

This is an electronic appendix to the Biology Letter by Vidal & Hedges 2004 Molecular evidence for a terrestrial origin of snakes. *Proc. R. Soc. Lond. B (Suppl.)* **271**, S226–S229. (DOI 10.1098/rsbl.2003.0151.)

Electronic appendices are refereed with the text. However, no attempt has been made to impose a uniform editorial style on the electronic appendices.

Electronic Appendix A

(Molecular Evidence for a Terrestrial Origin of Snakes)

Materials and Methods

Tissue samples (tissue homogenate, liver, blood, tail tip, or shed skin) were obtained from the tissue collections of Nicolas Vidal and S. Blair Hedges (see next section for list of taxa, localities, and accession numbers). DNA extraction was performed using Winnepenninckx et al.'s protocol (Winnepenninckx et al. 1993) or using the Nucleospin tissue kit from Biotech. Amplification was performed using the following sets of primers: G73, 5'-GCG-GTA-AAG-CAG-GTG-AAG-AAA-3', G74, 5'-TGA-GCA-TCC-AAA-GTC-TCC-AAT-C-3', G78, 5'-AGR-GTG-ATR-WCA-AAN-GAR-TAR-ATG-TC-3' (Saint et al. 1998) and L39, 5'-CTG-SAR-YTT-TCT-YCA-TCT-GT-3' (Vidal & Hedges 2002) for the C-mos gene; and L2408, 5'-TGCACTGTGACATTGGCAA-3', H2928, 5'-GACTGCYTG GCATTCATTTT-3' and H2920, 5'-GCCATTCATTTTYCGAA-3' for the RAG1 gene. Both strands of the PCR products were sequenced using the BigDye sequencing kit (Applied Biosystems) in the ABI Prism 3100-Avant Genetic Analyser. The two strands obtained for each sequence were aligned using the BioEdit Sequence

Alignment Editor program (Hall 1999). Sequence entry and alignment (64 taxa) were performed manually with the MUST2000 software (Philippe 1993). For the C-mos gene, amino acid properties were used while there were no indels for the RAG1 gene, resulting in 378 C-mos sites (252 variable sites, 217 of which are informative for parsimony) and 519 RAG1 sites (271 variable sites, 223 of which are informative for parsimony). In all further analyses, gaps were treated as missing data. We built phylogenies using Minimum Evolution (ME), Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian methods of inference. ME, MP, and ML analyses were performed with PAUP*4 (Swofford 1998). Bayesian analyses were performed with MrBayes 2.1 (Huelsenbeck & Ronquist 2001). For the model-based methods (ME, ML, and Bayesian), an appropriate model of sequence evolution was inferred using ModelTest (Posada & Crandall 1998). For the C-mos gene, this resulted in the selection of the HKY85+I+G model with a ti/tv ratio of 2.6386, a gamma parameter of 2.6233, a proportion of invariant sites of 0.2929, and base frequencies as A = 0.2792, C = 0.2212, G = 0.2235 and T = 0.2761. For the RAG1 gene, this resulted in selection of the GTR+I+G model with substitution parameters as A-C = 1.6814, A-G = 5.8987, A-T = 0.8247, C-G = 1.1577, and C-T = 7.9477, a proportion of invariant sites of 0.4039, a gamma parameter of 1.4615, and base frequencies as A = 0.349, C = 0.1872, G = 0.1999, and T = 0.2638. For the concatenated C-mos/RAG1 data set, this resulted in the selection of the TrN+I+G model with substitution parameters as A-C/A-T/C-G = 1, A-G = 5.2024, and C-T = 6.2819, a proportion of invariant sites of 0.3622, a gamma parameter of 1.9264, and base frequencies as A = 0.3202, C = 0.2091, G = 0.2098, and T = 0.2609. Bayesian analyses were run with model parameters estimated as part of the Bayesian analyses, and the best-fit model as inferred by Modeltest. For MP analyses, all sites were weighted equally. ME,

MP, and ML results are presented under the form of bootstrap consensus trees, which are considered to be reliable estimates of phylogeny (Nei & Kumar 2000) (2000, 2000, and 100 replicates respectively, random option with TBR branch swapping for MP analyses; NJ starting tree both for ME and ML analyses with TBR branch swapping for ME analyses and NNI branch swapping for ML analyses). Bayesian analyses were performed by running 1,000,000 generations in four chains, saving the current tree every 10 generations. The last 65,000 trees were used to construct a 50% majority rule consensus tree. Bayesian posterior probabilities may represent overestimates of the actual support for relationships (Suzuki et al. 2002; Cummings et al. 2003).

