

# Population density and genetic structure of the giant clams *Tridacna crocea* and *T. squamosa* on Singapore's reefs

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**ABSTRACT:** Giant clams in Singapore have been subjected to anthropogenic impacts such as fishing, coastal development and sediment pollution for decades, making their study a priority for local reef management. Between September 2009 and August 2010, 29 reefs among Singapore's Southern Islands were surveyed (total area = 87 515 m<sup>2</sup>). Both intertidal and subtidal zones were included, i.e. from shore to the edge of the upper reef slope (depths = 1 to 6 m). A total of 59 clams (but no juveniles) were found, resulting in a contemporary density of 0.067 per 100 m<sup>2</sup>. Only 2 species, *Tridacna crocea* and *T. squamosa*, were encountered, compared to the 4 species recorded historically. The genetic relatedness among individuals from these 2 species was examined using cytochrome *c* oxidase subunit I (COI). Higher levels of polymorphism and genetic diversity were observed among *T. crocea*; both were less pronounced in the *T. squamosa* population, where a single haplotype was present in half of the individuals. The reef conditions in Singapore, especially the lack of suitable substrate and high turbidity, create a poor environment for giant clam reproduction and recruitment. It appears to be low mortality among mature clams that maintains the present population, but with the adults exposed to ongoing stressors, Singapore's entire giant clam stock is endangered.

**KEY WORDS:** Giant clam · Sediment · Distribution · Survey · Restocking · Population genetics

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## INTRODUCTION

Coral reefs and their associated fauna are under intense anthropogenic pressure, especially from overexploitation and poor management of coastal resources (Wilkinson 2008). Intervention efforts, such as the formation of marine protected areas (MPAs) (Carleton Ray 1999, Palumbi 2001) and integrated coastal management schemes (Castilla 2000, Arkema et al. 2006), can ameliorate some of these impacts. One of the major challenges for such schemes, and for coral reef conservation in general, is accounting for the bipartite life history characteristic of many reef taxa—where the adult is either sedentary or

possesses a limited home range, while the larvae are dispersed by currents (Scheltema 1988). In order to effectively protect marine biodiversity, Carpenter et al. (2011) highlighted the need to manage MPAs on a larger spatial scale, such as including nearby reefs, since most species' ranges span over large distances. Data on connectivity among coral reefs, however, are often deficient or absent. Population genetics have provided the opportunity to investigate a variety of ecological interactions, including the linkages between spatially separated groups of conspecifics (Grosberg & Cunningham 2001, Hedgecock et al. 2007). Their genetic structures can provide information on larval dispersal and distribution patterns

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(Hedgecock 1986), providing insights on the probable sources and sinks of pelagic larvae (Juinio-Meñez et al. 2003, Selkoe et al. 2008).

Giant clams (Tridacnidae) are conspicuous bivalves that live in close association with coral reefs throughout the Indo-Pacific (Lucas 1988). They have a well-developed planktonic phase of ~9 d (Lucas 1988) and therefore possess substantial dispersal potential, which may explain their widespread distribution (Benzie & Williams 1992a, Othman et al. 2010). Even though these simultaneous hermaphrodites are highly fecund, releasing up to millions of gametes at each spawning (Lucas 1988), reproduction is sporadic (Solis 1987, Tan & Yasin 1998) and natural recruitment of juveniles is often very low (Gomez et al. 2000). Allozyme variation studies (e.g. Laurent et al. 2002, Juinio-Meñez et al. 2003) and, more recently, mitochondrial cytochrome *c* oxidase subunit I (COI) studies (e.g. DeBoer et al. 2008, Kochzius & Nuryanto 2008), have provided information on the possible gene flow/larval dispersal routes within and among various Indo-Pacific giant clam populations that can either be explained by ocean current patterns (Benzie & Williams 1992a, Macaranas et al. 1992) or geographic isolation (Benzie & Williams 1992b). Nothing is known, however, about the extent of larval dispersal or diversity of genetic material among the giant clams on Singapore's reefs.

During the mid-20th century, 4 species of tridacnids were reported in Singapore: *Tridacna crocea* Lamarck, *T. maxima* (Röding), *T. squamosa* Lamarck and *Hippopus hippopus* (Linnaeus) (Rosewater 1965). Since the early 1960s, however, local giant clam numbers have been severely depleted due to overexploitation for food (Harrison & Tham 1973, Dawson & Philipson 1989) and habitat degradation (Courtois de Vicoise & Chou 1999) leading to a density of just 0.24 per 100 m<sup>2</sup> (Guest et al. 2008). Only 3 species were found: *T. crocea*, *T. maxima* and *T. squamosa*; *H. hippopus* was not sighted in the 9670 m<sup>2</sup> area censused. Furthermore, no juveniles of any clam species were observed, indicating a lack of natural recruitment (Guest et al. 2008).

