

ซิสเต็มแมติกและชีวภูมิศาสตร์ของหอยต้นไม้ *Amphidromus atricallosus* (Gould, 1843) และ
A. inversus (Müller, 1774) ในประเทศไทยและพื้นที่ใกล้เคียง



นางสาวผ่องพรรณ ประสารก

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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SYSTEMATICS AND BIOGEOGRAPHY OF THE CAMAENID TREE
SNAILS *Amphidromus atricallosus* (Gould, 1843) AND *A. inversus*
(Müller, 1774) IN THAILAND AND NEARBY REGIONS



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สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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ผ่องพรรณ ประสารกค: ชีสเต็มแมติกและชีวภูมิศาสตร์ของหอยต้นไม้ *Amphidromus atricallosus* (Gould, 1843) และ *A. inversus* (Müller, 1774) ในประเทศไทยและพื้นที่ใกล้เคียง (SYSTEMATICS AND BIOGEOGRAPHY OF THE CAMAENID TREE SNAILS *Amphidromus atricallosus* (Gould, 1843) AND *A. inversus* (Müller, 1774) IN THAILAND AND NEARBY REGIONS) อ. ที่ปรึกษา: รศ. ดร. สมศักดิ์ ปัญหา, อ. ที่ปรึกษาร่วม: Prof. Dr. Hidetoshi Ota. 129 หน้า. ISBN 974-14-2716-6.

ได้ทำการศึกษาพันธุกรรมระหว่างกลุ่มประชากรในหอยต้นไม้สองชนิดคือ *Amphidromus atricallosus* (หอยนกกม้น) และ *A. inversus* (หอยชอคโกแลต) ซึ่งเป็นหอยที่มีการกระจายกว้างในภูมิภาคเอเชียตะวันออกเฉียงใต้ โดยเก็บตัวอย่างหอยนกกม้น 319 ตัวอย่าง จาก 12 พื้นที่ของไทยและ 1 พื้นที่ของสิงคโปร์ และหอยชอคโกแลต 144 ตัวอย่าง จาก 9 พื้นที่ของไทย 1 พื้นที่ของมาเลเซียและ 1 พื้นที่ของสิงคโปร์ จากนั้นนำเนื้อเยื่อจากตับและกล้ามเนื้อมาศึกษาด้วยวิธีทางอัลโลไซม์อิเล็กโตรโฟรีซิสเพื่อวิเคราะห์ความผันแปรในทางภูมิศาสตร์และความถี่ของอัลลีลในหอยแต่ละชนิด ผลการวิเคราะห์อัลโลไซม์ในหอยนกกม้นทั้งหมด 13 ตำแหน่ง พบว่า 11 ตำแหน่งมีความผันแปรทางพันธุกรรมในขณะที่ 1 ตำแหน่งไม่มีความผันแปรทางพันธุกรรม ในขณะที่หอยชอคโกแลตศึกษาอัลโลไซม์ทั้งหมด 18 ตำแหน่ง พบว่า 5 ตำแหน่ง มีความผันแปรทางพันธุกรรมในขณะที่ 13 ตำแหน่งไม่มีความผันแปรทางพันธุกรรม ผลการวิเคราะห์ความผันแปรทางพันธุกรรมในหอยชอคโกแลตพบว่า มีค่า heterozygosity (0-0.023, mean=0.002) ที่ต่ำกว่าหอยนกกม้น (0.018-0.201, mean=0.085) ในทางตรงกันข้าม กลับพบว่าค่า heterogeneity ระหว่างกลุ่มประชากรในหอยชอคโกแลต ($F_{st} = 0.965$) มีค่าสูงกว่าหอยนกกม้น (0.781) นอกจากนี้ยังพบว่ากลุ่มประชากรหอยนกกม้นทางภาคใต้ของประเทศไทย ($F_{st} = 0.551$) มีค่า heterogeneity ที่สูงกว่ากลุ่มประชากรภาคตะวันออกเฉียงใต้ของประเทศไทย ($F_{st} = 0.144$) อย่างมีนัยสำคัญ การที่กลุ่มประชากรของหอยชอคโกแลตมีค่า F_{st} สูง ในขณะที่ค่า heterozygosity ต่ำ สามารถอธิบายได้ว่า หอยชนิดนี้ได้รับผลกระทบที่รุนแรงจากปรากฏการณ์ bottleneck ที่เกิดขึ้นในอดีตร่วมด้วยกับการแบ่งแยกของสภาพทางภูมิศาสตร์อย่างชัดเจนของแต่ละประชากร สำหรับกลุ่มประชากรหอยนกกม้น ในภาคตะวันออกเฉียงใต้ของประเทศไทย พบว่ามีค่า F_{st} ต่ำแต่มีค่า heterozygosity สูง แสดงให้เห็นว่าในกลุ่มตัวอย่างนี้มีการแลกเปลี่ยนยีนระหว่างกันเกิดขึ้นอย่างต่อเนื่อง ในขณะที่กลุ่มประชากรภาคใต้ของประเทศไทยมีค่า F_{st} และ heterozygosity สูง ทั้งนี้อาจเป็นผลเนื่องมาจากการแบ่งแยกกันของถิ่นที่อยู่อาศัยที่จำเพาะในแต่ละพื้นที่พร้อมกับการเกิดกระบวนการทางวิวัฒนาการ เมื่อวิเคราะห์ค่าระยะห่างทางพันธุกรรมระหว่างกลุ่มตัวอย่างหอยนกกม้นภาคใต้และภาคตะวันออกเฉียงใต้พบว่ามีค่าระยะห่างทางพันธุกรรมสูงมาก [$D=0.485-0.946$] และมีความแตกต่างของอัลลีลระหว่างกลุ่มประชากรทั้ง 2 ผลการวิจัยดังกล่าวแสดงให้เห็นอย่างเด่นชัดถึงการเกิดขึ้นของสปีชีส์ที่แยกออกจากสปีชีส์เดิมของหอย 2 บริเวณ

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PONGPUN PRASANKOK: SYSTEMATICS AND BIOGEOGRAPHY OF THE CAMAENID TREE SNAILS *Amphidromus atricallosus* (Gould, 1843) AND *A. inversus* (Müller, 1774) IN THAILAND AND NEARBY REGIONS. THESIS ADVISOR: ASSOC. PROF. SOMSAK PANHA, Ph.D. THESIS COADVISOR : PROF. HIDETOSHI OTA, Ph.D. 129 pp. ISBN 974-14-2716-6.

The genetic variation of the two camaenid tree snails, *Amphidromus atricallosus* and *A. inversus* which broadly distributed in Southeast Asia was examined. A total of 319 individuals of *A. atricallosus* were collected from 12 localities in Thailand and one in Singapore, and 144 of *A. inversus* from 9 localities in Thailand, one in Malaysia and one in Singapore. Tissues from these specimens were subjected to horizontal starch gel electrophoresis. As a result, 13 allozyme loci (including 11 polymorphic) were screened for *A. atricallosus* and 18 allozyme loci (including five polymorphic) for *A. inversus*. The degree of heterozygosity was higher in *A. atricallosus* ($H_{exp}=0.018-0.201$, mean=0.085) than in *A. inversus* ($H_{exp}=0-0.023$, mean=0.002). In contrast, overall genetic heterogeneity among local samples was higher in *A. inversus* ($F_{st}=0.965$) than in *A. atricallosus* ($F_{st}=0.781$). Within *A. atricallosus*, the heterogeneity was distinctly higher among the southern Thailand samples ($F_{st} = 0.551$) than among the eastern Thailand samples ($F_{st} = 0.144$). The high F_{st} and low heterozygosity values in *A. inversus* suggest that this species, chiefly occurring off-shore continental-shelf islands experienced a series of strong bottlenecks and subsequent range extensions. The low F_{st} and high H_{exp} values for the eastern Thailand of *A. atricallosus* suggest the frequent gene flows among populations in this region. The southern Thailand populations indicate relative large values in both F_{st} and H_{exp} . This may have been involved in an extensive local fragmentation under various selection forces. The southern and eastern samples of *A. atricallosus* exhibit fixed allele differences at four loci and great genetic distance [$D=0.485-0.946$]. The results strongly suggest the separated biological species of snail samples between the two regions.

Field of study..... Biological Science..... Student's signature..... P. Prasankok

Academic year..... 2006..... Advisor's signature.....

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CHAPTER I

INTRODUCTION

Amphidromus atricallosus (Gould, 1843) and *A. inversus* (Müller, 1774) are tree snails of the family Camaenidae which share a number of ecological features, such as the obligately arboreal habits and consuming microflora on trees (Sutcharit and Panha, 2006a). Both species broadly occur in the Indochina-Malay Peninsula region. *Amphidromus inversus* also reported occurring in Borneo, Sumatra and Sulawesi, where *A. atricallosus* seems to have narrow range (Pilsbry, 1900; Gude, 1903b, 1914; Laidlaw and Solem, 1961; Solem, 1965; Sutcharit and Panha, 2006a).

The tree snails, *Amphidromus atricallosus* and *A. inversus*, occur allopatrically in lowland areas, sea coasts and islands of eastern, southern Thailand to Singapore and Indonesia. The former species is mainly specific to forest on the mainland, whereas the latter species occurs mainly in the forest along sea coasts and islands (Laidlaw and Solem, 1961; Solem, 1965). Results of field observations and published information (Sutcharit and Panha, 2006 a, b) indicating their comparable high population densities in suitable habitats and relatively low fecundity, suggest that the two species perform different demographic traits. The only prominent ecological difference between *A. atricallosus* and *A. inversus* recognized so far resides in their habitat preferences. *Amphidromus atricallosus* prefers rain forest or deciduous forest while *A. inversus* prefers of coastal localities such as beach forest (Chirasak Sutcharit, 2004).

Within such broad overall ranges, each of the two species occupies discrete patchy areas (Panha, unpublished data: see Fig. 7), and this predicts the presence of complicated geographic genetic structure in each species as in a few other land snails from other regions (Hillis *et al.*, 1987; Woodruff and Solem, 1990). Moreover, because *A. atricallosus* and *A. inversus* mostly inhabit, respectively, the continental part and small continental-shelf islands (i.e., islets that are currently isolated from the continent by shallow straits but experienced several periods of dry land connections to the continent during the Pleistocene: Voris, 2000; Sathiamurthy and Voris, 2006) within the Indochina-Malay Peninsula region, they are expected to provide a good opportunity to examine effects of the Quaternary geohistory involving insularization of habitats upon the current geographic genetic structure in terrestrial organisms with low vagility.

Besides these, subspecific classifications of the two *Amphidromus* species need re-examinations most desirably on the basis of genetic data. Several subspecies are currently recognized for each of these species on the basis of variation in shell (size, shell shape and shell coloration), radula and genitalia as well as geographic isolation (see Laidlaw and Solem [1961] and Sutcharit and Panha [2006a, b] for subspecies recognition of *A. atricallosus* and *A. inversus*). However, the validity of these hypotheses still need verification, because some of the morphological characters used in diagnose them apparently show extensive within-population variation (Solem, 1965) and the general lack of detailed field data have more than caused taxonomists to be indecisive about, which view is more appropriate: multiple species or geographic variation within a single species (Sutcharit and Panha, 2006a).

As from the latest classical classification as most reliable information by Sutcharit and Panha (2006 a, b), Sutcharit *et al.* (2006) using from shell and radular morphology, anatomy of genitalia to mitochondrial DNA phylogenetic analysis concluded the new classification of *A. (A.) atricallosus* and *A. (A.) inversus* of Thailand and nearby areas as follows; 4 subspecies of *A. (A.) atricallosus* were classified. They are *A. (A.) atricallosus atricallosus* (Gould, 1843), *A. (A.) atricallosus leucoxanthus* (von Martens, 1864), *A. (A.) perakensis* Fulton, 1901 and *A. (A.) classiarius* (Sutcharit and Panha, 2006a). The four subspecies consist of similar characters such as shell colour. In general, however there are some distinct characters using for examples the white parietal callus appears in *leucoxanthus*, but exhibits dark brown colour in nominotypical subspecies, and columellar plait very prominent in *perakensis*, and the only left handed coiling population of isolated *classiarius*. The mitochondrial sequences analysis show the separation of three analyzed subspecies but still be sister which *aticallosus* sister to *leucoxanthus* followed by *perakensis* (Sutcharit *et al.*, 2006). The most important data is that the remarkable isolation of the locality of the four subspecies from southern Thailand and Myanmar of nominotypical, subspecies *leucoxanthus* eastern of Thailand, Malaysia and Singapore of subspecies *perakensis* and an isolated island in Andaman Sea, western Thailand of subspecies *classiarius*.

Three subspecies of *A. (A.) inversus* were also classified. They are *A. (A.) inversus inversus* (Müller, 1774), *A. (A.) inversus annamiticus* (Crosse and Fischer, 1863) and *A. (A.) albulus* Sutcharit and Panha, 2006. The three subspecies consist of some specific shell characters such as whitish to creamy spire in *inversus* but exhibit rose-coloured, with lighter or dark purplish suprasutural band, rosaceous or dark purplish apex in

annamiticus, and possession of whitish shell and the absence of brownish radial streaks or brownish sub-peripheral band as normally present in the three recognized subspecies. The two subspecies of *inversus* and *albulus* perform dimorphic shell coiling while *annamiticus* has only dextral morphology. The habitats of *Amphidromus inversus* are unique along the sea side or coast on the trees of the beach forest. It may have the sea influence to its life history. The molecular systematic analysis using mitochondrial DNA sequences confirmed the sister relationship of *inversus* and *annamiticus* but the *albulus* has not been included yet (Sutcharit *et al.*, 2006). The locality of each subspecies is also remarkable, *inversus* found in Singapore, many islands of Indonesia and Borneo; *annamiticus* found in Vietnam, Cambodia and southern Thailand; *albulus* was recently classified from an island off coast in the South China Sea of Malaysia. Schilthuizen *et al.* (2005) has done marked and recaptured on *Amphidromus inversus albulus* of the population in Kapas Island off the east coast of Malaysia and conclude that population structure alone cannot stabilize the coil dimorphism in *Amphidromus*.

The above mentioned literatures are mostly complete on both revision of classical taxonomy and analysis on modern systematics to be referred concerning *Amphidromus* systematics. However both *Amphidromus* species are quite complex and very interesting on their genetic structures at the population level. The basic genetic distances will show how the gene flow in each species and each locality do. This will imply to their dispersal or vicariance hypotheses and will finally be an assistant on confirming systematic decision.

Objectives

1. To establish a historical biogeographical hypothesis on two widely distributed *Amphidromus* species (*A. atricallosus* and *A. inversus*) by investigating allozyme variation among a number of populations from Thailand, Malaysia and Singapore.

2. To revise the population systematics of the two species on the basis of information regarding reproductive isolation and genetic divergence obtained through allozyme electrophoresis.

Anticipated Benefit

The results will be used to verify and revise the conventional classification, and can be utilized firmly for further biogeographical and evolutionary investigations.



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CHAPTER II

LITERATURE REVIEW

Biology of *Amphidromus atricallosus* and *A. inversus*

The tree snails, *Amphidromus atricallosus* and *A. inversus* belong to the family Camaenidae. The activities of these two species particularly occurring all of their lives on the tree including feeding microflora, mating and laying egg (Fig. 1). These two species found in quite vary habitat characteristics from primary forest to fruit plantations close by the forest. The highly disturbed forests show no snails or even the extinction evidences were discovered, but *Amphidromus inversus* was proved to be extinct from koh Kang Kao, upper Gulf eastern Thailand (Panha, unpublished data). The active season, in which feeding and reproductive take place in eastern and southern Thailand, starts at the onset of the rain, around mid May to June, respectively, and ends when the dry season starts, in late October (Panha, unpublished data). It aestivates conspicuously during dry periods with a sheet of mucus closing the aperture. During the 4-7 month dry season, they are inactive and depend on stored food for survival. Predators of these snails can be assumed from the broken shell. Field observation indicated that small mammal, such as birds, rats and squirrels are the common predators (Fig 1D, H).

Land pulmonate snails like *Amphidromus* perform simultaneous hermaphrodites by cross-fertilization. The frequent field observations of many, simultaneously copulating pair (Panha, unpublished data, see Fig. 1G) suggest that outcrossing is the rule. Courtship and copulation are reciprocal, with both animals acting as male and female at the same time

(Woodruff and Solem, 1990). Sutcharit and Panha (2006a) found *A. atricallosus* making egg nests using tree leaves including pomelo, banyan and rambutan. The foot is used to hold two leaves facing each other and secrete sticky mucus that traps the lower surface of leaves to form a nest. The edges of the leaves are glued by sticky mucus making a cavity, opened at both ends, in which the snail gradually lays between 100-150 eggs in the nest cavity. Polymorphism in shell coiling has been found in these species (see Fig. 6G-H, 11, 12, 13). The dimorphism of chirality either clockwise (right handed or dextral), or counter-clockwise (left handed or sinistral). The chirality of snail is determined by maternal genotype at a single locus (Murray and Clarke, 1976; Freeman and Lundelius, 1982; Ueshima and Asami, 2003). In many of studies, authors suggest that the different chiral types are unable to mate and so could be reproductively isolated from one other (Gittenberger, 1988; Orr, 1991). In Thailand, both left and right handed coiling are found in the same population of *A. atricallosus*, even in approximately equal numbers, or with a distinct predominance in one phase (Panha *et al.*, 2001). In comparison, *Amphidromus inversus* found only right handed coiling in all populations.

Diagnosis

1. *Amphidromus atricallosus*

Recognition of this species has been based on shape, size, and coloration of the shell (Pilsbry, 1900; Laidlaw and Solem, 1961; Solem, 1965). Basically, *A. atricallosus* has a moderately large (40-55 mm.), ovate or conic, solid and highly polished shell. Usually, they have yellow, green-yellow to yellow or white to brown ground color shell with

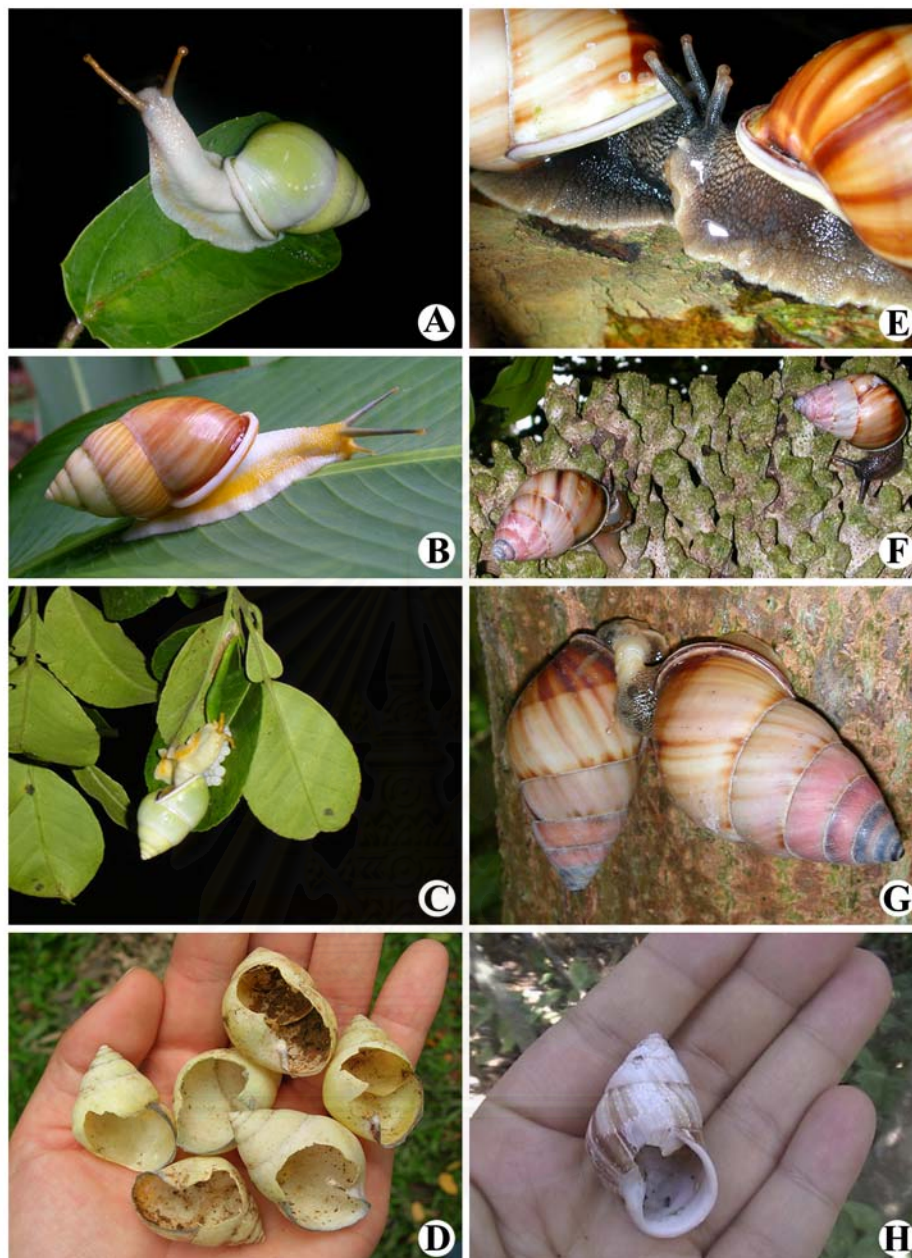


Fig. 1. Biology of *Amphidromus atricallosus* (Left) and *A. inversus* (Right). **A-B, E-F.** Feeding. **C.** Egg laying of *A. atricallosus* from Ban Takhun, Suratthani observed in September, 2003. **G.** Mating pair of *A. inversus* from Koh Tan, Suratthani observed in August, 2002. **D, H.** Broken shells of *A. atricallosus* and *A. inversus* were found after killing by some small mammals.

or without varices (Fig. 2) and white columella which is generally straight but sometimes folded and twisted. There is black to dark brown or white parietal callus with spiral bands or shaded zones. Shell exhibits chiral dimorphism (see Fig. 2A, B).

The male reproductive system exhibits conical penis. The epiphallus shows large and convoluted structure. The flagellum is a long and folded into single coil near the appendix. Appendix is relatively long and narrows (Sutcharit and Panha, 2006a).

The female reproductive system displays relatively short, slender and cylindrical vagina. Gametolytic sac reveals swollen and connected to long gametolytic duct. There is shortly free, enlarge and compact oviduct which, fuses with prostate gland, locating under oviduct. The albumen gland shows a large with curved lingulate shape (Sutcharit and Panha, 2006a).

Amphidromus atricallosus distributes in the Malay Peninsula and the Southeastern part of Thailand (Fig. 3). Previously, the description of *A. atricallosus* had been accomplished based on shell morphology. *Amphidromus atricallosus* shows extensive variations in size, shape, and shell coloration (see Fig. 11, 12, 13). Such evidences have lead to considerable inconsistency in specific and subspecific classifications for each species among taxonomists (Gould, 1843; Morlet, 1889; Pilsbry, 1900; Fulton, 1900; Laidlaw and Solem, 1961; Solem, 1965; Sutcharit and Panha, 2006a). *Amphidromus atricallosus* was formerly divided into three species, *A. atricallosus* (Gould, 1843), *A. leucoxanthus* (von Marten, 1864), and *A. perakensis* Fulton, 1901. Of these, *A. atricallosus* was defined as having a shell with brown or black callus in parietal area

and broad white subsutural zone. *Amphidromus leucoxanthus* has no dark parietal callus, while *A. perakensis* has a distinct twisted plait columella (Fig. 2).

Later, Laidlaw and Solem (1961) rendered those species into subspecies of *A. atricallosus*. Material collected show that variation occur in different combinations in all parts of the range but never indicate of speciation. However, specimens are too few in number.

Solem (1965) examined shell specimens from several sets of collection from Thailand and concluded that, based on shell characters, such as the subsutural white zone, varices, black parietal callus, white parietal callus and pale brown radial streaks, recognized four distinctive color phases in *A. atricallosus* as *atricallosus*, *leucoxanthus*, *perakensis* forms, and a new color form, characterized by pale brown radial streaks along the growth lines as *laidlawi* form. Because these forms were recognized almost throughout the range of the species, however he did not recognize these forms as subspecies.

