

1 **Spatial scales of genetic patchiness in the western rock lobster**
2 **(*Panulirus cygnus*)**

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31 **Abstract**

32 In planktonic dispersers, impediments to dispersal, local selection or large variance in the
33 reproductive success among individuals (sweepstakes reproductive success) can create
34 genetic heterogeneity at local scales. While these processes are well recognized, relatively
35 few studies have investigated the spatial scales over which genetic heterogeneity occurs and
36 how it is distributed across species' ranges. We investigate population structure in the
37 western rock lobster (*Panulirus cygnus*), a commercially exploited species found in shallow
38 and deep water reef habitats along the Western Australia coastline. We screened 631
39 individuals from nine locations across the species' range for genetic variation at 22
40 microsatellite loci. Consistent with expectations of extensive larval mixing during an
41 extended planktonic stage, we found no significant genetic differentiation among the nine
42 locations ($F_{ST} = 0.003$, $G''_{ST} = 0.007$). Despite the lack of large-scale geographic structure,
43 small but significant positive spatial autocorrelation (SA) was detected over distances up to
44 40 km. Two-dimensional local SA analysis confirmed that fine-scale genetic heterogeneity
45 was common throughout the species' range. An intriguing aspect of these results was that
46 they were based on juvenile and adult lobsters, suggesting restricted movement or spatial
47 cohesion of individuals after settlement.

48

49 **Introduction**

50 The spatial extent of genetic structure is largely dependent on the dispersal capacity of
51 individuals (Bohonak 1999). This is especially apparent in the marine environment, where
52 species with planktotrophic larvae that spend months in the water column tend to maintain
53 low levels of genetic structure across large geographic scales, while those with short
54 planktotrophic larval phases or direct developers usually have much higher levels of
55 subdivision (Waples 1987, Palumbi 1994, Johnson & Black 2006a, Lee & Boulding 2009).
56 However, the role of dispersal capacity in structuring marine populations is complex, and can
57 be strongly influenced by other factors. For example, in spiny lobsters, most species are
58 characterized by a lack of genetic differentiation among localities, consistent with their high
59 dispersal capability during an extended planktonic larval stage (e.g., Ovenden et al. 1992,
60 Silberman et al. 1994, Tolley et al. 2005, García-Rodríguez & Perez-Enriquez 2008, Naro-
61 Maciel et al. 2011), but in some species, barriers to dispersal created by topographic or
62 oceanographic features can lead to moderate to high levels of population structure (Perez-
63 Enriquez et al. 2001, Gopal et al. 2006, Palero et al. 2008).

64 Impediments to dispersal are not always obvious. For example, Johnson and Black
65 (2006b) showed that over short distances (< 2 km) genetic subdivision increased fivefold
66 between populations on different islands compared to different populations on the same
67 island in both a direct developing snail and a planktonic disperser. Such genetic heterogeneity
68 at local scales can occur even when there is little genetic subdivision over large distances
69 (e.g., Hedgecock 1986, Benzie & Stoddart 1992, Johnson et al. 1993, Ayre & Hughes 2000).
70 Adaptation to local environments (e.g. low salinity or temperature) can also lead to genetic
71 differentiation at selected and linked neutral loci, despite high levels of gene flow (see
72 Nielsen et al. 2009). The extent of population structure can therefore vary considerably
73 among species and is not always determined by life-history characteristics alone. For

74 commercially exploited species, failure to detect underlying population structure is a concern,
75 because it may result in overexploitation and depletion of localized subpopulations, with a
76 corresponding loss of genetic variation (Carvalho & Hauser 1994, Begg et al. 1999).

77 The western rock lobster *Panulirus cygnus* (Decapoda: Palinuridae) is found in shallow
78 and deep water reef habitats along the Western Australia coastline, from Cape Leeuwin (34°
79 22' S) to North West Cape (21° 45' S). It supports one of the most economically important
80 single species fisheries in Australia, with until recently, an annual commercial catch of
81 between 8 000 and 14 500 t (Fletcher et al. 2005). A key assumption underlying the
82 management of *P. cygnus* is that the breeding stock comprises a single, demographically
83 united population. This assumption is based on the extended pelagic larval stage of *P. cygnus*,
84 which is thought to ensure high dispersal throughout the species' range. Larvae hatch in
85 spring and early summer, and spend the next nine to eleven months in the plankton, with mid
86 stages being found up to 1500 km offshore. The late-stage larvae metamorphose into pueruli
87 and swim inshore to start the juvenile stage of their life-cycle (Phillips et al. 1979). Allozyme
88 studies also suggest *P. cygnus* is a single panmictic population, but with ephemeral genetic
89 patchiness (small-scale genetic heterogeneity among local populations) caused by temporal
90 variation in allele frequencies of recruits (Thompson et al. 1996, Johnson & Wernham 1999).
91 It therefore represents an extreme model for testing for subtle fine-scale genetic structure
92 over a large geographic range. The aim of this study was to investigate the spatial scale of
93 genetic patchiness in juvenile and adult *P. cygnus* across the main geographic distribution of
94 the species. To achieve a resolution beyond previous genetic studies, we sampled at finer
95 spatial scales, and used 22 microsatellite loci for our study. Microsatellites have proven to be
96 a powerful tool for detecting genetic subdivision within marine species with high larval
97 dispersal capabilities (e.g., Knutsen et al. 2003, Riccioni et al. 2010, White et al. 2010) and

