



Understanding the formation of ancient intertropical disjunct distributions using Asian and Neotropical hinged-teeth snakes (*Sibynophis* and *Scaphiodontophis*: Serpentes: Colubridae)

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ABSTRACT

Numerous taxa show ancient intertropical disjunct distributions. Many can be explained by well-known processes of historical vicariance, such as the breakup of Gondwanaland. Others, such as Asian–Neotropical divergences are not as well understood. To clarify the phylogenetic position and understand biogeographic and temporal origins of the geographically disjunct and morphologically unique genera of hinged-teeth snakes, *Scaphiodontophis* ($n = 1$) and *Sibynophis* ($n = 9$; Colubridae), we inferred a time-calibrated phylogeny with additional 107 taxa representing the superfamily Colubroidea using four genes (c-mos, cyt-b, ND2, RAG-1; 3085 bp). We used this tree to estimate ancestral areas for the group. The results show that *Scaphiodontophis* is sister to *Sibynophis*, both originated in the late Eocene/Oligocene in Asia and likely dispersed through Beringia to the New World, but unlike other snake groups left no extant species in temperate North America. Current recognition of Scaphiodontophiinae renders Colubrinae paraphyletic, and we resurrect the previously named subfamily Sibynophiinae to encompass both genera and use the tribes Sibynophiini (*Sibynophis*) and Scaphiodontophiini (*Scaphiodontophis*) to highlight the geographically distinct areas occupied by these taxa. These results suggest that intercontinental dispersal with extinction in intermediate areas can explain puzzling patterns of ancient intertropical disjunct distributions.

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1. Introduction

Historical processes responsible for modern spatial patterns of biodiversity may be complex, are often ancient, and are usually difficult to properly understand without a fossil record and credible phylogenetic estimates for the groups in question (Blackburn and Gaston, 2002; Cox and Moore, 2010; Lomolino et al., 2010). Organisms with disjunct distributions, where closely related taxa are distributed in geographically discontinuous regions are not uncommon, but the processes generating these patterns are not necessarily straightforward, and can easily be obscured by

dispersal and extinction (Ree et al., 2005). Several processes could account for discontinuities in group ranges. For the most part, three general hypotheses explain patterns of disjunction: (1) vicariance of formerly wide spread taxa; (2) extinction of species/populations in the intermediate areas of formerly widespread taxa; (3) dispersal from one region to another (Cox and Moore, 2010; Lomolino et al., 2010; Sanmartin et al., 2001). Further, it is possible that the entire distribution of a group is the result of more than one of these three causes, therefore increasing the difficulty of estimating process from pattern.

To test these three basic hypotheses, it is first necessary to construct a time-calibrated phylogeny and estimate ancestral areas, while accounting for dispersal, vicariance, and extinction (Ree and Smith, 2008; Ronquist, 1997). With this information it is possible to eliminate potential explanations for given disjunct distributions. For example, if a group is found on two continents and

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credible divergence dates suggest that the most recent common ancestor (MRCA) of the group is younger than the split between these landmasses, then dispersal from one area to another must account for their current distribution (see de Queiroz, 2005). In the case where the MRCA is older than the split between continents, this might indicate that vicariance was the most likely driver of their current distributions (Lomolino et al., 2010), though dispersal and extinction could still account for this (Ree and Smith, 2008).

Disjunct distributional patterns appear repeatedly in many reptile groups, such as Iguanidae (Keogh et al., 2008; Noonan and Chippindale, 2006b; Noonan and Sites, 2010; Okajima and Kumazawa, 2009), Boidae (Noonan and Chippindale, 2006a; Noonan and Sites, 2010), Dibamidae (Townsend et al., 2011) and true crocodiles (Oaks, 2011). These patterns could be explained by either vicariance due to the breakup of Gondwanaland (provided groups are sufficiently old), long distance dispersal, the extinction of wide spread taxa, or any combination of effects. However, the appearance of similar patterns among unrelated taxa may be evidence of a single process impacting many groups (Lomolino et al., 2010).

One pattern that has emerged for some squamate reptiles is the unidirectional dispersal from the Old World (OW) to the New World (NW). In particular, the land bridges connecting Eurasia and North America during the Cenozoic (65–14 Ma) have been considered a main factor shaping the present NW faunas (Ronquist, 1997; Sanmartin et al., 2001). Two possible dispersal routes exist: the trans-Atlantic land bridges (de Geer and Thulean) connecting Europe with eastern North America (Liebherr, 1991; McKenna, 1983; Tiffney, 1985) and the trans-Beringian land bridge connecting eastern Asia with western North America (Lafontaine and Wood, 1988; Matthews, 1979; McKenna, 1983; Nordlander et al., 1996; Tangledler, 1988).

In particular, Beringia has been important for seeding the NW with multiple squamate groups from the OW, including vipers (Wüster et al., 2008), ratsnakes (Burbrink and Lawson, 2007), watersnakes (Guo et al., 2012), eublepharid geckos (Gamble et al., 2011), and *Plestiodon* skinks (Brandley et al., 2011). Because the origins of all of these taxa are in the OW and divergence date estimates indicate that the MRCA of OW and NW taxa occurred in the late Oligocene or early Miocene, then dispersal must have happened unidirectionally from the OW through Beringia to the NW, barring extinction which would erase the patterns of return colonization or multiple colonizations in the same direction. In this study, we mainly focus on two morphologically unique colubroid genera, *Scaphiodontophis* and *Sibynophis* as well as multiple species representing all families in Colubroidea and subfamilies in Colubridae to examine and explain patterns of disjunct distributions of close related taxa.

