

Impacts of Early Pleistocene glacial vicariance among refugial lineages and Mid-Late Pleistocene interglacial dispersal and expansion on forging population genetic structure of the giant clam *Tridacna squamosa* (Bivalvia: Cardiidae: Tridacninae) across the Red Sea and Indo-West Pacific oceans

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Abstract

This study aims at identifying the microevolutionary processes responsible for the onset of the remarkable phylogeographic structure already recorded for the endangered giant clam *Tridacna squamosa* across its distribution range. For this purpose, the evolutionary, biogeographic and demographic histories of the species were comprehensively reconstructed in a mitochondrial dataset comprising nearly the whole available published cytochrome c oxidase 1 gene sequences of *T. squamosa*. Relatively higher level of genetic diversification was unveiled within *T. squamosa*, in comparison to earlier macro-geographic investigations, whereby five mitochondrial clusters were delineated. The resulting divergent gene pools in the Red Sea, western Indian Ocean, Indo-Malay Archipelago and western Pacific were found to be driven by Early Pleistocene glacial vicariance events among refugial lineages. Accentuated genetic diversification of the species across the Indo-Malay Archipelago was successively triggered by historical dispersal event during the Mid-Pleistocene MIS19c interglacial. This latter historical event might have also enabled genetically distinct giant clams from the Indo-Malay Archipelago to subsequently colonize the western Pacific, accounting for the genetic diversity hotspot detected within this region (comprising three divergent mitochondrial clusters). Late Pleistocene demographic expansion of *T. squamosa*, during the Last Interglacial period, could have contributed to forging spatial distribution of the so far delineated genetic entities across the Indo-Western Pacific. Overall, being resilient to major climate shifts during the Pleistocene through adaptation and consequent diversification, *T. squamosa* could be used as a model species to track the impact of climate change on genetic variability and structure of marine species. In particular, the new information, provided in this investigation, may help with understanding and/or predicting the consequences of ongoing global warming on genetic polymorphism of endangered coral reef species among which *Tridacna* sp. are listed as ecologically important.

Key words: Mollusks, Red Sea and Indo-Pacific, evolutionary and biogeographic histories, mitochondrial DNA, Pleistocene glacial refugia, interglacial dispersal and expansion

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1 Introduction

Understanding the relationships between patterns of spatial distribution of genetic diversity and the potential evolutionary processes, involved in shaping these patterns, is a central and longstanding goal for evolutionary biology. Recently, numerous phylogeographical investigations have started to clarify such kind of links by highlighting the significant impact of Pleistocene climatic oscillations on sculpting contemporary genetic diversification patterns within marine and terrestrial biota (Deli et al., 2016, 2018, 2019; Machado et al., 2022; Ye et al., 2021).

During the Pleistocene, extensive climatic fluctuations had a significant impact on the species distribution (Zhao et al., 2019; Bernardi et al., 2020). Repeated range contractions were followed by range expansions from climatically more favourable regions (the so-called glacial refugia). These historical cyclic events have markedly influenced population dynamics and genetic di-

versity (Albaina et al., 2012; Evangelisti et al., 2017; Jeon et al., 2021), and consequently left a signature within the genome of many species (Deli et al., 2019; Fernández Iriarte et al., 2020; Jeon et al., 2021). Although Pleistocene glaciations have been shown to exert significant impact on population genetic structure by impeding gene flow among refugial lineages and promoting allopatric differentiation (Deli et al., 2018; Fernández Iriarte et al., 2020), it is still not clear how these effects translate into lineage divergence and therefore into population genetic structuring. Knowles and Richards (2005) postulated that the potential genetic diversification of species during the Pleistocene might have been driven by either divergent selection or genetic drift associated with displacements into glacial refugia and/or recolonization of previously glaciated areas.

The Indo-Pacific is listed among the world's major biodiversity hotspots and has long been considered as a region of

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great biogeographic interest across a wide taxonomic range of species (Farhadi et al., 2017; Guo et al., 2021). The Pleistocene glaciations have left pronounced evolutionary footprints in a wide range of Indo-Pacific marine fauna and flora (Crandall et al., 2008a, 2008b; DiBattista et al., 2013; Ludt and Rocha, 2015; Farhadi et al., 2017; Hu et al., 2018). Notably, significant palaeogeographic shifts markedly occurred during cyclic glacial maxima following significant reduction of coastal marine habitats (i.e., between the Red Sea and the Indian Ocean at Bab El Mandeb Strait). These historical glaciations events also led to the emergence of land barriers such as the Indo-Pacific Barrier separating the Indian and Pacific oceans (Fleminger, 1986; Voris, 2000). Such physical barriers have been postulated to stem from the dry exposure of the Sunda shelf, surrounding the Malay Peninsula and western islands of Indonesia, and the Sahul shelf off northern Australia and New Guinea (Voris, 2000). These exposed shelves might have acted as vicariant barriers potentially causing genetic divergence between Indian and Pacific oceans populations of many taxa. For instance, these physiographic alterations, potentially associated with habitat loss and population isolation, might have led to dramatic population bottlenecks (Ludt and Rocha, 2015). Consequently, they could have played a crucial role in promoting genetic differentiation among allopatric populations resulting in high genetic structuring and marked phylogeographic breaks (Timm and Kochzius, 2008; Timm et al., 2008, 2012; Wörheide et al., 2008; Knittweis et al., 2009; Gaither et al., 2011; Dohna et al., 2015; Farhadi et al., 2017; Yamamoto et al., 2019). Furthermore, adaptation to disparate environmental features might have generated selective pressures, therefore, accelerating divergence between isolated populations in allopatry (Froukh and Kochzius, 2008; Wörheide et al., 2008; DiBattista et al., 2016; Farhadi et al., 2017). The cyclic population expansions and contractions, primed by the Quaternary climatic oscillations, have been postulated to play crucial role in shaping the contemporary spatial distribution of genetic variation across different geographic areas encompassing the Indian (including the Red Sea) and Pacific oceans (Crandall et al., 2008a, 2008b; Gaither et al., 2011; DiBattista et al., 2013; Ludt and Rocha, 2015; Farhadi et al., 2017; Yamamoto et al., 2019). Nevertheless, it remains unclear and poorly understood how these evolutionary events have shaped genetic diversification of marine organisms in these regions.

The giant clam *T. squamosa* represents a good model to explore this topic. Its historical origin can be traced back to the Late Pliocene-Early Pleistocene (Schneider and Foighil, 1999; Harzhauser et al., 2008). Besides, this bivalve species has a wide geographic distribution, stretching from the Red Sea and Indian Ocean across the Indo-Malay Archipelago to the western Pacific (Gilbert et al., 2007; Andréfouët et al., 2014). Moreover, adults of *T. squamosa* are sedentary and have a short pelagic larval dispersal of about twelve days (Lucas, 1988). On account of having all of these life history traits and historical background, *T. squamosa* is ideal for unraveling the potential impact of Pleistocene glacial and interglacial cycles on sculpting contemporary patterns and levels of population genetic diversity and structure. It is postulated that species, characterized by low dispersal capability, are expected to bear more signatures of the Pleistocene glaciations in their genomes owing to reduced gene flow and increased population differentiation (Remerie et al., 2009).

While population genetic studies of *T. squamosa* across micro-geographic spectra unveiled different levels of population genetic connectivity, ranging from genetic panmixia (i.e., in Singapore's reefs; Neo and Todd, 2012) to mid genetic differentiation

(i.e., on the fringing reefs of Perhentian Islands, Malaysia; Lee et al., 2022), the so far accumulated large-scale phylogeographic investigations of *T. squamosa* provided firm evidences of significant population genetic structuring across its geographic distribution area. The study of DeBoer et al. (2014) revealed strong phylogeographic patterning coinciding with biogeographic barriers and marine eco-regions across the Indo-West Pacific. Later on, the phylogeographic investigation of Hui et al. (2016) unveiled restricted gene flow between the Red Sea, the western Indian Ocean, the Indo-Malay Archipelago and the western Pacific. The four mitochondrial clades corresponding to these aforementioned regions were defined based on 10–23 mutations (Hui et al., 2016). The very recent study by Fauvelot et al. (2020) unveiled a deep divergence of the Red Sea clade suggesting the existence of potential cryptic species within the giant clam *T. squamosa*. All of these macro-geographic studies agreed in proposing that this noticeable level and pattern of spatial distribution of genetic diversity could be likely triggered by the impact of Pleistocene climate oscillations and later maintained by the effects of contemporary hydrographic isolation barriers and environmental gradients roaming across the region. However, no study has so far tried to elucidate the evolutionary history and microevolutionary processes responsible for the onset of this remarkable phylogeographic structure of *T. squamosa* across its distribution range. It needs to be clarified that information on the evolutionary and biogeographic histories of this giant clam species across its distribution range is still lacking in the literature. Such specific knowledge gap and persisting lack of information allow raising the following questions. First, what is the true content and scale of geographic distribution of genetic diversity? Second, what is the tempo (diversification time) and mode (microevolutionary processes, i.e., vicariance or dispersal) of divergence of the delineated genetic types? In this context, have the Pleistocene climate oscillations (during glacial and interglacial periods) played a crucial role in triggering genetic divergence and shaping spatial distribution of genetic diversity and phylogeographic patterning? Besides, do divergence and distribution of the lineages reflect survival in or dispersal from disjunct glacial refuge areas? Third, have historical demographic events also contributed to forging spatial distribution of the so far delineated genetic entities or mitochondrial lineages within *T. squamosa*?

