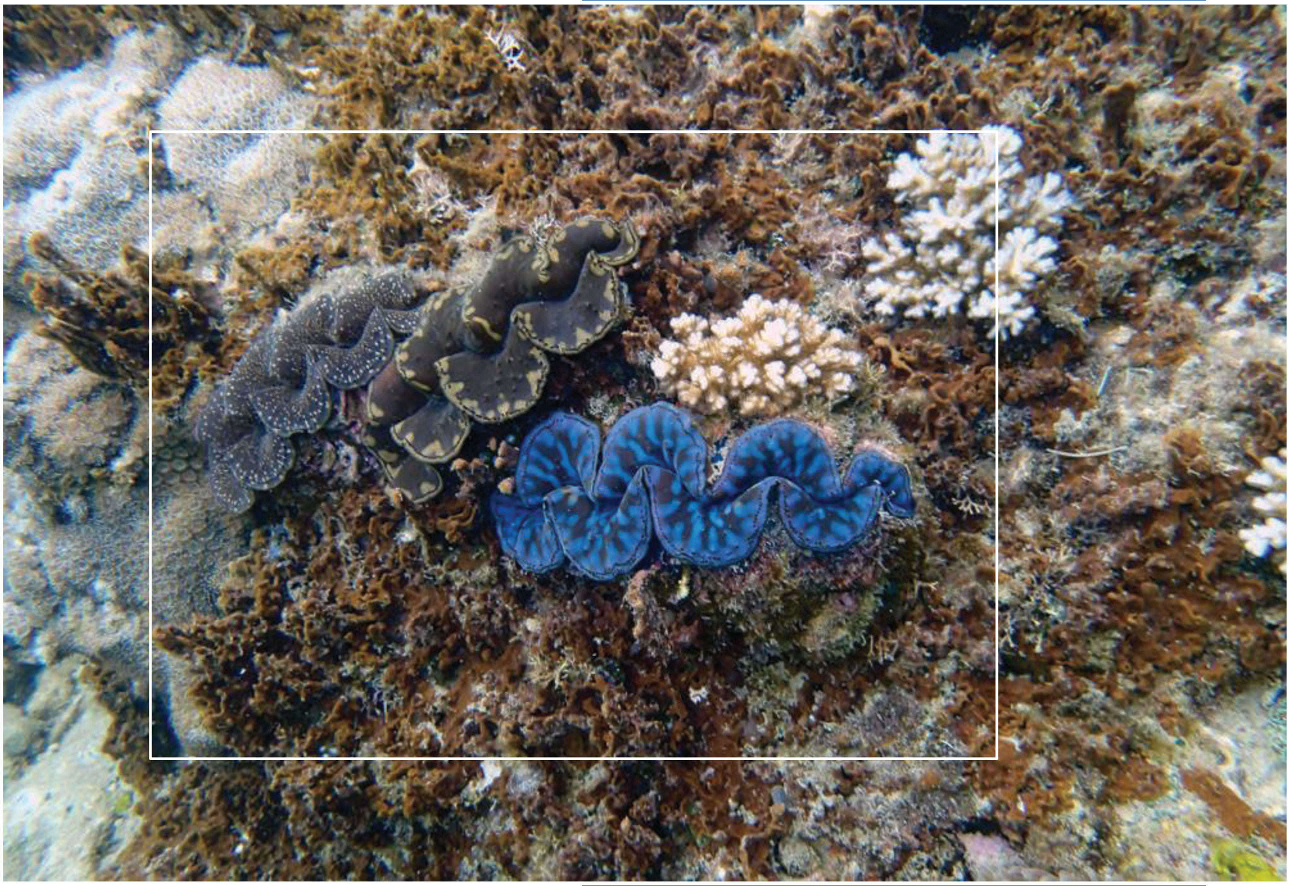
TECHNICAL REPORT



DISTRIBUTION, ABUNDANCE AND POPULATION STRUCTURE OF TWO GIANT CLAM SPECIES (CARDIIDAE: BIVALVIA) OF PERHENTIAN ISLANDS MARINE PARK

Lim Po Teen
Leaw Chui Pin
Md Nizam Ismail
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KIMA

Locally, the giant clam is called “Kima laut“ or “kima”.

Technical Report: Distribution, Abundance and Population Structure of Two Giant Clam Species (Cardiidae: Bivalvia) of Perhentian Islands Marine Park

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Project Information

RESEARCH LOCATION: Perhentian Islands Marine Park, Terengganu

PROJECT TITLE: Distribution, abundance and population structure of giant clams (Cardiidae: Bivalvia) of Perhentian Islands Marine Park, Terengganu

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Summary

Field surveys were undertaken at 13 sites of Perhentian Islands Marine Park, Terengganu, to assess the diversity and distribution of the giant clam *Tridacna* species. Biopsy of tissue samples was carried out to examine the population structuring of *Tridacna* at Perhentian Islands Marine Park. Two species of *Tridacna* were observed inhibiting the bottom substrata of Perhentian Islands: ***Tridacna maxima*** and ***Tridacna squamosa***. The abundance and density of both *Tridacna* species were recorded over the estimated survey areas. The abundance of *T. maxima* was higher than *T. squamosa*. Teluk Gadung (TG) was found with the highest clam density of **17.4 ind. 100 m^{-2}**. Giant clam was not observed at Lighthouse (LH) during the survey. A total of **185** tissue samples from both species were collected, and genomic DNA isolated. The mitochondrial *COI* gene sequences of **41** samples of *T. maxima* and **39** samples of *T. squamosa* were successfully obtained. Evidence of recent recruitment of giant clams in the survey sites was reassuring, as it stands for healthy and replenishing of the giant clam populations in Perhentian Islands. This study provides an update data inventory on the distribution and abundance of two giant clams in Perhentian Islands Marine Park, as well insights into the population genetic connectivity of the two species of Tridacna of Malaysia.

Introduction

Giant clams are one of the important coral reef species, as they contribute to the overall reef biodiversity and functionality. These large charismatic mollusks play numerous key ecological roles such as supplying calcium carbonates to the reef framework, acting as food for predators, and increasing topographic complexity. Unfortunately, human-mediated threats have exacerbated the pervasive loss of giant clams across the Indo-Pacific. The populations are in the state of decline due to the combined effects of pollution, environmental degradation and harvesting for meat (Hviding, 1993) and shells (Brown & Muskanofola, 1985; Dawson & Philipson, 1989). Removing of large clams from the reefs disrupts their natural reproductive cycle and lead to population decline (Munro, 1992). And, they are in pressing need of conservation action.

Populations of giant clams in Malaysia were previously assessed Pulau Redang (Mohamed-Pauzi et al., 1994), Pulau Tioman (Tan et al., 1998), Johore Islands (Zulfigar and Tan, 2000; Tan and Zulfigar, 2001), and East Sabah (Montague et al., 2013). There are at least seven species of giant clams previously recorded in Malaysia, with *T. crocea* with a stable population (Tan and Zulfigar, 2003). *Tridacna gigas*, the world largest giant clam species, has been found extinct in Peninsular Malaysia, *Hippopus porcellanus* and *T. derasa* are restricted to Sabah (Tan and Zulfigar, 2003). Species of giant clams are protected under the Malaysian Department of Fisheries. However, much of the information has not been updated, and no genetic information was obtained from these populations.

Marine populations have traditionally been assumed to be well connected via the dispersal of pelagic larvae stages, but recent studies have shown that they are more structured than previously thought with higher natal retentions near source populations. Modern tridacnine populations across Coral Triangle showed strong genetic structure with little or no connectivity among regions, and a strong concordance with ecoregion boundaries. Given the smaller geographic scales, tridacnine populations in Singapore were also found to be relatively well structured (Neo et al. 2012).

Genetic data are very powerful for highlighting boundaries in marine environments and findings can help identify common patterns shaping biodiversity and regional limits to connectivity. Importantly, they can help to identify evolutionarily distinct source populations of giant clams potentially contributing to the regional gene pool, thus facilitating the development of trans-boundary management strategies. Various Indo-Pacific populations of giant clams have been well studied in this aspect (Indonesia, Philippines, Singapore, French Polynesia, and Australia) but lack consolidated information of populations within Malaysian waters. Distribution in other Southeast Asian countries including Brunei Darussalam, Cambodia, Laos, Myanmar and Vietnam remained unclear (reviewed in Othman et al. 2010).

The aim of the study is to evaluate the diversity and distribution of giant clams around Perhentian Islands, and examine the population genetic connectivity between *Tridacna* species of Perhentian Islands and other populations regionally. The genetic data will be integrated in a global-based integrated risk assessment study (combining evolutionary history, ecological information and extinction threats) to help draw researchers' attention to remarkable species from lesser known localities and endangered lineages. The loss of species viability requires new robust and sustainable conservation solutions, and the integrated risk assessments can help identify unique lineages for introducing back to viable gene pool.

Objectives

Objectives of the study are as follow:

1. To investigate the distribution and abundance of giant clams at islands of Perhentian Marine Park.
2. To determine the population structures of two *Tridacna* species, *T. maxima* and *T. squomosa*.
3. To investigate the genetic relatedness of tridacnine populations utilizing molecular approach.

