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Genetic signatures of rafting dispersal in algal-dwelling brooders *Limnoria* spp. (Isopoda) along the SE Pacific (Chile)

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ABSTRACT: Brooding marine isopods of the genus *Limnoria* inhabit and feed on kelp holdfasts and wood. These substrata have high floating potential, making these species ideal organisms to study the effects of rafting-mediated connectivity on the population structure of brooders living on rafting substrata. It is hypothesized that rafting leaves particular genetic signatures such as low differentiation among distant local populations and absence of isolation by distance (IBD) at a macro-geographic scale (thousands of km). Using cytochrome oxidase I (COI) sequences, we tested the effects of rafting-mediated gene flow with respect to genetic differentiation on *L. quadripunctata* (from wood and also the holdfasts of the giant kelp *Macrocystis pyrifera*) and *L. chilensis* (mainly from the bull kelp *Durvillaea antarctica*) sampled across 2400 km of the Chilean coast. Analyses of COI data for both species indicated low differentiation between distant locations along the Chilean coast and lack of IBD, bearing the expected genetic signatures of rafting dispersal. Phylogenetic analyses were performed with COI and the nuclear gene 28S to place the genetic diversity of Chilean *Limnoria* spp. into a wider geographical context. Both markers revealed that *L. quadripunctata* from Chile is a sister clade to other *Limnoria* spp. analyzed (*L. chilensis, L. segnis*, and *L. stephenseni*), which mainly inhabit *D. antarctica*. *L. chilensis* from Chile and subantarctic islands form a tight monophyletic group. Phylogenetic and phylogeographic analyses show that along the studied area, *L. quadripunctata* and *L. chilensis* have the genetic signatures of relatively recent or ongoing rafting.

KEY WORDS: Peracarids · Rafting · Biogeography · Mitochondrial DNA · COI · 28S · Phylogeny · Phylogeography

INTRODUCTION

While benthic marine brooding species lack a larval dispersal stage, they may have even wider geographic distributions than sympatric congeners with planktonic larvae (Thiel & Haye 2006). For example, Rockall Island in the North Atlantic has been colonized by the brooding snail *Littorina saxatilis*, but not by its broadcast-spawning congener *L. littorea*, which has a pelagic larval stage lasting 4 wk (Johannesson 1988). While the duration of larval stages is frequently used as a valuable proxy for potential dispersal distances (Jablonski & Lutz 1983, Díaz 1995, Grantham 1995, but see Lester et al. 2007), the prediction of dispersal distances for direct developers is more complicated. One of the most important natural mechanisms of passive dispersal for benthic organisms is rafting on floating algae or other objects (Helmuth et al. 1994, Ingólfsson 1995, Hobday 2000). Passive dispersal of brooders over long distances depends on the stochastic transport of juveniles and/or adults on discrete patches of rafting material. Organisms traveling on floating algae have a high potential to be dispersed over long distances and col-

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onize new geographic areas or maintain population connectivity with distant areas (e.g. Waters & Roy 2004, Donald et al. 2005, Fraser et al. 2009, 2011, Nikula et al. 2010). Such individuals may form colonizing or founder groups over a wide range of distances, including isolated localities (Johannesson 1988, Cunningham & Collins 1998).

Based on the rafting hypothesis, Johannesson (1988) proposed that colonization success would be higher for taxa that brood offspring than for planktonic developers, as she had observed in Rockall with *Littorina* species. Organisms that live and feed on their raft may persist throughout the voyage, including brooding females, whose juveniles recruit nearby and experience extended parental care. These features increase the probability of long-distance dispersal and successful colonization (Davenport & Stevenson 1998, Thiel & Haye 2006).

Species with a low potential for autonomous dispersal (such as brooders and organisms with very short planktonic larval stages) may have a spatial distribution of the genetic diversity conforming to an isolation by distance (IBD) pattern. This pattern is expected when the dispersal potential of species is lower than their range of geographic distribution, leading to a strong relationship between genetic differentiation and geographic distance (e.g. Hoelzer et al. 2008). An extensive literature review by Thiel & Haye (2006) underlined that rafting facilitates low to moderate levels of gene flow between populations of marine benthic brooders (see also Le Gac et al. 2004, Colgan et al. 2005, Hart et al. 2006). Taxa for which rafting had been inferred as a potential dispersal mechanism often displayed high levels of connectivity among both adjacent and distant populations and did not show an IBD pattern. Intermittent and frequent rafting allows high connectivity between localities, breaking the IBD pattern of genetic diversity that is otherwise expected. Thus, rafting may shape local population and metapopulation dynamics (see Thiel & Haye 2006).

Some of the most common species in floating kelps are boring isopods from the genus *Limnoria* Leach that brood their offspring (Edgar 1987, Edgar & Burton 2000, Thiel & Vásquez 2000). Kelp-boring species of *Limnoria* excavate extensive burrows in the holdfasts and haptera of large kelps (Menzies 1957, Jones 1971, Edgar 1987, Thiel & Vásquez 2000, Thiel 2003a). They feed on holdfast tissue that they rasp with their mandibles from within their burrows (Cragg et al. 1999). Present knowledge suggests that individual isopods reside for long time periods within the same burrow if undisturbed (Thiel 2003a). Most burrows harbor only 1 individual, but females may cohabit with males and with their offspring (Menzies 1957). Reproductive females care for their developing offspring within their burrows, and upon reaching subadult size, juveniles recruit directly into their natal holdfasts (Thiel 2003a). Extended parental care and local recruitment favor population persistence in floating substrata during extended rafting journeys (Thiel 2003b). These behaviors also make limnoriids very efficient colonizers of new habitats. For example, Nikula et al. (2010) inferred that the brooders *L. stephenseni* and *Parawaldeckia kidderi* colonized the subantarctic region by rafting dispersal on their kelp host *Durvillaea antarctica.* Similarly, Fraser et al. (2011) reported long-distance rafting dispersal (400–600 km) from subantarctic islands to the southern coast of New Zealand (NZ). Ten epifaunal invertebrate species, including *Limnoria* spp., were transported attached or inside the kelp *D. antarctica.*

