CHAPTER VI

PHYLOGENETIC RELATIONSHIPS OF THE TREE SNAIL GENUS *AMPHIDROMUS* (PULMONATA: CAMAENIDAE) INFERRED FROM MITOCHONDRIAL DNA SEQUENCES

6.1 Introduction

Southeast Asia has been recognized as one of the most important 'hotspots' for biodiversity (Myers, *et al.*, 2000). In addition, it also provided a rich resource of geological diversity and complex geological history with an array of exposes and submergence of land through time providing dynamic patterns for geographical isolation that form the basis for biogeographical records. The most importance studies have focused on vertebrate and some plant groups (e.g. Inger and Voris, 2001; Cannon and Manos, 2003; Hughes *et al.*, 2003; Woodruff, 2003; Gorog *et al.*, 2004), but invertebrate groups, including land snails, have been largely neglected.

Amphidromus is a genus of tree snails belonging to the Cameanidae composed of 150 genera in Southeast Asia. In the genus, the scale of distribution range greatly varies depending on species; some are distributed throughout the Southeast Asia, while others are limited to some provinces (Pilsbry, 1900; Solem, 1959; Laidlaw and Solem, 1961). As commonly known in tree snails, shells of *Amphidromus* exhibit a great deal of variation in complicated color patterns within and between populations. Thus the conchological taxonomy of *Amphidromus* has often been confused and subjected to repeated revisions. Over 300 nominal species proposed since the early 19th century, only 85 species have remained. Of those, 28 are dimorphic for chirality, 6 dextral and 46 sinistral (Fulton, 1896; Pilsbry, 1900; Laidlaw and Solem, 1961; Richardson, 1985).

The current taxonomy based on shell and genital morphologies separates *Amphidromus* into two subgenera: *Amphidromus* and *Syndromus* (Pilsbry, 1900). Members of the subgenus *Amphidromus* are all chirally dimorphic, except for dextral *A. givenchyi*. On the other hand, *Syndromus* are all fixed for sinistrality, except for one dimorphic species *A. glaucolarynx* (Zilch, 1953; Abbot, 1989). Shells and genital organs greatly differ between the subgenera. Their relationships, however, have not been tested by means of molecular phylogeny.

In gastropods, more than 90% of species are dextral. Chiral variation is known to be determined by a maternal effect of a single locus in several pulmonates (Freeman and Lundelius, 1982; Ueshima and Asami, 2003).

Sinistral and dextral snails have left-right reversed bodies from each other, including the genital opening on body side. Thus copulation between the two chiral morphs is physically difficult (Lipton and Murray, 1978; Johnson, 1982; Gittenberger, 1988; Asami et al. 1998; Ueshima and Asami, 2003). Within a dimorphic population, the less frequent morph suffers mating disadvantage, resulting in positive frequency-dependent selection against the minority (Johnson, 1982). For this reason, sinistral and dextral morphs could not coexist within populations, unless any additional factor counterbalances selection pressure on the minority. The presence of multiple sinistral and dimorphic species in *Amphidromus*, therefore, prompts questions on evolutionary history of chirality; how sinistrality and chiral dimorphism have evolved.

Analyses of sequence variation in mitochondrial DNA genes in several gastropods have become an effective tool for resolving systematic and phylogenetic problems (e.g. Remigio and Blair, 1997; Douris *et al.*, 1998a, b; Chiba, 1999; Thacker and Hadfield, 2000; Pfenninger and Magnin, 2001; Goodacre, 2002; Valdés, 2003; Rundell *et al.*, 2004; Holland and Hadfield, 2004). In this study, we analyzed data for mitochondrial 16S ribosomal RNA (rRNA) gene sequences of 15 *Amphidromus* species in both subgenera. Objectives are to elucidate a phylogenetic hypothesis of their relationship and to test the evolutionary history of chiral diversity in the genus. This will allow us to assess previous systematic hypotheses and discuss the historical biogeography of *Amphidromus*.

6.2 Results of Molecular Phylogeny

6.2.1 Sequence Variation

The aligned sequences of 16S rRNA gene consisted of 845 sites including insertions and deletions, 469 bp (55.5 %) of which were variable positions. In the average base frequency of all taxa, A and T were higher (36.5 and 30.9 %, respectively) than C and G (13.7 and 18.9 %, respectively). The base frequencies did not differ significantly across taxa (chi-square test implemented in PAUP*, $P = 0.99$). The mean transition:transversion ratios with and without the outgroups were 1.47:1 and 1.59:1 respectively.

Sequence divergence between the taxa of *Amphidromus* and each of the outgroups, *Beddomea*, *Camaena* and *Chloritis* ranged from 32.6 to 38.2%. Within the genus *Amphidromus*, it ranged from 8.2 to 24.0%. Within the subgenera, it was between 8.2 and 24.0% in *Amphidromus*, and between 0.4

and 29.3% in *Syndromus*. Divergence between the taxa of two subgenera ranged from 0.4 to 29.3%.

Nucleotide sequences differed in 10.0 to 12.4% between three subspecies of *A. atricallosus* and 1.8 to 5.0% between two subspecies of *A. inversus*. Within subspecies, divergence ranged from 5.1 to 5.5% in *A. atricallosus atricallosus*, 6.9 to 7.1% in *A. atricallosus leucoxanthus*, 1.8% in *A. inversus inversus*, and 4.9% in *A. inversus annamiticus*. Divergence within species of *Syndromus* varied from 5.2 to 8.8% in *A. glaucolarynx*, 2.6 to 10.3% in *A. xiengensis*, 0.7 to 22.5% in *A. semitessellatus*, and 17.5% in *A. areolatus*.

