



A Fossil Snake with Limbs

Eitan Tchernov, *et al.*

Science **287**, 2010 (2000);

DOI: 10.1126/science.287.5460.2010

The following resources related to this article are available online at www.sciencemag.org (this information is current as of April 9, 2008):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/cgi/content/full/287/5460/2010>

This article has been **cited by** 64 article(s) on the ISI Web of Science.

This article has been **cited by** 4 articles hosted by HighWire Press; see:

<http://www.sciencemag.org/cgi/content/full/287/5460/2010#otherarticles>

This article appears in the following **subject collections**:

Paleontology

<http://www.sciencemag.org/cgi/collection/paleo>

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce this article** in whole or in part can be found at:

<http://www.sciencemag.org/about/permissions.dtl>

introduction of the reactive partners into transiently associated biopolymers might allow their covalent trapping within a cell and, as a result, the identification of previously unobservable interactions.

References and Notes

1. K. A. Winans and C. R. Bertozzi, *Chem. Biol.* **5**, R313 (1998).
2. L. K. Mahal, K. J. Yarema, C. R. Bertozzi, *Science* **276**, 1125 (1997).
3. K. J. Yarema, L. K. Mahal, R. E. Bruehl, E. C. Rodriguez, C. R. Bertozzi, *J. Biol. Chem.* **273**, 31168 (1998).
4. G. A. Lemieux, K. J. Yarema, C. L. Jacobs, C. R. Bertozzi, *J. Am. Chem. Soc.* **121**, 4278 (1999).
5. J. H. Lee et al., *J. Biol. Chem.* **274**, 21878 (1999).
6. B. A. Griffin, S. R. Adams, R. Y. Tsien, *Science* **281**, 269 (1998).
7. H. Staudinger and J. Meyer, *Helv. Chim. Acta* **2**, 635 (1919).
8. Y. G. Gololobov and L. F. Kasukhin, *Tetrahedron* **48**, 1353 (1992).
9. Synthesis of *N*-azidoacetylmannosamine (**3**) and acetylated **3**. A solution of mannosamine hydrochloride (250 mg, 1.16 mmol) and sodium methoxide (1.16 ml of a 1 M methanolic solution) in dry MeOH (10 ml) was stirred for 1 hour at room temperature, after which chloroacetic anhydride (991 mg, 5.80 mmol) was added. The resulting solution was stirred overnight at room temperature under an atmosphere of N₂ and then quenched with H₂O (5 ml) for 1 hour. The solution was neutralized with saturated NaHCO₃ and concentrated, and the residue was filtered through a plug of silica gel eluting with 5:1 CHCl₃/MeOH. The crude product obtained was dissolved in dimethylformamide (10 ml) and NaN₃ (78 mg, 1.39 mmol) was added. After heating at reflux overnight, the solution was cooled and concentrated. Purification by silica gel chromatography eluting with a gradient of 50:1 to 6:1 CHCl₃/MeOH afforded 179 mg of compound **3** (59% over two steps). The compound was peracetylated before incubation with cells as follows. A solution of **3** (25 mg, 0.095 mmol), acetic anhydride (1.0 ml, 11 mmol), and a catalytic amount of 4-dimethylaminopyridine in pyridine (2 ml) was cooled to 0°C. The mixture was stirred overnight, warmed to room temperature, then diluted with CH₂Cl₂ (100 ml) and washed with 1 N HCl (3 × 50 ml), saturated NaHCO₃ (1 × 50 ml), water (1 × 50 ml), and saturated NaCl (1 × 50 ml). The combined organic layers were dried over Na₂SO₄ and concentrated. The crude product was purified by silica gel chromatography eluting with a gradient of 1:10 to 1:2 EtOAc/hexanes to afford 39 mg (95%) of acetylated **3**.
10. Synthesis of intermediate phosphine **4**. A solution of NaNO₂ (180 mg, 2.64 mmol) in 1 ml of H₂O was added dropwise to a solution of 1-methyl-2-aminoterephthalate (500 mg, 2.56 mmol) in 5 ml of cold concentrated HCl. The mixture was stirred for 30 min at room temperature and then filtered through glass wool into a solution of KI (4.30 g, 25.0 mmol) in 7 ml of H₂O. The dark red solution was stirred for 1 hour and then diluted with CH₂Cl₂ (100 ml) and washed with saturated Na₂SO₃ (2 × 10 ml). The organic layer was washed with water (2 × 20 ml) and saturated NaCl (1 × 20 ml). The combined aqueous layers were back extracted with CH₂Cl₂ (20 ml). The combined organic layers were dried over Na₂SO₄ and concentrated. The crude product was dissolved in a minimum amount of MeOH and H₂O was added until the solution appeared slightly cloudy. Cooling to 4°C and subsequent filtration afforded 449 mg (57%) of a yellow solid. To a flame-dried flask was added this product (300 mg, 1.00 mmol), dry MeOH (3 ml), triethylamine (0.3 ml, 2 mmol), and palladium acetate (2.2 mg, 0.010 mmol). While stirring under an atmosphere of Ar, diphenylphosphine (0.17 ml, 1.0 mmol) was added to the flask by means of a syringe. The resulting solution was heated at reflux overnight, and then allowed to cool to room temperature and concentrated. The residue was dissolved in 250 ml of a 1:1 mixture of CH₂Cl₂/H₂O and the layers were