In an attempt to provide answers to these questions, the evolutionary, biogeographic, and demographic histories of *T. squamosa* were reconstructed based on the mitochondrial cytochrome c oxidase subunit 1 (Cox1 gene). Due to its smaller effective population size, in comparison to nuclear loci markers, mtDNA has been postulated to be the appropriate molecular marker for reconstructing species evolutionary histories (Avice, 1994). Indeed, mtDNA-based phylogeographies have not ceased to clarify the impact of the Quaternary climate oscillations on the distribution dynamics and the build-up of genetic divergence within marine biota (Deli et al., 2018; Zhong et al., 2020). In particular, the assessment of Cox1 gene variation in evolutionary and demographic histories investigations has allowed unveiling the potential impacts of Pleistocene glacial and interglacial episodes on forging contemporary patterns and levels of population genetic diversity and structure of marine species (Ibáñez and Poulin, 2014; Barahona et al., 2017; Zelada-Mázmela et al., 2022). All available Cox1 sequences, derived from the so far conducted phylogeographic examinations and covering the whole distribution area of *T. squamosa*, were used in this study. In order to recover the maximum amount of information contained in the relatively small portion of analyzed mtDNA genome, several analyt-

ical approaches were implemented at different hierarchical levels including Bayesian clustering, calibrated phylogeny, ancestral area reconstruction, inference of inter-lineage divergence by means of approximate Bayesian computation, and demographic history.

2 Materials and methods

2.1 Data analysis procedure

In order to comprehensively reconstruct the evolutionary, biogeographic, and demographic histories of *T. squamosa*, a total of 256 Cox1 sequences, available so far for the giant clam species in GenBank, were retrieved and analyzed. These sequences were derived from previous phylogeographic investigations (DeBoer et al., 2008, 2014; Neo and Todd, 2012; Huelsken et al., 2013; Lizano and Santos, 2014; Su et al., 2014; Hui et al., 2016; Findra et al., 2017; Keyse et al., 2018; Fauvelot et al., 2020; Liu et al., 2020; Mamat et al., 2021; Lee et al., 2022). They were also found to be representative of the whole distribution area of *T. squamosa* (Red Sea, western Indian Ocean, Indo-Malay Archipelago, and western Pacific). Out of the 256 published sequences in GenBank, only nine sequences were excluded owing to ambiguous nucleotide composition (KF446518, KF446521, KF446522, KF446528, KF446529, KF446545, KF446554), uncertainty regarding the geographic origin (KF446591) or unreliable sequence identification (EU003615). Thus, a total of 247 Cox1 sequences were used in the present investigation. It has to be mentioned that apart from these sequences, six new Cox1 sequences, recently generated for specimens of *T. squamosa* from the Republic of Mauritius and deposited in GenBank under the accession numbers (OP143746–OP143751), were not included in the study owing to their very short lengths ranging from 192 bp to 222 bp. Detailed information on the analyzed sequences (including source investigation, geographic origin and corresponding accession numbers) is exhibited in Fig. 1 and Table 1. The downloaded Cox1 sequences with different lengths were aligned with Clustal W, included in

BIOEDIT (Hall, 1999). The final sequences alignment resulted in a cropped and finally adjusted Cox1 fragment of 319 bp, which subsequently served to conduct statistical analyses.

2.2 Statistical analyses

2.2.1 Bayesian inference of mitochondrial genetic clusters

The Bayesian clustering approach, included in the software BAPS version 6.0 (Corander et al., 2008, 2013), was used to delineate genetic clusters within the examined Cox1 dataset of *T. squamosa*. The Bayesian approach, adopted by BAPS, is characterized by a stochastic optimization algorithm for analyzing models of population structure. It greatly improves the speed of the analysis compared to traditional Markov Chain Monte Carlo (MCMC)-based algorithms (Corander and Marttinen, 2006). This method determines the most likely number of clusters (K) within a given set of sequences, by clustering the recorded haplotypes into monophyletic clusters of haplotypes (haplogroups). The only given prior information was the geographic origin of the analyzed Cox1 sequences. The “Clustering with linked loci” function was selected for the BAPS analysis. The upper bound K values (i.e., the number of clusters) were set to 10. Five replicates were run for each K value (1–10). The likely number of genetic clusters, defined in the examined mitochondrial dataset, was selected by the software according to the log marginal likelihood of optimal partition and the maximum-associated probability (P) of the number of groups in optimal partition.

2.2.2 Reconstruction of evolutionary and biogeographic histories

Reconstruction of genetic diversification, through inference of sequential divergence events from a common ancestor through time, can be accurately achieved if appropriate calibrations or substitution rates are provided and applied. In order to reliably reconstruct the evolutionary history of *T. squamosa*, a mutation rate of the Cox1 gene was specifically determined in this study. Noteworthy, the use of appropriately determined spe-

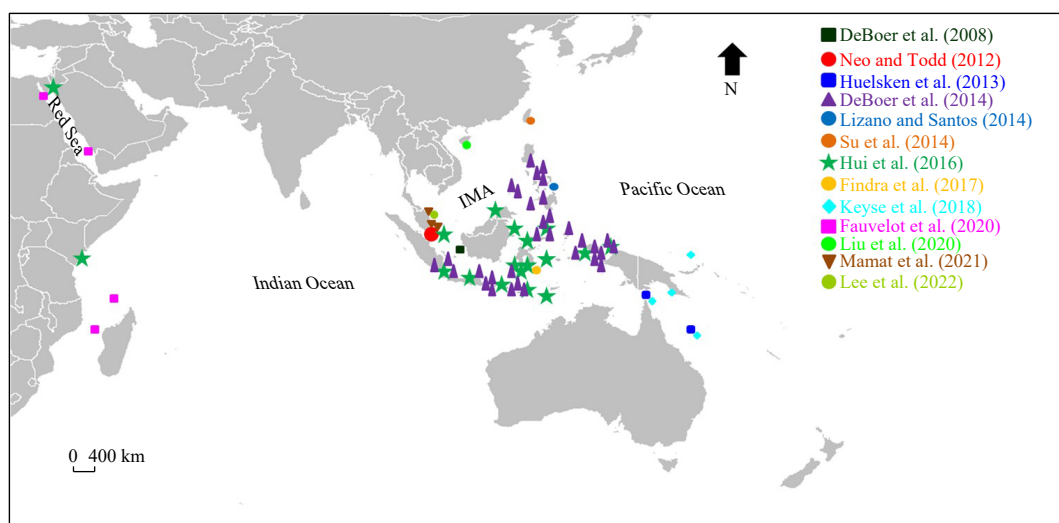


Fig. 1. Sampling locations of the examined Cox1 sequences of *Tridacna squamosa* (retrieved from previous phylogeographic investigations) along the Red Sea and Indo-West Pacific. Sampling sites are denoted with symbols specific for each population genetic study (indicated in the top right of the map). IMA: Indo-Malay Archipelago. Further details on the geographic origins and names of the giant clam sampling sites [including the exact number of sampling sites across micro-geographic spectra, i.e., in the three studies of Neo and Todd (2012), Liu et al. (2020) and Lee et al. (2022)], as well as information on the number of captured specimens at each sampling site, are included in the thirteen mentioned investigations. Construction of the base map was carried out by the software DIVA-GIS 7.5.0 (<http://www.diva-gis.org>).

Table 1. Information on Cox1 (cytochrome c oxidase subunit 1) dataset used to examine evolutionary and biogeographic histories of the giant clam *Tridacna squamosa*