Knowledge on the spatial distribution of genetic variation within a species is an important tool for the management of marine resources (Palumbi 2003, Carpenter et al. 2011). To forestall the decline of giant clam numbers, it is necessary to gather information on the current population status and its genetic structure. The 2003 survey by Guest et al. (2008) was limited to 7 reef sites and no genetic work was undertaken. Here we present results from a much larger survey conducted to definitively quan-

tify the distribution and abundance of giant clams among Singapore's Southern Islands. In addition, genetic relatedness among individuals from 2 species, *Tridacna crocea* and *T. squamosa*, was examined using COI—a commonly used gene marker for studies on bivalve population genetics (e.g. Luttkhuizen et al. 2003, Nuryanto et al. 2007).

## MATERIALS AND METHODS

### Study area

Singapore (1° 22' N, 103° 48' E) is a small island nation (land area = 710.2 km<sup>2</sup>) situated at the southern tip of Peninsular Malaysia. The climate is equatorial, with high temperatures and rainfall throughout the year, but there exists a relatively wet northeast monsoon (December to February) and a relatively dry southwest monsoon (June to August) (Thia-Eng et al. 2000). Coral reefs are either patch or fringing, and generally distributed around the islands to the south of the mainland (Chou 1999), known locally as the Southern Islands. Singapore's reefs were previously described as having wide reef flats and steep reef slopes but many reef flat areas, for example Pulau Sudong (Pulau = island, abbreviated to P.) and P. Hantu, have been reclaimed, i.e. filled in to create new land area (Chou 1999). The remaining reefs continue to experience direct sediment impacts from coastal construction and dredging of shipping lanes (Chou 2008), while associated turbidity has severely reduced light penetration (Low & Chou 1994, Dikou & van Woesik 2006).

### Survey method and tissue sampling

Between September 2009 and August 2010, 29 Southern Island reefs were surveyed. Sites represent all of Singapore's reef types: patch versus fringing, restricted versus accessible, upstream versus downstream, as well as reefs experiencing various levels of human impact. Both intertidal and subtidal zones, i.e. from shore to the edge of the upper reef slope (depths of 1 to 6 m below mean sea level), were included within the area surveyed. To calculate giant clam density, 6 m wide belt transects, covering almost the entire length of the target reefs, were used for SCUBA searches, while intertidal surveys were conducted by censusing large portions of the reef flat, using quadrats of 10 × 10 to 20 × 20 m<sup>2</sup>. All clams found were identified to species level and their sizes,

and GPS positions when possible, recorded. A labelled stainless steel stake was also hammered into the nearby substrate for future reference. Areas searched at each site varied from 100 to 7000 m<sup>2</sup>, but all were surveyed with equal unit effort (search time m<sup>-2</sup>). Biopsies of mantle tissue were taken whenever possible (i.e. when the clams did not close their valves completely) and preserved in 96% ethanol.

### DNA extraction, amplification and sequencing

Tissue samples for DNA extraction were subsampled, air-dried, ground, and incubated for 24 h at 55°C in CTAB buffer with 20 µg ml<sup>-1</sup> proteinase K. The mixture was agitated with 500 µl phenol/chloroform mixture for 1 min before extraction of aqueous supernatant. This procedure was repeated once before an equal volume of absolute ethanol was added and stored at -80°C overnight to elucidate the DNA pellet. The pellet was subsequently washed with 70% ethanol. RNase-free water was used to dissolve the DNA before storage at -80°C.

Approximately 500 base pair (bp) of the mitochondrial COI gene was amplified via polymerase chain reaction (PCR) using *Tridacna*-specific primers (DeBoer et al. 2008, Kochzius & Nuryanto 2008). PCR was carried out in a total volume of 23 µl, and contained 2 µl DNA template, Thai TAQ buffer, 10 mM dNTPs, BSA additive, 10 µM of each primer and 0.8 µl Thai TAQ. The COI gene for *T. crocea* was amplified using COI-Tricro-Frwd 5-GGG TGA TAA TTC GAA CAG AA-3 and COI-Tricro-Rev 5 -TAG TTA AAG CCC CAG CTA AA-3 (Kochzius & Nuryanto 2008), initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 52°C for 1.5 min, 72°C for 1 min, and a final extension of 72°C for 5 min. The COI gene for *T. squamosa* was amplified using 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), with PCR parameters of 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 success was determined by means of gel electrophoresis in 1% agarose gel using 2 µl of reaction mix. PCR products were purified using Bioline Quick-Clean DNA (Randoph), and each sequencing reaction volume comprised 2 µl of PCR product, 0.5 µl of BigDye Terminator v3.1. (Applied Biosystems), 2 µl of 5× BigDye sequencing buffer, 1.75 µl of sequencing primer (10 µM) and topped up to 10 µl with RNase-free water. The cycle sequencing profile

comprised 30 cycles for 30 s at 95°C, 15 s at 50°C and 4 min at 60°C, followed by another purification step using 5 µl of Agencourt CleanSeq solution. Direct sequencing was carried out using an ABI 3130 genetic analysis sequencer (Perkin Elmer Applied Biosystems).