separated. The organic layer was washed with 1 M HCl (1 × 10 ml) and concentrated. The crude product was dissolved in a minimum amount of methanol and an equal amount of H₂O was added. The solution was cooled to 4°C for 2 hours and the resulting solid was collected by filtration. The pure product, compound **4**, was isolated in 69% yield (245 mg). This compound can be coupled with amines by using standard procedures [such as EDC [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] or DCC [1,3-dicyclohexylcarbodiimide] coupling reactions].

11. Acetylated monosaccharides are metabolized 200-fold more efficiently than the free sugars owing to improved cellular uptake, which is followed by deacetylation by cytosolic esterases [C. L. Jacobs and C. R. Bertozzi, unpublished results; A. K. Sarkar, T. A. Fritz, W. H. Taylor, J. D. Esko, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 3323 (1995)].

12. E. Saxon and C. R. Bertozzi, data not shown.
13. I. L. Cartwright, D. W. Hutchinson, V. W. Armstrong, *Nucleic Acids Res.* **3**, 2331 (1976).
14. Retrovir (zidovudine), Glaxo Wellcome.
15. J. C. M vanHest and D. A. Tirrell, *FEBS Lett.* **428**, 68 (1998).
16. M. W. Nowak et al., *Science* **268**, 439 (1995).
17. We acknowledge generous funding from the W. M. Keck Foundation, Glaxo Wellcome, and the Burroughs Wellcome Fund. E.S. was supported by a Howard Hughes Medical Institute Predoctoral Fellowship. This research was supported by the Office of Naval Research, grant N00014-98-1-0605 and order N00014-98-F-0402 through the U.S. Department of Energy under contract DE-AC03-76SF00098, and by the National Institutes of Health (GM58867-01).

1 December 1999; accepted 2 February 2000

A Fossil Snake with Limbs

Eitan Tchernov,¹ Olivier Rieppel,^{2*} Hussam Zaher,³ Michael J. Polcyn,⁴ Louis L. Jacobs⁴

A 95-million-year-old fossil snake from the Middle East documents the most extreme hindlimb development of any known member of that group, as it preserves the tibia, fibula, tarsals, metatarsals, and phalanges. It is more complete than *Pachyrhachis*, a second fossil snake with hindlimbs that was recently portrayed to be basal to all other snakes. Phylogenetic analysis of the relationships of the new taxon, as well as reanalysis of *Pachyrhachis*, shows both to be related to macrostomatans, a group that includes relatively advanced snakes such as pythons, boas, and colubroids to the exclusion of more primitive snakes such as blindsnakes and pipesnakes.

