Journal of Sea Research 102 (2015) 1–9

Contents lists available at ScienceDirect

Journal of Sea Research

journal homepage: www.elsevier.com/locate/seares

Population genetics of the invasive cryptogenic anemone, *Anemonia alicemartinae*, along the southeastern Pacific coast

C.B. Canales-Aguirre [a,b,c], A. Quiñones [a], C.E. Hernández [b], P.E. Neill [a,d], A. Brante [a,*]

[a] Departamento de Ecología. Facultad de Ciencias. Universidad Católica de la Santísima Concepción, Casilla 294, Concepción, Chile
[b] Laboratorio de Ecología Evolutiva y Filoinformática, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción Casilla 160-C, Concepción, Chile
[c] Laboratorio de Genética y Acuicultura, Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción Casilla 160-C, Concepción, Chile
[d] The Thomas Jefferson School, Department of Applied Sciences, Ave. Jorge Alessandri, Parcela 26, Concepción, Chile

ARTICLE INFO

Article history:
Received 5 March 2014
Received in revised form 24 March 2015
Accepted 26 March 2015
Available online 3 April 2015

Keywords:
COI
Demographic Expansion
Human-mediated Transport
Humboldt Current Ecosystem
Invasion Genetics
Marine Invasions

ABSTRACT

One of the most important issues in biological invasions is understanding the factors and mechanisms determining the invasion success of non-native species. Theoretical and empirical works have shown that genetic diversity is a determinant of invasion success; thus, studying spatial patterns of genetic diversity, and exploring how biological and physical factors shape this population trait, are fundamental for understanding this phenomenon. Coastal marine ecosystems are one of the most susceptible habitats to invasion given the complex network of maritime transport. In this work we study the cryptogenic anemone, *Anemonia alicemartinae*, which has rapidly increased its geographical range southward during the last 50 years (approx. 2000 km) along the southeastern Pacific coast. Based on COI mtDNA sequences we evaluated three main hypotheses: a) the genetic diversity of *A. alicemartinae* decreases according to the direction of invasion (from north to south); b) there is biogeographic–phylogeographic concordance at the 30°S biogeographic break; and c) the demographic history is coherent with a recent geographic expansion. A total of 161 individual samples of *A. alicemartinae* were collected along the southeastern Pacific coast range of distribution, covering more than 2000 km, including samples along the 30°S biogeographical break. Results showed low genetic diversity ($H_d = 0.253$; $\pi = 0.08$) and a lack of geographic population genetic structure ($F_{ST} = -0.009$, p-value = 0.656). The highest genetic diversity was observed in Peru (Chero and Mesas) and at localities close to the main Chilean seaports. We did not observe concordance between biogeographic and phylogeographic patterns or isolation by distance. Demographic indices ($D = -2.604$, $p < 0.001$; Fu's $= -26.619$, $p < 0.001$), as well as a star-like configuration of the haplotype network support recent population expansion of this species. Our results, together with historical field observations, support the idea that the current distribution of *A. alicemartinae* may be explained by an increase in population size from one small ancestral population probably from the south of Peru, with subsequent human-mediated southward transport, probably associated with regional-scale maritime activities.

1. Introduction

Invasion biology seeks to identify the evolutionary mechanisms underlying the success of invasive species (Sakai et al., 2001). Genetic diversity is one of the main population traits determining the evolutionary and adaptive potential of a species to new habitat conditions (e.g. Lavergne and Molofsky, 2007; Reed and Frankham, 2003; Tsutsui et al., 2000). In this sense, studying geographic patterns of genetic diversity in an invasive species, and exploring how biological and physical factors shape genetic diversity during the invasion process, are fundamental for understanding this phenomenon. Also, revealing the underlying factors of invasions may help managers to implement policies and mitigation actions.

Theoretical models and experimental data have shown that small populations, which characterize the initial processes of invasion, are subject to the founder effect, dominated by low genetic diversity and strong genetic drift (England et al., 2003; Leberg, 1992; McCommas and Bryant, 1990; Nei et al., 1975). Genetic drift is expected to act strongly at the invasion front, given the reduced size of the newly colonizing population. Once the invader population is established in the new habitat, a signature of recent genetic expansion may be evident at the first stage of population spread (Sakai et al., 2001). In this way, assuming only one invasion event, three main expectations may arise: (1) populations of invaders show chronic low genetic diversity, (2) genetic diversity decreases toward the front of the invasion, and (3) a signature of genetic expansion is observed in the invader population.

* Corresponding author. Tel.: +56 41 234 5642.
E-mail addresses: cristiancanales@udec.cl (C.B. Canales-Aguirre), adriana.quinonestoloza@gmail.com (A. Quiñones), cristianhernand@udec.cl (C.E. Hernández), pneill@jefferson.cl (P.E. Neill), abrante@ucsc.cl (A. Brante).

http://dx.doi.org/10.1016/j.seares.2015.03.005
1385-1101/

In addition to the demographic dynamic of invasions (e.g. the founder effect), the combination of abiotic local environmental characteristics (e.g. current patterns, thermal gradients), as well as biological characteristics of the invader (e.g. reproductive strategies, dispersal capacity) also shape the spatial distributional pattern of genetic diversity (Chomsky et al., 2009; Severance and Karl, 2006; Sherman and Ayre, 2008; Sherman et al., 2007). In general, invasive species with high dispersal potential should have faster invasion rates and higher invasion success (Schreiber and Lloyd Smith, 2009), showing low or null population genetic structure in the new habitat (Marrs et al., 2008; Roderick and Navajas, 2003). In contrast, strong barriers for dispersal, such as natural biogeographic barriers, would retard or stop the advance of the invaders. In this case, when human-mediated transport is not involved in the subsequent invasion process, the invasive species population should present genetic structure concordant with the biogeographic–phylogeographic break (Zardi et al., 2007).

Coastal marine ecosystems are one of the most susceptible habitats to invasion given the complex network of maritime transport (Kaluza et al., 2010; Molnar et al., 2008), which can override the effect of natural biogeographical barriers, sometimes resulting in the introduction of small invading populations. *Anemonia alicemartinae* is an invasive cryptogenic anemone on the Chilean coast, described in 2001, and characterized by its deep red color, high population densities in intertidal and shallow subtidal zones, and rapid geographic expansion southward along the southeastern Pacific coast (Häussermann and Försterra, 2001). In early marine biodiversity records of the Chilean coast (i.e. 1959 to 1965) this species was absent; with the first record in northern Chile (20°16′S, Iquique) in 1975 (see Castilla et al., 2005). During the 1980s, *A. alicemartinae* was reported further south, in Coquimbo (30°S), in high abundances, but was absent at sites south of this point. Later, in 1998 a small population was recorded in central-southern Chile at Cocholgüe (36°34′S), where it had previously been reported absent (1994, 1995), and later observations at the site showed an increase in population abundance (Häussermann and Försterra, 2001). Currently, the austral limit of *A. alicemartinae* is in south-central Chile at Lirquén (36°42′S; pers. obs. P. Neill). The northernmost limit has not been determined, although specimens have been obtained from southern Peru (in this study; locality of Ilo: 17°38′S), but no historical records are available. The origin of *A. alicemartinae* is unknown, however Häussermann and Försterra (2001) suggested different hypotheses, including a recent introduction from an undetermined site (e.g. a different continent) or range extension from an ancestral population in the north of Chile, followed by a southward invasion.

Experimental works, as well as observations from the field, suggest that *A. alicemartinae* individuals have a high local dispersal capacity. Weak adherence of the pedal disc to the substrate, together with the ability to quickly reattach (Häussermann and Försterra, 2001; López et al., 2013), could be an effective dispersal strategy, where an entire individual may act as the dispersing unit. As in other anemone species, *A. alicemartinae* appears to combine asexual and sexual reproduction. However, the high frequency of fission in adults suggests that asexual reproduction may be an important reproductive strategy in populations of this species on the Chilean coast (Häussermann and Försterra, 2001).