### Sequence editing

Sequence contigs were proofread in Sequencher v4.0 (GenCodes Corporation) and subsequently aligned using ClustalX Multiple Sequence Alignment Program v2.0.12 (Thompson et al. 1997). Alignments were edited and translated to amino acids to check for stop codons in MEGA (Tamura et al. 2007) before being exported into Arlequin v3.5 (Excoffier & Schneider 2005) and TCS v1.21 (Clement et al. 2000) for further analyses.

### Population genetic diversity and haplotype parsimony network

Molecular diversity measures for both clam populations were calculated in Arlequin v3.5 (Excoffier & Schneider 2005), including haplotype diversity and nucleotide diversity. To determine the genetic structure within each *Tridacna* species population, the relationships among haplotypes were inferred using parsimony networks generated by TCS v1.21 (Clement et al. 2000), and the statistical parsimony procedure followed Crandall (1994) and Templeton et al. (1992). This method estimates the maximum number of differences among haplotypes due to single substitutions with a 95% parsimony connection limit (Posada & Crandall 2001). Outgroup weights were calculated following Castelleo & Templeton (1994); this predicts the oldest haplotype based on the neutral coalescent theory applied to intraspecific networks (Crandall & Templeton 1993, Posada & Crandall 2001).

### Historical demography

The null hypothesis for neutral evolution of the COI marker was tested using Tajima's *D* test (Tajima 1989) and Fu's *F<sub>s</sub>* test (Fu 1997) with 10 000 permutations implemented in Arlequin v3.5. Harpending's raggedness index (HRI; Harpending 1994), based on mismatch distribution analyses, was assessed using Arlequin v3.5 (10 000 permutations). The sudden

population expansion model is rejected when a significant HRI value ( $p < 0.05$ ) is obtained (Schneider & Excoffier 1999).

## RESULTS

### Survey data

Altogether 16 fringing and 13 patch reefs were surveyed. From the 87 515 m<sup>2</sup> censused (intertidal = 76 470 m<sup>2</sup>; subtidal = 11 045 m<sup>2</sup>) 59 giant clams were found, comprising 2 species: *Tridacna crocea* ( $n = 31$ , size range = 94 to 160 mm) and *T. squamosa* ( $n = 28$ , 150 to 418 mm) (Fig. 1, Table 1). No *T. maxima* or *Hippopus hippopus* were encountered within (or outside) the transects or quadrats. Based on information on shell sizes (e.g. Lucas 1988) none of the clams were juveniles (Fig. 1).

Densities for *Tridacna crocea* and *T. squamosa* were 0.035 and 0.032 per 100 m<sup>2</sup> respectively. While *T. crocea* densities were slightly higher on the intertidal areas compared to the subtidal ones (0.037 versus 0.027 per 100 m<sup>2</sup>), the *T. squamosa* intertidal density was ~3.2× lower than its subtidal density (0.025 versus 0.081 per 100 m<sup>2</sup>) (Table 1). Our study

included a total of 22 previously un-surveyed reefs, and clams were found at 16 of these new sites. Of the previously surveyed 7 reefs (Guest et al. 2008), one (Terumbu Bayan) has been completely reclaimed, while clams were found on all of the others.

### Genetics

Sequence alignments for *Tridacna crocea* (27 individuals) and *T. squamosa* (20 individuals) were deposited in GenBank (accession numbers JN392020 to JN392066). Haplotype diversity was similar for both species populations, but *T. crocea* had a higher nucleotide diversity than *T. squamosa* (Table 2). From the 27 *T. crocea* individuals, 10 unique COI haplotypes were obtained, with 20 polymorphic sites and 20 substitutions (18 transitions, 2 transversions) from 453-bp fragments (i.e. 4.42% variable sites). From the 20 *T. squamosa* individuals, 6 unique COI haplotypes were obtained, with 10 polymorphic sites and 10 substitutions (9 transitions, 1 transversion) from 471-bp fragments (i.e. 2.12% variable sites).

### Historical demography

Tests of neutrality using Tajima's *D* and Fu's *F<sub>s</sub>* tests were negative for both giant clam populations, but this was only significant for *Tridacna squamosa*, and only for Tajima's *D*. Negative values suggest that *T. crocea* and *T. squamosa* populations have undergone recent population expansions, and the mismatch distribution analysis supported this. The non-significant HRI values (Table 2) mean that the sudden population expansion model cannot be rejected for either population.

