Journal of Herpetology, Vol. 39, No. 3, pp. 500–503, 2005

A Phylogenetic Study of the Emerald Treeboa (*Corallus caninus*)

NICOLAS VIDAL,[1,2] ROBERT W. HENDERSON,[3,4] ANNE-SOPHIE DELMAS,[5] AND S. BLAIR HEDGES[1]

[1]*Department of Biology, 208 Mueller Lab, Pennsylvania State University, University Park, Pennsylvania 16802, USA*
[3]*Vertebrate Zoology, Milwaukee Public Museum, Milwaukee, Wisconsin 53233-1478, USA*
[5]*7, rue d'Arsonval, 75015 Paris, France*

ABSTRACT.—**Phylogenetic relationships of *Corallus caninus* phylogeny were examined with DNA samples from five geographically disparate localities from across the range of the species (Guyana, Venezuela, Brazilian states of Pará and Rondônia, and Peru). The Peruvian sequence was the most divergent (16.2%) and the closest relative of a clade including Brazilian, Guyanan, and Venezuelan populations. Within the latter clade, the most basal lineage was from the state of Pará, followed by the state of Rondônia, which was the closest relative of populations from the Guiana Shield. Preliminary morphological data paralleled molecular results, and it is likely that a separate species of *Corallus* currently included in *C. caninus* occurs in the upper Amazon.**

The Emerald Treeboa (*Corallus caninus*) is one of the most easily recognized snakes in the world. It has an extensive distribution on the South American mainland (Fig. 1) that encompasses most of the Guianas, a large portion of Venezuela, Amazonian Colombia, Ecuador and Peru, northern Bolivia, and much of the Brazilian Amazon (Henderson, 1993; unpubl.). In addition, records of *C. caninus* in Colombia exist from north of the Cordillera Central in the departments of Córdoba (Renjifo and Lundberg, 1999) and Antioquia (J. M. Daza R., unpubl. data), and on the west slope of the Cordillera Oriental in Boyacá (W. Lamar, unpubl. data). Distribution is for the most part confined to rain forest, and altitudinal distribution for *C. caninus* over most of its range (Guianas, Amazonia) is < 200 m a.s.l., but in Peru, the species occurs at elevations of 850–1000 m (Schulte, 1988; Lehr, 2001), and in Colombia it occurs up to 500 m (W. Lamar, unpubl. data).

Corallus caninus has maintained a rather remarkable taxonomic stability since described by Linnaeus nearly 250 years ago (with type-locality "America"). Although other "emerald" species were subsequently described (e.g., *Boa aurantiaca* Laurenti, 1768), all were eventually relegated to the synonymy of *C. caninus*. More recently, Gray (1860) described *Chrysensis batesii* based on a juvenile specimen from the "Upper Amazon," and Boulenger (1893) placed it into the synonymy of *C. caninus*. Since then, surprisingly little attention has been paid to *C. caninus*, despite its enigmatic juvenile coloration, its eye-catching adult coloration, and its popularity as an exhibit animal in zoos and among herpetoculturists. The latter have for many years recognized two or more "morphs" of *C. caninus*: one from the Guiana Shield and one or more from the Amazon basin (e.g., Chiras, 1998; Kivit and Wiseman, 2000).

As part of an ongoing review of *Corallus* biology, we investigated the phylogeny of *C. caninus* by examining DNA samples from five geographically disparate localities. The analysis raises taxonomic questions based on the population genetics.

