

MOLECULAR SYSTEMATICS OF AFRICAN COLUBROIDEA (SQUAMATA: SERPENTES)

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Abstract: Phylogenetic relationships between African representatives of the Colubroidea have been relatively little investigated. In this paper, DNA sequences of three marker genes were used to identify phylogenetically relevant groups. Viperids represent a basal clade among Colubroidea. The two monophyletic families Elapidae and Atractaspidae are nested within the paraphyletic family Colubridae. African colubroid snakes are found within the cosmopolitan subfamilies Colubrinae and Natricinae, the mainly African subfamilies Lamprophiinae, Psammophiinae and Pseudoxyrhopiinae, and also within the families Elapidae and Atractaspidae. The clade comprising Lamprophiinae, Psammophiinae, Pseudoxyrhopiinae, Elapidae and Atractaspidae is likely to have an African origin, whereas the Colubroidea probably originated in Asia.

Key words: Africa; Serpentes; Colubroidea; phylogeny; mtDNA; *c-mos*

1. INTRODUCTION

The African snake fauna has remained one of the biggest challenges for systematic herpetology. High diversity in general and low representation in collections have been the main obstacles for comprehensive analyses. For these reasons, the African taxonomic sampling used in previous molecular systematic investigations (e.g., Heise et al., 1995; Kraus and Braun, 1998; Gravlund, 2001) was insufficient. Moreover, those studies used mitochondrial genes only.

In a collaborative effort, we increased the taxonomic sampling among African colubroids, and used a combination of genes which have already been shown to be suitable at this level of diversification (Nagy et al., 2003).

2. MATERIALS AND METHODS

We tried to include representatives of as many afrotropical genera of colubrid and atractaspidid snakes as possible. A choice of African elapid and viperid taxa were also included, as well as some Eurasian representatives of the genera *Hemorrhoids*, *Psammophis*, *Telescopus*, *Platyceps*, *Ptyas* and *Vipera*. Three taxa were used as hierarchical outgroups in the phylogenetic analyses: a scolecophidian snake (*Rhinotyphlops schlegelii*) and two henophidians (*Sanzinia madagascariensis* and *Calabaria reinhardtii*).

Three molecular markers known to have different evolutionary and functional characteristics were amplified in polymerase chain reactions and then sequenced directly (see Nagy et al., 2003 for details): (1) the entire mitochondrial cytochrome *b* gene; (2) part of the mitochondrial 16S rRNA gene; (3) part of the nuclear *c-mos* gene. The partition homogeneity test was performed for the combined set of sequences.

For phylogenetic reconstructions, maximum parsimony (MP) analyses were carried out with PAUP* 4.0b10 (Swofford, 2002), as well as Bayesian inference of phylogeny (BI) using MrBayes 3.0b4 (Huelsenbeck and Ronquist, 2001). For likelihood based methods, an appropriate model of nucleotide substitution was inferred with Modeltest 3.06 (Posada and Crandall, 1998).

All DNA sequences – including collection data of the investigated specimens – were deposited in GenBank and will be published later.