98 have revealed spatial genetic structure on finer scales than found with allozymes and mtDNA
99 (e.g., Ruzzante et al. 1996, Jørgensen et al. 2005).

100

101 **Materials and methods**

102 *Sample collection*

103 In 2009, tissue samples were collected from juvenile and adult *P. cygnus* (carapace length >
104 45 mm) at nine locations spanning nearly 660 km along the Western Australian coastline
105 (Fig. 1). A total of 631 individuals were captured using commercial lobster pots set over
106 distances up to 27.4 km apart within each location. Sample sizes at each location ranged
107 between 19 and 64 individuals. To allow investigation of fine-scale patterns within the
108 Houtman Abrolhos Islands, samples were collected from an additional seven sites (eight sites
109 in total) between four and 82 km apart (sample sizes ranged between 40 and 68 individuals).
110 The spatial coordinates for each individual were recorded at the time of capture.

111

112 *DNA extraction and microsatellite genotyping*

113 DNA was extracted from the middle lobe of the tail fan stored in 100% ethanol, using a
114 QIAGEN Dneasy Blood and Tissue kit, following the manufacturer's recommendations.
115 After the DNA was extracted, each sample was analysed using a NanoDrop ND-1000
116 spectrophotometer to determine the concentration and quality of the DNA. All DNA samples
117 were stored at -20 °C until genotyping. Genotypes at 22 microsatellite loci (S3, S8, S28, S50,
118 W25, Pcyg1 – 9 and 11– 18) were determined for each individual using primers and PCR
119 running conditions described in Groth et al. (2009) and Kennington et al. (2010). PCR
120 products were analyzed on an ABI 3700 sequencer using a GeneScan-500 LIZ internal size
121 standard and scored using GENEMARKER (SoftGenetics, State College, PA, USA) software.

122

123 *Data analysis*

124 Microsatellite variation at each location was quantified by calculating allelic richness (a
125 measure of the number of alleles independent of sample size) and Nei's (1987) estimator of
126 gene diversity. The presence of null alleles was tested for each locus using MICROCHECKER
127 (van Oosterhout et al. 2004). Tests for a deficit or excess in heterozygotes at each location
128 were carried out using randomisation tests, and the results were characterized using the F_{IS}
129 statistic. Significantly positive F_{IS} values indicate a deficit of heterozygotes relative to
130 random mating, and negative values indicate an excess of heterozygotes. Linkage
131 disequilibrium between each pair of loci was assessed by testing the significance of
132 association between genotypes. Corrections for multiple comparisons were carried out using
133 the Sequential Bonferroni method (Rice 1989).

134 Genetic differentiation among locations was assessed by calculating Weir and
135 Cockerham's (1984) estimator of F_{ST} and G''_{ST} , a version of Hedrick's (2005) standardized
136 G_{ST} corrected for bias when the number of populations is small (Meirmans & Hedrick 2011).
137 Microsatellite R_{ST} values (Slatkin 1995) were also calculated, but were qualitatively similar
138 to F_{ST} values so are not reported. Tests for genetic differentiation were performed by
139 permuting genotypes among samples. Estimates of allelic richness, gene diversity, F_{IS} ,
140 deficits in heterozygotes and linkage disequilibrium were calculated using the FSTAT version
141 2.9.3 software package (Goudet 2001). Estimates of F_{ST} , G''_{ST} and tests for genetic
142 differentiation using were performed using the software package GENALEX version 6 (Peakall
143 & Smouse 2005). Differences in estimates of genetic variation and F_{IS} among locations were
144 tested using Friedman's ANOVA. To test for a relationship between genetic and geographical
145 distance, we compared a matrix of G''_{ST} with a matrix of geographical distance (ln km),
146 using a Mantel test with 10 000 permutations.