The advanced snakes (Colubroidea) include the majority of snake species (>2500 species) and have been divided into seven families (Xenodermatidae, Pareatidae, Viperidae, Homalopsidae, Elapidae, Lamprophiidae, Colubridae; Uetz, 2009; Pyron et al., 2011). Among these families, Colubridae includes over half of the species in Colubroidea (>1600 species) and covers the largest range of distribution. Colubridae has been subdivided into seven subfamilies (Natricinae, Pseudoxenodontinae, Dipsadinae, Calamariinae, Grayiinae, Colubrinae and Scaphiodontophiinae; Uetz, 2009; Pyron et al., 2011). The Neotropical neckband snakes in the genus *Scaphiodontophis* (Scaphiodontophiinae, Colubridae), currently including two species, are presumed mimics of coral snakes and occur in Central and South America, from southern Mexico to northern Colombia (Savage and Slowinski, 1996; Uetz, 2009; Fig. 1). The Asian black-headed snakes in the genus *Sibynophis* (Colubrinae, Colubridae), with nine recognized species, are found in tropical Asia and southern China (Uetz, 2009; Fig. 1). Because of the hinged and spatulate teeth, *Scaphiodontophis* has been associated with *Sibynophis* and *Liophidium* found in Madagascar; the three genera

were placed in Sibynophiinae, Colubridae (Bogert, 1940; Dunn, 1928; Taylor and Smith, 1943). However, Savitzky (1981, 1983) argued the similarities were likely the result of convergence, which would invalidate Sibynophiinae. Other studies suggested that, because of the distinct pterygoid articulation and hemipenial morphology, *Liophidium* should be excluded from the group (Underwood, 1967; Zaher, 1999). Molecular phylogenies demonstrate that *Liophidium* is closely related to other Malagasy genera and should be placed in family Lamprophiidae (Nagy et al., 2003; Pyron et al., 2011), but the relationship among *Scaphiodontophis*, *Sibynophis*, and other colubrids is still unclear based primarily on morphological data (Zaher, 1999).

Pyron et al. (2011) placed *Scaphiodontophis* into a new subfamily, Scaphiodontophiinae, because the group is apparently unrelated to other established groups of colubrids and possesses unique features such as hinged maxillary teeth and the presence of fracture planes between caudal vertebrae (Savage and Slowinski, 1996). Nevertheless, species of *Sibynophis* were not included in that study, which left the position of the genus within Colubridae unclear. In this study, we clarify the relationships of *Scaphiodontophis* and *Sibynophis* and test whether the two genera are sister groups with disjunct distributions or, alternatively, unrelated but sharing remarkable morphological convergence in unusual traits. We then estimate divergence times and reconstruct ancestral areas to test hypotheses regarding the biogeographic origin of these snakes to ultimately understand the processes responsible for this disjunct distribution.

2. Materials and methods

2.1. Sampling and DNA sequencing

We obtained nine tissue samples from four species of *Sibynophis* (Appendix Table S1). We amplified and sequenced the mitochondrial protein-coding genes cytochrome *b* (cyt-*b*; 1000 bp), NADH subunit 2 (ND2; 994 bp), and the nuclear protein-coding genes oocyte maturation factor Mos (*c-mos*; 542 bp) and recombination-activating gene 1 (RAG-1; 549 bp) following the steps in Pyron and Burbrink (2009b). The primers we used are listed in Appendix Table S2. Sequences of *Scaphiodontophis annulatus* were obtained from Pyron et al. (2011). In addition, we used sequences from 107 taxa representing multiple species from all families of Colubroidea and subfamilies of Colubridae (Xenodermatidae, Pareatidae, Viperidae, Homalopsidae, Elapidae, Lamprophiidae, Natricinae, Pseudoxenodontinae, Dipsadinae, Calamariinae, Grayiinae, and Colubrinae) and the outgroup Acrochordidae (GenBank accession numbers are given in Appendix Table S1). Sequences were aligned in Geneious Pro 4.7.5 using the MUSCLE algorithm with default parameters (Edgar, 2004), and checked by eye for ambiguities. All genes are protein-coding and maintained open reading frame in the final alignments. The completeness of taxa for cyt-*b*, *c-mos*, ND2, and RAG-1, are 100%, 99%, 79% and 23%, respectively. Although some missing data were present in our data set, all sampled species *Sibynophis* and *Scaphiodontophis* were represented for every gene, except *Scaphiodontophis annulatus* which lacked the ND2 sequence.

2.2. Phylogeny and divergence time inference

We first determined the appropriate substitution models for each gene in jModelTest 0.1.1 (Posada, 2008), using the Bayesian information criterion (BIC). We used relaxed phylogenetic methods to infer the time-calibrated phylogeny in BEAST v1.6.2 (Drummond et al., 2006; Drummond and Rambaut, 2007). We ran two analyses for 50 million generations, partitioning the substitution model and

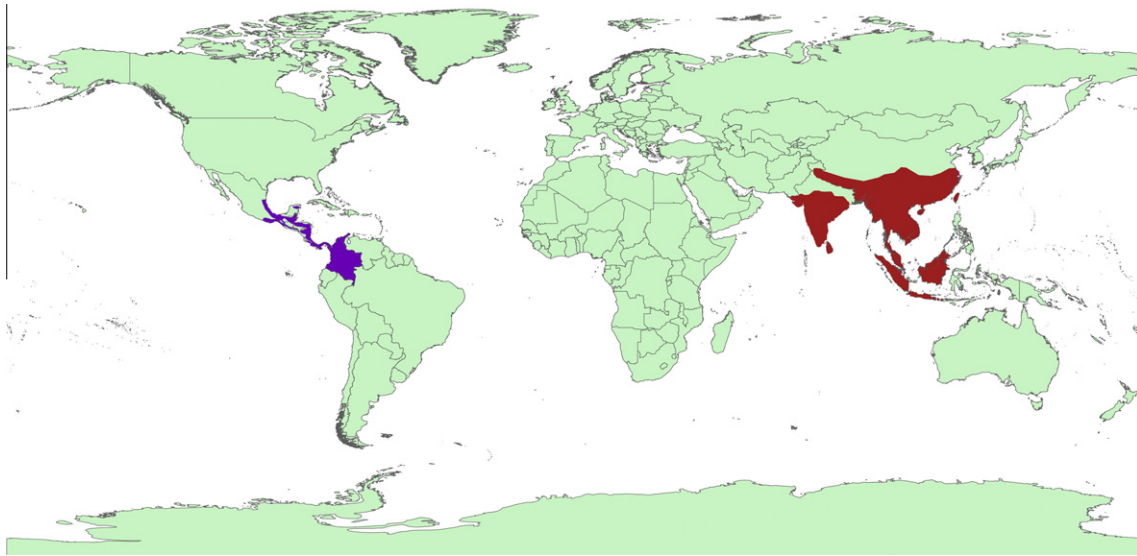


Fig. 1. Distribution of *Scaphiodontophis* (purple) and *Sibynopsis* (red) (Köhler, 2008; Khan, 2002; Manthey and Groosmann, 1997; Uetz, 2009; Whitaker and Captain, 2007; Zhao and Adler, 1993). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Time calibrations and the tree prior distribution used for divergence time estimation in BEAST 1.6.2 (Drummond et al., 2006; Drummond and Rambaut, 2007).

