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Tridacna noae (Röding, 1798) – a valid giant clam species separated from *T. maxima* (Röding, 1798) by morphological and genetic data

Y. Su[1], J.-H. Hung[2], H. Kubo[3] & L.-L. Liu[2*]

Abstract. The taxonomic status of a giant clam species *Tridacna noae* (Röding, 1798) in relation to its congener *T. maxima* (Röding, 1798) was examined by a combination of molecular and morphological analyses. Until recently, *T. noae* was considered conspecific with *T. maxima*. However, in this study, among the four genes (COI, 16S rRNA, 18S rRNA, 28S rRNA) examined, two (i.e., 16S rRNA and COI) genes grouped *T. maxima* into two distinct clades, suggesting that a cryptic species was involved. The genetic distance of the 16S rRNA gene between *T. maxima* and cryptic species was 0.042–0.048. In contrast, the differences within the clades comprising *T. maxima* and the cryptic species were 0–0.003 and 0–0.005, respectively. A careful comparison of morphological traits revealed differences between *T. maxima* and the cryptic species, and the shells of the latter species were consistent with the figures referred to by Röding for the species he named '*noae*'. *Tridacna noae* differs from *T. maxima* in the appearance and arrangement of hyaline organs on the mantle in living specimens. In contrast, shell characters such as numbers of radial ribs and prominence of rib scales, were relatively unreliable. Occasionally, prominent rib scales only grew in one valve of an individual, e.g., in one *T. maxima* specimen from Hongchia. Based on previous and the present studies, here, a formal taxonomic description of *Tridacna noae* (Röding, 1798) with the designation of a neotype is provided.

Key words. Tridacninae, CO1, 16S rRNA, 18S rRNA, 28S rRNA, lectotype, Taiwan

INTRODUCTION

Members of the cardiid bivalve subfamily Tridacninae (Schneider & O'Foighil, 1999; WoRMS, 2013) are a small but conspicuous group of large bivalves more commonly known as giant clams. These bivalves are exploited extensively for their flesh, shell, and the living organism resulting in a decline of populations throughout their Indo-Pacific range (Brown & Muskanofola, 1985; Juinio et al., 1989; Othman et al., 2010). Giant clams are listed as vulnerable in the IUCN (International Union for Conservation of Nature) list and all species are categorised in Appendix II of CITES (United Nations Convention on International Trade in Endangered Species of Wild Fauna and Flora).

Currently, two species in the genus *Hippopus* and eight species in the genus *Tridacna* are recognised, i.e., *Hippopus porcellanus* Rosewater, 1982, and *H. hippopus* (Linnaeus, 1758); *Tridacna squamosa* Lamarck, 1819, *T. crocea* Lamarck, 1819, *T. maxima* (Röding, 1798), *T. gigas* (Linnaeus, 1758), *T. derasa* (Röding, 1798) (Rosewater, 1965, 1982), *T. tevoroa* Lucas, Ledua & Braley, 1991, *T. rosewateri* Sirenko & Scarlato, 1991, and *T. costata* Roa-Quiaoit, Kochzius, Jantzen, Zibdah & Richter, 2008 (Rosewater, 1965, 1982; Lucas et al., 1991; Richter et al., 2008). Recently *T. costata* was synonymised with *Tridacna squamosina* Sturany, 1899 (Huber & Eschner, 2011). In Taiwan, six species of giant clams have been reported, i.e., *H. hippopus*, *T. crocea*, *T. gigas*, *T. squamosa*, *T. maxima*, and *T. derasa* (Catalogue of Life in Taiwan, http://taibnet.sinica.edu.tw/). Of these, *T. derasa* and *T. gigas* have not been recorded over the last three decades and may now be locally extinct in Taiwan. Other giant clam species in Taiwan waters are also uncommon, occurring in densities between 1–5 individuals 100 m^{-2} (pers. obs.).

Among members of the subfamily Tridacninae, *T. maxima* is the most common and widely distributed species in the Indo-Pacific, ranging from the Red Sea, Madagascar, and East Africa to the Tuamotu Archipelago and Pitcairn Island in the South Pacific, as well as from southern Japan in the north to Lord Howe Island, off the coast of New South Wales in the south (Othman et al., 2010). It is a reef-top inhabitant, living either on the surface of the reef or sand, usually seen with its coloured mantle exposed.

[1]Department of Marine Sport and Recreation, National Penghu University, Magong, Penghu 880, Taiwan, R.O.C.

[2]Department of Oceanography, National Sun Yat-sen University, Kaohsiung 804, Taiwan, R.O.C.; Email: lilian@mail.nsysu.edu.tw (*corresponding author LLL)

[3]Okinawa Prefectural Fisheries and Ocean Research Center, Kyan 1528, Itoman, Okinawa, Japan

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Rosewater (1965) regarded several species including *T. elongata* Lamarck, *T. noae* (Röding), *T. imbricata* (Röding), *T. lanceolata* Sowerby, and *T. fossor* Hedley as variants of *T. maxima* in view of their close morphological relationships. More recently, however, the existence of a cryptic species amongst *T. maxima* individuals was reported by Tang (2005) in Taiwan and Kubo & Iwai (2007) in Okinawa and the Ishigaki Islands, Japan. Both studies observed that mantle colours of *T. maxima* varied considerably, from brilliant to subdued grayish yellow, bluish green, blackish blue, to purple and brown. These colours occurred medially on the mantle and were sometimes spotted and streaked with other colours. Often there is a peripheral-banded pattern with different colours on the mantle margin, and black hyaline organs (eyes) formed a distinct continuous line along the mantle margin. In contrast, the mantle pattern of *T. noae* (designated as *T. maxima* type A in Tang, 2005) had one to several obvious layers of oval patches bounded by white margins, and the black hyaline organs along the border of the mantle formed a discontinuous line. The shell of *T. maxima* usually had 4–5 ribs with round projections on the upper margins, while *T. noae* had 6–7 ribs with sharp projections.

In addition, Kubo & Iwai (2007) reported that *T. maxima* were commonly distributed at the edges and crests of reefs, whilst *T. noae* were frequently observed in the reef flat. They suggested that *T. noae* is not a synonym of *T. maxima* but a valid species based on the number of shell ribs that was originally illustrated and described by Chemnitz (1798). Through Tang's (2005) molecular examination of a few individuals of each species, it was found that shared nucleotides of the mitochondrial 16S rRNA (16S) and cytochrome c oxidase subunit-1 (COI) genes between them were 97.6% and 87.2%, respectively.

In this study, we provide additional molecular and morphological evidence to support the taxonomic separation of *Tridacna noae* from *T. maxima*.

MATERIAL AND METHODS

Sample collection. Giant clams were sampled at depths between 5–20 m by either scuba diving or snorkeling around Taiwan and adjacent islands from March–October 2007 and again from April–November 2008, including Naliao, Kaiyuan, Hsiaoliuchiu, Hongchia, Houbihu, and Chuanfanshr (Fig. 1). Collected samples were stored in dry ice and shipped back to laboratory where they were transferred to –70°C freezer until used. Voucher specimens were deposited in the National Museum of Natural Science, Taiwan, with catalogue numbers: NMNS-6928-001~018.

