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Authors: Tan, K-S., and Liu, L-L.

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Description of a New Species of *Thais* (Mollusca: Neogastropoda: Muricidae) from Taiwan, Based on Morphological and Allozyme Analyses

K.-S. Tan[1]* and L.-L. Liu[2]

[1] *Tropical Marine Science Institute, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260*
[2] *Institute of Marine Biology, National Sun Yat-Sen University, Kaohsiung 804, Taiwan, Republic of China*

ABSTRACT—*Thais keluo* sp. nov. is described from intertidal shores of southwest Taiwan. The new species is differentiated from five other closely related species, namely *T. bitubercularis* (Lamarck), *T. jubilaea* Tan and Sigurdsson, *T. clavigera* (Küster), *T. luteostoma* (Holten) and *T. rufotincta* Tan and Sigurdsson, all of which occur in the South China Sea, on the basis of shell, radula and penis morphology. *Thais keluo* is also distinguished from the latter three species based on allozyme electrophoresis. The shell of *T. keluo* is characterized by four raised, spiral bands on the last whorl, one or two small, oblique columellar plica(e) on the inner lip, a finely crenate, thin, narrow, reddish-brown outer lip edge and four white, papillate denticles inside the outer lip of the aperture. In males, the penis is curved with a long, simple flagellum. The UPGMA cluster analysis based on 9 enzyme loci revealed that *T. luteostoma* is more closely related to *T. clavigera* than to *T. keluo* n.sp. The Nei's genetic distance (D) obtained between the new species and *T. clavigera*/*T. luteostoma* was 0.31, while *T. clavigera* and *T. luteostoma* were separated by a distance of 0.16. *Thais rufotincta* was separated from the other species by a distance of 0.78. In contrast, phylogenetic analysis of morphological data by maximum parsimony suggested that *T. luteostoma* was more closely related to *T. keluo* than to *T. clavigera*. However, both analyses indicated the close relationship amongst *T. clavigera*, *T. luteostoma* and the new species in relation to *T. rufotincta*.

Key words: systematics, taxonomy, Neogastropoda, Rapaninae, new species

INTRODUCTION

Members of the predatory neogastropod family Muricidae are common and well-known on intertidal rocky shores of Chinese, Japanese and Korean coastlines (Yen, 1933; Habe and Kosuge, 1967; Lai, 1981; Morton and Morton, 1983; Qi *et al*., 1983; Tan *et al*., 1986; Huang, 1994; Choe and Park, 1997; Lai, 1998). Often found in high densities, they incur much damage and loss to the substantial oyster culture industries in Taiwan (Lin and Hsu, 1979) and Japan (Koganezawa, 1963) because of their efficient predatory habits. Muricid gastropods also exert a significant effect on the structure of subtropical, intertidal rocky shore communities (Taylor and Morton, 1996). At the same time, recent work has indicated that they can be effective bioindicators of organotin pollution (e.g., Horiguchi *et al*., 1997; Liu *et al*., 1997). Despite their economic and ecological significance, there is still much confusion over their identities. For example, inherent variation in their shell morphology has resulted in at least five synonyms for the common East Asian oyster predator *Thais clavigera* (Küster) (see Tan, 1995). Apart from a checklist (Kuroda, 1941) and observations of radulae (Wu, 1965), there are no recent studies in Taiwan addressing the identities of these taxonomically difficult gastropods. These problems in identification hamper further environmental studies especially as bioindicators, since species-specific factors are likely to be important determinants of response to organotin pollutants (Stroben *et al*., 1995; Liu *et al*., 1997; Tan, 1997; Gibbs *et al*., 1997; Tan, 1999). In view of this, preliminary morphological examination of several samples of muricid gastropods from southwest Taiwan was carried out to determine species composition of muricid molluscs present. This paper reports on the occurrence of a new species of *Thais* in southwestern Taiwan, as revealed by morphological and allozyme analyses.

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* Corresponding author: FAX. +65-7749654.
E-mail: tmstanks@nus.edu.sg

MATERIALS AND METHODS

Morphology

Some 25 *Thais keluo* n.sp. were collected from Tsaishan in Kaohsiung, Taiwan in December 1998 and June 1999, and a further 20 specimens were obtained from Tungkang about 50 km south of Kaohsiung in June 1999. All were found on rocks or metal plates at or below low tide level with *T. rufotincta* Tan and Sigurdsson, a common Southeast Asian species (Tan and Sigurdsson, 1996a). To determine shell microstructure, the acetate peel technique was used (Kennish, Lutz and Rhoads, 1980). Four shells of the new species were vacuum-embedded in epoxy and sectioned either parallel or perpendicular to the shell axis using a low speed diamond saw (Buehler Isomet). The cut surfaces were then polished using different grades of lapping paper (3M) until a mirror-like surface was obtained. The polished surfaces were subsequently etched with dilute hydrochloric acid (1 ml HCl in 100 ml distilled water) for about one minute, after which the blocks were rinsed in distilled water and air-dried. To obtain a replica of the shell section, a small amount of acetone was first applied to the acid-etched surface over which a sheet of cellulose acetate (Agar Scientific) of 35 µm thickness was placed. The imprint obtained was then mounted on a glass slide and examined under a microscope. The microstructure observed in these imprints was compared with those previously prepared from shells of *Nucella lapillus*, whose crystalline composition and mineralogy were earlier determined using an X-ray diffractometer (Philips PW1710). The shell microstructure of other *Thais* species were similarly determined (see also Tan and Sigurdsson, 1996a, b).

Anatomical observations were made on 10 animals that were relaxed in excess 1:1 seawater: 7.5% magnesium chloride solution for about 6–8 hr before they were examined and dissected under a stereomicroscope. Spermatozoa from five mature male specimens were obtained from the testes and seminal vesicles, mounted on slides and their lengths measured under a light microscope using an eyepiece micrometer. The remaining samples were frozen immediately after collection in a –70°C freezer. Dissected animals were preserved either in 5% formaldehyde in seawater or 80% ethanol for further observations. Radulae extracted from 20 snails were cleaned in 1M potassium hydroxide solution and measurements made prior to mounting on slides or brass stubs for light and scanning electron microscopy respectively, as detailed in Tan and Sigurdsson (1996b). Three other species, *Thais clavigera* (Küster), *T. luteostoma* (Holten) (both from Hong Kong) and *T. rufotincta* (from Taiwan) were examined alive for comparison with the new species. Three specimens of *T. bitubercularis* were identified and examined from Chiku, Taiwan, and numerous other specimens of *T. bitubercularis*, *T. clavigera*, *T. jubilaea* and *T. rufotincta* from Malaysia and Singapore were dissected. Observations of radulae follow methods as detailed in Tan and Sigurdsson (1996a, b).

Cladistic analysis

A total of seven species using 11 morphological characters coded as 24 discrete character states were used to construct a data matrix (Table 1) in MacClade 4.0 (Maddison and Maddison, 2000), which was in turn analysed using PAUP* 4.0 beta 8 test version (Swofford, 1998). All characters were unweighted and unordered. The branch-and-bound option was employed to find the shortest trees. *Thais malayensis* Tan and Sigurdsson was selected to be the outgroup taxon. Selection of the outgroup is problematic because relationships amongst species in the genus *Thais* are not established. We have used *T. malayensis* because it clearly does not belong to the ingroup comprising *T. bitubercularis*, *T. clavigera*, *T. jubilaea*, *T. keluo* n.sp. and *T. luteostoma* based on shell features but could possibly be more related to *T. rufotincta*. The type species of *Thais* (*T. nodosa*, a tropical Atlantic species) was re-described by Kool (1993) but preliminary analyses provided little resolution in the trees generated by PAUP*. The use of other rapanine genera as outgroup taxa was considered but only a few characters proved useful in resolving the trees and was therefore excluded.

Table 1. Morphological data matrix used for phylogenetic analysis. Characters 1, 3, 4 and 8, marked with an asterisk (*), are non-informative.

	Character										
Thais species	1*	2	3*	4*	5	6	7	8*	9	10	11
bitubercularis	0	0	0/1	1	1	1	0	0	1	1	1
clavigera	0	0	0/1	1	1	0	1	0	0	0	1
jubilaea	0	0	0/1	1	1	0	2	0	0	0	1
keluo	0	1	1	1	1	0	0	0	1	1	?
luteostoma	1	0	0	0	–	1	2	0	1	1	1
rufotincta	0	1	0	1	0	0	0	1	1	0	0
malayensis	0	0	0	1	0	0	0	0	1	0	0

Bootstrap analysis was performed on 10,000 replicates using the branch-and-bound search option in PAUP*, and Bremer support (i.e., decay values; Bremer, 1994; Kitching *et al*., 1998) was calculated in PAUP* using the command instructions generated by MacClade 4.0.