The lower to middle Cenomanian (basal Upper Cretaceous) carbonates of 'Ein Yabrud near Jerusalem, deposited in a low-energy shallow marine platform environment (1), have yielded two species of fossil snakes, *Pachyrhachis problematicus* (2–4) and the new taxon reported here. Because of the presence of relatively well-developed hindlimbs and a supposedly primitive skull structure, a series of recent publications (5–7) have interpreted *Pachyrhachis* to be basal to all other snakes, indeed to represent “an excellent example of a transitional taxon” (8) linking snakes to an extinct group of “lizards,” the mosasauroids. On the basis of this pattern of phylogenetic relationships, it was claimed that snakes had a marine origin (8) and that the mosasauroid jaws provided the starting point for the evolution of the ophidian feeding mechanism (9). The transitional position of *Pachyrhachis* influenced a scenario explaining the origin and

evolution of limblessness in snakes, based on the analysis of underlying developmental mechanisms as revealed by patterns of *Hox* gene expression in *Python* (10). The basal position of *Pachyrhachis* and the putative relationships of snakes to mosasauroids were tested by a review of the character evidence and the methods of phylogenetic analysis used, and were found to be refuted by the position of *Pachyrhachis* as the sister taxon of relatively advanced (i.e., macrostomatan) snakes (11–15).

Here, we describe the second snake from 'Ein Yabrud, which is better preserved than *Pachyrhachis* in the skull and hindlimb, and which highly corroborates the macrostomatan affinities of these fossil snakes.

Haasiophis, gen. nov.

Genotypical species: *Haasiophis terrasancus*, sp. nov.

Diagnosis: A snake with a snout-vent length of 717 mm; premaxilla small and narrow, edentulous; 24 tooth positions on the maxilla, 8 on the palatine, 15 to 17 on the pterygoid, and 26 on the dentary; enamel surface of teeth distinctly striated; mandibular nerve foramen underlapped by distinct prootic process; quadrate slender and vertically oriented; coronoid process on mandible small, formed by coronoid bone only; 155 precloacal vertebrae; at least 12 proximal caudal vertebrae with distally expanded and bifurcated lymph-

¹Department of Evolution, Systematics, and Ecology, Hebrew University of Jerusalem, Berman-Lubin Buildings, Givat Ram, Jerusalem 91904, Israel. ²Department of Geology, Field Museum, 1400 South Lake Shore Drive, Chicago, IL 60605, USA. ³Instituto de Biociencias, Departamento de Zoologia, Universidade de Sao Paulo, Rua do Matao, Travessa 14, Cidade Universitaria, 05508-900 Sao Paulo SP, Brazil. ⁴Shuler Museum of Paleontology, Southern Methodist University, Dallas, TX 75275, USA.

*To whom correspondence should be addressed. E-mail: rieppel@fmmh.org

REPORTS

apophyses; expanded hemapophyses on posterior tail vertebrae.

Distribution: Early Upper Cretaceous, Middle East.

Haasiophis terrasanctus, sp. nov.

Holotype: Hebrew University of Jerusalem, Paleontological Collections, HUU-Pal. EJ 695.

Stratum typicum: Aminadav Formation or the slightly younger Bet-Meir Formation, middle part of the Judea Group, early to middle Cenomanian, basal Upper Cretaceous.

Locus typicus: Limestone quarries of 'Ein Yabrud, Judean hills, 20 km north of Jerusalem.

Diagnosis: Same as for genus, of which this is the only known species (specimen).

Etymology: *Haasiophis*, in honor of Prof. G. Haas, who initiated research on vertebrate fossils from 'Ein Yabrud; *ophis* (Greek, snake); *terrasanctus* (Latin, Holy Land).

