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Phylogeny, evolutionary history, and biogeography of Oriental–Australian rear-fanged water snakes (Colubroidea: Homalopsidae) inferred from mitochondrial and nuclear DNA sequences

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Abstract

Homalopsid snakes are widely distributed throughout Southeast Asia and form the ecologically dominant component of the herpetofauna over much of their range. Although they are considered well differentiated from other colubrid lineages, several aspects of their radiation including within-family relationships, temporal patterns of species diversification, and biogeographic history remain under studied. We analyzed sequence data from four genes (three mitochondrial and one nuclear) for 22 species of the Homalopsidae to generate the most comprehensive phylogeny of the family to date. We also estimated divergence times within the family using a model of independent but log-normally distributed rates of evolution in conjunction with two external fossil calibrations. Using this chronogram, we inferred historical patterns of species diversification within the family. Finally, we used previously published sequence data for 172 snake species to test for the monophyly of the Homalopsidae. Phylogenetic analysis reveals strong support for homalopsid monophyly with an estimate age of the crown group of ~22 MYA. The family comprises three major clades which all originated 18–20 MY. Lineage through time plots reveal that homalopsids experienced a significantly higher rate of effective cladogenesis in their early history, consistent with a hypothesis of adaptive radiation. We discuss several Miocene and Pliocene paleogeographic factors that might underlie observed patterns of temporal diversification and biogeography.

Keywords: Homalopsidae; Rear-fanged water snakes; Asian water snakes; Diversification; Divergence time; Relaxed clocks

1. Introduction

The Family Homalopsidae comprises approximately 34 species of aquatic to semi-aquatic snakes inhabiting mangrove forests, tidal mudflats, coastal waters, ponds, marshes, wetlands, and lakes across Southeast Asia (Fig. 1 and Table 1) (Readers interested in more detailed distributional data for the homalopsids are referred to Murphy, 2007, which contains range maps for each known homalopsid species). Morphologically, the family is defined by a suite of adaptations for aquatic life including crescentric, slit-like valvular nostrils, dorsally oriented eyes, a glottis that can be extended to fit into the internal nares, and a shallowly notched rostral that permits tight closure of the mouth. In addition, the posterior two or three maxillary teeth are enlarged and grooved and hypapophyses are present on most vertebrae (Gyi, 1970; Voris et al., 2002). Taxonomic reviews are provided in Gyi (1970) and Voris et al. (2002); we note briefly that the Homalopsidae were first attributed familial rank by Günther (1864), considered a

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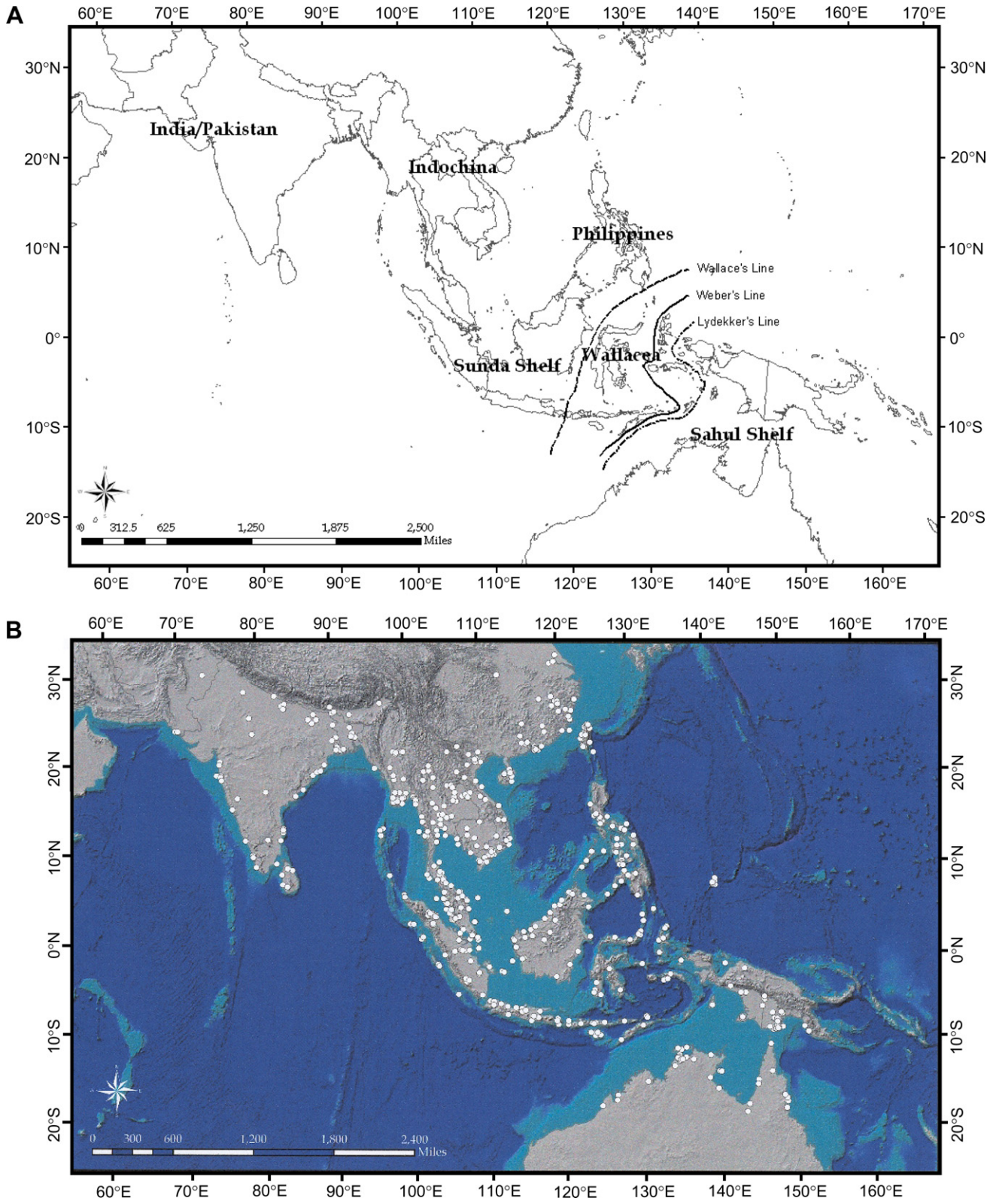

Fig. 1. Distribution of homalopsid snakes. (A) Map showing the six geographic regions used and defined in Table 2. These regions are defined as follows: India/Pakistan (including Bangladesh and Nepal but not the Andaman and Nicobar Islands), Indochina (Cambodia, southern China, Laos, Malaysia, Myanmar, Andaman and Nicobar Islands, Singapore, Taiwan, Thailand, and Vietnam), Sunda Shelf (Greater Sunda Islands of Borneo, Java, and Sumatra, and adjacent islands), Wallacea (Sulawesi and associated islands between the Sunda Shelf [Wallace's Line] and the Sahul Shelf [Lydekker's Line]), Philippines, and Sahul Shelf (New Guinea, Australia, and adjacent islands). (B) Map showing the distribution of the Homalopsidae. Each dot represents a documented collection site for a homalopsid snake. The distributional data are based on 893 locality records drawn from Gyi (1970), Murphy (2007), and museum records.

Table 1
Specimens examined in this study

Species	GenBank Accession Number				Voucher No.
	12S	16S	cyt *b*	c-*mos*	
Bitia hydroides	EF395872	EF395847	EF395896	EF395921	CAS 204955
Cantoria violacea	EF395873	EF395848	EF395897	EF395922	FMNH 250117
Cerberus australis	EF395874	EF395849	EF395898	EF395923	NTM R22721
Cerberus microlepis	EF395875	EF395850	EF395899	EF395924	USNM 197851
Cerberus rynchops 1	EF395876	EF395851	EF395900	EF395925	HKV 32404
Cerberus rynchops 2	Missing	EF395852	EF395901	EF395926	USNM 497590
Enhydris bocourti	EF395877	EF395853	EF395902	EF395927	FMNH 252500
Enhydris chinensis	EF395878	EF395854	EF395903	EF395928	ROM 31031
Enhydris enhydris	EF395879	EF395855	EF395904	EF395929	FMNH 250119
Enhydris innominata	EF395880	EF395856	EF395905	EF395930	FMNH 259247
Enhydris subtaeniata	EF395881	EF395857	EF395906	EF395931	FMNH 252505
Enhydris longicauda	EF395882	EF395858	EF395907	EF395932	FMNH 257256
Enhydris matannensis	EF395883	EF395859	EF395908	EF473654	Djoko Iskandar
Enhydris plumbea 1	EF395884	EF395860	EF395909	EF395933	HKV 31858
Enhydris plumbea 2	EF395885	EF395861	EF395910	EF395934	FMNH 250123
Enhydris polylepis	EF395886	EF395862	EF395911	EF473655	NTR 22716
Enhydris punctata	EF395887	EF395863	EF395912	EF395935	FMNH 250112
Enhydris sp. 'Lake Towuti, Sulawesi'	EF395894	EF395870	EF395919	EF395942	ZFMK 77797
Erpeton tentactulatus	EF395888	EF395864	EF395913	EF395936	FMNH 252504
Fordonia leucobalia 1	EF395889	EF395865	EF395914	EF395937	NTM R22714
Fordonia leucobalia 2	EF395890	EF395866	EF395915	EF395938	NTM R22715
Gerarda prevostiana	EF395891	EF395867	EF395916	EF395939	ZRC 2.346
Homalopsis buccata	EF395892	EF395868	EF395917	EF395940	FMNH 252514
Myron richardsonii	EF395893	EF395869	EF395918	EF395941	NTM R22718
Outgroups					
Xenochrophis vittata	EF395871	EF395846	EF395895	EF395920	FMNH 257460
Natrix maura/natrix	AF402623	AF158530	AF402906	AF544697	
Rhabdophis nuchalis/subminiatus/tigrinus	AF402624	AF544805	AF402907	AF471119	
Heterodon simus	AY577020	AY577029	AF217840	AF471142	
Micrurus fulvius/surinamensis	U96805	AF544799	U69846	AY058935	
Dinodon semcarinatus/rufozonatum	NC001945	NC001945	NC001945	AF471163	
Pareas carinatus/macularius	AF544773	AF544802	AF471082	AF471150	

subfamily by the majority of later authors, and have recently been reassigned familial status (Lawson et al., 2005). Recent molecular studies place the Homalopsidae as the sister group to most other members of the Colubroidea (Kelly et al., 2003; Lawson et al., 2005; Vidal et al., 2007).