Morphological analysis. *Tridacna* specimens were firstly classified based on morphological characteristics of Tridacninae based on Rosewater (1965), Lucas et al. (1991), Tang (2005), and Kubo & Iwai (2007). Tissue was then sampled from each individual and four gene regions, i.e., two mitochondrial (CO1, 16S) and two nuclear (18S, 28S) rRNA, were amplified and sequenced per individual.

DNA extraction. Crude DNA extraction in general followed the method of Sambrook et al. (1989) with modifications. A small portion of the adductor muscle (50–100 mg) was taken from a giant clam and put in liquid nitrogen before grounding in a TE buffer (Tris-HCl pH 8.0 10mM, EDTA 1 mM). After homogenisation, samples were incubated for 1–2 hours with 100 µl 10% SDS and 4 µl RNase A at 60°C. Nuclei and debris were precipitated by centrifugation at 8,000 rpm for 5 min at 4°C. The supernatant was removed into another tube and precipitated with one volume of phenol and chloroform (1:1) by centrifugation at 8,000 rpm for another 5 min at 4°C twice. The supernatant was then transferred into another tube, with the addition of 10 volumes of 3M NaOAc (pH7) and isopropanol (1:2). The resulting mixture was incubated for 8 hours at –20°C and then centrifuged at 13,500 rpm for 15 min at 4°C. The pellet was collected, washed with isopropanol, and centrifuged at 13,500 rpm for another 15 min at 4°C. The precipitated DNA was dried at 45°C for 1 hour. Afterwards, a total of 50 µl distilled deionised water was added to the DNA pellet and stored at –20°C for later use.

DNA amplification and sequencing. The primers used for PCR amplification of COI, 16S, 18S, and 28S rRNA genes are listed in Table 1. Amplification was conducted in a Biometra UnoII Thermocycler (Whatman Biometra). Conditions for COI and 16S were (30 cycles): 94°C, 6 min (denaturation); 53°C, 1 min (annealing); and 72°C, 1 min (elongation). Afterward, set at 72°C, 5 min for final elongation. Conditions for 18S and 28S were (30 cycles): 94°C, 6 min (denaturation); 53°C, 1 min (annealing); and 72°C, 1.5 min (elongation). Final elongation was carried

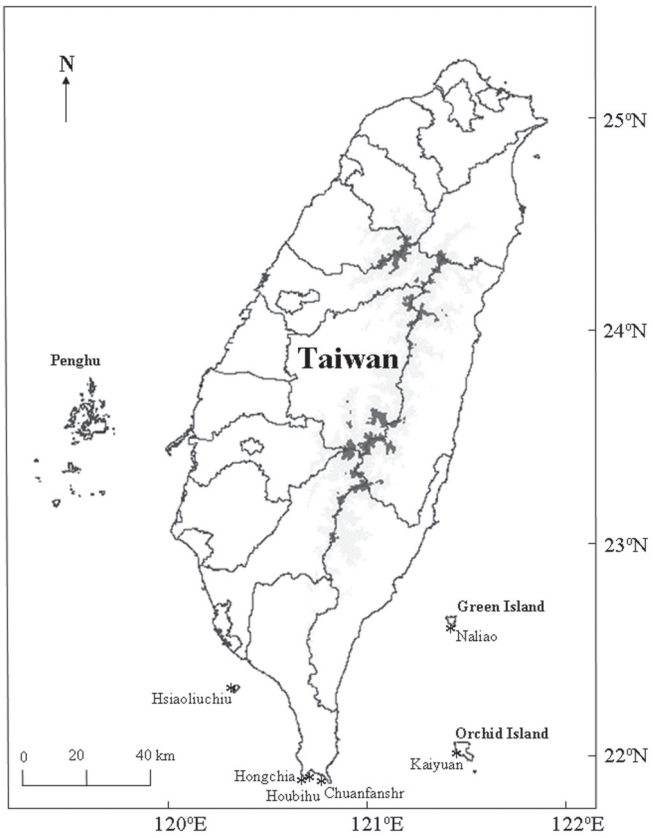

Fig. 1. Map of Taiwan showing sampling sites for giant clams.

Table 1. Forward (F) and reverse (R) PCR primers used to amplify portions of COI, 16S, 18S and 28S rRNA genes.

Name	Sequence 5′–3′	Source
COI		Modified from Folmer et al., 1994
LCO1490 (F)	GGT CAA CAA ATC ATA AAG ATA	
HCO2198 (R)	TAA ACT TCA GGG TGA CCA AAA	
16S		Kessing et al., 1989
16Sar (F)	CGC CTG TTT ATC AAA AAC AT	
16Sbr (R)	CCG GTC TGA ACT CAG ATC ACG T	
18S		
18S–5 (F)	CTG GTT GAT YCT GCC AGT	Winnepenninckx et al., 1998
18S1100 (R)	CTT CGA ACC TCT GAC TTT CG	Williams et al., 2003
28S		
LSU900 (F)	CCG TCT TGA AAC ACG GAC CAA G	Olsen et al., 2003
LSU1600 (R)	AGC GCC ATC CAT TTT CAG G	Williams et al., 2003

Table 2. Species sampled, genes sequenced, and GenBank accession numbers. *: this study.

Species	Locality	CO1	16S rRNA	18S rRNA	28S rRNA
C. cardissa	Canada	EU733136	EU733078		EU733039
C. cardissa	Palau			D88909	
T. maxima	Australia			AJ581874	AJ581907
T. maxima	Palau			D84659	
T. maxima	Taiwan	DQ155301	DQ115320		
T. maxima 6	Taiwan*	KC456021	KC456034	KC456043	KC456026
T. maxima 8	Taiwan*	KC456022	KC456035	KC456044	KC456027
T. maxima 15	Taiwan*	KC456024	KC456037	KC456046	KC456028
T. gigas	Indonesia	EU003616			
T. gigas	USA		AF122975		
T. gigas	Palau			D84189	
T. crocea	Indonesia	EU341379	EU341349		
T. crocea	USA	HM188392			
T. crocea	Japan			D88908	
T. squamosa	Indonesia	EU346364			
T. squamosa	USA		EU341338		
T. squamosa	Palau			D84190	
T. squamosa 30	Taiwan*	KC456025	KC456038	KC456047	KC456029
T. squamosa 33	Taiwan*		KC456039	KC456038	KC456030
T. squamosa 44	Taiwan*		KC456042	KC456051	KC456033
T. costata	Red Sea		AM909741		
T. noae	Taiwan	DQ168140			
T. noae 13	Taiwan*	KC456023	KC456036	KC456045	
T. noae 36	Taiwan*		KC456040	KC456049	KC456031
T. noae 38	Taiwan*		KC456041	KC456050	KC456032
T. derasa	USA		AF122976		
T. derasa	Palau			D84658	
H. hippopus	Palau			D84660	
H. hippopus	USA		AF122973		

out at 72°C for 10 min. The amplified DNA was directly sequenced on an automated DNA sequencer (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems). Additional sequences from GenBank were also used for comparison and 31 new sequences were deposited in GenBank (Table 2).