Sutcharit and Panha (2006a), on the basis of shell and anatomical characters data from some *Amphidromus* specimens including *A. atricallosus* and *A. inversus* followed with Laidlaw and Solem (1961)'s classification. A new subspecies, *Amphidromus atricallosus classarius* was recognized. It's distinct from nominotypical species by 35 to 40 mm of shell height (Fig. 2), elongate-conic shape, sinistral, invariably yellow shell, and slightly short penis. They also constructed a key to species and subspecies for *A. atricallosus* and *A. inversus* in Indochina-Malay Peninsula region as follows:

- 1a Shell yellowish, whitish, greenish colour.....2
- 2a Parietal callus white; whorl convex; straight or twisted columella.....3
- 3a Collumella straight or twisted, monochrome yellow or white and one or more varices usually present.....*A. a. leucoxanthus*
- 3b Columella always twisted, monochrome yellow and varix absent.....*A. a. perakensis*
- 2b Parietal callus black or brownish; whorl relative flat; columella straight. Shell conic or elongate-conic.....4
- 4a Parietal callus black. Shell height 40-50 mm, conic and usually chrially dimorphic, yellow or white, penis long
.....*A. a. atricallosus*
- 4b Parietal callus blackisk. Shell height 35 to 40 mm, elongate-conic, always sinistral invariably yellow, and penis slightly short.....*A. a. classarius*
- 1b Shell brown or radial streak.....5
- 5a Shell with irregular redial streaks, distinct brown spiral band present at lower periphery of the last whorl. Penial appendix absent.....6
- 6a Shell height 50 to 55mm, usually chirally dimorphic. Spire slightly convex, pale brown or whitish*A. i. inversus*
- 6b Shell height 40 to 50mm, usually dextral. Spire pink to brownish.....*A. i. annamticus*
- 5b Shell with parietal streak, brown spiral band absent. Long penial appendix present.....*A. a. leucoxanthus* var.laidlawi

2. *Amphidromus inversus*

Amphidromus inversus displays a moderately large shell (50 mm).

There is a dull surface with brown streaks and zones or varices. The lip is strong and reflex with white color. The apex has a white, brown or dark purple color (Fig. 5, 6). Dimorphism occurs in this species (Fig. 6G, H). In Thailand, all investigated shell specimens are dextral (right handed coiling) (Fig. 5, 6).

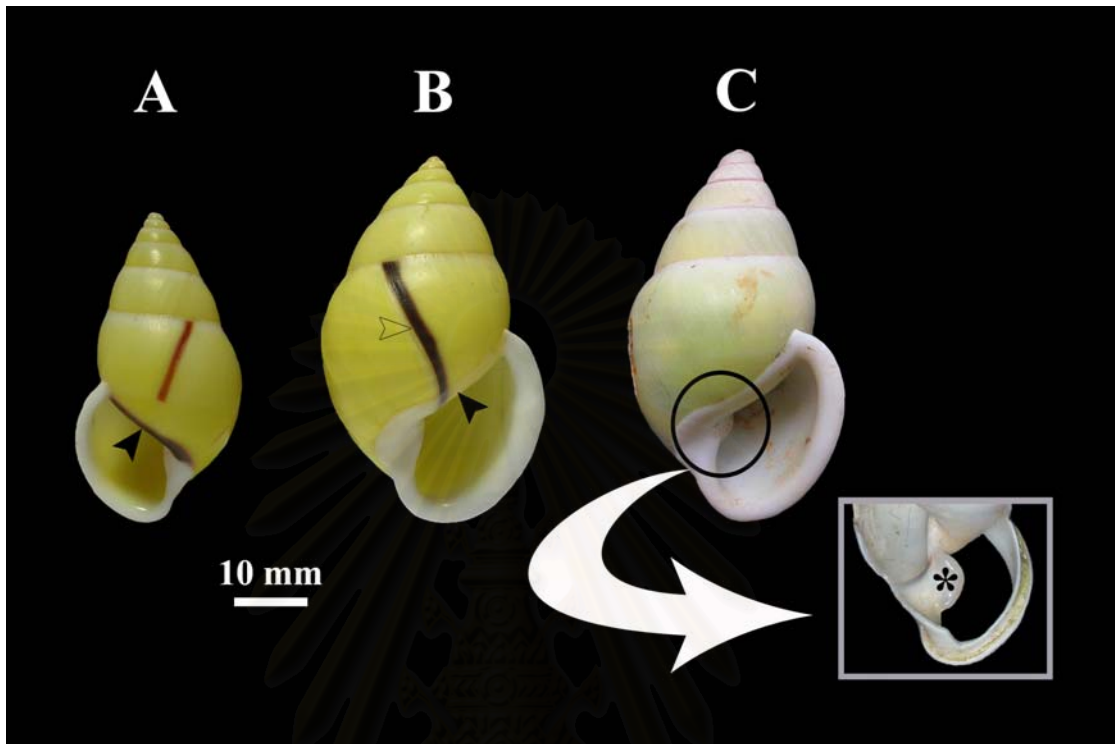


Fig. 2. Shell characters of *Amphidromus atricallosus*. **A.** Left handed coiling. **B-C.** Right handed coiling. Close arrows, open arrow and star indicate parietal callus, varix and twist plate columella.

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The male reproductive system contains cylindrical penis which distally folds at penial base. Epiphallus has long with variable coiling. Flagellum is smaller than epiphallus and terminates with folded coiled portion. Appendix is very short or absent.

The female reproductive system contains relatively long and cylindrical vagina about twice the length of penis. Gametolytic duct has a long folded and proximal convoluted to vagina, and terminates with swollen gametolytic sac. There is shortly free, enlarge and compact oviduct which fused with prostate gland locating under oviduct. Albumen gland curves like lingulate shape (Sutcharit and Panha, 2006a).

Amphidromus inversus (Müller, 1774), four subspecies were recognized for populations from Vietnam to Sulawesi of Indonesia (Laidlaw and Solem, 1961)(Fig. 4): the nominotypical subspecies were recorded from Borneo, Sumatra, Java and Singapore performing whitish to creamy spire and several black to dark-brown varices (Fig. 6F-H); the subspecies *annamiticus* from Cambodia, Vietnam and Thailand exhibits rose-coloured spire with lighter or dark-purplish suprasutural band and rosaceous or dark purplish apex (Fig. 5A-E) (von Möllendorff, 1894), however, morphologically, the eastern populations have no dark border above the sutures of the early whorls (Fig. 5); the subspecies *andamensis* from Borneo has smaller shell size with flame-like color pattern; the subspecies *koperbergi* from the northern Sulawesi (Celebes) has flat-sided shell with light brown colour, and the dark zone of last whorl is bright chestnut (Laidlaw and Solem, 1961). These records show very interesting convergence research questions which should be proved in the future.

Recently, On the basis of shell and anatomical character data, a new subspecies *A. inversus albulus* (Sutcharit and Panha, 2006b) was described from Peninsular Malaysia (Fig. 6G-H). Interestingly, the dimorphic shell coiling was observed in these snail populations.

Allozyme electrophoretic analysis

One of the most widely used procedures for revealing genetic variation in enzyme and other protein is electrophoresis. Protein electrophoresis is the migration of protein under the influence of an electric field. Enzymes that differ in electrophoretic mobility as a result of allelic differences at a single locus are called allozyme that can be separated on the basis of net charge and size. The allozyme variation in the population is an indication of genetic variation, and such genetic variation is very common. Allozyme electrophoretic analysis is a powerful technique which can be used to detect taxonomic and phyletic diversity in a group of organisms exhibiting complicated patterns of morphological variation (Nevo, 1978; Hillis *et al.*, 1987)

Nevo (1978) reviewed electrophoretic patterns in both plant and animal species based on studies published prior to early 1976 and involving 243 species. He suggested that the amounts of genetic polymorphism and heterozygosity varied nonrandomly between loci, population, species, habitats and life zones, and are correlated with ecological heterogeneity. Natural selection, in some form, may often be the major determinant of genetic population structure and differentiation.

Hillis and Patton (1982), who examined two forms electrophoretically and morphologically of *Corbicula* to determine their

degree of differences, reported that the two color forms differed in morphological evidence, and showed fixed allelic differences at six of 26 genetic loci. They concluded that two color forms actually represented separated species.

Tatarenkov and Johnnesson (1999) investigated the microscale transitions between exposed and sheltered parts of two continuous populations of *Littorina fabalis* by using allozyme. They found that snails from different microhabitats have almost diagnostic differences in one allozyme locus, and differ in adult size. However, between exposed and sheltered groups showed a mixing of two genetically separated populations. They suggested that the hybridization supported that observation.

Katoh and Foltz (1994) investigated genetic variation in a freshwater snail species complex which formerly referred to as *Viviparus georgianus* (Lea) in southern Georgia and Florida. They found that 11 populations clustered into three genetic isolations. Canonical discriminant analysis of shell morphological measurements also separated all three species with the little overlapping.

In addition, allozymic approach has an advantage over mitochondrial DNA approach in delimiting reproductive units in nature and quantifying the degrees of gene flows among them, because results of this approach usually reflect variation in nuclear DNA (Tatarenkov, 1995; Kemperman and Degenaaars, 1992).

Advantageous properties of allozyme electrophoresis in the context of our objectives are as follows

1. Usually divergences in allozyme alleles progress largely, neutrally and thus are not much noised from natural selection through locally variable environment. (contra morphological variations that are usually vulnerable to noises from natural selection under divergent local environment)
2. Because results of allozyme electrophoresis reflect nucleic gene variation, they can clearly illustrate gene flows among populations. (contra mitochondrial genes that may reflect past maternal gene flows only)
3. By allozyme electrophoresis, data for variation at a large number of nuclear loci can be obtained relatively easily, and swiftly with low cost.

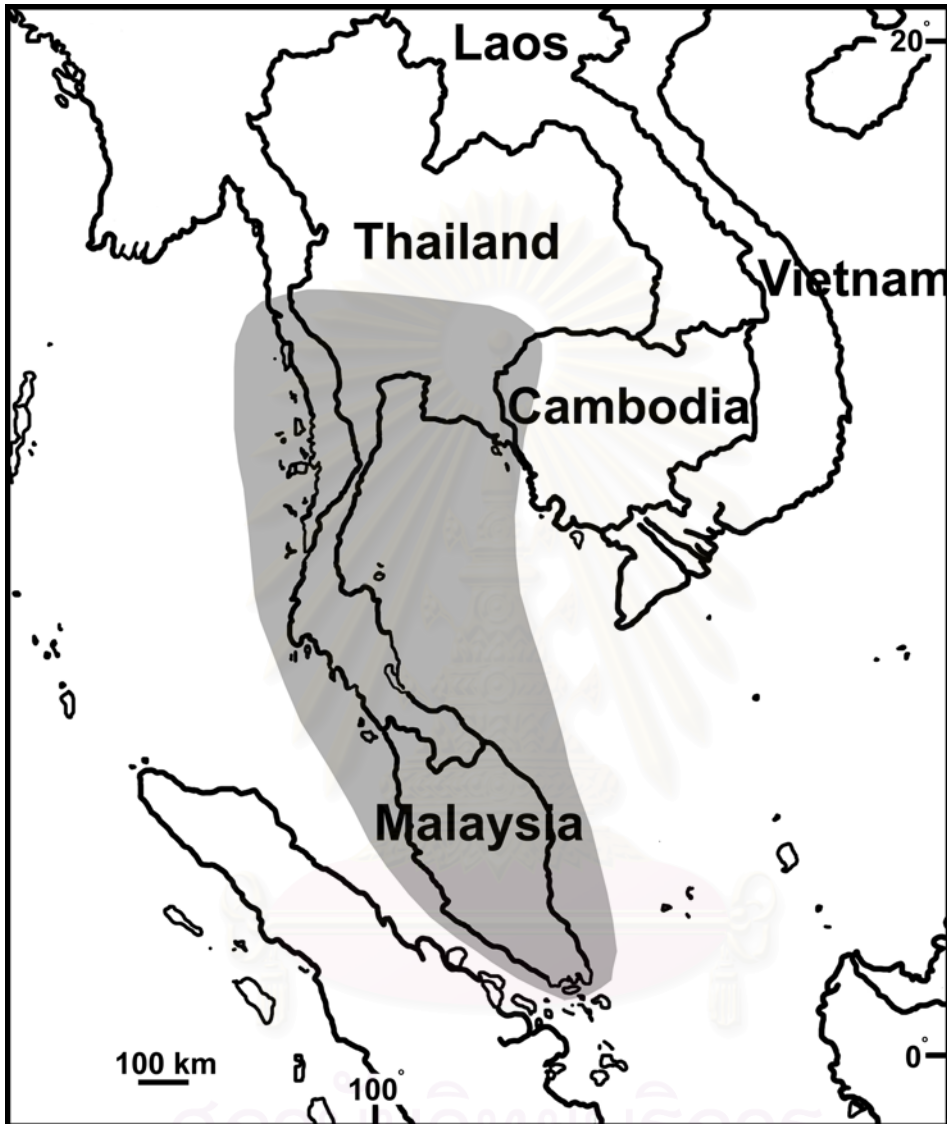


Fig. 3. The distribution range of *Amphidromus atricallosus*.

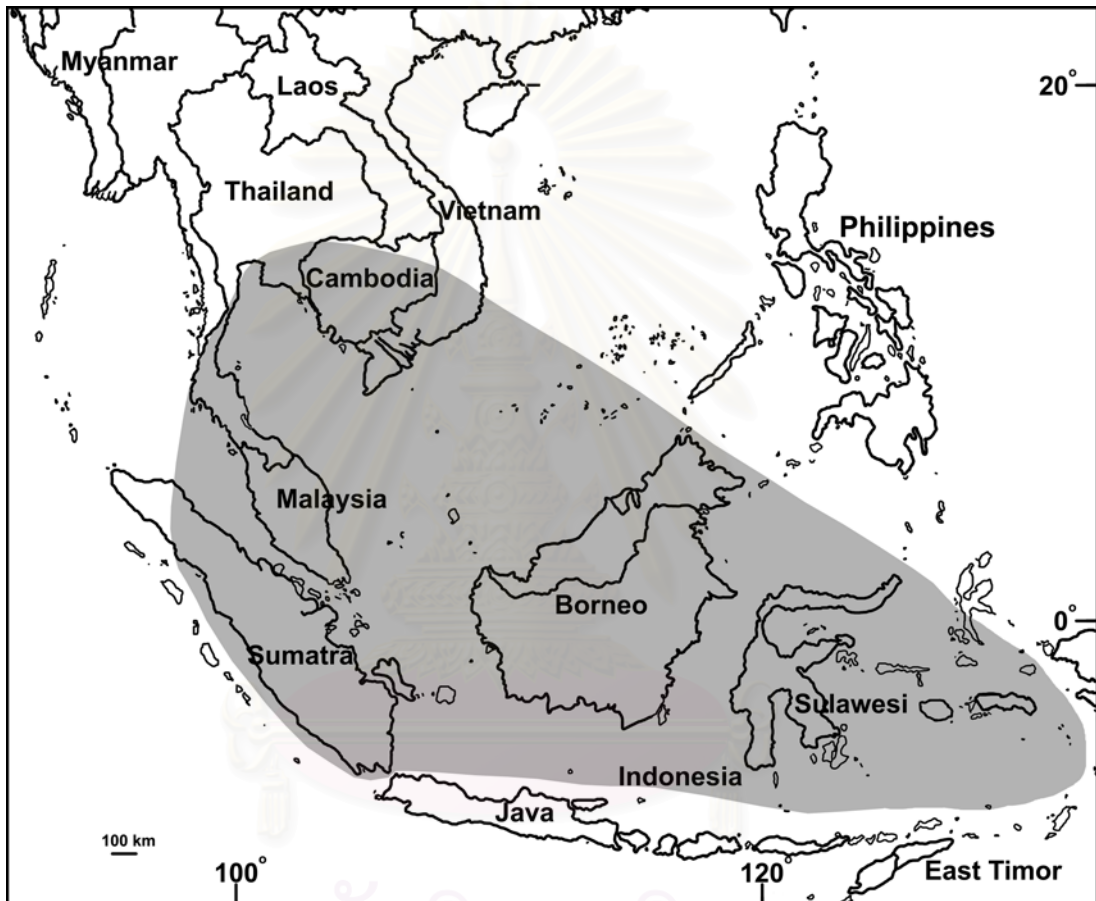


Fig. 4. Map of South East Asia showing the distribution range of *Amphidromus inversus*.

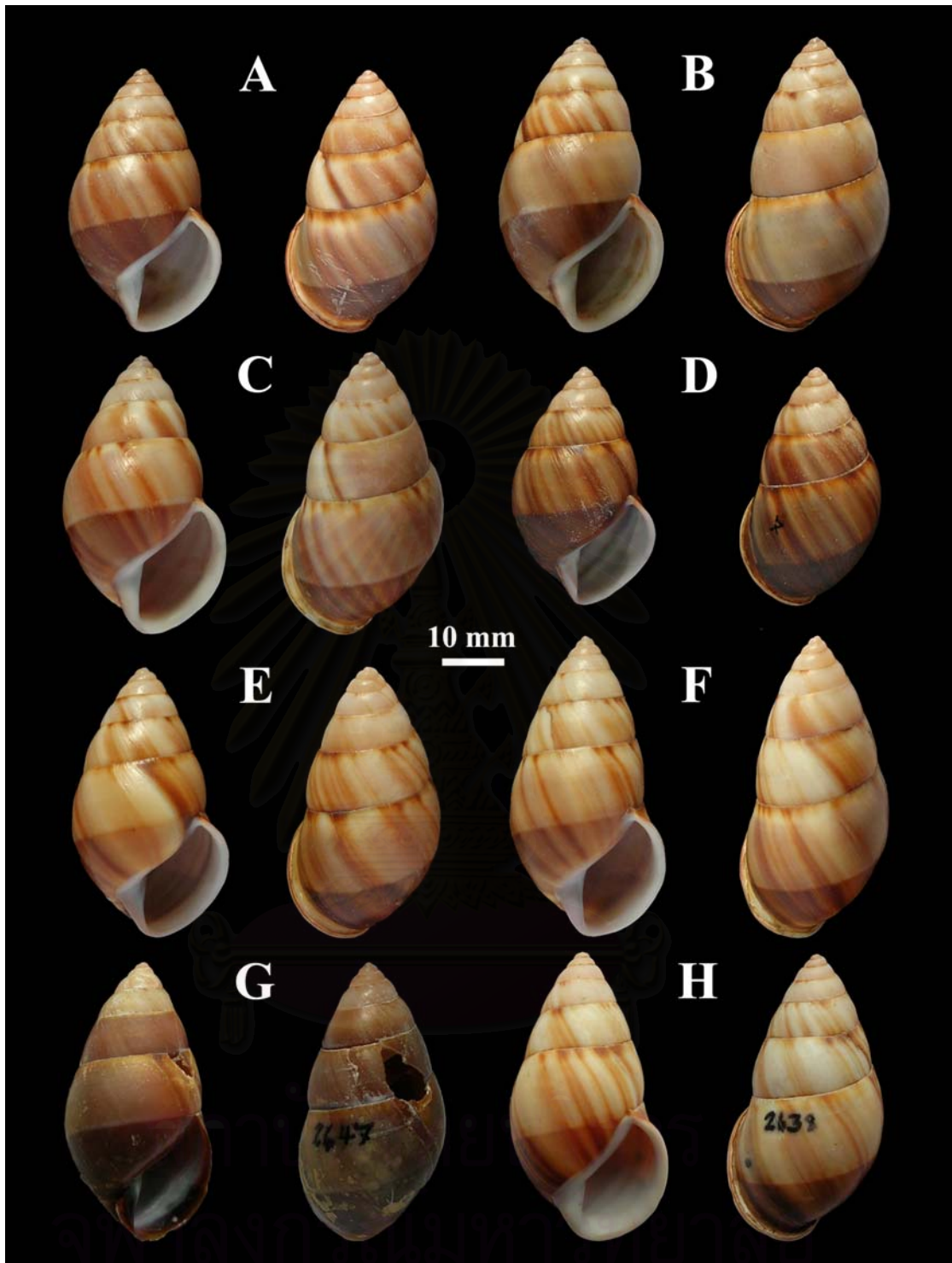


Fig. 5. Shell characteristic of *Amphidromus inversus*. **A-B.** *A. i. annamiticus* from Koh Kham, Chonburi. **C-D.** *A. i. annamiticus* from Koh Lueam, Chonburi. **E.** *A. i. annamiticus* from Koh Elar, Chonburi. **F.** *A. i. annamiticus* from Koh Pai, Chonburi. **G.** *A. i. annamiticus* from Koh Samet, Rayong. **H.** *A. i. annamiticus* from Koh Jarn, Chonburi.

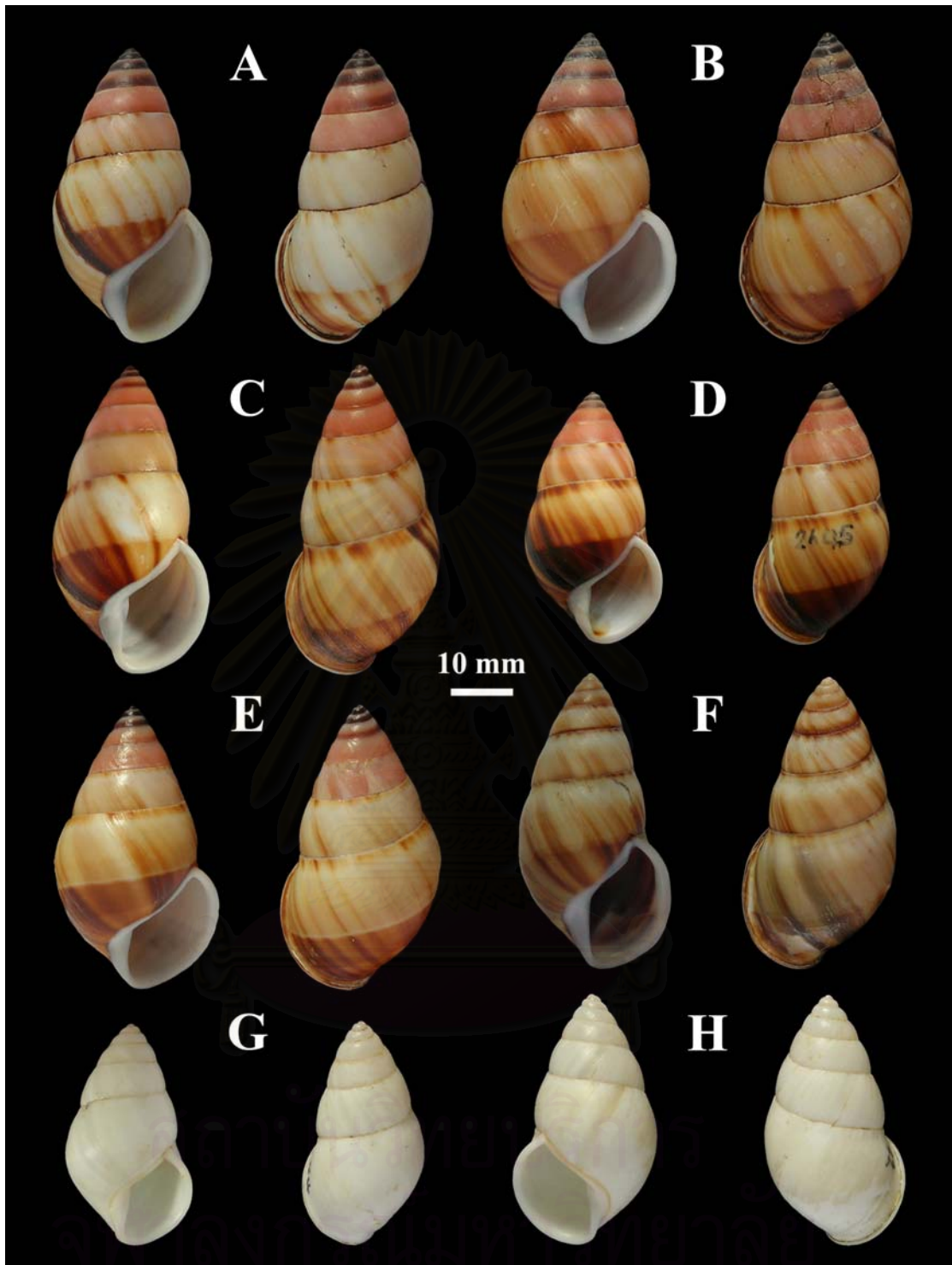


Fig. 6. Shell characteristic of *Amphidromus inversus*. **A-B.** *A. i. inversus* from Chaiya, Suratthani. **C-D.** *A. i. inversus* from Koh Samui, Suratthani. **E.** *A. i. inversus* from Koh Tan, Suratthani. **F.** *A. i. inversus* from Singapore Botanic garden, Singapore. **G-H.** *A. i. albulus* from Pulau Kapas, Malaysia represent left and right handed coiling.

Analysis

1. Calculation of genetic variability

In order to compare different genes and different populations, it is necessary to have some convenient quantitative measure of genetic variation. Genetic variation can be quantified using the concept of allele frequency. The allele frequency of assigned allele among a group of individuals is simply the proportion of all alleles of the gene that are of the assigned type. The frequency of any assigned allele in a sample is therefore equal to twice the number of homozygotes for the allele (because each homozygote carries two copies of the allele) plus the number of heterozygotes for that allele (because each heterozygote carries one copy) divided by two times the number of individuals in the sample (because each individual carries two alleles of the gene).

