ORIGINAL ARTICLE

Little shrimp left on the shelf: the roles that sea-level change, ocean currents and continental shelf width play in the genetic connectivity of a seagrass-associated species

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ABSTRACT

Aim Caridean shrimp are diverse and abundant inhabitants of seagrass beds. Anthropogenic disturbances have already reduced and fragmented seagrass habitat, and the rate of change is likely to increase in the future. It is therefore becoming increasingly important to build a basis of understanding of connectivity among populations of seagrass-associated fauna. *Phycomenes zostericola* is closely associated with seagrass and makes an ideal study species with which to explore patterns of connectivity and the influence of biogeographic boundaries and historical sea-level changes on seagrass-associated species. We hypothesized that strong currents and the high potential of *P. zostericola* for dispersal and adult movement would result, for the most part, in panmixia. We also hypothesized that if structure was evident, it would occur close to known biogeographic boundaries in Queensland.

Location *Phycomenes zostericola* is an abundant shrimp species distributed throughout Queensland's seagrass habitats. Nineteen seagrass sites from the Torres Strait Islands and Queensland coastlines were sampled.

Methods Molecular sequence data for a 590 base pair fragment of the mitochondrial gene cytochrome *c* oxidase subunit I (COI) was analysed for 279 specimens of *P. zostericola*. Phylogeographic patterns were analysed using nested clade phylogeographic analysis (NCPA); an isolation-by-distance effect was tested using a Mantel test; the effect of biogeographic boundaries was tested using an analysis of molecular variance (AMOVA), and also a spatial analysis of molecular variance (SAMOVA); demographic expansions were tested for using Tajima's D, Fu's F_S and timing estimated using mismatch analysis; the timing of vicariant events was estimated using coalescent analysis (IM program).

Results Contrary to our original hypothesis, the strong marine currents are not a connective influence among populations of *P. zostericola*. Regional genetic structure and an isolation-by-distance effect are enhanced by existing coastal biogeographic boundaries. Population genetic structure and demographic history are intricately linked to the effects of a tumultuous Pleistocene sea-level history on the Queensland continental shelf.

Main conclusions Connectivity diminishes among populations of *P. zostericola* over scales larger than a few hundred kilometres. As seagrass habitats world-wide become increasingly fragmented, low levels of connectivity will result in an isolated future for *P. zostericola* and other species reliant on seagrass as habitat.

Keywords

Biogeographic boundary, continental shelf, Decapoda, East Australian Current, habitat fragmentation, *Phycomenes zostericola*, Pontoniinae, Queensland, sea level, seagrass.

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INTRODUCTION

In marine ecosystems, patterns of genetic connectivity between populations of inshore species are influenced on intermediate spatial scales (10–100 km) by their biology and life history (e.g. larval duration) (Palumbi, 2003), and over large spatial scales (> 1000 km) by biogeographic boundaries and currents (Sotka *et al.*, 2004). Planktotrophic larvae, persisting from weeks to months, have great opportunity for dispersal. If dispersal potential is realized, which is often the case (Doherty *et al.*, 1995), little genetic structure exists between populations (Palumbi, 1995).

Ocean currents either enhance connectivity, producing homogenizing effects where flows are continuous, or they act as biogeographic boundaries, reducing connectivity where currents divide or are diverted. The largest ocean current close to Australian shores is the East Australian Current (EAC). The EAC moves up to 30 million cubic metres of water per second, reaching speeds of 9 km h^{-1} in summer (Church, 1987). The strongest flow exists between 25 and 30° S at the southern end of the Great Barrier Reef (GBR), gradually weakening as it makes its way southwards to Tasmania. The EAC is responsible for high levels of population connectivity (resulting in genetic panmixia) among populations of fish (Sumpton *et al.*, 2008), portunid crabs (Gopurenko & Hughes, 2002) and other invertebrates (Murray-Jones & Ayre, 1997; Hoskin, 2000) along the east coast of Australia. The EAC is the southern arm of the divergent South Equatorial Current which flows westward from the Coral Sea and diverges at around 15° S. The northern arm is known as the Hiri Current and is much weaker, heading north past the Torres Strait to Papua New Guinea. The divergence of the South Equatorial Current is thought to have maintained genetic divergence in otherwise highly connected populations of crabs (Gopurenko & Hughes, 2002).

Warm waters carried south by the EAC mix with cooler southern waters near the Tropic of Capricorn (latitude 23°26′22″ S, Burrage *et al.*, 1996). This transition zone delimits the southern extent of the GBR and tropical species and the northern extent for many temperate species (Hopley, 1982). The biogeographic boundary surrounding 23° S is a divergence area for species of fish (Chenoweth *et al.*, 2002), sponges (Wörheide *et al.*, 2002) and mangroves (Duke *et al.*, 1998). Several factors individually or together might have reduced connectivity between southern Queensland and northern Queensland, namely currents, sea surface temperature transition, sea-level history, shelf conditions and the influence of Fraser Island on local oceanographic processes.

The growth and decay of ice sheets during the Pleistocene epoch (2.6 Ma to 10 ka) caused rapid and vast fluctuations in sea level of up to 200 m (Galloway & Kemp, 1981). In the Southern Hemisphere, low sea levels repeatedly exposed the Sunda (Southeast Asia) and Sahul (Australia and New Guinea) shelves (Galloway & Kemp, 1981; Voris, 2000; and see Fig. 1), causing divergence in many marine populations, including prawns (Benzie *et al.*, 2002), crabs (Lavery *et al.*, 1996) and seastars (Crandall *et al.*, 2008). In northern Australia, the repeated closure of the Torres Strait reportedly separated populations of fish (Chenoweth *et al.*, 1998), crabs
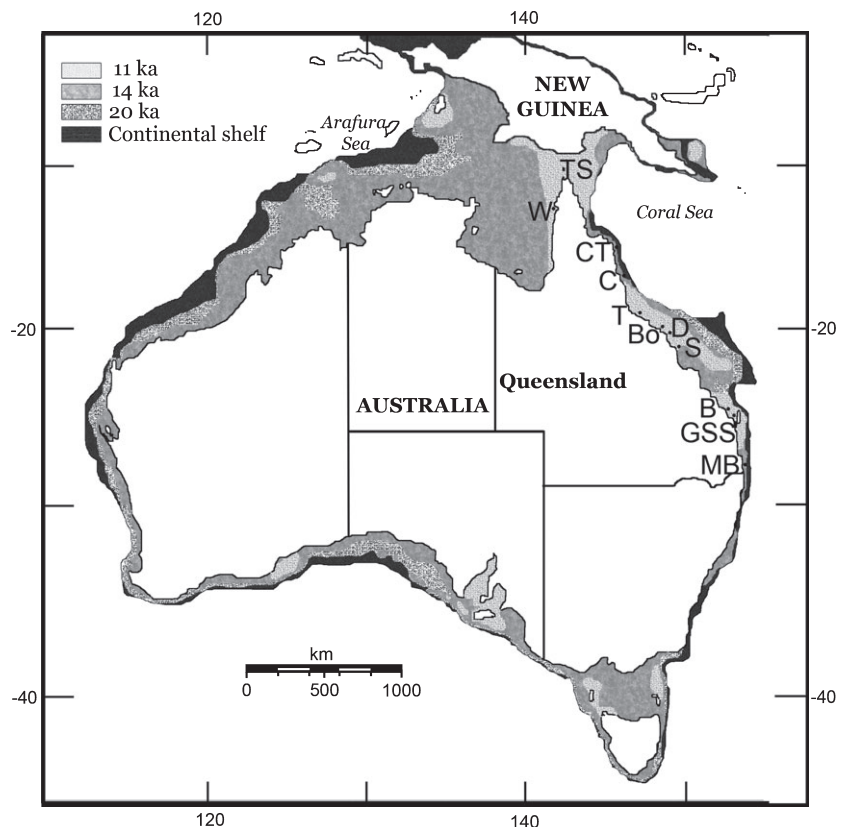

