Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support

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Abstract

Four New World genera of dwarf boas (*Exiliboa*, *Trachyboa*, *Tropidophis*, and *Ungaliophis*) have been placed by many systematists in a single group (traditionally called Tropidophiidae). However, the monophyly of this group has been questioned in several studies. Moreover, the overall relationships among basal snake lineages, including the placement of the dwarf boas, are poorly understood. We obtained mtDNA sequence data for 12S, 16S, and intervening tRNA–val genes from 23 species of snakes representing most major snake lineages, including all four genera of New World dwarf boas. We then examined the phylogenetic position of these species by estimating the phylogeny of the basal snakes. Our phylogenetic analysis suggests that New World dwarf boas are not monophyletic. Instead, we find *Exiliboa* and *Ungaliophis* to be most closely related to sand boas (Erycinae), boas (Boinae), and advanced snakes (Caenophidea), whereas *Tropidophis* and *Trachyboa* form an independent clade that separated relatively early in snake radiation. Our estimate of snake phylogeny differs significantly in other ways from some previous estimates of snake phylogeny. For instance, pythons do not cluster with boas and sand boas, but instead show a strong relationship with *Loxocemus* and *Xenopeltis*. Additionally, uropeltids cluster strongly with *Cylindrophis*, and together are embedded in what has previously been considered the macrostomatan radiation. These relationships are supported by both bootstrapping (parametric and nonparametric approaches) and Bayesian analysis, although Bayesian support values are consistently higher than those obtained from nonparametric bootstrapping. Simulations show that Bayesian support values represent much better estimates of phylogenetic accuracy than do nonparametric bootstrap support values, at least under the conditions of our study.
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Keywords: Bayesian analysis; Bootstrapping; Phylogenetic support; Phylogeny; Dwarf boas; Snakes; Serpentes; Tropidophiidae; Ungaliophiidae; Booidea; Macrostomata; Caenophidea; *Exiliboa*; *Trachyboa*; *Tropidophis*; *Ungaliophis*

1. Introduction

Four New World genera of dwarf boas (*Exiliboa*, *Trachyboa*, *Tropidophis*, and *Ungaliophis*) traditionally have been placed in a single group of snakes, the Tropidophiidae. About 21 species of these small terrestrial and arboreal snakes are distributed in the West Indies (Greater Antilles and Bahama Islands) and in isolated areas of Central and South America. The biology of species in this group is very poorly known, and the phylogenetic relationships of dwarf boas to other snakes are not well resolved.

Part of the uncertainty surrounding the relationships of dwarf boas is a result of the poor understanding of basal snake lineages in general. Most recent authors support a primary division in snakes between Scolecophidea (the blind snakes) and Alethinophidea (all other snakes), and most authors also recognize the groups Macrostomata, Caenophidea, and Colubroidea (Fig. 1a). However, there is considerable disagreement among authors about the relationships within Macrostomata. Some authors (Cundall et al., 1993; Groombridge, 1979; Rieppel, 1988) recognize various subsets of *Loxocemus*, *Xenopeltis*, Boinae, Erycinae, Pythonidae, Bolyeriidae, and Tropidophiidae as a monophyletic Booidea (or Henophidia), whereas other evidence (e.g., Kluge, 1991, 1993) suggests that the "booids" are a paraphyletic group of basal macrostomatans. Several recent summaries of

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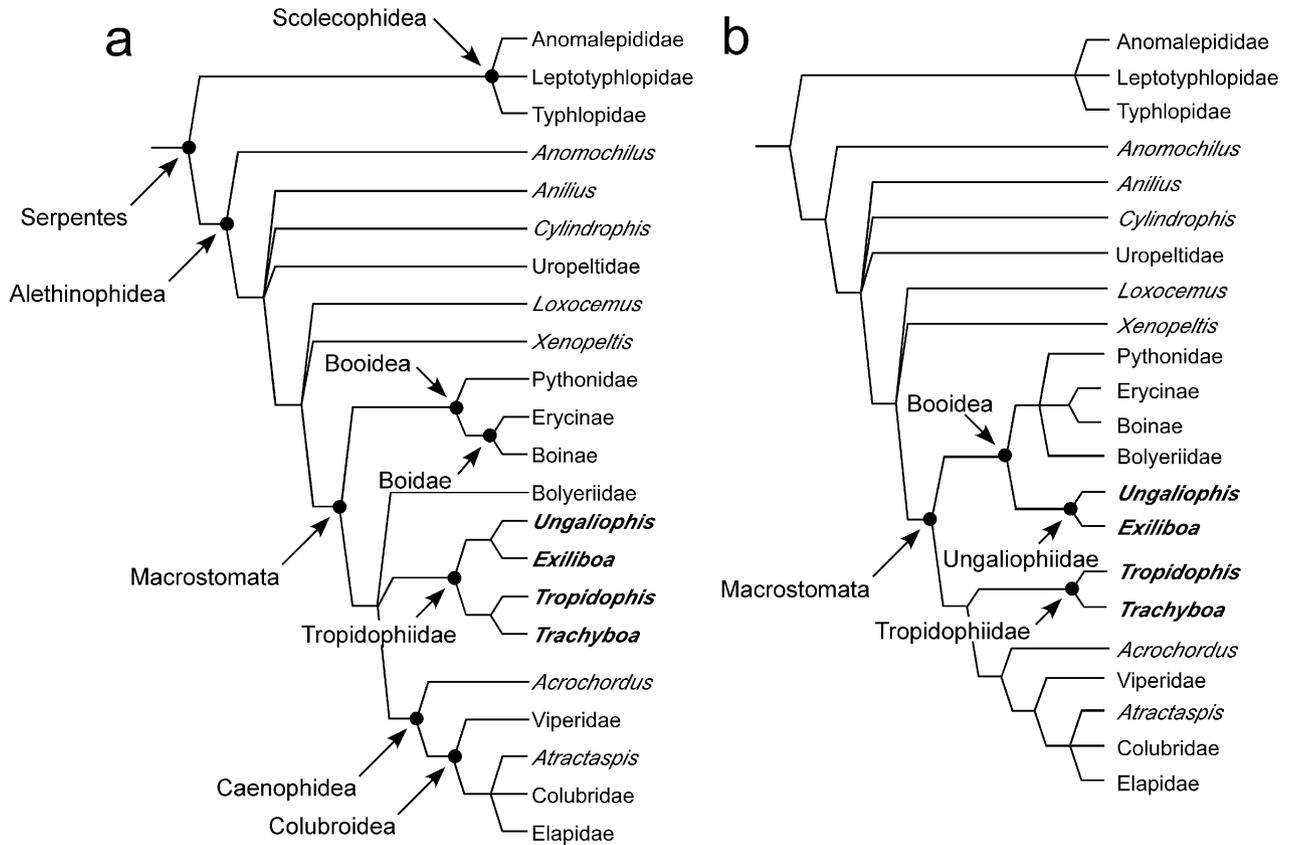
Fig. 1. Hypothesized relationships among the major snake Lineages. (a) Traditional phylogeny after Greene (1997). (b) Traditional phylogeny as modified by Zaher (1994). Taxa in bold were traditionally placed in the Tropidophiidae.

