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Comparative genetic population structure of three endangered giant clams (Cardiidae: *Tridacna* species) throughout the Indo-West Pacific: implications for divergence, connectivity and conservation

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ABSTRACT

Information on the genetic population structure of endangered giant clams is important for conservation programmes and the understanding of ecological and evolutionary processes. In this study, the genetic population structures of three codistributed and ecologically similar giant clam species (*Tridacna crocea*, *T. maxima* and *T. squamosa*) are compared. A fragment of the cytochrome *c* oxidase I gene was sequenced as a genetic marker in three giant clam species sampled throughout the Indo-West Pacific, from the Western Indian Ocean (WIO) and Red Sea (RS) to the Eastern Indian Ocean (EIO), across the centre of marine biodiversity in the Indo-Malay Archipelago (IMA) to the Western Pacific (WP) and the Society Islands in the Central Pacific (CP). All three species showed limited gene flow and a highly significant genetic population structure. The Φ_{st}-values ($P < 0.001$) are 0.46, 0.81 and 0.68 for *T. crocea*, *T. maxima* and *T. squamosa*, respectively. Based on a hierarchical AMOVA they could be divided into three to six groups from West to East: (1) WIO (*T. maxima* and *T. squamosa*), (2) RS (*T. maxima* and *T. squamosa*), (3) EIO (including Java Sea in *T. maxima*), (4) central IMA, (5) WP and (6) CP (*T. maxima*). The distribution of the haplotype clades in the populations and the pairwise Φ_{st}-values between populations indicated a high level of gene flow in the central IMA for the three species. The concordant patterns suggest that geological history, sea-level changes during glacial periods of the Pliocene and Pleistocene, and oceanography are important factors shaping the genetic population structure of giant clams. The observed deep evolutionary lineages in the peripheral areas of the IMA might include cryptic species.

INTRODUCTION

Congruent patterns of genetic population structures across multiple codistributed taxa indicate that the taxa examined might be subjected to the same historical biogeographic processes and environmental conditions (Bermingham & Moritz, 1998; Avise, 2000). In contrast, a lack of congruence in genetic structure suggests that the species concerned might respond differently to the same biogeographic processes (Cartens *et al.*, 2005; Crandall *et al.*, 2008).

The Indo-West Pacific (IWP) comprises the tropical waters of the Red Sea (RS), Western Indian Ocean (WIO), Eastern Indian Ocean (EIO), seas in the Indo-Malay Archipelago (IMA), as well as the Western Pacific (WP) and Central Pacific (CP). Many species, such as giant clams, are restricted to this biogeographic region, which is characterized by an exceptionally high diversity (Briggs, 1995). In this region, the East African reefs located in the WIO exhibit high levels of species diversity similar to those of the Central Indian Ocean, but with many endemic species, which has led to the recognition of a WIO centre of diversity (Spalding, Ravilious & Green, 2001). Also the RS is considered to be an important secondary centre of evolution, because of its special oceanographic characteristics, high number of endemic species and a large number of coral taxa (Klausewitz, 1989; Veron,

2000; DiBattista *et al.*, 2015). The Coral Triangle, located in the IMA, hosts the greatest diversity of marine species (Hoeksema, 2007), while coral reefs in the Society Islands (CP), due to their isolation, show a relatively low species diversity, especially on a unit-area basis (Spalding *et al.*, 2001).

The IWP and especially the IMA provide an excellent study area for investigating the contribution of historical and ongoing processes to the high degree of biodiversity. The IMA has experienced a complicated geological history (Hall, 2002). In particular, sea-level lowstands during glacial periods in the Pliocene and Pleistocene exposed the Sunda and Sahul continental shelves (Voris, 2000). These exposed shelves acted as a vicariant barrier that has been hypothesized to cause genetic divergence between Indian and Pacific Ocean populations of many taxa. However, marine species in this region exhibit different patterns, ranging from deep divergence to shallow genetic population structure or lack of differentiation. Strong genetic divergence can be observed e.g. in populations of anemonefish (*Amphiprion ocellaris*: Timm & Kochzius, 2008; Timm, Figiel & Kochzius, 2008; Timm, Planes & Kochzius, 2012; *A. perideraion*: Dohna *et al.*, 2015) and the mushroom coral *Heliofungia actiniformis* (Knittweis *et al.*, 2009). The blue seastar *Linckia laevigata* is an example of a species with a shallow genetic population structure (Kochzius *et al.*, 2009; Alcazar & Kochzius, 2015). In contrast, genetic differentiation is absent in three surgeonfishes (*Naso brevirostris*, *N. unicornis* and *N. vlamingii*: Horne *et al.*, 2008) and two moray eels (*Gymnothorax flavimarginatus* and *G. undulatus*: Reece *et al.*, 2010, 2011).

Giant clams of the family Cardiidae (formerly Tridacnidae; Herrera *et al.*, 2015) are economically and ecologically important coral reef species. *Tridacna maxima* and *T. squamosa* are widely distributed from the RS and WIO across the IMA to the Society Islands in the CP, while *T. crocea* occurs from the EIO across the IMA to the WP (Rosewater, 1965; Knop, 1996; Gilbert *et al.*, 2007; Andréfouët *et al.*, 2014). The high commercial value of giant clams for food and as marine ornamentals attracts large-scale collection from the wild and aquaculture of the species (Lucas, 1988). Tridacnid species are listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). To provide background information for the conservation of the species, it is important to understand their genetic population structure and connectivity. Adults are sessile and connectivity among population is only possible by their pelagic larval stages. Due to a pelagic larval duration (PLD) of 9–12 d (Jameson, 1976; Lucas, 1988) giant clams can potentially disperse over long distances and might possess low genetic differentiation. However, oceanographic or geographic barriers might limit their dispersal and lead to considerable genetic differentiation among populations. There have been many previous genetic population studies on giant clams, but most did not cover the full species range (Benzie & Williams, 1992a, b; Macranas *et al.*, 1992; Benzie & Williams, 1995; Benzie & Williams, 1997; Kittiwattanawong, 1997; Yu, Juinio-Meñez & Monje, 2000; Kittiwattanawong, Nugranad & Sriswat, 2001; Laurent, Planes & Salvat, 2002; Juinio-Meñez *et al.*, 2003). Recently, large-scale studies on genetic population structure were performed for giant clams in the IMA and a genetic break between the EIO and Pacific Ocean was documented (DeBoer *et al.*, 2008, 2014a, b; Kochzius & Nuryanto, 2008; Nuryanto & Kochzius, 2009). However, none of these studies has yet covered multiple species across their full geographic range.

This study compares the genetic population structure of three codistributed species of giant clams (*T. crocea*, *T. maxima* and *T. squamosa*) across the IWP, which covers most of their geographic distributional range. The study aims to reveal if concordant barriers exist that prevent gene flow among populations and to identify factors that influence connectivity.

