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Deep phylogeographic divergence among populations of limpet *Siphonaria lessoni* on the east and west coasts of South America

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Abstract The historical processes that have influenced the genetic structure of many species are often associated with environmental changes of the Pleistocene glacial cycles. These climate changes involve temperature oscillation, marine currents and loss of coastal habitats, which could have affected the abundance and geographic distribution of marine species in temperate coastal habitats. In this work, a 552-bp mtDNA fragment of COI locus of 92 individuals was sequenced to analyze the genetic structure of the limpet *Siphonaria lessoni*. Individuals were collected on the intertidal coast of the Southern Atlantic (Mar del Plata, San Antonio Oeste, Puerto Madryn and Ushuaia in Argentina) and the Southern Pacific (Valdivia and Valparaíso in Chile). *S. lessoni* displayed two distinct lineages that were nearly reciprocally monophyletic between the Atlantic and Pacific coasts. AMOVA tests revealed the existence of strong population genetic structure. The Pacific coasts yielded more haplotypes and polymorphic sites as well as higher haplotype and nucleotide diversity than the Atlantic clade did. Both Tajima's D and Fu's F_s were significant and negative, suggesting that limpet populations are in population expansion or have recently expanded. Accordingly, the haplotype network for each clade showed a star-like phylogeographic pattern. From IMa analysis, the divergence time between Pacific and Atlantic populations was 100,000–1,000,000 ybp with gene flow occurring from Pacific to Atlantic populations. The Bayesian Skyline analysis revealed an older coalescence in the Pacific clade (30,000–300,000 ybp) as compared to that in the Atlantic clade (4,000–40,000 ybp). This work reports evidence of Pacific–Atlantic geographic isolation with asymmetric migration, which is probably related to changes in sea level and temperature due to the extended glaciation periods that occurred in the region throughout the Pleistocene.

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Introduction

Glacial cycles, especially those of the Pleistocene (1,800,000–11,500 ybp), have affected and shaped the genetic structure of both terrestrial and marine species (Avise 2000; Muellner et al. 2005; Ruzzante et al. 2006; Túnez et al. 2010; Fernández Iriarte et al. 2011; Sérsic et al. 2011; Fraser et al. 2012). The climate changes associated with the glaciations led not only to a reduction in global and sea temperature, including changes at sea level during the full glacial episodes, but also to changes in ocean current patterns and to the displacement or eradication of coastal habitats (e.g., Rabassa et al. 2005, 2011; Grant and Bowen 2006). In this context, the biogeographic contraction expansion model (CE) (Provan and Bennett 2008) describes the species geographic responses to past climate changes induced by the glacial–interglacial cycles. Under the basic CE model, most of the marine littoral species inhabiting the cold-template waters of the southern hemisphere would have survived in northern refuges and re-colonized higher latitudes after the Last Glacial Maximum (LGM, 18,000–23,000 ybp) (Hewitt 2004). The CE model predicts that the recently colonized sites would present lower genetic diversity than source populations and maintain other genetic imprints of population expansion, such as a star-like

haplotype network, negative Tajima's D and Fu F_s indices, lower R_2 index and an unimodal mismatch distribution, among others (e.g., Hewitt 2000, 2001, 2004; Hewitt and Ibrahim 2001; Emerson and Hewitt 2005). In the marine environment, coastal species reveal different responses to past climate change than offshore species. While coastal-benthic species provide strong evidence of recent (i.e., LGM) climate changes (e.g., Wares and Cunningham 2001; Marko et al. 2010; González-Wevar et al., 2013), offshore-pelagic species show patterns of genetic differentiation and divergence consistent with long-term regional persistence (Janko et al. 2007; Fernández Iriarte et al. 2011). These contrasting biogeographic histories could reflect fundamental differences in the way in which glaciations impacted on coastal and offshore scenarios (e.g., Wares and Cunningham 2001; Janko et al. 2007; Marko et al. 2010). Aside from the historical climate changes, other factors may promote different levels of phylogeographic structure and differentiation in marine species. Indeed, the interconnection among populations could be influenced by the ontogeny of marine organisms (e.g., Palumbi 2003; Riginos et al. 2011; Fraser et al. 2012). For instance, species with planktonic phase could increase their dispersion ability and connectivity, displaying, in some cases, a weaker population structure than that of species with direct development (e.g., Palumbi 1994; Thorrold et al. 2002; Kinlan and Gaines 2003; Wares 2002; Marko et al. 2010).