snake phylogeny have suggested that dwarf boas (all or a subset) are relatively closely related to Caenophidea—the group that includes most of world's snakes, including colubrids, elapids, and viperids (Greene, 1997; Kluge, 1991; Pough et al., 1998). Nonetheless, as noted by Pough et al. (1998), all alternative phylogenetic hypotheses for basal snakes are weakly supported at present.

Even the monophyly of Tropidophiidae is not well supported. Dessauer et al. (1987) presented immunological data that suggested that tropidophiids were not monophyletic. Zaher (1994) argued on the basis of morphological evidence that *Trachyboa* and *Tropidophis* form the sister-group to Caenophidea, but that *Exiliboa* and *Ungaliophis* are more closely related to other groups that he considered part of Booidea (erycines, boines, pythonids, and bolyeriids; Fig. 1b). As a result, Zaher (1994) placed *Exiliboa* and *Ungaliophis* in Ungaliophiidae, and considered Tropidophiidae to contain only *Trachyboa* and *Tropidophis* (Fig. 1b).

To address the relationships of the dwarf boas we obtained sequences from three mitochondrial genes (12S and 16S ribosomal RNA genes, and the valine tRNA gene) from representatives of *Exiliboa*, *Ungaliophis*, *Trachyboa*, and four species of *Tropidophis*, as well as from representatives of most of the snake families.

Specifically, we wished to test the following three hypotheses suggested by Zaher (1994):

1. *Exiliboa* and *Ungaliophis* form a monophyletic group (Ungaliophiidae), and are not closely related to Tropidophiidae *sensu* Zaher (*Trachyboa* and *Tropidophis*).
2. *Trachyboa* and *Tropidophis* together form the sister-group of Caenophidea.
3. *Exiliboa* and *Ungaliophis* are part of a monophyletic Booidea that includes boines, erycines, and pythonids, but not *Loxocemus* or *Xenopeltis*.

In testing these phylogenetic hypotheses, we compared several commonly used methods for assessing phylogenetic support. To test our specific a priori hypotheses, we used parametric bootstrapping to perform likelihood-ratio tests of the alternative hypotheses (Goldman et al., 2000; Hillis et al., 1996b; Huelsenbeck et al., 1996). However, we also assessed support for other groups that we found during the course of our study. The two methods that we used for this purpose, nonparametric bootstrapping (Felsenstein, 1985) and Bayesian analysis (Larget and Simon, 1999; Rannala and Yang, 1996), supported the same groups. However, the Bayesian support values were consistently higher than the values obtained from bootstrapping—a pattern that we, and others, have noticed across several phylo-

genetic analyses (e.g., Buckley et al., 2002; Leaché and Reeder, 2002; Whittingham et al., 2002). Nonparametric bootstrapping is known to produce highly conservative estimates of phylogenetic accuracy (Hillis and Bull, 1993). To assess if Bayesian support values are better measures of phylogenetic accuracy than those obtained through nonparametric bootstrapping, we conducted a simulation study using our estimated snake phylogeny as a model tree.

2. Materials and methods

2.1. Samples, DNA extraction, and sequencing

We obtained tissues (liver, muscle, or blood) from 23 species of snakes, representing most of major snake lineages (Greene, 1997): *Acrochordus javanicus* (UTA 17064), *Anilius scytale* (LSUMNS H-8033), *Azemiops feae* (UTA 18701), *Boa constrictor* (UTA 24752), *Crotalus polystictus* (UTA 14514), *Cylindrophis ruffus* (UTA 24902), *Eryx conicus* (TNHC 61448), *Exiliboa placata* (UTA 12233), *Leptotyphlops dulcis* (TNHC 61447), *Loxocemus bicolor* (UTA 14494), *Morelia boeleni* (UTA 11396), *Pituophis lineaticolis* (UTA 25116), *Rhinophis philippinus* (LSUMNS H-6179), *Trachyboa boulengeri* (UTA 12958), *Tropidophis feicki* (POE 89), *Tropidophis greenwayi* (JAC 9649), *Tropidophis pardalis* (KdQ 2020), *Tropidophis melanurus* (KdQ 2018), *Typhlops jamaicensis* (TNHC 61449), *Typhlops ruber* (PNM/CMNH H-1538), *Ungaliophis continentalis* (UTA 12239), *Uropeltis melanogaster* (LSUMNS H-5696), and *Xenopeltis unicolor* (MVZ 226505). Definitions for abbreviations for museum/tissue collections are given in Acknowledgments. *Xenophidion*, a Malaysian genus known only from holotypes, may also be allied with the Tropidophiidae (Günther and Manthey, 1995; Wallach and Günther, 1998) but tissues were not available for analysis. We did not include representatives of Anomalepididae, *Anomochilus*, Bolyeriidae, Elapidae, or *Atractaspis*.

DNA was extracted from tissues using either the DNeasy Tissue Extraction Kit (Qiagen) or standard phenol:chloroform extraction of SDS/proteinase K-treated tissues (Hillis et al., 1996a). Using a series of nested primers (Fig. 2), we then amplified approximately 1.9 kb of mitochondrial DNA, spanning portions of the 12S and 16S genes and the intervening valine–tRNA (corresponding to positions 2475–4574 in the complete mitochondrial sequence of *Xenopus laevis* [GB M10217]). Standard PCR conditions were used (Palumbi, 1996) with the following thermal cycle profile: 2 min at 94 °C, followed by 35 cycles of: (94 °C for 30 s, 42 °C for 30 s, and 72 °C for 1 min). Amplification products were purified from agarose gel slices using the Qiaquick Gel Extraction Kit (Qiagen) and sequenced using fluorescent thermal cycle sequencing and an ABI 377 automated sequencer (Perkin–Elmer).

