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Historical divergences associated with intermittent land bridges overshadow isolation by larval dispersal in co-distributed species of *Tridacna* giant clams

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Running header: Historical *versus* contemporary influences on giant clam population structure

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ABSTRACT

Aim

To test historical and contemporary influences on population structure in the giant clams, *Tridacna maxima* (Röding, 1798) and *T. crocea* Lamarck, 1819. To refine the location of clade boundaries within a newly-resurrected species, *Tridacna noae* (Röding, 1798).

Location

Indo- Australian archipelago, including Indonesia, the Philippines, Australia, Papua New Guinea, the Solomon Islands, Republic of Kiribati, the Line Islands, and Taiwan.

Methods

We used isolation-migration (IMa) coalescent models and distance-based redundancy analyses (dbRDA) to test the relative influence of barriers and continuous distances on historical divergence, gene flow and population structure of *Tridacna maxima* and *T. crocea*. Continuous metrics of distance included present-day and Last Glacial Maximum overwater distances along with probability of larval dispersal among sampling sites. We combined new mitochondrial cytochrome oxidase subunit I (mtDNA COI) sequences with existing data to compile the largest data set of these species yet analysed.

Results

The Pleistocene land barriers of the Sunda Shelf and Torres Strait were associated with old (> 0.5 million years) divergence times. The western and eastern boundaries of the Halmahera Eddy were also locations of significant, but more recent, divergence. No gene flow was detected across any of the four barriers tested. Larval dispersal distances between sampling sites were significant predictors of *T. crocea* population structure, accounting for differentiation above and beyond the contribution of barriers. We

further delineated the species range of *T. noae* and showed that its two known clades are sympatric in central Indonesia.

Main Conclusions

The strong signature of historical barriers on genetic differentiation argues against the assumption that Indo-Pacific *Tridacna* are open meta-populations. Despite similar life histories, *T. maxima* and *T. crocea* differ in their mtDNA population structure. The widespread species (*T. maxima*) exhibits population structure linked solely with historical factors, whereas *T. crocea's* population structure reflects both historical factors and larval dispersal distances.

INTRODUCTION

The Indo-Australian Archipelago (IAA) is the global epicentre of shallow-water marine biodiversity. More species of tropical shallow-water marine animals live in the IAA than anywhere else on Earth (Briggs et al. 2013) and the geological complexity of the region may have directly promoted diversification (Bowen et al. 2013). Sea level changes during the Pleistocene Epoch (Voris 2000) caused significant and intermittent reductions in IAA shallow-water marine habitat (Fig. 1). During the later part of the Pleistocene, sea levels reached ~ 120 metres below present on multiple occasions, the most recent ~18 ka (Voris 2000). Many species inhabiting this region show genetic patterns characteristic of population expansions and differentiation, consistent with colonizing new habitat on re-flooded shelf areas (Lukoschek et al. 2007, Crandall et al. 2012). Several regions of genetic discontinuity appear to be concordant across species (reviewed by Carpenter et al. 2010, Briggs et al. 2013).

Here we focus on three widely distributed species of giant clams in the genus *Tridacna*, whose species richness peaks in the IAA. These species capture attention for their brilliant colours, massive size and cultural significance (Heslinga 1996). All species are regulated by the Convention on International Trade in Endangered Species (CITES) and some populations are dramatically reduced (Tisdell and Menz 1992, Wells 1997). *Tridacna maxima* (Röding 1798) has a wide ranging distribution from the western Indian Ocean and Rea Sea to the Pitcairn Islands in the central Pacific (Lucas 1988); *Tridacna crocea* (Lamark 1819) is restricted to the central Indo-Pacific from northern Australia to southern Japan, and from western Indonesia east to Palau (IUCN 1996); and *Tridacna noae* (Röding 1798) was recently resurrected as a distinct species (Su et al. 2014; Borsa et al. 2015a) with a range that minimally includes Taiwan, the Philippines, western Australia and the Solomon Islands (Huelsken et al. 2013; Lizano and Santos 2014; Penny and Willan 2014, Su et al. 2014; Borsa et al. 2015b). All *Tridacna* species are broadcast spawners with planktotrophic (feeding) larvae that are competent to settle within a week (Fitt et al. 1984, Lucas 1988). Larvae can spend up to 19 days (Jameson 1976) drifting with the currents before settling on hard substrate. This planktonic life stage can potentially connect distant populations.

