Journal of Biogeography **WILEY**

First come, first served: Possible role for priority effects in marine populations under different degrees of dispersal potential

Christiaan A. de Leeuw[1] [ID] | Katja T. C. A. Peijnenburg[2,3] [ID] | Rosemary G. Gillespie[4] [ID] | Diede L. Maas[1] | Naoto Hanzawa[5] | Yosephine Tuti[6] | Abdul Hamid A. Toha[7] [ID] | Ludi Parwadani Aji[1,6] | Leontine E. Becking[1,2,4] [ID]

[1]Wageningen University & Research, Wageningen, The Netherlands

[2]Naturalis Biodiversity Center, Leiden, The Netherlands

[3]Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Amsterdam, The Netherlands

[4]Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA, USA

[5]Faculty of Science, Yamagata University, Yamagata, Japan

[6]Indonesian Institute of Sciences, Research Centre for Oceanography, Jakarta, Indonesia

[7]Department of Fisheries, Faculty of Fisheries and Marine Sciences, University of Papua, Indonesia

Correspondence

Christiaan A. de Leeuw and Leontine E. Becking, Department of Marine Animal Ecology, Wageningen University & Research, Droevendaalsesteeg 1, 6708PB Wageningen, The Netherlands.
Emails: christiaan.deleeuw@wur.nl (C.A.L.) and lisa.becking@wur.nl (L.E.B.)

Abstract

Aim: Studying clearly delineated populations in marine lakes, islands of sea, we investigated the interplay of habitat size, dispersal potential, and priority effects in shaping marine population genetic structure.

Location: Marine lakes and coastal locations in Indonesia, Palau, Papua New Guinea and Australia.

Taxon: Mussels (Mytilidae, *Brachidontes* spp.)

Methods: Populations were sampled from four coastal locations and 22 marine lakes of similar age (~8,000 years), yet differing in size (0.04–4.7 km^2) and degree of connection to the adjacent sea. While some lakes are highly connected, allowing potential influx of larvae from the sea, others have very limited water exchange. We assessed the phylogeographical structure and demographic history using mitochondrial and nuclear DNA sequence data, and combined this with geometric morphometrics. The effects of lake characteristics on population genetic diversity and structure were tested using linear regression and Mantel tests.

Results: Each lake contained one of six distinct genetic lineages, which were characterized by deep phylogenetic splits and significant morphometric differences. These lineages likely represent separate species. The lineages showed similar demographic patterns, with lakes containing founder populations that rapidly expanded and diverged. Genetic diversity within lake populations was significantly correlated with lake area, but not with physical connection to the adjacent sea. Within lineages that occurred in multiple lakes there was strong population structure (average Φ_{ST} 0.65), which did not conform to an isolation-by-distance pattern or to the degree of dispersal potential.

Main Conclusions: Marine lakes across a gradient of physical isolation show strong population structure and evidence for in situ divergence. We hypothesize that the observed genetic structure is the result of priority effects. In addition, reduction of

habitat size appears to reduce genetic diversity, even at very small spatial scales. Our findings are relevant in the context of ongoing alterations to coastal hydrodynamics, which lead to habitat reduction and influence migration among populations at fine spatial scales.

KEYWORDS

anchialine ecosystems, *Brachidontes* mussels, coral triangle, isolation-by-distance, marine biodiversity, phylogeography

1 | INTRODUCTION

The Coral Triangle (located between the Philippines, Indonesia and Papua New Guinea) houses the global maximum of marine biodiversity (Hoeksema, 2007; Roberts et al., 2002), yet the origins of this high diversity remain unresolved. Phylogeographical studies have shown that there are high levels of genetic structuring in populations of many marine taxa in the Coral Triangle, even at scales of less than 100 km (e.g. Kochzius & Nuryanto, 2008; Tornabene, Valdez, Erdmann, & Pezold, 2015). These observations indicate that isolating mechanisms over small spatial scales may contribute to the patterns of high global marine diversity in the Coral Triangle. However, barriers to dispersal in the sea and the timing of population divergences are elusive (Bowen et al., 2016; DeBoer et al., 2014; Peijnenburg & Goetze, 2013). Genetic breaks in the Coral Triangle are often considered to be linked to intermittent periods of isolation caused by Pleistocene glacial cycles, which resulted in multiple smaller basins and heterogeneous environments (e.g. variation in temperature, salinity and pH) (e.g. DeBoer et al., 2014; Hoeksema, 2007). Furthermore, multiple marine barriers related to ocean currents have been proposed (Ayre, Minchinton, & Perrin, 2009; Barber, Palumbi, Erdmann, & Moosa, 2000; Bowen et al., 2016; Peijnenburg & Goetze, 2013). It has also been hypothesized that priority effects combined with rapid local adaptation may underlie striking patterns of genetic differentiation, as seen in the Coral Triangle (De Meester, Gómez, Okamura, & Schwenk, 2002; De Meester, Vanoverbeke, Kilsdonk, & Urban, 2016). However, this remains to be identified with empirical data. A key issue of the majority of phylogeographical studies in the marine realm is that dispersal barriers and subsequent populations are defined a posteriori, based on the structures found in the molecular data, sometimes combined with computer simulations of ocean currents and larval survival and settlement rates (e.g. Cowen, Paris, & Srinivasan, 2006; Romero-Torres, Treml, Acosta, & Paz-Garciá, 2018; Treml, Roberts, Halpin, Possingham, & Riginos, 2015). Defining populations a posteriori based on genetic data can lead to circular reasoning because the population's identification and subsequent genetic characterization are coupled (Lowe & Allendorf, 2010; Waples & Gaggiotti, 2006). To accurately define populations and the area they reside in, marine biotopes with clear delineations are required.

Marine lakes, islands of sea, provide a setting of clearly defined marine populations residing in basins of different sizes and levels of porosity of landscape barriers, which influence dispersal potential among populations. Marine lakes are anchialine systems, small bodies of landlocked seawater, that are connected to the surrounding ocean only through underground caverns and fissures (Hamner & Hamner, 1998; Holthuis, 1973). Similar to island systems, marine lakes provide discrete and replicated microcosms of biological communities. They are connected to the sea by varying degrees, as reflected by reduced tidal fluctuation in the lakes. At one end of the spectrum are marine lakes that have high water exchange with the adjacent sea. These lakes have physical characteristics and biological communities that resemble lagoons, such as diverse reef fish communities and scleractinian corals. On the other end of the spectrum are highly isolated lakes, with limited water inflow from the sea, containing depauperate species communities. A relatively large number of marine lakes (10s–100 s) are located in Indonesia and Palau (Becking et al., 2011; Dawson, Martin, Bell, & Patris, 2009), and more are being discovered in remote areas of Indonesia (Becking, de Leeuw, & Vogler, 2015). The great majority of marine lakes are less than 50 m deep. Since seawater levels were approximately 110–140 m lower during the Last Glacial Maximum than at present (Geyh, Streif, & Kudrass, 1979; Voris, 2000), these lakes must have been dry or contained fresh water at that time (Dawson, 2006). When sea levels rose, the lakes filled up with seawater, which has been estimated to have occurred around 8,000 years before present for the great majority of lakes (Dawson, 2006; Sathiamurthy & Voris, 2006). Despite being young environments, marine lakes harbour unique, possibly endemic, genetic and species diversity (Becking et al., 2011; Dawson & Hamner, 2005; Maas et al., 2018).

Selection of marine lakes with different sizes, degrees of connection to the adjacent sea and at different spatial scales offers an opportunity to assess the influence of area, geographical distance and porosity of landscape barriers in shaping marine population genetic structure. A key question is whether reductions in area of available habitat at small scales may influence genetic diversity. A larger lake could allow multiple colonization events of haplotypes or divergence of populations following colonization, resulting in a positive interaction between size of the lake and genetic diversity. A decrease in the rate of immigration due to distance or landscape barriers can lead to lower local genetic

diversity, but diminished gene flow can also allow populations to diverge and ultimately form local diversity (Hendry, Bolnick, Berner, & Peichel, 2009; Vellend, 2005). When a lake has a higher connection to the sea, that is, is closer to the source pool, the potential influx of genetically different propagules could be more frequent, resulting in a positive correlation between genetic diversity and the degree of connection. If, however, a reduction in connection causes a time lag in the arrival of immigrants, then priority effects could in theory prevent a correlation between connection and genetic diversity. Priority effects arise when first colonizers have an advantage and can shape subsequent population genetic structure (De Meester et al., 2002; Fukami, 2015; Orsini, Vanoverbeke, Swillen, Mergeay, & De Meester, 2013). Rapid population growth and local adaptation upon colonization of a new habitat could result in the effective monopolization of resources. Following population dominance, fewer genotypes will establish than expected under ongoing immigration, promoting strong genetic differentiation even at fine spatial scales (De Meester et al., 2016). Priority effects are, however, stochastic and therefore hard to prove directly, requiring assessment by a process of elimination.

One of the most dominant invertebrates in marine lakes are mussels of the genus *Brachidontes*, which are found almost ubiquitously in marine lakes in Palau and Indonesia (Becking et al., 2016; Goto, Tamate, & Hanzawa, 2011; Hanzawa et al., 2012; Maas et al., 2018, this study). *Brachidontes* spp. are sessile organisms that disperse through broadcast spawning and have a pelagic larval stage of approximately 3 weeks in the tropics (Monteiro-Ribas, Rocha-Miranda, Romano, & Quintanilha, 2006). Phylogeographical studies of *Brachidontes* populations in marine lakes in Palau (Goto et al., 2011) and Indonesia (Becking et al., 2016; Maas et al., 2018) indicated that there are multiple deeply diverged lineages with significantly different shell-morphology and that these lineages constitute a species complex of at least four undescribed species. Becking et al. (2016) showed that three neighbouring lakes each harboured a different lineage and found possible evidence of local divergence. With respect to the population genetic structure of *Brachidontes* sp., Maas et al. (2018) examined one lineage that inhabited seven marine lakes in Indonesia, which were similar in age and size but varied in environmental regimes and degrees of connection to the sea. The authors hypothesized that even incomplete dispersal barriers may cause sufficient isolation to allow priority effects to influence the population genetic structure.

