Phylogeny of South American and Malagasy Boine Snakes: Molecular Evidence for the Validity of *Sanzinia* and *Acrantophis* and Biogeographic Implications

MIGUEL VENCES, FRANK GLAW, JOACHIM KOSUCH, WOLFGANG BÖHME, AND MICHAEL VEITH

A total of 1981 bp of the mitochondrial 16S rRNA, 12S rRNA and cytochrome *b* genes were analyzed in five boine and one pythonine snake species to determine phylogenetic relationships between Malagasy and Neotropical taxa included in the genus *Boa*. The obtained cladograms significantly grouped *Boa constrictor* with the Neotropical genera *Eunectes* and *Epicrates*, whereas the Malagasy species were the sister group of the clade of the three Neotropical taxa. Based on these results, *Sanzinia* and *Acrantophis* should be considered as valid generic names for the Malagasy boas. Their origin may be a result of a Cenozoic dispersal from Africa or Asia.

↑IANT snakes (family Boidae) contain, be-G side the largely burrowing Erycinae, two main clades: the pythons (Pythoninae; sometimes also regarded as own family Pythonidae), distributed in Australia, Asia, and Africa (Kluge, 1993); and the boas (Boinae), which are distributed in Middle and South America, Pacific region (from American Samoa in the East, through Melanesia, to Sulawesi in the West) and Madagascar (Kluge, 1991; Austin, 2000). In Madagascar, three boas occur, Sanzinia madagascariensis, Acrantophis madagascariensis, and Acrantophis dumerili. In a cladistic analysis based on 79 morphological characters, Kluge (1991) found a sister-group relationship of Sanzinia and Acrantophis to the Neotropical Boa constrictor, which is member of a monotypic genus; he included these four species in the genus Boa and proposed the replacement name Boa manditra for S. madagascariensis.

Presence of boas in Madagascar and the Neotropics, and their absence in Africa and most of Asia, is paralleled by two other reptile groups (oplurine iguanid lizards and podocnemine turtles; Schulte et al., 1998; Georges et al., 1998). Taking into account the long geographical isolation of Madagascar (Storey, 1995), the classification of Malagasy and Neotropical taxa in one genus is unusual. Therefore, we applied molecular methods to test whether the genus *Boa* sensu Kluge (1991) actually constitutes a monophyletic group and to discuss biogeographic origins of the Malagasy reptiles with Neotropical affinities.

MATERIALS AND METHODS

We sequenced fragments of the 16S rRNA (468 bp) and 12S rRNA genes (400 bp) in six

boid species (GenBank accessions AF21572-AF21577 and AF368055-AF368060) using primers and protocols given in Vences et al. (2000). These data were complemented by sequences of the cytochrome b gene (1113 bp) available from GenBank, which were obtained by Campbell (1997). Taxa studied by us were A. madagascariensis, Boa constrictor, Epicrates cenchria crassus, Eunectes notaeus, Python curtus, and S. madagascariensis. Cytochrome b sequences were available for A. madagascariensis, B. constrictor, E. notaeus, and S. madagascariensis (U69736, U69746, U69810, U69866); instead of E. cenchria crassus and P. curtus, we used Epicrates cenchria maurus (U69779) and Python reticulatus (U69859) in the cytochrome b analyses. A P. reticulatus 16S sequence (Z46478) and two additional B. constrictor 16S sequences (Z46495, L10675) were also included in the separate 16S analysis. We used snakes of the family Typhlopidae, one of the most basal ophidian clades according to Heise et al. (1995), as outgroups: Typhlops lumbricalis (16S: Z46475; 12S: Z46444) and Ramphotyphlops braminus (cytochrome b: U69865). In addition to the boid taxa, we sequenced 16S gene fragments of two iguanid lizards (Oplurus cuvieri, AF215260; Phymaturus somuncurensis, AF215261) and compared sequences of the turtles Erymnochelys madagascariensis (AF113640) and Podocnemis expansa (AF113642) available from GenBank.