### Haplotype relationship

*Tridacna crocea* showed relatively high diversity with 3 widespread haplotypes. Haplotype 3 was the most common, in 8 of 27 individuals at 6 reefs; 5 haplotypes occurred as singletons (Fig. 2). Outgroup weight calculations suggest that Haplotype 4 (outgroup weight = 0.464) is the ancestral haplotype for *T. crocea* in Singapore. The *T. crocea* haplotypes were heterogeneously distributed throughout the reefs (Fig. 2a). Haplotype diversity within locality either differed by one mutational step (i.e. P. Salu) or occurred in separate clades (i.e. P. Senang,

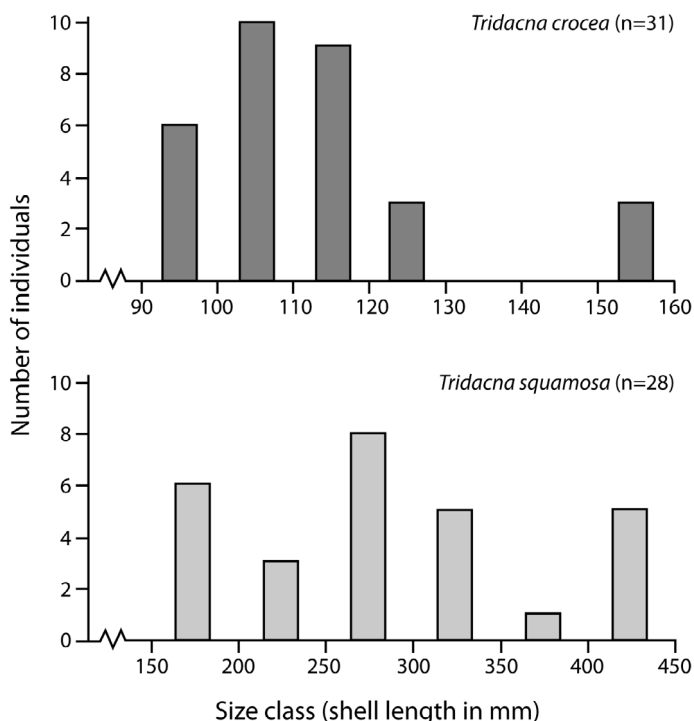


Fig. 1. *Tridacna crocea* and *T. squamosa*. Size frequency distribution on Singapore reefs

Table 1. *Tridacna crocea* and *T. squamosa*. Giant clam numbers for the 29 reefs surveyed. Sites are ranked with furthest straight-line distance to mainland at the top

Sites surveyed	Area (m <sup>2</sup> )	Number of giant clams		
		Total	<i>T. crocea</i>	<i>T. squamosa</i>
<b>Fringing reefs</b>				
Raffles Lighthouse (Pulau Satumu) <sup>a</sup>	1175	4	2	2
Pulau Biola <sup>b</sup>	3590	6	3	3
Pulau Senang <sup>b</sup>	4850	4	3	1
Pulau Pawai <sup>b</sup>	8780	1	0	1
Pulau Sudong <sup>b</sup>	8080	1	0	1
Pulau Jong	3800	2	0	2
Pulau Salu <sup>b</sup>	2100	5	4	1
Pulau Semakau/Sakeng <sup>a</sup>	6165	11	6	5
Sisters' Islands (Pulau Subar Darat, Pulau Subar Laut) <sup>a</sup>	2320	2	0	2
St John's Island (Pulau Sakijang Bendara)	4620	0		
Lazarus Island (Pulau Sakijang Pelepah)	1350	0		
Kusu Island (Pulau Tembakul) <sup>a</sup>	1890	2	0	2
Pulau Tekukor	3510	0		
Pulau Hantu <sup>a</sup>	3750	2	1	1
Labrador	1770	0		
<b>Patch reefs</b>				
Terumbu Berkas <sup>b</sup>	800	0		
Pulau Berkas <sup>b</sup>	2300	1	0	1
Terumbu Berkas Besar <sup>b</sup>	3000	3	3	0
Terumbu Palat <sup>b</sup>	250	0		
Beting Bemban Besar	3750	2	0	2
Terumbu Bemban	1000	0		
Terumbu Salu <sup>b</sup>	3000	4	4	0
Terumbu Semakau	5200	3	2	1
Terumbu Raya	1900	2	1	1
Terumbu Pempang Darat	1590	1	1	0
Terumbu Pempang Laut	2800	1	1	0
Terumbu Pempang Tengah	2175	1	0	1
Cyrene (Terumbu Pandan)	2000	1	0	1
<b>Total area</b>	87 515	59	31	28
Density of clams per 100 m <sup>2</sup>		0.067	0.035	0.032
<b>Intertidal area</b>	76 470	47	28	19
Density of clams per 100 m <sup>2</sup>		0.062	0.037	0.025
<b>Subtidal area</b>	11 045	12	3	9
Density of clams per 100 m <sup>2</sup>		0.109	0.027	0.081