MATERIAL AND METHODS

Sampling and sequencing

Small pieces of mantle tissue were collected from *Tridacna crocea* ($n = 344$, 20 localities), *T. maxima* ($n = 317$, 20 localities) and *T. squamosa* ($n = 182$, 18 localities) by SCUBA diving, across their distribution range in the IWP (Table 1; Fig. 1) from 2004 to 2011. Tissues were preserved in 96% ethanol. From these samples, cytochrome *c* oxidase I (COI) sequences had already been obtained from 300 individuals of *T. crocea* (Kochzius & Nuryanto, 2008) and 211 individuals of *T. maxima* (Nuryanto & Kochzius, 2009).

Genomic DNA was extracted from the new samples using the Chelex method (Walsh, Metzger & Higuchi, 1991). A fragment of COI was amplified using tridacnid-specific primers (Kochzius & Nuryanto, 2008). PCRs were carried out in 50 µl volumes containing 10–100 ng template DNA, 10 mM Tris-HCl (pH 9), 50 mM KCl, 0.2 mM dNTPs, 0.2 µM forward and reverse primers, 2 mM $MgCl_2$ and 1 U *Taq* DNA polymerase. PCR amplification was conducted under the following conditions: 5 min initial denaturation at 94 °C, 35 cycles of 1 min at 94 °C, 1.5 min at 45 °C, 1 min at 72 °C and a final extension at 72 °C for 5 min. PCR products were purified with the QIAquick PCR purification kit (Qiagen, Germany). Sequencing of both strands was conducted with an ABI PRISM 310 and an ABI 3730 XL automated sequencer (Applied Biosystems).

Sequences were edited with the program Sequence Navigator v. 1.0.1 (Applied Biosystem) and aligned by ClustalW using the software BioEdit v. 7.0. The sequences were compared with sequences in GenBank using BLASTN to check for orthology to tridacnids. Using the programme Squint (Goode & Rodrigo, 2007), the DNA sequences were translated to amino acid sequences to confirm that a functional mitochondrial DNA sequence had been obtained. The new sequences were combined with those from previous studies (Kochzius & Nuryanto, 2008; Nuryanto & Kochzius, 2009) for further analysis.

Genetic diversity

Molecular diversity indices, such as the number of haplotypes, haplotype diversity *h* (Nei, 1987) and nucleotide diversity л (Nei & Jin, 1989) were obtained using the program Arlequin v. 3.5 (Excoffier & Lischer, 2010).

Demographic history

To compare demographic histories of mtDNA in the three species, two different approaches were used to test each population for departures from the neutral model due to selection or population growth. First, Tajima *D* (Tajima, 1989) and Fu's F_S tests (Fu, 1997) were used to test for neutrality. Significant negative *D* and F_S values can be interpreted as signatures of selection or demographic expansion. Historic demographic expansions were further explored based on the distribution of pairwise differences between sequences (mismatch distribution; Rogers & Harpending, 1992). The concordance of the observed with the expected distribution under Rogers' model of sudden population expansion was also tested using Arlequin. The values of τ (units of mutational time) were converted to estimate time since expansion with the equation $\tau = 2$ ut (Rogers & Harpending, 1992), where $t =$ number of generations since expansion and $u = 2\mu \times$ number of nucleotides sequenced, with μ the mutation rate of complete COI sequences (0.6% per million years; Marko, 2002). Then the time since expansion was calculated by the $T = t \times$ generation time, with a minimum generation time of 2 years in giant clams (Lucas, 1988).

Genetic population structure and gene flow

To reveal genetic differentiation between populations, pairwise Φ_{st} values (Excoffier, Smouse & Quattro, 1992) were calculated

Table 1. Summary statistics for each population of the three *Tridacna* species.