Figure 1 Map of Australia and Papua New Guinea showing latitude and longitude of sampling locations along the Queensland coast and Pleistocene mean sea level on the Australian continental shelf 11, 14 and 20 ka (Last Glacial Maximum). Sea-level outlines are redrawn from maps produced by GLOSS (2009): TS, Torres Strait; W, Weipa; CT, Cooktown; C, Cairns; T, Townsville; Bo, Bowen; D, Dingo Beach; S, Seaforth; B, Baffle Creek; GSS, Great Sandy Strait (Poona, Inskip Point, Fraser Island); MB, Moreton Bay (Pumicestone Passage and Loders Creek).

(Gopurenko & Hughes, 2002) and sponges (Hooper & Ekins, 2004) long enough for significant genetic differences to have accumulated between northern and eastern populations. Along the east coast of Queensland, low sea levels completely exposed the continental shelf (Galloway & Kemp, 1981), leaving shallow-water systems such as reef lagoon systems and river channels (Hopley, 1982) on the edge of the continental shelf as potential refugial habitats for shallow-water marine species. Broad offshore plateaus in the Coral Sea adjacent to Queensland were potential refuge sites during periods of low sea level. Sponges show evidence of recolonization and population growth since the Last Glacial Maximum (LGM) and are hypothesized to have occupied offshore refugia in the Coral Sea during periods of low sea level (Wörheide *et al.*, 2002).

Few studies have explored broad-scale population patterns of small marine crustaceans, especially those of no direct commercial value. Small shrimp, especially carideans, are diverse in seagrass habitats (De Grave, 1999) and represent a large contribution to higher trophic levels. The abundance and palatability of small shrimp enhances the nursery function of seagrass habitats for juvenile fish (Heck *et al.*, 2003), thus having a valuable indirect contribution to commercial catches. Small freshwater shrimp species have been used extensively in Australian phylogeographic studies to describe connectivity (e.g. Hughes *et al.*, 1995; Hurwood & Hughes, 2001) and have proved useful in broad spatial and temporal studies. Broad-scale connectivity studies on small marine shrimp have been undertaken in South Africa (Teske *et al.*, 2007). However, little other phylogeographic research has been undertaken on shrimp in seagrass systems.

Phycomenes zostericola Bruce (Decapoda, Palaemonidae) is a small (< 150 mm), extremely abundant pontoniine shrimp inhabiting seagrass habitats in shallow (< 2 m) marine waters from northern Western Australia, Northern Territory, Queensland and New South Wales (Bruce, 2008). Like many caridean shrimp, *P. zostericola* females brood their eggs until the first zoel stage, which undergoes several moults prior to a final metamorphosis into the subadult stage some 2 weeks later (J. Haig, unpublished data). Larval movement/dispersal in *P. zostericola* has not been measured. Based on the larval duration and the high abundance of *P. zostericola* in seagrass, we hypothesized panmixia among most sites. However, if structure was present, we predicted it would occur either side of the Torres Strait, and possibly at latitudes 15 and 23° S along the east Queensland coastline.

MATERIALS AND METHODS

Sampling strategy

Specimens of *P. zostericola* were collected from 19 seagrass beds along 2800 km of the Queensland coast (Fig. 1). The smallest distance between adjacent sites was 18 km (Poona to Inskip Point) and the greatest was 650 km (Torres Strait Islands to Cooktown; Fig. 1). Sample sites were spread along the known range of *P. zostericola* in Queensland in order to test hypotheses of genetic breaks near known biogeographic boundaries. Sites were < 100 m from shore and < 2 m deep.

DNA extraction

The distal abdominal segments of shrimp were sectioned to avoid the potential contamination by food contents partially digested in the foregut. Total genomic DNA was extracted using a modification of the cetyl trimethyl ammonium bromide (CTAB)/phenol-chloroform DNA extraction protocol (Doyle & Doyle, 1987) and stored in 50 μL of water at < 4 °C.

Polymerase chain reaction amplification and sequencing

An *c.* 800 bp fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified by polymerase chain reaction (PCR). The COI fragment of the mitochondrial genome was chosen due the high level of intraspecific diversity for crustaceans. PCRs were run in an Eppendorf Mastercycler Gradient or an Applied Biosystems Geneamp PCR System 2700 and 9700 (Applied Biosystems, Mulgrave, Vic., Australia). PCRs contained approximately 40 ng of template DNA, 0.5 of each 1 M primer CR COI forward (5′-CWACMAAYCATAA-GAYATTGG-3′) and CR COI reverse (5′-GCRGANGTRAAR-TARGCTCG-3′) (Cook *et al.*, 2008), 0.5 mM dNTP (Astral Scientific, Caringbah, NSW, Australia), 2 mM MgCl$_2$, 1 μL of 10 × polymerase reaction buffer and 0.02 units of *Taq* polymerase (Fisher Biotech, Subiaco, WA, Australia) adjusted to a final volume of 10 μL. PCR followed the thermocycling profile of: 5 min at 94 °C; 15 cycles of 30 s at 94 °C, 30 s at 50 °C and 30 s at 72 °C; then 25 cycles of 30 s at 94 °C, 30 s at 50 °C and 30 s at 72 °C; followed by an extension step of 5 min at 72 °C and a final hold at 4 °C. The success of the PCR was visualized by running product through a 1% agarose gel containing ethidium bromide. The PCR products were run alongside a 1 kb DNA size ladder (Invitrogen, Carlsbad, CA, USA). The PCR product were purified with the exonuclease I-shrimp alkaline phosphatase (exo-sap) method, using 7 μL PCR product, 1 μL shrimp alkaline phosphatase (Promega, Fitchburg, WI, USA) and 0.25 μL exonuclease I (Fermentas, Burlington, ON, Canada) and a two-step thermal cycling profile of: 35 min at 35 °C and 20 min at 80 °C. Sequencing reactions contained 1 μL purified product, 1 μL forward primer, 0.5 μL BigDye v1.1 (Applied Biosystems) and 2 μL BigDye 5 × sequencing buffer (Applied Biosystems) and 5.5 μL of distilled water at the following thermal cycling profile: 1 min at 96 °C; 30 cycles of 10 s at 96 °C, 5 s at 50 °C and 4 min at 60 °C; and a hold at 4 °C. Sequencing was conducted on a 3130xl Capillary Electrophoresis Genetic Analyser (Applied Biosystems).

