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Phylogenetic Relationships of the Oriental-Australian Rear-Fanged Water Snakes (Colubridae: Homalopsinae) Based on Mitochondrial DNA Sequences

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The Homalopsinae (Oriental-Australian rear-fanged water snakes) is a small (34 species, 10 genera) colubrid subfamily notable for its ecological and morphological diversity. Despite considerable interest in the ecology and evolution of this group, phylogenetic relationships within the subfamily are poorly resolved. We present the results of a molecular phylogenetic study of the homalopsines based on partial sequence of three mitochondrial genes (12S and 16S ribosomal RNA and cytochrome *b*) from 14 ingroup species, five Old and New World natricines and the Old World colubrid, *Dinodon semicarinatus*. Maximum likelihood analysis in combination with bootstrapping and Bayesian Markov chain Monte Carlo methods for assessing phylogenetic confidence revealed that the single most likely topology contained a number of well-supported nodes. Homalopsine monophyly was strongly supported with respect to the outgroups included in our study. *Cantoria violacea*, a morphologically distinctive marine crustacean eater, formed the sister group to the rest of the homalopsines. *Enhydris*, the most species-rich genus in the subfamily, was polyphyletic with respect to other homalopsines although five morphologically and ecologically similar species formed a well-supported clade. The marine crustacean eaters *Fordonia leucobalia* and *Gerarda prevostiana*, also formed a novel clade. We discuss the evolutionary and ecological implications of this phylogeny for the Homalopsinae.

THE majority of colubroid snakes are placed in the Colubridae (approximately 290 genera, 1700 species), a family considered to be paraphyletic (Heise et al., 1995; Kraus and Brown, 1998). The Homalopsinae (Oriental-Australian rear-fanged water snakes) have been generally recognized as a valid monophyletic clade within the Colubridae (e.g., Greene, 1997; Pough et al., 1998; Zug et al., 2001) and assigned subfamilial rank (nomenclatural history described in Gyi, 1970), although they have been attributed familial (Günther, 1864) or tribal status (Dowling and Duellman, 1978) by some authors. However, the hypothesis of monophyly has not been convincingly demonstrated on the basis of either morphological (e.g., McDowell, 1987; Underwood, 1967; Zaher, 1999) or molecular evidence (e.g., Schwaner and Dessauer, 1982; Dowling et al., 1996; Kraus and Brown, 1998), nor has the relationship of homalopsine snakes to other colubroid lineages been resolved (e.g., Dessauer et al., 1987; Dowling and Duellman, 1978; Zaher, 1999). The most recent taxonomic treatment of the subfamily is by Gyi (1970). Based on morphological criteria, Gyi recognized 10 genera and 34 species. Gyi placed 22 species in the genus *Enhydris* and proposed species groupings within *Enhydris*, but he did not attempt a phylogenetic analysis of the subfamily Homolopsinae.

The Homalopsinae is widely distributed from the Indus Valley of Pakistan eastward into India and Nepal, across Myanmar into the Indochinese Peninsula, and northward into southern China. The subfamily extends southward across the islands of Indonesia and eastward to the Philippines, New Guinea, Australia, and the Palau Islands (Gyi, 1970; Murphy and Voris, 1994). All homalopsines are aquatic and eight of the 34 species (24%) are marine and live in mangrove forests, tidal mudflats, near-shore coastal waters, and estuarial habitats (Heatwole, 1999; but see Discussion). The freshwater species are found in ponds, streams, marshes, agricultural wetlands (e.g., rice paddies), and lakes (Gyi, 1970). Most homalopsines eat fish, frogs, and tadpoles, but crustacean feeding is well documented in three of the marine species (Voris and Murphy, 2002).

The Homalopsinae is defined by a suite of adaptations for aquatic life including crescentric, slitlike valvular nostrils, dorsally oriented eyes (most species), a glottis that can be extended to fit into the internal nares, and a shallowly notched rostral that permits tight closure of the mouth (Gyi, 1970). The posterior two or three maxillary teeth are enlarged and grooved and hypapophyses are present on most vertebrae. The species for which reproduction has

been described are all viviparous and they are all considered mildly venomous (Minton, 1990). Homalopsines are usually associated with mud substrates, relatively small in size (most species < 1 m adult size), primarily nocturnal, and live at low elevations. The homalopsines exhibit considerable morphological and ecological diversity for a small clade.