Despite planktonic dispersal, previous phylogeographic studies of giant clams (and other coral reef species with planktonic larvae) have identified genetic discontinuities in the IAA associated with locations where dispersal may have been historically, or is contemporaneously, impaired. The most sizeable historical barrier is the shallow Sunda Shelf that was exposed land during Pleistocene low sea level stands, reducing exchange between the Indian and Pacific Ocean at the Last Glacial Maximum (LGM). Mitochondrial clades of *T. maxima* and *T. crocea* that are geographically restricted to western Sunda may reflect Indian Ocean isolation associated with this historical barrier (DeBoer et al. 2008, Kochzius and Nuryanto 2008, Nuryanto and Kochzius 2009, DeBoer et al. 2014b, Hui et al. 2016). The same pattern is also observed in genetic clusters based on microsatellites in *T. crocea* (DeBoer et al. 2014a, Hui et al. 2017).

Another prominent historical land barrier is represented by the modern-day Torres Strait, connecting the Gulf of Carpenteria with the Coral Sea (Fig. 1). This area formed a land bridge during the Pleistocene and remained closed for approximately 80 % of the past ~ 250 kyr (Voris 2000). Previous work has discovered genetic differentiation consistent with separation of populations either side of the Torres Strait land bridge in many marine taxa, but with a lack of structure found in some species (reviewed in Mirams et al. 2011). Modelling of larval dispersal, guided by modern oceanographic data, indicates a low probability of gene flow across the Torres Strait (Treml et al. 2015b). Mitochondrial DNA-based phylogenetic trees of *T. maxima* and *T. crocea* suggest deep divisions between Indonesian and western Australian locations in line with the Torres Strait land bridge being a likely long-standing barrier to larval dispersal (Huelsken et al. 2013).

Other putative barriers in the IAA are associated with ocean circulation. For example, the Halmahera Eddy (Fig 1) is a seasonally strong eddy just north of Halmahera and the Bird's Head Peninsula of West Papua, which limits water flow and, potentially, larval exchange into and out of Indonesia (Kool et al. 2011, Treml et al. 2015b). Previous studies have shown the congruence of this eddy with genetic differentiation in fishes (Timm et al. 2008, Liu et al. 2012, Ackiss et al. 2013, Dohna et al. 2015), mantis shrimps (Barber and Boyce 2006), seastars (Crandall et al. 2008, Kochzius et al. 2009), *Tridacna squamosa* (Hui et al. 2016), and in both *T. crocea* (DeBoer et al. 2008, Hui et al. 2016; Kochzius and Nuryanto 2008, DeBoer et al. 2014a) and *T. maxima* (Nuryanto and Kochzius 2009, DeBoer et al. 2014b, Hui et al. 2016), although the position of the disjunction shifts slightly between species. For this reason, we test barriers at both the western and eastern boundaries of the eddy (Schiller et al. 2008). Our western boundary falls where most of the abovementioned authors designate the Halmahera Eddy (for example, Eastern Barrier in Barber et al. 2006; see also Fig. 1). Our eastern boundary extends north approximately 500 km from the northern shore of the Bird's Head Peninsula (Kashino et al. 1999). This eastern boundary approximates the disjunction to the east of Cenderawasih Bay reported within *T. crocea* and *T. maxima* (Huelsken et al. 2013).

Although all four IAA barriers considered here (Sunda Shelf, Torres Strait, western and eastern Halmahera Eddy) are known to approximately align with mtDNA clade turnover for both *T. maxima* and *T. crocea*, the relative contributions of these features to genetic structuring have not been evaluated nor tested against distance-related hypotheses of genetic structure. Whereas barriers can create geographically circumscribed restrictions to gene flow, diminished gene flow over distance should also result in genetic differentiation among populations (i.e. IBD, isolation by distance: Wright 1943). The dispersal paths of larvae are likely to be strongly influenced by oceanography and thus distance measures based on biophysical models where larval characteristics are combined with oceanographic flows are often chosen to represent contemporary dispersal patterns (White et al. 2010). In the IAA, in fact, modern biophysical distances alone predict many of the commonly observed phylogeographic patterns (Treml et al. 2015b). Biophysical distances can be statistically evaluated against overwater distances with the latter representing a null IBD expectation. Of course, we are not able to predict past (Pleistocene) biophysical distances, however, we can extend overwater distances to an historical framework by altering the coastline to reflect the lowest sea level stands and therefore can approximate Pleistocene larval dispersal routes.