The austral edge of South America underwent strong climatic fluctuations during the Pleistocene glaciations (Rabassa et al. 2011). Thus, the biogeographic regions of Peru, Central Chile and Magellan in the Pacific Ocean (Fig. 1) are defined by contrasting the geologic and climate history (Harrison 2004) that could be reflected in the phylogeographic structure of littoral species. In this sense, the barnacle *Notochthamalus scabrosus* (Zakas et al. 2009), the alga *Macrocystis pyrifera* (Macaya and Zuccarello 2010) and the sleeper limpet *Crepipatella dilatata* (Brante et al. 2012) displayed a break at 30°S between the Central and Peruvian regions, while the kelp *Durvillaea antarctica* presented a phylogeographic break between the Central and Magellan regions at 44°S (Fraser et al. 2009, 2010). In contrast, the barnacle *Jehlius cirratus* (Zakas et al. 2009), the gastropods *Concholepas concholepas* (Cárdenas et al. 2009) and the fish *Eleginops maclovinus* (Ceballos et al. 2012) did not show genetic structure linked to biogeographic coastal regions of southern South America. A recent comparison of the limpet *Nacella magellanica* with the Pacific and Atlantic Patagonia (Magellan region) indicates that this species did not suffer a phylogeographic break (probably due to the long duration of its larval phase). In spite of that, *N. magellanica* is in postglacial expansion on the Atlantic and Pacific shores (de Aranzamendi et al. 2011; González-Wevar et al. 2012).

Siphonaria lessoni (Blainville 1824) is a pulmonate limpet (Pulmonata, Siphonariidae) that inhabits intertidal crevices and tide pools on rocky shores (Penchaszadeh 2004). Many of the ecological and life history traits of this species turn it into an interesting study system to address questions of historical events driving the current geographic distribution of species living in the littoral of South America. To begin with, *S. lessoni* features a wide distribution range from the Peruvian coast (Alamo and Valdivieso 1997) to Cabo de Hornos (Dell 1971) in the Pacific Ocean and from Islas Malvinas (Falkland Islands) and the Patagonian coast (Castellanos et al. 1993) to Santa Catarina, Brazil (Penchaszadeh et al. 2007), which allows to analyze demographic and population responses to climate changes in an extended geographic range. Secondly, planktonic larval duration of *S. lessoni* is about 7 days (Olivier and Penchaszadeh 1968), which affords the opportunity to explore whether persistence of larvae in the water column influences the historic connectivity and gene flow among populations. Thirdly, despite fossil records indicating that *S. lessoni* is present in the southwestern Atlantic from the Holocene (5,000–8,000 ybp), records from the Pleistocene (125,000 ybp) account for their presence in the coast of Golfo Nuevo, Argentina (42–44°S, Fig. 1) (Aguirre et al. 2008, 2009). This finding suggests that the Golfo Nuevo could have functioned as a refuge to littoral species during the Pleistocene glaciations, a hypothesis that can be tested for *S. lessoni* by conducting phylogeographic analyses. Fourthly, palaeoenvironmental and geographic evidence suggests that part of the molluscan fauna of the Argentinean Patagonia radiated from the southeastern Pacific (see Aguirre et al. 2008). Therefore, *S. lessoni* could be used to determine whether Chilean populations are, in fact, the "ancestral populations" that originated the Atlantic populations.

This study aimed to analyze the genetic structure and demographic history of *S. lessoni* in the southwestern Atlantic and southeastern Pacific, using the mitochondrial marker cytochrome oxidase I (COI). The major hypotheses formulated were: (1) glacial expansion isolated, at some point during the Pleistocene, the Pacific and Atlantic populations. On the basis of this hypothesis, a strong phylogeographic structure should be found between the Pacific and Atlantic populations with a divergence time between these two populations in the Pleistocene, (2) Pacific populations have acted as a colonization source for Atlantic populations on at least one occasion, if this hypothesis held true, Pacific populations would be expected to reveal more genetic diversity than Atlantic populations do; also, we would be expected similar diversity between Pacific and ancestral populations. Finally, we would be expected that migration of Pacific individuals into to Atlantic would be greater than the opposite.

Materials and methods

Sample collection

Individuals from *S. lessoni* were collected from rocky areas of the middle intertidal zone in Mar del Plata (MDP; 38°S 57°W), San Antonio Oeste (SAO; 40°S 64° W), Puerto Madryn (MAD; 42°S, 65°W) and Ushuaia (USH; 54°S, 68°W) on the Argentinean coast, and from Valdivia (VLD; 39°S, 73°W) and Valparaíso (VLP; 30°S, 71°W) on the Chilean coast. These sites comprise the following biogeographic provinces: Argentinean province (MDP + SAO), Magellan province (MAD + USH) and Central Chile province (VLD + VLP) (Fig. 1). At each site, 20 individuals were removed from the upper intertidal and stored in 70 % ethanol until further processing.