Methodology

Sampling sites

The study was undertaken at Perhentian Islands Marine Park: consists of two main islands (Pulau Perhentian Besar dan Pulau Perhentian Kecil) and five uninhabited islands (Fig. 1). A total of 13 sites were surveyed (Table 1; Fig. 1) for the giant clam abundance and density from March to June 2017.

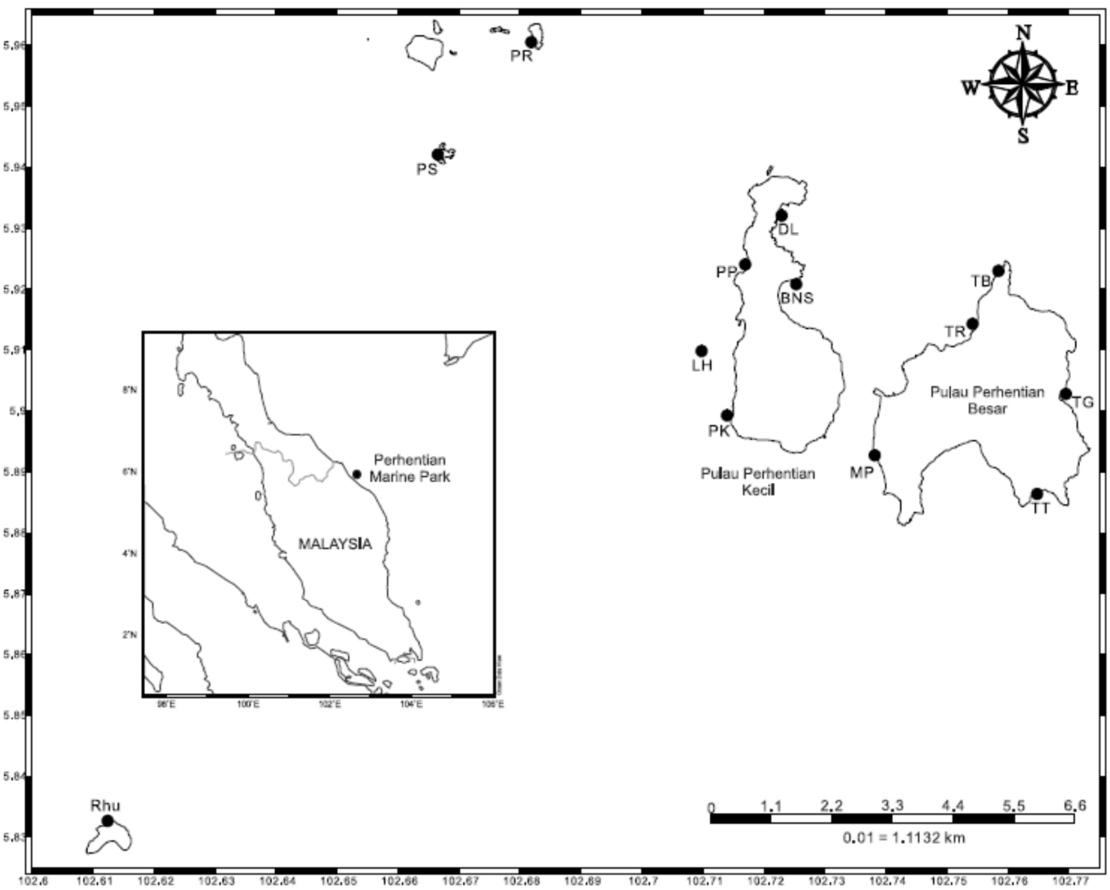


Fig. 1. Map of Perhentian Islands, Terengganu showing the 13 selected survey sites.

Table 1.

Sampling sites of Perhentian Islands, Terengganu in this study, with coordinates and estimated surveyed areas.

Site	Site code	Coordinates	Survey area (m^2)
D. Lagoon	DL	5°55'55.49"N 102°43'22.45"E	800
Batu Nisan	BNS	5°55'15.00"N 102°43'31.03"E	800
Pasir Pengalau	PP	5°55'26.68"N 102°43'0.95"E	800
Lighthouse	LH	5°54'35.22"N 102°42'35.22"E	800
Pasir Keranji	PK	5°53'57.24"N 102°42'50.23"E	800
Tanjung Basi	TB	5°55'22.84"N 102°45'30.40"E	800
Marine Park Jetty	MP	5°53'33.67"N 102°44'17.41"E	800
Tanjung Tukas	TT	5°53'10.89"N 102°45'53.19"E	500
Teluk Gadung	TG	5°54'9.90"N 102°46'10.14"E	500
Tiga Ruang	TR	5°54'51.25"N 102°45'15.10"E	500
Rawa Island	PR	5°57'37.83"N 102°40'54.72"E	2400
Seringgih Island	PS	5°56'31.47"N 102°40'0.08"E	800
Rhu Island	Rhu	5°49'57.62"N 102°36'44.72"E	800

Field survey

During each dive, belt transects of 100 m × 8 m or 50 m × 10 m (depends on the water visibility) were lay parallel to shoreline at the depth of 5 to 10 m in each selected site. Field survey log is presented in Appendix A.

Exhaustive search method was used, where divers were looking specifically for giant clams present within the belt transects (Fig. 2). Giant clams were individually photographed and shell length estimated, and their depth occurrence was noted.

Fig. 2.

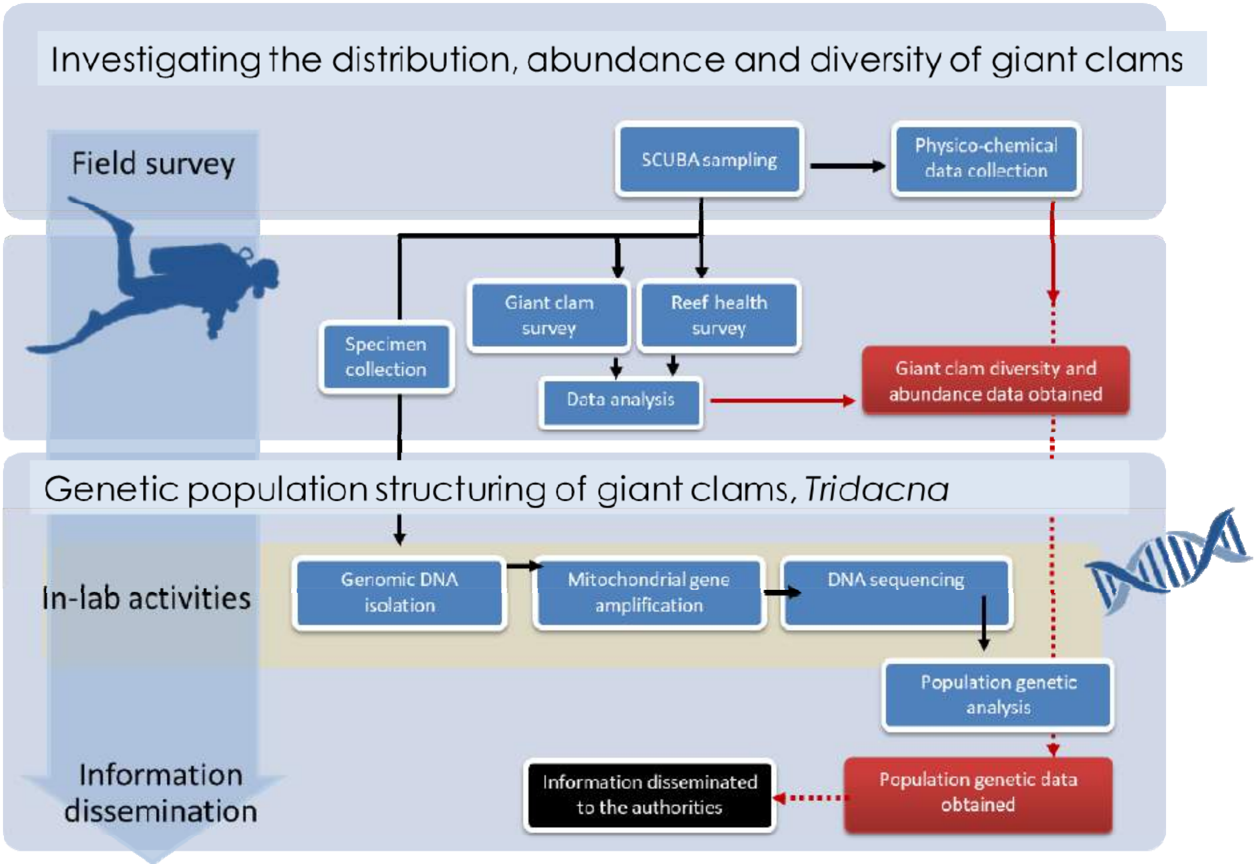
(A) Laying transect on the reef for abundance count. (B) Spotting giant clams. (C) Photographing giant clams for shell length measurements.