Description of characters used:

Character 1. Shell: external calcitic layer [(0)=absent; (1)=present]. An external calcitic layer is present in some species of *Thais* (e.g., Petitjean, 1965; Kool, 1993; Tan, 1995). This configuration apparently provides some protection from erosion in seawater due to its lower solubility compared to aragonite (Taylor and Reid, 1990) although other factors such as the proportion of organic matrix and crystal structure of the shell may be more important (Harper, 2000). The presence of a thin calcitic layer is an autapomorphy for *Thais luteostoma* in the present analysis. It is absent in *T. bitubercularis*, *T. clavigera*, *T. jubilaea*, *T. keluo*, *T. rufotincta* and the outgroup species *T. malayensis*.

Character 2. Shell: number of aragonitic crossed-lamellar layers [(0)=two layers; (1)=three layers]. Many tropical rapanines possess shells with two or three layers of crossed-lamellar aragonite (Petitjean, 1965; Tan, 1995). *Thais rufotincta* and *T. keluo* have three layers while the other species have shells with only two distinct layers.

Character 3. Shell aperture: columellar plicae [(0)=absent; (1)=present]. Four species may have a small columella plica in the mid-region of the columella, although the condition appears to be somewhat variable in *Thais bitubercularis*, *T. clavigera* and *T. jubilaea*. Almost invariably the columella of the new species *T. keluo* has one or two small plicae (see Fig. 1b, f), while these are absent in *T. luteostoma*, *T. rufotincta* and the outgroup species *T. malayensis*.

Characters 4 and 5. Shell aperture: outer lip denticles [character 4: (0)=absent; (1)=present; character 5: (0)=pustulate; (1)=lirate]. All but one species (*T. luteostoma*) examined have denticles on the inside edge of the outer lip. Four species possess pustulate denticles, while in *T. rufotincta* and *T. malayensis*, the denticles are lirate and extend into the aperture.

Character 6. Radula. Marginal denticles on rachidian [(0)=five or less; (1)=six or more]. Species examined in this study can be divided into two groups based on the number of marginal denticles present between the marginal and lateral cusps of the rachidian teeth. In *T. bitubercularis* and *T. luteostoma*, there are usually more than six denticles on either side of the rachidian (Tan and Sigurdsson, 1990; Tan, 1995), as compared to the other species which have five or fewer denticles.

Character 7. Penis: flagellum shape (males) [(0)=simple; (1)=hooked tip; (2)=barbed]. Penis morphology can be species-specific and thus can be used for species identification (Tan and Sigurdsson, 1990). Amongst the species examined in this study, four species have penes with simple flagella, two species (*T. jubilaea* and *T. luteostoma*)

Table 2. *Thais* species assayed by starch gel electrophoresis.

Species/Site	Sample size	Shell length (mm)±SD	Collection date
Thais clavigera (Küster)			
Chiku, Taiwan (120°20'E; 23°25'N)	66	34.8±4.2	May 2000
Cape d'Aguilar, Hong Kong (114°15.5'E, 22°12.5'N)	22	24.4±3.9	Jun 1999
T. luteostoma (Holten)			
Cape d'Aguilar, Hong Kong	30	35.5±4.9	Jun 1999
Thais keluo new species			
Tungkang, Taiwan (120°26'E; 22°31'N)	29	38.0±2.7	Feb 2000
T. rufotincta Tan and Sigurdsson			
Chiku, Taiwan	34	25.6±4.8	Jul 1999
Kaohsiung, Taiwan (120°17'E; 22°38'N)	44	29.0±1.3	Aug 1999
Tungkang, Taiwan	30	25.9±1.1	Mar 2000

have barbed flagella, and in an autapomorphic case, the tip of the flagellum is in the form of a small hook in *T. clavigera*.

Character 8. Pallial vas deferens (males) [(0)=marginal; (1)=central]. The pallial vas deferens that is associated with the prostate gland takes a marginal route along the ventral edge of the gland in all but one species. In *Thais rufotincta* its vas deferens runs through the prostate gland centrally.

Character 9. Sperm length (males) [(0)=short, less than 100 μm; (1)=long, more than 100 μm]. *Thais clavigera* and *T. jubilaea* have short euspermatozoa that are between 96–98 μm in length, while the other species have slightly longer sperm measuring between 106 to 110 μm.

Character 10. Ventral channel of capsule gland (females) [(0)=grooves absent; (1)=grooves present]. The capsule gland, when viewed in cross-section, has a ventral channel that is partially isolated from the lumen of the capsule gland by a ventral flap which is used as a passageway for sperm (Fretter, 1941; Fretter and Graham, 1994). This ventral channel may be grooved along the length of the capsule gland, as in *T. bitubercularis*, *T. luteostoma* and *T. keluo*, or generally devoid of these grooves as seen in *T. clavigera*, *T. jubilaea*, *T. rufotincta* and *T. malayensis*.

Character 11. Egg capsules [(0)=exit orifice occupies only a part of apex; (1)=exit orifice occupies entire apex]. The species considered in this analysis can be divided into two groups based on the position of the "escape hatch" present on the top surface of the egg capsule. In *T. bitubercularis*, *T. clavigera*, *T. jubilaea* (see Tan and Sigurdsson, 1990) and *T. luteostoma* (illustrated in Tan, 1995), the hatch or orifice occupies only a part of the surface of the capsule. In contrast, one end of the capsule is entirely occupied by the escape hatch in *T. rufotincta* and *T. malayensis*, as illustrated in Tan and Sigurdsson (1996a, b).

Allozyme electrophoresis

Adult samples of *Thais keluo* n. sp. and *T. rufotincta* were collected fromTaiwan. *Thais luteostoma* was obtained from Hong Kong, while *T. clavigera* samples were collected from both Taiwan and Hong Kong (see Table 2). They were frozen alive and kept at –70°C before use. Foot tissue was homogenized in a Tekmar tissumizer with an equal volume of 10mM Tris-HCl buffer (pH 7.0) containing 1% Triton X-100. Homogenates were centrifuged at 5,000g for ten minutes, and the resulting supernatants were stored at –70°C for later use. Horizontal starch-gel electrophoresis was used with buffer systems Tris-citrate (pH 7.0), Tris-malate-EDTA (pH 7.4) and lithium hydroxide (pH 8.1/8.3). Enzyme staining methods follow Richardson *et al*. (1986) and Murphy *et al*. (1990).

Multiple loci encoding the same enzymes (i.e., isozymes) were designated by consecutive numbers, with 1 denoting the slowest migrating isozyme. Nine enzyme loci were scored: arginine kinase (ARK, EC 2.7.3.3), esterase (EST, EC 3.1.1.1), malate dehydrogenase 1, 2 and 3 (MDH-1, MDH-2 and MDH-3, EC.1.1.1.37), leucine aminopeptidase (LAP, EC 3.4.11.-), phosphoglucomutase 1 and 2 (PGM-1 and PGM-2, EC 2.7.5.1) and xanthine dehydrogenase (XDH, EC 1.1.1.204). Alleles at each locus were scored by designating the most common allele of *T. clavigera* as 100. All other alleles were numbered according to their relative anodal distance from the reference allele. Data analyses were performed with POPGENE (Yeh *et al*., 1997), while UPGMA cluster analyses were calculated according to Nei's (1978) unbiased minimum genetic distance.

Abbreviations used in the text: ABO—accessory boring organ; AH—apertural height; asg—accessory (=tubular) salivary gland; msg—main (=acinous) salivary gland; RL—radula length; RW—rachidian width; SH—shell height; SW—shell width.

RESULTS

Systematic Descriptions

Family: Muricidae Rafinesque, 1815

Thais keluo new species

(Figs 1, 3, 4–6)

Holotype (Figs 1a–d) (NMNS-IN-3505001) SH=33.8 mm, AH=22.2 mm, SW=20.7 mm, male. Type locality: Tungkang, Taiwan. On metal plates at mouth of estuary, collected by K.S. Tan, 17 June 1999; five paratypes (NMNS-IN-3505002) from Tsaishan and Tungkang, Taiwan, deposited in the National Museum of Natural Science, Taichung, Taiwan and three paratypes (ZRC.2001.1094–1096) deposited in the Zoological Reference Collection, Raffles Museum of Biodiversity Research, Department of Biological Sciences, National University of Singapore.

Diagnosis: Shell—Low, spirally elongate, dark brown or black tubercles borne on four prominent, raised spiral rows on last whorl; yellowish-white columella bears one or two oblique plicae; outer lip inside edge has a thin, finely crenulated reddish-brown border. Animal—Sides of foot yellowish-white with mottled dark grey and black surface pigment; tentacles with distinct, broad brownish-black transverse pigment band near eye; penis base gradually tapers towards flagellum, whose tip is simple; accessory boring organ located dorsal to the ventral pedal gland in females; sperm length 104–106 μm.