Currently, the distribution of *A. alicemartinae* covers over 2000 km of the Chilean coastline, from 17°S to 37°S, crossing a well-documented oceanographic break at 30°S (Hormazabal, 2004), which defines two biogeographic provinces: the Peruvian Province and the Intermediate Province (Camus, 2001). In addition, at an intraspecific level, this oceanographic discontinuity affects the dispersal patterns of many marine invertebrate species, including organisms with both, high and low dispersal potentials, resulting in an important phylogeographic barrier (e.g. Haye et al., 2014; Sánchez et al., 2011). No study has evaluated whether this strong environmental and ecological break at 30°S may have some effect on *A. alicemartinae* ecology or on spatial patterns of genetic diversity.

The purpose of this study is to describe the spatial pattern of population genetic diversity in the cryptogenic anemone invader, *A. alicemartinae*. Based on mitochondrial DNA (mtDNA) sequences, we evaluated whether: a) the genetic diversity of *A. alicemartinae* decreases according to the direction of invasion (from north to south); b) populations are genetically structured along the species' distribution or whether there is a consistency between biogeographic and phylogeographic boundaries along the southeastern Pacific coast; and c) the demographic history is coherent with a recent geographic expansion.

2. Materials and methods

2.1. Sampling zone

A total of 161 individuals of *A. alicemartinae* were collected from the shallow subtidal and rocky intertidal zone, at eight locations throughout its geographical distribution, covering a range of 2200 km of coastline, from southern Peru to south-central Chile (Table 1; Fig. 1). Pedal disc tissue (0.5 cm^2) from each individual was fixed in ethanol at 95% for subsequent analyses.

For species recognition, we used morphological and behavioral traits reported by Häussermann and Försterra (2001). They describe *A. alicemartinae* as a conspicuous, red anemone (i.e. red column, oral disc, tentacles, and pedal disc) of noticeable size, inhabiting the intertidal and shallow subtidal zone, easy to remove from the substrate given the weak adhesion of its pedal disc. Also, when adrift, *A. alicemartinae* is characterized by a floating appendage.

2.2. Extraction, amplification and DNA sequencing

Total genomic DNA from pedal disc tissue was isolated using the salting-out protocol (Miller et al., 1988). The Cytochrome Oxidase I (COI) gene was amplified using the universal primers HCO2198 and LCO1491 as described by Folmer et al. (1994). The COI fragment was amplified in 30 μL reactions containing 3 μL of PCR buffer (1×), 3.6 μL $MgCl_2$ (3 mM), 1.5 μL dNTPs (0.125 mM), 0.3 μL BSA (1×), 0.3 μL of primers (0.1 μM), 0.25 μL Taq polymerase TopTaq (Qiagen, 1.25 U)

Table 1
Molecular diversity indices for the Cytochrome Oxidase I (COI) at the sampled locations. N = sample size; N_{HAP} = number of haplotypes; S = segregating sites; H_d = haplotype diversity; π = nucleotide diversity; D = Tajima's test; F_S = Fu's test. Significance level: ns = non-significant; *p < 0.05, **p < 0.01 and ***p < 0.001. N.A. = not applicable.

	Latitude	Longitude	N	N_{HAP}	S	H_d	π (%)	D	F_S
Ilo	−17.6338	−71.3434	5	1	0	0.000	0.00	N.A.	N.A.
Mesas	−18.0507	−70.8037	18	6	10	0.562	0.23	−1.860*	−1.442 (ns)
Chero	−18.0517	−70.8026	5	2	1	0.400	0.07	−0.816 (ns)	0.090 (ns)
Iquique	−20.2330	−70.1482	20	5	4	0.368	0.08	−1.638 (ns)	−3.053 (ns)
Antofagasta	−23.6168	−70.3929	31	3	3	0.185	0.04	−1.543 (ns)	−1.182 (ns)
Huasco	−28.4500	−71.2000	34	2	1	0.059	0.01	−1.137 (ns)	−1.315 (ns)
Las Cruces	−34.5847	−72.0437	20	6	10	0.447	0.16	−2.256**	−2.261 (ns)
Quiriquina Island	−36.6333	−73.0500	22	5	6	0.338	0.10	−1.917*	−2.225 (ns)
All localities	–	–	155	19	27	0.253	0.08	−2.604***	−26.619***

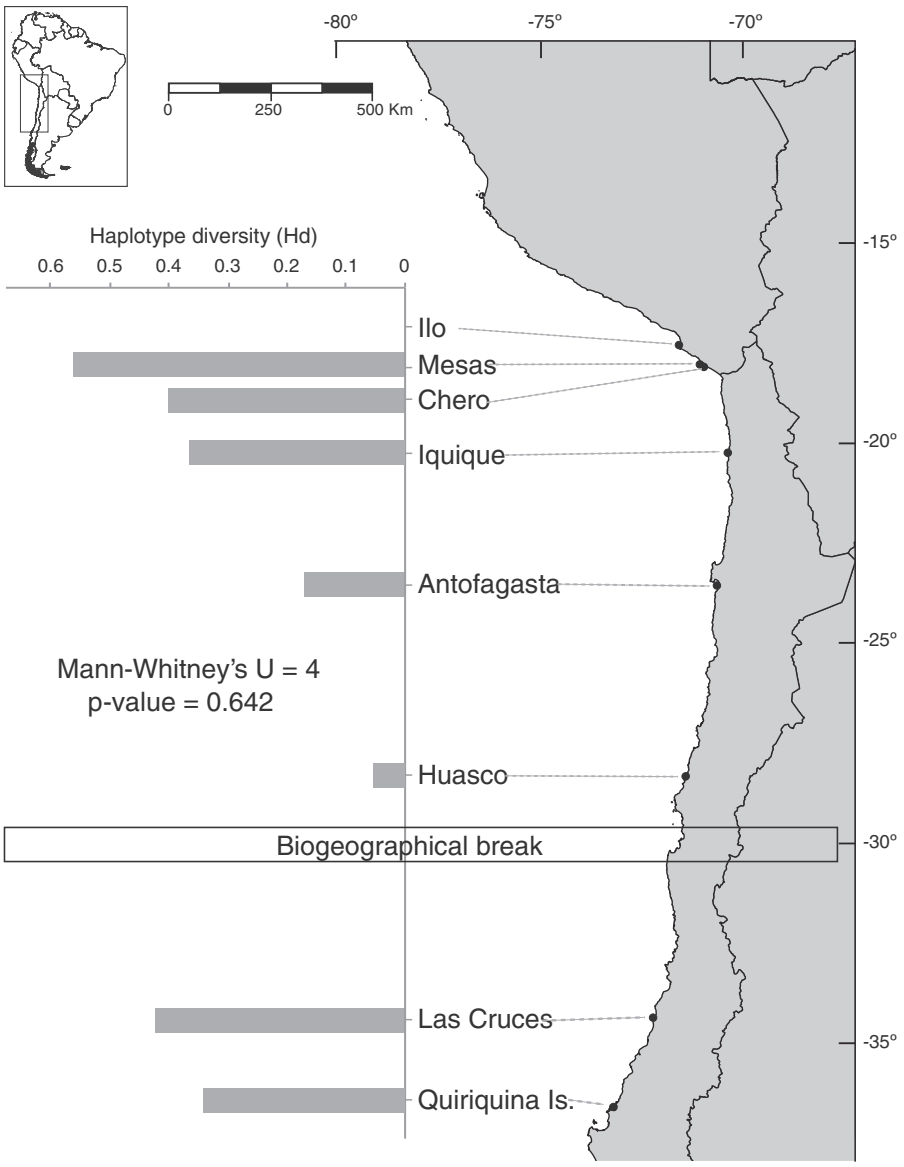

Fig. 1. Map showing sampling locations and distribution of haplotype diversity along a latitudinal gradient. The transparent box shows the well-documented biogeographical break around 30°S.