Genetic analysis. Molecular phylogenetic analysis was based on the collected *Tridacna* species with additional sequences from GenBank (Table 2) and *Corculum cardissa* used as an outgroup (Canapa et al., 2001). Sequence alignment was performed using CLUSTAL W (Thompson et al., 1994) as

implemented in Bioedit (ver. 7.0.9; Hall, 2001). Sequence analysis by the distance method was done using Molecular Evolution Genetic Analysis (MEGA) version 4.0 (Kumar et al., 2004; Tamura et al., 2007). Distances were calculated according to the two-parameter method of Kimura (1980), and the resulting matrix was used to develop a phylogeny following the neighbour-joining method of Saitou & Nei (1987). Maximum parsimony analyses were also performed with the MEGA-4 programme using the close-neighbour-interchange as the search method. The robustness of the phylogeny produced was tested by re-analysis of 1,000 bootstrap replicates of the resultant data set (Felsenstein, 1985).

RESULTS

Sequence data. A total of 12, 20, 20, and 15 giant clams were examined for the genes of CO1, 16S, 18S, and 28S respectively. Thirty-one sequences were deposited in GenBank (accession numbers KC456021–KC456051; Table 2). We had more difficulty obtaining sequences for COI gene compared to other genes, and we were not successful at all in extracting the COI gene from some specimens. This suggested that our choice of primers for CO1 were not optimal. The resulting sequences of CO1, 16S, 18S, and 28S for *T. maxima*, *T. squamosa*, and *T. noae* collected in this study were approximately 450, 435, 900, and 655 bp nucleotides, respectively. Species sequence divergences among the genes was considerable in COI, especially in *T. squamosa*, which ranged between 0.020 and 0.125 (Table 3). Although misidentification in this *T. squamosa* specimen (EU003615) was a possibility, further investigation is needed to rule it out. In contrast, little variation was obtained in 16S, i.e., 0–0.005.

Phylogenetic data. Neighbour-Joining (NJ) analysis on CO1 gene from five *Tridacna* species grouped the same species into one sub-clade with strong bootstrap support

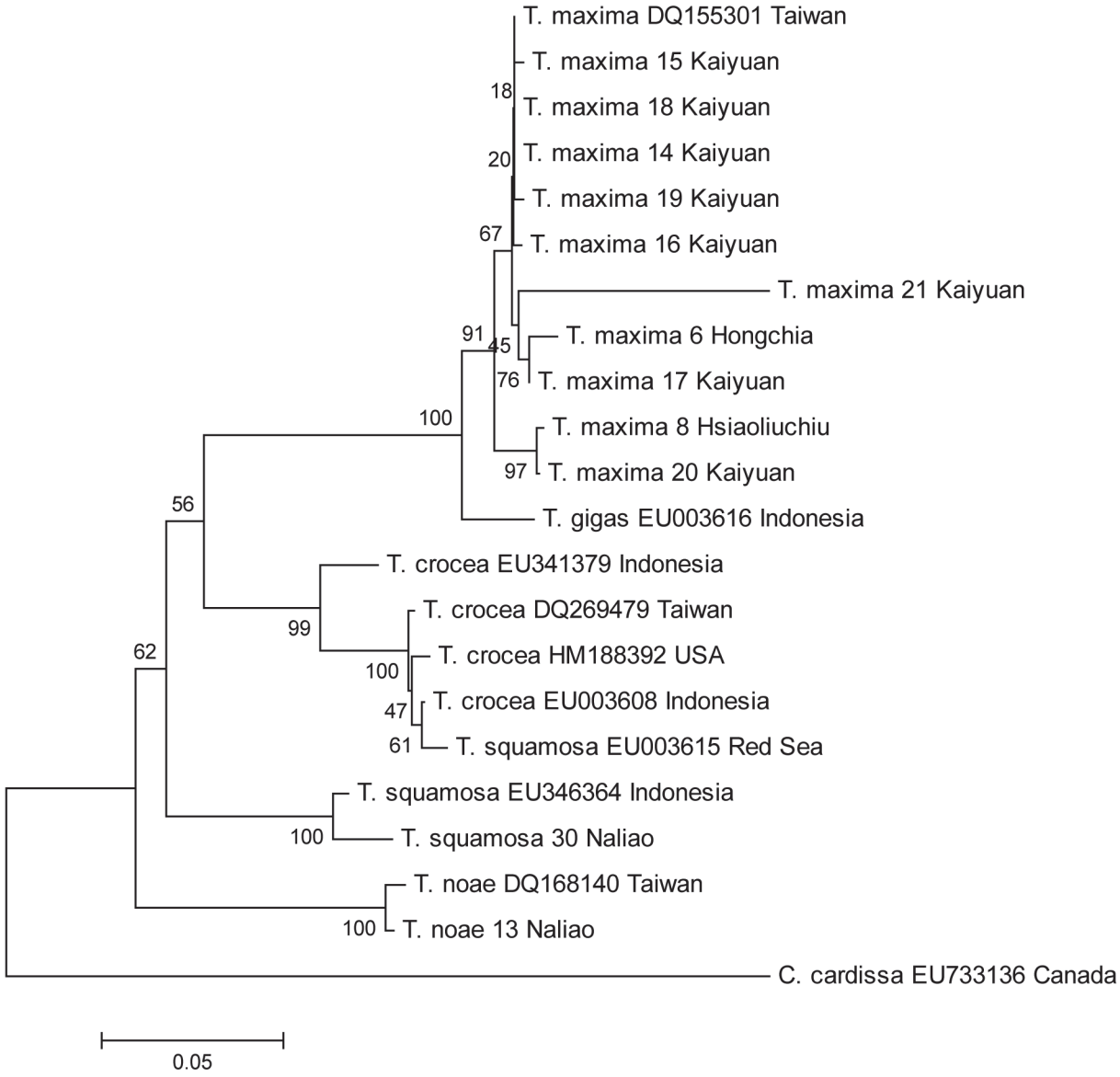

Fig. 2. Neighbour joining tree of Tridacninae using Kimura 2-parameter model based on Cytochrome c oxidase subunit 1(COI) gene sequence. Bootstrap values: 1,000; outgroup: *Corculum cardissa*.

Table 3. Genetic distances among species with different classification levels. —: not examined.

	COI	16S	18S	28S
Within species				
T. crocea	0.008–0.045	—	—	—
T. maxima	0–0.085	0–0.003	0–0.009	0–0.003
T. noae	—	0–0.005	0–0.004	0.002–0.121
T. squamosa	0.020–0.125	0–0.005	0–0.006	0–0.002
Between species				
T. maxima / *H. hippopus*	—	0.138–0.141	0.100–0.105	—
T. maxima / *H. porcellanus*	—	0.138–0.142	—	—
T. maxima / *T. costata*	—	0.031–0.034	—	—
T. maxima / *T. crocea*	0.127–0.224	0.054–0.060	0.002–0.007	—
T. maxima / *T. derasa*	—	0.082–0.085	0.008–0.016	—
T. maxima / *T. gigas*	0.034–0.103	0.103–0.106	0.025–0.033	—
T. maxima / *T. noae*	0.172–0.261	0.042–0.048	0.003–0.011	0.020–0.021
T. maxima / *T. squamosa*	0.147–0.315	0.048–0.054	0.003–0.018	0.016–0.020
T. maxima / *T. tevoroa*	—	0.106–0.109	—	—
T. noae / *H. hippopus*	—	0.134–0.137	0.0097–0.108	—
T. noae / *H. porcellanus*	—	0.138–0.141	—	—
T. noae / *T. costata*	—	0.028–0.034	—	—
T. noae / *T. crocea*	0.153–0.172	0.037–0.042	0–0.010	—
T. noae / *T. derasa*	—	0.057–0.063	0.006–0.016	—
T. noae / *T. gigas*	0.182–0.185	0.106–0.109	0.023–0.033	—
T. noae / *T. squamosa*	0.130–0.166	1.019–0.028	0–0.011	0.007–0.010
T. noae / *T. tevoroa*	—	0.096–0.099	—	—
T. squamosa / *H. hippopus*	—	0.138–0.141	0.097–0.112	—
T. squamosa / *H. porcellanus*	—	0.141–0.145	—	—
T. squamosa / *T. costata*	—	0.048–0.051	—	—
T. squamosa / *T.crocea*	0.008–0.115	0.017–0.019	0–0.014	—
T. squamosa / *T. derasa*	—	0.072–0.075	0.006–0.020	—
T. squamosa / *T. gigas*	0.148–0.172	0.112–0.115	0.023–0.035	—
T. squamosa / *T. tevoroa*	—	0.099–0.102	—	—