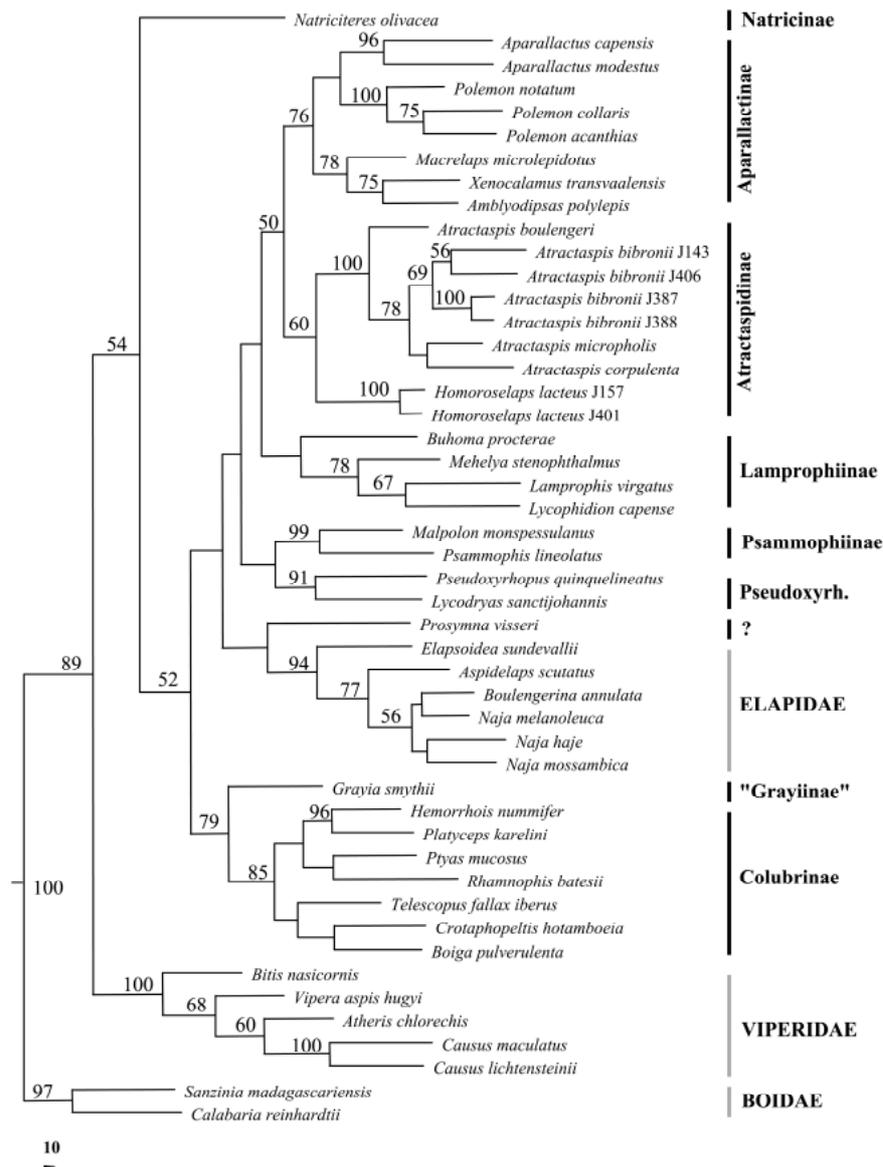


Figure 1. Maximum Parsimony phylogram of African Colubroidea, based on cytochrome *b*, 16S rRNA and *c-mos* sequences (one most parsimonious tree, heuristic search, TBR branch swapping). The most basal outgroup *Rhinotyphlops* is not shown. Numbers are bootstrap values in percentages and given for clades that received support values over 50% (1000 replicates). The actually recognized taxa on family-level are capitalized and indicated with grey bars. Subfamilial taxa are indicated with black bars. *Incertae sedis* are marked with question marks.

