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Allozyme Variation in the Camaenid Tree Snails *Amphidromus atricallosus* (Gould, 1843) and *A. inversus* (Müller, 1774)

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We examined allozyme variation in two camaenid tree snails, *Amphidromus atricallosus* and *A. inversus*, across two principal regions of Thailand and from Singapore, plus for *A. inversus*, one site in peninsular Malaysia. Using horizontal starch gel electrophoresis, 13 allozyme loci (11 polymorphic) were screened for *A. atricallosus* and 18 (5 polymorphic) for *A. inversus*. Heterozygosity was higher in *A. atricallosus* ($H_{exp}=0.018\text{--}0.201$, mean=0.085) than in *A. inversus* ($H_{exp}=0\text{--}0.023$, mean=0.002). Genetic heterogeneity among samples was higher in *A. inversus* ($F_{st}=0.965$) than in *A. atricallosus* ($F_{st}=0.781$). Within *A. atricallosus*, populations were more differentiated in southern Thailand ($F_{st}=0.551$) than in eastern Thailand ($F_{st}=0.144$). The high F_{st} and low H_{exp} in populations of *A. inversus* suggest that this species is likely to have experienced a series of strong bottlenecks, perhaps occurring chiefly on offshore continental-shelf islands. The low F_{st} values of *A. atricallosus* in eastern Thailand suggest frequent gene flows among populations in this region. The southern and eastern samples of *A. atricallosus* exhibited fixed allele differences at four loci and great genetic distance (Nei's $D=0.485\text{--}0.946$), suggesting that these two samples may actually represent, or else be evolving into, separate species.

Key words: *Amphidromus atricallosus*, *Amphidromus inversus*, allozyme, geographic genetic structure, taxonomy

INTRODUCTION

Amphidromus atricallosus (Gould, 1843) and *A. inversus* (Müller, 1774) are arboreal snails in the family Camaenidae and feed on microflora (Sutcharit and Panha, 2006). Both species occur in the Indochina-Malay Peninsula region of Southeast Asia. *Amphidromus inversus* also occurs in Borneo, Sumatra, and Sulawesi (Pilsbry, 1900; Laidlaw and Solem, 1961; Solem, 1965).

Each species often occupies discrete patchy areas (Panha, unpublished data; see Fig. 1), and thus may exhibit geographically complex genetic structure. *Amphidromus atricallosus* and *A. inversus* inhabit continental areas and small continental-shelf islands, respectively. These islands are isolated from the continent by shallow straits but experienced dry land connections to the continent for several periods during the Pleistocene (Vorisi, 2000). Thus they potentially provide a good opportunity to examine the effects of Quaternary geological history involving insularization of hab-

itats on the current genetic structure of distinct geographic populations.

For both of these species, several subspecies have been recognized on the basis of shell morphology, radula morphology, and anatomical characters of the genitalia (Laidlaw and Solem, 1961; Sutcharit and Panha, 2006). However, given that these traits may be homoplastic, these described subspecies need to be verified by additional means such as assessment of fixed genetic traits between populations. Indeed, extensive morphological variation within *Amphidromus* has often confused the taxonomy of subspecies (Solem, 1965).

Allozyme electrophoresis is one such tool to estimate relatively large changes in the geographic genetic structure of a broadly ranging species, since both overall genetic differentiation and gene flow amongst populations can be estimated on the basis of variation in non-synonymous (nuclear) coding genes. We analyzed allozymic variation among 12 populations of *A. atricallosus* and nine populations of *A. inversus* across two geographically disparate regions of Thailand, plus one site each in Singapore and in the case of *A. inversus*, additionally one site in peninsular Malaysia. Our goals were (1) to clarify the genetic structure of the two species around the Indochina-Malay Peninsula region; (2) to

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Fig. 1. Map of Southeast Asia (left), with insets (right) showing sampling localities of *Amphidromus atricallosus* and *A. inversus*. 1=Soidao, 2=Makham, 3=Traknong, 4=Makok, 5=Ranong, 6=Koh Tachai, 7=Takhun, 8=Klongsang, 9=Khaosok, 10=Suwankuha, 11=Poungchang, 12=Bangkram, 13=Singapore Nee Soon, 14=Koh Samet, 15=Koh Kham, 16=Koh Jarn, 17=Koh Elar, 18=Koh Pai, 19=Koh Lueam, 20=Chaiya, 21=Koh Samui, 22=Koh Tan, 23=Pulau Kapas, 24=Singapore Botanic garden. Gray and dark shaded areas indicate land higher than 50 and 500 m asl, respectively.

use the geographic genetic structure to help elucidate the processes involved in the formation of the structure; and (3) to revise classification of the (sub)species accordingly.

