Origin of West Indian Populations of the Geographically Widespread Boa *Corallus enydris* Inferred from Mitochondrial DNA Sequences

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Corallus enydris (Serpentes: Boidae: Boinae) is an arboreal snake with an extremely wide mainland distribution from southern Costa Rica to southeastern Brazil and is one of two boine species that has invaded the Lesser Antilles (Grenada Bank and St. Vincent). Mitochondrial DNA sequences of samples from seven geographically disparate localities provided evidence of phylogenetic relationships. The monophyly of *C. enydris* is corroborated and a major dichotomy between northern samples (Panama and Trinidad) and southern samples (Guyana, Perú, southeastern Brazil) was found and corresponds to the two currently recognized subspecies. Unexpectedly, the two samples from the West Indies (southern Lesser Antilles) cluster with the southern rather than the geographically closer northern samples (e.g., Trinidad). The results imply a fairly recent Guianan–Amazonian origin of West Indian populations.   © 1995 Academic Press, Inc.

INTRODUCTION

The neotropical mainland boine snake (Serpentes: Boidae: Boinae) fauna, currently comprised of 10 species, includes five species with widely sympatric ranges in Amazonia and the Guianas: *Boa constrictor, Corallus caninus, Corallus enydris, Epicrates cenchria,* and *Eunectes murinus.* The ranges of *B. constrictor, C. enydris,* and *E. cenchria* are even broader, extending from Mexico and/or southern Central America to southeastern Brazil and/or northern Argentina; only *B. constrictor* and *C. enydris* have invaded the West Indies. Only *C. caninus* is regarded as monotypic, whereas *B. constrictor* and *E. cenchria* have 9 or 10 currently recognized subspecies. They are poorly defined geographically, taxonomically, and systematically, although recent advances have been made (e.g., Tolson, 1987; Kluge, 1991) or are in progress (R.W.H., in preparation).

C. enydris, an arboreal species ranging from southern Costa Rica to southern Brazil, has two currently recognized subspecies (Fig. 1): *C. e. enydris* which occurs throughout most of the Amazonian lowlands, the Guianas, and the Atlantic coastal forest of Brazil and *C. e. cooki* which occurs in southern Central America, northern Colombia, northern Venezuela (including Isla Margarita), Trinidad and Tobago, and the Lesser Antilles as far north as St. Vincent. *C. enydris* is more variable in color and pattern than any other boine (e.g., Figs. 1–7 in Henderson, 1993). In this paper we present data regarding phylogenetic relationships in *C. enydris* based on mitochondrial DNA sequences of samples from seven geographically disparate localities and evidence for the origin of the West Indian populations.