Tissue samples and sequence data used

The 70 new sequences have been deposited in GenBank under Accession Numbers AY487346 to AY487414, and AY491999. They include 64 RAG1 sequences from all taxa and six C-mos sequences from *Amphisbaena cubana*, *Anniella pulchra*, *Dibamus novaeguinea*, *Heloderma suspectum*, *Rhineura floridana*, and *Trachyboa gularis*. All are listed below.

The following list identifies the sequence accession number of all samples. For new sequences, the accession number is followed by the collection number of the sample and the locality. Collection numbers have the following prefixes: LACM (Los Angeles County Museum), MNHN (National Museum of Natural History, France), MVZ (University of California Museum of Vertebrate Zoology, Berkeley), NV (Nicolas Vidal, ethanol preserved tissue collection), SBH (S. Blair Hedges, frozen tissue collection), and

USNM (United States National Museum, Smithsonian, Washington, D.C.). The family-level classification follows Zug et al. (2001).

Chelonia: RAG1: *Pelusios subniger* (AY487412, SBH191580, locality unknown), C-mos: *Pelomedusa subrufa* (AF109208), **Sphenodontia:** *Sphenodon punctatus* (AY487362, SBH266085, New Zealand), *Sphenodon punctatus* (AF039483), **Squamata:** **Xantusiidae:** *Xantusia vigilis* (AY487366, LACM 136813, California, San Bernadino County, Hesperia), *Xantusia vigilis* (AF148703), **Amphisbaenidae:** *Amphisbaena cubana* (AY487365, AY487346, SBH161956, Cuba, Guantánamo Bay Naval Station), **Rhineuridae:** *Rhineura floridana* (AY487361, AY487347, SBH172913, Florida, Plant City), **Bipedidae:** *Bipes biporus* (AY487358, MVZ137543, Baja California Sur), *Bipes biporus* (AF039482), **Trogonophidae:** *Trogonophis wiegmanni* (AY487355, MVZ11124, North Africa), *Diplometopon zarudnyi* (AF148709), **Lacertidae:** *Lacerta hispanica* (AY487363, SBH266666, Morocco, Asilah), *Lacerta kulzeri* (AF148712), **Teiidae:** *Ameiva auberi* (AY487359, USNM306540, Cuba, Guantánamo, 8.9 km SW Hatibonico), *Kentropyx calcarata* (AF420864), **Gymnophthalmidae:** *Gymnophthalmus underwoodi* (AY487364, SBH102274, Guadeloupe, Playa Anse Laborde), *Gymnophthalmus leucomystax* (AF420824), **Agamidae:** *Agama agama* (AY487356, SBH194522, locality unknown), *Agama agama* (AF137530), **Chamaeleonidae:** *Chamaeleo senegalensis* (AY487370, SBH266079, locality unknown), *Chamaeleo jacksonii* (AF137528), **Iguanidae:** *Sceloporus undulatus* (AY487367, SBH266667; Arizona), *Sceloporus grammicus* (AF039478), **Gekkonidae:** Gekkoninae: *Gecko gecko* (AY487368, SBH266077, locality unknown), *Tarentola mauritanica* (AF363566); Pygopodinae: *Lialis burtonis* (AY487353, SBH266057, Papua New Guinea, Wipim),

