Journal of Biogeography (J. Biogeogr.) (2013)

ORIGINAL ARTICLE

Phylogeography of the supralittoral isopod *Ligia occidentalis* around the Point Conception marine biogeographical boundary

Renate Eberl[1]*, Mariana Mateos[2], Richard K. Grosberg[1], Carlos A. Santamaria[2] and Luis A. Hurtado[2]*

[1]*Department of Evolution and Ecology, College of Biological Sciences, University of California, Davis, CA, 95616, USA,* [2]*Department of Wildlife and Fisheries Sciences, College of Agriculture and Life Sciences, Texas A&M University, College Station, TX, 77843-2258, USA*

*Correspondence: Renate Eberl, Department of Evolution and Ecology, College of Biological Sciences, One Shields Avenue, University of California, Davis, CA 95616, USA.
E-mail: reberl@ucdavis.edu
Luis A. Hurtado, Department of Wildlife and Fisheries Sciences, College of Agriculture and Life Sciences, Texas A&M University, College Station, TX 77843-2258, USA.
E-mail: lhurtado@tamu.edu

ABSTRACT

Aim The Point Conception (PC) biogeographical boundary is defined by a transition between cold northern and warm southern water masses, accompanied by shifts in numerous ecological and environmental parameters. While these factors can potentially contribute to the genetic differentiation of lineages whose distributions span this boundary, few organisms exhibit genetic signatures of restricted gene flow across this region. We examine the effects of the PC boundary and other potential dispersal barriers on phylogeographical patterns in the rocky supralittoral isopod *Ligia occidentalis*. This isopod has a limited dispersal potential, its distribution spans the PC region, and it exhibits high levels of allopatric genetic differentiation south of PC.

Location Eastern Pacific coastline between southern Oregon and the Baja California Peninsula.

Methods We conducted a thorough sampling of *L. occidentalis* from the PC area to its northern range limit in southern Oregon, and increased previous sampling coverage south of PC. We obtained sequences of the mitochondrial genes cytochrome *c* oxidase subunit I (*COI*) and 16S rDNA for the new localities and combined them with a previously published data set, resulting in a total of 58 sampling locations. We also obtained sequences of the nuclear gene sodium–potassium ATPase α-subunit (*NaK*). We conducted phylogenetic and population-genetic analyses.

Results The geographical limit between the two most divergent clades of *L. occidentalis* (20–25% divergence for *COI*) distributed between southern Oregon and the Baja California Peninsula occurs at the PC biogeographical boundary. Levels of allopatric genetic divergences were greatly reduced north of PC (≤ 3.0% divergence for *COI*).

Main conclusions The geographical limit between the two main *L. occidentalis* clades in California largely reflects the changes in sea surface temperature that define the PC biogeographical boundary. Reduced levels of genetic divergence among *L. occidentalis* lineages found north of PC suggest a recent expansion in the northern range of this isopod.

Keywords

Bayesian, direct developer, genetic divergence, low vagility, maximum likelihood, Oniscidea, Pleistocene, post-glacial, sea surface temperature, western US coastline.

INTRODUCTION

The area around Point Conception (PC), USA (34.4° N; Fig. 1), has traditionally been considered the biogeographical boundary between the Oregonian and Californian marine zoogeographical provinces (Briggs, 1974; Seapy & Littler, 1980). This area is defined by a transition between cold northern and warm southern water masses (Fig. 1; Newman,