Shell length was measured for each individual clam (Fig. 3) and categorized according to three size classes. These size classes may generically represent the life stages of the clams. They are:

- mature adult clams, ≥ 10 cm
- sub-adult clams, 5.1 – 9.9 cm
- juvenile clams, ≤ 5 cm

Fig. 3. Measurement of giant clams shell lengths.

Flow chart of Methodology



Biopsy of giant clam mantle tissue

Biopsy of giant clam mantle tissues was undertaken for genetic population study. A small piece of mantle tissue (<0.5 cm) was cut from each individual clam using scissors and forceps without causing any harm to the animals (Figs 4, 5). All tissues collected was labelled and brought back to the laboratory in a cool box filled with ice packs. Tissues were kept at –80°C in a deep freezer prior to lyophilization.

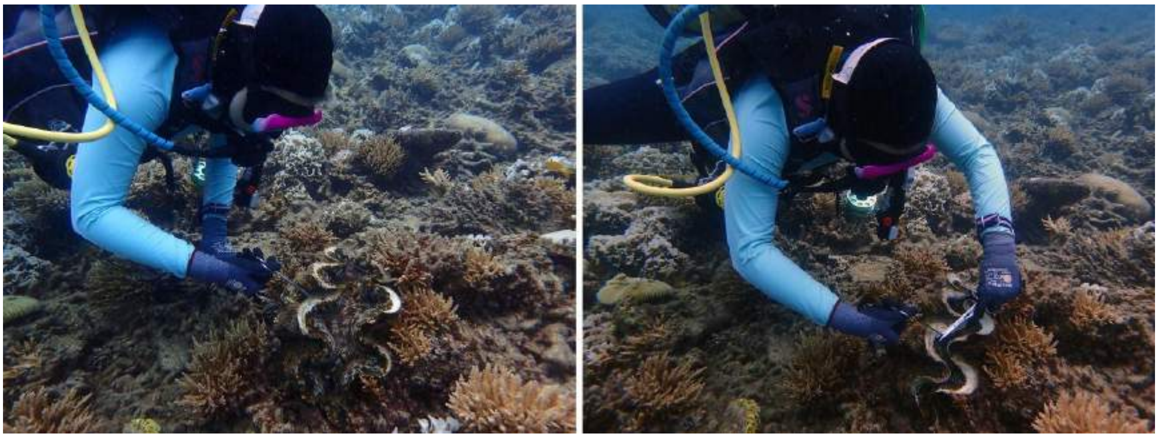


Fig. 4. Procedure of biopsy: clipping the mantle with forceps and cutting the tissue.

Fig. 5. Close-up of the procedure to conducting biopsy of giant clam mantle tissue.

DNA isolation and gene amplifications

For genomic DNA (gDNA) extraction, freeze-dried tissue samples were sub-sampled, and gDNA was extracted using DNeasy® Blood & Tissue kit (QIAGEN, Hilden, Germany). Genomic DNAs were kept at –20°C for further analysis.

Mitochondrial cytochrome c oxidase I (*COI*) gene was amplified via polymerase chain reaction (PCR). PCR was carried out in a total volume of 25 μL containing 2 μL of gDNA, 10× *Taq* buffer, 10mM dNTPs, 50mM $MgCl_2$, 25 μM of each primer and 0.2 μL *Taq* DNA Polymerase (Invitrogen, Life Technologies, USA). Primers SQUA-R1 (5´−ATG TAT AAA CAA AAC AGG ATC−3´) and SQUA-F3 (5´−CAT CGT TTA GAG TAA TAA TTC G−3´) (DeBoer et al. 2008) were used in this study. The PCR condition is as follow: initial denaturation at 94°C for 15 s, followed by 38 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 45 s, and a final extension of 72°C for 3 min. PCR product was visualized by gel electrophoresis in a 1% agarose gel. Amplicons were further purified using an UltraClean® PCR Clean-Up Kit (QIAGEN) prior to DNA sequencing using an ABI 3700XL automated DNA sequencer (Applied Biosystems, USA). Both strands were sequenced.

Findings

Species of giant clams at Perhentian Islands

Two species of giant clams were found: *Tridacna maxima* (commonly known as the small giant **c**lam) and *T. squamosa* (commonly known as the fluted giant clam). The two species were identified based on the following morphological characteristics:

Tridacna maxima (Fig. 6)

- Grows up to 35 cm in shell length.
- Close-set scutes/flutes
- Mantle coloration range from blue, purple, brown.
- Can be distinguished by the presence of 'hyaline organs' (black) dotted along the margin of mantle.

Habitats: Partially embedded, sometimes fully embedded into the reef substrates (i.e. dead coral rubble, live coral heads).

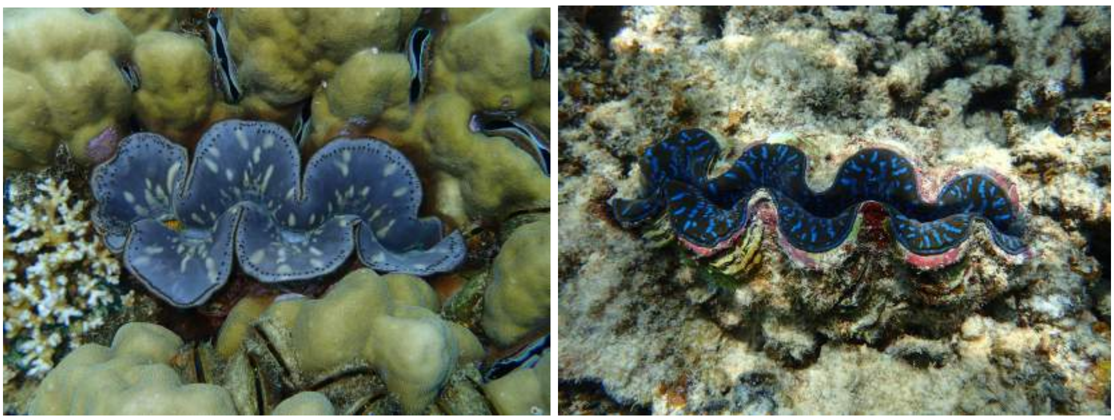


Fig. 6.

Tridacna maxima

The species was recognized by the presence of "hyaline organs" dotted along the mantle edge and mostly found partially embedded or fully embedded into reef substrata.

Tridacna squamosa (Fig. 7)

- Grows up to 40 cm in shell length.
- Widely spaced scutes/flutes
- Mantle coloration mostly brown with occasional mottles of green and blue.

Habitats: Free-living as adults, mostly found in coral reefs and rubble. Juvenile are usually byssally attached to the reef substrates.

Fig. 7.

Tridacna squamosa

The species was differentiated by the widely spaced scutes/flutes and less organized "hyaline organs" along the mantle. It was found free living in adult stage where juvenile were usually byssally attached to reef substrata.

Abundance and density of giant clams on Perhentian Islands

The numbers of *T. maxima* was higher than *T. squamosa* at Perhentian Islands; total counts for *T. squamosa* were 195 ind. whereas *T. maxima* was almost doubled, with 401 ind, across the 13 sites. Giant clams were not observed in one site, LH, during the surveys (Table 2). Giant clams had been spotted in the range of depths of 1.4 m - 13.76 m.

