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Phylogeny of the Colubroidea (Serpentes): New evidence from mitochondrial and nuclear genes

R. Lawson [a], J.B. Slowinski [b,†], B.I. Crother [c], F.T. Burbrink [d,*]

[a] *Osher Foundation Laboratory for Molecular Systematics, California Academy of Sciences, 875 Howard Street, San Francisco, CA 94103, USA*
[b] *Department of Herpetology, California Academy of Sciences, 875 Howard Street, San Francisco, CA 94118, USA*
[c] *Department of Biological Sciences, Southeastern Louisiana University, Hammond, LA 70402, USA*
[d] *College of Staten Island/City University of New York, 2800 Victory Blvd, Staten Island, NY 10314, USA*

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Abstract

The Colubroidea contains over 85% of all the extant species of snakes and is recognized as monophyletic based on morphological and molecular data. Using DNA sequences (cyt *b*, c-mos) from 100 species we inferred the phylogeny of colubroids with special reference to the largest family, the Colubridae. Tree inference was obtained using Bayesian, likelihood, and parsimony methods. All analyses produced five major groups, the Pareatidae, Viperidae, Homalopsidae, the Elapidae, and the Colubridae. The specific content of the latter two groups has been altered to accommodate evolutionary history and to yield a more stable taxonomy. We propose an updated classification based on the reallocation of species as indicated by our inferred phylogeny.

Keywords: Colubroidea; cyt *b*; c-mos; Bayesian; Likelihood; Parsimony; Classification

1. Introduction

The Colubroidea represents nearly 2500 species of extant snakes (Pough et al., 2004) and is understood to be monophyletic based on both morphological (Lee and Scanlon, 2002; Rieppel, 1988; Zaher, 1999) and molecular data (Cadle, 1988; Gravlund, 2001; Heise et al., 1995; Kraus and Brown, 1998; Slowinski and Lawson, 2002, 2005; Wilcox et al., 2002). A trend among herpetological lexicographers is to subdivide Colubroidea into the families Viperidae, Elapidae, Atractaspididae, and Colubridae (Pough et al., 2004), although Dowling and Jenner (1988) restricted the superfamily to just the Colubridae and Natricidae and in the process erected four other superfamilies that contain traditional colubroid groups. The proposed classification by Dowling and Jenner (1988) was not accompanied by supporting data and is not considered further. Morphological characters related to their respective venom-delivery systems (Cadle, 1992; Jackson and Fritts, 1995; Kochva, 1978; Underwood and Kochva, 1993; Zaher, 1999) and several gene sequences (Heise et al., 1995; Kelly et al., 2003; Scanlon and Lee, 2004; Slowinski and Lawson, 2002, 2005; Vidal and Hedges, 2002) identify Elapidae and Viperidae as monophyletic groups. Within the Colubroidea, the Viperidae may be the sister group to all other colubroids (Cadle, 1988; Kelly et al., 2003; Kraus and Brown, 1998). However, other molecular studies by Dowling et al. (1996), Kraus and Brown (1998), and Gravlund (2001) are ambiguous with regard to the deepest divisions within the Colubroidea. Additionally, the recognition of Elapidae may render the largest colubroid

* Corresponding author. Fax: +1 718 982 3852.
E-mail addresses: rlawson@calacademy.org (R. Lawson), bcrother@selu.edu (B.I. Crother), burbrink@mail.csi.cuny.edu (F.T. Burbrink).
† Deceased

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family, Colubridae, paraphyletic (Heise et al., 1995; Kelly et al., 2003; Kraus and Brown, 1998). The monophyly of the other family, the Atractaspididae, is supported by some studies (Bourgeois, 1968; Heymans, 1975; McDowell, 1968, 1987; Underwood and Kochva, 1993; Zaher, 1999) but rejected by others (Cadle, 1988, 1994; Kelly et al., 2003). The generic composition of Atractaspididae with respect to the inclusion of *Homoroselaps* in this family or Elapidae has been debated for over three decades (Cadle, 1994; Kelly et al., 2003; McCarthy, 1985; McDowell, 1968; Slowinski and Keogh, 2000; Underwood and Kochva, 1993; Zaher, 1999). As with Elapidae, the Atractaspididae may also render Colubridae paraphyletic.