Lialis burtonis (AF090850), **Cordylidae**: *Cordylus giganteus* (AY487371, SBH266055, South Africa), *Cordylus cordylus* (AF148711), **Scincidae**: *Tiliqua scincoides* (AY487414, NV, Indonesia), *Tiliqua scincoides* (AF039462); *Eumeces inexpectatus* (AY487413, SBH191579, Florida), *Eumeces skiltonianus* (AF315396), **Helodermatidae**: *Heloderma suspectum* (AY487352, AY487348, SBH194118, locality unknown), **Dibamidae**: *Dibamus novaeguinea* (AY487360, AY487349, SBH266054, Philippines), **Anguidae**: *Diploglossus delasagra* (AY487372, SBH191541, Cuba, Pinar del Río, San Vicente), *Anguis fragilis* (AY099972); *Anniella pulchra* (AY487357, AY487350, SBH194106, California, Santa Barbara County, Santa Maria), **Varanidae**: *Varanus dumerilii* (AY487354, SBH266058, locality unknown), *Varanus rosenbergi* (AY099976), **Xenosauridae**: *Shinisaurus crocodilurus* (AY487369, SBH194119, locality unknown), *Shinisaurus crocodilurus* (AY099976), **Leptotyphlopidae**: *Leptotyphlops columbi* (AY487383, SBH192936, Little Fortune Hill, San Salvador, Bahamas), *Leptotyphlops columbi* (AF544718), **Typhlopidae**: *Typhlops lumbricalis* (AY487387, SBH191018, La Fangosa, Guantánamo, Cuba), *Typhlops jamaicensis* (AF544733); *Ramphotyphlops braminus* (AY487410, NV, Phang-Nga City, Muang District, Phang-Nga Province, Thailand), *Ramphotyphlops braminus* (AF544717), **Aniliidae**: *Anilius scytale* (AY487382, SBH 267100, locality unknown), *Anilius scytale* (AF544722), **Tropidophiidae**: *Tropidophis melanurus* (AY487384, SBH172610, Soroa, Pinar del Rio, Cuba), *Tropidophis melanurus* (AF544725); *Trachyboa gularis* (AY487409, AY491999, SBH194899, locality unknown), **Boidae**: *Acrantophis madagascariensis* (AY487401, NV, captive born), *Acrantophis madagascariensis* (AF544707); *Boa constrictor* (AY487351, SBH266668, unknown locality), *Boa constrictor* (AF544676); *Calabaria reinhardtii* (AY487391, NV, Togo), *Calabaria*

reinhardtii (AF544682); *Candoia carinata* (AY487402, NV, Halmahera Island, Indonesia), *Candoia carinata* (AF544674); *Charina bottae* (AY487390, NV, captive born), *Charina bottae* (AF544681); *Eryx miliaris* (AY487393, NV, unknown locality), *Eryx miliaris* (AF544683); *Gongylophis colubrinus* (AY487392, NV, captive born), *Gongylophis colubrinus* (AF544716); *Lichanura trivirgata* (AY487394, NV, captive born), *Lichanura trivirgata* (AF544687); *Ungaliophis continentalis* (AY487407, SBH1946421, locality unknown), *Ungaliophis continentalis* (AF544724), **Pythonidae**: *Apodora papuana* (AY487405, NV, Irian Jaya), *Apodora papuana* (AF544720); *Liasis savuensis* (AY487397, NV, Savu Island, Indonesia), *Liasis savuensis* (AF544726); *Python reticulatus* (AY487396, NV, captive born), *Python reticulatus* (AF544675), **Xenopeltidae**: *Xenopeltis unicolor* (AY487400, NV, CUB MZ R 1998.12.11.30, Ban Salakern, Ban Lat District, Phetchaburi Province, Thailand), *Xenopeltis unicolor* (AF544689), **Loxocemidae**: *Loxocemus bicolor* (AY487406, HGD145976, “Mexico”), *Loxocemus bicolor* (AF544730), **Bolyeriidae**: *Casarea dussumieri* (AY487408, SBH267099, Round Island), *Casarea dussumieri* (AF544731), **Uropeltidae**: *Rhinophis drummondhayi* (AY487386, SBH194102, north of Namunukula, Pindarawatta, Sri Lanka), *Rhinophis drummondhayi* (AF544719); *Uropeltis melanogaster* (AY487399, NV, Kardy, Sri Lanka), *Uropeltis phillipsi* (AF471100), **Acrochordidae**: *Acrochordus granulatus* (AY487388, NV, MNHN 1997.6576, Ko Mai Phai Island, Muang District, Phang-Nga Province, Thailand), *Acrochordus granulatus* (AF544706), **Xenodermatidae**: *Stoliczkaia borneensis* (AY487398, NV, Crocker Range National Park, Sabah, East Malaysia), *Stoliczkaia borneensis* (AF544721), **Viperidae**: *Bothriechis schlegelii* (AY487374, NV, captive born), *Bothriechis schlegelii* (AF544680), **Elapidae**: *Bungarus fasciatus* (AY487389, NV, “Malaysia”), *Bungarus fasciatus* (AF544732); *Dendroaspis*