MATERIALS AND METHODS

Sampling was carried out in Thailand, Malaysia, and Singapore (Fig. 1). In total, 319 *A. atricallosus* were collected from 11 localities in continental areas and from two offshore islets. For *A. inversus*, 144 specimens were collected from ten offshore islets and one continental locality.

Liver and muscles tissues were removed from each specimen and homogenized. Protein extracts from each homogenate were subjected to horizontal starch gel electrophoresis following the methods of Murphy *et al.* (1996) with slight modifications. Voucher specimens were deposited in the Chulalongkorn University Museum of Zoology.

Electrophoretic methods largely followed Clayton and Tretiak

(1972), Boyer *et al.* (1963), and Ridgway *et al.* (1970). Enzyme nomenclature and E.C. numbers follow those proposed by the International Union of Biochemistry.

Data analyses were performed using BIOSYS-1 (Swofford and Selander, 1981). Genetic variability within each population was assessed by calculating the mean expected heterozygosity (H_{exp}), the mean number of alleles per locus (A), and the percentage of polymorphic loci (P). The genotypic frequency at each polymorphic locus was tested for agreement with Hardy-Weinberg expectation using the exact test.

To estimate heterogeneity among samples for each species, Wright's (1965) F_{st} was calculated for each locus. Each F_{st} value was tested for significant departure from zero following Workman and Niswander (1970). Nei's (1978) unbiased genetic distance and Rogers' (1972) genetic distance were calculated for all pairwise comparisons of samples in order to estimate the extent of differentiation among populations. A neighbor-joining (NJ) tree (Saitou and Nei, 1987) was created for *A. atricallosus* from Rogers genetic distances.

RESULTS

In the samples of *A. atricallosus*, 11 out of 13 loci for ten enzymes were polymorphic (Table 1). In the samples of *A. inversus*, five out of 18 loci for 11 enzymes were polymorphic (Table 2 and 3).

Genetic variation in *Amphidromus atricallosus*

In *A. atricallosus*, genotype frequencies appeared to deviate from Hardy-Weinberg expectation in only one sample, Singapore Nee Soon, at one locus, *Aat-1*. However, considering the total number of loci examined, this was not statistically significant because of multiple comparisons involved.

Different alleles were fixed at four of the 11 polymorphic loci (*Gpi*, *Me*, *Mpi*, and *Lgg-2*) between the southern and the eastern Thailand samples. The Singapore Nee Soon sample was distinct from all Thailand samples by invariably having allele "a" at *Mpi*. Also, this sample showed fixed allelic differences from the eastern Thailand samples at *Hbdh*, *Me*, and *Pgd*, and from the southern Thailand samples at *Gpi* and *Lgg-2*. Within the southern Thailand samples, fixed differences were observed between the Koh Tachai sample and the Bangkram-Ranong samples at *Aat-1*, between the Koh Tachai sample and the Klongsang-Suwankuha-Poungchang-Bangkram samples at *Hbdh*, and between the Bangkram sample and the Koh Tachai-Klongsang-Khaosok-Suwankuha-Poungchang samples at *Lgg-2*. There were no such fixed differences among the eastern Thailand samples.

Matrices of Nei's (1978) genetic distance (*D*) and Rogers (1972) distance among the samples of *A. atricallosus* are presented in Table 4. Large *D* values were obtained between the southern Thailand samples and the Singapore Nee Soon sample (mean±S.D., 0.841±0.105; range, 0.590–0.915), between the Singapore sample and the eastern Thailand samples (0.580±0.018; range, 0.557–0.597), and between the southern Thailand samples and the eastern Thailand samples (0.729±0.148; range, 0.485–0.946). The *D* values between the southern Thailand samples (0.157±0.073; range, 0.012–0.306) were smaller, and larger than those between the eastern Thailand samples (0.017±0.011; range, 0.005–0.031), with the exception of the value between the Suwankuha and Poungchang samples (0.012).