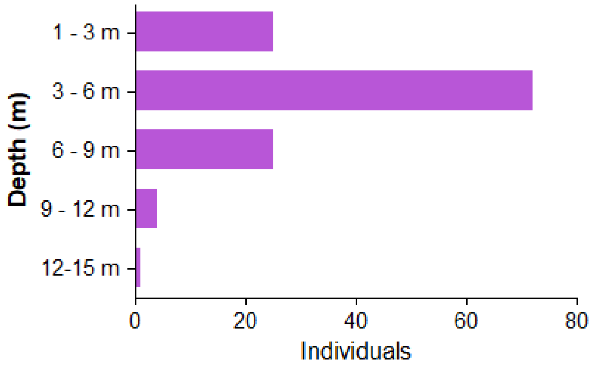
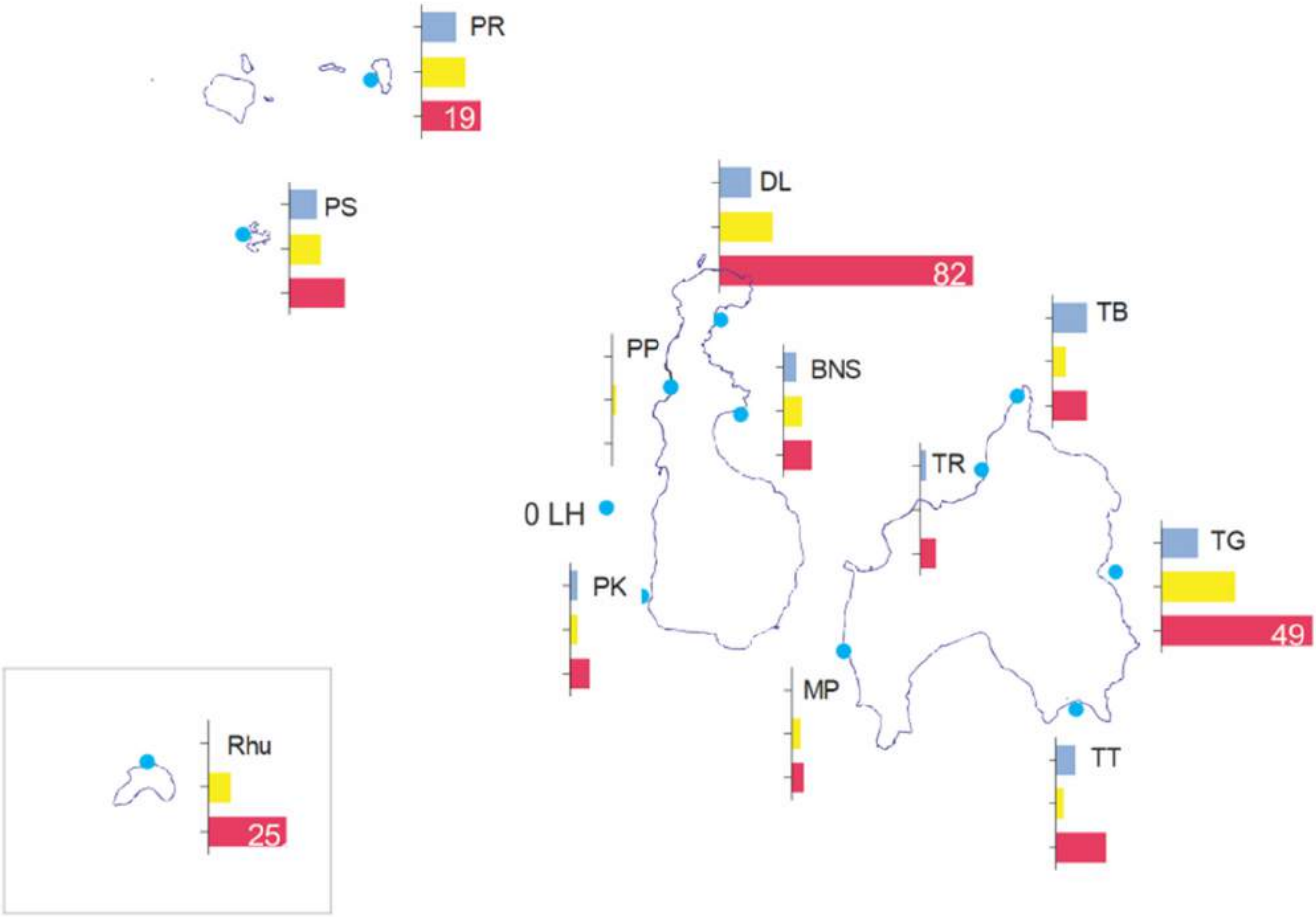


Table 2. Density of giant clams (individuals 100 m^{-2}) at Perhentian Islands.

Site	*T. squamosa*	*T. maxima*
D. Lagoon (DL)	0.75	**13.63**
Batu Nisan (BNS)	1.88	2.38
Pasir Pengalau (PP)	1.38	0.13
Lighthouse (LH)	**0**	**0**
Pasir Keranji (PK)	0.75	1.25
Tanjung Basi (TB)	0.25	3.25
Marine Park Jetty (MP)	**8.25**	0.88
Tanjung Tukas (TT)	**0**	4.80
Teluk Gadung (TG)	0.40	**17.00**
Tiga Ruang (TR)	1.60	1.40
Rawa Island (PR)	2.33	1.83
Seringgih Island (PS)	0.38	4.63
Rhu Island (Rhu)	2.50	4.00

Abundance of T. maxima

The highest density of *T. maxima* (17 ind. 100 m^{-2}) was observed at site TG, followed by site DL with 13.63 ind. 100 m^{-2}. The lowest density of *T. maxima* was observed at site PP, with 0.13 ind. 100 m^{-2} (Table 2).

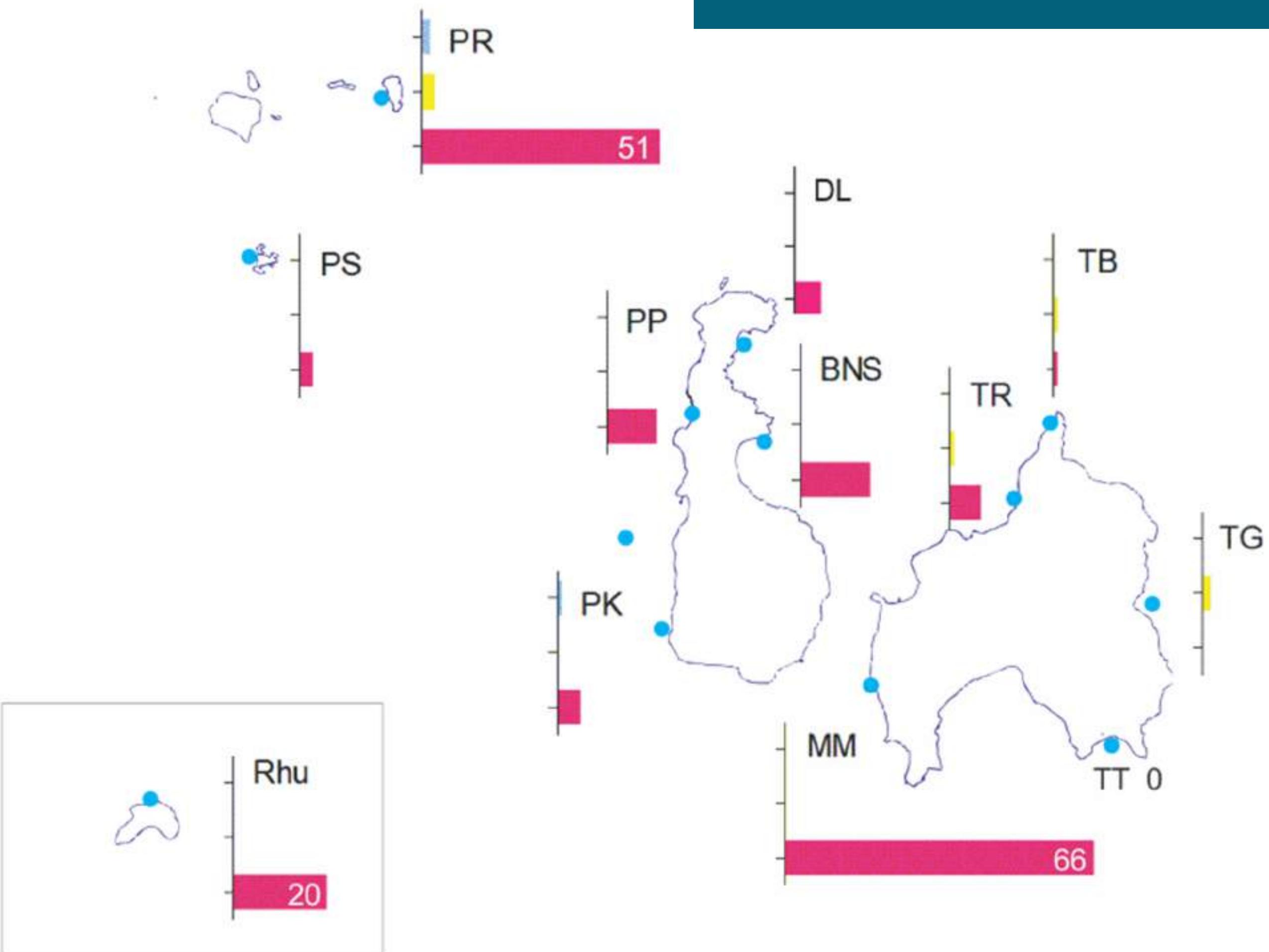


Abundances of *T. maxima* in three size classes throughout Perhentian Islands.

Abundance of T. squamosa

For *T. squamosa*, the highest density was found at site MP (8.25 ind. 100 m^{-2}), followed by site Rhu (2.50 ind. 100 m^{-2}) (Table 2). However, the giant clam population at site MP was translocated from Rhu by the Marine Park Department; hence it did not reflect the existing natural population. *Tridacna squamosa* was either absent or low in number at site TT.

Abundances of *T. squamosa* in three size classes throughout Perhentian Islands.



For juvenile clams (< 5 cm in shell length), *T. squamosa* young recruits were considered rare in the survey, as only three individuals were found throughout the 13 sites. On the other hand, *T. maxima* juveniles were more abundant with 67 individuals recorded. This observation may clue in on the recruitment constraints in *T. squamosa* populations, but not so much for *T. maxima*. The genetic analyses may provide additional insights on the recruitment situation.

0.1%

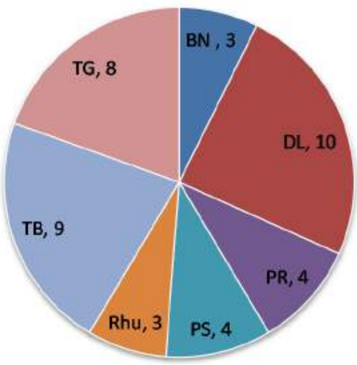
Less than one in a thousand of the small drifted larvae could survive and become a juvenile settling down on the sea floor.