Sinistral and dextral morphs exhibited differences at only a few base pairs in three of six taxa where samples of both morphs were available from the same localities (Table 6.1). In those cases, haplotype sequences differed only equivalently within and between the morphs. Thus, essentially no sequence difference was detected between the chiral morphs, although the sample sizes were limited.

6.2.2 Phylogenetic Relationship

The MP analysis supported the monophyly of the genus *Amphidromus* and a close affinity between *Beddomea* and *Camaena* (Fig. 6.2). In the genus *Amphidromus*, *A. glaucolarynx* was first separated from the rest of members clustered in a single clade. The latter was divided into two clusters; one consisted of all the members examined in the subgenus *Amphidromus* and the other included those in *Syndromus* other than *A. glaucolarynx*.

The subgenus *Amphidromus* was split into two clusters; one included *A. inversus*, *A. givenchyi* and *A. schomburgki*, and the other *A. atricallosus*, *A. palaceus*, *A. perversus*, *A. martensi* and *A. similis*. Within the latter clade, samples of *A. atricallosus* did not constitute a monophyletic cluster and suggested the presence of three lineages, each of which supported by a high bootstrap value. In one of those lineages, *A. atricallosus perakensis* from Singapore was clustered with *A. palaceus* from Java and *A. perversus natunensis* from Natuna island.

Table 6.1 Number of base differences between chiral morphs.

Taxa	Locality	Sample size	No. sites	Within		Between D & S	
				D	S	no.	%
A. atricallosus atricallosus	17	6D, 3S	781	0	0	0	0.00
A. atricallosus leucoxanthus	10	3D, 1S	802	2	-	2	0.26
A. atricallosus perakensis	28	4D, 1S	807	2	-	2	0.26
A. inversus inversus	29	4D, 2S	800	3	0	3	0.38
A. similis	26	2D, 2S	755	0	0	0	0.00
A. palaceus	29	1D, 2S	805	-	0	0	0.00

Note: D: dextral; S: sinistral.

Syndromus exclusive of *A. glaucolarynx* was separated into two groups, into which the two haplotypes of *A. semitessellatus* were split. Of the two groups, the larger one accommodated two clusters, where the two haplotypes of *A. areolatus* were separated. One of those clusters was further split into three distinct lineages, each of which included one or two haplotypes of *A. xiengensis*. These separations of conspecific samples were all supported by high bootstrap values. The present results, therefore, indicate that mtDNA haplotypes of *A. semitessellatus, A. areolatus* and *A. xiengensis,* defined by shell morphology, are not monophyletic.

The results of the NJ and ML analyses were congruent with those of the MP analysis (Figs. 6.3 and 6.4). Cluster topologies with *A. similis, A. martensi* and *A. perversus* appear to be slightly different between the three trees. Those differences are, however, not informative because of small bootstrap repeatabilities. In *Syndromus*, the ML analysis resolved the relationship of *A. areolatus, A. porcellanus* and *A. xiengensis* differently from the MP and NJ analyses. In the latter topology, a cluster of *A. areolatus* and *A. xiengensis* was separated from a cluster of *A. porcellanus* and *A. xiengensis*. On the other hand, the ML analysis resolved the cluster of *A. porcellanus* and *A. xiengensis* as the most basal at the highest probability. The ML analysis also differently resolved the relationship of the three outgroup taxa from the MP and NJ methods.

6.2.3 Chiral Phylogeny

The mtDNA phylogeny obtained by the three analyses demonstrated that chiral dimorphism is a synapomorph of the genus *Amphidromus* (Figs. 1, 2 and 3). The present outgroups and most camaenids are dextral. Thus, the dimorphism of *Amphidromus* has most likely evolved in a dextral ancestor. In the classical taxonomy, *Syndromus* includes *A. galucolarynx* as only dimorphic species. Thus the dimorphism has been thought to have evolved in a sinistral ancestor within *Syndromus*, independently of the dimorphism of the genus *Amphidromus*. Our results, however, indicates that the dimorphism of *A. glaucolarynx* is instead the ancestral status of chirality. Thus the sinistral clade of *Syndromus* has evolved from a single dimorphic ancestor. Similarly the dextrality of *A. givenchyi* has evolved from the dimorphic status within the subgenus *Amphidromus*.

6.3 Results and Discussion for Morphological Phylogeny

The cladistic analysis using Branch and bound option was resulted in 42 equally parsimonious trees. The two confamily species were used as outgroup, and of 17 characters were parsimony-informative. The 50% majority rule consensus tree cladogram (Fig. 6.5A) has 55 steps, CI=0.42 and RI=0.60. The dendogram revealed the monophyletic of ingroup taxa, which supported by 68% bootstrap values. The ingroup taxa composed of two clades; one included 5 species of subgenus *Amphidromus*, and other included 6 species of subgenus *Syndromus* (Fig. 6.5). These two clades were marginally supported with 51% and 69% bootstrap values (Fig. 6.5A). Only the relationship of *A. (S.) flavus* and *Amphidromus (S.)* sp. was supported with slightly high values (78% bp). However, the present morphological analysis revealed that *A. (S.) glaucolarynx* was clustered with subgenus *Syndromus*, but its relationship still uncertain (Fig. 6.5A). This result is slightly contradicted with the mtDNA phylogeny, which *A. (S.) glaucolarynx* resolved at the most basal clade of the ingroup taxa (Figs 6.2-6.4). The species level relationships within these two subgenera are mostly solved as polytomies.