MATERIALS AND METHODS

DNA was extracted from specimens of *C. caninus* from the following localities (Fig.1): Guyana: unspecified locality (private collection, Joseph Polanco, Cincinnati, Ohio); Venezuela: Amazonas, Neblina (USNM 559977); Brazil: Pará: Agropecuaria Treviso LTDA, approximately 101 km south and 18 km east of Santarem (LSUMZ H-14433); Brazil: Rondônia: Rio Formosa, Parque Estadual Guajara-Mirím, approximately 90 km north of Nova Mamore (LSUMZ H-17648-17650); and Peru: Loreto: Yarinacocha, Pacaya-Samiria Reserve (private collection, Lima, Peru [upon death, to be deposited in the Universidad Mayor de San Marcos in Lima]). *Boa constrictor* tissue originated from French Guiana, Piste de Petit-Saut (NV tissue collection). DNA extraction was performed using the DNeasy Tissue Kit from Qiagen. Amplification was performed using the following sets of primers: ND4, 5′-TGA-CTA-CCA-AAA-GCT-CAT-GTA-GAA-GC-3′ (Forstner et al., 1995) and LEU, 5′-TAC-TTT-TAC-TTG-GAT-TTG-CAC-CA-3′ (Forstner et al., 1995) for the ND4 gene; L14910, 5′-GACCTGTGATMTGAAAAACCAYCGTTGT-3′ (Burbrink et al., 2000), L14919, 5′-AACCACCGTTGT-TATTCAACT-3′ (Burbrink et al., 2000), and H16064, 5′-CTTTGGTTTACAAGAACAATGCTTTA-3′ (Burbrink et al., 2000) for the cytochrome *b* gene; L4437b, 5′-CAG-CTA-AAA-AAG-CTA-TCG-GGC-CCA-TAC-C-3′ (Kumazawa et al., 1996), H5382, 5′-GTG-TGG-GCR-ATT-GAT-GA-3′ (de Queiroz et al., 2002), and tRNA-trpR, 5′–GGC-TTT-GAA-GGC-TMC-TAG-TTT-3′ (de Queiroz et al., 2002) for the ND2 gene. Both strands of the PCR products were sequenced using the BigDye sequencing kit (Applied Biosystems) in the ABI Prism 3100-Avant Genetic Analyser.

Two strands obtained for each sequence were aligned using the BioEdit Sequence Alignment Editor program (Hall, 1999). Sequence entry and alignment were performed manually with MUST2000 software (Philippe, 1993). Alignment was straightforward for the three genes as no indels were included.

[2] Present address: Département Systématique et Evolution, Muséum National d'Histoire Naturelle, 25 rue Cuvier 75005 Paris, France.