Biopsy of giant clams

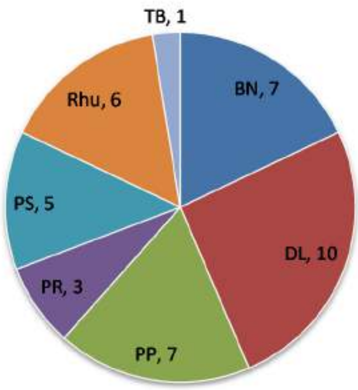
A total of 185 tissue samples were collected from 11 sites across Perhentian Islands, 78 tissues samples from *T. squamosa* and 107 from *T. maxima*.

Molecular characterization of giant clams of Perhentian Island

80 tissue samples from both species were processed, and genomic DNAs isolated. The mitochondrial COI gene sequences of ~580 base pairs length were obtained from 41 samples of *T. maxima* and 39 samples of *T. squamosa*. Genbank accession number of each individual are listed in **Appendix B**.



T. maxima



T. squamosa

Genetic diversity

For *T. squamosa*, COI alignment data yielded 397 characters, 268 characters are constant and 72 are parsimony-informative; while for *T. maxima*, the alignment data yielded 433 characters, with 284 are constant and 91 are parsimony-informative. Both analyses used *T. derasa* and *T. gigas* as outgroup.

The COI alignments of *T. squamosa* and *T. maxima* yielded 73 and 138 haplotypes, respectively. The two species revealed a degree of polymorphism with high intraspecific divergences, 8.3% and 8.1%, respectively. The interspecific sequence divergence between T. squamosa and T. maxima was 12.9 – 17.2%.

Species	*T. maxima*	*T. squamosa*
T. maxima	0-8.1%	
T. squamosa	12.9-17.2%	0-8.3%

Genetic population structuring of *Tridacna squamosa*

The resulted tree showed that *T. squamosa* grouped into one major clade, Clade I, comprised of sequences from the Indo-Malay Philippines Archipelago (IMPA), two specimens from Indian Ocean (Kenya) were grouped into Clade II and Clade III were specimens from Red Sea. In *T. squamosa*, from the defined clades, up to 11–39 mutations were observed **(Fig. 8)**. The most likely number of *K* inferred using the method as described in Evanno et al. (2005) showed a peak at *K* = 2. STRUCTURE revealed two genetic clusters in *T. squamosa* populations where Indian Ocean and Red Sea represent distinct populations. FST revealed high degree of gene flow across all the populations (Appendix C). High FST ranged from 0.72–1.00 were observed in some populations. For example, high FST was observed between Malacca Strait and Makassar Strait with populations from Romblon Pass, Ticao Pass and Leyte Gulf, indicating no genetic exchange. However, the samples size was too small to conclusively elucidate the gene flow.

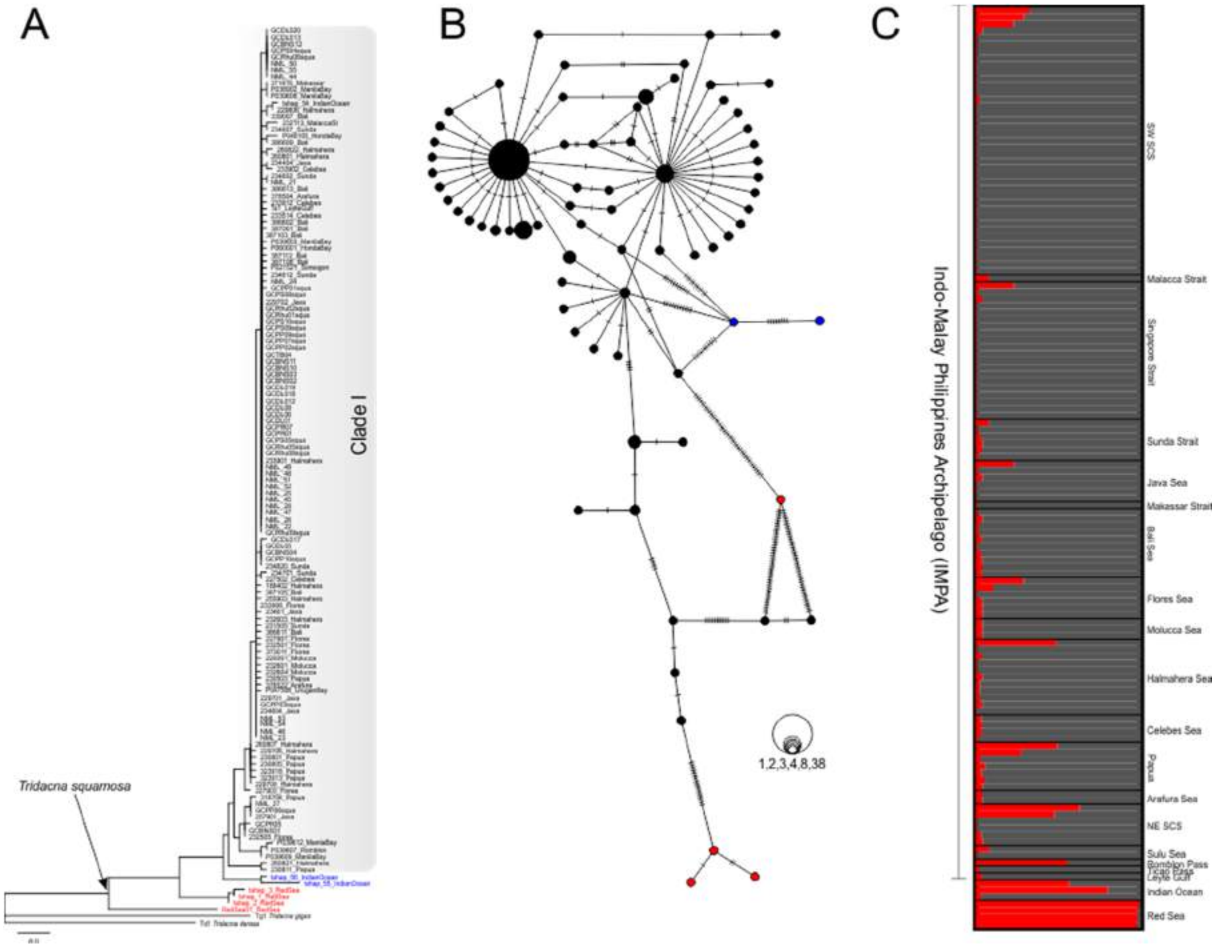
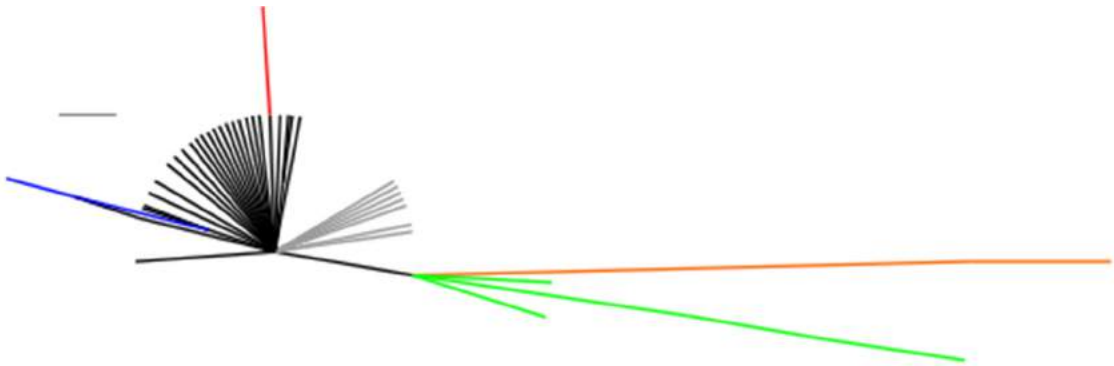
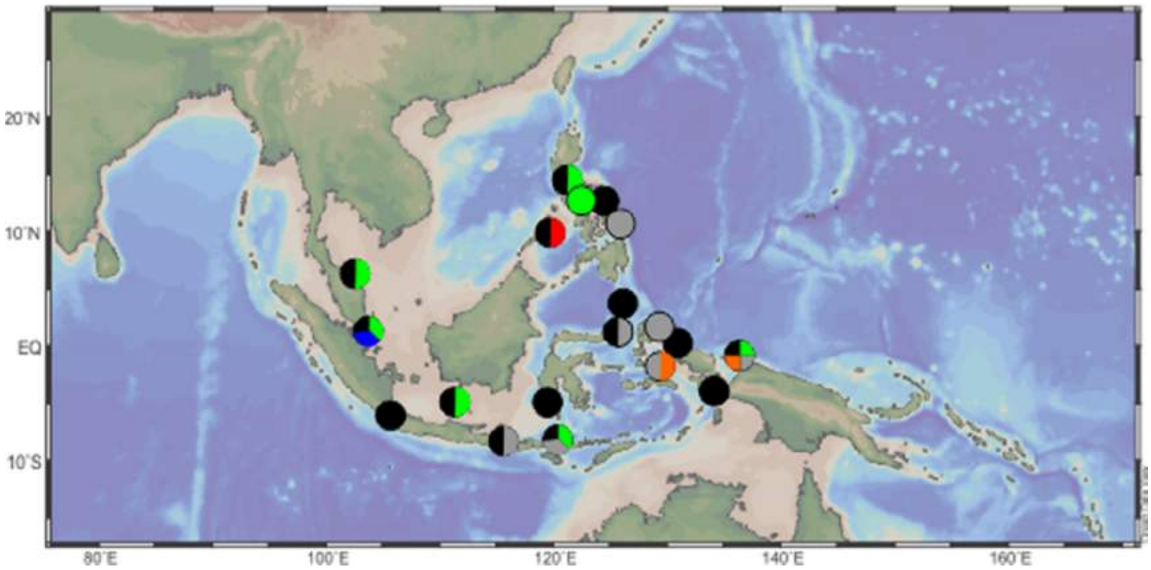


Fig. 8. Genetic population structuring of *Tridacna squamosa*.