2.2. Phylogenetic analyses

Sequences were aligned using Clustal W (Thompson et al., 1994) and adjusted to accommodate conserved secondary structures (Cannone, 2002; http://www.rna.icmb.utexas.edu/). Aligned sequences were analyzed with PAUP* (version 4.0b8; Swofford, 2000) using maximum-likelihood and a GTR + Γ + PINVAR model of sequence evolution with four Γ-distributed rate classes (for parameter values see Appendix B) (Swofford et al., 1996). This model of sequence evolution was chosen based on results from Modeltest (v3.06, Posada and Crandall, 1998). Tree searches were conducted via TBR branch-swapping (Swofford et al., 1996) on five stepwise-addition trees (assembled in random order of taxa). We estimated initial model parameters on maximum parsimony trees and then refined the parameters via successive approximation on trees recovered using likelihood (Swofford et al., 1996). These final model parameters were used in all successive analyses and simulations.

We assessed support for each branch using both bootstrap and Bayesian analysis. Nonparametric bootstrap proportions (nbp; Felsenstein, 1985) were estimated from 100 pseudo-replicate datasets analyzed using maximum-likelihood. Bayesian posterior probabilities (bpp) were estimated as the proportion of trees sampled after burn-in that contained each of the observed bipartitions (Larget and Simon, 1999). Analyses were performed with MrBayes (v2.01; Huelsenbeck and Ronquist, 2001), with

a) 12L1 - AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT
b) 12sm - GGCAAGTCGTAACATGGTAAG
c) 16sc - GTRGGCCTAAAAGCAGCCAC
d) 16sh - GCTAGACCATKATGCAAAAGGTA
e) 16sa - ATGTTTTTGGTAAACAGGCG
f) 16H1 - CTCCGGTCTGAACTCAGATCACGTAGG
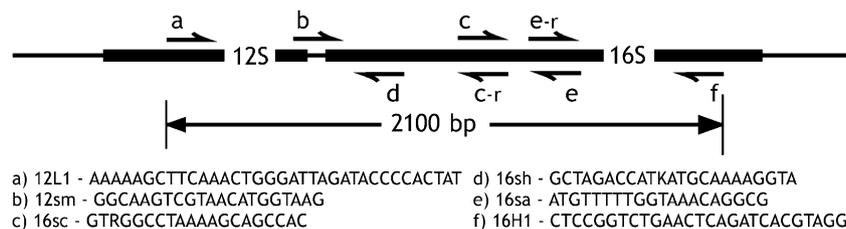
Fig. 2. Primers used to amplify the mtDNA 12S–16S region from 23 species of snakes. The two reverse primers (designated by -r) were only used in sequencing. All others were used in various combinations for PCR amplification. Primer descriptions can be found in Goebel et al. (1999). Base-pair distances are based on the *Xenopus* complete mitochondrial sequence (GB M10217).

GTR + Γ + PINVAR parameters being estimated during the run, and using the default value of four Markov chains. Multiple chains can assist in more easily traversing tree-space and help avoid entrapment in local topological optima. The "temperature" parameter was set to 0.2, resulting in incremental heating of each chain. Higher temperature values result in greater differences in heating between chains, and hotter chains are less constrained by likelihood scores in moving through tree-space. We found that a temperature parameter of 0.2 allowed for more rapid convergence. The Monte Carlo Markov chain (MCMC) length was 10^6 generations, and we sampled the chain every 100 generations. Log-likelihood values for sampled trees stabilized after approximately 200,000 generations. Therefore, we used the last 5000 sampled trees to estimate Bayesian posterior probabilities, also called Bayesian support values. If $\geqslant 95\%$ of the sampled trees contained a given clade, we considered it to be significantly supported by our data.

2.3. Hypothesis testing

We used parametric bootstrap analysis (Hillis et al., 1996b; Huelsenbeck et al., 1996) to test our three main phylogenetic hypotheses. Parametric bootstrapping is preferable over other tree comparison methods that are prone to type II statistical error (Goldman et al., 2000). Model trees (Appendix A) were estimated using maximum-likelihood searches with taxa constrained to be compatible with each hypothesis. For each model tree (with branch lengths) we used the model of sequence evolution estimated above (see Appendix B) to generate

100 replicate simulated datasets of the same size as the original dataset (1545 aligned sites). Sequence evolution was modelled using a version of Siminator (Huelsenbeck, 1995) modified by M.T. Holder to allow use of the GTR + Γ + PINVAR model of sequence evolution. Two heuristic searches were conducted on each replicate dataset: once to find the overall optimal tree and again to find the best tree compatible with the constraint used to generate the model tree. Scores of these likelihood trees were then used to construct an expected distribution of likelihood differences under the null hypothesis being tested. Significance of the test statistic (the difference in log-likelihood between the constrained and optimal trees) was assessed by direct comparison to the expected distribution (Goldman et al., 2000; Hillis et al., 1996b).