The family Colubridae is the most diverse, widespread, and species-rich family within all of Serpentes, occupying all continents except Antarctica and consisting of greater than 1800 species (Pough et al., 2004). The composition of this group, the putative paraphyly of the family, and the hierarchical structuring into subfamilies remain contentious issues (Dowling and Duellman, 1978; Heise et al., 1995; Kelly et al., 2003; Kraus and Brown, 1998; McDowell, 1987; Meirte, 1992; Nagy et al., 2003a; Vidal and Hedges, 2002; Williams and Wallach, 1989). Zaher (1999) and Zug et al. (2001) each published recent taxonomic allocations of all colubrid genera into subfamilies, based in part on lists and research by Dowling and Duellman (1978), McDowell (1987), Williams and Wallach (1989), and Meirte (1992). The 12 subfamilies comprising the Colubridae referred to in Zaher (1999) are Xenodermatinae, Pareatinae, Calamariinae, Homalopsinae, Boodontinae, Pseudoxyrhophiinae, Colubrinae, Psammophiinae, Pseudoxenodontinae, Natricinae, Dipsadinae, and Xenodontinae. The monophyly of the subfamilies Colubrinae, Natricinae, Psammophiinae, and Xenodontinae appears to be common to several molecular studies (Cadle, 1988; Dowling et al., 1996; Gravlund, 2001; Kelly et al., 2003; Kraus and Brown, 1998, (in part)).

Assessment of the monophyly and relationships among the families and subfamilies of Colubroidea has been hampered in the past by lack of thorough sampling and collection of data from independent sources. Therefore, our goals in this paper are to determine whether the four colubroid families each represent monophyletic groups and, if so, to examine the relationships among them using a diverse sampling of taxa. We also investigate relationships among the members of the family Colubridae and assess whether they conform to the subfamilies of Zaher (1999). To meet these goals, we infer a phylogeny for the group from a taxonomically wide range of species within the Colubroidea using the nucleotide sequences of two unlinked and independently evolving genes: the single-copy nuclear c-mos gene (Graybeal, 1994; Harris et al., 1999; Saint et al., 1998) and the mitochondrial cytochrome *b* gene. We have analyzed the genes separately and together using Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP). Separate analyses of unlinked genes allows the identification of areas of congruence, which, because of the low probability of shared clades by chance alone, can be considered supported with a high degree of confidence (de Queiroz et al., 1995; Hendy et al., 1988; Miyamoto and Fitch, 1995). Combined datasets were analyzed using BI, ML, and MP. Current computational implementations of Bayesian methods allow phylogenetic inference of combined datasets where separate model parameters may be applied to individual genes. Recommendations for taxonomic changes that support monophyletic arrangements within Colubroidea are constrained by phylogenies produced here and previous evidence from independent studies of morphology and molecular data.

2. Methods and materials

Snakes collected for this study were humanely euthanized following protocols approved by the California Academy of Sciences Animal Welfare Committee and the three North American herpetological societies (American Society of Ichthyologists and Herpetologists 1987).

2.1. DNA extraction, amplification, and sequencing

We extracted DNA from liver tissue, tail tip biopsies, or shed skin from 89 species representing all families of Colubroidea and all subfamilies of Colubridae (Table 1). All tissues were treated by the standard method of proteinase K digestion in lysis buffer followed by phenol/chloroform extraction (see Burbrink et al., 2000; for details). Template DNA for the polymerase chain reaction (PCR) was also prepared as in Burbrink et al. (2000). For amplification of the entire mitochondrial cytochrome *b* gene we used primers L14910 (de Queiroz et al., 2002) and H16064 (Burbrink et al., 2000). Our cycle sequencing protocol for the cytochrome *b* gene was identical to that given in Burbrink et al. (2000). For sequencing we used primers L15584 (de Queiroz et al., 2002) H16064 and L14919 (Burbrink et al., 2000), H15149 (Kocher et al., 1989), and H15716 (Slowinski and Lawson, 2002). Taxon-specific sequencing primers (available from the senior author) were required for *Alsophis portoricensis*, *Bitis nasicornis*, *Pareas macularius*, and *Coronella girondica*. This combination of primers allowed us to sequence both strands of the approximately 1116 nucleotides making up the cytochrome *b* gene of snakes.

For the c-mos gene, we developed primers that allow the amplification and sequencing in snakes of a 570–576 bp segment exclusive of the primers. In the develop-