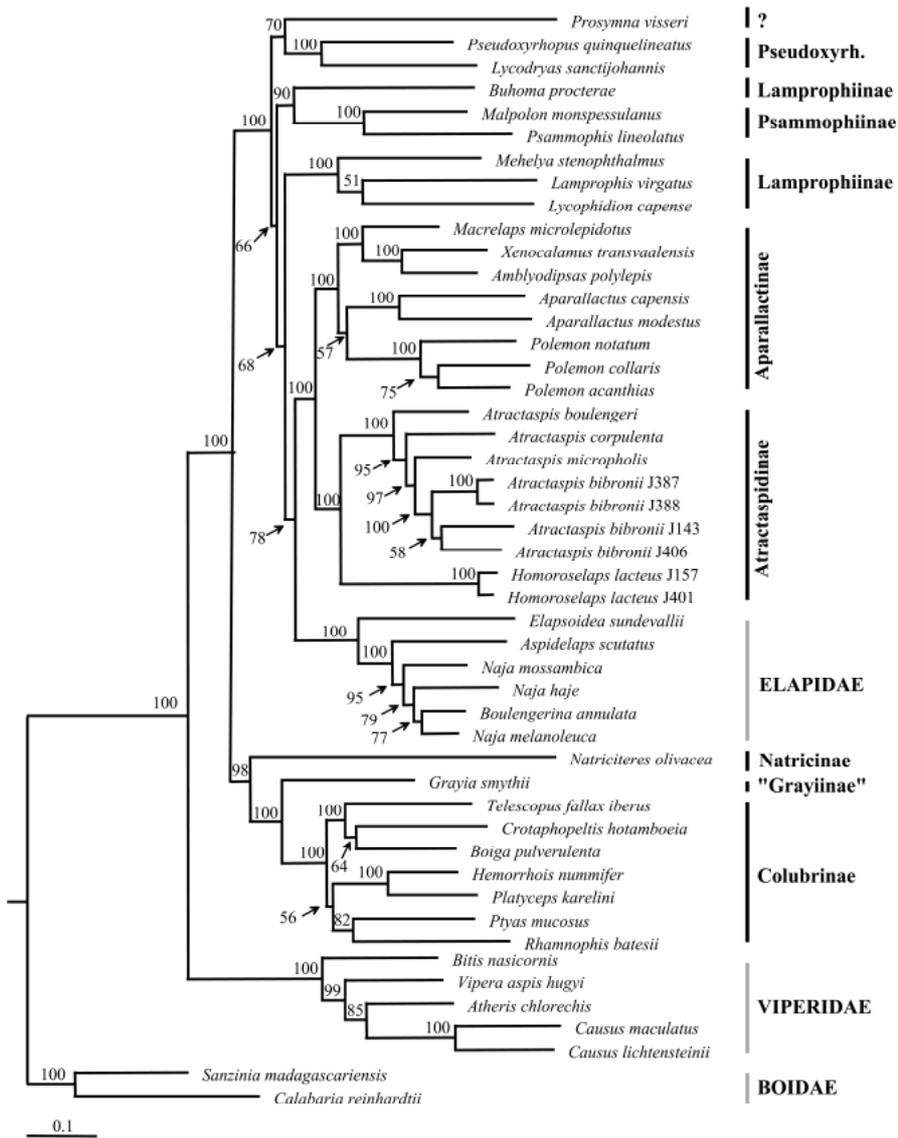


Figure 2. Bayesian Inference phylogram of African Colubroidea, calculated on the nucleotide substitution model GTR+I+G (one million generations, sampling of every 100th generation, the first 500 of 10000 trees were discarded). Numbers are clade credibility values in percentages and given for clades that received support values over 50%. For further details, see figure caption of Fig. 1.

3. RESULTS

The partition homogeneity test did not contradict the congruence of the three investigated genes ($p=0.742$), thus all sequences were used in the combined analyses. The combined data set comprises 2186 bp for 49 taxa (after the exclusion of the hypervariable regions of 16S rRNA).

The most important result of our phylogenetic analyses is that the colubrid snakes are paraphyletic – even considering African taxa only. Colubrids form a monophyletic unit only with the inclusion of elapid and atractaspidae snakes. The family Viperidae appears to be the sister taxon of this entire group. This observation is in agreement with recent molecular studies (Cadle, 1994; Knight and Mindell, 1994; Dowling et al., 1996; Slowinski and Lawson, 2002; Vidal and Hedges, 2002a, b).

The overall topology of the combined trees (MP: Fig. 1 and BI: Fig. 2) is well supported by high bootstrap and posterior probability values, respectively. Moreover, repetitions of the Bayesian analysis were in excellent agreement with the first one. The main difference between the results of the two reconstruction methods are found (a) in the positions of Elapidae and Atractaspidae among colubrid subfamilies, and (b) in the phylogenetic status of the subfamily Lamprophiinae. A high degree of congruence is found (a) in the monophyly of the Viperidae, Elapidae, Atractaspidae, Psammophiinae, Pseudoxyrhopiinae, and Colubrinae and (b) in the relationships within those clades.

The summarizing tree of all combined analyses is shown in Figure 3.

4. DISCUSSION

4.1 A diversified picture of African venomous snake evolution

The basal position of viperids is congruent with results of other authors (see Results). However, the assumption of earlier studies (e.g. Cadle, 1987; Lenk et al., 2001) that the genus *Causus* represents the most basal lineage within Old World viperids (based on a presumably primitive condition of scalation, unusually round pupils and the morphology of the venom apparatus) is not supported by our data.