Although the homalopsids are generally treated as a monophyletic lineage (Günther, 1864; Pough et al., 2003; Zug et al., 2001), this hypothesis has never been tested explicitly. Modern phylogenetic analyses of higher-level snake relationships have used only a few species to represent the family (Kelly et al., 2003; Lawson et al., 2005; Vidal et al., 2007). A substantial degree of morphological diversity exists within the group and efforts to identify synapomorphies have not been successful. Given their current position as the sister group to most other colubroids, the question of homalopsid monophyly or paraphyly has important consequences for our understanding of snake phylogenetic relationships.

Although divergence time estimation is becoming increasingly common in phylogenetic studies, there have been relatively few applications of modern, relaxed clock methods to the inference of divergence times in snakes. Some snake divergence time studies are consistent with the fossil record in showing the Miocene to be a period of heightened cladogenesis within colubroids (Holman, 2000; Rage, 1987). For example, substantial diversification among whipsnake and racer lineages occurred during the late Oligocene and early-to-mid Miocene (30 to 18 MY) (Nagy et al., 2004) and diversification among *Natrix* species appears also to have occurred during this interval (Guicking et al., 2006). In contrast, diversification events within the ratsnakes appear to be older with a concentration of events in the late Eocene and throughout the Oligocene (41 to 24 MY) (Burbrink and Lawson, 2006).

The fossil record has suggested several hypotheses regarding the tempo of diversification within snakes. An initial pulse of diversification is hypothesized to have occurred during the late Cretaceous; diversification within booids is thought to have peaked during the Eocene, and the Miocene is regarded as the 'age of colubroids" (Holman, 2000; Rage, 1987). Somewhat surprisingly, no studies of snakes have implemented any of the temporal or tree-based diversification methods avail-

able that might be used to explicitly test these or other hypotheses. Lawson et al. (2005) and Voris et al. (2002) have suggested that homalopsids diverged anciently from other colubroids. Their present day diversity might reflect an ancient origin of the group coupled with a constant speciation rate. Alternatively, if the present-day ecological diversity in homalopsids reflects an initial adaptive radiation of the clade, we would expect that cladogenetic events would be concentrated in their early history. Distinguishing between these two hypotheses would provide important insights into the evolution of this ecologically diverse group as well as provide data for future comparative study of diversification in other snake lineages.

Here we present a statistical phylogenetic study of homalopsid evolutionary history. Using nuclear and mitochondrial markers, we construct the most inclusive phylogeny of the family to date. We also use previously published data to explicitly test for the monophyly of the Homalopsidae. To infer the tempo of homalopsid diversification, we implement a recently described relaxed-clock method that assumes independent but log-normally distributed rates on branches. Finally, we test for departure from a constant rates model of lineage accumulation using a range of diversification statistics. We interpret present day patterns of homalopsid distribution in light of these data and suggest possible scenarios for the evolutionary history of the family.

2. Materials and methods

2.1. Choice of taxa

We expanded the scope of our previous study on homalopsids (Voris et al., 2002) by sampling a new nuclear gene (c-*mos*), sequencing the remaining two-thirds of cyt *b*, and adding seven new species to the matrix. In total, we sampled 20 of the 34 named species and nine of the 10 genera in the Homalopsidae. In addition, we included an unnamed species from Lake Towuti, Sulawesi (Table 2) collected during a field expedition in 2002. The largest genus in the family, *Enhydris*, contains 23 named species and is represented by 10 species in this study. The identity of the sister group to homalopsids remains uncertain despite several recent studies of colubroid relationships (Kelly et al., 2003; Lawson et al., 2005; Vidal et al., 2007). We selected seven taxa as outgroups to represent both recent and traditional hypotheses of homalopsid affinity (Voris et al., 2002). These included the slug snake, *Pareas*, the natricines *Xenochrophis vittata, Natrix*, and *Rhabdophis*, the non-natricine colubrids *Dinodon*, and *Heterodon*, and the elapid *Micrurus*. We obtained a tissue sample for *Xenochrophis vittata* and used it to generate sequences for all of the loci examined in our study. For the other six outgroups, GenBank sequences from a single species was not available for all loci. Therefore, to minimize the amount of missing data in the matrix, we concatenated GenBank sequences from two or more species within the same genus to construct exemplars of outgroup genera. To test the monophyly of the Homalopsidae, we also downloaded c-*mos* sequences from 161 outgroup species (Appendix A).

Homalopsid tissue was collected fresh from the field. Samples were taken from the heart, liver, or tail and were preserved in saturated EDTA or in 95% EtOH.

2.2. DNA isolation, amplification, and sequencing

Total genomic DNA was extracted from tissues using PureGene Animal Tissue DNA Isolation Protocol (Gentra Systems, Inc.). All genes were amplified by PCR reactions with the following protocol: 94 °C 45 s, 50–60 °C 30 s, 72 °C 1 min for 35 cycles using the primer pairs indicated in Table 3. PCR products were electrophoresed in a 1% low-melt agarose TALE gel stained with ethidium bromide and visualized under ultraviolet light. The bands containing DNA were excised and agarose was digested from bands using GELase (Epicentre Technologies). PCR products were sequenced in both directions by direct double strand cycle sequencing using Big Dye version 3 chemistry (Perkin Elmer) and the amplifying primers. Internal primers were also used in sequencing reactions of 12S and cyt *b* (Table 3). Cycle sequencing products were precipitated with ethanol, 3 M sodium acetate, and 125 mM EDTA, and sequenced with a Prism 3100 Genetic Analyzer (ABI). Sequences were edited using Sequencher v. 4.1 (Genecodes Corp.).

2.3. Phylogenetic analysis

We obtained sequence data for most of the outgroup species in our analyses from GenBank (Table 2 and Appendix A). Our study also incorporates GenBank sequences for homalopsid 12S, 16S, and cyt *b* that we produced as part of an earlier study (Table 2), (Voris et al., 2002). Sequencher v. 4.1 (Genecodes Corp.) was used to assemble contigs and produce preliminary alignments of the sequence data. We aligned 12S and 16S sequences to previously published vertebrate models of secondary structure for natricine snakes (Alfaro and Arnold, 2001) and teleosts (Orti and Meyer, 1997) using the text editor BBEdit (Bare Bones Software). Ambiguously aligned regions were excluded from further analysis. We used Se–Al (A. Rambaut) to align cyt *b* and c-*mos* sequences by eye. After exclusion of ambiguous regions, our data matrix consisted of 2896 characters. We deposited this matrix on Treebase (www.treebase.org, Accession No. SN3613).

2.3.1. Conflict among partitions

Several processes are known that can cause different loci to estimate significantly different phylogenies (e.g. Bull et al., 1993). We assessed concordance among genes by

Table 2
Regional distribution of the 21 species of Homalopsidae included in this study

Species	Regions					
	India/Pakistan	Indochina	Sunda Shelf	Wallacea	Philippines	Sahul Shelf
Bitia hydroides		x				
Cantoria violacea		x	x			
Cerberus australis						x
Cerberus microlepis					x	
Cerberus rynchops	x	x	x	x	x	x
Enhydris bocourti		x				
Enhydris chinensis		x				
Enhydris enhydris	x	x	x			
Enhydris innominata		x				
Enhydris longicauda		x				
Enhydris matannensis				x		
Enhydris plumbea		x	x	x		
Enhydris polylepis						x
Enhydris punctatus		x	x			
Enhydris subtaeniata		x				
Erpeton tentaculatus		x				
Fordonia leucobalia	x	x	x	x	x	x
Gerarda prevostiana	x	x	x		x	
Homalopsis buccata	x	x	x			
Lake Towuti, Sulawesi				x		
Myron richardsonii						x

The regions used below are defined as follows: India/Pakistan (including Bangladesh and Nepal but not the Andaman and Nicobar Islands), Indochina (Cambodia, southern China, Laos, Malaysia, Myanmar, Andaman and Nicobar Islands, Singapore, Taiwan, Thailand, and Vietnam), Sunda Shelf (Greater Sunda Islands of Borneo, Java, and Sumatra, and adjacent islands), Wallacea (Sulawesi and associated islands between the Sunda Shelf [Wallace's Line] and the Sahul Shelf [Lydekker's Line]), Philippines, and Sahul Shelf (New Guinea, Australia, and adjacent islands). These distributional data were drawn from Gyi (1970), Murphy (2007), and museum records.