2.4. Simulations

The accuracy of Bayesian branch support values was assessed by simulation experiment using an approach similar to that used by Hillis and Bull (1993) in their analysis of nonparametric bootstrapping. We simulated 120 datasets of 500 characters each using Seq-Gen (Rambaut and Grassly, 1997), based on our maximum-likelihood estimated tree and associated model parameters (Fig. 3 and Appendix B). To obtain a broad distribution of support values, we limited our simulated data to 500 bp in length (at sequence lengths similar to our original dataset, almost all bipartitions were supported in 100% of trees sampled). These datasets were analyzed using MrBayes with the same MCMC
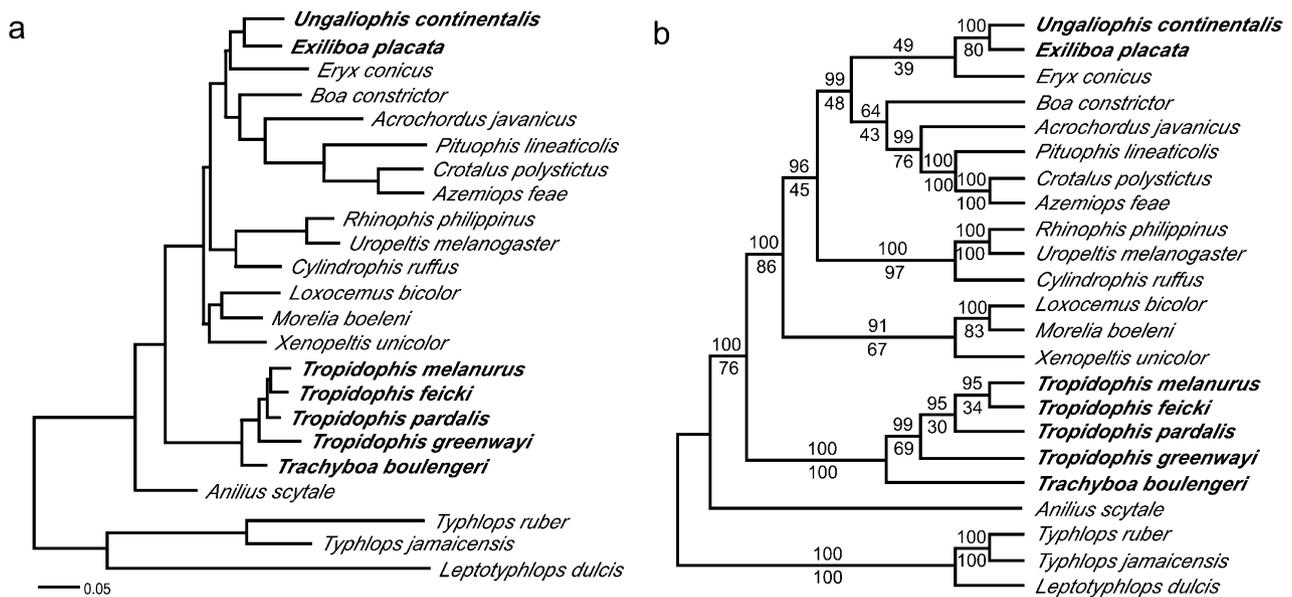
Fig. 3. Maximum-likelihood tree recovered from analysis of 1545 bp of mitochondrial DNA sequence spanning the 12S and 16S genes. Taxa in bold traditionally were placed in Tropidophiidae. (a) Tree shown with branch lengths proportional to estimated divergence. (b) Tree shown with support values from Bayesian analysis (above the branches) and nonparametric bootstrapping (below the branches).

parameters used in analysis of the observed data. Each dataset was run for 100,000 generations to allow adequate time for convergence. We sampled the Markov chain every 100 generations after the initial burn-in period of 80,000 generations.

These same datasets were then subjected to likelihood-based nonparametric bootstrap analysis (Felsenstein, 1985). Bootstrap values were generated for all bipartitions obtained from analyses of pseudo-replicate datasets. Parsimony searches on each bootstrap replicate were heuristic, with five stepwise-addition starting trees of random-addition order, followed by TBR branch-swapping, and without swapping on multiple trees.

We then examined the accuracy of Bayesian posterior probabilities, as well nonparametric bootstrap proportions, as estimates of the probability of reconstructing the correct bipartitions. Bipartitions were grouped according to their support values in bins with 10% intervals. Frequencies of true bipartitions in each bin were then calculated based on presence or absence of the respective bipartition in the model tree, and then compared to respective support values from Bayesian and nonparametric bootstrap analysis.

3. Results

Our mitochondrial DNA sequences for the ribosomal 12S and 16S genes and the intervening tRNA–val are deposited in GenBank (Accession Nos. AF12726–12748). Sequences ranged from 1747 to 1850 bp in length, and were AT-biased (mean %AT = 57.8). Examination of sequences for the presence of conserved nucleotide sequences and secondary structural elements, and BLAST searches against GenBank, indicated that all sequences were functional mitochondrial sequences from snakes. Aligned sequences were adjusted to accommodate ribosomal RNA secondary structure (Cannone et al., 2002), but due to considerable length differences in known highly variable regions (Cannone et al., 2002), we could not confidently assign nucleotide homology for approximately 300–400 bp of the sequences (depending on the individual sequence). These regions were excluded from the phylogenetic analyses. Our aligned dataset has been deposited in TreeBase (http://www.treebase.org/treebase/index.html; Accession No. M1169). Of the 1545 nucleotide positions in the final alignment, 712 were variable sites, and 536 of these were phylogenetically informative under the parsimony criterion.

Our maximum-likelihood analysis supported a single best tree (log-likelihood, −11988.79). In this tree, dwarf boas are not monophyletic (Fig. 3). Instead, *Tropidophis* and *Trachyboa* form a well-supported clade near the base of Alethinophidea (bpp = 100), whereas *Exiliboa* and

Ungaliophis form a well-supported clade (bpp = 100) related to boines, erycines, and caenophideans. The early emergence of *Tropidophis* plus *Trachyboa* is well supported, but the exact placement of *Exiliboa* plus *Ungaliophis* among boines, erycines, and caenophideans is not clear. Parametric bootstrap analysis clearly rejects monophyly of the traditional Tropidophiidae (*p* < 0.01; Fig. 4a), as well as the placement of *Tropidophis* and *Trachyboa* as sister to the Caenophidea (*p* < 0.01; Fig. 4b). Additionally, we can reject a monophyletic Booidea (*sensu* Zaher, 1994; Fig. 1b) including *Boa, Eryx, Morelia, Exiliboa,* and *Ungaliophis* (*p* < 0.01; Fig. 4c). Monophyly of Booidea *sensu* Zaher is not supported by our analysis, as constraining *Morelia* to join Zaher's (1994) other "booids" results in an optimal tree 21 log-likelihood units worse than the optimal unconstrained tree.