The mean *Fst* for all the *A. atricallosus* samples was 0.781. Among the four eastern Thailand samples and among the eight southern Thailand samples, mean *Fst* values were much smaller, 0.144 and 0.551, respectively. Nevertheless, *Fst* values were significantly higher than zero for five polymorphic loci of the former and for ten polymorphic loci of the latter.

The eastern Thailand samples showed moderate *P* and *Hexp* values (15.4–38.5 and 0.021–0.098, respectively; Table 6). Likewise, the southern Thailand samples exclusive of those from Koh Tachai and Bangkram displayed moderate to high *P* and *Hexp* values (23.1–61.5 and 0.057–0.201, respectively). In the Koh Tachai sample and the Bangkram sample, both *P* and *Hexp* values were much lower, 7.7 and 0.025 for the former, and 7.7 and 0.018 for the latter, respectively (Table 6).

Table 1. Enzymes and presumptive loci with tissues and buffer systems used.

Species	Enzyme	E.C. Number	Locus	Tissue*	Buffer system**
<i>A. atricallosus</i>					
	Aspartate aminotransferase	2.6.1.1	<i>Aat-1, 2</i>	L	TC8
	Esterase	3.1.1–	<i>Est-2</i>	L	LioH
	Glucose-6-phosphate isomerase	5.3.1.9	<i>Gpi</i>	L	LioH
	3-Hydroxybutyrate dehydrogenase	1.1.1.30	<i>Hbdh</i>	L	TBE8.7
	Peptidase (leucyl-glycyl-glycine)	3.4.–.–	<i>Lgg-1, 2</i>	L	TC8
	Malate dehydrogenase	1.1.1.37	<i>Mdh</i>	M	CAPM6
	Malate dehydrogenase (NADP+)	1.1.1.40	<i>MdhP</i>	M	TC8, TBE8.7
	Mannose-6-phosphate isomerase	5.3.1.8	<i>Mpi</i>	M	CAPM6
	Phosphoglucomutase	2.7.5.1	<i>Pgm-1, 2</i>	L	TC8
	Phosphogluconate dehydrogenase	1.1.1.44	<i>Pgd</i>	M	CAPM6
<i>A. inversus</i>					
	Aspartate aminotransferase	2.6.1.1	<i>Aat</i>	L	CAPM6
	Esterase	3.1.1–	<i>Est-1, 2, 3, 4</i>	M, L	CAPM6, TBE8.7
	Glucose-6-phosphate isomerase	5.3.1.9	<i>Gpi</i>	L	TC8
	3-Hydroxybutyrate dehydrogenase	1.1.1.30	<i>Hbdh</i>	L	TBE8.7
	Peptidase (leucyl-glycyl-glycine)	3.4.–.–	<i>Lgg-1, 2, 3</i>	L	TC8
	Malate dehydrogenase	1.1.1.37	<i>Mdh-1, 2</i>	M	CAPM6
	Malate dehydrogenase (NADP+)	1.1.1.40	<i>MdhP</i>	M	TBE8.7
	Mannose-6-phosphate isomerase	5.3.1.8	<i>Mpi</i>	L	CAPM6
	Phosphoglucomutase	2.7.5.1	<i>Pgm-1, 2</i>	L	TC8, CAPM6
	Phosphogluconate dehydrogenase	1.1.1.44	<i>Pgd</i>	M	CAPM6
	Superoxide dismutase	1.15.1.1	<i>Sod</i>	L	TC8

* Tissues: L, liver; M, muscle

** Buffer systems: TC8, tris-citrate, pH 8.0 (Clayton and Tretiak, 1972); TBE8.7, tris-borate-EDTA, pH 8.7 (Boyer *et al.*, 1963); CAPM6, citrate-aminopropylmorpholine, pH 6.0 (Clayton and Tretiak, 1972); LioH, lithium hydroxide-boric acid, pH 8.1 (Ridgway *et al.*, 1970)

Table 2. Allele frequencies at polymorphic loci in *Amphidromus atricallosus*. Locality numbers correspond to those in Fig. 1. Notations of alleles are alphabetical in order of anodal mobilities.