147 Spatial genetic structure was also investigated using two Bayesian clustering methods,
148 implemented with the software packages STRUCTURE (Pritchard et al. 2000) and GENELAND
149 (Guillot et al. 2005). Both these programs group individuals into the most likely number of
150 clusters (K) that maximizes the within cluster Hardy-Weinberg and linkage equilibria.
151 However, GENELAND differs from STRUCTURE in that geographical information can be
152 incorporated to produce more accurate inferences of population structure based on the spatial
153 distribution of individuals. Analyses involving STRUCTURE were based on an ancestry model
154 that assumed admixture and correlated allele frequencies. No prior information about the
155 origin of the samples was used. Ten independent runs were performed for each value of K (1–
156 10), with a burnin of 10 000 followed by 100 000 MCMC iterations. The most likely number
157 of clusters was assessed by comparing the likelihood of the data for different values of K and
158 using the ΔK method of Evanno et al. (2005). For the GENELAND analysis, the spatial
159 coordinates (latitude and longitude) of each individual were used to run the spatial model.
160 The uncertainty of coordinates was set at zero. Ten independent runs were performed for
161 each value of K (1–10) using the uncorrelated and null allele models. Each run consisted of
162 100 000 MCMC iterations with a thinning of 100 and a burnin of 200. The most likely
163 number of clusters was chosen as the modal K (from each independent run) with the highest
164 posterior probability.

165 We also carried out spatial autocorrelation (SA) analysis to evaluate the genetic
166 similarity of individuals over varying spatial scales. We used GENALEX to calculate a spatial
167 autocorrelation (r) coefficient for a range of distance classes. The results from the SA
168 analysis were presented in two ways. Firstly, r was plotted as a function of distance class to
169 produce a spatial genetic autocorrelogram. Secondly, because estimates of spatial
170 autocorrelation are influenced by the size of distance classes (see Peakall et al. 2003), r was
171 calculated for series of increasing distance class sizes. When significant positive spatial

172 structure is present, r will decrease with increasing distance class sizes. The distance class
173 where r no longer differs significantly from zero provides an approximation of the extent of
174 detectable positive spatial genetic structure (Peakall et al. 2003). Tests for statistical
175 significance were performed by random permutation and calculating the bootstrap 95%
176 confidence limits (CL) of r , using 1000 replicates in each case. We also performed a two-
177 dimensional local spatial autocorrelation analysis using GENALEX. With this analysis, the local
178 autocorrelation (lr) is estimated by comparing an individual with its n nearest neighbours,
179 allowing investigation of local patterns of spatial autocorrelation within the two dimensional
180 landscape (Double et al. 2005). Calculations of lr were made using the nearest five, 10, 20
181 and 50 individuals. As with the global autocorrelation analysis, statistical significance was
182 determined using permutation tests.

183 Finally, tests for selection acting on marker loci were carried out using the F_{ST} outlier
184 approach (Beaumont & Nichols 1996, Beaumont 2005), implemented with the LOSITAN
185 software package (Antoa et al. 2008). The method evaluates the relationship between F_{ST} and
186 expected heterozygosity in an island model of migration with neutral markers. This
187 distribution is used to identify loci with excessively high or low F_{ST} values compared to
188 neutral expectations. These loci are candidates for being subject to directional and balancing
189 selection respectively. Simulations were run using 10 000 replications, 99% confidence
190 intervals and the neutral and forced mean options. For this analysis, individuals were grouped
191 by location and both the stepwise and infinite allele mutation models were performed.

192

193 **Results**

194 Thirteen loci (S3, S8, S50, Pcyg02, Pcyg04, Pcyg06, Pcyg07, Pcyg09, Pcyg12, Pcyg13,
195 Pcyg14, Pcyg16 and Pcyg17) were identified as having null alleles in at least one location
196 using MICROCHECKER and excluded from further analyses unless specified otherwise. The

197 remaining loci showed high levels of genetic diversity at each location (Table 1). There were
198 no significant differences in allelic richness ($\chi^2 = 11.57$, $P = 0.172$), gene diversity ($\chi^2 = 6.89$,
199 $P = 0.548$) or F_{IS} ($\chi^2 = 3.11$, $P = 0.927$) among locations. Nor was there genotypic
200 disequilibrium between pairs of loci after adjusting for multiple comparisons or deviations
201 from Hardy-Weinberg Equilibrium (HWE).

202 There was no significant genetic differentiation among the nine sampling locations (F_{ST}
203 $= 0.003$, $G''_{ST} = 0.007$, $P = 0.249$). Most tests of population differentiation between pairs of
204 locations were non-significant (Table 2), and divergences between most locations were
205 comparable to those observed between sampling sites within the Houtman Abrolhos Islands
206 (pairwise G''_{ST} ranged from -0.015 to 0.020), which were separated by much smaller
207 geographical distances. There was no evidence for isolation-by-distance using pairwise G''_{ST}
208 values calculated between locations (broad-scale) or between sampling sites within the
209 Houtman Abrolhos Islands (local-scale) (Mantel tests, $P = 0.170$ and 0.111 respectively). No
210 significant genetic divergences among locations were also found when analyses were
211 performed using all 22 loci ($F_{ST} = 0.000$, $G''_{ST} = 0.000$, $P = 0.595$). We also failed to detect
212 isolation-by-distance when analyses were performed using all 22 loci (Mantel tests, $P = 0.170$
213 and 0.453 for broad and local spatial scales respectively).