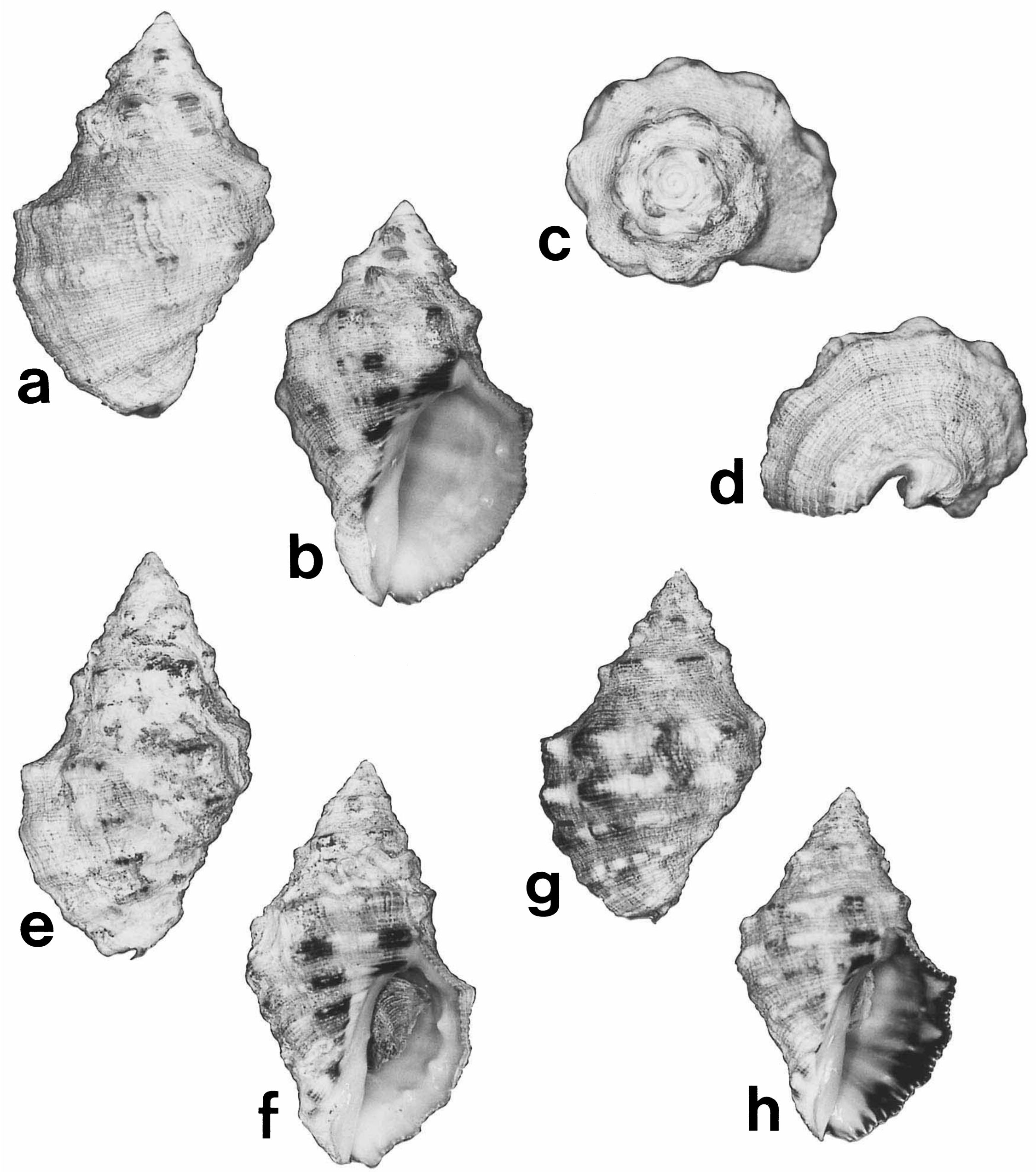

Fig. 1. *Thais keluo* new species. (a–d) Holotype (NMNS-IN-3505001) Tungkang, Taiwan, SH=33.8 mm, SW=20.7 mm, male; (e, f) paratype, Tsaishan, Kaohsiung, Taiwan (NMNS-IN-3505002) SH=34.1 mm; (g, h) paratype, Tungkang, Taiwan, SH=26.1 mm, juvenile.

Etymology: This species is named for the generic Chinese term used in Taiwan for *Thais* species.

Material examined (for morphology): Taiwan: Tsaishan, Kaohsiung (23 specimens); Tungkang (30 specimens). See Table 2 for locality coordinates.

Shell (Fig. 1a–h): Height up to 37 mm, high-spired; entire shell lined with fine, narrow, primary spiral cords each 0.2–0.5 mm wide, crossed collabrally by axial threads. The *protoconch* was not examined. The last whorl bears between 5 and 10 (mode=6, n=27) elongate, anterior-posteriorly compressed tubercles on each of the four raised spiral rows present. Tubercles on the first and second rows are the largest, while those on the third and fourth rows are smaller and less prominent. There are between 60 and 70 primary spiral cords on the last whorl, which are generally flat and narrow, with the widest cords crossing the tubercles. The grooves separating the cords are narrow and are less than one-third the width of the cords. The suture is formed at or just below the second row of tubercles on the penultimate whorl. About 11–15 primary spiral cords form the sutural shelf. The region between the axial rows of tubercles is white or yellow, which alternate with black bands that correspond to axial rows of tubercles. As most tubercles are black (except the most recently formed ones which are white), they form black axial bands on the last

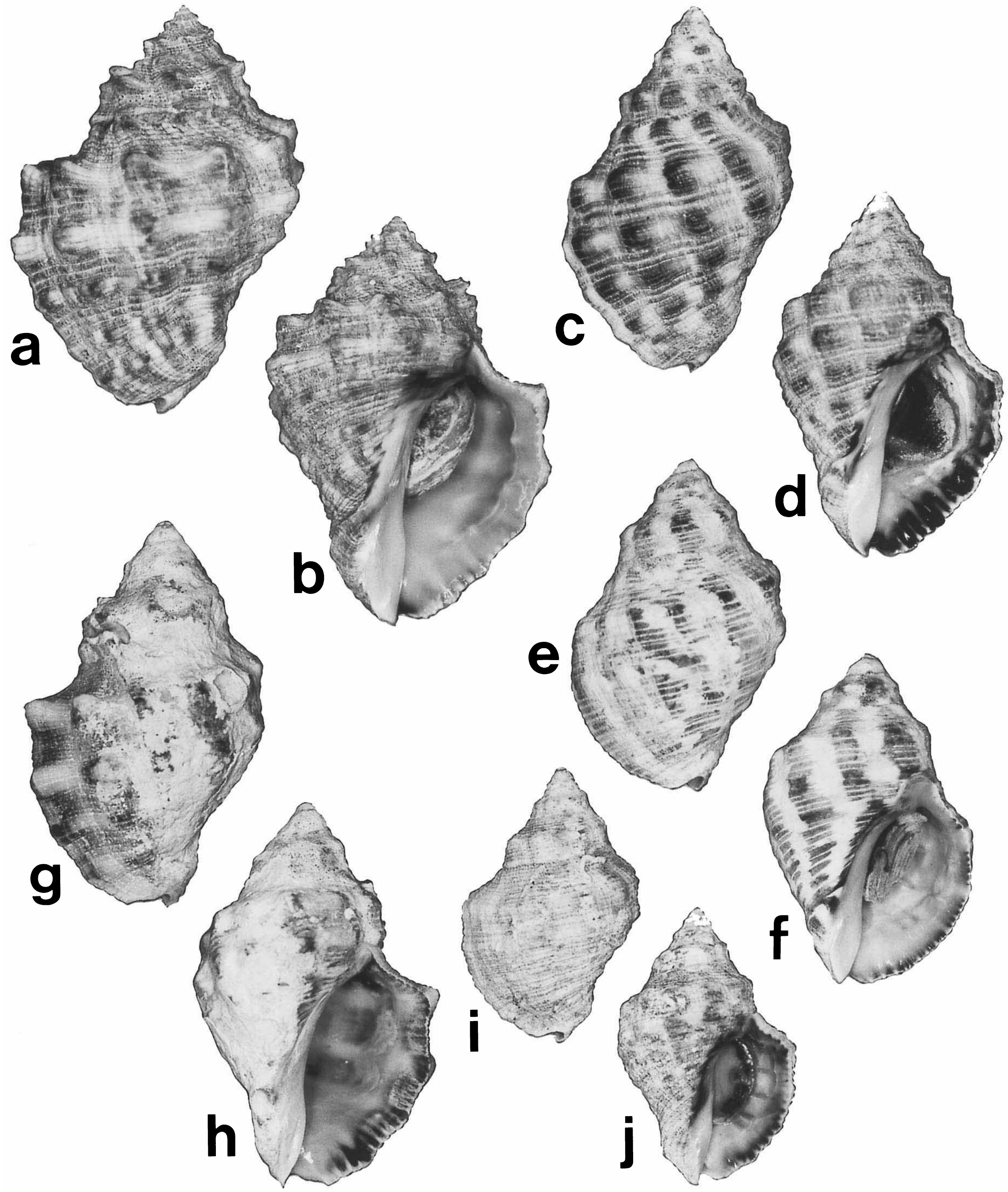

Fig. 2. Shells of *Thais* species. (a, b) *Thais bitubercularis* (Lamarck), Chiku, Taiwan, SH=36.7 mm; (c, d) *Thais clavigera* (Küster), Ta-an (24 21’N, 120 34’E), Taiwan, SH=33.2 mm; (e, f) *Thais jubilaea* Tan and Sigurdsson, Kuantan, Malaysia, SH=29.9 mm; (g, h) *Thais luteostoma* (Holten), Cape d’Aguilar, Hong Kong, SH=34.9 mm; (i, j) *Thais rufotincta* Tan and Sigurdsson, Tungkang, Taiwan, SH=25.2 mm.