Study	Examined regions	Cox1 GenBank accession number
DeBoer et al. (2008)	Indo-Malay Archipelago (Indonesia)	EU346361–EU346364
Neo and Todd (2012)	Indo-Malay Archipelago (Singapore)	JN392020–JN392039
Huelsken et al. (2013)	western Pacific (Australia: Torres Strait, Australia: Lihou Reef)	JX974924–JX974925
DeBoer et al. (2014)	Coral Triangle (Indo-Malay Archipelago, western Pacific)	KF446517, KF446519–KF446520, KF446523–KF446527, KF446530–KF446544, KF446546–KF446553, KF446555–KF446590
Lizano and Santos (2014)	Indo-Malay Archipelago (Guiuan Samar, Philippines)	KJ202117
Su et al. (2014)	western Pacific (Taiwan Island)	KC456025
Hui et al. (2016)	Red Sea, western Indian Ocean, Indo-Malay Archipelago, western Pacific	HE995488–HE995532
Findra et al. (2017)	Indo-Malay Archipelago (Indonesia: Wangi-wangi, Wakatobi, South East Sulawesi)	KY769523
Keyse et al. (2018))	western Pacific (Australia: Lihou Reef, Papua New Guinea: Motupore Island, Papua New Guinea: Kavieng, Australia: Torres Strait)	MG385481–MG385484
Fauvelot et al. (2020)	Red Sea, western Indian Ocean	MN068796–MN068807
Liu et al. (2020)	western Pacific (Hainan Island)	MF969178–MF969181
Mamat et al. (2021)	Indo-Malay Archipelago (Peninsular Malaysia: Terengganu Islands, Pahang Island and Johor Islands)	MT499028–MT499030
Lee et al. (2022)	Indo-Malay Archipelago [Malaysia: Terengganu (Perhentian Besar, Perhentian Island, Perhentian Kecil, Rhu Island, Pulau Rhu, Pulau Serenggeh)]	MT502189–MT502191 MH018138–MH018176 MG700615–MG700652 MG600589 MW599769–MW599770

cific mutation rate, alongside adequate demographic priors, for genetic divergence estimation in intraspecific data has been proven successful in accurately inferring evolutionary events (i.e., glacial vicariance or interglacial/postglacial demographic expansions; see for examples: Marino et al., 2011; Deli et al., 2018, 2019). The genetic split between the two closely giant clam species *T. squamosa* and *Tridacna crocea*, occurring approximately 2.6 million years ago (Mya), as estimated from the time-calibrated maximum clade credibility tree of *Tridacna* sp. (Fauvelot et al., 2020), was chosen as calibration point to infer Cox1 mutation rate within *Tridacna*. Cox1 haplotypes, stemming from a total dataset of 147 sequences of *T. crocea* (comprehensively covering the extent of genetic variation of the species across the distribution range (Kochzius and Nuryanto, 2008; Hui et al., 2016); GenBank accession numbers: FM253431–FM253562, HE995439–HE995453) and 247 sequences of *T. squamosa* (examined in this study, see Table 1), were used in the analysis. The software BEAST version 1.7.5 (Drummond et al., 2012) was used to estimate the Cox1 substitution rate. The best-fit nucleotide substitution model for the examined dataset was determined with the software MODELTEST version 3.7 (Posada and Crandall, 1998). The two parameter Birth-Death model (regarded as an appropriate null model for species diversification; Nee et al., 1994), along with an uncorrelated lognormal relaxed clock, and the selected HKY nucleotide substitution model (Hasegawa et al., 1985) by MODELTEST, were used as priors for the analysis. A total run of 40 million generations were specified for the MCMC simulations. The software TRACER version 1.5 (Rambaut and Drummond, 2009) was used to check the convergence of the runs (effective sample sizes, ESS, of all parameters > 200) and exhibit the outcome of the designed Bayesian analysis.

Episodes of historical divergence among mitochondrial Cox1 lineages (as delineated by BAPS) were estimated with the software BEAST version 1.7.5. As the coalescent tree prior model was suggested to better fit the examined Cox1 data of *T. squamosa*, the Bayesian Skyline coalescent model, fitting a wide range of demographic scenarios while considering phylogenetic uncertainty (Drummond et al., 2005), was used as tree prior, jointly

with a strict molecular clock and the HKY model of sequence evolution. The determined Cox1 gene mutation rate for *Tridacna* was used to calibrate the haplotype phylogeny and to estimate the time to the most recent common ancestor (tMRCA) of BAPS Cox1 clusters. MCMC simulations were run for 50 million generations (sampled every 1 000 generations). The generated output of the Bayesian analysis was checked for chain convergence in TRACER version 1.5, ensuring that all ESS parameters were higher than 200. After summarizing the resulting trees in TreeAnnotator version 1.7.5 (Drummond et al., 2012), using maximum clade credibility, the obtained calibrated Cox1 haplotype genealogy was visualized and edited in FigTree version 1.4.0 (Rambaut, 2012).

The biogeographic history of *T. squamosa*, throughout the distribution range (encompassing the Red Sea and the Indo-Pacific oceans), was reconstructed by inference of ancestral geographic distributions of Cox1 haplotypes using the software RASP version 3.2 (Yu et al., 2015). Provided the crucial role played by Pleistocene glacial vicariance and interglacial recolonization in shaping contemporary population genetic structure of marine biota in the Indo-West Pacific (DiBattista et al., 2013; Ludt and Rocha, 2015; Farhadi et al., 2017; Yamamoto et al., 2019), the Statistical Dispersal-Vicariance Analysis (S-DIVA) method (Ronquist, 1997), included in RASP, was implemented in the analysis. According to the extant geographic distribution of Cox1 haplotypes, four biogeographic regions were considered allowing for a comprehensive overview on historical biogeography of *T. squamosa* across its distribution area. These delineated areas included: the Red Sea, the western Indian Ocean, the Indo-Malay Archipelago, and the western Pacific. In order to conduct the S-DIVA analysis, the MCMC-based condensed tree, exported from TreeAnnotator (included in BEAST), was loaded as “trees file”; while the distribution of the recorded Cox1 haplotypes through all defined biogeographical areas was loaded as “distribution file”. The likelihood of possible ancestral range at each generated node of the phylogeny was determined by means of the Most Likely States (MLS) function, included in RASP.

2.2.3 Unraveling the microevolutionary process responsible for the onset of phylogeographic patterning within the Indo-Malay Archipelago

Because the RASP analysis failed in yielding accurate information on the microevolutionary process (vicariance or dispersal) generating divergence between the two BAPS clusters 1 and 2 (see results), the approximate Bayesian computation (ABC) method, implemented in the software DIYABC version 2.0.1 (Cornuet et al., 2014), was used to investigate the origin of the genetic differentiation among these mitochondrial clusters. The potential involvement of vicariance or dispersal event in promoting such pattern of genetic separation allowed testing three genetic diversification scenarios, corresponding to two evolutionary processes (allopatric divergence through vicariance event and historical colonization followed by founder effect). The first scenario (null model of population divergence) assumed an allopatric origin of the recorded genetic divergence among BAPS cluster 1 and BAPS cluster 2. In this scenario, the two mitochondrial haplogroups, BAPS cluster 1 (effective population size: N_1) and BAPS cluster 2 (effective population size: N_2), are postulated to split simultaneously from a common ancestral population (effective population size: N_A) at historical time t_2 . The second scenario considered divergence of BAPS cluster 2 clams from their ancestral BAPS cluster 1 counterparts, following historical colonization event at historical time t_2 . The third scenario postulated an inverse pathway of diversification, in which BAPS cluster 1 clams split from their ancestral BAPS cluster 2 counterparts following historical colonization event at historical time t_2 . A set of summary statistics, describing genetic diversity and differentiation, were employed to determine the most similar simulated dataset to the observed dataset, allowing estimation of posterior distributions of the parameters of interest. The implemented summary statistics for each examined mitochondrial dataset included the number of haplotypes, the number of segregating sites, the mean of pairwise differences, and the number of private segregating sites. Regarding the summarizing statistics of population pairs, the number of haplotypes, the number of segregating sites, the mean of pairwise differences (W) and (B), and F_{ST} were used. A total of three million simulations (one million datasets for each scenario) were run for the ABC analysis. The posterior probabilities, corresponding to the three tested scenarios, were determined using the logistic regression method, as implemented in DIYABC. The most likely evolutionary scenario was identified as the one with the highest posterior probability (Beaumont, 2010).

2.2.4 Demographic history reconstruction

Taking into account the number of examined sequences per region and the distribution patterns of delineated mitochondrial clusters, trends of population dynamic through time and space can be only comprehensively assessed in this study case across the geographic scale encompassing the Indo-Malay Archipelago and western Pacific. In particular, aiming at unraveling the origin and cause of the geographic prevalence of BAPS cluster 1 across the aforementioned extended region, the demographic history of the giant clam species, throughout the Indo-West Pacific, was comprehensively reconstructed for both BAPS clusters 1 and 2 (found to co-occur across the region) using various approaches. The highly divergent BAPS cluster 5 [geographically confined to the western Pacific and defined in small dataset (only two specimens)] was omitted from the analysis.

Imprints of historical demographic changes within each dataset were discerned through assessment of departure from mutation-drift equilibrium, by means of the analysis of three neutral-

ity tests [Tajima's D (Tajima, 1989), Fu's F_s (Fu, 1997), and Ramos-Onsins and Rozas's R_2 (Ramos-Onsins and Rozas, 2002)]. The two statistics D and F_s were determined with ARLEQUIN version 3.1 (Excoffier et al., 2007). The R_2 index was calculated with DnaSP version 5.10 (Librado and Rozas, 2009). A total of 1 000 coalescent simulations were used to estimate these three demographic parameters as well as their level of significance. Significantly negative outputs of D and F_s , along with a significant value of the R_2 index, are indicators of a significant trend of demographic expansion.

Evidences of demographic and spatial expansion events were further assessed within each examined dataset by a mismatch distributions analysis, via the sum of squared deviations (SSD) (Rogers and Harpending, 1992; Rogers, 1995) between observed and expected distributions. The level of significance of this statistical procedure was computed in ARLEQUIN, using 1 000 random permutations. Non-significant outcomes ($P > 0.05$) for SSD allowed acceptance of the tested expansion (demographic or spatial) model.