<sup>a</sup>Surveyed in 2003 by Guest et al. (2008); <sup>b</sup>Military live firing areas

Terumbu Berkas Besar, P. Semakau, Terumbu Salu, Terumbu Semakau). There existed a partial genetic difference of up to 9 mutational steps (4.9% uncorrected differences) between the ancestral Haplotype 4 with Haplotypes 5 and 6 found at Terumbu Berkas Besar and P. Senang (Fig. 2b).

In 20 *Tridacna squamosa* individuals sampled, Haplotype 13 (Fig. 3) predominated, being found in 10 individuals at 8 reefs. It differed from 4 other haplotypes by just 1 mutational step. Haplotypes 11, 14 and 16 represented unique individuals from their respective localities (Fig. 3a). Haplotype 16 from P. Sudong was especially distinct, differing by 7 mutational steps (2.9% uncorrected differences) (Fig. 3b). Outgroup weight calculations obtained from TCS suggest that Haplotype 13 (outgroup weight = 0.528) is the ancestral haplotype for *T. squamosa* in Singapore.

## DISCUSSION

Giant clams in Singapore have been subjected to anthropogenic impacts such as fishing, coastal development and sediment pollution for decades (Dawson & Philipson 1989, Chou 1999), making their study, and conservation, a priority for local reef management. The current survey covered an additional 22 sites, and an area 9 times greater (87 515 m<sup>2</sup>) than the 2003 study (Guest et al. 2008). We found a total of 59 giant clams resulting in a

Table 2. *Tridacna crocea* and *T. squamosa*. Number of sequences (n), number of haplotypes (N<sub>hp</sub>), haplotype diversity (hp; mean ± SD), nucleotide diversity (π; mean ± SD), Tajima's *D*, Fu's *F*<sub>s</sub> and Harpending's raggedness index (HRI). \*p < 0.05, NS: not significant

Species	Genetic diversity			Neutrality tests		Mismatch distribution	
	n	N <sub>hp</sub>	hp	π (%)	Tajima's <i>D</i>	Fu's <i>F</i> <sub>s</sub>	HRI
<i>T. crocea</i>	27	10	0.86 ± 0.041	0.76 ± 0.45	-1.19 <sup>NS</sup>	-1.21 <sup>NS</sup>	0.053 <sup>NS</sup>
<i>T. squamosa</i>	20	6	0.72 ± 0.088	0.31 ± 0.22	-1.68*	-1.14 <sup>NS</sup>	0.169 <sup>NS</sup>