Node number	Calibration	Age range (95% HPD; Ma)	Tree prior distribution	Reference
2	The root age of Colubroidea	48.6–95	Normal	Pyron et al. (2011) and Rage et al. (2008)
25	The root age of Colubridae	33–65	Normal	Holman (2000) and Pyron and Burbrink (2012)
100	The MRCA of Lamprodelphini	11.4–37.1	Lognormal	Holman (2000)
102	The divergence of <i>Pantherophis</i> and <i>Pituophis</i>	9.5–25.3	Lognormal	Holman (2000)
112	The divergence of <i>Lampropeltis</i> and <i>Cemophora</i>	8.4–24.4	Lognormal	Holman (2000)

relative rate among genes and codons and linking the clock and tree model for each gene. We used uncorrelated lognormally distributed, branch-specific rates under a birth–death process and discarded the first 5 million generations as burn-in.

We used five fossil calibrations with log-normally and normally distributed priors to date the phylogeny (Table 1). These have been applied in the past to estimate divergence dates across various colubroid groups (Pyron and Burbrink, 2009b, 2012). We also compared tree topology and support from BEAST with those estimated using maximum likelihood. We obtained the maximum likelihood (ML) tree and assessed the tree support with the rapid-bootstrapping algorithm using 1000 non-parametric bootstrap replicates in RAxML 7.2.8 (Stamatakis, 2006b; Stamatakis et al., 2008). Finally, we performed the SH test (Anisimova and Gascuel, 2006; Shimodaira and Hasegawa, 1999) in both RAxML 7.2.8 and PhyML 3.0 (Guindon et al., 2010). All ML estimates and tests were run under the GTRCAT model (Stamatakis, 2006a).

2.3. Reconstructed ancestral areas

We used the maximum likelihood-based program Lagrange 2.01 (Ree et al., 2005; Ree and Smith, 2008) and the Bayesian stochastic search variable selection (BSSVS) approach (Lemey et al., 2009) to estimate ancestral areas across the time-calibrated tree. Six regions were coded for all taxa including Nearctic (NA), Neotropical (NT), Palearctic (PA), Oriental (O), Australian (AU), and Ethiopian (E), following Wallace's biogeographic regions (Barry, 2001; Kreft and Jetz, 2010; Wallace, 1876). Because the root age of Colubroidea ranges 48.6–95 Ma, after the breakup of Laurasia and Gondwanaland (Lomolino et al., 2010; Sanmartin et al., 2001), dispersal probabilities in Lagrange were constrained to zero between NT/PA, NT/O, NT/AU, NT/E, NA/O, NA/AU, NA/E,

PA/AU, O/E and AU/E. Initial extinction and dispersal probabilities were unconstrained and the rates of dispersal and extinction were constant and equal among areas. The BSSVS allows the ancestral reconstruction of discrete states in a Bayesian statistical framework, while accounting for phylogenetic uncertainty. Although this procedure was originally applied to phylogeographic models, it is also available for general ancestral state reconstruction (Lemey et al., 2009). The XML code for this analysis follows the standard discrete phylogeographic analysis with the number of locations/geographic areas (K) described above for Lagrange. Following Lemey et al. (2009), the dimension of the frequencies in the Continuous-time Markov Chain (CTMC) has been set to 6, matching the number of geographic areas. The dimension of rates and indicators of the CTMC model has been set to $K * (K - 1) / 2 = 15$. Two chains were run for 50 million generations each and the first 5 million generations were discarded as burn-in. The XML code for the time-calibrated phylogenetic and BSSVS are available from Dryad: <http://dx.doi.org/10.5061/dryad.g85fb>.

3. Results

3.1. Phylogenetic inference and divergence time estimation

Using jModeltest, BIC selected GTR+I+I as the best-fitting model for cyt-b and ND2, GTR+I for c-mos and HKY+I as the best-fitting model for RAG-1. We removed the first 5 million generations as burn-in and combined the two runs of BEAST analyses, which produced an estimated sample sizes (ESS) of all parameters above 200 except ND2 codon1 transition ratio (ESS = 175) and cyt-b codon 3 frequency (ESS = 187). The ESS of the likelihood and posterior is above 3000. The resulting topology is congruent with trees from