Since both BAPS clusters 1 and 2 were found to co-occur across the Indo-Malay Archipelago and western Pacific, assessing trends of population dynamic through time across the geographic scale encompassing these two regions will provide insights into the potential impact of Pleistocene climatic fluctuations on forging spatial distribution of genetic polymorphism within *T. squamosa*. Detailed reconstruction of the historical demography, via assessment of evolution of population size through time, was assured by means of the coalescent-based Bayesian Skyline Plot approach (BSP; Drummond et al., 2005) implemented in the software package BEAST version 1.7.5 (Drummond et al., 2012). Given the sensitivity of the BSP analysis to the number of examined sequence data (Grant, 2015), and based on the outcomes of neutrality tests and mismatch distribution analyses, two kinds of Cox1 datasets of *T. squamosa* from the Indo-Malay Archipelago and western Pacific (with statistically and reliably representative sequences data) were analyzed. The first dataset included only BAPS cluster 1; while the second one was represented by both BAPS clusters 1 and 2. It has to be mentioned that BSP plot was not constructed separately for BAPS cluster 2 provided that the corresponding low number of representative sequences may underestimate pattern and time of population expansion. The BSP analyses were conducted using the HKY substitution model and a strict molecular clock as priors. The Cox1 gene mutation rate, specifically determined for *Tridacna*, was used to estimate time since expansion for each examined mitochondrial dataset. In order to ensure convergence of the posterior distributions, two independent Markov Chain Monte Carlo (MCMC) runs of 60 millions generations each were performed. These two MCMC simulations were combined by means of LogCombiner version 1.7.5 (Drummond et al., 2012), after discarding the first 10% iterations (6 millions) as burn-in. Bayesian Skyline Plots for both Cox1 datasets were generated in TRACER version 1.5, after confirming data convergence [effective sample size (ESS) > 200 for each parameter].

3 Results

3.1 Bayesian inference of mitochondrial genetic clusters

The Bayesian clustering approach, implemented in BAPS, detected five clusters (Fig. 2) within the examined Cox1 dataset of *T. squamosa* (number of groups in optimal partition ($K = 5$), log marginal likelihood of optimal partition is $-1\,902.05$) with maximum-associated probability value ($P = 1$). Clusters 1 and 2 were

sampling is needed to confirm the retrieved results.

Inference of the historical biogeography of *T. squamosa*, through reconstruction of ancestral geographic distributions of Cox1 haplotypes (Fig. 4), unveiled a geographically wide ancestral range for the giant clam encompassing the Red Sea, the western Indian Ocean, the Indo-Malay Archipelago and the western Pacific. From this postulated ancestral area, successive events of allopatric divergence through vicariance were responsible for the onset of the three BAPS genetic clusters 5, 4, and 3 corresponding to the western Pacific, Red Sea and western Indian Ocean, respectively (Fig. 4). According to the outcome of S-DIVA analysis, both BAPS clusters 1 and 2, co-occurring in the Indo-Malay Archipelago and western Pacific, were found to originate in the Indo-Malay Archipelago (Fig. 4). Dispersal events were recorded from the Indo-Malay Archipelago to the western Pacific and also inside the Indo-Malay Archipelago (Fig. 4). These events were more pronounced in BAPS cluster 2 (Fig. 4).

3.3 Origin of genetic divergence between BAPS clusters 1 and 2

Based on the outcome of the logistic regression, the estimated posterior probability for each tested evolutionary scenario (Fig. 5a) indicated unambiguous support for scenario 2 (Fig. 5b). According to this latter scenario, giant clams corresponding to BAPS cluster 2 split from their BAPS cluster 1 counterparts following a potential historical colonization event with a founder effect. The generated probability for the most supported scenario of genetic divergence (logistic approach: PP = 0.728 2, 95% confidence intervals (CI): 0.670 4–0.786 1) was found to lack overlap with those recorded for scenario 1 (PP = 0.060 2, 95% CI: 0.040 9–0.079 4) and scenario 3 (PP = 0.211 6, 95% CI: 0.157 0–0.266 2). According to these results, the significantly higher posterior probability, detected for scenario 2, allowed discarding with no doubt the possible allopatric origin of genetic divergence among both mitochondrial haplogroups (scenario 1). A founder effect

following a historical colonization could be rather a likely driver of the detected phylogeographic patterning within the Indo-Malay Archipelago.

3.4 Demographic history reconstruction

Negative values of Tajima's D and Fu's F_s were revealed for both examined mitochondrial datasets (Table 2). Significant outputs for the three analyzed neutrality tests (D , F_s , and R_2) were recorded for BAPS cluster 1 (Table 2). Only Fu's F_s was found to be significant for BAPS cluster 2 (Table 2).

Statistical analyses of the mismatch distributions allowed accepting both models of demographic (SSD = 0.004, P = 0.370) and spatial (SSD = 0.003, P = 0.540) expansion for BAPS cluster 1 (Table 2). These historical population dynamic models were found to be rejected for BAPS cluster 2 provided the recorded significant SSD values (Table 2).

Detailed examination of the evolution of effective population size through time was found to corroborate previous analyses of demographic history (namely for BAPS cluster 1) and yielded a comprehensive overview on the time since expansion. The generated Bayesian skyline plots, for the two kinds of examined datasets of *T. squamosa* within the Indo-West Pacific (BAPS cluster 1 and combined BAPS clusters 1 and 2), provided clear evidence of historical demographic expansion with marked increase in the effective population size (Fig. 6). In particular, significant increase in the effective population sizes, following a phase of relatively constant size, was shown for both kinds of datasets. The expansion of BAPS cluster 1 started approximately at about 145 000 years ago (CI: 130 000–180 000 years ago). For the combined dataset including both BAPS clusters 1 and 2, the expansion event occurred roughly at about 130 000 years ago (CI: 120 000–155 000 years ago). Notably, these recorded times of historical population dynamic events coincide with the Last Interglacial period (between 130 000–115 000 years ago; Wilson et al., 1998).

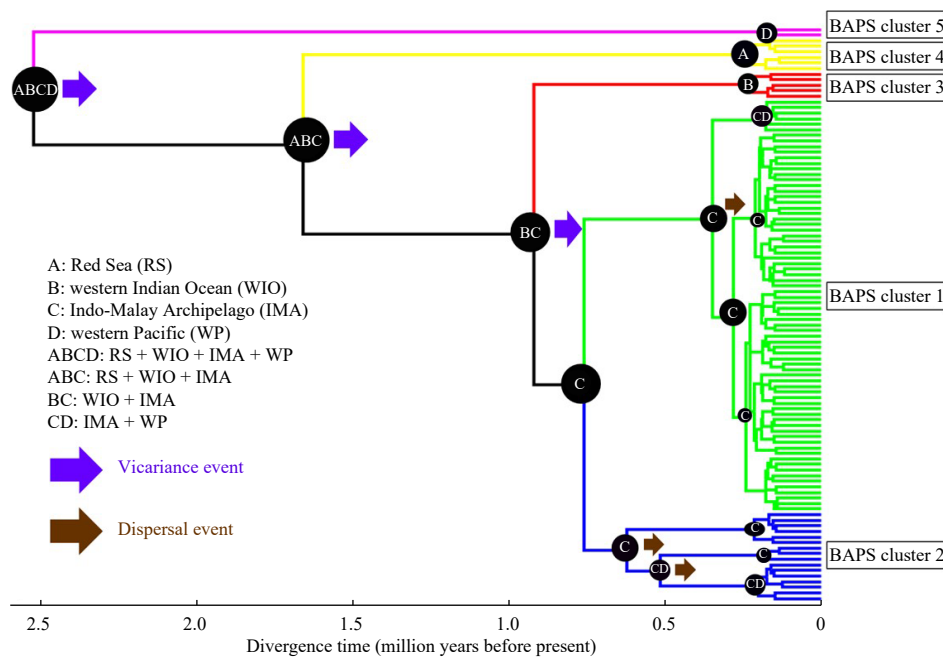


Fig. 4. Historical biogeography of *Tridacna squamosa*, highlighting ancestral areas reconstruction of Cox1 BAPS clusters. Circles on each main node show the likelihood of occurrence of each ancestral haplotype at an inferred ancestral biogeographic region. Graphical results of ancestral distributions at each main node of the phylogeny were obtained by the Statistical Dispersal-Vicariance Analysis (S-DIVA) method, as implemented in RASP version 3.2. Biogeographic regions are denoted with alphabetic letters. Purple arrows indicate vicariance events at the corresponding nodes, while brown arrows highlight possible dispersal events.