whorl. *Aperture*: the edge of the outer lip is finely crenate and has a thin, narrow reddish-brown border at the edge. Four white, papillate denticles are present inside the outer lip, which correspond in position to regions between tubercles (of an axial row of tubercles) on the last whorl. The interior of the aperture is off-white, while the inside edges of the outer and inner lips may be tinged yellowish-orange. The columella is entirely off-white, narrow, straight with one or two prominent oblique plicae present. One plica is located at the mid-region of the columella, while the other is slightly anterior to the first. The parietal region is black due to the tubercles showing through. The anal canal is not well-developed. *Shell microstructure* comprises three aragonitic crossed-lamellar layers. Operculum (Fig 3) Reddish-brown, opaque, attached surface

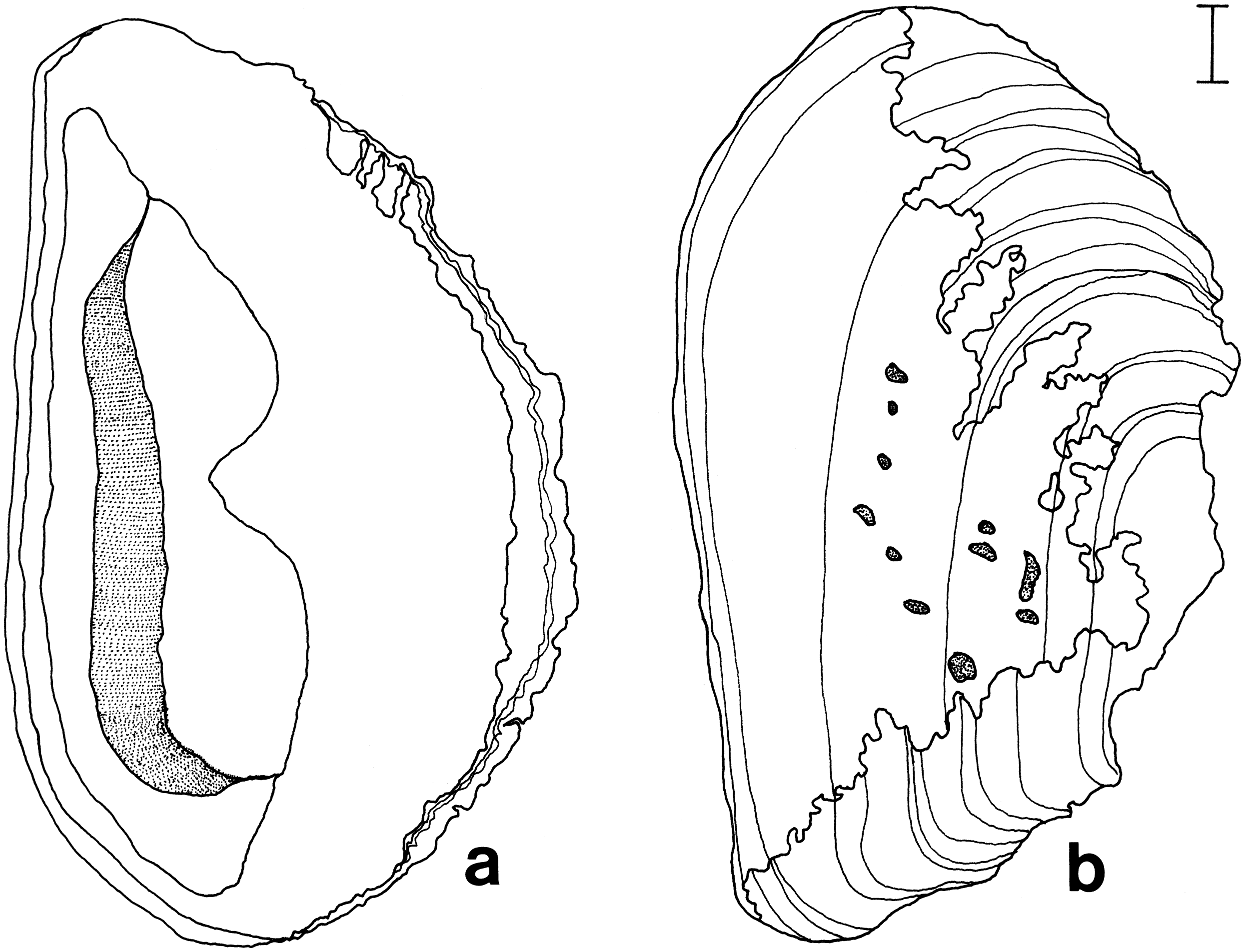

Fig. 3. *Thais keluo* new species: Operculum. (a) attached surface; (b) external surface. Scale bar=1 mm

with single adventitious layer. The external surface bears a lateral nucleus.

External anatomy: The sole of foot is yellowish-white to grey, with no subcutaneous pigment grains, while the edges of the sole have sparse yellow and white subcutaneous pigment. The sides of foot is yellowish-white with mottled dark grey and black surface pigment. Large aggregations of subcutaneous yellow pigment grains and smaller quantities of white pigment grains can be seen through the surface pigmentation. The head region is mottled grey and black on the surface. *Tentacles*: from base to eyes, same as sides of foot; from eye to tip, there is a distinct, broad brownish-black transverse pigment band across the tentacle, while the tip is devoid of surface pigment, revealing the subcutaneous white and yellow pigment grains underneath. The penis is curved and flagellate. The base of the penis gradually tapers towards flagellum; the flagellum tip is simple (Fig. 4). On the dorsal surface of the mantle, the black, prominent rectal gland can be seen to contrast with the bright yellow hypobranchial gland. ABO is located dorsal to ventral pedal gland in females. The osphradium is symmetrical (i.e., left and right leaflets are of about the same width) and is about 0.75 times the length of the ctenidium. The free edges of the ctenidial leaflets are rounded.

Internal anatomy: Proboscis about 6 mm long, 2 mm in diameter (SH=32 mm), not pigmented. Accessory salivary glands are translucent yellow; both left and right asg are convoluted and of equal size, about 3 mm in length, 0.6 mm diameter; left asg is embedded in msg whilst the right asg is attached to floor of body cavity, not attached to msg. A glande framboisée is present but somewhat small and elongate, while the gland of Leiblein is large and enmeshed in a fibrous coat. In females, the ventral channel in the capsule gland of females is formed by a single, folded left flange that is grooved (Fig. 5). Posterior to the capsule gland, the yellowish-brown sperm ingesting gland comprises multiple chambers. Several posterior seminal receptacles are attached to the dorsal region of the anterior fold of the albumen gland. In males, the pallial vas deferens was not examined. Sperm length 104–106 μm (n=10).

Radula (Fig. 6): Radula length 9.5 to 10.3 mm (SH=28–32 mm), rachidian width 99–108 μm. Rachidian teeth: Central cusp is long, slightly curved while the lateral cusps point slightly outwards, with a broad base; the marginal cusps are promi-