Given the possible importance of priority effects for low levels of isolation (de Meester et al., 2016; Maas et al., 2018), the current study set out to examine the interplay between dispersal potential, habitat size and priority effects in marine lake populations by significantly increasing the number of sample sites. Here, we compare mussel populations of 22 marine lakes in Indonesia and Palau and 4 coastal locations from Indonesia, Papua New Guinea and Australia. The distance between lakes ranges from 100 to 1,400 km. The lakes are of similar age (~8,000 years), but differ in size (0.04–4.7 km^2) and

degree of connection to the adjacent sea (from high water exchange to very little exchange). By analysing population genetics and geometric morphometrics, we aimed to (a) identify the distribution of lineages of *Brachidontes* mussels in the Indo-Pacific, (b) test whether marine genetic diversity conforms to the prediction of accumulation of diversity with area and (c) assess which levels of dispersal potential can lead to local divergence and structure among populations at small spatial and temporal scales.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Mussels were collected from a total of 26 sampling locations, resulting in a total of 773 samples (Table 1, Figure 1). The present study incorporates 12 new locations and 578 new samples. In all, 22 marine lakes were sampled, eight are located in the Raja Ampat region, West-Papua, Indonesia, three in Berau (East Kalimantan, Indonesia) and 11 in Palau. Four coastal locations are included, which likely represent ancestral populations and provide a baseline for genetic structure: two in Berau (Sea 1 and Sea 2), one in Darwin, Australia (Sea 3) and one in Madang, Papua New Guinea (Sea 4). The samples from Berau and a small part of those from Palau have been analysed previously by Becking et al. (2016, Genbank accession numbers KX346179–KX346214) and Goto et al. (2011, AB465555–AB465572 and AB509361), respectively. In most marine lakes, mussels were found in large abundances, forming dense beds in and beneath the intertidal zone along the rocky shoreline and mangrove roots. Mussels were sampled along the perimeter of the lakes, from multiple beds. Degree of water exchange between the marine lakes and the adjacent sea was assessed by placing a water level logger inside each lake, as well as one just outside in the surrounding sea, during a 48-hr period. The measurements of these Onset® HOBO U20L loggers were converted from pressure (Pa) to depth (m) using Hoboware® Pro 3.7.16 software (Fig. S5). The 'relative tidal amplitude' is calculated by $(Lake_{max}-Lake_{min})/(Sea_{max}-Sea_{min})$, where $Lake_{max}$ and $Lake_{min}$ refer to the maximum and minimum water level in the marine lake during a 48-hr period, and Sea_{max} and Sea_{min} represent the maximum and minimum water level in the adjacent open sea during that period. The maximum value of 1 would indicate no or very limited obstruction to water flow in and out of the lake. Conversely, the minimum of 0 would mean that there is no water exchange with the sea.

2.2 | DNA extraction and gene amplification

DNA was extracted from the posterior adductor muscles of the mussels, using the DNeasy Blood&Tissue kit (Qiagen), following the manufacturers protocol. Using gel electrophoresis, DNA extracts were viewed under UV light on 1% agarose gel stained with ethidium bromide to check for quality. Subsequently, polymerase

TABLE 1 Sample locations with genetic diversity indices. Per locality is provided: number of individuals sequenced for cytochrome oxidase 1 (N), number of CO1 haplotypes, nucleotide diversity (π), haplotype diversity (h), mean pairwise differences and Tajima's D. SDs are shown in brackets. Tajima's D values are in bold when significant. Several environmental conditions are also shown: Max depth in meters, relative tidal amplitude and surface area (m²). Relative tidal amplitude is defined as the ratio of the maximum tidal amplitude in a lake divided by the maximum tidal amplitude of the adjacent open sea. A higher ratio indicates more water exchange with the sea, the maximum value of 1 would indicate an identical tidal regime to the surrounding sea

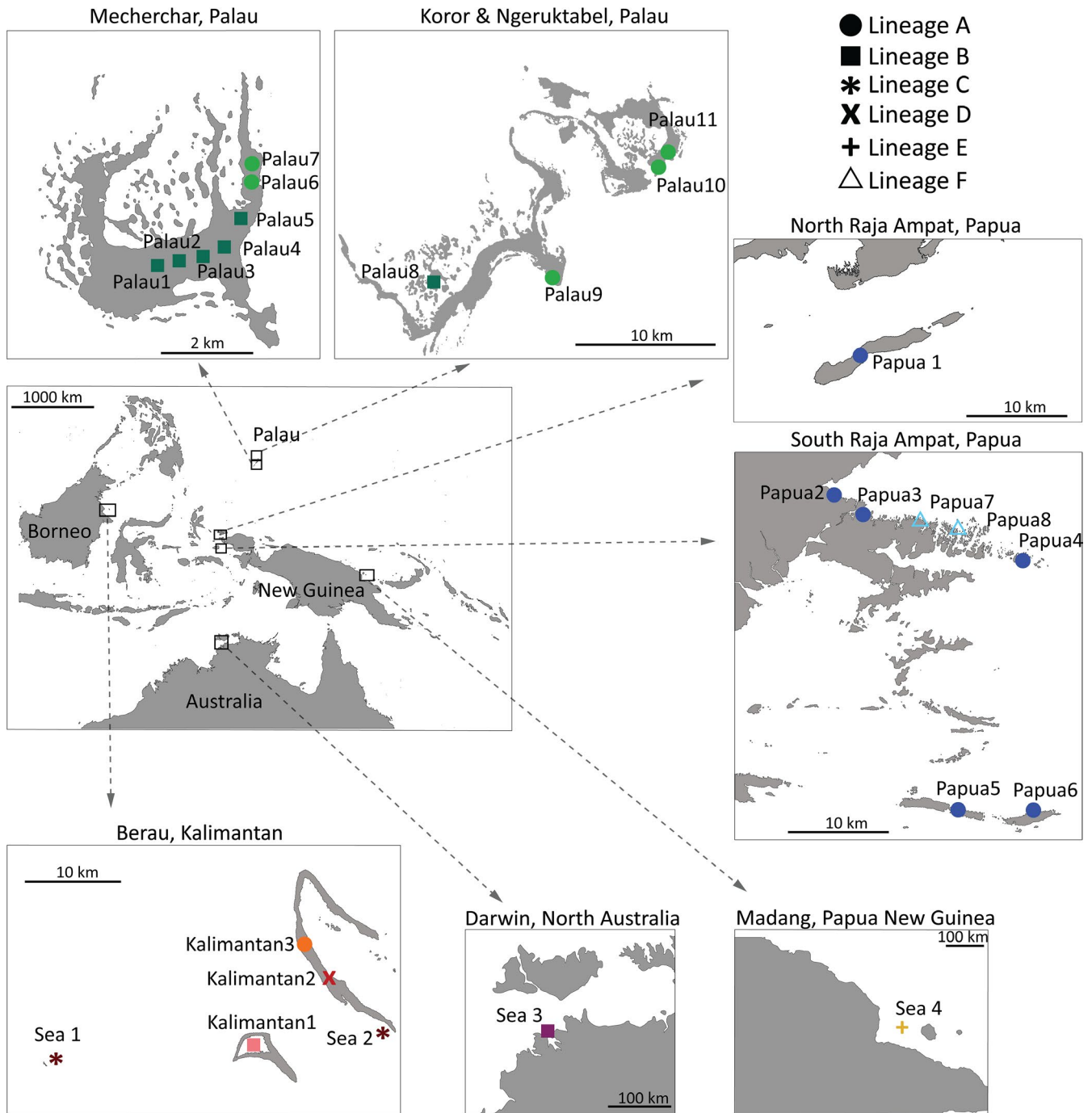| Code	Location	N	#CO1	π % (SD)	h (SD)	Mean pairwise differences (SD)	Tajima's D	Max depth (m)	Relative tidal amplitude	Surface area m²
Coastal										
Sea3	Madang, Papua New Guinea	30	10	0.27 (0.19)	0.67 (0.092)	1.4 (0.89)	−1.58	NA	—	—
Sea1	Berau, East-Kalimantan	15	9	0.48 (0.30)	0.88 (0.061)	2.5 (1.4)	−1.45	NA	—	—
Sea2	Berau, East-Kalimantan	7	5	0.72 (0.47)	0.86 (0.14)	3.7 (2.1)	−1.32	NA	—	—
Sea4	Darwin, Australia	8	6	2.5 (1.5)	0.93 (0.084)	13 (6.6)	0.159	NA	—	—
Lake										
Kalimantan1	Berau, East-Kalimantan	34	11	0.21 (0.16)	0.62 (0.096)	1.1 (0.74)	**−1.87**	12	0.2	466.8 × 10⁴
Kalimantan2	Berau, East-Kalimantan	25	10	0.33 (0.22)	0.73 (0.093)	1.7 (1.0)	**−2.13**	17	0.9	16.3 × 10⁴
Kalimantan3	Berau, East-Kalimantan	21	3	0.070 (0.079)	0.34 (0.12)	0.36 (0.36)	−0.804	17	0.75	10.3 × 10⁴
Papua1	Mansuar, West-Papua	46	12	0.15 (0.12)	0.55 (0.089)	0.76 (0.57)	**−2.17**	7	0.1	7.7 × 10⁴
Papua2	Misool, West-Papua	32	6	0.98 (0.54)	0.67 (0.074)	5.1 (2.5)	1.85	7	0.15	2.0 × 10⁴
Papua3	Misool, West-Papua	18	6	0.25 (0.18)	0.78 (0.066)	1.3 (0.84)	−0.875	8	0.5	1.7 × 10⁴
Papua4	Misool, West-Papua	15	4	0.078 (0.085)	0.37 (0.15)	0.40 (0.39)	**−1.68**	8	0.83	1.2 × 10⁴
Papua5	Misool, West-Papua	25	2	0.078 (0.083)	0.080 (0.072)	0.40 (0.38)	−1.99	20	0.37	0.6 × 10⁴
Papua6	Misool, West-Papua	37	3	0.021 (0.039)	0.11 (0.068)	0.11 (0.18)	**−1.49**	5	0.81	0.3 × 10⁴
Papua7	Misool, West-Papua	16	3	0.094 (0.095)	0.34 (0.14)	0.48 (0.44)	**−0.45**	12	NA	0.9 × 10⁴
Papua8	Misool, West-Papua	16	2	0.045 (0.062)	0.23 (0.13)	0.23 (0.29)	**−1.35**	NA	NA	0.2 × 10⁴
Palau1	Palau	46	11	0.13 (0.11)	0.49 (0.091)	0.65 (0.51)	**−2.195**	30	0.2	5.1 × 10⁴
Palau2	Palau	38	4	0.031 (0.048)	0.15 (0.078)	0.16 (0.22)	**−1.72**	14	0.25	1.3 × 10⁴
Palau3	Palau	40	3	0.038 (0.054)	0.19 (0.081)	0.20 (0.25)	−1.1	22	0.2	8.2 × 10⁴
Palau4	Palau	42	6	0.056 (0.067)	0.23 (0.086)	0.29 (0.31)	**−1.9**	20	0.33	3.5 × 10⁴
Palau5	Palau	36	5	0.12 (0.11)	0.53 (0.72)	0.61 (0.49)	−0.918	32	0.33	6.3 × 10⁴
Palau6	Palau	36	3	0.23 (0.17)	0.11 (0.070)	1.2 (0.78)	**−1.77**	4	NA	0.4 × 10⁴
Palau7	Palau	37	1	0	0	0	0	4	NA	0.9 × 10⁴
Palau8	Palau	38	5	0.13 (0.11)	0.43 (0.087)	0.66 (0.52)	**−1.7**	4	NA	1.2 × 10⁴
Palau9	Palau	38	3	0.030 (0.048)	0.15 (0.077)	0.16 (0.22)	−1.3	5	NA	0.5 × 10⁴
Palau10	Palau	38	4	0.16 (0.13)	0.45 (0.078)	0.83 (0.61)	0.33	15	0.3	2.1 × 10⁴
Palau11	Palau	39	11	0.31 (0.21)	0.56 (0.095)	1.6 (0.97)	**−2.1**	38	0.25	4.4 × 10⁴