Table 3
Oligonucleotide primers used to amplify and sequence homalopsid mitochondrial and nuclear DNA in this study

Primer	Product	Use	Sequence	Source
L-12Shom	12S	A, S	5′-ATACCCATACATGCAAGCCTC-3′	This study
H-12Shom	12S	A, S	5′-CACACTTTCCAGTACGCTTACC-3′	This study
L-12Shomint	12S	S	5′-ATTGCTCGCCAAATAACTACGAG-3′	This study
H-12Shomint	12S	S	5′-GTTGTTGTGAAGTACCGTCAAGTC-3′	This study
16SAR	16S	A, S	5′-GCGCTGTTTATCAAAAACAT-3′	Voris et al. (2002)
16SBR	16S	A, S	5′-CCGGTCTGAACTCAGATCACGT-3′	Voris et al. (2002)
L-gluhom	cyt *b*	A, S	5′-ACCGTTGTTAATCAACTACAAAAAT-3′	This study
H-thrhom	cyt *b*	A, S	5′-ACAATGCTTTAGTGGTTAAGCTAC-3′	This study
H-16064	cyt *b*	A, S	5′-CTTTGGTTTACAAGAACAATGCTTTA-3′	Burbrink et al. (2000)
L-15584	cyt *b*	S	5′-TCCCATTYCACCCATACCA-3′	de Queiroz et al. (2002)
H-15149	cyt *b*	S	5′-CCCTCAGAATGATATTTGTCCTCA-3′	Kocher et al. (1989)
L-homcyt bint	cyt *b*	S	5′-TATGTCCTACCATGAGGACAAATATC-3′	This study
L-homc-mos2	c-*mos*	A, S	5′-AATGCACGTCCCTGCAGTAG-3′	This study
H-homc-mos	c-*mos*	A, S	5′-TTAAGAAGTTCAGGAGCACG-3′	This study
H-homc-mos2	c-*mos*	A, S	5′-CCTTTAAGAAGTTCAGGAGCACG-3′	This study
S77	c-*mos*	A, S	5′-CATGGACTGGGATCAGTTATG-3′	Lawson et al. (2005)
S78	c-*mos*	A, S	5′-CCTTGGGTGTGATTTTCTCACCT-3′	Lawson et al. (2005)

'L' and 'H' refer to light and heavy strands, respectively. 'A' and 'S' refer to amplifying and sequencing, respectively.

running preliminary Bayesian analyses of each gene independently and comparing the resulting Bayesian consensus topologies. We found no evidence for strongly supported conflicts among individual gene partitions (pp $\geqslant$ 95%) and so concatenated gene sequences for subsequent analysis.

2.3.2. Likelihood analysis

We assessed the fit of common phylogenetic models to the concatenated data set using ModelTest 3.6 (Posada, 2003; Posada and Crandall, 1998) in conjunction with PAUP* 4.0 b 10.0 (Swofford, 2003). The GTR + G + I model received a decisive amount of the Akaike weight

(~1.0) (Burnham and Anderson, 2003), and we used this model for all subsequent analyses of the concatenated data set. We performed a heuristic search using this model in PAUP* with TBR branch swapping and 10 random addition sequence replicates. Strength of statistical support for phylogenetic results was assessed using nonparametric bootstrapping (Felsenstein, 1985) with 300 pseudoreplicates each with two random addition sequences.

2.3.3. Bayesian analysis

We partitioned our data by gene and assigned separate GTR + I + G models to each partition using the unlinked command in MrBayes 3.1. Default priors were used for all model parameters (topology: uniform, revmat: Dirichlet(1.0,1.0,1.0,1.0,1.0,1.0), pinvar: Uniform(0.0,1.0), brlengths: Exp(10.0)). We set the Markov chain to run for a maximum of 10 million generations but also implemented a stopping rule so that the analysis would halt when the average deviation of the split frequencies was less than 0.001%. We ran four independent analyses each with one cold and four heated chains with the default heating parameter (temp = 0.2). We sampled every 100 generations and discarded the first 25% of MCMC samples as burnin.

2.3.4. Hypothesis testing

We evaluated the monophyly of the Homalopsidae with respect to the other major clades of snakes using parametric bootstrapping (the SOWH test, Goldman et al., 2000) and the SH test (Shimodaira and Hasegawa, 1999). We downloaded 161 snake c-*mos* sequences from GenBank (Appendix A) and added them to a matrix of 18 homalopsids distributed across our ML tree (Fig. 2). We focused on c-*mos* sequences for this test because samples from a large number of coluboid lineages were available in GenBank and because sequences were generally length invariant, facilitating alignment and minimizing the amount of missing data (which could affect the SOWH test statistic) in the matrix. We trimmed all sequences to the size of the smallest fragment to eliminate missing data and produced a final matrix for analysis of 179 taxa and 516 sites. We used Modeltest 3.6 (Posada and Crandall, 1998) in conjunction with PAUP to select an appropriate phylogenetic model for the data set (GTR + I + G) and performed a heuristic search with twenty random addition sequence replicates in PAUP* 4.0 under the model to find the maximum likelihood topology.

To test the hypothesis that the Homalopsidae appear as monophyletic in the c-*mos* analysis by chance, we first created a constraint tree with a monophyletic Homalopsidae in MacClade 4.0 (Maddison and Maddison, 2000). Then we used PAUP* to search for the maximum likelihood tree not compatible with the monophyly constraint. We used the difference in likelihood between the constrained and unconstrained trees as our test statistic for homalopsid monophyly. We constructed a null distribution for this statistic by simulating the evolution of 100 data sets along the constrained (paraphyletic Homalopsidae) topology using Seq-Gen (Rambaut and Grassly, 1997) and SG Runner (T. Wilcox) under a GTR + I + G model (with parameters reoptimized on the constrained tree). Next we performed heuristic searches on each data set in PAUP* with no constraint and with an enforced monophyletic Homalopsidae and recalculated the test statistic. If the observed difference in likelihood between the best tree and constrained tree exceeded 95% of the values from our null distribution, we took this as evidence to reject the hypothesis that homalopsid monophyly occurred by chance. We also tested the hypothesis that a paraphyletic Homalopsidae was not significantly worse than homalopsid monophyly using the unconstrained and constrained topologies in an SH test (Shimodaira and Hasegawa, 1999) in PAUP*.

2.3.5. Divergence time estimation

We used BEAST (Drummond and Rambaut, 2003) to estimate divergence times under a model of uncorrelated but log-normally distributed rates of molecular evolution (Drummond et al., 2006). We calibrated two nodes of the tree using available fossil information (Table 4) using priors with soft upper bounds (meaning that the fossils provided a hard minimum age of the node but no hard maximum age). Priors with soft upper bounds offer a measure of robustness in cases where there is strong conflict among the constrained nodes in a divergence time analysis (Yang and Rannala, 2006). We assigned the split of *Pareas* from the remaining taxa in the tree to the Eocene (35–55 MYA). Although there are no fossils that can be specifically assigned to either the lineage leading to *Pareas* or the lineage that gives rise to the remaining taxa in our study, this broad prior encompasses the first occurrence of several colubrid and colubroid fossils (Rage, 1987; Rage et al., 1992). We also explored the influence of the root prior on our analysis by assigning it a normal distribution with a mean age of 55 MY and SD of 10 MY. This corresponds to the age of the earliest fossils that can be assigned to the Colubroidea (Rage et al., 2003) in the Ypresian of the Eocene. We followed Guicking et al. (2006) in assigning a more narrow prior to the split between natricine and colubrine snakes at 35–45 MY on the basis of the natricine and colubrine fossil record (Ivanov, 2001; Szyndlar, 1991). We used lognormal prior distributions for both of the calibrations in our analysis so that 95% of the prior weight fell on the specified interval. We also specified a Yule prior on rates of cladogenesis. The concatenated data set was assumed to have evolved under a GTR model (Rodriguez et al., 1990) with invariant sites and gamma-distributed rate heterogeneity (Yang, 1994). Five independent analyses of 10,000,000 generations each were run and their output analyzed using TRACER 1.2 (A. Rambaut and A.J. Drummond).

2.3.6. Diversification statistics

Using the chronogram in Fig. 4 we computed the rate of net diversification for crown-group homalopsids using