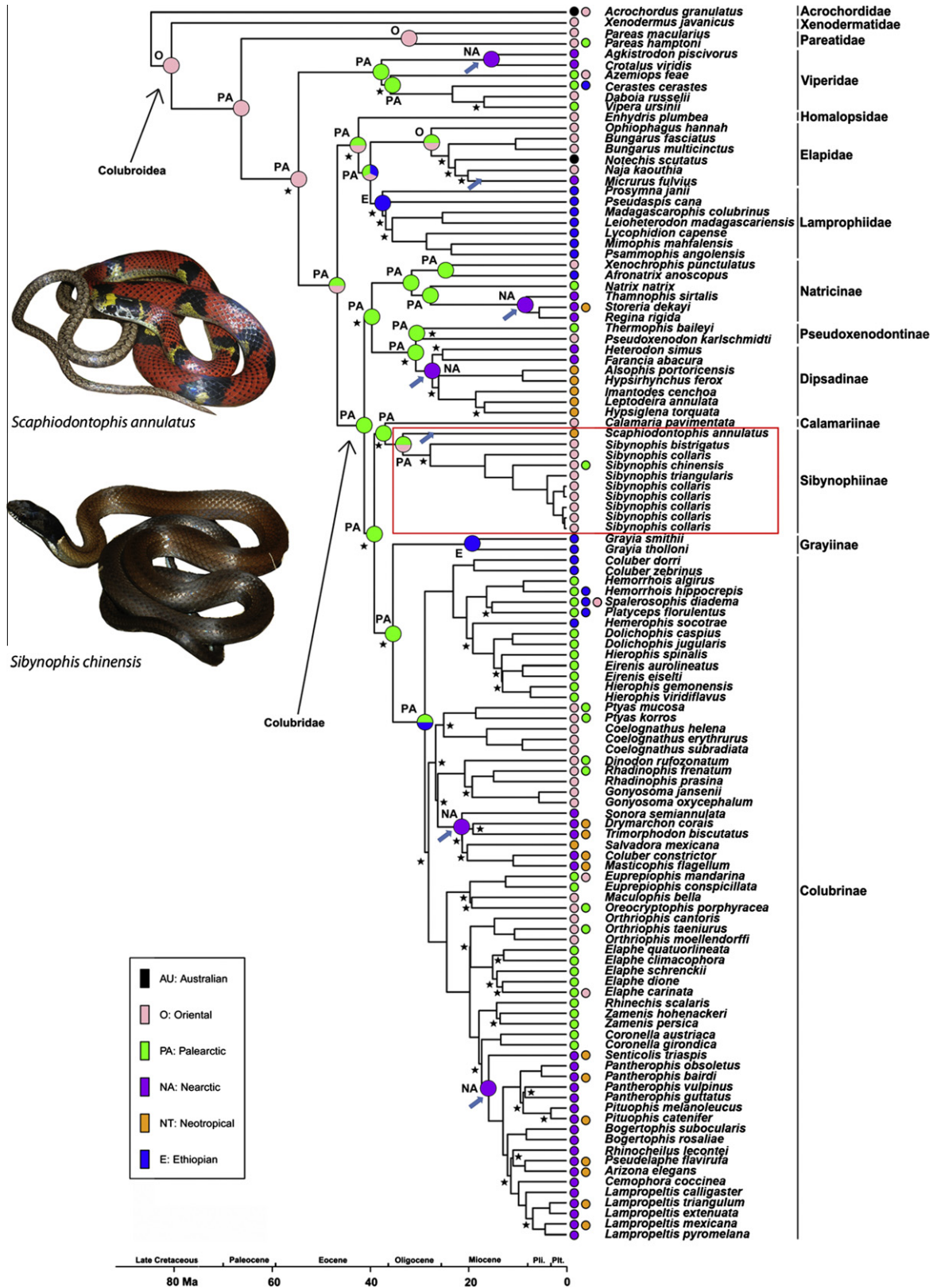


Fig. 2. Dated phylogeny of Colubroidea, including *Scaphiodontophis* and *Sibynophis*, estimated in BEAST 1.6.2 with reconstructed ancestral areas (Drummond et al., 2006; Drummond and Rambaut, 2007). The internodes with “star” label have posterior probability (pp) below 0.95 and all unmarked nodes indicate pp > 0.95. The pp, bootstrap and SH test support value for all the internodes are listed in Appendix Table S2. “Pli.” refers to the Pliocene; “Plt.” refers to the Pleistocene. Current distribution ancestral states of the taxa are labeled at tips of phylogeny (pink: Oriental; green: Palearctic; purple: Nearctic; black: Australian; orange: Neotropical; blue: Ethiopian). Estimated ancestral states of internodes are partially labeled (see Appendix Table S3 for the complete list). The ancestral states with the highest pp from BSSVS are labeled in letters (PA: Palearctic; O: Oriental; E: Ethiopian; NT: Neotropical; AU: Australian; NA: Nearctic) and the states of internodes from Lagrange with the highest likelihood value are indicated in the colored pie charts. The seven dispersals from the OW to the NW are highlighted with blue arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Pyron et al. (2011) and yielded strong support for most recognized families and subfamilies (Fig. 2; Appendix Fig. S1; Appendix Table S3). Phylogenetic relationships among some lineages such as Grayiinae, Calamariinae, and Scaphiodontophiinae are still uncertain given low support values. However, this tree strongly supports that *Sibynophis* is sister to *Scaphiodontophis* (pp = 0.99).

The maximum likelihood trees also support the sister relationship of *Scaphiodontophis* and *Sibynophis*, with a moderately high bootstrap value of 84 and SH test support values of 100 and 98 using RAxML and PhyML, respectively. This monophyletic group is related to Grayiinae, Colubrinae and Calamariinae, but the relationship of these groups are unclear in BEAST and ML trees with low support values (Fig. 2; Appendix Table S3). The BEAST results and the ML trees from RAxML shows that *Scaphiodontophis* and *Sibynophis* are the sister group to Calamariinae (pp = 0.36, bootstrap = 18, SH support value = 49), while the ML tree from PhyML indicates that they are sister to Colubriinae + Grayniinae with an SH support value of 53.

Divergence date estimates suggest that the timing of origin of most families and subfamilies are concordant with the results in Pyron and Burbrink (2012; Fig. 2). Our results indicate that *Sibynophis* and *Scaphiodontophis* last shared a common ancestor at 33.6 Ma (95% HPD: 40.0–22.9 Ma) and that this group may have diverged from Calamariinae at 37.3 Ma (95% HPD: 47.6–29.7 Ma; Fig. 2).

3.2. Reconstructed ancestral areas

The results of ancestral area estimation from both Lagrange and BSSVS demonstrate that Colubroidea originated in tropical Asia, and the ancestral areas of all families and subfamilies (except Dipsadinae) originated in the OW (Fig. 2; Appendix Table S3). Although the sampling is incomplete, the chosen taxa in our study represent the distributions of both OW and NW taxa in each family and subfamily. Because most clades originated well after all continents had separated and ancestral areas were located in the OW, we estimate that seven dispersals from the OW to the NW occurred among the following groups: Viperidae, Elapidae, Natricinae, Dipsadinae, *Scaphiodontophis*, Colubrinae (twice; Fig. 2). Results from Lagrange suggest that the common ancestor of *Sibynophis* and *Scaphiodontophis* is likely to have originated in the OW (Fig. 2; Appendix Table S3) and the area of origin for this group and Calamariinae occurred in the OW (Fig. 2; Appendix Table S3). Similarly, the BSSVS analyses also indicate an OW origination of the MRCA of *Scaphiodontophis* and *Sibynophis* (PA, pp = 0.52; O, pp = 0.47; Fig. 2; Appendix Table S3).