FIGURE 1 Sample locations of mussels: centre left map is an overview of Indonesia, Papua New Guinea, northern Australia and Palau. The seven smaller maps show a detailed view of sampling locations, grey indicates land and white indicates sea. Note that scale is different in each map. Locations are indicated with symbols correlating to the genetic lineages found in the CO1 phylogeny (see legend and Figure 2). Symbol colours correspond to the CO1 haplotype networks in Figure 2

chain reaction (PCR) primers were used to partially amplify the informative marker cytochrome oxidase subunit one (CO1, 516bp). Primers designed by Goto et al. (2011) specifically for the female type (F-type) (CB1F 5′-GTGTTAAGCCTGAGATGTTG-3′, CO1R 5′-CCACCWCCRATAGGRTCA-3′) were used initially. The jgLCO/HCO primers from Geller, Meyer, Parker, and Hawk (2013) for marine invertebrates (jgLCO1490 5′-TITCIACIAAYCAYAARGAYATTGG-3′,

jgHCO2198 TAIACYTCIGGRTGICCRAARAAYCA) were used for samples that did not successfully amplify with the Goto primers. PCR reactions for both primers were performed in 25 µl volumes containing 15.25 µl ddH2O, 1 µl 25 mM MgCl$_2$, 2 µL dNTPs (1 mM each), 2.5 µl 10× PCR Buffer (Qiagen), 1 µl of both primers (10 µM), 0.25 µl Taq polymerase (Qiagen, 5 units/µl) and 2 µl template DNA. Amplification was performed through an initial denaturation step
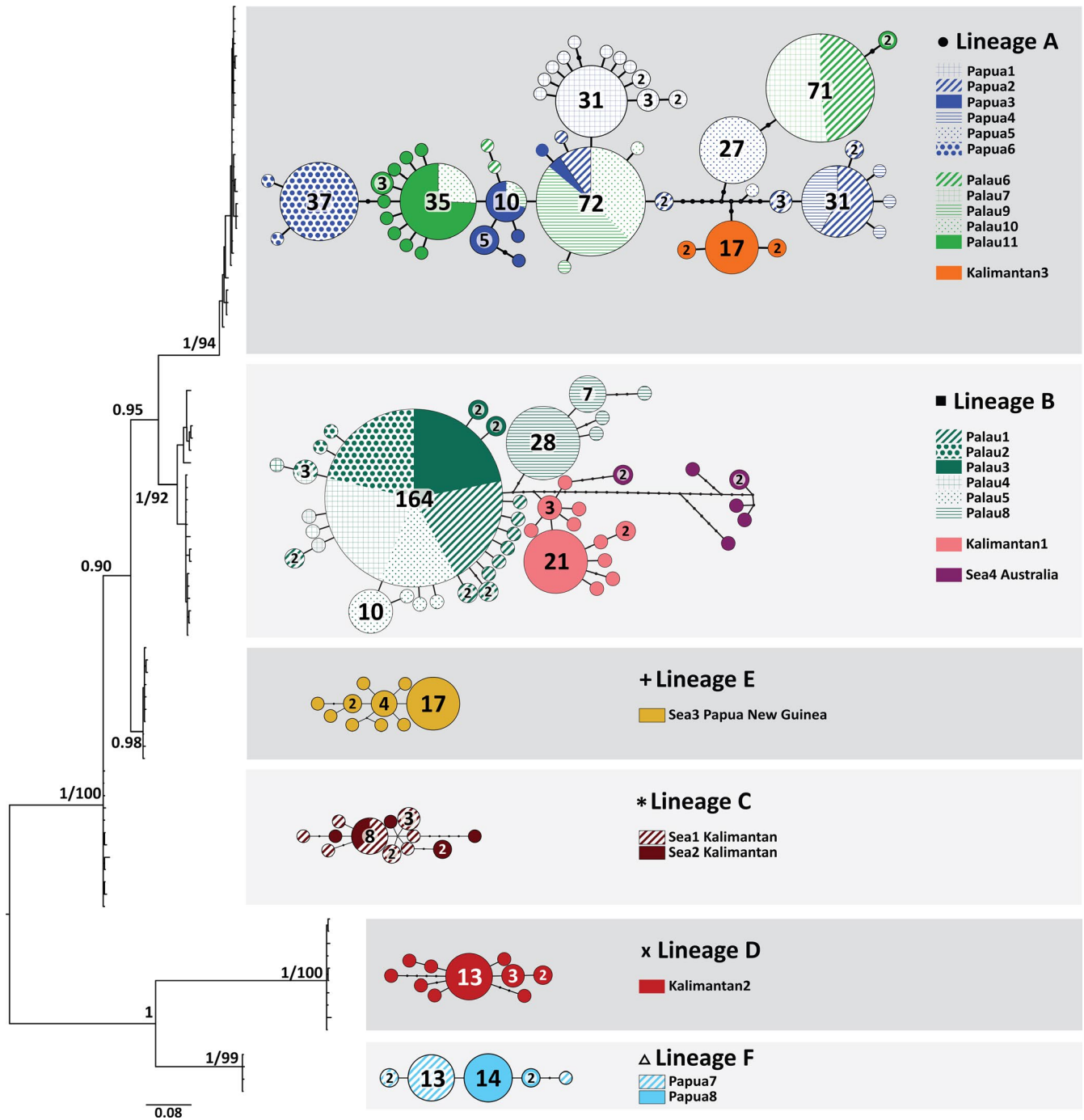
FIGURE 2 Midpoint rooted phylogeny of mussels based on 516 base pairs of cytochrome oxidase 1. Posterior probabilities (>0.9) as well as maximum likelihood bootstrap values (>70%) are shown for all major splits. For each lineage, a haplotype network with haplotype frequencies is shown. Haplotypes are connected by single mutations and black dots denote missing haplotypes. Within lineages, haplotypes are coloured according to sampling region. Patterns differentiate sampling locations within regions. Across lineages, sampling regions are coloured with different hues of the same colour: Kalimantan = red, Palau = green, Papua = Blue, Papua New Guinea (PNG) = Yellow, Northern Australia = Purple. All colours and symbols correspond to Figure 1

of 95°C, followed by 35 PCR cycles of 95°C for 30 s, 48° for 30 s and 72°C for 45 s, and a final extension step of 72°C for 5 m.

To verify different lineages, for a subset of two individuals per lineage, we amplified the large ribosomal subunit (28S), of 976 bp (aligned length), and the small subunit rRNA (18S), of 1,323 bp

(aligned length), using the primers D23F/D6R (Park & Foighil, 2000) and 22F/1789R (Medlin, Elwood, Stickel, & Sogin, 1988), respectively. PCR products were purified and sequenced in both directions, using PCR primers, by Macrogen Inc. (Korea and The Netherlands).