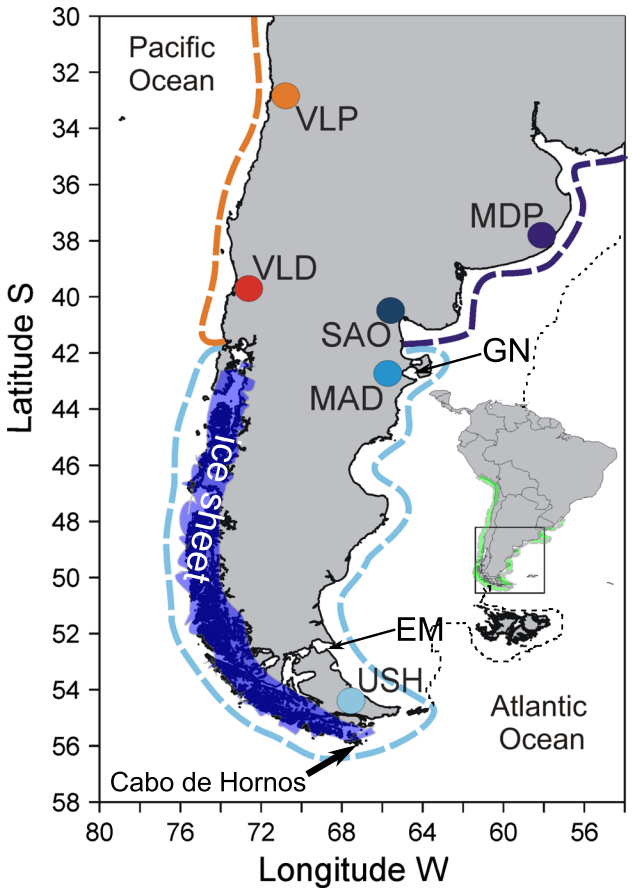

Fig. 1 Map of sampling scheme for *S. lessoni* along almost all its distribution range. *Dashed lines* indicate the different biogeographic provinces: Argentina (MDP + SAO); Magellan (MAD + USH); Central Chile (VLD + VLP). MDP, Mar del Plata; SAO, San Antonio Oeste; MAD, Puerto Madryn; USH, Ushuaia; VLP, Valparaíso; VLD, Valdivia; GN, Golfo Nuevo; EM, Estrecho de Magallanes. The *diffuse line* over the Andes mountain range shows the Patagonian ice sheet in the Last Glacial Maximum (LGM) from McCulloch et al. (2000). The *dotted line* in the ocean shows the sea level at the LGM according to Rabassa et al. (2005)

DNA extraction, PCR amplification and sequencing

DNA was extracted from small muscle pieces using the Chelex 100 (Biorad) method sensu Walsh et al. (1991). A fragment of the mitochondrial gene COI was amplified using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). Reactions were performed in 30 µl final volume, containing 3 µl of 10× buffer, 2 µl $MgCl_2$ (25 mM), 2.5 µl DNTPs (2.5 mM), 3 µl of each primer (2 mM), 0.08 units/ml of Taq polymerase and 3 µl of DNA template. PCR cycling was performed starting with denaturation for 1 min at 96 °C, followed by 40 cycles of 30 s at 94 °C, 1 min at 50 °C and 1 min at 72 °C, with a final extension of 14 min at 72 °C. Successful PCR reactions were confirmed in a 2 % agarose gel and purified with kits. PCR products were sequenced in Macrogen Inc. (Korea). The sequences were aligned, corrected and edited with PROSEQ (Filatov 2002). Sequences were deposited in GenBank under the access numbers XXX to XXX.

Genetic diversity indexes and population genetic structure

For each sampled population, haplotype (h) and nucleotide (π) diversities were estimated using DNAsp v5 (Librado and Rozas 2009). An analysis of molecular variance (AMOVA) was performed using Arlequin, with 10,000 permutations to test significance (Excoffier et al. 2005). To infer the spatial genetic structure of *S. lessoni,* the pairwise values of Phi_{ST} were calculated. A nested AMOVA was also conducted by placing biogeographic samples (CHI, MAG and ARG) in different groups.

The genealogical relationships between sequences were inferred by the haplotype network obtained using "median-joining" with Network 4.6 software (http://www.fluxus-engineering.com). The substitution model of the COI fragment was estimated with the jModelTest 0.1.1 software (Posada 2008).

An intraspecific phylogeny was constructed with *S. lateralis* as the outgroup with the neighbor-joining tree using software MEGA 5 (Tamura et al. 2011). The evolutionary distance was calculated with the Maximum Composite Likelihood method, and node support was evaluated with 1,000 bootstrap replicates (Felsenstein 1985).

Demographic analysis

The history of demographic changes was assessed by calculating the Tajima's D test (Tajima 1989) and Fu's F_s test (Fu 1997), using DnaSP. Tajima's and Fu's neutrality tests are currently used to verify whether a population

is in a neutral mutation/drift equilibrium (Ramos-Onsins and Rozas 2002). Negative and significant values of these parameters are indicative of population expansion and/or negative selection. The demographic history of *S. lessoni* was also studied with "mismatch" distributions (differences in distributions between haplotype pairs) (Rogers and Harpending 1992). This method discriminates between stability and sudden population expansion. The observed mismatch distributions were compared to those expected at each site, using a model of sudden population expansion. Any deviation from the model was evaluated with the R_2 (Ramos-Onsins and Rozas 2002) calculated in DNAsp. After large expansion, populations should present unimodal mismatch distributions with low R_2, while stable ones with high R_2. The significance of each index (F_s, D and R_2) was tested with 10,000 simulations based on the coalescent process in DNAsp.