angusticeps (AY487395, NV, Southeast Tanzania), *Dendroaspis angusticeps* (AF544735); *Elapsoidea semiannulata* (AY487373, NV, Central African Republic), *Elapsoidea semiannulata* (AF544678); *Laticauda colubrina* (AY487404, NV, MNHN 1999.7692, Ko Khai Nai Island, Ko Yao District, Phang-Nga Province, Thailand), *Laticauda colubrina* (AF544702); *Micrurus surinamensis* (AY487411, NV, RN1 road, pk 68, French Guiana), *Micrurus surinamensis* (AF544708), **Lamprophiidae**: *Lamprophis fuliginosus* (AY487378, NV, Burundi), *Lamprophis fuliginosus* (AF544686); *Leioheterodon madagascariensis* (AY487377, NV, Madagascar), *Leioheterodon madagascariensis* (AF544685); *Mehelya capensis* (AY487379, NV, Burundi), *Mehelya capensis* (AF544703); *Psammophylax variabilis* (AY487380, NV, Burundi), *Psammophylax variabilis* (AF544709), **Dipsadidae**: *Diadophis punctatus* (AY487403, NV, USA), *Diadophis punctatus* (AF544705); *Leptodeira annulata* (AY487375, NV, Kaw, French Guiana), *Leptodeira annulata* (AF544690); *Alsophis cantherigerus* (AY487376, NV, Cuba), *Alsophis cantherigerus* (AF544694), **Colubridae**: *Hapsidophrys smaragdina* (AY487381, NV, MNHN 1997.6516, Ivindo River, Ogooué, Gabon), *Hapsidophrys smaragdina* (AF544691); *Phyllorhynchus decurtatus* (AY487385, SBH266669, locality unknown), *Phyllorhynchus decurtatus* (AF544728).

Sequence alignment

The aligned sequence data for both genes is available at this location:

<http://www.evogenomics.org/Publications/Data/SnakeOrigin/>

Supplementary Figures. Consensus bootstrap trees obtained from ML (Figure 3), MP (Figure 4) and ME (Figure 5) analyses (bootstrap confidence values (>50%) are shown at nodes).