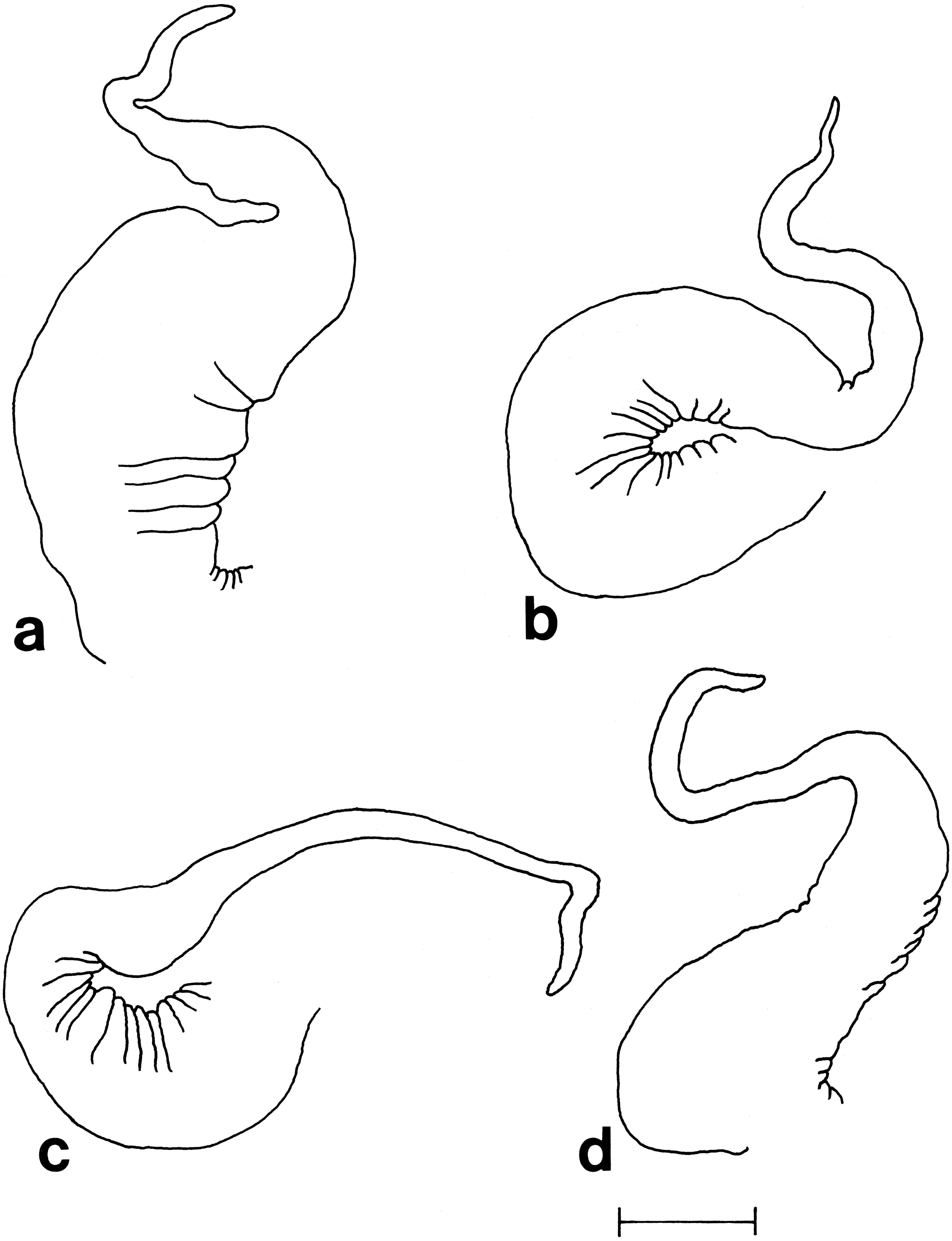

Fig. 4. *Thais keluo* new species. Penes. (a–d) Scale bar=1 mm.

nent, sharp, pointing outwards; there are between four and five (rarely six) marginal denticles, of which two or three are lateral cusp serrations; lateral teeth have a narrow base. No structural differences were discernible between the radulae of males and females.

Egg capsules: Unknown.

Habitat: Intertidal exposed rocky shores, generally occurring together with *Thais rufotincta* and/or *T. clavigera*.

Distribution: The new species is known from Taichung, Chiku, Kaohsiung and Tungkang along the coast of western and southwestern Taiwan.

Similar species: The shell of *Thais keluo* n.sp. closely resembles those of *T. bitubercularis*, *T. clavigera*, *T. jubilaea*, *T. luteostoma* and to a lesser extent, to *T. rufotincta* (see Fig. 2) in terms of general shell morphology. The latter species, *T. rufotincta*, can be immediately singled out from all the others by the presence of about ten (up to 13) small, reddish-brown tubercles along the carina of the last whorl, a dark, brownish-black parietal region that extends halfway anteriorly along the

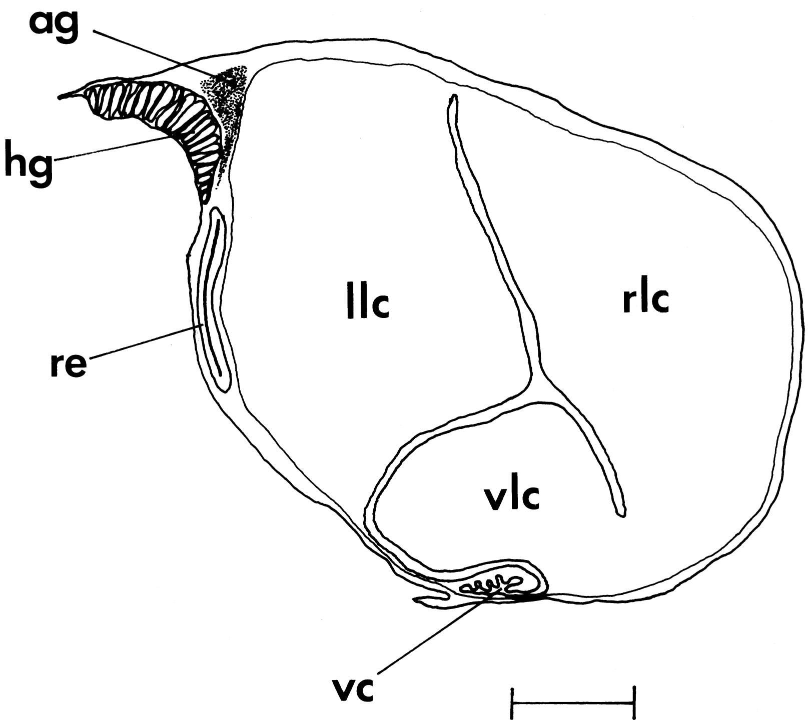

Fig. 5. *Thais keluo* new species. Cross section of capsule gland at mid-region, viewed anteriorly. Abbreviations: ag–anal gland; hg–hypobranchial gland; llc–left lobe of capsule gland; re–rectum; rlc–right lobe of capsule gland; vc–ventral channel; vlc–ventral lobe of capsule gland. Scale bar=1 mm.

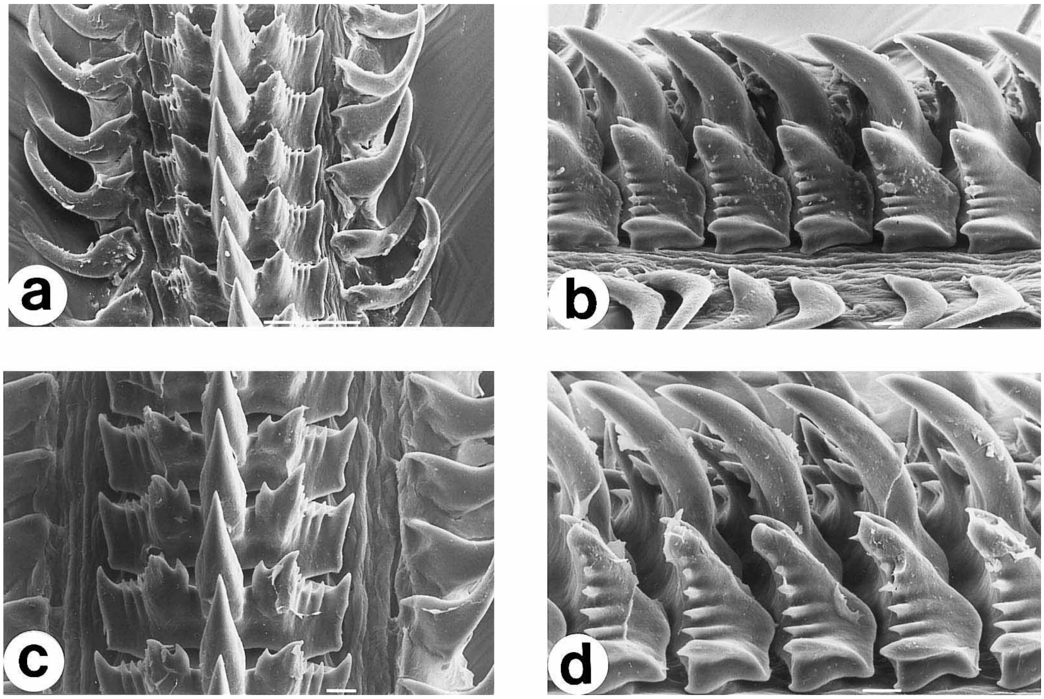

Fig. 6. *Thais keluo* new species. Radula. (a, b) male; (c, d) female. (a, c) and (b, d) show perpendicular and side (60°) views, respectively. Scale bar for (a)=50 μm; Scale bar for (b,c,d)=10 μm.

columella towards the siphonal canal, and between 4 and 8 spirally elongate, thin apertural denticles inside the outer lip (Tan and Sigurdsson, 1996a). All of the others, including the new species, have generally less than 10 (modal value) black and/or grey tubercles/knobs around the carina on the last whorl, an orange, yellow or white columella, and between 4 to 6 papillate denticles on the inside of the outer lip (except in *T. luteostoma* where these denticles are absent). In a recent

