



Phylogeny of *Cerberus* (Serpentes: Homalopsinae) and phylogeography of *Cerberus rynchops*: diversification of a coastal marine snake in Southeast Asia

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ABSTRACT

Aim The biogeography of Southeast Asia has been greatly affected by plate tectonic events over the last 10 Myr and changing sea levels during the Quaternary. We investigated how these events may have influenced the evolution of *Cerberus* Cuvier, a marine coastal snake belonging to the Homalopsinae (Oriental-Australian Rear-fanged Water Snakes). This study is an expansion of a previous study on the biogeography and systematics of *Cerberus*.

Location We obtained species from localities across the range of the widely distributed *Cerberus*: India, Sri Lanka, the Andaman islands, Myanmar, the Philippines, Borneo, Sulawesi, Sumatra, Vietnam, Thailand, Singapore and Australia.

Methods We analysed mtDNA sequences (12S, ND3, ATPase, 2338 nucleotide characters) from 21 localities. The sample consisted of 65 *Cerberus rynchops* (Schneider), three *Cerberus australis* (Gray) and four *Cerberus microlepis* Boulenger. One *Homalopsis buccata* (Linnaeus), one *Bitia hydroides* Gray, one *Enhydryis enhydryis* (Schneider), and two *Enhydryis plumbea* (Boie) were used as outgroups.

Results We produced phylogenetic trees based on parsimony, maximum likelihood and Bayesian analysis. We did not find unambiguous support for the monophyly of *Cerberus*. *Cerberus australis*, *H. buccata* and all other *Cerberus* populations formed a three-way basal polytomy under parsimony and *C. australis* formed the sister group to a clade consisting of *H. buccata* and all other *Cerberus* in likelihood and Bayesian analysis. The non-Australian *Cerberus* were monophyletic and consisted of four primary biogeographical clades: Indian and Myanmar, Philippines, Greater Sunda Islands and Sulawesi, and the Thai-Malay peninsula and Gulf of Thailand. The range of genetic divergence between these clades and Australian *Cerberus* was 0.06–0.12. Genetic divergence among clades to the west of Australia was less pronounced (Thai-Malay peninsula and Gulf of Thailand = 0.02–0.05; Sunda Islands and Sulawesi = 0.02–0.05; Philippines = 0.02–0.06; India and Myanmar = 0.04–0.06, Philippines = 0.02–0.5).

Main conclusions Gyi [University of Kansas Publications, Museum of Natural History 20 (1970), 47] recognized three species of *Cerberus*: *C. australis* (from Australia), *C. microlepis* (known only from Lake Buhi in the Philippines), and the widely distributed *C. rynchops* (India to Wallacea). We did not find strong support for the monophyly of the genus. *Cerberus australis* is highly divergent from all other *Cerberus* lineages sampled from this region. The geographically widespread *C. rynchops* is resolved into four biogeographical clades (Indian and

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Myanmar, Philippines, Greater Sunda Islands and Suluwesi, and the Thai-Malay Peninsula and Gulf of Thailand). We discuss how the dispersal biology of a salt-water tolerant, coastal marine taxon and the complex geological history of the region (Tertiary plate tectonic movements and Quaternary sea-level changes) could produce the observed patterns of diversification.

Keywords

Cerberus rynchops, Homalopsinae, Asian water snake, mtDNA, Southeast Asia, mangroves, phylogeography.

INTRODUCTION

The Oriental and Australian zoogeographical regions have been of special interest to evolutionary biologists and biogeographers since Wallace (1860) first formalized the faunal affinities of these areas. Over the last 2 Myr the area has been profoundly affected by changes in sea levels, shorelines, climate and vegetation associated with Quaternary glacial episodes (Flenley, 1985; Bloom & Yonekura, 1990; Pirazzoli, 1996; Voris, 2000; Woodruff, 2003). On a longer time scale, Cenozoic tectonic events such as the collision of India with Asia and the movement of the Australian plate towards the equator have brought floras and faunas with very different histories into juxtaposition (Hall & Holloway, 1998; Hall, 2002). The entire area from the Asian mainland to New Guinea has had an exceedingly complex history due to Quaternary and long-term tectonic events (Hall, 1998). These processes have produced dynamic and diverse opportunities for dispersal and vicariance events and probably account for the spectacular terrestrial and marine biodiversity of the region. There is a large literature documenting how the historical biogeography of the Oriental-Australian region may have affected the evolutionary history of a variety of taxa (e.g. Austin, 1999; Brown & Guttman, 2002; Cannon & Manos, 2003; de Bruyn *et al.*, 2004). In these and other studies molecular techniques have provided a powerful set of tools to rigorously test biogeographical hypotheses.

The colubrid subfamily Homalopsinae Jan (Oriental-Australian rear-fanged water snakes) includes 10 genera and 34 species of medium-sized snakes distributed from India across Southeast Asia to northern Australia (Gyi, 1970; Murphy & Voris, 1994; Greer, 1997; Voris *et al.*, 2002). The group is diagnosed by a small suite of morphological characters (Greer, 1997) but despite a long history of morphological work (e.g. Underwood, 1967; Dowling & Duellman, 1978; McDowell, 1987) there are no known unambiguous synapomorphies that define the Homalopsinae (Zaher, 1999; Voris *et al.*, 2002). However, molecular data sets have recently provided growing evidence of the monophyly of the Homalopsinae (Voris *et al.*, 2002; M.E. Alfaro *et al.*, in review).

As part of an ongoing study of the ecology and evolution of homalopsine snakes (e.g. Karns *et al.*, 2002; Voris *et al.*, 2002) we are interested in how the complex biogeographical history of

this region might differentially affect the evolutionary history of homalopsine species with different physiological and ecological characteristics. Members of the subfamily share several features that are frequently observed among semi-aquatic snakes, including dorsally placed valvular nostrils, relatively small eyes, and live birth (Kathariner, 1900; Greer, 1997; Heatwole, 1999; Costa & Hofstadler-Deiques, 2002). All homalopsines are amphibious, usually associated with mud substrates, primarily nocturnal, and live at relatively low elevations. Eight of the 34 species (24%) are coastal marine species living in mangrove forests, tidal mudflats, near-shore coastal waters and estuarial habitats (Heatwole, 1999; Karns *et al.*, 2000). The freshwater species are found in ponds, streams, wetlands, agricultural wetlands (e.g. rice paddies) and lakes (Gyi, 1970; Voris & Karns, 1996). Most homalopsines eat fish, frogs and tadpoles, but feeding on crustaceans is well documented in three of the coastal marine species (Jayne *et al.*, 2002; Voris & Murphy, 2002). Overall, the homalopsines exhibit considerable morphological and ecological diversity for a small ophidian clade.

The coastal marine and freshwater species of homalopsine snakes should be differentially affected by the biogeographical history of the Oriental-Australian region. Taking the Thai-Malay peninsula as an example, the potential for dispersal and gene flow among populations of freshwater species should be facilitated during periods of low sea level when land mass connectivity is greatest. Avenues along major river systems would theoretically allow mixing between populations in Thailand, Sumatra and Borneo (Karns *et al.*, 2000; Voris, 2000). In contrast, low sea level and concomitant greater land mass connectivity might be expected to create new vicariant barriers for coastal marine populations, resulting in reduced gene flow for these species. Thus, marine populations on the east and west coast of the Thai-Malay peninsula would be effectively isolated when sea level was low. Alternatively, falling sea levels might produce novel coastline corridors that would, for example, increase gene flow between populations on the east coast of the Thai-Malay peninsula and Borneo (e.g. see Voris, 2000; Woodruff, 2000). Additionally, the probability of successful long-distance dispersal across marine barriers (e.g. from the Sunda Shelf to the Sahul Shelf via Wallacea) should be greater for coastal marine species because they live along coasts and are salt water tolerant.