Sequences were aligned using the Clustal option of SEQUENCE NAVIGATOR (Applied Biosystems). The presence of indels accounted for the addition of gaps to one or more sequences at a total of 11 bp positions of the 12S and 16S rRNA fragments. The aligned sequences were submitted to separate and combined analysis using PAUP*, version 4.062, written by D. L. Swofford. MODELTEST (Posada and Crandall, 1998) was used to test the goodness of fit of various substitution models. We performed maximum parsimony (MP) analyses (with gaps as fifth character) using exhaustive searches, and neighbor joining (NJ) analyses (with gaps treated as missing data). Homogeneity of base frequencies across taxa and partition homogeneity were tested using algorithms implemented in PAUP*. Log likelihood tests were performed using PHYLIP, written by J. Felsenstein. The morphological character matrix of Kluge (1991:15-16) was reanalyzed using PAUP* using MP algorithms; characters were either included as ordered or unordered.

RESULTS

A chi-square test did not contradict homogeneity of base frequencies across taxa in the combined dataset nor in any of the separate sets (16S, 12S, cytochrome b; df = 18; P > 0.75). The partition homogeneity test did not reject the null hypothesis of congruence of the included gene fragments (1000 replicates; P =(0.83), thus not contradicting their suitability for combination in phylogenetic analysis. The hierarchical likelihood tests implemented in MO-DELTEST selected a general time-reversible substitution model with a proportion of invariable sites of 0.4861 and a gamma distribution shape parameter of 0.6645 as best fitting the combined dataset. This substitution model was consequently used in the NJ analysis.

The genus Boa sensu Kluge (1991) did not result as monophyletic group in any of the analyses performed using the separate and combined molecular datasets. In the combined NJ analysis of all three gene fragments (Fig. 1), B. constrictor was the sister group of the (Eunectes, *Epicrates*) clade, and these Neotropical taxa were the sister group of the (Sanzinia, Acrantophis) clade. This topology corresponded to the single most parsimonious tree (357 parsimony-informative characters of 800 variable characters and 1981 total characters; 1353 steps; consistency index 0.79; retention index 0.39). The alternative hypothesis, with Boa as sister group of the (Sanzinia, Acrantophis) clade (1367 steps) was significantly worse according to the results of a log likelihood test as implemented in PHYLIP. Bootstrap values of the placement of B. constrictor as sister group of the (Eunectes, Epicrates) clade were high (87% and 99% in the NJ and MP analysis, respectively).

Methodical variations in the phylogenetic analyses (results not shown) did not have rele-



Fig. 1. Maximum parsimony phylogram based on 1981 bp of the mitochondrial 16S rRNA, 12S rRNA and cytochrome *b* genes. Numbers are bootstrap values in percent (2000 replications) of the MP and NJ analyses, respectively. The arrow indicates the node supporting nonmonophyly of *Boa* sensu Kluge (1991).

vant effects on the phylogenetic position of *Boa*, *Sanzinia*, and *Acrantophis* in the obtained phylograms. This was true for (1) NJ calculations with other substitution models, (2) MP and NJ analyses after exclusion of all sites with gaps in one or several sequences, (3) analyses which included further cytochrome *b* sequences available from GenBank (e.g., *Loxocemus, Candoia*, and erycine boids).

In a reanalysis of the available morphological data (Kluge, 1991), *Boa* was the sister group of the Malagasy taxa (bootstrap value 64% with characters defined as ordered, 73% with characters defined as unordered; 2000 replications). The alternative hypothesis (*Boa* being the sister group of *Eunectes* and *Epicrates*) needed three additional steps in the analysis assuming ordered state of characters and two additional steps assuming unordered character states.

DISCUSSION

We found significant molecular evidence for the nonmonophyly of the genus *Boa* sensu Kluge (1991), whereas only few morphological characters indicate their monophyly. Nonmonophyly of *Boa* was also found by Austin (2000) who studied 270 bp of the cytochrome *b* gene in five boine genera. To avoid a paraphyletic classification, the Malagasy taxa should therefore not be included in the same genus as *Boa constrictor*.

The karyotype of *Sanzinia* and *Acrantophis* (2n = 34) is derived and unique among boines, whereas *B. constrictor* has the primitive number of 2n = 36 as *Epicrates* and *Eunectes* (Mengden and Stock, 1980; Branch, 1980; Kluge, 1991). Although this character is not phylogenetically in-

formative regarding the relationships of the Malagasy taxa to *Boa*, it nevertheless emphasizes their differentiation.

The hemipenes of the Malagasy taxa share two derived features that are lacking in other boines including *B. constrictor* but are also known in the enigmatic Pacific genus *Candoia* (Branch, 1981). Together with the possible molecular affinities of *Candoia* and *Sanzinia* (Austin, 2000), this indicates that relationships between the Malagasy and the Pacific island boas should be reinvestigated.