Despite their abundance and widespread distribution in South and Southeast Asia and northern Australia, the homalopsines remain poorly known. However, there is a growing literature on homalopsine ecology (Karns et al., 1999–2000; Murphy et al., 1999; Voris and Karns, 1996), conservation (Murphy and Voris, 1994; Stuart et al., 2000), diet and feeding behavior (Jayne et al., 1988; Mori, 1998; Voris and Murphy, 2002), predation (Voris and Jeffries, 1995), physiology (Dunson and Dunson, 1979; Heatwole, 1977; Heatwole and Seymour, 1978), and biogeography (Karns et al., 2000). Phylogenies provide a framework in which to test evolutionary and ecological hypotheses (e.g., Brooks and MacLennan, 1991; Harvey and Pagel, 1991), and a phylogeny of the homalopsine snakes is needed to provide historical context for ongoing and future studies and to help interpret past work.

Here, we report the results of a phylogenetic analysis of the Homalopsinae based on mitochondrial DNA sequences from the 12S and 16S ribosomal RNA and cytochrome *b* (cyt*b*) genes. Our objective was to investigate relationships among the genera and species of homalopsine snakes. We provide a summary of the systematic literature of the homalopsines, discuss the implications of our phylogeny for further evolutionary and ecological studies, and compare the homalopsine radiation with other ophidian lineages.

Materials and Methods

Outgroup justification.—Outgroup selection for phylogenetic analysis was potentially problematic because the relationship of homalopsines to other colubrid lineages is uncertain (see Discussion). In a preliminary analysis of sequences from 16S and cyt*b* gene fragments (E. Thompson, unpubl. data) homalopsine monophyly was supported with respect to a variety of colubrids including *Xenopeltis unicolor* (Xenopeltinae), *Tropidophis haetianus* (Tropidophiidae), *Heterodon nasicus* and *Carphophis amoenus* (Xenodontinae), as well as *Acrochordus granulatus* (Acrochordidae), *Hydrophis brookii* (Elapidae), *Trimeresurus albolabris* (Viperidae), *Boa constrictor* (Boidae), and *Python molurus* (Pythonidae). For this study, we chose three Old World (*Amphiesma*, *Natrix*, *Rhabdophis*) and two New World (*Nerodia*, *Thamnophis*) natricines as outgroups because natricines and homalopsines have been hypothesized to be close relatives (Dowling, 1967) and because sequence data for these taxa was available from a previous study (Alfaro and Arnold, 2001). We also included the Old World colubrid *Dinodon semicarinatus* to provide a nonnatricine and potentially more distant outgroup comparison.

Tissues and DNA extraction.—We used a sample of 14 homalopsine and six outgroup species of snakes in this analysis (see Materials Examined and Fig. 1). Samples included heart, liver, or tail tissue, and tissues were preserved in a saturated solution of EDTA or 93% ethanol. We extracted DNA obtained from the tissue using the Puregene DNA extraction kit (Gentra Systems, Minneapolis, MN). PCR amplification of most samples was accomplished with standard protocols, Ready-to-Go® Beads (Pharmacia Biotech) were used for samples that were difficult to amplify with traditional PCR cocktails. We used a combination of previously published and newly designed primers to amplify a 312 bp fragment of cyt*b,* a 877 bp fragment of 12S and a 550 bp fragment of 16S (Table 1). We purified successful amplification products with the Geneclean kit (BIO 101, Vista, CA) and cycle-sequenced the gene fragments using the Big Dye Ready Reaction Mix (ABI, Foster City, CA). Fluorescently labeled products were electrophoresed on an ABI Prism 377 Automated DNA Sequencer. Sequencher™ 3.0 (Gene Codes Corp.) was used to assemble contigs and create consensus sequences for each taxon. Gene sequences were deposited in GenBank; accession numbers are provided in Material Examined.