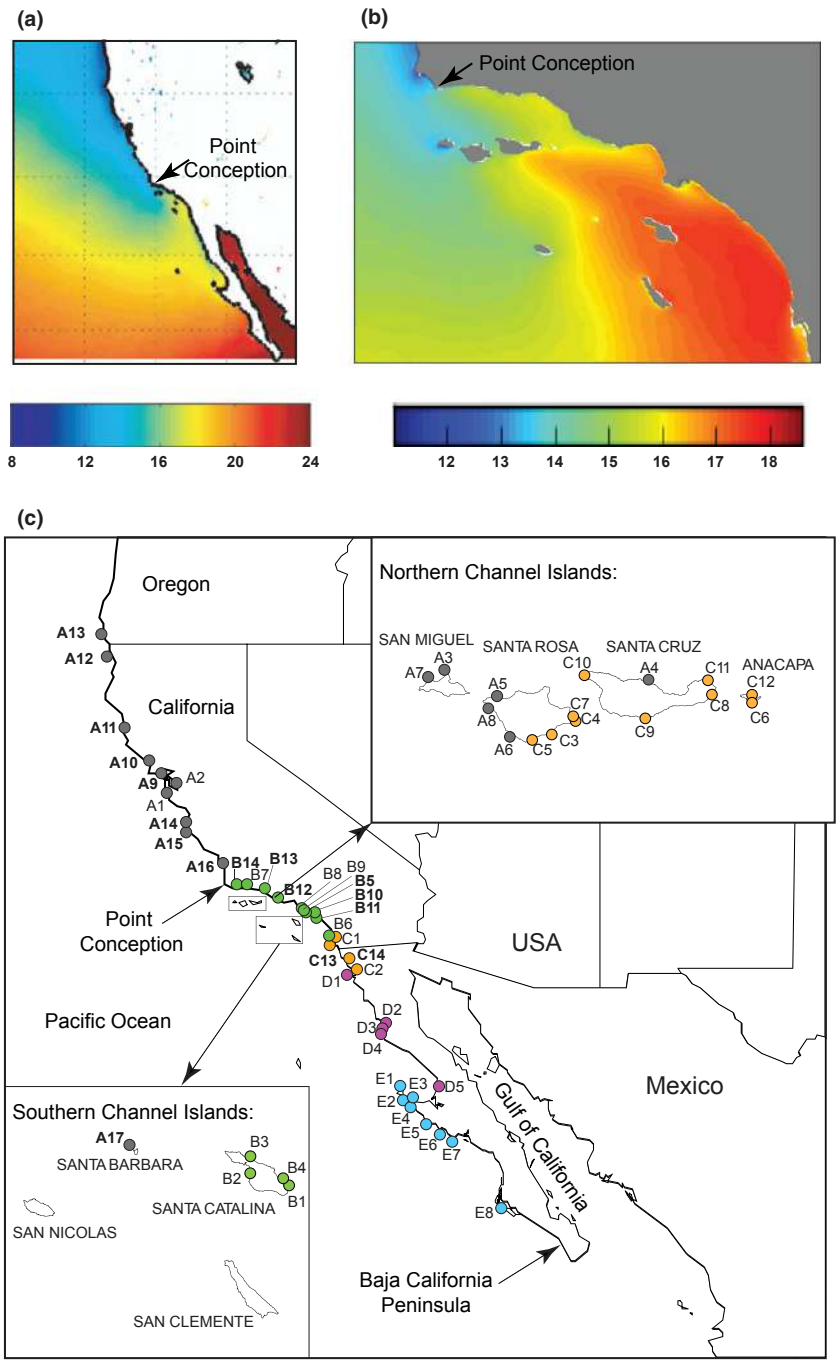

Figure 1 Study area in western North America, with sampling locations of *Ligia occidentalis* and sea surface temperature (SST) information. (a) Monthly averaged SST with a 4-km resolution in the study area and the Gulf of California from July 2002 to October 2010 (modified from Amaral *et al.*, 2012, Figure 5). (b) Monthly averaged SST in the Southern California Bight for the period 1997–2008 from the Pathfinder 5 Advanced Very-High Resolution Radiometer (Kahru & Mitchell, 2002). (c) Study area with sampling locations. Different colours and letters correspond to the clades in the phylogenetic tree depicted in Fig. 2. All new locations are marked in bold. Sample IDs of previously published locations appear as in Hurtado *et al.* (2010). A1, Princeton; A2, Coyote Point; A3, Harris Point; A4, Orizaba; A5, NW Talcott; A6, China Point; A7, Otter Harbor; A8, Fossil Reef; **A9, Kirby Cove; A10, McClures Beach; A11, Van Damme; A12, Point St George; A13, Cape Ferrelo; A14, Point Lobos; A15, Soberanes Point; A16, Avila State Beach; A17, Santa Barbara Island**; B1, Industrial Area; B2, Little Harbor; B3, Isthmus Cove; B4, Descanso; **B5, Long Beach** (one new sample and one old sample included); B6, San Diego; B7, Refugio; B8, Malaga Cove; B9, Cabrillo Beach; **B10, Crystal Beach; B11, Dana Point; B12, Point Mugu; B13, Ventura; B14, Gaviota**; C1, San Diego; C2, Corona Ensenada; C3, Ford Point; C4, East Point; C5, Johnsons Lee; C6, Side Frenchys; C7, Sandy Beach; C8, Smugglers Cove; C9, Willows; C10, Fraser Cove; C11, Scorpion; C12, Frenchys; **C13, Cabrillo National Monument; C14, Campo López**; D1, Bufadora, Ensenada; D2, San Quintín; D3, Punta Baja; D4, Arroyo Ancho; D5, Tomatal; E1, Isla Cedros (2 localities); E2, Punta Eugenia; E3, (includes three localities) El Chevo, El Queen and Malarrimo; E4, Tortugas; E5, Bahía Asunción; E6, San Hipólito; E7, Punta Abreojos; E8, Puerto San Carlos.

1979). Furthermore, marked changes in coastal hydrography, dissolved oxygen, salinity and topography, as well as convergent ocean currents, occur in the vicinity of PC (Briggs, 1974; Seapy & Littler, 1980; Browne, 1994). The same factors that govern the biogeographical distribution of different species in this region could also contribute to the genetic differentiation of lineages whose distributions span this boundary, but surprisingly few organisms exhibit genetic signatures of restricted gene flow across PC (Dawson, 2001; Hohenlohe, 2004; Pelc *et al.*, 2009). This has led to the proposal that neither disruption of gene flow by ocean circulation patterns, nor divergent selection due to physical differences between water masses around PC, has been sufficient to cause strong concordant patterns of genetic differentiation (Burton, 1998). Nonetheless, several intertidal organisms sharing limited dispersal potential show signatures of either ancient phylogeographical breaks or recent range expansions across the PC area.

Among the many marine Californian species whose ranges span PC, only the harpacticoid copepod *Tigriopus californicus* exhibits a deep phylogeographical break [> 20% Kimura two-parameter distance (K2P) for *COI*] coincident with PC (Burton, 1998; Dawson, 2001; Edmands, 2001). This copepod, which is restricted to pools in the high rocky intertidal, shows extraordinarily high levels of allopatric differentiation in the Pacific region between the central Baja California Peninsula and California, suggesting very limited dispersal (Burton, 1998; Edmands, 2001). Two other rocky intertidal species, also with limited dispersal potential, show genetic signatures of recent northern expansions across PC, suggestive of late Pleistocene post-glacial range expansions: the snails *Nucella emarginata* (Marko, 1998) and *Acanthinucella spirata* (Hellberg *et al.*, 2001). The occurrence of both deep and recent genetic signatures associated with PC in these three species suggests that past events (e.g. tectonics and palaeoclimate) associated with the PC biogeographical boundary may have operated at several times in this region. Furthermore, similarities among the three species, such as their restriction to the patchy rocky intertidal habitat and limited dispersal potential, imply that other animals with these characteristics may also show genetic signatures associated with PC.

The isopod *Ligia occidentalis* (Dana, 1853) occupies a narrow vertical portion of the rocky supralittoral (splash zone to high intertidal) along the Pacific coast of North America between southern Oregon and central Mexico (Schmalfuss, 2003; Eberl, 2012). Like all peracarids, *L. occidentalis* is a direct developer that lacks a dispersive larval stage. This isopod actively avoids fully aquatic and terrestrial environments adjacent to its patchy rocky intertidal habitat (Carefoot & Taylor, 1995). Consistent with its limited dispersal ability, this isopod exhibits high levels of allopatric genetic differentiation in the region between southern California, south of PC, and central Mexico, including the Gulf of California (Hurtado *et al.*, 2010). Numerous highly divergent lineages suggest the presence of multiple species within what has been regarded as *L. occidentalis* (Hurtado *et al.*, 2010). The phylogeographical patterns of *L. occidentalis* are consistent with hypothesized geological events in southern California and the Baja California Peninsula–Gulf of California region, and with phylogeographical and biogeographical patterns of several other taxa (discussed in Hurtado *et al.*, 2010).

Given the phylogeographical patterns previously observed in the southern part of its range, high levels of allopatric genetic differentiation in *L. occidentalis* might also occur in the northern part of its range. Besides PC, this region encompasses areas such as Cape Mendocino, San Francisco Bay and Monterey Bay, where phylogeographical breaks occur in other coastal organisms (Marko, 1998; Dawson, 2001; Pelc *et al.*, 2009; Kelly & Palumbi, 2010; Sivasundar & Palumbi, 2010). Alternatively, due to recent post-glacial expansions, the levels of genetic differentiation in the northern part of the range of *L. occidentalis* may be far less, as has been reported for other marine intertidal organisms (Marko, 1998; Edmands, 2001; Kelly & Eernisse, 2007).

Except for two localities around the species' type locality of San Francisco (Dana, 1853, pp. 742–743 and plate 49), no populations of *L. occidentalis* north of PC have been genetically characterized. The San Francisco samples group closely with samples from several localities of the Northern Channel Islands, but are highly divergent (20–25% K2P divergence for *COI*) from all other populations sampled south of PC (Hurtado *et al.*, 2010). The sampling gaps north of PC preclude the identification of the geographical limit between the two most divergent lineages of this isopod in California. Furthermore, whether other highly divergent lineages occur north of PC is unknown.

In this study, we used sequences from two mitochondrial genes and one nuclear gene to: (1) characterize the phylogenetic relationships of *L. occidentalis* lineages north of PC, and thus infer the distribution of the two main clades that inhabit the California coast; and (2) examine patterns of genetic diversity within its northern range. We expanded the geographical coverage of previous analyses, by comprehensively sampling populations in the region between PC and the northern range limit of *L. occidentalis* in southern Oregon. We also increased the sampling coverage south of PC. Our results indicate that the geographical limit between the two most divergent clades of *L. occidentalis* distributed in California occurs around the PC biogeographical boundary. The levels of allopatric genetic divergence were greatly reduced north of PC.

MATERIALS AND METHODS

Sampling

We collected *L. occidentalis* from Cape Ferrelo, Oregon (42.1° N) to Ventura, California (34.3° N), between December 2006 and January 2010. In June 2010, we sampled additional locations between the US–Mexico border and Monterey, California. Isopods were collected by hand and preserved in 95% ethanol. Figure 1c shows the new locations

sampled in this study (marked in bold in Fig. 1c) and the locations previously sampled by Hurtado *et al.* (2010) that we included in this study.

DNA extraction, PCR and sequencing

We extracted DNA from leg segments of individual isopods either with the DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA) or with a cetyltrimethylammonium bromide (CTAB) extraction protocol (von der Schulenburg *et al.*, 2001). For each new locality, we obtained partial sequences of the mitochondrial genes cytochrome *c* oxidase subunit I (*COI*) and 16S rDNA (*c.* 490 bp) for at least one individual, as described in Hurtado *et al.* (2010). For some individuals, we did not obtain the above *COI* PCR product; instead, a 620-bp region of this gene that overlaps with the 710-bp region used in Hurtado *et al.* (2010) was amplified using isopod-specific primers (Wilson *et al.*, 2009). In addition, we amplified a *c.* 709-bp fragment of the nuclear locus sodium–potassium ATPase α-subunit (*NaK*), using the primers NaK-for-b and NaK-rev2 (Tsang *et al.*, 2008), from 49 individuals, representing all five of the major clades identified (see Results). We used Sequencher 4.8 (Gene Codes, Ann Arbor, MI, USA) for sequence editing and primer removal. None of the *COI* or *NaK* sequences had premature stop codons or frame shifts.