and 1 μL of genomic DNA. PCR amplification was performed in a PTC200 MJ research thermal cycler with the following parameters: one cycle at 94 °C for 60 s, followed by 35 cycles at 94 °C for 30 s, 49 °C for 55 s and 72 °C for 90 s, and a final extension at 72 °C for 600 s. PCR products were sequenced with the forward primer, following the ABI 3730xl BigDye Terminator Cycle Sequencing 3.1 (Applied Biosystems) standard protocol in Macrogen Inc. In order to recheck for polymorphism in variable sequences, we re-sequenced such individuals two or three times. We checked each chromatogram of haplotypes in order to ensure the quality of the sequences and reduce the possibility of low quality readings. To be conservative, we eliminated more divergent sequences that showed some ambiguous nucleotide positions. In total, we discarded 6 sequences. We also evaluated genetic divergence based on K2P distance, to compare the levels of divergence of *A. alicemartinae* compared with other anthozoans. All haplotype sequences were deposited in GenBank under the following accession numbers KM520739–KM520757.

2.3. Alignment and prior analyses for species verification

Taking into to account that an accurate alignment matrix adds robustness to further analyses, we conducted a multiple sequence alignment in MUSCLE 3.8.31 software (http://www.drive5.com/muscle/) (Edgar, 2004) and checked final alignments visually. Prior to conducting population analyses we carried out several analyses to avoid erroneous conclusions in population outcomes. First, to avoid any contaminant that could incorporate erroneous variants into our intraspecific dataset, we checked each haplotype obtained using the Basic Local Alignment Search Tool (BLAST). To optimize the BLAST protocol, searches for each haplotype were restricted to the Anthozoa (taxid:6101) using the Megablast option. Second, given that a homing endonuclease gene (HEG) has been found in several black corals and sea anemones (e.g. Beagley et al., 1996; Brugler et al., 2013; Fukami et al., 2007; Goddard et al., 2006; Sinniger and Pawlowski, 2009), we checked for the presence of HEG in *A. alicemartinae* before conducting further analyses. Specifically, we isolated the conserved structural motif LAGLIDADG (HEG) from *Anemonia viridis* (GenBank accession number DQ831333) and then we conducted an alignment, using MUSCLE, in order to identify sequences that matched it. Finally, we evaluated genetic divergence based on K2P distance to compare the levels of divergence of *A. alicemartinae* at interspecific (Supplementary Table 1) and intraspecific levels (Supplementary Table 2).

2.4. Phylogenetic analyses

In order to evaluate the specific taxonomic status of *A. alicemartinae* and discard the inclusion of any closely related species, we conducted phylogenetic analyses comparing sequences of species of the Anthozoa Class obtained from GenBank (excluding those with HEG; Supplementary Table 3). First, we performed Xia's test to evaluate whether the sequences used displayed saturation by substitution, and hence could be useful in the phylogenetic analyses (Xia et al., 2003). We found that our sequences did not show saturation by substitution (Iss = 0.043 < Iss.c = 0.699), therefore they have a phylogenetic signal, suitable for phylogenetic analyses.

To evaluate the monophyly of our ingroup (*A. alicemartinae*) we incorporated a maximum likelihood (ML) phylogenetic analysis based on model selection (Tamura 3-parameters based on Bayesian Information Criterion) and bootstrap support (1000 replicates) in MEGA-6 (Tamura et al., 2013). To root the tree we used *Metridium senile* as the outgroup. Finally, to resolve putative species delimitated in a phylogenetic context, we conducted an analysis based on the Poisson Tree Processes model (PTP; http://sco.h-its.org/exelixis/web/software/PTP/index.html) (Zhang et al., 2013), using the ML tree resulting from the MEGA-6 analysis.

2.5. Population analyses

Standard genetic diversity indices were calculated for each location: number of haplotypes (N_{HAP}), number of segregating sites (S), nucleotide diversity (π) and haplotype diversity (H_d). We estimated all these indices in the DNAsp v5.0 software (Librado and Rozas, 2009).

To evaluate whether locations were geographically structured we estimated pairwise F_{ST} and performed hierarchical analyses of molecular variance (AMOVA). AMOVA analyses were used to evaluate two likely hypotheses: a) panmixis and b) presence of a phylogeographic break at the 30°S of latitude. F_{ST} pairwise comparisons and AMOVA were carried out in ARLEQUIN v3.0 software (Excoffier et al., 2005). A third hypothesis, the stepping stone hypothesis, was evaluated using a model of isolation by distance. Under this model we expected a positive correlation between genetic distance ($F_{ST} / 1 - F_{ST}$) and geographical distance (log km). The significance of the relationship between genetic and geographical distance was evaluated using 10,000 permutations in the PASSaGE v2 software package (Rosenberg and Anderson, 2011). F_{ST} values were estimated based on haplotypic frequency. We applied a sequential Bonferroni correction (Rice, 1989) for multiple comparisons when necessary.

To determine the genealogical relationship between the haplotypes (i.e. the network) we used a Median-Joining algorithm implemented in the program NETWORK 4.5 (http://www.fluxus-technology.com) (Bandelt et al., 1999). In order to evaluate the possible events of population expansion, we performed the Tajima's D test of neutrality (Tajima, 1983) and Fu's F_S test (Fu, 1997) in DNAsp v5.0 software.

3. Results

3.1. Prior analyses for species verification and phylogenetic analyses

We analyzed a total of 155 sequences of 612 bp of individuals of *A. alicemartinae*, from three locations in Peru and five locations in Chile. The outcome of the BLAST tool matched with species from the Family Actiniidae, which were used in the interspecific analyses (Supplementary Table 1). Alignment of the conserved structural motif LAGLIDADG (HEG) did not match with our dataset, confirming the absence of HEG in our sequences. Genetic divergence based on K2P distance ranged between 0.16 and 1.19% at the intraspecific level (Supplementary Table 1) and between 3 and 13% at the interspecific level (Supplementary Table 2). The ML phylogenetic tree showed that all sequences of *A. alicemartinae* correspond to a monophyletic clade (bootstrap = 96%, Fig. 2). The species delimitation based on the PTP approach recovered 12 putative species, where all sequences of *A. alicemartinae* correspond to a single species (support of 0.28; Fig. 2).

3.2. Population analyses

From the total of the analyzed sequences we identified 27 segregating sites and 19 different haplotypes, ranging between 1 and 6 haplotypes per location (Table 1). Nucleotide diversity ranged from 0% at the locality of Ilo to 0.23% at Mesas. Haplotype diversity decreased northward from Mesas (0.562) to Ilo (0.0), and southward up to the northern side of the 30°S biogeographic break (Huasco = 0.059; Table 1). At the two localities south of the biogeographic break, haplotype diversity increased again (Table 1). When evaluating the northern grouping of sites vs. the southern group, the Mann–Whitney test (Table 2) indicated no significant differences in the genetic diversity indices of S, N_{HAP}, π, and H_d between localities located north and south of the 30°S biogeographic break.