We chose *Cerberus* Cuvier, to initiate a study of these biogeographical questions because it is (1) widespread, (2) marine and (3) locally abundant over much of its range. *Cerberus rynchops* (Schneider) has the widest distribution of any species of homalopsine (from India across Southeast Asia to New Guinea); indeed, the range of this one species is almost coincident with the range of the entire subfamily. This wide geographical range is most certainly due to a suite of factors that includes habitat (inter-tidal mud flats and mangrove forests) and ecological and physiological versatility (diverse diet, wide thermal tolerance, salt-water tolerance, viviparity). *Cerberus rynchops* is most often associated with mangrove forests and mudflats along the shores of brackish estuarine and marine coastal environments (Greer, 1997; Karns *et al.*, 2000); however, it is occasionally found in freshwater as well and on sheltered sandy coasts. It feeds on a wide variety of fish including eight families and 15 genera (Jayne *et al.*, 1988), and has also been reported to eat crustaceans, tadpoles and frogs (Voris & Murphy, 2002). It is the only homalopsine species in which a salt gland (premaxillary and rudimentary compared with other marine reptiles) has been documented (Dunson & Dunson, 1979).

Studies on the spatial ecology of *C. rynchops* (Jayne *et al.*, 1988; D.R. Karns & H.K. Voris, unpubl. obs.) indicate that this species is closely associated with aquatic edges, sedentary, and does not normally venture into deeper water. Thus, gene flow among *Cerberus* populations is most likely to occur in small incremental steps along coasts between adjacent local populations. Although it avoids deep water, it is plausible that *C. rynchops* could occasionally survive long-distance transport in open water via rafts if accidentally carried out to sea and picked up by suitable currents. As a live bearing, ecological generalist, a gravid female might well survive landfall and become established under less than ideal conditions. Human-mediated dispersal (e.g. in the bilge water of boats) also cannot be discounted, especially considering the widespread local fishing practices in this part of the world.

Gyi (1970) recognized three species of *Cerberus*: the widely distributed *C. rynchops* (coastal India, across Southeast Asia to New Guinea), *C. australis* (Gray) (northern coastal Australia), and *C. microlepis* Boulenger (known only from freshwater Lake Buhi, Luzon Island, Philippines). In a preliminary phylogeographical study we used mitochondrial DNA sequences of 12S, 16S, and cytochrome B (Karns *et al.*, 2000) to examine genetic variation among eight populations (representing all three *Cerberus* species recognized by Gyi) of *Cerberus*: two populations on the Thai-Malay peninsula and in the Philippines, and one on each of Sumatra, Sulawesi, Borneo and Australia. We found that *C. rynchops* populations from the Sunda Shelf localities (Thai-Malay peninsula, Sumatra, Borneo), the Philippines, and Sulawesi were genetically similar compared with genetically divergent *Cerberus* from northern Australia (*C. australis*); *C. microlepis* was very similar to Sunda Shelf *Cerberus* populations. These results support the biogeographical importance of Weber's line (the boundary of approximate faunal balance between Asian and Australian faunas, located to

the east of Wallace's Line) to snake assemblages in the region (In den Bosch, 1985; How & Kitchener, 1997).

In this report, we expand the phylogeographical study of *Cerberus* by substantially increasing both the number of localities sampled and the number of molecular characters used. We focus on *Cerberus* populations west of Weber's line, especially Sundaland and adjacent islands. We sample populations from India and Sri Lanka, and more localities in continental Southeast Asia, the islands of the Sunda Shelf, the Philippines, and northern Australia. Using sequence data from three mitochondrial genes (12S, ND3, ATPase) we assess the hypothesis of an Indo-Chinese homalopsine origin and subsequent dispersal to the east and west from Indochina (Voris *et al.*, 2002). In addition, we develop new hypotheses to explain the recovered phylogeographical pattern of *Cerberus* populations found to the west of Weber's line.

MATERIALS AND METHODS

Tissue samples and DNA sequencing

Homalopsine snake tissue samples were obtained from 21 localities distributed from India to northern Australia (Fig. 1). The sample consisted of 65 *C. rynchops*, three *C. australis*, four *C. microlepis*, one *Bitia hydroides* Gray, one *Homalopsis buccata* (Linnaeus), one *Enhydris enhydris* (Schneider) and two *Enhydris plumbea* (Boie) (Appendix 1). DNA was extracted from heart, liver and tail tissue using the PUREGENE 1 Extraction kit (Gentra Systems). The polymerase chain reaction (PCR) was used to amplify the ND3 and ATPase mitochondrial genes. Specific primers were used to ensure that the gene (or portion of a gene) of interest was amplified 2 (Table 1). READY-TO-GO Beads (Pharmacia Biotech) were used for samples that were difficult to amplify. Amplified PCR products were cleaned using either the GELASE method or a 3 GENCLEAN kit (Bio 101, Inc.). Cycle-sequencing reactions were performed using fluorescent dyes. The sequencing data were visualized following electrophoresis using a 377 DNA 4 Sequencer or the 3100 Genetic Analyzer. The sequence analysis 5 program SEQUENCHER 4.0 (Gene Codes Corporation) was used to align and edit the data and construct consensus sequence strands.

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. ND3 and ATPase sequences were aligned by eye using Sequencer 3.0 (Gene Codes Corporation). Base pairs of 12S sequences were aligned to a previously published secondary structure model for thamnophiine snakes (Alfaro & Arnold, 2001) using the data editor in PAUP*4.0b10 (Swofford, 2000). Ambiguously alignable regions, usually corresponding to loops, were excluded from further analysis. After excluding unalignable regions and trimming sequence ends the ND3 gene partition contained 235 characters, the ATPase partition contained 701 characters, and the 12S partition contained 764 characters for a total of 1702 nucleotide characters.

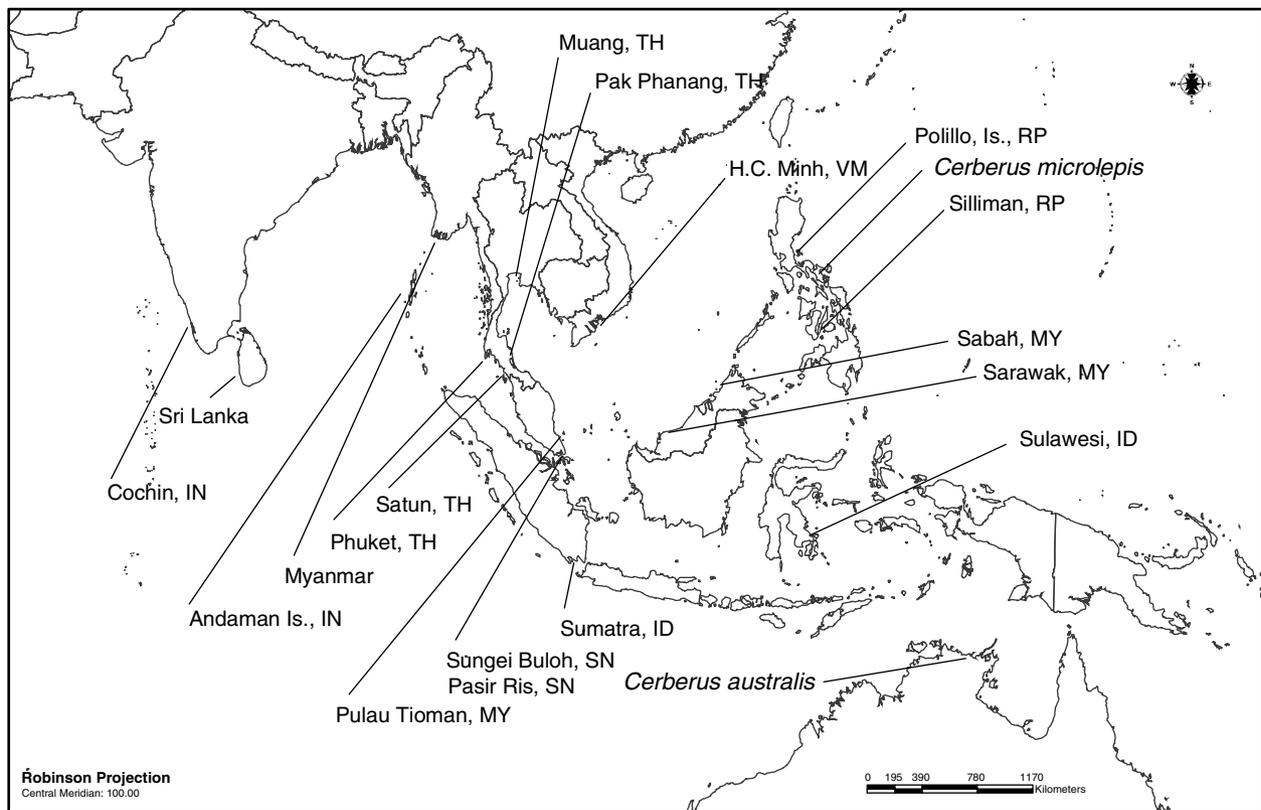


Figure 1 Map showing collection locations for *Cerberus* (Schneider) tissue samples included in this study. See Appendix 2 for detailed locality data, sample sizes at each location, and the storage location of voucher specimens.