The Chilean mainland coast extends over 37 degrees of latitude (4100 km) along the SE Pacific. Limnoriids occur along most of this extensive coastline, where they are abundant in floating algae (Hinojosa et al. 2007). To date, 2 species of *Limnoria* are reported for the Chilean coast. *L. quadripunctata* has a worldwide distribution and lives primarily in wood (Antezana 1968), but also occasionally occurs in algae (Cookson 1991), while *L. chilensis* inhabits kelp holdfasts (Menzies 1962). Even though only *L. chilensis* has been reported in kelp holdfasts along the Chilean coast, a suite of microsatellite loci only amplified on a subset of local populations of kelp-dwelling *Limnoria* spp., suggesting that there might be greater diversity of *Limnoria* dwelling in kelps (Haye & Marchant 2007). One goal of this study was to shed light on the number of kelp-dwelling species of *Limnoria* present along the coast of Chile.

The large abundances of floating macroalgae (mainly from the genera *Macrocystis* and *Durvillaea*) found along the Chilean coast (Macaya et al. 2005, Hinojosa et al. 2011) offer ample opportunity for rafting dispersal. The high dispersal potential of floating substrata along the Chilean coast suggests a high connectivity between distant locations. Conservative estimates have suggested that patches of *Macrocystis* can float for more than 2 wk (Macaya et al. 2005), and a recent study showed very high growth rates for algae floating at temperatures <15°C, indicating that under these conditions, kelp rafts may even stay afloat for several months (Rothäusler et al. 2009). During this time period, and considering typical current velocities of 10 to 20 cm s^{-1} in the surface waters of the Humboldt Current System (Marin & Delgado

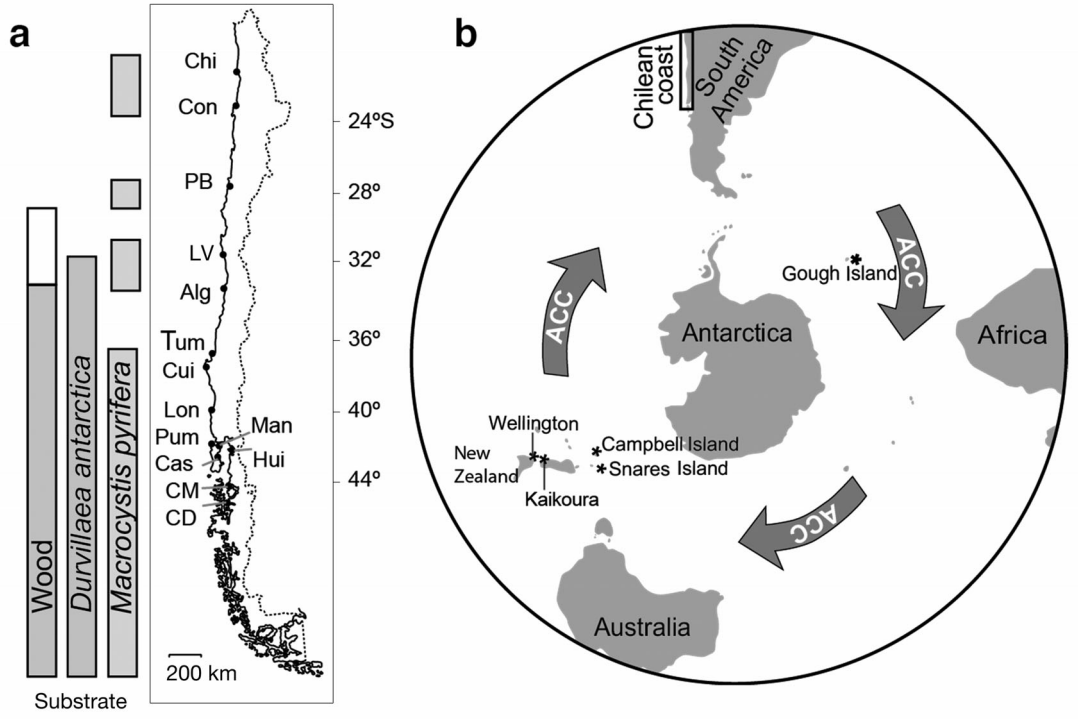

Fig. 1. (a) Chile, showing sampling locations and the distribution of the main rafting substrata. For wood, the grey area indicates abundant wood and many rivers while the white area corresponds to regions with less wood available and few rivers. Codes for sites as in Table 1. (b) Subantarctic region. The approximate position and direction of the Antarctic Circumpolar Current (ACC) are shown. Locations highlighted include the main study area, the Chilean coast (rectangle), and other sites for which limnoriids were included in the analyses (all marked with an asterisk). From Wellington, New Zealand, *Limnoria* spp. samples and sequences were obtained de novo. From Gough, Snares, and Campbell Islands, sequences were obtained from GenBank

2007), kelp rafts together with their long-term inhabitants may easily be transported over 100s of km along the Chilean coast (Thiel 2003b).

Most previous studies have *a posteriori* suggested rafting as a means of dispersal when species with a low potential of autonomous dispersal display high levels of genetic connectivity between distant populations with no IBD (e.g. Waters & Roy 2004). In the present study, we followed a different approach by predicting rafting effects *a priori*. For species of the genus *Limnoria*, which have direct development with high potential of passive dispersal, we hypothesized that recent and/or ongoing rafting-mediated gene flow leads to low genetic differentiation over large spatial scales and a lack of IBD.