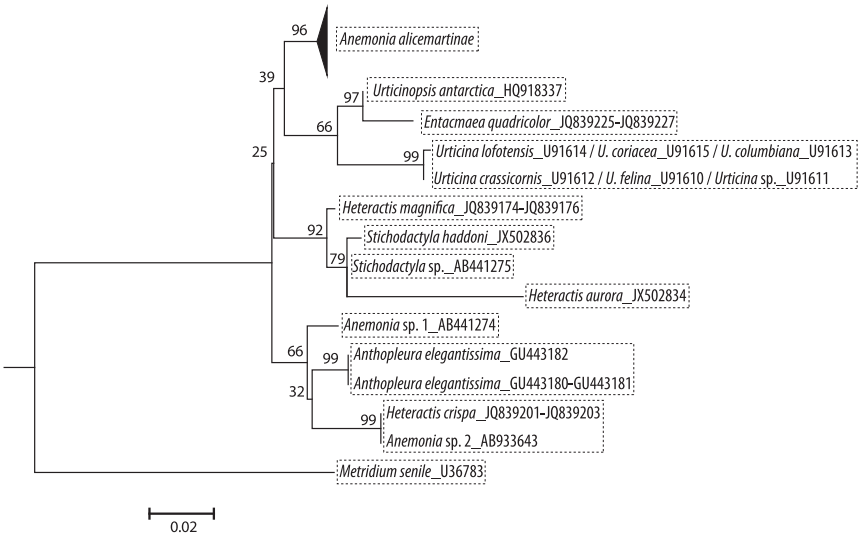

Fig. 2. Maximum likelihood tree resulting from the analysis of sea anemone species, including the individuals collected for this study and COI fragment gene sequences available in the GenBank database. Numbers above nodes correspond to bootstrap support. Dashed boxes correspond to putative phylogenetic species resulting from PTP analyses. The analyses assigned the species *Urticina lofotensis*, *U. coriacea* and *U. columbiana* to the same branch. Additional information on the species analyzed is provided in Supplementary Table 3.

Table 2
Mann–Whitney test for comparing diversity indices on both sides of 30°S biogeographical break.

Diversity index	U	North	South	p-Value
N_{HAP}	2	6	2	0.321
S	1.5	6	2	0.178
H_d	4	6	2	0.642
π	2	6	2	0.285

Table 4
Results from AMOVAs based on COI sequences for *A. alicemartinae* samples to evaluate the differences between all localities (panmixis hypothesis) and between groups of localities separated by the biogeographical break at 30°S. All p values are based on 10,000 permutations.

Hypothesis	Groups	F-statistic	p-Value
Panmixis	Group 1 (all localities)	$F_{ST} = -0.00987$	0.656
Biogeographic break (30°S)		$F_{ST} = -0.00857$	0.644
	Group 1 (Ilo, Mes, Che, Iqu, Ant, Hua)	$F_{SC} = -0.01102$	0.606
	Group 2 (LCr, QIs)	$F_{CT} = 0.00242$	0.521

F_{ST} values varied between −0.109 and 0.019. None of the pairwise F_{ST} comparisons between locations were significant (Table 3). The F_{ST} index of AMOVA suggested no genetic structure when we tested the panmixis hypothesis ($F_{ST} = -0.009$; $p = 0.656$), nor were there significant differences between localities on either side of the 30°S biogeographic break ($F_{CT} = 0.002$; $p = 0.521$; Table 4). The Mantel test did not show a significant correlation between genetic distance ($F_{ST} / 1 - F_{ST}$) and the log of geographical distance when all data were included ($r = -0.035$; $p = 0.857$; Fig. 3), nor when one outlier point was excluded from the analysis (see Fig. 3; $r = 0.050$; $p = 0.803$), demonstrating the absence of isolation by distance in this species.

The haplotype network showed a star-like configuration, which is a characteristic of rapidly growing populations (Fig. 4). Haplotype H1 was the most common, and was observed at all localities. H1 could likely be the ancestral haplotype, given its high frequency and central position in the network. These results indicate that under a neutral model, *A. alicemartinae* populations have experienced a recent expansion event. The negative values of Tajima's D and Fu's indices also support recent population expansion of this species ($D = -2.604$, $p < 0.001$; Fu's $= -26.619$, $p < 0.001$; Table 1).

4. Discussion

Our observation of low genetic diversity and recent genetic expansion in the invading population of *A. alicemartinae* along the southeastern Pacific coast agrees with theoretical models and empirical data for the genetic dynamics of invasive species. We did not observe a consistent, decreasing pattern of genetic diversity southward, isolation by distance, or genetic structure resulting from biogeographic barriers. Together with historical distribution records, this evidence suggests that the current spatial pattern of genetic diversity of *A. alicemartinae* on the southeastern Pacific coast is the result of a combination of life-history characteristics (e.g. reproductive strategy and dispersal ability), human-mediated transport, and demographic features of the invasive anemone.

4.1. Genetic diversity in A. alicemartinae

Phylogenetic analyses showed that the sequences we studied in *A. alicemartinae* corresponded to an intraspecific monophyletic clade. However, the phylogenetic relationships between other species of the genus *Anemonia* are less clear, resulting in a polyphyletic group (Fig. 2). The low genetic diversity of the COI gene, which has been reported for Anthozoa in general (e.g. Chen et al., 2008; France and Hoover, 2002; Hellberg, 2006), does not allow for robust conclusions at the interspecific level, and requires inclusion of other genes (mitochondrial and nuclear genes) to resolve the phylogenetic relationship of the genus *Anemonia*.

The population genetic diversity of *A. alicemartinae* ranged from low to intermediate values at the sampled localities. This level of genetic diversity is higher than the general pattern observed in the class Anthozoa for mitochondrial genes, where reported values of mitochondrial diversity are very low or null (e.g. *Balanophyllia elegans* and *Tubastraea coccinea* (Hellberg, 2006); *Acanthogorgia* sp., *Corallium ducale*, and *Paramuricea* sp. (France and Hoover, 2002); *Montastraea cavernosa* (Fukami and Knowlton, 2005; Snell et al., 1998); twenty-seven scleractinian coral species (Shearer and Coffroth, 2008); four gorgonian species (Calderón et al., 2006); the subfamily Keratoisidinae (Brugler and France, 2008); *Porites* species (Forsman et al., 2009); *Phymanthus crucifer* (González-Muñoz et al., 2015); *Stylophora pistillata*, *Pocillopora damicornis*; *Madracis mirabilis* (Chen et al., 2008)). Nonetheless, it is important to note that widely cited studies using mtDNA are based on small sample sizes (less than 5 individuals in some cases; (Chen et al., 2008; France et al., 1996; Shearer and Coffroth, 2008; Snell et al., 1998; but see Shearer et al., 2002)), which could mask the actual levels of genetic diversity of mitochondrial genes.

The low levels of diversity reported for mitochondrial genes from the Anthozoa group indicate a very low mutational rate of mtDNA compared to other metazoans (Huang et al., 2008). For instance, Fukami and Knowlton (2005) estimated a mutational rate between 0.03 and 0.04%/site/year^{-6} in a *Montastraea annularis* complex. Hellberg (2006) demonstrated that substitution rates in anthozoan species are 50–100 times lower than most animals, suggesting that the low mutational rate could be an ancestral condition of this group. Additionally, Shearer et al. (2002) suggest that the low diversity observed in mtDNA may be explained by an efficient mechanism of molecular reparation. For example, in octocorals researchers have identified a bacterial MutS homolog gene with a repair function (McFadden et al., 2011; Pont-Kingdon et al., 1998). This gene probably plays a specific role in slow mitochondrial evolution (Bilewitch and Degnan, 2011). In addition, it has been proposed that gene duplication, followed by random losses, results in an extreme shuffling of genes in mtDNA (Brockman and McFadden, 2012). This mechanism would result in low genetic diversity because of random losses of mtDNA duplications. In addition, an intron homing endonuclease gene (HEG), acting as a selfish conservative gene, has been described in the COI gen for several anthozoan species (Beagley et al., 1996; Brugler et al., 2013; Fukami et al., 2007;

Table 3
F_{ST} pairwise comparison among sampled localities.