Sequence alignment and analysis.—Sequences from all genes were trimmed to the size of the smallest fragment to minimize the amount of missing data that was introduced to the data matrix. cyt*b* sequences were aligned by eye using Sequencher 3.0 (Gene Codes Corp). 12S and 16S sequences were aligned to previously published secondary structure models for natricine snakes (Alfaro and Arnold, 2001) and teleosts (Orti, 1997) using the data editor in PAUP*4.0b8 (D. L. Swofford, Sinauer Assoc., Sunderland, MA, 2000). Ambiguously alignable regions, usually corresponding to loops, were excluded from further analysis. After excluding unalignable regions and trimming sequence ends the cyt*b* gene partition contained 312 characters, the 12S partition contained 751 charac-

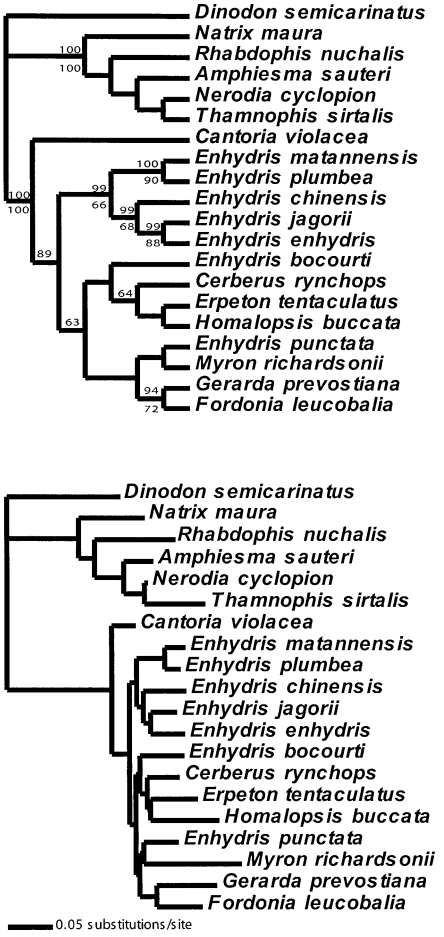

Fig. 1. Phylogeny of the Homalopsinae. (A) Shown is the single most likely tree ($-\ln(l) = 7551.34$) based on sequence from three mitochondrial genes: 12S, 16S, and cyt*b* under a GTR model of sequence evolution with a shape parameter of 0.6723 for gamma-distributed rate heterogeneity and a proportion of invariant sites equal to 0.5334. Numbers above the nodes indicate Bayesian posterior probabilities calculated from the last 900,000 generations (sampling every 100 trees) of a 1,000,000 generation Markov Chain. Numbers below nodes indicate the bootstrap proportion calculated from 200 bootstrap replicates. Values below 50 are not reported. *Enhydris* is shown to be polyphyletic with respect to other homalopsines. Bayesian posteriors provide support for an *Enhydris* complex as well as for the topology within the *Enhydris* clade and strong support for two novel clades: (*Gerarda* + *Fordonia*) and *Cerberus* + (*Erpeton* + *Homalopsis*). *Cantoria violacea* receives moderate support as the sister group to the rest of the homalopsines. (B) Phylogram indicating maximum likelihood branch lengths. Shown is the maximum likelihood topology with branch lengths equal to the expected number of substitutions per site.

ters and the 16S partition contained 425 characters. Although all three genes examined in this study come from the mitochondrion and, thus, would be expected to reflect a shared evolutionary history, it is possible that evolutionary rates among genes have differed enough that there would be apparent conflict in their phylogenetic signal (Bull et al., 1993). The incongruence length difference (ILD) test (Farris et al., 1995), as implemented in PAUP*4.0b8, was used to determine whether significant conflict existed among the three data partitions (12S, 16S, and cyt*b*). We generated the null distribution for this test using 500 replications of heuristic searching with two random addition sequence replicates. Following Cunningham (1997) invariant sites were excluded, and a significance level of 0.01 was adopted for this test. The ILD test indicated that there was no significant conflict among any of the partitions, and we combined our data for phylogenetic analyses.