Table 4. Morphological comparison between *Tridacna maxima* and *T. noae*. Modified from Tang (2005) and Kubo & Iwai (2007).

Feature	***T. maxima***	***T. noae***
Byssal orifice	moderately wide	moderately wide
Valve	inequilateral	inequilateral
Radial ribs	4–6	5–7
Ribs scales	relative closed	relative spaced
Guard tentacles of incurrent siphon	presence	presence
Pattern of mantle edge	one to several layers of spots or streaks with other colours	one to several layers of oval patches with different colours bounded by white margins
Hyaline organs in mantle margin	concentrated	sparse

(91–100%), except for *T. squamosa*, where one individual was clustered within the *T. crocea* clade (Fig. 2). For the 16S, 10 giant clam species were analysed. NJ clustering separated different species into different groups although some with weak bootstrap support, e.g., 31% between *T. gigas* and others (Fig. 3). In addition, a closer relationship among *T. squamosa* (*T. crocea* + *T. noae*) than between *T. maxima* and *T. costata* was observed. In contrast, the 18S rRNA gene, *T. squamosa*, *T. crocea*, and *T. noae* were undifferentiated based on the comparison of seven giant clam species (Fig. 4). This limited its usability for identification of species-level taxa. With only three *Tridacna* species analysed, the 28S tree showed low bootstrap values in specific separation of *T. maxima*, *T. squamosa*, and *T. noae* (Fig. 5). Although the phylogenetic relationship between *T. maxima* and *T. noae* was different based on CO1 and 16S genes, the common outcome was that *T. noae* clade always separated from the clade of *T. maxima* (Figs. 2, 3).

Morphological data. Shell morphology was compared amongst different-sized individuals with shell length varying from 55–228 mm for *T. maxima* (N = 24) and 83–166 mm for *T. noae* (N = 4) collected from six sites (Fig. 1). The number of radial ribs (Fig. 6) in *T. maxima* ranged from 4–6 with a mean value of 5.1 ± 0.4 ribs. In contrast, it was between 5 and 7 with an average of 6.5 ± 1.7 ribs in *T. noae*. The distance between rib scales (Fig. 6) of *T. maxima* were relatively narrow compared to those of *T. noae*. However, the prominence of rib scales in both species varied extensively among individuals, whereas occasionally,

it happened between the two valves of an individual, e.g., the specimen of *T. maxima* collected from Hongchia. Based on the above results, it is concluded that rib characters are not reliable taxonomically.

It was well recognised that the mantle colour of *Tridacna* giant clams can vary considerably, ranging from brilliant to subdued grayish yellow, bluish green, blackish blue, purple, and brown. When comparing the mantle pattern, *T. maxima* usually had one to several layers of spots or streaks with other colours (Fig. 7). In contrast, *T. noae* possessed one to several layers of oval patches with different colours bounded by white margins. Along the mantle edge, black hyaline organs (eyes) formed a continuous line in *T. maxima*, i.e., about 24–30 eyes cm^{-2} but these were discontinuous and less than half of the density in *T. noae*.

Based on previous and the present studies, it is concluded that *T. maxima* and *T. noae* are different species. Thus, the formal taxonomic description of *T. noae* is as follows.

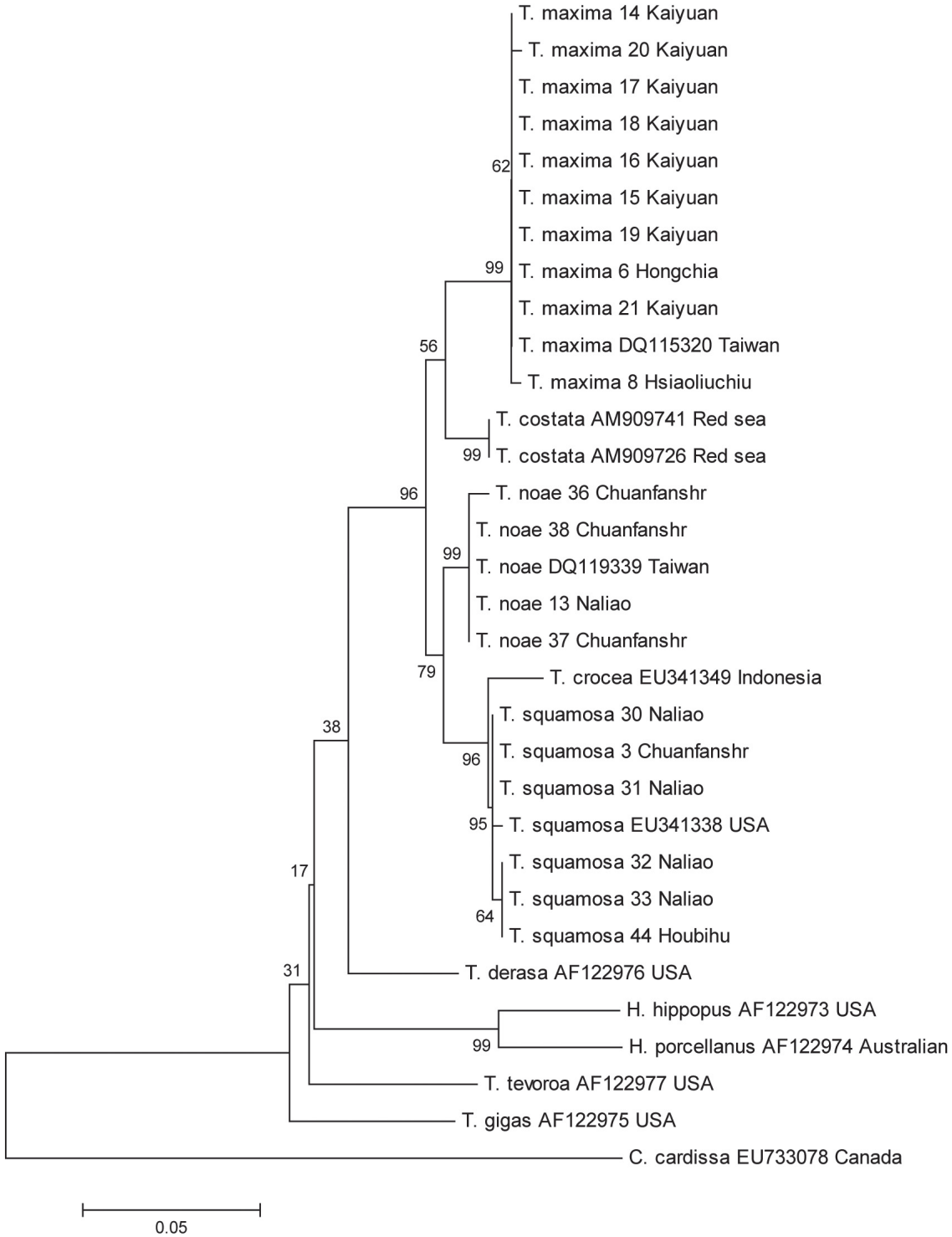

Fig. 3. Neighbour joining tree of Tridacninae using Kimura 2-parameter model based on 16S rRNA gene sequence. Bootstrap values: 1,000; outgroup: *Corculum cardissa*.