Locality	Ilo	Mes	Che	Iqu	Ant	Hua	LCr	QIs
Ilo	–							
Mesas	−0.087	–						
Chero	0.000	−0.047	–					
Iquique	−0.089	0.018	−0.004	–				
Antofagasta	−0.106	0.004	−0.071	−0.007	–			
Huasco	−0.109	0.019	−0.050	0.003	−0.018	–		
Las Cruces	−0.105	0.013	−0.051	0.004	−0.003	−0.001	–	
Quiriquina Island	−0.097	0.010	−0.033	0.000	−0.013	0.003	0.006	–

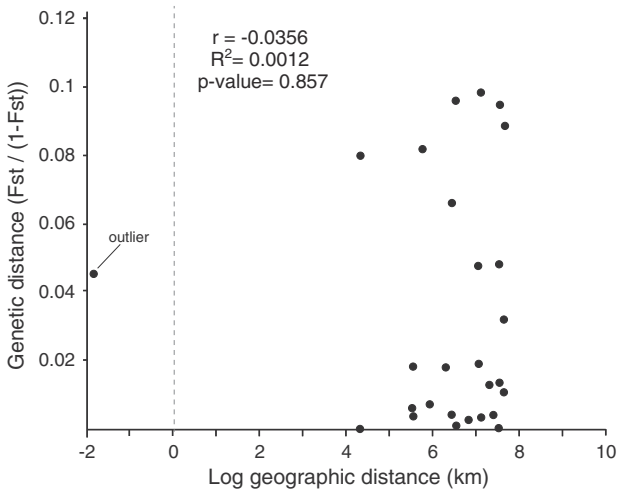

Fig. 3. Scatter plot between genetic distance (F_{ST} / (1 − F_{ST})) and the logarithm of geographical distance (km) of sampled localities. Each point corresponds to a pairwise comparison of localities.

Goddard et al., 2006; Kayal et al., 2013; Sinniger and Pawlowski, 2009). These characteristics of the COI mitochondrial gene could explain the low diversity observed in the Anthozoa.

Considering that some authors have suggested that mitochondrial DNA sequences less than 1 kb are not suitable for population level studies (France and Hoover, 2002; McFadden et al., 2004; Shearer et al., 2002; Thoma et al., 2009), the COI gene would not be useful for intraspecific analyses in anemone species (France and Hoover, 2002; Huang et al., 2008; Shearer and Coffroth, 2008). Nevertheless, in our study of *A. alicemartinae* the COI gene was shown to be suitable for population genetics given that: 1) we did not find the HEG; 2) we used a considerable number of individual sequences (n = 155) to capture the real genetic variability; and 3) the sequences obtained in *A. alicemartinae* showed higher intraspecific divergence values than other anthozoans (1.19% K2P genetic distances). In addition, we used TopTaq Polymerase (Qiagen), which has an error rate of 2–3 × 10^{-5} (per base, per cycle). Given that we worked with a sequence length of 612 bp, obtained from 35 PCR cycles, the error by amplification is approximately 0.069 bases, so, theoretically, we may be incorporating less than one amplification error due to base misincorporation during PCR. Based on this, we can assure that our results are exclusively resulting from genetic variation in *A. alicemartinae*, rather than the product of Taq DNA polymerase error. As in *A. alicemartinae*, other anthozoan species have shown similar levels of genetic variability for COI. In *Metridium meandrites* researchers recorded 1.07% K2P genetic distance and in *Oxypora lacera* a 1.15% K2P genetic distance (see Shearer and Coffroth, 2008). This difference suggests that *A. alicemartinae* is a species with exceptionally high genetic variability in COI, as compared with most other anthozoans, but it is not the only exception in the group (Shearer and Coffroth, 2008).

As a potential invader, the low genetic diversity observed along its distributional range could be explained by the small size of the founding ancestral population, followed by a rapid expansion, with few individuals originating from the area of introduction. Hellberg (2006) pointed out that low mtDNA diversity could result from range expansions, thereby enhancing the founder effect. Additionally, with small effective population sizes, genetic drift is an important evolutionary force, which erodes genetic diversity (Lonhart, 2009). For instance, Chandler et al. (2008) used mtDNA to demonstrate pronounced genetic bottleneck in introduced populations of the marine gastropod *Rapana venosa* in the Black Sea (i.e., only one haplotype of the 110 native haplotypes). Likewise, invasive populations of the colonial tunicate *Botrylloides violaceus* on the Pacific and Atlantic coasts of North America show low genetic diversity in mtDNA and microsatellite loci, in comparison with native populations from the Northwest Pacific (Bock et al., 2011). This effect has also been observed in other introduced sea anemones (e.g. Pearson et al., 2002; Reitzel et al., 2007; Shick and Lamb, 1977). For example, *Haliplanella luciae* showed extremely low genetic diversity resulting from individuals arising from a single clone, exemplifying an extreme founder effect (Shick and Lamb, 1977). In *Nematostella vectensis*, Pearson et al. (2002) suggested that a low number of individuals may

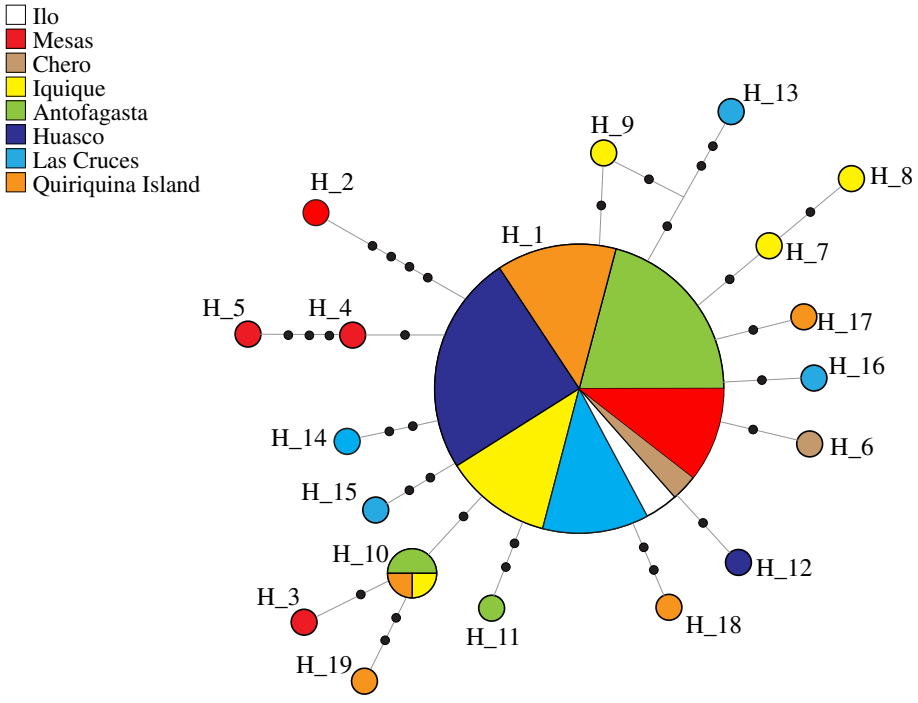

Fig. 4. Haplotype network for samples of *A. alicemartinae* from eight locations along the southeastern Pacific coast. Each haplotype is represented by a circle, where the size is proportional to its frequency. The different tones of gray represent different localities, and black dots represent the number of mutational steps between different haplotypes.

have been recently introduced into the United Kingdom, with reports of low genetic diversity.