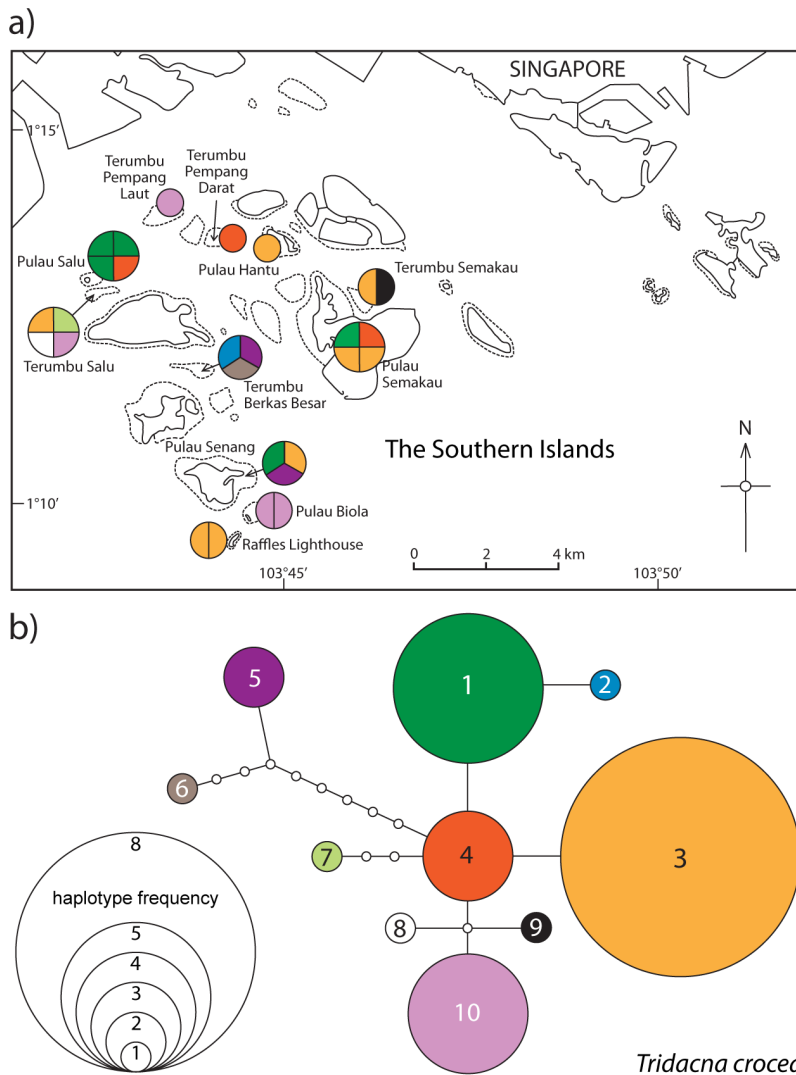
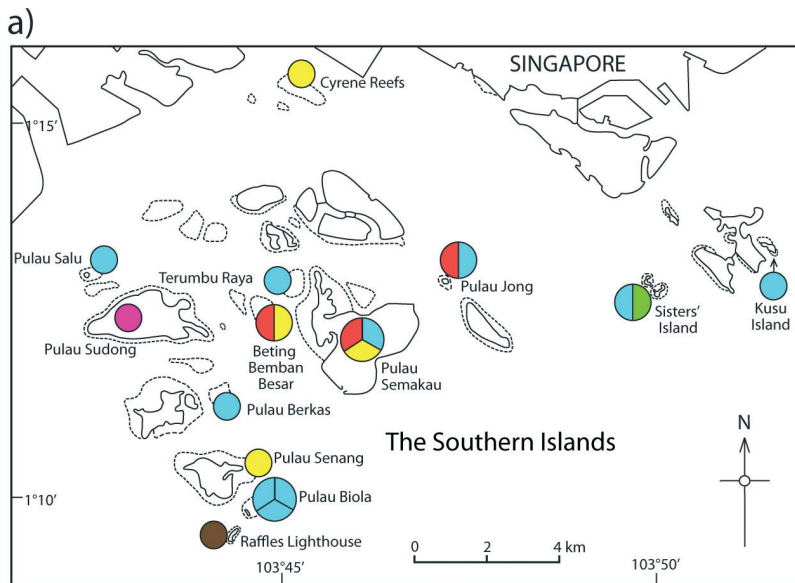


Fig. 2. *Tridacna crocea*. (a) Distribution of unique COI haplotypes. Individual clams represented by each section in the circle. (b) Minimum spanning network using 453-bp fragment from the COI gene. Colour-coded circles (plus reference number) = unique COI haplotype. Open circles on lines connecting haplotypes = single putative mutations. Lines join all haplotypes within a 95% statistical confidence parsimony network

contemporary density of 0.067 per 100 m<sup>2</sup> (~4 times lower than the 2003 figure of 0.24 per 100 m<sup>2</sup>); no juvenile clams were encountered. Furthermore, unlike Guest et al. (2008), we did not encounter any *Tridacna maxima* individuals, only *T. crocea* and *T. squamosa*. The density of *T. crocea* was quite similar in both intertidal and subtidal zones, but *T. squamosa* were more abundant in subtidal areas. The very small sample size made statistically meaningful genetic analyses impossible, but higher levels of polymorphism and genetic variation were observed within the *T. crocea* population compared to the *T. squamosa* population, where a single haplotype was present in half of the specimens.

The present survey estimates the *Tridacna crocea* density to be 0.035 per 100 m<sup>2</sup>. This may be a conservative figure due to this species' cryptic coloration (Todd et al. 2009) and boring behaviour that makes them hard to find (Guest et al. 2008). Nevertheless, *T. crocea* aggregations can reach up to 100s per m<sup>2</sup> (e.g. Chantrapornsyl et al. 1996, Tan et al. 1998), suggesting that numbers in Singapore are very low. The *T. squamosa* density in Singapore is established at 0.032 per 100 m<sup>2</sup>, lower than that in protected reefs elsewhere but higher than areas with intense exploitation (Kilada et al. 1998, Tan et al. 1998, Eliata et al. 2003). *Tridacna squamosa* intertidal density was 0.025 per 100 m<sup>2</sup> whereas its subtidal abundance was 0.081 per 100 m<sup>2</sup>. For *T. crocea*, the densities in these 2 habitats were more similar (Table 1). Similar depth distributions for both species have been reported for the Johore Islands, Malaysia (Yasin & Tan 2000) and the Red Sea (Jantzen et al. 2008), and are probably due to different light requirements, with *T. squamosa* being more tolerant to lower light and/or deeper depths (Yasin & Tan 2000). Harvesting for food could also contribute. Giant clam gleaning during low tide is easier than extraction via snorkelling or diving (Hviding 1993) and the larger and free-living *T. squamosa* are less demanding to remove from the reef than the bur-