Table 1 Primer sequences used for PCR amplification and sequencing

Primer	Mitochondrial gene	Sequence (5'–3')
12S-1	12S	GCT TCA AAC TGG GAT TAG ATA CC
12S-8	12S	CGA GTG TAG GTC GAG TGC TTT G
12S-M2	12S	TTA CTC GTA GTT ATT TGG CG
12S-E2	12S	AGG TCT TGG TCT TAA ACC
GLY1F*	ND3	AGTACAAATGCCTTCCAAGCA
ARG1R*	ND3	CGCCCTGTAATCACTCGTATA
ND3R2	ND3	GGYGGRTTRGTGTTAATGCTTCA
LYS2F*	ATPase	TAGCCTTTTAAGTTGAAGA
ATP65F	ATPase	AAAYATATTCGAACAATTTGCCAG
ATP66F*	ATPase	TACTAATCCCAGTTATAATAAT
CO31R*	ATPase	GTGGAGTTGGTGGGTCATTA
ATP610R	ATPase	ARGGTRRTGTGATAATGCTRAT

*Primers obtained from One Trick Pony Oligos (Ramona, CA, USA); all others were ordered from Great American Gene Company (Ramona, CA, USA).

Phylogenetic analyses

Congruence evaluation

Data set congruence and ‘combinability’ has been a topic of considerable controversy (see Bull *et al.*, 1993). The partition

homogeneity test is one approach for determining congruence among data sets. However, recent studies have suggested that this test performs poorly as an indicator for data combination (Barker & Lutzoni, 2002), possibly due to its sensitivity to the number of characters and levels of homoplasy within each partition (Dolphin *et al.*, 2000; Downton & Austin, 2002). Given the current lack of a satisfactory test of combinability, we assessed incongruence among the three gene partitions (12S, ND3, ATP) by comparing the bootstrap trees from each partition. We bootstrapped individual gene partitions 500 times using the heuristic search option and two random addition sequence replicates. We did not find any well-supported (BP > 70%) clades in conflict among the gene partitions and so combined our data for all further analysis.

Analysis

We analysed our data using parsimony, likelihood and Bayesian methods. Parsimony searches were performed in PAUP* (4.0b10). All sites were equally weighted and gaps treated as missing characters. Preliminary exploration of the data revealed that heuristic searches quickly converged on islands of trees 851 steps long. As a result of sampling each locality multiple times, our data matrix contained many sequences that were identical or nearly identical, and we suspected that the topological differences on these islands

were primarily confined to rearrangements of taxa at the tips of the tree. As we were most interested in relationships among localities, we adopted a search strategy to explore and potentially move beyond tree islands of 851 steps rather than exhaustively collect all of the equally parsimonious rearrangements on the islands. We enforced a time limit of 20,000 s per heuristic search and used 100 random addition

7 sequence replicates and TBR branch swapping. We used nonparametric bootstrapping (Felsenstein, 1985) to measure support of clades with 500 total pseudoreplicates and TBR branch-swapping with two random sequence addition replicates per pseudoreplicates and a time limit of 200 s per pseudoreplicate.

8 For the maximum likelihood analysis the TrN substitution model (rmat = 1.000, 14.667, 1.000, 1.000, 18.879) with invariable sites (pinvar = 0.446) and among-site rate heterogeneity ($\alpha = 0.506$) was selected using hierarchical likelihood ratio tests implemented in Modeltest 3.06 (Posada & Crandall, 1998). We used the heuristic search option in PAUP* with TBR branch swapping and 10 random addition sequence replicates. To keep the likelihood searches tractable with the large number of nearly identical sequences, we enforced a time limit of 200,000 s per random addition sequence replicate. To assess confidence in our likelihood analysis, we performed likelihood bootstrapping with 500 total pseudoreplicates and TBR branch-swapping with two random sequence addition replicates per pseudoreplicates and a 1000 s per pseudoreplicate time limit.

Bayesian analyses

To calculate posterior probabilities of clades, we used MrBayes 2.01 (Huelsenbeck, 2000) to run a 3,000,000 generation Markov chain under a HKY + G + I model sampling every 100 generations. We used the default (flat) priors for rate matrix (uniform 0–100), branch length (uniform 0–10), and tree topology parameters and ran one cold and five heated chains simultaneously. Visual inspection suggested that the Markov chain reached stationarity within 5000 generations but we discarded the first 200,000 generations to ensure that stationarity was reached. A majority rule consensus tree calculated from the 28,000 remaining trees was constructed and used to determine the posterior probabilities of clades. To help ensure that the Markov chain was sampling from the posterior distribution, we repeated the analysis and plotted posteriors for clades against one another. We found low variance in estimated posteriors for focal clades between these analyses (< 2%), suggesting that the chain from both analyses had reached stationarity (data not shown).

We calculated uncorrected genetic distances (p) within localities and within and between biogeographical regions. These distances provided an estimate of the relative divergence of taxa within the study and were also compared with molecular clocks calibrated for other squamates to estimate absolute divergence times.

RESULTS

Parsimony analysis

Our heuristic searches found 145,503 equally most-parsimonious trees (length = 851 steps, CI = 0.68; Fig. 1). Despite the large number of trees, we found a number of well-supported relationships among sampled localities. We did not find strong support for the monophyly of *Cerberus*, with *H. buccata*, the Australian species *C. australis* and the rest of the *Cerberus* localities forming a three-way basal polytomy. The *Cerberus* + *Homalopsis* clade was strongly supported with respect to *B. hydroides* and *E. enhydis* and *E. plumbea*, the other outgroup species included in our study.

The non-Australian *Cerberus* were monophyletic with respect to the rest of the species in our study and consisted of two well-supported groups: an India/Myanmar group and a clade containing all other *Cerberus* populations sampled (i.e. the Philippines, Thai-Malay Peninsula/Gulf of Thailand, Greater Sunda Islands and Sulawesi populations). Monophyly of each of the sampling localities within the India/Myanmar group was strongly supported. Three well-supported clades formed a trichotomy at the base of the non-sister to the India/Myanmar clade: a Sulawesi/Sumatra group, a Philippines group (including *C. microlepis*), and a Thai-Malay Peninsula/Gulf of Thailand + Borneo (Sarawak and Sabah) group. Within the Thai-Malay Peninsula/Gulf of Thailand + Borneo clade, the Bornean sites (Sarawak and Sabah) formed a well-supported sister to the Thai-Malay Peninsula/Gulf of Thailand sites. Within the Thai-Malay Peninsula/Gulf of Thailand group, the Ho Chi Minh group formed a well-supported sister to a clade consisting of the rest of the sampled localities. We found weak phylogeographical structuring among the west coast Thai-Malay Peninsula sites: Satun and Phuket (Andaman sea coast of Thailand), and S. Buloh and Pasir Ris (Singapore) localities. However, the Pak Phanang, Muang and the P. Tioman localities from the east coast of the Thai-Malay Peninsula formed a well-supported clade within this group.