Statistical analyses

Sequences were aligned and edited using SEQUENCHER version 4.1.2 (Gene Codes Corporation, Ann Arbor, MI, USA). The COI dataset was aligned at default settings. No gaps occurred

in any alignments. As crustaceans may contain pseudogenes of mitochondrial fragments (Song *et al.*, 2008), all sequences were transformed into codons and scanned for internal stop codons. No internal stop codons were found, permitting all fragments to be used in data analyses. Estimates of haplotype and nucleotide diversity were calculated using DnaSP 4.0 (Rozas *et al.*, 2003). Pairwise comparisons of F_{ST} and Φ_{ST} values were calculated in Arlequin 3.1 using 1000 bootstrap repetitions (Excoffier *et al.*, 2005) and a modified false discovery rate (FDR) correction (Narum, 2006) where alpha equalled 0.05 and probability values less than 0.00874 were considered significant. A Mantel test (1000 permutations) was used to test for isolation-by-distance. The input matrix compared distance values (in km) against Slatkin's linearized Φ_{ST} values. Geographic structure was analysed using a hierarchical analysis of molecular variance (Excoffier *et al.*, 1992). The significance of the following fixation indices (Φ_{ST}, Φ_{SC} and Φ_{CT}) was tested using permutation procedures outlined in Excoffier *et al.* (1992). Populations were grouped to test for the significant partitioning of among-group genetic variation (Φ_{CT}) based on the following hypotheses: (1) a land bridge formed between Australia and Papua New Guinea creating variation between Torres Strait island and Queensland populations; (2) the divergence of currents at 15° S; and (3) biogeographic boundaries at 23° S. The geographic structure was also investigated using a simulated annealing approach in SAM-OVA (spatial analysis of molecular variance, Dupanloup *et al.*, 2002), which was used to partition populations that are geographically similar and maximally differentiated (thus providing the highest Φ_{CT} values). SAMOVA was run for 10,000 iterations for $K = 2$ to 10 using 100 initial conditions.

Haplotype network and nested clade phylogeographic analysis (NCPA)

Haplotype networks were constructed using statistical parsimony (95% probability cut-off) in TCS software, version 1.21 (Clement *et al.*, 2000). Several ambiguous loops were resolved using some predictions from coalescent theory before clades were nested (Templeton & Sing, 1993; Posada & Crandall, 2001). Clades were nested by hand using rules from Templeton *et al.* (1987, 1995) and Templeton & Sing (1993). Input files for the geographic locations and nested haplotype design were run by Geodis 2.5 (Posada *et al.*, 2000). The output was assessed using the latest version of the Geodis inference key (15 December 2008) to infer biological explanations for clades found to display significant structure. Although nested clade phylogeographic analysis has been the subject of recent criticism (Petit, 2008; and references therein) it remains a valuable phylogeographic tool (Templeton, 2008), particularly when inferences are corroborated by independent analyses.

Population demographic expansion

Tajima's D (Tajima, 1989) and Fu's F_S (Fu, 1997) tests were used to determine if sequence variation was consistent with predictions under the neutral model. Fu's F_S is sensitive to population demographic expansion, which is indicated by large negative F_S values. *Phycomenes zostericola* haplotypes were placed into clade groups from nesting level four of the nested clade phylogeographic analysis. Parameters of the mismatch distributions, with 95% confidence intervals, were calculated using a generalized least-squares approach (Schneider & Excoffier, 1999; Excoffier, 2004) with the addition of Harpending's raggedness index (Harpending, 1994).

Coalescent analysis

The hypothesis that the populations may have experienced isolation and vicariance due to the LGM was tested using coalescent analysis. Population divergence times (t), effective population size (Ne) and the time to most recent common ancestor (TMRCA) were calculated using the IM program (Hey & Nielsen, 2004). Where there was an absence of genetic structure between populations, populations were grouped into location. The t value was estimated between each pair of adjacent locations, and well as the Ne value for each. Preliminary analyses using wide intervals were used to determine prior distributions. The analyses comparing Cooktown and Cairns with neighbouring sites were unable to estimate time of coalescence due to the lack of any genetic variation within the Cooktown and Cairns locations (all 22 individuals were of the same haplotype); for this reason comparisons were not made between these and neighbouring locations. Metropolis coupling was used to swap between 10 and 20 chains with heating of all chains set at g1 = 0.8, g2 = 0.9 to ensure the effective sampling of parameter space. All runs were executed for 3 million updates. Posterior distributions (including 90% credibility intervals) provided estimates of population divergence time (t) and contemporary and ancestral theta (θ_{1or2} and θ_A). To convert divergence time into years, a sequence divergence rate of 1.4% Myr^{-1} for COI was used, which is the calibrated evolutionary rate for COI in caridean shrimp (Knowlton & Weigt, 1998; Morrison *et al.*, 2004), and with a generation time of 1 year, with generation time taken to mean the mid point between becoming a reproductive individual and dying (A. J. Bruce, Queensland Museum, pers. comm.). Runs were repeated for each pairwise comparison of adjacent populations to ensure parameter estimates were consistent.

RESULTS

Sequencing usually resulted in good quality sequences that were easy to align. A number of sequences did not sequence well at the 5′ end and so all analyses were performed on the shorter sequence. A total of 279 individual specimens of *P. zostericola* from 19 populations were analysed for a 590-bp segment of the mitochondrial gene COI. An alignment of all the sequences obtained was submitted to GenBank (accession numbers GU576176–GU576454).

Table 1 Pairwise F_{ST} (upper matrix) and Φ_{ST} (lower matrix) estimates calculated in ARLEQUIN (Excoffier *et al.*, 2005) for *Phyconemes zostericola* populations from Queensland coastal seagrass habitats.