4. Discussion

4.1. Biogeography

Disjunct distributions among continents among closely related taxa, which on the surface may be somewhat unexpected, are not uncommon among squamates (Brandley et al., 2011; Burbrink and Lawson, 2007; Keogh et al., 2008; Noonan and Chippindale, 2006a,b; Noonan and Sites, 2010; Oaks, 2011; Townsend et al., 2011; Wüster et al., 2008). In particular, a connection between Eurasian and Nearctic/Neotropical taxa has been previously reported in viperids, ratsnakes, watersnakes, eublepharid geckos, and skinks (Brandley et al., 2011; Burbrink and Lawson, 2007; Gamble et al., 2011; Guo et al., 2012; Wüster et al., 2008). However, in colubroid snakes, it is usually the case that NW taxa with OW origins always have representatives in temperate regions of both areas. Here, we demonstrate an exception to this pattern and find support for an OW to NW tropical dispersal in colubroids that failed to leave extant temperate species.

Our results indicate that *Scaphiodontophis*, found only in the New World tropics of Central America and northern South America, and *Sibynophis*, generally distributed in the south and south-east Asian tropics, are sister genera within Colubridae. The current distribution of these sister groups is the result of two processes. First, the group likely originated in Asia 33.6 Ma (95% HPD: 40.0–22.9 Ma). At this time the relative positions of all continents were near their current positions. Therefore, the only route to the New World from Asia for ectothermic vertebrates would have been through Beringia, which, during this time, supported a coniferous/deciduous hardwood forest (mesophytic forests; Sanmartin et al., 2001; Wolfe, 1987). An alternative route, over the Greenland–Faeroes Bridge, during the same period of time, would not have provided suitable habitat for snake dispersal. Although unlikely, it is possible they rafted from Southeast Asia to Central America and northern South America, although little evidence exists for this route in any other terrestrial vertebrate group. Therefore, it is most likely that *Sibynophiinae* originated in the OW and dispersed across Beringia.

The second major process is linked to extinction or extirpation in the Holarctic. If these snakes colonized the Neotropics via the Nearctic as our results suggest, then this by definition indicates that taxa in northern latitudes were once present. *Sibynophis* generally lacks species distributed in temperate Asia, although a recent reported record suggests they occur as far north as Beijing (Hu et al., unpublished results), and *Scaphiodontophis* is completely absent in the Nearctic. This pattern is unlike the ratsnakes, crotaline viperids and watersnakes, which attain high diversities at temperate latitudes (Burbrink and Lawson, 2007; Guo et al., 2012; Wüster et al., 2008), particularly in the NW. Although not easily testable because of a lack of extant species, it is likely that *sibynophiines*, a mostly tropical group, became extinct or were extirpated in temperate regions as the tropics receded to their current latitudes (Lomolino et al., 2010; Sanmartin et al., 2001). This suggests that niche conservatism has played a major role in current distributions, as populations likely failed to adapt to temperate niches (Pyron and Burbrink, 2009a; Wiens and Graham, 2005).

Another peculiar pattern is the low diversity in the group, with only two species in *Scaphiodontophis* and nine in *Sibynophis* (Uetz, 2009). This contrasts with other species-rich assemblages such as the ratsnakes, crotaline viperids and watersnakes (Natricinae), which all originated at similar times (Burbrink and Lawson, 2007; Guo et al., 2012; Wüster et al., 2008). Given that there are only 11 extant species in the clade containing *Scaphiodontophis* and *Sibynophis*, a low rate of diversification must have occurred relative to the other groups. This could have been caused by a reduced speciation rate and/or increased extinction rate caused by competition or environmental pressures (Burbrink and Pyron, 2010; Rabosky, 2009). Reduced speciation rates may have been due to failure to diverge at barriers to gene flow seen in other snakes or a trade-off between morphological specialization in feeding apparatus and speciation, such as in fish where specialization on hard-bodied prey limits greater diversification on the more common soft-bodied prey (see Meyer, 1989).

Finally, our biogeographic analyses also reveal a general trend with regard to the origin and dispersal of colubroids overall. Our ancestral area estimates support an Asian tropical origin of Colubroidea consistent with the conclusions from fossil records (Head et al., 2005; Rage et al., 2008) and Pyron and Burbrink (2012), but also reveals seven independent dispersals from OW to NW. Our results support this unidirectional dispersal in Viperidae, Elapidae, Natricinae, Dipsadinae, *Scaphiodontophis* and Colubrinae (twice) from late Eocene to mid Miocene (see Section 3.2, Fig. 2). During this period, Beringia likely provided a more suitable intercontinental dispersal route than the Greenland–Faeroes Bridge. Thus, this result further indicates the importance of the Beringian

land bridge as a dispersal route between the OW and NW, which ultimately influenced the composition of flora and fauna in each region (Burbrink and Lawson, 2007; Lomolino et al., 2010; Sanmartin et al., 2001; Wen, 1999; Wüster et al., 2008).

4.2. Systematics

Our phylogenetic result indicates that hinged-teeth snakes *Scaphiodontophis* and *Sibynophis* are sister taxa, and occupy a phylogenetic position in Colubridae outside all other recognized subfamilies (Fig. 2). Currently, *Sibynophis* is placed in Colubrinae (Uetz, 2009) and *Scaphiodontophis* has been recognized as a new subfamily, Scaphiodontophiinae (Pyron et al., 2011). To maintain monophyly, we resurrect the subfamily Sibynophiinae, comprising *Sibynophis* and *Scaphiodontophis*. The genus *Liophidium*, previously included in this subfamily, has been demonstrated to be a part of the family Lamprophiidae (Nagy et al., 2003; Pyron et al., 2011). Thus, sharing the hinged-teeth between *Liophidium* and the other two genera should be considered morphological convergence. Due to ancient divergence of *Sibynophis* and *Scaphiodontophis*, disjunct distribution and distinctive features between the two genera, we suggest placing *Sibynophis* in the tribe Sibynophiini and *Scaphiodontophis* in the tribe Scaphiodontophiini, respectively.