As alternative to the inclusion of the Malagasy taxa in the genus Boa, Kluge (1991) proposed to synonymize Sanzinia with Acrantophis. Taking (to our knowledge) first reviser's action, he determined nomenclatural priority of A. madagascariensis over S. madagascariensis since both taxa were described at the same date (page priority as invoked by Kluge was not relevant according to the International Code of Zoological Nomenclature, third edition, in force in 1991). However, important differences are also found among Malagasy boas. Total pairwise sequence differentiation between Acrantophis and Sanzinia in the 16S fragment considered here was as high as between Eunectes and Epicrates (4.9% in both cases). The conspicuously enlarged supralabials of Sanzinia make its immediate identification possible. In addition, Sanzinia (partly arboreal) and Acrantophis (terrestrial) occupy different adaptive zones (see Glaw and Vences, 1994). It is therefore appropriate to consider these snakes as belonging to two different genera, Sanzinia and Acrantophis. The replacement name Boa manditra Kluge, 1991, is to be regarded as junior objective synonym of S. madagascariensis.

Our data did not support the close relationships between B. constrictor and Malagasy boas emphasized by Kluge (1991). Nevertheless, the molecular results confirmed that Sanzinia and Acrantophis are members of the boine lineage rather than the pythonines and thus have relationships to the Neotropics (and the Pacific region) rather than to Africa or continental Asia. A similar situation exists in two other reptile groups. The turtle genus Erymnochelys from Madagascar is the sister group of the Neotropical Podocnemis (Georges et al., 1998), and the Malagasy oplurines are related to South American iguanas rather than African or Asian agamas (Frost and Etheridge, 1989; Schulte et al., 1998). Sequence differentiation between Neotropical and Malagasy species in the 16S fragment was 8.5% in boas, 12.8% in podocnemine turtles, and 13.9% in iguanas. Different dispersal scenarios can be invoked to explain the unusual distribution patterns of these three groups: (1) They may have been present in Africa, reached Madagascar by rafting through the Mozambique channel, and were later replaced in Africa by pythons, agamas, and pelomedusine turtles (Mertens 1972); (2) They may have reached Madagascar from Asia, passing hypothetical land connections that possibly existed in the Early Cenozoic while the Indian plate drifted northward (Rage 1996); (3) They may have reached Madagascar in the Late Cretaceous through Antarctica, passing a land connection through the Kerguelen plateau (Sampson et al., 1998); and (4) They may have arrived by direct transmarine dispersal from southeastern Asia or the Pacific region to Madagascar, their raft having been favored by the Equatorial current and the southeast Trade winds as is probable for most autochthonous Mascarene reptile lineages which have Australasian affinities (Arnold, 2000).

To podocnemines, the first hypothesis may apply, because fossils believed to belong to this group are known from Africa (Rage, 1996). However, no African boa and iguana fossils are so far known, whereas both groups were apparently widespread in Laurasia during the early Tertiary. Their Cenozoic dispersal from Laurasia to Madagascar via the Indian subcontinent may therefore be taken into consideration (Rage, 1996), a hypothesis that at least is not contradicted by the relatively low genetic differentiation between Malagasy and Neotropical taxa. On the other hand, if close relationships of Candoia to Malagasy boas (Austin, 2000) were confirmed, the likelihood of their direct marine dispersal from southeastern Asia or the Pacific region would be rather strong.

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- (MVENCES, WB) ZOOLOGISCHES FORSCHUNGSIN-STITUT UND MUSEUM ALEXANDER KOENIG, AD-ENAUERALLEE 160, 53113 BONN, GERMANY; (FG)Zoologische STAATSSAMMLUNG, MÜNCHHAUSENSTR, 21, 81247 MÜNCHEN, GER-MANY; AND (JK, MVEITH) ZOOLOGISCHES IN-STITUT DER UNIVERSITÄT MAINZ, ABTEILUNG Ökologie, Saarstr. 21, 55099 Mainz, Ger-MANY. PRESENT ADDRESS: (MVENCES) MUSÉUM NATIONAL D'HISTOIRE NATURELLE, LABORA-TOIRE DES REPTILES ET AMPHIBIENS, 25 RUE CU-VIER, 75005 PARIS, FRANCE. E-mail: (MVences) m.vences@t-online.de. Send reprint requests to MVences. Submitted: 30 May 2000. Accepted: 15 May 2001. Section editor: J. D. Mc-Eachran.