Our analysis strongly supports a sister-group relationship between a *Tropidophis* plus *Trachyboa* clade and a clade that includes the remaining taxa that are usually considered "macrostomatans" plus *Cylindrophis, Loxocemus, Xenopeltis,* and Uropeltidae (bpp = 100). Within this latter clade, we found strong support (bpp = 100) for a Pythonidae plus *Loxocemus* clade and also for a *Cylindrophis* plus Uropeltidae clade (Fig. 3). *Xenopeltis* was also strongly, but not significantly, supported (bpp = 91) as the sister-lineage to the Pythonidae-*Loxocemus* clade. Our analysis shows a monophyletic Caenophidea emerging from within a paraphyletic group of "boids" (*Boa* and *Eryx*) plus Ungaliophiidae, with strong support for each node within Caenophidea (Fig. 3). As has been previously found, our data support *Acrochordus* as the sister-group to the remaining caenophideans (Dowling and Duellman, 1978; Kluge, 1991). Our finding of paraphyly for the "boids" is not well supported. Indeed, under model parameters optimized on our maximum-likelihood tree, the best tree with "boids" constrained to be monophyletic is only 0.41 log-likelihood units worse than the tree in Fig. 3. If the model parameters are optimized on the best tree that has boids monophyletic, then the difference in likelihood between the topology in Fig. 3 and boid monophyly is even smaller (0.11 log-likelihood units). This tree, with a very similar likelihood score, differs from the tree in Fig. 3 only in supporting the arrangement (((*Eryx, Boa*), (*Ungaliophis, Exiliboa*)), Caenophidea). Within this group, only the sister relationship between *Ungaliophis* and *Exiliboa* is strongly supported (bpp = 100) by our data (Fig. 3).

Levels of support for internal branches with nbp < 100 were much higher under Bayesian analysis than under nonparametric bootstrap analysis, with some branches of interest having Bayesian support values more than double their respective bootstrap values (Fig. 3b). Analysis of our simulation results indicates that Bayesian posterior probabilities provided a much closer estimate of
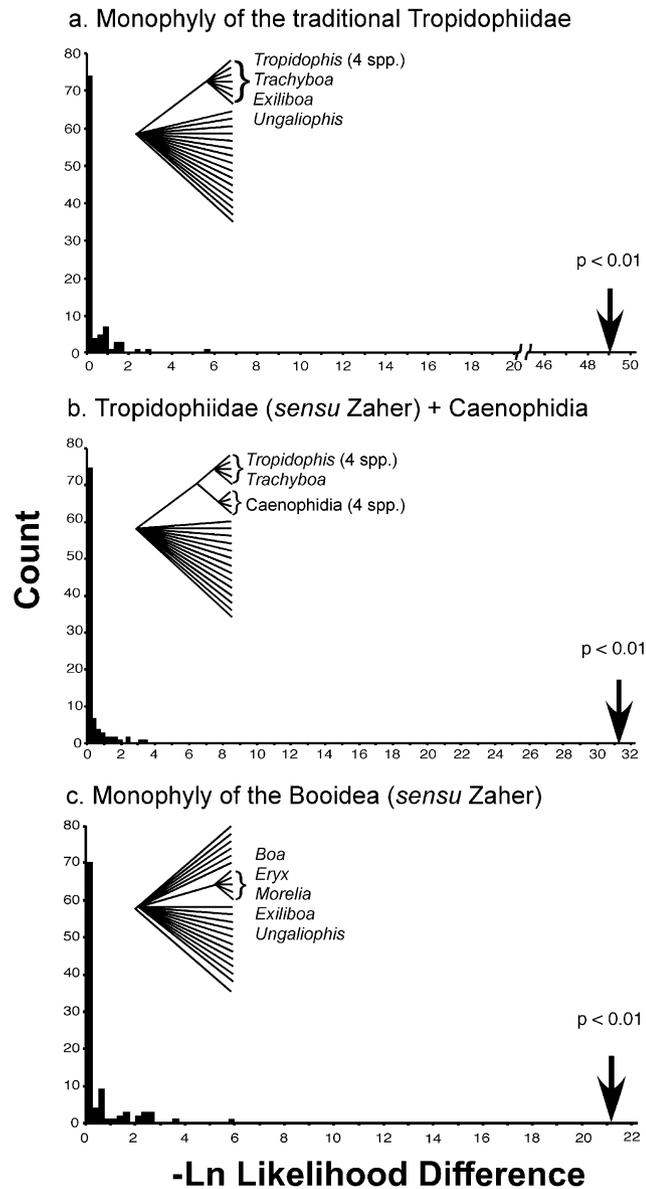
Fig. 4. Results of parametric bootstrap analysis. Model trees were constructed for each hypothesis by conducting maximum-likelihood searches with taxa constrained to be compatible with each hypothesis (constraints inset for each hypothesis). The distributions of the differences in likelihood scores between the optimal trees and the best trees that fit the respective constraint are shown for 100 simulations for each tested hypothesis. In each case, the difference in likelihood scores between model and observed trees for the original data (arrows) was considerably greater than expected if the corresponding hypothesis were true. Therefore, all three of these hypotheses are rejected at $p < 0.01$. (a) Test of the monophyly of the traditional Tropidophiidae. (b) Test of the sister-group relationship between Tropidophiidae (*sensu* Zaher; *Tropidophis* plus *Trachyboa*) and Caenophidea. (c) Test of the monophyly of Booidea (*sensu* Zaher).

Fig. 5. Comparison of Bayesian and nonparametric bootstrap support values. (a) Histogram showing the proportion of bipartitions in each support bin from 120 simulations for Bayesian values and nonparametric bootstrap support values. Note that the average support values are higher for Bayesian compared to bootstrap analyses. (b) The relationship between the percentage of correct bipartitions and percentage support values for the respective bipartitions for Bayesian and nonparametric bootstrapping analyses. Error bars are the binomial standard errors around the proportion of correct bipartitions in each bin. The dotted diagonal line indicates perfect correspondence between phylogenetic accuracy (percentage of correct bipartitions) and percent support values. Both Bayesian and nonparametric bootstrapping underestimate phylogenetic accuracy at higher levels of support, but Bayesian support values are much better indicators of phylogenetic accuracy under the conditions we examined.

phylogenetic estimation accuracy than did bootstrap support values (Fig. 5). In our simulations, nonparametric bootstrapping significantly underestimated the probability of recovering a clade for all but the lowest support values, as has been previously reported by several

authors (Hillis and Bull, 1993; Rodrigo, 1993; Zharkikh and Li, 1995). In contrast, Bayesian support values provided much closer estimates of the true probabilities of recovering the respective clades, although they also were conservative measures of phylogenetic accuracy (Fig. 5).