Here, we consider the influence of historical *versus* contemporary factors on the population genetic structure of *T. maxima* and *T. crocea.* Specifically, we evaluate continuous measures of distance against potential barriers to gene flow and also compare historical divergence times across these barriers. Finally, we unite DNA-informed sampling for the recently-resurrected species *T. noae* to expand the knowledge of its phylogeographic structure. To achieve maximal geographic coverage, we combine all available mtDNA COI data from the Indo-Pacific in a single analysis alongside new data, allowing us to place the genetic structure of *Tridacna* from the IAA into the fullest geographic context.

MATERIALS AND METHODS

SAMPLING AND DNA SEQUENCING

Mitochondrial COI sequences for *Tridacna maxima, T. crocea* and *T. noae* were obtained from published (DeBoer et al. 2008, Kochzius and Nuryanto 2008, Nuryanto and Kochzius 2009, Gardner et al. 2012, Huelsken et al. 2013, DeBoer et al. 2014b, Su et al. 2014) and unpublished surveys (full details including permits in supplementary materials). These combined data yield a data set spanning the majority of the species ranges for *T. maxima* and *T. crocea*: analyses focus on these two species. Progress has been made towards clarifying the range of *T. noae* (Huelsken et al. 2013; Borsa et al. 2015b; Johnson et al. 2016), and here we update phylogeographic relationships within this species but do not undertake any quantitative analyses for *T. noae* as the number of sampling locations is insufficient for formal analyses. DNA extractions, PCR amplifications, sequencing and sequence editing follow Huelsken *et al.* (2013), and further details can be found in Supplementary Methods, Appendix S1.

GENETIC DATA ANALYSES

Combining data from multiple sources can require pooling samples from different lab groups that were collected from the same general location. Locations were defined based on geographic proximity: if sampling sites were within 100 km overwater distance from each other, they were combined, provided pairwise Φ_{ST} was not significant between them (See Supplementary Methods, Appendix S1, for details).

Molecular diversity indices were calculated in ARLEQUIN 3.5.1.3 (Excoffier and Lischer 2010), including haplotype diversity (h), the likelihood of identical haplotypes being drawn from a population, and nucleotide diversity (π), the average number of pairwise sequence differences within a population. Genetic differentiation was measured using Φ_{ST}, based on the Tamura-Nei (1993) distance between haplotypes, and by F_{ST} where the number of substitutions between different haplotypes does not affect the statistic. Significance of both Φ_{ST} and F_{ST} was assessed by 10,000 permutations of individual identity with respect to population. COI differentiation between sampling locations was visualised using maximum parsimony median joining haplotype networks using Network 4.611 (Bandelt et al. 1999). Singleton haplotypes were excluded for ease of viewing; this did not change the main structure of the networks.

EFFECTS OF HISTORICAL AND CONTEMPORARY BARRIERS TO GENE FLOW

Four barrier locations were tested for their effects on genetic divergence within *T. maxima* and *T. crocea*: two intermittent Pleistocene land barriers, the Sunda Shelf (1, hereafter SS) and the Torres Strait land bridge (2, hereafter TS) along with two putative contemporary oceanographic barriers, the western (3, hereafter HE_{west}) and eastern (4, hereafter HE_{east}) limits of the Halmahera Eddy (Fig. 1). We estimated the age of divergence between population pairs spanning each barrier and also tested for significant migration using the coalescent method implemented in IMA 2.0 (Hey and Nielsen 2007). Pilot runs were used to select appropriate priors and heating schemes along with stable and mixed run lengths. Final searches used a burnin of 300,000 Markov Chain steps followed by 200,000 steps sampled every 20 steps (representing a total of 50 million steps per search across 100 chains). All resultant ESS values were greater than 1000. Searches were undertaken both allowing migration and with no migration (isolation model). Divergence times were translated into approximate chronological time using the calibration points from Crandall et al. (2012) for *T. crocea*.