Location code & location		n	nh	h	π	Torres Strait Islands							Far north				Central			South-east				
						SA	M	MA	E	CO	Y	U	W	CT	C	T	BO	D	S	B	PO	I	P	L
SA	Saibai Island	15	9	0.876	0.007		−0.004	0.124	0.124	0.017	−0.031	−0.018	0.172	0.305	0.605	0.141	0.120	0.090	0.085	0.077	0.086	0.082	0.070	0.073
M	Moa Island	13	9	0.936	0.01	0.040		0.064	0.064	−0.001	0.030	−0.001	0.116	0.219	0.564	0.090	0.071	0.035	0.068	0.048	0.060	0.053	0.041	0.044
MA	Mabuig Island	1	1	1	0	0.525	0.278		1.000	0.058	0.167	0.044	0.231	1.000	1.000	0.176	0.124	0.048	0.071	0.033	0.057	0.043	0.020	0.025
E	Erub Island	1	1	1	0	0.436	0.341	1.000		0.058	0.167	0.044	0.231	1.000	1.000	0.176	0.124	0.048	0.071	0.033	0.057	0.043	0.020	0.025
CO	Coconut Island	16	12	0.942	0.01	−0.010	−0.021	0.356	0.347		0.057	0.002	0.142	0.260	0.563	0.116	0.091	0.054	0.065	0.045	0.057	0.050	0.039	0.041
Y	Yam Island	9	6	0.833	0.008	−0.062	0.011	0.506	0.396	−0.022		−0.018	0.180	0.353	0.712	0.165	0.137	0.110	0.099	0.077	0.085	0.089	0.081	0.084
U	Ugar Island	14	11	0.956	0.009	−0.042	0.040	0.446	0.368	−0.007	−0.060		0.122	0.254	0.578	0.105	0.080	0.046	0.049	0.027	0.037	0.037	0.028	0.031
W	Weipa	13	6	0.769	0.008	0.307*	0.120*	0.263	0.420	0.217*	0.272*	0.290*		−0.031	0.356	−0.029	0.003	0.003	0.103	0.093	0.093	0.112	0.110	0.113
CT	Cooktown	2	1	0	0	0.563*	0.385	1.000	1.000	0.453	0.550*	0.502*	0.127		0.000	−0.041	0.031	0.051	0.259	0.241	0.251	0.244	0.228	0.231
C	Cairns	20	1	0	0	0.773*	0.679*	1.000	1.000	0.697*	0.818*	0.744*	0.492*	0.000		0.335	0.388	0.554	0.493	0.532	0.499	0.515	0.491	0.488
T	Townsville	14	8	0.824	0.009	0.294*	0.147*	0.119	0.391	0.219*	0.272*	0.273*	−0.001	0.005	0.376*		−0.015	−0.007	0.085	0.092	0.095	0.100	0.091	0.094
BO	Bowen	15	9	0.876	0.01	0.202*	0.074*	0.151	0.342	0.133	0.185*	0.186*	0.020	0.122	0.465*	−0.035		−0.007	0.061	0.067	0.074	0.067	0.067	0.070
D	Dingo Beach	7	6	0.952	0.011	0.232	0.061	−0.122	0.281	0.135	0.194	0.207	−0.035	0.125	0.609*	−0.031	−0.028		0.036	0.039	0.053	0.045	0.032	0.035
S	Seaforth	28	16	0.929	0.008	0.213*	0.070*	0.190	0.459	0.124*	0.192*	0.207	0.095*	0.385*	0.588*	0.114*	0.072*	−0.015		0.029	0.029	0.041	0.040	0.043
B	Baffle Creek	18	14	0.967	0.01	0.386*	0.287*	0.369	0.463	0.320*	0.361*	0.366	0.281*	0.394*	0.643*	0.284*	0.256*	0.218*	0.288		−0.002	−0.005	0.000	0.008
PO	Poona	25	17	0.943	0.098	0.391*	0.292*	0.300	0.459	0.326*	0.367*	0.373	0.269*	0.367*	0.586*	0.265*	0.245*	0.203*	0.266	−0.008		0.008	0.011	0.010
I	Inskip	21	15	0.957	0.009	0.391*	0.296*	0.334	0.471	0.325*	0.371*	0.371	0.273*	0.366	0.605*	0.258*	0.238*	0.204*	0.277*	−0.016	−0.024		−0.004	0.006
P	Pumicestone	23	19	0.98	0.008	0.479*	0.413*	0.443	0.566	0.428*	0.472*	0.460	0.398*	0.446*	0.652*	0.357*	0.340*	0.326*	0.397*	0.042	0.051	0.022		−0.010
L	Loders Creek	24	18	0.975	0.01	0.426*	0.358*	0.348	0.505	0.376*	0.415*	0.408*	0.354*	0.389	0.606*	0.310*	0.293*	0.269*	0.351*	0.054	0.050	0.021	−0.014	

n, number of individuals; *nh*, number of haplotypes; *h*, haplotype diversity; *π*, nucleotide diversity; **bold type** = probability ≤ 0.05; significant Φ_{ST} probability values under false discovery rate (FDR) correction (Narum, 2006) were ≤ 0.008 and are indicated with an asterisk.
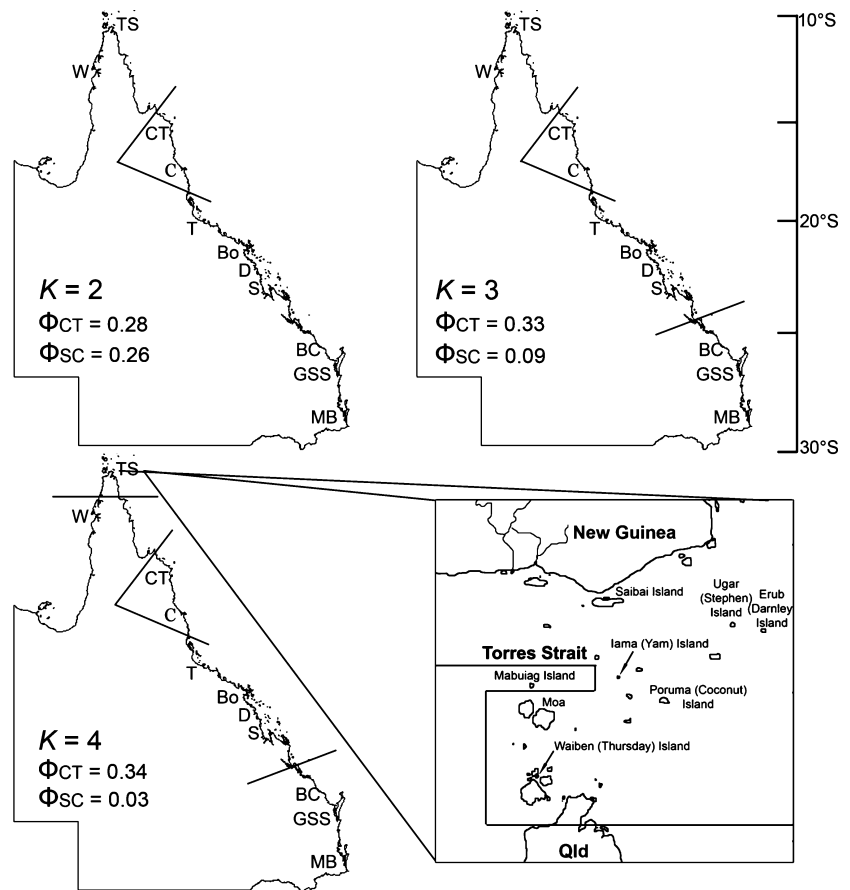
Figure 2 Results of spatial analysis of molecular variance (SAMOVA) of *Phycomenes zostericola* populations from Queensland seagrass habitats. Lines indicate genetic breaks resulting from SAMOVA groupings of $K = 2$, 3 and 4. Probability values for Φ_{CT} and Φ_{SC} ≤ 0.001. Where $K = 4$, Mabuiag Island falls in with W, T, Bo, D and S. TS, Torres Strait; W, Weipa; CT, Cooktown; C, Cairns; T, Townsville; Bo, Bowen; D, Dingo Beach; S, Seaforth; B, Baffle Creek; GSS, Great Sandy Strait (location for sites Poona and Inskip Point, west of Fraser Island); MB, Moreton Bay (location for sites Pumicestone Passage and Loders Creek).