Data sets and phylogenetic analyses

Our mitochondrial phylogenetic analyses included sequences of individuals from the newly sampled localities (bold labels in Fig. 1c) and the sequences reported in Hurtado *et al.* (2010) from localities along the Pacific coast of California and the Baja California Peninsula; in this region, Hurtado *et al.* (2010) reported two main well-supported and highly differentiated (20–25% K2P for *COI*) monophyletic groups of *L. occidentalis*. The 16S rDNA sequences were aligned with ClustalX 2.0 (Thompson *et al.*, 1997), and the alignment was revised in MacClade 4.08 (Maddison & Maddison, 2005). We used all positions obtained for this gene, as the size of the fragment was conserved among the taxa analysed and homology was easy to assess. We excluded other lineages of *Ligia*, because their high divergence from the two clades of interest introduced many alignment ambiguities to the 16S rDNA gene data set, resulting in the removal of numerous informative positions (Hurtado *et al.*, 2010). Furthermore, our aim was to establish the phylogenetic relationships of the new samples in the context of the two previously identified, highly divergent clades in California (Hurtado *et al.*, 2010).

We concatenated the *COI* and 16S rDNA genes for maximum likelihood (ML) and Bayesian phylogenetic analyses. To determine the most appropriate model of DNA substitution, we used jModelTest 0.1.1 (Posada, 2008) to evaluate 88 substitution models with full likelihood optimization, under the Akaike information criterion (AIC). The top three models, which collectively accounted for 99% of the AIC weight, were TVM+G (weight = 56.48%), GTR+G (40.84%) and TPM3uf+G (1.64%).

For the ML analyses, we used two different programs with nonparametric bootstrapping (1000 replicates): (a) RAxML 7.2.6 (Stamatakis *et al.*, 2008); and (b) garli 0.96beta8 (Zwickl, 2006). A single substitution model is available in RAxML (GTR+G). The top three models selected by the AIC (above) were used in the garli analyses. Fifty-percent majority-rule consensus trees of the bootstrap replicates were obtained for each analysis with the SumTrees command implemented in DendroPy 3.7.1 (Sukumaran & Holder, 2010). For the Bayesian analyses, we used MrBayes parallel version 3.1.2 (Ronquist & Huelsenbeck, 2003) under the GTR+G model. We conducted four independent runs (with four chains each) for 300 million generations, sampling every 5000 generations (after a burn-in of 30,000 generations). To determine whether the Markov chain converged on the stationary distribution and whether a sufficient sample of the stationary distribution had been obtained, we used the following criteria: (a) stable posterior probability values; (b) a high correlation between the split frequencies of independent runs as implemented in awty (Nylander *et al.*, 2008); (c) small and stable average standard deviation of the split frequencies of independent runs (< 0.002); (d) potential scale reduction factor close to 1; and (e) an effective sample size (ESS) > 200 for the posterior probabilities, as evaluated in Tracer 1.5 (Rambaut & Drummond, 2007). Samples prior to reaching the stationary posterior distribution were discarded as burn-in. All trees were rooted at the branch joining the two main clades.

Owing to the low number of *NaK* alleles and low degree of divergence among them, we used maximum parsimony (exhaustive search) as implemented in paup* 4.0b10 (Swofford, 2003) for the phylogenetic analyses of this gene fragment.

Population genetics analyses

Localities north of PC and in some Northern Channel Island localities formed a clade with very low genetic divergences (Clade A; see Results). To test whether the low levels of genetic diversity within this clade are consistent with ongoing gene flow, we obtained a preliminary estimate of genetic differentiation among populations within this clade. We computed pairwise F_{ST} with Arlequin 2.0 (Schneider *et al.*, 2000) for a subset of six localities for which we had sample sizes ≥ 8 individuals (A5, A9, A10, A12, A13 and A14; Fig. 1). In addition, we analysed genetic diversity for the *COI* gene with Arlequin 2.0 and DnaSP 5 (Rozas, 2009). K2P distances within and between clades were calculated in mega 5 (Tamura *et al.*, 2011). Finally, as phylogenetic reconstructions show poor resolution within this clade, we inferred a haplotype network with the software tcs 1.21, which calculates the most parsimonious connections between haplotypes (Clement *et al.*, 2000).

RESULTS

In this study, we sequenced 17 individuals for the 16S rDNA gene, representing each of the new localities sampled, and obtained 15 new haplotypes. In addition, we sequenced 117 individuals for the *COI* gene and obtained 48 new haplotypes. Finally, we sequenced a fragment of the *NaK* gene from 49 individuals, which yielded five unique alleles. The new haplotypes obtained in this study were deposited in GenBank under accession numbers JQ894946–JQ895008, JX134582–JX134584 and KC953096–KC953143 (see Appendix S1 in Supporting Information).

Phylogenetic analyses

Our *COI* + 16S rDNA concatenated data set contains 44 taxa (alignment in Appendix S2). A maximum-likelihood bootstrap consensus tree (Fig. 2) showed that all new samples (bold in Fig. 2) belonged to the previously identified clades in Hurtado *et al.* (2010). Thus, we retained the names and letter coding of different clades (clades A to E) as previously defined in Hurtado *et al.* (2010).

All populations north of PC belonged to Clade A (grey in Figs 1 & 2), whereas all mainland populations south of PC formed a southern cluster composed of the geographically delineated clades B, C, D and E (henceforward Clade BCDE). The geographical distance between the northernmost and southernmost mainland localities containing members of Clade A – Cape Ferrelo (A13) and Avila State Beach (A16; *c.* 83 km north of PC) – spans *c.* 830 km. Clade A also included samples from the Northern Channel Islands [San Miguel Island (A3, A7), western Santa Rosa Island (A5, A6, A8) and one locality on Santa Cruz Island (Orizaba, A4)], as well as a previously unsampled locality on Santa Barbara Island (A17) in the Southern Channel Islands. Within Clade A, a relatively deep divergence (*COI* K2P range = 10.69–11.57%) occurs between Santa Barbara Island and the remaining localities, which cluster in turn into a shallow group (< 3.1% *COI* K2P) with little internal resolution. This clade includes the samples from San Francisco Bay, the type locality of *L. occidentalis*.

All samples from mainland populations collected south of PC belonged either to Clade B or Clade C (green and yellow, respectively, in Figs 1 and 2). Clade B includes samples from Santa Catalina Island (B1–4) and localities on the mainland coast between Gaviota State Park (B8; *c.* 20 km east of PC) and San Diego (B6). Clade B is divided into two groups. The first one includes previously sampled localities from San Diego (B6) and Catalina Island (B1–4). The second one comprises old and new localities from Dana Point (B12) to Gaviota State Park (B8). Within this second mainland group, the samples from Ventura to Gaviota State Park (B7–B9) formed a shallow but well-supported clade, suggesting a recent phylogeographical break in the region between Point Mugu State Park (B10) and Ventura (B9).

On the mainland, Clade C is restricted to the region between San Diego, California, and Ensenada, Baja California, Mexico. The new samples collected within this range also fell within Clade C: the sample from Cabrillo National Monument (C2) represented a new haplotype, whereas the one from Campo López (C3) was identical to the ones from Corona Beach in Ensenada. Clade C also includes samples from the Channel Islands (Anacapa, Santa Cruz and the east of Santa Rosa). None of the new samples fell into clades D or E, which occur along the Pacific coast of the Baja California Peninsula (Hurtado *et al.*, 2010).