16S sequence was not available for the natricine taxa, and as a result the final data matrix contained missing entries for these five taxa and 425 characters. Preliminary analyses to examine the effects of missing data on the resultant maximum likelihood topology by excluding both the 16S gene partition and the natricine outgroups revealed that ingroup relationships were insensitive to these matrix perturbations so we used the combined matrix (20 taxa by three genes and 1490 characters) for all subsequent analyses. To justify an appropriate model for maximum likelihood analysis we used the program MODELTEST 3.06 (Posada and Crandall, 1998), which performs a series of likelihood ratio tests (Huelsenbeck and Crandall, 1997; Huelsenbeck and Rannala, 1997) on nested likelihood models. This procedure revealed that a GTR model with parameters for invariant sites (pinv = 0.5334) and gamma distributed rate heterogeneity ($\alpha = 0.6723$) best fit our data. We used PAUP*4.0b8 (D. L. Swofford, Sinauer Assoc., Sunderland, MA, 2000) to perform a heuristic search with 25 random addition sequences and TBR branch swapping under this model. We used two methods to estimate support for nodes within the likelihood topology. Bootstrapping (Felsenstein, 1985) with 200 total pseudoreplicates under the fixed likelihood model, two random addition sequences per replicate, and TBR swapping was performed using PAUP*4.0b8. We also used MrBayes 2.01 (Huelsenbeck, 2000) to calculate the posterior probabilities of clades. We ran the Markov chain using the same model as we used in our likelihood analysis (GTR + G + I) for 1,000,000 genera-

TABLE 1. PRIMER SEQUENCES FOR AMPLIFICATION AND SEQUENCING PRIMERS USED IN THIS STUDY.

Primer	Mitochondrial gene	Sequence (5′–3′)
12S-1	12S	GCTTCAAACTGGGATTAGATACC
12S-2	12S	GCAGAGGGTGACGGGCGGTGTGT
12S-8	12S	CGAGTGTAGGTCGAGTGCTTTG
12S-m	12S	CGACGGCGGTATATAGACTG
12S-m2	12S	TTACTCGTAGTTATTTGGCG
12S-3	12S	TATACATGCAAGCCTCACCA
12S-e2	12S	AGGTCTTGGTCTTAAACC
16S-ar	16S	GCGCTGTTTATCAAAAACAT
16S-br	16S	CCGGTCTGAACTCAGATCACGT
B1a	cytochrome *b*	CCATCCAACATCTCAGCATGATGAAA
B2	cytochrome *b*	AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA
B3	cytochrome *b*	GGCAAATAGGAARTATCATTC
GLUDG-L	cytochrome *b*	TGACTTGAARAACCAYCGTTG

tions sampling every 100 trees. Visual inspection revealed that model parameters reached stationarity within 20,000 generations; however we discarded the first 100,000 generations to ensure that the Markov chain was sampling from the target distribution. Posterior probabilities were calculated from the majority-rule consensus trees constructed from the remaining 9000 trees (Larget and Simon, 1999). For complex, multiparameter problems like Bayesian phylogenetic inference, it can be difficult to determine whether the Markov chain is mixing well and whether the chain has run a sufficient number of generations to approximate the joint posterior distribution (Li et al., 2000). We monitored convergence of the Markov chain by repeating our Bayesian analysis five times and examining the variance associated with nodal posterior probabilities. Standard deviation of posterior probabilities reported in this paper were less than 1%, strongly suggesting the Markov chain was performing adequately for our data. From a theoretical perspective, standard nonparametric bootstrapping and Bayesian Markov chain Monte Carlo sampling share some similarities in that both approaches may be regarded as methods for sampling the posterior probabilities of trees (Efron et al., 1996; Larget and Simon, 1999). However the comparative performance and behavior of these two methods is not yet well understood.

RESULTS

We found a moderate amount of genetic differentiation across the taxa in our study. Within the Homalopsinae uncorrected genetic distances ranged from 4–10% and within the genus *Enhydris* they ranged from 4–7%. Distances between the ingroup and *Dinodon* ranged from 13–15%, between the ingroup and *Natrix* from 14–17%, and between the ingroup and *Nerodia* from 15–18%.