214 No genetic subdivision was found using Bayesian clustering analysis. The STRUCTURE
215 analysis revealed decreasing log probability estimates with increasing values of K and there
216 were no large fluctuations in ΔK , suggesting that the probable number of clusters was one.
217 Further, when $K > 1$, the proportion of individuals assigned to each cluster was fairly even
218 and most individuals were admixed, consistent with inferred population structure not being
219 real (Pritchard et al. 2010). The analysis involving GENELAND gave a similar result, with
220 posterior distributions of the estimated number of populations indicating a clear mode at $K =$
221 1 in nine out of 10 replicates. Similar results were obtained when clustering analysis were

222 performed using all 22 loci, with the STRUCTURE analysis revealing only slight increases in
223 log probability estimates with increasing values of K and no large fluctuations in ΔK , while
224 the GENELAND analysis indicated a clear mode at $K = 1$ in all 10 replicates.

225 In contrast to the Mantel tests, spatial genetic structure was detected with the SA
226 analyses. Significantly positive r values were found within the first four distance classes (0–
227 10, 11–20, 21–30 and 31–40 km), after which r decreased and oscillated between being non-
228 significantly different from zero and significantly negative (Fig. 2A). This pattern is
229 indicative of fine-scale spatial genetic structure generated by discrete patches of similar
230 multilocus genotypes (Smouse & Peakall 1999, Diniz-Filho & Telles 2002). Positive spatial
231 genetic structure at local geographical scales was confirmed when estimates of r were
232 calculated with increasing distance class sizes. Figure 2B shows little change in r between 10
233 and 100 km, after which r decreased, but remained significant until 150 km. It also appears
234 that positive genetic structure was not confined to one geographical area. Two-dimensional
235 local spatial autocorrelation analysis revealed clusters of positive lr at most sampling
236 locations (Fig. 3). The close proximity of significantly positive and non-significant values
237 suggests that local patches were not genetically uniform. A similar number and distribution of
238 positive lr values were obtained when calculations were based on sampling the nearest five,
239 10, 20 and 50 individuals, confirming the consistency of the result. Again, similar results to
240 these were found when all 22 loci were used. The only exceptions being that in the SA
241 analysis positive r values were found within the first two distance classes only and none of
242 the remaining distance classes were significantly negative. The outlier analyses failed to
243 detect any loci with higher than expected F_{ST} values.

244

245 **Discussion**

246 The major finding of this study was the significant genetic heterogeneity among local
247 populations in *P. cygnus*, over very short spatial scales, without the presence of large-scale
248 geographic structure. We found extremely low levels of differentiation among locations
249 sampled across the species' range ($F_{ST} = 0.003$, $G''_{ST} = 0.007$), consistent with extensive
250 gene flow over large geographic distances. The lack of geographic pattern was emphasized
251 by genetic divergences between locations separated by distances over 650 km being no larger
252 than the divergences between sites at the Houtman Abrolhos Islands, which are separated by
253 distances less than 85 km. We also found no evidence of isolation-by-distance using pairwise
254 G''_{ST} estimates and no genetic subdivision using Bayesian clustering analysis. Our results,
255 therefore, add weight to the findings of previous allozyme (Thompson et al. 1996, Johnson &
256 Wernham 1999) and microsatellite studies (Kennington et al. 2013), which suggest that *P.*
257 *cygnus* is a single, panmictic population.

258 Fine-scale population structure in *P. cygnus* was most clearly evident with spatial
259 autocorrelation analysis. Significant genetic structure was observed when lobsters were
260 sampled over distances up to 40 km, with detectable positive spatial genetic structure
261 extending out to 150 km when distance classes were pooled. Further, two-dimensional local
262 SA analysis indicates that these patterns were not driven by the strong influence of one region
263 alone, but were a common feature throughout the species' range. Such microgeographic
264 genetic patchiness has been demonstrated in other marine species with planktonic larvae
265 (e.g., Hedgecock 1994b, Knutsen et al. 2003, Pujolar et al. 2006), including several species
266 along the Western Australian coast (Johnson & Black 1982, Watts et al. 1990, Johnson et al.
267 1993, Johnson et al. 2001). Genetic patchiness has also been observed in *P. cygnus* using
268 allozymes (Johnson & Wernham 1999), but the scale of the genetic heterogeneity reported

269 here is much smaller than shown previously. This likely reflects the increased genetic
270 sensitivity and fine-scale geographic information of this study.