4.2. Reproductive mode: sexual versus asexual

Reproductive strategy also plays a key role in shaping population genetic structure, with asexual reproduction decreasing both genetic diversity and population genetic differentiation (Balloux et al., 2003). Häussermann and Försterra (2001) recorded mainly infertile individuals of *A. alicemartinae* (92%) together with frequent evidence of fission, suggesting that asexual reproduction could be the main reproductive strategy in this species. In accordance with this observation, the low diversity and high frequency of the predominant haplotype found in our study may result from the predominance of asexual reproduction in *A. alicemartinae*; nevertheless to corroborate this hypothesis it is necessary to use a codominant molecular marker, such as microsatellite loci. The asexual reproductive mode has been observed in other anthozoan genera (e.g. *Actinia tenebrosa*, Sherman et al., 2007; Veale and Lavery, 2012; *Actinia bermudensis*, Monteiro et al., 1998; *Actinia ebhayiensis*, Schama et al., 2012; *N. vectensis*, Hand and Uhlinger, 1995; Reitzel et al., 2007). This predominance of asexual reproduction has been explained by environmental selective forces. For instance, reproductive mode in *Actinia equina* depends on water temperature, with warmer water triggering sexual reproduction and colder water promoting asexual reproduction (Chomsky et al., 2009). More studies on the reproduction of *A. alicemartinae* are necessary to evaluate its potential role in the invasion success and population genetic diversity of this species.

4.3. Genetic population structure and phylogeographic pattern

The hypothesis of concordance between biogeographic and phylogeographic breaks suggests that the same environmental factors that define species distribution may also determine genetic population patterns (Avise et al., 1987). Our results did not support this hypothesis in *A. alicemartinae*, nor was there evidence of population genetic structure along the southeastern Pacific coast. The absence of isolation by distance and biogeography–phylogeographic concordance around 30°S in *A. alicemartinae* indicates that the well-recognized oceanographic break (Hormazabal, 2004) does not affect the invasion dynamic of this species, at least at a population genetic level, reinforcing the idea of high dispersal potential for this species through the floating and drifting of individuals and/or through anthropogenic vectors, such as, ballast water or fishing activities. Furthermore, despite strong differences in environmental conditions northward and southward of 30°S (Camus, 2001; Hormazabal, 2004), *A. alicemartinae* is able to inhabit a wide range of habitats. High population connectivity has also been observed in marine invertebrate species with long dispersing larval stages (e.g. Cárdenas et al., 2009; Lee and Boulding, 2007; Porobić et al., 2013; Uthicke and Benzie, 2003). Although an early larval stage has not yet been reported for *A. alicemartinae*, post-larval individuals have been observed drifting near the shore (Häussermann and Försterra, 2001), and exhibit the ability to detach and reattach to substrata (López et al., 2013), suggesting that post-larval individuals may act as a dispersing unit. However, the dispersal distance of juveniles and adults in *A. alicemartinae* has not been evaluated to be able to distinguish whether this is a local or regional scale phenomenon. The ability to detach and reattach to the substratum has also been recorded in the anemone *M. senile*, which uses this mechanism to avoid local scale fluctuations in oxygen (Shumway, 1978). Additionally, *A. alicemartinae*, as an efficient disperser, could use human-mediated transport as dispersal vectors, which could allow haplotypes to randomly migrate, homogenizing genetic variability. It is likely that more variable molecular markers, such as microsatellite loci, will help to unmask the underlying population genetic patterns in this species.

4.4. Population expansion and human-mediated dispersal in *A. alicemartinae*

Analyzing biodiversity reports of different surveys since 1959 for the coast of Chile and Peru, Häussermann and Försterra (2001) and information in current databases (OBIS, 2014) suggest that *A. alicemartinae* first appeared in northern Chile and spread to the south as a result of favorable environmental changes and/or human activity, such as maritime transport. However, these authors lacked samples of *A. alicemartinae* from Peru in determining the actual northern limit of the range of distribution. Our results showed the highest genetic diversity in the Peruvian locality of Chero (18°9′S; 70°48′W), decreasing both to the north and south of this locality up to the 30°S biogeographic break, although there is no statistical support for isolation by distance (Fig. 3). Similarly, at 30°S one can observe an increase in haplotype diversity at more southern localities (Fig. 1), however there is no statistical support for population genetic structure in the studied range of distribution.

The historical records of the distribution of *A. alicemartinae*, and the observed spatial structure of genetic diversity may suggest, first, a possible origin or introduction of this species in the south of Peru, close to the border with Chile; and second, the potential effect of anthropogenic vectors. For example, shipping activities may move haplotypes over both sides of this well-documented biogeographical barrier. In fact, there are big seaports close to (less than 10 km) the sampling localities of Iquique and the two most southern localities of Las Cruces and Quiriquina Island (Iquique, San Antonio and San Vicente seaports, respectively), where the main shipping activity of Chile is concentrated. These results agree with the global observation that maritime activity is one of the main vectors of transportation for non-indigenous marine species (Carlton, 1985; Molnar et al., 2008; Ruiz et al., 2000). For instance, the anemone *H. luciae* has been transported over long distances attached to boat hulls (Gollasch and Riemann-Zürneck, 1996). Nevertheless, given the easy detachment of *A. alicemartinae*, other aspects of maritime transport may play an important role in the dispersal of this species on a regional scale, such as transport in ballast water, in sea chests, or on ropes of small-scale fishing boats.

5. Conclusion

In summary, our results complement the ecological observation of a southward invasion of *A. alicemartinae* along the coast of the Humboldt Current Ecosystem, with a strong genetic signal of demographic expansion. In addition, we propose two main hypotheses with respect to the potential origin and posterior expansion of *A. alicemartinae*: first, although we cannot yet refute that *A. alicemartinae* is a non-indigenous species from the west coast of South America that has not been recorded in its native distribution, it is clear that given the ecological history of this species (i.e. first records in the north of Chile) and the highest genetic diversity observed in the Peruvian localities of Chero and Mesas, with decreasing diversity toward north and southern latitudes up to the 30°S biogeographic break, it is possible that the point of origin or introduction of *A. alicemartinae* is the south of Peru. Second, the current distribution of *A. alicemartinae* may be explained by an increase in population size from one small ancestral population, with subsequent human-mediated transport (probably associated with regional-scale maritime activities), where haphazard transport results in the random distribution of the haplotypes.

Acknowledgments

This work forms part of the DIN 02/2009 project. We thank Sofia Paz for contributing samples of *A. alicemartinae* from Chile. We thank two anonymous reviewers for helpful comments and suggestions on the manuscript. Cristian B. Canales-Aguirre was supported by a Doctoral Fellowship for the "Programa de Doctorado en Sistemática y Biodiversidad",

from the graduate school of the Universidad de Concepción. Antonio Brante is grateful to funding from FONDECYT grant 1130868.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.seares.2015.03.005.

References

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A., Saunders, N.C., 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. 18, 489–522.

Balloux, F., Lehmann, L., de Meeûs, T., 2003. The population genetics of clonal and partially clonal diploids. Genetics 164, 1635–1644.

Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.

Beagley, C.T., Okada, N.A., Wolstenholme, D.R., 1996. Two mitochondrial group I introns in a metazoan, the sea anemone *Metridium senile*: one intron contains genes for subunits 1 and 3 of NADH dehydrogenase. Proc. Natl. Acad. Sci. 93, 5619–5623.

Bilewitch, J.P., Degnan, S.M., 2011. Unique horizontal gene transfer event has provided the octocoral mitochondrial genome with an active mismatch repair gene that has potential for an unusual. BMC Evol. Biol. 11, 228.

Bock, D.G., Zhan, A., Lejeusne, C., MacIsaac, H.J., Cristescu, M.E., 2011. Looking at both sides of the invasion: patterns of colonization in the violet tunicate *Botrylloides violaceus*. Mol. Ecol. 20, 503–516.

Brockman, S.A., McFadden, C.S., 2012. The mitochondrial genome of *Paraminabea aldersladei* (Cnidaria: Anthozoa: Octocorallia) supports intramolecular recombination as the primary mechanism of gene rearrangement in octocoral mitochondrial genomes. Genome. Biol. Evol. 4, 994–1006.