In order to quantify the genetic variation of specimen from each locality, the population genetic variability was determined using mean expected heterozygosity (H_{exp}) that represents the adaptive potential of the species and is an extremely powerful evolutionary force, mean number of alleles per locus (A), and percentage of polymorphic loci (P) which is simply the percentage of loci examined that show evidence of more than one allele.

2. Testing the hypothesis of Hardy-Weinberg equilibrium

To estimate the genetic variability in a population, a method will involve with the Hardy-Weinberg law. Daniel (1988) suggested that the assumptions made so far in developing the model for predicting genotype frequencies can be summarized as follows:

1. The organism in question is diploid.
2. Reproduction is sexual.
3. Generations are nonoverlapping.
4. Mating is random.
5. Population size is very large.
6. Migration is negligible
7. Mutation can be ignored.
8. Natural selection does not affect the gene under consideration.

The expected number of each genotype is obtained by multiplying the Hardy-Weinberg frequencies by the sample size. The goodness of fit between the observed numbers and the expected number can be judged quantitatively by means of the chi-squared value (χ^2), calculated as follows:

$$\chi^2 = \sum \frac{(\text{observed number} - \text{expected number})^2}{(\text{expected number})}$$

with the sum taken of all genotypic classes, producing $\chi^2 = 0.005$. The test based on this statistic is called the “chi-square test”. Associated with any χ^2 value is a second number called the degree of freedom for that χ^2 . In general, the number of degree of freedom associated with a χ^2 equals the number of classes of data minus one. If the probability value for a particular χ^2 is P , this means that if we were to take 1000 samples of the same size from a population in Hardy-Weinberg equilibrium and a calculation for each, a fraction P would be greater than our observed test value. Typically, if $P < 0.05$, the test is considered “significant”, which means the null hypothesis is rejected, and the test indicates significant departure from Hardy-Weinberg proportions.

3. Interpopulation analysis

3.1 Genetic distance (D)

The Genetic distance (D) was used to estimate genetic differentiation among populations and to compare local population within a species to estimate the amount of gene flow and genetic drift between populations. These forces along with natural selection lead to the creation of new species. It is sometimes necessary to determine the amount of genetic differentiation taking place during speciation. Various genetic distance measures used for gene frequency data have been described by Nei (1978). D ranges from 0 to infinity with zero indicating that a pair of populations is genetically identical.

3.2 The fixation index (F_{st})

The fixation index (F_{st}) expresses (on a scale of 0 to 1) the amount of variation detected among subpopulations as a fraction of that expected if the same set of genetic markers were randomly distributed (i.e. panmixia). An F_{st} of zero indicates that there is no heterogeneity of allele frequencies amongst populations.

The fixation index (F_{st}) was used to estimate genetic differentiation among populations. Although F_{st} has a theoretical minimum of 0 (indicating genetic divergence) and a theoretical maximum of 1 (indicating fixation for alternative alleles in the subpopulations), the observed maximum is usually much less than 1. Wright (1978) suggests the following qualitative guidelines for the interpretation of F_{st} :

1. The range 0 to 0.05 may be considered as indicating little genetic differentiation.
2. The range 0.05 to 0.15 indicates moderate genetic differentiation.
3. The range 0.15-0.25 indicates great genetic differentiation.
4. Values of F_{st} above 0.25 indicate very great genetic differentiation.

However, to quote Wright (1978), who developed the concept of F_{st} , “Differentiation is by no means negligible if F_{st} is as small as 0.05 or even less”.

In this study, our purposes are: (1) to clarify geographic genetic structures in the two *Amphidromus* species around the Indochina-Malay Peninsula region; (2) to infer process of formation of such structures; and (3) to revise classifications of these species accordingly.



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CHAPTER III

MATERIALS AND METHODS

1. Sampling

Sampling was carried out in Thailand, Malaysia, and Singapore (Fig. 7). As a result, 319 *A. atricallosus* were collected from 11 localities in the continental part and two off-shore islets. For *A. inversus*, 144 specimens were collected from ten off-shore islets and one continental locality (Table 1). The field collected snails were maintained in our laboratory at the Animal Systematic Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, and then stored at -70°C until analysis.

2. Characteristics in shell morphology

For each specimen, states of following shell morphological characters were recorded.

- Color
- Varix
- Shell size
- Aperture size
- Color of parietal callus
- Collumellar plait
- Direction of coiling

3. Electrophoresis

Individual snails were thawed and soft parts were removed from the shells. Liver and muscle tissues were removed from each specimen and homogenized. The supernatant was absorbed onto Whatman No. 3

filter paper. Protein extracts from the homogenates were subjected to horizontal starch gel electrophoresis following Murphy *et al.* (1996) with slight modifications. Voucher shell and several preserved specimens were deposited in the Zoological Collection of the Natural History Museum, Chulalongkorn University (CUMZ: see Table 2). Buffer system reported here was described in Table 3. Electrophoresis was carried out by which time a bromophenol blue marker dye had migrated 100-120 mm anodally. The gels were sliced into seven or eight slabs for staining. Agar overlaying was employed for all enzyme assays.

In the electrophoresis, migration and staining procedures largely followed those described by Clayton and Tretiak (1972), Boyer *et al.* (1963), and Ridgway *et al.* (1970). Enzyme nomenclature and E.C. number follow those proposed by the International Union of Biochemistry. The esterase substrate was alpha-naphthyl acetate and the peptidase substrate was leucyl-glycyl-glycine. Snails from different samples were run on each gel to facilitate comparison. Photographs of gels at various stages of staining were taken, to record the relative mobilities and intensities of all alleles in the adjacent slots, and the absolute position of each band within each samples.

Allozyme phenotypes were scored directly from the gel or digital image. Activity zones in the zymograms were interpreted as a product of gene loci. Heterozygote banding patterns corresponded with the subunit compositions of the enzymes. Italicized abbreviations of the enzymes designated the loci. Multiple loci are designated by number, beginning with the locus responsible for the most anodally migrating form of the enzyme. Alleles were labeled alphabetically according to decreasing

mobility (see Fig. 8). The notations for loci encoding peptidase with abbreviations of the substrates have been made.

4. Analysis

The scoring of the loci resulted in a single individual genotype dataset. Data analyses were performed using BIOSYS-1 (Swofford and Selander, 1981). Genetic variability within each population was assessed by calculating mean expected heterozygosity (H_{exp}), mean number of alleles per locus (A), and percentage of polymorphic loci (P). Genotypic frequency at each polymorphic locus was tested for its agreement with the Hardy-Weinberg expectation by exact probability test ($p \leq 0.05$).

To estimate heterogeneity among local samples in each species, Wright's (1965) F_{st} was calculated for each locus. Statistical significance in the difference from zero was then tested for each F_{st} value following Workman and Niswander (1970). Following Weir and Cockerham (1984), F_{st} was also applied to analyze population structure using FSTAT (Goudet, 1995). Nei's (1978) unbiased genetic distance and Rogers' (1972) genetic distance were calculated for all pairwise comparisons of the samples in order to estimate the extent of differentiations among populations. The Nei (1978) unbiased genetic distance was clustered in to the form of a dendrogram using the Unweighted Pair Group Method of Analysis (UPGMA) of Sneath and Sokal (1973). Rogers' (1972) distance matrix for the *A. atricallosus* samples was clustered by the neighbor-joining (NJ) method (Saitou and Nei, 1987).

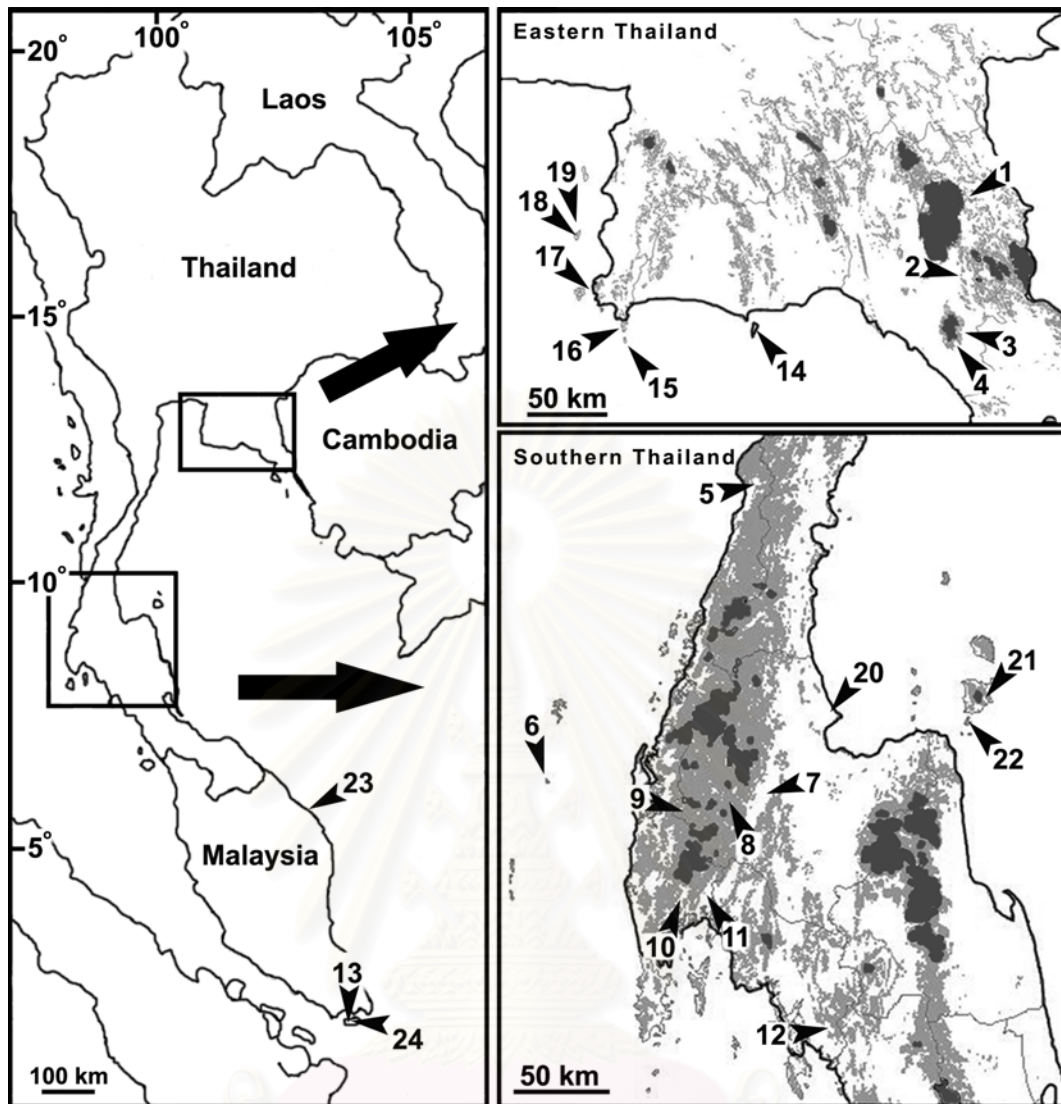


Fig. 7. Map of Southeast Asia, showing sampling localities of *Amphidromus atricallosus* (1-13) and *A. inversus* (14-24). Numerals correspond to locality numbers used in Table 1. Gray and dark shaded areas enclose portions of mountain range higher than 50 and 500 m asl., respectively. These portions are considered to have been effectively interrupting gene flows among *A. atricallosus* populations in this region (see text).

Table 1. Localities and sizes of samples of *Amphidromus atricallosus* and *A. inversus* used in this study. The sample numbers correspond to those in Fig. 7.

Species	Sample	
	Locality	n
<i>A. atricallosus</i>	1. Soidao	14
	2. Makham	37
	3. Troknong	16
	4. Makok	44
	5. Ranong	23
	6. Koh Tachai	20
	7. Takhun	14
	8. Klongsang	42
	9. Khaosok	33
	10. Suwankuha	31
	11. Pungchang	19
	12. Bangkram	8
	13. Singapore Nee Soon	18
<i>A. inversus</i>	14. Koh Samet	8
	15. Koh Kham	10
	16. Koh Jarn	4
	17. Koh Elar	8
	18. Koh Pai	13
	19. Koh Lueam	19
	20. Chaiya	23
	21. Koh Samui	18
	22. Koh Tan	14
	23. Pulau Kapas	19
	24. Singapore Botanic garden	8

Table 2. Catalog of the studies taxa, sampling localities and voucher specimen information (CUMZ: Chulalongkorn University, Museum of Zoology)

Species	Locality	Catalog No.
<i>Amphidromus atricallosus leucoxanthus</i>	Soidao, Chantaburi	CUMZ 2621
<i>A. atricallosus leucoxanthus</i>	Makham, Chantaburi	CUMZ 2622
<i>A. atricallosus leucoxanthus</i>	Troknong, Chantaburi	CUMZ 2623
<i>A. atricallosus leucoxanthus</i>	Makok, Chantaburi	CUMZ 2624
<i>A. atricallosus atricallosus</i>	Ranong	CUMZ 2625
<i>A. atricallosus classarius</i>	Koh Tachai, Pang Nga	CUMZ 2626
<i>A. atricallosus atricallosus</i>	Takhun, Suratthani	CUMZ 2627
<i>A. atricallosus atricallosus</i>	Klongsang, Suratthani	CUMZ 2628
<i>A. atricallosus atricallosus</i>	Khaosok, Suratthani	CUMZ 2629
<i>A. atricallosus atricallosus</i>	Suwankuha, Pang Nga	CUMZ 2630
<i>A. atricallosus atricallosus</i>	Poungchang, Pang Nga	CUMZ 2631
<i>A. atricallosus atricallosus</i>	Bangkram, Krabi	CUMZ 2632
<i>A. atricallosus perakensis</i>	Singapore Nee Soon	CUMZ 2633
<i>A. inversus inversus</i>	Singapore Botanic garden	CUMZ 2636
<i>A. inversus albulus</i>	Pulau Kapas, Malaysia	CUMZ 2637
<i>A. inversus annamiticus</i>	Koh Jarn, Chonburi	CUMZ 2638
<i>A. inversus annamiticus</i>	Koh Pai, Chonburi	CUMZ 2639
<i>A. inversus annamiticus</i>	Koh Kham, Chonburi	CUMZ 2640
<i>A. inversus annamiticus</i>	Koh Lueam, Chonburi	CUMZ 2641
<i>A. inversus annamiticus</i>	Koh Elar, Chonburi	CUMZ 2642
<i>A. inversus annamiticus</i>	Koh Tan, Suratthani	CUMZ 2643
<i>A. inversus annamiticus</i>	Chaiya, Suratthani	CUMZ 2644
<i>A. inversus annamiticus</i>	Koh Samui, Suratthani	CUMZ 2645
<i>A. inversus annamiticus</i>	Koh Samet, Rayong	CUMZ 2647

Table 3. Correspondence of 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>Me</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>Me</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)

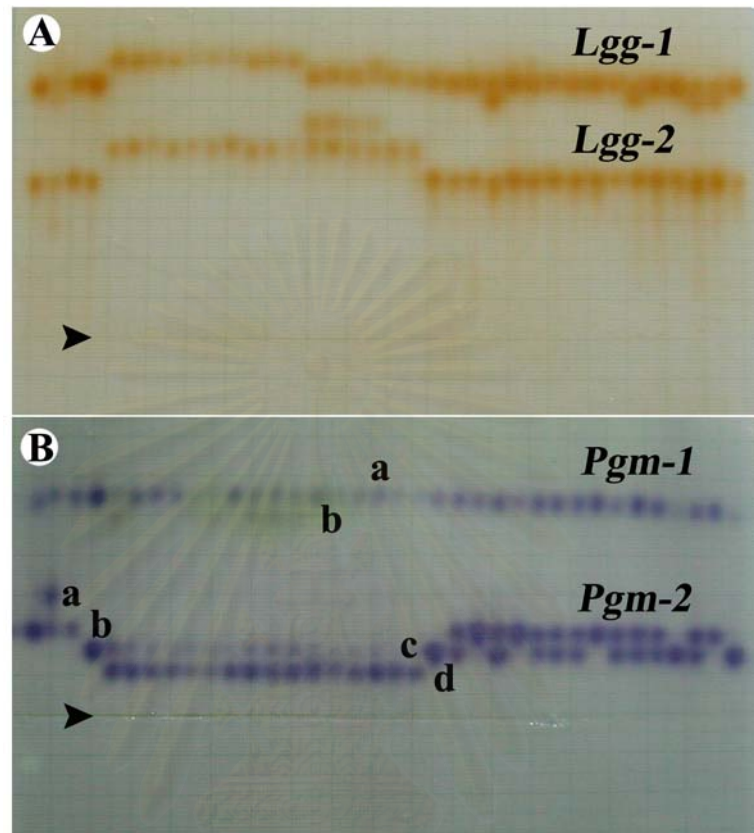


Fig. 8. Examples demonstrate the activity of enzymes. A: peptidase; leucyl-glycyl-glycine, B: phosphoglucomutase. Arrows indicate the origin.

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CHAPTER IV

RESULTS

Thirteen allozyme loci of ten enzyme systems were screened for *A. atricallosus*, and 18 loci of 11 systems for *A. inversus* (Table 3). Of these, 11 and five loci were polymorphic, respectively (Tables 4 and 5).

Genetic variation in *Amphidromus atricallosus*

In *A. atricallosus* genotype frequencies at the 11 polymorphic loci showed no significantly deviate from the Hardy-Weinberg expectation in all but one from Singapore Nee Soon samples. In the Singapore Nee Soon, 2 individuals exhibit aa, 2 for bb, 6 for bd, and 8 for dd at *Aat-1* (Appendix I), and deviation from the expectation was statistically significant ($p < 0.01$) (Appendix II). This may suggest the presence of sympatric cryptic species or long standing population subdivision within Singapore population. However, the number of available Singapore specimens (18) was not large enough to examine these possibilities. Moreover, allelic frequencies at the other ten polymorphic loci showed no significantly deviate from the expected ranges (see above). We, therefore, continued to treat the Singapore specimens collectively as a single sample.

At four out of the 11 polymorphic loci (*Gpi*, *Me*, *Mpi* and *Lgg-2*), allelic compositions completely differed 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 with the eastern Thailand

samples at *Hbdh*, *Me* and *Pgd*, and with the southern Thailand samples at *Gpi* and *Lgg-2*. Within the southern Thailand, complete allelic displacements 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 complete allelic displacements among the eastern Thailand samples.

Matrices of Nei's (1978) distance (D) and Rogers (1972) distance between samples of *A. atricallosus* are presented in Table 6. Large D values were obtained between the southern Thailand samples and the Singapore Nee Soon sample ($\bar{x} \pm \text{SD} = 0.841 \pm 0.105$, range = 0.590-0.915), between the Singapore sample and the eastern Thailand samples (0.580 ± 0.018 , 0.557-0.597), and between the southern Thailand samples and the eastern Thailand samples (0.729 ± 0.148 , 0.485-0.946). The D values between the southern Thailand samples (0.157 ± 0.073 , 0.012-0.306) were distinctly smaller, but, with the exception of the value between the Suwankuha and Poungchang samples (0.012), were still distinctly larger than those between the eastern Thailand samples (0.017 ± 0.011 , 0.005-0.031).

Distance phenogram of *A. atricallosus* constructed by UPGMA and NJ methods were similar in topology; the former are present in Fig. 9. The phenogram shows three major groups: ones containing all six samples along southern Thailand, the other containing the four samples in the eastern Thailand and the last containing the one sample from Singapore.

The mean F_{st} for the whole *A. atricallosus* was so large (0.781). Among the four eastern Thailand samples and among the eight southern Thailand samples, means of F_{st} were much smaller, 0.144 and 0.551, respectively. Nevertheless, F_{st} values were significantly higher than zero for five polymorphic loci of the former, and for ten polymorphic loci of the latter (Table 9).

The eastern Thailand samples showed moderate P and H_{exp} values (15.4-38.5 and 0.021-0.098, respectively: Table 8). Likewise, the southern Thailand samples exclusive of those from Koh Tachai and Bangkram displayed moderate to high P and H_{exp} values (23.1-61.5 and 0.057-0.201, respectively). In the Koh Tachai sample and the Bangkram sample, both P and H_{exp} values were much lower, 7.7 and 0.025 for the former, and 7.7 and 0.018 for the latter, respectively (Table 8).

Genetic variation in *Amphidromus inversus*

In *A. inversus*, within-sample variations were detected only at *Lgg-3* of the Koh Samui sample and the Koh Tan sample: no variations were recognized at all in the other samples or at the other loci (Table 5). The P and H_{exp} values of those samples were 5.6 and 0.023 (Koh Samui), and 5.6 and 0.004 (Koh Tan), respectively (Table 8).

Samples from Pulau Kapas, Malaysia and Singapore Botanic Garden showed fixed allelic differences with all Thailand samples at *Lgg-1* and *Lgg-2*. The Singapore sample was also distinct from the remainder in having allele “a” at *Est-4*. Fixed allelic difference was also recognized at *Pgm-2* between the eastern Thailand samples and the remainder (Table 5).

Between-sample D varied from 0-0.260 (Table 7), with particularly large values between the Singapore sample and the southern Thailand samples (0.254 ± 0.005 , 0.251-0.260). The values were also relatively large between the former and the eastern Thailand samples (invariably 0.182), and between the Pulau Kapas sample and the southern (0.185 ± 0.004 , 0.182-0.189) and eastern Thailand samples (invariably 0.118). Pairwise comparisons of the southern Thailand samples and the eastern Thailand samples, and of the Singapore sample and the Pulau Kapas sample yielded moderate D values (0.059 ± 0.002 , 0.057-0.062; and invariably 0.057, respectively). In contrast, the values were so 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. *Fst* values were significantly higher than zero at all five polymorphic loci ($x=0.965$), of which four (*Est-4*, *Lgg-1*, *Lgg-2* and *Pgm-2*) exhibited particularly high heterogeneity (i.e., fixed allelic differences between several combinations of local samples: see above; also see Tables 5 and 9).

Table 4. Allele frequencies at polymorphic loci of the *Amphidromus atricallosus* samples. Locality numbers correspond to those in Fig. 7. Notations of alleles are made alphabetically 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	1.000	0.986	1.000	0.966	0.957		0.714	0.976	0.242	0.274	0.079	1.000	0.278
c						1.000	0.250	0.024	0.682	0.726	0.921		
d		0.014		0.034									0.611
<i>Est-2</i>													
a	0.929	1.000	1.000	0.989	1.000	1.000	0.964	0.833	0.530	1.000	0.974	1.000	1.000
b	0.071			0.011			0.036	0.167	0.470		0.026		
<i>Gpi</i>													
a					0.652			0.036					
b		0.108		0.045									
c					0.348	1.000	1.000	0.964	1.000	1.000	1.000	1.000	
d	1.000	0.892	1.000	0.955									1.000
<i>Hbdh</i>													
a													0.944
b		0.014			0.364		0.769	0.095	0.424	1.000	0.816	1.000	
c		0.243									0.184		
d					0.636	1.000	0.231		0.576				0.056
e	1.000	0.743	1.000	1.000				0.905					
<i>Me</i>													
a	1.000	1.000	1.000	1.000									
b					1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
<i>Mpi</i>													
a													1.000
b	1.000	1.000	1.000	1.000									
c					0.804	1.000	1.000	1.000	1.000	1.000	0.947	1.000	
d					0.196						0.053		
<i>Lgg-1</i>													
a						0.800		0.012	0.030				0.875
b					0.239	0.200	0.179	0.607	0.394	1.000	0.765	0.125	
c	1.000	1.000	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						1.000
b					0.978	1.000	0.714	1.000	1.000	1.000	1.000		
c	1.000	1.000	1.000	1.000									1.000
<i>Pgm-1</i>													
a	1.000	1.000	0.781	1.000	1.000	1.000	1.000	0.976	1.000	0.821	1.000	1.000	1.000
b			0.219					0.024		0.179			
<i>Pgm-2</i>													
a									0.212				
b	0.071	0.554	0.594	0.580	0.109				0.015				
c	0.929	0.446	0.406	0.420	0.696				0.015	0.016	0.026		1.000
d					0.196	1.000	1.000	1.000	0.758	0.984	0.974	1.000	
<i>Pgd</i>													
a	1.000	1.000	1.000	1.000	0.761	1.000	1.000	1.000	0.984	1.000	1.000	1.000	
b					0.239				0.016				1.000

Table 5. Allele frequencies at polymorphic loci of the *Amphidromus inversus* samples. Locality numbers correspond to those in Fig. 7. Notation of alleles is made alphabetically 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												1.000
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	
<i>Lgg-1</i>												
a											1.000	1.000
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000			
<i>Lgg-2</i>												
a											1.000	1.000
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000			
<i>Lgg-3</i>												
a								0.278	0.036			
b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.722	0.964	1.000	1.000	
<i>Pgm-2</i>												
a	1.000	1.000	1.000	1.000	1.000	1.000						
b							1.000	1.000	1.000	1.000	1.000	

Table 6. 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	0.610	0.582	0.537	0.443
2 Makham	0.024	-	0.070	0.035	0.414	0.587	0.460	0.449	0.542	0.584	0.558	0.513	0.474
3 Troknong	0.031	0.015	-	0.040	0.450	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	0.220	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	0.140	0.237	0.592
7 Takhun	0.627	0.582	0.647	0.611	0.130	0.164	-	0.152	0.153	0.156	0.140	0.162	0.558
8 Klongsang	0.560	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	0.740	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	0.150	0.089	0.133	0.085	-	0.068	0.215	0.600
11 Pongchang	0.855	0.801	0.860	0.829	0.239	0.116	0.076	0.136	0.057	0.012	-	0.230	0.581
12 Bangkram	0.765	0.719	0.750	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	0.590	0.903	0.821	0.864	0.889	0.915	0.880	0.864	-

Table 7. 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	-	0.000	0.000	0.000	0.000	0.000	0.056	0.071	0.058	0.111	0.167
15. Koh Kham	0.000	-	0.000	0.000	0.000	0.000	0.056	0.071	0.058	0.111	0.167
16. Koh Jarn	0.000	0.000	-	0.000	0.000	0.000	0.056	0.071	0.058	0.111	0.167
17. Koh Elar	0.000	0.000	0.000	-	0.000	0.000	0.056	0.071	0.058	0.111	0.167
18. Koh Pai	0.000	0.000	0.000	0.000	-	0.000	0.056	0.071	0.058	0.111	0.167
19. Koh Lueam	0.000	0.000	0.000	0.000	0.000	-	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	0.000	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	0.260	0.252	0.057	-

Table 8. Localities, 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. 7.