Tridacna noae (Röding, 1798)

Types. Species figures were provided in Chemnitz, 1784, vol. 7, pl. 49, fig. 494 and McLean, 1947, fig. 12. Since no type material is extant and no types have been designated to date, a neotype of *Tridacna noae* is hereby designated. The neotype specimen (length 94.4 mm; height 58.4 mm) was collected on 28 August 2007 by Jhih-Hui Hung at Naliao, Green Island, Taiwan. The specimen is deposited in the National Museum of Natural Science, Taiwan, with catalogue number NMNS-6928-001 (Fig. 6G–L). The soft body parts were preserved in 70% alcohol. Two additional specimens (NMNS-6928-002, length, 97.9mm; height, 52.5mm and NMNS-6928-003, length, 87.7mm; height, 45.6mm) were collected on 1 November 2008 by Jhih-Hui Hung at Chuanfanshr, Taiwan and the soft body parts were preserved in 70% alcohol.

Description. Shell inflated, strongly inequilateral, usually elongate-ovate. Shell length commonly between 6–20 cm. Umbo markedly anterior in position, margin with a moderately wide byssal orifice. Shell has 5–7 radial ribs, mostly 6, with round to sharp projections on the upper margins. Scales are relatively abundant and the intervals between them on each rib are narrow. In the living animal, mantle colours vary considerably from brilliant to subdued brown yellow, bluish green, blackish blue, blue, and brown. The mantle pattern has one to several obvious layers of oval

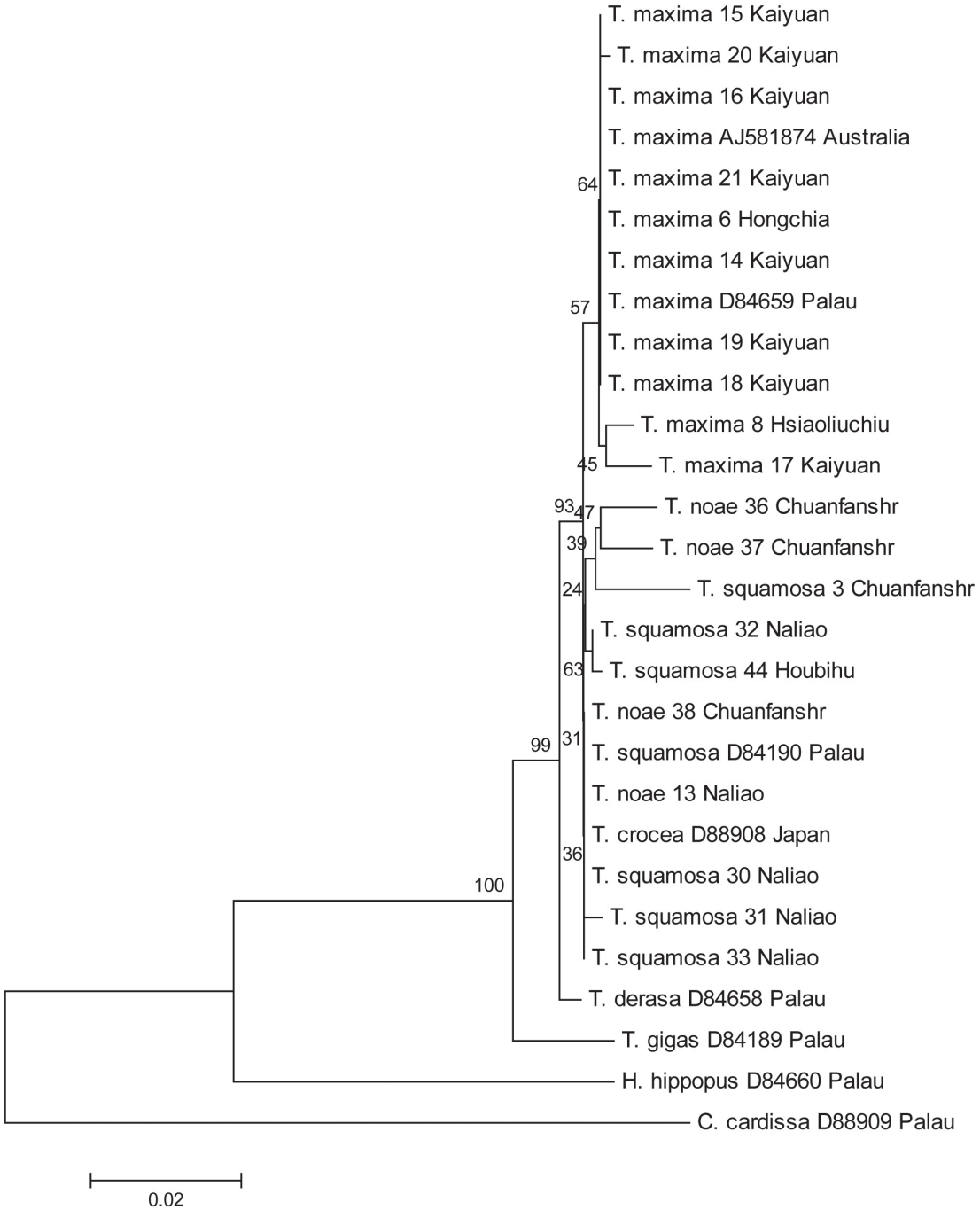

Fig. 4. Neighbour joining tree of Tridacninae using Kimura 2-parameter model based on 18S rRNA gene sequence. Bootstrap values: 1,000; outgroup: *Corculum cardissa*.

patches bounded by white margins. Black hyaline organs (eyes) along the border of the mantle are relatively sparse forming a discontinuous line.

Distribution and ecology. Known from northern and Southern Taiwan, Orchid Island, Green Island, Hsiaoliuchiu, Penghu (Fig. 1; Tang, 2005 and this study), Okinawa, and the Ishigaki Islands of Japan (Kubo & Iwai, 2007). Individuals are attached by a byssus and bore into coral, living in littoral and shallow waters to a depth of 20 m.