Locus and allele	Locality												
	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>Aat-1</i>													
a					0.043		0.036		0.076				0.111
b	1	0.986	1	0.966	0.957		0.714	0.976	0.242	0.274	0.079	1	0.278
c						1	0.25	0.024	0.682	0.726	0.921		
d		0.014		0.034									0.611
<i>Est-2</i>													
a	0.929	1	1	0.989	1	1	0.964	0.833	0.53	1	0.974	1	1
b	0.071			0.011			0.036	0.167	0.47		0.026		
<i>Gpi</i>													
a					0.652			0.036					
b		0.108		0.045									
c					0.348	1	1	0.964	1	1	1	1	
d	1	0.892	1	0.955	1								
<i>Hbdh</i>													
a													0.944
b		0.014			0.364		0.769	0.095	0.424	1	0.816	1	
c		0.243									0.184		
d					0.636	1	0.231		0.576				0.056
e	1	0.743	1	1				0.905					
<i>Me</i>													
a	1	1	1	1									
b					1	1	1	1	1	1	1	1	1
<i>Mpi</i>													
a													1
b	1	1	1	1									
c					0.804	1	1	1	1	1	0.947	1	
d					0.196						0.053		
<i>Lgg-1</i>													
a						0.8		0.012	0.03			0.875	
b					0.239	0.2	0.179	0.607	0.394	1	0.765	0.125	
c	1	1	0.719	0.909	0.761		0.821	0.381	0.576		0.235		0.028
d			0.281	0.091									0.972
<i>Lgg-2</i>													
a					0.022		0.286					1	
b					0.978	1	0.714	1	1	1	1		
c	1	1	1	1									1
<i>Pgm-1</i>													
a	1	1	0.781	1	1	1	1	0.976	1	0.821	1	1	1
b			0.219					0.024		0.179			
<i>Pgm-2</i>													
a									0.212				
b	0.071	0.554	0.594	0.58	0.109				0.015				
c	0.929	0.446	0.406	0.42	0.696				0.015	0.016	0.026		1
d					0.196	1	1	1	0.758	0.984	0.974	1	
<i>Pgd</i>													
a	1	1	1	1	0.761	1	1	1	0.984	1	1	1	
b					0.239				0.016				1

Table 3. Allele frequencies at polymorphic loci in *Amphidromus inversus*. Locality numbers correspond to those in Fig. 1. Notation of alleles are alphabetical in order of anodal mobilities.

Locus and allele	Locality										
	14	15	16	17	18	19	20	21	22	23	24
<i>Est-4</i>											
a											1
b	1	1	1	1	1	1	1	1	1	1	
<i>Lgg-1</i>											
a										1	1
b	1	1	1	1	1	1	1	1	1		
<i>Lgg-2</i>											
a										1	1
b	1	1	1	1	1	1	1	1	1		
<i>Lgg-3</i>											
a								0.278	0.036		
b	1	1	1	1	1	1	1	0.722	0.964	1	1
<i>Pgm-2</i>											
a	1	1	1	1	1	1					
b							1	1	1	1	1