MATERIALS AND METHODS

Study region and sampling procedure

Samples for this study were taken from 14 sites in Chile between Chipana in the north (21° S) and the fjord region (45° S) in the south (Fig. 1a). The study extended over a wide geographic range that crosses at least 1 biogeographic break situated by most recent authors around 30° S (Camus 2001) that has congruent phylogeographic breaks in populations of organisms with limited dispersal potential (e.g. Tellier et al. 2009, Sánchez et al. 2011). Furthermore, 6 individuals of *Limnoria segnis* were obtained from the Wellington area, NZ (Fig. 1b). We focused on holdfasts of *Macrocystis pyrifera* and *Durvillaea antarctica* because these algae are positively buoyant, float at the sea surface when detached, and have a wide distribution along the Chilean coast (Fig. 1a). Some species of *Limnoria* have been reported from both wood and kelp holdfasts (e.g. *L. quadripunctata*; Antezana 1968, Cookson 1991) and might have been dispersed by wooden ships. Consequently, in the northern fjord region (about 42° S) where abundant driftwood can be found, we also obtained samples from wood.

Samples were taken from subtidal kelp beds in central (30° to 36° S) and northern Chile, while in southern Chile most samples came from kelp hold-

Table 1. *Limnoria* spp. Collection sites and number of individuals of *L. chilensis* (*Lc*) and *L. quadripunctata* (*Lq*) collected along the coasts of Chile and of *L. segnis* (*Ls*) from Wellington, New Zealand (NZ). Substrata from which samples were obtained are indicated and numbers correspond to sample size analyzed

Country	Location	Code	Latitude (°S)	Tidal zone	Substratum			Total
					Macrocystis pyrifera	*Durvillaea antarctica*	Wood	
Chile	Chipana	Chi	21.33	Subtidal	23 *Lq*			23
	Caleta Constitución	Con	23.42	Subtidal	14 *Lq*			14
	Playa Blanca	PB	28.19	Subtidal	23 *Lq*			23
	Los Vilos (south)	LV	32.02	Subtidal	22 *Lq*			22
	Algarrobo	Alg	33.36	Subtidal	27 *Lq*			27
	Tumbes	Tum	36.64	Intertidal	5 *Lq*	2 *Lc*		7
	Cuidico	Cui	37.37	Intertidal	6 *Lq*	1 *Lc*		7
	Punta Loncoyén	Lon	39.82	Intertidal	18 *Lq*	8 *Lc*		26
	Pumillahue	Pum	41.95	Intertidal	15 *Lc*	2 *Lc*		17
	Huinay	Hui	42.39	Subtidal			41 *Lq*	41
	Castro	Cas	42.48	Intertidal			8 *Lq*	8
	Manao	Man	41.90	Intertidal			9 *Lq*	9
	Moraleda Channel	CM	44.87	Floating		16 *Lc*		16
	Darwin Channel	CD	45.44	Intertidal		7 *Lc*		7
NZ	Wellington	NZ	41.30	Subtidal	6 *Ls*			6
Total					159	36	58	253

fasts collected in the low intertidal zone (Table 1). In accordance with substratum availability (Fig. 1a), in northern and central Chile we collected individuals of *Limnoria quadripunctata* from *Macrocystis pyrifera*. In the southern part of the study area, from 36 to 42° S, individuals of both species were collected: *L. chilensis* from *Durvillaea antarctica*, *L. quadripunctata* from *M. pyrifera* (1 southern population of *M. pyrifera* contained *L. chilensis*) and wood (Table 1). To collect individuals, holdfasts were carefully detached from the benthic substratum and placed in individual plastic bags. At each site, we collected isopods from several holdfasts (at least 10 holdfasts). On the shore, holdfasts were dissected to expose and collect the isopods which were immediately placed in 95% ethanol. Similarly, wood was collected from the intertidal and subtidal zone and dissected for isopods. Whenever possible, we collected only adult isopods, because these have likely migrated between holdfasts (see Miranda & Thiel 2008), thereby reducing the risk of sampling siblings. After 24 h, the ethanol from all vials was exchanged with fresh 95% ethanol. Collected individuals were maintained at ambient temperatures during fieldwork (for a maximum of 20 d), and subsequently returned to the lab where they were stored at −20°C after changing the preserving alcohol.

Individuals collected from wood were morphologically identified as *Limnoria quadripunctata*, and those from *Macrocystis pyrifera* as *L.* cf. *quadripunctata*, because even if highly similar to *L. quadripunctata* from wood, the ones from *M. pyrifera* lacked a rasp on the left mandible (L. Cookson pers. comm.). All individuals collected from *Durvillaea antarctica* were identified as *L. chilensis*. This latter species was also found in *M. pyrifera* in the locality of Pumillahue (41° S). Summarizing, for the coast of Chile, 79.4% of the individuals analyzed were *L. quadripunctata*, and 20.6% were *L. chilensis*.

Data collection

Whole individuals were homogenized (after the removal of embryos in the case of ovigerous females) and their genomic DNA was extracted using a QIAamp DNA mini kit (Qiagen) following the instructions of the manufacturer. Polymerase chain reaction (PCR) was used to amplify a portion of the mitochondrial DNA cytochrome oxidase I (COI) gene using the universal primers HCO and LCO of Folmer et al. (1994). Sequences of the more conserved nuclear 28S rDNA (28S) gene were obtained for 13 individuals using the primers 28SniphF1 and 28SniphR1 (Lefébure et al. 2006). We used DNA from 1 individual of *Limnoria segnis* from NZ, and 6 individuals each for both *L. chilensis* and *L. quadripunctata* from the Chilean coast.

The PCR mix consisted of ~20 ng of DNA, 1× PCR buffer, 2 mM $MgCl_2$ for COI and 1 mM for 28S, 0.4 µM of each primer, 0.22 mM of each dNTP, 1.5 U of *Taq* polymerase, and 1.5 mg ml^{-1} bovine serum

albumin. Cycling conditions consisted of an initial denaturing at 94°C for 10 min followed by 35 cycles of 1 min denaturing at 94°C, 1 min annealing at 51°C for COI and 57°C for 28S, and a 2 min extension at 72°C. Purification of 45 µl of the amplicon was achieved by adding 28.8 µl of shrimp alkaline phosphatase and 7.2 µl of Exonuclease I and incubating for 15 min at 37°C and 15 min at 80°C. Subsequently, purified amplicons were concentrated through evaporation at 50°C for 4 h. Sequencing was performed in both directions with an ABI 3730XL capillary automated sequencer. The software GENEIOUS 5.5.4 (Biomatters; www.geneious.com) was used to obtain a unique sequence for each individual and to perform alignment. The alignment was verified through translation into amino acid sequences that were generated using the invertebrate mitochondrial genetic code in GENEIOUS. The 28S gene sequences were aligned in CLUSTAL-W (Thompson et al. 1994). Final aligned sequences for both genes were truncated at each extreme to produce equal length sequences.