2.3 | Sequence assembly and alignment

Forward and reverse sequences were aligned and edited using CODONCODE aligner 5.1.5 (CodonCode Corporation), using the Muscle algorithm (Edgar, 2004). Chromatograms of the resulting alignments were checked by eye and primer ends were trimmed. The bivalve origin of the obtained sequences was verified through BLAST searches (http://blast.ncbi.nlm.nih.gov). To create a second dataset with only unique sequences, the final alignment was collapsed using DAMBE 5.5.29 (Xia, 2013). Many bivalve molluscs, including *Brachidontes* spp., have a sperm-transmitted mitochondrial genome (M), along with the standard egg-transmitted one (F). This system of mtDNA transmission is called doubly uniparental inheritance (DUI; Zouros, Oberhauser Ball, Saavedra, & Freeman, 1994). M-type sequences resulting from DUI were filtered out using methods described in Goto et al.(2011) and Becking et al. (2016), and subsequent analyses were focused on F-type sequences only. Genbank numbers of Mytilidae samples that were used in the analysis are shown in Figures S1–S3.

2.4 | Model selection, maximum likelihood and Bayesian inference

To classify the different lineages, phylogenetic trees were made. The best-fit DNA substitution model was estimated under the Akaike information criterion, using JMODELTEST 2.1.6 (Darriba, Tab oada, Doallo, & Posada, 2012) for each of the genetic markers (CO1, 18S and 28S). The best-fit model for each dataset was employed to construct a maximum likelihood tree using MEGA 6.06 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013), with a 1,000 replicate bootstrap analysis to test the support of phylogenetic clades. For the CO1 data, Bayesian inference methods using MRBAYES 3.2.5 (Ronquist & Huelsenbeck, 2003) were applied under the same model. Two runs with four metropolis-coupled chains each were run, until the *SD* of split frequencies dropped below 0.01 (1.5 million generations). The resulting phylogenetic trees were drawn with FIGTREE 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Net nucleotide divergence (Nei & Li, 1979) between the main lineages in the resulting phylogenetic tree were calculated in MEGA 6.06, using the model with the best fit available (Tamura & Nei, 1993) and 1,000 bootstrap replicates to compute *SE*s. To create haplotype networks for each lineage, a separate maximum likelihood analysis was run for the samples of each lineage. The resulting phylogenetic trees were used as input to create haplotype networks using haploviewer (Salzburger, Ewing, & Von Haeseler, 2011).

2.5 | Morphometric analysis

To identify morphospecies, geometric morphometric analyses were processed for a total of 419 digital images of 13 mussel populations in Indonesia (lineage A–D, F). The shells of lineage E were not available for analysis. The analyses were performed following the procedure described in Becking et al. (2016). Briefly, mussels were positioned in a standardized way and photographed. Shell outlines were drawn via curves starting from the beak of the mussel using tpsDig (Rohlf, 2010). Curves were converted to semi-landmarks and standardized for size and orientation in tpsRelw (Rohlf, 2010). We obtained centroid size and relative warp scores for each specimen. Furthermore, repeatability was confirmed for the first three relative warp axes; thus, subsequent analyses were performed only on the first three axes. To study differences among groups, we performed a nonparametric permutational multivariate analysis of variance (PERMANOVA, 1,000 permutations) implemented in PAST 2.11 based on Euclidean distances (Hammer, Harper, & Ryan, 2001). We corrected for multiple testing by calculating Bonferroni-corrected *P* values.

2.6 | Genetic diversity and demographic history

Genetic diversity was estimated using haplotype (h) (Nei, 1987) and nucleotide (π) (Tajima, 1983) diversities and mean pairwise differences in ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010) for each sampling location. To assess recent demographic histories, two methods were used to test the data for signatures of recent population expansions. First, Tajima's *D* (Tajima, 1989) was calculated, with significance determined by 10,000 random permutations using Arlequin. A significantly negative Tajima's *D* is consistent with recent population expansion, or a selective sweep (Tajima, 1989). Second, mismatch distributions were calculated. For each sampling location, the observed distribution of pairwise differences between sequences was compared with a theoretical distribution, as expected under a sudden expansion model (Rogers & Harpending, 1992). This theoretical distribution was computed in DnaSP (Librado & Rozas, 2009) with values for τ and θ calculated and tested in Arlequin. Harpending's raggedness index (rg; Harpending, Sherry, Rogers, & Stoneking, 1993) was determined as well. This is a measure for the smoothness of the observed mismatch distribution, which can be used to distinguish between expanded and stationary populations. The value of the raggedness index will be low and non-significant in expanding populations, while it is usually high and significant in stationary populations (Harpending, 1994; Harpending et al., 1993).

2.7 | Population structure of lineage A

To investigate the population structure within lineage A, pairwise Φ_{ST} values, which include information on haplotype frequency and genetic distances, were calculated among the marine lakes using the Tamura–Nei substitution model (in Arlequin). The significance was estimated from 10,000 permutations among pairs of populations. To visualize the genetic differences between populations, principal coordinate analysis (PCoA) was conducted on the Φ_{ST} dissimilarity matrix, as implemented in R (VEGAN package, Oksanen et al., 2012).

We transformed the Φ_{ST} values to calculate pairwise genetic distance between populations ($\Phi_{ST}/(1 - \Phi_{ST})$) (Slatkin, 1995) and correlate this with geographical distance. The geographical distance matrix was calculated using lake coordinates and determining the minimum pairwise geographical distances between lakes via the R function *distm* implemented in the geosphere package (Hijmans, 2016). We used Mantel tests (Mantel, 1967; Slatkin, 1993) to assess the correlation between genetic and geographical distances. The test was run with 1,000 permutations, as implemented in the function *mantel* (VEGAN package). A correlation of *r* > 0.6 was considered strong, and significance was assigned when the *P* value was smaller than 0.05.

3 | RESULTS

3.1 | Phylogenetic lineages

A total of 773 COI sequences of 516 base pairs were collected, resulting in 132 F-type haplotypes (Genbank accession numbers MN578257–MN579037). No indels or stop codons were detected in the sequences. Phylogenetic analysis shows six main lineages (A–F, net nucleotide distance 9.3%–52%), which are supported by both maximum likelihood analysis and Bayesian inference (Fig. S1). Lineages A–D correspond to lineages found earlier in marine lakes in Palau and Indonesia by Goto et al. (2011) and Becking et al. (2016). Two new lineages were found in this study (E and F). The lineages are divided into two well-supported clades, with the first clade containing lineages A, B, C, E and the second D and F. A phylogeny including 24 other species of the family Mytilidae reveals that the lineages in the first clade are closely related to species of the genus *Brachidontes*, while the second clade is most closely related to the genus *Mytilus* (Fig. S1). Lineages D and F (clade two) are more diverged from each other (nucleotide divergence 42%, Table S1) than the lineages within the first clade (net nucleotide divergence 9.3%–21%) and likely represent distinct species. Lineages A, B, C and E are relatively closely related, and fit in the *Brachidontes s.s.*

clade as established by Trovant, Orensanz, Ruzzante, Stotz, and Basso (2015) (Fig S1). In contrast, lineages D and F do not fall within the subfamily Brachidontinae and are most closely related to the genus *Mytilus*. Lineages C and E only occur in the sea, A, D, F only in the lakes and lineage B was found in several lakes and a sea location. Each sampling location contained just one genetic lineage (Figures 1 and 2). Lineage A was found in Raja Ampat (six lakes), Palau (five lakes) and Berau (one lake), lineage B in Berau (one lake), Palau (six lakes) and Australia (one coastal location). The other lineages were found in a single sampling region: lineage C and D in Berau, lineage E in Papua New Guinea and lineage F in Raja Ampat.

A subset of individuals representing each lineage was sequenced for the nuclear markers 28S and 18S, which resulted in identical sequences for lineages A, B, C and E (Genbank accession numbers MN579041–MN579048 and MN579038–MN579040). These lineages fall within the clade *Brachidontes* s.s. as established by Trovant et al. (2015). Lineages D and F, from Berau and Misool, respectively, are confirmed by the nuclear markers to be closely related to species from the genus *Mytilus* (see Figures S2 and S3). However, *blastn* searches for CO1, 28S and 18S sequences of lineage D and F did not result in high similarity (all <90%) with any sequences in the genbank database.