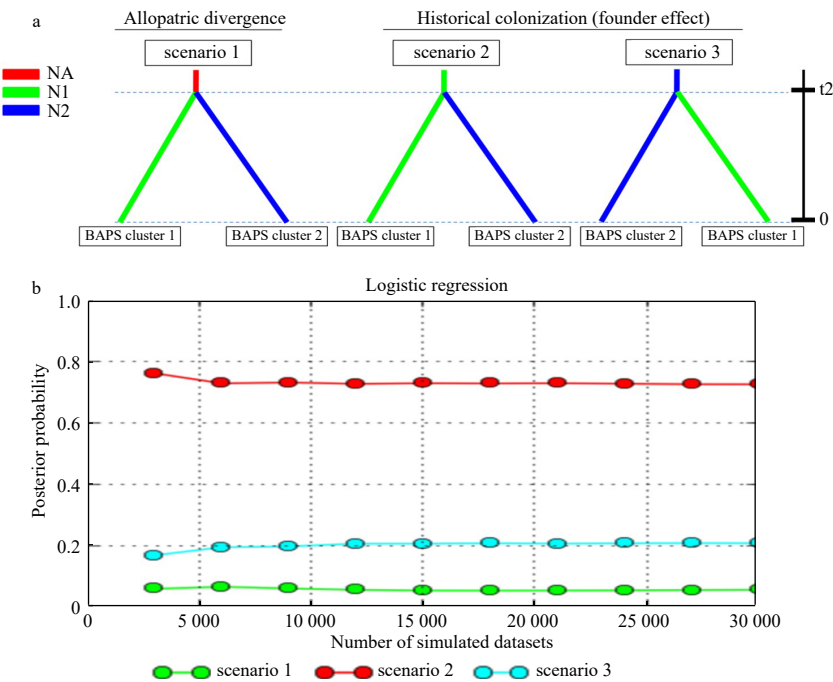


Fig. 5. Alternative tested evolutionary scenarios by means of the approximate Bayesian computation (ABC) method, implemented in the software DIYABC, to explain the origin of genetic divergence between BAPS clusters 1 and 2 of *Tridacna squamosa*, based on mtDNA data. a. Three tested scenarios corresponding to two evolutionary processes: allopatric divergence through vicariance event (scenario 1) and historical colonization followed by founder effect (scenarios 2 and 3). NA: effective population size of the common ancestral population; N1 and N2 represent the effective population sizes of both BAPS clusters 1 and 2 respectively; t_2 : the historical time at which both BAPS clusters 1 and 2 are postulated to diverge. b. Logistic regression plot for the simulated scenarios. The x-axis represents the number of simulated datasets used to calculate the posterior probability of each scenario, as expressed in the y-axis.

Table 2. Analyses of neutrality tests and mismatch distributions (assessing both demographic and spatial expansion models) for the examined mitochondrial BAPS clusters 1 and 2 within *Tridacna squamosa*

Examined BAPS cluster	N	Neutrality tests			N	Mismatch distributions analyses			
		D	F_S	R_2		Demographic expansion		Spatial expansion	
						SSD	P	SSD	P
BAPS cluster 1	202	-2.459	-27.728	0.015	202	0.004	0.370	0.003	0.540
BAPS cluster 2	26	-0.083	-5.996	0.115	26	0.057	0.027	0.066	0.028

Notes: N: Sample size; D: Tajima's D test; F_S : Fu's F_S test; R_2 : Ramos-Onsins and Rozas's R_2 test. For these latter indices, significant values are marked in bold. SSD sum of squared deviations between observed and expected distributions under the tested expansion model. P: represents the significance of sum of squared deviations.

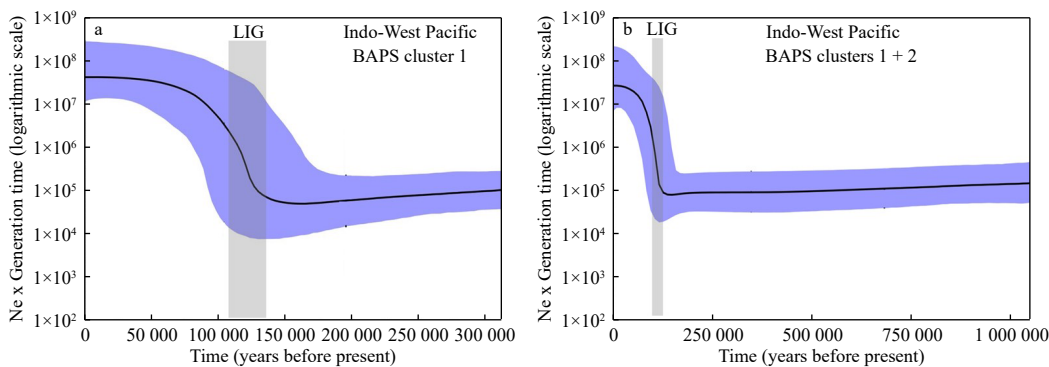


Fig. 6. Bayesian skyline plots (BSP) for specimens of *Tridacna squamosa* from the Indo-West Pacific [characterizing two kinds of datasets: BAPS cluster 1 (a) and the combined data including BAPS clusters 1 and 2 (b)], showing changes in effective population size (N_e multiplied with generation time) over time (measured in years before present). The thick solid line depicts the median estimate, and the margins of the blue area represent the highest 95% posterior density intervals. The enclosed grey-shaded box corresponds to the Last Interglacial (LIG) period (130 000–115 000 years ago; Wilson et al., 1998).

4 Discussion

The present study provides exclusive and novel insights into the evolutionary and biogeographic histories of the giant clam *T. squamosa* across its distribution range. It also allowed understanding the origin of the delineated patterns of phylogeographic structure already discerned in previous population genetic surveys of the species (DeBoer et al., 2014; Hui et al., 2016). Overall, the findings of this investigation provided a comprehensive overview on the genetic composition of *T. squamosa* in terms of mitochondrial lineages delineation and geographic distribution. They also unraveled the potential impact of cyclic Pleistocene palaeoclimate shifts on shaping such recorded patterns of spatial genetic variability and structure.

It should be indicated that the determined mutation rate of Cox1 gene for *Tridacna* sp. in this study (2.42% per Mya) was found to roughly approach the one previously determined within giant clam species (i.e., *T. crocea*: 2.30% per Ma) by Crandall et al. (2012). These very similar values obtained for the Cox1 mutation rate, inferred from two different approaches, strongly hint at the correctness of the calculated mutation rate within *Tridacna* sp. and the reliability of the implemented calibrated approach (adopted in this investigation). As a result of this reliable inference of mutation rate, the retrieved molecular divergence estimates (recorded diversification times in this investigation) were found to match well documented and known historical biogeographic events (i.e., Early Pleistocene glacial vicariance, as well as Middle and Late Pleistocene interglacial dispersal and expansion, see below for detailed discussion).

4.1 Geographic distribution of mitochondrial lineages and origin of the recorded hotspot of genetic diversity within the western Pacific

The outcome of BAPS Bayesian clustering approach allowed defining five mitochondrial clusters, three of which were endemic to the Red Sea (BAPS cluster 4), the western Indian Ocean (BAPS cluster 3) and the western Pacific (BAPS cluster 5). The other two BAPS clusters (1 and 2) were found to be co-distributed in both the Indo-Malay Archipelago and western Pacific. In comparison to earlier macro-geographic investigations, this is the first survey that unveiled such relatively higher level of genetic diversification within *T. squamosa*. In their comparative phylogeographic study, DeBoer et al. (2014) previously unraveled two Cox1 clades designated as dark and gray across the Indo-West Pacific. The former (dark clade) was found to be represented by two star-like clusters occurring throughout the studied area; while the latter (gray clade) included highly divergent haplotypes with low frequency. Later on, the study by Hui et al. (2016) allowed characterizing four clusters within *T. squamosa*. One of these defined clusters (clade 1) was present throughout the Indo-Malay Archipelago; while the others were restricted to the peripheral areas (clade 2 found in the western Pacific, clade 3 in the Red Sea and clade 4 in the western Indian Ocean). The potential origin of the level of genetic diversification patterns found within *T. squamosa* in this study could be linked to the fact that all published sequences for the giant clam were used and analyzed. This is in contrast with earlier investigations (DeBoer et al., 2014; Hui et al., 2016); whereby newly generated mitochondrial datasets were analyzed without being compared to previously examined sequences from other surveys. Hence, analysis of nearly all published Cox1 sequences by means of a robust statistical clustering procedure (i.e., the Bayesian clustering approach, included in BAPS, Corander et al., 2008) likely helped with the accurate identification of subtle genetic diversity within the mitochondrial dataset of *T. squamosa* that was previously confounded with or

assigned to other evolutionary lineages. For example, comparison of all available Cox1 sequences of *T. squamosa* showed that clade 2 as identified by Hui et al. (2016) (designated in yellow), comprising three haplotypes, was indeed composed of two subclades corresponding to the two identified BAPS clusters 2 and 5 in this study, with the most divergent two haplotypes (within clade 2; Hui et al., 2016) corresponding to BAPS cluster 5 and the remaining one assigning to BAPS cluster 2. Notably, this latter BAPS cluster has been found to occur across the Indo-Malay Archipelago (although with less frequency than that of the prevailing BAPS cluster 1) when investigating nearly all published Cox1 dataset (thereby covering much of the distribution area of the species). Hence, with the increase of sampled genetic diversity (all available sequences for *T. squamosa* stemming from previous phylogeographic surveys) and the use of appropriate and powerful genetic delineation tool (i.e., BAPS), comprehensive overview on the genetic composition of the studied giant clam species could be accurately inferred.