This specimen is identified as a fossil snake on the basis of its highly kinetic skull with anteriorly free ending maxillae and dentaries, slender and elongate tooth-bearing palatines and pterygoids, single mental foramen in the dentary, high number of presacral vertebrae, and the presence of hypapophyses or hemal ridges throughout the trunk, distally bifurcated lymphapophyses in the cloacal and proximal tail region, and paired hemapophyses on the tail vertebrae. Its cranial structure (Fig. 1) (16) displays relatively primitive characters, such as are present in anilioids (pipesnakes) with advanced macrostomatan features. The extended contact between the anteroventrally sloping prefrontal and the ascending process of the maxilla is plesiomorphic, as is the coronoid process on the lower jaw formed by the coronoid bone only. Advanced features include an elongate preorbital region recalling the condition seen in *Python*; a nearly complete postorbital arch; highly mobile connections among the elements of the dermal palate and upper jaw (vomer, palatine, pterygoid, ectopterygoid, maxilla, and premaxilla); the presence of well-developed [neomorph (17)] basipterygoid processes as revealed by radiographs; a slender, elongate, and vertically oriented quadrate suspended from a posteriorly free-ending supratemporal; the development of longitudinal crests for muscle attachment both on the skull roof (parietal, supraoccipital) and on the skull base (parabasisphenoid and basioccipital); and the anterior extent of the splenial and development of a deep fossa for the insertion of jaw adductor muscles on the lower jaw. The exoccipitals appear not to meet above the foramen magnum, but this may well be an artifact of preservation.

Except for pachyostosis and the well-developed hindlimb (Fig. 2), the postcranial

skeleton of *Haasiophis* is typically snake-like. Pachyostosis of vertebrae and ribs occurs between the 45th to 48th and the 105th to 108th vertebrae, with a distinct hypertrophy of the parapophysis separated by a furrow from the smaller, dorsal diapophyseal component of the rib articulation. Anterior hypapophyses are gradually transformed to distinct hemal ridges along the trunk. Broad and plate-like hemapophyses add to the lateral compression of the tail, which must have served as a propulsive organ.

The last dorsal rib is associated with the 154th vertebra. There is no evidence for the

suspension of rudimentary pelvic elements from the axial skeleton. Two poorly preserved, obliquely oriented, delicate rods of bone, located near the 155th vertebra, may represent the pubis and ilium of a rudimentary, originally triradiate pelvis. The left femur (Fig. 2) is a small (7.2 mm long), straight, slender element with moderately expanded proximal and distal ends, which emerges from below the last dorsal rib. The tibia (3.3 mm), characterized by a relatively broad proximal end, has been flipped across the fibula (3.1 mm) during fossilization. Three tarsal ossifications are identified as