4. Discussion

4.1. Snake phylogeny

Based on morphological analysis of cephalic soft-tissues, Zaher (1994) proposed that the traditional

Tropidophiidae is not monophyletic, and split the group into two clades: a re-defined Tropidophiidae (*Tropidophis* plus *Trachyboa*) and Ungaliophiidae (*Exiliboa* and *Ungaliophis*). He further proposed that Tropidophiidae is the sister-group to Caenophidea, and re-defined Booidea to include Ungaliophiidae, pythons, boines, erycines, and bolyeriids. Our analysis supports Zaher's conclusion that the traditional Tropidophiidae is not monophyletic (Fig. 4a). Another recent analysis of nuclear 28S ribosomal RNA genes also found evidence for a split between the Tropidophiidae (*sensu* Zaher) and Ungaliophiidae (White et al., in press). However, we find no support for a sister-group relationship between Tropidophiidae (*sensu* Zaher) and Caenophidea (Fig. 4b). Instead, our estimated phylogeny shows Tropidophiidae emerging near the base of the alethinophidean radiation (Fig. 3). We cannot confidently identify the placement of Ungaliophiidae relative to boines, erycines, and caenophideans, but our analysis indicates that these four lineages do form a well-supported monophyletic group (bpp = 99). Our optimal tree indicates that Ungaliophiidae shares a common ancestor with erycines, but this placement is weakly supported (Fig. 3, bpp = 49) and thus we cannot reject the possibility that boids (erycines plus boines, with or without Ungaliophiidae) are monophyletic. Given that there is a tree of nearly identical likelihood score (−11988.90 versus −11988.79) that differs from Fig. 3 only in supporting the arrangement (((*Eryx*, *Boa*), (*Ungaliophis*, *Exiliboa*)), Caenophidea), we do not believe that our data provide a clear resolution between these two possibilities.

Zaher (1994) based his conclusions about dwarf boa relationships on the placement of jaw muscles and the facial carotid artery. He found that *Tropidophis* and *Trachyboa* share with Caenophidea the loss of the *adductor mandibulae externus medialis pars anterior* as well as a derived position for the facial carotid artery. Likewise, *Exiliboa* and *Ungaliophis* have a jaw musculature and facial carotid artery placement most similar to pythons, boines, erycines, and bolyeriids. Based on our analysis, these characters are considerably more evolutionarily plastic than hypothesized by Zaher (1994). If our estimated phylogeny is correct, then the loss of the *adductor mandibulae externus medialis pars anterior* and the derived position of the facial carotid artery evolved independently in Tropidophiidae (*sensu* Zaher) and Caenophidea. Likewise, our estimated phylogeny indicates that shared states for these characters were derived independently in pythons compared to the remainder of Zaher's Booidea.

Perhaps the greatest difference between our analysis and previous analyses of snake phylogeny is the early emergence of the lineage that contains *Tropidophis* and *Trachyboa* (Fig. 3). Phylogenetic analysis of morphology has placed these genera either with boids or as sister to Caenophidea (Kluge, 1991; Scanlon and Lee, 2000;

Tchernov et al., 2000; Underwood, 1976). Underwood (1976, p. 172), who included bolyeriids in the Tropidophiidae, noted that "[f]eatures general to or common in the Caenophidea which are also found in the Tropidophiidae are: keeled scales, simple transverse scale rows, terminal entry of trachea, tracheal lung, left lung vestigial or absent, absence of coronoid, absence of basipterygoid processes, hyoid cornua parallel joined in midline, with a forward process, prootic fused around maxillary nerve, absence of pelvic spurs on both sexes, presence of paracotylar foramina, and posterior hypapophyses." Although some of these states are not found in *Tropidophis* and *Trachyboa* (e.g., pelvic spurs are present in the males of these genera), and states of some of the other characters are highly variable among both dwarf boas and caenophideans (e.g., keeled scales), it does appear that *Tropidophis* and *Trachyboa* have a number of morphological similarities to Caenophidea (Kluge, 1991). If our estimated phylogeny is correct, then this morphological similarity is either convergent in the two groups or represents retained pleisiomorphic similarity.

Tropidophis, *Trachyboa*, *Exiliboa*, and *Ungaliophis* were united in past studies based largely on the presence of a tracheal lung and the absence of a left lung. Based on the traditional, morphologically derived estimate of snake phylogeny, there were only two independent origins of tracheal lungs, once in Scolecophidea and once in the common ancestor of the traditional Tropidophiidae and Caenophidea, with secondary losses occurring in some colubrids (McDowell, 1987; Greene, 1997). In contrast, our analysis supports an alternative hypothesis in which the tracheal lung is a primitive characteristic of snakes that has been lost repeatedly (McDowell, 1987) (although multiple independent gains cannot be ruled out). Similarly, the presence of *Tropidophis* and *Trachyboa* near the base of Alethinophidea indicates that a relatively kinetic skull emerged early in snake evolution, with secondary reductions in skull flexibility occurring in lineages of fossorial snakes such as the uropeltid-*Cylindrophis* clade (Gans, 1973).

As in some previous molecular studies, we find that pythons and boas are not each other's closest relatives (Dessauer et al., 1987; Hiese et al., 1995). Unlike those studies, however, we find that pythons and *Loxocemus* are together a sister-group to *Xenopeltis*, and this clade is then the sister-group to a clade containing *Cylindrophis*, uropeltids, boines, erycines, ungaliophiids, and caenophideans. Early snake classifications considered *Loxocemus* as a New World python based on several morphological characters and the presence of oviparity (see Underwood, 1967, Discussion). Recent classifications, however, have considered *Loxocemus* distinct from pythons, but usually have placed *Xenopeltis* and *Loxocemus* in Booidea (Dowling, 1975; Dowling and Duellman, 1978). Nonetheless, in his analysis of morphological data, Underwood (1976) did find evidence

for xenopeltids as the sister-group to a clade containing pythons and *Loxocemus*, as in our tree. Additionally, recent phylogenetic analysis of osteological data placed *Xenopeltis* and *Loxocemus* as sister taxa (Scanlon and Lee, 2000). Heise et al. (1995), in an analysis of two small segments of the 12S and 16S mitochondrial rDNA, also found support for a close relationship between pythons and *Loxocemus* (although little resolution was apparent among other included primitive snakes in their study).