The isolation with the migration model implemented in the Bayesian-based IMa program (Hey and Nielsen 2007) was used to assess gene flow between Atlantic (USH + MAD + SAO + MDP) and Pacific populations (VLD + VLP). To fit the IM model to the data, a Bayesian coalescent method that integrates all possible genealogies with a Markov chain Monte Carlo approach was used. Three independent runs performed in "MCMC mode" with different random seed numbers were conducted with a burn-in period of 50,000 steps and running for 500,000 steps. To ensure that there are no obvious trends, the run of the program should be long enough for chains to converge and the effective sample size (ESS) value among the parameters should be greater than 50 (see Hey and Nielsen 2007). So we ran the program multiple times (with a different random number seed) and corroborated that the results were similar and with ESS greater than 50.

Runs were monitored by using estimates of the effective sample size based on the measured autocorrelation of parameter values over the course of the run.

Genealogies saved during the runs were analyzed in a "Load Trees mode" (*L*-mode) to determine whether the full model fit the data better than models that excluded the parameters. To convert scaled model parameter estimates into demographic parameters (including effective population size Ne and divergence time *T*), we assumed a generation time of 1 year and specified a COI mutation rate of 1–10 % per million years (Myr) (see below). Values of demographic parameters reported are the means from three runs (±SD) and means of 95 % confidence limits.

Past changes in the effective population size (Ne) of *S. lessoni* were characterized by generating COI Bayesian skyline plots (BSPs) with BEAST v1.4.8 (Drummond et al. 2005; Drummond and Rambaut 2007). These analyses were run under the HKY + I + G model selected by jModelTest using the Akaike information criterion. The analysis was performed with three runs of 10,000,000 generations each, in which trees and parameters were sampled every 1,000 generations.

To estimate population divergence times, the COI substitution rate of 1 % per million years was used, previously applied to *S. concinna*, *S. nigerrima* and *S. capensis,* which is a mean of divergence between gastropod species (Teske et al. 2011). This substitution rate was corrected based on theoretical studies that suggest that the substitution rate within lineages is 10 times higher than that between lineages (Ho et al. 2005, 2007, 2011) and recently used in limpets (de Aranzamendi et al. 2011; González-Wevar et al. 2011, 2012, 2013). In view of this, substitution rates of 1 and 10 % were run for both lineages, and the mean coalescence times from three runs of BEAST were corrected and plotted.

Results

Genetic diversity indexes and population genetic analysis

Sixty-one substitutions (42 transitions and 6 transversions) and 47 polymorphic sites in 92 samples of 520 bp of *S. lessoni* COI sequences obtained from Argentina and Chile defined 43 mitochondrial haplotypes. The mean number of pairwise differences was 5.899 (±2.843). Overall nucleotide diversity was 0.0107 (ranging from 0.0008 to 0.0074), and haplotype diversity was 0.801 (ranging from 0.419 in MDP to 0.992 in VLP) (Table 1). *S. lessoni* haplotypes belonged to two lineages, one comprising the Central Chilean province samples (LI) and the other including almost all the Magellan and Argentinean province samples (LII) with high bootstrap (Fig. 2). The Chilean and Magellan–Argentinean bioregions did not share haplotypes, and only in MAD did they present two haplotypes closely related to LI and five haplotypes assigned to LII (Figs. 2, 3). The AMOVA tests revealed the existence of strong population genetic structure between biogeographic provinces. Thus, CHI versus MAG–ARG Argentinean province groupings explained most of the genetic variations (Table 2). The other geographic levels explained lesser variation and were not significant among groups (Table 2). Also, the τ_{ST} between pair of sites indicated that the Central Chilean samples (VLD and VLP) were similar (yielded lower π_{ST}) and largely divergent from the Magellan (MAD and USH) and Argentinean (MDP and SAO) provinces. Likewise, within these regions, MDP was moderately divergent from SAO and MAD (Table 3).

The COI haplotype network for *S. lessoni* showed that the LI and LII lineages are connected by seven mutational steps (Fig. 3). LI, mostly samples from the Pacific Ocean, yielded a higher number of haplotypes, polymorphic sites,

Table 1 Genetic diversity and demographic parameters for 552 bp of cytochrome oxidase I (COI) mtDNA of *S. lessoni*

Sample	*N*	Nh	*S*	*h* (SD)	π (SD)	*D*	F_s	R_2	τ
MDP	17	4	3	0.419 (0.141)	0.0008 (0.0003)	−1.377	−1.936*	0.114	0.456
SAO	16	4	4	0.442 (0.142)	0.0013 (0.0006)	−1.312	−1.045	0.065**	0.166
MAD	14	7	19	0.813 (0.094)	0.0092 (0.0029)	−0.626	0.557	0.119	0.104
USH	15	6	10	0.571 (0.149)	0.0024 (0.0012)	−2.156**	−1.944*	0.149	0.000
VDL	14	13	20	0.989 (0.031)	0.0080 (0.0010)	−1.251	−8.510**	0.076**	4.407
VLP	16	15	22	0.992 (0.025)	0.0074 (0.0009)	−1.542	−11.716**	0.065**	4.108
Argentinean province (MDP, SAO)	33	7	7	0.428 (0.107)	0.0011 (0.0004)	−1.899**	−4.652**	0.060*	0.231
Magellan province (MAD, USH)	29	12	27	0.700 (0.095)	0.0059 (0.0020)	−1.885*	−2.900	0.055**	0.000
Central Chile province (VLD, VLP)	30	26	29	0.989 (0.013)	0.0078 (0.0024)	−1.562*	−25.124**	0.064*	4.299
LI	33	28	33	0.989 (0.011)	0.0083 (0.0007)	−1.623*	−26.433**	0.061*	4.587
LII	59	15	18	0.517 (0.080)	0.0015 (0.0004)	−2.412**	−14.942**	0.065	1.549
ALL	92	43	47	0.801 (0.043)	0.0107 (0.0008)	−1.202	−25.754**	0.059	1.668