Species	Sample	A	P	H_{exp}
<i>A. atricallosus</i>	1. Soidao	1.2	15.4	0.021 (0.014)
	2. Makham	1.4	30.8	0.086 (0.047)
	3. Troknong	1.2	23.1	0.098 (0.052)
	4. Makok	1.4	38.5	0.064 (0.038)
	5. Ranong	1.7	61.5	0.201 (0.058)
	6. Koh Tachai	1.1	7.7	0.025 (0.025)
	7. Takhun	1.5	38.5	0.124 (0.051)
	8. Klongsang	1.5	46.2	0.085 (0.041)
	9. Khaosok	1.8	46.2	0.186 (0.067)
	10. Suwankuha	1.2	23.1	0.057 (0.037)
	11. Pungchang	1.5	46.2	0.080 (0.035)
	12. Bangkram	1.1	7.7	0.018 (0.018)
	13. Singapore Nee Soon	1.3	23.1	0.055 (0.042)
<i>A. inversus</i>	14. Koh Samet	1.0	0.0	
	15. Koh Kham	1.0	0.0	
	16. Koh Jarn	1.0	0.0	
	17. Koh Elar	1.0	0.0	
	18. Koh Pai	1.0	0.0	
	19. Koh Lueam	1.0	0.0	
	20. Chaiya	1.0	0.0	
	21. Koh Samui	1.1	5.6	0.023 (0.023)
	22. Koh Tan	1.1	5.6	0.004 (0.004)
	23. Pulau Kapas	1.0	0.0	
	24. Singapore Botanic garden	1.0	0.0	

Table 9. Summary of *Fst* 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

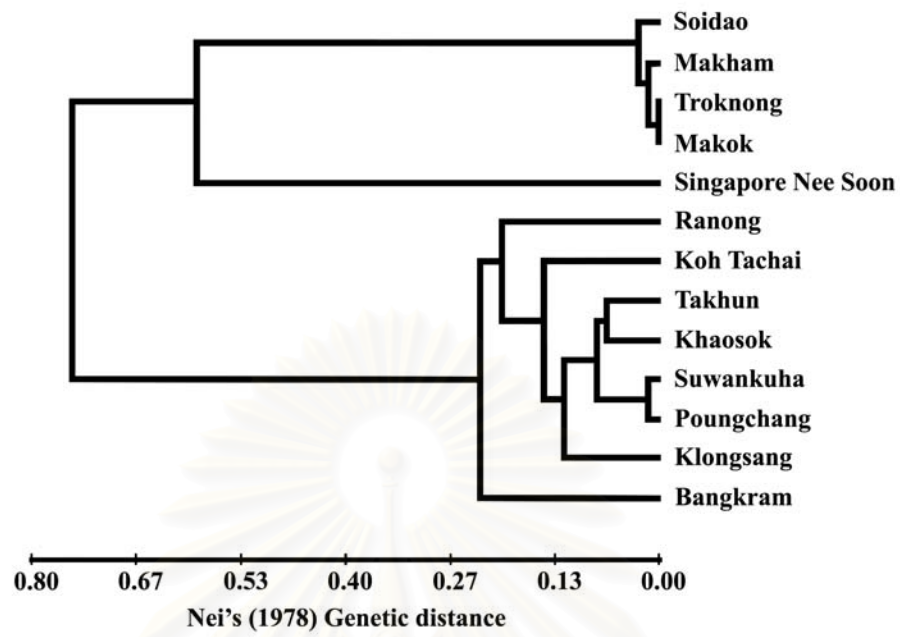


Fig. 9. Unweighted Pair Group Method of Analysis for the local samples of *Amphidromus atricallosus* based on Nei's (1978) genetic distance.

CHAPTER V

DISCUSSION

Geographic genetic structure

The statistical tests for deviation from Hardy-Weinberg equilibrium indicated insignificant in all case except the Singapore Nee Soon sample. We suggested that every sample considered representative of a single deme.

In our investigation, significant departure from Hardy-Weinberg equilibrium was observed at one locus (*Aat-1*) (see Table 23). Such deviations from panmixia have been frequently observed in *Helix aspersa* (Guiller *et al.*, 1996) and other simultaneous hermaphrodite land snails like *Cepaea* spp. (Guiller and Madec, 1993). They are interpreted as a result of sampling in genetically differentiated microlocal demes, rather than a consequence of selective pressures. Thus, a colony would be subdivided into interbreeding neighbourhoods (Shapcott, 1995). Samples formed by an admixture of local population with differing allelic frequencies which possibly come from the same family (intrademe Wahlund effect) could be additional factors explaining deviations from Hardy-Weinberg equilibrium.

In our populations of *A. atricallosus* and *A. inversus*, the percentage of polymorphic loci were 7.7% to 61.5%, mean 31.38% and 0% to 5.6%, mean 1.02%, respectively. In the majority of marine mollusks, the percentage of polymorphic loci is between 30% and 50% (Berger, 1983); in the freshwater mussels *Anodonta*, 11% to 36%,

depending on species (Kat, 1983); in the brackish water snails *Hydrobia*, 13% to 23% (Davis *et al.*, 1988); in freshwater gastropods, 14% to 62% (Brown and Richardson, 1988; Woodruff *et al.*, 1988). In land snail, Nevo (1978) listed mean estimated of polymorphism which range from 6% to 100% with an average of 31.5%. For example, in Australian camaenids it range from 19% to 71% (Woodruff and Solem, 1990), from 65% to 80% in *Partula* (Johnson *et al.*, 1977), but reaches only about 4% in *Liguus* (Hillis *et al.*, 1987). In *Cepaea*, it is about 60% (Clarke *et al.*, 1978). Thus, the value of polymorphic loci found in *A. inversus* is rather low for a polymorphic species, whereas, *A. atricallosus* was similar to those mollusks.

Heterozygosity in *A. atricallosus* and *A. inversus* in this study varied from 0.025 to 0.201, mean at 0.085 and 0 to 0.023, mean at 0.003, respectively. The heterozygosity values in *A. atricallosus* are similar to the reported by Woodruff and Solem (1990) for camaenids tree snail (0.08-0.24). Comparison to other tree snails, *A. atricallosus* was relatively higher heterozygosity than *Partula* (0.1 to 0.17) (Johnson *et al.*, 1977). In other land snails, the heterozygosity values in *A. atricallosus* are similar to ones given by Nevo (1978) for *Theba* (0.054 to 0.165), Brown and Richardson (1988) for *Cepaea nemoralis* (0.134), and by Brown and Richardson (1988) for *Bradybaena similaris* (0.083). In land slugs, average heterozygosity varies among species (0 to 0.19: Foltz *et al.*, 1984), but also among conspecific population from various parts of the range (0.006 to 0.19, *Milax*: Foltz *et al.*, 1984; 0.4 to 0.19, *Oncomelania*: woodruff *et al.*, 1988). On the other, *A. inversus* appeared to show relatively lower heterozygosity than the other land snails, as in *Samoana*, it does not excess 0.002 (Johnson *et al.*, 1986). Furthermore, the tree snail

species *Liguus fasciatus* from Florida was similar heterozygosity to *A. inversus* (Hillis *et al.*, 1987)

Allozymic variation among morphotypes and populations of *A. inversus* is surprisingly low. The level of polymorphic loci per population in *A. inversus* (0-0.010) is lower than any other gastropod reported (Nevo, 1978), except for several self-fertilizing species (Selander and Kaufman, 1973a, b; McCracken and Selander, 1980). This is surprising because the normally highly polymorphic esterase and peptidases were included in this study. Among other gastropod studies, degree of allozymic variation has been shown to be a strong indicator of the type of breeding system employed by the species. Among outcrossing gastropods, the percent of polymorphic loci and average individual heterozygosity are high, whereas in self-fertilization species, average individual heterozygosity is very low and polymorphic loci are rare or absent (Hillis *et al.*, 1987). This pattern has also been observed in several other groups of hermaphroditic organisms. It has been reported that a complete or near absence of genetic variability within populations may occur in facultative self-fertilizing breeding system, as is known, for example, for *Rumina decollata* (see Selander and Kaufman, 1973), *Partula gibba* (see Johnson *et al.*, 1977) and some *Arion* species (see McCracken and Selander, 1980). Hillis *et al.* (1987) investigated allozymic variability and heterozygosity deficiency among populations of tree snails genus *Liguus fasciatus*. The results showed low level of polymorphic loci (0-0.04) and the significant deficiencies of heterozygotes in four of five polymorphic populations which indicate typical of self-fertilizing species. Several other pulmonates have been shown to consist of both self-fertilization and outcrossing populations, or individual may be facultatively self-fertilizing; furthermore, reproduction following copulation in *Philomycus*

spp. can be either by self-fertilization or outcrossing (McCracken and Selander, 1980). Although the patterns of allozymic variability observed in this study was highly likely that multiple reproductive modes can be possible in populations of *A. inversus*. However, results of field observations and published information (Woodruff and Solem, 1990), indicated reciprocal mating for *A. inversus* (Fig. 1G).

Both geographic pattern in the number of fixed allelic differences and topology of the NJ tree (Fig. 10) from the genetic distance matrix indicate that the present samples of *A. atricallosus* are classified into three groups that correspond to their geographic locations---the eastern Thailand Group (ETG), southern Thailand Group (STG), and Singapore Group (SPG: consisting solely of the Singapore Nee Soon sample). High genetic heterogeneity among these groups was also supported by mean F_{st} value for the whole sample (0.781), which is much higher than those separately calculated for the ETG (0.144) and STG (0.551).

The ETG samples of *A. atricallosus* lacked fixed allelic differences and showed low F_{st} and small genetic distances with each other. These suggest frequent gene flows among the eastern Thailand populations. Absence of prominent geographic barriers among the sampling sites in this region corroborates this assumption.

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 almost distinctly larger than those between the ETG samples. These suggest less frequent gene flows among samples in this region.

Of the sampling localities of the STG specimens, Koh Tachai is unique in being a small island. This geographic feature may explain the scarcity of gene flows between the Koh Tachai population and other southern Thailand populations of *A. atricallosus* as implied by the presence of fixed allelic differences between this island sample and a few other STG samples. Also, it may explain the distinctly lower H_{exp} value (0.025) in the Koh Tachai sample than in the other STG samples but one from Bangkram (0.057-0.186), because the drift usually more effectively lowers genetic diversity in such small and strictly isolated populations as those on small islands through bottleneck (e.g., Gorman and Nevo, 1975). Another possible cause of low genetic diversity in the Koh Tachai population is such a tsunami in the past as that hitting the island after our sampling (on 26 December 2004). This recent tsunami directly wiped out a large proportion of the Koh Tachai population of *A. atricallosus* by its forceful waves and also perished the majority of host trees by causing high environmental salinity (Panha, 2005).

It looks more difficult to explain substantial geographic genetic heterogeneity, as indicated by a relatively large F_{st} value (0.415) in *A. atricallosus* from the remaining continental localities in southern Thailand. These localities surround a long north-south chain of granite mountain range, which reaches approximately 1000 m in height (Woodruff, 2003: Fig. 7). Because the habitat of the *Amphidromus* species usually ranges from 30-500 m in elevation (Panha *et al.*, 2001; Sutcharit and Panha, 2006a), this mountain range should have long been interrupting direct gene flows among *A. atricallosus* populations on different sides, enhancing independent genetic drift within each habitat patch. Similarly, Chiba (1998) found highly genetic differentiation for

Mpi and *Me* (0.13 to 0.24) indicating geographical variation in allozyme frequencies. Furthermore, within the area of the southern populations, there are some barriers to dispersal, such as roads, streams and rice field. Further work based on mitochondrial DNA sequence and more rapidly evolving nuclear markers such as microsatellite may resolve this issue.

It is not clear in general how effective such barriers must be to prevent or strongly limit gene flow. Even a small river may be a true barrier for land snail (e.g. Hillis *et al.*, 1987). In addition, sharp geographical differentiation over very short distances appears to be the rule in landsnails (Nevo, 1978). Microgeographical population genetic structure in the colonizing outbreeding landsnail *Helix aspersa* indicates highly significant genetic differentiation in allele frequencies within and between blocks, more so in small colonies, suggesting either selection in spatially heterogeneous environments or incidents of drift occurring in the demographic history of the populations (Selander and Kaufman, 1975). In another landsnail, *Cepaea nemoralis*, area effects reflect substantial genetic differentiation (Johnson, 1976). Relatively large genetic variability in each of those samples may reflect varying selection forces operating on the snails.

In *A. inversus*, the *H_{exp}* values were extremely low: within-sample variation was observed only at one locus in two samples (Table 5). In contrast, *F_{st}* values were extremely high, obviously due to the presence of fixed allelic differences at four of the five polymorphic loci detected (Table 9).

In Table 10, we list values of the average heterozygosity, range of heterozygosity and *F_{st}* estimated from multi-locus allozyme data for

populations of various other land snails by previous authors. It is likely from this table that the heterozygosity within population of *A. inversus* represents some of the lowest value described for land snails. Conversely, F_{st} values for these two species relatively higher than other land snails so far studies.

Nevo (1978) investigated the correlation between several biological parameters and the genetic variability observed in over 200 species of plant and animal. One might expect that the genetic variability depends on the mode of reproduction. In *A. inversus* material, there is a considerable deficiency of heterozygosity in most population (Table 5). This pattern has also been found in other hermaphroditic molluscs (Hillis *et al.*, 1987; Kemperman and Degenaaars, 1992). However, this factor can be excluded in the present case, because they are outcrossing snail (Woodruff and Solem, 1990).

Considering the mostly insular habitat of *A. inversus* (Fig. 7), occurrence of bottleneck appears to be responsible for the extremely low genetic variability in each sample (Gorman and Nevo, 1975) as in the case of the Koh Tachai sample of *A. atricallosus* (see above). However, it is obvious that the occurrence of independent bottleneck with or without mutations on each island (Slatkin, 1985) alone cannot explain such geographically clearly patterned allelic displacements in *A. inversus* (Table 5). Depths of straits laying between those islands having *A. inversus* populations and the continent is shallow enough to have been exposed above the sea during a series of the Pleistocene Glaciations (see Fig. 14; Voris, 2000; Sathiamurthy and Voris, 2006). We, therefore, suspect that the current characteristic geographic genetic structure in *A. inversus* has been formed through a series of bottlenecks on the islands

during the inter-glacier periods, coupled with substantial range extensions repeated during the glacier periods. However, the number of Koh Jarn sample specimens (4) was too small to examine genetic variability. Thus, this sample remains to be resolved on the basis of additional sampling sizes.

Taxonomic implications

In this study, a number of *A. inversus* populations including those representing a few of its recognized subspecies, such as *A. i. andamensis* from Borneo and *A. i. koperbergi* from northern Sulawesi, have not been examined. We, therefore, discuss taxonomic implications of the present results only for *A. atricallosus*.

Laidlaw and Solem (1961), in an annotated catalogue of the genus *Amphidromus* from Southeast Asia, recognized three geographically defined subspecies for *A. atricallosus* on the basis of a few previous studies (Gould, 1843; Pilsbry, 1900; Fulton, 1901). Of these, the nominotypical subspecies, restricted to southern Thailand and southern Myanmar, was diagnosed as having distinct black or dark-brown parietal callus and straight columella. The subspecies *A. a. leucoxanthus* was recognized from eastern Thailand and was differentiated from the nominotypical subspecies in possessing a white parietal callus. In contrast, the subspecies *A. a. perakensis* was recognized from Malaysia and Singapore on the basis of white parietal callus and twist plait columella (see Fig. 13G-H). However, these characters cannot use to describe distinct subspecies *A. a. perakensis* from other subspecies (see Fig. 11-13). Recently the Koh Tachai population was recently described as the fourth subspecies *A. a. classarius* on the basis of relatively small

shell size and brown or black parietal callus (Sutcharit and Panha, 2006a). States of shell characters in our samples of *A. atricallosus* from the continental part of southern Thailand, eastern Thailand, Singapore, and Koh Tachai largely coincide with those used to define the nominotypical subspecies, and the three other subspecies, *leucoxanthus*, *perakensis* and *classarius*, respectively, although within-sample variations are also evident to some extent.

Fixed allelic differences at several allozyme loci among the southern Thailand samples, eastern Thailand samples, and Singapore sample (Table 4) strongly suggest the absence of gene flows among populations in the three regions. Furthermore, the distance analysis of allozyme data showed relatively large divergences among these populations (Fig. 10). These results not only support the validity of *leucoxanthus* and *atricallosus*, but also suggest their being two full species as in the case of a few other land snails so far studied by similar methods [Woodruff *et al.*, 1988; Emberton, 1995; Manganelli *et al.*, 2001; Sutcharit *et al.* (inpress)].

Manganelli *et al.* (2001) examined land snail, *Cerneuella usticensis* based on the analysis of morphological characters and on the genetic study of allozyme pattern. The morphological and genetic studies demonstrated that *C. usticensis* can be distinguished from all the other *Cerneuella* species. They also reported that genetic distances between *Cerneuella* species ranged from 0.258-0.525.

Hillis and Patton (1982), who examined two forms electrophoretically and morphologically of *Corbicula* to determine their degree of differences, reported that the two color forms differed in

morphological evidence, and the two forms also have fixed allelic differences at six of 26 genetic loci. They concluded that two color forms actually represent separate species.

Hoagland and Davis (1987) investigated electrophoretic data of allozyme in Succineid snail from Chittenango Falls, New York. They found that *Oxyloma decompi gouldi* and *O. retusa* were different species with the distance 0.2. Furthermore, Results of fixed for alternate alleles also confirmed separate species status among *Succinea* species.

On the basis of allozyme data it is clear that two full species are occurred. Sutcharit and Panha (2006a), who examined a several sets of shell morphological and anatomical data, provided key to species and subspecies of *Amphidromus* including *A. atricallosus*. They recognized two subspecies, *leucoxanthus* and *atricallosus*. *Amphidromus atricallosus leucoxanthus* was defined as a group of subspecies with white parietal callus, straight or twisted columella, yellow or white monochrome and usually present one or more varices (Fig. 11). On the other hand, *A. a. atricallosus* described with black parietal callus, 40-50 mm of shell height, conic and usually chiral dimorphic, yellow or white and long penis (Fig. 12). It seems that *atricallosus* differs from *leucoxanthus* by possessing black parietal callus. However, representative shells from allozyme studied individuals of all 7 localities of southern Thailand branching in the divergence of *A. a. atricallosus* are illustrated in Figure 10. Of three electrophoresed localities from Suwankuha, Pongchang and Bangkram were close genetically to the other; the shells showed distinguishing characteristics (white parietal callus; Fig. 13). Sutcharit and Panha (2006a) diagnosed these populations are uncertain status

subspecies. These polymorphisms may rapidly evolve under strong selection forces from environment.

This time, we failed to access to any *A. atricallosus* sample from Malaysia (Laidlaw and Solem, 1961). So, further analyses incorporating data for Malaysian samples are needed to verify genetic discontinuity between the nominotypical subspecies and *perakensis*, which is essential to determine the validity and status of the latter with certainty.

With respect to *A. a. classarius*, the validity is not supported well, because the results of our allozyme analyses, while suggesting the absence of gene flows between the Koh Tachai population and a few continental southern Thailand populations (Table 4), located the Koh Tachai population a rather minor branch in the divergence of *A. atricallosus* in southern Thailand (Fig. 10). Some of the characteristics used to diagnose the subspecies *A. a. classarius* by Sutcharit and Panha (2006a), such as the small shell size (Fig. 12F), may have been rapidly evolved under strong selection forces from insular environment with or without prominent bottlenecks (see above). Thus, it seems to be more appropriate to regard the Koh Tachai population as a distinct evolutionary significant unit (Moritz, 1994; Karl and Bowen, 1998) rather than a subspecies. Alternatively, subspecific separations of the Ranong population and a few other populations might be possible if they have appropriate diagnostic features.

From the allozyme study, it was observed that the Koh Tachai population is in the same cluster with southern Thailand samples (Fig. 10). This may be explained through the fact that, the depth of the ocean lying between Tachai island and the mainland is 120 m deep. However,

during Pleistocene the sea level dropped more than 120 m, therefore exposing a land mass connecting Tachai island and the mainland (Fig. 14: Voris, 2000; Sathiamurthy and Voris, 2006). Thereafter, Koh Tachai may be separated from the mainland by the increase in sea level in the Gulf of Thailand and the Andaman Ocean after 15 ka (Somboon, 1988). The sea level reached a maximum height of 4-5 m above the present mean sea level around 6 ka and became close to the present level around 3.5 ka (Sinsakul, 2000).

Bangkram sample exhibited low heterozygosity of *A. atricallosus*. It is also very likely that the population had recently experienced strong bottleneck effects. Population of Bangkram provide outstanding example of such low heterozygosity. However, detailed additional field work will be required to understand the genetic variability between Bangkram to Pongchang population. Thus, to test effectively this assumption, additional samplings from Bangkram to Pongchang is necessary.

Table 9. Average heterozygosity and F_{st} for the populations of several land snail species estimated on the basis of multi-locus allozyme data.

