

Molecular evidence for a terrestrial origin of snakes

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Biologists have debated the origin of snakes since the nineteenth century. One hypothesis suggests that snakes are most closely related to terrestrial lizards, and reduced their limbs on land. An alternative hypothesis proposes that snakes are most closely related to Cretaceous marine lizards, such as mosasaurs, and reduced their limbs in water. A presumed close relationship between living monitor lizards, believed to be close relatives of the extinct mosasaurs, and snakes has bolstered the marine origin hypothesis. Here, we show that DNA sequence evidence does not support a close relationship between snakes and monitor lizards, and thus supports a terrestrial origin of snakes.

Keywords: evolution; phylogeny; reptiles; squamates; lizards; mosasaurs

1. INTRODUCTION

Living snakes (*ca.* 3000 sp.) are grouped together with lizards (*ca.* 4700 sp.) and amphisbaenians (*ca.* 160 sp.) in the reptilian order Squamata, named for their scaly skin (Zug *et al.* 2001; Uetz 2003). Snakes are divided into two main groups. The scolecophidians ('blindsnakes') are burrowing species with small gape size and feed on small prey (mainly ants and termites). The alethinophidians ('typical snakes') are more ecologically diverse and most species feed on relatively large prey, primarily vertebrates (Cundall & Greene 2000; Vidal & Hedges 2002*b*). In addition to the more obvious characteristics of body elongation and loss of limbs, other features of living snakes include absence of eyelids and external ears and the presence of deeply forked tongues (Coates & Ruta 2000).

Two main hypotheses have been proposed and debated concerning the ancestral mode of the life of snakes: a terrestrial (burrowing or semi-burrowing) origin (Camp 1923; Mahendra 1938; Walls 1940) and a marine origin (Cope 1869; Nopcsa 1923; Caldwell & Lee 1997; Lee 1997; Lee & Scanlon 2002). This controversy, which has implications for understanding the evolution of locomotor and feeding systems in squamate reptiles (Gans 1961; Cundall & Greene 2000), has recently been fuelled by the discovery or reanalysis of fossils of three marine snake species ('pachyophiids') with small but well-developed hindlimbs (genera *Pachyrhachis*, *Haasiophis* and *Eupodophis*) (Caldwell & Lee 1997; Rage & Escuillié 2000; Tchernov *et al.* 2000; Rieppel *et al.* 2003).

According to the terrestrial hypothesis, the characteristics that define snakes were acquired in ancestors that were burrowing or semi-burrowing (Walls 1940). A close

relationship between snakes and some burrowing squamates that are limbless or have reduced limbs, such as amphisbaenians and/or dibamids (Rieppel & Zaher 2000*b*; Zaher & Rieppel 2000; Kearney 2003), has been considered as support for the terrestrial hypothesis, but this is not required. Specializations shared by snakes, amphisbaenians and dibamids include the loss, reduction and consolidation of skull bones; braincase enclosure; loss or reduction of limbs and girdles; and increased uniformity along the vertebral column (Coates & Ruta 2000). The terrestrial origin hypothesis agrees with data derived from the sensory system of living snakes and in particular with the peculiarities of their eyes (Walls 1940). Under this hypothesis, the fossil marine snakes (pachyophiids) are considered to be derived alethinophidian snakes and therefore to have no particular bearing on snake origins. This derived position of pachyophiids implies either a re-evolution of the limbs or several independent losses of limbs in living snake lineages (Greene & Cundall 2000; Rieppel *et al.* 2003). The latter has been a common theme among vertebrates in general and squamates in particular (Greer 1991).