Sites (code)	Region	*n*	N_{hp}	*h*	Л(%)	*D*	F_s	SSD	HRI
Tridacna crocea									
Gulf of Thailand (GT)	IMA	7	3	0.67	0.48	1.08^{NS}	-1.32^{NS}	0.111^{NS}	0.283^{NS}
Phuket (Ph)	EIO	7	6	0.95	1.03	0.27^{NS}	-1.44^{NS}	0.019^{NS}	0.066^{NS}
Trang Islands (Tr)	EIO	10	6	0.84	0.82	-0.54^{NS}	-0.50^{NS}	0.023^{NS}	0.078^{NS}
Satun Islands (SI)	EIO	9	7	0.92	1.04	-0.09^{NS}	-1.57^{NS}	–	–
Padang (Pa)	EIO	7	6	0.95	1.00	-0.79^{NS}	-1.49^{NS}	–	–
Pulau Seribu (PS)	IMA	14	6	0.60	0.60	-0.79^{NS}	-0.38^{NS}	0.054^{NS}	0.148^{NS}
Karimunjava (Ka)	IMA	16	9	0.77	0.70	-1.71^{*}	-2.65^{NS}	0.014^{NS}	0.044^{NS}
Komodo (Ko)	IMA	26	17	0.89	0.96	-2.15^{**}	-8.55^{***}	0.017^{NS}	0.038^{NS}
Kupang (Ku)	IMA	9	7	0.92	0.67	-1.47^{NS}	-2.76^{*}	0.028^{NS}	0.101^{NS}
Spermonde (Sp)	IMA	40	23	0.96	1.04	-1.56^{*}	-13.4^{***}	0.004^{NS}	0.019^{NS}
Bira (Bi)	IMA	12	6	0.82	0.70	-0.48^{NS}	-0.35^{NS}	0.017^{NS}	0.055^{NS}
Sembilan Islands (Se)	IMA	20	14	0.94	1.23	-1.36^{NS}	-4.91^{*}	0.007^{NS}	0.014^{NS}
Kendari (Ke)	IMA	28	16	0.82	0.78	-1.69^{***}	-8.02^{***}	0.002^{NS}	0.010^{NS}
Luwuk (Lu)	IMA	23	12	0.78	0.68	-1.51^{NS}	-4.68^{*}	0.006^{NS}	0.021^{NS}
Togian Islands (TI)	IMA	46	21	0.71	0.65	-1.85^{*}	-12.3^{***}	0.011^{NS}	0.040^{NS}
Manado (Ma)	IMA	8	8	1.00	0.75	-0.92^{NS}	-5.45^{***}	–	–
Sangalaki (Sa)	IMA	16	12	0.96	1.09	-0.83^{NS}	-4.67^{*}	0.005^{NS}	0.017^{NS}
Kota Kinabalu (KK)	IMA	21	16	0.97	1.06	-1.49^{*}	-8.63^{***}	–	–
Misool (Mi)	IMA	11	8	0.93	1.08	-1.20^{NS}	-1.75^{NS}	0.010^{NS}	0.032^{NS}
Biak (Bk)	WP	14	13	0.99	3.30	0.94^{NS}	-3.05^{NS}	–	–
Overall		344	149	0.94	1.71	-1.90^{**}	-24.34^{**}	0.008^{NS}	0.008^{NS}
Tridacna maxima									
Kenya (Ky)	WIO	9	7	0.92	0.23	0.75^{NS}	-0.17^{NS}	0.070^{NS}	0.073^{NS}
Red Sea (RS)	RS	13	10	0.95	0.69	-0.94^{NS}	-5.61^{***}	–	–
Phuket (Ph)	EIO	34	13	0.91	0.46	-1.36^{NS}	-6.90^{***}	–	–
Trang Islands (Tr)	EIO	19	6	0.86	0.43	-0.017^{NS}	-0.80^{NS}	–	–
Satun Islands (SI)	EIO	24	17	0.97	0.69	-1.13^{NS}	-13.3^{***}	–	–
Padang (Pa)	EIO	15	9	0.88	0.56	-0.03^{NS}	-4.12^{**}	–	–
Pulau Seribu (PS)	IMA	12	4	0.45	0.12	-1.62^{*}	-2.12^{**}	–	–
Karimunjava (Ka)	IMA	20	6	0.52	0.59	-1.30^{NS}	-0.06^{NS}	0.069^{NS}	0.280^{NS}
Komodo (Ko)	IMA	12	4	0.56	0.16	-1.18^{NS}	-1.59^{*}	–	–
Kupang (Ku)	IMA	14	10	0.89	0.92	-1.37^{NS}	-4.09^{*}	–	–
Spermonde (Sp)	IMA	21	10	0.68	0.62	-2.24^{**}	-3.85^{*}	0.007^{NS}	0.028^{NS}
Bira (Bi)	IMA	10	9	0.98	0.85	-1.86^{*}	-5.31^{***}	–	–
Sembilan Islands (Se)	IMA	12	8	0.85	0.46	-2.07^{**}	-4.36^{**}	0.002^{NS}	0.046^{NS}
Luwuk (Lu)	IMA	16	9	0.86	0.44	-1.31^{NS}	-4.82^{NS}	–	–
Togian Islands (TI)	IMA	21	16	0.96	0.79	-2.18^{**}	-12.0^{***}	–	–
Manado (Ma)	IMA	22	15	0.90	0.48	-1.94^{*}	-13.5^{**}	–	–
Sangalaki (Sa)	IMA	7	3	0.52	0.62	-1.58^{*}	1.60^{NS}	0.078^{NS}	0.209^{NS}
Misool (Mi)	IMA	8	5	0.78	0.85	-1.57^{*}	-0.16^{NS}	0.056^{NS}	0.128^{NS}
Biak (Bk)	WP	16	13	0.97	4.78	-0.45^{NS}	-1.87^{NS}	0.043^{NS}	0.057^{NS}
Society Islands (So)	CP	12	6	0.68	0.79	-0.82^{NS}	-0.36^{NS}	0.053^{NS}	0.092^{NS}
Overall		317	135	0.94	2.78	-1.02^{NS}	-23.87^{**}	0.024^{NS}	0.014^{NS}
Tridacna squamosa									
Kenya (Ky)	WIO	2	2	1.00	0.21	0.00^{NS}	2.08^{NS}	–	–
Red Sea (RS)	RS	6	3	0.60	0.25	-1.23^{NS}	-0.19^{NS}	0.008^{NS}	0.062^{NS}
Batam (Bt)	IMA	2	2	1.00	1.31	0.00^{NS}	1.61^{NS}	–	–
Pulau Seribu (PS)	IMA	3	2	0.67	0.16	0.00^{NS}	0.20^{NS}	–	–
Karimunjava (Ka)	IMA	17	9	0.83	0.53	-1.51^{NS}	-3.68^{*}	0.010^{NS}	0.057^{NS}
Bali (Ba)	IMA	6	4	0.87	0.52	-0.31^{NS}	-0.44^{NS}	–	–
Komodo (Ko)	IMA	11	6	0.85	0.57	-1.27^{NS}	-1.37^{NS}	–	–
Kupang (Ku)	IMA	6	3	0.73	0.32	-0.18^{NS}	0.21^{NS}	0.022^{NS}	0.133^{NS}
Spermonde (Sp)	IMA	49	13	0.75	0.48	-1.73^{*}	-5.45^{**}	–	–
Bira (Bi)	IMA	14	11	0.96	0.52	-1.57^{*}	-8.53^{***}	–	–
Sembilan Islands (Se)	IMA	6	4	0.87	0.60	0.37^{NS}	-0.22^{NS}	0.075^{NS}	0.240^{NS}
Kendari (Ke)	IMA	13	4	0.52	0.17	-0.90^{NS}	-1.31^{NS}	0.001^{NS}	0.080^{NS}

Continued

Table 1. *Continued*

Sites (code)	Region	*n*	N_{hp}	*h*	Л(%)	*D*	F_s	SSD	HRI
Togian Islands (TI)	IMA	6	5	0.93	0.64	-1.01^{NS}	1.62^{NS}	–	–
Manado (Ma)	IMA	9	4	0.75	0.28	0.02^{NS}	-0.82^{NS}	–	–
Sangalaki (Sa)	IMA	9	5	0.81	0.96	−0.37	0.32	0.049^{NS}	0.119^{NS}
Kota Kinabalu (KK)	IMA	9	6	0.83	0.68	-1.64^{*}	-1.47^{NS}	0.020^{NS}	0.048^{NS}
Misool (Mi)	IMA	11	3	0.62	0.17	0.04^{NS}	-0.11^{NS}	–	–
Biak (Bk)	WP	3	3	1.00	3.68	0.00^{NS}	1.39^{NS}	–	–
Overall		182	56	0.83	1.08	-2.22^{***}	-25.44^{***}	0.012^{NS}	0.035^{NS}

Abbreviations: *n*, number of sequences; N_{hp}, number of haplotypes; Л, nucleotide diversity; D, Tajima's *D*; *Fs*, Fu's *Fs*; SSD, sum of square deviation; HRI, Harpending's raggedness index; RS, Red Sea; WIO, Western Indian Ocean; EIO, Eastern Indian Ocean; IMA, Indo-Malay Archipelago; WP, Western Pacific; CP, Central Pacific.

$^{*}0.05 \geq P \geq 0.01$; $^{**}0.01 > P > 0.001$; $^{***}P < 0.001$; ^{NS}: not significant.

(Weir & Cockerham, 1984). Significance of pairwise population comparisons was tested by 10,000 permutations and sequential Bonferroni correction (Rice, 1989) was conducted for the *P*-values. Hierarchical AMOVA (Excoffier *et al.*, 1992) was performed using Arlequin in order to define spatial groups of sample sites that were maximally differentiated from each other (Φ_{ct}). Minimum-spanning networks of the haplotypes were drawn based on results obtained with Arlequin and the haplotypes were divided into clades based on the number of mutational steps. Frequencies of the clades were calculated for each sample site and are shown as pie diagrams on the maps.

The correlation between genetic distance (pairwise Φ_{st} values) and geographic distance was investigated conducting isolation-by-distance (IBD) analysis with the reduced major axis regression method among all populations and populations in the central IMA, respectively. To represent the geographic distance the shortest way by sea between populations was measured using an electronic world atlas. A Mantel test was conducted to test the significance of the correlation with 30,000 permutations using the web service IBDWS v. 3.2.3 (http://ibdws.sdsu.edu; Jensen, Bohonak & Kelley, 2005).

