

Electronic Supplementary Material for “Novel phylogenomic inference and ‘Out of Asia’ biogeography of cobras, coral snakes, and their allies”

Tissues. Tissues were collected by J. Alesio, K. E. Allen, V. Arias, R. M. Brown, W. and J. Bulalacao, L. Canseco, A. C. Diesmos, E. Ducasse, W. E. Duellman, J. B. Fernandez, E. B. Greenbaum, C. Hayden, J. Guayasamin, B. Gurubat, D. S. McLeod, J. Porras, S. Pouy, C. Raxworthy, R. Reyes, D. Roldán-Piña, E. L. Rico, M. B. Sanguila, C. D. Siler, J. E. Simmons, W. P. Tapondjou N., I. Tigulu, S. L. Travers, L. J. Welton, E. and Z. Wickham, and J. Zafe during field expeditions in Cameroon (2015, 2018), China (2006, 2007), El Salvador (2000), Ghana (1998), Paraguay (1996), Peru (1990), Philippines (2000, 2001, 2005, 2007, 2008, 2009, 2011, 2013, 2014, 2017), Solomon Islands (2016), and Thailand (2005), and deposited in the University of Kansas Biodiversity Institute, or as loans from tissue collections at University of Texas El Paso and Villanova University (Villanova, PA).

Selecting loci to target for sequence capture. Using a probe-based sequence capture approach, we targeted rapidly evolving exons (REEs), ultraconservative elements (UCEs), ddRAD-like loci (inferred *in-silico*), and protein-coding regions of previously identified major histo-compatibility (MHC), vision, and scalation-associated genes. To identify REEs, we used the R package REEs v1.9 [1] and 11 previously published squamate genome assemblies (<https://github.com/JeffWeinell/SnakeCap>). To select ddRAD-like loci, we used the REEs function "proposeLoci.ddRADlike" to locate 900–1000bp regions in the genome of *Thermophis baileyi* that begin and end with the *EcoRI* and *SbfI* restriction enzyme site recognition sequences (CCTGCAGG and GAATTC), respectively. To select vision loci, we used BLASTn from BLAST+ v2.9 [2] to search within sampled snake genomes for matches to probe sequences used by [3] for vision-associated genes in *Anolis*, *Columba*, *Gallus*, *Pelodiscus*, *Sceloporus*, or *Python*, and our probes corresponded to the genomic hit sequences of best matches. To select scalation loci, we used tBLASTn from BLAST+ to search for *Ophiophagus* and *Python*. Epidermal Differentiation Complex protein sequences previously identified by [4] in genomes of *T. sirtalis*, *P. mucrosquamatus*, and *C. horridus*. Furthermore, we included a subset of the UCEs of *Micrurus fulvius* from the study by [5], and 27 sequence regions corresponding to annotated major histocompatibility complex genes from the genome of *Thamnophis sirtalis*. Arbor Sciences synthesized 120nt RNA probes for our target sequences with 2x tiling and ultrastringent filtering, as a custom MyBaits 20,000 RNA-probes kit, and conducted library preparation prior to sequencing by NovoGene on an Illumina HiSeq X sequencing (dual-index, paired-end, 150 bp inserts). The optimized probe set included 20,020 probes for 3128 loci (1,517,011 nt) selected as targets: 1652 REEs, 907 UCEs, 328 ddRAD-like, 27 MHC, 119 vision, and 95 scalation loci (Open Science Framework, project krhx3).

Geographic transition zones. We considered two geographic transitional zones when coding taxa as present or absent in our geographic range dataset used for biogeographic range evolution analyses. One transition zone comprises the area containing Anatolia, Armenian Highlands, and Iranian Plateau, where Africa, Asia, and Europe meet. A second transition zone comprises islands of Wallacea between Asia and Australasia (figure S1). Colubroid or elapoid genera are not endemic to either transition zone [6–11], and therefore when scoring genera as present or absent in regions, we ignored portions of their ranges intersecting transition zones. For example,

genera occurring both within the Africa-Asia-Europe transition zone and in Africa outside of the transition zone were coded in our geographic range dataset as occurring only in “Africa”.

Table S1. Individuals sampled using targeted sequence capture (bold font) or from previously published assembled genomes. Institution and field series abbreviations: KU = University of Kansas; Steinhardt Museum of Natural History, Tel Aviv University; MCZ = Museum of Comparative Zoology; PNM = Philippine National Museum; UTEP = University of Texas, El Paso. For sequence capture data, raw reads are available in the NCBI Sequence Read Archive with BioProject ID PRJNA926108. Sequence alignments used for phylogenetic analyses are available on Open Science Framework, project krhx3. Data ID is either the NCBI BioSample accession number (SAMN numbers), NCBI genome assembly accession number (GCA numbers), or 'snake_7C' for the *Boa constrictor* genome assembly (GigaDB repository; project: <http://dx.doi.org/10.5524/100060>).

Species	Family	voucher ID	other sample ID	data ID
<i>Acrochordus granulatus</i>	Acrochordidae	KU 302952	CDS 681	SAMN32850298
<i>Achalinus spinalis</i>	Xenodermidae	KU 312258	MCB 516	SAMN32850299
<i>Cerberus schneiderii</i>	Homalopsidae	KU 324534	CDS 5109	SAMN32850300
<i>Aplopeltura boa</i>	Pareidae	KU 315147	RMB 10058	SAMN32850301
<i>Ahaetulla prasina preocularis</i>	Colubridae	KU 323364	RMB 12330	SAMN32850302
<i>Calamaria gervaisii</i>	Calamariidae	KU 327406	ACD 1871	SAMN32850303
<i>Boiga irregularis</i>	Colubridae	KU 345021	SLT 833	SAMN32850304
<i>Scolecophis atrocinctus</i>	Colubridae	KU 289804	EBG 276	SAMN32850305
<i>Tantilla taeniata</i>	Colubridae	KU 289863	EBG 393	SAMN32850306
<i>Dipsas indica</i>	Dipsadidae	KU 214857	WED 59279	SAMN32850307
<i>Grayia smythii</i>	Grayiidae	KU 341872	RMB 19318	SAMN32850308
<i>Pseudoxenodon bambusicola</i>	Pseudoxenodontidae	KU 312221	KU-FS 734	SAMN32850309
<i>Sibynophis bivittatus</i>	Sibynophiidae	KU 309608	RMB 7779	SAMN32850310
<i>Elapoidea sundevallii</i>	Elapidae	MCZ R-190390	MCZ A-27807	SAMN32850311
<i>Micrurus corallinus</i>	Elapidae	KU 289205	JES 1814	SAMN32850312
<i>Hemibungarus mcclungi</i>	Elapidae	KU 303027	CDS 1486	SAMN32850313
<i>Buhoma marlieri</i>	incertae sedis	UTEP 22598	CFS 1528	SAMN32850314
<i>Aparallactus capensis</i>	Atractaspididae	MCZ R-193258	MCZ A-28808	SAMN32850315
<i>Cyclocorus lineatus lineatus</i>	Cyclocoridae	KU 335808	RMB 17698	SAMN32850316
<i>Oxyrhabdium leporinum</i>	Cyclocoridae	KU 346583	RMB 23647	SAMN32850318
<i>Oxyrhabdium modestum</i>	Cyclocoridae	KU 310866	CDS 3006	SAMN32850319
<i>Oxyrhabdium modestum</i>	Cyclocoridae	KU 338101	RMB 19059	SAMN32850317