Character transformations are shown on one of the most parsimonious cladogram obtained form outgroup rooted analysis (Fig. 6.5B). The present morphological analysis (Fig. 6.5B) is congruent with mtDNA phylogeny (Figs 6.2-6.4) in a monophyletic of the genus, which diagnosed by the

apertural shape (character 3), shell coiling direction (character 5), radula teeth and central tooth shapes (characters 13, 14). Only 3 places were supported with bootstrap values and morphological characters transformations (Fig. 6.5B). 1) The monophyletic of subgenus *Amphidromus* was diagnosed by only two characters of shell size (character 2) and the reproductive organ of flagellum (character 16). 2) The subgenus *Syndromus* clade (inclusive of *A. glaucolarynx*) was diagnosed by the thin and fragile shell (character 1), shell height smaller than 35 mm (character 2), variegated shell colour pattern (character 12) and internal sculpture of the vagina (character 17). 3) The relationship between *A. flavus* and *A. (Syndromus)* sp. was diagnosed by sub-circular shaped aperture (character 3) and the monochrome shell colour pattern (character 12). However, many of these characters appear to be either homoplasy or reversal. For example, the synapomorphies supported a subgenus *Syndromus* clade, character of shell colour pattern (character 12), was also the reversal character in (*A. flavus, Amphidromus* sp.) clade.

Using the shell morphology alone may contain more homoplastic, which increase the risk in similarity of the character observed. This may not reflect the common ancestry but rather than the parallel or convergent evolution, and this may caused the error in conclusions. In this study, the high level of homoplasy, unsolved polytomies or bootstrap values lower than 50% especially in the relationship among species levels were observed in both subgenera. Possibly, this situation may due to the highly shell colour variation for ecological adaptation among the studies species, in contrast to the low variation in the reproductive organ. Utilizing a combined analysis of nuclear and mtDNA data are needed to complete a comprehensive phylogeny especially in the subgenus Syndromus.

Table 6.2 Data matrix used in the morphological phylogeny

Taxa	Characters																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A. inversus inversus	1	2	2	1	2	0	1	0	0	1	0	1,2	1	1	0	0	0
A. inversus annamiticus	1	2	2	1	0	0	1	1	0	1	0	1,2	1	0	0	0	0
A. atricallosus atricallosus	1	2	2	1	2	0	1	0	0	1	2	0	1	0	0	1	0
A. atricallosus leucoxanthus	1	2	2	1	2	0	1	0	0	1	0	0,1	1	0	0	1	0
A. atricallosus perakensis	1	2	2	1	2	0	0	0	0	0	?	0	1	0	0	1	0
A. atricallosus ssp.	1	2	2	1	1	0	1	0	0	1	0,2	0	1	0	0	1	0
A. perversus natunensis	1	2	2	1	2	0	1	0	0	1	0	0,2	1	0	0	1	0
A. schomburgki schomburgki	1	2	1	1	2	1	0	2	1	1	1	1	1	0	0	1	0
A. schomburgki ssp.	1	2	2	1	0	1	0	0	0	1	0	1	1	0	0	1	0
A. givenchyi	1	2	2	1	0	1	0	1	0	0	?	1	1	0	0	1	0
A. xiengensis	0	1	2	0	1	0	0	1	0	0	?	3	1	1	1	0	1
A. semitessellatus	0	1	2	0	1	1	0	1	0	0	?	0,3	1	1	1	0	0
A. flavus	0	1	1	0	1	0	0	1	0	0	?	0	1	0	1	0	1
A. areolatus	0	1	2	0	1	0	0	1	0	0	?	3	?	?	?	?	?
A. (Syndromus) sp.	0	1	1	0	1	0	0	1	0	0	?	0	1	0	1	0	0
A. glaucolarynx	0	1	2	0	2	0	0	2	1	0	1	3	1	0	1	1	1
Camaena illustris	1	0	0	1	0	0	0	0	0	1	0	0	0	1	1	0	0
Chloritis siamensis	0	0	0	0	0	0	0	0	0	0	?	02	0	1	1	0	0

6.4 Discussion for Molecular Phylogeny

6.4.1 Phylogenetic relationships

The present results have demonstrated that the genus *Amphidromus* is a monophyletic clade. Based on morphological similarities, *Beddomea* was classified as a subgenus of *Amphidromus* (Pilsbry, 1901; Gude, 1914), and then raised to a distinct genus (Zilch, 1960; Richardson, 1985; Vaught, 1989). We have disclosed that *Beddomea* differs from *Amphidromus* in 36.2% of mtDNA sequence and is closer to *Camaena* and *Chloritis* than *Amphidromus*, supporting the current taxonomy of *Beddomea*.

The subgenus *Amphidromus* is clearly monophyletic. On the other hand, *Syndromus* is paraphyletic, because of the most basal separation of *A. glaucolarynx*. Although the bootstrap values for the node may not be sufficiently large, our posteriori evaluation of morphological traits has supported the unique differences of *A. glaucolarynx* from the rest of *Syndromus* in radula teeth, shell size and shape, shell-colour pattern and epiphallic caecum (unpublished data).

In the subgenus *Amphidromus*, three separate clusters were found in samples of *A. atricallosus* (clades A, B and C). Clade B corresponds to three localities within a range of the Malay Peninsula, whereas clade A included samples from relatively distant localities 10 and 23. Thus, these clusters are not necessarily corresponding to geographical proximity. Clade C has resulted from five individual sequences of *A. atricallosus perakensis* exhibiting closer affinity with two separate species than the two other subspecies of *A. atricallosus*. Samples of those taxa in clade C were from a southwestern range of the present survey. *A. atricallosus perakensis* differs from other members of *A. atricallosus* in shell morphology lacking a dark parietal callus and varices. It was formerly classified as a different species (Fulton, 1901), but has been a subspecies since Laidlaw and Solem (1961). *A. atricallosus perakensis* may be reproductively isolated from others of *A. atricallosus*. The current result could also result from introgressive hybridization or ancestral polymorphism of mtDNA. However, we have not found more than one species of the subgenus *Amphidromus* at single localities ever surveyed, even in numerous empty shells accumulated on the ground. Accordingly the chance of interspecific hybridization in the subgenus would not be large, although it does not reject the possibility throughout their history of radiation. Further examination of phylogeography is necessary to understand evolutionary relationships of the three clades detected.