Remarks. The shell of this species (Fig. 6G–L) is very similar in general appearance to *Tridacna maxima* (e.g., NMNS-6928-004, length, 93.0 mm; height, 77.3 mm) (Fig. 6A–F). Both species are extremely variable in outline as well as in the number and form of the ribs, and the abundance and shape of the scales. Key characters that distinguish *T. noae* and *T. maxima* are the mantle pattern and the appearance/arrangement of hyaline organs (eyes). *Tridacna maxima* often has a peripheral-banded pattern with a different colour on the mantle margin. However, the mantle pattern of *T. noae* is characterised by having one to several obvious layers of oval patches bounded by white margins (Fig. 7). The eyes are concentrated as a continuous line along the mantle margin in *maxima* but are discontinuous in *noae*.

DISCUSSION

Among the four analysed genes (CO1, 16S, 18S and 28S), the 16S and CO1 data strongly supported the separation of *T. noae* from *T. maxima* (Figs. 2, 3) which was consistent with literature data proposed by Tang (2005). Our study also verified the characters of mantle patterns, and the appearance and arrangement of the hyaline organs were distinct features that separated the two species. In addition, conchological differences, i.e., the number of ribs and distance between rib scales, were inconclusive characteristics.

Röding (1798) proposed the giant clam species '*noae*' and '*maxima*' based on Figs. 494 and 495 in Chemnitz (1784) (Fig. 8). McLean (1947) pointed out that *T. noae* was very similar in general appearance to *maxima*. Both species were extremely variable in shell outline, number and form of the ribs, shape and abundance of the scales. However, the *noae* species possessed well-spaced rib scales, particularly on the upper third of the shell, while in *maxima* they were closely crowded together. On the other hand, Rosewater (1965) treated *noae* as a variant of *T. maxima*. Basically, the classifications applied by McLean (1947) and Rosewater (1965) were based on shell morphology only.

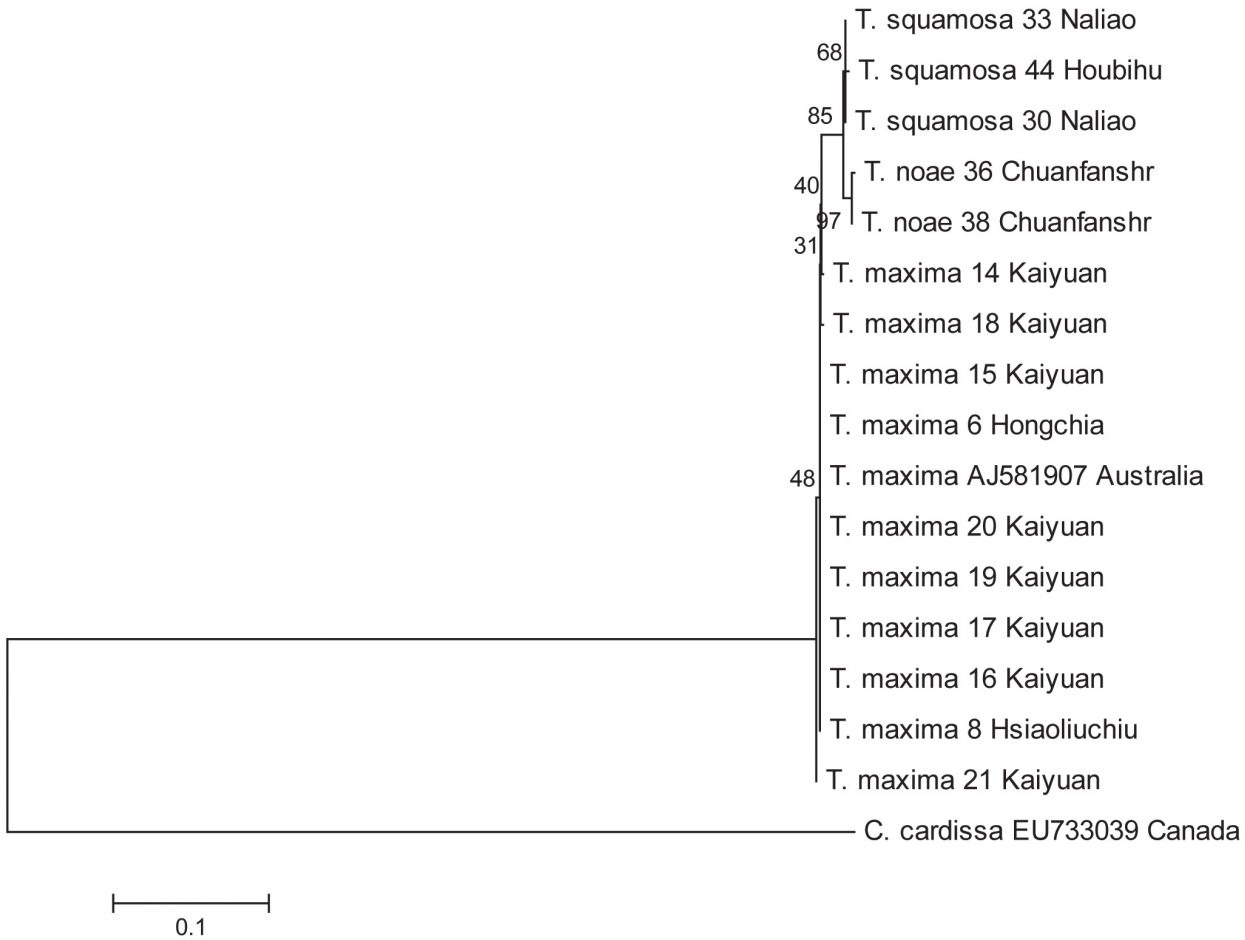

Fig. 5. Neighbour joining tree of Tridacninae using Kimura 2-parameter model based on 28S rRNA gene sequence. Bootstrap values: 1,000; outgroup: *Corculum cardissa*.

Our results revealed that morphological characteristics that can be used to distinguish *Tridacna maxima* and *T. noae* are the colour patterns on the mantle and the number and arrangement of hyaline organs (Table 4; Fig. 7). Although counts of radial ribs and rib scales are obvious features, a considerable overlap in numbers was observed for the two species in the current study, confirming Kubo & Iwai's (2007) observations. The mean number of radial ribs observed by Kubo & Iwai (2007) was 4.4 ± 0.5 in *T. maxima* (N = 165; shell length 75–270 mm) and 6.4 ± 0.6 in *T. noae* (N = 79; 60–290 mm). In our study, the mean number of radial ribs was 5.1 ± 0.4 (N = 24; 55–228 mm) for *T. maxima* and 6.5 ± 1.7 (N = 4; 83–166mm) for *T. noae*. Although Kubo & Iwai (2007) stated that *T. maxima* was commonly seen at reef edges whilst *T. noae* were observed on reef flats, the two species co-occurred in our sampling areas in Taiwan.