Population analyses

Populations displayed high levels of diversity both within and between populations, with significant support for regional structuring. There were 125 distinct haplotypes and 103 segregating sites were found. Haplotype variability within populations ranged from 0 to 0.98 (Table 1) and had an overall haplotype diversity of 0.96. During population range expansion, the expanding 'front' or newer populations would be expected to have lower genetic diversity when compared with ancestral populations (Hewitt, 1996); however, no relationships were found between nucleotide diversity and geographic location. In 171 pairwise population comparisons, 111 Φ_{ST} and 105 F_{ST} comparative values were significant (Table 1). After FDR correction, 81 of the 171 pairwise Φ_{ST} comparisons were found to be significant; this did not change any overall patterns of genetic differentiation observed prior to the correction. Mantel tests detected a positive correlation between genetic (Slatkin's linearized Φ_{ST} values) and geographic distance ($r = 0.29$; $P \leq 0.001$). The observed regional structure coincided with hypothesized biogeographic boundaries. AMOVA found a significant proportion of the genetic variance among populations regardless of hierarchy ($\Phi_{ST} = 0.3$, $P \leq 0.001$), with most variation either side of

Table 2 Analysis of molecular variance (AMOVA) results calculated in ARLEQUIN 3.1 (Excoffier *et al.*, 2005) for mitochondrial DNA cytochrome *c* oxidase subunit I (COI) using haplotype frequency differences between groups of *Phycomenes zostericola* populations.

AMOVA				Percentage variation		
Hypothesized genetic splits	Φ_{CT}	Φ_{SC}	Φ_{ST}	Among group	Among popn within group	Within Popn
1. TS/QLD	0.21	0.23	0.39	21.01	17.97	61.02
2. 15°S N/S	0.13	0.25	0.35	12.70	21.89	65.42
3. 23°S N/S	0.26	0.18	0.39	26.07	12.95	60.99
4. Regional structure	0.31	0.06	0.35	31.43	3.79	64.78

Groupings 1–3 represent potential barriers to dispersal along the Queensland (QLD) coastline; grouping 4 tests for regional structure into four areas: Torres Strait Islands (TS), north, central and south-east QLD. All *P* values are significant at ≤ 0.001 except Φ_{CT} in row 2 ($P \leq 0.01$).
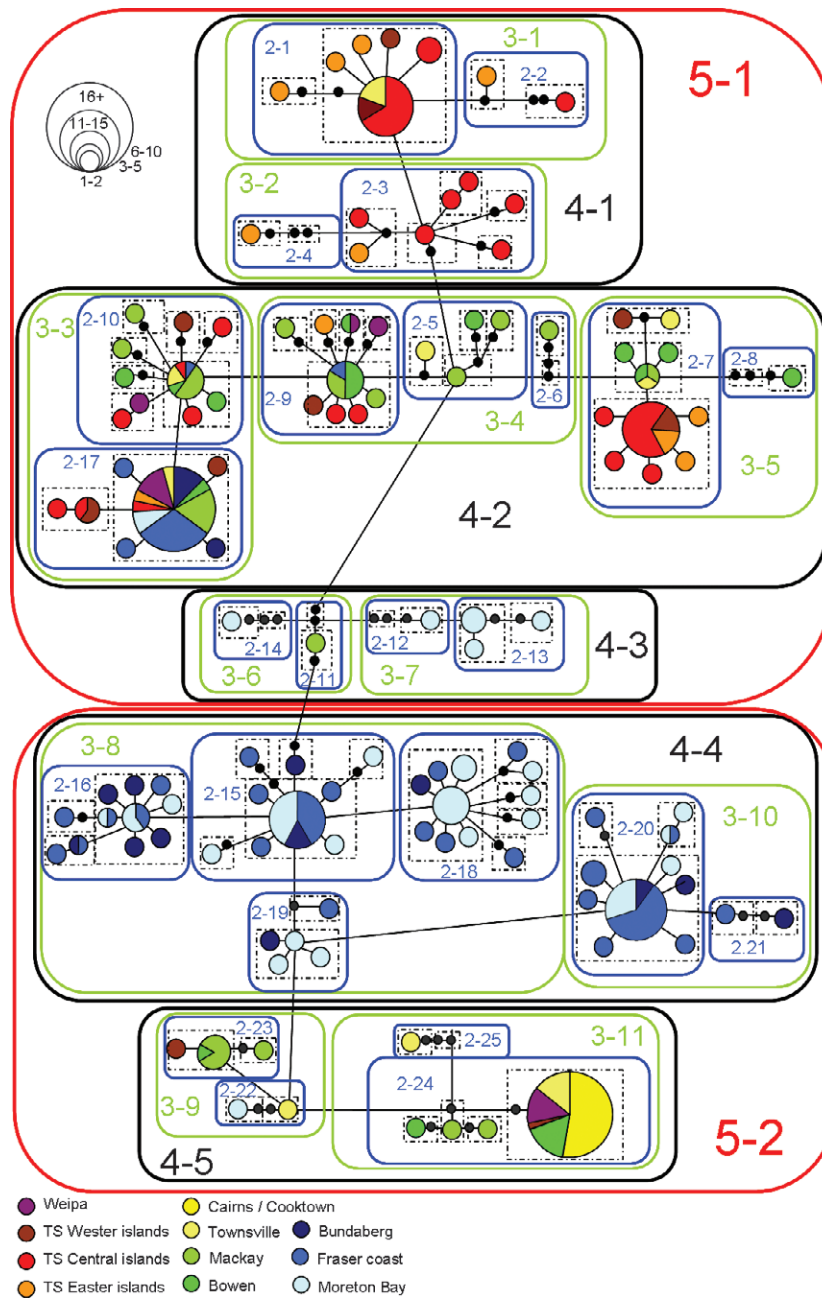
Figure 3 Nested parsimony network for 125 Queensland *Phycomenes zostericola* haplotypes, computed by TCS using 590 base pairs of the cytochrome *c* oxidase subunit I (COI) gene with 95% confidence interval. The circle size indicates the number of individuals sharing a haplotype; colour indicates sample location. Connecting bars indicate one base pair mutation and black circles indicate additional base pair mutations. Nesting levels are boxed into clade levels 1 (broken lines) to 5.