3.2 | Geometric morphometric analysis

Geometric morphometric analysis of shell outlines of lineages A, B, C, D and F showed results consistent with the genetic divergences (Figure 3). Since centroids were correlated to relative warp scores, indicating that there is a length effect, we corrected for this using the residuals of relative warp scores in subsequent analyses. The first three relative warp axes explained 90.37% of the total morphometric variation (52.83%, 32.4% and 5.13%, respectively). Variation in shell outline was found in the position of the umbo relative to the longitudinal axis of the shell (Figure 3, axis 1) and the shell length to width ratio (Figure 3, axis 2). Overall shell shape variation among populations was highly significant
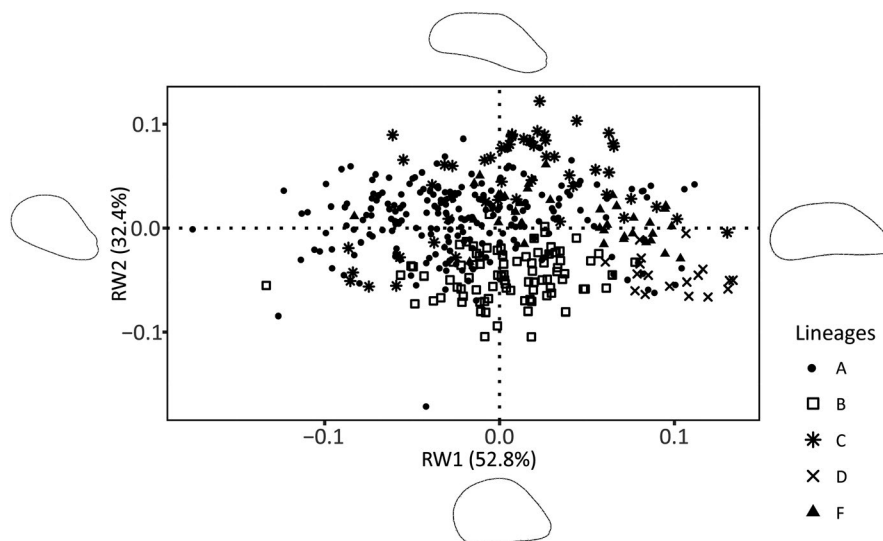

FIGURE 3 Geometric morphometric analysis of shell outlines. Ordination of mussel shell morphology identified by relative warp (RW) axes 1 and 2, together explaining 85.23% of the variance. *F*-values of a one-way PERMANOVA performed on this model are shown in Table S3. All *F*-values are significant (<0.001)
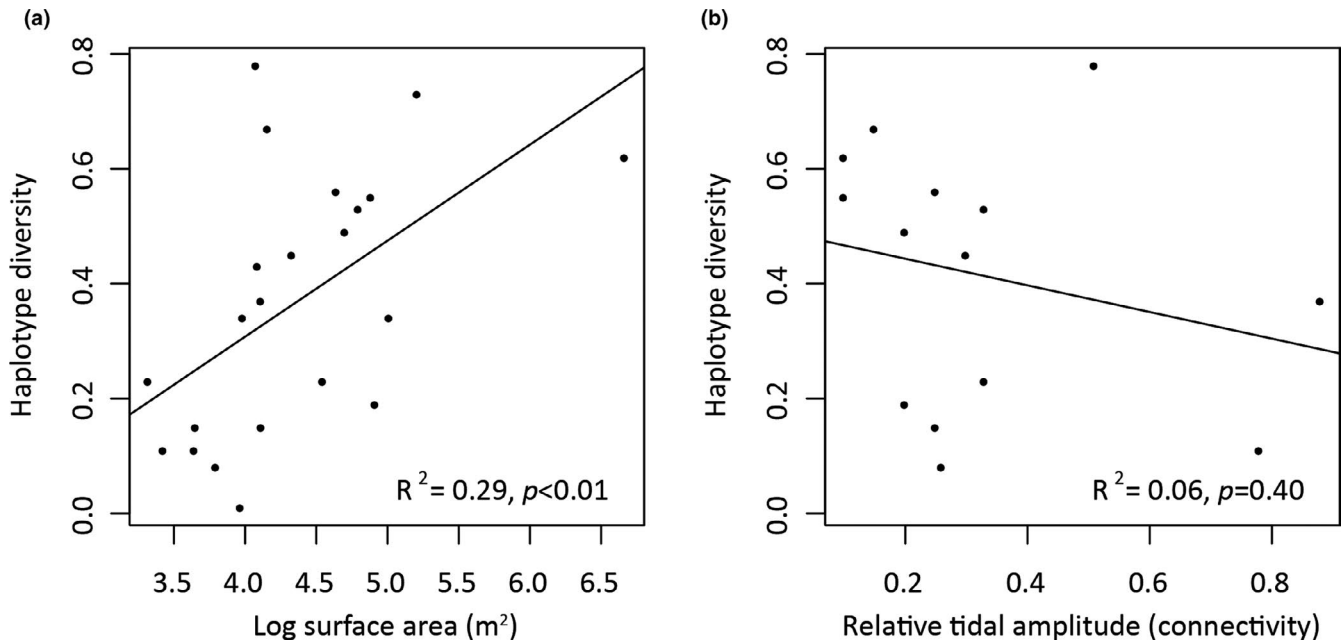
FIGURE 4 Haplotype diversity in relation to (a) area of lake and (b) degree of connection to adjacent sea. Linear regression was used to test effects, values for R^2 and P are shown in the bottom right corner. Relative tidal amplitude (b) is used as a proxy for connectivity with higher ratios (maximum = 1) indicating a higher degree of connection to the adjacent sea

(one-way PERMANOVA, overall F = 20.29, P = 0.0001) and all pairwise comparisons between locations showed significant differences (Table S3).

3.3 | Genetic diversity

Genetic diversity estimates (Table 1) were on average lower in the lakes (π: 0.16% ± 0.20; h: 0.37 ± 0.22; pairwise difference: 0.83 ± 1.0) than in the coastal areas (π: 0.99% ± 0.88; h: 0.84 ± 0.098; pairwise difference 5.2 ± 4.6). There was a significant and positive correlation between haplotype diversity and the surface area of the lake (linear regression R^2 = 0.29, P < 0.01, Figure 4a), yet no correlation with degree of connection of the lake to the adjacent sea (linear regression R^2 = 0.06, P = 0.40, Figure 4b). The relationship between haplotype diversity (H) and $\log_{10}$ transformed lake area (A) can be expressed by the formula H = 0.167A–0.36.

3.4 | Demographic history

The populations in the marine lakes show a genetic signature of recent population expansion or a selective sweep. Fourteen of the 22 marine lakes exhibit significant negative values of Tajima's D (Table 1), while none of the coastal locations do. Furthermore, for the majority of lakes, the haplotype network had a star-like shape, with a frequent dominant haplotype that differed from the other haplotypes in the lake by one or only a few nucleotide substitutions (Figure 2). The coastal locations, conversely, show more evenly distributed frequencies of haplotypes, separated

by multiple nucleotide substitutions. The mismatch distributions were unimodal for the majority of the lake populations, as supported by low and non-significant raggedness index and SSD values (Fig. S4).

3.5 | Population structure

Because of the high divergence between lineages, population genetic analyses were not performed on the complete dataset but only within lineage A, which was most thoroughly sampled (12 populations, n = 389). Within lineage A, a total of 51 haplotypes was found. Haplotype sharing between marine lakes is limited: two haplotypes are shared between Palau and Papua, two within Palau and one within Papua (Figure 2). Estimates of population differentiation between lakes (pairwise Φ_{ST}) range from 0.015 to 0.99 (average 0.64, Figure 5a, Table S2), indicating strong genetic structuring. All the values are significant (P < 0.01), except between Palau6 and Palau7, which share their dominant haplotype (Figure 2) and are on the same island (Mercherchar), less than 500 m apart (Figure 1). Many estimates are close to 1, indicating that marine lake populations are not connected by gene flow. There was no significant correlation between genetic distance and geographical distance (Figure 5b).

4 | DISCUSSION

Marine lakes represent relatively controlled biotopes where each lake can be seen as an independent replicate of eco-evolutionary dynamics
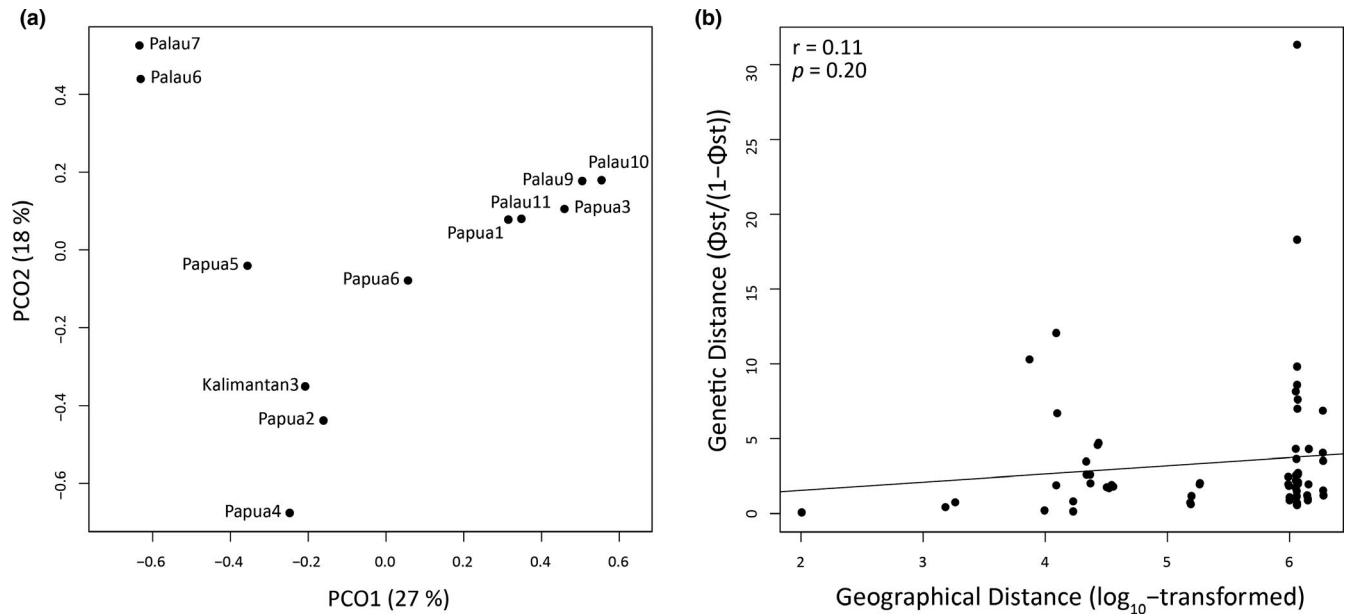
FIGURE 5 Genetic structure of populations of Lineage A from marine lakes and relationship between genetic distance and geographical distance. (a) Principal coordinate analysis ordination of the ΦST distance matrix (based on Table S2). The amount of variation captured per axis is indicated in the axis labels. Distances between points reflect relative genetic distances between populations. Location codes provided in Table 1. (b) Pairwise genetic differentiation [(ΦST/(1−ΦST)] of mussels as a function of $\log_{10}$ transformed geographical distance (m) between marine lakes. The correlation was tested with a Mantel test, values for r and P are shown in the top left corner

over time. By comparing mussel populations in Indonesian and Palauan marine lakes at different spatial scales, and varying in area and degree of connection to the sea, we were able to assess local drivers of genetic diversity and differentiation among marine lake populations. Our study shows that marine genetic diversity conforms to the prediction of accumulation of diversity with area and our results indicate that even a small reduction in area (10s of m^2) can lead to decreased population genetic diversity. On a local scale, we find repeated evidence that even when there is high water exchange, with the potential influx of larvae, there is strong population structure and local divergence of populations. We conclude that a reduction in connection can cause a time lag in the arrival of immigrants, which allows first colonizers to benefit from priority effects and gain a competitive advantage. This leads to an apparently random assignment of colonizing lineages and reduced gene flow between populations. Subsequent local divergence of founder populations results in rapid genetic differentiation.