Analysis of the spatial distribution of genetic diversity of *T. squamosa*, via the Bayesian delineation procedure, also revealed higher level of genetic diversification within the western Pacific (with the occurrence of three mitochondrial clades, BAPS clusters 1, 2, and 5) than that recorded across the Indo-Malay Archipelago (harboring only BAPS clusters 1 and 2). This finding clearly contradicts the general expectation of high biodiversity in the Indo-Malay Archipelago; whereby hotspots of genetic diversity have been frequently unraveled in this region (McMillan and Palumbi, 1995; Lavery et al., 1996; Benzie, 1999; Barber et al., 2006; Crandall et al., 2008a, 2008b; DeBoer et al., 2008, 2014; Kochzius and Nuryanto, 2008; Ackiss et al., 2013; Jackson et al., 2014; Guo et al., 2021). It has been postulated that the high level of genetic diversification recorded across the Indo-Malay Archipelago could stem from the fact that this region is considered as a biogeographic centre of origin (Briggs, 1999) or as a centre of biotic overlapping between the Indian and Pacific oceans (Woodland, 1983). Against this background, in this study, the surprisingly high genetic richness (in terms of mitochondrial lineages) recorded in the western Pacific, in comparison to that identified across the Indo-Malay Archipelago, could be attributed to different explanations. For instance, the occurrence of three BAPS clusters (1, 2, and 5) within the western Pacific could reflect the residual effect of historical allopatric population isolation due to the potential impact of Pleistocene glacial climate deterioration (Deli et al., 2018). In this context, the remarkable mitochondrial richness (in terms of Cox1 types) suggests the occurrence of climatically favorable repositories of genetic diversity (or the so called glacial refugia) that allowed preserving genetic variability of the species while promoting genetic divergence in isolation leading to the observable three mitochondrial lineages within the western Pacific. Nevertheless, the biogeographic history reconstruction of *T. squamosa*, as inferred from the outcome of RASP analysis, showed that among the three genetic types (BAPS clusters 1, 2, and 5) characterizing the gene pool of the western Pacific, only BAPS cluster 5 was postulated to originate in this region. The ancestral biogeographic region of the other BAPS clusters 1 and 2 was suggested to be located in the Indo-Malay Archipelago. Accordingly, the recorded hotspot of genetic diversity in the western Pacific could be likely explained by a secondary contact following range fragmentation and population historical isolation. As such, the western Pacific could be regarded as a contact zone and/or a reservoir of population mixing following range expansion and recolonization. This scenario is likely supported by the concordant outcomes of RASP and BSP

analyses. Indeed, not only historical dispersal events were recorded from the Indo-Malay Archipelago into the western Pacific, but also demographic and spatial expansion events were unveiled mainly in BAPS cluster 1 stretching across the Indo-Malay Archipelago and western Pacific.

4.2 Genetic imprints of Early Pleistocene glacial refugia and allopatric divergence within *Tridacna squamosa* across the Red Sea and Indo-Pacific oceans

Assessment of spatio-temporal origin of genetic diversification within *T. squamosa* across its distribution range unveiled complex evolutionary history. It also provided insights into how harsh Pleistocene climatic fluctuations drove genetic divergence and shaped phylogeographic structure within the giant clam species.

The geographic confinement of the three BAPS clusters 3, 4, and 5 to the western Indian Ocean, Red Sea, and western Pacific, respectively, alongside their time frame and mode of diversification, suggest undoubtedly the occurrence of glacial refugia within these regions. These potential refugia might have served as climatically favorable zones to preserve genetic diversity and subsequently allowed accumulation of significant genetic divergence in allopatry. Two lines of evidences are in favor with this scenario. First, the successive diversification episodes within *T. squamosa* [2.55 Mya (95% HPD: 1.66–3.67 Mya); 1.68 Mya (95% HPD: 1.06–2.42 Mya); 0.94 Mya (95% HPD: 0.59–1.37 Mya)], that led to the onset of these three mitochondrial lineages, coincide with glaciations periods of the Early Pleistocene (2.58 Mya to 0.8 Mya; [Riccardi, 2009](#)). Noteworthy, these estimated diversification times roughly echoed the major cooling-down steps progression during the Pleistocene occurring around 2.6 Mya at the start of the Quaternary, and followed by steps around 1.8 Mya, and 0.9 Mya ([Kleiven et al., 2002](#); [Riccardi, 2009](#)). Second, the outcome of S-DIVA analysis, as implemented in RASP, showed that vicariance events were at the origin of these separation events, approving the glacial refugium hypothesis as a potential process for driving the allopatric divergence. In light of these evidences, it is highly likely that marked dropping of sea levels during glacial maxima of the Pleistocene, resulting in the emergence of land barriers and disruption of oceanographic circulation systems, were initial drivers of the phylogeographic patterning as observed in *T. squamosa*.

4.2.1 Early Pleistocene glacial-driven exposure of the Sunda and Sahul shelves as land barriers to gene flow resulting in genetic distinctness of the western Pacific

The solely and restricted occurrence of the highly divergent BAPS cluster 5 in the western Pacific, associated with the Early Pleistocene vicariant divergence between this mitochondrial clade and the common ancestor of all remaining lineages (as revealed by the outcomes of BEAST and RASP analyses), hint at the potential role played by land barriers formation such as the Sunda shelf and the Sahul shelf during Pleistocene glaciations in driving genetic divergence among the western Pacific and Indian oceans. Shallow shelf areas, such as the Java Sea on the Sunda shelf, fell dry and created more or less isolated ocean basins, resulting in gene flow restriction between populations ([McManus, 1985](#); [Voris, 2000](#)). Recent findings on the geomorphology of the nowadays submerged Sunda shelf indicate that it subsided during the Pleistocene and that, over the Late Pliocene and Quaternary, was never submerged prior to the Marine Isotope Stage 11 (400000 years ago) ([Husson et al., 2020](#)). Hence, the so long period of exposure of this land barrier might have enabled the con-

tinuous separation of Indo-Pacific biota and allowed accumulation of significant genetic differences in allopatry. The resulting divergent lineages could have also persisted across later successive glacial and interglacial periods of the Pleistocene. This scenario is strongly supported by the following two main findings. First, the study of [Hui et al. \(2016\)](#) revealed high nucleotide divergence separating the two divergent haplotypes within clade 2 (corresponding to BAPS cluster 5 in this study) and the remaining clades (1, 3, and 4; [Hui et al., 2016](#)). Second, the current investigation showed that the retrieved time since divergence between BAPS cluster 5 and the common ancestor of all remaining clusters (1, 2, 3, and 4) occurred around 2.55 Mya (95% HPD: 1.66–3.67 Mya), corresponding to the Early Pleistocene.

4.2.2 Early Pleistocene glacial-induced disruption of oceanographic circulation and genetic exchange across the Strait of Bab El Mandeb at the origin of the genetic endemism of the Red Sea

The deep genetic divergence of the Red Sea haplogroup (BAPS cluster 4), occurring around 1.68 Mya (95% HPD: 1.06–2.42 Mya), confirms the findings of [Hui et al. \(2016\)](#) and [Fauvelot et al. \(2020\)](#). Notably, such pattern of genetic separation has been also noticed based on the recorded high Cox1 nucleotide divergence ([Hui et al., 2016](#)) and the outcome of concatenated mitochondrial Cox1 and 16S Bayesian phylogeny reconstruction ([Fauvelot et al., 2020](#)). This study is the first to determine the tempo and mode of diversification of the Red Sea lineage. This level of genetic differentiation could be likely associated with the key role played by the narrow and shallow Strait of Bab El Mandeb (connecting the Red Sea and Indian Ocean) in driving genetic endemism of this semi enclosed basin ([Klauewitz, 1989](#)). Over glacial maxima of the Pleistocene, the Red Sea was repeatedly isolated when the sea level lowered as much as 140 m ([Rohling et al., 1998](#)) which is about the current depth of the Strait of Bab El Mandeb. These historical episodes of efficient cut off of the Red Sea from the Indian Ocean could have been either achieved through physical isolation or as the result of the restriction of oceanographic circulation associated with elevated salinity and temperature ([Siddall et al., 2003](#); [Bailey, 2009](#)). In this context, it may be hypothesized that the deep genetic divergence of the Red Sea recorded in this investigation, taking root to the Early Pleistocene, could have been driven by the significant impact of these palaeogeographic and palaeoenvironmental isolating events. Notably, these historical events might have allowed sufficient time for accumulation of genetic differences and consequent population divergence owing to disruption of genetic exchange. The resulting distinct evolutionary lineage of the Red Sea might have been subsequently reinforced and/or maintained by the effect of contemporary isolating processes including (1) the lack of reef formation such as those documented between the north-east African and southern Arabian coasts as a result of the impact of the cold-water welling up off Somalia ([Smeed, 1997](#); [Kemp, 1998, 2000](#)), (2) impact of life history traits (manifested by the limited dispersal potential of *Tridacna squamosa*; [Lucas, 1988](#)) and (3) the occurrence of ecological barrier to dispersal evidenced by the strong latitudinal temperature and salinity gradients characterizing the Red Sea ([Sofianos et al., 2002](#); [Sofianos and Johns, 2007](#); [Raitos et al., 2013](#); [Kürten et al., 2014](#)).

