A Fossil Snake with Limbs
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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
9. Synthesis of N-azidocetylmannosamine (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 h 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 h. The reaction was concentrated with saturated NaHCO3 and concentrated, and the residue was filtered through a plug of silica gel eluting with 5:1 CHCl3/Methanol. The crude product obtained was dissolved in dimethylformamide (10 ml) and NaNO2 (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 (1.01 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 treated with CH3CN (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 1:1 to 1:2 EtOAc/hexanes to afford 39 mg (95%) of acetylated 3.
10. 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 mosasaurid jaws provided the starting point for the evolution of the opisthodont 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., macrostomatian) snakes (11–13).

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 macrostomatian affinities of these fossil snakes.

Haastiospis, gen. nov.
Genotypic species: Haastiospis terrasmactus, 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 and narrow, formed by coronoid bone only; 15 preoccipital vertebral; at least 12 proximal caudal vertebrae with distally expanded and bifurcated lymph-
apophyses; expanded hemapophyses on posterior tail vertebrae.

Distribution: Early Upper Cretaceous, Middle East.

Haasiophis terrasanctus, sp. nov.
Holotype: Hebrew University of Jerusalem, Paleontological Collections, HUJ-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 foramina 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) displays relatively primitive characters, such as are present in anilioids (pipersnakes) 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
as 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 macrostomatan (i.e., near pythons and booids). 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 macrostomatan, as well as the sister-group relationship of Haasiophis and Pachyrhachis, remain weakly supported, probably because of their divergent specialization. Boine 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. Pythoidean 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 styllopodial and zeugopodial limb elements. Haasiophis therefore cannot represent a juvenile specimen of the larger Pachyrhachis.

Given the relationships of Pachyrhachis and Haasiophis to macrostomatan, 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 macrostomatan 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 macrostomatan.

As macrostomatan snakes, Haasiophis and Pachyrhachis have no particular bearing on snake-mosasaourid relationships or snake origins. Instead, they represent the first unequivocal documentation of the incursion of macrostomatan snakes into the sea. Basal snakes—including basal macrostomatan—retain rudimentary hindlimbs, which, however, remain much more incomplete than those of Haasiophis. With Haasiophis and Pachyrhachis related to basal macrostomatan, 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
16. For anatomical details, see Science Online (www.sciencemag.org) (10/47373.shl).
18. The phylogenetic analysis was based on a data matrix compiled by H. Zehrer 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 considered monophyletic. Critical evaluation of currently available evidence (14) indicates two alternative potential sister groups of snakes among squamates, i.e., the amphibiomorph-bibibrachial clade [J.-C. Rage, C. R. Acad. Paris 294, 563 (1982)] and the varanoid clade (Lanthanotus, Varanus, and mosasaourids) (7). We rooted the analysis on a varanoid outgroup, because varanoids appear to be more widely favored as a probable snake 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-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 scolecophidian 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 = 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).
20. J. D. Scanlon and M. S. Y. Lee, Nature 403, 416 (2000). This study adds the Tertiary madidous 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.
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.
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