271 Spatial genetic patchiness in some species is due to temporal variation in the genetic
272 composition of recruits (Johnson & Black 1984, Watts et al. 1990, Hedgecock 1994a, Pujolar
273 et al. 2006). This also seems to be the case for *P. cygnus*, in which a combination of temporal
274 variation in allele frequencies and contrasting patterns of recruitment resulted in genetically
275 different cohorts of *P. cygnus* at two sites (Johnson & Wernham 1999). Furthermore, this
276 pattern was ephemeral, as it was not repeated in the subsequent two years. Under the
277 ‘sweepstakes reproductive success’ hypothesis (Hedgecock 1994a), temporal genetic
278 variance in recruits might be a by-product of large variance in the reproductive success of
279 individuals, owing to chance matching of reproductive activity with oceanographic
280 conditions conducive for larval survival. Other possible explanations for temporal genetic
281 variation in *P. cygnus* recruits are (1) origin from different source populations, (2) limited
282 mixing of larvae in the plankton, or (3) natural selection on larvae prior to settlement. Given
283 the low geographic structure in *P. cygnus*, it is unlikely that temporal genetic variation arises
284 from different source populations. The finding that *P. cygnus* larvae settling at the same time
285 at locations 350 km apart shared the same allele frequencies (Johnson & Wernham 1999) also
286 argues against temporal genetic variation being due to the cohesion of larvae in the plankton,
287 though this result was based on only three allozyme loci.

288 If a combination of temporal variation in allele frequencies and contrasting patterns of
289 recruitment is responsible for the genetic patchiness observed in this study, it would require
290 juvenile *P. cygnus* to be relatively sedentary. This appears to be the case. Studies on foraging
291 movements suggest juvenile *P. cygnus* forage over relatively small areas (~150 m radius),
292 though the extent of movement is variable (Jernakoff et al. 1987, Jernakoff & Phillips 1988).
293 The life-cycle of *P. cygnus* also includes a migratory phase, which occurs between four and

294 six years of age, just after many lobsters undergo a synchronised moult that changes their
295 normal red shell to a paler colour (Morgan et al. 1982). During this migration, lobsters leave
296 the coastal reefs and move into deeper water breeding grounds, where they become sedentary
297 again on deeper reefs. Because the lobsters we collected were predominantly from shallow
298 water locations, it is unlikely that they had undertaken these migratory movements.
299 Nevertheless, tag and release experiments have shown that while large movements (>200 km)
300 do occur, most lobsters (>87%) are recaptured within 10 km of their release site (Chubb et al.
301 1999), which is within the distance range we detected positive population structure. More
302 recently, a study using acoustic telemetry found that only a small proportion (13.6%) of
303 migratory phase lobsters emigrated from their resident reef, suggesting that a mass offshore
304 migration may not hold for all inshore reefs (MacArthur et al. 2008).

305 Another explanation for spatial genetic patchiness is natural selection acting after
306 settlement (Larson & Julian 1999). Given the broad latitudinal range of *P. cygnus* (> 1200
307 km), local populations are likely to experience highly varied environmental conditions,
308 providing the opportunity for local adaptations to develop across populations (Kawecki &
309 Ebert 2004). Indeed, several studies have found evidence for local adaptation in widely
310 distributed marine fish (see Nielsen et al. 2009). While we found no clear evidence of
311 directional selection using outlier analysis, genome scans involving many more neutral
312 markers, candidate genes or population transcriptomics would be needed to exclude
313 confidently this possibility. A study monitoring the genetic composition of cohorts of recruits
314 as they develop into adults would also yield valuable insights on post-settlement processes.

315

316 *Implications for fisheries management*

317 The implications of genetic patchiness for fisheries management have been discussed by
318 Larson and Julian (1999). If genetic patchiness is due to selection after settlement, they

319 suggest that the implications for fisheries management are minor, unless a fishery is
320 concentrated on one particular habitat or location, which might disproportionately affect a
321 certain portion of the gene pool. By contrast, factors affecting the genetic composition of
322 recruits prior to settlement may have greater consequences. The most relevant of these to *P.*
323 *cygnus* is the effect of stochastic spatial variation in the sources of successful larvae
324 (sweepstakes reproductive success). This effect implies that the sources of successful larvae
325 vary unpredictably over time. Fisheries management should therefore ensure that both the
326 distribution as well as the total spawning potential of the exploited population is protected.
327 Further, spatial stochasticity of successful spawning argues for either the spatial dispersion of
328 reserves (if they are involved in managing the exploitation), or suitably low exploitation rates
329 across the fishery, thereby increasing the chance that at least some larvae will be released into
330 conditions favourable for their survival. Local genetic patchiness also suggests juvenile and
331 adult lobsters are comparatively sedentary, so they may be more susceptible to
332 environmental/anthropogenic impacts at a finer scale than previously thought.

333

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338

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496

497 **Table 1.** Genetic variation at each location.