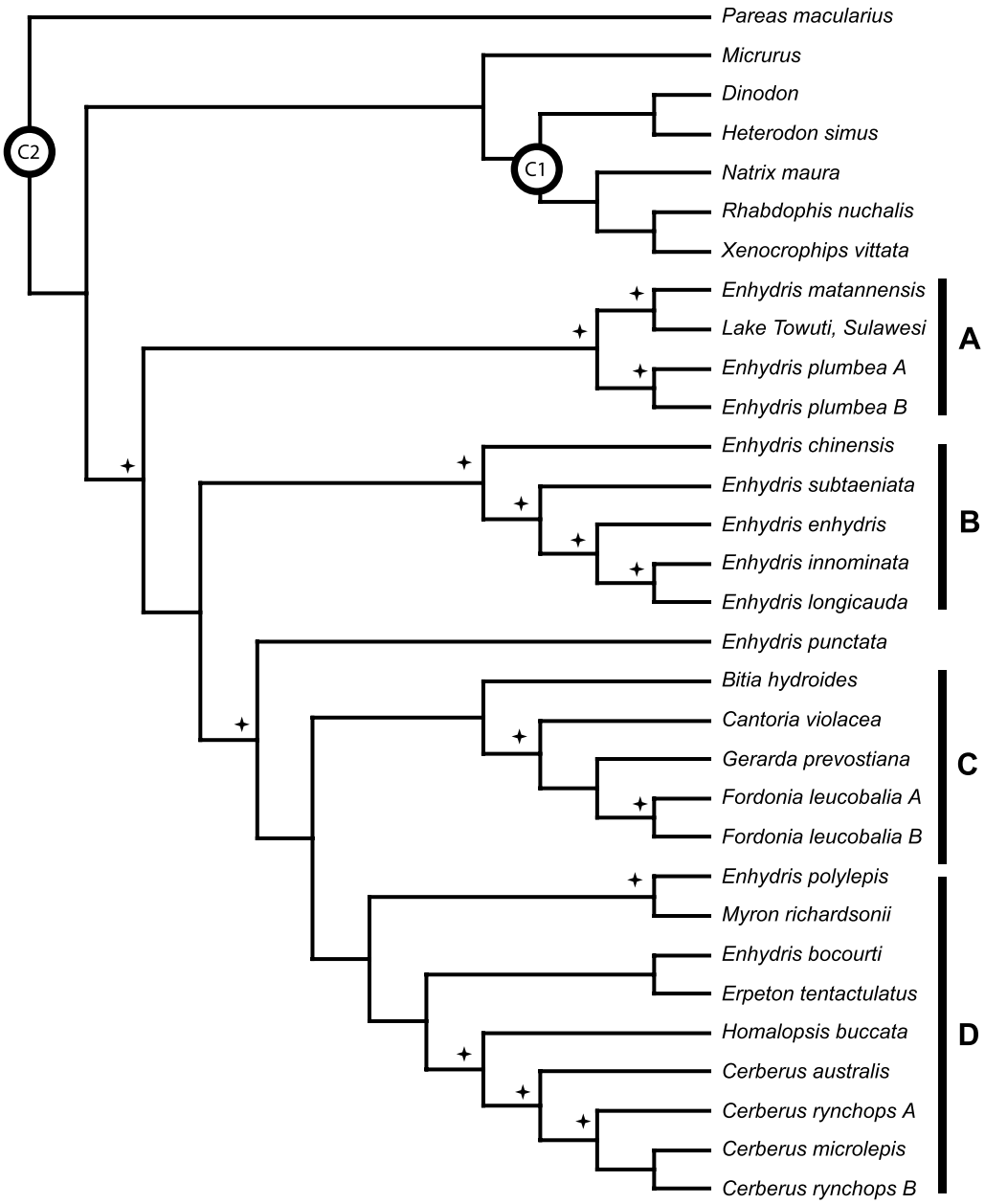

Fig. 2. Maximum likelihood topology of the Homalopsidae. Shown is the single most-likely tree ($-\ln L = 20658.07$) found during a heuristic search of the concatenated data set. Stars indicate nodes supported by $\geqslant 70\%$ BP and $\geqslant 95\%$ Bayesian PP.

Table 4
Fossil calibrations used in BEAST analysis

Calibration	Description	Min/95%	Reference
C1	Natricines vs. colubrines	35/45	Guicking et al. (2006)
C2	*Pareas* vs. other colubrids	36/55	Rage (1987)

Shown are the minimum age and 95% upper limit of two external calibrations used to constrain the ages of nodes external to the Homalopsidae (shown on Fig. 2). Min/95% indicates the minimum age/age of the 95% cumulative probability implied by the lognormal prior used in the analysis.

the methods of Magallon and Sanderson (2001). To test for elevated rates of lineage accumulation over a specific time interval, a lineage through time (LTT) plot (Nee et al., 1992) for the homalopsids was compared with that of the expectation for a pure birth model (see Harmon et al., 2003 for description of the construction of the null expectation plot). The area between the two plots was calculated for the first two-thirds of the duration of the group starting from the root (Harmon et al., 2003). To assess the significance of this area, we simulated 20,000 34 taxon trees under a Yule process with a diversification rate parameter equal to that estimated above. For each simulated tree, the area between the LTT and the pure birth expectation plot was calculated for the given interval, producing a null distribution of the test statistic. *p*-Values were calculated as the proportion of simulated trees that showed an area between curves greater than

that calculated for the homalopsid tree. Our area was considered significantly greater than expected by chance for a time interval when this proportion was <0.05 (one-tailed test). The above test was run with source code written in R language (R Development Core, 2006) by CDB. To test for a departure from a constant rate of cladogenesis throughout the history of the homalopsids, we used the Markov Chain Constant Rates (MCCR) test (Pybus and Harvey, 2000). Briefly, this test constructs a null distribution of node waiting times expected on pure birth trees (summarized by the γ statistic) and asks whether the observed γ is more extreme than expected. Incomplete taxonomic sampling is accommodated by calculating γ for each of 20,000 simulated 34-taxon pure birth trees randomly pruned to the number of taxa empirically sampled. This test assumes a constant rate of cladogenesis between lineages across any temporal slice of the tree. In order to test for violations of this assumption, we used the relative cladogenesis (RC) statistic of Nee et al. (1992). The MCCR test and the calculation of the relative cladogenesis statistic were performed in R (R Development Core, 2006) using routines written by L.J. Harmon, W. Challenger, and J. Weir.

3. Results

3.1. Phylogenetic analysis

Our maximum likelihood analysis produced a single tree ($-\ln L = 20658.07$), (Fig. 2) All Bayesian analyses terminated after approximately 1.8 million generations when they reached the stop rule threshold. Potential scale reduction factors (Gelman and Rubin, 1992) calculated among independent analyses approached 1.0 (all values <1.05), suggesting that the MCMC chains had converged on the same target distribution. All strongly supported nodes (PP $\geqslant$ 0.95) in the Bayesian consensus were perfectly congruent with the ML topology.

Bootstrap values and Bayesian posterior probabilities strongly support the monophyly of the Homalopsidae. *Enhydris* is strongly polyphyletic and comprises as many as five distinct lineages. The undescribed Lake Towuti species nests within one of these as the sister taxon to *Enhydris plumbea* (Fig. 2, Clade A). This clade, along with *Enhydris matannensis*, forms the sister taxon to the remaining homalopsids. Within this larger clade, a morphologically similar clade of *Enhydris* forms the sister taxon to the remaining homalopsids (Fig. 2, Clade B). *Enhydris punctata* forms the sister taxon to the remaining homalopsids although its position within this clade is not strongly supported. *Enhydris polylepis* is strongly supported as the sister taxon to *Myron richardsonii*. And *Enhydris bocourti* forms the sister taxon to the tentacled snake, *Herpeton tentaculatus*.

We found strong support for a morphologically and ecologically diverse clade of coastal marine, crustacean-eating snakes including *Cantoria*, *Gerarda*, and *Fordonia* (Fig. 2 Clade C). *Bitia* formed the sister group to this clade in all analyses though it's position was only strongly supported in our BEAST analysis (see below). We also found support for a clade consisting of *Homalopsis* + *Cerberus* although *Cerberus rynchops* is apparently paraphyletic with respect to *Cerberus microlepis*. All of the extant genera were separated from one another by relatively long branches with several long branches also present within *Enhydris*.

Heuristic searches of the c-*mos* data set recovered a single most likely tree ($-\ln L = 6599.93$) with a monophyletic Homalopsidae (Appendix B). This tree was 15.61 log likelihood units better than the most likely tree with a paraphyletic Homalopsidae. This value fell well outside of the null distribution (Fig. 3) suggesting that homalopsid monophyly is not likely to have arisen by chance ($p < 0.02$). The constrained topology was also significantly worse than the ML topology by the SH test ($p = 0.014$).

3.1.1. Divergence time estimation

Both the lognormal and the normal prior on the root of the tree yielded highly similar divergence time estimates, with mean ages from the normal root prior between 0.3 and 2.0 MY older than mean estimated with

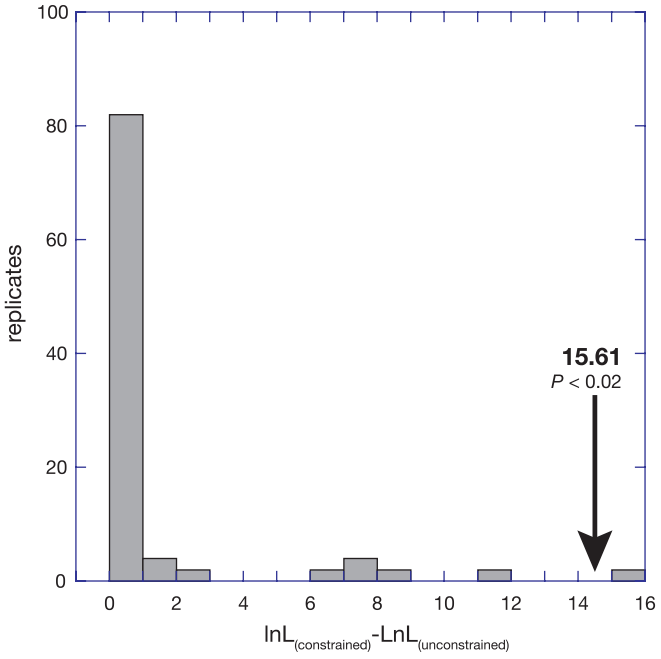

Fig. 3. Null distribution of the SOWH test of homalopsid monophyly. Shown is the expected distribution of the test statistic (the difference in likelihood scores of constrained and unconstrained trees) assuming the null hypothesis that homalopsids are not monophyletic (Goldman et al., 2000). The observed difference in likelihood scores (indicated by the arrow) was significantly different from null distribution, indicating that it is highly unlikely that homalopsid monophyly has appeared by chance ($p < 0.02$).

the lognormal prior (for the youngest and oldest nodes, respectively). In all cases, 95% credible intervals for node age estimates overlapped. Given the minor influence of the choice of the prior distribution on our phylogenetic inference, we report only the results from the lognormal root analysis.