Table 4. Matrix of genetic distances between samples of *Amphidromus atricallosus* from eastern Thailand, southern Thailand, and Singapore. Below diagonal: Nei's (1978) unbiased genetic distance. Above diagonal: Rogers (1972) genetic distance.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Soidao	—	0.071	0.084	0.057	0.421	0.612	0.481	0.446	0.559	0.61	0.582	0.537	0.443
2 Makham	0.024	—	0.07	0.035	0.414	0.587	0.46	0.449	0.542	0.584	0.558	0.513	0.474
3 Troknong	0.031	0.015	—	0.04	0.45	0.607	0.495	0.451	0.569	0.578	0.583	0.532	0.473
4 Makok	0.021	0.005	0.005	—	0.427	0.593	0.468	0.439	0.549	0.592	0.567	0.522	0.472
5 Ranong	0.488	0.485	0.547	0.515	—	0.307	0.22	0.249	0.241	0.318	0.293	0.333	0.474
6 Koh Tachai	0.946	0.898	0.936	0.912	0.269	—	0.203	0.219	0.163	0.175	0.14	0.237	0.592
7 Takhun	0.627	0.582	0.647	0.611	0.13	0.164	—	0.152	0.153	0.156	0.14	0.162	0.558
8 Klongsang	0.56	0.554	0.559	0.542	0.177	0.212	0.087	—	0.177	0.182	0.16	0.223	0.585
9 Khaosok	0.775	0.74	0.808	0.764	0.168	0.085	0.061	0.119	—	0.163	0.139	0.289	0.588
10 Suwankuha	0.908	0.859	0.883	0.877	0.251	0.15	0.089	0.133	0.085	—	0.068	0.215	0.6
11 Pongchang	0.855	0.801	0.86	0.829	0.239	0.116	0.076	0.136	0.057	0.012	—	0.23	0.581
12 Bangkram	0.765	0.719	0.75	0.738	0.306	0.268	0.114	0.215	0.254	0.204	0.223	—	0.583
13 Singapore Nee Soon	0.557	0.597	0.574	0.591	0.59	0.903	0.821	0.864	0.889	0.915	0.88	0.864	—

Table 5. Matrix of genetic distances between samples of *Amphidromus inversus* from eastern Thailand, southern Thailand, Malaysia, and Singapore. Below diagonal: Nei's (1978) unbiased genetic distance. Above diagonal: Rogers (1972) genetic distance.

Population	14	15	16	17	18	19	20	21	22	23	24
14. Koh Samet	—	0	0	0	0	0	0.056	0.071	0.058	0.111	0.167
15. Koh Kham	0	—	0	0	0	0	0.056	0.071	0.058	0.111	0.167
16. Koh Jarn	0	0	—	0	0	0	0.056	0.071	0.058	0.111	0.167
17. Koh Elar	0	0	0	—	0	0	0.056	0.071	0.058	0.111	0.167
18. Koh Pai	0	0	0	0	—	0	0.056	0.071	0.058	0.111	0.167
19. Koh Lueam	0	0	0	0	0	—	0.056	0.071	0.058	0.111	0.167
20. Chaiya	0.057	0.057	0.057	0.057	0.057	0.057	—	0.015	0.002	0.167	0.222
21. Koh Samui	0.062	0.062	0.062	0.062	0.062	0.062	0.004	—	0.013	0.182	0.238
22. Koh Tan	0.057	0.057	0.057	0.057	0.057	0.057	0	0.003	—	0.169	0.224
23. Pulau Kapas	0.118	0.118	0.118	0.118	0.118	0.118	0.182	0.189	0.183	—	0.056
24. Singapore Botanic garden	0.182	0.182	0.182	0.182	0.182	0.182	0.251	0.26	0.252	0.057	—

Table 6. Localities, sizes, mean number of alleles per locus (A), percentage of polymorphic loci (P), and expected heterozygosity (H_{exp}) in samples of *Amphidromus atricallosus* and *A. inversus*. Standard error of H_{exp} is indicated in parentheses. The sample numbers correspond to those in Fig. 1.

Species	Sample		A	P	H_{exp}
	Locality	n			
<i>A. atricallosus</i>	1. Soidao	14	1.2	15.4	0.021 (0.014)
	2. Makham	37	1.4	30.8	0.086 (0.047)
	3. Troknong	16	1.2	23.1	0.098 (0.052)
	4. Makok	44	1.4	38.5	0.064 (0.038)
	5. Ranong	23	1.7	61.5	0.201 (0.058)
	6. Koh Tachai	20	1.1	7.7	0.025 (0.025)
	7. Takhun	14	1.5	38.5	0.124 (0.051)
	8. Klongsang	42	1.5	46.2	0.085 (0.041)
	9. Khaosok	33	1.8	46.2	0.186 (0.067)
	10. Suwankuha	31	1.2	23.1	0.057 (0.037)
	11. Pongchang	19	1.5	46.2	0.080 (0.035)
	12. Bangkram	8	1.1	7.7	0.018 (0.018)
	13. Singapore Nee Soon	18	1.3	23.1	0.055 (0.042)
<i>A. inversus</i>	14. Koh Samet	8	1	0	
	15. Koh Kham	10	1	0	
	16. Koh Jarn	4	1	0	
	17. Koh Elar	8	1	0	
	18. Koh Pai	13	1	0	
	19. Koh Lueam	19	1	0	
	20. Chaiya	23	1	0	
	21. Koh Samui	18	1.1	5.6	0.023 (0.023)
	22. Koh Tan	14	1.1	5.6	0.004 (0.004)
	23. Pulau Kapas	19	1	0	
	24. Singapore Botanic garden	8	1	0	