In the sinistral clade of *Syndromus*, we have detected polyphyletic origins of mtDNA haplotypes within each of three species: *A. semitessellatus*, *A. xiengensis* and *A. areolatus*. First, the three haplotypes of *A. semitessellatus*, all from Thailand, were divided by 22.5% base substitution into the two largest sinistral clades of *Syndromus*. One haplotype from the central Thailand exhibited affinity to those of two other species from the Malay Peninsula and Borneo. On the other hand, the other two haplotypes from Chanthaburi (localities 10 and 11) near Cambodia were close to *A. areolatus* from Pangnga (locality 19) on the Peninsula. Thus, these divergent haplotypes of *A. semitessellatus* may not be easily ascribable to interspecific introgression possible only in sympatry. Instead, those two types of *A. semitessellatus* are likely to represent genetically independent populations exhibiting closely similar shell-color patterns. Second, the haplotypes of *A. xiengensis* were separated into three clusters, which are all supported by high bootstrap values. One of those included *A. xiengensis* from an island near Bangkok (locality 16) and *A. porcellanus* from Java (locality 30), which could hardly be explained by mtDNA introgression or ancestral polymorphism considering their remote localities and probable bottle-necks in an insular population. Third, one haplotype of *A. areolatus* was closer to that of *A. xiengensis* than the other conspecific haplotype, which exhibited

little divergence from the haplotypes of *A. semitessellatus*, in spite of the sampling localities of *A. areolatus* within a range across the Peninsula.

Polyphyly of mtDNA haplotypes repeatedly discovered within the three species of Syndromus open intriguing questions on the existence of cryptic species isolated reproductively from each other within the present morphological species. Shells of *Syndromus* commonly exhibit complicated mosaic color patterns which are likely to camouflage arboreal snails from predators. Because of this critical function for survival, diverging species likely happen to maintain similar color patterns under strong stabilizing selection. The present results of *Syndromus* may exemplify divergence of cryptic species behind closely similar shell-color patterns performing ecologically significant functions. This hypothesis of convergence can be tested by further surveys of divergence in nuclear loci and genital anatomy within the present species. Interspecfic introgression of mtDNA need to be also tested by phylogeographic analysis of genetic variation within and between populations.

The present results have disclosed a contrasting difference in specific phylogeny between the two subgenera, even though both have been classified based on morphological traits. The pattern of mtDNA phylogeny has supported specific taxonomy in the subgenus *Amphidromus* except for one subspecies of *A. atricallosus*. However, it has shown little correspondence to the specific taxonomy of *Syndromus*. Several reasons may account for this difference. Snails of the subgenus *Amphidromus* are far larger and more abundant than those of *Syndromus*. Thus the current taxonomy of the former has better incorporated genital anatomy in addition to conchology. The subgenus *Amphidromus* exhibits greater variation in shell shape as well as in shell-color pattern between species. Further, the shell-color pattern varies in conspicuously different manners between the two subgenera.

6.4.2 Chiral dimorphism

Oppositely coiled snails can not easily copulate with each other because of their lateral reversal in genital positions. In chirally dimorphic populations, however, chiral reversal does not cause reproductive isolation between the chiral morphs even if they are unable to mate at all, because of delayed inheritance. Thus chiral morphs exhibit little genetic differences in *Partula suturalis* and *P. otaheitana* (Goodacre, 2002). The present study has detected no significant differences in mtDNA sequence between dextral and sinistral morphs and supported that they share the same gene pools with no reproductive isolation. For this subject, however, genetic structure of dextrals

and sinistrals from single localities needs to be examined explicitly with sufficient population samples.

Amphidromus glaucolarynx is only chirally dimorphic species in *Syndromus*, where all other members are sinistral. The present study has revealed that the dimorphism of this species is the ancestral state for the genus *Amphidromus*, but not derived one within the sinistral clade. The pattern of chiral phylogeny indicates that chiral dimorphism has evolved only once in the ancestor. Chiral dimorphism has been maintained throughout the radiation of *Amphidromus*, except for the sinistral clade of *Syndromus* and for only dextral species *A. givenchyi*. Therefore, chiral dimorphism within species must be positively maintained by a certain mechanism that enables the two morphs to coexist stably over a long term. This is the first phylogenetic evidence of long-term stability of chiral dimorphism within species of snails. Contrary to stable monomorphism of chirality in most gastropods, the genus *Amphidromus* exhibits phylogenetic constraint for chiral dimorphism. It does not mean, however, that the dimorphism is necessarily maintained within populations across the genus. Parapatry of sinistral and dextral monomorphism could cause chiral dimorphism within species such as in *Partula* (Johnson *et al.*, 1990). To answer this line of questions on the stability of chiral dimorphism, we are investigating population dynamics.

Sinistrality has also evolved only once across the genus, resulting in the sinistral clade of *Syndromus*. This indicates that sinistral monomorphism can evolve in a dimorphic species and be stably sustained once it occurs. This evidence of sinistral stability and also dextral monomorphism of *A. givenchyi* further support the existence of positive maintenance of chiral dimorphism in the rest of congenic members against the stochastic evolution of chiral monomorphism.