The solenoglyphous dentition displayed by the genus *Atractaspis* is not homologous to the solenoglyphous dentition displayed by the viperids. According to our results (Figs. 1 and 2), Atractaspidae are monophyletic and consist of two monophyletic subfamilies, Atractaspinae and

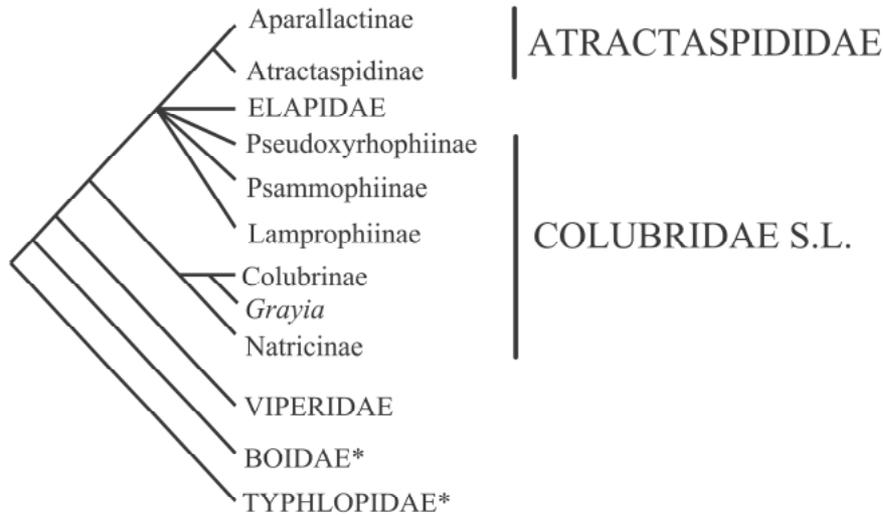


Figure 3. Overall (summarizing) tree showing the general phylogeny of African Colubroidea, based on combined analyses of cytochrome *b*, 16S rRNA and *c-mos* sequences. Asterisks mark outgroups.

Aparallactinae. Within Aparallactinae, the genera *Macrelaps*, *Xenocalamus* and *Amblyodipsas* form a strongly supported monophyletic group. Within Atractaspidinae, the genus *Homoroselaps* is the sister group to the genus *Atractaspis*. Moreover, we found considerable intraspecific variation within *Atractaspis bibronii*. Initially, *Homoroselaps* was considered to be a member of the nominative genus of the family Elapidae, *Elaps*. McDowell (1968) pointed to large morphological differences between elapids and *Homoroselaps* and postulated that the latter was rather a colubrid. Underwood and Kochva (1993) put it back into the family Elapidae. Slowinski and Keogh (2000) found *Homoroselaps* to be basal to the elapids, but unfortunately did not include atractaspidids in their study. Our investigations confirm earlier results (Vidal and Hedges, 2002a) that *Homoroselaps* is part of the Atractaspidinae.

We analysed only African representatives of the family Elapidae. With this limitation, elapids appear monophyletic. However, the relationships of elapids within our African colubroid data set remain unresolved. In the BI phylogram (Fig. 2), Atractaspididae are their sister group, while in the MP tree (Fig. 1), the enigmatic genus *Prosymna* appears closer, but with no significant support. Within African elapids, *Naja* species occupy a derived position, which is concordant with results by Slowinski and Keogh (2000). Moreover, *Naja* appears paraphyletic, as the aquatic cobra *Boulengerina annulata* branches off from within *Naja*. In accordance with results by

Slowinski et al. (1997), the genus name *Boulengerina* should therefore be synonymized with the genus name *Naja*.

4.2 Different origins of the diversity of the African colubrid snake fauna

In our trees, a clear separation of African colubrids into two major groups is evident. One of them contains only Old World subfamilies of African dominance. Species of the subfamily Psammophiinae are found in Africa (including Madagascar) and Asia. The Pseudoxyrhopiinae occupy Madagascar and Socotra (Nagy et al., 2003). The Lamprophiinae include African species only, with the exception of the Asiatic genus *Psammodynastes* (Vidal and Hedges, 2002a). The fact that the afro-tropical Atractaspididae and Elapidae branch off from the same origin as this assemblage indicates that it is of African origin.

On the other hand, the two other colubrid subfamilies including African members, Colubrinae and Natricinae, occur both in the Old and New Worlds. An Asian origin of these two lineages is generally assumed (e.g. Rage et al., 1992). According to our study and Vidal and Hedges (2002a), the position of the enigmatic African endemic genus *Grayia* as sister group to the colubrines is strongly supported. Presently, more comprehensive studies are in progress which may lead to the recognition of a new subfamily including the genus *Grayia* only.