[4] Corresponding Author. E-mail: henderson@mpm.edu

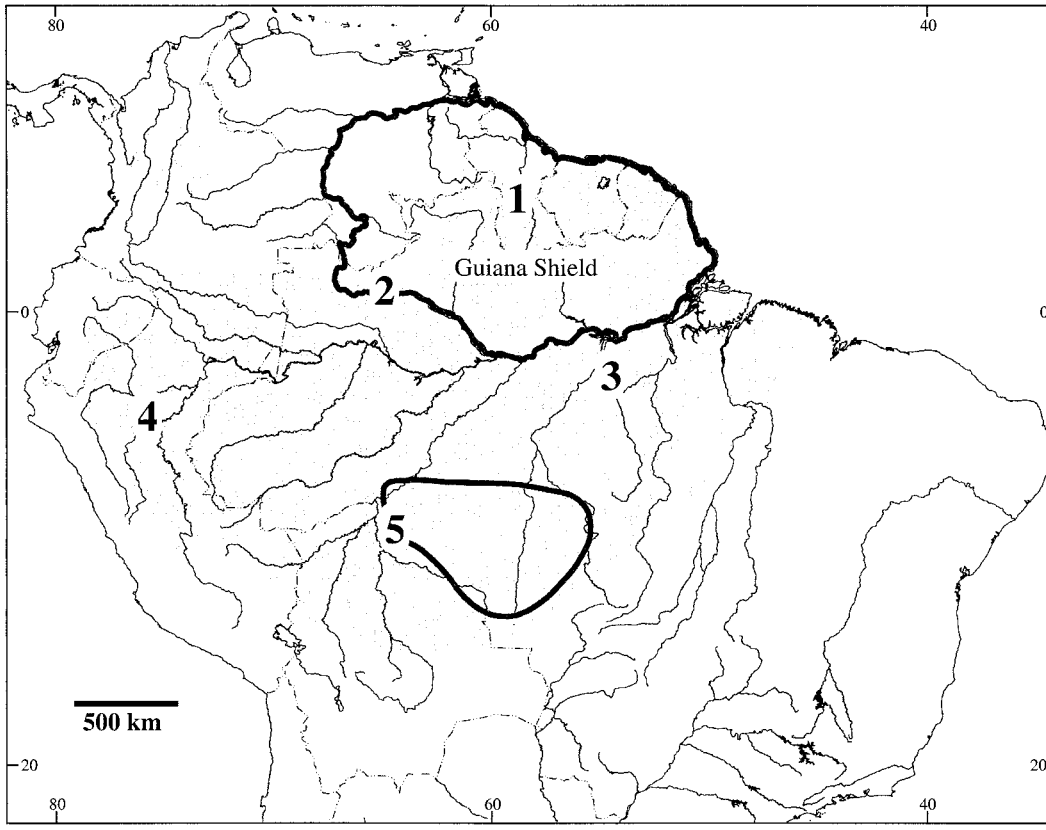

FIG. 1. Map illustrating the approximate range of *Corallus caninus*. Localities from which tissue samples used for DNA sequencing originated are designated by number: 1. GUYANA: unspecified locality; 2. VENEZUELA: Amazonas: Neblina; 3. BRAZIL: Para: 101 km south and 18 km east of Santarem; 4. PERU: Loreto: Yarinacocha: Pacaya-Samiria Reserve; 5. BRAZIL: Rondônia: Rio Formosa: Parque Estadual Guajara-Mirím. The enclosed area associated with locality 5 indicates where specimens of *C. caninus* exhibit pattern characteristics similar to material from the Guiana Shield.

A boid (*B. constrictor*) was used as an outgroup. The two *C. caninus* samples from the Brazilian state of Pará yielded only one cytochrome *b* haplotype as did the three *C. caninus* samples from the Brazilian state of Rondônia. Therefore, we used only one sample from each of these localities in phylogenetic analyses.

Alignment (six unique haplotypes) resulted in 1053 cytochrome *b* sites (276 variable sites; 89 were informative for parsimony), 672 ND4 sites (175 variable sites; 62 were informative for parsimony), and 540 ND2 sites (135 variable sites; 37 were informative for parsimony). Because mtDNA evolves as a single linkage unit, we concatenated the different gene sequences for each specimen and analyzed the data jointly (2265 sites, 586 variable sites; 188 were informative for parsimony).

We built phylogenies using Minimum Evolution (ME), Maximum Parsimony (MP), and Bayesian methods of inference. ME and MP analyses were performed with PAUP*4 (D. L. Swofford, Vers. 4. Sinauer Associates, Sunderland, MA, 1998). Bayesian analyses were performed with MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003; Nylander et al., 2004). For MP analyses (random option with TBR branch swapping, 2000 bootstrap replicates), all sites were weighted equally. For ME analyses (TBR branch swapping, 2000 bootstrap replicates), we chose the Kimura's two-parameter (K2P) model, following guidelines described in Nei and Kumar (2000); the K2P model is recommended when the Jukes-Cantor estimate of the number of nucleotide substitution per site (d) is between 0.05 and 1 and the transition/transversion ratio (R) is above 5. For Bayesian analyses, we followed the strategy advocated by Mueller et al. (2004) in partitioning our dataset by codon position across the three genes. Bayesian analyses were then run with model parameters estimated as part of the Bayesian analyses, and the best-fit model as inferred by Modeltest (Posada and Crandall, 1998) for each codon position across the three genes (GTR model for the first codon position, HKY model for the second and third codon positions). Bayesian analyses were performed by running two million generations in four chains, saving the current tree every 10 generations. The last 170,000 trees were used to construct a 50% majority rule consensus tree.

Results

Phylogenetic trees were well resolved with strong statistical support for all nodes (Fig. 2). The Peruvian haplotype was the most divergent and was the sister group to a clade including Brazilian, Guyanan, and Venezuelan populations. Among the latter clade, the most basal lineage was from the state of Pará, followed by the lineage from the state of Rondônia, which was the sister group to the populations from the Guiana Shield (Guyana and Venezuela in our study). Pairwise sequence differences were estimated using the K2P model. Mean levels of sequence divergence between *Boa* and *Corallus* was 20.2 ± 1, whereas sequence divergence within *Corallus* ranged from 1.7 ± 0.3 (between Guyana and Venezuela) to 16.2 ± 0.9 (between Peru and the remaining *Corallus*) (other pairwise sequence differences are 3.1 ± 0.4 between Rondônia and the Guiana Shield and 6.3 ± 0.5 between Pará and the clade including Rondônia and the Guiana Shield). The sequence divergence between Peru and the remaining *Corallus* (16.2%) was well above the range of values for sequence divergence between other closely related snakes, 1.6–5.3% (Zamudio and Greene, 1997; Burbrink et al., 2000; Ashton and de Queiroz, 2001; Keogh et al., 2001; Rawlings and Donnellan, 2003).