Species	No. of populations sampled	Average heterozygosity	Range	F_{st}	Reference
<i>Amphidromus atricallosus</i>	13	0.085	0.018-0.201	0.781	Present study
<i>A. inversus</i>	11	0.002	0.0-0.023	0.965	Present study
<i>Bradybaena fruticum</i>	16	0.287	0.138-0.373	0.224	Falniowski <i>et al.</i> , 2004
<i>B. similaris</i>		0.083			Brown and Richardson, 1988
<i>B. fruticum</i>	11	0.144	0.069-0.205		Falniowski <i>et al.</i> , 1993
Land slugs			0.0-0.190		Foltz <i>et al.</i> , 1984
<i>Theba</i>			0.054-0.165		Nevo, 1978
<i>Cepaea nemoralis</i>		0.134			Johnson, 1976
<i>Cristilabrum</i> sp.	5	0.19	0.15-0.22		Woodruff and Solem, 1990
<i>Liguus fasciatus</i>	7		0.0-0.016		Hillis <i>et al.</i> , 1987
<i>Arianta arbustorum</i>	14		0.107-0.236	0.146	Haase and Bisenberger, 2003
<i>Mandarina aureola</i>	23	0.046	0.0-0.11	0.28	Chiba, 1993
<i>M. ponderosa</i>	11	0.073	0.040-0.095	0.13	Chiba, 1993

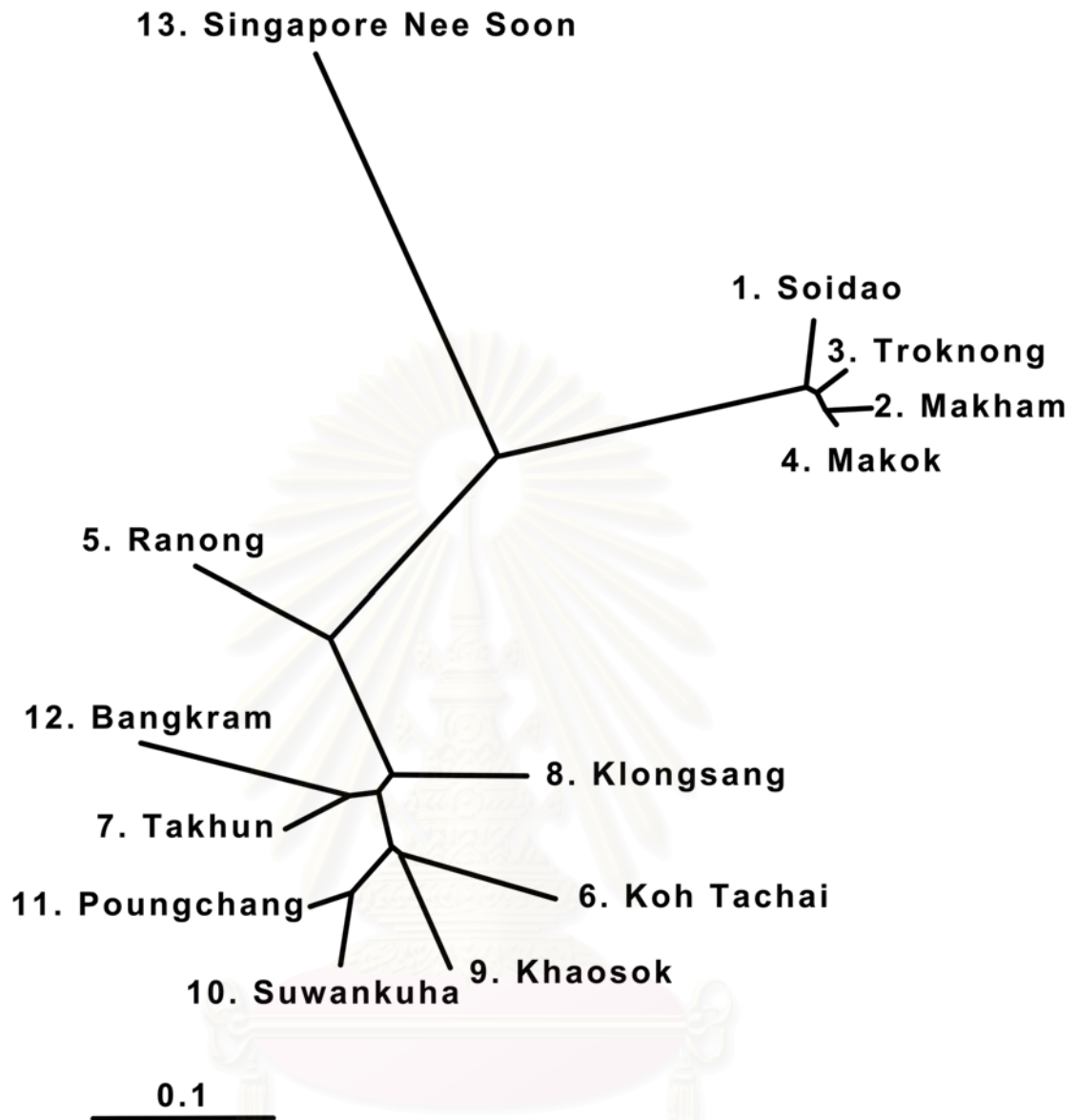


Fig. 10. Neighbor-joining network for the local samples of *Amphidromus atricallosus* based on Rogers' (1972) distance.

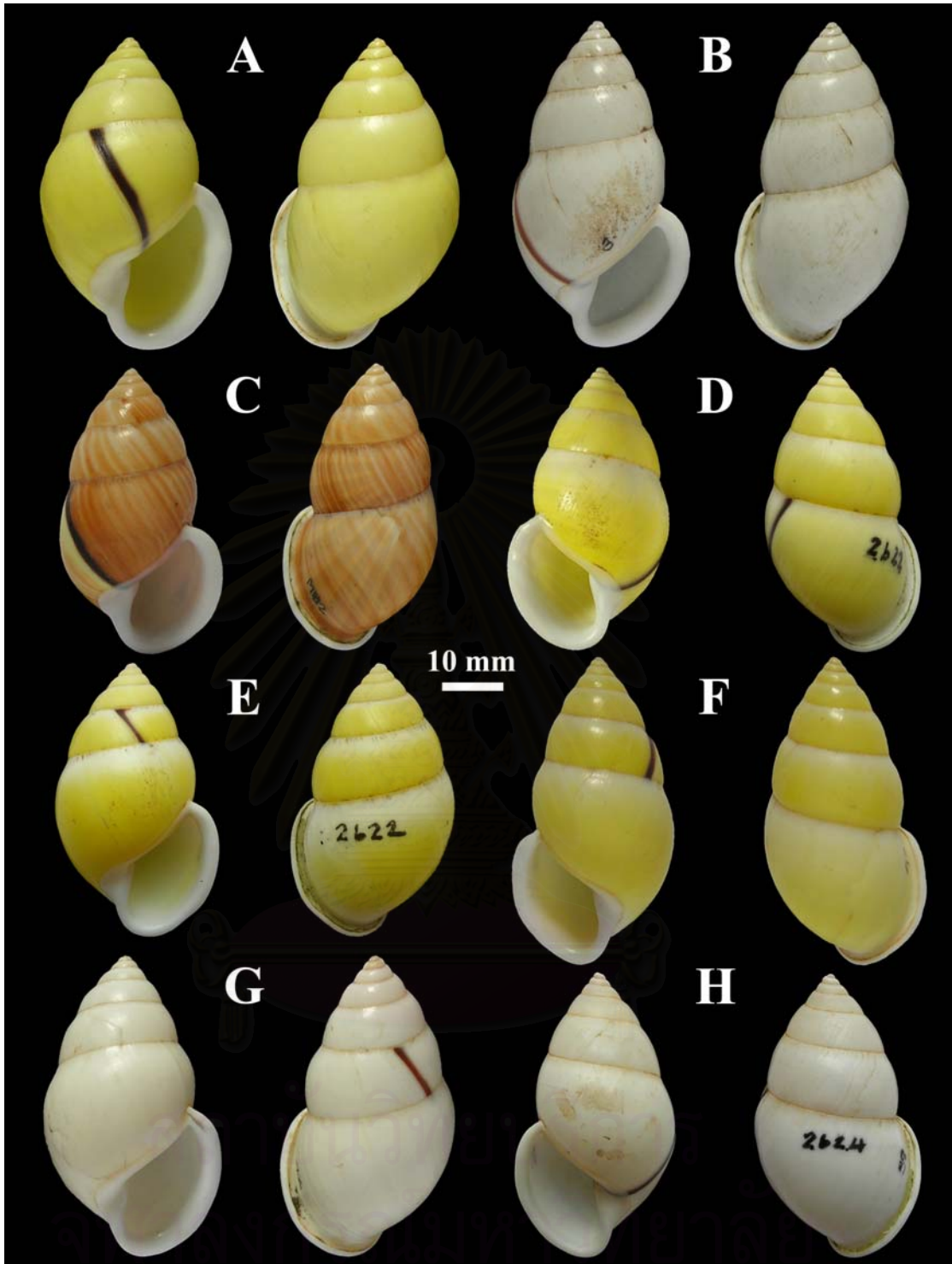


Fig. 11. Shell characteristic of *Amphidromus atricallosus*. **A-B.** *A. a. leucoxanthus* from Soidao, Chantaburi. **C-E.** *A. a. leucoxanthus* from Makham, Chantaburi. **F-H.** *A. a. leucoxanthus* from Makok, Chantaburi.

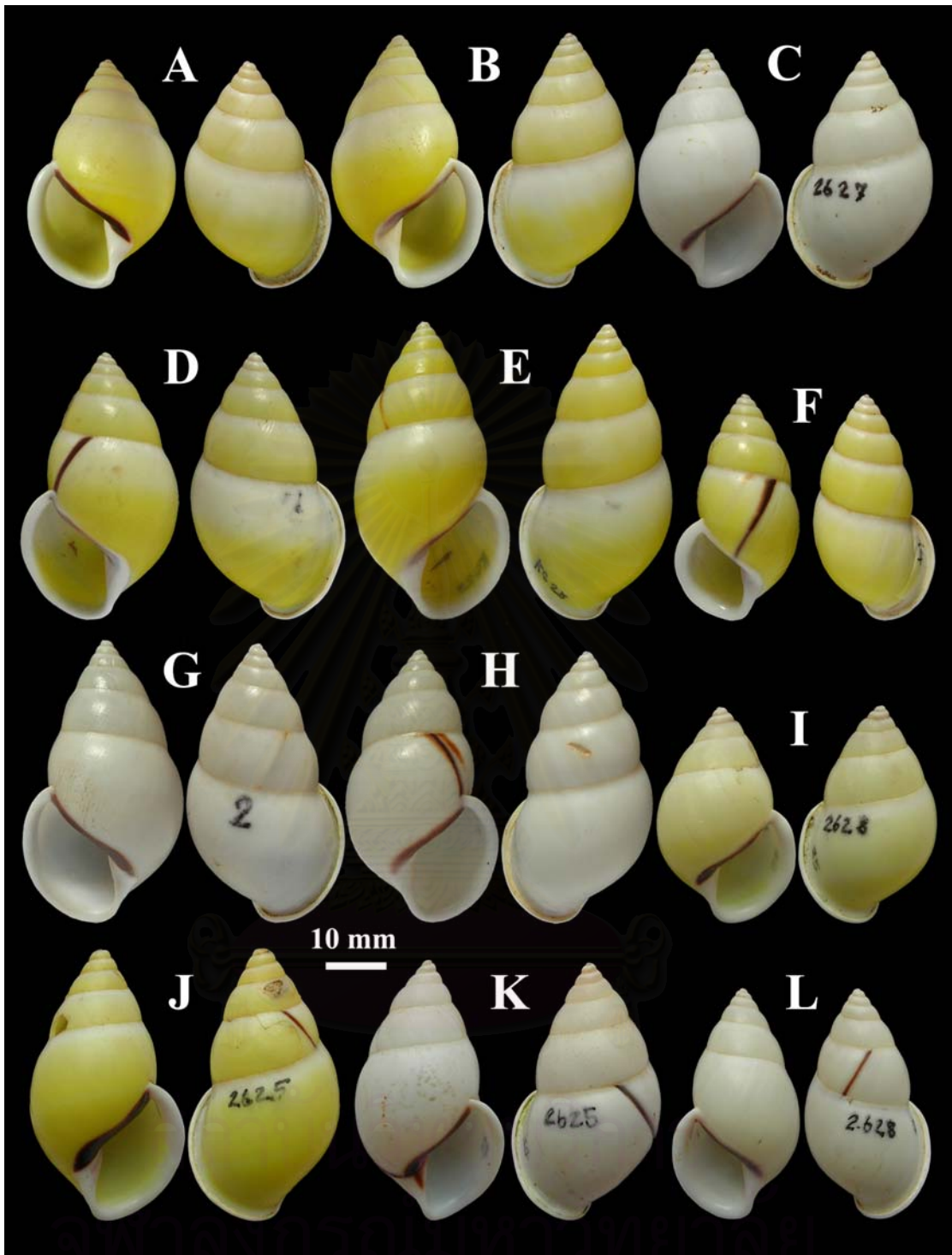


Fig. 12. Shell characteristic of *Amphidromus atricallosus*. **A-C.** *A. a. atricallosus* from Takhun, Suratthani. **D-E, G-H.** *A. a. atricallosus* from Khaosok, Suratthani. **F.** *A. a. classarius* from Koh Tachai, Pangnga. **I, L.** *A. a. atricallosus* from Klongsang, Suratthani. **J-K.** *A. a. atricallosus* from Ranong.

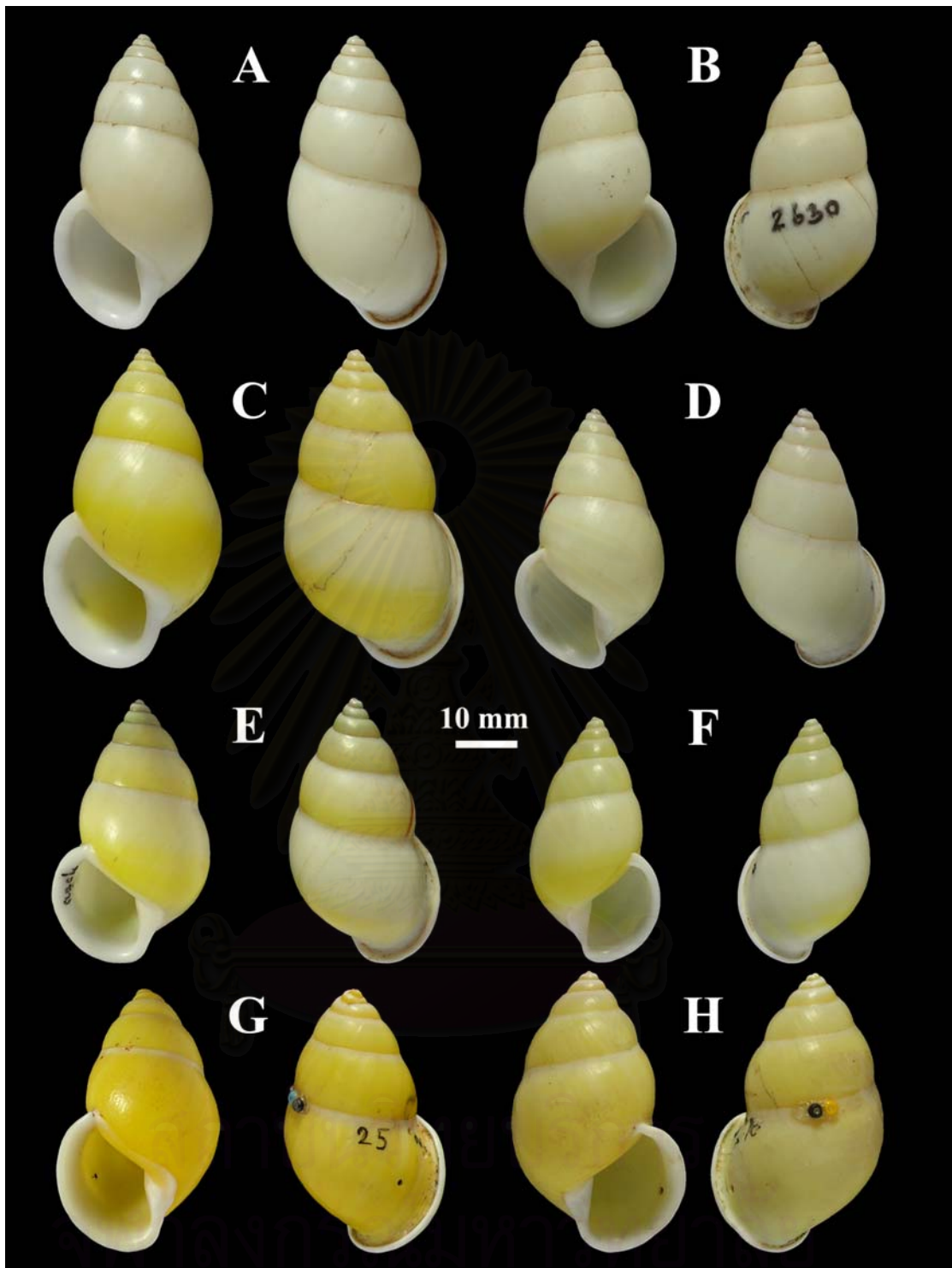


Fig. 13. Shell characteristic of *Amphidromus atricallosus*. **A-C.** *A. atricallosus*, an uncertain status from, Suwankuha, Pang Nga. **D.** *A. atricallosus*, an uncertain status from, Bangkram, Krabi. **E-F.** *A. atricallosus*, an uncertain status from Pongchang, Pang Nga. **G-H.** *A. perakensis* from Singapore Nee Soon, Singapore.

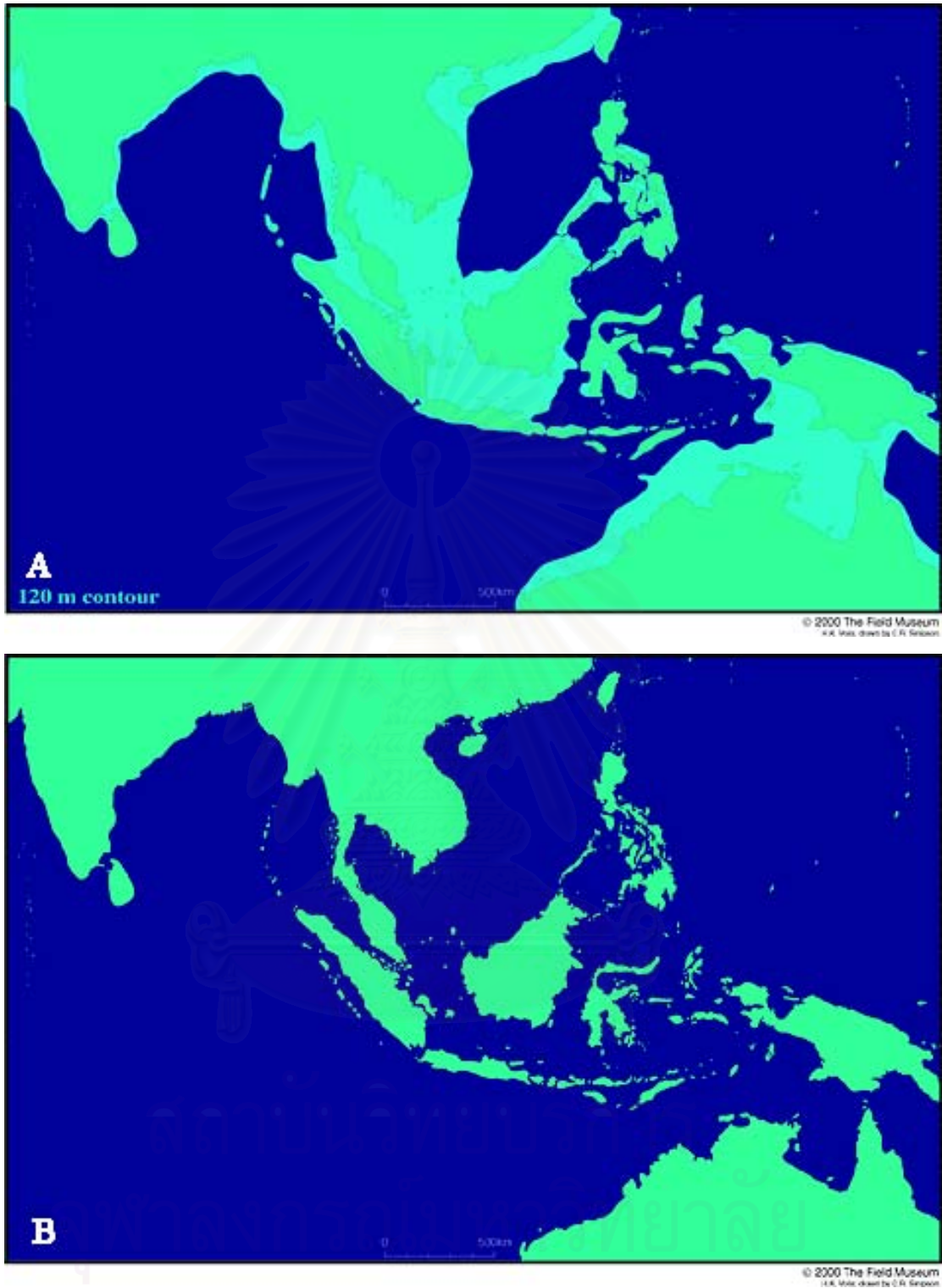


Fig. 14. Map of tropical Southeast Asia and illustrating depth contours. A. 120 m and B. present sea level (Sathiamurthy and Voris, 2006).

Amphidromus inversus is recorded in the Indochina-Malay Peninsula region, Borneo, Sumatra and Sulawesi which bounded by different Cenozoic plate tectonic (Hall, 1997). Moreover, this region was fragmented at various times in the past (Voris, 2000). As a result of biogeographical discontinuities, Southeast Asia is the centre of species diversity in plant and mammal (Roos *et al.*, 2004; Hughes *et al.* 2003). We therefore suspect that the current subspecies of *A. inversus* may have been isolated as distinct species. However, in order to confirm the distinct species boundary, more samples must be collected from other population within the distributional range of *A. inversus*.

Regarding the boundary evidence for biogeographic lines, there are various boundaries between the Australia and Oriental region. The most famous among these boundaries is Wallace's Line, which Alfred Wallace himself proposed to separate his Australian and Oriental region flora and fauna (Fig. 15). Australia has very different mammal fauna and somewhat distinctive when compared to mainland Asia and Oriental region. In general, plant and animal occur in the west of Wallace's Line are demonstrated as Oriental entities, while Australian entities occur in the east. Considering the subspecies *koperbergi*, it occurs in the west of Wallace's Line. We strongly suggest the separated biological species of *A. inversus*.

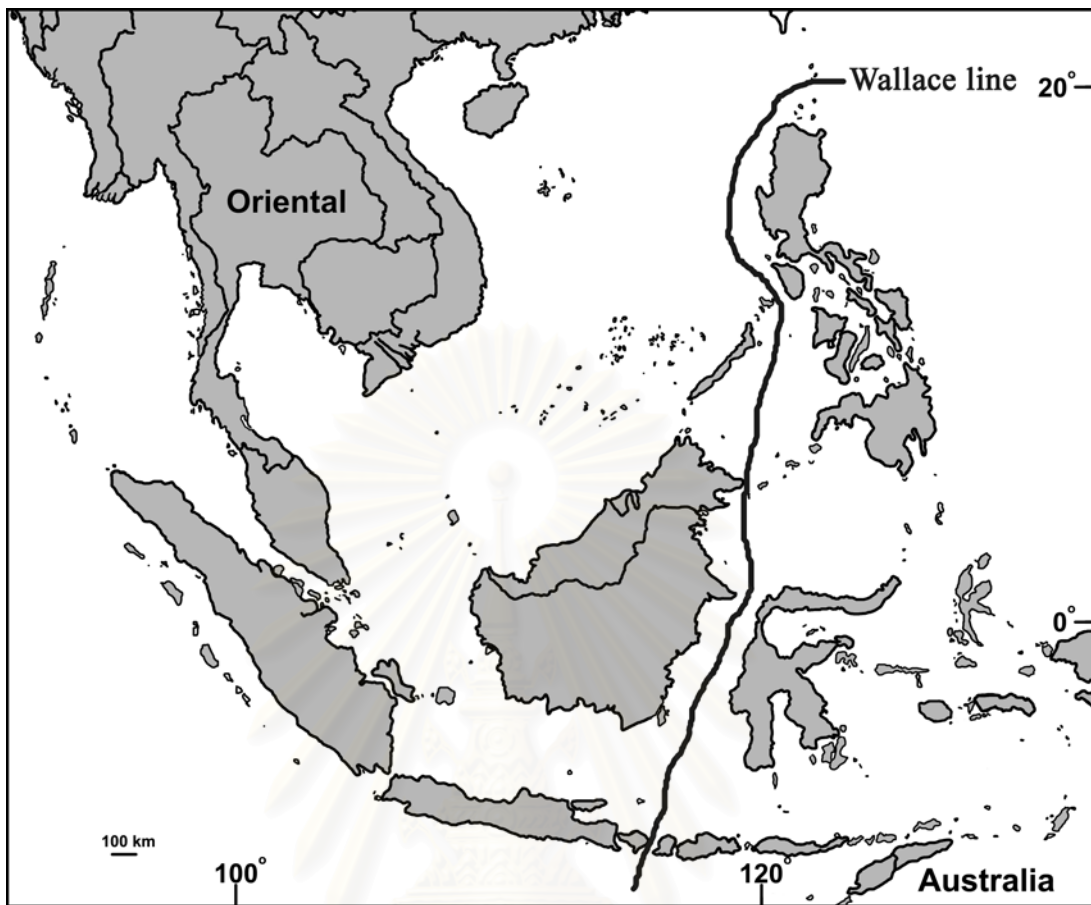


Fig. 15. The biogeographic line that has been proposed to separated the Australasian and Oriental regions.