Many previous authors have recognized a close relationship between uropeltids and *Cylindrophis*, and our analysis supports this conclusion (Cadle et al., 1990; Kluge, 1991; Scanlon and Lee, 2000; Tchernov et al., 2000). Furthermore, uropeltids have long been considered "primitive" snakes, based on their extremely rigid, and somewhat simplified, skull architecture (Cundall et al., 1993; Gans, 1973), and their relatively basal placement has been supported by several recent analyses of morphological characters (Cundall et al., 1993; Greene, 1997; Kluge, 1991; Scanlon and Lee, 2000; Tchernov et al., 2000). In our tree, however, this clade does not emerge near the base of the alethinophidean radiation. Instead, the uropeltids plus *Cylindrophis* emerge as the sister-group to a clade containing the boines, erycines, ungaliophiids, and caenophideans. Our results suggest that the rigidity of the skull, along with several other "primitive" features of uropeltids, arose secondarily as a function of their fossorial habits.

Although the phylogeny we estimated differs in some substantial ways from some recent phylogenetic estimates based on morphology, we did find several areas of agreement between morphological and molecular data. For instance, we found strong support for a monophyletic Caenophidea, with *Acrochordus* as the sister-group to the Colubroidea. *Acrochordus* was historically the source of much contention regarding its placement among snakes (McDowell, 1987). Recently, however, placement of *Acrochordus* as the sister-group to the vipers, elapids, and colubrids has been well supported (Cundall et al., 1993; Greene, 1997; Kluge, 1991; Scanlon and Lee, 2000; Tchernov et al., 2000).

Considerable uncertainty exists regarding the relationships among basal snakes, and the varied classification schemes proposed over the years certainly reflect this uncertainty. Our results indicate that some of this uncertainty is due to a complicated evolutionary history for many morphological characters used to estimate snake relationships. Our analyses suggest that some morphological characters previously thought to have evolved in a linear progression (e.g., those involved in the increasing kinesis of the skull) instead show considerable levels of homoplasy in snake evolution.

We recognize that this study is based on only one region of DNA, and thus we do not wish to add new names or phylogenetic definitions to snake classification at this
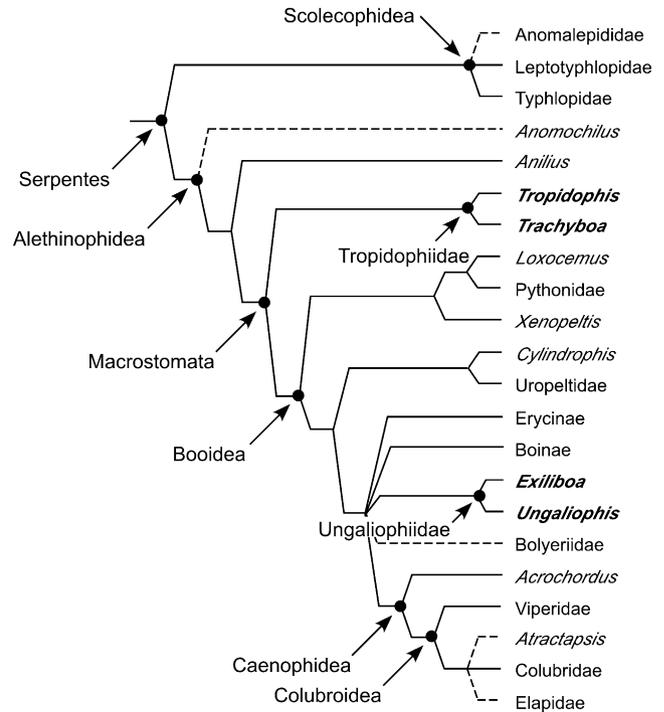
Fig. 6. A revised phylogenetic hypothesis and classification for the major groups of snakes. Taxa in bold were traditionally placed in the Tropidophiidae. Dashed branches represent taxa not included in this study.

time. However, our analysis does suggest a shift in content within some of the existing named groups of snakes (Fig. 6). For instance, if Macrostomata is defined to include the common ancestor of Tropidophiidae, Ungaliophiidae, Pythonidae, Boinae, Erycinae, Bolyeriidae, and Caenophidea, and all descendants of that common ancestor (see Fig. 1a and b), then our analysis suggests that *Loxocemus*, *Xenopeltis*, *Cylindrophis*, and Uropeltidae also are part of Macrostomata (Fig. 6). Furthermore, if Booidea includes Pythonidae, Boinae, and Erycinae, then our analysis indicates that it also includes the entire list of our expanded Macrostomata minus Tropidophiidae (*sensu* Zaher; i.e., *Tropidophis* and *Trachyboa*). The remaining traditional groups of higher snakes are largely supported in our analysis, although we do support Zaher's (1994) revised definitions of Ungaliophiidae and Tropidophiidae. Because of the similarity in the log-likelihood scores of alternative trees with "boids" (*Eryx* and *Boa*) monophyletic or paraphyletic, we show the relationships among boines, erycines, ungaliophiids, bolyeriids, and caenophidians as an unresolved polytomy in our recommended classification (Fig. 6).

4.2. Bootstrapping versus Bayesian support values

We used three methods for assessing support of our phylogenetic results. When clear a priori phylogenetic hypotheses exist (as, for example, Zaher's hypotheses for

dwarf boa relationships that we tested in this study), parametric bootstrapping is a reasonable way to generate expected distributions to assess significance of the differences in the optimal tree scores for each hypothesis (Goldman et al., 2000; Hillis et al., 1996b). If maximum-likelihood is used as the optimality criterion, then these distributions provide the basis for a likelihood-ratio test (Huelsenbeck et al., 1996). If other optimality criteria are used, then analogous tests can be used to assess the observed difference in scores between the alternative hypotheses using the generated null distribution to determine the appropriate critical values of the test statistic (Hillis et al., 1996b). However, in phylogenetic studies most results are not part of an explicit a priori phylogenetic hypothesis. In these cases, it is still desirable to indicate levels of support for various estimated clades. Nonparametric bootstrapping (Felsenstein, 1985) has been the most widely used method for assessing phylogenetic support for the past decade. Nonetheless, nonparametric bootstrapping proportions represent highly conservative estimates of phylogenetic accuracy (Hillis and Bull, 1993). Several remedies have been proposed for this problem, including iterated bootstrapping (Rodrigo, 1993) and the full-and-partial bootstrap method (Zharkikh and Li, 1995), but the considerable computational expense of these methods has inhibited their widespread use.