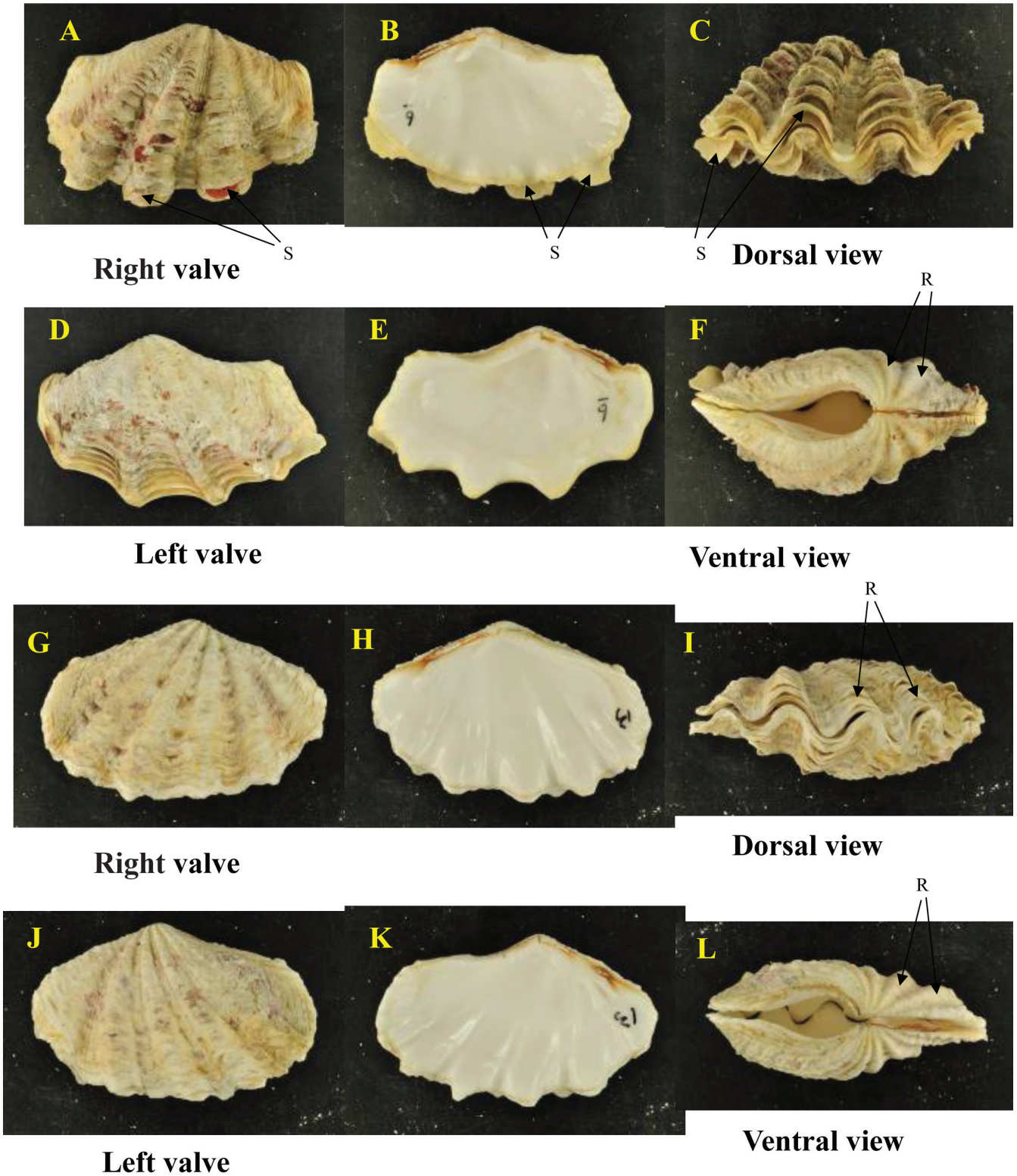

Fig. 6. Shell morphology of *Tridacna maxima* from Hongchia with prominent rib scales on right valve (A–F) and *Tridacna noae* from Naliao (G–L). R: rib; S: scale.

Tang (2005) suggested the presence of a cryptic species in the samples of "*T. maxima*" based on not only on the characteristics of shell radial ribs and mantle pattern but also 16S rRNA and CO1 genes. Kubo & Iwai (2007) examined the morphology and distribution of *T. maxima* in Okinawa and Ishigaki Islands and came to the same conclusion. Moreover, they pointed out that *noae* was not a synonym but a valid species name according to the description and illustration of giant clam shells in the book by Chemnitz (1798). These drawings clearly showed that the number of radial ribs was more in *T. noae* than that of *T. maxima*. The results of our molecular analysis were consistent with published data (Tang, 2005), especially for the 16S rRNA gene, as 10 giant clam species had been analysed and the genetic distance between *T. maxima* and *T. noae* ranged from 0.042–0.048 (Table 3) with the NJ tree separating them into different clades (Fig. 3).

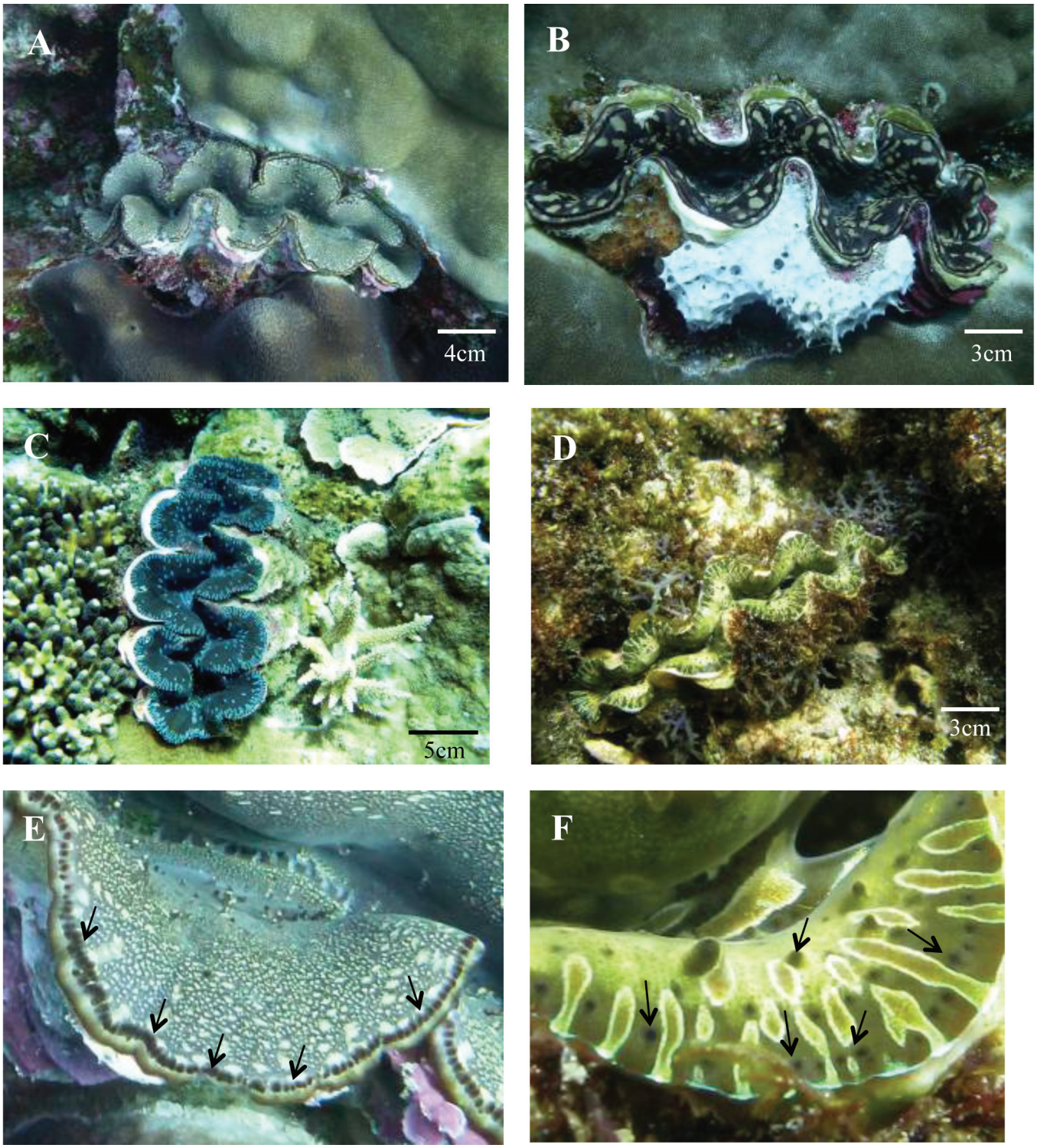

Fig. 7. Mantle colour pattern and hyaline organs of *Tridacna maxima* (A, B) and *Tridacna noae* (C, D). E, Enlarged hyaline organs of *T. maxima*; F, Enlarged hyaline organs of *T. noae*. →: Hyaline organs.