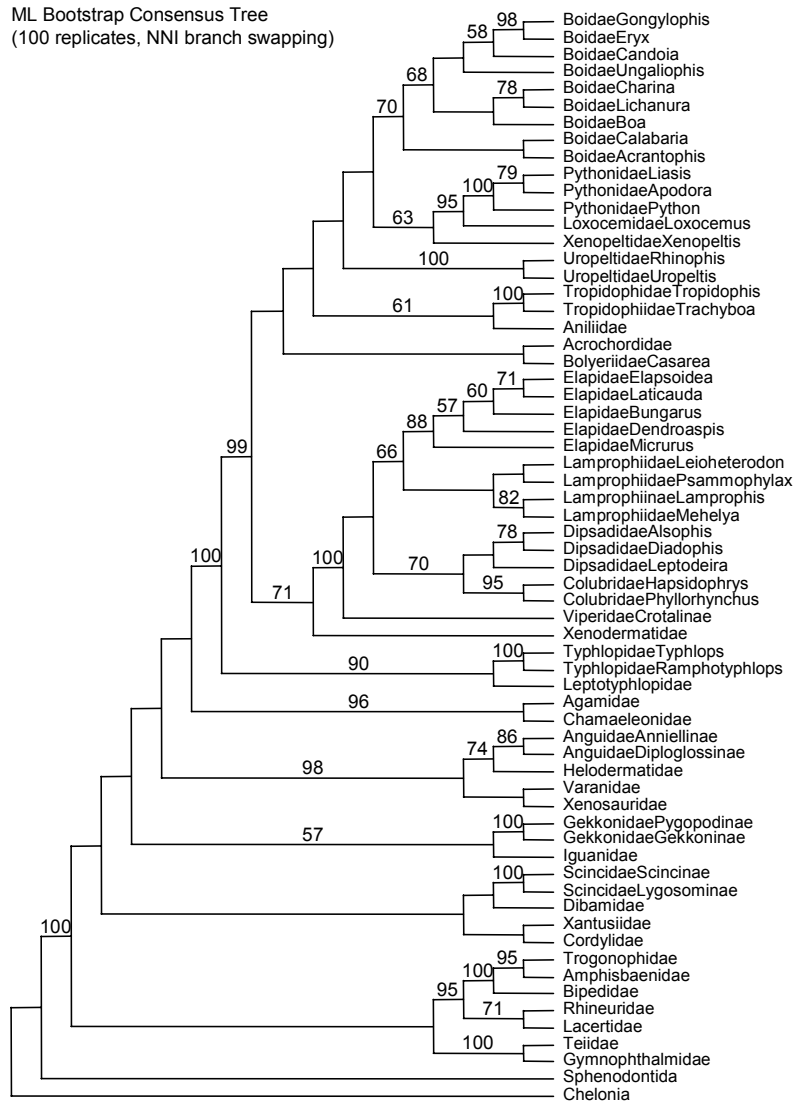


Figure 3

MP Bootstrap Consensus Tree
(2000 replicates, TBR branch swapping)