Six divergent lineages, that likely represent at least six distinct species, were detected based on the genetic and morphological characteristics of mussels found in 22 marine lakes and four coastal locations in the Indo-Pacific. The genetic distances between lineages are larger than previously reported between species in the genus *Brachidontes* (Lee & Foighil, 2004; Sirna Terranova, Lo Brutto, Arculeo, & Mitton, 2007), and comparable to interspecific distances in other bivalves (e.g. Shearer, Oppen, Romano, & Wörheide, 2002). Shell shape was found to be congruent with the pattern of genetic differentiation, with each lineage having distinctly different shell outlines. Morphological variation can be related to underlying genetic variation but can also be a result of phenotypic plasticity and thus reflect differences in environmental regimes (Burridge, Goetze,

Raes, Huisman, & Peijnenburg, 2015; Dawson & Hamner, 2005; Luttikhuizen, Drent, & Baker, 2003; Mariani, Peijnenburg, & Weetman, 2012). In marine lake jellyfish populations, morphological variation was found to correlate with environment rather than genetic lineage (Swift, Gómez Daglio, & Dawson, 2016).

Three similar patterns are observed in the marine lake populations: a single lineage per lake, accumulation of genetic diversity with area and demographic patterns of founder populations that have expanded. The similarity of these patterns found for different lineages and different lakes suggests general processes are at play. First, only one genetic lineage was found in each lake, and even lakes that are less than 5 km apart could harbour highly divergent lineages. We assume that the floodwaters that filled marine lakes during the Holocene sea level rise (<8,000 years before present) allowed for independent colonization of lakes by propagules from the surrounding sea (Dawson, 2006; Dawson & Hamner, 2005). Preliminary studies of sediment cores from the marine lakes indicate that *Brachidontes* was present at the onset of the lakes in Raja Ampat (Klei, 2015; Maas et al., 2018). A variety of taxa in marine lakes (jellyfish, fish, bivalves) show the same consistent pattern of a single genetic lineage per lake (Dawson & Hamner, 2005; Goto et al., 2011; Gotoh, Sekimoto, Chiba, & Hanzawa, 2009; Rose, Masonjones, & Jones, 2016). Inter-lineage competition could explain this pattern, and is expected for closely related species that occupy similar niches. Whichever lineage colonizes a lake first can have a competitive advantage from priority effects, resulting in dominance over any later arriving immigrants (Alford & Wilbur, 1985; Fukami, 2015; Wilbur & Alford, 1985). By significantly reducing successful immigration of other lineages, priority

effects are expected to substantially reduce gene flow. The initial chance arrival of propagules results in a largely stochastic outcome, since whatever lineage colonizes first can proliferate and out-compete any subsequent arrivals (Gillespie & Baldwin, 2009). To strengthen the indirect evidence for priority effects found in the current study, future research should analyse sediment cores to assess shell morphometric changes over time, which can give further insights into colonization history (Cole, 2017; Klei, 2015).

Second, there is an accumulation of genetic diversity with increasing area. It is striking that even a reduction at scales of a few hundred m^2 in marine lakes showed a decrease in haplotype diversity, irrespective of the degree of connection to the adjacent sea. Typically, a larger area will support a bigger population size. Given a set mutation rate, it is expected that genetic diversity will increase with population size (Hague & Routman, 2016; Kimura, 1983). Another possible effect of habitat size is the potential for multiple niches (Warren et al., 2014), which would enhance the maintenance of locally diverged haplotypes.

Third, each lake appears to show the genetic signature of a founder event followed by rapid population expansion (Rogers & Harpending, 1992; Slatkin & Hudson, 1991). Each lake, except Papua 2, shows a single colonization event with a single dominant haplotype and multiple private haplotypes separated by one or a few mutational steps. It is remarkable that of the 51 haplotypes of lineage A, only five are shared by two or more lakes. We therefore suggest this pattern did not result from random redistribution of haplotypes that existed in the sea during the formation of each lake, but that the majority of the within lineage diversity is due to local divergence within the marine lakes during the approximately 8,000 years of their existence. This would correspond to a genetic structure of evolution in peripatry, similar to oceanic islands and satellite lakes of ancient rift lakes (Becking, Cleary, & de Voogd, 2013, Becking et al., 2016; Chen & He, 2009; Dawson & Hamner, 2005; Emerson & Gillespie, 2008; Genner et al., 2007; Gotoh et al., 2011; Goto et al., 2011; Rosindell & Phillimore, 2011).

Moving beyond distinct genetic lineages, we tested to what extent marine genetic differentiation conforms to the decay of population similarity with geographical distance resulting in a pattern of isolation-by-distance (IBD, sensu Wright, 1943) using only lineage A. There was a strong population structure, yet no pattern of isolation-by-distance was observed, indicating that mechanisms other than only dispersal limitation by geographical distance are important in shaping marine lake populations. Maas et al. (2018) did find evidence of isolation by distance in Brachidontes from marine lakes at large spatial scales (1,400 km) (with double-digest restriction-site-associated DNA sequencing). However, similar to the present study, Maas et al. (2018) did not observe IBD patterns on smaller spatial scales (<100 km). As their study was limited to 7 lakes, the statistical power of the current study assessing 22 lakes provides additional evidence of the presence of other dispersal barriers. Phylogeographical studies spanning much larger areas and multiple dispersal barriers in the Coral Triangle show comparable (Kochzius & Nuryanto, 2008; Timm & Kochzius, 2008), or less (Barber, Cheng, Erdmann, & Tengardjaja, 2011; Knittweis, Kraemer, Timm, & Kochzius, 2008) population structure than found in marine lakes.

Depending on the degree of connection to the sea, there is high water flow with potential gene flow into many of the marine lakes in this study. The most isolated lakes have small fissures connecting the lakes to the surrounding sea, which likely obstruct the entrance of larvae. In contrast, the well-connected lakes have short, wide tunnels that presently allow the entry of even large organisms such as adult fish and turtles. These lakes harbour typical reef flat species communities and have similar tidal regimes compared to the adjacent sea, which would indicate the possibility of waterflow as a vector for larvae. Yet, we observed significant genetic structure even in these well-connected lakes. This structure, resulting from genetic drift and/or adaptation, would not be expected under ongoing dispersal. Remarkably, there was no relationship between genetic diversity and the degree of connection to the surrounding sea. Priority effects potentially provide an explanation for the population structure we observe (De Meester et al., 2016; Fukami, 2015). A key element of priority effects is that early-arriving colonizers are able to grow rapidly in population size, thereby substantially occupying or modifying niches before others arrive. As a result, novel migrants are not able to establish. Small passive dispersers with short generation times and high population growth, such as marine mussels, could monopolize resources and, subsequently, habitats (De Meester et al., 2016). Given the high growth rates and fecundity of Brachidontes mussels, a slight barrier to dispersal could be enough for a dominant role of priority effects in shaping populations (Cilia & Deidun, 2012; Fukami, 2015; Morton, 1988).

The scale at which we see structure and divergence is at the level at which decisions are made on marine protected areas (MPAs), policy and licensing (Agardy, di Sciara, & Christie, 2011; McLeod, Salm, Green, & Almany, 2009). Further human disturbances such as habitat degradation and fragmentation, eutrophication and climate change might strengthen priority effects, which can lead to decreased establishment of other genetic variants and species. In addition, insights in how populations respond to these disturbances and how they disperse to colonize new habitats are crucial in times of global climate change and habitat fragmentation. Our combined results are relevant for coastal conservation, where fragmentation, due to human activities, can lead to reduced habitat size and connectivity for marine populations.

On a final note, marine lakes are ecologically valuable systems that are currently not considered in the conservation zoning of the MPAs in Raja Ampat (West Papua, Indonesia) (Maas et al., 2020). Marine lakes are, however, threatened marine ecosystems due to aquaculture and unregulated tourism (Becking et al., 2011; Mangubhai et al., 2012). Some of the lakes from the current study are filled with jellyfish that are gaining the attention of tourists as the region becomes more accessible (Becking et al., 2015; Maas et al., 2020). In Palau, increased tourism pressure has been associated with the collapse of jellyfish populations and the introduction of invasive species (Dawson, Martin, & Penland, 2001). The data of the current study indicate that marine lakes in Raja Ampat contain unique genetic diversity and isolated populations.

PERMITS

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

The authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

All sequences are available on Genbank: accession numbers MN578257–MN579037; MN579038–MN579040; MN579041–MN579048. The morphometric dataset in Dryad, will be available online soon at doi:10.5061/dryad.573n5tb4n.