It is worth noting that COI sequences obtained from 90 of the 343 *Limnoria* specimens did not correspond to *Limnoria* spp. sequences and were not used for the analyses. The discarded sequences (GenBank accession numbers FJ541266–FJ541275, FJ541277, FJ541278, FJ541280, FJ541282, and FJ541283) were extremely divergent from the *Limnoria* spp. COI sequences (up to 66%) and did not match malacostracan species in GenBank. Instead, they matched DNA of gut contents found in the amphipod *Eurythenes gryllus* (GenBank accession number AY830420) and most likely represent sequences of gut content or epibionts of *Limnoria* spp. Care must be taken when using universal primers that will likely amplify genes of other organisms living in or on the target species.

Data analysis

COI sequences obtained for *Limnoria chilensis* and *L. quadripunctata* from the Chilean coast, and for *L. segnis* from NZ were analyzed together with sequences of *Limnoria* spp. obtained from GenBank. The included sequences were *L. segnis* from Kaikoura, NZ, *L. chilensis* from Snares Island (south of NZ), Gough Island (south Atlantic Ocean), and Chile (36° S; Fraser et al. 2011, GenBank accession numbers HQ161076, HQ161071, HQ161075, and HQ161073, respectively), and *L. stephenseni* from Campbell Island south of NZ (Nikula et al. 2010, GenBank accession number FJ608919) (Fig 1b). Sequences from 5 isopods and 1 amphipod species were used as outgroup taxa for COI analyses (Table S1 in the Supplement at www.int-res.com/articles/suppl/m455p111_supp.pdf). The 28S sequences obtained from individuals of *L. chilensis, L. quadripunctata*, and *L. segnis* were analyzed using 14 amphipod sequences as outgroup taxa (Table S2 in the Supplement). No isopods could be used as an outgroup because the sequence portion available for 28S aligned only partially to those of *Limnoria* spp. Their use would have considerably reduced the number of nucleotides for analyses.

COI data were analyzed at the nucleotide and amino acid levels, the latter to account for saturation at the nucleotide level. Maximum likelihood analyses (MLA) were conducted with PAUP* 4.0b10 (Swofford 2002) and Bayesian analyses (BA) with MRBAYES 3.1.2 (Ronquist & Huelsenbeck 2003). jModelTest 0.1.1 (Posada 2008) was used to select the model of nucleotidic substitution for each gene that was applied to MLA and BA. For BA of amino acid data, MRBAYES was set to estimate the best fixed-rate model during the analysis. For MLA, support for the nodes was obtained from 10 000 bootstrap replications. BA were performed using 5 000 000 iterations and sampling every 10 generations, ensuring that the average standard deviation of split frequencies was <0.001. The first 25% of the saved trees were discarded as burn-in.

For intra-specific analyses of Chilean specimens of *Limnoria chilensis* and *L. quadripunctata*, median-joining haplotype networks for COI sequences were built with NETWORK 4.5 (Bandelt et al. 1999). Unresolved loops were analyzed with the coalescent criteria of Crandall & Templeton (1993). ARLEQUIN 3.11 (Excoffier et al. 2005) was used to calculate population pairwise genetic differentiation through the haplotypic fixation index Φ_{ST} (significance was determined with 1000 permutations), and to perform Mantel tests between the genetic (linearized Φ_{ST}) and geographic (km) distance in order to test for IBD. DnaSP 5.10.00 (Librado & Rozas 2009) was used to estimate diversity indices. In order to test for a sudden population expansion (Rogers 1995), Tajima's *D* test and Mismatch frequency distribution analyses (Rogers & Harpending 1992) were performed in ARLEQUIN. The validity of the fit to the sudden expansion model was tested using parametric bootstrap analysis. The sum of squared deviations between the observed and expected distribution of pairwise differences was used as the test statistic. The probability value (p) represents the probability that the model fits the observed data. Graphic repre-

sentation of the frequency distribution of pairwise differences among haplotypes was obtained from DnaSP. If the mismatch distribution fit a sudden expansion model, time since the most recent expansion was obtained from τ ($\tau = 2ut$, t = time since expansion in number of generations, and u = mutation rate per generation, Rogers 1995). The mutation rates used were those proposed by Knowlton & Weigt (1998: 1.4×10^{-8}) for snapping shrimp (Caridea), based on the rise of the Isthmus of Panama, and Henzler (2006: 4.8×10^{-8}) for gammaridean amphipods (Peracarida) of the northern hemisphere, considering the opening of the Bering Strait. The latter is a conservative estimate based on a relatively fast mutation rate. However, both used rates are actually estimates of substitution rates, i.e. mutations that were fixed in each lineage. Substitution rates are much lower than mutation rates mainly because natural selection will remove a large proportion of spontaneous deleterious mutations (Ho et al. 2011).