MATERIALS AND METHODS

DNA was extracted from small amounts (<50 mg) of liver or red blood cells from the following specimens of *C. enydris:* MPM (Milwaukee Public Museum) 26290, Panama, Canal Zone; MPM 23596, St. Vincent, St. Patrick, 2.0 miles ENE of Layou; MPM 25445, Grenada, St. Andrew, Pearls; MPM 23595, Trinidad, Hollis Reservoir; LSUMZ (Louisiana State University Museum of Zoology) 43139, Guyana, Jonestown; KU (University of Kansas) 204895, Peru, Cuzco, Cuzco Amazonica; and IB (Instituto Butantan) 55042, Brazil, São Paulo, Iguape (Fig. 1). Considering the broad range of *Corallus enydris,* we acknowledge the relatively small number of samples in our analysis. Those we have, however, are geographically strategic (Fig. 1), being near (1) the northern edge of the mainland range (Panama), (2) the southern end of the mainland range (Atlantic coastal forest in southeastern Brazil), and (3) the western edge in the Upper Amazon of Peru and from (4) two West Indian islands, including one from the northernmost edge of the range (St. Vincent) and (5) Trinidad. The Trinidad sample (*C. e. cooki*) is critical because of its geographic proximity to the Lesser Antilles and because of its morphological similarity to Venezuelan populations occurring between
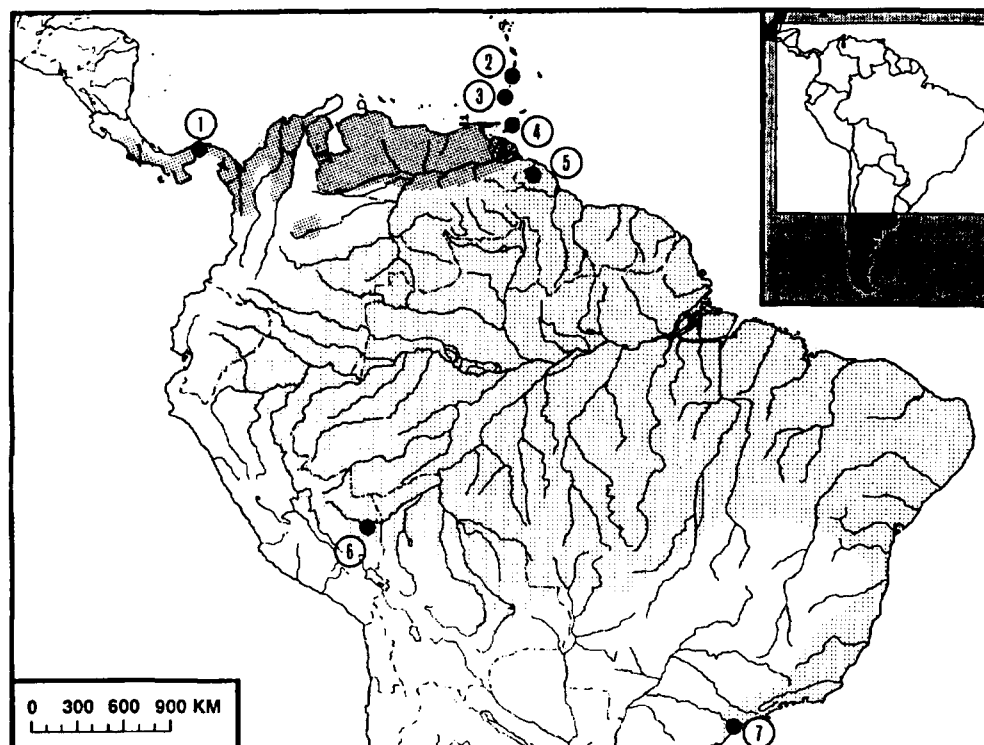
FIG. 1. Geographic distribution of the boid snake *Corallus enydris*, showing the locations of samples used in this study. The more northern, darker stippling indicates the range of *C. e. cooki*, and the more southern, lighter stippling indicates the range of *C. e. enydris*. The numbered localities indicate sites represented in our sample: (1) Panama, (2) St. Vincent, (3) Grenada, (4) Trinidad, (5) Guyana, (6) southern Peru, and (7) southeastern Brazil. See Materials and Methods for more precise locality data.

the Caribbean coast and the Rio Orinoco (Henderson and Boos, 1993). Despite long distances between Guianan–Amazonian sample localities on the South American mainland (Guyana–Brazil, ca. 3500 km; Guyana–Peru, ca. 2700 km) and the potential for wide rivers (e.g., Rio Amazonas) to act as barriers to dispersal, it is likely that the Guianan–Amazonian range (including Atlantic coastal forest in Brasil) of *C. enydris* is contiguous (Fig. 1; Henderson, 1993). An individual of *C. caninus* (Audubon Zoo 5902, locality unknown) was included for comparison, and the boid *Epicrates striatus* (S.B.H. Laboratory No. 103120, Dominican Republic, Samana, 7 km S. of Las Galeras) was included for the purpose of rooting the tree.

Methods of DNA extraction, amplification, and dideoxy sequencing are described elsewhere (Hedges *et al.*, 1991; Hedges and Bezy, 1993). Two oligonucleotide primers were used to amplify and sequence both complementary strands of a 307-bp region of the mitochondrial cytochrome b gene (Hedges *et al.*, 1992). In some cases, two other primers designed to amplify the same region (Kocher *et al.*, 1989) also were used. Aerosol-resistant tips were used in preparation of reagents in order to reduce the probability of contamination. There were no insertions or deletions and therefore alignment was straightforward.