According to the marine hypothesis, snakes are derived varanoids, a group that includes several families of extinct marine reptiles (mosasauroids) such as aigialosaurs, dolichosaurs and mosasaurs and two living families of terrestrial lizards, the Helodermatidae (genus *Heloderma*) and Varanidae (genera *Lanthanotus* and *Varanus*) (Lee 1997, 1998, 2000; Lee *et al.* 1999; Lee & Caldwell 2000). Under the marine hypothesis, the varanid lizards would be the closest living relatives of snakes. This result has also been suggested in molecular analyses using mitochondrial genes, although always with limited taxon sampling and usually not with robust statistical support (Forstner *et al.* 1995; Macey & Verma 1997; Rest *et al.* 2003). When mosasauroids are taken into account in morphological analyses, they form a paraphyletic branching pattern leading to snakes, and are thus closer to snakes than are varanids (Lee & Caldwell 2000). The group comprising snakes and mosasauroids, to the exclusion of other varanoids, is called Pythonomorpha (Cope 1869; Lee 1997) and is based on the sharing of presumably derived characters of the skull, the lower jaw and the dentition. According to this scheme, the extinct marine pachyophiids are considered to be intermediates between limbed marine squamates (mosasauroids) and living snakes (Lee & Caldwell 2000; Lee & Scanlon 2002). This basal position of both marine groups of squamates implies a marine-to-terrestrial transition leading to living snakes, an event otherwise unknown to have occurred within tetrapods.

2. METHODS

We used sequences from two protein-coding nuclear genes, recombination-activating gene 1 (*RAG1*) and oocyte maturation factor (*C-mos*), obtained from all 19 families of living lizards and amphisbaenians (Zug *et al.* 2001) and 17 out of the 25 families of living snakes (Vidal & Hedges 2002*a,b*). The usefulness of *C-mos* for resolving interfamilial squamate relationships is well known (Saint *et al.* 1998; Vidal & Hedges 2002*a,b*), although *RAG1* has until now never, to our knowledge, been sequenced in squamate reptiles despite its potential for resolving various higher-level vertebrate relationships (Venkatesh *et al.* 2001). Maximum-likelihood (ML), Bayesian inference, minimum evolution (ME) and maximum-parsimony (MP) methods were used to analyse the *RAG1* and *C-mos* sequences of 64 species, both separately and combined. Details of the samples used, methods for obtaining and sequencing the DNA and methods of phylogenetic analysis are detailed in electronic Appendix A, available on The Royal Society's Publications Web site.