Sibynophiinae Dunn, 1928 subfamily

Type genus *Sibynophis* Fitzinger (1848), species *Sibynophis geminates* (Boie, 1826).

Content: genus *Sibynophis* with nine recognized species (Uetz, 2009)—*S. bistrigatus* (Günther, 1868), *S. bivittatus* (Boulenger, 1894), *S. chinensis* (Günther, 1889), *S. collaris* (Gray, 1853), *S. geminatus* (Boie, 1826), *S. melanocephalus* (Gray, 1835), *S. sagittarius* (Cantor, 1839), *S. subpunctatus* (Duméril, Bibron & Duméril, 1854), *S. triangularis* Taylor, 1965; genus *Scaphiodontophis* Taylor and Smith, 1943 with two species (Uetz, 2009)—*S. annulatus* (Duméril, Bibron & Duméril, 1854), *S. venustissimus* (Günther, 1893).

Diagnosis and definition the distinctive features of Sibynophiinae include a posterior free dentary; hinged teeth and dentigerous bones attaching loosely; pterygoid not articulating to quadrate or articular; hypapophyses long and narrow with an acute point; hemipenes unilobed and calyculate; sulcus spermaticus single and simple with a U-shaped convolution in proximal region of hemipenial body; presence of spines on basal part of the hemipenes and distal calyces (Bogert, 1940; Savage and Slowinski, 1996; Savitzky, 1981; Underwood, 1967; Zaher, 1999). These traits support the position of Sibynophiinae in Colubridae, but render it distinct to other subfamilies. Other features include body size from 300 to 1000 mm in total length; numerous small teeth, from 35 to over 50, on each maxilla; dorsal scales smooth without apical pits arranged in 15–17 rows; pupil round and an extremely long tail (Das, 2010; Leviton, 1963; Taylor and Smith, 1943).

Sibynophiini Zaher, 1999 tribe

Type: genus *Sibynophis*, species *Sibynophis geminates*.

Content: genus *Sibynophis* with nine species listed above.

Diagnosis and definition: body size from 300 to 1000 mm total length; numerous teeth (35–48 on each maxilla), the first and the last two or three slightly smaller than the rest; hinged teeth; maxillary bone beyond ectopterygoid-maxillary articulation; maxillary process of palatine articulating with palatine process of maxilla; posterior free dentary bone; body cylindrical; extremely long tail; dorsal scales smooth in 15–17 rows without apical pits; ventrals rounded; subcaudals paired; anal plate divided; pupil round; hemipenes unforked, spinose and calyculate; hypapophyses present on posterior vertebrae; striped or uniform coloration; alternating modes (first even number, then odd number) of teeth replacement (Leviton, 1963; Savage and Slowinski, 1996).

Scaphiodontophiini tribe nov.

Type genus *Scaphiodontophis*, species *Scaphiodontophis annulatus* (Duméril and Bibron, 1854).

Content: genus *Scaphiodontophis* with two species listed above.

Diagnosis and definition: highly variable color patterns considered as coral snake mimics; hinged teeth; extremely long and disproportionately thick tail with fracture planes between the caudal vertebrae allowing non-regenerative breakage between vertebrae rather than within them; teeth replacement simultaneously; moderate body size about 920 mm; dorsal scales smooth, in 17 rows, without apical pits; subcaudals single; anal divided; one loreal, one preocular, two postoculars; one or two anterior temporal; maxillary teeth numerous (>50), scaphoid or spatulate, smaller at anterior and posterior ends of maxilla, arranged in groups of two or three teeth with different size and length (Savage and Slowinski, 1996; Taylor and Smith, 1943).

4.3. Relationships across Colubroidea

Besides Sibynophiinae, our phylogeny supports the validation of all other families in Colubroidea and subfamilies in Colubridae and is mostly consistent with Pyron et al. (2011). Nevertheless, the phylogenetic positions of Homalopsidae, Elapidae and Lamprophiidae are not well supported (Fig. 2; Appendix Table S3), and the relationship of Grayiinae, Calamarinae, Sibynophiinae, and Colubrinae within Colubridae are still unclear (see Section 3.1; Fig. 2; Appendix Table S3).

5. Conclusion

Our results demonstrate that *Scaphiodontophis* and *Sibynophis* are sister taxa, possessing geographically disjunct distributions in the NW and OW tropics. To maintain the monophyly of all subfamilies in Colubridae, we suggest including these two genera in the subfamily Sibynophiinae and recognize the genera *Scaphiodontophis* and *Sibynophis* in the tribes Scaphiodontophiini and Sibynophiini, respectively, to reflect the deep divergence, distant and separate distributions and distinctive morphological characteristics. Divergence time estimates suggest that these two tribes diverged in the Late Eocene or Early Oligocene, during which only Beringia would provide a climate suitable for snake dispersal. The results of the reconstructed ancestral areas indicate an OW origin of Sibynophiinae. This group of snakes follows an OW to NW trans-Beringian pattern dispersal also found for six other colubroid groups, indicating the importance of Beringia in shaping the present faunal composition of both the OW and NW. However, this group demonstrates the unusual pattern of overland dispersal between the OW and NW tropics, followed by the subsequent extinction of any temperate representatives in the NW or OW. This is in contrast to most other trans-Beringian squamate dispersals, all of which still have temperate representatives in either the OW, NW, or both regions.

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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.2012.09.032>.