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REFERENCES

- Cadle, J. E., 1987, Geographic distributions: problems in phylogeny and zoogeography, in: *Snakes: ecology and evolutionary biology*, R. A. Siegel, J. T. Collins, and S. S. Novak, eds., MacMillan, New York, pp. 77-105.
- Cadle, J. E., 1994, The colubrid radiation in Africa (Serpentes: Colubridae): phylogenetic relationships and evolutionary patterns based on immunological data, *Zool. J. Linn. Soc.* **110**:103-140.
- Dowling, H. G., Haas, C. A., Hedges, S. B., and Highton, R., 1996, Snake relationships revealed by slow evolving proteins: a preliminary survey, *J. Zool.* **240**:1-28.

- Gravlund, P., 2001, Radiation within the advanced snakes (Caenophidia) with special emphasis on African opisthopholid colubrids, based on mitochondrial sequence data, *Biol. J. Linn. Soc.* **72**:99-114.
- Heise, P. J., Maxson, L. R., Dowling, H. G., and Hedges, S. B., 1995, Higher-level snake phylogeny inferred from mitochondrial DNA sequences of 12S rRNA and 16S rRNA genes, *Mol. Biol. Evol.* **12**:259-265.
- Huelsenbeck, J. P., and Ronquist, F., 2001, MrBayes: Bayesian inference of phylogenetic trees, *Bioinformatics* **17**:754-755.
- Knight, A., and Mindell, D. P., 1994, On the phylogenetic relationship of Colubrinae, Elapidae, and Viperidae and the evolution of front-fanged venom systems in snakes, *Copeia* **1994**:1-9.
- Kraus, F., and Brown, W. M., 1998, Phylogenetic relationships of colubroid snakes based on mitochondrial DNA sequences, *Zool. J. Linn. Soc.* **122**:455-487.
- Lenk, P., Kalyabina, S., Wink, M., and Joger, U., 2001, Evolutionary relationships among the true vipers (Viperinae) suggested by mitochondrial DNA sequences, *Mol. Phylogenet. Evol.* **19**:94-104.
- McDowell, S. B., 1968, Affinities of the snakes usually called *Elaps lacteus* and *E. dorsalis*, *Zool. J. Linn. Soc.* **47**:561-578.
- Nagy, Z. T., Joger, U., Wink, M., Glaw, F., and Vences, M., 2003, Multiple colonization of Madagascar and Socotra by colubrid snakes: evidence from nuclear and mitochondrial gene phylogenies, *Proc. R. Soc., Lond. B* **270**:2613-2621.
- Posada, D., and Crandall, K. A., 1998, Modeltest: testing the model of DNA substitution, *Bioinformatics* **14**:817-818.
- Rage, J.-C., Buffetaut, E., Buffetaut-Tong, H., Chaimanee, Y., Ducrocq, S., Jaeger, J.-J., and Suteethorn, V., 1992, A colubrid snake in the late Eocene of Thailand: The oldest known Colubridae (Reptilia, Serpentes), *C. R. Acad. Sci. Paris* **314**:1085-1089.
- Slowinski, J. B., and Keogh, J. S., 2000, Phylogenetic relationships of elapid snakes based on cytochrome *b* mtDNA sequences, *Mol. Phylogenet. Evol.* **15**:157-164.
- Slowinski, J. B., Knight, A., and Rooney, A. P., 1997, Inferring species trees from gene trees: a phylogenetic analysis of the Elapidae (serpents) based on the amino acid sequences of venom proteins, *Mol. Phylogenet. Evol.* **8**:349-362.
- Slowinski, J. B., and Lawson, R., 2002, Snake phylogeny: evidence from nuclear and mitochondrial genes, *Mol. Phylogenet. Evol.* **24**:194-202.
- Swofford, D. L., 2002, *PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods)*, Version 4.0b10, Sinauer, Sunderland, MA.
- Underwood, G., and Kochva, E., 1993, On the affinities of the burrowing asps *Atractaspis* (Serpentes: Atractaspididae), *Zool. J. Linn. Soc.* **107**:3-64.
- Vidal, N., and Hedges, S. B., 2002a, Higher-level relationships of caenophidian snakes inferred from four nuclear and mitochondrial genes, *C. R. Biologies* **325**:987-995.
- Vidal, N., and Hedges, S. B., 2002b, Higher-level relationships of snakes inferred from four nuclear and mitochondrial genes, *C. R. Biologies* **325**:977-985.