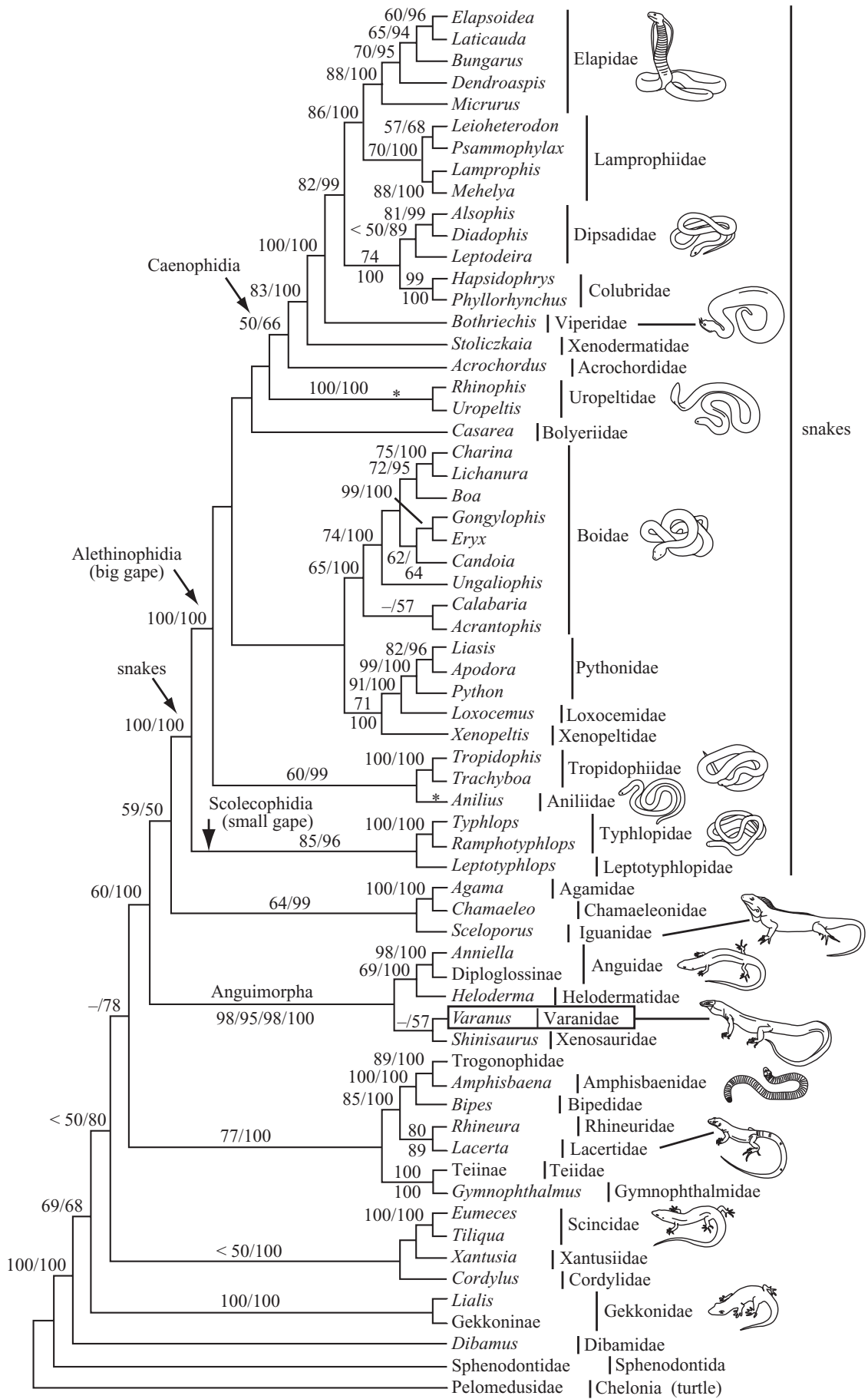


Figure 1. (Caption overleaf.)

Figure 1. Phylogenetic relationships of snakes, lizards and amphisbaenians inferred from DNA sequences of the nuclear genes *RAG1* and *C-mos*. For the critical node (Anguimorpha), values for all four tree-building methods are shown in the following order: bootstrap support values from ML, MP, ME and Bayesian posterior probabilities. For other nodes, bootstrap support values from MP are shown, followed by Bayesian posterior probabilities. Support values above 50% are shown at nodes in the Bayesian consensus tree; a dash indicates a node not appearing in the bootstrap consensus MP tree. An asterisk identifies a lineage with small gaped species, and the box indicates the taxon (Varanidae; monitor lizards) believed to be the closest relative of snakes under the marine hypothesis. The tree is rooted with a tuatara (*Sphenodon*) and turtle. Detailed results for all methods are in electronic Appendix A.

3. RESULTS

The resulting phylogenetic trees show remarkable consistency among different methods of analysis (figure 1; electronic Appendix A). Snakes are monophyletic and the basic division of Scolecophidia and Alethinophidia is retrieved. Relationships within snakes are similar to that seen in more focused analyses of snake phylogeny with additional genes (Vidal & Hedges 2002*a,b*). Among lizards, the Gekkonidae (including gekkonines and limbless pygopodiniines) is monophyletic. The large infraorder Iguania (Agamidae, Chamaeleonidae, Iguanidae) also forms a single clade in all analyses except ML. Other significant groupings are Anguimorpha (Varanidae, Helodermatidae, Xenosauridae, Anguidae), a cluster containing Teiidae, Gymnophthalmidae and Lacertidae with the four families of amphisbaenians (Amphisbaenidae, Trogonophidae, Bipedidae and Rhineuridae), and another group containing Cordylidae, Xantusiidae and Scincidae. The Dibamidae ('blindskinks') are the closest relatives of the Scincidae in the ME and ML trees, while they appear as a basal lineage in the MP and Bayesian trees (see electronic Appendix A).

Our results (figure 1) show that *Varamus* is allied with the remaining anguimorph lizards (Helodermatidae, Xenosauridae and Anguidae) to the exclusion of snakes (support values for the ML, MP, ME and Bayesian analyses are 98, 95, 98 and 100, respectively). Our phylogeny also rejects the association of snakes and amphisbaenians (support values of 95, 85, 79 and 100). The clustering of snakes with iguanian lizards (figure 1) is not strongly supported, but is curious because of their presumed Gondwanan ancestry and some shared similarities of the chromosomes, skull, vertebral column, inner ear, urinary bladder (absence) and oral glands (Bellairs & Underwood 1950; Rieppel 1988).