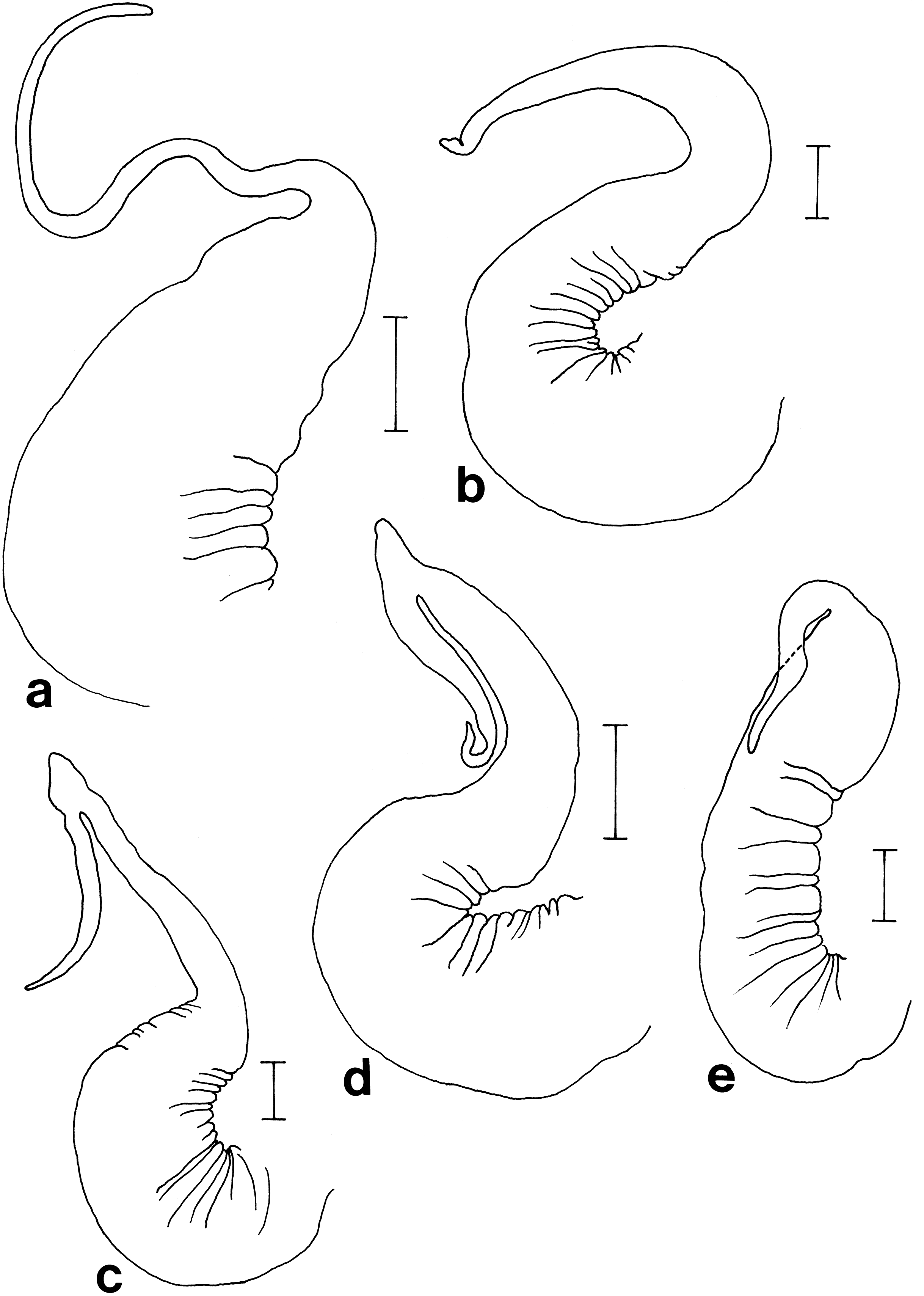

Fig. 7. Penes of *Thais* species. (a) *Thais bitubercularis*; (b*) Thais clavigera*; (c) *Thais jubilaea*; (d) *Thais luteostoma*; (e) *Thais rufotincta*. Scale bar=1 mm

publication, Tsuchiya in Okutani (2000) illustrated two shells labelled *T. bronni* (Dunker) and *T.* "*kyteistina* (Holten)" (page 398, species nos 181 and 182, respectively in Okutani, 2000). *Thais bronni* is a well-known species found in Japan, Korea and northern China with large, off-white bulbous tubercles on the last whorl (the lectotype of *T. bronni* is illustrated in Janssen, 1993: plate 4, fig. 27; see also Dunker, 1861, plate 1, fig. 23; Kira, 1965, plate 24, fig. 6; Habe and Kosuge, 1967, plate 28, fig. 7; Habe and Okutani, 1975, page 112; Fujioka, 1986, page 137). The specimen (species no. 181) illustrated in Okutani (2000), which was collected in Aichi prefecture, central Honshu, Japan (K. Tsuchiya, pers. comm.), is therefore not *T. bronni*, but appears to resemble *T. keluo*, although this requires anatomical confirmation. The label *T.* "*kyteistina* (Holten)" for species no. 182 is a gross typographical error for *T. luteostoma* (K. Tsuchiya, pers. comm.) and the name "kyteistina" does not exist.

Amongst *T. keluo* n.sp., *T. bitubercularis*, *T. clavigera*, *T. jubilaea* and *T. luteostoma*, penis morphology is diagnostic for all except the latter two species which possess barbed penes (Fig. 7c, d; see also Tan and Sigurdsson, 1990; Proud and Richardson, 1997); a somewhat similar barbed construction is also characteristic of *T. bronni* (see Fujioka, 1986: p. 136 for an illustration of its penis). The penis of *T. keluo* has a flagellum that is long with a simple tip, while that of *T. bitubercularis* is very long and drawn almost into a thread but also has a simple tip (Fig 7a; Tan and Sigurdsson, 1990). In comparison, the distal end of the flagellum in the penis of *T. clavigera* is in the form of a hook (Fig. 7b, this study; Tan and Sigurdsson, 1990; Proud and Richardson, 1997). The penis of *T. rufotincta* has a distinct short flagellum attached to a broad, elongate proximal region (Fig. 7e; Tan and Sigurdsson, 1996a).

There are also minor differences in the structure of the rachidian teeth in the four species, the most striking being the number of marginal denticles, which in *T. bitubercularis* and also in *T. luteostoma*, there are between 5 and 8 denticles. This is in contrast to those of *T. keluo* n.sp. which has only four or five on either side of its rachidian teeth. However, the rachidian teeth of *T. clavigera* and *T. jubilaea* resemble closely to those of *T. keluo* and are difficult to distinguish.

Except for the new species, almost all the above men-

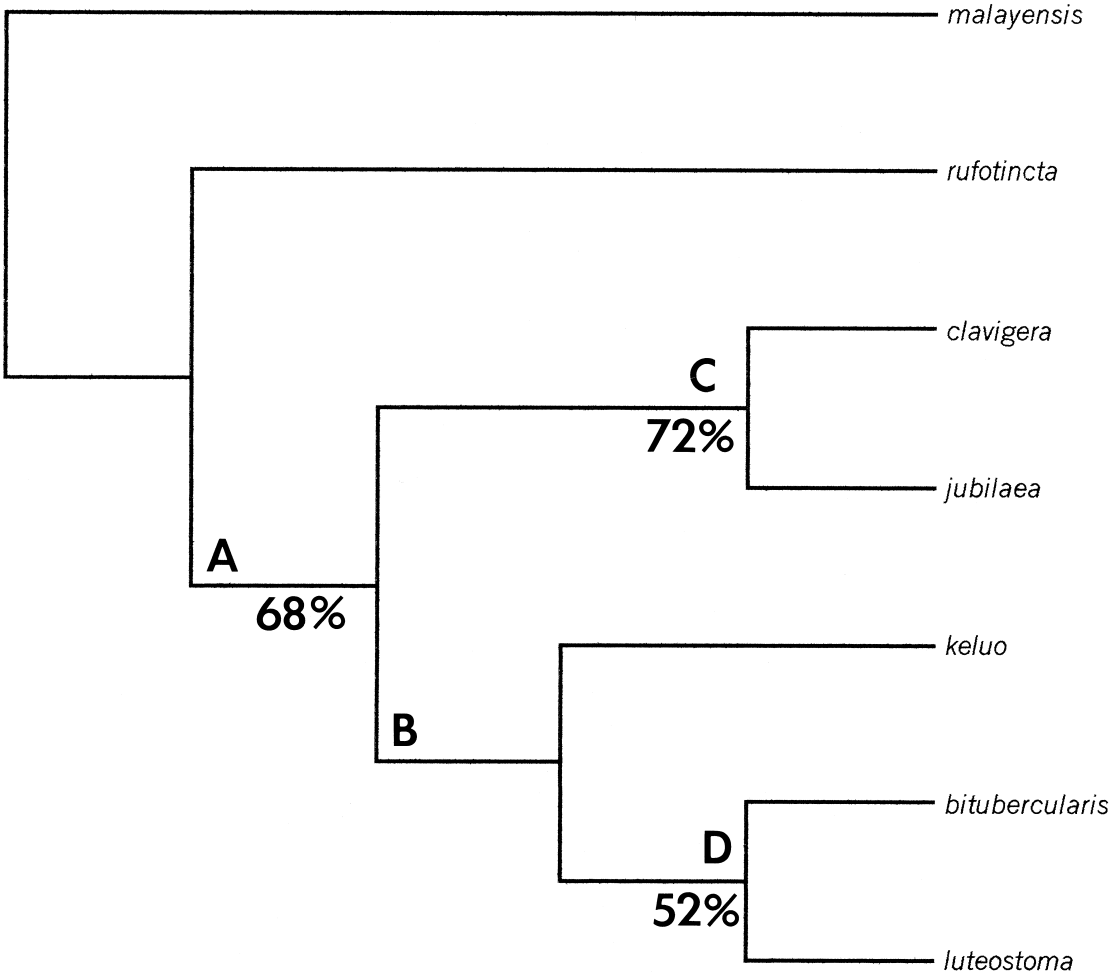

Fig. 8. Phylogenetic tree obtained by PAUP* based on the morphological data matrix as provided in Table 1. *Thais malayensis* was the outgroup taxon. A–D are monophyletic clades. Percentages beside these clades indicate bootstrap support based on 10,000 replicates.