Likelihood and Bayesian analysis

Our maximum likelihood search produced two equally likely topologies ($-\ln(L) = 6361.8188$, Fig. 2). The likelihood topology was similar to the MP tree but was more fully resolved. *Cerberus* was paraphyletic with respect to *H. buccata*. The Australian *Cerberus* formed the sister group to *H. buccata* + the rest of *Cerberus*. The India/Myanmar localities formed the sister to the rest of the *Cerberus* sampled, just as in the MP analysis. Within this group, the position of the Philippines clade (including *C. microlepis*) and the Sunda Islands/Sulawesi clade as successive sister groups to the Thai-Malay Peninsula and Gulf of Thailand clade received high and moderate posterior probability but a low bootstrap proportion. Within the Thai-Malay Peninsula/Gulf of Thailand group, the Ho Chi Minh group formed a well-supported sister to an east coast (South China Sea, Gulf of Thailand) Thai-Malay Peninsula



Figure 2 Phylogenetic relationship of *Cerberus* (Schneider) populations inferred under parsimony, based on ND3, ATPase, and 12S gene sequences. Shown is a consensus of 145,503 equally most-parsimonious trees (length = 851 steps, CI = 0.68). Numbers above branches indicate the bootstrap support for the clade following 500 pseudoreplicates. See Appendix 2 for detailed locality data.

group. This group consisted of a well-supported Pak Phanang/Muang + P. Tioman group. In addition, we found a high posterior probability for the P. Tioman group. This east coast group was in turn sister to a group containing west coast (Andaman Sea) and Singapore samples that, similar to the MP analysis, showed weak phylogeographical structure.

Relationships among sampling localities based on likelihood and Bayesian analyses are shown in Fig. 4. In general, Bayesian analysis and likelihood bootstrapping found support for the same clades although the posterior probability was usually more extreme than the bootstrap proportion. Our empirical result is consistent with findings in other papers (Murphy *et al.*, 2001; Wilcox *et al.*, 2002; Alfaro *et al.*, 2003) although the implications of divergent support values are currently a topic of debate (Murphy *et al.*, 2001; Suzuki *et al.*, 2002; Wilcox *et al.*, 2002; Alfaro *et al.*, 2003). We did find a single node where the bootstrap proportion exceeded the posterior probability: for the clade consisting *H. buccata* + non-Australian *Cerberus*.

DISCUSSION

Phylogeographical patterns

Broad scale patterns

The phylogeographical analysis of *Cerberus* by Karns *et al.* (2000) showed genetic similarity (mean divergence = 2.1%, range = 0.7–2.8%) among populations from Sunda Shelf localities, the Philippines, and Sulawesi (biogeographical region west of Weber's line) compared with *C. rynchops* from northern Australia (mean divergence = 6.6%, range = 6.0–7.3%), suggesting a relatively high degree of movement and gene flow among populations in the biogeographical region west of Weber's line and ancient isolation and genetic divergence of the Australian (Sahul Shelf) *Cerberus*. The results reported here support and extend that work.

The large-scale phylogeographical patterns observed in the trees (Figs 2–4) are consistent with the hypothesis that the

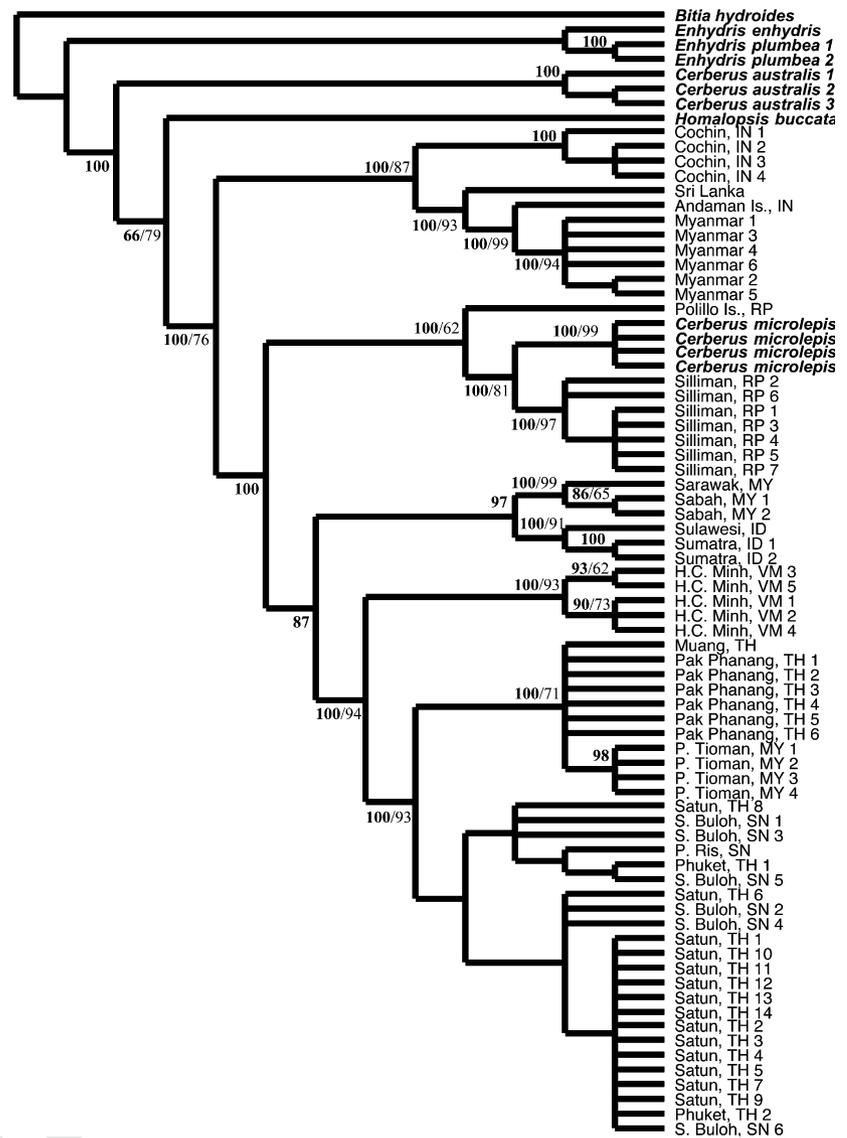


Figure 3 Phylogenetic relationship of *Cerberus* (Schneider) populations inferred under maximum likelihood, based on ND3, AT-Pase, and 12S gene sequences. Shown is the consensus of two equally most-likely trees ($-\ln(L) = 6361.8188$). Numbers in bold indicate the posterior probability based on a 3,000,000 generation MCMC analysis. Bootstrap values greater than 60, calculated from 500 pseudoreplicates, follow the posterior probability. See Appendix 2 for detailed locality data.

Homalopsinae originated in continental Indochina and that *C. rynchops* spread westward to India and eastward to the Greater Sunda Islands, the Philippines, Wallacea, and Australia from Indochina. The results show the greatest degree of genetic similarity between the populations of continental Southeast Asia (Thai-Malay peninsula and the Gulf of Thailand) and the adjacent Greater Sunda Islands and Sulawesi (2.0–3.9% genetic divergence, Table 2) indicating the greatest degree of movement and gene flow in this region. Compared with these central *Cerberus* populations of Indochina and the Sunda Islands, the Philippines (2.1–4.9% genetic divergence), India/Myanmar (3.5–5.4%), and Australia (5.6–11.8%) show progressively greater levels of genetic differentiation, consistent with decreasing levels of gene flow correlated with distance, marine barriers, dispersal and vicariance events radiating out from Indochina, and with the biogeographical history of the region.

Thai-Malay Peninsula and Gulf of Thailand

This clade consists of populations that are found along the coast of present-day continental Southeast Asia, from Phuket, Thailand, to Singapore, and to southern Viet Nam (Fig. 1). We observed phylogeographical structure in this clade (Fig. 4) that corresponds to both geographical distance and the historical biogeography of the Quaternary. The Vietnam locality at the mouth of the Mekong is the most geographically and genetically distant from the Thai-Malay Peninsula sites. Our trees (Figs 2–4) resolve the Thai-Malay Peninsula sites into east coast (Pulau Tioman and Gulf of Thailand) and west coast (Singapore and Andaman Sea coast). We suggest that our samples from the Gulf of Thailand (Muang, Pak Phanang and Pulau Tioman (see 'M's in Fig. 5) are nearly identical because they were displaced as coastal mangrove habitat moved outward from the present-day coastline into the South China Sea to form an emergent