ORCID

Christiaan A. de Leeuw https://orcid.org/0000-0003-1128-5390
Katja T. C. A. Peijnenburg https://orcid.org/0000-0001-7544-1079
Rosemary G. Gillespie https://orcid.org/0000-0003-0086-7424
Abdul Hamid A. Toha https://orcid.org/0000-0002-4522-6842
Leontine E. Becking https://orcid.org/0000-0002-4584-3912

REFERENCES

Agardy, T., di Sciara, G. N., & Christie, P. (2011). Mind the gap: Addressing the shortcomings of marine protected areas through large scale marine spatial planning. *Marine Policy*, *35*(2), 226–232.

Alford, R. A., & Wilbur, H. M. (1985). Priority effects in experimental pond communities: Competition between Bufo and Rana. *Ecology*, *66*(4), 1097–1105.

Ayre, D. J., Minchinton, T. E., & Perrin, C. (2009). Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? *Molecular Ecology*, *18*(9), 1887–1903.

Barber, P. H., Cheng, S., Erdmann, M., & Tengardjaja, K. (2011). Evolution and conservation of marine biodiversity in the coral triangle: Insights from Stomatopod Crustacea. *Crustacean Issues*, *19*, 129–156.

Barber, P. H., Palumbi, S. R., Erdmann, M. V., & Moosa, M. K. (2000). Biogeography: A marine Wallace's line? *Nature*, *406*(6797), 692–693.

Becking, L. E., Cleary, D. F. R., & de Voogd, N. J. (2013). Sponge species composition, abundance, and cover in marine lakes and coastal mangroves in Berau, Indonesia. *Marine Ecology Progress Series*, *481*, 105–120.

Becking, L. E., de Leeuw, C. A., Knegt, B., Maas, D. L., de Voogd, N. J., Abdunnur, … Peijnenburg, K. T. C. A. (2016). Highly divergent mussel lineages in isolated Indonesian marine lakes. *PeerJ*, *4*, e2496.

Becking, L. E., de Leeuw, C., & Vogler, C. (2015). Newly discovered "jellyfish lakes" in Misool, Raja Ampat, Papua, Indonesia. *Marine Biodiversity*, *45*(4), 597–598.

Becking, L. E., Renema, W., Santodomingo, N. K., Hoeksema, B. W., Tuti, Y., & de Voogd, N. J. (2011). Recently discovered landlocked basins in Indonesia reveal high habitat diversity in anchialine systems. *Hydrobiologia*, *677*(1), 89–105.

Bowen, B. W., Gaither, M. R., DiBattista, J. D., Iacchei, M., Andrews, K. R., Grant, W. S., … Briggs, J. C. (2016). Comparative phylogeography of the ocean planet. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(29), 201602404.

Burridge, A. K., Goetze, E., Raes, N., Huisman, J., & Peijnenburg, K. T. C. A. (2015). Global biogeography and evolution of Cuvierina pteropods phylogenetics and phylogeography. *BMC Evolutionary Biology*, *15*(1), 1–16.

Chen, X. Y., & He, F. (2009). Speciation and endemism under the model of island biogeography. *Ecology*, *90*(1), 39–45.

Cilia, D. P., & Deidun, A. (2012). Branching out: Mapping the spatial expansion of the lessepsian invader mytilid *Brachidontes pharaonis* around the Maltese Islands. *Marine Biodiversity Records*, *5*, e28.

Cole, S. R. (2017). Phylogeny and morphologic evolution of the Ordovician Camerata (Class Crinoidea, Phylum Echinodermata). *Progress in Echinoderm Paleobiology*, *91*(4), 815–828.

Cowen, R. K., Paris, C. B., & Srinivasan, A. (2006). Scaling of connectivity in marine populations. *Science*, *311*(5760), 522–527.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*(8), 772.

Dawson, M. N. (2006). Island evolution in marine lakes. *JMBA Global Marine Environment*, *3*, 26–29.

Dawson, M. N., & Hamner, W. M. (2005). Rapid evolutionary radiation of marine zooplankton in peripheral environments. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(26), 9235–9240

Dawson, M. N., Martin, L. E., Bell, L. J., & Patris, S. (2009). Marine Lakes. In R. G. Gillespie, & D. A. Clague (Eds.), *Encyclopedia of Islands* (pp. 603–607). Berkeley, CA: University of California Press.

Dawson, M. N., Martin, L. E., & Penland, L. K. (2001). Jellyfish swarms, tourists, and the Christ-child. *Hydrobiologia*, *451*(1), 131–144.

De Meester, L., Gómez, A., Okamura, B., & Schwenk, K. (2002). The monopolization hypothesis and the dispersal-gene flow paradox in aquatic organisms. *Acta Oecologica*, *23*(3), 121–135.

De Meester, L., Vanoverbeke, J., Kilsdonk, L. J., & Urban, M. C. (2016). Evolving perspectives on monopolization and priority effects. *Trends in Ecology and Evolution*, *31*(2), 136–146.

DeBoer, T. S., Naguit, M. R. A., Erdmann, M. V., Ablan-Lagman, M. C. A., Ambariyanto, C., Carpenter, K. E. … Barber, P. H. (2014). Concordance between phylogeographic and biogeographic boundaries in the coral triangle: Conservation implications based on comparative analyses of multiple giant clam species. *Bulletin of Marine Science*, *90*(1), 277–300.

Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797.

Emerson, B. C., & Gillespie, R. G. (2008). Phylogenetic analysis of community assembly and structure over space and time. *Trends in Ecology and Evolution*, *23*(11), 619–630.

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*, 564–567.

Fukami, T. (2015). Historical contingency in community assembly: Integrating niches, species pools, and priority effects. *Annual Review of Ecology Evolution and Systematics*, 46, 1–23.

Geller, J., Meyer, C., Parker, M., & Hawk, H. (2013). Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular Ecology Resources*, 13(5), 851–861.

Genner, M. J., Nichols, P., Carvalho, G. R., Robinson, R. L., Shaw, P. W., Smith, A., & Turner, G. F. (2007). Evolution of a cichlid fish in a Lake Malawi satellite lake. *Proceedings of the Royal Society B: Biological Sciences*, 274(1623), 2249–2257.

Geyh, M. A., Streif, H., & Kudrass, H.-R. (1979). Sea-level changes during the late Pleistocene and Holocene in the Strait of Malacca. *Nature*, 278(5703), 441–443.

Gillespie, R. G., & Baldwin, B. G. (2009). Island biogeography of remote archipelagoes: Interplay between ecological and evolutionary processes. In J. Losos, & R. E. Ricklefs (Eds.), *The theory of island biogeography revisited* (pp. 358–387). Princeton, CA: Princeton University Press.

Goto, T. V., Tamate, H. B., & Hanzawa, N. (2011). Phylogenetic characterization of three morphs of mussels (Bivalvia, Mytilidae) inhabiting isolated marine environments in Palau Islands. *Zoological Science*, 28(8), 568–579.

Gotoh, R. O., Sekimoto, H., Chiba, S. N., & Hanzawa, N. (2009). Peripatric differentiation among adjacent marine lake and lagoon populations of a coastal fish, *Sphaeramia orbicularis* (Apogonidae, Perciformes, Teleostei). *Genes & Genetic Systems*, 84(4), 287–295.

Hague, M. T. J., & Routman, E. J. (2016). Does population size affect genetic diversity? A test with sympatric lizard species. *Heredity*, 116(1), 92–98.

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1), 1–9.

Hamner, W. M., & Hamner, P. P. (1998). Stratified marine lakes of Palau. *Physical Geography*, 19(3), 175–220.

Hanzawa, N., Gotoh, R. O., Sekimoto, H., Goto, T. V., Chiba, S. N., & Kuriiwa, K. (2012). Genetic diversity and evolution of marine animals isolated in marine lakes. In M. Caliskan (Ed.), *Analysis of genetic variation in animals* (pp. 121– 150). Rijeka, Croatia: InTech Open Access Publisher.

Harpending, H. C. (1994). Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology*, 66(4), 591–600.

Harpending, H. C., Sherry, S. T., Rogers, A. R., & Stoneking, M. (1993). The genetic structure of ancient human populations. *Current Anthropology*, 34(4), 483–496.

Hendry, A. P., Bolnick, D. I., Berner, D., & Peichel, C. L. (2009). Along the speciation continuum in sticklebacks. *Journal of Fish Biology*, 75(8), 2000–2036.

Hijmans, R. J. (2016). geosphere: Spherical trigonometry. https://CRAN.R-Project.Org/Package=geosphere

Hoeksema, B. W. (2007). Delineation of the Indo-Malayan centre of maximum marine biodiversity: The coral triangle. In W. Renema (Ed.), *Biogeography, time, and place: Distributions, barriers, and islands* (pp. 117–178). Dordrecht, The Netherlands: Springer.

Holthuis, L. (1973). Caridean shrimps found in land-locked saltwater pools at four Indo-West Pacific localities (Sinai Peninsula, Funafuti Atoll, Maui and Hawaii Islands), with the description of one new genus and four new species. *Zoologische Verhandelingen*, 128, 1–48.

Kimura, M. (1983). *The neutral theory of molecular evolution*. Cambridge, UK: Cambridge University Press.

Klei, K. (2015). Naturalis biodiversity Center, Leiden, The Netherlands, Msc Thesis. Naturalis Biodiversity Centre, Leiden, The Netherlands.

Knittweis, L., Kraemer, W. E., Timm, J., & Kochzius, M. (2008). Genetic structure of *Heliofungia actiniformis* (Scleractinia: Fungiidae) populations in the Indo-Malay Archipelago: Implications for live coral trade management efforts. *Conservation Genetics*, 10(1), 241–249.