Table 3. Allele frequencies and heterozygosities for 9 loci of *Thais* species. n: sample size; H_o, H_e: observed and expected heterozygosity, respectively; **: significant deviation from Hardy-Weinberg proportion at p=0.01.

Locus allele	*Thais clavigera*		*Thais luteostoma*	*Thais keluo* n.sp.	*Thais rufotincta*		
	Chiku	Cape d'Aguilar	Cape d'Aguilar	Tunkang	Chiku	Kaohsiung	Tungkang
ARK							
n	55	13	24	23	25	32	30
100	0.6182	0.8462	0.6458	0.9783	1.0000	0.9844	0.9833
55	0.3818	0.1538	0.3542	0.0217	0.0000	0.0156	0.0167
H_o	0.4727	0.1538	0.4583	0.0435	0.0000	0.0313	0.0333
H_e	0.4764	0.2708	0.4672	0.0435	0.0000	0.0313	0.0333
EST							
n	66	22	30	29	34	44	30
100	1.0000	0.9773	1.0000	1.0000	0.0588	0.0000	0.0500
86	0.0000	0.0227	0.0000	0.0000	0.9118	1.0000	0.9500
50	0.0000	0.0000	0.0000	0.0000	0.0294	0.0000	0.0000
H_o	0.0000	0.0000	0.0000	0.0000	0.1765	0.0000	0.0333
H_e	0.0000	0.0000	0.0000	0.0000	0.1668	0.0000	0.0966**
LAP							
n	66	22	29	29	36	44	30
130	0.0000	0.0000	0.0517	0.0000	0.0139	0.0000	0.0000
120	0.0000	0.0227	0.0000	0.0000	0.0139	0.0455	0.0833
110	0.0000	0.0000	0.0000	0.0000	0.9722	0.9205	0.9167
105	0.0455	0.0455	0.0172	0.0000	0.0000	0.0000	0.0000
100	0.9318	0.8864	0.8448	0.0345	0.0000	0.0227	0.0000
90	0.0076	0.0227	0.0517	0.7069	0.0000	0.0000	0.0000
83	0.0152	0.0227	0.0345	0.2586	0.0000	0.0114	0.0000
H_o	0.1364	0.2273	0.3103	0.3793	0.0556	0.1591	0.1667
H_e	0.1303	0.2156	0.2843	0.4398	0.0552	0.1518	0.1554
MDH-1							
n	66	22	30	29	36	44	30
500	0.0000	0.0000	0.0000	1.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	0.0000	0.0000	0.0000	0.0000
MDH-2							
n	66	22	30	29	36	44	30
100	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.0000
14	0.0000	0.0000	0.0000	0.0000	1.0000	1.0000	1.0000
MDH-3							
n	66	22	30	29	36	44	30
156	0.0000	0.0000	0.0000	0.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.0000
PGM-1							
n	66	22	30	29	32	39	30
163	0.0000	0.0000	0.0000	0.0517	0.0000	0.0000	0.0000
150	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000
129	0.0000	0.0000	0.0000	0.9138	0.0000	0.0000	0.0000
118	0.0606	0.0455	0.7500	0.0000	0.0000	0.0000	0.0000
100	0.8712	0.7955	0.1833	0.0345	0.0938	0.0769	0.1333
82	0.0000	0.0000	0.0000	0.0000	0.9063	0.9231	0.8667
64	0.0682	0.1591	0.0500	0.0000	0.0000	0.0000	0.0000
H_o	0.2576	0.4091	0.3000	0.1724	0.1875	0.1538	0.2667
H_e	0.2344	0.3487	0.4079	0.1639	0.1726	0.1439	0.2350
PGM-2							
n	66	22	30	29	36	44	30
110	0.0000	0.0227	0.0000	0.0000	0.0000	0.0000	0.0000
100	1.0000	0.9773	1.0000	1.0000	0.0000	0.0000	0.0000
80	0.0000	0.0000	0.0000	0.0000	1.0000	1.0000	0.9833
69	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0167
H_o	0.0000	0.0455	0.0000	0.0000	0.0000	0.0000	0.0333
H_e	0.0000	0.0455	0.0000	0.0000	0.0000	0.0000	0.0333
XDH							
n	66	22	30	29	36	44	30
117	0.0000	0.0000	1.0000	0.0000	1.0000	1.0000	1.0000
100	1.0000	1.0000	0.0000	1.0000	0.0000	0.0000	0.0000

tioned species have a relatively wide and overlapping geographical distribution. *Thais bitubercularis* ranges from Southeast Asia and Taiwan through the Philippines and the Malay archipelago to northeastern Australia (as *T. kieneri*), while *T. clavigera* has a remarkable north-south distribution from Singapore through Hong Kong and Taiwan into northern Japan (Tan, 1995). *Thais jubilaea* appears to have a restricted range in the Gulf of Thailand and Malacca Straits, and *T. luteostoma* is likewise confined to the subtropical Chinese coast, including Hong Kong and Kinmen (Tan, pers. obs.).

Not withstanding the above mentioned differences in morphology, there are notable similarities between the new species and four other species mentioned (namely, *T. bitubercularis*, *T. clavigera*, *T. jubilaea* and *T. luteostoma*). Besides their remarkable resemblance in overall shell shape, size, and colour, the pigmentation patterns on the foot are surprisingly similar, with white and yellow subcutaneous pigment grains distributed under the mottled black surface pigmentation. All five species possess opercula with a single adventitious layer, have pigmented bands near the eyes on their tentacles and, internally, have long, convoluted, paired accessory salivary glands. Sperm length in males of all five species is confined to a narrow range of between 96 and 106 µm. Females all possess multiple-chambered sperm ingesting glands, and for those whose egg capsules are known (i.e., all except the new species) their shape and size appear remarkably similar. All of these suggest that they are closely related, but the superspecific relationships amongst species based on phylogenetic analysis are still unclear (e.g., Kool, 1993; Vermeij and Carlson, 2000) and generic assignment is therefore not entirely satisfactory at this time.

Cladistic analysis

The maximum parsimony analysis of 7 taxa and 11 characters (see Table 1) using PAUP* yielded a single tree (Fig. 8) that is 14 steps long (CI=0.800; RI=0.750; RC=0.643; HI=0.200). Four characters (characters 1, 3, 4 and 8) were uninformative and were excluded from the analysis. Except for characters 2 (number of aragonitic crossed-lamellar layers) and 7 (penis shape) with CI values of 0.500 and 0.667 respectively, CI values for all other characters were 1.000. Bootstrap values obtained for clades A, C and D (Fig. 8) were

Table 4. Summary of genetic variation in *Thais* species. N: average number of individuals examined per locus; No. of alleles: average number of alleles per locus, with standard errors. Percent polymorphism was determined using the 0.95 criterion; heterozygosity data show mean heterozygosities across loci, with standard errors.

Species	N	No of alleles	% polymorphism	Heterozygosity	
				Observed	Expected
Thais clavigera					
Chiku, Taiwan	64.8	1.7±0.1	33.3	0.0963±0.0208	0.0935±0.0206
Cape d' Aguilar, Hong Kong	21.0	2.0±0.3	33.3	0.0979±0.0309	0.1028±0.0298
T. luteostoma					
Cape d'Aguilar, Hong Kong	29.2	1.9±0.3	33.3	0.1187±0.0340	0.1288±0.0368
T. keluo n.sp.					
Tungkang, Taiwan	28.3	1.6±0.2	22.2	0.0661±0.0245	0.0719±0.0279
T. rufotincta					
Chiku, Taiwan	34.1	1.6±0.2	22.2	0.0466±0.0135	0.0438±0.0126
Kaohsiung, Taiwan	42.1	1.6±0.2	22.2	0.0382±0.0105	0.0363±0.0099
Tungkang, Taiwan	30.0	1.6±0.1	33.3	0.0593±0.0172	0.0615±0.0154

Table 5. Nei's (1978) Genetic identities (above diagonal) and genetic distances (below diagonal) among *Thais* species.