Brugler, M.R., France, S.C., 2008. The mitochondrial genome of a deep-sea bamboo coral (Cnidaria, Anthozoa, Octocorallia, Isididae): genome structure and putative origins of replication are not conserved among octocorals. J. Mol. Evol. 67, 125–136.

Brugler, M.R., Opresko, D.M., France, S.C., 2013. The evolutionary history of the order Antipatharia (Cnidaria: Anthozoa: Hexacorallia) as inferred from mitochondrial and nuclear DNA: implications for black coral taxonomy and systematics. Zool. J. Linnean Soc. 169, 312–361.

Calderón, I., Garrabou, J., Aurelle, D., 2006. Evaluation of COI and ITS markers as tools for population genetic studies of temperate gorgonians. J. Exp. Mar. Biol. Ecol. 336, 184–197.

Camus, P.A., 2001. Biogeografía marina de Chile continental. Rev. Chil. Hist. Nat. 74, 587–617.

Cárdenas, L., Castilla, J.C., Viard, F., 2009. A phylogeographical analysis across three biogeographical provinces of the south-eastern Pacific: the case of the marine gastropod *Concholepas concholepas*. J. Biogeogr. 36, 969–981.

Carlton, J.T., 1985. Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. Oceanogr. Mar. Biol. Annu. Rev. 23, 313–371.

Castilla, J.C., Uribe, M., Bahamonde, N., Clarke, M., Desqueyroux-Faúndez, R., Kong, I., Moyano, H., Rozbaczylo, N., Santelices, B., Valdovinos, C., Zavala, P., 2005. Down under the southeastern Pacific: marine non-indigenous species in Chile. Biol. Invasions 7, 213–232.

Chandler, E.A., McDowell, J.R., Graves, J.E., 2008. Genetically monomorphic invasive populations of the rapa whelk, *Rapana venosa*. Mol. Ecol. 17, 4079–4091.

Chen, C., Chiou, C.-Y., Dai, C.-F., Chen, C.A., 2008. Unique mitogenomic features in the Scleractinian Family Pocilloporidae (Scleractinia: Astrocoeniina). Mar. Biotechnol. 10, 538–553.

Chomsky, O., Douek, J., Chadwick, N.E., Dubinsky, Z., Rinkevich, B., 2009. Biological and population—genetic aspects of the sea anemone *Actinia equina* (Cnidaria: Anthozoa) along the Mediterranean coast of Israel. J. Exp. Mar. Biol. Ecol. 375, 16–20.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

England, P.R., Osler, G.H.R., Woodworth, L.M., Montgomery, M.E., Briscoe, D.A., Frankham, R., 2003. Effects of intense versus diffuse population bottlenecks on microsatellite genetic diversity and evolutionary potential. Conserv. Genet. 4, 595–604.

Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol. Bioinformatics Online 1, 47–50.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

Forsman, Z.H., Barshis, D.J., Hunter, C.L., Toonen, R.J., 2009. Shape-shifting corals: molecular markers show morphology is evolutionarily plastic in Porites. BMC Evol. Biol. 9, 45.

France, S.C., Hoover, L.L., 2002. DNA sequences of the mitochondrial COI gene have low levels of divergence among deep-sea octocorals (Cnidaria: Anthozoa). Hydrobiologia 471, 149–155.

France, S.C., Rosel, P.E., Agenbroad, J.E., Mullineaux, L.S., Kocher, T.D., 1996. DNA sequence variation of mitochondrial large-subunit rRNA provides support for a two-subclass organization of the Anthozoa (Cnidaria). Mol. Mar. Biol. Biotechnol. 51, 15–28.

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147, 915–925.

Fukami, H., Knowlton, N., 2005. Analysis of complete mitochondrial DNA sequences of three members of the *Montastraea annularis* coral species complex (Cnidaria, Anthozoa, Scleractinia). Coral Reefs 24, 410–417.

Fukami, H., Chen, C.A., Chiou, C.-Y., Knowlton, N., 2007. Novel group I introns encoding a putative homing endonuclease in the mitochondrial cox1 gene of Scleractinian corals. J. Mol. Evol. 64, 591–600.

Goddard, M.R., Leigh, J., Roger, A.J., Pemberton, A.J., 2006. Invasion and persistence of a selfish gene in the Cnidaria. PLoS ONE 1, e3.

Gollasch, S., Riemann-Zürneck, K., 1996. Transoceanic dispersal of benthic macrofauna: *Haliplanella luciae* (Verill, 1898) (Actiniaria) found on a ship's hull in a shipyard dock in Hamburg Harbour, Germany. Helgoländer Meeresunters 50, 253–258.

González-Muñoz, R., Simões, N., Mascaró, M., Tello-Musi, J.L., Brugler, M.R., Rodríguez, E., 2015. Morphological and molecular variability of the sea anemone *Phymanthus crucifer* (Cnidaria, Anthozoa, Actiniaria, Actinoidea). J. Mar. Biol. Assoc. UK 95, 69–79.

Hand, C., Uhlinger, K.R., 1995. Asexual reproduction by transverse fission and some anomalies in the sea anemone *Nematostella vectensis*. Invertebr. Biol. 114, 9–18.

Häussermann, V., Försterra, G., 2001. A new species of sea anemone from Chile, *Anemonia alicemartinae* n. sp. (Cnidaria: Anthozoa). An invader or an indicator for environmental change in shallow water? Org. Divers. Evol. 1, 211–224.

Haye, P.A., Segovia, N.I., Muñoz-Herrera, N.C., Gálvez, F.E., Martínez, A., Meynard, A., Pardo-Gandarillas, M.C., Poulin, E., Faugeron, S., 2014. Phylogeographic structure in benthic marine invertebrates of the southeast Pacific coast of Chile with differing dispersal potential. PLoS ONE 9, e88613.

Hellberg, M.E., 2006. No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. BMC Evol. Biol. 6, 24.

Hormazabal, S., 2004. Coastal transition zone off Chile. J. Geophys. Res. 109 (C01021).

Huang, D., Meier, R., Todd, P.A., Chou, L.M., 2008. Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding. J. Mol. Evol. 66, 167–174.

Kaluza, P., Kölzsch, A., Gastner, M.T., Blasius, B., 2010. The complex network of global cargo ship movements. J. R. Soc. Interface 7, 1093–1103.

Kayal, E., Roure, B., Philippe, H., Collins, A.G., Lavrov, D.V., 2013. Cnidarian phylogenetic relationships as revealed by mitogenomics. BMC Evol. Biol. 13, 5.

Lavergne, S., Molofsky, J., 2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proc. Natl. Acad. Sci. U. S. A. 104, 3883–3888.

Leberg, P.L., 1992. Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. Evolution (N Y) 46, 477–494.

Lee, H.J., Boulding, E.G., 2007. Mitochondrial DNA variation in space and time in the northeastern Pacific gastropod, *Littorina keenae*. Mol. Ecol. 16, 3084–3103.

Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.

Lonhart, S.I., 2009. Natural and climate change mediated invasions. In: Rilov, G., Crooks, J. (Eds.), Biological Invasions in Marine Ecosystems. Springer-Verlag, Berlin, pp. 57–70.

López, D.N., Arancibia, P.A., Neill, P.E., 2013. Potential dispersal mechanisms of the cryptogenic anemone, *Anemonia alicemartinae*. Rev. Chil. Hist. Nat. 86, 369–372.

Marrs, R.A., Sforza, R., Hufbauer, R.A., 2008. When invasion increases population genetic structure: a study with *Centaurea diffusa*. Biol. Invasions 10, 561–572.

McCommas, S.A., Bryant, E.H., 1990. Loss of electrophoretic variation in serially bottlenecked populations. Heredity 64, 315–321.