Genetic variation in *Amphidromus inversus*

In *A. inversus*, variation within samples was detected only at *Lgg-3* in the Koh Samui and Koh Tan samples; no variation at all was observed in the other samples or at the other loci (Table 3). The P and H_{exp} values of the Koh Samui samples were 5.6 and 0.023, respectively, and those of the Koh Tan samples, 5.6 and 0.004 (Table 6).

Samples from Pulau Kapas, Malaysia, and Singapore Botanic Garden showed fixed allelic differences from all the Thailand samples at *Lgg-1* and *Lgg-2*. The Singapore sample was also distinct from the others in having allele “a” at *Est-4*. There were also fixed differences at *Pgm-2* between the eastern Thailand samples and the others (Table 3).

The values of D between the samples varied from 0–0.260 (Table 5), with particularly large values between the Singapore and southern Thailand samples (0.254 ± 0.005 ; range, 0.251–0.260). The values were also relatively large between the Singapore and eastern Thailand samples (invariably 0.182), between the Pulau Kapas and southern Thailand samples (0.185 ± 0.004 ; range, 0.182–0.189), and between the Pulau Kapas and eastern Thailand samples (invariably 0.118). Pairwise comparisons between southern and eastern Thailand samples, and between the Singapore and Pulau Kapas samples, yielded moderate D values (0.059 ± 0.002 ; range, 0.057–0.062; and invariably 0.057, respectively). In contrast, the values were very small between the southern Thailand samples (0.002 ± 0.002) and were invariably zero between the eastern Thailand samples.

Amphidromus inversus exhibited a high level of genetic heterogeneity among the samples. F_{st} values were signifi-

Table 7. Summary of F_{st} values for 13 samples of *Amphidromus atricallosus* and 11 samples of *A. inversus*. ^a: significant at level of $p < 0.01$.

Locus	<i>A. atricallosus</i>	<i>A. inversus</i>
<i>Aat-1</i>	0.659 ^a	–
<i>Est-2</i>	0.284 ^a	–
<i>Est-4</i>	–	1.000 ^a
<i>Gpi</i>	0.886 ^a	–
<i>Hbdh</i>	0.754 ^a	–
<i>Me</i>	1.000 ^a	–
<i>Mpi</i>	0.941 ^a	–
<i>Lgg-1</i>	0.626 ^a	1.000 ^a
<i>Lgg-2</i>	0.940 ^a	1.000 ^a
<i>Lgg-3</i>	–	0.228 ^a
<i>Pgm-1</i>	0.164 ^a	–
<i>Pgm-2</i>	0.678 ^a	1.000 ^a
<i>Pgd</i>	0.826 ^a	–
Mean	0.781	0.965

cantly higher than zero at all five polymorphic loci (mean = 0.965), of which four loci (*Est-4*, *Lgg-1*, *Lgg-2*, and *Pgm-2*) exhibited particularly high heterogeneities (i.e., fixed allelic differences between several combinations of local samples; Tables 3 and 7).

DISCUSSION

Geographic genetic structure

Both the geographic pattern of fixed allelic differences and the topology of the NJ tree (Fig. 2) grouped the samples

Fig. 2. Unrooted neighbor-joining tree for *Amphidromus atricallosus* based on Rogers' (1972) genetic distance.

of *A. atricallosus* according to their geographic location: an eastern Thailand group (ETG), a southern Thailand group (STG), and a Singapore group (SPG, consisting solely of the Singapore Nee Soon sample). High genetic heterogeneity among these groups was also supported by the mean F_{st} value for the combined samples (0.781), which was much higher than those obtained separately for the ETG (0.144) and STG (0.551).