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APPENDICES

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX I

Genotype data set of *Amphidromus atricallosus*

	<i>Aat-1</i>	<i>Aat-2</i>	<i>Est-2</i>	<i>Gpi</i>	<i>Hbdh</i>	<i>Mdh-2</i>	<i>Me</i>	<i>Mpi</i>	<i>Lgg-1</i>	<i>Lgg-2</i>	<i>Pgm-1</i>	<i>Pgm-2</i>	<i>Pgd</i>
Soidao (SD)													
SD-1	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
SD-10	BB	AA	AB	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
SD-11	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
SD-12	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
SD-13	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
SD-14	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
SD-2	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
SD-3	BB	AA	AA	DD	EE	AA	0	BB	CC	CC	AA	BC	AA
SD-4	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
SD-5	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
SD-6	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
SD-7	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
SD-8	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
SD-9	BB	AA	AB	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
Makham (MH)													
MH-10	BB	AA	AA	DD	CE	AA	AA	BB	CC	CC	AA	BC	AA
MH-11	BB	AA	AA	DD	CE	AA	AA	BB	CC	CC	AA	BC	AA
MH-12	BD	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MH-13	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MH-14	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
MH-15	BB	AA	AA	BD	BE	AA	AA	BB	CC	CC	AA	BB	AA
MH-16	BB	AA	AA	DD	CE	AA	AA	BB	CC	CC	AA	BC	AA
MH-17	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MH-18	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MH-19	BB	AA	AA	BD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MH-20	BB	AA	AA	DD	CE	AA	AA	BB	CC	CC	AA	BC	AA
MH-21	BB	AA	AA	BD	CE	AA	0	BB	CC	CC	AA	BB	AA
MH-22	BB	AA	AA	DD	CE	AA	AA	BB	CC	CC	AA	BC	AA
MH-23	BB	AA	AA	DD	CE	AA	0	BB	CC	CC	AA	BC	AA
MH-24	BB	AA	AA	DD	CC	AA	AA	BB	CC	CC	AA	CC	AA
MH-25	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
MH-26	BB	AA	AA	BD	CE	AA	AA	BB	CC	CC	AA	BC	AA
MH-27	BB	AA	AA	DD	CE	AA	AA	BB	CC	CC	AA	BC	AA
MH-28	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MH-29	BB	AA	AA	DD	CE	AA	AA	BB	CC	CC	AA	BB	AA
MH-30	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MH-31	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
MH-32	BB	AA	AA	BD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MH-33	BB	AA	AA	DD	CE	AA	AA	BB	CC	CC	AA	BC	AA
MH-34	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
MH-35	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MH-36	BB	AA	AA	DD	CE	AA	AA	BB	CC	CC	AA	BB	AA
MH-38	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MH-39	BB	AA	AA	BB	EE	AA	AA	BB	CC	CC	AA	BC	AA
MH-4	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
MH-40	BB	AA	AA	DD	CC	AA	AA	BB	CC	CC	AA	BC	AA
MH-41	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MH-5	BB	AA	AA	BD	EE	AA	AA	BB	CC	CC	AA	CC	AA
MH-6	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MH-7	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MH-8	BB	AA	AA	DD	CE	AA	AA	BB	CC	CC	AA	BB	AA
MH-9	BB	AA	AA	DD	CE	AA	AA	BB	CC	CC	AA	BC	AA

Troknong (TN)

TN-1	BB	AA	AA	DD	EE	AA	AA	BB	CD	CC	AA	BC	AA
TN-10	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	BB	BC	AA
TN-11	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
TN-12	BB	AA	AA	DD	EE	AA	AA	BB	CD	CC	AB	BB	AA
TN-13	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
TN-14	BB	AA	AA	DD	EE	AA	AA	BB	CD	CC	AA	CC	AA
TN-15	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
TN-16	BB	AA	AA	DD	EE	AA	AA	BB	CD	CC	AA	CC	AA
TN-2	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AB	BB	AA
TN-3	BB	AA	AA	DD	EE	AA	AA	BB	CD	CC	AB	BB	AA
TN-4	BB	AA	AA	DD	EE	AA	AA	BB	CD	CC	AA	BB	AA
TN-5	BB	AA	AA	DD	EE	AA	AA	BB	CD	CC	AA	BC	AA
TN-6	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
TN-7	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
TN-8	BB	AA	AA	DD	EE	AA	AA	BB	DD	CC	BB	BB	AA
TN-9	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA

Makok (MK)

MK-1	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MK-10	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-11	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-12	BB	AA	AA	BD	EE	AA	AA	BB	CD	CC	AA	BC	AA
MK-13	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	0	BB	AA
MK-14	BB	AA	AA	DD	EE	AA	AA	BB	CD	CC	AA	BC	AA
MK-15	BB	AA	AA	BD	EE	AA	AA	BB	CD	CC	AA	BC	AA
MK-16	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-17	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
MK-18	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-19	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MK-2	BB	AA	AA	BD	EE	AA	AA	BB	CD	CC	AA	BB	AA
MK-20	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MK-21	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	0	BB	AA
MK-22	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-23	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
MK-24	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	0	BB	AA
MK-25	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MK-26	BD	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-27	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-28	BB	AA	AB	DD	EE	AA	AA	BB	CD	CC	AA	BC	AA
MK-29	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-3	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MK-30	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MK-31	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-32	BB	AA	AA	BD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-33	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MK-34	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-35	BB	AA	AA	DD	EE	AA	AA	BB	CD	CC	AA	BC	AA
MK-36	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-37	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
MK-38	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MK-39	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-4	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
MK-40	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-41	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-42	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BC	AA
MK-43	BD	AA	AA	DD	EE	AA	AA	BB	CD	CC	AA	CC	AA

MK-44	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MK-5	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
MK-6	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
MK-7	BB	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	BB	AA
MK-8	BD	AA	AA	DD	EE	AA	AA	BB	CC	CC	AA	CC	AA
MK-9	BB	AA	AA	DD	EE	AA	AA	BB	CD	CC	AA	BB	AA

Ranong (HS)

HS-1	BB	AA	AA	AC	BD	AA	CC	CC	CC	BB	AA	BC	BB
HS-10	BB	AA	AA	AA	DD	AA	CC	CC	BC	BB	AA	CD	AA
HS-11	BB	AA	AA	AC	BB	AA	CC	CD	CC	BB	AA	CD	AB
HS-12	BB	AA	AA	AA	BD	AA	CC	CC	CC	BB	AA	CC	AA
HS-13	BB	AA	AA	CC	BB	AA	CC	CC	BC	BB	AA	CD	AA
HS-14	BB	AA	AA	AA	DD	AA	CC	CD	CC	BB	AA	BC	AA
HS-15	BB	AA	AA	AC	DD	AA	CC	CC	CC	BB	AA	CC	AA
HS-16	BB	AA	AA	AC	BD	AA	CC	CC	BC	BB	AA	CC	AB
HS-17	BB	AA	AA	AC	DD	AA	CC	CC	BC	BB	AA	CD	AA
HS-18	BB	AA	AA	AA	DD	AA	CC	CC	BC	BB	AA	CC	AA
HS-19	BB	AA	AA	AA	DD	AA	CC	CD	BC	BB	AA	BC	AB
HS-2	BB	AA	AA	AC	BD	AA	CC	CD	CC	BB	AA	CD	AA
HS-20	BB	AA	AA	AA	BD	AA	CC	CD	BC	BB	AA	CC	AB
HS-21	AB	AA	AA	AA	DD	AA	CC	CC	BC	AB	AA	CC	AB
HS-22	BB	AA	AA	AC	BD	AA	CC	CC	CC	BB	AA	CC	AB
HS-23	BB	AA	AA	AA	BD	AA	CC	CD	BC	BB	AA	CD	AA
HS-3	BB	AA	AA	AA	DD	AA	CC	CD	BC	BB	AA	CC	AB
HS-4	AB	AA	AA	AC	0	AA	CC	CC	CC	BB	AA	BC	AA
HS-5	BB	AA	AA	AC	BB	AA	CC	CC	CC	BB	AA	CD	AA
HS-6	BB	AA	AA	AC	BD	AA	CC	CC	CC	BB	AA	CC	AB
HS-7	BB	AA	AA	AC	BD	AA	CC	CC	CC	BB	0	DD	AA
HS-8	BB	AA	AA	CC	BD	AA	CC	CD	CC	BB	AA	CC	AA
HS-9	BB	AA	AA	AC	DD	AA	CC	CD	BC	BB	AA	BC	AB

Koh Tachai (TC)

TC-1	CC	AA	AA	CC	DD	AA	CC	CC	AA	BB	0	DD	AA
TC-10	CC	AA	AA	CC	DD	AA	CC	CC	AA	BB	AA	DD	AA
TC-11	CC	AA	AA	CC	DD	AA	CC	CC	AA	BB	0	DD	AA
TC-12	CC	AA	AA	CC	DD	AA	CC	CC	AA	BB	AA	DD	AA
TC-13	CC	AA	AA	CC	DD	AA	CC	CC	AB	BB	AA	DD	AA
TC-14	CC	AA	AA	CC	DD	AA	CC	CC	AB	BB	AA	DD	AA
TC-15	CC	AA	AA	CC	DD	AA	CC	CC	AA	BB	AA	DD	AA
TC-16	CC	AA	AA	CC	DD	AA	CC	CC	AA	BB	AA	DD	AA
TC-17	CC	AA	AA	CC	DD	AA	CC	CC	BB	BB	AA	DD	AA
TC-18	CC	AA	AA	CC	DD	AA	CC	CC	AB	BB	AA	DD	AA
TC-19	CC	AA	AA	CC	DD	AA	CC	CC	AB	BB	AA	DD	AA
TC-2	CC	AA	AA	CC	DD	AA	CC	CC	AA	BB	AA	DD	AA
TC-20	CC	AA	AA	CC	DD	AA	CC	CC	AA	BB	AA	DD	AA
TC-3	CC	AA	AA	CC	DD	AA	CC	CC	AA	BB	0	DD	AA
TC-4	CC	AA	AA	CC	DD	AA	CC	CC	AA	BB	AA	DD	AA
TC-5	CC	AA	AA	CC	DD	AA	CC	CC	AA	BB	AA	DD	AA
TC-6	CC	AA	AA	CC	DD	AA	CC	CC	AB	BB	AA	DD	AA
TC-7	CC	AA	AA	CC	DD	AA	CC	CC	AA	BB	AA	DD	AA
TC-8	CC	AA	AA	CC	DD	AA	CC	CC	AA	BB	AA	DD	AA
TC-9	CC	AA	AA	CC	DD	AA	CC	CC	AB	BB	AA	DD	AA

Takhun (TK)

TK-1	BC	AA	AA	CC	BD	AA	CC	CC	CC	BB	0	DD	AA
TK-10	CC	AA	AA	CC	BD	AA	CC	CC	BC	BB	AA	DD	AA
TK-11	BC	AA	AA	CC	BD	AA	CC	CC	BB	BB	AA	DD	AA

TK-12	BC	AA	AA	CC	0	AA	CC	CC	CC	BB	AA	DD	AA
TK-13	BC	AA	AA	CC	BB	AA	CC	CC	CC	BB	0	DD	AA
TK-14	BB	AA	AA	CC	BD	AA	CC	CC	BC	BB	AA	DD	AA
TK-2	BC	AA	AA	CC	BD	AA	CC	CC	CC	AB	0	DD	AA
TK-3	BB	AA	AA	CC	BB	AA	CC	CC	CC	AB	AA	DD	AA
TK-4	BB	AA	AA	CC	BB	AA	CC	CC	CC	AB	AA	DD	AA
TK-5	BB	AA	AB	CC	BB	AA	CC	CC	CC	BB	AA	DD	AA
TK-6	AB	AA	AA	CC	BB	AA	CC	CC	CC	AA	0	DD	AA
TK-7	BB	AA	AA	CC	BD	AA	CC	CC	CC	AB	0	DD	AA
TK-8	BB	AA	AA	CC	BB	AA	CC	CC	CC	AA	0	DD	AA
TK-9	BB	AA	AA	CC	BB	AA	CC	CC	BC	BB	AA	DD	AA

Klongsang (PP)

PP-1	BB	AA	AA	CC	BE	AA	CC	CC	BC	BB	AA	DD	AA
PP-10	BB	AA	AA	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-11	BB	AA	AB	CC	BE	AA	CC	CC	BB	BB	AA	DD	AA
PP-12	BB	AA	AA	AC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-13	BB	AA	AB	CC	EE	AA	CC	CC	BB	BB	AA	DD	AA
PP-14	BC	AA	AA	CC	BE	AA	CC	CC	BC	BB	AA	DD	AA
PP-15	BC	AA	AA	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-16	BB	AA	AA	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-17	BB	AA	AA	AC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-18	BB	AA	AA	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-19	BB	AA	AA	CC	EE	AA	CC	CC	CC	BB	AA	DD	AA
PP-2	BB	AA	AA	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-20	BB	AA	AA	CC	EE	AA	CC	CC	BC	BB	AA	DD	0
PP-21	BB	AA	AA	AC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-22	BB	AA	AA	CC	EE	AA	CC	CC	AB	BB	AA	DD	AA
PP-23	BB	AA	AA	CC	EE	AA	CC	CC	BB	BB	AA	DD	AA
PP-24	BB	AA	AA	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-25	BB	AA	AA	CC	EE	AA	CC	CC	BB	BB	AA	DD	AA
PP-26	BB	AA	AA	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-27	BB	AA	AA	CC	EE	AA	CC	CC	CC	BB	AA	DD	AA
PP-28	BB	AA	AB	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-29	BB	AA	AA	CC	EE	AA	CC	CC	BB	BB	AA	DD	AA
PP-3	BB	AA	AA	CC	EE	AA	CC	CC	BB	BB	AA	DD	AA
PP-30	BB	AA	AB	CC	EE	AA	CC	CC	BB	BB	AA	DD	AA
PP-31	BB	AA	AB	CC	EE	AA	CC	CC	BB	BB	AA	DD	AA
PP-32	BB	AA	AA	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-33	BB	AA	AA	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-34	BB	AA	AA	CC	EE	AA	CC	CC	BC	BB	AB	DD	AA
PP-35	BB	AA	AA	CC	EE	AA	CC	CC	BB	BB	AA	DD	AA
PP-36	BB	AA	AA	CC	EE	AA	CC	CC	CC	BB	AA	DD	AA
PP-37	BB	AA	AB	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-38	BB	AA	AB	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-39	BB	AA	AB	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-4	BB	AA	AA	CC	BE	AA	CC	CC	BC	BB	AA	DD	AA
PP-40	BB	AA	AB	CC	BE	AA	CC	CC	BC	BB	AA	DD	AA
PP-41	BB	AA	AB	CC	EE	AA	CC	CC	BC	BB	AA	DD	0
PP-42	BB	AA	AB	CC	BE	AA	CC	CC	BC	BB	AA	DD	AA
PP-5	BB	AA	AA	CC	BE	AA	CC	CC	BB	BB	AA	DD	AA
PP-6	BB	AA	AA	CC	BE	AA	CC	CC	BC	BB	AA	DD	AA
PP-7	BB	AA	AA	CC	EE	AA	CC	CC	BC	BB	AA	DD	AA
PP-8	BB	AA	BB	CC	EE	AA	CC	CC	BB	BB	AA	DD	AA
PP-9	BB	AA	AB	CC	EE	AA	CC	CC	BB	BB	AB	DD	AA

Khaosok (KS)

KS-1	CC	AA	AA	CC	BB	AA	CC	CC	CC	BB	AA	AA	AA
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KS-10	CC	AA	AB	CC	DD	AA	CC	CC	BB	BB	AA	DD	AA
KS-11	AB	AA	AB	CC	DD	AA	CC	CC	BC	BB	AA	DD	AA
KS-12	BC	AA	BB	CC	DD	AA	CC	CC	BB	BB	0	DD	AA
KS-13	BC	AA	AB	CC	DD	AA	CC	CC	BC	BB	AA	DD	AA
KS-14	CC	AA	AB	CC	BB	AA	CC	CC	BC	BB	AA	DD	BB
KS-15	AC	AA	AA	CC	BB	AA	CC	CC	BC	BB	0	AD	AA
KS-16	BC	AA	AA	CC	BD	AA	CC	CC	CC	BB	AA	DD	AA
KS-17	CC	AA	BB	CC	BD	AA	CC	CC	BC	BB	AA	DD	AB
KS-18	BC	AA	AB	CC	DD	AA	CC	CC	BC	BB	AA	DD	AA
KS-19	CC	AA	AB	CC	DD	AA	CC	CC	AC	BB	AA	BD	AA
KS-2	AC	AA	AB	CC	BB	AA	CC	CC	CC	BB	AA	DD	AA
KS-20	CC	AA	AB	CC	DD	AA	CC	CC	CC	BB	AA	AD	AA
KS-21	BB	AA	AB	CC	BD	AA	CC	CC	BC	BB	AA	AA	AA
KS-22	CC	AA	AA	CC	DD	AA	CC	CC	BC	BB	AA	DD	AA
KS-23	BC	AA	BB	CC	BD	AA	CC	CC	BC	BB	AA	DD	AA
KS-24	BB	AA	AA	CC	BD	AA	CC	CC	CC	BB	AA	DD	AA
KS-25	BC	AA	AB	CC	BD	AA	CC	CC	AC	BB	0	AD	AA
KS-26	CC	AA	AB	CC	BD	AA	CC	CC	BC	BB	0	DD	AA
KS-27	BC	AA	AB	CC	BD	AA	CC	CC	BC	BB	AA	DD	AA
KS-28	CC	AA	AA	CC	BB	AA	CC	CC	CC	BB	0	DD	AA
KS-29	BC	AA	AA	CC	DD	AA	CC	CC	BB	BB	0	AA	AA
KS-3	AC	AA	AB	CC	BD	AA	CC	CC	BC	BB	AA	AD	AA
KS-30	CC	AA	BB	CC	BD	AA	CC	CC	BC	BB	AA	DD	AA
KS-31	CC	AA	BB	CC	BB	AA	CC	CC	CC	BB	AA	DD	AA
KS-32	BC	AA	AB	CC	DD	AA	CC	CC	BC	BB	AA	DD	AA
KS-33	AC	AA	AA	CC	DD	AA	0	CC	CC	BB	AA	AD	AA
KS-4	BC	AA	BB	CC	DD	AA	CC	CC	BC	BB	AA	DD	AA
KS-5	CC	AA	AB	CC	BD	AA	CC	CC	BB	BB	AA	DD	AA
KS-6	CC	AA	BB	CC	BB	AA	CC	CC	BC	BB	AA	AC	AA
KS-7	CC	AA	AB	CC	BD	AA	CC	CC	BC	BB	AA	DD	AA
KS-8	BC	AA	AA	CC	BB	AA	CC	CC	CC	BB	0	AD	AA
KS-9	CC	AA	AB	CC	DD	AA	CC	CC	BC	BB	AA	AD	AA

Suwankuha (SW)

SW-1	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-10	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-11	BC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AB	DD	AA
SW-12	BC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AB	DD	AA
SW-13	BC	AA	AA	CC	BB	AA	CC	CC	BB	BB	BB	DD	AA
SW-14	BB	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-15	BC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-16	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-17	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-18	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AB	DD	AA
SW-19	BC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-2	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	0	DD	AA
SW-20	BC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AB	DD	AA
SW-21	BC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AB	DD	AA
SW-22	BC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AB	DD	AA
SW-23	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-24	BC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-25	BC	AA	AA	CC	BB	AA	CC	CC	0	BB	0	DD	AA
SW-26	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-27	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-28	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-29	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-3	BC	AA	AA	CC	0	AA	CC	CC	BB	BB	0	DD	AA
SW-30	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA

SW-31	BB	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-4	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	CD	AA
SW-5	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-6	BB	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-7	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AB	DD	AA
SW-8	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
SW-9	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AB	DD	AA

Poungchang (PC)

PC-1	CC	AA	AA	CC	BB	AA	CC	CC	BC	BB	0	DD	AA
PC-10	CC	AA	AA	CC	BC	AA	CC	CC	BB	BB	0	DD	AA
PC-11	CC	AA	AA	CC	BC	AA	CC	CC	CC	BB	AA	DD	AA
PC-12	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
PC-13	BC	AA	AA	CC	BB	AA	CC	CC	0	BB	AA	DD	AA
PC-14	CC	AA	AA	CC	BB	AA	CC	CD	BB	BB	AA	DD	AA
PC-15	CC	AA	AA	CC	BC	AA	CC	CC	BB	BB	AA	DD	AA
PC-16	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
PC-17	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
PC-18	BC	AA	AA	CC	BB	AA	CC	CC	BC	BB	AA	DD	AA
PC-19	BC	AA	AA	CC	BB	AA	CC	CC	BC	BB	AA	DD	AA
PC-2	CC	AA	AA	CC	BC	AA	CC	CC	BB	BB	AA	DD	AA
PC-3	CC	AA	AA	CC	BC	AA	CC	CC	BB	BB	AA	CD	AA
PC-4	CC	AA	AA	CC	BC	AA	CC	CC	BC	BB	0	DD	AA
PC-5	CC	AA	AA	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
PC-6	CC	AA	AB	CC	BB	AA	CC	CC	BB	BB	AA	DD	AA
PC-7	CC	AA	AA	CC	BC	AA	CC	CD	BB	BB	AA	DD	AA
PC-8	CC	AA	AA	CC	BB	AA	CC	CC	0	BB	AA	DD	AA
PC-9	CC	AA	AA	CC	BB	AA	CC	CC	CC	BB	AA	DD	AA

Bangkram (BK)

BK-1	BB	AA	AA	CC	BB	AA	CC	CC	AA	AA	AA	DD	AA
BK-2	BB	AA	AA	CC	BB	AA	CC	CC	AA	AA	AA	DD	AA
BK-3	BB	AA	AA	CC	BB	AA	CC	CC	AB	AA	AA	DD	AA
BK-4	BB	AA	AA	CC	BB	AA	CC	CC	AA	AA	AA	DD	AA
BK-5	BB	AA	AA	CC	BB	AA	CC	CC	AA	AA	AA	DD	AA
BK-6	BB	AA	AA	CC	BB	AA	CC	CC	AA	AA	AA	DD	AA
BK-7	BB	AA	AA	CC	BB	AA	CC	CC	AA	AA	AA	DD	AA
BK-8	BB	AA	AA	CC	BB	AA	CC	CC	AB	AA	AA	DD	AA

Singapore Nee Soon (SIN)

SIN-1	BD	AA	AA	DD	AA	AA	CC	AA	DD	CC	AA	CC	BB
SIN-10	DD	AA	AA	DD	AA	AA	CC	AA	DD	CC	AA	CC	BB
SIN-11	BD	AA	AA	DD	AA	AA	CC	AA	CD	CC	AA	CC	BB
SIN-12	BD	AA	AA	DD	AA	AA	CC	AA	DD	CC	AA	CC	BB
SIN-13	BD	AA	AA	DD	AA	AA	CC	AA	DD	CC	AA	CC	BB
SIN-14	BD	AA	AA	DD	AD	AA	CC	AA	DD	CC	AA	CC	BB
SIN-15	DD	AA	AA	DD	AA	AA	CC	AA	DD	CC	AA	CC	BB
SIN-16	BB	AA	AA	DD	AA	AA	CC	AA	DD	CC	AA	CC	BB
SIN-17	DD	AA	AA	DD	AD	AA	CC	0	DD	CC	AA	CC	BB
SIN-18	BD	AA	AA	DD	AA	AA	CC	AA	DD	CC	AA	CC	BB
SIN-2	BB	AA	AA	DD	AA	AA	0	AA	DD	CC	0	CC	BB
SIN-3	DD	AA	AA	DD	AA	AA	CC	AA	DD	CC	AA	CC	BB
SIN-4	DD	AA	AA	DD	AA	AA	CC	AA	DD	CC	AA	CC	BB
SIN-6	DD	AA	AA	DD	AA	AA	CC	AA	DD	CC	AA	CC	BB
SIN-7	DD	AA	AA	DD	AA	AA	CC	AA	DD	CC	AA	CC	BB
SIN-9	DD	AA	AA	DD	AA	AA	CC	AA	DD	CC	AA	CC	BB
SIN-5	AA	AA	AA	DD	AA	AA	CC	AA	DD	CC	0	CC	BB
SIN-8	AA	AA	AA	DD	AA	AA	CC	AA	DD	CC	AA	CC	BB

Genotype data sets of *Amphidromus inversus*

	<i>Aat-1</i>	<i>Est-1</i>	<i>Est-2</i>	<i>Est-3</i>	<i>Est-4</i>	<i>Gpi</i>	<i>Hbdh</i>	<i>Mdh-1</i>	<i>Mdh-2</i>	<i>Me</i>	<i>Mpi</i>	<i>Lgg-1</i>	<i>Lgg-2</i>	<i>Lgg-3</i>	<i>Pgm-1</i>	<i>Pgm-2</i>	<i>Pgd</i>	<i>Sod</i>
Chaiya (CY)																		
CY1	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY10	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY11	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY12	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY13	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY14	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY15	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY16	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY17	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY18	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	0	AA
CY19	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY2	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY20	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	0	AA
CY21	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY22	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY23	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY3	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY4	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY5	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY6	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY7	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY8	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
CY9	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
Koh Elar (KE)																		
KE1	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KE2	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KE3	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA

KE4	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KE5	AA	AA	AA	AA	BB	AA	AA	AA	AA	0	AA	BB	BB	BB	AA	AA	AA	AA
KE6	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KE7	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KE8	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA

Koh Jarn (KJA)

KJA1	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KJA2	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KJA3	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KJA4	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA

Koh Kham (KK)

KK1	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KK10	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KK2	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KK3	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KK4	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KK5	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KK6	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KK7	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KK8	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KK9	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA

Koh Luarm (KL)