MEASURES OF DISTANCE

To investigate the effects of geographic separation on populations of giant clams (i.e. isolation by distance), we assessed the explanatory power of five continuous measures of distance. The first three measures were derived from the probability of contemporary larval dispersal from one sampling site to another as modelled using an advection-transport approach (Treml et al. 2012, hereafter LD model). See Supplementary Methods in Appendix S1 and Treml et al (2012) for details of this model. Because LD model predictions arising from direction movements are asymmetrical and genetic distances are symmetrical, we summarized LD predictions using minimum (1), maximum (2) and mean (3) dispersal distances. The fourth distance measure was based on the modern overwater distance, which was calculated using a cost distance analysis in ArcMap 10 (ESRI, Redlands, CA) where the least cost path between sampling sites was forced around land (hereafter Mod o/w). The fifth distance measure was based on overwater distance during the LGM (hereafter LGM o/w) and was estimated using the same technique but with the land boundary shifted to the 120 metre isobath. Because most present-day locations would have been dry land at low sea level stands, we moved the site to the closest cell connected to the ocean, provided this did not put the site on the other side of an existing landmass. These five predictors of isolation span a gradient from contemporary (LD model) to historical (LGM o/w). To put the relationship between these measures of geographic distance and estimates of population genetic structure into context of traditional isolation-by-distance where distances are univariate predictors of genetic differentiation, we undertook a series of standard Mantel tests involving all populations for each of the five distance predictors in turn.

MULTIVARIATE MODELS ASSESSING THE RELATIVE EFFECTS OF DISTANCE AND BARRIERS

In order to evaluate the effect on population structure of both geographic distance and barriers, we used distance-based redundancy analysis (dbRDA: Legendre and Anderson 1999) using the *capscale* function in the R package 'vegan' (Oksanen et al. 2008). In dbRDA, the genetic distance matrix (values of Φ_{ST} or F_{ST} between population pairs) is ordinated to yield population values along orthogonal eigenvectors and these vectors serve as the response variables in a redundancy analysis (RDA). Further ordination in the dbRDA reduces collinearity among predictors allowing the effects of each predictor variable to be assessed in the context of the others (Borcard and Legendre 2002). Among our predictive variables, the five measures of distance were also initially formatted as distance matrices; here again we used ordination to convert these distance matrices to eigenvectors, choosing two dimensions as a reasonable representation of locations along the Earth's surface (employing principal coordinate analysis, with the *cmdscale* function in *vegan*). To predict the effects of barriers, we constructed a matrix describing the biogeographic assignment of each population with respect to the barrier locations. All populations on one side of the barrier were coded as 0 and populations on the other side were coded as 1 (i.e. dummy variables). Under this coding scheme, the effects of the Torres Strait could not be differentiated from the effects of either the eastern or western Halmahera Eddy. Thus, for each RDA model we had 13 predictive variables: three representing barriers and 10 representing the two principal coordinates (PCO's) of the five continuous distance measures.

Because initial examination of phylogeographic patterns indicated substantial genetic divisions associated with barriers, we were especially interested in whether isolation-by-distance might predict genetic structure when barriers were also considered. To investigate distance in the context of barriers we first conducted standard dbRDAs with all 13 predictors and used forward model selection based on a null model (intercept only, no predictive variables) to determine the most parsimonious set of predictive variables. This was undertaken using an adjusted R^2 method appropriate for permutated data (Blanchet et al. 2008) with the *ordiR2step* function in *vegan*. Second, we undertook partial dbRDAs where models were conditioned upon barrier variables. The significance of the constraining variables (namely the 10 distance measures) was assessed using ANOVA-like permutations (*anova.cca* function).

RESULTS

GENETIC DATA AND VISUALISATION

We combined 114 novel COI sequences for *Tridacna maxima* (385 base pairs), 78 for *T. crocea* (332 bp), and 19 for *T. noae* (353 bp) with previously published sequence data. The resulting data set comprised 614 sequences for *T. maxima* containing 283 unique haplotypes across 43 populations, 721 for *T. crocea* with 311 unique haplotypes from 46 populations and 58 for *T. noae* with 28 unique haplotypes from five populations (see Table S1 in Appendix S3). In general, the *T. maxima* network showed more sharing of haplotypes across the IAA than *T. crocea* (Fig. 2), although both showed the deep subdivisions characteristic of previous work. *T. noae,* for which phylogeographic information is relatively lacking, showed sharing of regional clades at Tanjung Jerawai, just west of the Halmahera Eddy (Fig. S2 in Appendix S2). The collection of *T. noae* at numerous sites refines the range of this species based on DNA data from that reported in Huelsken et al (2013). The apparent range of *T. noae* includes Taiwan to the north, the Solomon Islands to the east, Ningaloo Reef, western Australia to the south-west and encompasses Indonesia, Papua New Guinea and the Philippines.