Site	Sample size	A_R (SE)	H (SE)	F_{IS}
Kalbarri	39.4 (0.3)	7.1 (1.9)	0.58 (0.11)	-0.05 ^{NS}
HA1	38.9 (0.4)	7.8 (2.0)	0.62 (0.10)	0.01 ^{NS}
Dongara	38.2 (0.7)	7.2 (1.8)	0.62 (0.10)	-0.01 ^{NS}
Jurien Bay	38.3 (0.6)	7.3 (2.0)	0.60 (0.10)	-0.01 ^{NS}
North Lancelin	17.7 (0.6)	7.2 (2.0)	0.56 (0.11)	-0.04 ^{NS}
Lancelin	35.9 (0.7)	7.0 (1.8)	0.59 (0.11)	0.03 ^{NS}
Rottneest Island	63.0 (0.2)	7.5 (1.9)	0.64 (0.11)	-0.03 ^{NS}
Fremantle	20.4 (0.2)	6.8 (1.8)	0.61 (0.10)	-0.01 ^{NS}
Mandurah	21.4 (0.2)	7.3 (1.8)	0.61 (0.10)	0.00 ^{NS}

498 A_R : allelic richness (based on a sample size of 14 individuals); H : gene diversity. ^{NS}

499 designates no significant deviation from Hardy-Weinberg equilibrium.

500

501 **Table 2.** Pairwise G''_{ST} estimates (below diagonal) and P -values from tests of differentiation (above diagonal) between geographic locations.

502 The adjusted significance level for multiple comparisons is 0.0014. Significant divergences are highlighted in bold text.

	Kalbarri	HA1	Dongara	Jurien Bay	N. Lancelin	Lancelin	Rottnest Is.	Fremantle	Mandurah
Kalbarri	–	0.000	0.005	0.157	0.065	0.120	0.845	0.176	0.308
HA1	0.045	–	0.128	0.137	0.252	0.205	0.002	0.022	0.099
Dongara	0.023	0.010	–	0.333	0.006	0.157	0.591	0.201	0.650
Jurien Bay	0.007	0.009	0.003	–	0.413	0.661	0.705	0.341	0.922
North Lancelin	0.017	0.007	0.037	0.002	–	0.431	0.025	0.352	0.251
Lancelin	0.009	0.006	0.008	–0.004	0.001	–	0.433	0.110	0.603
Rottnest Island	–0.005	0.026	–0.002	–0.003	0.023	0.001	–	0.278	0.789
Fremantle	0.009	0.026	0.009	0.004	0.005	0.015	0.005	–	0.888
Mandurah	0.004	0.015	–0.005	–0.013	0.008	–0.004	–0.007	–0.015	–

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507 **Figure legends**

508

509 **Fig. 1** Locations where *P. cygnus* samples were collected.

510

511 **Fig. 2** Spatial autocorrelation analyses. (A) Correlogram plot of the genetic correlation

512 coefficient (r) as a function of distance. (B) Multiple distance class plot, showing the

513 influence of different distance class sizes on genetic correlation. Permuted 95% confidence

514 interval (dashed lines) and the bootstrap 95% confidence error bars are shown.

515

516 **Fig. 3** Plot of two-dimensional local spatial autocorrelation analyses. Symbols represent

517 geographical coordinates with significantly positive (red circles) or non-significant (crosses)

518 lr values. Calculations of lr were based on sampling the nearest 20 individuals. For clarity

519 significantly positive values were offset by -0.1° longitude.