McFadden, C.S., Tullis, I.D., Hutchinson, M.B., Winner, K., Sohm, J.A., 2004. Variation in coding (NADH dehydrogenase subunits 2, 3, and 6) and noncoding intergenic spacer regions of the mitochondrial genome in Octocorallia (Cnidaria: Anthozoa). Mar. Biotechnol. (N Y) 6, 516–526.

McFadden, C.S., Benayahu, Y., Pante, E., Thoma, J.N., Nevarez, P.A., France, S.C., 2011. Limitations of mitochondrial gene barcoding in Octocorallia. Mol. Ecol. Resour. 11, 19–31.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.

Molnar, J.L., Gamboa, R.L., Revenga, C., Spalding, M.D., 2008. Assessing the global threat of invasive species to marine biodiversity. Front. Ecol. Environ. 6, 485–492.

Monteiro, F.A., Russo, C.A.M., Solé-Cava, A.M., 1998. Genetic evidence for the asexual origin of small individuals found in the coelenteron of the sea anemone *Actinia bermudensis* McMurrich. Bull. Mar. Sci. 63, 257–264.

Nei, M., Maruyama, T., Chakraborty, R., 1975. The bottleneck effect and genetic variability in populations. Evolution (N Y) 29, 1–10.

OBIS, 2014. Data from the Ocean Biogeographic Information System. Intergovernmental Oceanographic Commission of UNESCO (Web. http://www.iobis.org (consulted on 2014/10/05).).

Pearson, C.V.M., Rogers, A.D., Sheader, M., 2002. The genetic structure of the rare lagoonal sea anemone, *Nematostella vectensis* Stephenson (Cnidaria; Anthozoa) in the United Kingdom based on RAPD analysis. Mol. Ecol. 11, 2285–2293.

Pont-Kingdon, G., Okada, N. a, Macfarlane, J.L., Beagley, C.T., Watkins-Sims, C.D., Cavalier-Smith, T., Clark-Walker, G.D., Wolstenholme, D.R., 1998. Mitochondrial DNA of the coral *Sarcophyton glaucum* contains a gene for a homologue of bacterial MutS: a possible case of gene transfer from the nucleus to the mitochondrion. J. Mol. Evol. 46, 419–431.

Porobić, J., Canales-Aguirre, C.B., Ernst, B., Galleguillos, R., Hernández, C.E., 2013. Biogeography and historical demography of the Juan Fernández Rock Lobster, *Jasus frontalis* (Milne Edwards, 1837). J. Hered. 104, 223–233.

Reed, D.H., Frankham, R., 2003. Correlation between fitness and genetic diversity. Conserv. Biol. 17, 230–237.

Reitzel, A.M., Burton, P.M., Krone, C., Finnerty, J.R., 2007. Comparison of developmental trajectories in the starlet sea anemone *Nematostella vectensis*: embryogenesis, regeneration, and two forms of asexual fission. Invertebr. Biol. 126, 99–112.

Rice, W.R., 1989. Analyzing tables of statistical test. Evolution (N Y) 43, 223–225.

Roderick, G.K., Navajas, M., 2003. Genes in new environments: genetics and evolution in biological control. Nat. Rev. Genet. 4, 889–899.

Rosenberg, M.S., Anderson, C.D., 2011. PASSaGE: Pattern Analysis, Spatial Statistics and Geographic Exegesis. Version 2. Methods Ecol. Evol. 2, 229–232.

Ruiz, G.M., Fofonoff, P.W., Carlton, J.T., Wonham, M.J., Hines, A.H., 2000. Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. Annu. Rev. Ecol. Syst. 31, 481–531.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N., Weller, S.G., 2001. The population biology of invasive species. Annu. Rev. Ecol. Syst. 32, 305–332.

Sánchez, R., Sepúlveda, R.D., Brante, A., Cárdenas, L., 2011. Spatial pattern of genetic and morphological diversity in the direct developer *Acanthina monodon* (Gastropoda: Mollusca). Mar. Ecol. Prog. Ser. 434, 121–131.

Schama, R., Mitchell, M., Solé-Cava, A.M., 2012. *Actinia ebhayiensis* sp. nov., a new species of sea anemone (Anthozoa: Actiniaria: Actiniidae) from South Africa. J. Mar. Biol. Assoc. U. K. 92, 885–894.

Schreiber, S.J., Lloyd Smith, J.O., 2009. Invasion dynamics in spatially heterogeneous environments. Am. Nat. 174, 490–505.

Severance, E.G., Karl, S.A., 2006. Contrasting population genetic structures of sympatric, mass-spawning Caribbean corals. Mar. Biol. 150, 57–68.

Shearer, T.L., Coffroth, M.A., 2008. DNA barcoding: barcoding corals: limited by interspecific divergence, not intraspecific variation. Mol. Ecol. Resour. 8, 247–255.

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

Sherman, C.D.H., Ayre, D.J., 2008. Fine-scale adaptation in a clonal sea anemone. Evolution (N Y) 62, 1373–1380.

Sherman, C.D.H., Peucker, A.J., Ayre, D.J., 2007. Do reproductive tactics vary with habitat heterogeneity in the intertidal sea anemone *Actinia tenebrosa*? J. Exp. Mar. Biol. Ecol. 340, 259–267.

Shick, J.M., Lamb, A.N., 1977. Asexual reproduction and genetic population structure in the colonizing sea anemone *Haliplanella luciae*. Biol. Bull. 153, 604–617.

Shumway, S.E., 1978. Activity and respiration in the anemone, *Metridium senile* (L.) exposed to salinity fluctuations. J. Exp. Mar. Biol. Ecol. 33, 85–92.

Sinniger, F., Pawlowski, J., 2009. The partial mitochondrial genome of *Leiopathes glaberrima* (Hexacorallia: Antipatharia) and the first report of the presence of an intron in COI in black corals. *Galaxea*, J. Coral Reef Stud. 11, 21–26.

Snell, T.L., Foltz, D.W., Sammarco, P.W., 1998. Variation in morphology vs conservation of a mitochondrial gene in *Montastraea cavernosa* (Cnidaria, Scleractinia). Gulf. Mex. Sci. 2, 188–195.

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

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.

Thoma, J., Pante, E., Brugler, M., France, S.C., 2009. Deep-sea octocorals and antipatharians show no evidence of seamount-scale endemism in the NW Atlantic. Mar. Ecol. Prog. Ser. 397, 25–35.

Tsutsui, N.D., Suarez, A.V., Holway, D.A., Case, T.J., 2000. Reduced genetic variation and the success of an invasive species. Proc. Natl. Acad. Sci. U. S. A. 97, 5948–5953.

Uthicke, S., Benzie, J.A.H., 2003. Gene flow and population history in high dispersal marine invertebrates: mitochondrial DNA analysis of *Holothuria nobilis* (Echinodermata: Holothuroidea) populations from the Indo-Pacific. Mol. Ecol. 12, 2635–2648.

Veale, A.J., Lavery, S.D., 2012. The population genetic structure of the waratah anemone (*Actinia tenebrosa*) around New Zealand. N. Z. J. Mar. Freshw. Res. 46, 523–536.

Xia, X., Xie, Z., Salemi, M., Chen, L., Wang, Y., 2003. An index of substitution saturation and its application. Mol. Phylogenet. Evol. 26, 1–7.

Zardi, G.I., McQuaid, C.D., Teske, P.R., Barker, N.P., 2007. Unexpected genetic structure of mussel populations in South Africa: indigenous *Perna perna* and invasive *Mytilus galloprovincialis*. Mar. Ecol. Prog. Ser. 337, 135–144.

Zhang, J., Kapli, P., Pavlidis, P., Stamatakis, A., 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29, 2869–2876.