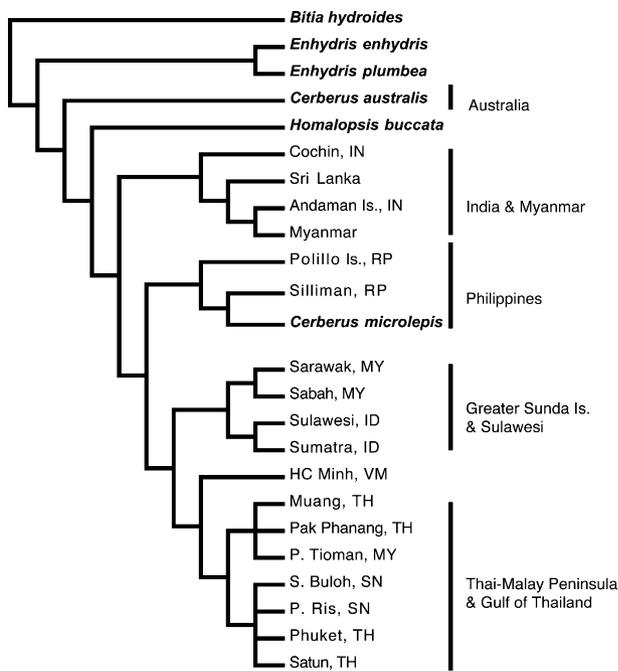


Figure 4 Summary of phylogenetic relationships among sampling localities and biogeographical regions. Tree reflects the maximum likelihood topology in Fig. 3 with localities rather than sampled individuals at the tips.

north-eastern Sundaland coastline (NE coastline in Fig. 5) during the last glacial maximum. The effect of this coastline shifting was to connect the coast of present-day Borneo to Indochina, reduce the amount of coastline, and bring populations into closer contact. A similar displacement likely occurred from east to west ('A' to 'NW' coastline, Fig. 5) in the Straits of Malacca when coastal habitat connected Sumatra and the Thai-Malay peninsula across the Andaman Sea forming an emergent north-western Sundaland coastline.

The differentiation in the Thai-Malay clade as a whole is consistent with periodic interruption of movement and inhibition of gene flow around the Malay Peninsula due to the periodic connection of the Asian mainland to the Greater Sunda Islands during the Pleistocene caused by lowered sea levels and emergence of the Sunda Shelf (see Fig. 5). Our data, the observations of Brown *et al.* (1951) and Hall's (2002) models do not support a sea-level connection between the Gulf of Thailand and the Andaman Sea in the Neogene as suggested

by Woodruff (2003). Movement among populations and gene flow around the Thai-Malay peninsula may be inhibited even under present-day sea levels due to the constant north-flowing current in the Straits of Malacca (Great Britain Hydrographic Office, 1958). In addition, there are currently significant vegetational and hydrographical differences between the east coast of the Malay Peninsula and the Straits of Malacca. The east coast of the Thai-Malay peninsula is largely exposed beach ridges punctuated by sheltered river mouths (Nossin, 1961, 1964; Hill, 1966). Mangroves and mud flats are infrequent along this coast (Chapman, 1977; Spalding *et al.*, 1997). In addition, this coast is directly exposed to the strong winds and wave action of the north-east monsoon for several months each year (Dale, 1959). In contrast, the coastlines of the Straits of Malacca are sheltered due to its narrow nature and the shores consist of nearly uninterrupted mangroves, mudflats and estuaries (Chapman, 1977).

In light of these factors it is interesting to note that *C. rynchops* from Pulau Tioman (a small volcanic island located 56 km off the south-eastern coast of peninsular Malaysia, Fig. 1) is more genetically similar to distant Gulf of Thailand populations (e.g. Pak Phanang = 816 km straight-line distance from Pulau Tioman; Muang = 1,318 km) than it is to closer populations in Singapore at the tip of the Thai-Malay peninsula (Pasir Ris = 125 km; Sungei Buloh = 120 km). Recent reports of extensive endemism on Pulau Tioman support the assertion that coastal habitat fragmentation and the marine barrier has contributed to the isolation of Pulau Tioman's herpetofauna (Das & Grismer, 2003; Grismer *et al.*, 2003a,b).

Greater Sunda Islands

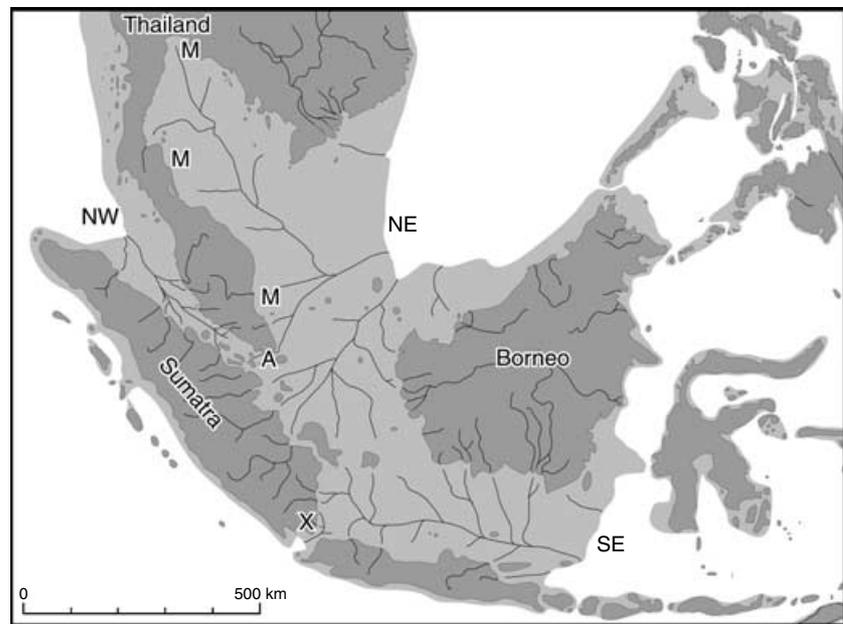
This clade consists of populations from two of the Greater Sunda Islands (Sumatra and Borneo) and Sulawesi (Figs 1–4). We observed two clades in this group. The localities in the Sabah and Sarawak clade on Borneo are geographically close (491 km) and mangrove forest is found all along the northern coast of Borneo (Chapman, 1977). Thus, incremental coastal movements would promote gene flow along this coastline and genetic similarity would result. Conversely, the other clade in this group, Lampung (Sumatra) and Kendari (Sulawesi), are geographically distant (straight-line geographical distance = 1945 km) from each other compared with Sundaland sites that are geographically closer (e.g. Singapore = 793 km; Sarawak = 1306 km), but less genetically similar.

	TMP	GSS	PP	IM	OG	Australia
TMP	0.0–0.018	0.020–0.039	0.021–0.049	0.037–0.054	0.085–0.192	0.056–0.118
GSS		0.0–0.028	0.023–0.041	0.035–0.047	0.088–0.185	0.057–0.089
PP			0.0–0.025	0.035–0.063	0.085–0.201	0.064–0.089
IM				0.0–0.040	0.094–0.178	0.064–0.095
OG					0.0–0.206	0.081–0.191

TMP, Thai-Malay Peninsula & Gulf of Thailand; GSS, Greater Sunda Is. & Sulawesi; PP, Philippines; IM, India & Myanmar; OG, Out Group.

Table 2 Maximum uncorrected (*p*) distance within and between geographical regions. Geographical regions correspond to clades labelled in Fig. 3

Figure 5 Map of tropical Southeast Asia illustrating the depth contour at 120 m. This 120 m contour illustrates the maximum sea-level drop during the last 17,000 years. The primary drowned river systems on the Sunda Shelf are illustrated (from Voris, 2000). The single letter abbreviations represent sampled localities: A = Singapore localities (Sungei Buloh, Pasir Ris); M = Thai and Malaysian localities (Muang, Pak Phanang, Pulau Tioman); X = the Indonesian locality (Sumatra). The double-lettered abbreviations identify emergent Sundaland coastlines: NW (north-west), NE (north-east), SE (south-east). Coastal populations were displaced from present-day locations (A → NW, M's → NE, X → SE) as the coastline moved outward in response to sea-level drops during the Pleistocene.