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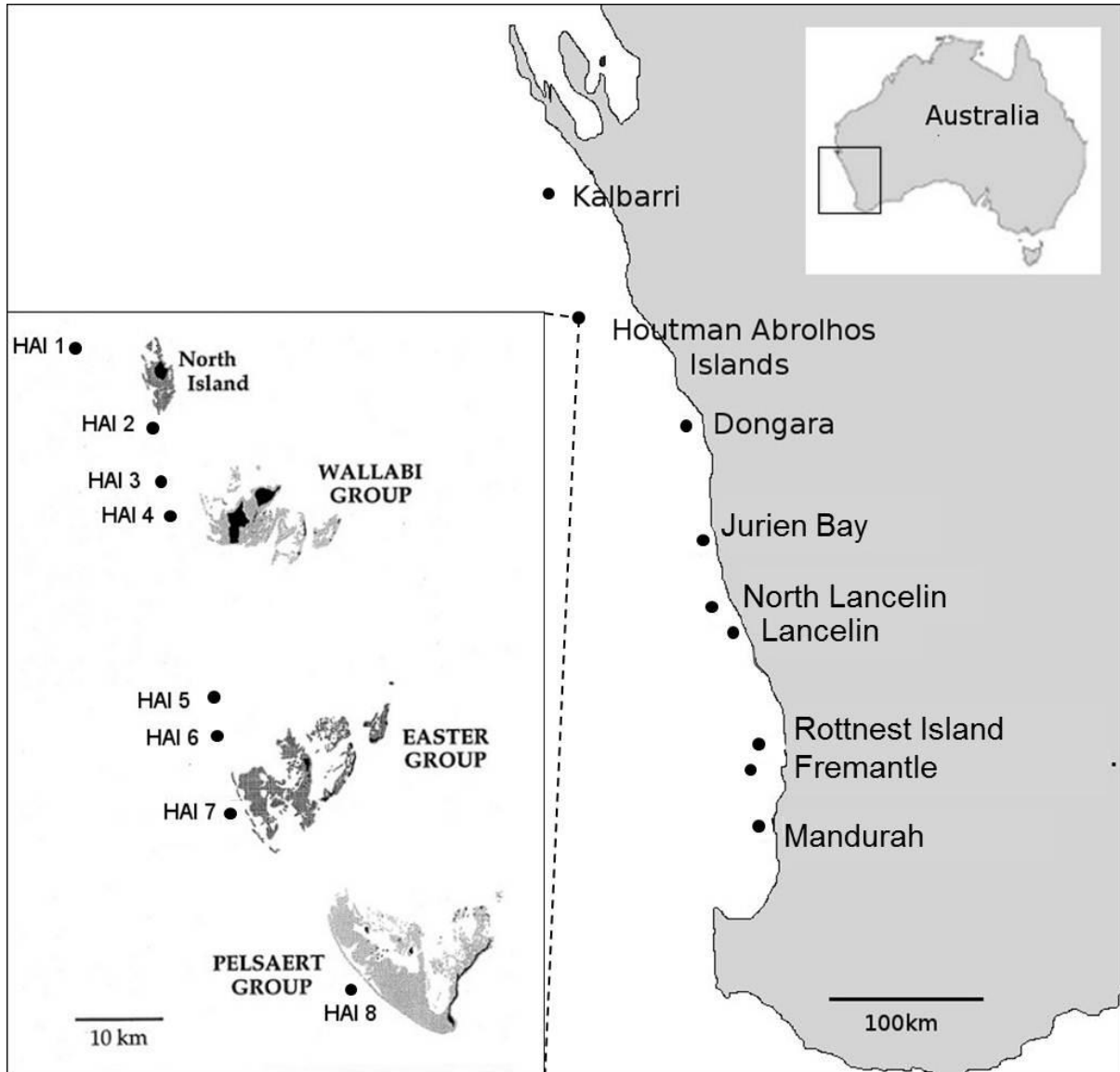
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Fig. 1

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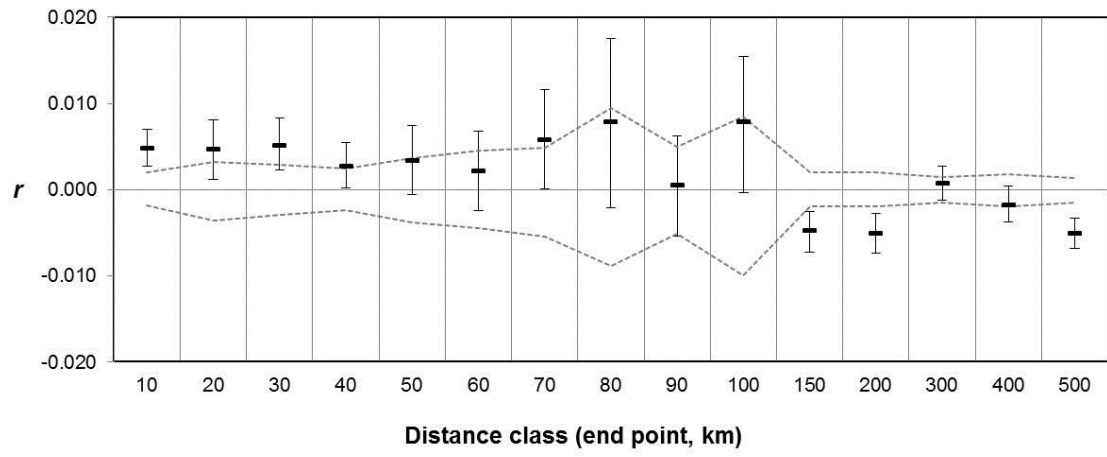
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Fig. 2