EFFECTS OF HISTORICAL AND CONTEMPORARY BARRIERS TO GENE FLOW

For the four barriers tested within the two species, there was no evidence for migration. Initial IMa2 runs showed that zero migration was the highest posterior probability point; thus, for final estimates of divergence, we undertook searches with a prior of $m = 0$. Posterior estimates of divergence times were fairly concordant for TS, SS, and $\mathrm{HE}_{\mathrm{west}}$ with modes of posterior probability for both species from 0.5-2.0 million years for TS and SS and <0.3 million years for $\mathrm{HE}_{\mathrm{west}}$ (Fig. 3). For $\mathrm{HE}_{\mathrm{east}}$, divergence dates were notably different, with <0.3 for *T. maxima* and ~0.5-2.0 million years for *T. crocea*. Together these data suggest that migration has been minimal across all barriers for a long time including the recent past, and that divergences associated with the episodic emergence of land (SS and TS) are quite old.

MEASURES OF DISTANCE

The five measures of geographic distance were highly correlated ($r \geq 0.91$ for both species) and thus cannot be considered as independent predictors of differentiation. Using Φ_{ST} values in Mantel tests, strong and significant relationships of genetic structure with all distance measures were detected for both species ($r \geq 0.56$, $P < 0.001$). Using F_{ST}, there was no IBD association for *T. maxima*, whereas for *T. crocea* there were significant but weak relationships ($r \geq 0.30$, $P < 0.01$). Notably, LGM o/w was the distance metric returning the highest measures of correlation for these univariate analyses (i.e. all Φ_{ST} results and F_{ST} for *T. crocea*).

MULTIVARIATE MODELS ASSESSING THE RELATIVE EFFECTS OF DISTANCE AND BARRIERS

Standard dbRDAs using forward model selection to identify the most important variables yielded quite different outcomes using Φ_{ST} or F_{ST} as the genetic response variable (Table 1). For Φ_{ST}-based analyses, HE_{west}/TS and SS barrier predictors were retained for both species. Additionally for *T. crocea,* HE_{east} and four predictors based on the larval dispersal model were retained. For F_{ST}-based analyses, no variables were retained in forward model selection for *T. maxima*, indicating little improvement in model outcome by the addition of predictive variables as compared to a null intercept model; for *T. crocea* the first PCO of minimal larval dispersal distance (minLD1) was a small but significant predictor of F_{ST} values ($R^2 = 0.039$; $P = 0.015$). Partial dbRDAs, where distance-derived PCO predictors were evaluated conditioned upon barriers, provided largely similar results to standard dbRDAs. The proportion of conditioned inertia for Φ_{ST} models was very high (*T. maxima*: 0.86; *T. crocea*: 0.73) indicating that the majority of variance in Φ_{ST} values among populations was attributable to barriers. Substantially less proportional conditioned inertia was found using F_{ST} (*T. maxima*: 0.02; *T. crocea*: 0.07). The proportion of constrained inertia, representing variance attributable to the distance-derived predictors, was low across all models (≤ 0.22) indicating little influence on variance in population genetic structure and was only significant in the case of Φ_{ST} in *T. crocea* (proportion of constrained inertia = 0.096; $F_{10,40} = 3.31$; $P < 0.001$).

In summary, across RDA analyses for *T. crocea* a modest but statistically significant amount of variance in Φ_{ST} was attributable to two-dimensional aspects of LD (with marginal support for LD explaining variance in F_{ST}). For *T. maxima* there was no evidence that any distance measure explained genetic variance above and beyond the effect of barriers. To illustrate the contrasting relationships with minimum LD for the two species, Fig. 4 shows these relationships for population-pairs not spanning barriers, namely populations between the SS and HE_{west} + TS barriers and east of the HE_{east} + TS barriers. Within *T. crocea*, the positive correlation between minimum LD and Φ_{ST} is evident. For *T. maxima*, there is no simple relationship and the large Φ_{ST} values arise predominantly from comparisons involving KRI, which contains a high proportion of divergent haplotypes (symbolized in black in Fig. 2a). *Post hoc* repeated analyses of the above RDAs and partial RDAs with KRI removed did not change findings.

In all RDA analyses, it is not possible to discriminate the effect of the Torres Strait barrier from effects of the Halmahera Eddy. For example, in the population pair comparison of HLM to SOL (Supp. Fig. 1) all three barriers (TS, HE_{west}, and HE_{east}) arguably separate the two populations. In the dbRDA outcomes, differentiation attributable to the Torres Strait is combined with the HE_{west} measure.