Phylogenetic constraint for chiral dimorphism suggests that dimorphic members of the genus share some particular trait that allows stable maintenance of dimorphism. *A. glaucolarynx* and the subgenus *Amphidromus* excluding *A. givenchyi* are most divergent in phylogeny and largely differ in shell traits, but have commonly maintained chiral dimorphism. Thus some trait common between them would likely be a crucial key for stable maintenance of chiral dimorphism against positive frequency-dependent selection. The chiral dimorphism of *Partula suturalis* has been ascribed to migration of the two morphs into the boundary between dextral and sinistral monomorphic populations. However, we have not found probable source populations of sinistrals and of dextrals surrounding dimorphic populations in

species we have surveyed in *Amphidromus* (unpublished). Easy copulation between the two morphs is not good enough; interchiral copulation has to be easier than ordinary intrachiral copulation for chiral dimorphism to be maintained. To be achieved by apostatic selection, predation pressure needs to be strong enough to overcome positive frequency-dependent selection resulting from interchiral mating difficulty. To determine a mechanism responsible for stable dimorphism, we are continuing investigation of their life history traits and spatial distributions of the two morphs.

6.4.3 Biogeography

The present results suggest that mtDNA haplotypes from the continent tend to be basal in phylogeny, whereas those from islands (localities 23-30) are mostly the relatively derived. In the subgenus *Amphidromus*, the continental samples of *A. givenchyi* and *A. schomburgki* were basal to *A. inversus* (localities 16-28), which is widely distributed on Indonesian islands and elsewhere in relatively southern ranges. *A. atricallosus perakensis*, *A. placaeus*, *A. perversus natunensis* (localities 24, 27, 29), *A. martensi* and *A. similis* (localities 25, 26) may be derived from continental *A. atricallosus*. In Syndromus, *A. adamsi*, *A. pictus* and *A. porcellanus* (localities 26, 30) were resolved at the tip of clades. Only exception was that a relatively southern insular sample of *A. atricallosus leucoxanthus* (locality 23) was ancestral to the continental samples. However, *A. atricallosus leucoxanthus* is not particularly insular but distributed across the continent as well. Thus, the ancestor of the genus *Amphidromus* may likely have originated in the continent of Indochina.

Similarly to the present results, high rates of mtDNA base substitution have been reported in camaenid *Mandarina* (Chiba, 1999), bradybaenid *Euhadra* (Hayashi and Chiba, 2000) and achatinellid *Achatinella* (Thacker and Hadfield, 2000). Dated fossils of *Amphidromus* have not been recorded. Thus, we adopted a conservative range of 1-10% per million years as the approximate base substitution rate in divergence of closely related groups. Then mtDNA divergence observed in this study suggests that the minimum divergence time of the genus *Amphidromus* would likely be between Oligocene (32 to 38 MYA) and Pliocene (3.2 to 3.8 MYA).

Distribution of *Amphidromus* would have been aided by the reduction of sea level and extension of the continental area around the mid Pliocene (Haq *et al.*, 1987) allows terrestrial passage between Indochina, Sundaland and among the Sunda islands. According to the low level of dispersal and sedentary behavior of land snails, the high genetic divergence within *A.*

atricallosus, *A. xiengensis*. The phylogenetically close relationship found in terminal taxa at nodes 9, 12 and clades A, E, F is not reflected in their geographical distributions that are widely separated. This pattern of widely disjunct distribution for terminal clade is likely to be the result of contraction in what were previously wider distribution. The similar situation have been reported in *Euhadra* spp. in Japan (Hayashi and Chiba, 2000; Watanabe and Chiba, 2001) and some species of *Partula* spp. (Goodcare, 2002).

Amphidromus inversus exhibits a wide distribution from central Vietnam to Sumatra and Celebes and is restricted to island and maritime locations. The close genetic affinities with this species is an indication that it has recently spread through this distribution range. Change in the sea level have reflected in complex patterns of land expose and submergence with repeated opportunities for dispersal across land followed by periods of land fragmentation and isolation of populations.