Maximum likelihood analysis produced a single most likely tree (Fig. 1). This tree contains eight of the 10 genera and approximately half of the described species of homalopsine and thus represents the most thorough hypothesis of relationship within the subfamily to date. We found strong support for a number of novel and interesting clades within the subfamily. Homalopsine monophyly was strongly supported by both bootstrapping and Bayesian analysis. The position of *Cantoria violacea*, a morphologically unusual, crustacean-eating, marine species, as most basal member of the subfamily, received a moderate posterior probability. The genus *Enhydris* was polyphyletic with respect to other homalopsines although we found a high posterior probability for a clade consisting of five morphologically and ecologically similar *Enhydris*. Bayesian support for all relationships within this clade was 100% and bootstrap proportions were also generally high (Fig. 1). We also found a high posterior probability for a sister group relationship between *Gerarda* and *Fordonia*, both crustacean eaters. The clade consisting of *Erpeton tentaculatus*, *Homalopsis buccata*, and *Cerberus rynchops* contains three morphologically and ecologically very distinctive taxa and represents a novel hypothesis of relationship. However, the posterior probability of this clade is low, suggesting that this clade is weakly supported by the current data.

For our data the posterior probability of a clade always equaled or exceeded the bootstrap proportion. Other studies which have used both support methods have similarly found that pos-

terior probabilities for clades are often more extreme than the bootstrap proportion (Murphy et al., 2001; Bollback and Huelsenbeck, 2001; Streelman et al., 2002). A discussion of the reasons for the differential behavior of these methods is beyond the scope of this paper. However, we note that in simulation over a range of conditions, the Markov-chain based Bayesian approach finds support for a greater number of true clades than does bootstrapping (M. Alfaro et al., unpubl. data) which is consistent with the observation that bootstrapping is a conservative measure of phylogenetic accuracy in some situations (Hillis and Bull, 1993).

Discussion

Intergeneric relationships of the Homalopsinae.—Our results are largely incongruent with the most recent revision of the subfamily (Gyi, 1970). For example, Gyi proposed that (*Fordonia* + *Gerarda* + *Cantoria*) formed a clade. Although our results support a sister group relationship between *Gerarda* and *Fordonia*, we find *C. violacea* to be the sister to the rest of the homalopsines. Furthermore, our *Cerberus* + (*Erpeton* + *Homalopsis*) clade is incongruent with Gyi's (*Enhydris* + *Homalopsis* + *Cerberus*) group [although our arrangement is congruent with another previously proposed morphological hypothesis (Underwood, 1967)]. Notably, these three taxa are morphologically and ecologically distinctive: *Erpeton tentaculatus* is a freshwater sit-and-wait fish predator with unique rostral appendages, *Homalopsis buccata* is a stout, large-headed, freshwater fish-eater, and *Cerberus rynchops* is a coastal marine fish-eater. *Cerberus* has a wide geographic distribution (India to Australia) and may contain several species (Karns et al., 2000). The most striking difference between our results and those of Gyi concern the status of the genus *Enhydris*. Of the 34 species of homalopsines recognized by Gyi, he placed 22 into *Enhydris*. Gyi further recognized nine species groups within *Enhydris* based on squamation, body proportions, coloration, and dentition. Our study included eight species of *Enhydris* (spread across four of Gyi's nine *Enhydris* groupings) and found that the genus, as currently recognized, is polyphyletic. Five freshwater species, *E. jagorii*, *E. chinensis*, *E. enhydris*, *E. matannensis*, and *E. plumbea*, are relatively similar morphologically and form a well-supported clade in our analysis. The subgroup *E. chinensis*, *E. jagorii*, and *E. enhydris* within this group are morphologically very similar to each other. Notably, the two *Enhydris* species that were not part of the five-species clade, *E. punctata* and *E. bocourti*, are the largest and stoutest of the homalopsines, and differ from other members of the genus in squamation and skull anatomy (Gyi, 1970). Our study highlights the need for more inclusive sampling of this genus to further elucidate the relationship of *Enhydris* members to the rest of the subfamily.