rowing *T. crocea*. Exploitation of clams in Singapore has ceased but other anthropogenic inputs, especially sediment pollution from dredging of shipping lanes and nearshore construction activities (e.g. Chou 1988, Chou et al. 2004), continue to be a major problem. Sediment can deleteriously affect giant clams in numerous ways, for example, by interfering with their filter feeding (Purchon 1955), by increasing turbidity and thus reducing light reaching the photosynthetic symbiotic zooxanthellae in the clam's mantle tissues (Guest et al. 2008), and by covering reef substrates with a layer of sediment that makes it difficult for clam larvae to settle (Rogers 1990, Neo et al. 2009).



b) *Tridacna squamosa*

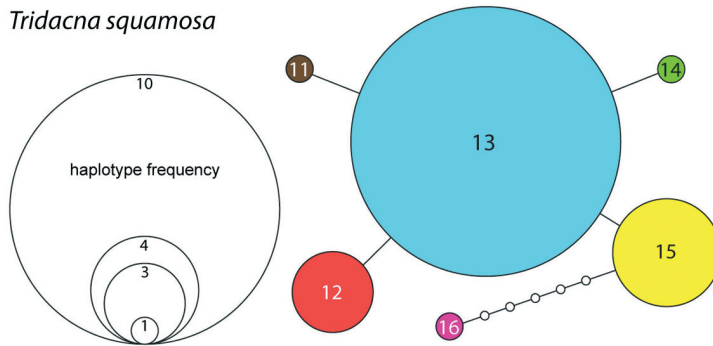


Fig. 3. *Tridacna squamosa*. (a) Southern Islands of Singapore showing distribution of unique COI haplotypes for *T. squamosa*. Individual clams represented by each section in the circle. (b) Minimum spanning network of *T. squamosa* using 471-bp fragment from the COI gene. See Fig.2 for definitions

In concordance with Guest et al. (2008), we found the highest clam density (0.340 per 100 m<sup>2</sup>) at Raffles Lighthouse, a reef generally considered Singapore's healthiest due to its high coral diversity and abundance (Huang et al. 2009) and low sedimentation levels (Todd et al. 2004). This density, however, was still considerably lower than that recorded in the 2003 survey (1.290 per 100 m<sup>2</sup>). It should be noted that Guest et al.'s (2008) objectives and survey methods were different to ours. Their work was preliminary and the sites chosen did not represent all the reef types found in Singapore. Our study aimed to provide the definitive status of giant clams in Singapore via extensive and dedicated surveys. Guest et al. (2008) employed a SCUBA zig-zag swim search that, having no fixed boundaries, could have resulted in an underestimation of actual survey area (and therefore an overestimation of clam density). We used a combination of 6 m belt transects (SCUBA) and large

quadrats (intertidal surveys), all measured out with nylon tapes. Our survey was scaled up to a total of 29 sites, and we were able to gain access to some rarely visited patch reefs (e.g. Terumbu Pempang Laut, Terumbu Raya) plus fringing reefs found within Live Firing Areas (e.g. P. Pawai, P. Sudong, P. Senang) managed by the Republic of Singapore Air Force that required permission to enter. Many of these more inaccessible reefs hosted giant clams. Clearly, the 2003 survey was not as comprehensive or rigorous as the one presented here, so direct comparisons of results need to be treated with a degree of caution. Nevertheless, we expect that natural mortality (albeit in an impacted environment) coupled with zero recruitment would amply explain any genuine decline since 2003.

*Hippopus hippopus* was last recorded in Singapore in the 1960s (Lee 1966), whereas the most recent record for *Tridacna maxima*, a single individual, was in 2003 (Guest et al. 2008). *H. hippopus* are usually found at shallow depths (Yasin & Tan 2000), but many shallow reef flats around Singapore have been reclaimed (Chou 2008), removing a substantial portion of this species' natural habitat. *T. maxima* is a lithophagic species

that exhibits partial burrowing (Rosewater 1965). Singapore's deteriorating coral reefs are now characterised by loose rubble (Chou 2008), which may not be able to sustain populations of mature *T. maxima* (shell lengths up to 300 mm) that require a more consolidated substrate for stable attachment (McMichael 1974). No historical abundance data for these 2 species exist, nor any precise information on their exploitation. We can only propose that 3 factors, either individually, but more likely synergistically, have caused their extirpation from Singapore's waters: habitat loss, overexploitation, and/or the effects of sediment.