RESULTS

Genetic diversity

A 417-bp unambiguous COI alignment was obtained based on 843 specimens of three species. Sequence comparison of the segment from 344 *Tridacna crocea*, 317 *T. maxima* and 182 *T. squamosa* individuals resulted in 149, 135 and 56 haplotypes, respectively. The sequences of new haplotypes were submitted to the EMBL database and have the accession numbers HE995439-HE995453 (14 *T. crocea*), HE995454-HE995487 (34 *T. maxima*) and HE995488-HE995532 (44 *T. squamosa*). Intrapopulation diversity indices of the three species are shown in Table 1. All three species revealed a high level of polymorphism and genetic diversity, with high haplotype and nucleotide diversity (the overall haplotype and nucleotide diversity were higher than 0.83 and 1.08%, respectively). However, in the Java Sea, populations from Karimunjava and Pulau Seribu showed a much lower genetic diversity compared with other populations of the three species.

Demographic history

Both neutrality test and mismatch distribution were performed for each population of the three species. Many populations showed significant negative *D* and F_S values (Table 1), which indicated significant departure from mutation-drift equilibrium, especially Fu's F_S. Compared with Tajima's *D*, Fu's F_S has more power to detect population growth and genetic hitchhiking (Fu, 1997), indicating departures from neutral expectations of the utilized marker, while the opposite is true for background selection. The mismatch distribution analysis and Rogers' test of sudden population expansion indicated population expansion (Rogers, 1995; Table 1). The estimated time of initiation of expansion (*T*) for all species was in the range of 46,500–33,000 years ago, close to the last glacial maximum.

Genetic population structure and gene flow

Overall, all three giant clam species showed a strong genetic structure and restricted gene flow. *Tridacna maxima* and *T. squamosa* populations exhibited the largest genetic differentiation, with overall Φ_{st} values of 0.81 and 0.68 ($P < 0.001$), respectively. If only the region of codistribution (the IMA) was considered, all three species revealed a high differentiation, with Φ_{st}-values of 0.46 in *T. crocea*, 0.77 in *T. maxima* and 0.40 in *T. squamosa* ($P < 0.001$). The star-like haplotype networks can be partitioned into three (*T. crocea*), four (*T. squamosa*) and seven (*T. maxima*) clades (Fig. 1A3, B3, C3).

In *T. crocea* (Fig. 1A1–A3) three clades were separated by 14 and 18 mutations. Clade 1 was distributed throughout the IMA and in the Gulf of Thailand, while clade 2 was the dominant clade in populations of the EIO (Padang, Phuket, Trang Islands and Satun Islands). Clade 3 was only observed in the WP (Biak).

In *T. maxima*, 7 clades were defined, with up to 27 mutations difference and a minimum differentiation of 7 mutations. These clades showed a phylogeographic pattern in populations of the WIO, RS, WP and CP (Fig. 1B1–B3). Clade 1 was restricted to the central and eastern IMA, clade 2 occurred in the western IMA and EIO, while the other clades were found only in the peripheral areas. Clade 3 was present in the WP, clade 4 in the RS, clades 5 and 6 in the WIO and clade 7 in the CP.

In *T. squamosa*, four clades were defined based on 10–23 mutations. Clade 1 was present throughout the IMA, while the others were restricted to the peripheral areas. Clade 2 was found in the WP, clade 3 in the RS and clade 4 in the WIO (Fig. 1C1–C3).

The observed genetic structures based on the distribution of clades were further verified by a hierarchical AMOVA and pairwise Φ_{st} values. In *T. crocea*, the populations from the EIO were the most divergent populations with pairwise Φ_{st} values from 0.61 to 0.89, followed by the WP, with values ranging from 0.23 to 0.66 (Table 2), while the Φ_{st} values were low between most of the populations in the IMA. Pairwise Φ_{st} values for populations of *T. maxima* and *T. squamosa* were high for the populations in the WIO ($\Phi_{st} = 0.47–0.96$), RS ($\Phi_{st} = 0.71–0.98$), WP ($\Phi_{st} = 0.64–0.93$) and CP ($\Phi_{st} = 0.79–0.97$) (Tables 3, 4). In *T.*