A, phylogenetic tree; B, network of haplotypes; C, STRUCTURE constructed based on COI gene sequences.





Tridacna squamosa populations from Perhentian Islands shared a degree of genetic homogeneity with other sub-populations within the IMPA zone (Philippines, Singapore and Indonesia), revealing **high genetic exchange** in this species.

Genetic population structuring of *Tridacna maxima*

In *T. maxima*, the sequences were clustered into three clades: Clades I – III. Clade I comprised sequences from IMPA and one sequence was from Taiwan. Clade II was mainly sequences from Java Sea, Indian Ocean, Halmahera Sea, Andaman Sea and Malacca Strait; while Clade III comprised some sequences from Papua and Halmahera Sea (**Fig. 9**). The three clades were separated by 8 and 25 mutations (Fig. 9). The STRUCTURE revealed three genetic clusters in *T. maxima* populations, with Malacca Strait, Java Sea, Andaman Sea and Indian Ocean formed a distinct population; Papua revealed another distinct population, while the rest of the sub-populations in within the IMPA as the major population. *Tridacna maxima* from Halmahera Sea comprised all the three populations; while Papua also comprised two populations. The distinct populations were supported by the FST values indicating limited gene flow. *Tridacna maxima* from Perhentian Islands exhibited high gene flow with sub-populations within IMPA, except sub-populations of Papua, Andaman Sea and Indian Ocean, Malacca Strait and Java Sea (Appendix D).

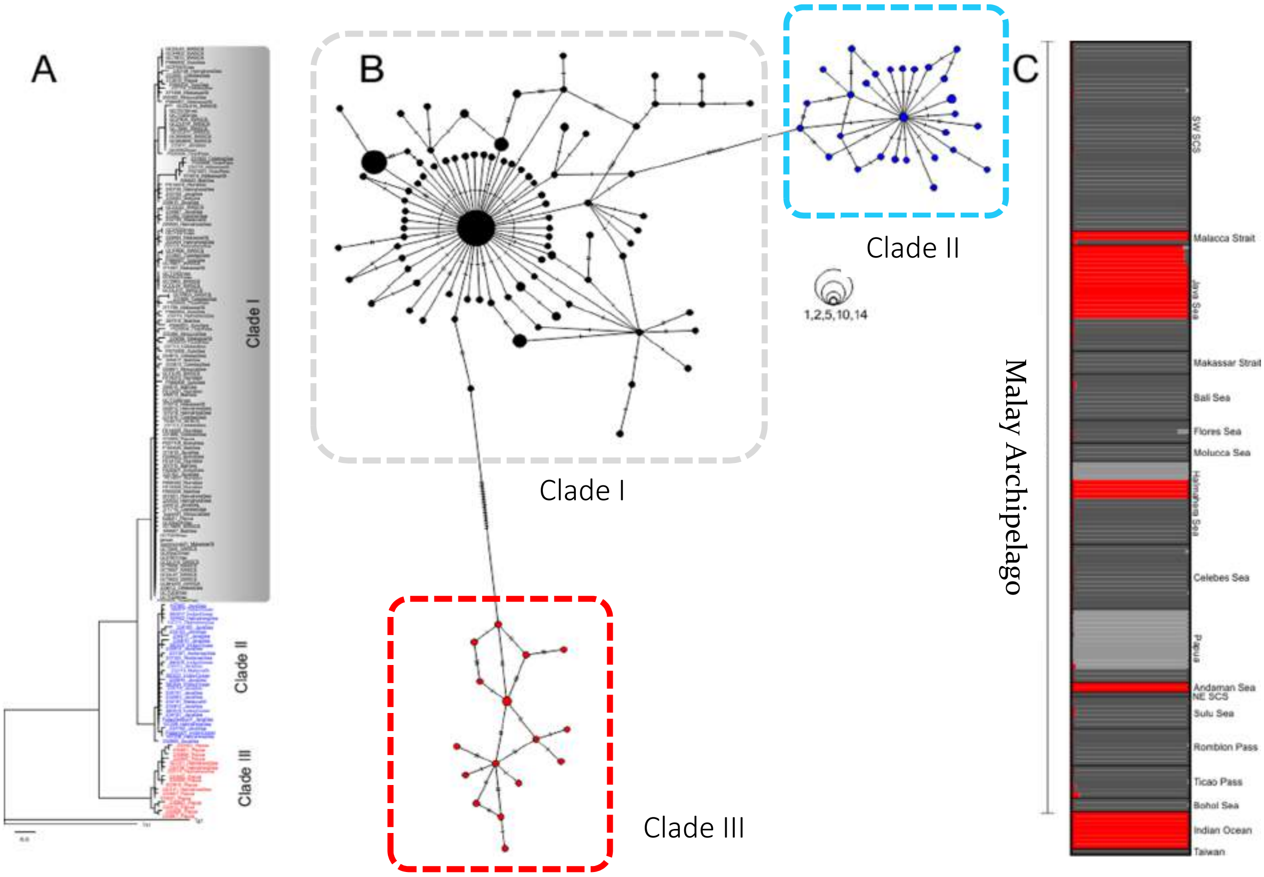
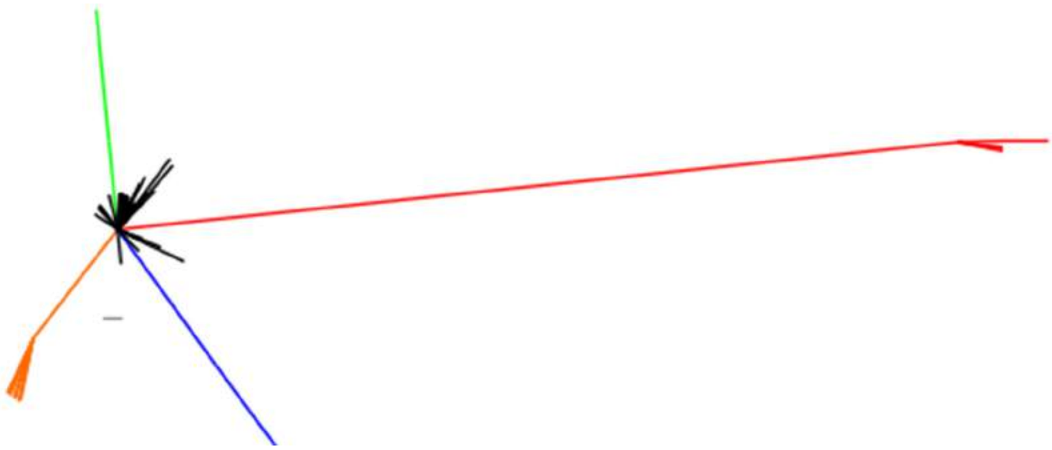
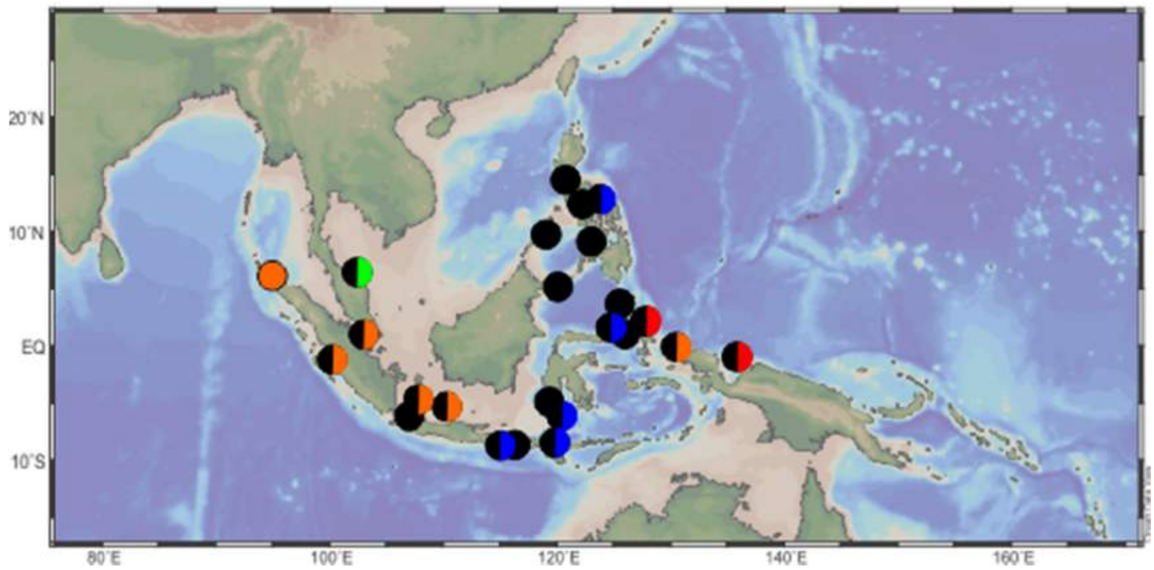


Fig. 9. Genetic population structuring of *Tridacna maxima*.