6.4.4 Taxonomic implications

The present results of mtDNA phylogeny based on partial 16S rDNA sequences provided clear evidence for the necessity of taxonomical revision. First, *A. glaucolarynx* should not be included within *Syndromus*, which supports the current classification by Laidlaw and Solem (1961) than the earlier taxonomy by Zilch (1953). On this evidence further careful studies of morphology should reveal synapomorphic characters of *Syndromus* s. str. Second, the present results support separation of *A. schomburgki* from *A. givenchyi* as distinct species by Sutcharit and Panha (2005). Third, the current specific taxonomy of *Syndromus* should be revised based on additional studies of anatomy and reproductive isolation between populations. Remarkable confusion of taxonomy due to complicated shell-color pattern in *Syndromus* and contrasting usefulness of shell traits in the subgenus *Amphidromus* may provide a unique opportunity to disclose the adaptive functions of shell-color patterns.
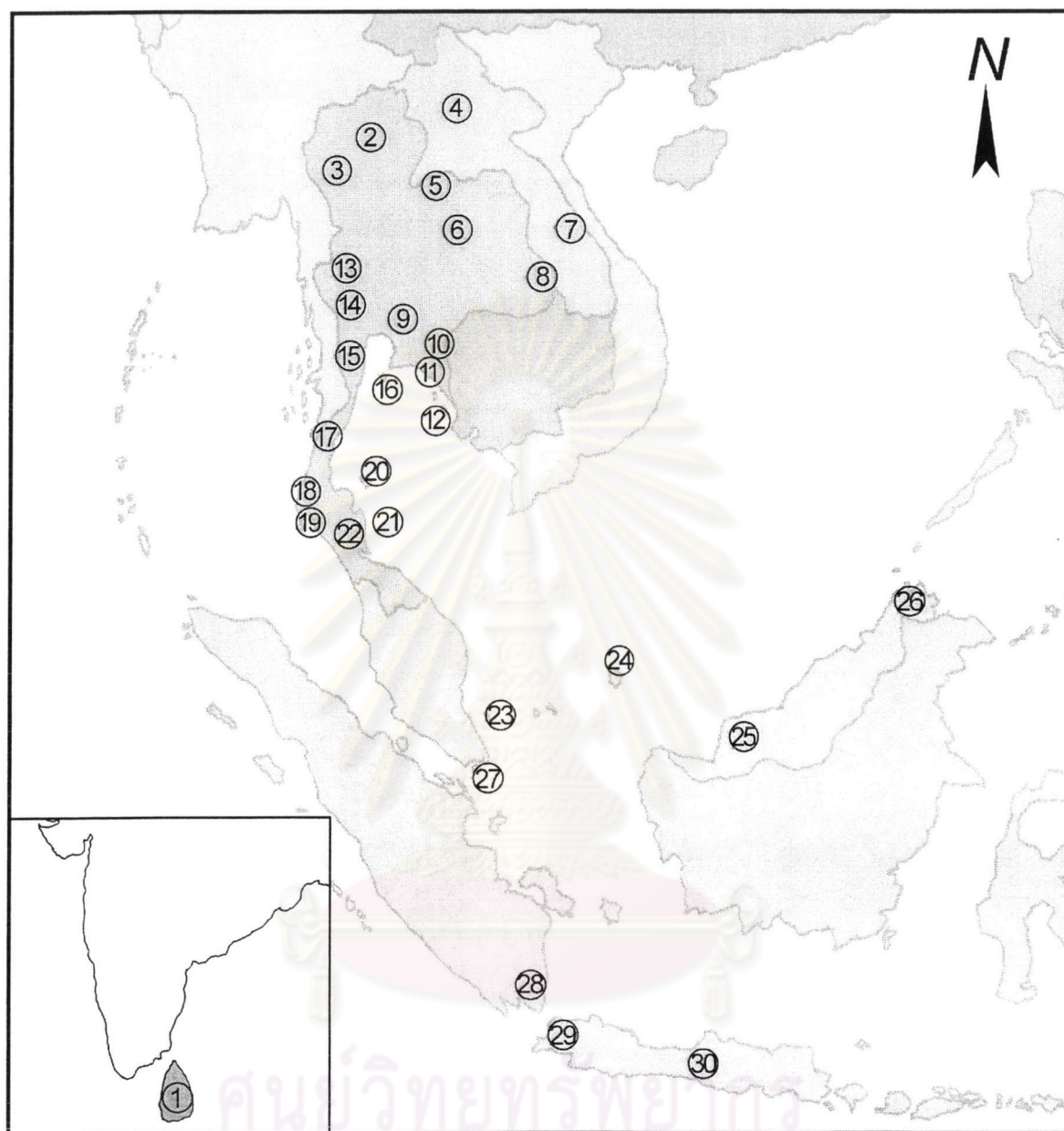
Figure 6.1 Collecting localities in Southeast Asia and Sri Lanka (lower left). Numbers correspond to locality numbers indicated in Table 1. 