The ETG samples of *A. atricallosus* lacked fixed allelic differences and exhibited a low F_{st} and small genetic distances. This suggests frequent gene flow among the eastern Thailand populations. Absence of prominent geographic barriers among the sampling sites in this region supports this inference.

The STG samples of *A. atricallosus* exhibited fixed allelic differences in a few combinations, mostly involving the Koh Tachai sample. Also, the STG showed a much higher F_{st} value than the ETG samples. Genetic distances between samples were larger than those between the ETG samples. This suggests less frequent gene flow among populations in this region.

Among the STG localities, Koh Tachai is unique in being a small island. This geographic feature may explain the

apparent limited gene flow between this population and other southern Thailand populations of *A. atricallosus*, as implied by the fixed allelic differences from other STG samples. The low H_{exp} value (0.025) in the Koh Tachai population, excluding a single sample from Bangkram, may have resulted from genetic drift in isolated populations on the small island, where habitats may have been perturbed by tsunamis, such as the one of 26 December 2004, and by the death of the majority of host trees through raised salinity levels (Panha, 2005).

The most continental localities in southern Thailand are surrounded by a long, north-south orientated granite mountain range that reaches approximately 1,000 m in height (Woodruff, 2003: Fig. 1). Because the habitat of *A. atricallosus* usually occurs at 30–500 m in elevation (Sutcharit and Panha, 2006), this mountain range may have acted as a barrier to gene flow among *A. atricallosus* populations, resulting in a relatively large F_{st} value (0.415) through frequent genetic drift within each habitat patch. Further work based on mitochondrial DNA sequences and more rapidly evolving nuclear markers such as microsatellites may resolve this issue.

In *A. inversus*, the H_{exp} values were extremely low:

within-sample variation was observed only at one locus in two samples (Table 3). In contrast, *F_{st}* values were extremely high, because of the fixed allelic differences at four of the five polymorphic loci (Table 7).

Considering the mostly insular habitat of *A. inversus* (Fig. 1), bottleneck effects may be responsible for the low genetic variability in the samples, as in the case of the Koh Tachai sample of *A. atricallosus*. However, bottlenecks cannot alone explain such a geographically clear pattern of allelic displacement in *A. inversus* (Table 3). The straits between the islands with *A. inversus* populations and the continent are shallow enough to have been exposed during Pleistocene glaciations (Voris, 2000). Therefore, we suspect that the current characteristic geographic genetic structure in *A. inversus* was formed through a series of bottlenecks on the islands during interglacial periods, coupled with substantial repeated range extensions during the periods of lowered sea level.

Taxonomic implications

In this study, a number of *A. inversus* populations, including those representing certain recognized subspecies such as *A. inversus andamensis* from Borneo and *A. inversus kopperbergi* from northern Sulawesi (Laidlaw and Solem, 1961), were not examined. Therefore, we discuss taxonomic implications only for *A. atricallosus*.

In their annotated catalogue of the genus *Amphidromus* from Southeast Asia, Laidlaw and Solem (1961) recognized three geographically defined subspecies for *A. atricallosus* on the basis of morphology (Pilsbry, 1900; Fulton, 1901). Of these, the nominotypical subspecies, restricted to southern Thailand and southern Myanmar, has been diagnosed as having a black or dark-brown parietal callus and a straight columella. The subspecies *A. atricallosus leucoxanthus* has been recognized from eastern Thailand and differentiated from the nominotypical subspecies in possessing a white parietal callus. In contrast, the subspecies *A. atricallosus perakensis* has been recognized from Malaysia and Singapore on the basis of both its white parietal callus and a twisted, plaited columella. The Koh Tachai population was recently described as a fourth subspecies, *A. atricallosus classarius*, on the basis of its relatively small shell size and brown or black parietal callus (Sutcharit and Panha, 2006). The shell characters in our samples of *A. atricallosus* from the continental part of southern Thailand, eastern Thailand, Singapore, and Koh Tachai correspond to those that define the nominotypical subspecies and the three other subspecies, *leucoxanthus*, *perakensis* and *classarius*, respectively, although variation within the samples is evident.