We suggest that geographical displacement of populations caused by sea-level changes provides an explanation for this similarity. The Greater Sunda Islands were periodically connected by land to the Asian mainland due to lowered sea levels throughout the late Quaternary (Dawson, 1992). During periods of falling sea levels and Sundaland emergence coastal mangrove populations of *C. rynchops* on the eastern end of Sumatra ('X' in Fig. 5) would be passively displaced c. 1200 km. to the east across what is currently the Java Sea and create an emergent south-eastern Sundaland coastline connecting Java and Borneo (e.g. 'SE' coastline in Fig. 5). This displacement would bring *Cerberus* populations to the eastern edge of the Sunda Shelf, adjacent to the east coast of Borneo, and within 30 km of Suluwesi by way of the deep, yet narrow Makassar Strait at Cape Onkona (see Fig. 4 in Umbgrove, 1949). Under this scenario we further predict high genetic similarity among today's coastal populations of eastern Sumatra, northern Java, and southern Borneo due to the coalescence of populations along the emergent coastline associated with the East Sunda River System and during the Pleistocene (see Fig. 4 in Tjia, 1980; Voris, 2000). Likewise we also predict that present-day populations of *Cerberus* in the rivers along the south-eastern coast of Sumatra were displaced north-east via the North Sunda River system and at glacial maxima were only c. 200 km north-west of Sarawak populations (see Fig. 3 in Tjia, 1980 and Fig. 5 here). Additional sampling of these localities is already underway and will allow us to evaluate these hypotheses.

Philippines

Parsimony, likelihood and Bayesian analyses all recovered a clade of Philippines taxa. However, we note that this node did not receive high likelihood bootstrap support, although both parsimony bootstrapping and Bayesian posterior probabilities

were high. Thus some caution is in order for the interpretation of the biogeography of this clade.

The Philippines have an exceedingly complex geological history (Hall, 1998). The present-day Philippines were tectonically 'assembled' during the Cenozoic. Palawan and Mindoro are very old (> 20 Myr) and are the only Philippine islands that originated as part of the Asian mainland while the other Islands originated as a series of island arcs far out in the Pacific Ocean (Heaney & Regaldo, 1998). Today, two island chains extend south from Luzon to the Sunda Shelf and present-day Borneo. These two island chains have largely been in place for the past 4–5 Myr (Fig. 9 in Moss & Wilson, 1998; Fig. 24 in Hall, 2002) and either or both of these island chains likely provided the dispersal route for *C. rynchops* from the Sunda Shelf (north Borneo) to Luzon.

We suggest that *Cerberus* reached the Philippines by a combination of incremental coastal movements and occasional open ocean waif dispersal along one or both of the island chains. The position of the Philippine populations as the sister group to the Greater Sunda Island populations and Asian mainland populations at intermediate levels of genetic divergence (2.1–4.9%) strongly supports this interpretation. This scenario does not require the existence of a continuous land bridge between the Philippines and the Sunda Shelf at maximum low Quaternary sea levels. An alternative scenario having an isolated population of *Cerberus* 'ride' to the Philippines from the mainland of Asia on Palawan over the past 50 Myr would imply much higher levels of genetic divergence between Philippine and Asian populations and is thus not supported by the present data.

The relationships of the populations within the Philippine clade do not appear to be entirely consistent with past and present-day Philippine geography. *Cerberus microlepis* is known only from the freshwater Lake Buhi on Luzon. Lake Buhi is a small (7 × 5 km) freshwater lake that lies in the valley between two ancient, inactive volcanoes, Mt Malimas and

Mt Iriga. The lake lies *c.* 200 m above sea level and *c.* 15 km from the coast on the east flank of Mt Iriga (Heaney *et al.*, 1999; Karns *et al.*, 2000). The lake was apparently formed in 1641 when a strong earthquake caused the side of Mt Iriga to collapse, forming a natural dam. Thus, the differentiation that has occurred between the Lake Buhi *Cerberus* and those collected at Silliman on the south end of Negros Island is likely a product of both distance and time. Samples of *Cerberus* from the sea coast near Lake Buhi could help resolve these factors.

The Polillo population is strongly supported as the sister group to *C. microlepis* (Lake Buhi, Luzon) + Silliman population (Figs 2–4). Polillo is a large island 20 km off the eastern coast of Luzon. It is on the Luzon Shelf and has been connected by land to Luzon during Pleistocene sea-level drops (Heaney, 1991; Steppan *et al.*, 2003) however, it has been isolated from Luzon by sea for the past 8–10 ka. This marine barrier may account for some of the differentiation between the Lake Buhi population and the Polillo population but it does not explain why the Lake Buhi and Silliman populations are more similar to each other despite both greater distance (602 km vs. 240 km between Lake Buhi and Polillo) and narrow inter-island deep sea barriers. Once again, human-mediated dispersal cannot be ruled out.

India/Myanmar

India began to collide with the Asian continent 50 Ma and since *c.* 30 Ma there has been a continuous coastline connection between Southeast Asia and the Indian subcontinent around the Bay of Bengal (Hall, 2002). It is most likely that *Cerberus* reached India via incremental dispersal along this coastline. Although waif dispersal scenarios cannot be ruled out, the prevailing surface current in the Bay of Bengal is clockwise (Couper, 1983) and would favour dispersal to the east and not the west. Another factor to consider is coastal vegetation, historically major mangrove and mudflat areas are found in isolated fragments around the Bay of Bengal (e.g. Irawaddy, Ganges and Brahmaputra deltas; Blasco, 1975) separated by coastal habitats less suitable for colonization by *Cerberus*. This factor has undoubtedly affected homalopsine dispersal along the east coast of India.

The India–Myanmar clade is well supported (Figs 2 and 3). The Myanmar samples from the Irawaddy Delta and the sample from the Andaman Islands (513 km straight-line distance) form a sister group. The Andaman Islands are a submarine mountain chain of islands that have never been connected to the mainland (Hall, 2002). The distance today from the river mouth to the most northern of the Andaman Islands is 230 km. However, Preparis island sits halfway between these points on the edge of the continental shelf. In addition, at the last glacial maximum with sea levels at *c.* 120 m below present-day levels the distance separating the northern islands and the mouth of the Irawaddy was likely only 70 km (Voris, 2000). Discharge of debris from the Irawaddy delta peaks during the strong south-east monsoon season (van der Leeden, 1975; Nieuwolt, 1981) and has

certainly provided a major source of potential rafts that are discharged towards the Andaman Island chain to the south. These factors greatly increase the probability of waif dispersal between the Asian mainland and the Andaman Islands, although human-mediated dispersal cannot be ruled out (Austin, 1999).

Based on geographical distance alone it would be predicted that the *Cerberus* population from Sri Lanka would be more similar to the population at Cochin, India, near the south-western tip of India (Sri Lanka to Cochin coastline distance *c.* 550 km) than to the Myanmar + Andaman Island clade (Sri Lanka to Myanmar coastline distance *c.* 3,000 km); however, our data suggest the opposite. The Sri Lankan sample came from the south-western side of the island and a shallow strait (*c.* 10 m depth) connects Sri Lanka to India. In fact, a coastal corridor between Sri Lanka and India would have been present repeatedly during the Quaternary, presumably further facilitating movement and gene flow from the south-western coast of India to the east and Sri Lanka (Voris, 2000). However, it is possible that the highly exposed rocky coast of Cape Comorin at the southern tip of India may prove to be a major habitat barrier and explain the genetic distance between populations on the east and west coasts of India. Further samples are needed to test this hypothesis.

Australia

Karns *et al.* (2000) found that Australian *Cerberus* were genetically divergent from *Cerberus* populations found west of Weber's line. In this study the divergence of Australian *Cerberus* was confirmed; we found that *H. buccata*, one of the homalopsine outgroups used, was the sister group to all the *Cerberus* populations found west of Weber's line, and that Australian *Cerberus* was the sister group to that clade, making *Cerberus* a paraphyletic group (systematic implications discussed below). The extensive genetic divergence of Australian *Cerberus* is explained by both biogeographical history and, to a lesser extent, by geographical distance.

Australia, New Guinea and islands associated with the Sahul Shelf have moved north from Antarctica towards the equator for the last 55 Myr (Hall, 2002) and during this time they have always been separated from Sundaland and continental Asia by deep water marine barriers and north–south currents. According to the figures in Hall (2002), Australia's Sahul Shelf and the Sunda Shelf have been at about the same distance apart for the past 20–25 Myr. Thus, Australia has likely been 'available' for colonization by *Cerberus* from the Sunda Shelf for at least 15–20 Myr.