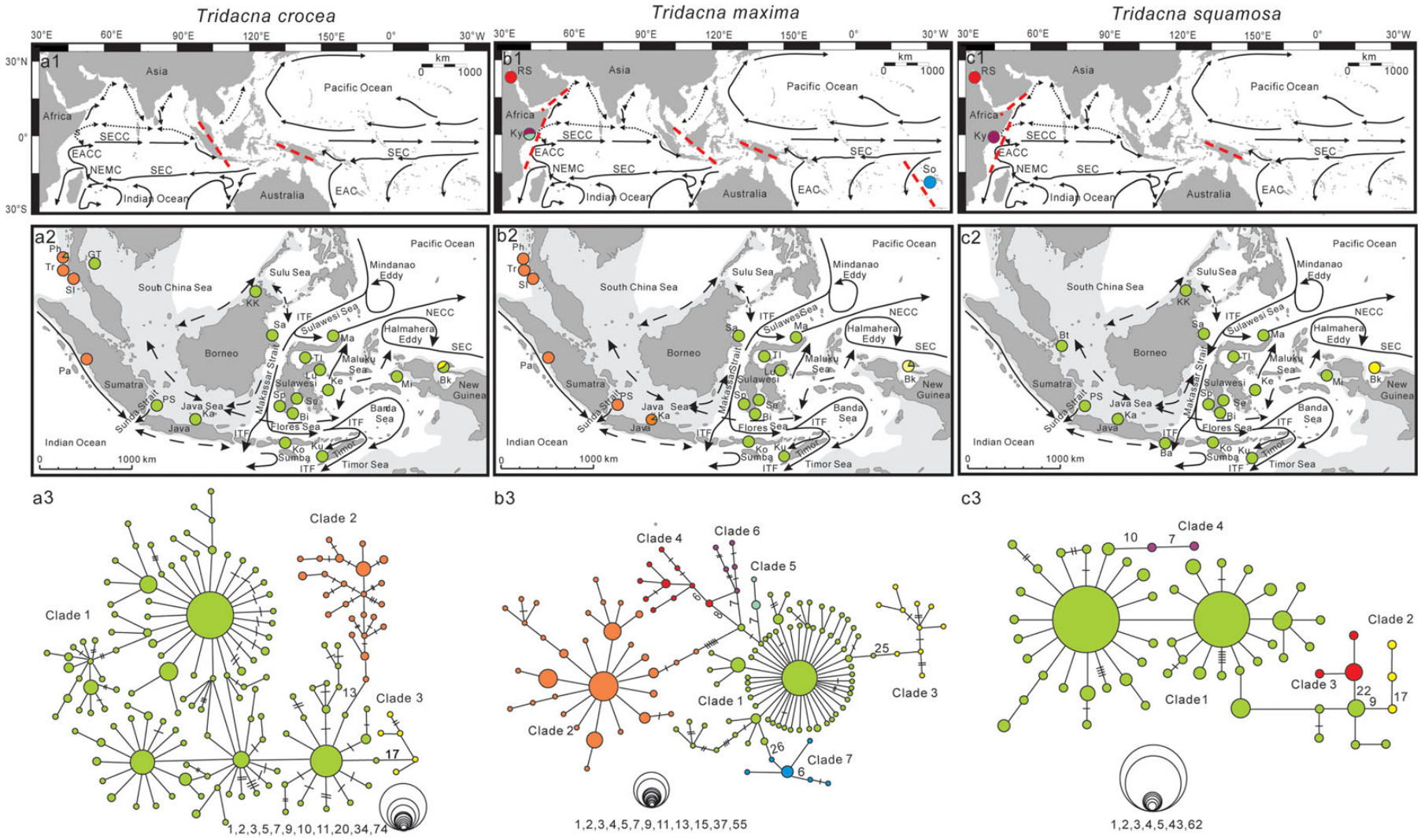

Figure 1. Maps of the Indo-West Pacific (**A1, B1, C1**) and the Indo-Malay Archipelago (**A2, B2, C2**) with sampling sites (see Table 1 for abbreviations). **A1–3.** *Tridacna crocea*. **B1–3.** *T. maxima*. **C1–3.** *T. squamosa*. **A1, B1, C1.** Major genetic breaks in Indo-West Pacific (red dashed lines) for the three giant clam species. Surface currents with constant (solid arrows) and seasonally changing flows (dashed arrows) (Wyrtki, 1961; Gordon & Fine, 1996; Carpenter, 1998; Schott & McCreary, 2001; Gordon, 2005) are shown: South Equatorial Current (SEC), Northeast Madagascar Current (NEMC), East African Coast Current (EACC), South Equatorial Counter Current (SECC), Indonesian Throughflow (ITF), North Equatorial Counter Current (NECC) and East Australian Current (EAC). **A2, B2, C2.** Land emergent during Pleistocene low sea-level stand (120 m below datum; Voris, 2000) is shown in pale grey. **B1, C1, A2, B2, C2.** Pie charts on maps represent proportions of clades (as defined in haplotype networks, **C1–C3**) at different sites for each. **C1–C3.** Networks of COI haplotypes of *T. crocea*, *T. maxima* and *T. squamosa*, respectively. Sizes of circles are proportional to haplotype frequencies. Lines between circles represent one mutational step. The hatches and numbers indicate additional mutational steps.

Table 2. Pairwise Φ_{st} values between populations of *Tridacna crocea* in the Indo-West Pacific.

	Ph	TR	SI	Pa	GT	PS	Ka	Ko	Ku	Sp	Bi	Se	Ke	Lu	TI	Ma	Sa	KK	Mi
TR	0.18																		
SI	0.06	0.09																	
Pa	0.07	0.07	−0.00																
GT	**0.86**	**0.88**	**0.87**	**0.86**															
PS	**0.86**	**0.87**	**0.87**	**0.86**	0.09														
Ka	**0.85**	**0.87**	**0.86**	**0.86**	0.07	−0.05													
Ko	**0.84**	**0.85**	**0.84**	**0.84**	**0.28**	**0.23**	**0.20**												
Ku	**0.86**	**0.88**	**0.86**	**0.86**	**0.40**	**0.30**	**0.24**	−0.04											
Sp	**0.83**	**0.84**	**0.84**	**0.83**	0.11	0.08	**0.07**	0.07	0.08										
Bi	**0.86**	**0.87**	**0.86**	**0.85**	0.22	0.20	0.16	0.03	0.06	−0.01									
Se	**0.82**	**0.83**	**0.83**	**0.81**	0.29	0.21	**0.21**	0.06	0.07	0.09	0.12								
Ke	**0.86**	**0.87**	**0.87**	**0.86**	**0.34**	**0.24**	**0.21**	−0.01	−0.02	0.08	0.08	0.03							
Lu	**0.87**	**0.88**	**0.87**	**0.87**	**0.36**	**0.27**	**0.24**	−0.02	−0.02	0.07	0.06	0.04	−0.02						
TI	**0.87**	**0.88**	**0.89**	**0.87**	**0.37**	**0.27**	**0.24**	−0.00	−0.01	**0.09**	0.09	0.04	−0.01	−0.01					
Ma	**0.84**	**0.86**	**0.85**	**0.84**	0.14	0.14	0.11	0.09	0.16	−0.03	−0.01	0.14	0.16	0.16	0.17				
Sa	**0.82**	**0.84**	**0.83**	**0.82**	0.11	0.09	0.08	0.06	0.10	−0.02	−0.00	0.06	0.08	0.08	0.10	−0.01			
KK	**0.82**	**0.83**	**0.83**	**0.82**	0.11	0.06	0.05	0.05	0.07	−0.02	0.01	0.05	0.06	0.06	0.08	−0.00	−0.02		
Mi	**0.82**	**0.84**	**0.83**	**0.82**	0.12	0.03	0.08	0.10	0.11	0.07	0.11	0.11	0.11	0.12	0.15	0.11	0.06	0.02	
Bk	**0.61**	**0.66**	**0.65**	**0.60**	0.25	0.30	0.31	**0.37**	0.29	**0.39**	**0.28**	**0.37**	**0.41**	**0.40**	**0.47**	0.23	0.31	**0.32**	0.28

For abbreviations of sites see Table 1. With sequential Bonferroni correction: $P < 0.0005$ indicated in bold.

Table 3. Pairwise Φ_{st} values between populations of *Tridacna maxima* in the Indo-West Pacific.