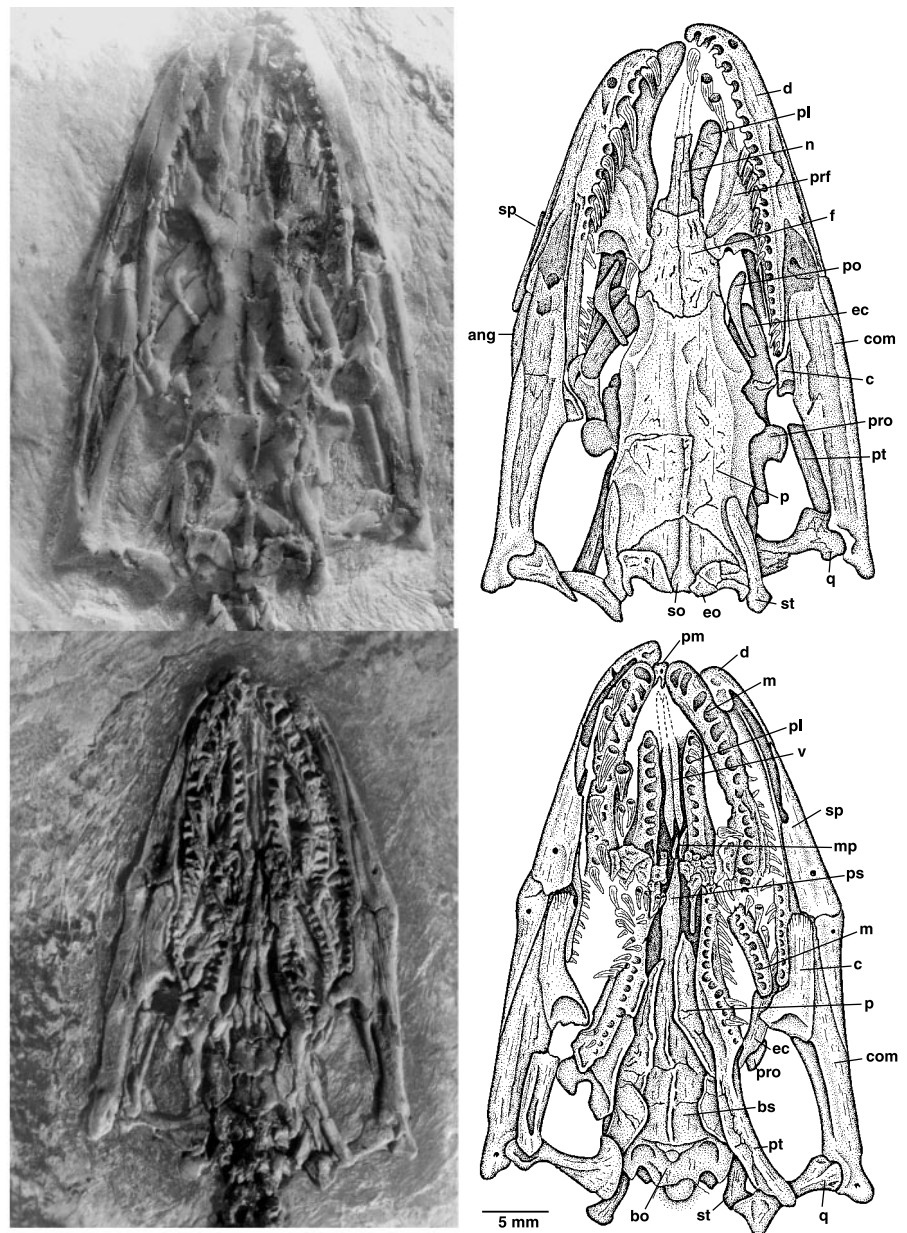


Fig. 1. The skull of *H. terrasanctus* in dorsal (above) and ventral (below) views. Abbreviations: ang, angular; bo, basioccipital; bs, basisphenoid (parabasisphenoid); c, coronoid; com, compound bone; d, dentary; ec, ectopterygoid; eo, exoccipital; f, frontal; m, maxilla; mp, medial process of palatine; n, nasal; p, parietal; pl, palatine; pm, premaxilla; po, postorbital; prf, prefrontal; pro, prootic; ps, parasphenoid rostrum; pt, pterygoid; q, quadrate; so, supraoccipital; sp, splenial; st, supratemporal; and v, vomer.

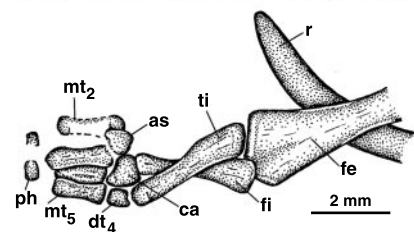


Fig. 2. The limb of *H. terrasanctus*. Abbreviations: as, astragalus; ca, calcaneum; dt4, fourth distal tarsal; fe, femur; fi, fibula; ph, phalanx; mt, metatarsal; r, rib (of 154th vertebra); ti, tibia.

astragalus, calcaneum, and the fourth distal tarsal. The straight metatarsals of digits two through five are at least partially preserved, as are two partial phalanges.

Phylogenetic analysis (Fig. 3) (18) shows *Haasiophis* to be the sister taxon of *Pachyrhachis*, both nested within basal macrostomatans (i.e., near pythons and boids). Statistical support for the position of *Haasiophis* and *Pachyrhachis* within Alethinophidia (Fig. 3), and for their relationship to Macrostomata, is strong (18–20). By contrast, the position of these taxa within basal macrostomatans, as well as the sister-group relationship of *Haasiophis* and *Pachyrhachis*, remain weakly supported, probably because of their diver-