The islands found between Wallace's line and Lydekker's line (the boundary of the Sahul continental shelf) are collectively known as Wallacea (Whitmore, 1981); Wallacea is the zone of transition between the Oriental and Australian zoogeographical provinces recognized by Wallace (1860). *Cerberus* most likely colonized the Sahul Shelf by a combination of open ocean waif dispersal and incremental coastal movements across Wallacea sometime in the later Cenezoic

(age estimates discussed below). In isolation on the Sahul Shelf, *Cerberus* has genetically diverged from the *Cerberus* populations found to the west of Weber's line, while remaining morphologically very similar to its Oriental relatives.

Samples of *Cerberus* from the Northern Territory, Australia are relatively close to, but genetically divergent from, a number of Oriental sites (e.g. Sulawesi = 1518, Sabah = 2813, Sarawak = 2835 km). In contrast, *Cerberus* populations that are geographically more distant (e.g. Sri Lanka: Silliman, Philippines = 4830 km) are genetically more similar. This indicates that, overall, there is greater opportunity for movement and gene flow among the *Cerberus* populations found west of Weber's line, despite the geographical distances involved. Strong north–south trending currents through Wallacea, drier climates, and lack of suitable habitat along the lesser Sunda island chain are factors that likely contribute to the isolation of Sahul Shelf populations. The best opportunity for gene flow across Weber's line would appear to be in northern Wallacea where open ocean distances are reduced and suitable *Cerberus* habitat is found. Here again, further samples will allow these ideas to be tested.

Systematic implications

The analyses presented here do not unambiguously support the monophyly of *Cerberus*. Parsimony analysis suggest that there is a three-way polytomy among *H. buccata*, the Australian *Cerberus*, and the non-Australian *Cerberus*, while likelihood and Bayesian analysis support a paraphyletic *Cerberus* with respect to *H. buccata*. On the basis of morphological and behavioural data, we are suspicious of this result because the two genera are strikingly different in size, body proportions and scalation. A recent molecular study (Voris *et al.*, 2002) suggests that *C. rynchops*, *H. buccata*, and the tentacled snake, *Erpeton tentaculatus* Lacepede, form a distinct clade within the subfamily. A full examination of the phylogenetic relationships within the homalopsinae is beyond the scope of this paper but is a focus of our current research (M.E. Alfaro *et al.*, in review).

Gyi (1970), in the most recent monographical treatment of the Homalopsinae (based solely on classical morphological characteristics) recognized three species of *Cerberus*. He followed Gray (1842) and recognized Australian *Cerberus* as a separate species (*C. australis*); however, most subsequent authorities (e.g. Cogger, 1975, 2000; Cogger *et al.*, 1983; Storr *et al.*, 1986; Shine, 1991; Greer, 1997) have not recognized *C. australis* as a separate species, but include it in *C. rynchops* (an exception to this is found in Wilson & Swan, 2003). Our data strongly indicate that *C. australis* is a valid taxon. Despite the large genetic differences, *C. australis* is morphologically and ecologically very similar to Oriental *C. rynchops*.

Gyi (1970) also recognized *C. microlepis*, a taxon known only from freshwater Lake Buhi on Luzon in the Philippines. The inclusion of the Lake Buhi population within the *C. rynchops* Philippines clade does not support its recognition as a separate species. However, this population does have a

distinctive habitat for *Cerberus* (a freshwater lake) and does possess distinctive morphological traits (e.g. males develop tubercles on the head during reproduction). Thus, *C. microlepis* may prove to be an isolated freshwater ecotype of *C. rynchops* rather than a separate species, but we will continue to recognize *C. microlepis* for the present.

Gyi also recognized two subspecies of *C. rynchops*: the widely distributed *Cerberus rynchops rynchops* (Schneider) and a New Guinea subspecies, *Cerberus rynchops novaeguineae* Loveridge. In contrast to Gyi's (1970) morphological study, our analysis indicates a high degree of diversification within *C. rynchops*. The widely distributed *C. r. rynchops* of Gyi is split into four to five phylogenetic species correlated with biogeographical regions and *C. microlepis* is included in the Philippines *Cerberus* clade.

Timing of divergence events

Several studies on the phylogeography of squamates have attempted to determine the timing of phylogenetic splits based on molecular sequence divergences through the identification of external calibration events (e.g. appearance and colonization of an island). We have searched for geological or biological events that could be used to calibrate our genetic divergence data, including tectonic events (Choubert & Faure-Muret, 1976; Hall, 2002), climatic changes (Heaney, 1991; Kershaw *et al.*, 2001), Quaternary sea-level changes (e.g. Pirazzoli, 1991, 1996; Dawson, 1992), volcanic eruptions (Ninkovich *et al.*, 1978; Dawson, 1992; Zielinski *et al.*, 1996), and extraterrestrial impact events (Lee & Wei, 2000). We have not been able to determine any reliable calibration events because potential markers for our localities consisted of either widespread, gradual and reoccurring events (e.g. tectonic changes, sea-level changes, climatic changes) or singular events that were too localized to be useful (volcanic eruption, impact event).

In the absence of well-defined calibration events for *Cerberus* localities, we used estimated rates of change in mitochondrial DNA from other squamate studies to suggest divergence times for the two major lineages of *Cerberus* (*C. rynchops* from the Sunda Shelf and Indian Ocean and *C. australis*). Zamudio & Green (1997) drew on a number of lizard and snake studies to establish a range of 0.47–1.32% divergence per million years for mitochondrial genes. This range spans values used by Gravlund (2002) for snakes and Schulte *et al.* (2003) used for lizards. Genetic divergence values between *C. australis* and the geographically adjacent Greater Sunda Islands and Sulawesi clade range from 5.7% to 8.9% (Table 2). Use of the higher rate of divergence (1.32%) from Zamudio & Green (1997) provides a minimum range of time since divergence for Australian *Cerberus* from the Sunda shelf clade of 4.3–6.7 Ma; the slower rate of divergence (0.47%) provides a maximum range of time since divergence of 12.1–18.9 Ma. Although these time range estimates fit within the time period when the Sunda and Sahul shelves were nearing present-day positions (Hall, 2002), the range is so large as to have very limited use. However, it is interesting to note that even the shortest

divergence time of 4.3 Ma substantially predates the ice ages of the Quaternary period.

FUTURE RESEARCH

This study expands our understanding of the phylogeographical history of *Cerberus*, especially in the Oriental zoogeographical region. It would be particularly instructive to obtain more samples from the islands of Wallacea, from New Guinea, from more Sunda Shelf sites, and from Oceanic islands (e.g. Palau); this study is weak in representation from those areas. We are currently in the process of obtaining morphological data to help address some of the systematic questions raised by our data (e.g. status of *C. australis* and *C. microlepis*). We noted several instances where relationships among populations were not correlated with either geographical distance or biogeographical history (e.g. Philippines clade). More samples from these sites would help clarify the situation. Lastly, we have made a number of predictions for relationships among populations based on the processes of inhibition, facilitation, and displacement with respect to movement routes and changing sea levels (e.g. the differentiation of east and west coast *Cerberus* populations along the Thai-Malay Peninsula). More testing of these and other predictions could be done with targeted sampling in the region. We are currently analysing comparable mitochondrial DNA sequence data from populations of *E. plumbea*, a freshwater homalopsine, from sites in Indochina, Borneo and Sulawesi to compare phylogeographical structure between species with different ecological characteristics (terrestrial freshwater vs. coastal marine). These two species would have been differentially affected by the complex biogeographical history of the region.

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APPENDIX 1

List of homalopsine snake samples from which mitochondrial DNA was sampled. The locality and museum or field designations are given for each sample. For each species the localities are listed in alphabetical order. The localities for *Cerberus rynchops* (Schneider) samples match those in Figs 1 and 2 where country abbreviations are used. The museum and field designation abbreviations used below are elucidated as follows: Ajit, Ajit, V. P. field number; CAS, California Academy of Sciences; DAS, Indraneil Das field number; FMNH, Field

Museum of Natural History; HKV, Harold K. Voris field number; Iskandar, Djoko Iskandar field number; NTR, Museum and Art Gallery of the Northern Territory, Australia; RMB, Raffe M. Brown field number; ROM, Royal Ontario Museum; Silliman, Siliman University specimen, Philippines; SM & SSM, Sabah State Museum; WHT, Wildlife Heritage Trust of Sri Lanka field number, Sri Lanka; USNM, United States National Museum; ZRC, Zoological Reference Collection, Raffles Museum of Biodiversity, Singapore.