The mean evolutionary rate was 0.04692 substitutions per site per million years (95% high posterior density, HPD: 0.03694–0.05846). The Yule process birth rate was 0.037 (HPD: 0.00188–0.065). The data showed moderate clocklike behavior with a coefficient of variation of 0.26 (95% HPD: 0.174–0.366). Parent and daughter branches showed slight covariation although this was not significantly different from 0 (mean covariance: 0.0211, 95% HPD: −0.243–0.283). The BEAST consensus chronogram differed from the ML topology in uniting clades A and B. The lack of precise within-homalopsid calibrations resulted in a chronogram with relatively wide confidence intervals on the deeper nodes (Fig 4 and Table 5). Crown homalopsids appeared in the Early Miocene (~22 MYA) and clades A + B, C and D all appeared shortly thereafter in the early to mid-Miocene.

3.1.2. Diversification statistics

The relative cladogenesis statistic showed no significant differences in rates of cladogenesis between lineages within the homalopsids, suggesting that diversification rates across the tree for any given time slice have been relatively uniform. The lineage through time plot demonstrates a greater rate of lineage accumulation early in the history of the homalopsids (the first two-thirds of the tree) than expected under our null pure-birth model (Fig. 3). This

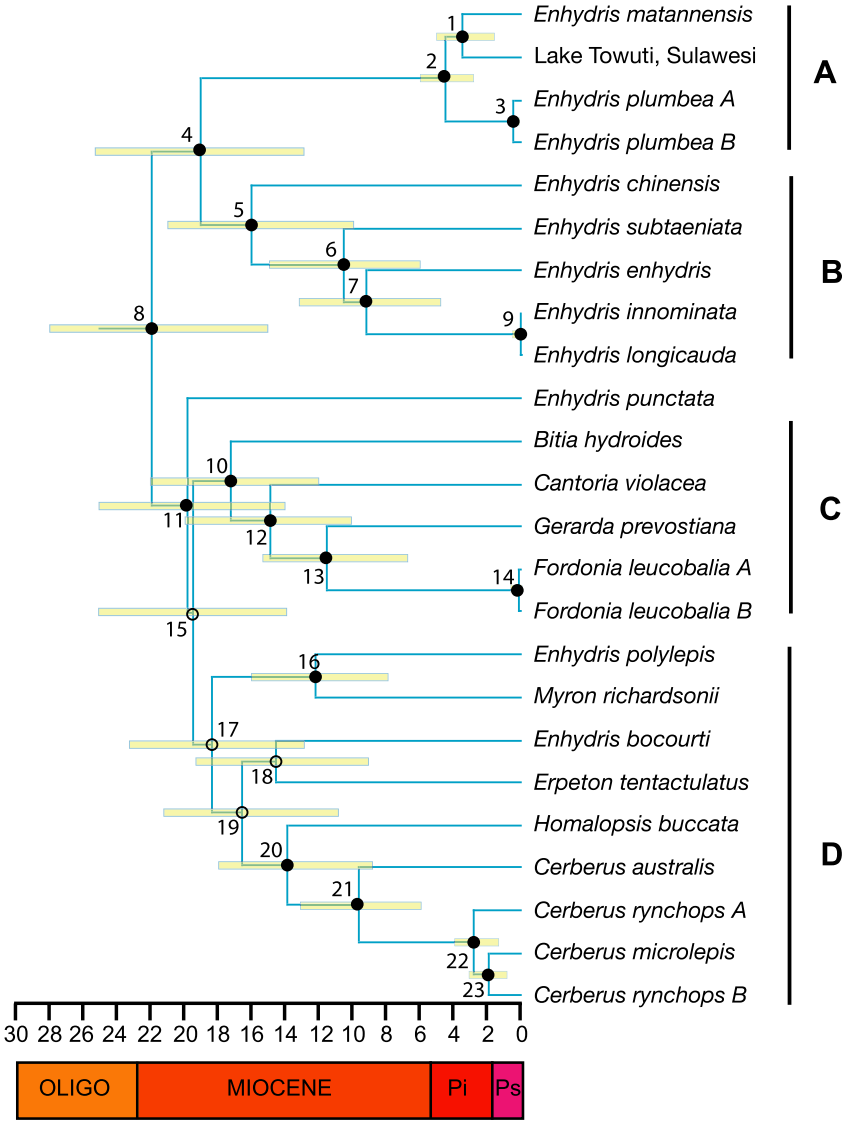

Fig. 4. Divergence time estimation in the Homalopsidae. Shown is the Bayesian consensus topology following analysis of the concatenated data set in BEAST (Drummond and Rambaut, 2003) using node calibrations in Table 4. Node heights indicate mean ages and node bars indicate the 95% HPD. Filled circles indicate nodes with ⩾95% PP, hollow circles show PP ⩾ 90%.

Table 5
Mean and 95% HPD of homalopsid divergence times

Node	Mean (MY)	95% HPD (MY)
1	3.4	1.8–5.0
2	4.5	2.7–6.3
3	0.5	0.1–0.9
4	18.9	12.9–24.9
5	15.9	10.4–21.4
6	10.5	6.4–14.6
7	9.1	5.2–12.9
8	21.8	15.6–28.0
9	0.1	0.0–0.2
10	17.0	12.0–22.0
11	19.7	14.2–25.1
12	14.9	10.2–19.5
13	11.5	7.4–15.7
14	0.1	0.0–0.3
15	19.2	13.8–24.5
16	12.1	7.9–16.3
17	18.0	12.8–23.2
18	14.1	9.3–18.8
19	16.2	11.4–21.1
20	13.8	9.4–18.2
21	9.6	5.8–13.4
22	2.9	1.6–4.2
23	2.0	1.0–3.1

Node refers to labels in Fig. 4.

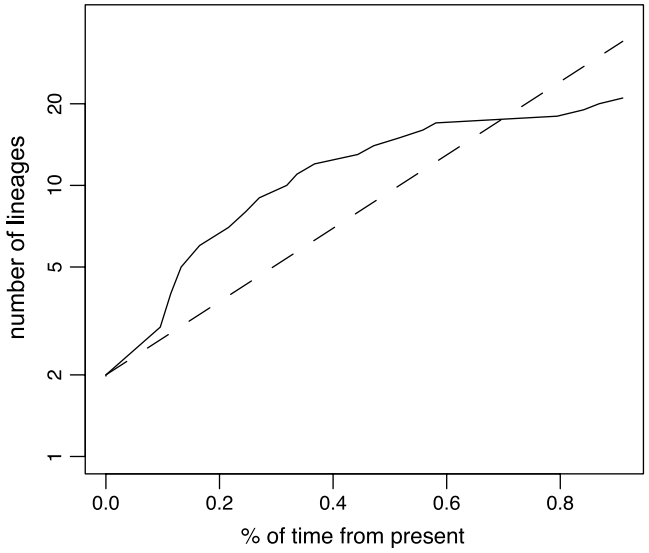

Fig. 5. Homalopsid lineages through time. Dashed line indicates number of expected lineages under the pure birth model. Solid line shows the inferred number of lineages conditional on the chronogram in Fig. 4. Over much of their history, homalopsids show a greater than expected number of lineages.

result was significant by our parametric test (p-value = 0.030; Fig. 6A). In addition, the MCCR test revealed a significantly faster rate of diversification early in the history of the homalopsids (Fig. 6B) (corrected p-value = 0.001), further supporting the results of our parametric test. Together, these results suggest that diversification in the homalopsids was initially rapid, with the rate of effective

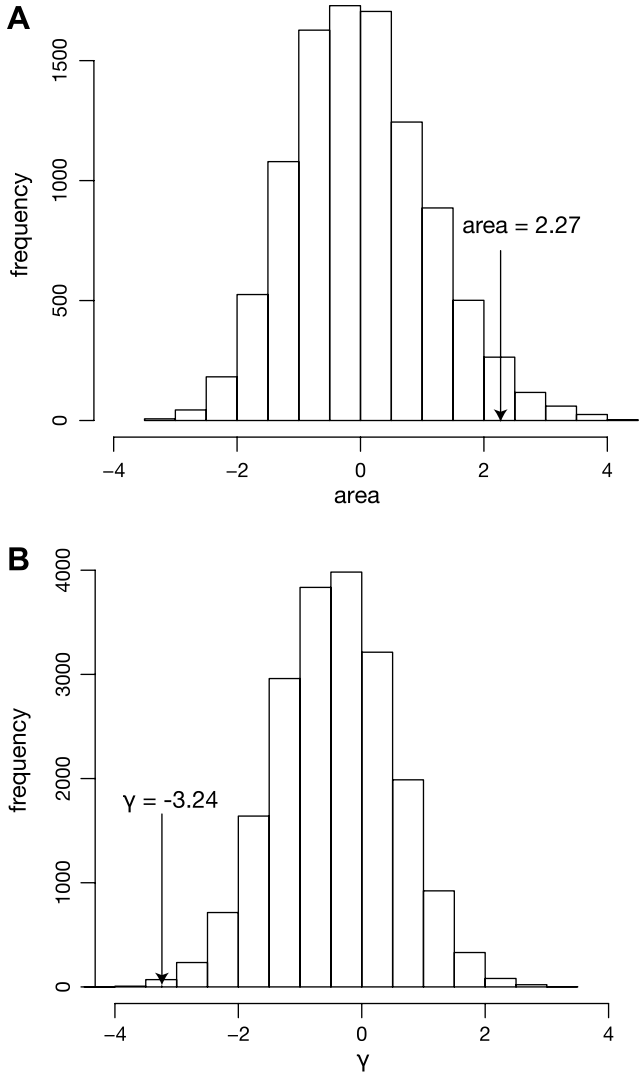

Fig. 6. Null distributions to test the significance of (A) area above the pure-birth process plot in Fig. 5 and (B) gamma statistic value for lineage distribution through time. Both results are significant (area $p < 0.030$; MCCR $p < 0.001$) and suggest either rapid initial speciation or a more recent increase in the extinction rate within the homalopsids.

cladogenesis (speciation-extinction) declining towards the present.