<i>Levitonius mirus</i>	Cyclocoridae	PNM 9872	KU 337269	SAMN32850320
<i>Hologerrhum philippinum</i>	Cyclocoridae	KU 330752	CDS 5735	SAMN32850321
<i>Myersophis alpestris</i>	Cyclocoridae	KU 308684	ELR 1149	SAMN32850322
<i>Chamaelycus fasciatus</i>	Lamprophiidae	KU 348632	KAE 243	SAMN32850323
<i>Boaedon lineatus</i>	Lamprophiidae	no voucher	EBG 705	SAMN32850324
<i>Prosymna visseri</i>	Prosymnidae	MCZ R-190223	AMB 8075	SAMN32850325
<i>Psammophis sibilans</i>	Psammophiidae	KU 290424	RAX 2053	SAMN32850326
<i>Pseudaspis cana</i>	Pseudaspididae	no voucher	MCZ A-27068	SAMN32850327
<i>Pseudoxyrhopus tritaeniatus</i>	Pseudoxyrhophiidae	KU 340969	SML 029	SAMN32850328
<i>Rhamphiophis oxyrhynchus</i>	Rhamphiophiidae	KU 290451	RAX 2104	SAMN32850329
<i>Psammodynastes pulverulentus</i>	<i>incertae sedis</i>	KU 329686	RMB 14526	SAMN32850330
<i>Psammodynastes pulverulentus</i>	<i>incertae sedis</i>	KU 328547	DSM 1384	SAMN32850331
<i>Micrelaps muelleri</i>	Micrelapidae	SMNH TAU R-16738	TAU R-16738	SAMN32850332
<i>Boa constrictor</i>	Boidae	—	ERS218597	snake_7C
<i>Chrysopelea ornata</i>	Colubridae	—	—	GCA_018340635.1
<i>Pantherophis guttatus</i>	Colubridae	—	—	GCA_019457695.1
<i>Pantherophis obsoletus</i>	Colubridae	—	—	GCA_012654085.1
<i>Pituophis catenifer</i>	Colubridae	—	—	GCA_019677565.1
<i>Ptyas mucosa</i>	Colubridae	—	—	GCA_012654045.1
<i>Thamnophis elegans</i>	Natricidae	—	—	GCF_009769535.1
<i>Thamnophis sirtalis</i>	Natricidae	—	—	GCA_001077635.2
<i>Thermophis baileyi</i>	Natricidae	—	—	GCA_003457575.1
<i>Emydocephalus ijimae</i>	Elapidae	—	—	GCA_004319985.1
<i>Ophiophagus hannah</i>	Elapidae	—	—	GCA_000516915.1
<i>Hydrophis curtus</i>	Elapidae	—	—	GCA_019472885.1
<i>Hydrophis cyanocinctus</i>	Elapidae	—	—	GCA_019473425.1
<i>Hydrophis hardwickii</i>	Elapidae	—	—	GCA_004023765.1
<i>Hydrophis melanocephalus</i>	Elapidae	—	—	GCA_004320005.1
<i>Laticauda colubrina</i>	Elapidae	—	—	GCA_015471245.1

<i>Laticauda laticaudata</i>	Elapidae	—	—	GCA_004320025.1
<i>Naja naja</i>	Elapidae	—	—	GCA_009733165.1
<i>Notechis scutatus</i>	Elapidae	—	—	GCF_900518725.1
<i>Pseudonaja textilis</i>	Elapidae	—	—	GCF_900518735.1
<i>Myanophis thanlyinensis</i>	Homalopsidae	—	—	GCA_017656035.1
<i>Python molurus</i>	Pythonidae	—	—	GCA_000186305.2
<i>Bothrops jararaca</i>	Viperidae	—	—	GCA_018340635.1
<i>Crotalus adamanteus</i>	Viperidae	—	—	GCA_018446365.1
<i>Crotalus horridus</i>	Viperidae	—	—	GCA_001625485.1
<i>Crotalus pyrrhus</i>	Viperidae	—	—	GCA_000737285.1
<i>Crotalus tigris</i>	Viperidae	—	—	GCA_016545835.1
<i>Crotalus viridis</i>	Viperidae	—	—	GCA_003400415.2
<i>Protobothrops flavoviridis</i>	Viperidae	—	—	GCA_003402635.1
<i>Protobothrops mucrosquamatus</i>	Viperidae	—	—	GCA_001527695.3
<i>Vipera berus</i>	Viperidae	—	—	GCA_000800605.1

Table S2. Individuals with novel Sanger sequence data. Sequence alignment available on Open Science Framework, project krhx3.

taxon	voucher	field tag ID	GenBank (CYTB)	GenBank (CMOS)
<i>Acrochordus granulatus</i>	KU 302951	CDS 357	OQ290841	OQ290863
<i>Aplopeltura boa</i>	KU 319950	ACD 3924	OQ290842	OQ290864
<i>Boiga cynodon</i>	KU 344107	RMB 21632	OQ290843	OQ290865
<i>Calliophis bilineatus</i>	KU 309511	RMB 7884	OQ290844	OQ290866
<i>Cerberus schneiderii</i>	KU 302983	CDS 309	OQ290845	OQ290867
<i>Chrysopela paradisi</i>	KU 310165	RMB 8473	OQ290846	OQ290868
<i>Coelognathus erythrurus</i>	KU 303024	CDS 1126	OQ290847	OQ290869
<i>Cyclocorus nuchalis</i>	KU 334469	RMB 16039	OQ290848	OQ290870
<i>Gonyosoma oxycephalum</i>	KU 304104	RMB 5388	OQ290849	OQ290871
<i>Hemibungarus gemianulis</i>	—	CMNH H794	OQ290850	OQ290872
<i>Hologerrhum philippinum</i>	KU 328837	RMB 13628	OQ290851	OQ290873
<i>Laticauda colubrina</i>	KU 341149	SLT 197	OQ290852	—
<i>Laticauda colubrina</i>	KU 351734	NDR 145	OQ290853	OQ290874
<i>Naja samarensis</i>	KU 320521	CDS 3662	OQ290854	OQ290875
<i>Opisthotropis typica</i>	KU 327424	RMB 3111	OQ290855	OQ290876
<i>Oxyrhabdium leporinum</i>	KU 306307	CDS 1769	OQ290856	OQ290877
<i>Pseudorabdion taylori</i>	KU 327215	ACD 5357	OQ290857	OQ290878
<i>Sibynophis geminatus</i>	no voucher	ELR 198	OQ290858	OQ290879
<i>Stegonotus muelleri</i>	KU 328844	RMB 8541	OQ290859	OQ290880
<i>Trimeresurus flavomaculatus</i>	KU 310865	CDS 2895	OQ290860	OQ290881
<i>Tropidolaemus subannulatus</i>	KU 334489	RMB 16352	OQ290861	OQ290882
<i>Tropidonophis spilogaster</i>	KU 329682	RMB 14471	OQ290862	OQ290883