Phylogenetic analysis (distance and parsimony) was performed with MEGA (Kumar *et al.*, 1993). The Jukes–Cantor (1969) distance was used with the neighbor-joining method (Saitou and Nei, 1987), and statistical confidence of the nodes on the trees was inferred by a t test for branch-length significance from zero, expressed as the complement of the probability, or confidence probability (CP; Rzhetsky and Nei, 1992; Kumar *et al.*, 1993), and by the bootstrap method (Felsenstein, 1985) using 2000 replications (Hedges, 1992). Statistical significance was assessed at the 95% level. Maximum parsimony analysis was done with the modified branch and bound algorithm in MEGA. Sites containing ambiguities were not included in the analyses.

RESULTS

A total of 271 aligned sites (nine taxa) could be scored from autoradiograms and were used in the phylogenetic analyses (Fig. 2). Of these, 74 were variable and 28 were parsimony sites (those sites informative under the conditions of parsimony). Pairwise corrected distances among the specimens of *C. enydris* were low (<0.08), supporting the use of a Jukes–Cantor distance rather than a more complicated correction (Nei, 1991).
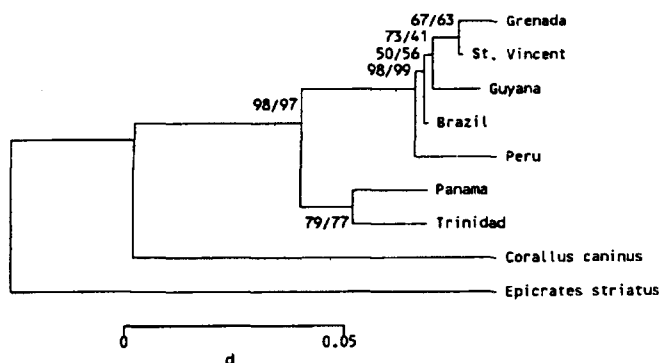
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                                                                        46
1 E.striatus  C TTC GGA TCC ATA CTA CTT GCT TGC TTA TCT CTA CAA CTA CTT ACA
2 caninus     T ... ... ..T ... ..T ..C A.C ..T .C. G.C ... ... G.. ..A ...
3 Panama      . ... ..G ... ... ... ..C A.C ... .C. G.C T.G ... G.. ..G ...
4 Trinidad    . ... ..G ... ... ... ... A.C ... .C. G.C T.. ... G.. ..A ...
5 Guyana      . ... ... ..A ... ... ..C A.C ... .CG G.C ... ... G.. ..G ...
6 Brazil      . ... ... ..A ... ... ..C A.C ... .CG G.C ... ... G.. ..G ...
7 Peru        . ... ... ..A ... ... ..C A.C ... .CG G.C ... ... G.. ..G ...
8 Grenada     . ... ... ..A ... ... ..C A.C ... .CG G.C ... ... G.G ..G ...
9 St.Vincent  . ... ... ..A ... ... ..C A.C ... .CG G.C ... ... G.. ..G ...


                                                                        91
1 E.striatus  GGA TTC TTC TTA GCT GTA CAC TAC ACA GCA AAT ATT AAC CTA GCA
2 caninus     ..C ... ..T C.. ..C ... ..T ... ... ... ..C ..C G.. T.. ...
3 Panama      ..C ... ..T C.. ... ... ... ... ... ... ..C ..C G.. T.. ..G
4 Trinidad    ..C ... ... C.. ... ... ... ... ... ... ..C ..C ... T.. ..G
5 Guyana      ..C ... ... C.. ... ... ... ... ... ... ..C ..C G.. T.. ...
6 Brazil      ..C ... ... C.. ... ... ... ... ... ... ..C ..C G.. T.. ...
7 Peru        ..C ... ... C.. ... ... ... ... ... ... ..C ..C G.. T.. ...
8 Grenada     ..C ... ... C.. ... ... ... ... ... ... ..C ..C G.. T.. ...
9 St.Vincent  ..C ... ... C.. ... ... ... ... ... ... ..C ..C G.. T.. ...


