PALEONTOLOGY

Four legs too many?

A long-bodied fossil snake retains fore- and hindlimbs

By Susan Evans

A classic Gary Larson cartoon shows a robed and bearded figure rolling out clay strips, with the caption: "God makes the snake." Body elongation was certainly fundamental in the evolution of snakes from lizards, as was the shrinking and ultimately the loss of limb pairs (limb reduction). However, informative early fossils are rare, and many details of the transition remain unresolved. A remarkable fossil described on p. xxx of this issue by Martill *et al.* (*1*) brings fresh perspective to the debate. The aptly named *Tetrapodophis* combines a snakelike body with fore- and hindlimbs bearing five well-developed digits.

Snakelike bodies evolved several times through geological history. Among amniotes (reptiles, birds, and mammals), they occur only in Squamata, the group comprising lizards and snakes. Within Squamata, however, this body form has arisen independently at least 26 times (*2*) (see the figure). Body elongation is always correlated with limb reduction (*2*), and the forelimbs are usually lost first (*Bipes* and *Bachia* are rare exceptions). One explanation is that as the body lengthens, coordination of limb movements becomes increasingly difficult. Moreover, a serpentine body moves most effectively by lateral undulation, a movement in which limbs can become a hindrance, especially in narrow spaces. Researchers have identified a threshold body length at which limb reduction begins, and no known squamate with more than 70 precaudal (before tail) vertebrae retains four complete limbs (*2*). T*etrapodophis* (*1*),

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with around 160 precaudals, is therefore exceptional.

Efforts to reconstruct the evolutionary stages in the snake body plan are hampered by a lack of consensus on snake relationships and ancestral lifestyle. Analyses using molecular data group snakes with terrestrial lizards like iguanas and Komodo Dragons (Iguania and Anguimorpha) (*3*) and generally assume a burrowing or semi-burrowing ancestry (*1*–*3*). However, some analyses that include anatomical characters place them with extinct Cretaceous (~100 to 66 million years ago) marine lizards, the mosasaurs (*3*, *4*). This has prompted the suggestion of a marine swimming ancestry for snakes (*4*).

Molecular divergence estimates date snake origins to the Jurassic (~150 million years ago) (*5*), but the earliest uncontested fossils are isolated vertebrae from the mid-Cretaceous (~113 million years ago) of North America (*5*). These vertebrae come from terrestrial deposits but are otherwise fairly uninformative. More instructive are several articulated skeletons or partial skeletons from slightly younger (~100 million-year-old) deposits. The largest set consists of several related marine snakes from the Middle East, North Africa, and southern Europe. These fossil snakes have 140 to 155 precaudal vertebrae and a short tail. They show no trace of forelimbs or shoulder girdle but do have small hindlimbs; only one [*Haasiophis* (*6*)] preserves digits. The relationships of these limbed marine snakes remain controversial, but many analyses (*1*, *3*, *6*, *7*) nest them among modern snakes, rather than nearer the base of the snake evolutionary tree. This implies either that hind limbs were reduced more than once within snakes, or that the limbs redeveloped in some lineages (6).

A second set of early fossil snakes comes from terrestrial deposits in South America. The most complete, [illegible]0-million-year-old *Najash* (*7*), resembles the fossil marine snakes in having small hind legs without preserved digits but is more primitive (*1*, *7*). *Tetrapodophis* is also from South America, and from a deposit that yields a mix of freshwater and terrestrial species, but it is older (~113 million years old). Martill *et al.* (*1*) place it on the stem of the snake evolutionary tree, below *Najash* and close to another early terrestrial snake, the North American *Coniophis*, represented by vertebrae and attributed jaw elements.

Whereas fossils can yield information on the sequence of anatomical changes involved in any major transition, developmental biology helps to explain how these changes occurred. Evolution of the snake body form combined axial elongation, limb loss, and reduced regionalization (*8*, *9*). Whether and how these components are linked developmentally remains uncertain. In all vertebrate embryos, individual vertebrae develop from segments (somites) that form at regular intervals. To increase vertebral numbers, somite formation must either continue for longer or occur at a faster rate. Snakes use both strategies (*8*). Individual vertebrae then acquire positional identity along the body axis through the overlapping expression domains of *Hox* genes. In a typical tetrapod, the boundaries between major vertebral regions (such as the neck and the trunk) coincide with *Hox* gene expression boundaries.

In a pioneering study of *Python* development, Cohn and Tickle (*10*) reported a marked expansion of the typical Hox expression domains, particularly those normally associated with the neck-trunk boundary. They argued that the neck had been lost in snakes and that this loss disrupted the molecular signals required for forelimb positioning and outgrowth. However, in another snake, *Pantherophis*, the Hox expression domains, although expanded and without sharp boundaries, retain a regionalized pattern comparable to that of lizards with a distinct neck (*9*, *11*). A parallel study of vertebral anatomy across a wide range of snakes (*12*) revealed a similar regionalized pattern, implying that snakes have a neck of 10 to 12 segments.

Like that of a lizard, the vertebral column of Tetrapodophis has distinct regions, including 10 to 11 short-ribbed neck vertebrae adjacent to the tiny forelimbs. This neck length is within the range of some generalized terrestrial lizards and matches that proposed by the developmental (*9*, *11*) and anatomical (*12*) studies. Thus, as in long-bodied lizards, elongation of the snake skeleton occurred in the trunk region and not the neck. Moreover, if Tetrapodophis is correctly interpreted as a stem-snake, that elongation preceded loss of the forelimbs.

Love them or loathe them, snakes have long fascinated humans. The combined efforts of paleontology and developmental biology have gone some way toward unravelling the early history of snakes, but many questions remain as to their origins, relationships, character evolution, and ancestral lifestyle. Resolution of these questions depends, ultimately, depends on the recovery of further fossils and their thorough and objective analysis.

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A four-limbed snake from the Cretaceous. *Tetrapodophis* retains four limbs, each with five digits, in an elongated body with 160 before-tail vertebrae.

Credit: TKTKTKTKTK

Limbs or no limbs. (A) Martill *et al.* report the discovery of a four-limbed snake, *Tetrapodophis amplectis*, from the Cretaceous. **(B)** Schematic showing independent development of the long bodied, limb-reduced body plan amongst squamates (not to scale).

Credit: panel A, D. M. Martill/University of Portsmouth