4.2.3 Impact of the Mid-Pleistocene Transition (at the end of the Early Pleistocene) on driving allopatric genetic divergence among the western Indian Ocean and the Indo-Malay Archipelago

The outcome of the calibrated Bayesian phylogeny (as in-

ferred from BEAST) showed a deep separation of the western Indian Ocean giant clams (BAPS cluster 3) from their Indo-Malay Archipelago counterparts (BAPS clusters 1 and 2) dating back to approximately 0.94 Mya. It also unraveled a closer genetic relationship of the western Indian Ocean (WIO) mitochondrial lineage to the Indo-Malay Archipelago (IMA) clusters than to the Red Sea mitochondrial clade, suggesting that the WIO and IMA were genetically connected in historical times prior to their eventual divergence. This finding has been recently highlighted by the study of Fauvelot et al. (2020) who also noticed such pattern of clustering based on the outcome of concatenated mitochondrial Cox1 and 16S Bayesian phylogeny reconstruction. The evolutionary process of diversification of both genetic entities, as inferred from the outcomes of BEAST and SDIVA analyses, strongly favours the scenario of vicariant separation of these clusters following potential allopatric divergence among glacial refugial lineages (with the ancestral ranges of BAPS cluster 3 and the others BAPS clusters 1 and 2 were found to be strictly confined to the WIO and IMA respectively). This postulated scenario is likely supported by the recorded time frame for the genetic diversification coinciding with the Middle Pleistocene Transition between 1.25 Mya and 0.6 Mya (Mudelsee and Schulz, 1997; Clark et al., 2006). During this historical period, the global climate changes were characterized by marked shift in the amplitude and frequency of cold and arid periods, the decreases in sea surface temperatures, as well as the significant increase in ocean levels dropping compared with those of the very Early Pleistocene (Mudelsee and Schulz, 1997; Clark et al., 2006). In light of these insights, it can be hypothesized that these palaeoclimatic and palaeogeographic conditions, along with the emergence of the Mid-Indian Ocean Barrier, might have been likely involved in affecting and shaping the genetic make-up of Indo-Pacific marine biota (Ludt and Rocha, 2015; Borsa et al., 2016; Bowen et al., 2016; Hodge and Bellwood, 2016; Farhadi et al., 2017; Hubert et al., 2017), including the recorded pattern of deep genetic divergence between western Indian Ocean specimens of *T. squamosa* and their Indo-Malay Archipelago counterparts. The resulting deep genetic distinctness of the western Indian Ocean lineage from that corresponding to the Indo-Malay Archipelago, initially driven by historical isolating processes, as previously discussed, could be currently maintained and/or accentuated by the effect of contemporary eco-biological features. For instance, the noticeable difference between coral reefs of the western Indian Ocean and those from other regions in the Indo-Pacific (Spalding et al., 2001), alongside the limited larval dispersal potential of *T. squamosa* (Lucas, 1988), might have driven the independent evolution of endemic gene pools in the western Indian Ocean.

4.3 Historical Mid-Pleistocene interglacial dispersal and colonization driving genetic diversification across the Indo-Malay Archipelago and shaping genetic diversity hotspot in the western Pacific

The outcomes of both RASP and DIYABC analyses showed that genetic diversification of both BAPS clusters 1 and 2 took place in the Indo-Malay Archipelago. Notably, the giant clams corresponding to BAPS cluster 2 were found to split from their counterparts representing BAPS cluster 1 following a potential historical colonization event with a founder effect. Although it is difficult, at this stage, to precise the potential location of refugial areas within the Indo-Malay Archipelago as well as the direction and pathways of dispersal within this region, the divergence time between both BAPS clusters 1 and 2 (as inferred from the outcome of BEAST calibrated phylogeny) was found to occur around

0.77 Mya during the Mid-Pleistocene. Interestingly, this temporal frame of genetic separation roughly coincides with an historical interglacial period corresponding to the Marine Isotope Stage 19c (MIS19c), occurring approximately 777 000 years ago (Ferretti et al., 2015; Vavrus et al., 2018). The MIS19c interglaciation has been considered as possible analog to the current Holocene Interglacial (MIS1) (Yin and Berger, 2012; Tzedakis et al., 2012; Vavrus et al., 2018). Noteworthy, during the MIS1 period, the post glacial re-establishment of favorable environmental conditions might have led to the range expansion of marine species and recolonization of new territories from potential glacial refugia, enhancing the foundation of postglacial divergent lineages following dispersal and genetic drift (Tarnowska et al., 2010; Evangelisti et al., 2017). As such, an analogous scenario during the MIS19c interglacial might have led to the genetic diversification pattern recorded in *T. squamosa* across the Indo-Malay Archipelago. Following this event of diversification, both BAPS clusters 1 and 2 were suggested to have subsequently colonized the western Pacific by means of historical dispersal episodes originating from the Indo-Malay Archipelago (as revealed by the outcome of SDIVA analysis, implemented in RASP). Such historical colonization pattern was found to be more pronounced in BAPS cluster 2. Overall, the obtained findings hint at the complex evolutionary history of *T. squamosa* across the Indo-Pacific.

It has to be clarified that the results of genetic diversification across the Indo-Malay Archipelago may not be influenced by the data size difference between the BAPS cluster 1 and BAPS cluster 2. Indeed, the frequency of occurrence of both BAPS clusters 1 and 2 was determined based on statistically well supported data (after examining all published Cox1 sequences). According to the outcome of this investigation, BAPS cluster 1 was found to be geographically the most common and prevalent across the Indo-Malay Archipelago. This distribution pattern has been previously recorded in the study of Hui et al. (2016), although the authors did not implement the powerful Bayesian clustering approach (included in BAPS) as used in this study and have instead delineated mitochondrial clusters based on the outcome of haplotypes network. So, even if more data would be examined in future investigations, similar proportions would be always figured out. As such, even if more genetic diversity would be sampled for BAPS cluster 2, spatial and temporal frames of genetic diversification across the IMA would not significantly change. Most importantly, the striking congruence between molecular divergence estimates (diversification time across the Indo-Malay Archipelago) and documented historical biogeographic process (dispersal event during an historical interglacial period), as found in this study, could be only retrieved if reliably representative genetic diversity is sampled.

4.4 Last Interglacial population expansion accentuating the impact of historical dispersal and accounting for admixture among refugial lineages within the Indo-Malay Archipelago and western Pacific

While the Mid-Pleistocene historical dispersal and colonization events could explain the origin of gene pool composition in the Indo-Malay Archipelago and western Pacific, they may not provide comprehensive explanation to the extent of contemporary geographic distribution pattern of both BAPS clusters 1 and 2 recorded along the geographic spectrum encompassing the Indo-Malay Archipelago and western Pacific (i.e., the geographic prevalence of BAPS cluster 1 across this area). Detailed reconstruction of demographic history of both BAPS clusters 1 and 2 has provided some clues to explain the origin of contemporary

distribution patterns of both lineages across the Indo-Pacific. Indeed, the outcome of BSP analysis for two kinds of examined datasets of *T. squamosa* from the Indo-West Pacific (BAPS cluster 1 and combined BAPS clusters 1 and 2) provided clear evidence of historical demographic expansion dating back to 145 000–130 000 years ago. These recorded times of historical population expansion events nearly coincide with the Last Interglacial period during the Eemian epoch (between 130 000–115 000 years ago; Wilson et al., 1998). The Last Interglacial (LIG) was a period characterized by higher global sea level and reduction in ice sheet area (Zagwijn, 1996). It was also marked by warmer climate conditions, which might have been favourable for extended colonization and diversification of marine fauna (Muhs et al., 2002). In light of these considerations, it may be hypothesized that this noticeable expansion event, driven by favorable environmental conditions during the LIG, could be at the origin of secondary contact and admixture among BAPS clusters 1 and 2 (potentially surviving previous glaciations in isolation within refugial areas). Therefore, the discerned demographic expansion might have accentuated the impact of previous historical dispersal during the MIS19c interglaciation on population genetic structure and connectivity. Statistical assessment of both demographic and spatial expansion models showed that these historical population dynamic scenarios were only accepted for BAPS cluster 1. Such recorded patterns of demographic and range expansions allow clarifying the origin of the geographic prevalence of this mitochondrial lineage across the Indo-Malay Archipelago and western Pacific. They also provide explanation to the extent of geographic mixing between BAPS clusters 1 and 2 stretching to the western Pacific. The detected differences in demographic tendencies (manifested by disparate trends of demographic and spatial expansions) among both BAPS clusters 1 and 2 could be attributed to the potential impact of Pleistocene climate fluctuations (alternating glacial and interglacial cycles) on habitat availability and suitable ecological niche (Kousteni et al., 2015).