Table S3. Polymerase chain reaction and sequencing primers used in this study. Novel CYTB primers (72.15 and 987R.20) were designed using Primer3 v0.4.0 [12].

locus	primer name	sequence	direction	source
CYTB	L14910	GACCTGTGATMTGAAAACCAYCGTTGT	forward	[13]
CYTB	L14919	AACCACCGTTGTTATTCAACT	forward	[13]
CYTB	H16064	CTTGTTTACAAGAACAAATGCTTA	reverse	[13]
CYTB	H15720	TGGTGTGTTGTAATTGGTCT	reverse	[13]
CYTB	72F.18	AGCAATYCCTACTAYACAGC	forward	This study
CYTB	337F.23	TTCTGAGCAGCAACAGTAATCAC	reverse	[14]
CYTB	732R.21	YTCTGGTTAATGTGTTGKGG	forward	[14]
CYTB	987R.20	AATGGAGGTTGTTGACCAA	reverse	This study
C-MOS	S77	CATGGACTGGATCAGTTATG	forward	[15]
C-MOS	S78	CCTTGGGTGTGATTTCTCACCT	reverse	[15]

Table S4. Fossils used to calibrate node ages for divergence time estimation.

Fossil description	Calibrated node	Fossil age (Ma)
<i>Vipera</i> cf. <i>V. antiqua</i> Szyndlar & Böhme, 1993	stem-Viperinae	22.1
Elapidae indet. McCartney, Stevens, & O'Connor, 2014	stem-Elapidae	24.9
<i>Paleoheterodon tiheni</i> Holman, 1964	stem-Dipsadidae	12.5
Colubridae indet. Smith, 2013	stem-Colubroidea	35.2
<i>Incongruelaps iteratus</i> Scanlon, Lee, & Archer, & 2003	stem-Oxyuraninae	10

Table S5. Number of reticulations, complexity, and maximum pseudolikelihood of estimated phylogenetic networks; complexity (C) considered equal to number of identifiable (= number of internal branches + number of reticulations). Best model selected using slope heuristics estimated with capushe R package shown in bold, and using data-driven slope estimation (DDSE) and dimension jump (Djump) algorithms; MPL = maximum pseudolikelihood; MPL networks had at 0–4 reticulations, although maximum number of reticulations allowed was 0–9; HMAX = maximum number of reticulations allowed during network optimization; HMPL = number of reticulations in MPL tree.

HMAX	HMPL	C	MPL	Notes
0	0	9	87.172	<u>Results:</u> This was the model chosen by capushe as optimal, under the jump_model algorithm, when complexity was set to observed (rather than maximum) number of hybridizations.
1	1	10	72.340	<i>Buhoma + Elapsoidea</i> = stem <i>Psammophis + Aparallactus</i>
2	2	11	65.567	rerooting not possible
3	3	12	52.966	(<i>Elapsoidea + Aparallactus</i> = <i>Psammophis</i>); (<i>Boaedon</i> contribution to ancestral <i>Pseudoxyrhopus</i>); (<i>Psammodynastes</i> contribution to ancestral Africa-Malagasy lineage) <u>Results:</u> capushe selected this as the optimal model under both the DDSE_model and jump_model algorithms, when complexity was set to the maximum number of reticulations rather than to the observed number.
4	4	13	54.421	Re-rooting not possible on <i>Pseudoxenodon</i> , so rooted on <i>Hologerrhum</i> . <i>Hologerrhum + Elapsoidea</i> = <i>Pseudoxenodon</i> ; <i>Buhoma + Psammophis</i> = <i>Aparallactus</i> ; <i>Boaedon + Pseudaspis</i> = <i>Prosymna</i> ; <i>Boaedon + Pseudaspis + Prosymna</i> stem contributed to <i>Buhoma + Psammophis + Aparallactus</i> stem.
5	3*	12	52.943	Not possible to rerooot
6	4*	13	54.171	Not possible to rerooot; <u>Results:</u> This was the model chosen by capushe as optimal, under the DDSE_model algorithm, when complexity was set to observed (rather than maximum) number of hybridizations.
7	3*	12	50.547	<i>Elapsoidea + Aparallactus</i> = <i>Psammophis</i> ; <i>Prosymna + Pseudoxyrhopus</i> = <i>Boaedon</i> ; <i>Hologerrhum</i> + stem Africa-Malagasy lineage = <i>Psammodynastes</i>
8	3*	12	53.344	<i>Psammophis</i> + stem Africa-Malagasy lineage = <i>Elapsoidea</i> ; <i>Psammodynastes</i> contributed to <i>Hologerrhum</i> ; <i>Prosymna + Pseudoxyrhopus</i> = <i>Boaedon</i>
9	3*	12	50.812	<i>Hologerrhum</i> + stem-Africa Malagasy lineage = <i>Psammodynastes</i> ; <i>Prosymna + Pseudoxyrhopus</i> = <i>Boaedon</i> ; <i>Elapsoidea + Aparallactus</i> = <i>Psammophis</i> .
10	3*	12	50.658	identical to max H = 9 network