Kochzius, M., & Nuryanto, A. (2008). Strong genetic population structure in the boring giant clam, *Tridacna crocea*, across the Indo-Malay Archipelago: Implications related to evolutionary processes and connectivity. *Molecular Ecology*, 17(17), 3775–3787.

Lee, T., & Foighil, D. Ó. (2004). Hidden Floridian biodiversity: Mitochondrial and nuclear gene trees reveal four cryptic species within the scorched mussel, *Brachidontes exustus*, species complex. *Molecular Ecology*, 13(11), 3527–3542.

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics (Oxford, England)*, 25(11), 1451–1452.

Lowe, W. H., & Allendorf, F. W. (2010). What can genetics tell us about population connectivity? *Molecular Ecology*, 19(15), 3038–3051.

Luttikhuizen, P. C., Drent, J., & Baker, A. J. (2003). Disjunct distribution of highly diverged mitochondrial lineage clade and population subdivision in a marine bivalve with pelagic larval dispersal. *Molecular Ecology*, 12(8), 2215–2229.

Maas, D. L., Capriati, A., Ahmad, A., Erdmann, M. V., Lamers, M., de Leeuw, C. A., … Becking, L. E. (2020). Recognizing peripheral ecosystems in marine protected areas: A case study of golden jellyfish lakes in Raja Ampat, Indonesia. *Marine Pollution Bulletin*, 151, 110700.

Maas, D. L., Prost, S., Bi, K., Smith, L. L., Armstrong, E. E., Aji, L. P., … Becking, L. E. (2018). Rapid divergence of mussel populations despite incomplete barriers to dispersal. *Molecular Ecology*, 27(7), 1556–1571.

Mangubhai, S., Erdmann, M. V., Wilson, J. R., Huffard, C. L., Ballamu, F., Hidayat, N. I., … Wen, W. (2012). Papuan Bird's Head Seascape: Emerging threats and challenges in the global center of marine biodiversity. *Marine Pollution Bulletin*, 64(11), 2279–2295.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research*, 27(2), 209–220.

Mariani, S., Peijnenburg, K. T. C. A., & Weetman, D. (2012). Independence of neutral and adaptive divergence in a low dispersal marine mollusc. *Marine Ecology Progress Series*, 446, 173–187.

McLeod, E., Salm, R., Green, A., & Almany, J. (2009). Designing marine protected area networks to address the impacts of climate change. *Frontiers in Ecology and the Environment*, 7(7), 362–370.

Medlin, L., Elwood, H. J., Stickel, S., & Sogin, M. L. (1988). The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene*, 71(2), 491–499.

Monteiro-Ribas, W., Rocha-Miranda, F., Romano, R. C., & Quintanilha, J. (2006). Larval development of *Brachidontes solisianus* (Bivalvia, Mytilidae), with notes on differences between its hinge system and that of the mollusk *Perna perna*. *Brazilian Journal of Biology*, 66(1A), 109–116.

Morton, B. (1988). The population dynamics and reproductive cycle of *Brachidontes variabilis* (Bivalvia: Mytilidae) in a Hong Kong mangrove. *Malacological Review*, 21(1–2), 109–118.

Nei, M. (1987). *Molecular evolutionary genetics*. New York, NY: Colombia University Press.

Nei, M., & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*, 76(10), 5269–5273.

Oksanen, A. J., Blanchet, F. G., Kindt, R., Legen-, P., Minchin, P. R., Hara, R. B. O., Stevens, M. H. H. (2012). Community ecology package.

Orsini, L., Vanoverbeke, J., Swillen, I., Mergeay, J., & De Meester, L. (2013). Drivers of population genetic differentiation in the wild: Isolation by dispersal limitation, isolation by adaptation and isolation by colonization. *Molecular Ecology*, 22(24), 5983–5999.

Park, J.-K., & Foighil, D. Ò. (2000). Sphaeriid and corbiculid clams represent separate heterodont bivalve radiations into freshwater environments. *Molecular Phylogenetics and Evolution*, 14(1), 75–88.

Peijnenburg, K. T. C. A., & Goetze, E. (2013). High evolutionary potential of marine zooplankton. *Ecology and Evolution*, 3(8), 2765–2783.

Roberts, C. M., McClean, C. J., Veron, J. E. N., Hawkins, J. P., Allen, G. R., McAllister, D. E., … Werner, T. B. (2002). Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science (New York, N.Y.)*, *295*(5558), 1280–1284.

Rogers, A. R., & Harpending, H. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, *9*(3), 552–569.

Rohlf, F. J. (2010). Tpsdig, digitize landmarks and outlines. Version 2.12. Stony Brook: Department of Ecology and Evolution, State University of New York at Stony Brook.

Romero-Torres, M., Treml, E. A., Acosta, A., & Paz-García, D. A. (2018). The eastern tropical pacific coral population connectivity and the role of the eastern pacific barrier. *Scientific Reports*, *8*(1), 1–13.

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, England)*, *19*(12), 1572–1574.

Rose, E., Masonjones, H. D., & Jones, A. G. (2016). A DNA-based assessment of the phylogenetic position of a morphologically distinct, anchialine-lake-restricted seahorse. *Journal of Heredity*, *107*(6), 553–558.

Rosindell, J., & Phillimore, A. B. (2011). A unified model of island biogeography sheds light on the zone of radiation. *Ecology Letters*, *14*(6), 552–560.

Salzburger, W., Ewing, G. B., & Von Haeseler, A. (2011). The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Molecular Ecology*, *20*, 1952–1963.

Sathiamurthy, E., & Voris, K. H. (2006). Maps of Holocene sea level transgression and submerged lakes on the Sunda shelf. *The Natural History Journal of Chulalongkorn University*, Supplement, *2*, 1–44.

Shearer, T. L., Van Oppen, M. J. H., Romano, S. L., & Wörheide, G. (2002). Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology*, *11*, 2475–2487.

Sirna Terranova, M., Lo Brutto, S., Arculeo, M., & Mitton, J. B. (2007). A mitochondrial phylogeography of *Brachidontes variabilis* (Bivalvia: Mytilidae) reveals three cryptic species. *Journal of Zoological Systematics and Evolutionary Research*, *45*(4), 289–298.

Slatkin, M. (1993). Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, *47*(1), 264–279.

Slatkin, M. (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, *139*(1), 457–462.

Slatkin, M., & Hudson, R. R. (1991). Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, *129*(2), 555–562.

Swift, H. F., Gómez Daglio, L., & Dawson, M. N. (2016). Three routes to crypsis: Stasis, convergence, and parallelism in the Mastigias species complex (Scyphozoa, Rhizostomeae). *Molecular Phylogenetics and Evolution*, *99*, 103–115.

Tajima, F. (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics*, *105*(2), 437–460.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, *123*(3), 585–595.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*(3), 512–526.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725–2729.

Timm, J., & Kochzius, M. (2008). Geological history and oceanography of the Indo-Malay Archipelago shape the genetic population structure in the false clown anemonefish (*Amphiprion ocellaris*). *Molecular Ecology*, *17*(18), 3999–4014.

Tornabene, L., Valdez, S., Erdmann, M., & Pezold, F. (2015). Support for a "Center of Origin" in the Coral Triangle: Cryptic diversity, recent speciation, and local endemism in a diverse lineage of reef fishes (Gobiidae: Eviota). *Molecular Phylogenetics and Evolution*, *82*(PA), 200–210.

Treml, E. A., Roberts, J., Halpin, P. N., Possingham, H. P., & Riginos, C. (2015). The emergent geography of biophysical dispersal barriers across the Indo-West Pacific. *Diversity and Distributions*, *21*(4), 465–476.

Trovant, B., Orensanz, J. M. (Lobo), Ruzzante, D. E., Stotz, W., & Basso, N. G. (2015). Scorched mussels (Bivalvia: Mytilidae: Brachidontinae) from the temperate coasts of South America: Phylogenetic relationships, trans-Pacific connections and the footprints of Quaternary glaciations. *Molecular Phylogenetics and Evolution*, *82*, 60–74.

Vellend, M. (2005). Species diversity and genetic diversity: Parallel processes and correlated patterns. *The American Naturalist*, *166*(2), 199–215.

Voris, H. K. (2000). Maps of Pleistocene sea levels in Southeast Asia: Shorelines, river systems and time durations. *Journal of Biogeography*, *27*(5), 1153–1167.

Waples, R. S., & Gaggiotti, O. (2006). What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, *15*(6), 1419–1439.

Warren, B. H., Simberloff, D., Ricklefs, R. E., Aguilée, R., Condamine, F. L., Gravel, D., … Thébaud, C. (2014). Islands as model systems in ecology and evolution: Prospects fifty years after MacArthur-Wilson. *Ecology Letters*, *18*, 200–217.

Wilbur, H. M., & Alford, R. A. (1985). Priority effects in experimental pond communities: Responses of Hyla to Bufo and Rana. *Ecology*, *66*(4), 1106–1114.

Wright, S. (1943). Isolation by distance. *Genetics*, *28*(2), 114–138.

Xia, X. (2013). DAMBE5: A comprehensive software package for data analysis in molecular biology and evolution. *Molecular Biology and Evolution*, *30*(7), 1720–1728.

Zouros, E., Oberhauser Ball, A., Saavedra, C., & Freeman, K. R. (1994). An unusual type of mitochondrial DNA inheritance in the blue mussel Mytilus. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(16), 7463–7467.

BIOSKETCH

Christiaan de Leeuw is interested in the distribution of biodiversity over time and space, in particular in the marine realm. This work is part of his PhD at Wageningen University & Research on eco-evolutionary dynamics in isolated marine environments.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.