RESULTS

Phylogenetic analyses

Partial sequences of the COI gene of 539 base pairs (bp) were obtained for a total of 253 individuals of *Limnoria* (Table 1): 51 *L. chilensis*, 196 *L. quadripunctata*, both from Chile, and 6 *L. segnis* from NZ. The MLA and BA of COI nucleotide and amino acid data (Table S3 in the Supplement) resulted in similar tree topologies (Fig. 2b), although branch support was higher for the topology derived from the amino acid data. Phylogenetic analyses of a 777 bp sequence of 28S rDNA (Table S4 in the Supplement) of 1 individual of *L. segnis* from NZ, 6 from *L. quadripunctata,* and 6 from *L. chilensis*, are highly consistent with the COI gene tree (Fig. 2c). The results of both data sets indicate that Chilean *L. quadripunctata* are monophyletic and are a sister clade of the other *Limnoria* species analyzed. Se-

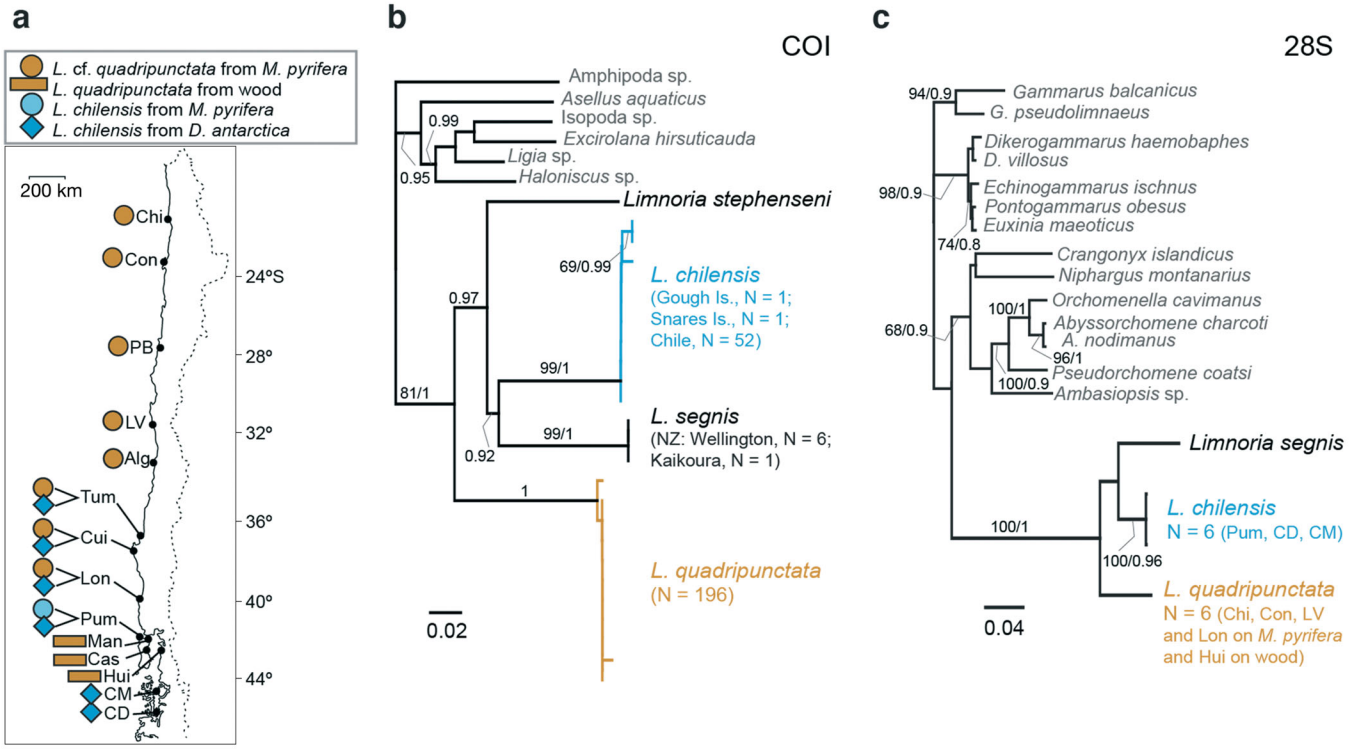

Fig. 2. *Limnoria* spp. (a) Geographic distribution of the 2 species sampled in Chile, *L. quadripunctata* and *L. chilensis,* represented with different colors on the map. Codes for sites are given in Table 1. Shapes symbolize the substratum where samples were collected (*Macrocystis pyrifera, Durvillaea antarctica*, and wood). (b) Rooted maximum likelihood (ML) phylogram of the 18 cytochrome oxidase I haplotypes found in the 247 Chilean individuals analyzed including 2 haplotypes of *L. segnis* and other sequences of *Limnoria* spp. obtained by Nikula et al. (2010) and Fraser et al. (2011) as well as of other peracarids as outgroup taxa from GenBank. N: number of individuals sampled of each clade. The 2 numbers along branches correspond to bootstrap values >65% (10 000 replicates) and Bayesian posterior probabilities >0.8, respectively. For a few nodes, only the posterior probability is shown. (c) ML phylogram of three 28S sequences found among the 13 specimens analyzed, 12 from the coast of Chile and 1 from New Zealand (NZ), and of amphipod sequences used as outgroup. In (b) and (c), the scale bar represents the number of substitutions per site

quences of *L. chilensis* obtained from distant localities (coast of Chile and subantarctic islands) form a well-defined monophyletic group.

Geographic diversity of Chilean *Limnoria* spp.

In Chile, *Limnoria quadripunctata* is widely distributed from Chipana (21° S) to Castro (42° S; Fig. 2a). Individuals were collected from *Macrocystis pyrifera* in northern localities and from wood in some southern locations. *L. chilensis* mostly inhabited *Durvillaea antarctica* and are therefore restricted to the south of Chile (Fig. 2a). Only in Pumillahue, *L. chilensis* was found both in *M. pyrifera* and *D. antarctica*. *L. chilensis* and *L. quadripunctata* are sympatric kelp dwellers in Tumbes, Cuidico, and Loncoyén (between 36 and 39° S). Throughout their sympatric geographic range, the 2 species use different substrata (*L. chilensis* dwells in *D. antarctica* and *L. quadripunctata* in *M. pyrifera*; Fig. 2a).

Intra-species analyses of the COI data along the Chilean coast were performed for both species (Table 2). Haplotype diversity was similar between species, while nucleotide diversity was lower in *Limnoria quadripunctata* (0.0015) than in *L. chilensis* (0.0040). Individuals of *L. quadripunctata* collected from wood had the greatest haplotypic and nucleotidic diversity (Table 2, Fig. 3).