Phylogenetic position and status of the Homalopsinae.—This study included only two distinct colubrid lineages (*Dinodon* + Old and New World natricines) and thus provides only a weak test of the monophyly of the Homalopsinae. Nevertheless, our results are consistent with those of a number of biochemical studies (e.g., Dessauer et al., 1987; Dowling et al., 1983, 1996; George and Dessauer, 1970) and molecular studies (Heise et al., 1995; Kraus and Brown, 1998) that have generally supported the monophyly of the subfamily. Although the available biochemical and molecular evidence support homalopsine monophyly, it has proved problematic to identify morphological synapomorphies for the group. Despite a long history of morphological work (e.g., Dowling and Duellman, 1978; McDowell, 1986, 1987; Underwood, 1967) and a comprehensive descriptive review of the group (Gyi, 1970), there are no known unambiguous synapomorphies that define the Homalopsinae (Zaher, 1999). The subfamily is recognized by a shared suite of characters largely related to the aquatic adaptive zone inhabited by these snakes, and these characters occur in other aquatic snakes as well (e.g., valvular nostrils, dorsally located eyes). A better understanding of homalopsine morphology is necessary to properly evaluate the affinities of a number of colubrids described from southeast Asia that have tentatively been placed in the Homalopsinae. For example, McDowell (1987) suggested that *Brachyorrhos* (*B. albus*, *B. jobiensis*), a terrestrial, aglyphous (without fangs) genus from New Guinea and adjacent islands, is a member of the Homalopsinae and Zaher (1999) noted this genus as *incertae sedis* within Homalopsinae. *Anoplohydrus aemulans*, (described by Werner, 1909), a snake from Sumatra known only from the holotype, was placed in the Homalopsinae by Iskandar and Coljin (2001) based on a suggestion by Van Wallach in David and Vogel (1996). Also, an undescribed, paddle-tailed, freshwater snake from Sulawesi will soon be described as a new homalopsine genus (D. Iskander, pers. comm.). Formal justification for the inclusion of any of these taxa in the Homalopsinae is severely hampered by the present lack of a systematic study of homalopsine morphology.

The question of the phylogenetic position of

the Homalopsinae within the Colubridae remains unresolved. Recent studies have invalidated some of the older suggested taxonomic arrangements. For example, based on morphological criteria, Underwood (1967) recognized the Homalopsidae which included three subfamilies: Homalopsinae, Boiginae, and Dasypeltinae, and Dowling and Duellman (1978) lumped three largely aquatic taxa into what would currently be considered a paraphyletic taxon, the Natricinae (watersnakes and their allies), consisting of three tribes: the homalopsines, the Old and New World natricines, and the acrochordids. More recently, McDowell (1986), based on details of the architecture of the corner of the mouth, suggested that proteroglyphous (fixed front-fanged) snakes are primitive remnants of the basal stock of the Colubroidea and that homalopsines are closely related to proteroglyphs. A linkage between the homalopsines and *Acrochordus*, currently considered to be the sister group to the colubroids (Greene, 1997; Pough et al., 1998), has been suggested (Dowling, 1996; Dowling and Duellman, 1978) but is not supported on biochemical grounds (Dessauer et al., 1987; Kraus and Brown, 1998). Analysis of the ND 4 gene (Kraus and Brown, 1998) showed the Pareatinae (an Old World clade dominated by snail-eaters) to be the sister group of their representative homalopsine (*Cerberus* + *Enhydris*) clade. Zaher (1999) suggested a clade comprised of the ''Pseudooxyrhophiinae'' (Malagasy colubrids) + ''Homalopsinae'' based on hemipenal synapomorphies (quotes used by Zaher indicate groups that he considers problematic). This sample of divergent hypotheses demonstrates the current uncertainty regarding the higher-level relationships of the Colubroidea and the phylogenetic position of Homalopsinae.

Origin and biogeography.—The center of species richness and abundance for the Homalopsinae is Indochina and the adjacent Sunda Shelf area (Borneo, Java, Sumatra, and adjacent islands). In this region, 19 species of homalopsines are found. The number of species decreases to the west (seven species in India, five species in Sri Lanka) and to the east (six species known from the Sahul Shelf: Australia, New Guinea, and adjacent islands). In the phylogeny presented here, 12 of the 14 homalopsine species are found in the Indochina/Sunda Shelf region. Based on these patterns of species diversity, it is reasonable to hypothesize that the Indochina/Sunda Shelf region is the geographical site of origin for the homalopsine radiation, although caution is required in using species numbers to infer centers of origins (Brown and Lomolino, 1998). The Homalopsinae have been variously referred to as ''Oriental Rear-fanged Snakes'' (Dowling and Duellman, 1978), ''Asian Water Snakes'' (Murphy and Voris, 1994), and ''Poisonous River Snakes'' (Ditmars, 1922). Because of their geographic distribution, we propose the ''Oriental-Australian rear-fanged water snakes'' as a common name.