Population	cl-Chiku	cl-HK	lu-HK	ke-Tungkang	ru-Chiku	ru-Kaohsiung	ru-Tungkang
cl-Chiku		0.9934	0.8160	0.6882	0.0905	0.0835	0.0940
cl-HK	0.0066		0.8151	0.7134	0.1198	0.1123	0.1225
lu-HK	0.2034	0.2045		0.5827	0.2095	0.2030	0.2106
ke-Tungkang	0.3737	0.3377	0.5401		0.2405	0.2313	0.2400
ru-Chiku	2.4023	2.1223	1.5629	1.4249		0.9993	0.9996
ru-Kaohsiung	2.4826	2.1865	1.5948	1.4640	0.0007		0.9997
ru-Tungkang	2.3649	2.0994	1.5578	1.4272	0.0004	0.0003	

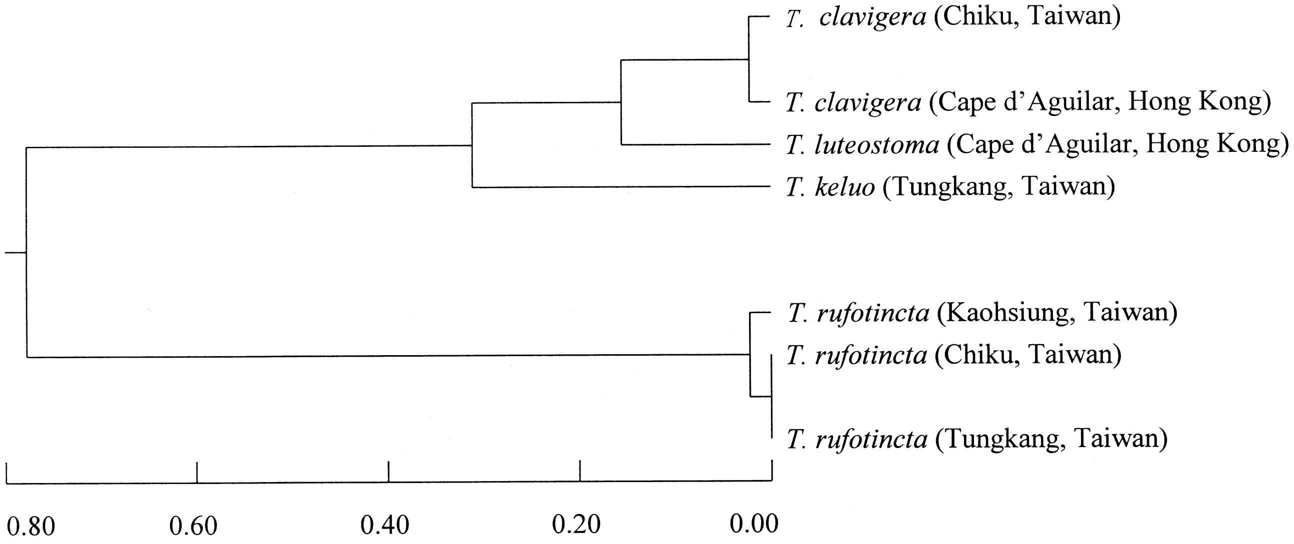

Fig. 9. The UPGMA cluster analysis tree based on Nei's (1978) unbiased minimum distance (D) amongst four *Thais* species.

between 52 and 72%, and Bremer support was calculated to be one (D=1) for clades A, B, C and D. Using *Thais malayensis* as the outgroup taxon, *T. rufotincta* is shown to be a sister species to clade A, which contains *T. clavigera*, *T. jubilaea*, *T. keluo* n.sp., *T. bitubercularis* and *T. luteostoma*. This group is supported by two synapomorphies: pustulate outer lip denticles (character 5) and the partial occupation of the capsule surface by the exit orifice of egg capsules laid (character 11). Clade B containing the new species forms a monophyletic group with Clade C (*T. clavigera*, *T. jubilaea*). Clade B is supported by a single synapomorphy: the possession of a grooved ventral channel in the female capsule gland (character 10). *Thais clavigera* and *T. jubilaea* (Clade C) share one unambiguous synapomorphy: the possession of short euspermatozoa (character 9), while *T. bitubercularis* and *T. luteostoma* (Clade D) have six or more marginal denticles on either side of their rachidian teeth (character 6), a synapomorphy that the two species share.

A similar analysis using *T. malayensis* as the outgroup taxon but excluding taxa which were not examined in the allozyme analysis produced one tree with a similar distribution of clades.

Allozyme electrophoresis

Among the nine loci examined, four loci (ARK, EST, LAP and PGM-1) were polymorphic, using 0.95 as the criterion for polymorphism. Detailed allelic frequencies of the four *Thais* species are given in Table 3. Fixed allelic differences were observed for PGM-1 and XDH between *T. clavigera* and *T. luteostoma*, for LAP, MDH-1 and PGM-1 between *T. clavigera* and *T. keluo* n. sp, and finally for EST, LAP, MDH-1, MDH-2, MDH-3, PGM-1, PGM-2 and XDH between *T. clavigera* and *T. rufotincta*. The mean heterozygosities (see Table 4) were considerably higher in *T. clavigera* and *T. luteostoma* (between 0.0963 and 0.1187) than for *T. keluo* and *T. rufotincta*, which was calculated to range between 0.0382 and 0.0661.

Table 5 shows Nei's (1978) genetic identities and distances based on nine loci from four *Thais* species. The UPGMA cluster analysis (Fig. 9) using POPGENE based on Nei's unbiased minimum distance (D) values indicated that minor differentiation existed amongst Taiwan and Hong Kong populations of *T. clavigera* ($D<0.005$) and amongst Taiwan populations of *T. rufotincta* ($D<0.0002$). The genetic distance between *T. clavigera* and *T. luteostoma* was 0.16 units. *Thais keluo* n.sp. was separated from *T. clavigera* and *T. luteostoma* by 0.31 units, while *T. rufotincta* was separated from the other three *Thais* species by 0.78 units.

DISCUSSION

Our results indicate that Nei's genetic distances and levels of fixed allelic differences among *Thais* species were well above the range of values generally expected at the specific level. *Thais rufotincta* is clearly a different species from the rest, as apparent from its genetic distance value of 0.78 and its distinctive shell and penis. The new species described in this study is also distinguished from *T. clavigera* and *T. luteostoma* in terms of its genetic distance and morphological characters. *Thais keluo*, separated by a genetic distance of 0.31 from *T. clavigera* and *T. luteostoma*, may have previously been confounded with the two species, but allozyme and anatomical differences provide sufficiently compelling evidence for its recognition as a separate species.

Although there is no straightforward relationship between genetic distance and taxonomic distinctness, Thorpe (1983) found that Nei's genetic distances ranged between 0.19 and 2.59 for an overwhelming majority (95%) of congeneric invertebrates. Richardson *et al.* (1986) also suggested that loci with fixed allelic differences can be diagnostic in separating species if these loci are more than 20% that of the examined loci. Three widespread *Drupella* (Gastropoda: Muricidae) species examined by Johnson and Cumming (1995) were separated by an average D value of 0.25. In other allozyme studies of congeneric *Thais* species in the NW Pacific, D values of 0.1161–0.1175 (in Japan; Hayashi, 1999) and 0.363–0.473 (in Korea; Park and Choe, 1999) separated *T. clavigera* and *T. bronni*. The North American oyster drills *T.* (*Stramonita*) *haemastoma canaliculata* and *T.* (*S.*) *h. floridana* are considered to be separate species with a D value of 0.30 (Liu *et al.*, 1991). It is apparent that even amongst congeneric morphospecies, genetic distances vary considerably.

In this study, it is shown that *T. clavigera* and *T. luteostoma* are morphologically distinct, with clearly different shell, radula and penis morphologies which are reflected in their placement in different clades in the phylogenetic analysis. Allozyme electrophoresis, however, show that the two species are separated by a genetic distance (Nei's standard D) of 0.16 units. The resulting relationship as derived from an UPGMA analysis is that *T. luteostoma* is more closely related to *T. clavigera* as compared to *T. keluo* n.sp. In contrast, PAUP suggests that *T. luteostoma* forms a monophyletic group with *T. keluo*, although this clade (Clade B; see Fig. 8) is only supported by a single, somewhat weak synapomorphy (presence of grooves in the ventral channel of the female capsule gland). The phylogenetic tree also indicates that another closely related species, *T. bitubercularis*, to be monophyletic with *T. luteostoma* and *T. keluo* n.sp. and forms a clade with the former. Currently there is no additional evidence to support either hypothesis, and the resolution of this problem will require an allozymic assessment of *T. bitubercularis*. Indeed, *T. keluo* and *T. bitubercularis* may be closely related. It is possible to distinguish the two species based on their shells (e.g. the shell of *T. keluo* is narrow and the spire is tall) and radula (there are more numerous marginal denticles on the rachidian teeth of *T. bitubercularis*) but both have a simple, long flagellum on their penes. It is generally long and thin in the case of *T. bitubercularis* but because of its contractile ntractile nature it can resemble those of *T. keluo*.

The allozyme survey is consistent with the conclusions of the morphological analysis described in this study, which showed that *Thais keluo* n.sp. is closely related but clearly separate from *T. clavigera* and *T. luteostoma*. *Thais rufotincta* is also shown to be farthest in relationship from these three species. The results of this study bring the total number of *Thais* species present in the South China Sea (Tan, 2000) to 21 species.

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