N = sampled size; Nh = haplotype number; *S* = number of polymorphic sites; *h* = haplotype diversity; π = nucleotide diversity; Tajima's *D*; Fu's F_s; R_2 and Tau (τ)

* $p < 0.05$; ** $p < 0.01$

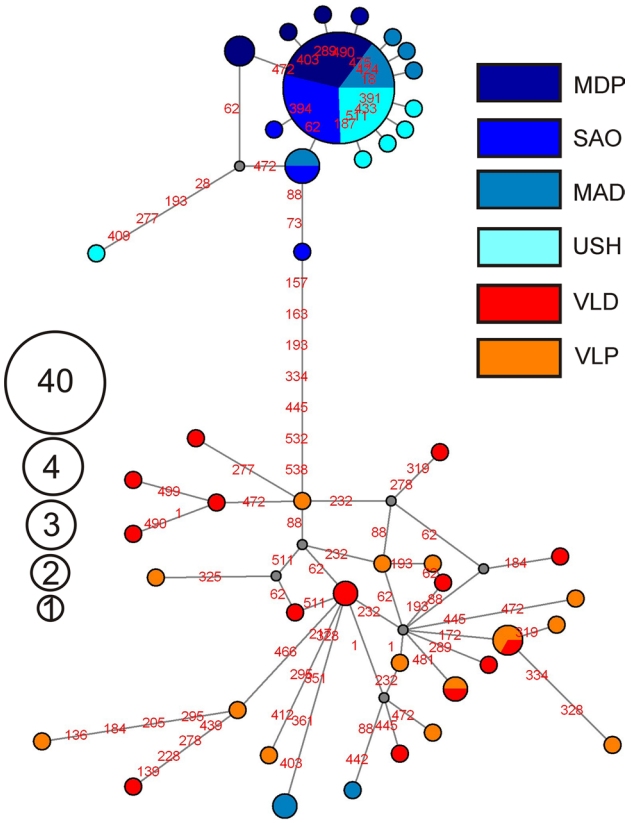

Fig. 2 Median-joining haplotype network for cytochrome oxidase I (COI) mtDNA sequences of *S. lessoni*. The *circles' area* is proportional to the number of individuals in each haplotype found. Numbers between *circles* represent additional mutational steps

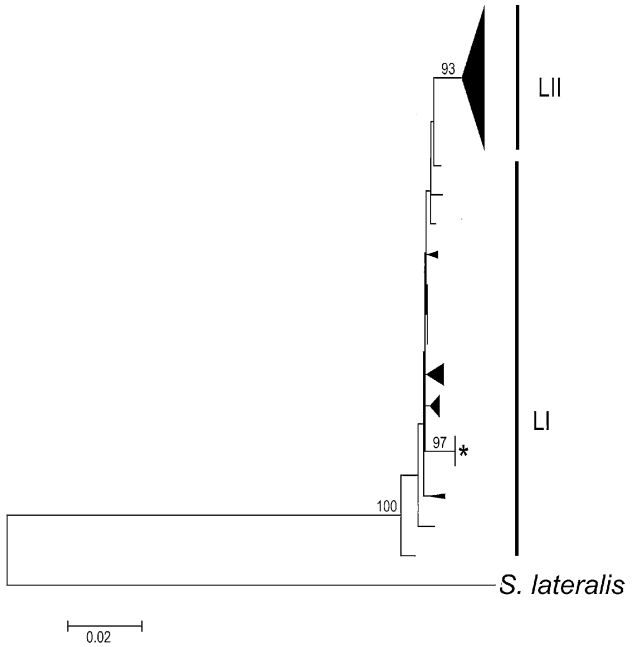

Fig. 3 Neighbor-joining tree from the haplotypes rooting the tree with a sequence of *S. lateralis*. The *asterisk* in the branch indicates the two haplotypes of MAD belonging to lineage I. Bootstrap values are shown in above nodes. LI: lineage I, LII: lineage II

and haplotype and nucleotide diversity than clade II did (most samples from the Atlantic Ocean) (see Table 1). *S. lessoni* from the Argentinean province showed the lowest haplotype and nucleotide diversity (Table 1), and the haplotype network comprised one central and common haplotype and few rare haplotypes that differed from the central one by one to three different base pairs. The Magellan province displayed intermediate values of haplotype and nucleotide diversity (Table 1), and the haplotype network consisted in one central and common haplotype and few rare haplotypes that, in this particular case, differed from