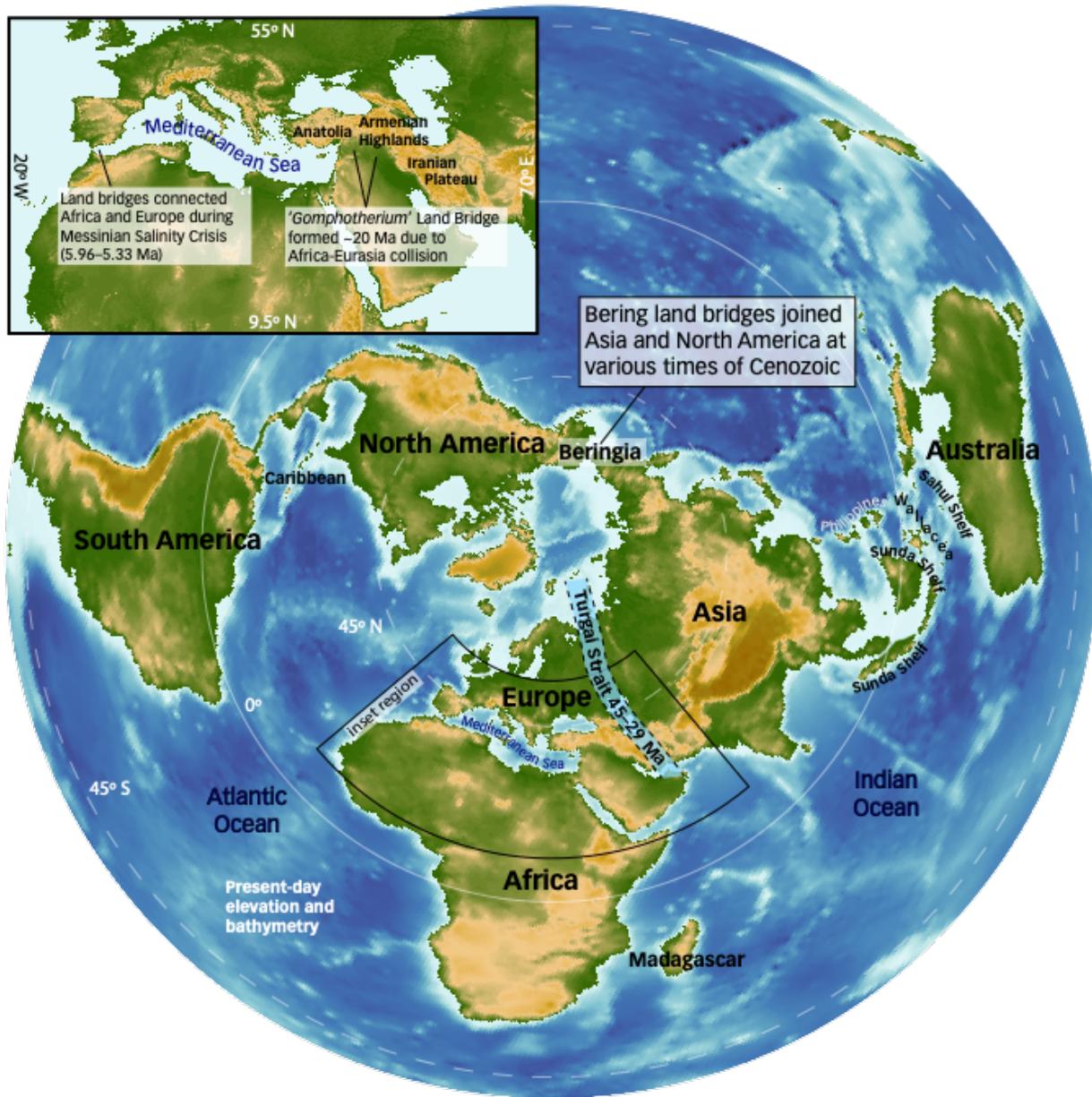


Figure S1. Places mentioned in this article. Colors indicate elevation above sea level (increasing from green to yellow to brown) and marine depth (blues; darker = deeper). Paleogeographical features hypothesized as facilitating or limiting historical faunal exchanges are described and indicated in the context of modern geography.