DISCUSSION

Pleistocene land barriers were the strongest predictors of mtDNA differentiation in two species of giant clams. Both of the barriers arising from land exposure during low sea level stands, namely the Sunda Shelf and Torres Strait, were associated with old divergence times (> 0.5 million years, Fig. 3) and were consistent predictors of population structure as estimated by Φ_{ST} (Table 1). In contrast, divergence times across the western and eastern sides of the Halmahera Eddy were not consistent between species (Fig. 3). New samples from Papua New Guinea, along with increased sample sizes from the SW Pacific region (Solomon Islands and Coral Sea), highlight the lack of shared haplotypes (Fig. 2) and hence the substantial divergence times for *T. crocea*, in contrast to more complex mixing associated with the Halmahera Eddy (and therefore more recent divergence estimates) for *T. maxima*. Using dbRDAs, we quantified effects of continuous distance measures while accounting for genetic structure associated with these land and oceanographic barriers: larval dispersal (LD) distances explained a significant proportion of genetic structure in *T. crocea* Φ_{ST} values. Thus, for *T. crocea* there appears to be a subtle effect of contemporary dispersal in addition to the very obvious effects of long-standing historical barriers to larval exchange. For *T. maxima*, however, only historical signals associated with barriers were significant. We also demonstrate sympatry of the two major clades within *T. noae* in eastern Indonesia, a site of overlap in other species. Below, we discuss our findings for *T. maxima, T. crocea* and *T. noae* within the context of previous work in the region.

THE EFFECT OF BARRIERS

Putative barriers were assessed using both coalescent (IMa2) and frequentist, multivariate (dbRDA) approaches. We expected to find a strong signature of divergence associated with historical land bridges at the Sunda Shelf and Torres Strait as reported by previous studies of *Tridacna maxima* and *T. crocea* (Sunda Shelf: DeBoer et al. 2008; Kochzius and Nuryanto 2008; Nuryanto and Kochzius 2009; DeBoer et al. 2014b; Torres Strait: Huelsken et al. 2013). Here, for the first time, we can directly assess the ages and effects of these barriers on population genetic structure. Both barriers have been long-standing impediments to gene flow (Fig. 3) and are significant predictors of population structure (Table 1). Of course, the contemporary structure of currents and resultant dispersal barriers (Treml et al. 2015b) may play a significant role in maintaining this signal of divergence, particularly associated with the Sunda Shelf. This finding is also concordant with there being limited water flow (and therefore also larval dispersal) across the Torres Strait today (Wolanski et al. 2013).

Both the western and eastern edges of the Halmahera Eddy were areas of significant divergence, although with recent age estimates for HE_{west} and varying age estimates for HE_{east} (Fig. 3). These boundaries reinforce previous suggestions that Cenderawasih Bay, which is bounded by both oceanographic barriers and is set back from the open ocean and characterised by strong environmental gradients (DeBoer et al. 2012), encloses a relatively isolated set of sites (DeBoer et al. 2008; Kochzius and Nuryanto 2008; Nuryanto and Kochzius 2009a; DeBoer et al. 2014b). Maintaining genetic isolation between Sulawesi and eastern Indonesia requires limited dispersal across both the Torres Strait and the Halmahera Eddy (both western and eastern boundaries) because individuals could disperse along the northern or southern shores of New Guinea. Neither *T. maxima* clade 4 haplotypes nor clade 3 haplotypes of *T. crocea* were found across the Maluku Sea (i.e. they were absent in locations MAN, SAN, LUW and TOG: Fig. 2 and Fig. S1 in Appendix S2), indicating limits to dispersal in this region, with similar results found for sea stars (Crandall et al. 2014), mantis shrimp (Barber et al 2006), and clownfish (Timm and Kochzius 2008, Timm et al. 2012, Dohna et al. 2015). For such consistent historical signals to be preserved in phylogeographic patterns of co-distributed species implies that contemporary exchange is also minimal.

EFFECTS OF DISPERSAL DISTANCE DIFFER BY SPECIES

Although the strong effect of barriers on genetic differentiation was evident simply from inspecting phylogeographic distributions (Fig. 2), the dbRDA framework allowed us to quantify the added influence of dispersal distances, spanning a continuum from contemporary (LD model) through to historical (LGM o/w) routes. For *T. maxima*, continuous distances were not significant predictors of population structure and indeed there was no evidence for an isolation-by-distance pattern within regions bounded by barriers (Fig. 4). For *T. crocea*, however, LD distances were small but significant predictors of population structure (based on Φ_{ST}). Thus, the biophysical larval dispersal model, based on a combination of oceanography and biological attributes, is a superior predictor of population structure than overwater distance alone. Notably, univariate Mantel tests evaluating dispersal distances in the absence of information regarding barriers, can yield incomplete results: here, LGM o/w distances were the strongest predictors of population structure, undoubtedly because they inherently included barrier effects (i.e. historical distances required to connect populations by traversing around exposed land masses). While high isolation-by-distance correlations using LGM o/w do correctly uncover the very strong effects of Pleistocene events, the predictive ability of the LD model for *T. crocea* is obscured without statistically accounting for barrier effects (here using dbRDA). Thus, joint considerations of multiple predictive variables provide more nuanced results that implicate both contemporary and historical factors.