The *NaK* data set (trimmed to 448 bp to remove positions containing missing data for several taxa) had nine parsimony-informative sites and yielded five unique alleles. A single most parsimonious tree (Fig. 3), obtained after an exhaustive maximum parsimony search, depicts results congruent with the mitochondrial data. Allele Nak_A was restricted to populations identified as Clade A by the mitochondrial data, and was 6–7 steps divergent from the remaining alleles, which were restricted to localities occupied by Clade BCDE, as defined by the mitochondrial data. The remaining four alleles differed from each other by 1–4 steps. Two alleles differing from each other at a single position (Nak_B1 and Nak_B2) were restricted to populations identified as Clade B by the mitochondrial data. The other two alleles (Nak_CD and Nak_CDE) also differed from each other by a single position and were restricted to populations identified as members of Clade CDE by the mitochondrial data. No further resolution within Clade CDE was obtained. Two individuals from this clade (C5 Johnsons Lee and C11 Scorpion) exhibited a double peak at the position that distinguishes the Nak_CD and Nak_CDE haplotypes. Therefore, we assumed that these two individuals were heterozygous for these alleles. No other individuals exhibited evidence of heterozygosity.

Genetic diversity and differentiation within Clade A populations

We inferred a *COI* haplotype network for all individuals belonging to Clade A, with the exception of Santa Barbara Island, which was very divergent, and Fossil Reef, for which we were unable to amplify *COI* (Fig. 4). For the remaining 15 populations, we observed 30 different *COI* haplotypes among 101 individuals (Appendix S3); only nine of these haplotypes were shared between two or more sampling locations. The maximum uncorrected pairwise (p) distance within this clade was 3.0% (K2P, 3.1%). Seven haplotypes that were not shared with any other locality occurred at Cape Ferrelo, with a maximum K2P divergence of 2.6% (uncorrected $P = 2.6\%$) among them. No haplotypes were shared between mainland localities and the Northern Channel Islands. Two haplotypes were shared among Channel Island populations and seven haplotypes were shared among mainland populations. Haplotype sharing within the mainland occurred among geographically close localities (Fig. 4,

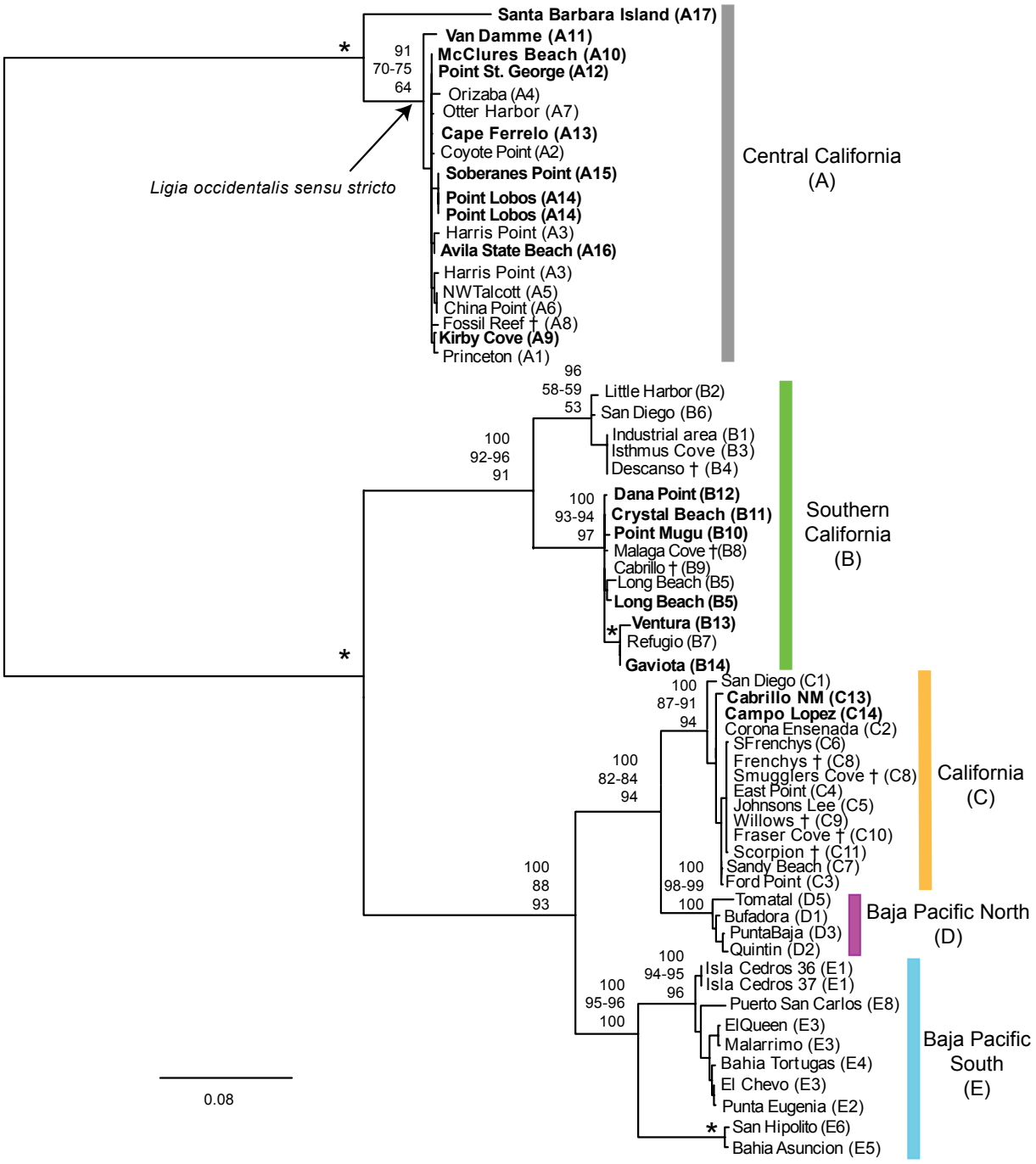

Figure 2 Maximum-likelihood bootstrap consensus tree of *Ligia* samples from localities in Fig. 1 (southern Oregon, USA to Baja California Sur, Mexico), obtained by RAxML analyses (GTR+G model), for the 16S rDNA and *COI* genes. Tree was rooted at the branch joining Clade A and Clade BCDE. Taxon identifiers and clade colours correspond to those in Fig. 1, with new samples in bold. Numbers by nodes indicate the corresponding range of node support values obtained for each method: top, Bayesian posterior probabilities; middle, GARLI bootstrap support; bottom, RAxML bootstrap support. Asterisks (*) denote nodes that received 100% support for all methods. Nodes with no corresponding support values were of little relevance or had low support value. Obelisks (†) denote relationship based on 16S rDNA sequence only. The scale bar represents the mean number of nucleotide substitutions per site.

Appendix S3), but not across the entire mainland region. At the six localities within Clade A for which we sequenced ≥ 8 individuals, 3–7 haplotypes were observed per locality; haplotype diversity (*Hd*) was 0.32–0.86; nucleotide diversity (π) was 0.001–0.007; and the average number of nucleotide differences (*k*) was 0.8–6.5 (Table 1). With one exception (Point St George versus McClures Beach), all pairwise F_{ST} values were large (0.144–0.626) and significant (Table 2), suggesting a high level of isolation among these populations.