Fixed allelic differences at several allozyme loci between the southern and eastern Thailand and Singapore populations (Table 2) strongly suggest the absence of gene flow among these three regions. Furthermore, Roger's genetic distance based on the allozyme data showed relatively large divergences among these regions (Fig. 2). These results not only support the validity of the subspecies classifications of *A. atricallosus leucoxanthus* and *A. atricallosus atricallosus*, but also may suggest that they are two full species, although this requires further work, especially to verify the genetic discontinuity between the nominotypical subspecies and *A. atricallosus perakensis*.

The validity of *A. atricallosus classarius* as a distinct

subspecies is not well supported by our allozyme data, which placed the Koh Tachai population as a minor branch in the southern Thailand group of *A. atricallosus* (Fig. 2). It may be more appropriate to regard the Koh Tachai population as a distinct evolutionarily significant unit (Moritz, 1994; Karl and Bowen, 1998) rather than a subspecies. The Ranong population might be separable if it shows appropriate diagnostic features.

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REFERENCES

- Boyer SH, Fainer DC, Watson EJ (1963) Lactate dehydrogenase variation from human blood: evidence for molecular subunit. *Science* 141: 642–643
- Clayton JW, Tretiak DN (1972) Amine-citrate buffers for pH control in starch gel electrophoresis. *J Fish Res Board Can* 29: 1169–1172
- Fulton H (1901) Descriptions of new species of *Xesta*, *Amphidromus* and *Cyclostoma* from Madagascar and Perak. *J Malacol* 8: 103–104
- Karl SA, Bowen BW (1998) Evolutionary significant units versus geopolitical taxonomy: molecular systematics of an endangered sea turtle (genus *Chelonia*). *Conserv Biol* 13: 990–999
- Laidlaw FF, Solem A (1961) The land snail genus *Amphidromus*, a synoptic catalogue. *Fieldiana Zool* 41: 507–677
- Moritz C (1994) Defining "evolutionary significant units" for conservation. *Trends Ecol Evol* 9: 373–375
- Murphy RW, Sites Jr JW, Buth DG, Haufler CH (1996) Protein: isozyme electrophoresis. In "Molecular Systematics" 2nd ed, Ed by DM Hillis, C Moritz, BK Mable, Sinauer Associates, Sunderland, Massachusetts, pp 51–120
- Nei M (1978) Estimation of average heterozygosity and genetic distance from a small sample number of individuals. *Genetics* 89: 583–590
- Panha S (2005) A survey of the impacts of the Southeast Asian tsunami on some terrestrial invertebrates including molluscs. *Unitas Malacol Newslett* 22: 3
- Pilsbry HA (1900) Manual of Conchology, Structural and Systematic with Illustrations of the Species, Ser 2 Vol 13. Academy of Natural Sciences of Philadelphia, Philadelphia
- Ridgway GJ, Sherbrune SW, Lewis RD (1970) Polymorphisms in the esterase of Atlantic herring. *Trans Am Fish Soc* 99: 147–151
- Rogers JS (1972) Measures of genetic similarity and genetic distance. *Stud Genet Univ Texas Publ* 7213: 145–153
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4: 406–425
- Solem A (1965) Land snails of the genus *Amphidromus* from Thailand (Mollusca: Pulmonata: Camaenidae). *Proc US Natl Mus* 117: 615–627
- Sutcharit C, Panha S (2006) Taxonomic review of the tree snail

- Amphidromus* Albers, 1850 (Pulmonata: Camaenidae) in Thailand and adjacent areas: Subgenus *Amphidromus*. J Moll Stud 72: 1–30
- Swofford DL, Selander RB (1981) BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J Hered 72: 281–283
- Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J Biogeogr 27: 1153–1167
- Woodruff DS (2003) Neogene marine transgressions, palaeogeography and biogeographic transitions on the Thai-Malay Peninsula. J Biogeogr 30: 551–567
- Workman PL, Niswander, JD (1970) Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Am J Hum Genet 22: 24–49
- Wright S (1965) The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19: 395–420

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