Bitia hydroides Gray, Myanmar, CAS 211898.

Cerberus australis (Gray), Northern Territory, Australia (1), NTR 22721; Northern Territory, Australia (2), NTR 22722; Northern Territory, Australia (3), NTR 22723.

Cerberus microlepis Boulenger, Lake Buhi, Philippines (1), USNM 197851; Lake Buhi, Philippines (2), USNM 197852; Lake Buhi, Philippines (3), USNM-FS 197853; Lake Buhi, Philippines (4), USNM-FS 197854.

Cerberus rynchops, Andaman Islands, India, DAS 2; Cochin, India (1), Ajit A; Cochin, India (2), Ajit B; Cochin, India (3), Ajit C; Cochin, India (4), Ajit D; Ho Chi Minh, Vietnam (1), ROM 37943; Ho Chi Minh, Vietnam (2), ROM 37944; Ho Chi Minh, Vietnam (3), ROM 37945; Ho Chi Minh, Vietnam (4), ROM 37946; Ho Chi Minh, Vietnam (5), ROM 37947; Muang, Thailand, HKV SM1/14251; Myanmar (1), CAS 211979; Myanmar (2), CAS 211980; Myanmar (3), CAS 211981; Myanmar (4), CAS 211982; Myanmar (5), CAS 211984; Myanmar (6), CAS 211985; Pak Phanang, Thailand (1), HKV 32404; Pak Phanang, Thailand (2), HKV 32405; Pak Phanang, Thailand (3), HKV 32406; Pak Phanang, Thailand (4), HKV 32407; Pak Phanang, Thailand (5), HKV 32408; Pak Phanang, Thailand (6), HKV 32409; Pasir Ris, Singapore, ZRC 2.4577; Phuket, Thailand (1), FMNH 250126; Phuket, Thailand (2), FMNH 250125; Polillo, Philippines, USNM 497589; Pulau Tioman, Malaysia (1), ZRC 2.4594; Pulau Tioman, Malaysia (2), ZRC 2.4595; Pulau Tioman, Malaysia (3), ZRC 2.4596; Pulau Tioman, Malaysia (4), ZRC 2.4597; Sabah, Borneo (1), SSM A009042; Sabah, Borneo (2), SSM 438; Sarawak, Malaysia, DAS 1; Satun, Thailand (1), HKV 39; Satun, Thailand (2), HKV 40; Satun, Thailand (3), HKV 41; Satun, Thailand (4), HKV 42; Satun, Thailand (5), HKV 43; Satun, Thailand (6), HKV 32309; Satun, Thailand (7), HKV 32310; Satun, Thailand (8), HKV 32311; Satun, Thailand (9), HKV 32312; Satun, Thailand (10), HKV 32313; Satun, Thailand (11), HKV 32314; Satun, Thailand (12), HKV 32315; Satun, Thailand (13), HKV 32316; Satun, Thailand (14), HKV 32317; Silliman, Philippines (1), Silliman A; Silliman, Philippines (2), Silliman B; Silliman, Philippines (3), Silliman C; Silliman, Philippines (4), Silliman D; Silliman, Philippines (5), Silliman E; Silliman, Philippines (6), Silliman F; Silliman, Philippines (7), Silliman G; Sri Lanka, WHT 5013; Sulawesi, Indonesia, Iskandar A; Sumatra, Indonesia (1), Iskandar B; Sumatra, Indonesia (2), Iskandar C; Sungei Buloh, Singapore (1), HKV 32438; Sungei Buloh, Singapore (2), HKV

32439; Sungei Buloh, Singapore (3), HKV 32440; Sungei Buloh, Singapore (4), HKV 32441; Sungei Buloh, Singapore (5), HKV 32443; Sungei Buloh, Singapore (6), HKV 32444.

Enhydris enhydris (Schneider), Bangkok, Thailand, FMNH 250119.

Enhydris plumbea (Boie), Sulawesi, Indonesia (1), RMB 1725; Sulawesi, Indonesia (2), RMB 1694.

Homalopsis buccata (Linnaeus), Thailand, FMNH 252514.

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Appendix 2. The mean genetic distances between localities and taxa are provided for the localities shown in Fig. 1.

	Number	Satun C.	<i>atralis</i> B.	<i>hydroides</i> Cochin	<i>E. enhydris</i>	Andaman	H. C. Minh	<i>H. buccata</i>	<i>C. microlepis</i>	Muang	Myanmar Pak	Phanang	MY	P. Tioman	Ris	SN	RP	TH	Phuket	Poilillo	Sabah	Sarawak	<i>E. plumbea</i>	Sri Lanka	Sulawesi	Sumatra	IN
	TH	TH	IN	IN	VM	VM	VM	TH	TH	TH	TH	TH	TH	TH	TH	TH	TH	TH	TH	TH	TH	TH	TH	TH	TH	TH	TH
Satun	91.00	0.00																									
TH																											
<i>C. australis</i>	45.00	0.08	0.00																								
<i>B. hydroides</i>	17.00	0.18	0.17	0.00																							
Cochin IN	78.00	0.04	0.08	0.18																							
<i>E. enhydris</i>	22.00	0.13	0.14	0.21	0.00																						
Andaman	23.00	0.04	0.08	0.17	0.04	0.13	0.00																				
Is. IN																											
H. C. Minh	130.00	0.01	0.08	0.18	0.04	0.14	0.04	0.00																			
VM																											
<i>H. buccata</i>	29.00	0.09	0.10	0.19	0.09	0.15	0.10	0.09	0.00																		
<i>C. microlepis</i>	61.00	0.03	0.10	0.17	0.05	0.16	0.05	0.03	0.10	0.00																	
Muang TH	34.00	0.00	0.08	0.18	0.05	0.14	0.05	0.01	0.09	0.03	0.00																
Myanmar	225.00	0.04	0.08	0.17	0.04	0.14	0.01	0.04	0.10	0.04	0.00																
Pak Phanang	261.00	0.00	0.08	0.18	0.04	0.14	0.05	0.01	0.09	0.03	0.00	0.04	0.00														
TH																											
P. Tioman	194.00	0.00	0.08	0.18	0.04	0.14	0.04	0.01	0.09	0.03	0.00	0.04	0.00	0.00													
MY																											
Pasir Ris	51.00	0.00	0.08	0.19	0.04	0.13	0.05	0.01	0.08	0.03	0.00	0.04	0.00	0.00													
Silliman RP	385.00	0.03	0.08	0.18	0.05	0.14	0.05	0.03	0.09	0.02	0.03	0.04	0.03	0.02	0.00												
Phuket TH	119.00	0.00	0.09	0.18	0.05	0.16	0.05	0.02	0.11	0.03	0.01	0.05	0.01	0.01	0.00	0.04	0.00										
Poilillo RP	61.00	0.02	0.08	0.17	0.04	0.14	0.04	0.02	0.09	0.02	0.02	0.04	0.02	0.02	0.02	0.02	0.03	0.00									
Sarawak MY	125.00	0.02	0.08	0.17	0.04	0.13	0.04	0.02	0.09	0.03	0.02	0.04	0.02	0.02	0.02	0.03	0.03	0.02	0.00								
Sarawak MY	64.00	0.02	0.08	0.17	0.04	0.14	0.04	0.02	0.09	0.03	0.02	0.04	0.02	0.02	0.02	0.03	0.03	0.02	0.00	0.00							
Sarawak MY	131.00	0.10	0.09	0.17	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
<i>E. plumbea</i>	131.00	0.10	0.09	0.17	0.10	0.10	0.10	0.10	0.10	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sri Lanka	67.00	0.04	0.07	0.16	0.04	0.12	0.02	0.04	0.10	0.04	0.04	0.02	0.04	0.04	0.04	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.00
Sulawesi ID	68.00	0.03	0.08	0.18	0.04	0.14	0.05	0.03	0.09	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00
Sumatra IN	139.00	0.03	0.08	0.18	0.04	0.14	0.05	0.02	0.09	0.03	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.00
S. Buloh	441.00	0.00	0.08	0.18	0.04	0.13	0.04	0.01	0.09	0.03	0.00	0.04	0.00	0.00	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03

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