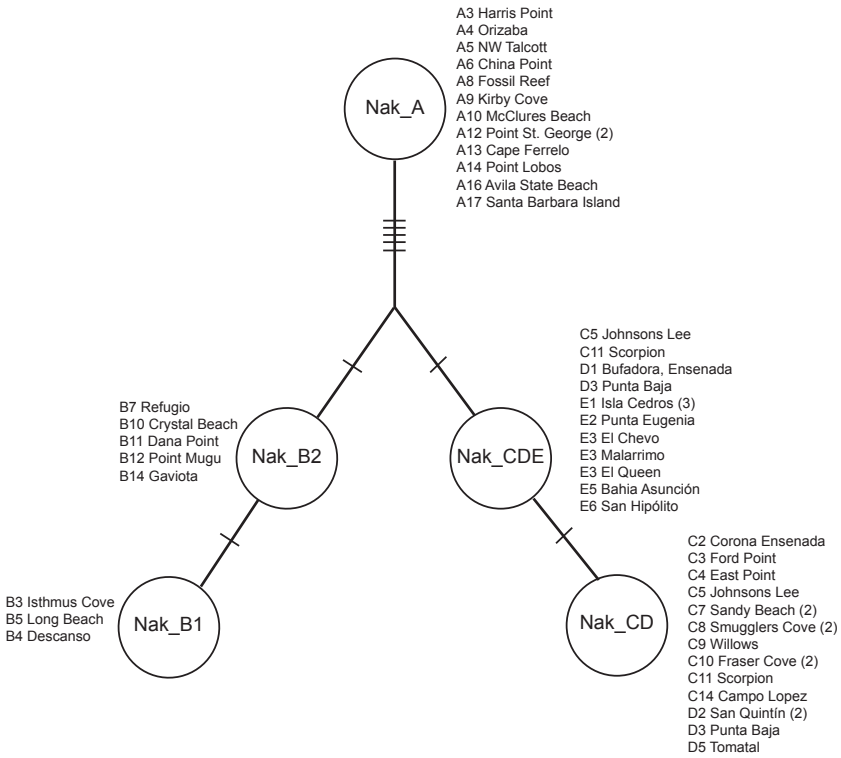

Figure 3 Single most parsimonious tree of the five *NaK* alleles (represented by circles) identified among 49 individuals of *Ligia occidentalis* from 43 localities from southern Oregon, USA to Baja California Sur, Mexico, representing members from clades A, B, C, D and E as identified by the mitochondrial data. Slashes indicate number of parsimony steps. Localities where each allele was found are listed by each allele. For localities where more than one individual was examined, the number of individuals appears in parentheses.

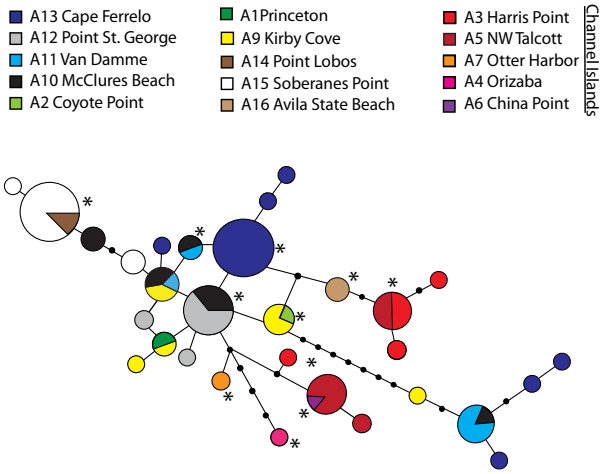

Figure 4 Mitochondrial *COI* haplotype network for *Ligia occidentalis* Clade A (southern Oregon to southern California, USA), excluding the sample from Santa Barbara Island. The size of the circles is proportional to the number of individuals carrying the respective haplotype. Asterisks (*) indicate the *COI* sequences that were used for the phylogenetic reconstructions in Fig. 2.

Table 1 Mitochondrial *COI* genetic diversity among locations of *Ligia occidentalis* Clade A from southern Oregon to southern California, USA.

Location	*I*	*H*	*Hd*	π	*k*
Cape Ferrelo (A13)	22	7	0.481 ± 0.131	0.006	3.208
Point St George (A12)	12	3	0.439 ± 0.158	0.001	0.773
Van Damme SP (A11)	6	3	0.600 ± 0.215	0.012	6.467
McClure Beach (A10)	11	5	0.782 ± 0.107	0.007	3.691
Kirby Cove (A9)	8	5	0.857 ± 0.108	0.007	3.679
Coyote Point (A2)	1	1			
Princeton (A1)	1	1			
Point Lobos (A14)	2	1			
Soberanes Point (A15)	17	3	0.324 ± 0.136	0.001	0.779
Avila State Beach (A16)	2	1			
Harris Point (A3)	6	4	0.810 ± 0.130	0.007	3.714
Otter Harbor (A7)	1	1			
Orizaba (A4)	1	1			
NW Talcott (A5)	10	3	0.600 ± 0.131	0.006	3.000
China Point (A6)	1	1			
Santa Barbara Island (A17)	1	1			

I, number of individuals; *H*, number of haplotypes; *Hd*, haplotype diversity; π, nucleotide diversity; *k*, average number of nucleotide differences.

DISCUSSION

Deep phylogeographical break around the PC biogeographical boundary

The identification of geographical boundaries between genetically differentiated lineages is a crucial step towards understanding the factors that shape their evolutionary histories and limit their distributions. Our results reveal that the boundary between the two most divergent (20–25% K2P distance for *COI*) clades of *L. occidentalis* present in the Pacific region from Baja California Sur to southern Oregon (Clade A and Clade BCDE; Fig. 2) closely corresponds to the PC

Table 2 Pairwise F_{ST} values for populations of *Ligia occidentalis* Clade A from southern Oregon to southern California, USA. Populations with fewer than eight individuals sampled were not included.

	Cape Ferrelo (A13)	Point St George (A12)	McClures Beach (A10)	Kirby Cove (A9)	Soberanes Pt (A15)
Point St George	0.567*				
McClures Beach	0.424*	0.076			
Kirby Cove	0.406*	0.375*	0.144*		
Soberanes Pt	0.620*	0.626*	0.477*	0.468*	
NW Talcott	0.504*	0.485*	0.307*	0.278*	0.563*

*$P < 0.05$ obtained after 1000 permutations.

biogeographical boundary, a region characterized by a transition between cold northern and warm southern water masses (Fig. 1a,b) and convergent patterns of oceanographic circulation. The harpacticoid copepod *T. californicus* is the only other marine organism for which a deep phylogeographical break (*c.* 21% K2P distance for *COI*) coincident with PC has been reported (Burton, 1998; Edmands, 2001). Nonetheless, whereas for *L. occidentalis* the deepest phylogeographical break along this coastline occurs in the PC region, additional breaks of equal or greater magnitude occur in *T. californicus* (Burton, 1998; Edmands, 2001).

The distribution of the two main *Ligia* lineages, at both mainland and insular localities, generally matches differences in sea surface temperature (SST): Clade A occupies localities with colder SST and Clade BCDE occupies localities with warmer SST (Fig. 1). On the mainland, Clade A is distributed north of PC, extending *c.* 830 km to southern Oregon, whereas Clade BCDE is distributed south of PC, extending *c.* 1330 km to the southern Baja California Peninsula. On the Southern California Islands, the distribution of the two main *Ligia* clades also corresponds with the cold–warm oceanic circulation patterns that define the PC biogeographical boundary. Neushul *et al.* (1965) and Seapy & Littler (1980) suggest that: (1) the coasts of the California Channel Islands that are strongly influenced by the cold California Current should show north-of-PC biogeographical affinities; (2) the coasts strongly influenced by the warm, northerly-flowing Southern California Eddy should show south-of-PC biogeographical affinities; and (3) coasts with a mixture of cold and warm waters should have intermediate affinities. Consistent with these expectations, Clade A occupied sites in the Northern Channel Islands that are strongly affected by the cold California Current (San Miguel, the western half of Santa Rosa and the northern side of Santa Cruz). San Nicolas Island (not sampled in our study) is strongly affected by the cold California Current and also appears to harbour members of Clade A according to allozyme data (McGill, 1978; discussed below). A divergent lineage of Clade A was found on the small (2.6 km^2), relatively isolated Santa Barbara Island, which has a mixture of cold and warm waters. Clade C was found in Northern Channel Islands localities that have a mixture of cold and warm waters from the California Current and Southern California Eddy (Anacapa, the southern side of Santa Cruz and the eastern half of Santa Rosa). Clade B was found on Santa Catalina Island, in the path of the warm, northerly-flowing Southern California Eddy.