1) Sri Lanka; 2) Phrae; 3) Chiengmai; 4) Luang Prabang; 5) Loei; 6) Kalasin; 7) Savanakhet, Laos; 8) Ubonratchathani; 9) Chachoengsao; 10) Makok waterfall, Chanthaburi; 11) Trongnong waterfall, chanthaburi; 12) Trat; 13) Thong Pha Phum, Kanchanaburi; 14) Sai Yok waterfall, Kanchanaburi; 15) Phetchaburi; 16) Elar island, Chonburi; 17) Ranong; 18) Ban Takun, Suratthani; 19) Pangnga; 20) Samui island, Suratthani; 21) Nakhonsithammarat; 22) Phattalung; 23) Tioman island, Malaysia; 24) Natuna island, Indonesia; 25) Sarawak, Malaysia; 26) Sabah, Malaysia; 27) Singapore; 28) Sumatra, Indonesia; 29) Pangandaran, Indonesia, and 30) Bogor, Indonesia.
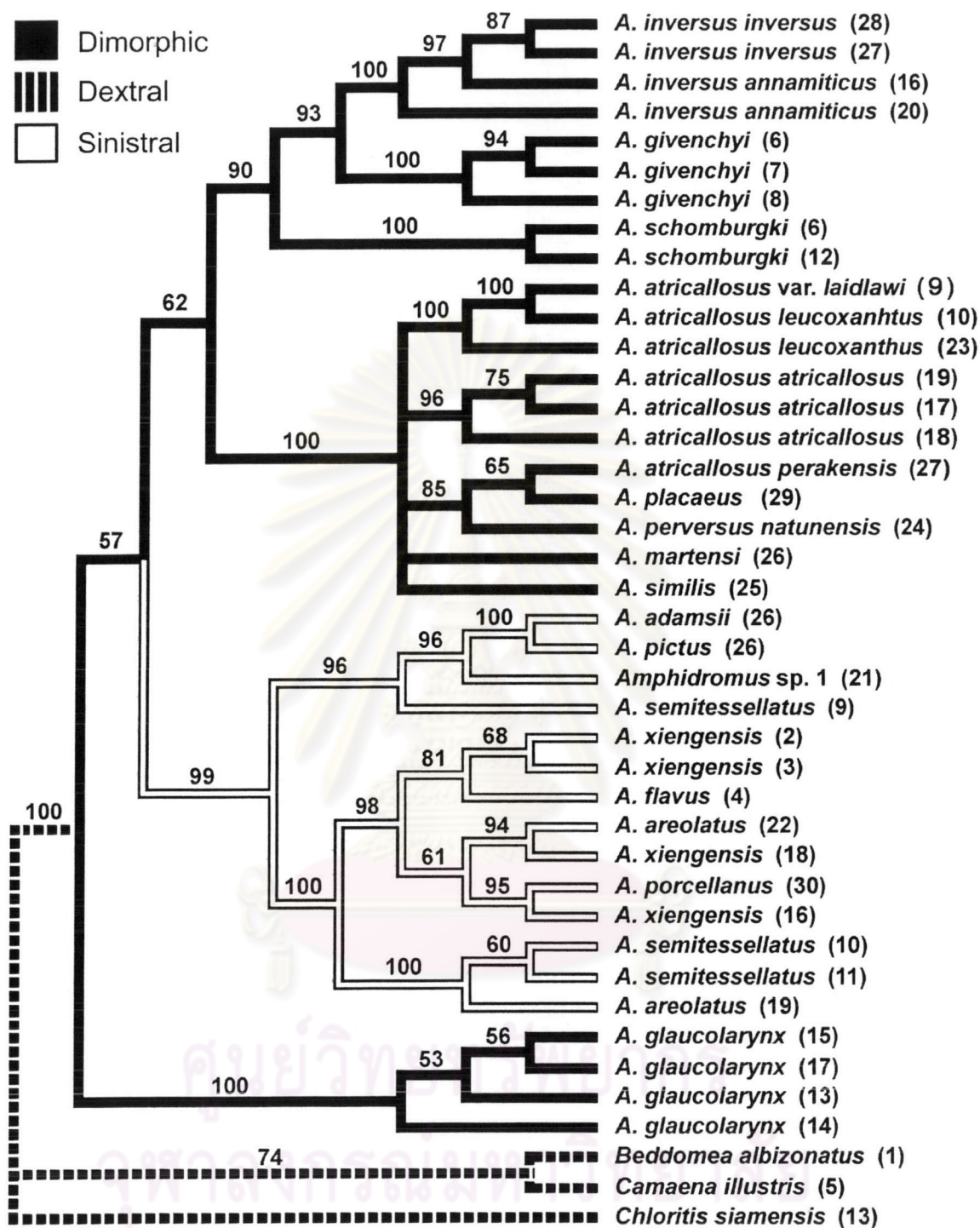
Figure 6.2 Maximum parsimony cladogram using heuristic option (transition:transversion = 2:1, strict consensus of 12 parsimonious tree, 2371 steps, 469 bp informative under the condition of parsimony, CI = 0.43 and RI = 0.71). Numbers above branches are bootstrap probabilities higher than 50% in 1,000 replications. Numbers in parentheses indicate collecting localities shown in Figure 6.1.
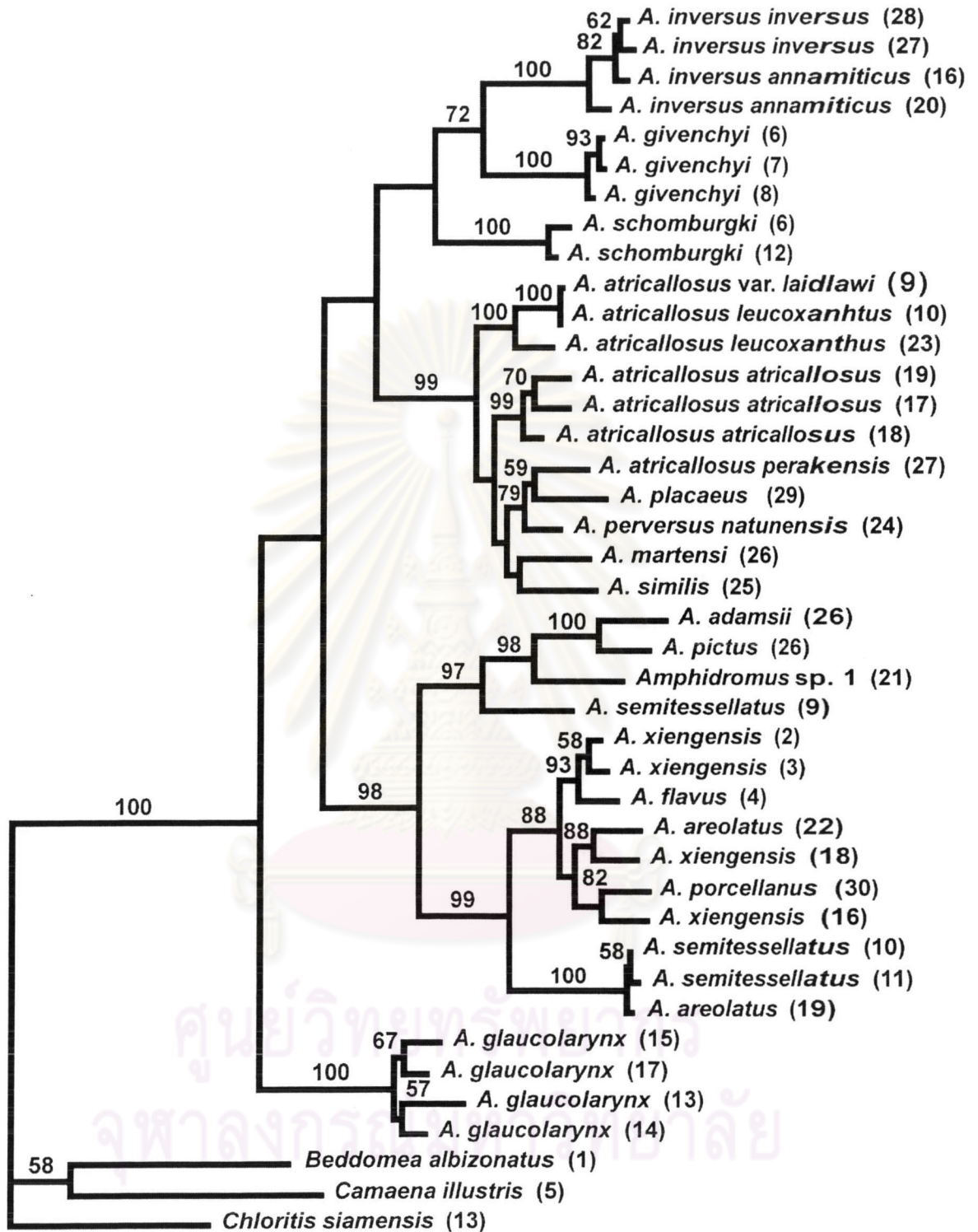
Figure 6.3 Neighbor-joining dendrogram derived from Kimura's two parameter model. Bar equals 0.1 units. Numbers above branches are bootstrap probabilities higher than 50% in 1,000 replications. Numbers in parentheses indicate collecting localities shown in Figure 6.1.