	Ky	RS	Ph	Tr	SI	Pa	PS	Ka	Ko	Ku	Sp	Bi	Se	Lu	TI	Ma	Sa	Mi	Bk
RS	**0.57**																		
Ph	**0.77**	**0.90**																	
TR	**0.72**	**0.89**	−0.01																
SI	**0.70**	**0.87**	−0.00	−0.03															
Pa	**0.68**	**0.88**	0.10	0.89	0.04														
PS	**0.70**	**0.91**	0.04	0.06	0.02	0.08													
Ka	**0.68**	**0.87**	0.07	0.06	0.05	0.03	−0.02												
Ko	**0.59**	**0.88**	**0.90**	**0.91**	**0.87**	**0.89**	**0.96**	**0.87**											
Ku	**0.51**	**0.79**	**0.86**	**0.84**	**0.82**	**0.81**	**0.86**	**0.80**	0.02										
Sp	**0.57**	**0.83**	**0.86**	**0.85**	**0.82**	**0.82**	**0.87**	**0.81**	−0.02	0.04									
Bi	**0.49**	**0.81**	**0.86**	**0.86**	**0.82**	**0.82**	**0.88**	**0.81**	−0.00	−0.05	0.02								
Se	**0.56**	**0.85**	**0.88**	**0.89**	**0.85**	**0.86**	**0.92**	**0.84**	−0.01	0.02	−0.00	0.00							
Lu	**0.60**	**0.86**	**0.89**	**0.89**	**0.85**	**0.87**	**0.92**	**0.85**	−0.04	0.04	0.02	0.02	0.02						
TI	**0.57**	**0.82**	**0.86**	**0.85**	**0.83**	**0.83**	**0.86**	**0.82**	−0.03	0.02	0.03	−0.01	0.00	−0.02					
Ma	**0.62**	**0.86**	**0.88**	**0.88**	**0.85**	**0.86**	**0.90**	**0.85**	−0.04	0.05	0.03	0.01	0.02	−0.02	−0.01				
Sa	**0.48**	**0.82**	**0.88**	**0.88**	**0.83**	**0.84**	**0.91**	**0.83**	0.02	−0.08	−0.00	−0.08	0.00	0.03	−0.02	0.018			
Mi	**0.47**	**0.81**	**0.87**	**0.86**	**0.82**	**0.83**	**0.89**	**0.81**	0.03	−0.07	0.02	−0.09	0.02	0.04	0.01	0.05	−0.09		
Bk	**0.66**	**0.71**	**0.86**	**0.82**	**0.83**	**0.80**	**0.79**	**0.81**	**0.74**	**0.73**	**0.77**	**0.70**	**0.74**	**0.76**	**0.76**	**0.78**	**0.68**	**0.69**	
So	**0.90**	**0.95**	**0.96**	**0.96**	**0.95**	**0.96**	**0.97**	**0.96**	**0.96**	**0.94**	**0.95**	**0.94**	**0.95**	**0.95**	**0.94**	**0.95**	**0.94**	**0.94**	**0.79**

For abbreviations of sites see Table 1. With sequential Bonferroni correction: $P < 0.0008$ indicated in bold.

maxima, populations from the EIO and Java Sea were also highly divergent ($\Phi_{st} = 0.79–0.91$).

Based on geography and oceanography, a hierarchical AMOVA was carried out with different groupings (Table 5). In all species, AMOVA revealed the highest fixation index ($\Phi_{ct} = 0.728$, $\Phi_{ct} = 0.864$, $\Phi_{ct} = 0.935$, $P < 0.001$ for *T. crocea*, *T. maxima* and *T. squamosa*, respectively) when the populations were grouped as follows: (1) WIO (*T. maxima* and *T. squamosa*), (2) RS (*T. maxima* and *T. squamosa*), (3) EIO (including Java Sea in *T. maxima*), (4) central IMA, (5) WP and (6) CP (*T. maxima*).

IBD was verified by a significant positive correlation between genetic and geographic distances for all populations of all species (*T. crocea*: $r = 0.70$, $P < 0.001$; *T. maxima*: $r = 0.52$, $P < 0.001$; *T. squamosa*: $r = 0.75$, $P < 0.001$). However, the correlation was reduced when highly divergent populations (Kenya, RS, Padang, Phuket, Trang Islands, Satun Islands, Biak and Society Islands) were excluded from the analysis and only populations from the central IMA were included (*T. crocea*: $r = 0.36$, $P = 0.009$; *T. maxima*: $r = 0.26$, $P = 0.05$ and *T. squamosa*: $r = 0.35$, $P = 0.042$).

DISCUSSION

The three congeneric species of giant clams share the same habitat and life history, living on tropical coral reefs of shallow

Table 4. Pairwise Φ_{st} values between populations of *Tridacna squamosa* in the Indo-West Pacific.

	Ky	RS	Bt	PS	Ka	Ba	Ko	Ku	Sp	Bi	Se	Ke	Tl	Ma	Sa	KK	Mi
RS	**0.96**																
Bt	**0.64**	**0.96**															
PS	**0.82**	**0.98**	0.56														
Ka	**0.87**	**0.95**	0.54	−0.02													
Ba	**0.84**	**0.96**	0.51	0.00	−0.02												
Ko	**0.85**	**0.95**	0.46	0.00	0.01	−0.02											
Ku	**0.88**	**0.97**	**0.65**	0.52	0.16	0.24	0.20										
Sp	**0.89**	**0.96**	0.56	0.02	0.01	−0.00	−0.01	0.20									
Bi	**0.87**	**0.96**	0.55	−0.16	−0.03	−0.04	−0.00	0.16	−0.01								
Se	**0.83**	**0.96**	0.51	0.09	0.14	0.11	0.11	0.23	0.13	0.11							
Ke	**0.93**	**0.98**	**0.76**	0.06	0.01	0.04	0.03	0.49	0.01	−0.01	0.29						
Tl	**0.82**	**0.96**	0.49	−0.06	0.04	0.03	0.06	0.29	0.10	0.03	0.20	0.08					
Ma	**0.90**	**0.97**	**0.67**	0.28	0.03	0.04	0.02	0.22	−0.01	−0.01	0.09	0.16	0.17				
Sa	**0.77**	**0.93**	0.14	−0.06	0.06	0.04	−0.00	0.23	0.08	0.06	0.15	0.10	0.04	0.13			
KK	**0.83**	**0.95**	0.49	0.20	0.09	0.07	0.07	0.00	0.10	0.07	0.10	0.24	0.17	0.03	0.15		
Mi	**0.92**	**0.98**	**0.74**	0.14	−0.03	−0.01	−0.02	0.41	−0.03	−0.04	0.23	−0.05	0.09	0.08	0.08	0.15	
Bk	**0.73**	**0.91**	**0.64**	**0.79**	**0.90**	**0.84**	**0.87**	**0.86**	**0.93**	**0.89**	**0.84**	**0.92**	**0.84**	**0.89**	**0.82**	**0.86**	**0.91**

For abbreviations of sites see Table 1. With sequential Bonferroni correction: $P < 0.0005$ indicated in bold.

Table 5. Hierarchical analysis of molecular variance (AMOVA) of COI sequences in *Tridacna crocea*, *T. maxima* and *T. squamosa* from Indo-West Pacific.