23° S (Φ_{CT} = 0.28, $P \leq 0.001$). A smaller, though significant, level of variation was divided either side of 15° S (Φ_{CT} = 0.13, $P \leq 0.01$) and also between the Torres Strait and Queensland (Φ_{CT} = 0.21, $P \leq 0.001$) (Table 2). SAMOVA values of Φ_{CT} increased from group partitioning of K = 2 to 4 (Fig. 2) and then remained the same from K = 5 to 9 (Φ_{CT} = 0.35, $P \leq 0.001$) and decreased again at K = 10 (Φ_{CT} = 0.34, $P \leq 0.001$). Multiple genetic barriers were observed at all groupings, but the barrier at latitude 23° S [between south-east

Queensland (SEQ) and all other populations] was significant in all partitions greater than 2.

Nested clade phylogeographic analysis

The nesting of the haplotype network identified 125 haplotypes, nested into 75 one-step clades, 25 two-step clades, 11 three-step clades, 5 four-step clades, 2 five-step clades and 1 six-step clade (Fig. 3). Six of the eight nested clades were

Table 3 Nested clade phylogeographic analysis haplotypes of *Phycomenes zostericola* from Queensland seagrass habitats, with a significant χ^2 statistic.

Clade	χ^2 (P)	Clade	Position	D_c	D_n	Chain of inference
2–7	34.65 (0.005)	1–15	Interior	47	899[L]	RGF with IBD
		1–16	Tip	551	555	1–2a–3–4
		1–19	Tip	56[S]	373[S]	
		I–T		−54	509[L]	
2–17	27.96 (0.003)	1–34	Tip	51	1006	Either IBD with SDM or LDD
		1–35	Interior	704	791	1–2–3–5–6–7–8
		I–T		652[L]	−216	
2–24	51.61 (0.054)	1–62	Interior	0	543	RGF with IBD
		1–63	Tip	0	543	1–2–3–4
		1–64	Tip	0	420	
		1–67	Tip	270[S]	270	
		I–T		−257[L]	263	
4–2	42.31 (0.029)	3–3	Tip	738	765[L]	Either LDD or gradual SDM during past range expansion and fragmentation
		3–4	Interior	518[S]	589[S]	1–2–3–5–6–13–21–mismatch distributions
		3–5	Tip	589	680	(also see Clade 2–17)
		I–T		−172	−148[S]	
4–5	35.26 (0.000)	3–9	Interior	631[L]	700[L]	RGF with IBD
		3–11	Tip	285[S]	298[S]	1–2–3–4
		I–T		346[L]	402[L]	
5–1	46.61 (0.000)	4–1	Tip	62[S]	566[S]	RGF with IBD
		4–2	Interior	702	733[L]	1–2–3–4
		I–T		639[L]	166[L]	

Significant values for D_c (clade distance), D_n (nested clade distance) and I–T (interior versus tip) are denoted by S (significantly small) or L (significantly large). Inference key results abbreviations are as follows: RGF, restricted gene flow; IBD, isolation-by-distance; SDM, short-distance movements; LDD, long-distance dispersal.

significant, the remaining two clades (1–2 and 1–50) had inconclusive outcomes using the inference key (Table 3). Significant clades (2–7, 2–24, 4–5 and 5–1) all gave inferences of restricted gene flow with isolation-by-distance. The inference key suggested that clade 2–17, consisting predominantly of Torres Strait and Central Queensland haplotypes, had inadequate sampling to discriminate between isolation-by-distance with short-distance movements or long-distance dispersal. Clade 4–2, consisting of haplotypes from all Queensland locations, required further investigation using mismatch distributions to distinguish between long-distance dispersal or gradual short-distance movements during range

expansion, followed by fragmentation/extinction in intermediate areas.

Neutrality tests and mismatch distributions

Tajima's D compares two estimates of diversity that should be the same under neutrality whilst Fu's F_S compares the observed number of haplotypes with that expected under neutrality; negative values in both analyses result from an excess of substitutions relative to expectations for a constant-sized population and may be interpreted as evidence for recent population expansion. Tests of neutrality

Table 4 Results of neutrality tests, Tajima's D and Fu's F_S and parameters of the mismatch distribution for four-step clades from a nested clade phylogeographic analysis (NCPA) of *Phycomenes zostericola* populations from Queensland.

NCPA clade	Tajima's D (P)	Fu's F_S (P)	Tau (range)	t (ka) (range)	Hri (P)
4–1	−2.29 (0.00)	−10.29 (0.00)	3.70 (0.33–7.90)	448 (40–955)	0.04 (0.76)
4–2	−1.79 (0.01)	−25.79 (0.00)	6.81 (1.08–11.78)	824 (131–1426)	0.01 (0.86)
4–3	−0.37 (0.34)	−1.15 (0.19)	No expansion	No expansion	0.07 (0.88)
4–4	−2.15 (0.00)	−26.16 (0.00)	3.78 (1.96–4.86)	457 (238–588)	0.02 (0.60)
4–5	−1.45 (0.05)	−2.11 (0.18)	No expansion	No expansion	0.35 (0.94)