References

- Anisimova, M., Gascuel, O., 2006. Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. *Syst. Biol.* 55, 539–552.
- Barry, C., 2001. The biogeographic regions reconsidered. *J. Biogeogr.* 28, 511–523.
- Blackburn, T.M., Gaston, K.J., 2002. Macroecology is distinct from biogeography. *Nature* 418, 723.
- Bogert, C.M., 1940. Herpetological results of the Vernay Angola Expedition. *Bull. Am. Mus. Nat. Hist.* 77, 1–107.
- Brandley, M.C., Wang, Y., Guo, X., Montes de Oca, A.N., Fería-Ortiz, M., Hikida, T., Ota, H., 2011. Accommodating heterogeneous rates of evolution in molecular divergence dating methods: an example using intercontinental dispersal of *Plestiodon* (*Eumeces*) lizards. *Syst. Biol.* 60, 3–15.
- Burbrink, F.T., Lawson, R., 2007. How and when did Old World ratsnakes disperse into the New World? *Mol. Phylogenet. Evol.* 43, 173–189.
- Burbrink, F.T., Pyron, R.A., 2010. How does ecological opportunity influence rates of speciation, extinction, and morphological diversification in new world ratsnakes (tribe Lampropeltini)? *Evolution* 64, 934–943.
- Cox, C.B., Moore, P.D., 2010. *Biogeography: An Ecological and Evolutionary Approach*. Wiley, Hoboken, NJ.
- Das, I., 2010. *A Field Guide to the Reptiles of South-East Asia: Myanmar, Thailand, Laos, Cambodia, Vietnam, Peninsular Malaysia, Singapore, Sumatra, Borneo, Java*. Bali, New Holland, London.
- de Queiroz, A., 2005. The resurrection of oceanic dispersal in historical biogeography. *Trends Ecol. Evol.* 20, 68–73.
- Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4, 699–710.
- Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7, 214.
- Dunn, E.R., 1928. A tentative key and arrangement of the American Colubridae. *Bull. Antivenin Inst. Am.* 2, 18–24.
- Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32, 1792–1797.
- Gamble, T., Bauer, A.M., Colli, G.R., Greenbaum, E., Jackman, T.R., Vitt, L.J., Simons, A.M., 2011. Coming to America: multiple origins of New World geckos. *J. Evol. Biol.* 24, 231–244.
- Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* 59, 307–321.
- Guo, P., Liu, Q., Xu, Y., Jiang, K., Hou, M., Ding, L., Alexander Pyron, R., Burbrink, F.T., 2012. Out of Asia: Natricine snakes support the Cenozoic Beringian Dispersal Hypothesis. *Mol. Phylogenet. Evol.* 63, 825–833.
- Head, J.J., Holroyd, P.A., Hutchison, J.H., Cocchon, R.L., 2005. First report of snakes (Serpentes) from the late middle Eocene Pondaung Formation, Myanmar. *J. Vertebr. Paleontol.* 25, 246–250.
- Holman, J.A., 2000. *Fossil Snakes of North America: Origin, Evolution, Distribution, Paleogeology*. Indiana University Press, Bloomington.
- Hu, L., Fu, S., Ning, J., Bao, W., 2012. A New Record of Snake in Beijing: *Sibynophis chinensis* (unpublished results).
- Köhler, G., 2008. *Reptiles of Central America*. Herpton, Verlag Elke Köhler, Germany.
- Keogh, J.S., Edwards, D.L., Fisher, R.N., Harlow, P.S., 2008. Molecular and morphological analysis of the critically endangered Fijian iguanas reveals cryptic diversity and a complex biogeographic history. *Philos. Trans. Roy. Soc.* 363, 3413–3426.
- Khan, M.S., 2002. *A Guide to the Snakes of Pakistan*. Edition Chimaira.
- Kreft, H., Jetz, W., 2010. A framework for delineating biogeographical regions based on species distributions. *J. Biogeogr.* 37, 2029–2053.
- Lafontaine, J.D., Wood, D.M., 1988. A zoogeographic analysis of the Noctuidae (Lepidoptera) of Beringia, and some inferences about past Beringian habitats. *Mem. Entomol. Soc. Can.* 120, 109–123.
- Lemey, P., Rambaut, A., Drummond, A.J., Suchard, M.A., 2009. Bayesian phylogeography finds its roots. *PLoS Comput. Biol.* 5, e1000520.
- Leviton, A.E., 1963. Contributions to a review of Philippine snakes, II. The snakes of the genera *Liopeltis* and *Sibynophis*. *Philipp. J. Sci.* 92, 367–381.
- Liebherr, J.K., 1991. Phylogeny and revision of the *Anchonemus* clade: the genera *Tetraleucus*, *Anchonemus*, *Sericoda* and *Elliptoleus* (Coleoptera: Carabidae: Platynini). *Bull. Am. Mus. Nat. Hist.* 39, 175–176.
- Lomolino, M.V., Riddle, B.R., Whittaker, R.J., Brown, J.H., 2010. *Biogeography*. Sinauer Associates, Sunderland, MA.
- Manthey, U., Groosmann, W., 1997. *Amphibien & Reptilien Südostasiens*. Natur und Tier-Verlag, Münster.
- Matthews Jr, J.V., 1979. Tertiary and Quaternary environments: historical background for an analysis of the Canadian insect fauna. *Mem. Entomol. Soc. Can.* 111, 31–86.
- McKenna, M.C., 1983. Cenozoic paleogeography of North Atlantic land bridges. In: Bott, M.H., Saxov, S.P., Talwani, M., Thiede, J. (Eds.), *Structure and Development of the Greenland–Scotland Bridge: New Concepts and Methods*. Plenum Press, New York, pp. x, 685 p.
- Meyer, A., 1989. Cost of morphological specialization: feeding performance of the two morphs in the trophically polymorphic cichlid fish, *Cichlasoma citrinellum*. *Oecologia* 80, 431–436.
- Nagy, Z.T., Joger, U., Wink, M., Glaw, F., Vences, M., 2003. Multiple colonization of Madagascar and Socotra by colubrid snakes: evidence from nuclear and mitochondrial gene phylogenies. *Proc. Roy. Soc. B* 270, 2613–2621.
- Noonan, B.P., Chippindale, P.T., 2006a. Dispersal and vicariance: the complex evolutionary history of boid snakes. *Mol. Phylogenet. Evol.* 40, 347–358.