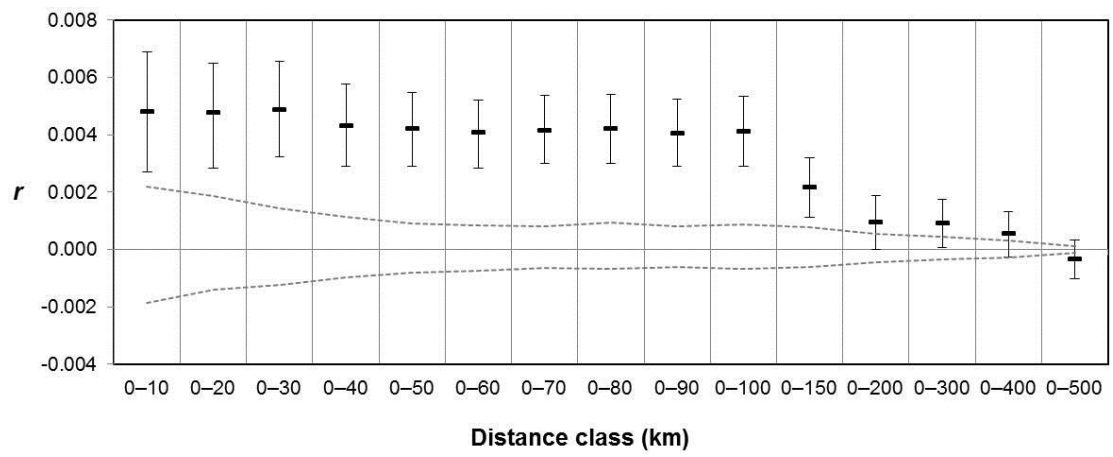
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A



B



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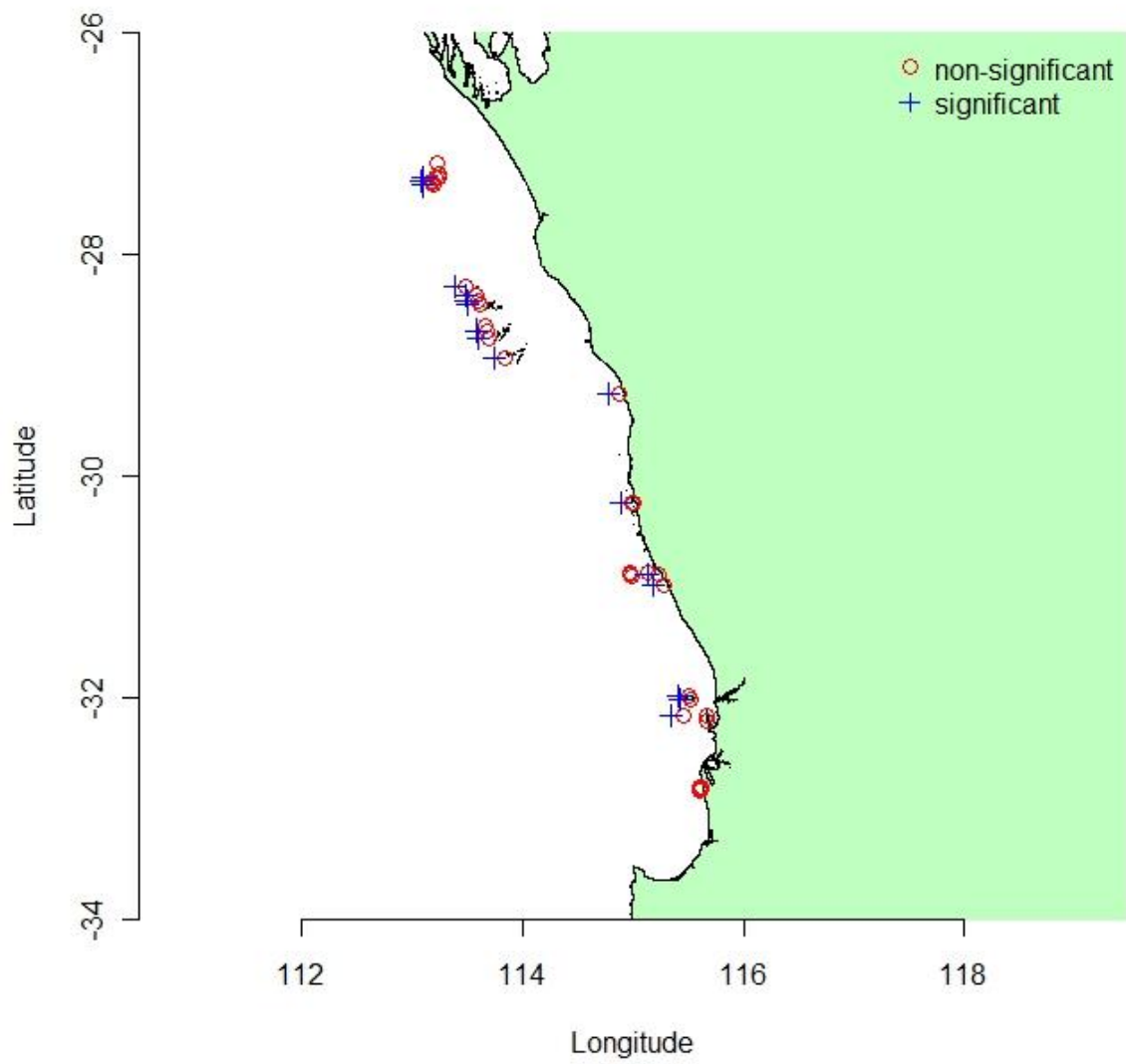
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Fig. 3

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