The overall distribution of the two main *L. occidentalis* clades suggests that SST might exert a selective pressure on this isopod. SST affects the distribution, abundance and community assemblages of invertebrates, macrophytes and fishes in the subtidal and intertidal of the Northern Channel Islands and the Southern California Bight (Hewatt, 1946; Blanchette *et al.*, 2006; Hamilton *et al.*, 2010; Watson *et al.*, 2011). Although *L. occidentalis* is practically terrestrial and does not venture into open water at any stage (Carefoot & Taylor, 1995), SST influences abiotic factors that are likely to be relevant to its survival and reproduction, such as air temperature, sea and land breezes, atmospheric humidity and coastal fog (van den Dool & Nap, 1985; Rayner *et al.*, 2003; Kawai & Wada, 2007; Tang, 2012). Differences in thermal tolerance could explain the distribution of the two main *L. occidentalis* clades. For example, in the supralittoral pool-dweller *T. californicus*, increased tolerance to high temperature stress is associated with decreasing latitude (Willett, 2010). We conducted an analysis of variance and observed a significant association of *L. occidentalis* clade identity (Clade A versus Clade BCDE) and SST ($F\text{-ratio}_{1,59} = 103$, $P < 0.0001$; SST values from sources in Fig. 1). A significant association was also observed between clade identity and both maximum and minimum air temperature for coastal mainland localities within the USA (minimum: $F\text{-ratio}_{1,24} = 61$, $P < 0.0001$; maximum: $F\text{-ratio}_{1,24} = 21$, $P = 0.0001$; values from http://www.wrcc.dri.edu/Climsum.html). Tolerance to low air temperatures is expected to be highly relevant to *L. occidentalis,* because this isopod is more active at night and tends to hide during the day. The relative contribution of abiotic factors to the distribution of the two main *L. occidentalis* clades, however, needs further study.

In addition to SST, the convergent ocean currents around the PC biogeographical boundary may have played a role in shaping the distribution of Clade A and Clade BCDE. Although *Ligia* is effectively terrestrial throughout its life cycle (Carefoot & Taylor, 1995), it could potentially use surface currents to disperse on floating debris. The presence of Clade A populations on the Channel Islands and the absence of Clade BCDE populations north of PC is consistent with the asymmetrical southward migration pattern around PC reported for several pelagically dispersing intertidal

invertebrates (Wares *et al.*, 2001; Hohenlohe, 2004; Cassone & Boulding, 2006). Nonetheless, colonization of the Northern Channel Islands could have occurred over land, as the four present-day Northern Channel Islands presumably formed a large contiguous land mass *c.* 17,000 years ago, the eastern end of which may have been connected to the mainland (Schoenherr *et al.*, 1999). In contrast, the colonization of Santa Barbara Island by a member of Clade A probably represents a relatively old event that occurred via rafting. This is suggested by the high sequence divergence of the Santa Barbara Island population, as well as the apparently long-standing isolation of this small island from other landmasses (Graham *et al.*, 2010).

Our results are consistent with an earlier allozyme study of *L. occidentalis* populations in California (McGill, 1978). The mainland populations examined by McGill between Los Angeles and Gaviota (Clade B in our study) were virtually identical, but were highly differentiated [Nei's (1972) $D = 0.412–0.427$] from four other populations, including three that fell within Clade A in our study (San Francisco, San Miguel Island and northern Santa Cruz Island), and one that we did not sample (San Nicolas Island). Allozyme distances among these last four populations were relatively low ($D = 0.001–0.015$), consistent with the low observed mitochondrial diversity among Clade A populations in the present study, thus suggesting that San Nicolas Island also harbours members of Clade A. An additional population examined by McGill but not included in our study – from San Clemente Island – was *c.* 0.17 and *c.* 0.20 distant (D) from populations in the putative clades A and B, respectively.

High genetic divergences among clades of *Ligia occidentalis*, as well as the outcome of laboratory crosses performed by McGill (1978), imply the presence of multiple *Ligia* species. Reciprocal crosses between San Francisco (Clade A) and Carpenteria [presumably belonging to Clade B according to allozyme distances (McGill, 1978) and its geographical location between Ventura and Santa Barbara] were unsuccessful, indicating that Clade A is reproductively incompatible with Clade B, and probably with the other members of Clade BCDE. Other reciprocal crosses attempted by McGill (1978) that failed were: San Nicolas Island (presumably Clade A) × Carpenteria; San Clemente Island × Carpenteria; and San Clemente Island × San Nicolas Island. McGill (1978) only observed F_1 offspring in reciprocal crosses between Los Angeles and Carpenteria, two putative Clade B populations with K2P *COI* divergence below 2%.

Based on our results, as well as the mating experiments and allozyme data of McGill (1978), we suggest that only the lineages within Clade A, excluding the Santa Barbara Island lineage, be referred to as *Ligia occidentalis sensu stricto* (Fig. 2), as this clade includes the type locality of *L. occidentalis*. The Santa Barbara Island lineage is 11% divergent from the other Clade A members, a divergence that appears to correspond better with species-level differentiation in marine metazoans (Bucklin *et al.*, 2011). Maximum K2P divergence among the remaining members of Clade A is 3.1%, a value more commonly observed within marine metazoan species (Bucklin *et al.*, 2011). South of PC, the numerous allopatric lineages that are highly divergent from *L. occidentalis sensu stricto* and from each other, are likely to represent a complex of undescribed species (Hurtado *et al.*, 2010). No junior synonyms have been described in this region (Schmalfuss, 2003).

The lack of reliable calibration points (e.g. from fossils or vicariant events) and knowledge of substitution rates in the mitochondrial genome of this isopod precludes the estimation of divergence times of *L. occidentalis* lineages. The observed genetic divergences between the two main California clades, however, suggest that this event occurred in the late Miocene–early Pliocene. We are unaware of tectonic events around PC that could explain this deep phylogenetic break. Furthermore, the divergence between these clades may have occurred somewhere else, with their present-day distributions shifting according to historical changes in SST. The California coast has had a dynamic history, during which SST, ocean currents, sea level and the distribution of rocky intertidal habitat have changed dramatically over short evolutionary periods, affecting the distributions of marine species (Graham *et al.*, 2010). For example, during the Last Glacial Maximum (*c.* 18,500 years ago), SST in the area between PC and San Diego is estimated to have been 6–10 °C lower than presently (Mortyn *et al.*, 1996). Thus, if SST indeed influences the distribution of Clade A and Clade BCDE, the geographical boundary between these two clades was probably located at that time south of its present location at PC.

Drastic reduction of allopatric genetic divergences north of Point Conception

We observed a dramatic decline in genetic divergences among lineages of *L. occidentalis* north of the PC biogeographical boundary, in a region that spans *c.* 830 km. This observation contrasts sharply with the extraordinarily high levels of allopatric genetic divergences and numerous deep phylogeographical breaks among *L. occidentalis* populations in the region between PC and central Mexico, including the Gulf of California (Hurtado *et al.*, 2010). The high and significant F_{ST} values detected in most of the Clade A pairwise comparisons suggest that the shallow genetic divergences observed within this clade (excluding Santa Barbara Island) resulted from a recent colonization or range expansion, rather than from high levels of contemporary gene flow. Accordingly, the poor dispersal abilities of this isopod and the patchy distribution of its habitat effectively isolate the northern populations from each other, but a relatively recent colonization history of the region might explain the lower levels of allopatric divergence.

An abrupt decline in the levels of allopatric genetic divergences north of PC has been observed in other rocky intertidal species with limited dispersal potential, and has been

attributed to recent late-Pleistocene post-glacial range expansions. The snail *Nucella emarginata* shows significantly lower genetic diversity north of PC, a shallow phylogeographical break (0.34–0.51% in *COI*) at this location, and a phylogeographical pattern consistent with a recent northward range expansion across this locality (Marko, 1998). Similarly, the snail *Acanthinucella spirata* exhibits a drastic reduction in genetic diversity north of PC, as well as a genetic signature indicating a recent and rapid northern expansion (Hellberg *et al.*, 2001). The sharp reduction in the levels of genetic divergences among lineages of *L. occidentalis* north of PC probably also reflects recent post-glacial range expansions. Post-glacial range expansions have also been hypothesized to explain abrupt declines in the levels of genetic differentiation in the northern range of *T. californicus* (Edmands, 2001). The location of the shift in this copepod, however, occurs around northern California to central Oregon (Burton, 1998; Edmands, 2001). Past glacial events thus appear to have influenced the evolutionary histories of non-vagile rocky intertidal invertebrates with distributions spanning PC differently. Further north, rocky intertidal invertebrates also appear to have been differentially affected by past glacial events (Marko *et al.*, 2010).