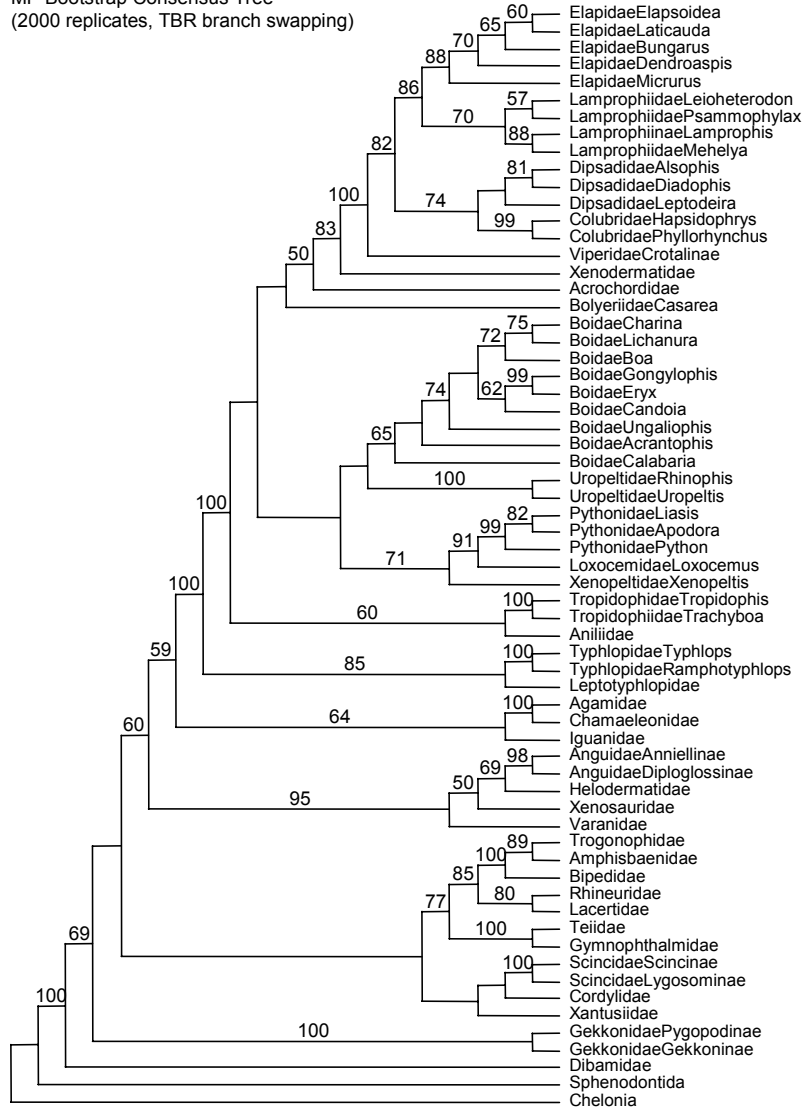


Figure 4

ME Bootstrap Consensus Tree
 (2000 replicates, TBR branch swapping)

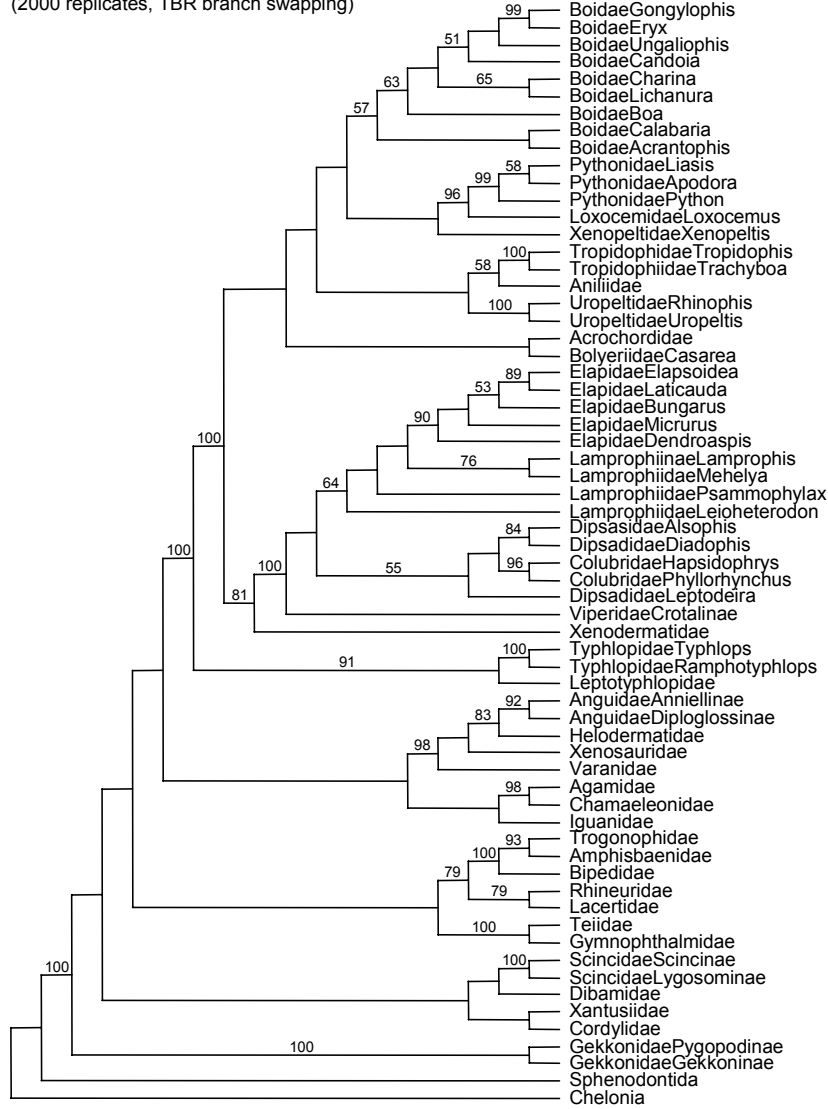


Figure 5

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