Levels of genetic diversity for both species here are similar or slightly lower than other populations within the Indo-Malay Archipelago (e.g. DeBoer et al. 2008, Kochzius & Nuryanto 2008, Nuryanto & Kochzius 2009). We expect that any loss of genetic variation will have coincided with the overall reduc-

tion in clam numbers Singapore has experienced during the last few decades. The majority of neutrality tests (Tajima's  $D$  and Fu's  $F_s$ ) did not reject the null hypothesis of neutral evolution of COI for populations in Singapore (Table 2). Significant Tajima's  $D$  for *Tridacna squamosa* reflects an excess of rare polymorphisms in the population, and these values indicate either positive selection (rejected by the mismatch analysis) or a recent population expansion (Tajima 1989, Fu 1997). Loss of habitats during low sea levels of the last glacial period (Bird et al. 2006) can result in population bottlenecks, whereas new habitats associated with sea level rise facilitate expansion of relict populations (Fauvelot et al. 2003). Similar signs of population expansions were found in *T. crocea* (DeBoer et al. 2008, Kochzius & Nuryanto 2008) and *T. maxima* (Nuryanto & Kochzius 2009) in the Indo-Malay Archipelago.

The qualitative results presented in Figs. 2 & 3 suggest relatively higher levels of genetic diversity for *Tridacna crocea* than for *T. squamosa*. Genetic studies on *T. crocea* have shown strong population structure across the Indo-Malay Archipelago, indicating restricted gene flow between almost all the sample sites (DeBoer et al. 2008, Kochzius & Nuryanto 2008). No similar studies exist for *T. squamosa*, but in Singapore, a single haplotype present in half of the individuals collected suggests gene flow and possibly panmixis (except Haplotype 16, which may have derived from a neighboring reef). Patterns of ocean currents affect the frequency of larval exchange (White et al. 2010) and *T. squamosa* populations may be connected due to the current regimes within the Straits of Singapore (Bird et al. 2006). If larvae, however, are simply being well dispersed among islands, the same degree of connectivity should also be seen in *T. crocea*. A single spawning event can explain the dominant haplotype in *T. squamosa*; for example, a pulse of genetically related larvae, potentially originating from beyond Singapore's waters, could have settled and persisted. If this population then matured with little or no additional input of genetic material from elsewhere, the outcome would be difficult to distinguish from panmixis. Alternatively, as *T. crocea* are usually found burrowed into rubble habitats (Hamner & Jones 1976), while *T. squamosa* is free-living (Rosewater 1965), human exploitation would be biased to removing the more easily harvestable *T. squamosa*, resulting in higher levels of genetic diversity in the less exploited species.

Even if there is some connectivity among Singapore's giant clams, the population may not be viable. Wada (1952) highlighted that, as the distance be-

tween spawning adults increases, the likelihood of fertilization decreases rapidly (the Allee effect), and there is almost certainly a minimum population level at which no successful reproduction can take place—even when adult clams are still present (Nash et al. 1988). Natural recruitment of giant clam larvae has been observed only in a few locations, including the Michaelmas Reef, central Great Barrier Reef (Braley & Muir 1995) and Rose Atoll, Samoan Archipelago (Green & Craig 1999). Rose Atoll has up to 225 *Tridacna maxima* ind.  $m^{-2}$  (Green & Craig 1999) and is thought to have contributed to recruitment at nearby reefs. Other areas such as Palau (Hirschberger 1980); P. Tioman, Malaysia (Tan et al. 1998); Java, Indonesia (Brown & Muskanofola 1985); and Lee-Pae Island, Thailand (Chantrapornsyl et al. 1996), support low giant clam densities of sparsely distributed mature adults, with few or no juveniles—a similar situation to Singapore. Even if the clams in Singapore did somehow manage to reproduce, the very low numbers and star-like parsimony networks (Viñas et al. 2004) indicate a population genetic bottleneck that reduces recovery.

Low giant clam densities coupled with Singapore's contemporary reef conditions, especially the lack of suitable substrate and reduced water quality (Burke et al. 2002), create a poor environment for reproduction and recruitment. It appears to be low mortality among the mature clams that maintains the present population (Yamaguchi 1977) but, with the adults exposed to ongoing stressors (Courtois de Vicoise & Chou 1999), Singapore's entire giant clam stock is endangered. Plans to restock these large and colourful bivalves in Singapore, through mariculture and subsequent out-planting, are underway. Larval dispersal models are being used to help identify nursery sites that have the greatest potential as sources of larvae for other Southern Island reefs. Improving water quality, however, continues to be the major challenge for local marine managers and substantial reductions in turbidity and sediment load will be required to ensure the successful conservation of giant clams in Singapore.

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