Available evidence supports the hypothesis that not only the Homalopsinae but the entire colubroid radiation originated and diversified in Southeast Asia (Kraus and Brown, 1998). This region supports the highest number of basal colubroid taxa in the world, two primitive taxa, the Xenoderminae (primitive colubrids) and the Acrochordidae (sister group to the colubroids; Greene, 1997), are endemic to the region, and the oldest known colubrid fossil is from the Eocene of Thailand (Rage et al., 1992). Thus, the Homalopsinae may represent an adaptive radiation that occurred during the early evolution of colubroid snakes in Southeast Asia. If true, the antiquity of the lineage would help explain some of the ecological and morphological diversity found in this relatively small clade. Colubroid snakes appear in the early Tertiary, but there is no fossil record or reliable geological age estimate available for the Homalopsinae (Rage, 1987).

In the phylogeny presented here, *Cantoria violacea* receives support as the sister group to the other homalopsines. With this placement, simple parsimony reconstruction of homalopsine ancestral body type and habit would yield an ambiguity between two possible ecomorphs: medium to stout bodied freshwater snakes (the most common state in the subfamily) and elongate, slender, marine snakes (the condition in *Cantoria*). Although homalopsines have generally been considered to have descended from a stout-bodied freshwater form, the *Cantoria*-like reconstruction is consistent with at least one previously proposed evolutionary scenario for the group. McDowell (1986) suggested that homalopsines are closely related to proteroglyphs and that proteroglyphous snakes are remnants of the basal stock of the Colubroidea. He further suggested that the original habitat of the Colubroidea was seacoast and mangrove habitats, which describes the current habitat of *Cantoria*. Further molecular and morphological analyses of more homalopsines are needed to confirm the relationship of *C. violacea* to the rest of the homalopsines, to resolve the position of *Cantoria annulata* (not included in this study) within the Homalopsinae, and to address ques-

tions surrounding the origin and adaptive radiation of the homalopsines.

Southeast Asia has a complex geological and climatic history (references in Hall and Holloway, 1998) that has influenced the biogeographical and evolutionary history of the homalopsine snakes. Some species of homalopsines are extremely widespread; among the species included in this study, *Cerberus rynchops*, *Fordonia leucobalia*, *Homalopsis buccata*, *Gerarda prevostiana*, and *Enhydris enhydris* are found from India or near India, across Southeast Asia, into or adjacent to Wallacea (the region between the Sunda and Sahul Shelves), and *C. rynchops* and *F. leucobalia* extend further into Australia, New Guinea, and adjacent islands. These widely distributed taxa raise interesting questions concerning dispersal (marine vs freshwater taxa), vicariance, gene flow, speciation, and phylogeography that we are currently investigating within the context of the complex historical biogeography of the region (Voris, 2000). Using mitochondrial DNA variation, Karns et al. (2000) found significant genetic divergence between *C. rynchops* from Australia and *C. rynchops* populations from the Sunda Shelf region, which is consistent with the ancient separation and isolation of the Oriental and Australian biogeographic regions.

Adaptive zone of the Homalopsines.—Examination of ophidian clades reveals considerable variation in ecological and morphological diversity (Greene, 1997). Many smaller clades (e.g., Pareatinae, Xenoderminae, Calamarinae) are ecologically and morphologically conservative. Homalopsines have not entered into other major adaptive zones such as the terrestrial or arboreal; however, the Homalopsinae do exhibit an interesting diversity for a relatively small clade. They are morphologically diverse. *Bitia hydroides* (not included in this study) is a marine species with a small head, enlarged palatine teeth, narrow neck, reduced ventral scales, and somewhat flattened tail that resembles true sea snakes (Jayne et al., 1995). The homalopsine clade exhibits considerable variation in the morphology of the head, including the bizarre *Erpeton tentaculatus* with its unique rostral appendages, and head sizes ranging from the almost microcephalic (e.g., *Enhydris enhydris*, *E. indica*) to the massive (e.g., *E. bocourti*, *H. buccata*). Homalopsine body size and shape varies from the extremely long and slender (*C. violacea*) to the extremely stout (*H. buccata*).