The contrasting explanatory ability of dispersal distances for explaining genetic differentiation between the two species is concordant with relative dispersal propensity indicated by the patterns of haplotype distribution seen in Fig. 2. Haplotypes of *T. maxima*'s clade 2 are spread throughout the IAA and clade 3 spreads as far as the central Pacific (i.e. no clear relationship exists between genetic identity and geographic distance). Clades within *T. crocea*, in contrast, only mix in north-west Papua where the highly restricted clade 3 mixes with wide-ranging clade 2, suggesting limited larval dispersal over long periods of time. The lack of association between distances and genetic structure in *T. maxima* begs the question: is dispersal easier for *T. maxima*? While difference in pelagic larval duration is minimal between these species (19 days max. for *T. maxima*, 17 days max. for *T. crocea:* Jameson 1976) differences in life history and ecology, such as local abundance, may influence successful dispersal (Treml et al. 2015a). These factors deserve further investigation, but there are few data on life-history characteristics or ecological preferences of wild giant clams, most data coming from aquaculture studies (Jameson 1976, Lucas 1988). Moreover, the recent recognition of *T. noae* as a distinct species raises questions regarding the accuracy of previous surveys based on morphology alone (Johnson et al. 2017).

LOW GENETIC CONNECTIVITY AMONG REGIONS IN THE CENTRAL INDO-PACIFIC

Patterns of genetic structure among the three species were generally concordant and consistent with previous work (Benzie and Williams 1995, 1997, Kittiwattanawong et al. 2001, Juinio-Meñez et al. 2003, Ravago-Gotanco et al. 2007, DeBoer et al. 2008, Kochzius and Nuryanto 2008, Nuryanto and Kochzius 2009, Tisera et al. 2011, Huelsken et al. 2013). Coexistence of divergent clades within all three species, newly shown here for *T. noae*, adds to considerable evidence for eastern Indonesia as a region of overlap between clades in other marine species (Barber et al. 2006, Crandall et al. 2008, Liu et al. 2012). Our addition of data from Papua New Guinea allows us to refine placement of genetic discontinuities within two of these species. Huelsken et al. (2013) reported deep differentiation within *T. maxima* and *T. crocea* between the Solomon Islands and Cenderawasih Bay. With samples from Kavieng in Papua New Guinea, we are able to shift this boundary ~1000 km north-west and clarify the porosity of this disjunction. IAA sampling sites of *T. crocea* do not share haplotypes with those to the east and that these regions are populated by different clades (Fig. 2b). For *T. maxima*, we found shared haplotypes between Halmahera and Cenderawasih Bay and sites in the Coral Sea, Solomon Islands and Papua New Guinea.

Previous work on *T. maxima* identified deep differentiation between the central Pacific and the IAA (Gardner et al. 2012, Hui et al. 2016). By combining all existing data, we find that clade 5 is absent from the Coral Sea, Papua New Guinea and the Solomon Islands. However, we identified two Kiribati individuals of *T. maxima* with a haplotype shared throughout the Coral Triangle and Taiwan (Fig. 2a). The possibility of stepping stone dispersal from the IAA via Micronesia could be tested in the future. Additionally, human assisted translocations of clams (either via Polynesian settlement and trade or modern introductions such as associated with aquaculture) cannot be ruled out. Future studies employing multiple independently assorting markers would be useful for exploring these competing scenarios and dating divergence times.