Mismatch parameters are: time as scaled by mutation (tau) and time since expansion (t) calculated using the 1.4% Myr^{-1} mutation rate. Harpending's raggedness index (Hri) was also applied to mismatch data. Probability values (P) or upper and lower bounds of the 95% confidence intervals are in parentheses.
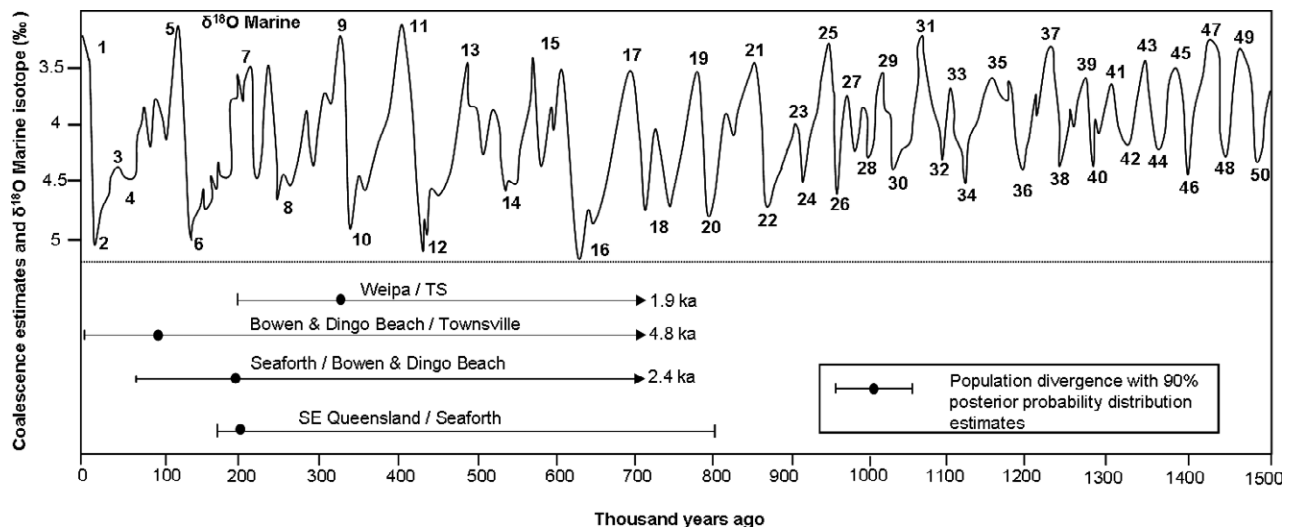
Figure 4 Estimated coalescent time between pairs of populations of *Phycomenes zostericola* (below) and their relationship to Pleistocene glacial maxima inferred from 57 globally distributed $\delta^{18}O$ records (above; Lisiecki & Raymo, 2005). Closed circles indicate the peak of the posterior density distribution for the time of splitting parameter *t* calculated in the Isolation with Migration (IM) program using $\mu = 1.4 \times 10^{-8}$ (Knowlton & Weigt, 1998); also displaying 90% highest (and lowest) posterior densities illustrated by error bars (or arrows if distribution was more than 1500 years ago). Locations: TS (Torres Strait Islands); SE Queensland, Baffle Creek, Poona, Inskip Point, Pumicestone Passage and Loders Creek.

for Tajima's *D* were significantly negative for nested clade levels 4–1 (-2.29 $P \le 0.003$), 4–2 (-1.79 $P \le 0.009$), 4–4 (-2.15 $P \le 0.002$) and 4–5 (-1.45 $P \le 0.047$). Fu's F_S neutrality tests were significantly negative for 4–1 (-10.29 $P \le 0.001$), 4–2 (-25.79 $P \le 0.001$), 4–4 (-26.16 $P \le 0.001$), though not significant for 4–5 (-2.11 $P \le 0.175$) (Table 4). Nested clade level 4–3 was negative yet not significant for Tajima's *D* (-0.37 $P \le 0.34$) and Fu's F_S (-1.15 $P \le 0.189$). The parameters of the mismatch distribution for nested clade levels 4–1, 4–2, 4–4 and 4–5 could not reject a sudden population expansion model (Table 4). Population expansions estimated using tau values all date well within the Pleistocene epoch: *c.* 450 ka for nested clade levels 4–1 and 4–4 and to *c.* 820 ka for nested clade level 4–2 (Table 4). Clade level 4–5 was only just significantly negative for Tajima's *D* ($P = 0.047$) and non-significant for Fu's F_S ($P = 0.175$). We propose that this probably does not result from a population expansion as Fu's F_S is more sensitive to population expansions than Tajima's *D* (Fu, 1997).

Coalescent analysis

The TMRCA for all pairs of adjacent locations occurred within the last 2 Myr. Population divergence times and effective population size estimates for some locations have very wide posterior densities, which is probably due, in part, to small sample sizes and the use of a single genetic locus. Regardless of this, the majority of locations were found to have diverged within the Pleistocene epoch (Fig. 4). The peak estimates of the posterior density distribution for neighbouring locations were: Weipa versus Torres Strait (329 ka); Townsville versus Bowen/

Dingo (90 ka); Bowen/Dingo versus Seaforth (198 ka); Seaforth versus south-east Queensland (200 ka). Calculations estimated effective population size to be between millions to hundreds of millions and ancestral effective population size ranged in the tens of thousands to hundreds of thousands (data not shown).

DISCUSSION

From the high abundance of adults of *P. zostericola*, their great potential for movement between habitats and their long planktonic larval life (*c.* 20 days; J. Haig, unpublished data), we expected to find low levels of genetic structure. Contrary to our original hypotheses, *P. zostericola* displayed both high within- and among-population diversity with a significant isolation-by-distance effect, shown by a Mantel test. A distinct regional structuring was observed in the haplotype network, which was also supported by AMOVA and SAMOVA. The high level of genetic structure suggests that connectivity between adjacent populations of *P. zostericola* may be limited to scales of hundreds of kilometres rather than the hypothesized thousands of kilometres.

Assuming that a sequence divergence rate of 1.4% Myr^{-1} (Knowlton & Weigt, 1998) is reasonable for *P. zostericola*, we can estimate that the structure observed in the haplotype network is a product of persistent influences over hundreds of thousands of years. The use of molecular clocks is not without controversy (Ho *et al.*, 2005; but see Emerson, 2007), and molecular rates have been shown to vary between taxonomic groups (Britten, 1986). The 1.4% divergence Myr^{-1} has been calibrated against multiple caridean sister taxa separated by the

closure of the Isthmus of Panama (Knowlton & Weigt, 1998; Morrison *et al.*, 2004) and was considered the most suitable to apply to *P. zostericola*. Other studies have reported sequence divergence rates of 1.25% Myr^{-1} for isopods (Ketmaier *et al.*, 2003), 5.16% Myr^{-1} for freshwater shrimp (Page *et al.*, 2008), 1.66% Myr^{-1} for terrestrial crabs (Schubart *et al.*, 1998) and even 20% Myr^{-1} for a species of cave shrimp (Craft *et al.*, 2008).

The continual and strong connective influence of the EAC appears to have had little effect on the contemporary connectivity between regions for *P. zostericola*. Similarly, the stomatopod shrimp *Haptosquilla pulchella* has a 4–6-week larval duration and lives adjacent to strong ocean currents through Indonesia, yet shows considerable genetic structure between localities as little as 300 km apart (Barber *et al.*, 2000). Like some other caridean species (Bauer, 2004), larval migration behaviours and limited movement by adults of *P. zostericola* may be responsible for their observed genetic structure.

Currents can enhance or inhibit connectivity among populations. Populations adjacent to strong currents may have increased connectivity, whereas populations either side of a divergent current may have connectivity reduced for long enough for genetic divergence to occur (Murray-Jones & Ayre, 1997). The divergence of the South Equatorial Current into the northern Hiri Current and the southern EAC probably influenced the genetic structure of portunid crabs (Gopurenko & Hughes, 2002). For *P. zostericola*, however, evidence that the divergent current has structured populations at 15° S is unconvincing. Isolation-by-distance effects and low genetic diversity in Cooktown and Cairns populations undoubtedly have a stronger influence on the structure between northern populations of *P. zostericola*, though only further sampling between Cooktown and the Torres Strait will resolve this definitively.