Both haplotype networks showed a dominant haplotype shared among most sampled locations (Fig. 3). The haplotype network of *Limnoria quadripunctata* has a star-like shape, suggesting that all samples conform to 1 population that has experienced a rapid expansion. The most common and putative ancestral haplotype is present at almost all localities, with the exception of Huinay (Hui), where only haplotypes unique to wood were found (Fig. 3a). For *L. chilensis*, only Cuidico and Darwin Channel lack the most common haplotype (Fig. 3b). Haplotypes from Darwin Channel, the southernmost location sampled, differ in 2 and 3 mutational steps from the most common haplotype.

Population pairwise Φ_{ST} values showed evidence of very low genetic differentiation between distant populations for *Limnoria quadripunctata*, such as between Punta Loncoyén and Chipana to the north, which are separated by ca. 2000 km. In contrast, Algarrobo and the wood samples Manao, Huinay, and Castro were significantly differentiated from most other populations (Table 3), consistent with the presence of unique haplotypes at these localities (Fig. 3a).

Table 2. *Limnoria chilensis* and *L. quadripunctata.* Intra-species cytochrome oxidase I genetic diversity indices along the coast of Chile. Codes for sites are given in Table 1. N: number of individuals, *S*: number of segregating (variable) nucleotide positions, *H*: number of haplotypes, *h*: haplotype diversity, π: nucleotide diversity, *k*: average number of nucleotide differences between sequences

Site	N	*S*	*H*	*h*	π	*k*
L. chilensis						
Tum	2	0	1	0.000	0.0000	0.000
Cui	1	0	1	–	–	–
Lon	8	3	2	0.571	0.0032	1.714
Pum *M*[a]	15	3	2	0.343	0.0019	1.029
Pum *D*[a]	2	0	1	0.000	0.0000	0.000
CM	16	5	3	0.542	0.0036	1.958
CD	7	1	2	0.476	0.0008	0.476
Total	51	7	7	0.704	0.0040	2.155
L. quadripunctata						
Chi	23	0	1	0.000	0.0000	0.000
Con	14	1	2	0.143	0.0003	0.143
PB	23	0	1	0.000	0.0000	0.000
LV	22	1	2	0.312	0.0006	0.312
Alg	27	1	2	0.074	0.0001	0.074
Tum	5	0	1	0.000	0.0000	0.000
Cui	6	1	2	0.533	0.0009	0.533
Lon	18	1	2	0.111	0.0002	0.111
Man	9	3	4	0.694	0.0019	1.000
Hui	41	5	5	0.582	0.0021	1.156
Cas	8	2	3	0.714	0.0016	0.857
Total	196	10	11	0.640	0.0015	0.807

[a]Substratum, *M*: *Macrocystis pyrifera* and *D*: *Durvillaea antarctica*

Most of the population pairwise Φ_{ST} values of *Limnoria chilensis* showed low genetic differentiation among populations (Table 4), even for the most distant populations of Tumbes and the Darwin Channel (~1000 km). However, the fjord region is significantly differentiated from Loncoyén and Pumillahue (in the latter, only for individuals collected from *Macrocystis pyrifera*). In addition, differentiation is high between both channels (Darwin and Moraleda; $\Phi_{ST} = 0.483$, $p < 0.05$), and between Cuidico and other localities (values are non-significant). This is likely due to the fact that we were only able to collect 1 individual of *L. chilensis* in Cuidico. Correlation analyses of genetic and geographic distance matrices through a Mantel test indicate that there is no IBD pattern for either of the 2 species (*L. chilensis*, $p = 0.248$; *L. quadripunctata*, $p = 0.291$).

The mismatch frequency distributions of species did not deviate significantly from the null hypothesis of a sudden population expansion model (Fig. 4), with τ values of 1.236 and 1.355 for *Limnoria chilensis* and *L. quadripunctata*, respectively. Only the mismatch frequency distribution of *L. quadripunctata* is clearly unimodal (Fig. 4), consistent with the star-like

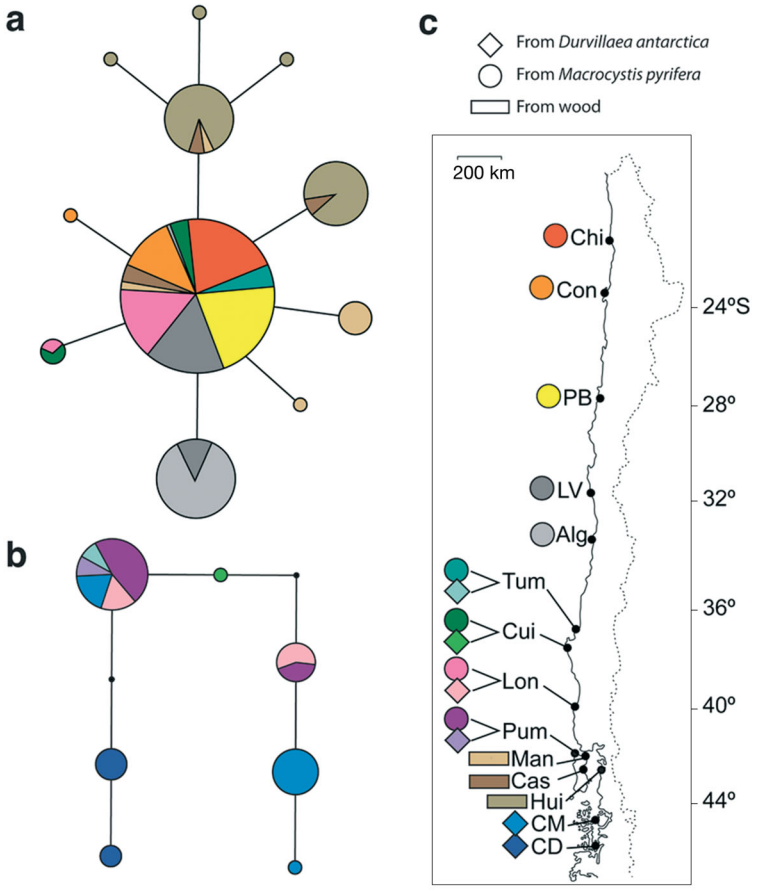

Fig. 3. *Limnoria quadripunctata* and *L. chilensis*. (a,b) Median-joining cytochrome oxidase I haplotype networks of haplotypes found along the coast of Chile. Circles represent each haplotype, and their size is proportional to their frequency. The smallest colored circles represent haplotypes found in 1 individual. Lines connecting haplotypes represent a mutational step; small black dots along the lines indicate hypothetical or undetected haplotypes. The circles representing haplotypes are subdivided when the haplotype was found in more than 1 locality. (c) Each locality is represented by a different color in the map. Codes for sites are given in Table 1. Shapes symbolize the substratum where samples were collected (*Macrocystis pyrifera, Durvillaea antarctica*, and wood)