MTDNA ENABLED DATA SYNTHESIS

Given the scale and logistical difficulties associated with working in the Indo-Pacific, most research includes a limited set of locations, either densely packed or widely spaced (Keyse et al. 2014). The present study, in the vein of recent studies taking a synthetic approach to existing DNA sequence data (Vogler et al. 2013, Crandall et al. 2014, Selkoe et al. 2014), would have been impossible without the cooperation of multiple researchers. The shortcomings of single locus mtDNA studies are well known: inability to 1) estimate evolutionary variance from a single non-recombining locus; 2) leverage assignment methods to identify contemporary structuring or migration; 3) detect hybridisation and introgression, and so forth. Despite these restrictions, however, DNA sequence data enables synthesis and collaboration among research groups; here, we have been able to span a significant portion of the extensive geographic ranges of three co-distributed species by combining data to make new inferences regarding both historical and contemporary influences on phylogeographic structure.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Supplementary Methods and Results.
Appendix S2. Figures S1 and S2.
Appendix S3. Supplementary Tables S1-S3.
Appendix S4: Arelequin files for each species.

Biosketch: Jude Keyse is interested in the factors affecting the movement and accumulation of biodiversity in the world's oceans. Her work spans the fields of population genetics and ecology. She recognises the benefits of genetic tools to reveal hidden patterns and believes that genetic diversity data are under-used in studies mapping diversity.

Data accessibility: Original sources of sequence data are reported in Supplementary Tables S1-S3. New sequences arising from the present study have been deposited in NCBI (MG385266 - MG385538) and associated sampling metadata are recorded in GeOMe (Deck et al. 2017: www.geome-db.org). ARLEQUIN formatted infiles for each species are provided in Appendix 4.

TABLES

Table 1. Distance-based redundancy analyses evaluated for the giant clams *Tridacna maxima* and *Tridacna crocea*: final models following forward selection whereby biogeographic barriers (Sunda Shelf: SS; Halmahera Eddy western edge: HE_{west}; and Halmahera Eddy eastern edge HE_{east})[a] and principal coordinates of dispersal distances[b] were the predictive variables evaluated.

Species	**Genetic distance**	**Variables retained**[c]	**Adj R^2**[d]	**P**[d]
T. maxima	Φ_{ST}	HE_{west}/TS, SS	0.917	<0.001
T. maxima	F_{ST}	-	0	NS
T. crocea	Φ_{ST}	HE_{west}/TS, HE_{east}, minLD2, maxLD1, SS, meanLD2, meanLD1	0.851	<0.001
T. crocea	F_{ST}	minLD1	0.039	0.015

[a] The influence of the Torres Strait (TS) barrier is combined with HE_{west}. See text for explanation.

[b] Two orthogonal PCOs were created for each of five distance measures, where meanLD1 and meanLD2 are the first and second PCO's of the mean larval dispersal distance and minLD1 is the first PCO of the minimum larval distance.

[c] In order of influence.

[d] For the whole model.

FIGURE LEGENDS

Figure 1. Indo- Australian archipelago and nearby locations in the Indo-Pacific Ocean region. Light grey outline indicates boundary of larval dispersal model. Dark grey outline indicates land area at last glacial maximum. Grey arrows indicate ocean currents (after Gordon, 2005). Putative barriers to dispersal are shown with thick lines and labelled SS: Sunda Shelf, $\mathrm{HE}_{\mathrm{west}}$: Halmahera Eddy western edge, $\mathrm{HE}_{\mathrm{east}}$: Halmahera Eddy eastern edge and TS: Torres Strait.

Figure 2. Sampling sites, haplotype frequencies, and maximum parsimony haplotype networks for *Tridacna maxima* (a) and *Tridacna crocea* (b). On the map, the light grey outline indicates the boundary of the larval dispersal model; dark grey outlines indicate land area at last glacial maximum. Network circles represent unique mtDNA COI haplotypes, sized by frequency. Numbers on edges indicate number of base pair differences between haplotypes. Haplotype frequencies based on clade identity are shown by pie graphs. Red haplotypes are from the Red Sea.

Figure 3. Posterior probabilities of divergence times across boundary regions for *Tridacna maxima* (a) and *Tridacna crocea* (b) based on IMA2. Boundaries are labelled: SS: Sunda Shelf, $\mathrm{HE}_{\mathrm{west}}$: Halmahera Eddy western edge, $\mathrm{HE}_{\mathrm{east}}$: Halmahera Eddy eastern edge and TS: Torres Strait.

Figure 4. Isolation by distance patterns within regions for *Tridacna maxima* (a) and *Tridacna crocea* (b) using minimum distance predicted from the larval dispersal model. Light grey dots represent population pairs within the central Coral Triangle, bounded by the Sunda Shelf and Halmahera Eddy western edge. Black dots represent population pairs from the western Pacific, bounded by the Torres Strait and Halmahera Eddy eastern edge.