                                                                        136
1 E.striatus  TTC TCA TCT ATC ATC CAC ATT ACC CGA GAT GTC CCA TAT GGA TGA
2 caninus     ... ... ..C G.. G.. ..T ... .T. ... ... ... ..C ..C ... ...
3 Panama      ... ... ... G.T G.. ... ... .TA ... ..C ... ... ..C ... ...
4 Trinidad    ... ... ... G.T G.. ... ... .TA ... ..C ... ... ..C ... ...
5 Guyana      ... ... ... G.T G.. ... ... .TA ... ..C ... ... ..C ... ...
6 Brazil      ... ... ... G.T G.. ... ... .TA ... ..C ... ... ..C ... ...
7 Peru        ... ... ... G.T G.. ... ... .TA ... ..C ... ... ..C ... ...
8 Grenada     ... ... ... G.T G.. ... ... .TA ... ..C ... ... ..C ... ...
9 St.Vincent  ... ... ... G.T G.. ... ... .TA ... ..C ... ... ..C ... ...


                                                                        181
1 E.striatus  ATA ATA CAA AAC CTT CAC GCT ATT GGG GCC TCA GTA TTA TTT ATT
2 caninus     ... ... ... ... ... ... ..A ..C ..A ... ... A.. ..C ..C ..C
3 Panama      ... ... ... ..T ..C ... ..C ..A ... ... ... A.. ..C ..C ..C
4 Trinidad    ... ... ... ..T ..C ... ..C ..G ... ... ... A.. ..C ..C ..C
5 Guyana      T.. ... ... ..T ..C ..T ..C ..A ... ..A ... A.. ..C ..C ..C
6 Brazil      ... ... ... ..T ..C ..T ..C ..A ... ... ... A.. ..C ..C ..C
7 Peru        ... ... ... ..T ..A ..T ..C ..A ... ... ... A.. ..C ..C ..C
8 Grenada     ... ... ... ..T ..C ..T ..C ..A ... ... ... A.. ..C ..C ..C
9 St.Vincent  ... ... ... ..T ..C ..T ..C ..A ... ... ... A.. ..C ..C ..C


                                                                        226
1 E.striatus  TGT ATT TAT ATC CAT ATC GCA CGA GGC TTG TAT TAT GGG TCG TAC
2 caninus     ... ..C ..C ... ... ... ... ..C ... C.A ..C ... ... ..A ..T
3 Panama      ... ... ..C ... ..C ..T ... ... ... C.. ..C ..C ..C ..C ...
4 Trinidad    ... ... ..C ... ..C ... ... ... ... C.. ..C ..C ..C ..C ...
5 Guyana      ... ... ..C ..T ..C ..T ... ... ... C.A ..C ..C ..T ..T ...
6 Brazil      ... ... ..C ..T ..C ... ... ... ... C.A ..C ..C ..T ..T ...
7 Peru        ... ..C ..C ..T ..C ... ... ... ... C.. ..C ..C ..T ..T ...
8 Grenada     ..C ... ..C ..T ..C ..T ... ... ... C.A ..C ..C ..T ..T ...
9 St.Vincent  ..C ... ..C ..T ..C ... ... ... ... C.A ..C ..C ..T ..T ...


                                                                        271
1 E.striatus  TTA AAC AAA GAA ACC TGA CTC TCA GGT ACC ACA TTA CTA ATC ATA
2 caninus     C.. ... ... ... ... ... ..A ... ..C ... T.T ... ... ... ...
3 Panama      C.. ... ... ... ... ... ..A ... ..G ..A ... ... T.. ..T ...
4 Trinidad    C.. ... ... ... ... ... ..A ... ..A ..A ... ... ??? ??? ???
5 Guyana      C.. ... ... ... ... ... ..A ... ..A ..A ... ... T.. ..T ...
6 Brazil      C.. ... ... ... ... ... ..A ... ..A ..A ... ... T.. ..T ...
7 Peru        C.. ... ... ..G ... ... ..A ... ..A ..A ... C.. T.. ..T ...
8 Grenada     C.. ... ... ..G ... ... ..A ... ..A ..A ... ... T.. ..T ...
9 St.Vincent  C.. ... ... ..G ... ... ..A ... ..A ..A ... ... T.. ..T ...
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FIG. 2. DNA sequences of a portion of the mitochondrial cytochrome b gene in nine boid snakes: (1) *Epicrates striatus*, (2) *Corallus caninus*, and (3–9) geographic representatives of the species *C. enydris*.