Figure 6.4 Maximum likelihood dendrogram with (ln likelihood = -11982). All branches were statistically significant (P < 0.01). Bar equals 0.1 unit. Numbers above branches are bootstrap probabilities higher than 50% in 100 replications. Numbers in parentheses indicate collecting localities shown in Figure 6.1.
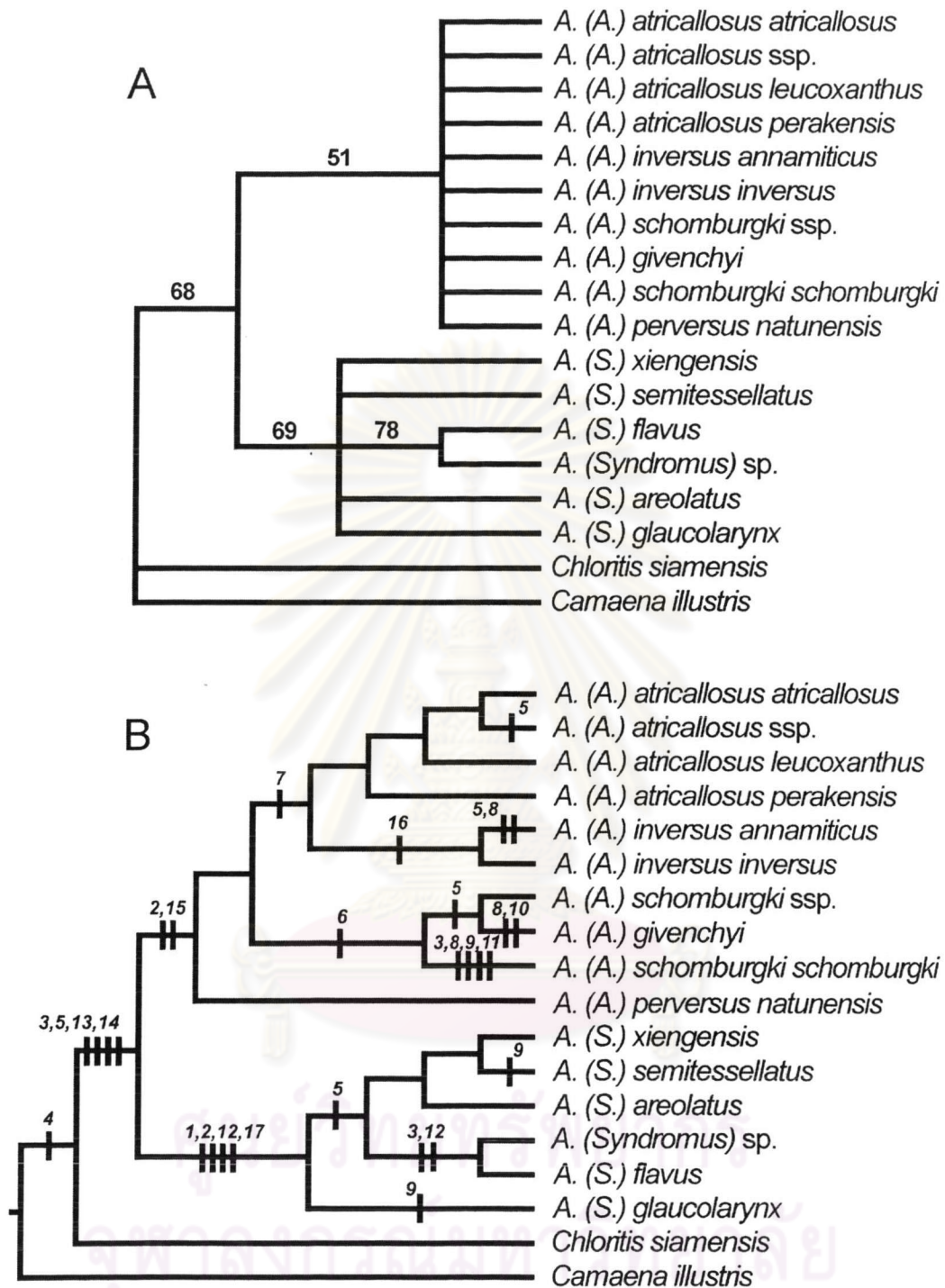
Figure 6.5 A. Maximum parsimony dendrogram derived from morphological characters using Branch-and-bound option (50% majority-rule consensus tree of 42 parsimonious tree, 55 steps, CI = 0.42 and RI = 0.60). The numbers above the branches are bootstrap probabilities higher than 50% in 1000 replications. **B.** One of the 42 equally parsimonious trees showed the characters transformations. The italic numbers are the character changing along that branch.