Table 2 Analysis of molecular variance (AMOVA) for COI from *S. lessoni* populations

Percentage of variation (degree of freedom)			
Source	Among groups	Among populations within groups	Within populations
Central Chile province (VLD, VLP) versus Magellan province (MAD, USH)–Argentinean province (MDP, SAO)	72.61 (1)$^{+}$	1.22 (4)**	26.16 (86)**
Central Chile province (VLD, VLP) versus Magellan province (MAD, USH) versus Argentinean province (MDP, SAO)	63.40 (2)	1.85 (3)*	34.75 (86)**
Central Chile province (VLD, VLP)–Magellan province (MAD, USH) versus Argentinean province (MDP, SAO)	17.55 (1)	45.76 (4)**	36.68 (86)**
Magellan province (MAD, USH) versus Central Chile province (VLD, VLP)–Argentinean province (MDP, SAO)	0.00 (1)	66.15 (4)**	41.22 (86)**
All populations (MDP, SAO, MAD, USH, VLD, VLP)		60.19 (5)**	39.81 (86)**

$+ = 0.06$; * $p < 0.05$; ** $p < 0.01$

Table 3 Population pairwise genetic differentiation (τ_{ST}) between sampled populations of *S. lessoni*

	MDP	SAO	MAD	USH	VDL	VLP
MDP		0.050*	0.118*	−0.006	0.786**	0.785**
SAO			0.087	0.001	0.760**	0.762**
MAD				0.082	0.509**	0.523**
USH					0.739**	0.742**
VDL						0.023
VLP						

* $p < 0.05$; ** $p < 0.01$

the central one by one to twelve different base pairs. The variability of this region was mainly ascribed to the high haplotype and nucleotide diversity values found in MAD site (Table 1). Finally, the Central Chilean province was characterized by high haplotype and nucleotide diversity, and the haplotype network revealed a high frequency of unique haplotypes (28 out of 33).

Demographic analysis

The Tajima's D and Fu's F_s values were negative and significant in both lineages (Table 1). R_2 did not yield a significant deviation from expectations under the spatial expansion model. The Tau index for LI was three times higher than that for LII (4.59 and 1.55, respectively. See Table 1). All regions presented significant and negative Fu's F_s except for the Magellan region (Table 1), and almost all indicated unimodal mismatch distributions (data not showed), thus suggesting population expansion in Argentinean and Central Chile regions. Nevertheless, the Magellan region exhibited a bi-modal curve in its mismatch distribution (data not showed) that is due to the two Pacific haplotypes, which are likely to be explained by the high h and π showed in MAD (Table 1).

IMa runs reached good convergence and ESS values as suggested by Hey and Nielsen (2007). Likelihood IMa curves provided strong unimodal posterior distributions for all parameter estimates and bounds fell within the prior distribution for the COI data of *S. lessoni*. Population size, divergence time and migration rates showed distinct peaks between the Pacific and Atlantic populations (Fig. 4a–c). The estimated population size (N) using 10 % mutation rate (1 % mutation rate showed an N value with a greater order of magnitude) yielded a higher number of individuals in the Pacific group ($N_1 = 270 \pm 0.33$ millions, 95 % confidence limit 183–326 millions) than the Atlantic group ($N_2 = 71 \pm 0.53$ millions, 95 % confidence limit 37–121); also, the ancestral size (N_A) was higher than N_2 but similar to N_1 ($N_A = 319 \pm 0.01$ millions, 95 % confidence limit 296–328) (Fig. 4a). Between Atlantic and Pacific limpet population was a divergence time of 113,000 and 1,130,000 ybp for 1 and a 10 % mutation rate, respectively (Fig. 4b). Migration rate was higher toward the Atlantic than toward the Pacific (Fig. 4c).

IMa runs indicated that, on the one hand, the model that excluded Atlantic gene flow was the nested model that fit data better ($p = 0.999$; Table 4). While, on the other hand, the other model that also fit data better ($p = 0.802$; Table 4) indicated that the population size was different between the Atlantic and Pacific ancestral population. The first model had the following parameters: θ_1 (Pacific), θ_2 (Atlantic), θ_A (Ancestral), m_{AP} (migration to the Pacific) equal to zero and m_{PA} (migration to the Atlantic). The second model accepted showed: $\theta_1 = \theta_A$, θ_2 and m_{AP}, m_{PA} (Table 4).