A cyclical pattern of rising and falling global temperatures and changing sea levels during the Quaternary has provided ample opportunities for range expansions/contractions and allopatric genetic differentiation in rocky intertidal organisms of the California coast. Fossil evidence indicates that marine faunas shifted polewards during high SST periods, such as the Pleistocene–Holocene transition (Addicott, 1969; Moore *et al.*, 1980). In addition, with each glacial–interglacial cycle, the southern California coast experienced essentially continuous fluctuations in reef distribution and size, cycling between sandy and rocky shores, and between cold and warm hydrographical conditions (Graham *et al.*, 2003). During most of the late Pleistocene and early Holocene, the Southern California Bight probably consisted of extensive cold, rocky, cobbled shores, interrupted by estuarine embayments (Graham *et al.*, 2003). The minimum sea level of this region during the Last Glacial Maximum (*c.* 18,500 years ago) was *c.* 117 m below present (Graham *et al.*, 2003). The present-day sand-dominated coastlines of the Southern California Bight appear to have developed only over the past 4000–6000 years (Inman, 1983). These fluctuations would have dramatically affected the upper intertidal rocky habitat, and thus the evolutionary history of *Ligia* isopods and other rocky intertidal organisms in this region.

ACKNOWLEDGEMENTS

We thank Evelyne Kuo, Guillermo Torres, Alejandro Hurtado and Daniela Hurtado for help with collecting. Dan Richards (Channel Islands National Park) collected the specimen from Santa Barbara Island. James Watson produced the map in Fig. 1b. We thank Doug Eernisse and two anonymous referees for valuable input. This work was supported by National Science Foundation (NSF) grants DEB 0743782 to L.A.H. and M.M., OCE 0929057 to R.K.G., and University of California Davis grants to R.E., who was partly supported through NSF grant DGE 0841297 (PI: S.L. Williams). Collections were conducted under scientific collecting permits: California Department of Fish and Game No. 9881 and No. 9005; United States Department of the Interior National Park Service GOGA-2008-SCI-0015 and PORE-2008-SCI-0023; and CONAPESCA DGOPA.10337.020908.2952. This manuscript is Contribution Number 227 of the Center for Biosystematics and Biodiversity, Texas A&M University. Contribution from the Bodega Marine Laboratory, University of California, Davis. The authors acknowledge the Texas A&M University Brazos HPC cluster (http://brazos.tamu.edu/), which was used for analyses in this project.

REFERENCES

Addicott, W.O. (1969) Tertiary climatic change in the marginal northeastern Pacific Ocean. *Science*, **165**, 583–586.

Amaral, A.R., Beheregaray, L.B., Bilgmann, K., Boutov, D., Freitas, L., Robertson, K.M., Sequeira, M., Stockin, K.A., Coelho, M.M. & Möller, L.M. (2012) Seascape genetics of a globally distributed, highly mobile marine mammal: the short-beaked common dolphin (genus *Delphinus*). *PLoS ONE*, **7**, e31482.

Blanchette, C.A., Broitman, B.R. & Gaines, S.D. (2006) Intertidal community structure and oceanographic patterns around Santa Cruz Island, CA, USA. *Marine Biology*, **149**, 689–701.

Briggs, J.C. (1974) *Marine zoogeography*. McGraw-Hill, New York.

Browne, D.R. (1994) Understanding the oceanic circulation in and around the Santa Barbara Channel. *The Fourth California Islands Symposium: Update on the Status of Resources* (ed. by W.L. Halvorson and G.J. Maender), pp. 27–34. Santa Barbara Museum of Natural History, Santa Barbara, CA.

Bucklin, A., Steinke, D. & Blanco-Bercial, L. (2011) DNA barcoding of marine Metazoa. *Annual Review of Marine Science*, **3**, 471–508.

Burton, R.S. (1998) Intraspecific phylogeography across the Point Conception biogeographic boundary. *Evolution*, **52**, 734–745.

Carefoot, T.H. & Taylor, B.E. (1995) *Ligia*: a prototypical terrestrial isopod. *Terrestrial isopod biology* (ed. by M.A. Alikhan), pp. 47–60. A.A. Balkema, Rotterdam.

Cassone, B.J. & Boulding, E.G. (2006) Genetic structure and phylogeography of the lined shore crab, *Pachygrapsus crassipes*, along the northeastern and western Pacific coasts. *Marine Biology*, **149**, 213–226.

Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Dana, J. (1853) *United States exploring expedition during the years 1838, 1839, 1840, 1841, 1842 under the command of Charles Wilkes, U.S.N.* Vol. 13, *Crustacea, Part II. Isopoda.* C. Sherman, Philadelphia, PA.

Dawson, M.N. (2001) Phylogeography in coastal marine animals: a solution from California? *Journal of Biogeography*, **28**, 723–736.

van den Dool, H.M. & Nap, J.L. (1985) Short and long range air temperature forecasts near an ocean. *Monthly Weather Review*, **113**, 878–887.

Eberl, R. (2012) Distribution, habitat and food preferences of sympatric high intertidal isopod species *Ligia occidentalis* and *Ligia pallasii* (Ligiidae: Oniscidea). *Journal of Natural History*, **46**, 1779–1797.

Edmands, S. (2001) Phylogeography of the intertidal copepod *Tigriopus californicus* reveals substantially reduced population differentiation at northern latitudes. *Molecular Ecology*, **10**, 1743–1750.

Graham, M.H., Dayton, P.K. & Erlandson, J.M. (2003) Ice ages and ecological transitions on temperate coasts. *Trends in Ecology and Evolution*, **18**, 33–40.

Graham, M.H., Kinlan, B.P. & Grosberg, R.K. (2010) Post-glacial redistribution and shifts in productivity of giant kelp forests. *Proceedings of the Royal Society Biological Sciences B: Biological Sciences*, **277**, 399–406.

Hamilton, S.L., Caselle, J.E., Malone, D.P. & Carr, M.H. (2010) Incorporating biogeography into evaluations of the Channel Islands marine reserve network. *Proceedings of the National Academy of Sciences USA*, **107**, 18272–18277.

Hellberg, M.E., Balch, D.P. & Roy, K. (2001) Climate-driven range expansion and morphological evolution in a marine gastropod. *Science*, **292**, 1707–1710.

Hewatt, W.G. (1946) Marine ecological studies on Santa Cruz Island, California. *Ecological Monographs*, **16**, 185–210.

Hohenlohe, P.A. (2004) Limits to gene flow in marine animals with planktonic larvae: models of *Littorina* species around Point Conception, California. *Biological Journal of the Linnean Society*, **82**, 169–187.

Hurtado, L.A., Mateos, M. & Santamaria, C.A. (2010) Phylogeography of supralittoral rocky intertidal *Ligia* isopods in the Pacific region from central California to central Mexico. *PLoS ONE*, **5**, e11633.

Inman, D.L. (1983) Application of coastal dynamics to the reconstruction of palaeocoastlines in the vicinity of La Jolla, California. *Quaternary coastlines and marine archaeology* (ed. by P.M. Masters and N.C. Fleming), pp. 1–49. Academic Press, New York.

Kahru, M. & Mitchell, B.G. (2002) Influence of the El Niño–La Niña cycle on satellite-derived primary production in the California Current. *Geophysical Research Letters*, **29**, 1846. doi:10.1029/2002GL014963.