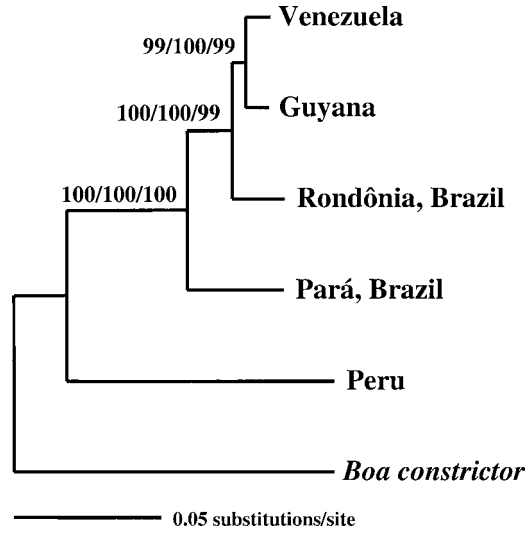

FIG. 2. ME tree obtained from the combined cytochrome *b*, ND4, and ND2 dataset. Values are ME bootstrap values (2000 replicates), MP bootstrap values (2000 replicates), and Bayesian posterior probabilities (two million generations).

Discussion

Corallus caninus from the Guiana Shield (Hoogmoed, 1979, 1982) and those from elsewhere across the range are discernible from each other (based on usually obvious scale and color pattern characters), but the results of the DNA sequence analysis indicate a major dichotomy between our sample from the upper Amazon in Peru and the rest of the samples. That *C. caninus* displays geographic variation, or that it may comprise more than one species, is not surprising based on its extensive geographic distribution. According to Bush (1994), pre-Quaternary vicariance events (e.g., formation of major rivers) in Amazonia probably established the major regional divisions of species complexes, and he concluded that any Amazonian speciation model "will almost certainly be complex and to some extent species-specific."

Based on examination of 120 *C. caninus* from throughout the range, the results of our genetic analysis parallel the morphological data collected to date. The close relationship between samples from Guyana and Venezuela was not unexpected, because both occur in the Guiana Shield and share scale (e.g., enlarged scales across the top of the snout) and pattern (e.g., no or very few white lateral blotches; no middorsal striping) characteristics. Individual *C. caninus* from eastern Brazil south of the Amazon have conspicuous lateral blotches and a middorsal stripe connecting the white triangle-like markings that lie on either side of the dorsal midline. Snakes from Rondônia and western Mato Grosso, like those from the Guiana Shield, either lack lateral blotches or have them greatly reduced, and the middorsal stripe is absent. Snakes from the upper Amazon (Peru, Ecuador) always have lateral blotches and they are more numerous than elsewhere in the range, as are the dorsal triangles; a middorsal stripe may or may not be present. The scales across the top of the muzzle are more numerous here than elsewhere in the range. Based on molecular and preliminary morphological evidence, it is likely that a separate species of *Corallus* currently included in *C. caninus* occurs in the upper Amazon. Examination of additional preserved material is ongoing (R. W. Henderson and M. S. Hoogmoed, unpubl.) and should provide critical insights regarding geographic variation and possible taxonomic partitioning.

Acknowledgments.—For providing *C. caninus* tissue samples, we are indebted to W. W. Lamar (Peru); J.Polanco (Guyana); L. J. Vitt (Brazil; result of National Science Foundation grants DEB-9200779 and DEB-9505518); R. Wilson and G. Zug of the National Museum of Natural History (USNM; Venezuela); the Louisiana State University Museum of Zoology (LSUMZ); and Natural Science Collection of Genetic Resources (via D. L. Dittmann; additional Vitt samples from Brazil). R. Powell provided the map in Figure 1, and W. Lamar offered several suggestions for improving the manuscript. Analysis of morphological variables by RWH has, to date, entailed loans from many institutions; all will be acknowledged accordingly in a future publication.

Literature Cited

ASHTON, K. G., AND A. DE QUEIROZ. 2001. Molecular systematics of the western rattlesnake, *Crotalus viridis* (Viperidae), with comments on the utility of the D-Loop in phylogenetic studies of snakes. Molecular Phylogenetics and Evolution 21:176–189.

BOULENGER, G. A. 1893. Catalogue of Snakes in the British Museum (Natural History). Vol. 1, Containing the Families Typhlopidae, Glauconiidae, Boidae, Ilysiidae, Uropeltidae, Xenopeltidae, and Colubridae aglyphae, Part. British Museum (Natural History), London.