shape of the haplotype network. Tajima's *D* values were –0.2144 and 0.5528 ($p > 0.1$) for *L. quadripunctata* and *L. chilensis*, respectively, indicating that the COI gene is neutral to natural selection and is in mutation-drift equilibrium. Sudden population expansion can be inferred for *L. quadripunctata* but not for *L. chilensis*, which seems to have been under a stable effective population size for the last hundreds of thousands of years. Using the mutation rates proposed by Knowlton & Weigt (1998) and Henzler (2006), the estimated time for the latest population expansion for *L. quadripunctata* was between 90 000 and 26 000 yr ago.

DISCUSSION

The 2 species of *Limnoria* detected along the coast of Chile, *L. chilensis* and *L. quadripunctata*, have the expected genetic signatures of relatively recent or ongoing rafting: there is low genetic differentiation and a lack of strong geographic structure of the genetic diversity at a macrogeographic scale.

When *Limnoria chilensis* and *L. quadripunctata* are sympatric along the Chilean coast, they bore into different substrata. Host use seems to be an important phylogenetic driver in *Limnoria* spp. The phylogenetic relationships among the *Limnoria* spp.

Table 3. *Limnoria quadripunctata*. Cytochrome oxidase I population pairwise Φ_{ST} values among the 11 surveyed populations. Significant pairwise values ($p < 0.05$) are marked in **bold**. Codes for sites are given in Table 1

	Con	PB	LV	Alg	Tum	Cui	Lon	Man	Hui	Cas
Chi	0.037	0.000	**0.147**	**0.958**	0.000	**0.505**	0.014	**0.711**	**0.652**	**0.501**
Con		0.037	0.042	**0.898**	–0.098	0.199	–0.031	**0.523**	**0.570**	**0.264**
PB			**0.147**	**0.958**	0.000	**0.505**	0.014	**0.711**	**0.652**	**0.501**
LV				**0.770**	0.004	0.115	0.063	**0.440**	**0.530**	**0.186**
Alg					**0.935**	**0.826**	**0.908**	**0.741**	**0.637**	**0.746**
Tum						0.161	–0.103	**0.462**	**0.554**	0.200
Cui							0.207	**0.269**	**0.432**	0.057
Lon								**0.576**	**0.594**	**0.323**
Man									**0.342**	**0.182**
Hui										**0.174**

Table 4. *Limnoria chilensis*. Cytochrome oxidase I population pairwise Φ_{ST} values among the 6 surveyed populations. In Pum, samples were obtained both from *Macrocystis pyrifera* (*M*) and *Durvillaea antarctica* (*D*). Significant pairwise values ($p < 0.05$) are marked in **bold**. Codes for sites are given in Table 1

	Cui *D*	Lon *D*	Pum *M*	Pum *D*	CM	CD
Tum	1.000	0.158	−0.188	0.000	0.364	0.620
Cui		0.429	0.657	1.000	0.458	0.524
Lon			0.106	0.158	**0.343**	**0.474**
Pum *M*				−0.188	**0.408**	**0.609**
Pum *D*					0.364	0.620
CM						**0.483**

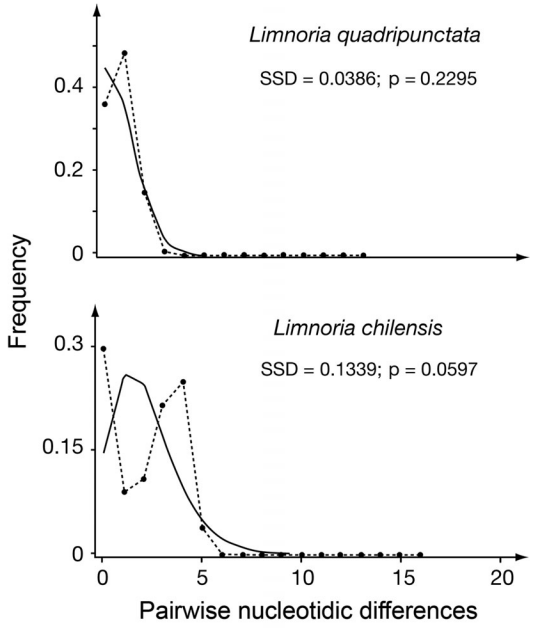

Fig. 4. *Limnoria quadripunctata* and *L. chilensis*. Cytochrome oxidase I mismatch distributions of pairwise sequences found on the coast of Chile. The continuous line represents the expected curve under a sudden expansion model while the dashed line corresponds to the observed data. SSD: sum of squared deviations between observed and expected distributions

included in the study seem to be strongly associated with their preferred substrata. The mitochondrial COI and nuclear 28S gene trees largely agree on the placement of *Limnoria* spp., suggesting that *L. quadripunctata* is sister species of the species dwelling in *Durvillaea antarctica* at mid and high latitudes of the southern hemisphere, which form a monophyletic group.