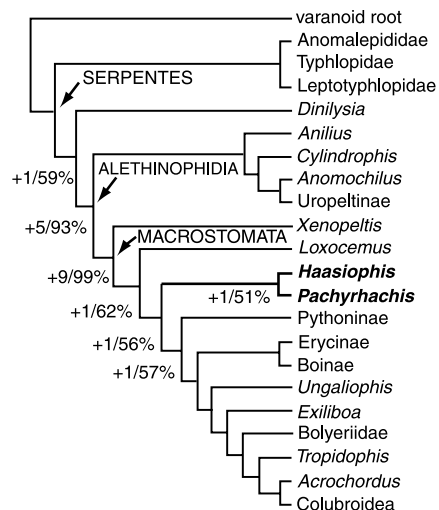


Fig. 3. The phylogenetic relationships of *H. terrasanctus*. A strict consensus tree of two equally parsimonious trees is shown [(18); for list of apomorphies, see (16)].

gent specialization. Boiné characters of *Haasiophis* are the laterally projecting process of the prootic (underlapping the mandibular nerve foramen) and the posteriorly dilated free-ending process of the supratemporal. Pythonine characters of *Pachyrhachis* are the straight frontoparietal suture and the nature of the postorbital-parietal contact. *Haasiophis* and *Pachyrhachis* differ in other respects as well, such as tooth counts, shape and relative size of the coronoid process and quadrate, size of the neural spines on the anterior (“cervical”) vertebrae, differentiation of the ribs, and relative proportions of the stylopodial and zeugopodial limb elements. *Haasiophis* therefore cannot represent a juvenile specimen of the larger *Pachyrhachis*.

Given the relationships of *Pachyrhachis* and *Haasiophis* to macrostomatans, the presence of well-developed hindlimbs optimizes unequivocally as a reversal (Fig. 3). Implicit weight can be added to the (plesiomorphic) presence of limbs by splitting those into discrete characters numerous enough to pull the fossils to the base of the ophidian tree. The number of limb characters required to break *Haasiophis* and *Pachyrhachis* away from macrostomatans is 14, and 15 limb characters are required to pull these fossils to a basal position. Loss of resolution throughout the cladogram, caused by the addition of more than 13 limb characters, is significant, indicating that the overall data set matches the prediction of a redevelopment of the hindlimbs better than it matches the assumption that the skulls of *Pachyrhachis* and *Haasiophis* are convergent on macrostomatans.

As macrostomatans snakes, *Haasiophis* and *Pachyrhachis* have no particular bearing on snake-mosasauroid relationships or snake origins. Instead, they represent the first unequivocal documentation of the incursion of macrostomatans snakes into the sea. Basal snakes—including basal macrostomatans—retain rudimentary hindlimbs, which, however, remain much more incomplete than those of *Haasiophis*. With *Haasiophis* and *Pachyrhachis* related to basal macrostomatans, the conclusion based on parsimony must be that these limbs redeveloped from rudiments such as those present in *Python* (10). The assumption of a multiple loss of hindlimbs among basal snakes is less parsimonious but remains a possibility, given the incompleteness of the fossil record of snakes (21) and the recognition of multiple loss of limbs among squamates in general (22).

References and Notes

1. M. G. Polcyn, E. Tchernov, L. L. Jacobs, *Nat. Sci. Mus. Monogr. Tokyo* **15**, 259 (1999).
2. G. Haas, *Bull. Mus. Natl. Hist. Nat. Ser. 4* **1**, 51 (1979).
3. _____, *Bull. Mus. Natl. Hist. Nat. Ser. 4* **2**, 87 (1980).
4. _____, in *Aspects of Vertebrate History*, L. L. Jacobs, Ed. (Museum of Northern Arizona Press, Flagstaff, AZ, 1980), pp. 177–202.