KL1	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KL10	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	0	AA	AA	AA
KL11	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KL12	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	0	AA	AA	AA
KL13	AA	AA	AA	AA	BB	AA	AA	AA	AA	0	AA	BB	BB	BB	0	AA	AA	AA
KL14	AA	AA	AA	AA	BB	AA	AA	AA	AA	0	AA	BB	BB	BB	0	0	AA	AA
KL15	AA	AA	AA	AA	BB	AA	AA	AA	AA	0	AA	BB	BB	BB	0	AA	AA	AA

KL16	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KL17	AA	AA	AA	AA	BB	AA	AA	AA	AA	0	AA	BB	BB	BB	0	AA	0	AA
KL18	AA	AA	AA	AA	BB	AA	AA	AA	AA	0	AA	BB	BB	BB	AA	AA	AA	AA
KL19	AA	AA	AA	AA	BB	AA	AA	AA	AA	0	AA	BB	BB	BB	0	AA	AA	AA
KL2	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KL3	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KL4	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KL5	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KL6	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KL7	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KL8	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KL9	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	0	AA	AA	AA

Koh Pai (KP)

KP1	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KP10	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KP11	AA	AA	AA	AA	BB	AA	AA	AA	AA	0	AA	BB	BB	BB	AA	AA	0	AA
KP12	AA	AA	AA	AA	BB	AA	AA	AA	AA	0	AA	BB	BB	BB	0	AA	AA	AA
KP13	AA	AA	AA	AA	BB	AA	AA	AA	AA	0	AA	BB	BB	BB	0	AA	AA	AA
KP2	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KP3	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KP4	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KP5	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KP6	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KP7	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KP8	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KP9	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA

Pulau Kapas (KPM)

KPM1	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA
KPM10	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA
KPM12	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	0	AA	AA	AA

KPM13	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA
KPM14	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	0	AA	AA	AA
KPM17	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA
KPM19	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	0	AA	AA	AA
KPM29	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA
KPM38	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	0	AA	AA	AA
KPM42	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	0	AA	AA	AA
KPM44	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA
KPM45	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	0	AA	AA	AA
KPM48	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	0	AA	AA	AA
KPM50	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA
KPM54	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	0	AA	AA	AA
KPM57	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA
KPM63	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA
KPM71	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA
KPM9	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	AA	AA	BB	0	AA	AA	AA

Koh Samet (KSM)

KSM1	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KSM2	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KSM3	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KSM4	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KSM5	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KSM6	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KSM7	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA
KSM8	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	AA	AA	AA

Koh Samui (KSS)

KSS1	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	AA	AA	BB	AA	AA
KSS10	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KSS11	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	AB	AA	BB	AA	AA
KSS12	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA

KSS13	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	AB	AA	BB	AA	AA
KSS14	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KSS15	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	AB	AA	BB	AA	AA
KSS16	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	AB	AA	BB	AA	AA
KSS17	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KSS18	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	AB	AA	BB	AA	AA
KSS2	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	AB	AA	BB	AA	AA
KSS3	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KSS4	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KSS5	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KSS6	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KSS7	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KSS8	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KSS9	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	AA	AA	BB	AA	AA

Koh Tan (KT)

KT1	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KT10	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KT11	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KT12	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KT13	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KT14	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KT2	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KT3	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KT4	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	AB	AA	BB	AA	AA
KT5	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KT6	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KT7	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KT8	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	AA	BB	AA	AA
KT9	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA	AA	BB	BB	BB	0	BB	AA	AA

Singapore Botanic Garden (SIN)

SIN1	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	0	AA	AA	AA
SIN2	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	0	AA	AA	AA
SIN3	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA
SIN4	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA
SIN5	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	0	AA	AA	AA
SIN6	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	0	AA
SIN7	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	0	AA
SIN8	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	BB	0	AA	0	AA



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APPENDIX II

Table 11. Chi-square test for deviation from Hardy-Weinberg equilibrium in Soidao.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
<i>Est-2</i>	A-A	12	12.071	0.083	1	0.773
	A-B	2	1.857			
	B-B	0	0.071			
<i>Pgm-2</i>	B-B	0	0.071	0.083	1	0.773
	B-C	2	1.857			
	C-C	12	12.071			

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Table 12. Chi-square test for deviation from Hardy-Weinberg equilibrium in Makham.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
<i>Aat-1</i>	B-B	36	36.007	0.007	1	0.934
	B-D	1	0.986			
	D-D	0	0.007			
<i>Gpi</i>	B-B	1	0.432	0.936	1	0.333
	B-D	6	7.135			
	D-D	30	29.432			
<i>Hbdh</i>	B-B	0	0.007	0.393	3	0.942
	B-C	0	0.243			
	B-E	1	0.743			
	C-C	2	2.189			
	C-E	14	13.378			
	E-E	20	20.439			
<i>Pgm-2</i>	B-B	11	11.358	0.057	1	0.812
	B-C	19	18.284			
	C-C	7	7.358			

Table 13. Chi-square test for deviation from Hardy-Weinberg equilibrium in Troknong.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
<i>Lgg-1</i>						
	C-C	8	8.266			
	C-D	7	6.469			
	D-D	1	1.266			
				0.108	1	0.743
<i>Pgm-1</i>						
	A-A	11	9.766			
	A-B	3	5.469			
	B-B	2	0.766			
				3.261	1	0.071
<i>Pgm-2</i>						
	B-B	7	5.641			
	B-C	5	7.719			
	C-C	4	2.641			
				1.985	1	0.159

Table 14. Chi-square test for deviation from Hardy-Weinberg equilibrium in Makok.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
<i>Aat-1</i>	B-B	41	41.051	0.055	1	0.815
	B-D	3	2.898			
	D-D	0	0.051			
<i>Est-2</i>	A-A	43	43.006	0.006	1	0.939
	A-B	1	0.989			
	B-B	0	0.006			
<i>Gpi</i>	B-B	0	0.091	0.100	1	0.752
	B-D	4	3.818			
	D-D	40	40.091			
<i>Lgg-1</i>	C-C	36	36.364	0.440	1	0.507
	C-D	8	7.273			
	D-D	0	0.364			
<i>Pgm-2</i>	B-B	15	14.778	0.019	1	0.891
	B-C	21	21.443			
	C-C	8	7.778			

Table 15. Chi-square test for deviation from Hardy-Weinberg equilibrium in Ranong.

Locus	Class	Observed frequency	Expected frequency	Chi- square	DF	P
<i>Aat-1</i>	A-A	0	0.043	0.048	1	0.827
	A-B	2	1.913			
	B-B	21	21.043			
<i>Gpi</i>	A-A	9	9.783	0.517	1	0.472
	A-C	12	10.435			
	C-C	2	2.783			
<i>Hbdh</i>	B-B	3	2.909	0.007	1	0.933
	B-D	10	10.182			
	D-D	9	8.909			
<i>Mpi</i>	C-C	14	14.880	1.361	1	0.243
	C-D	9	7.239			
	D-D	0	0.880			
<i>Lgg-1</i>	B-B	0	1.315	2.272	1	0.132
	B-C	11	8.370			
	C-C	12	13.315			
<i>Lgg-2</i>	A-A	0	0.011	0.011	1	0.915
	A-B	1	0.978			
	B-B	22	22.011			
<i>Pgm-2</i>	B-B	0	0.272	2.134	3	0.545
	B-C	5	3.478			
	B-D	0	0.978			
	C-C	10	11.130			
	C-D	7	6.261			
	D-D	1	0.880			
<i>Pgd</i>	AA	13	13.315	0.130	1	0.718
	AB	9	8.370			
	BB	1	1.315			

Table 16. Chi-square test for deviation from Hardy-Weinberg equilibrium in Tachai.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
<i>Lgg-1</i>						
	A-A	13	12.800			
	A-B	6	6.400			
	B-B	1	0.800			
				0.078	1	0.780



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Table 17. Chi-square test for deviation from Hardy-Weinberg equilibrium in Takhun.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
<i>Aat-1</i>	A-A	0	0.018	0.403	3	0.940
	A-B	1	0.714			
	A-C	0	0.250			
	B-B	7	7.143			
	B-C	5	5.000			
	C-C	1	0.875			
<i>Est-2</i>	A-A	13	13.018	0.019	1	0.890
	A-B	1	0.964			
	B-B	0	0.018			
<i>Hbdh</i>	B-B	7	7.692	1.170	1	0.279
	B-D	6	4.615			
	D-D	0	0.692			
<i>Lgg-1</i>	B-B	1	0.446	1.017	1	0.313
	B-C	3	4.107			
	C-C	10	9.446			
<i>Lgg-2</i>	A-A	2	1.143	1.260	1	0.262
	A-B	4	5.714			
	B-B	8	7.143			

Table 18. Chi-square test for deviation from Hardy-Weinberg equilibrium in Klongsang.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
<i>Aat-1</i>	B-B	40	40.024	0.025	1	0.874
	B-C	2	1.952			
	C-C	0	0.024			
<i>Est-2</i>	A-A	29	29.167	0.034	1	0.853
	A-B	12	11.667			
	B-B	1	1.167			
<i>Gpi</i>	A-A	0	0.054	0.058	1	0.810
	A-C	3	2.893			
	C-C	39	39.054			
<i>Hbdh</i>	B-B	0	0.381	0.465	1	0.495
	B-E	8	7.238			
	E-E	34	34.381			
<i>Lgg-1</i>	A-A	0	0.006	5.219	3	0.156
	A-B	1	0.607			
	A-C	0	0.381			
	B-B	12	15.482			
	B-C	26	19.429			
	C-C	3	6.095			
<i>Pgm-1</i>	AA	40	40.024	0.025	1	0.874
	AB	2	1.952			
	BB	0	0.024			

Table 19. Chi-square test for deviation from Hardy-Weinberg equilibrium in Khaosok.

Locus	Class	Observed frequency	Expected frequency	Chi- square	DF	P
<i>Aat-1</i>	A-A	0	0.189	0.339	3	0.953
	A-B	1	1.212			
	A-C	4	3.409			
	B-B	2	1.939			
	B-C	11	10.909			
	C-C	15	15.341			
<i>Est-2</i>	A-A	9	9.280	0.038	1	0.845
	A-B	17	16.439			
	B-B	7	7.280			
<i>Hbdh</i>	B-B	8	5.939	2.157	1	0.142
	B-D	12	16.121			
	D-D	13	10.939			
<i>Lgg-1</i>	A-A	0	0.030	2.646	3	0.449
	A-B	0	0.788			
	A-C	2	1.152			
	B-B	4	5.121			
	B-C	18	14.970			
	C-C	9	10.939			
<i>Pgm-2</i>	A-A	3	1.485	7.000	6	0.321
	A-B	0	0.212			
	A-C	1	0.212			
	A-D	7	10.606			
	B-B	0	0.008			
	B-C	0	0.015			
	B-D	1	0.758			
	C-C	0	0.008			
	C-D	0	0.758			
	D-D	21	18.939			
<i>Pgd</i>	A-A	31	31.008	0.008	1	0.928
	A-B	1	0.984			
	B-B	0	0.008			

Table 20. Chi-square test for deviation from Hardy-Weinberg equilibrium equilibrium in Suwankuha.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
<i>Aat-1</i>	B-B	3	2.331	0.365	1	0.546
	B-C	11	12.339			
	C-C	17	16.331			
<i>Pgm-1</i>	A-A	19	18.893	0.019	1	0.890
	A-B	8	8.214			
	B-B	1	0.893			
<i>Pgm-2</i>	C-C	0	0.008	0.008	1	0.927
	C-D	1	0.984			
	D-D	30	30.008			

Table 21. Chi-square test for deviation from Hardy-Weinberg equilibrium in Pongchang.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
<i>Aat-1</i>	B-B	0	0.118	0.140	1	0.709
	B-C	3	2.763			
	C-C	16	16.118			
<i>Est-2</i>	A-A	18	18.013	0.014	1	0.906
	A-B	1	0.974			
	B-B	0	0.013			
<i>Hbdh</i>	B-B	12	12.645	0.969	1	0.325
	B-C	7	5.711			
	C-C	0	0.645			
<i>Mpi</i>	C-C	17	17.053	0.059	1	0.809
	C-D	2	1.895			
	D-D	0	0.053			
<i>Lgg-1</i>	B-B	11	9.941	2.037	1	0.154
	B-C	4	6.118			
	C-C	2	0.941			
<i>Pgm-2</i>	C-C	0	0.013	0.014	1	0.906
	C-D	1	0.974			
	D-D	18	18.013			

Table 22. Chi-square test for deviation from Hardy-Weinberg equilibrium in Bangkram.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
<i>Lgg-1</i>						
	A-A	6	6.125			
	A-B	2	1.750			
	B-B	0	0.125			
				0.163	1	0.686



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Table 23. Chi-square test for deviation from Hardy-Weinberg equilibrium in Singapore Nee Soon.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
<i>Aat-1</i>						
	A-A	2	0.222			
	A-B	0	1.111			
	A-D	0	2.444			
	B-B	2	1.389			
	B-D	6	6.111			
	D-D	8	6.722			
				18.292	3	0.000
<i>Hbdh</i>						
	A-A	16	16.056			
	A-D	2	1.889			
	D-D	0	0.056			
				0.062	1	0.803
<i>Lgg-1</i>						
	C-C	0	0.014			
	C-D	1	0.972			
	D-D	17	17.014			
				0.015	1	0.904

Table 24. Chi-square test for deviation from Hardy-Weinberg equilibrium in Koh Samui.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
<i>Lgg-3</i>						
	A-A	2	1.389			
	A-B	6	7.222			
	B-B	10	9.389			
				0.516	1	0.473

Table 25. Chi-square test for deviation from Hardy-Weinberg equilibrium in Koh Tan.

Locus	Class	Observed frequency	Expected frequency	Chi-square	DF	P
<i>Lgg-3</i>						
	A-A	0	0.018			
	A-B	1	0.964			
	B-B	13	13.018			
				0.019	1	0.890

Chi-square test for deviation from Hardy-Weinberg equilibrium

Population: Koh Samet
*** No polymorphic loci ***

Chi-square test for deviation from Hardy-Weinberg equilibrium

Population: Koh Ela
*** No polymorphic loci ***

Chi-square test for deviation from Hardy-Weinberg equilibrium

Population: Koh Jarn
*** No polymorphic loci ***

Chi-square test for deviation from Hardy-Weinberg equilibrium

Population: Koh Kharm
*** No polymorphic loci ***

Chi-square test for deviation from Hardy-Weinberg equilibrium

Population: Koh Luarm
*** No polymorphic loci ***

Chi-square test for deviation from Hardy-Weinberg equilibrium

Population: Koh Pai
*** No polymorphic loci ***

Chi-square test for deviation from Hardy-Weinberg equilibrium

Population: Chaiya
*** No polymorphic loci ***

Chi-square test for deviation from Hardy-Weinberg equilibrium

Population: Puala Kapas Malaysia
*** No polymorphic loci ***

Chi-square test for deviation from Hardy-Weinberg equilibrium

Population: Singapore botanic garden
*** No polymorphic loci ***

APPENDIX III

Table 26. Significance test using exact probabilities in Soidao.

Locus	R1	R2	R3	P
<i>Est-2</i>	12	2	0	1.000
<i>Pgm-2</i>	12	2	0	1.000

Table 27. Significance test using exact probabilities in Makham.

Locus	R1	R2	R3	P
<i>Aat-1</i>	36	1	0	1.000
<i>Gpi</i>	30	6	1	0.344
<i>Hbdh</i>	20	15	2	1.000
<i>Pgm-2</i>	11	19	7	1.000

Table 28. Significance test using exact probabilities in Troknong.

Locus	R1	R2	R3	P
<i>Lgg-1</i>	8	7	1	1.000
<i>Pgm-1</i>	11	3	2	0.104
<i>Pgm-2</i>	7	5	4	0.292

Table 29. Significance test using exact probabilities in Makok.

Locus	R1	R2	R3	P
<i>Aat-1</i>	41	3	0	1.000
<i>Est-2</i>	43	1	0	1.000
<i>Gpi</i>	40	4	0	1.000
<i>Lgg-1</i>	36	8	0	1.000
<i>Pgm-2</i>	15	21	8	1.000

Table 30. Significance test using exact probabilities in Ranong.

Locus	R1	R2	R3	P
<i>Aat-1</i>	21	2	0	1.000
<i>Gpi</i>	9	12	2	0.662
<i>Hbdh</i>	9	10	3	1.000
<i>Mpi</i>	14	9	0	0.544
<i>Lgg-1</i>	12	11	0	0.279
<i>Lgg-2</i>	22	1	0	1.000
<i>Pgm-2</i>	10	12	1	0.619
<i>Pgd</i>	13	9	1	1.000

Table 31. Significance test using exact probabilities in Tachai.

Locus	R1	R2	R3	P
<i>Lgg-1</i>	13	6	1	1.000

Table 32. Significance test using exact probabilities in Takhun.

Locus	R1	R2	R3	P
<i>Aat-1</i>	7	6	1	1.000
<i>Est-2</i>	13	1	0	1.000
<i>Hbdh</i>	7	6	0	1.000
<i>Lgg-1</i>	10	3	1	0.326
<i>Lgg-2</i>	8	4	2	0.258

Table 33. Significance test using exact probabilities in Klongsang.

Locus	R1	R2	R3	P
<i>Aat-1</i>	40	2	0	1.000
<i>Est-2</i>	29	12	1	1.000
<i>Gpi</i>	39	3	1	1.000
<i>Hbdh</i>	34	8	1	1.000
<i>Lgg-1</i>	12	27	3	0.050
<i>Pgm-1</i>	40	2	0	1.000

Table 34. Significance test using exact probabilities equilibrium in Khaosok.

Locus	R1	R2	R3	P
<i>Aat-1</i>	15	15	3	1.000
<i>Est-2</i>	9	17	7	1.000
<i>Hbdh</i>	13	12	8	0.160
<i>Lgg-1</i>	9	20	4	0.286
<i>Pgm-2</i>	21	8	4	0.060
<i>Pgd</i>	31	1	0	1.000

Table 35. Significance test using exact probabilities in Suwankuha.

Locus	R1	R2	R3	P
<i>Aat-1</i>	17	11	3	0.650
<i>Pgm-1</i>	19	8	1	1.000
<i>Pgm-2</i>	30	1	0	1.000

Table 36. Significance test using exact probabilities in Pongchang.

Locus	R1	R2	R3	P
<i>Aat-1</i>	16	3	0	1.000
<i>Est-2</i>	18	1	0	1.000
<i>Hbdh</i>	12	7	0	1.000
<i>Mpi</i>	17	2	0	1.000
<i>Lgg-1</i>	11	4	2	0.177
<i>Pgm-2</i>	18	1	0	1.000

Table 37. Significance test using exact probabilities in Bangkram.

Locus	R1	R2	R3	P
<i>Lgg-1</i>	6	2	0	1.000

Table 38. Significance test using exact probabilities in Singapore Nee Soon.

Locus	R1	R2	R3	P
<i>Aat-1</i>	8	6	4	0.315
<i>Hbdh</i>	16	2	0	1.000
<i>Lgg-1</i>	17	1	0	1.000

Table 39. Significance test using exact probabilities in Koh Samui.

Locus	R1	R2	R3	P
<i>Lgg-3</i>	10	6	2	0.559

Table 40. Significance test using exact probabilities in Koh Tan.

Locus	R1	R2	R3	P
<i>Lgg-3</i>	13	1	0	1.000

APPENDIX IV

Table 41. Shell morphology and measurement of *Amphidromus atricallosus*

No./Locality	SH	S W	AW	A/J	Shell color	Coiling	Twist columella	Parietal callus color	Varix	Remark
Soidao										
1	50.34	30.47	28.77	A	Y	R	-	WHITE	/	
2	47.16	29.14	26.51	A	Y	R	/	WHITE	/	
3	53.04	28.62	27.96	A	W	R	-	WHITE	/	
4	48.72	28.87	26.91	A	W	R	-	WHITE	/	
5	46.52	26.68	25.15	A	Y	R	-	WHITE	/	
6	49.86	28.06	26.51	A	Y	R	-	WHITE	/	
7	51.84	30.67	27.9	A	Y	R	/	WHITE	/	
8	43.82	24.96	22.73	J	W	R	-	WHITE	-	
9	45.3	28.37	25.64	A	W	R	-	WHITE	/	
10	46.44	28.75	26.26	A	Y	R	-	WHITE	/	
11	36.86	23.38	20.29	J	Y	R	-	WHITE	-	
12				J	Y	L				broken shell
13	46.54	24.63	24.19	SA	B	L	-	WHITE	/	
14	24.54	19.35	15.35	J	B	R	-	WHITE	-	
Makham										
1	46.16	25.07	22.35	A	Y	L	/	WHITE	/	
2	46.43	26.41	23.71	A	Y	R	-	WHITE	-	
3	45.12	23.95	21.61	A	Y	R	-	WHITE	-	

4	41.15	25.04	21.95	A	Y	R	-	WHITE	-	
5	46.82	23.6	21.53	A	Y	R	/	WHITE	-	
6	42.38	24.94	21.02	A	Y	R	-	WHITE	-	
7	44.73	26.45	23.33	A	Y	R	-	WHITE	-	
8	47.24	26.06	21.5	A	Y	R	-	WHITE	-	
9	43.26	24.95	21.36	A	Y	L	-	WHITE	-	
10	43.75	25.53	20.68	A	Y	R	-	WHITE	-	
11	43.47	24.93	18.91	A	Y	R	-	WHITE	-	
12	44.85	23.8	20.83	J	Y	R	-	WHITE	-	
13	37.63	22.2	19.57	J	Y	R	-	WHITE	-	
14	48.42	27.49	22.47	A	Y	R	-	WHITE	-	
15	46.53	27.34	21.19	A	Y	L	-	WHITE	-	
16	43.89	25.76	21.26	A	Y	R	-	WHITE	-	
17	44.72	25	22.02	A	Y	R	-	WHITE	-	
18	45.57	24.33	22.83	A	Y	L	-	WHITE	-	
19	40.77	25.26	21.48	A	Y	R	-	WHITE	-	
20	44	24.99	21.34	A	Y	R	-	WHITE	-	
21	34.73	21.64	19.02	J	Y	R	-	WHITE	-	
22	33.23	20.87	16.54	J	Y	R	-	WHITE	-	
23	40.29	22.13	17.8	J	Y	R	-	WHITE	-	
24	35.55	21.85	17.7	J	Y	R	-	WHITE	-	
25	42.11	24.74	21.14	A	Y	R	-	WHITE	-	
26	35.33	22.82	18.13	J	Y	R	-	WHITE	-	
27	37.29	22.13	19.45	J	Y	R	-	WHITE	-	
28	34.47	20.58	18.71	J	Y	L	-	WHITE	-	

13	46.47	23.79	22.89	A	W	R	/	WHITE	/	
14	48.12	24.38	22.42	A	W	R	/	WHITE	/	
15	47.5	23.61	22.66	A	W	R	/	WHITE	/	
16	32.73	22.04	19.69	J	Y	R	-	WHITE	-	
17	48.11	23.97	22.51	A	Y	L	/	WHITE	/	
18	36.19	22.95	20.35	J	W	R	-	WHITE	-	
19	34.42	23.06	20.13	J	W	R	-	WHITE	-	
20	37.15	22.56	19.37	J	W	L	-	WHITE	-	
21	44.88	24.28	22.37	A	Y	L	-	WHITE	/	
22	46.47	24.77	22.85	A	Y	L	-	WHITE	/	
23	46.4	25.63	23.06	A	Y	R	-	WHITE	/	
24	46.46	23.73	21.41	A	Y	R	-	WHITE	-	
25	34.97	21.66	19.47	J	W	R	-	WHITE	-	
26	30.12	19.81	17.07	J	Y	L	-	WHITE	-	
27	44.95	25.02	21.81	A	W	L	-	WHITE	/	
28	42.4	22.26	21.4	A	W	L	/	WHITE	/	
29	35.45	21.91	19.09	J	W	L	-	WHITE	/	
30	45.02	25.61	22.65	A	W	L	-	WHITE	/	
31	44.37	24.18	21.99	A	Y	R	/	WHITE	/	
32	37.31	23.1	21.44	J	W	R	-	WHITE	-	
33	35.96	22.78	20.77	J	W	R	-	WHITE	-	
34	36.09	23.18	20.54	J	W	R	-	WHITE	-	
35	27.91	19.49	16.7	J	Y	R	-	WHITE	-	
36	31.61	21.03	16.47	J	W	L	-	WHITE	-	
37	20.04	18.83	14.27	J	W	L	-	WHITE	-	