4. Discussion

4.1. Topological Differences between the Likelihood and BEAST trees

The BEAST consensus topology and maximum likelihood topology disagree over whether clade B forms the sister group to clade A or to the remaining homalopsids. Furthermore, the statistical support for the ML result is weak but it is quite high for the BEAST placement (PP > 95%). We suspect that this difference in topology and support stem largely from differences between the unconstrained rates model used in both PAUP and

MrBayes and the log-normal rates model implemented in BEAST (Drummond et al., 2006). Our results suggest that the homalopsid data is relatively clocklike compared to some other data sets (Alfaro et al., 2007; Drummond et al., 2006) possibly indicating that the unconstrained model is an overparameterization of the data. However additional research on the comparative performance of these models as well as additional homalopsid sequence data are necessary before differences like this can be explained. For the purposes of our study, we focus on the BEAST topology when assessing the evolutionary history of the group.

4.2. Evolutionary history of the homalopsids

Our divergence time analysis relies on two calibrations external to the homalopsid radiation and should thus be interpreted cautiously. In general, multiple, precise, and consistent calibrations points across the phylogeny yield the most reliable estimates of divergence times (Near et al., 2005a,b; Near and Sanderson, 2004; Yang and Rannala, 2006). We note that our most precise calibration has a prior interval of approximately 10 MY and that this corresponds to the 95% HPD found on the most basal nodes in our study (Fig. 4). This fits with the expected relationship between prior constraints and posterior divergence time estimation (Stanley et al., 1996; Yang and Rannala, 2006). More precise external calibrations and/or within-homalopsid calibration points would improve the precision of our divergence time estimates. However, although several snake fossils have been discovered in SE Asia (Head et al., 2004; Rage, 1987; Rage et al., 1992), none of them have specific affinities to the homalopsids. Until such fossils are found or until other calibrations based on biogeographic events within homalopsids are described, divergence time analysis of the group will have to incorporate external calibration points.

The crown group containing the core *Enhydris* species in clades A + B (Fig. 4) arose in the Miocene approximately 18 MYA. Crown members of clade A arose at the beginning of the Pliocene and thus have a relatively long independent evolutionary history from members of clade B. The crown group containing *E. subtaeniata*, *E. enhydris*, and *E. innominata*/*E. longicauda* arose ~10 MYA and represents an important radiation of morphologically similar taxa that, today, comprise a significant portion of the vertebrate biomass in the wetlands of Southeast Asia (e.g. Stuart et al., 2000).

The oldest clade of homalopsids (containing our clades C and D) arose shortly after the origin of the crown group (~20 MYA). *Enhydris punctata*, a poorly known Sunda shelf species, split from the remaining members of the clade shortly thereafter. Somewhat later (~18 MYA), crown members of the ecologically diverse clade C evolved, eventually giving rise to the morphologically distinct *Bitia hydroides* (keeled ventral scales, somewhat flattened tail, true sea-snake like appearance) and the crustacean-eating lineages *Cantoria*, *Gerarda*, and *Fordonia*.

The morphologically and geographically diverse Clade D also appeared at the same time as clade C (~19 MYA) and over the next five million years almost all of the modern day lineages appeared. These include the freshwater *E. polylepis* and the mangrove and coastal marine *M. richardsoni* now found in Australia and New Guinea, the stout-bodied *E. bocourti* and the unusual sit-and-wait predator *H. tentaculatus* (both endemic to the Indochinese peninsula), the freshwater Indochinese *Homalopsis buccata* and the coastal marine *Cerberus*.

4.3. Causes of Miocene homalopsid diversification

Divergence time estimates coupled with the MCCR and parametric tests (Figs. 3–5) suggest that the homalopsids underwent extensive and rapid diversification during the Miocene. This pattern is consistent with the fossil record for many colubrid snakes in establishing the Miocene as a period of accelerated speciation and radiation (reviewed in Holman, 2000; Rage, 1987; Stanley, 1979). Although diversification statistics have yet to be calculated for other snake groups, recent chronograms for racers and their allies (Nagy et al., 2004) and natricines (Guicking et al., 2006) are qualitatively similar in showing a concentration of deeper divergences between the early and mid-Miocene. Ratsnakes and their allies also appear to show a concentration of cladogenetic events early in their history (Burbrink and Lawson, 2006) although the age of most of these splits is older (late Eocene and Oligocene instead of Miocene).

Potential reasons for this distinct spike in effective cladogenesis in homalopsids during this timeframe are not readily apparent. One possibility is that we have preferentially sampled the oldest homalopsid lineages and undersampled the youngest. Such a bias could theoretically produce an inflated gamma statistic since more old than young nodes will contribute to its calculation. The magnitude of this effect is currently not understood but deserves attention as workers often make special effort to include morphologically distinct forms, which are likely to be the most divergent, while cryptic lineages are prone to being undersampled. In our study, we sampled as many species from the Homalopsidae as possible, including several morphologically similar forms. We do not suspect that our bias toward older lineages is especially strong but future sampling across geographic ranges of widespread species (e.g. Alfaro et al., 2004) would be extremely helpful in clarifying this issue.

Historical explanations of elevated homalopsid diversification in the Miocene are plausible, though several different views of the paleogeographic history of the region exist. Some authors depict the Sunda shelf as either relatively well connected subaerially (excluding Sumatra and Java until ~10 MYA; Hall, 1998) or broken-up periodi-

cally by marine transgressions (Woodruff, 2003). Biogeographic evidence for the latter scenario has recently accumulated (e.g. de Bruyn et al., 2005; Hughes et al., 2003; Woodruff, 2003), though the extent, location, and frequency of these marine transgressions is still unclear.

One plausible hypothesis is that an Indochinese ancestor of the homalopsid radiation invaded the Sunda shelf approximately 22 MYA (the inferred homalopsid crown group age) when it was believed to be contiguous with Indochina. Periodic increases in sea level throughout the Miocene (Haq et al., 1987; Mitchum et al., 1994; Abreu and Anderson, 1998; Woodruff, 2003) may have isolated populations and thus facilitated allopatric speciation. This pattern of speciation would be analogous to Pleistocene refugia models often invoked to describe patterns of diversity in the tropics (e.g. Haffer, 1969; Haffer and Prance, 2001; Moritz et al., 2000; Prance, 1973). Interestingly, previous researchers have reported elevated rates of diversification during the Miocene in other SE Asian taxa, including callosciurine squirrels (Mercer and Roth, 2003) and *Tropidophorus* skinks (Honda et al., 2006).

4.4. Implications for homalopsid biogeography

Our divergence time analysis provides a framework for the study of homalopsid biogeography. Although an exhaustive analysis of distributional patterns is beyond the scope of the present study, we find at least two examples where our estimates of clade divergence offer insights into the historical factors underlying dispersal and speciation in Southeast Asia.

First, the close relationship of the endemic and morphologically distinct Lake Towuti and *E. matannensis* (also endemic to Sulawesi, known only from Lake Matana on Muna Island in SE Sulawesi) and their sister-group relationship to the geographically widespread *E. plumbea* (Fig. 3) is consistent with the hypothesis that *E. matannensis* and the Lake Towuti species are derived from a single dispersal event of *E. plumbea* from Borneo across the Makassar Strait to Sulawesi. The timing of this dispersal event roughly coincides with the shift from the early Pliocene highstands (~90 m above present level from 5.5 to 4.2 MYA) to the dramatically lowered sea levels (20 m below present level [BPL] *c.* 3.5 MYA; 100 m BPL *c.* 2.7 MYA) of the late Pliocene (Woodruff, 2003). This inferred trans-Makassar dispersal events is biogeographically significant as it represents a crossing of Wallace's original zoogeographic line (1863). A similar trans-Makassar Strait dispersal event has been inferred to explain the distribution of frogs of the *Limnonectes* sp. Complex 1 by Evans et al. (2003).

Block faulting and subsidence led to the formation of the Makassar strait in the middle Eocene (~42 MYA) and it has remained a relatively deep (>2000 m) seaway ever since (Kuenen, 1950; Moss and Wilson, 1998). Despite the lack of direct subaerial connections, glacioeustatic fluctuations during the late Pliocene/Pleistocene substantially narrowed the separation between Borneo and Sulawesi (Voris, 2000), possibly facilitating dispersal across the strait. Although the biotic contrast between the two sides of Wallace's line is well-documented (e.g. *Scomberomorus* spp.: Collette and Russo, 1984; Helfman et al., 1997; *Hippocampus trimaculatus*: Lourie and Vincent, 2004; *Echinolittorina trochoides*: Reid et al., 2006) our results add to a growing number of studies that suggest that it is not impermeable (e.g. Balke and Ribera, 2004; Brown and Guttman, 2002; de Boer and Duffels, 1996; Emerson et al., 2000; Evans et al., 2003; Heaney, 1986; How and Kitchener, 1997; Mcguire and Alcala, 2000).