4.5 Evolutionary history scenario of *T. squamosa* across the Red Sea and Indo-Pacific oceans

Overall, based on the obtained findings, the following evolutionary history scenario, that could explain the recorded phylogeographic patterning within *T. squamosa* across its distribution range, may be proposed (Fig. 7). The onset of Pleistocene glaciations cycles, occurring approximately 2.588 Mya (± 0.005) (Riccardi, 2009), might have triggered a series of allopatric divergences among populations of *T. squamosa* as a result of their retreatment to specific refugia following significant climate cooling and dropping of sea levels. Notably, the harsh palaeoenvironmental and palaeogeographic changes during the Early Pleistocene might have led to the restriction of biotic exchange among these refugia accounting for the emergence of endemic divergent lineages in the western Pacific (BAPS cluster 5), Red Sea (BAPS cluster 4), western Indian Ocean (BAPS cluster 3) and Indo-Malay Archipelago (common ancestor of BAPS clusters 1 and 2, postulated to have originated in this area according to the outcome of S-DIVA analysis). Later on, an historical dispersal during the Mid-Pleistocene MIS19c interglacial could have accentuated the pattern of genetic diversification within *T. squamosa* giving rise to a new divergent mitochondrial clade in the Indo-Malay Archipelago. The newly forged mitochondrial lineage (BAPS cluster 2) has been suggested to split from the postulated ancestral BAPS cluster 1 following a potential colonization with a founder effect. This trend of historical dispersal event might have

also enabled giant clams, assigning to both gene pools (BAPS clusters 1 and 2), to subsequently colonize the western Pacific through successive dispersal episodes, accounting for the recorded hotspot of genetic diversity as currently observed within the western Pacific (comprising at least three divergent mitochondrial clades: BAPS clusters 1, 2, and 5). Late Pleistocene demographic expansion of *T. squamosa* from the Indo-West Pacific, during the Last Interglacial period, could have promoted range extension of BAPS cluster 1 leading to the contemporary geographic prevalence of this lineage across the Indo-Malay Archipelago and western Pacific. It should be indicated that, against a background of seemingly homogenous genetic composition in both the Indo-Malay Archipelago and western Pacific (as evidenced by the outcome of BAPS clustering approach), the resulting genetic divergence among giant clams from the Red Sea, western Indian Ocean, and Indo-Malay Archipelago-West Pacific (in terms of their distinct mitochondrial lineages) is still likely maintained by the impact of contemporary isolating processes. These isolating mechanisms could stem from particular oceanographic circulation patterns across the Indo-Pacific, as well as from specific ecological requirements and life history traits of the species.

4.6 Conclusions

The results of the present study provide novel insights into the evolutionary history and microevolutionary processes that allowed genetic diversification of the giant clam *T. squamosa* across its distribution range. Notably, they contribute to deepening knowledge about the impact of historical climate fluctuations on forging contemporary spatial patterns of population genetic diversity and structure. Furthermore, being resilient to major climate shifts during the Pleistocene by adapting to these palaeoenvironmental conditions and diversifying, *T. squamosa* could be used as a model species to track the impact of climate change on genetic variability and structure of marine species. In particular, the new information, provided in this investigation, may help with understanding and/or predicting the consequences of ongoing global warming on genetic polymorphism of endangered coral reef species among which *Tridacna* sp. are listed as ecologically important. Indeed, climate deterioration during Pleistocene glacial cycles could be equivalent to the potential climate stressful conditions likely driven by the current global warming. Provided that *T. squamosa* has survived these historical climate deteriorations through adaptation and diversification, it may be expected that the giant clam species would be able to withstand the imposed threat of ongoing global warming on coral reefs and adapt to the newly established environmental conditions. This would certainly pave the way for alternative evolutionary pathways of the species and give rise, after considerable amount of time (when suitable climate conditions will be restored), to new phylogeographic patterns or even cryptic species. Further evolutionary histories reconstructions of a wider array of Indo-Pacific coral reef biota are required in order to clarify whether these species might have responded similarly to the harsh Pleistocene palaeoclimatic shifts.

On the other hand, against a background of scarce sampling in some regions (such as in the Red Sea, Indian Ocean or the western Pacific), the striking congruence between molecular divergence estimates (recorded diversification times) and documented historical biogeographic processes (vicariance and dispersal events during historical glacial and interglacial periods), as found in this study, could be only retrieved if reliably representative genetic diversity is sampled. Therefore, with the samples at

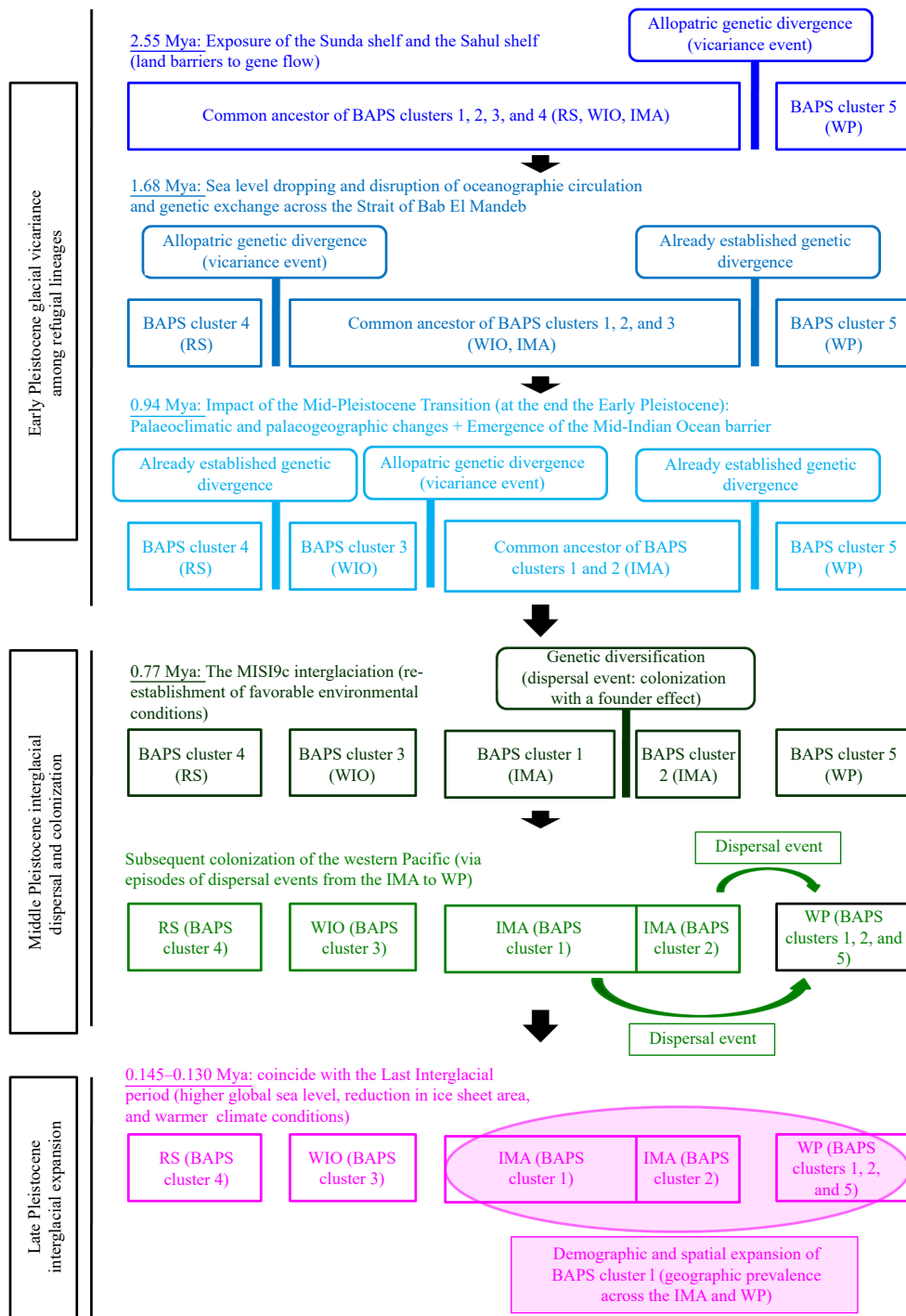


Fig. 7. A detailed and illustrative schematic diagram exhibiting the evolutionary history scenario of the giant clam *Tridacna squamosa* across its distribution range. RS: Red Sea, WIO: western Indian Ocean, IMA: Indo-Malay Archipelago, WP: western Pacific.

hand, at this stage, the obtained findings are relevant and reliable. However, more sampling of the highly genetically diversified species *T. squamosa* needs to be carried out across its distribution area in future investigation as more lineages for example could be identified when stretching the sampling spectrum in poorly surveyed geographic areas such as the Red Sea, Indian Ocean or western Pacific. Furthermore, because different markers (especially those independent ones such as mitochondrial and nuclear markers) may yield different evolutionary rates and coalescent histories, the outcomes of evolutionary, biogeograph-

ic and demographic histories reconstructions of the species (at this stage) across its distribution range (based on the single *Cox1* gene, although found to be informative) should be regarded as tentative. Other variable nuclear markers need to be examined and analyzed in future investigation in order to confirm the obtained findings. Overall, the use of several independent genetic markers in conjunction with well representative sampling (covering the whole distribution range) will provide a complete view on pattern and the historical origin of population genetic diversity and structure of *T. squamosa*.

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