38	45.06	24.93	21.95	A	W	L	-	WHITE	/	
39	21.94	18.31	16.97	J	W	L	-	WHITE	-	
40	48.19	26.73	20.16	A	Y	L	-	WHITE	/	
41	30.92	20.94	16.02	J	W	R	-	WHITE	/	
42	48.4	28.6	23.86	A	W	R	-	WHITE	/	
43	49.54	26	22.36	A	Y	L	-	WHITE	/	
44	44	24.11	20.76	A	Y	L	-	WHITE	/	
Ranong										
1	45.59	26.46	25.09	A	Y	R	-	BROWN	/	
2	40.13	22.09	19.89	J	Y	R	-	BROWN	/	
3	41.62	22.09	18.55	J	Y	R	-	BROWN	/	
4	38.19	21.23	17.59	J	W	R	-	BROWN	-	
5	44.13	27.53	26.32	A	Y	R	-	BROWN	/	broken shell
6	39	21.61	18.34	J	W	R	-	BROWN	/	
7	47.07	26.41	19.04	A	Y	R	-	BROWN	/	
8	45.44	25.3	23.01	A	Y	R	-	BROWN	/	
9	47.65	25.56	24.89	A	Y	R	-	BROWN	/	
10	43.77	24.68	21.85	A	Y	R	-	BROWN	/	
11	35.6	21.23	19.11	J	Y	R	-	BROWN	-	
12	28.53	18.13	15.79	J	Y	R	-	BROWN	-	
13	41.17	23.35	18.88	J	W	R	-	BROWN	-	
14	42.54	25.1	21.16	A	W	R	-	BROWN	/	
15	33.55	21.2	19.02	J	W	R	-	BROWN	/	
16	30.39	20.82	18.12	J	Y	R	-	BROWN	/	

17	45.02	27.33	24.11	A	Y	R	-	BROWN	/	
18	27.99	18.84	16.2	J	W	R	-	BROWN	/	
19	46.45	25.5	23.49	A	W	R	-	BROWN	/	
20	35.05	19.86	18.95	J	W	R	-	BROWN	-	
21	29.19	18.97	16.71	J	Y	R	-	BROWN	-	
22	34.25	20.78	16.54	J	W	R	-	BROWN	/	
23	32.62	20.09	18.34	J	Y	R	-	BROWN	-	
24	34.22	20.5	18.23	J	Y	L	-	BROWN	-	
Koh Tachai										
1	35.83	18.12	16.71	A	Y	L	-	BROWN	/	
2	34.45	18.63	17.16	A	Y	L	-	BROWN	/	broken apex
3	40.01	19.61	17.95	A	Y	L	-	BROWN	/	
4	38.6	20.75	17.47	A	Y	L	-	BROWN	/	
5	35.84	18.26	17.15	A	Y	L	-	BROWN	/	
6	35.2	17.9	16.31	A	Y	L	-	BROWN	/	
7	38.32	20.09	18.33	A	Y	L	-	BROWN	/	
8	36.65	18.3	17.33	A	Y	L	-	BROWN	/	
9	35.86	18.29	17.1	A	Y	L	-	BROWN	/	
10	35.75	18.5	16.73	A	Y	L	-	BROWN	/	
11	35.8	18.21	16.34	A	Y	L	-	BROWN	/	
12	34.35	18.39	16.15	A	Y	L	-	BROWN	/	
13	39.8	20.31	18.29	A	Y	L	-	BROWN	/	
14	37.76	18.82	16.72	A	Y	L	-	BROWN	/	
15	24.44	15.9	13.18	J	Y	L	-	BROWN	/	

16	25.82	16.56	14.47	J	Y	L	-	BROWN	/	
17	25.16	16.05	13.64	J	Y	L	-	BROWN	/	
18	24.27	15.85	13.27	J	Y	L	-	BROWN	/	
19	25.11	15.78	13.57	J	Y	L	-	BROWN	/	
20	21.36	14.81	12.08	J	Y	L	-	BROWN	/	
21	21.01	15.08	12.6	J	Y	L	-	BROWN	/	
22	27.71	17.02	14.58	J	Y	L	-	BROWN	/	
23	22.71	14.91	12.99	J	Y	L	-	BROWN	/	
24	22.13	14.76	12.67	J	Y	L	-	BROWN	/	
Takhun										
1	42.37	24.09	21.9	A	Y	R	-	BROWN	-	
2	40	24.26	22.12	A	W	R	-	BROWN	-	
3	38.06	21.28	18.77	A	Y	R	-	BROWN	/	
4	36.62	24.29	19.05	A	Y	L	-	BROWN	-	
5	37.89	23.69	20.27	A	Y	L	-	BROWN	/	
6	40.16	24.33	21.3	A	Y	R	-	BROWN	/	
7	41.88	24.5	22.25	A	Y	R	-	BROWN	/	
8	31.75	21.53	17.81	A	Y	R	-	BROWN	/	
9	43.6	21.27	24.25	A	Y	L	-	BROWN	/	
10	38.16	24.45	21.3	A	Y	L	-	BROWN	/	
11	42.95	23.27	21.25	A	Y	R	-	BROWN	-	
12	47.67	24.15	22.41	A	Y	R	-	BROWN	-	
13	41.6	23.04	21.09	A	Y	L	-	BROWN	-	
14	43.58	24.23	21.75	A	Y	R	-	BROWN	/	

Klongsang										
1	42.67	23.47	21.39	A	Y	L	-	BROWN	-	
2	37.08	25.41	20.19	A	Y	L	-	BROWN	/	
3	34.48	19.44	19.2	A	Y	L	-	BROWN	-	
4	34.99	22.62	19.63	A	Y	L	-	BROWN	-	
5	39.55	22.19	20.09	J	Y	L	-	BROWN	/	
6	39.23	24.01	19.97	A	Y	L	-	BROWN	/	
7	44.45	25.03	21.25	SA	Y	L	-	BROWN	-	
8	40.66	21.89	19.96	SA	Y	L	-	BROWN	-	
9	37.7	22.48	20.56	A	Y	L	-	BROWN	-	
10	42.77	24.17	20.07	A	Y	L	-	BROWN	/	
11	38.31	22.12	19.29	A	W	L	-	BROWN	/	
12	42.27	24.83	21.73	A	W	L	-	BROWN	-	
13	37.99	22.86	19.38	A	Y	L	-	BROWN	-	
14	38.08	24.5	21.79	A	Y	L	-	BROWN	/	
15	45.64	26.07	22.69	A	Y	L	-	BROWN	/	
16	36.33	21.64	20.12	A	Y	L	-	BROWN	-	
17	37.75	23.05	20.37	A	W	L	-	BROWN	-	
18	39.97	23.46	19.56	A	Y	L	-	BROWN	-	
19	39.63	23.02	21.79	A	Y	L	-	BROWN	-	
20	39.14	24.34	20.51	A	Y	L	-	BROWN	-	
21	34.8	23.31	19.21	A	Y	L	-	BROWN	-	
22	35.72	22.38	21.17	A	Y	L	-	BROWN	-	
23	37.43	21.78	19.54	A	Y	R	-	BROWN	/	

5	39.36	22.55	19.15	A	W	L	-	BROWN	/	
6	42.9	22.59	22.25	A	Y	L	-	BROWN	/	
7	40.45	22.39	20.74	A	Y	L	-	BROWN	/	
8	36.73	20.45	17.45	J	Y	L	-	BROWN	/	
9	42.84	24.24	21.67	A	Y	L	-	BROWN	-	
10	40.28	21.3	20.9	A	Y	L	-	BROWN	/	
11	43.26	24.71	22.55	A	Y	L	-	BROWN	-	
12	38.67	22.18	19.54	A	Y	L	-	BROWN	/	
13	44.37	23.67	22.95	A	Y	L	-	BROWN	-	
14	42.86	22.55	21.03	A	Y	L	-	BROWN	/	
15	43.75	24.5	22.68	A	Y	L	-	BROWN	-	
16	44.66	24.67	22.96	A	Y	L	-	BROWN	/	
17	40.38	24.04	20.56	A	Y	L	-	BROWN	-	
18	38.85	21.08	17.96	J	Y	L	-	BROWN	-	
19	37.76	20.25	14.66	J	Y	R	-	BROWN	/	
20	44.35	23.85	22.27	A	Y	R	-	BROWN	/	
21	45.77	25.37	24.68	A	Y	R	-	BROWN	/	
22	46.79	23.99	22.33	A	Y	R	-	BROWN	/	
23	47.93	27.33	22.46	A	W	R	-	BROWN	/	
24	43.59	23.61	21.06	A	Y	R	-	BROWN	-	
25	49.32	24.84	22.91	A	Y	R	-	BROWN	/	
26	50.67	25.91	25.51	A	Y	R	-	BROWN	/	
27	46.88	25.49	23.64	A	W	R	-	BROWN	/	
28	45.4	25.38	21.44	A	W	R	-	BROWN	/	
29	40.52	22.5	20.91	A	W	R	-	BROWN	-	

30	36.31	21.24	19.49	J	Y	R	-	BROWN	/	
31	32.22	19.98	17.12	J	Y	R	-	BROWN	/	
32	42.99	21.9	22.11	A	W	R	-	BROWN	-	
33	41.99	24.13	23.05	A	W	R	-	BROWN	-	
Suwankuha										
1	52.05	26.38	22.94	A	Y	L	-	WHITE	-	
2	49.84	25.07	20.1	A	Y	L	-	WHITE	-	
3	35.47	21.13	18.97	J	Y	L	-	WHITE	-	
4	45.54	23.32	21.13	A	Y	L	-	WHITE	-	
5	38.33	22.59	17.78	A	Y	L	-	WHITE	-	
6	46.6	22.31	20.67	A	Y	R	-	WHITE	-	
7	47.38	25.48	19.75	A	Y	L	-	WHITE	-	
8	47.8	24.33	21.14	A	W	L	-	WHITE	-	
9	46.57	23.17	21.56	A	Y	L	-	WHITE	-	
10	47.81	23.22	19.74	A	Y	L	-	WHITE	-	
11	44.91	23.37	18	A	Y	L	-	WHITE	-	
12	43.64	24.54	20.17	SA	Y	L	-	WHITE	-	
13	46.35	24.06	21.13	A	Y	L	-	WHITE	-	
14	48.32	24.34	21.13	A	Y	L	-	WHITE	-	
15	46.96	24.03	19.79	A	Y	L	-	WHITE	-	
16	49.54	26.77	20.09	A	Y	L	-	WHITE	-	
17	46.42	27.66	25.5	A	Y	L	-	WHITE	-	
18	51.5	28.75	27.31	A	Y	L	-	WHITE	-	
19	41.79	25.21	20.84	A	Y	L	-	WHITE	-	
20	53.31	28.39	25.79	A	Y	L	-	WHITE	-	

21	46.1	27.72	23	A	Y	L	-	WHITE	-	
22	44.97	23.64	19.9	J	Y	L	-	WHITE	-	
23	43.82	26.06	22.91	A	Y	L	-	WHITE	-	
24	48.04	26.07	22.6	A	Y	L	-	WHITE	-	
25	37.59	22.39	19.26	J	Y	L	-	WHITE	-	
26	47.01	25.25	20.95	A	Y	L	-	WHITE	-	
27	46.77	26.13	21.5	A	Y	L	-	WHITE	-	
28	44.7	24.69	21.76	A	Y	L	-	WHITE	-	
29	43.64	25.39	21.96	A	Y	L	-	WHITE	-	
30	37.22	22.7	19.18	J	Y	L	-	WHITE	-	
31	28.87	18.66	15.82	J	Y	L	-	WHITE	-	
Poungchang										
1	38.14	22.17	17.12	A	y	L	-	WHITE	/	
2	37.68	19.66	19.03	A	y	L	-	WHITE	/	
3	38.98	22.17	21.33	A	y	L	-	WHITE	-	
4	37.01	21.22	18.72	A	y	L	-	WHITE	/	
5	34.34	18.4	17.49	A	y	R	-	WHITE	-	
6	35.42	20.55	18.29	A	y	L	-	WHITE	-	
7	36.36	20.34	18.63	A	y	L	-	WHITE	-	
8	36.88	20.15	18.06	A	y	L	-	WHITE	-	
9	39.52	19.7	18.6	A	y	R	-	WHITE	-	
10	37.49	19.23	18.8	A	y	L	-	WHITE	-	
11	39.24	19.48	18.14	A	y	L	-	WHITE	-	
12	18.33	14.06	10.78	J	y	L	-	WHITE	-	

13	16.92	12.89	10.47	J	y	L	-	WHITE	-	
14	20.68	15.79	12.83	J	y	L	-	WHITE	-	
15	17.11	13.31	10.91	J	y	R	-	WHITE	/	
16	16.58	12.86	10.01	J	y	L	-	WHITE	-	
17	16.67	12.23	10.41	J	y	R	-	WHITE	-	
18	17.74	13.34	10.76	J	y	L	-	WHITE	-	
19	14.03	10.84	8.63	J	y	L	-	WHITE	-	
20	13.93	11.86	8.58	J	y	L	-	WHITE	-	
Bangkram										
1	41.79	24.34	22.35	A	Y	L	-	WHITE	/	
2	40.39	23.31	21.36	J	Y	R	-	WHITE	/	
3	31.53	18.62	17.97	J	Y	R	-	WHITE	-	
4	40.55	22.34	21.17	J	Y	L	-	WHITE	/	
5	39.41	22.11	20.39	J	Y	R	-	WHITE	/	
6	41.02	22.7	20.63	J	Y	L	-	WHITE	/	
7	42.41	22.92	22.03	J	Y	R	-	WHITE	-	
8	39.12	22.4	20.42	J	Y	R	-	WHITE	-	
Singapore Nee Soon										
1	45.24	27.06	23.96	A	Y	R	-	WHITE	-	
2	42.39	26.51	23.61	A	Y	L	-	WHITE	-	
3	40.15	23.23	21.26	SA	Y	L	-	WHITE	-	
4	44.08	26.87	24.77	A	Y	R	-	WHITE	-	
5	44.83	27.13	24.29	A	Y	R	-	WHITE	-	
6	42.44	25.23	23.01	A	Y	R	-	WHITE	-	

7	41.41	24.69	20.89	A	Y	R	-	WHITE	-	
8	48.25	26.15	23.32	A	Y	R	-	WHITE	-	
9	43.62	25.51	22.97	A	Y	R	-	WHITE	-	
10	33.52	20.72	18.21	J	Y	L	-	WHITE	-	
11	26.2	18.25	15.68	J	Y	R	-	WHITE	-	
12	30.66	20.37	18.14	J	Y	R	-	WHITE	-	
13	32.04	21.67	18.29	J	Y	L	-	WHITE	-	
14	27.42	19.06	15.28	J	Y	R	-	WHITE	-	
15	17.62	14.7	10.96	J	Y	R	-	WHITE	-	
16	17.84	14.86	11.52	J	Y	R	-	WHITE	-	
17	18.11	15.09	11.02	J	Y	L	-	WHITE	-	
18	19.25	15.67	12.49	J	Y	L	-	WHITE	-	

SH: Shell height

SW: Shell width

AW: Aperture width

Shell color: W, white, B, brown, Y, yellow

A: Adult

J: Juvenile

SA: Subadult

Coiling: L, left handed coiling, R, right handed coiling

Table 42. Shell morphology and measurement of *Amphidromus inversus*

No/Locality	SH	SW	AW	A/J	Shell color	Coiling	Remark
Singapore botanic garden							
1	39.84	25.01	19.72	SA	B	R	
2	41.87	23.62	21.52	SA	B	R	
3	48.25	25.97	25.12	A	B	R	
4	42.94	26.78	23.08	A	B	R	
5	42.82	23.68	22.32	SA	B	R	
6	41.1	24.22	20.23	SA	B	R	
7	33.05	20.82	16.72	J	B	R	
8	18.68	14.84	11.27	J	B	R	
9	12.14	10.69	6.82	J	B	R	
10	13.89	11.77	7.64	J	B	R	
Koh Tan							
1	22.05	37.96	19.86	J	B	R	broken apex, pink apex
2	23.76	43.84	20.75	J	B	R	pink apex
3	22.21	36.87	18.57	J	B	R	pink apex
4	23.16	42.78	19.98	A	B	R	pink apex
5	22.5	36.21	19.87	J	B	R	pink apex
6	19.97	30.75	17.76	J	B	R	pink apex
7	18.87	26.77	14.66	J	B	R	pink apex
8	21.59	33.33	18.2	J	B	R	pink apex
9	24.92	43.37	20.66	A	B	R	pink apex
10	21.62	36.15	20.27	J	B	R	pink apex

9	40.74	21.47	17.65	A	B	R	pink apex
10	44.79	21.58	19.39	A	B	R	pink apex
11	40.58	21.82	18.86	A	B	R	pink apex
12	48.43	24.33	20.4	A	B	R	broken apex, pink apex
13	44.52	21.69	20.24	A	B	R	broken apex, pink apex
14	42.16	21.41	18.94	A	B	R	pink apex
15	43.89	21.87	19.18	A	B	R	broken apex, pink apex
16	41.56	20.55	18.4	A	B	R	pink apex
17	39.53	20.4	18.29	A	B	R	pink apex
18	42.55	22.08	20.28	A	B	R	pink apex
19	40.35	20.92	18.61	A	B	R	pink apex
20	42.56	21.37	19.29	SA	B	R	broken apex
21	44.86	22.83	19.47	A	B	R	pink apex
22	42.53	22.16	19.63	A	B	R	pink apex
23	44.59	22.56	19.48	A	B	R	pink apex
24	40.13	20.06	17.74	A	B	R	pink apex
25	47.48	23.02	20.43	A	B	R	pink apex
26	49.07	24.1	20.12	A	B	R	pink apex
27	41.29	20.94	18.17	A	B	R	pink apex
28	48.55	23.38	20.08	A	B	R	pink apex
29	38.71	20.2	17.46	A	B	R	pink apex
30	37.81	20.64	17.65	A	B	R	pink apex
31	38.79	20.9	18.68	A	B	R	broken apex, pink apex
32	44.02	21.77	17.6	A	B	R	pink apex
33	38.8	20.85	19.12	J	B	R	pink apex

Chaiya							
1	46.64	24.27	21.01	A	B	R	pink apex
2	42.99	23.64	19.84	A	B	R	pink apex
3	43.64	23.39	19.8	A	B	R	pink apex
4	48.81	24.31	21.26	A	B	R	pink apex
5	45.77	22.71	20.23	A	B	R	pink apex
6	50.49	25.14	22.73	A	B	R	pink apex
7	45.51	24.97	21.36	A	B	R	pink apex
8	42.29	22.78	19.14	A	B	R	pink apex
9	43.15	22.46	20.11	A	B	R	pink apex
10	45.18	22.45	20.16	A	B	R	pink apex
11	43.96	22.37	19.48	A	B	R	pink apex
12	39.59	22.04	17.86	A	B	R	pink apex
13	48.74	24.07	20.14	A	B	R	broken apex, pink apex
14	46.42	22.95	20.52	A	B	R	pink apex
15	49.34	24.69	21.39	A	B	R	pink apex
16	42.68	22.9	19	A	B	R	pink apex
17	43.39	22.42	19.03	A	B	R	pink apex
18	41.86	22.08	18.89	A	B	R	pink apex
19	43.58	22.19	20.14	A	B	R	pink apex
20	46.69	23.42	20.38	A	B	R	pink apex
21	44.3	23.96	20.7	A	B	R	pink apex
22	49.81	25.61	21.55	A	B	R	pink apex
23	44.09	23.11	19.51	A	B	R	pink apex
24	45.55	22.8	19.6	A	B	R	pink apex
25	43.18	21.91	18.84	A	B	R	pink apex

26	40.55	22.26	18.7	A	B	R	pink apex
27	47.07	23.73	21.33	A	B	R	pink apex
28	47.75	23.81	21.34	A	B	R	pink apex
29	42.49	21.9	19.03	A	B	R	pink apex
30	48.96	23.66	21.15	A	B	R	pink apex
31	43.89	22.22	19.72	A	B	R	pink apex
32	45.88	22.93	21.3	A	B	R	pink apex
33	49.97	24.53	21.83	A	B	R	pink apex
34	49.36	24.5	22.15	A	B	R	pink apex
35	47.55	23.55	21.01	A	B	R	pink apex
36	49.57	23.95	22.63	A	B	R	pink apex
Pulau Kapas, Malaysia							
1	41.16	23.17	20.1	A	W	R	
2	37.15	22.33	19.37	A	W	R	
3	30.97	21.43	17.22	A	W	L	
4	34.39	20.39	16.93	A	W	L	
5	36.85	21.84	19.13	A	W	L	
6	37.19	20.89	18.52	A	W	L	
7	33.26	19.29	16.33	A	W	L	
8	40.56	22.5	20.27	A	W	L	
9	34.4	21.34	18.75	A	W	L	
10	40.5	22.17	19.64	A	W	L	
11	35.67	20.45	17.65	A	W	R	
12	32.7	20.25	17.87	A	W	L	
13	34.01	19.38	16.07	A	W	L	

14	37.25	22.41	19.37	A	W	L	
15	36.86	21.18	18.76	A	W	L	
16	28.72	18.58	14.57	A	W	R	
17	33.33	18.93	16.05	SA	W	L	
18	37.24	21.86	18.95	A	W	L	
19	34.33	21.04	17.28	A	W	R	

SH: Shell height

SW: Shell width

AW: Aperture width

A: Adult

J: Juvenile

SA: Subadult

Shell color: W, white, B, brown

Coiling: L, left handed coiling, R, right handed coiling

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APPENDIX V

The studies specimens deposited in the Chulalongkorn University, Museum of Zoology (CUMZ)

CUMZ	Species	Date	Locality	Specimen		Remark
				D	S	
2621	<i>Amphidromus atricallosus leucoxanthus</i>	22/08/2004	Soidao, Chantaburi	12	1	Shell, wet, -30 C
2622	<i>A. atricallosus leucoxanthus</i>	30/09/2003	Makham, Chantaburi	30	6	Shell, wet, -30 C
2623	<i>A. atricallosus leucoxanthus</i>	22/08/2004	Troknong, Chantaburi	27		Shell, wet, -30 C
2624	<i>A. atricallosus leucoxanthus</i>	04/09/2003	Makok, Chantaburi	22	21	Shell, wet, -30 C
2625	<i>A. atricallosus atricallosus</i>	17/09/2003	Ranong (hot spring)	23	1	Shell, wet, -30 C
2626	<i>A. atricallosus classarius</i>	21/12/2003	Koh Tachai, Pang Nga		14	Shell, wet, -30 C
2627	<i>A. atricallosus atricallosus</i>	13/09/2003	Takhun, Suratthani	7	5	Shell, wet, -30 C
2628	<i>A. atricallosus atricallosus</i>	04/10/2003	Klongsang, Suratthani	20	22	Shell, wet, -30 C
2629	<i>A. atricallosus atricallosus</i>	22/09/2003	Khaosok, Suratthani	15	18	Shell, wet, -30 C
2630	<i>A. atricallosus atricallosus</i>	11/05/2003	Suwankuha, Pang Nga	1	33	Shell, wet, -30 C
2631	<i>A. atricallosus atricallosus</i>	21/12/2003	Poungchang, Pang Nga	4	17	Shell, wet, -30 C
2632	<i>A. atricallosus atricallosus</i>	15/05/2004	Bangkram, Krabi	5	3	Shell, wet, -30 C
2633	<i>A. atricallosus perakensis</i>	04/03/2004	Nee Soon, Singapore	8	3	Shell, wet, -30 C
2636	<i>A. inversus inversus</i>	14/03/2004	Botanic garden, Singapore	5		Shell, wet, -30 C
2637	<i>A. inversus inversus</i>	08/03/2004	Pulau Kapas, Malaysia	2	7	Shell, wet, -30 C
2638	<i>A. inversus annamiticus</i>	27/02/2003	Koh Jarn, Chonburi	13		Shell, wet, -30 C
2639	<i>A. inversus annamiticus</i>	28/02/2003	Koh Pai, Chonburi	15		Shell, wet, -30 C
2640	<i>A. inversus annamiticus</i>	26/02/2003	Koh Kham, Chonburi	17		Shell, wet, -30 C
2641	<i>A. inversus annamiticus</i>	26/02/2003	Kok Lueam, Chonburi	23		Shell, wet, -30 C

CUMZ	Species	Date	Locality	Specimen		Remark
				D	S	
2642	<i>A. inversus annamiticus</i>	30/05/2003	Koh Elar, Chonburi	15		Shell, wet, -30 C
2643	<i>A. inversus annamiticus</i>	30/05/2003	Koh Tan, Suratthani	18		Shell, wet, -30 C
2644	<i>A. inversus annamiticus</i>	26/08/2002	Chaiya, Suratthani	30		Shell, wet, -30 C
2645	<i>A. inversus annamiticus</i>	26/02/2003	Koh Samui, Suratthani	23		Shell, wet, -30 C
2647	<i>A. inversus annamiticus</i>	26/08/2003	Koh Samet, Rayong	16		Shell, wet, -30 C

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BIOGRAPHY

Miss Pongpun Prasankok was born on the 11st of November 1977. She graduated both Bachelor and Master Degree of Science from Department of Biology, Faculty of Science, Chulalongkorn University in 1998 and 2001, respectively. In 2002, she was awarded the Royal Golden Jubilee (RGJ) Ph.D. Program scholarship of the Thailand Research Fund (TRF) for her Ph.D. study in Biological Science Ph.D. Program at Faculty of Science, Chulalongkorn University.



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