Second, our study provides further insight into the biogeographic events surrounding the evolution of *Cerberus microlepis*. Alfaro et al. (2004) provided phylogenetic evidence that *C. microlepis*, the only freshwater member of the genus *Cerberus*, is derived from a Sunda shelf population of *C. rynchops* that dispersed across the Palawan and Mindoro islands to Luzon. Our estimated date for this split (~2.9 MYA) is congruent with the estimated age of the island chains themselves which are thought to be no older than 4–5 MY. This date corresponds roughly to the beginning of the relatively rapid glacioeustatic fluctuations with 23-, 41-, and 100-kyr periodicities attributable to variation in insolation and associated with changes in Northern Hemisphere ice volumes (Abreu and Anderson, 1998; Haq et al., 1987; Mitchum et al., 1994; reviewed in Woodruff, 2003). During these fluctuations, subaerial connections between Palawan and the Sunda shelf occurred periodically until ~160,000 YA (Evans et al., 2003; Heaney, 1986; Mcguire and Alcala, 2000; Mcguire and Kiew, 2001).

Alternatively, waif dispersal during this period might have led to the colonization of Luzon by *C. microlepis*. Recent studies of frogs and *Draco* lizards provide evidence for possible waif dispersal events between Borneo and the Palawan islands and the latter and the Mindoro islands (*Rana signata* complex: Brown and Guttman, 2002; *Limnonectes* spp. Evans et al., 2003; *Draco* spp.: Mcguire and Alcala, 2000). Waif dispersal may be especially likely for *Cerberus* spp. which are live-bearing, relatively saltwater tolerant (Dunson and Dunson, 1979) and ecological generalists (Alfaro et al., 2004).

Further insight into the biogeographic and evolutionary history of homalopsids will be gained through additional fossil and biogeographic calibrations and finer-scale geographic sampling. In addition, as our understanding of the geographic history of the Sunda region itself improves, we will develop a more coherent understanding of underlying causes of early homalopsid diversification as well as the effects of inferred marine transgressions on homalopsid evolution. Given the phylogenetic position of homalopsids within snakes, future studies of diversification in this group may also shed light on an important phase of colubroid historical biogeography.

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Appendix A

GenBank taxa used to test homalopsid monophyly

Species	GenBank Accession No.
Acrantophis dumerili	AY099963
Acrochordus granulatus	AF471124
Agkistrodon piscivorus	AF471096
Ahaetulla fronticincta	AF471161
Alluaudina bellyi	AY187966
Alsophis portoricensis	AF471126
Amphiesma stolata	AF471097
Antaresia childreni	AY099967
Aparallactus werneri	AF471116
Arrhyton exiguum	AF471117
Aspidelaps scutatus	AY058923
Atheris nitschei	AF471125
Atractaspis bibronii	AY187969
Bitis nasicornis	AF471130
Boa constrictor	AF471115
Boiga dendrophila	AF471128
Bothrophthalmus lineatus	AF471129
Boulengerina annulata	AY058925

Appendix A (*continued*)

Species	GenBank Accession No.
Bungarus fasciatus	AY058924
Calabaria reinhardtii	AY099978
Calamaria pavimentata	AF471103
Candoia carinata	AF039473
Casarea dussumieri	AF471114
Cemophora coccinea	AF471132
Cerastes cerastes	AF471131
Cerberus rynchops	AF471162
Charina bottae	AY099971
Charina trivirgata	AY099974
Coluber constrictor	AY486938
Coluber dorri	AY188001
Coluber zebrinus	AY188004
Compsophis albiventris	AY187972
Contia tenuis	AF471134
Coronella austriaca	AY486954
Coronella girondica	AF471113
Crotalus viridis	AF471135
Crotaphopeltis tornieri	AF471112
Cylindrophis ruffus	AF471133
Daboia russellii	AF471156
Dasypeltis atra	AF471136
Demansia atra	AY058927
Dendroaspis polylepis	AY058928
Diadophis punctatus	AF471122
Dinodon rufozonatum	AF471163
Dipsadoboa unicolor	AF471139
Dispholidus typus	AY187973
Ditypophis vivax	AY187974
Dromicodryas quadrilineatus	AY187976
Drymarchon corais	AF471137
Drysdalia coronata	AY058929
Duberria lutrix	AF471138
Eirenis aurolineatus	AY376807
Eirenis barani	AY376804
Eirenis collaris	AY376824
Eirenis coronelloides	AY376816
Eirenis decemlineata	AY376818
Eirenis eiselti	AY376805
Eirenis levantinus	AY376808
Eirenis lineomaculata	AY376820
Eirenis medus	AY376825
Eirenis modestus	AY376809
Eirenis persicus	AY376815
Eirenis punctatolineatus	AY376813
Eirenis rothi	AY376817
Eirenis thospitis	AY376819
Elaphe obsoleta	AF471140
Elaphe quatuorlineata	AY486955
Elapsoidea nigra	AY058930
Epicrates striatus	AY099966
Eryx johnii	AY099975
Eunectes murinus	AY099964

Appendix A (*continued*)

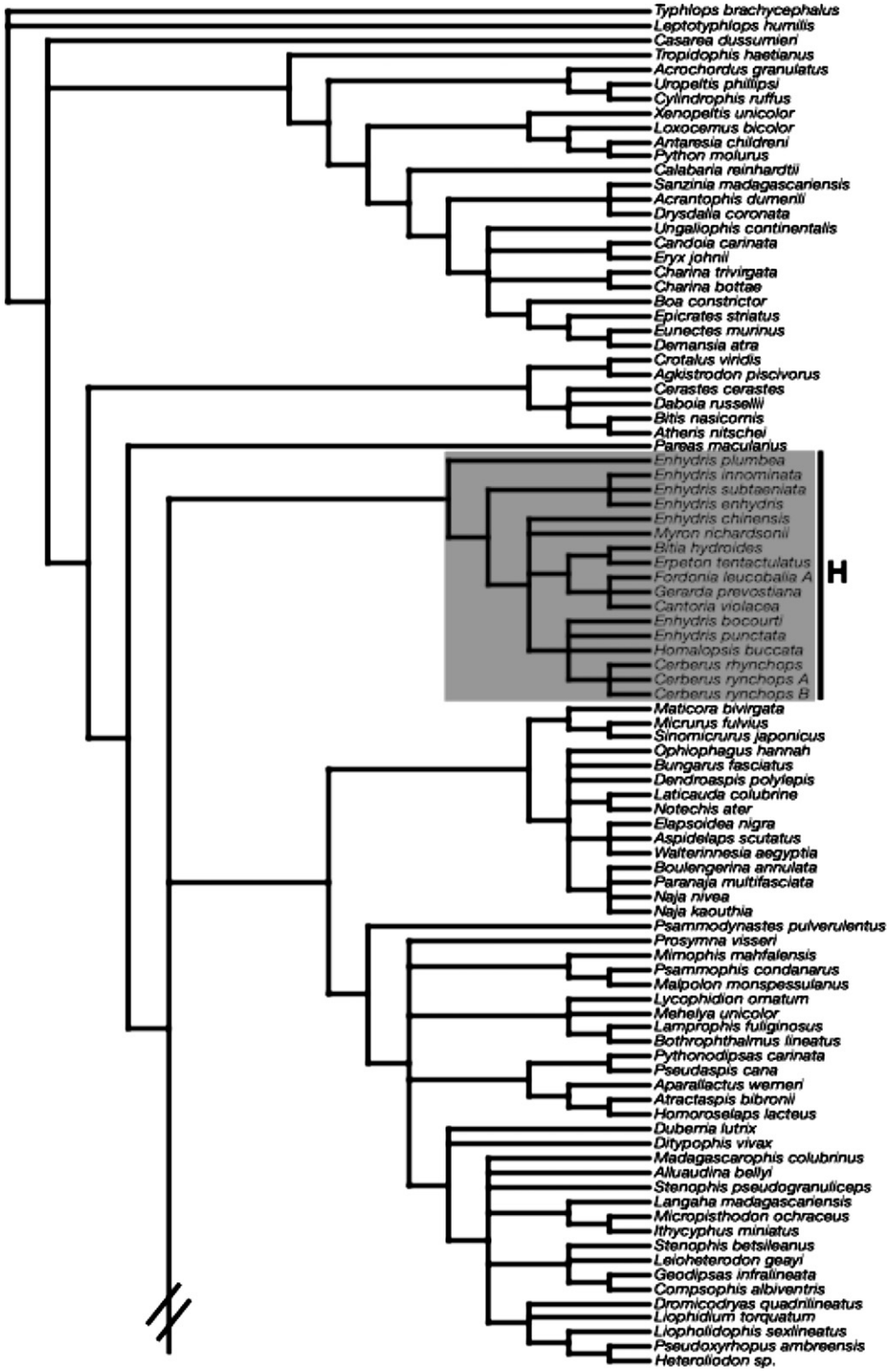
Species	GenBank Accession No.
Farancia abacura	AF471141
Geodipsas infralineata	AY187978
Gonyosoma oxycephalum	AF471105
Helicops angulatus	AF471160
Hemerophis socotrae	AY188003
Hemorrhois ravergieri	AY486944
Heterodon simus	AF471142
Heteroliodon sp.	AY187979
Hierophis caspius	AY376797
Hierophis gemonensis	AY376799
Hierophis jugularis	AY486941
Hierophis spinalis	AY376802
Hierophis viridiflavus	AY376803
Homoroselaps lacteus	AY058931
Hydrops triangularis	AF471158
Hypsiglena torquata	AF471159
Ithycyphus miniatus	AY187980
Lamprophis fuliginosus	AF471143
Langaha madagascariensis	AY187981
Laticauda colubrine	AY058932
Leioheterodon geayi	AY187982
Leptotyphlops humilis	AY099979
Liophidium torquatum	AY187984
Liopholidophis sexlineatus	AY187985
Loxocemus bicolor	AY444035
Lycodon zawi	AF471111
Lycophidion ornatum	AF471144
Lytorhynchus diadema	AY187986
Macroprotodon cucullatus	AY187987
Madagascarophis colubrinus	AY187989
Malpolon monspessulanus	AY187990
Masticophis flagellum	AY234228
Maticora bivirgata	AY058934
Mehelya unicolor	AF471099
Micropisthodon ochraceus	AY187991
Micrurus fulvius	AY058935
Mimophis mahfalensis	AY187993
Naja kaouthia	AY058938
Naja nivea	AY058939
Natriciteres olivacea	AF471146
Natrix natrix	AF471121
Notechis ater	AY058937
Oligodon cinereus	AF471101
Opheodrys aestivus	AF471147
Ophiophagus hannah	AY058940
Oxybelis aeneus	AF471148
Paranaja multifasciata	AY058941
Pareas macularius	AF471150
Philothamnus heterodermus	AF471149
Phyllorhynchus decurtatus	AF471098
Platyceps atayevi	AY486936