Bayesian support values offer an alternative to nonparametric bootstrapping for assessing the phylogenetic support of estimated clades (Larget and Simon, 1999). Ideally, Bayesian support values can be interpreted as posterior probabilities that the underlying clade has been correctly recovered, given the assumptions of the model. We have observed that Bayesian support values are usually higher than corresponding measures of support derived from nonparametric bootstrapping. Our simulation analysis (Fig. 5) indicates that these higher levels of support are appropriate, and that Bayesian support values provide much closer estimates of phylogenetic accuracy (even though they are still somewhat conservative) than the estimates provided by corresponding bootstrap proportions. Therefore, we recommend that when available, Bayesian posterior probabilities should be used in preference to bootstrap proportions to assess support for estimated clades in phylogenetic trees. Clearly, however, this preference for Bayesian posterior probabilities does not obviate the need for close attention to the appropriateness of the assumed model of evolution.

Appendix A. Model trees used in the parametric bootstrap analyses shown in Fig. 4

A.1. Test of the monophyly of the traditional Tropidophiidae

((*Leptotyphlops*:0.3772, (*Typhlops jamaicensis*:0.0836, *Typhlops ruber*:0.2162):0.1775):0, (*Anilius*:0.0901, (((((*Trachyboa*: 0.0318, (*Tropidophis greenwayi*:0.0537, (*Tropidophis pardalis*:0.0184, (*Tropidophis feicki*:0.0242, *Tropidophis melanurus*:0.0261):0.0052):0.0098):0.0222):0.1581, (*Ungaliophis*:0.0507, *Exiliboa*:0.0486):0.0246):0, (*Eryx*:0.1004, (*Boa*: 0.0771, (*Acrochordus*:0.1251, (*Pituophis*:0.1258, (*Crotalus*: 0.0574, *Azemiops*:0.0596):0.0664):0.0730): 0.0286):0.0181): 0.0081):0.0144, (*Xenopeltis*:0.0736, (*Morelia*:0.0504, *Loxocemus*:0.0769):0.0143):0.0147): 0.0071, (*Cylindro- phis*:0.0576, (*Uropeltis*:0.0431, *Rhinophis*: 0.0372):0.0856): 0.0278):0.0698):0.2102).

A.2. Test of the monophyly of Trodidophiidae (sensu Zaher) plus Caenophidea

((*Leptotyphlops*:0.3765, (*Typhlops jamaicensis*:0.0787, *Typhlops ruber*:0.2171):0.1689):0, (*Anilius*:0.0880, (((*Trachyboa*: 0.0316, (*Tropidophis greenwayi*: 0.0534, (*Tropidophis pardalis*: 0.0182, (*Tropidophis feicki*:0.0238, *Tropidophis melanurus*: 0.0259):0.0051):0.0091):0.0218):0.1216, (*Acrochordus*: 0.1273, (*Pituophis*:0.1282, (*Crotalus*:0.0563, *Azemiops*:0.0588): 0.0616):0.0592):0.0415):0, ((*Xenopeltis*: 0.0725, (*Morelia*: 0.0509, *Loxocemus*:0.0745):0.0147): 0.0097, ((*Cylindrophis*:0.0565, (*Uropeltis*:0.0429, *Rhinophis*:0.0362):0.0845): 0.0323, ((*Ungaliophis*:0.0511, *Ex-*

iliboa:0.0465):0.0186, (*Eryx*:0.0911, *Boa*:0.0856):0.0110): 0.0168):0.0079):0.0205):0.0547): 0.2020).

A.3. Test of the monophyly of Booidea (sensu Zaher)

((*Leptotyphlops*:0.3885, (*Typhlops jamaicensis*:0.0769, *Typhlops ruber*:0.2224):0.1700):0, (*Anilius*:0.0786, ((*Trachyboa*:0.0331, (*Tropidophis greenwayi*:0.0545, (*Tropidophis pardalis*:0.0183, (*Tropidophis feicki*:0.0239, *Tropidophis melanurus*:0.0263):0.0052):0.0089):0.0213):0.0927, ((*Xenopeltis*:0.0694, *Loxocemus*:0.0814):0.0082, (((*Morelia*:0.0667, ((*Ungaliophis*:0.0519, *Exiliboa*:0.0473): 0.0189, (*Eryx*:0.0913, *Boa*:0.0885): 0.0101): 0.0217): 0.0017, (*Acrochordus*:0.1214, (*Pituophis*:0.1300, (*Crotalus*:0.0580, *Azemiops*:0.0585):0.0629):0.0683):0.0435): 0.0044, (*Cylindrophis*:0.0576, (*Uropeltis*:0.0432, *Rhinophis*: 0.0369):0.0859):0.0327):0.0070):0.0481):0.0386): 0.2139).

Appendix B. Branch lengths and estimated parameters for the maximum-likelihood tree shown in Fig. 3. This was also the model tree used to assess bootstrapping and Bayesian support values (Fig. 5)

((*Leptotyphlops*:0.3880, (*Typhlops jamaicensis*:0.0770, *Typhlops ruber*:0.2201):0.1685):0, (*Anilius*:0.0787, ((*Trachyboa*: 0.0317, (*Tropidophis greenwayi*:0.0539, (*Tropidophis pardalis*:0.0183, (*Tropidophis feicki*:0.0239, *Tropidophis melanurus*:0.0262):0.0052):0.0092):0.0222): 0.0929, ((*Xenopeltis*:0.0723, (*Morelia*:0.0524, *Loxocemus*:0.0739):0.0156): 0.0075, ((*Cylindrophis*:0.0579, (*Uropeltis*:0.0433, *Rhinophis*: 0.0366):0.0851):0.0311, (((*Ungaliophis*:0.0504, *Exiliboa*: 0.0483):0.0172, *Eryx*:0.0982): 0.0061, (*Boa*:0.0781, (*Acrochordus*:0.1216, (*Pituophis*:0.1259, (*Crotalus*:0.0572, *Azemiops*:0.0589):0.0654):0.0729):0.0308):0.0175):0.0173): 0.0094):0.0463):0.0372): 0.2131).

Parameters used in simulations:

1. GTR + continuous gamma rate heterogeneity (Γ) + invariant sites (PINVAR):

Rate matrix:	A → C: 11.8852	A → G: 17.70631	A → T: 7.8201
		C → G: 2.5×10^{-7}	C → T: 58.3060
			G → T: 1.0000

Base frequencies: A: 0.3797, C: 0.2362, G: 0.1838, T: 0.2003.

Shape parameter for gamma distribution: 0.505086. Proportion of invariant sites: 0.301775.

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