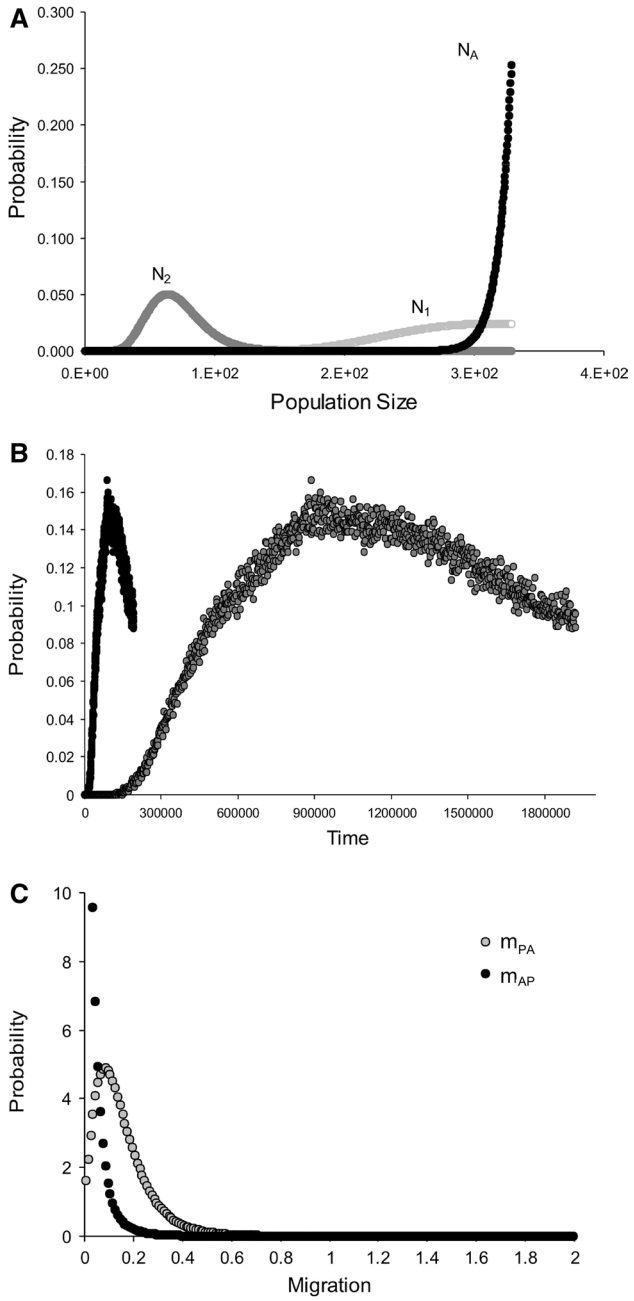

Fig. 4 Likelihood plots from IMa analyses based on data from the COI of *S. lessoni*. The marginal posterior probability distributions for model parameters were scaled by the neutral mutation rate: **a** Effective population sizes of Atlantic and Pacific groups (N_1 and N_2, respectively) and population sizes of ancestral populations (N_A); **b** divergence time, *black circle* is 10 % of mutation rate and *gray circle* is 1 %. **c** Migration rates, m_{AP} migration to the Pacific, m_{PA} migration to the Atlantic

Both analyses of population divergence times using 1 and 10 % of million years^{-1} (see "Materials and methods") revealed an older coalescence time for the *S. lessoni* in LI: 300,000 and 30,000 ybp, respectively, when a significant decline in population size occurred (Fig. 5).

Table 4 Model selection in IMa based on COI sequence data of *S. lessoni*

Model	d.f.	2LLR	*p* value
$\theta_1, \theta_2, \theta_A, m_1 = m_2$	1	3.599	0.058
$\boldsymbol{\theta_1, \theta_2, \theta_A, m_1 = 0, m_2}$	1	**−0.002**	**0.999**
$\theta_1, \theta_2, \theta_A, m_1, m_2 = 0$	1	5.062	0.024
$\theta_1, \theta_2, \theta_A, m_1 = 0, m_2 = 0$	2	5.059	0.080
$\boldsymbol{\theta_1 = \theta_A, \theta_2, m_1, m_2}$	**1**	**0.063**	**0.802**
$\theta_1 = \theta_A, \theta_2, m_1 = m_2$	2	3.598	0.165
$\theta_1 = \theta_A, \theta_2, m_1 = 0, m_2 = 0$	3	5.059	0.167

The models with highest probability of explanation are in bold

2LLR = log-likelihood ratio statistic, d.f. = degrees of freedom for each model. *p* values for 2LLR statistics were calculated using the x^2 calculator available at http://www.stat.tamu.edu/~west/applets/chisqdemo.html. θ_1 (Pacific), θ_2 (Atlantic), θ_A (Ancestral), m_1 (migration to the Pacific) and m_2 (migration to the Atlantic). Only the models with probabilities higher than 0.01 are shown

On the contrary, the haplotypes in LII coalesced nearly 40,000 and 4,000 ybp, respectively (Fig. 5). Overall, BSP and mismatch results suggested that LII probably started to expand as the total ice melt after the LGM period (18,000–23,000 ybp).