4. DISCUSSION

The exclusion here of snakes from varanoids undermines the marine hypothesis of snake origins because it breaks the proposed transition from marine squamate reptiles (mosasauroids) to early marine snakes (pachyophiids) (Caldwell & Lee 1997; Lee 1997; Lee & Caldwell 2000). Based on morphology, the mosasauroids are closely associated with varanoids, while the pachyophiids are closely associated with living snakes (either basal or derived within Alethinophidia) (Baur 1890; Camp 1923;

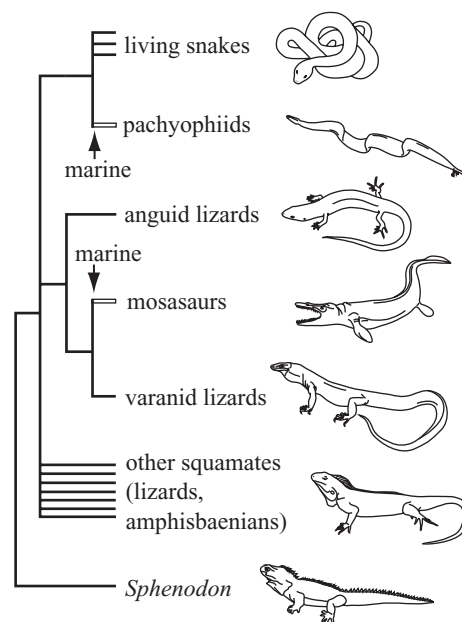


Figure 2. Summary tree showing the phylogenetic relationships, inferred in this study, of snakes to other living squamate reptiles. The positions of the two groups of fossil marine squamates are shown (hollow bars), as based on morphological evidence. The fossil marine snakes (pachyophiids) are related to living snakes, either basally or as members of a derived lineage. The mosasaurs, however, are closely related to varanoid lizards. The common ancestors of all lineages shown are believed to be terrestrial, with two inferred transitions (arrows) to the marine environment.

McDowell & Bogert 1954; Carroll & deBraga 1992; deBraga & Carroll 1993; Lee 1997; Lee *et al.* 1999; Lee & Caldwell 2000; Rieppel & Zaher 2000*b*; Tchernov *et al.* 2000), implying two independent terrestrial to marine transitions under this new phylogenetic scheme (figure 2).

By itself, a morphological connection between snakes and varanoids was not robust because snakes are so highly modified that they cannot be coded for some characters diagnostic of varanoids and they lack a unique character of varanoids (a surangular with a blunt anterior tip) (Lee 1997). Likewise, a close relationship between snakes and mosasaurs also was not well supported. Similarities in the structure of their jaws, such as the intramandibular joint, have been shown to be convergent (Rieppel & Zaher 2000*b*) and other presumed shared traits have been questioned (Fraser 1997; Zaher & Rieppel 1999; Rieppel & Zaher 2000*a*; Rieppel *et al.* 2003). Of course, without a mosasauroid–varanoid link, the disassociation of snakes and varanids would not challenge the marine hypothesis. However, with rare exceptions (Caldwell *et al.* 1995; Caldwell 1999), morphological analyses have unambiguously supported a close relationship between mosasaurs and varanoids (Baur 1890; Camp 1923; Nopcsa 1923; McDowell & Bogert 1954; Russell 1967; Carroll & deBraga 1992; deBraga & Carroll 1993; Lee 1997; Lee *et al.* 1999; Lee & Caldwell 2000; Rieppel & Zaher 2000*b*).

The current molecular evidence (figure 1) is unable to clarify all of the branches of the squamate phylogenetic tree or robustly identify the closest lineage to snakes, except to exclude varanoids, amphisbaenians and probably dibamids (assuming the association of the latter with

skinks). Nonetheless, the significant exclusion of snakes from varanoids is in itself sufficient to support a terrestrial origin of snakes, because no other marine connection has been suggested or is likely. This is true regardless of the affinities of snakes among remaining lineages of squamates. Within the terrestrial environment, the origin of snakes has been most often associated with the underground niche by drawing parallels with characteristics of burrowing (fossorial) lizards. However, it has been debated as to whether the ancestors of snakes were fully fossorial and constructed their own burrows or were semi-fossorial and occupied burrows constructed by other animals (Camp 1923; Walls 1940; Bellairs & Underwood 1950; Rieppel 1988). Further insight into this question, and the evolution of the locomotor and feeding systems of snakes in general, may come with a more robust phylogeny of squamates and additional Mesozoic fossils.

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