Differences in the two species are also supported by the developmental study by Su et al. (2013). The diameters of fertilised eggs of *T. maxima*, *T. noae*, and hybrids were 113.75 ± 18.5 µm, 99.04 ± 8.1 µm, and 116.3 ± 6.2 µm, respectively. Although the hybrids developed successfully after fertilisation, they died within two days. This cross-breeding experiment strongly suggests that *T. noae* is not an ecotype of *T. maxima* but a valid species.

Several phylogenetic studies had been conducted on giant clams, and the major groups recognised within Tridacninae were the two genera *Hippopus* and *Tridacna*, and the following subgenera within *Tridacna*: *Chametrachea* (comprising *T. squamosa*, *T. crocea*, and *T. maxima*), *Tridacna* (containing *T. gigas*), and *Persikima* (comprising *T. derasa* and *T. tevoroa*) based on the analysis of allozymes (Benzie & Williams, 1998) and 18S rRNA gene (Maruyama et al., 1998). However, inconsistent tree topologies were produced on low-level taxa. For example, within the subgenus *Chametrachea*, Maruyama et al. (1998) obtained the 18S NJ tree of *T. squamosa* (*T. crocea* + *T. maxima*), but in Schneider & O'Foighil (1999) and Richter et al. (2008), *T. maxima* was placed as the sister taxon of *T. squamosa* + *T. crocea* based on the 16S rRNA gene. Subsequently the recently proposed species *T. costata* was added and the relationship was *T. costata* (*T. maxima*(*T. squamosa* + *T. crocea*)) (Richter et al., 2008). Our results also indicated that the subgenus *Chametrachea* included *T. squamosa*, *T. maxima,* and *T. crocea* (Fig. 3). After the inclusion of *T. noae*, the constructed tree within the subgenus *Chametrachea* placed *T. maxima* and *T. costata* as sister taxa and sister group to (*T. noae* (*T. squamosa* + *T. crocea*)).

Cryptic biodiversity shown by the most widely distributed giant clam *T. maxima* suggests that the molecular approach is a useful tool in taxonomic research, especially for organisms with inconclusive conchological characteristics. From the views of conservation, natural resource protection and management, efforts to gather species-specific biological information such as the reproduction, distribution and abundance of *noae* species are definitely still needed.

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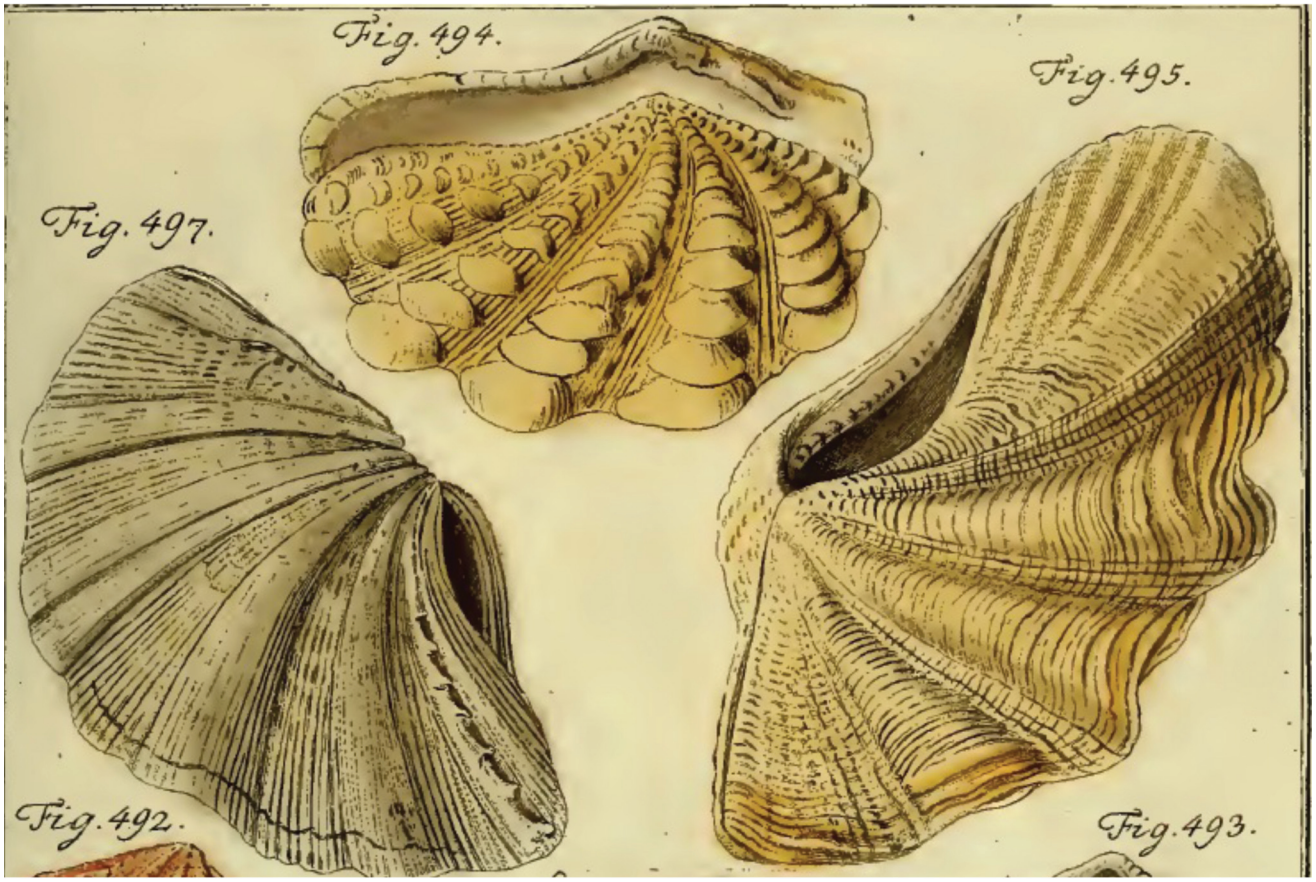

Fig. 8. Photos in Chemnitz (1784) referred by Röding (1798) firstly named the giant clam species "*noae*" (no. 494), "*maxima*" (no. 495), and *Tridacna derasa* (no. 497).

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