A, phylogenetic tree; B, network of haplotypes; C, STRUCTURE constructed based on COI gene sequences.

Perhentian populations





Perhentian populations of *T. maxima* showed some degrees of **genetic heterogeneity**. This may imply the local gene pool and was supported by the high abundances of juveniles at Perhentian Islands.

Status of giant clam populations at Perhentian Islands

The population across Perhentian Islands is considered to be healthy (Fig. 10, 11) although signs of poaching, or undesirable habitats for giant clams were observed throughout the survey. Observation of recruitment of both species in the reefs (Fig. 12) suggests that the populations maintain their ability to replenish, or healthy broodstock (Fig. 11) were in proximity to supply source larvae. The sighting of juveniles of *T. squamosa* and *T. maxima* in the reefs was noteworthy (Figs. 13, 14).

Fig. 10.

Aggregation of *T. maxima* in close proximity is common behavior of the species.

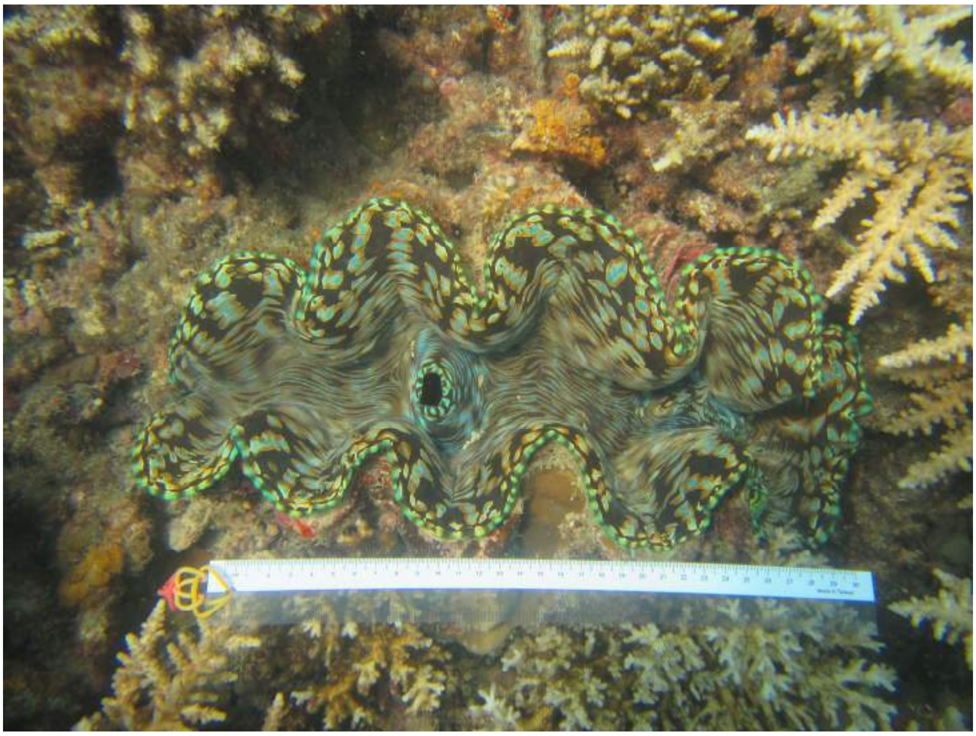


Fig. 11. Giant clams with a shell length > 20 cm are considered sexually matured, and are potential hermaphrodite broodstocks.

Fig. 12. Recruit of *T. squamosa* was noted on a massive boulder coral (approximately 1 cm; estimated to be a few months old).

Fig. 13. Young juvenile of *T. squamosa* with the distinct feature of widely spaced scutes/flutes.

Fig. 14. Juvenile of *T. maxima* burrowed into substrate, the dotted line "hyaline organs" along the mantle was noticeable.

Mortality of giant clams may be caused by natural disturbance, predation, parasitism or anthropogenic factors. The bleaching phenomenon on giant clams may suggest expulsion of zooxanthellae (*Symbiodinium*) which is usually caused by elevated sea temperature or other similar environmental stressors. Predation on both adult and juvenile giant clams by fishes such as wrasse, triggerfish and pufferfish are common (Alcazar, 1986; Richardson, 1991; Govan, 1992) and noted by the numerous bite marks along the mantle edges. However, the biggest threat to existence of giant clams come from anthropogenic factor of exploitation, where giant clam shells were commonly collected for antique display or increase demand by aquarist and flesh as local delicacies.

A small population of giant clams in Perhentian Islands has been experiencing bleaching (Fig. 15). Empty shells with clean inner surface were occasionally spotted, suggesting recent mortality either due to poaching for flesh as delicacy or natural mortality (Fig. 16). Empty shells found at Marine Park Jetty could be due to mortality after translocation (Fig. 17).

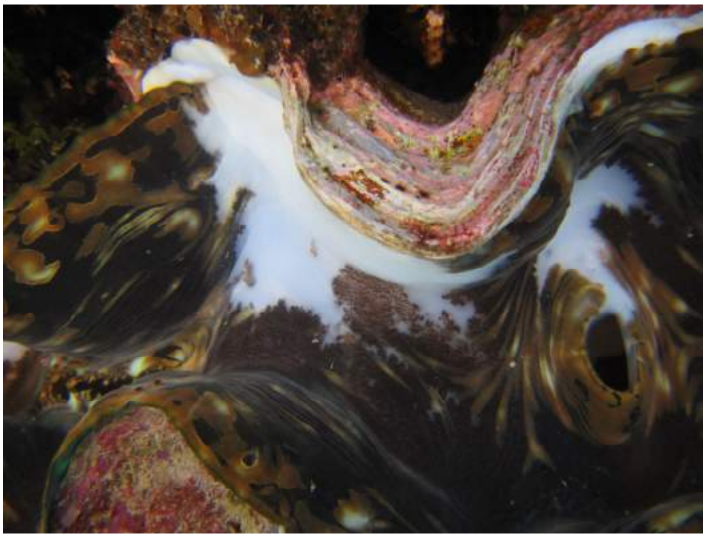


Fig. 15. Bleaching happened on giant clams as this indicates devoid of zooxanthellae.

Fig. 16. An empty shell with clean inner surface suggests recent mortality.

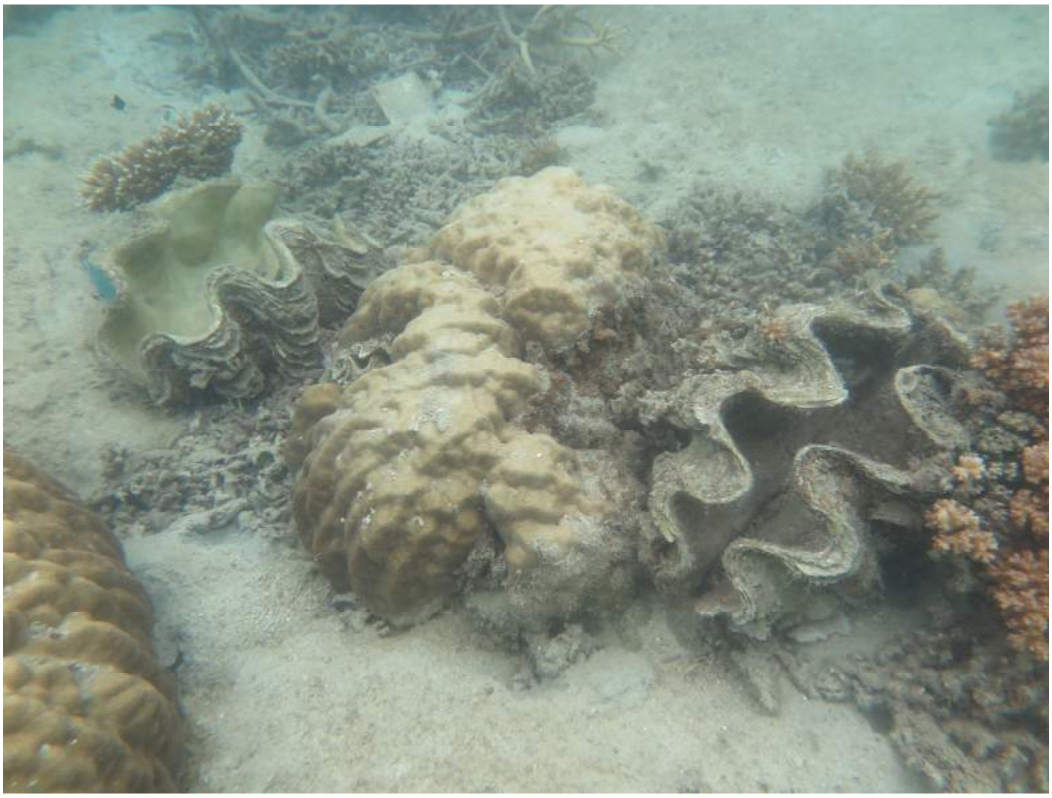


Fig. 17. Empty shells observed at site MP (Marine Park Jetty) that was covered up with sediments.