5. M. W. Caldwell and M. S. Y. Lee, *Nature* **386**, 705 (1997).
6. M. S. Y. Lee and M. W. Caldwell, *Philos. Trans. R. Soc. London Ser. B* **353**, 1521 (1998).
7. M. S. Y. Lee, *Biol. J. Linn. Soc.* **65**, 369 (1998).
8. J. D. Scanlon, M. S. Y. Lee, M. W. Caldwell, R. Shine, *Hist. Biol.* **13**, 127 (1999).
9. M. S. Y. Lee, G. L. Bell, M. W. Caldwell, *Nature* **400**, 655 (1999).
10. M. J. Cohn and C. Tickle, *Nature* **399**, 474 (1999).
11. H. Zaher, *J. Vertebr. Paleontol.* **18**, 1 (1998).
12. _____ and O. Rieppel, *Am. Mus. Novit.* **3271**, 1 (1999).
13. _____, *C.R.A.S. Sci. Terre* **329**, 831 (1999).
14. O. Rieppel and H. Zaher, *Feldiana Geol.* **43**, 1 (2000).
15. _____, *Zool. J. Linn. Soc.*, in press.
16. For anatomical details, see Science Online (www.sciencemag.org/feature/data/1047737.shl).
17. A. G. Kluge, *Misc. Publ. Mus. Zool. Univ. Michigan* **178**, 1 (1991).
18. The phylogenetic analysis was based on a data matrix compiled by H. Zaher and O. Rieppel. The matrix contains 89 cranial characters (to which up to 15 hypothetical limb characters were added) and 21 terminal taxa (16). The analysis was performed using the software package PAUP version 3.1.1 (D. Swofford, Laboratory of Molecular Systematics, Smithsonian Institution, 1993). With reference to earlier work (17–15, 17), the ingroup Serpentes was constrained to be monophyletic. Critical evaluation of currently available evidence (14) indicates two alternative potential sister groups of snakes among squamates, i.e., the amphisbaenian-dibamid clade [J.-C. Rage, *C. R. Acad. Sci. Paris* **294**, 563 (1982)] and the varanoid clade (*Lanthanotus*, *Varanus*, and mosasauroids) (7). We rooted the analysis on a varanoid outgroup, because varanoids appear to be more widely favored as a probable snake sister group [H. W. Greene, *Snakes* (Univ. of California Press, Berkeley, CA, 1997)]. We alternatively rooted the analysis on a dibamid outgroup, as well as on an hypothetical “all-0-ancestor,” with no effects relevant to this study. The heuristic search option implemented invariably used random stepwise addition (10 or 20 replications), and branch swapping (on minimal trees only) was effected by tree bisection and reconnection. All searches were run with all multistate characters unordered except for character 68 (ordered); all characters were informative. We obtained two equally parsimonious trees (a single tree with resolved scoleophidian relationships is obtained using dibamids as a root) with a tree length of 230 steps, an ensemble consistency index of 0.648, and a retention index of 0.796. Decay indices and bootstrap percentages (1000 replications) are given in Fig. 3. Constraining *Haasiophis* and *Pachyrhachis* to a basal position relative to all other snakes resulted in an increase of tree length by 18 steps. The Wilcoxon signed-rank test as implemented by Templeton rejects this alternative significantly by comparison to the most parsimonious solution ($T_s = 52, n = 26, P < 0.002$).
19. M. S. Y. Lee, M. W. Caldwell, J. D. Scanlon, *J. Zool. London* **248**, 509 (1990). These authors included *Pachyophis* (23) with *Pachyrhachis* in the Pachyophiidae. However, we interpret *Pachyophis* cautiously because of its preservation and incompleteness (14, 21, 23).
20. J. D. Scanlon and M. S. Y. Lee, *Nature* **403**, 416 (2000). This study adds the Tertiary madtsoiid genus *Wonambi* to the analysis of snake relationships. We disagree with many codings for *Pachyrhachis* (14, 15), and we disagree with many codings for *Wonambi* with reference to previously described material [D. J. Barrie, *Mem. Queensl. Mus.* **28**, 139 (1990)]. Final evaluation of the Scanlon and Lee report must await the monographic description of the new material of *Wonambi*.
21. J. C. Rage, in *Encyclopedia of Paleoherpptology*, P. Wellnhofer, Ed. (Fischer, Stuttgart, 1984), pp. 1–80.
22. A. E. Greer, *J. Herpetol.* **25**, 166 (1991).
23. F. Nopcsa, *Palaeontographica* **65**, 99 (1923).
24. We have greatly benefited from discussions with D. Cundall, H. W. Greene, R. Etheridge, N. C. Fraser, D. Frost, M. Kearney, A. G. Kluge, C. Bell, and J. Head in the formulation of the ideas presented in this paper.

7 December 1999; accepted 4 February 2000