Groupings	Φ_{ct}	Percentage of variation (%)
T. crocea		
(Ph, TR, Sl, Pa) (GT, PS, Ka, Ko, Ku, Sp, Bi, Se, Ke, Lu, Tl, Ma, Sa, KK, Mi) (Bk)	0.728***	72.80
(Ph, TR, Sl, Pa) (PS, Ka) (GT, Ko, Ku, Sp, Bi, Se, Ke, Lu, Tl, Ma, Sa, KK, Mi) (Bk)	0.645***	64.52
(Ph, TR, Sl, Pa) (GT, PS, Ka,) (Ko, Ku, Sp, Bi, Se, Ke, Lu, Tl, Ma, Sa, KK, Mi) (Bk)	0.634***	63.49
T. maxima		
(Ky) (RS) (Ph, TR, Sl, Pa, PS, Ka) (Ko, Ku, Sp, Bi, Se, Lu, Tl, Ma, Sa, Mi) (Bk)(So)	0.864***	86.38
(Ky, RS) (Ph, TR, Sl, Pa, PS, Ka) (Ko, Ku, Sp, Bi, Se, Lu, Tl, Ma, Sa, Mi) (Bk) (So)	0.850***	85.06
(Ky)(RS) (Ph, TR, Sl, Pa, PS, Ka) (Ko, Ku, Sp, Bi, Se, Lu, Tl, Ma, Sa, Mi) (Bk, So)	0.729***	72.94
(Ph, TR, Sl, Pa, PS, Ka) (Ko, Ku, Sp, Bi, Se, Lu, Tl, Ma, Sa, Mi)(Bk)	0.816***	81.65
(Ph, TR, Sl, Pa) (PS, Ka) (Ko, Ku, Sp, Bi, Se, Lu, Tl, Ma, Sa, Mi) (Bk)	0.797***	79.74
T. squamosa		
(Ky) (RS) (Bt, PS, Ka, Ba, Ko, Ku, Sp, Bi, Se, Ke, Tl, Ma, Sa, KK, Mi) (Bk)	0.935***	93.54
(Ky, RS) (Bt, PS, Ka, Ba, Ko, Ku, Sp, Bi, Se, Ke, Tl, Ma, Sa, KK, Mi) (Bk)	0.879***	87.89
(Bt, PS, Ka, Ba, Ko, Ku, Sp, Bi, Se, Ke, Tl, Ma, Sa, KK, Mi) (Bk)	0.896***	89.64
(Bt) (PS, Ka, Ba, Ko, Ku, Sp, Bi, Se, Ke, Tl, Ma, Sa, KK, Mi) (Bk)	0.853***	85.28

For abbreviations of sites see Table 1.

***$P < 0.001$.

seas and having planktonic larvae and sessile adults. Therefore, concordant patterns of population genetic structure could be expected and this study has indeed revealed concordant patterns of *Tridacna crocea*, *T. maxima* and *T. squamosa* across their distributional range in the IWP. The populations of the three species could be divided into the following groups from West to East: (1) WIO (*T. maxima* and *T. squamosa*), (2) RS (*T. maxima* and *T. squamosa*), (3) EIO (including Java Sea in *T. maxima*), (4) central IMA, (5) WP and (6) CP (*T. maxima*). The exact locations of the different genetic breaks vary slightly among species (Fig. 1A1, B1, C1). The utilization of the maternally inherited COI marker gene might be a limitation of this study, but other studies on giant clams (DeBoer *et al.*, 2014b) and anemonefish (Timm *et al.*, 2012; Dohna *et al.*, 2015) in the IMA have shown concordant patterns between mtDNA sequences and nuclear microsatellites. Therefore, COI is considered a suitable genetic marker for studying connectivity and evolution.

Genetic divergence between the WIO and EIO

This is the first study to investigate the genetic population structure of giant clams in the WIO in comparison with other regions in the IWP. It shows that the populations of *T. maxima* and *T. squamosa* in Kenya represent divergent lineages, indicating isolation. The coral reefs of the WIO are distinct from other reefs in the IWP, with predominantly fringing reefs along the east African coast, which are separated by deep ocean from reefs in the EIO. This might have supported the evolution of a distinct coral reef fauna with endemic species in the WIO (Spalding *et al.*, 2001). Even though surface currents cross the Indian Ocean, they obviously do not connect giant clam populations of the WIO and EIO (Fig. 1B1, C1). This might be due to the long distance and limited larval dispersal capabilities of giant clams. Studies carried out on the whole geographical range of the tiger prawn (*Penaeus monodon*; Benzie *et al.*, 2002),

bullethead parrotfish (*Chlorurus sordidus*; Bay *et al.*, 2004) and skunk clownfish (*Amphiprion akallopisos*; Huyghe & Kochzius, 2016) also show a genetic separation of populations from the WIO and EIO.

Genetic endemism in the RS

The populations of *T. maxima* and *T. squamosa* from the RS were highly divergent. These populations have specific haplotypes, with nine and 23 mutational steps from the nearest clades in *T. maxima* and *T. squamosa*, respectively (Fig. 1B, C). In comparison with other populations, both showed high Φ_{st} values. In the hierarchical AMOVA, the highest Φ_{ct} values were reached in both species when the populations from the RS were regarded as a separate group (Table 5). Such a pattern was also found in a previous study on *T. maxima* (Nuryanto & Kochzius, 2009). This divergence might be caused by limited exchange with the Indian Ocean through the Straits of Bab-el-Mandeb. This reduces connectivity of populations in the RS with their counterparts in the Indian Ocean, leading to a separate evolutionary path (DiBattista *et al.*, 2015). A genetic differentiation of the RS populations was also found in the mud crab *Scylla serrata* (Fratini & Vannini, 2002), the damselfish *Chromis viridis* (Froukh & Kochzius, 2008) and the sponge *Leucetta chagosensis* (Wörheide, Epp & Macis, 2008). However, in the lionfish *Pterois miles* (Kochzius *et al.*, 2003; Kochzius & Blohm, 2005), such genetic differentiation could not be detected. These deep evolutionary lineages of giant clams in the RS might be cryptic species, which supports the perspective that the RS is an important secondary centre of evolution (Klausewitz, 1989). This is supported by the recent discovery of a new species of giant clam (*T. costata*) in the RS using integrated taxonomy (Richter *et al.*, 2008). Such an integrated taxonomy approach, combining morphology, ecology and genetics, would be needed to verify if the divergent mitochondrial linages of *T. maxima* and *T. squamosa* are cryptic species. The specimens of *T. maxima* and *T. squamosa* investigated in this study are identical with the ones used by Richter *et al.* (2008) and analysis of 16S mtDNA sequences clearly shows that the divergent lineages of these species are not *T. costata*.

Genetic divergence between the EIO and the IMA

In *T. crocea* a genetic break was detected between the populations from the EIO and the central IMA (Fig. 1A2), while in *T. maxima* a deep divergence was shown for specimens from the Java Sea and EIO in comparison with the central IMA (Fig. 1B2). This or similar patterns have also been detected in previous studies of giant clams (DeBoer *et al.*, 2008, 2014a, b; Kochzius & Nuryanto, 2008; Nuryanto & Kochzius, 2009). Genetic breaks in the same region were also shown for populations of the crown-of-thorns starfish *Acanthaster planci* (Benzie, 1999), the anemonefish *A. ocellaris* (Nelson *et al.*, 2000; Timm & Kochzius, 2008; Timm *et al.*, 2008, 2012) and *A. perideraion* (Dohna *et al.*, 2015), as well as the seahorse *Hippocampus spinosissimus* (Lourie, Green & Vincent, 2005). It was hypothesized that this differentiation was caused by sea-level lowstands of up to 130 m during glacials, which created isolated ocean basins (McManus, 1985; Voris, 2000). Similar patterns were detected in two Indo-Pacific gastropods (*Nerita albicilla* and *N. plicata*) (Crandall *et al.*, 2008). In *T. maxima*, gene flow could be found between populations in the Java Sea and EIO (Padang) through the Sunda Strait, while in *T. crocea* connectivity was restricted. This might be explained by subtle differences between the species, including their size, living depth and planktonic life stages (Jameson, 1976). There is also a genetic break among the Thai populations of *T. crocea* from the Andaman Sea (Ph, Tr, SI) and Gulf of Thailand, which are separated by the Malay Peninsula and obviously have limited connectivity via the Malacca Strait. Such a separation of populations has also been observed in the giant clam *T. squamosa* (Kittiwattanawong *et al.*, 2001).