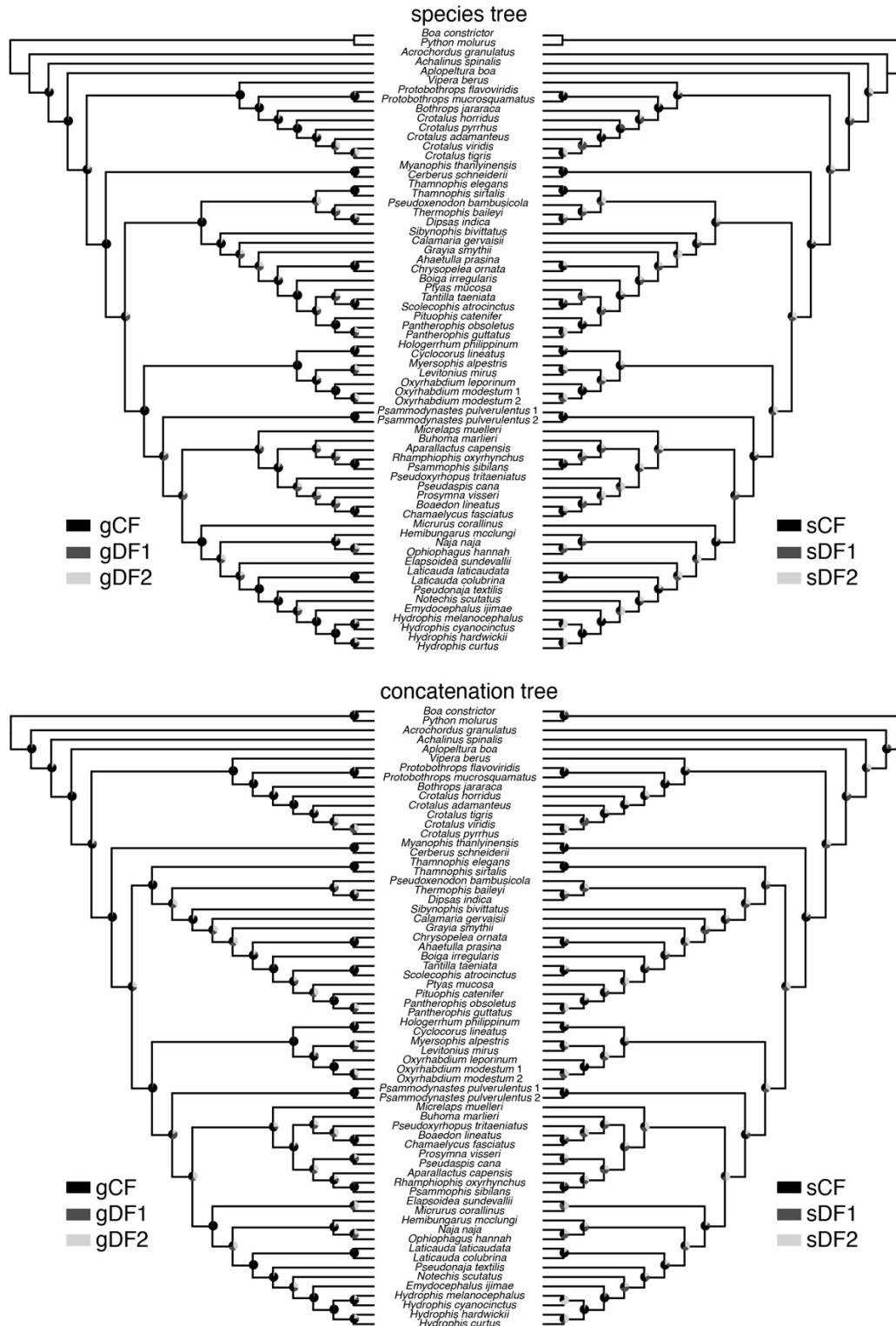


Figure S2. Species tree (top row) and concatenation tree (bottom row) with pie charts at nodes indicating gene (left) and site (right) concordance and discordance factors; gCF = gene concordance factor, gDF1 = gene discordance factor 1, gDF2 = gene discordance factor 2; sCF = site concordance factor, sDF1 = site discordance factor 1, sDF2 = site discordance factor 2.

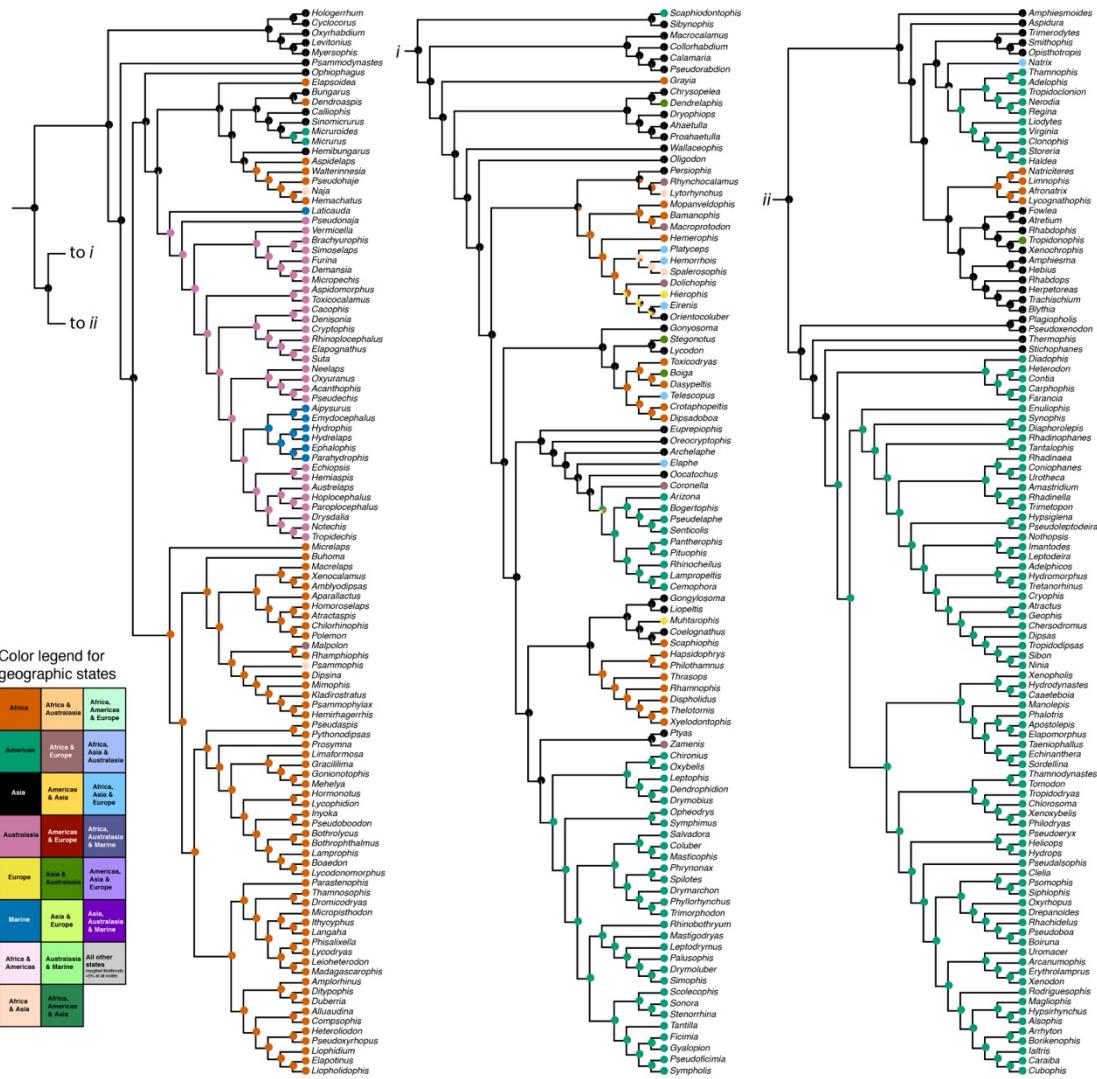


Figure S3. BioGeoBEARS geographic range evolution results of Elapoidea and Colubroidea under the biogeographic model “BAYAREALIKE+J”. Node pie charts depict marginal probability estimates of ancestral geographic states. Tip circles depict geographic states of extant lineages provided as input during analysis. Phylogeny used during analysis is visualized here as a cladogram.