Another type of predation or parasitism were caused by ectoparasitic pyramidellids which have bigger impact on mariculture of giant clams but non-threatening in reefs due to presence of natural predator of the ectoparasites (Cumming and Alford, 1994; reviewed in Neo et al. 2015). In our survey, the ectoparasitic gastropod, pyramidellid was spotted on juvenile clams (Fig. 18).

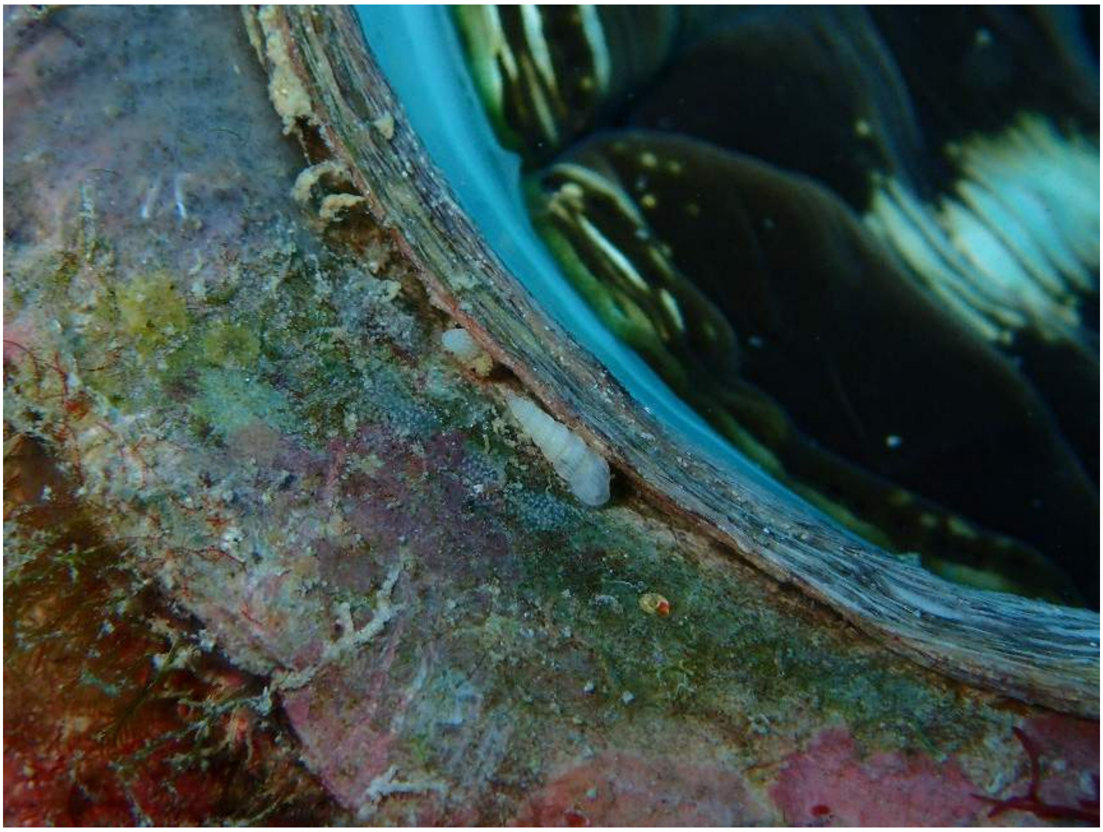


Fig. 18.

Minute ectoparasitic gastropods (Pyramidellidae) were known for parasitism on juvenile giant clams but less threatening to adult giant clams.

Recommendations

The field survey results to date showed healthy populations of two *Tridacna* species in Perhentian Islands and its surrounding islands. In addition, giant clams were moderately abundant in Rhu Island, an island that is not within the boundaries of Perhentian Marine Park. Notwithstanding the previously translocated of giant clams from Rhu Island to the Marine Park Jetty, the population at Rhu Island remained healthy. It is therefore recommended that Rhu Island be considered for designation as part of the Marine Park to provide marine protection to the stocks.

In general, sightings of giant clam juvenile recruitment have signified the presence of replenishing populations and that the adult giant clams may be broodstock material supplying the source larvae.

Given the geographical proximity, giant clams from Perhentian Islands may be used for restocking purposes (if necessary) to aid in restocking selected marine parks along East Coast of Peninsular Malaysia, such as Tioman Island, which have low numbers of giant clams. Such restocking endeavors help to conserve genetic diversity within the same region as well as to prevent diminishing populations of giant clams. The populations of these two giant clams in Perhentian Islands are potentially contributing to the regional gene pool; this information could facilitate the development of trans-boundary management strategies.

Health status of giant clams such as ectoparasite and bleaching – could serve as useful indicators to monitor heath status of giant clams in a particular reef area.

The five-month closure of the east coast Marine Parks during northeast monsoons has minimized the impacts of tourism to the reef ecosystems and its inhabitants, including the giant clams.

Outputs from this project

1. An update data inventory on the distribution and abundance of two giant clams in Perhentian Marine Park.
2. Insights into the population genetic connectivity of the two species of *Tridacna* of Malaysia.
3. The molecular data is served as barcodes of the two species of giant clams in Perhentians.

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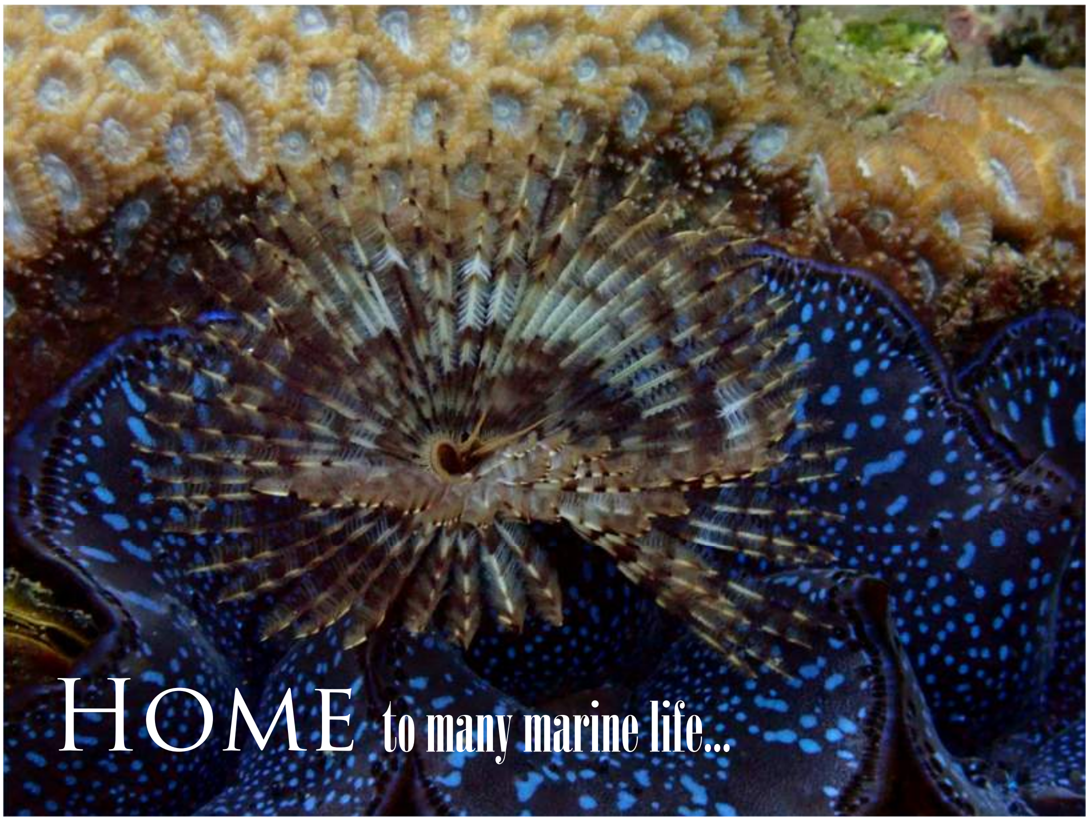
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