Genetic divergence between the IMA and the WP

Populations of the three species showed high level of gene flow along the Indonesian Throughflow (ITF) in the Sulawesi Sea, Makassar Strait, Flores Sea, Banda Sea and Timor Sea. Seasonally changing currents in the seas around Borneo also connect populations in this area to the populations along the ITF (Fig. 1A2–C2). Most pairwise Φ_{st} values were not significant, further emphasizing the high connectivity among these populations. In contrast, all species showed genetic separation of the IMA and the WP. These patterns have also been observed in other studies on species of *Tridacna* (DeBoer *et al.*, 2008, 2014a, b; Kochzius & Nuryanto, 2008; Nuryanto & Kochzius, 2009; Huelsken *et al.*, 2013). A similar genetic structure was detected in the anemonefish *A. perideraion* (Dohna *et al.*, 2015), the blue starfish *Linckia laevigata* (Kochzius *et al.*, 2009) three species of mantis shrimps (*Haptosquilla pulchella*, *H. glyptocerus* and *Gonodactylellus viridis*, Barber, Erdmann & Palumbi, 2006), as well as in *Nautilus* (Wray *et al.*, 1995). The results of these studies support that there is an important biogeographic barrier between the IMA and the WP, which is supposed to be located at the edge of the Sahul continental shelf (of the Australian-New Guinea continent), which was exposed as dry land when sea level fell during the Pleistocene ice ages (McManus, 1985; Voris, 2000). Another reason for the restricted genetic exchange between the IMA and WP might be the Halmahera eddy, which transforms the westward South Equatorial Current (SEC) into the eastward North Equatorial Countercurrent, and therefore limits water transport from New Guinea to the central IMA (Fig. 1; Wyrtki, 1961; Gordon & Fine, 1996). The populations of the giant clams in the WP were so divergent that they might be cryptic species. Recent taxonomic research has shown that *T. noae* is not a synonym of *T. maxima*, but a valid species (Su *et al.*, 2014), which occurs in the eastern IMA (Borsa *et al.*, 2015). However, a BLAST search on GenBank indicated that the divergent populations of *T. maxima* in this study are not *T. noae*.

Genetic divergence between the WP and CP

So far, fewer genetic population studies have been performed for giant clams in the CP and of their connectivity with other areas. In this study, the Society Islands population (CP) of *T. maxima* harbours haplotypes of a unique clade, with a distance of more than 27 mutational steps from the next closest clade, which suggests little or no genetic connectivity with other sites. A similar divergence in *T. maxima* from the CP was observed in specimens from Kiribati and Palmyra (Gardner *et al.*, 2012).

Even though there are no physical barriers between the CP and WP, limited dispersal capability, lack of stepping stones and IBD could be the explanation for this divergence. Many studies have shown that even if there are no apparent barriers to dispersal, some reef fishes with a high dispersal capacity show high genetic divergence among distant populations (Fauvelot & Planes, 2002; Taylor & Hellberg, 2003). Moreover, in the Pacific Ocean, the westward SEC has a southward component (Fig. 1), which might be another reason for the restricted gene flow between the CP and WP (Carpenter, 1998).

Implications for conservation

Giant clams are harvested commercially for food, shells and the aquarium trade, and stocks are severely over-exploited (Lucas, 1994; Wells, 1997), which calls for urgent conservation management. In this study, the genetically distinct groups within each species might be defined as separate evolutionary significant

units (ESUs). It is suggested that ESUs are important units for conservation (Moritz, 1994; Vogler & DeSalle, 1994). They are defined as "populations that are reciprocally monophyletic for mtDNA" (Moritz, 1994). However, the definition has been argued to be too restrictive and unique haplotypes, low gene flow or concordance between phylogenetic divergence and geographic barriers provide new criteria for defining ESUs (Crandall *et al.*, 2000). Therefore, the restricted gene flow between groups in each species of giant clams, as well as concordance between genetic and geographic barriers, might indicate six ESUs as follows: WIO, RS, EIO, central IMA, WP and CP. Management should put more effort into maintaining the diversity within these ESUs and preservating the genetic connectivity among populations (Crandall *et al.*, 2000).

In Indonesia, large amounts of coral reef species are traded, which makes Indonesia the most important exporter in the marine ornamental trade. In this study, populations of all the three *Tridacna* species in the Java Sea showed a low genetic diversity, which was also observed in other studies on giant clams (DeBoer *et al.*, 2008; Kochzius & Nuryanto, 2008; Nuryanto & Kochzius, 2009). This might be explained by overexploitation. Java is the home of 60% of the Indonesian population, and overexploitation could be caused by fishery and the marine ornamental trade (Wells, 1997; Nuryanto & Kochzius, 2009). However, low genetic diversity could also be due to natural causes, such as a genetic bottleneck after recolonization following the last glacial or a bleaching event in the Java Sea (Wilkinson, 2002; Leggat *et al.*, 2003). Low genetic diversity was also identified in East Africa and the RS, which might result from severe bleaching events (Wilkinson *et al.*, 1999), reef-top gathering (Ashworth, Ormond & Sturrock, 2004), pollution and over-exploitation (Obura *et al.*, 2004). Special attention should be paid to these regions.

In the IMA all populations along the ITF are very well connected over large distances, most probably due to the strong current. The three species have a PLD of 9–12 d and they should be able to travel about 400–700 km, given the speed of the ITF (Susanto & Gordon, 2005). However, on a large scale, connectivity is limited, which suggests a low potential for larval dispersal (Kyle & Boulding, 2000; Fievet *et al.*, 2007). This is also reflected in the IBD analysis. When considering all sample sites there is a strong and significant signal of IBD, but while considering only the sample sites in the central IMA the correlation is less strong, but still significant. Therefore, IBD is certainly an important factor shaping the genetic structure in all giant clam species studied.

Management efforts should consider smaller and local scales to maintain and enable population connectivity within the separated regions (Palumbi, 2003). For example, 51 marine protected areas (MPAs) in Indonesia with an area of 58,000 km^2 cover only about 1% of the country's marine area and in Kenya 11 MPAs of 1,585 km^2 only account for 1.3% (Spalding *et al.*, 2001). They are most probably not sufficient for the protection of giant clams. In the RS, only a MPA network along the coastlines of Egypt, Israel and Jordan in the Gulf of Aqaba (Kochzius, 2002) and along the Egyptian coast of the northern RS matches the dispersal capability of a reef fish (Froukh & Kochzius, 2007).

The distinct ESUs, connectivity patterns among populations and genetic diversity data revealed in this study could serve as helpful information for the design of MPA networks, with the ultimate goal of adequate protection of endangered giant clams.

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