BURBRINK, F. T., R. LAWSON, AND J. B. SLOWINSKI. 2000. Mitochondrial DNA phylogeography of the polytypic North American Ratsnake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution 54: 2107–2118.

BUSH, M. B. 1994. Amazonian speciation: a necessarily complex model. Journal of Biogeography 21:5–17.

CHIRAS, S. 1998. Identification and husbandry of Amazon Basin Emerald Tree boas (*Corallus caninus*). Reptiles 69:48–67, 70–75.

DE QUEIROZ, A., R. LAWSON, AND J. A. LEMOS-ESPINAL. 2002. Phylogenetic relationships of North American garter snakes (*Thamnophis*) based on four mitochondrial genes: how much DNA sequence is enough? Molecular Phylogenetics and Evolution 22:315–329.

FORSTNER, M. R. J., S. K. DAVIS, AND E. ARÉVALO. 1995. Support for the hypothesis of anguimorph ancestry for the suborder Serpentes from phylogenetic analysis of mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 4:93–102.

GRAY, J. E. 1860. Description of a new genus of Boidae discovered by Mr. Bates on the upper Amazon. Proceedings of the Zoological Society of London 28:132–133.

HALL, T. A., 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

HENDERSON, R. W. 1993. *Corallus caninus*. Catalogue of American Amphibians and Reptiles 574:1–3.

HOOGMOED, M. S. 1979. The herpetofauna of the Guianan region. *In* W. E. Duellman (ed.), The South American Herpetofauna: Its Origin, Evolution, and Dispersal, pp. 241–279. Museum of Natural History, Univ. of Kansas Monograph 7, Lawrence.

———. 1982 [1983]. Snakes of the Guianan region. Memórias do Instituto Butantan 46:219–254.

KIVIT, R., AND S. WISEMAN. 2000. The Green Tree Python and Emerald Tree Boa: Their Captive Husbandry and Reproduction. Kirschner and Seufer Verlag, Keltern-Weiler, Germany.

KEOGH, J. S., D. BARKER, AND R. SHINE. 2001. Heavily exploited but poorly known: systematics and biogeography of commercially harvested pythons (*Python curtus* group) in Southeast Asia. Biological Journal of the Linnean Society 73:113–129.

KUMAZAWA, Y., H. OTA, M. NISHIDA, AND T. OZAWA. 1996. Gene rearrangements in snake mitochondrial genomes: highly concerted evolution of control-region-like sequences duplicated and inserted into a tRNA gene cluster. Molecular Biology and Evolution 13:1242–1254.

LEHR, E. 2001. New records for amphibians and reptiles from departamentos Pasco and Ucayali, Peru. Herpetological Review 32:130–132.

MUELLER, R. L., J. R. MACEY, M. JAEKEL, D. B. WAKE, AND J. L. BOORE. 2004. Morphological homoplasy, life history evolution, and historical biogeography of plethodontid salamanders inferred from complete mitochondrial genomes. Proceedings of the National Academy of Science, USA 101:13820–13825.

NEI, M., AND S. KUMAR. 2000. Molecular Evolution and Phylogenetics. Oxford Univ. Press, Oxford.

NYLANDER, J. A. A., F. RONQUIST, J. P. HUELSENBECK, AND J. L. NIEVES-ALDREY. 2004. Bayesian phylogenetic analysis of combined data. Systematic Biology 53: 47–67.

PHILIPPE, H. 1993. MUST2000: A computer package of management utilities for sequences and trees. Nucleic Acids Research 21:5264–5272.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

RAWLINGS, L. H., AND S. C. DONNELLAN. 2003. Phylogeographic analysis of the green python, *Morelia viridis*, reveals cryptic diversity. Molecular Phylogenetics and Evolution 27:36–44.

RENJIFO, J. M., AND M. LUNDBERG. 1999. Anfíbios y Reptiles de Urrá. Skanska, Sweden.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

SCHULTE, R. 1988. Observaciones sobre la boa verde, *Corallus caninus*, en el Departamento San Martín-Perú. Boletin de Lima 55:21–26.

ZAMUDIO, K. R., AND H. W. GREENE. 1997. Phylogeography of the bushmaster (*Lachesis muta*, Viperidae). Implications for neotropical biogeography, systematics, and conservation. Biological Journal of the Linnean Society 62:421–442.

Accepted: 26 April 2005.