Variation in head and body size and shape is an indicator of dietary diversity. The majority of homalopsines feed on fish, several species feed on frogs and tadpoles, and at least three species are crustacean specialists (Jayne et al., 1988, 1995; Shine and Schwaner, 1985; Voris and Murphy, 2002). All of these diets occur among the species in our phylogeny. Crustacean eating is an unusual diet among snakes (Greene, 1997), and only homalopsine snakes are known to specialize in marine crustaceans. Three species in our phylogeny are well-documented marine crustacean eaters. *Fordonia leucobalia* (a hard shell crab-eater) and *G. prevostiana* (a specialist on recently molted crabs) comprise a well-supported clade in our phylogeny and *Myron richardsonii*, reported to feed on fish and crabs, is weakly supported as a sister taxon. *Cantoria violacea* feeds on snapping shrimp but is not part of the *Fordonia* + *Gerarda* clade. Therefore, our phylogenetic results indicate that crustacean-specialization has evolved at least twice in the Homalopsinae.

Another important aspect of the diversity of the Homalopsinae is saltwater tolerance and the ability to live in marine habitats. The Homalopsinae exhibit the largest marine radiation of any colubrid lineage. Eight of the 34 species (24%) live in marine habitats (Heatwole, 1999) and five of these species are included in this study: *C. rynchops*, *G. prevostiana*, *F. leucobalia*, *M. richardsoni*, and *C. violacea* (*Enhydris bennetti*, *C. annulata*, and *B. hydroides* are not included in this study; Heatwole also characterizes *E. chinensis* as a marine homalopsine, but we do not). We have documented saltwater tolerance in *F. leucobalia*, *G. prevostiana*, *C. rynchops*, and *C. violacea* in laboratory tolerance tests (H. K. Voris, D. R. Karns, and J. C. Murphy, unpubl.). Thus, for our tree the ancestral state of the homalopsines with respect to saltwater tolerance is equivocal since *Cantoria* is saltwater tolerant and basal. All marine reptiles that have been studied possess a salt gland of some type, and the marine homalopsines presumably possess salt glands as well; but to date, this has been documented only in *C. rynchops* (Dunson and Dunson, 1979). Our phylogeny suggests that salt glands may have evolved multiple times in the Homalopsinae or been lost multiple times.

Materials Examined

Amphieisma sauteri, FMNH 232808, Hongya Xian, Sichuan, China; *Cantoria violacea*, FMNH 250116, Phuket, Thailand, GenBank accession AF499292; *Cerberus rynchops*, USNM 497590, Polillo, Philippines, GenBank accession AF499289; *Dinodon semicarinatus*, GenBank accession NC 001945; *Enhydris bocourti*, FMNH 252500, Thailand; *Enhydris chinensis*, ROM 31031, Tam Dao,

Vietnam, GenBank accession AF499280; *Enhydris enhydris*, FMNH 250119, Thailand, GenBank accession AF499285; *Enhydris jagorii*, FMNH 252506, Thailand, GenBank accession AF499284; *Enhydris matannensis*, D. Iskandar, GenBank accession AF499281; *Enhydris plumbea*, FMNH 250123, Terengganu, Malaysia, GenBank accession AF499282; *Enhydris punctata*, FMNH 250112, Selangor, Malaysia, GenBank accession AF499283; *Erpeton tentaculatus*, FMNH 252504, Phattalung, Thailand, GenBank accession AF499286; *Fordonia leucobalia*, NTM R22714, Northern Territories, Australia, GenBank accession AF499290; *Gerarda prevostiana*, ZRC 2.346, Singapore, GenBank accession AF499287; *Homalopsis buccata*, FMNH 252514, Thailand, GenBank accession AF499288; *Myron richardsonii*, NTM R22718, Northern Territories, Australia, GenBank accession AF499291; *Natrix maura*, MEA 510, St. Laurent le Minier, Dept. du Gard, France; *Nerodia cyclopion*, Steven J. Arnold (SJA) 7995B, Baton Rouge Parish, LA; *Rhabdophis nuchalis*, HKV 36838, Hongya Xian., Sichuan, China; *Thamnophis sirtalis*, SJA 4545, Humboldt County, CA.

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