Limnoria quadripunctata from Chile was found in the kelp *Macrocystis pyrifera* and in wood, forming a monophyletic clade according to both the molecular markers. This is the first report of *L. quadripunctata* boring in kelp holdfasts along the coast of Chile. Presumably both speciation and phenotypic plasticity play a role in limnoriid evolution. The one consistent morphological difference (rasp on mandible) between individuals inhabiting *M. pyrifera* and wood may be indicating adaptive phenotypic plasticity related to different ecological environments (e.g. Thibert-Plante & Hendry 2011), i.e. host use being a selective factor.

For both species, rafting-mediated connectivity explains their ample range of distribution, as also suggested for other species from the genus *Limnoria* (Hill & Kofoid 1927, Johnson 1935, Svavarsson 1982). *L. quadripunctata* has a worldwide distribution, and in Chile it was found throughout almost the entire geographic extension studied (>2350 km from Chipana, 21° S, to Castro, 42° S). *L. chilensis* also has a wide geographic distribution with individuals found in southern Chile from Tumbes (36° S) to Darwin Channel (45° S) and in the subantarctic Snares and Gough Islands. There is high abundance of *Durvillaea* rafts in the path of the West Wind Drift (a strong west to east current), suggesting that dispersal events in the subantarctic region are common (Smith 2002, Waters & Roy 2004, Donald et al. 2005, Fraser et al. 2009, Nikula et al. 2010) and may maintain connectivity of *L. chilensis.*

Low levels of genetic differentiation and lack of IBD for limnoriid species along the Chilean coast may be consequence of past and/or ongoing rafting dispersal supporting our *a priori* hypothesis. Even though sample size may be biasing the intra-species analyses (mainly because of unequal sample size per site), both the limnoriid species studied have a common haplotype that is shared among most sampled localities. Distant local populations (i.e. ~1000 km for *Limnoria chilensis* and >2000 km for *L. quadripunctata*) have low levels of genetic differentiation. Instead of an IBD pattern, as expected for species with limited dispersal potential, along the Chilean coast both *Limnoria* species show a genetic structure that reflects occasional and stochastic incorporation of migrants of diverse geographic origins into local populations (e.g. Buchanan & Zuccarello in press). Past and/or current gene flow between local populations of limnoriids is consistent with the high abundance of floating algae along the coast of Chile (Macaya et al. 2005, Hinojosa et al. 2011), many of which harbor limnoriids (e.g. Hinojosa et al. 2007),

and the high potential of successful colonization of limnoriids (Thiel 2003a,b, Miranda & Thiel 2008). Using additional (variable nuclear) markers should allow us to determine whether the detected shared COI haplotypes represent ancestral polymorphisms or current gene flow, or a mixture of both (Marko & Hart 2011). If they correspond mainly to ancestral polymorphisms, ongoing gene flow would be more limited than expected given the abundance of floating algae and the low COI differentiation between localities. Regardless of whether gene flow is still ongoing, our data suggest that gene flow has occurred until relatively recently.

The nucleotide mismatch frequency distribution analyses of the 2 species along the coast of Chile were not coincident. Only *Limnoria quadripunctata* shows evidence of a sudden population expansion event, which was estimated to have occurred between 90 000 and 26 000 yr ago. These estimations are likely to be overestimated by 10-fold since the rates we used approximate substitution rates rather than mutation rates (Ho et al. 2005). The precise timing of the population expansion event is uncertain; however, it is likely that it occurred some time during the Late Pleistocene period (~175 000 to 10 000 yr ago) or even more recently. The population expansion in *L. quadripunctata* may have been enhanced by glaciation cycles during the Pleistocene period as inferred for the spiny lobster *Palinurus elephas* (Palero et al. 2008). Also, oceanographic events such as the intensification of the northward flowing Humboldt Current during the Last Glacial Maximum (Feldberg & Mix 2002) could have facilitated population expansion of *L. quadripunctata* along the coast of Chile via algal/wood rafting. It is also possible that during the expansion period additional introductions of *L. quadripunctata* might have occurred, which (if derived from similar source populations) could lead to the detected pattern. Furthermore, there may be very recent or ongoing dispersal of *L. quadripunctata* by wooden ships (in addition to floating *M. pyrifera*) along the Chilean coast (see e.g. Antezana 1968) and possibly across the Southern Ocean, a scenario previously discussed for *L. chilensis* rafting on *D. antarctica*. Future studies should examine the global phylogeography of *L. quadripunctata* and other putative cosmopolitan species of *Limnoria* spp. using a battery of DNA markers.

The average nucleotide diversity of *Limnoria chilensis* was 2.66 times greater than for *L. quadripunctata*. Even though comparing nucleotide diversity between taxa may be arguable, there are some biological factors that may be leading to this difference, acting separately or together, such as a comparably higher effect of genetic drift in *L. quadripunctata* than in *L. chilensis* along the coast of Chile. These may be due to events such as bottlenecks and/or founder effects, and a relatively smaller effective population size of *L. quadripunctata.* A lower diversity could be a consequence of a more recent colonization of the Chilean coast by *L. quadripunctata* than *L. chilensis*, not giving enough time for the accumulation of nucleotide diversity in *L. quadripunctata*. Along the Chilean coast the greatest haplotype diversity of *L. quadripunctata* was found south of 36° S (southern Chile). Kelp forests along the northern coast of Chile experience frequent local extinction due to El Niño events (Castilla & Camus 1992, Camus et al. 1994, Vega et al. 2005), and local limnoriid populations may temporarily go extinct; recurrent bottlenecks and colonization events would lead to genetic diversity loss. Alternatively, or in combination with the above scenario, the genetic diversity pattern of *L. quadripunctata* can be skewed because of a more recent colonization of the northern coast of Chile where the species has not yet had enough time to accumulate the same degree of genetic diversity as in the south, or because founding populations were small and subject to strong genetic drift. Another consideration is that in the south *L. quadripunctata* came from 2 different substrata, *Macrocystis pyrifera* and wood, which could lead to the overall pattern of lower genetic diversity in the north where only algal-dwelling limnoriids were collected. Phylogenetic and phylogeographic analyses of both species, considering their range of distribution, are necessary to determine the main factors that lead to their difference in genetic diversity along the coast of Chile.

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