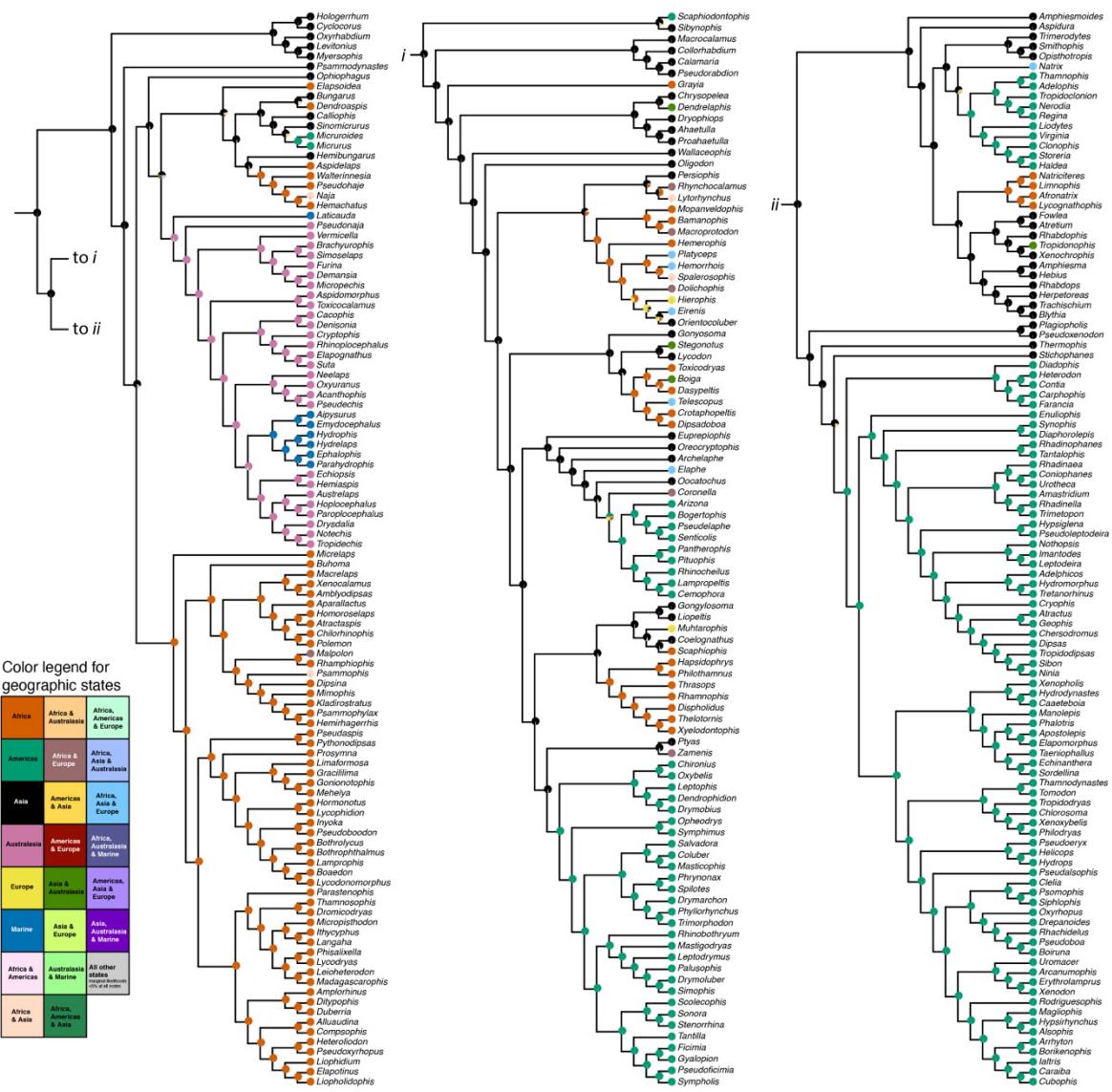


Figure S4. BioGeoBEARS geographic range evolution results of Elapoidea and Colubroidea under the biogeographic model “DIVALIKE+J”. Node pie charts depict marginal probability estimates of ancestral geographic states. Tip circles depict geographic states of extant lineages provided as input during analysis. Phylogeny used during analysis is visualized here as a cladogram.

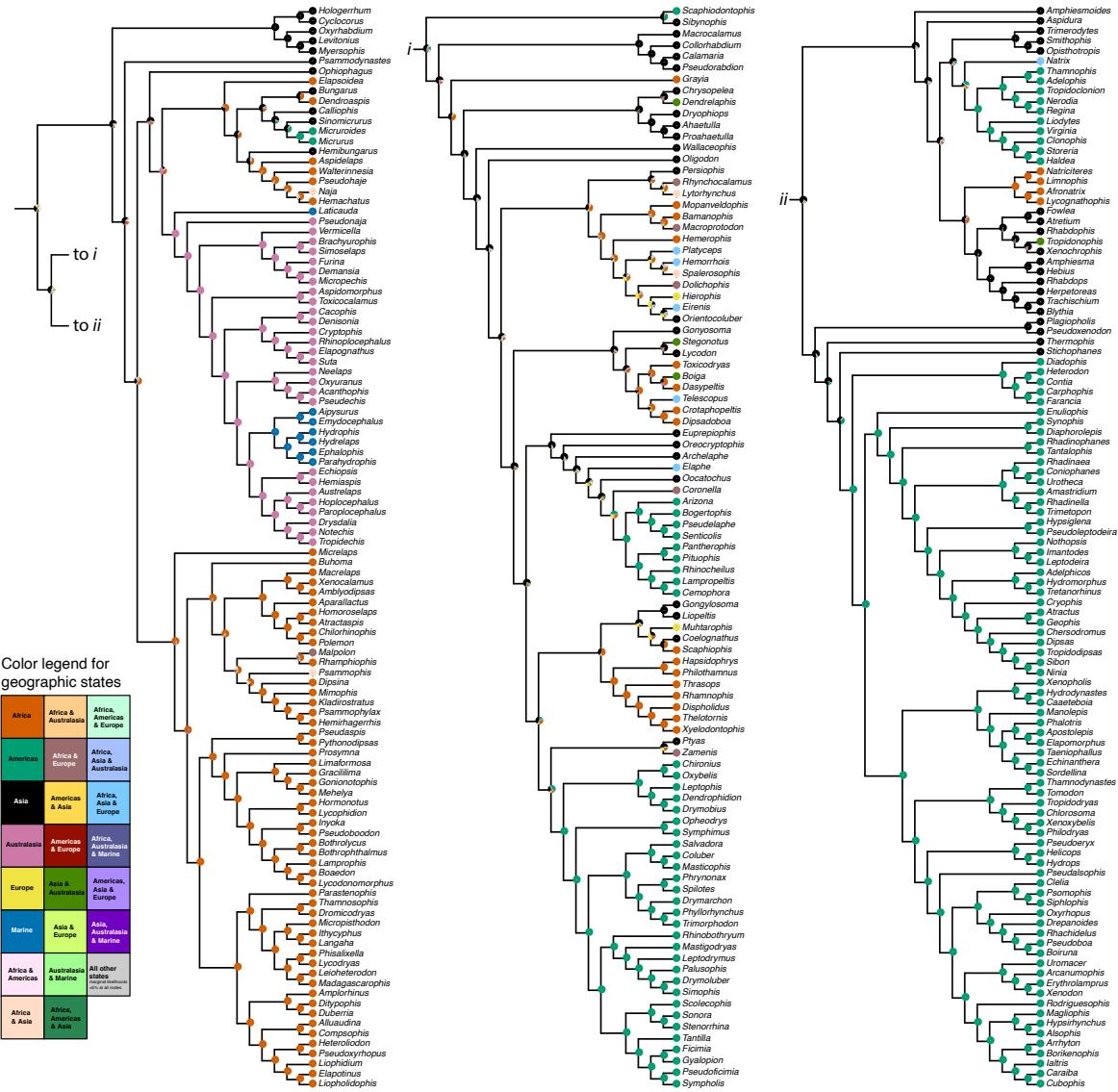


Figure S5. BioGeoBEARS geographic range evolution results of Elapoidea and Colubroidea under the biogeographic model “DEC+J”. Node pie charts depict marginal probability estimates of ancestral geographic states. Tip circles depict geographic states of extant lineages provided as input during analysis. Phylogeny used during analysis is visualized here as a cladogram.

