocene and Miocene. The times of these speciation events suggest that factors such as marine incursions and/or the uplift of the Andes may have been important drivers of diversification (Haffer, 2008; Webb, 1995). However, inconsistent with the SAC model, we found that *C. annulatus* arrived in Central America during the Late Miocene, prior to the formation of the Panamanian land bridge. The closure of the Panamanian Isthmus in the Pliocene (∼3 Ma) was pivotal for faunal exchange between North and South America in groups such as mammals, but earlier events may have also been important in taxa such as squamates (Savage, 2002). For example, the Proto-Antilles in the Early Paleocene may have provided a dispersal route between continents or dispersal via rafting or island hopping may have been possible in the Miocene before final closure of the Panamanian Isthmus as lower CA formed off the coast of northern SA (Savage, 2002). In contrast, *C. ruschenbergerii* appears to have dispersed from SA to CA during the Pleistocene via the traditionally recognized Panamanian land bridge, although the 95% HPD (Table 3) of our results allow for the possibility that *C. ruschenbergerii* may have arrived in CA prior to the closure of the Panamanian Isthmus.

Our results support previous studies (Henderson and Hedges, 1995) suggesting over-water dispersal of *C. grenadensis* and *C. cookii* from a northern, Guianan Shield ancestor. However, inconsistent with the SAC model, divergence estimates place the age of the MRCA of these two species and *C. hortulanus* from Guyana at approximately ~2 Ma (Early Pleistocene), well after the Late Tertiary. There is no evidence for a land connection between the Guyana Shield and the Lesser Antilles at any time and ancestral area reconstructions place the distribution of the common ancestor of *C. grenadensis* and *C. cookii* in the Lesser Antilles during the Pleistocene, thus indicating over-water dispersal by these species from South America. This result is likely due to the two species being nested within *C. hortulanus,* which occurs only in South America.

The biogeography of *Corallus* shows a similar pattern to other codistributed Neotropical squamate and amphibian taxa. Phylogenetic and biogeographic evidence has repeatedly revealed multiple dispersal events between Central and South America prior to the formation of the Panamanian land bridge and subsequent dispersal events back to South America after its closure (Daza et al., 2009; Koepfli et al., 2007). For example, evidence for colonization of Central America prior to the closure of the Panamanian Isthmus has been found in frog taxa with clear South American origins (Heinicke et al., 2007; Santos et al., 2009), and over-water dispersal to the Antilles rather than via the Proto-Antilles or Aves Ridge has been found in frogs and lizards (Censky et al., 1988; Heinicke et al., 2007; Hower and Hedges, 2003). The uplift of the Andes in the Miocene has been shown to have been both a dispersal barrier and provided areas of colonization of montane habitat in anurans (Graham et al., 2004). However, it seems that the Andean uplift predates diversification of *Corallus* lineages spanning its extent as is found in dendrobatid frogs (Noonan and Wray, 2006), and has since not been a significant dispersal barrier. Repeated marine incursions of South America during the Tertiary fragmented populations and have been implicated in driving diversification in anurans (Garda and Cannatella, 2006; Noonan and Wray, 2006). The geography and timing of these incursions corresponds with the patterns we recovered in some *Corallus* lineages (e.g. *Corallus caninus*).

4.3. Conclusion

Our study indicated that *Corallus* diversified multiple times within mainland South America (*C. annulatus*, *C. caninus*, *C. cropanii*, *C. hortulanus, C. ruschenbergerii*), dispersed over water once to Central America (*C. annulatus*) and once to the Lesser Antilles (*C. cookii, C. grenadensis*), and dispersed once to Central America via the traditionally recognized Panamanian land bridge (*C. ruschenbergerii*). The taxonomic validity of *C. grenadensis* and *C. cookii* are questionable and additional study of these taxa with respect to *C. hortulanus* is needed. We find incomplete support for the South American–Caribbean Track as a general biogeographic model for *Corallus,* and instead highlight the importance of over-water dispersal. The timing of speciation events within South America suggest possible roles for marine incursions, and the uplift of the Andes, but all South American species arose prior to the Pleistocene rejecting a role for refugia in speciation. The biogeographic history of *Corallus* further illustrates the difficulty of forming broadly applicable hypotheses regarding patterns of diversification in the Neotropical region.

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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.2012.11.027.

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