- Noonan, B.P., Chippindale, P.T., 2006b. Vicariant origin of Malagasy reptiles supports late Cretaceous antarctic land bridge. *Am. Nat.* 168, 730–741.
- Noonan, B.P., Sites, J.W., 2010. Tracing the origins of iguanid lizards and boine snakes of the Pacific. *Am. Nat.* 175, 61–72.
- Nordlander, G., Liu, Z., Ronquist, F., 1996. Phylogeny and historical biogeography of the cynipoid wasp family Ibalidae (Hymenoptera). *Syst. Entomol.* 21, 151–166.
- Oaks, J.R., 2011. A time-calibrated species tree of Crocodylia reveals a recent radiation of the true crocodiles. *Evolution* 65, 3285–3297.
- Okajima, Y., Kumazawa, Y., 2009. Mitogenomic perspectives into iguanid phylogeny and biogeography: Gondwanan vicariance for the origin of Madagascar oplurines. *Gene* 441, 28–35.
- Posada, D., 2008. JModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* 25, 1253–1256.
- Pyron, R.A., Burbrink, F.T., 2009a. Can the tropical conservatism hypothesis explain temperate species richness patterns? An inverse latitudinal biodiversity gradient in the New World snake tribe Lampropeltini. *Glob. Ecol. Biogeogr.* 18, 406–415.
- Pyron, R.A., Burbrink, F.T., 2009b. Neogene diversification and taxonomic stability in the snake tribe Lampropeltini (Serpentes: Colubridae). *Mol. Phylogenet. Evol.* 52, 524–529.
- Pyron, R.A., Burbrink, F.T., 2012. Extinction, ecological opportunity, and the origins of global snake diversity. *Evolution* 66, 163–178.
- Pyron, R.A., Burbrink, F.T., Colli, G.R., de Oca, A.N.M., Vitt, L.J., Kuczynski, C.A., Wiens, J.J., 2011. The phylogeny of advanced snakes (Colubroidea), with discovery of a new subfamily and comparison of support methods for likelihood trees. *Mol. Phylogenet. Evol.* 58, 329–342.
- Rabosky, D.L., 2009. Ecological limits and diversification rate: alternative paradigms to explain the variation in species richness among clades and regions. *Ecol. Lett.* 12, 735–743.
- Rage, J.C., Folie, A., Rana, R.S., Singh, H., Rose, K.D., Smith, T., 2008. A diverse snake fauna from the early Eocene of Vastan Lignite Mine, Gujarat, India. *Acta Palaeontol. Pol.* 53, 391–403.
- Ree, R.H., Moore, B.R., Webb, C.O., Donoghue, M.J., 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution* 59, 2299–2311.
- Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.* 57, 4–14.
- Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Syst. Biol.* 46, 195–203.
- Sanmartin, I., Engloff, H., Ronquist, F., 2001. Patterns of animal dispersal, vicariance and diversification in the Holarctic. *Biol. J. Linn. Soc.* 73, 345–390.
- Savage, J.M., Slowinski, J.B., 1996. Evolution of coloration, urotomy and coral snake mimicry in the snake genus *Scaphiodontophis* (Serpentes: Colubridae). *Biol. J. Linn. Soc.* 57, 129–194.
- Savitzky, A.H., 1981. Hinged teeth in snakes: an adaptation for swallowing hard-bodied prey. *Science* 212, 346–349.
- Savitzky, A.H., 1983. Coadapted character complexes among snakes: fossoriality, piscivory, and durophagy. *Am. Zool.* 23, 397–409.
- Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* 16, 1114.
- Stamatakis, A., 2006a. Phylogenetic models of rate heterogeneity: a high performance computing perspective. In: *Proceedings of the 20th IEEE/ACM International Parallel and Distributed Processing Symposium*.
- Stamatakis, A., 2006b. RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690.
- Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAXML Web servers. *Syst. Biol.* 57, 758–771.
- Tangelder, I.R.M., 1988. The biogeography of the holarctic *Nephrotoma dorsalis* group (Diptera, Tipulidae). *Beaufortia* 38, 1–35.
- Taylor, D.J., Smith, H.M., 1943. A review of American Sibynophine snakes, with a proposal of a new genus. *Univ. Kans. Sci. Bull.* 29, 301–337.
- Tiffney, B.H., 1985. The Eocene North Atlantic land bridge: its importance in Tertiary and modern phytogeography of the Northern Hemisphere. *J. Arnold Arbor.* 66, 243–273.
- Townsend, T.M., Leavitt, D.H., Reeder, T.W., 2011. Intercontinental dispersal by a microendemic burrowing reptile (Dibamidae). *Proc. Roy. Soc. B* 278, 2568–2574.
- Uetz, P., 2009. *The Reptile Database*. <<http://www.reptile-database.org>>.
- Underwood, G., 1967. *A Contribution to the Classification of Snakes*. Trustees of the British Museum (Natural History), London.
- Wallace, A.R., 1876. *The Geographical Distribution of Animals*. 2 vols. Macmillan, London.
- Wen, J., 1999. Evolution of eastern Asian and eastern North American disjunct distributions in flowering plants. *Annu. Rev. Ecol. Syst.* 30, 421–455.

- Whitaker, R., Captain, A., 2007. Snakes of India, The Field Guide. Draco Books, India.
- Wiens, J.J., Graham, C.H., 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. *Ann. Rev. Ecol. Evol. Syst.* 36, 519–539.
- Wolfe, J.A., 1987. Late Cretaceous–Cenozoic history of deciduousness and the terminal Cretaceous event. *Paleobiology* 13, 215–226.
- Wüster, W., Peppin, L., Pook, C.E., Walker, D.E., 2008. A nesting of vipers: phylogeny and historical biogeography of the Viperidae (Squamata: Serpentes). *Mol. Phylogenet. Evol.* 49, 445–459.
- Zaher, H., 1999. Hemipenial morphology of the South American xenodontine snakes, with a proposal for a monophyletic Xenodontinae and a reappraisal of colubroid hemipenes. *Bull. Am. Mus. Nat. Hist.*, 1–168.
- Zhao, E., Adler, K., 1993. Herpetology of China. Society for the Study of Amphibians and Reptiles, USA.