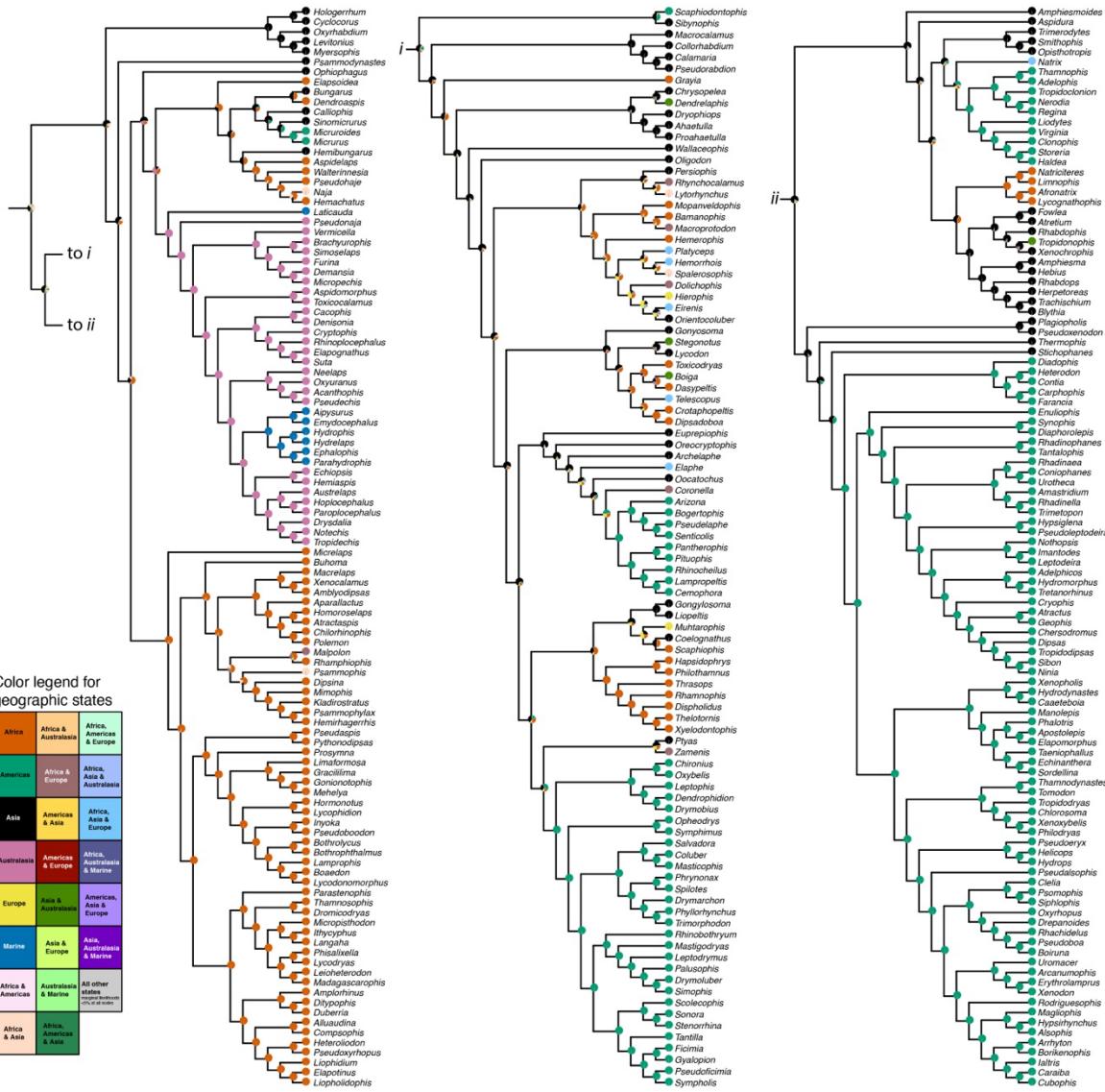


Figure S6. BioGeoBEARS geographic range evolution results of Elapoidea and Colubroidea under the biogeographic model “DEC”. Node pie charts depict marginal probability estimates of ancestral geographic states. Tip circles depict geographic states of extant lineages provided as input during analysis. Phylogeny used during analysis is visualized here as a cladogram.