A recognized species 'transition zone' exists around 23° S, where tropical and temperate waters mix and mark the southern boundary of the GBR. The Queensland continental shelf deepens and widens from north to south, having a maximum width of 290 km at *c*. 22° S (Hopley, 1982). At 24° S the northern spit of Fraser Island juts out from near the mainland to only 21 km from the shelf edge, greatly influencing coral reef growth in this area (Hopley, 1982) and altering southern flows of the EAC (Burrage *et al.*, 1996). *Phycomenes zostericola* populations south of 23° S are similar to each other and distinctly different from populations north of 23° S. Similarly, the seagrass-associated pipefish *Urocampus carinirostris* is distributed between 8 and 45° S and displays a large genetic break (9% mitochondrial cytochrome *b* sequence divergence) at approximately 23° S (Chenoweth *et al.*, 2002). Widely distributed populations of the sea sponge *Leucetta chagosensis* (Wörheide *et al.*, 2002) and mangrove *Avicennia* species (Duke *et al.*, 1998) also show similar genetic distinctions between south-east and north Queensland. South-east Queensland populations of *P. zostericola* coalesce with Seaforth populations approximately 200 ka, well within the turbulent Pleistocene epoch. The

observed patterns of vicariance between inshore marine populations at 23° S could be due to a complex of influences, predominantly local current conditions, sea-level changes on a broad and deep continental shelf and a transition between warm northern and cooler southern waters.

The rapid and vastly fluctuating sea levels during the Pleistocene epoch (Fig. 4, and see Lisiecki & Raymo, 2005) have influenced the genetic structure of many inshore marine species (Palumbi, 1997). The repeated closure of the Torres Strait throughout the Pleistocene is thought to have shaped the divergent genetic structures of mud crab (Gopurenko & Hughes, 2002), prawn (Benzie *et al.*, 1992), starfish (Williams & Benzie, 1997), finfish (Chenoweth *et al.*, 1998) and mangrove (Duke *et al.*, 1998) species. Similarly, *P. zostericola* populations in the Torres Strait share only a few haplotypes with mainland Queensland locations. Torres Strait Islands were completely exposed during the LGM, so must have been recolonized within the last 6000–8000 years (Galloway & Kemp, 1981). Torres Strait Islands populations coalesced with Queensland populations more than 300 ka, suggesting that the persistent low sea levels since that time were the likely cause of the vicariance between these two regions. The small amount of haplotype sharing between Torres Strait Islands and mainland Queensland may be explained by concurrent recolonization from eastern refugia in the Coral Sea which were distributed both north and south by the divergent South Equatorial Current upon entry into Queensland waters.

Queensland offshore plateaus in the Coral Sea are thought of as sources for recolonization of GBR populations after periods of low sea level (Hopley, 1982; Davies, 1994). The scarcity of inshore marine habitats during sea-level fluctuations explains much of the fragmentation, isolation-by-distance and population bottleneck/colonization events observed in the nested clade analysis of *P. zostericola*. The lack of genetic diversity in Cairns and Cooktown populations reflects the extreme influences that fluctuating sea levels have had in northern Queensland. Periods of low sea level saw northern shallow and narrow shelf areas exposed for much longer than shelf zones further south (Hopley, 1982). Using a COI sequence divergence rate of 1.4% Myr^{-1} Cairns populations of *P. zostericola* underwent a major bottleneck or a recolonization event at any time prior to 250 ka, though it is likely to have occurred more recently, during the LGM (see point 2, Fig. 4; see also Lisiecki & Raymo, 2005). Further evidence for population expansion followed by fragmentation and extinction is evident from the nested clade analysis; this pattern reflects the kinds of growth and loss of populations that might follow fluctuations in shallow-water marine habitat during sea-level changes. A hypothesis for the long-distance dispersal observed in the nested clade analysis is that small numbers of *P. zostericola* made their way onto refugial offshore habitats during low sea levels and were broadly distributed by the EAC on their way back to mainland Queensland. Alternatively, populations persisting on the shoulders of the continental shelf during low sea levels were much closer to the influences

of the EAC, and periodically underwent long-distance movements.

Whilst there is some evidence within the *P. zostericola* genetic data to suggest population extinctions and reductions, there is also evidence of sustained population growth throughout the Pleistocene epoch. Population expansions, for three of the five level-four clades, contain multiple star-like phylogenies indicative of population expansion; these clades also have significantly negative values of Tajima's D and Fu's F_S. Mismatch analysis dates expansions between approximately 450 ka (clades 4–1 and 4–2) and 820 ka (clade 4–4), suggesting that some clades persisted through multiple low-sea-level events without major demographic losses. For the estuarine fish species *Salanx ariakensis*, the presence of palaeochannels and deltas of the Yellow and East China seas permitted periodic geographic mixing of heterogeneous haplotypes (Hua *et al.*, 2009). We propose that, at least for some Queensland coastal species, ample refugial habitat existed in Queensland during low Pleistocene sea levels. Continental shelf shoulders, lagoons, inlets and palaeochannels, such as the Burdekin (Fielding *et al.*, 2003) and Fitzroy palaeorivers (Ryan *et al.*, 2007), must have provided sufficient estuarine conditions at the edge of the shelf to support seagrass and its associated fauna. The pipefish *Urocampus carinirostris*, another obligate seagrass animal, experienced a demographic expansion prior to the LGM (Chenoweth *et al.*, 2002), suggesting that seagrass habitat was available in south-east Queensland throughout the LGM. Presumably seagrass habitats moved east as sea levels gradually dropped, and returned west as sea levels increased. Evidence for this sequence of events exists along the Pacific coast of North America; the seagrass-associated pipefish *Syngnathus leptorhynchus* and its seagrass habitat (*Zostera marina*) share a post-glacial genetic history of recolonization from refugial populations (Olsen *et al.*, 2004; Wilson, 2006).

The genetic analysis of *P. zostericola* shows how a small seagrass shrimp can respond to environmental processes over broad temporal scales. Carideans, in particular, are important food sources for commercially valuable fish that use seagrass habitats during early phases of their life history (Gillanders, 2006). Seagrass habitat has suffered major declines over the last 100 years in many parts of the world due to human activities, particularly eutrophication (Orth *et al.*, 2006). Seagrass is also vulnerable to anthropogenic impacts exacerbated by climate change, but it is the combined effect of these two factors that makes seagrass especially likely to suffer further reductions in extent. Whilst populations of the caridean seagrass shrimp *P. zostericola* coped well with historical climate change, they currently experience low regional connectivity and are unlikely to recover quickly in the event of habitat loss over large scales.

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