Kawai, Y. & Wada, A. (2007) Diurnal sea surface temperature variation and its impact on the atmosphere and ocean: a review. *Journal of Oceanography*, **63**, 721–744.

Kelly, R.P. & Eernisse, D.J. (2007) Southern hospitality: a latitudinal gradient in gene flow in the marine environment. *Evolution*, **61**, 700–707.

Kelly, R.P. & Palumbi, S.R. (2010) Genetic structure among 50 species of the northeastern Pacific rocky intertidal community. *PLoS ONE*, **5**, e8594.

Maddison, D.R. & Maddison, W. (2005) *MacClade 4: analysis of phylogeny and character evolution*. Sinauer Associates, Sunderland, MA.

Marko, P.B. (1998) Historical allopatry and the biogeography of speciation in the prosobranch snail genus *Nucella*. *Evolution*, **52**, 757–774.

Marko, P.B., Hoffman, J.M., Emme, S.A., McGovern, T.M., Keever, C.C. & Cox, L.N. (2010) The 'Expansion–Contraction' model of Pleistocene biogeography: rocky shores suffer a sea change? *Molecular Ecology*, **19**, 146–169.

McGill, T.J. (1978) *Genetic divergence of mainland and insular populations of* Ligia occidentalis *(Oniscoidea–Isopoda)*. PhD Thesis, University of California, Santa Barbara, CA.

Moore, T.C., Burckle, L.H., Geitzenauer, K., Luz, B., Molina-Cruz, A., Robertson, J.H., Sachs, H., Sancetta, C., Thiede, J., Thompson, P. & Wenkam, C. (1980) The reconstruction of sea surface temperatures in the Pacific Ocean of 18,000 B.P. *Marine Micropaleontology*, **5**, 215–247.

Mortyn, P.G., Thunell, R.C., Anderson, D.M., Stott, L.D. & Le, J. (1996) Sea surface temperature changes in the southern California borderlands during the last glacial-interglacial cycle. *Paleoceanography*, **11**, 415–429.

Nei, M. (1972) Genetic distance between populations. *The American Naturalist*, **106**, 283–292.

Neushul, M., Clarke, W.D. & Brown, D.W. (1965) Subtidal plant and animal communities of the Southern California Islands. *Proceedings of the Symposium on the Biology of the California Islands* (ed. by R.N. Philbrick), pp. 37–55. Santa Barbara Botanic Garden, Santa Barbara, CA.

Newman, W.A. (1979) Californian transition zone: significance of short-range endemics. *Historical biogeography, plate tectonics, and the changing environment* (ed. by J. Gray and A.J. Boucot), pp. 399–416. Oregon State University Press, Corvallis, OR.

Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics*, **24**, 581–583.

Pelc, R.A., Warner, R.R. & Gaines, S.D. (2009) Geographical patterns of genetic structure in marine species with contrasting life histories. *Journal of Biogeography*, **36**, 1881–1890.

Posada, D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.

Rambaut, A. & Drummond, A.J. (2007) *Tracer v1.4*. Available at: http://beast.bio.ed.ac.uk/Tracer.

Rayner, N.A., Parker, D.E., Horton, E.B., Folland, C.K., Alexander, L.V., Rowell, D.P., Kent, E.C. & Kaplan, A. (2003) Global analyses of sea surface temperature, sea ice,

and night marine air temperature since the late nineteenth century. *Journal of Geophysical Research: Atmospheres*, **108**, 4407.

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

Rozas, J. (2009) DNA sequence polymorphism analysis using DnaSP. *Bioinformatics for DNA sequence analysis* (ed. by D. Posada), pp. 337–350. Humana Press, Totowa, NJ.

Schmalfuss, H. (2003) World catalog of terrestrial isopods (Isopoda: Oniscidea). *Stuttgarter Beiträge zur Naturkunde, Serie A*, **654**, 1–341.

Schneider, S., Roessli, D. & Excoffier, L. (2000) *Arlequin: a software for population genetic analysis*. Genetics and Biometry Laboratory, University of Geneva, Geneva.

Schoenherr, A.A., Feldmeth, C.R. & Emerson, M.J. (1999) *Natural history of the islands of California*. University of California Press, Berkeley, CA.

von der Schulenburg, J.H., Hancock, J.M., Pagnamenta, A., Sloggett, J.J., Majerus, M.E.N. & Hurst, G.D.D. (2001) Extreme length and length variation in the first ribosomal internal transcribed spacer of ladybird beetles (Coleoptera: Coccinellidae). *Molecular Biology and Evolution*, **18**, 648–660.

Seapy, R.R. & Littler, M.M. (1980) Biogeography of rocky intertidal macroinvertebrates of the Southern California Islands. *The California islands: proceedings of a multidisciplinary symposium* (ed. by D.M. Powers), pp. 307–323. Santa Barbara Museum of Natural History, Santa Barbara, CA.

Sivasundar, A. & Palumbi, S.R. (2010) Life history, ecology and the biogeography of strong genetic breaks among 15 species of Pacific rockfish, *Sebastes*. *Marine Biology*, **157**, 1433–1452.

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, **57**, 758–771.

Sukumaran, J. & Holder, M.T. (2010) DendroPy: a Python library for phylogenetic computing. *Bioinformatics*, **26**, 1569–1571.

Swofford, D.L. (2003) *PAUP*: phylogenetic analysis using parsimony (*and other methods)*. Version 4.0b10. Sinauer Associates, Sunderland, MA.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.

Tang, Y.-M. (2012) The effect of variable sea surface temperature on forecasting sea fog and sea breezes: a case study. *Journal of Applied Meteorology and Climatology*, **51**, 986–990.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The Clustal_X Windows interface: flexible strategies for multiple sequence alignments aided by quality analysis tools. *Nucleic Acids Research*, **25**, 4876–4882.

Tsang, L.M., Ma, K.Y., Ahyong, S.T., Chan, T.-Y. & Chu, K.H. (2008) Phylogeny of Decapoda using two nuclear protein-coding genes: origin and evolution of the Reptantia. *Molecular Phylogenetics and Evolution*, **48**, 359–368.

Wares, J.P., Gaines, S.D. & Cunningham, C.W. (2001) A comparative study of asymmetric migration events across a marine biogeographic boundary. *Evolution*, **55**, 295–306.

Watson, J.R., Hays, C.G., Raimondi, P.T., Mitarai, S., Dong, C., McWilliams, J.C., Blanchette, C.A., Caselle, J.E. & Siegel, D.A. (2011) Currents connecting communities: nearshore community similarity and ocean circulation. *Ecology*, **92**, 1193–1200.

Willett, C.S. (2010) Potential fitness trade-offs for thermal tolerance in the intertidal copepod *Tigriopus californicus*. *Evolution*, **64**, 2521–2534.

Wilson, G.D.F., Humphrey, C.L., Colgan, D.J., Gray, K.-A. & Johnson, R.N. (2009) Monsoon-influenced speciation patterns in a species flock of *Eophreatoicus* Nicholls (Isopoda; Crustacea). *Molecular Phylogenetics and Evolution*, **51**, 349–364.

Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. PhD Thesis, University of Texas, Austin, TX.

SUPPORTING INFORMATION

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

Appendix S1 Sampling localities and corresponding GenBank accession numbers.

Appendix S2 *COI* and 16S rDNA sequence alignment.

Appendix S3 *COI* haplotypes of *Ligia occidentalis* Clade A with accession numbers and distribution information used in the network analysis in Fig. 4.

BIOSKETCH

The authors share research interests in the study of the ecology, biodiversity and evolution of marine invertebrates.

Author contributions: R.E., L.H., M.M. and R.K.G. conceived the study; R.E., L.H., and M.M. obtained the samples; R.E., L.H. and C.A.S. collected the data; R.E., L.H., M.M. and C.A.S. analysed the data; R.E., L.H., M.M. and R.K.G. wrote the manuscript.

Editor: David Bellwood