FIG. 3. Phylogenetic relationships of Corallus enydris from seven widely distributed localities, inferred from a neighbor-joining analysis of DNA sequences (Jukes–Cantor distance) of a portion of the mitochondrial cytochrome b gene. The numbers on the tree are statistical estimates of confidence of each node: the "confidence probability" (Rzhetsky and Nei, 1992; Kumar et al., 1993) derived from the standard error estimate of the branch length (left of slash) and the bootstrap P value (Felsenstein, 1985) based on 2000 replications (right of slash). The West Indian boine species Epicrates striatus was used to root the tree. d, distance.

FIG. 4. Phylogenetic relationships of Corallus enydris inferred from a maximum parsimony analysis (branch-and-bound). The tree is a strict consensus of six most-parsimonious trees, each of length 41.

A phylogenetic tree (Fig. 3) corroborates the monophyly of C. enydris (CP = 98%) and shows a major dichotomy between samples from Panama and Trinidad (79%) and those from other areas (98%). With two exceptions, this split corresponds to a morphological subdivision within the species recognized at the subspecific level. The Panama and Trinidad samples are assigned to the subspecies C. e. cooki, whereas the other samples are placed in C. e. enydris (Henderson, 1993). The exceptions are the two samples from the Lesser Antilles (St. Vincent and Grenada). Although these West Indian populations have been assigned to the subspecies C. e. cooki, they cluster strongly (98%) with the samples of C. e. enydris. The two Lesser Antillean samples cluster with a mainland population (Guyana) farther to the south rather than the geographically proximal Trinidad.

The maximum parsimony analysis resulted in six equally most-parsimonius trees of length 41 (Fig. 4). All showed the same major dichotomy within C. enydris as found in the neighbor-joining analysis.

DISCUSSION

The most unexpected result of the DNA sequence analysis was the implication of a fairly recent Guianan–Amazonian origin of West Indian populations of C. enydris as indicated by the very close genetic relationship between those samples and Guyana. Although it is possible that this result is due to allelic relationships rather than population relationships, the latter explanation is better supported when the biogeography of the region is considered. The strong South American influence in the Grenada Bank–St. Vincent fauna is
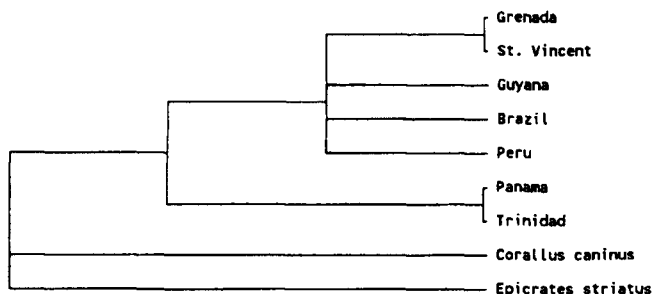
well-documented, but Trinidad or northern Venezuela (e.g., the Orinoco Delta), possibly via Trinidad, has been assumed to be the origin (e.g., Lescure, 1987; Lescure et al., 1991). Trinidad had a direct land connection to South America during the late Pleistocene low sea level stand (Eshelman and Morgan, 1985) and is now separated from Venezuela by about 10.5 km of water. There is no geological evidence to indicate that the Grenada Bank ever had a continental connection (Maury et al., 1990) and C. enydris almost surely reached the West Indies by overwater dispersal from South America on the South Equatorial Current. Although the Grenada Bank is situated closer to Venezuela and Trinidad (ca. 145 km) than to the Guyana coast (ca. 650 km), there is evidence for the potential overwater dispersal to the southern Lesser Antilles (the Grenada Bank and St. Vincent) from northeastern Brazil and the Guianas (e.g., the mouth of the Essequibo River in Guyana) (Guppy, 1917). The reptile fauna of the Grenada Bank and St. Vincent has a number of geographically wide-ranging species in common with Trinidad, northern Venezula, and the Guianas, but little is known regarding their relationships.

In light of these preliminary results, the relationships of the West Indian and mainland populations and the current taxonomy and systematics of C. enydris must be reevaluated.

ACKNOWLEDGMENTS

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