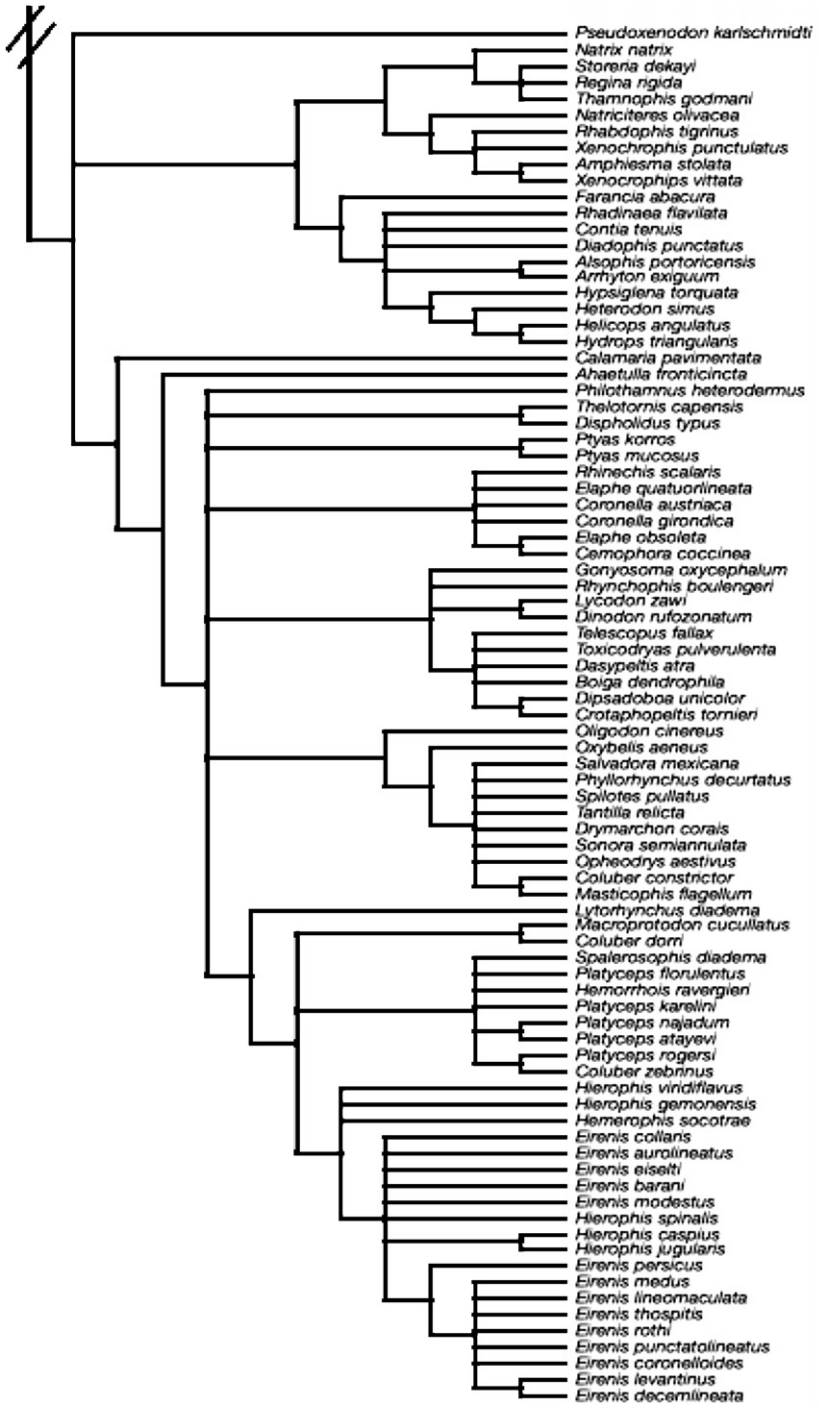
Appendix A (*continued*)

Species	GenBank Accession No.
Platyceps florulentus	AY486939
Platyceps karelini	AY486942
Platyceps najadum	AY486943
Platyceps rogersi	AY188002
Prosymna visseri	AY187994
Psammodynastes pulverulentus	AF471157
Pseudoxyrhopus ambreensis	AY187996
Ptyas korros	AY486953
Ptyas mucosus	AF471151
Python molurus	AY099968
Pythonodipsas carinata	AY187997
Regina rigida	AF471120
Rhabdophis tigrinus	AF471119
Rhadinaea flavilata	AF471152
Rhinechis scalaris	AY486956
Rhynchophis boulengeri	AF471153
Salvadora mexicana	AY486958
Sanzinia madagascariensis	AY099982
Sinomicrurus japonicus	AY058926
Sonora semiannulata	AF471164
Spalerosophis diadema	AY486950
Spilotes pullatus	AF471110
Stenophis betsileanus	AY187999
Stenophis pseudogranuliceps	AY187999
Storeria dekayi	AF471154
Tantilla relicta	AF471107
Telescopus fallax	AF471108
Thamnophis godmani	AF471165
Thelotornis capensis	AF471109
Toxicodryas pulverulenta	AF471118
Tropidophis haetianus	AY099962
Typhlops brachycephalus	AY099981
Ungaliophis continentalis	AY099970
Uropeltis phillipsi	AF471100
Walterinnesia aegyptia	AY058943
Xenochrophis punctulatus	AF471106
Xenopeltis unicolor	AY099977

Appendix B

ML phylogeny based on c-*mos* sequence data used to test homalopsid monophyly. Shown is the single most-likely topology ($-\ln L = 6599.93$) found during a heuristic search of the c-*mos* data set. Shaded region indicates the position of the Homalopsidae


Typhlops brachycephalus
Leptotyphlops humilis
Casarea dussumieri
Tropidophis haetianus
Acrochordus granulatus
Uropeltis phillipsi
Cylindrophis ruffus
Xenopeltis unicolor
Loxocemus bicolor
Antaresia childreni
Python molurus
Calabaria reinhardtii
Sanzinia madagascariensis
Acrantophis dumerili
Drysdalia coronata
Ungaliophis continentalis
Candoia carinata
Eryx johnii
Charina trivirgata
Charina bottae
Boa constrictor
Epicrates striatus
Eunectes murinus
Demansia atra
Crotalus viridis
Agkistrodon piscivorus
Cerastes cerastes
Daboia russellii
Bitis nasicornis
Atheris nitschei
Pareas macularius
Enhydris plumbea
Enhydris innominata
Enhydris subtaeniata
Enhydris enhydris
Enhydris chinensis
Myron richardsonii
Bitia hydroides
Erpeton tentactulatus
Fordonia leucobalia A
Gerarda prevostiana
Cantoria violacea
Enhydris bocourti
Enhydris punctata
Homalopsis buccata
Cerberus rhynchops
Cerberus rynchops A
Cerberus rynchops B
H
Maticora bivirgata
Micrurus fulvius
Sinomicrurus japonicus
Ophiophagus hannah
Bungarus fasciatus
Dendroaspis polylepis
Laticauda colubrine
Notechis ater
Elapsoidea nigra
Aspidelaps scutatus
Walterinnesia aegyptia
Boulengerina annulata
Paranaja multifasciata
Naja nivea
Naja kaouthia
Psammodynastes pulverulentus
Prosymna visseri
Mimophis mahfalensis
Psammophis condanarus
Malpolon monspessulanus
Lycophidion ornatum
Mehelya unicolor
Lamprophis fuliginosus
Bothrophthalmus lineatus
Pythonodipsas carinata
Pseudaspis cana
Aparallactus werneri
Atractaspis bibronii
Homoroselaps lacteus
Duberria lutrix
Ditypophis vivax
Madagascarophis colubrinus
Alluaudina bellyi
Stenophis pseudogranuliceps
Langaha madagascariensis
Micropisthodon ochraceus
Ithycyphus miniatus
Stenophis betsileanus
Leioheterodon geayi
Geodipsas infralineata
Compsophis albiventris
Dromicodryas quadrilineatus
Liophidium torquatum
Liopholidophis sexlineatus
Pseudoxyrhopus ambreensis
Heteroliodon sp.



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