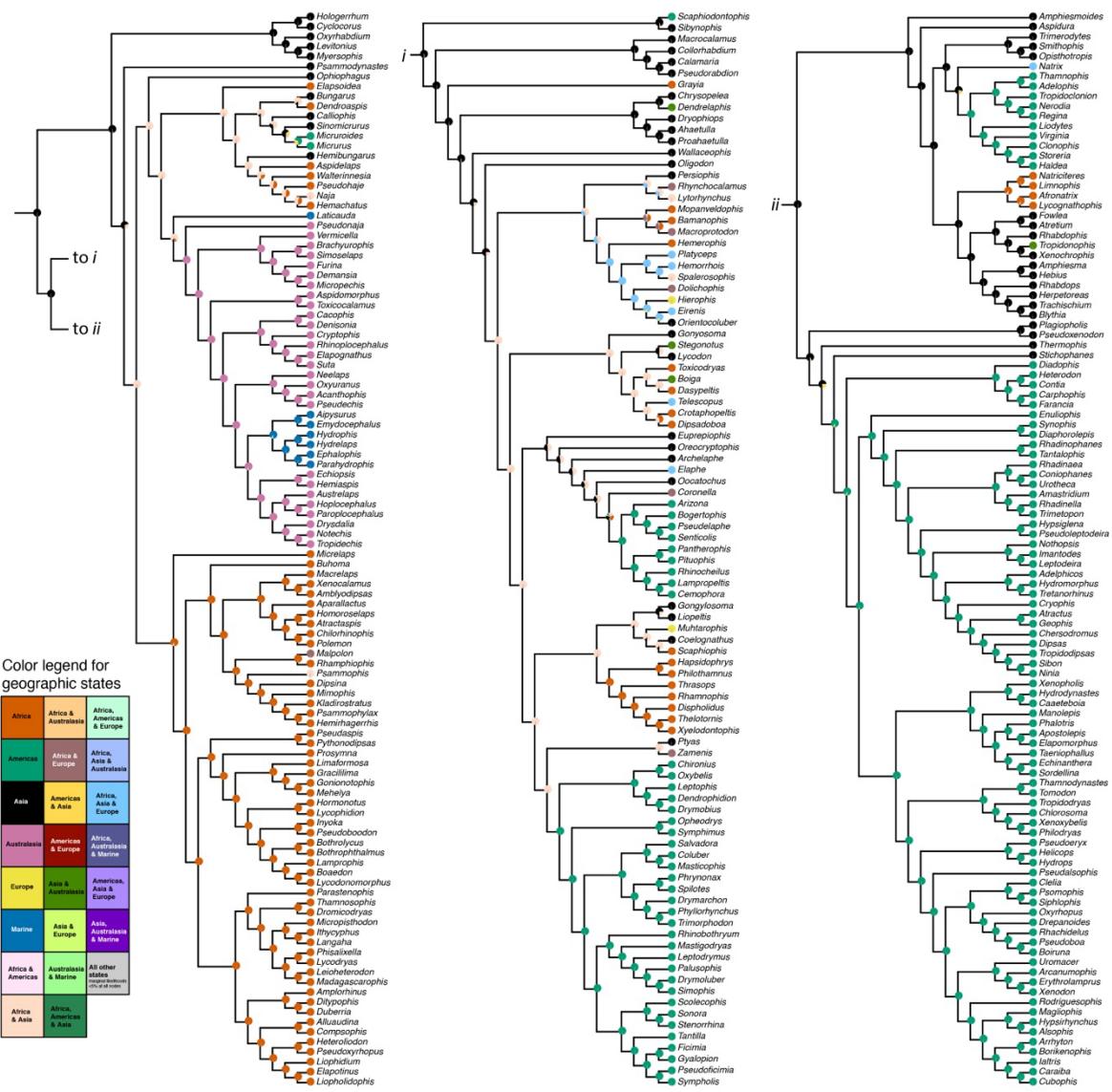


Figure S7. BioGeoBEARS geographic range evolution results of Elapoidea and Colubroidea under the biogeographic model “BAYAREALIKE”. Node pie charts depict marginal probability estimates of ancestral geographic states. Tip circles depict geographic states of extant lineages provided as input during analysis. Phylogeny used during analysis is visualized here as a cladogram.

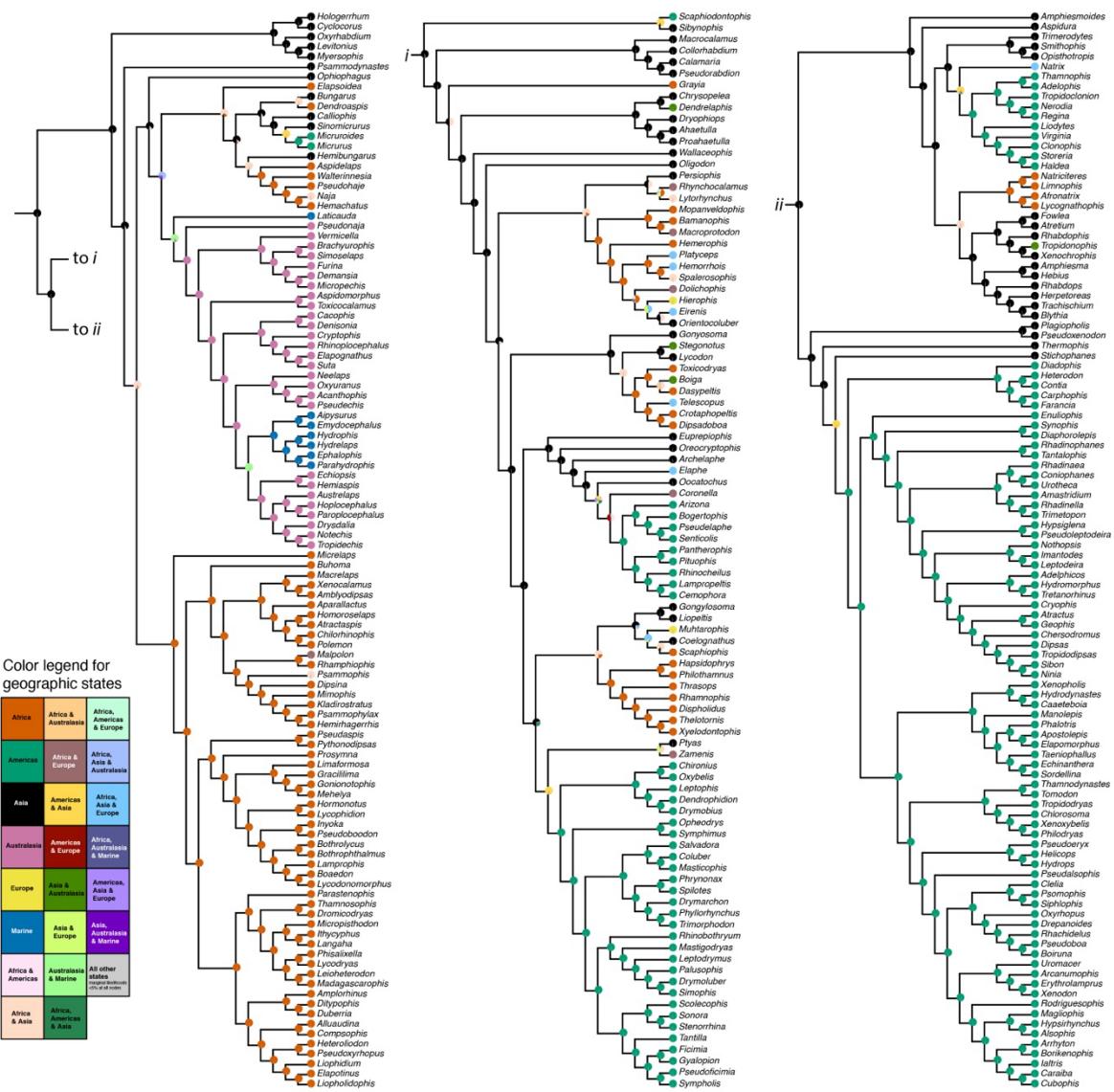


Figure S8. BioGeoBEARS geographic range evolution results of Elapoidea and Colubroidea under the biogeographic model “DIVALIKE”. Node pie charts depict marginal probability estimates of ancestral geographic states. Tip circles depict geographic states of extant lineages provided as input during analysis. Phylogeny used during analysis is visualized here as a cladogram.

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