Discussion

A high degree of population genetic structure was observed in *S. lessoni* along its distribution range. Two distinct mitochondrial lineages that were nearly reciprocally monophyletic between the Pacific and the Atlantic coasts could be identified in *S. lessoni*: LI included the populations from the Pacific (Central Chilean province: VLP and VLD) and some individuals from MAD; and LII, in turn, comprised the Atlantic population analyzed (Magellan and Argentinean provinces, USH–MAD and SAO–MDP, respectively). High levels of differentiation were revealed by high τ_{ST} values between the Pacific and the Atlantic populations. Also, data indicated that the Pacific populations could be considered as an ancestral population due to the higher number of haplotypes, polymorphic sites, as well as haplotype and nucleotide diversity as compared to the Atlantic populations. In this sense, the best model in IMa showed a similar θ value between the Pacific and ancestral samples, both different from the Atlantic limpet population. Thus, the second hypothesis predicting that the limpets from the Pacific (source population) would present more genetic diversity than those from the south Atlantic could not be rejected.

Within the Pacific population, the absence of a biogeographic structure (τ_{ST} not different from 0) could be attributed to a recent population expansion rather than to

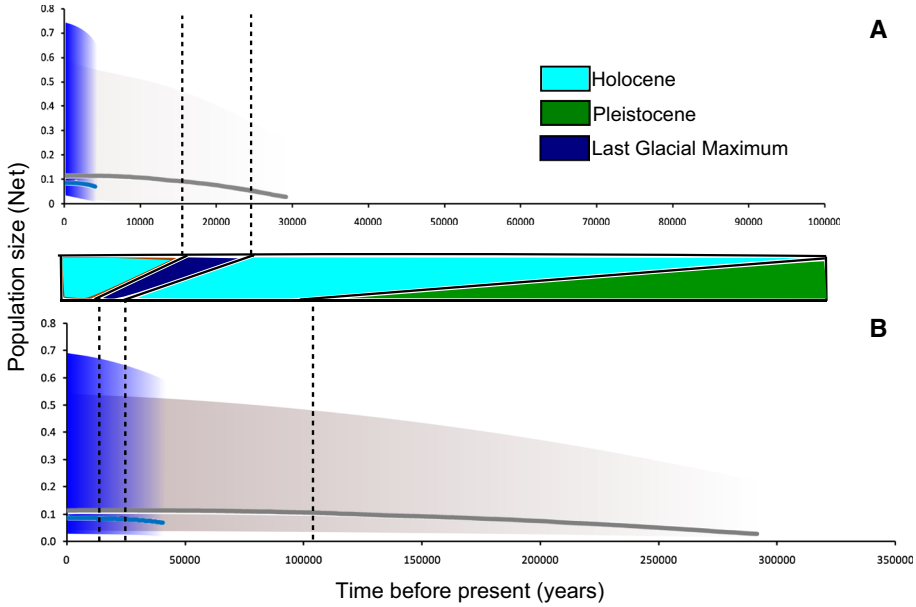

Fig. 5 Bayesian skyline plot (BSP) based on COI sequences of *S. lessoni* showing changes in population size through time above each scale. The *y-axis* is the product of effective population size (Ne) and generation length (*t*) on a logarithmic scale. The heavy *solid line* represents the median estimated under the assumption of a per site mutation rate of **a**: 10 % and **b**: 1 % of million years^{-1}. The *shaded zone* indicates 95 % higher density

population stability in the long term. This is evident in the topology of the Pacific haplotype network for VLD and VLP samples (Fig. 2), where almost all sampled individuals presented unique haplotypes and greater value of θ and N (population size) for the Pacific population. Likewise, these Central Chilean populations provided negative values in neutrality tests, indicating an excess of low frequency polymorphisms that revealed a population bottleneck followed by rapid population growth and accumulation of mutations.

With respect to samples from the Atlantic coast (Magellan and Argentinean provinces), they revealed low levels of pairwise differentiation but significant for the comparison between MDP and MAD–SAO. A weak population structure could be related to lesser population size and/or environmental instability. This is in line with the topology of the haplotype network, which shows a central haplotype with high frequency and many peripheral mutational steps. Additionally, all these populations, except MAD, also displayed negative values in neutrality tests indicating an excess of low frequency polymorphisms and revealing a recent population expansion.

From IMa analysis, the divergence time between the Pacific and the Atlantic populations was in agreement with our first hypothesis: estimating 100,000–1,000,000 ybp of divergence during the Pleistocene era. Thus, both the Atlantic and the Pacific populations showed evidence of population expansion but differed in the expansion time, being longer for LI than for LII lineages (Fig. 5). Therefore, the overall result supports the fact that the Atlantic populations would have been more severely affected during LGM.

The climatic oscillations that occurred during the Pleistocene in the austral edge of South America probably caused more conspicuous effects in the Atlantic coast than in the Pacific. This is because during the LGM, a continuous ice layer of 2,500 km in length extended from 56°S to 36°S (McCulloch et al. 2000) (Fig. 1). This ice layer covered almost entirely the Andean Patagonia and far to the east reaching the current marine platform in southern Argentina and to the west the edge of the continental platform (Hulton et al. 2002). This caused the mass extinction of several species living on rocky shores. In the Atlantic Ocean, glaciations not only lowered sea temperature and level, but also changed currents and eradicated habitats (Rabassa et al. 2005, 2011; Fraser et al. 2012). On the contrary, there is evidence that the Pacific, more precisely the Central Chilean coasts (until 42°S), was not significantly affected by the Pleistocene glaciations (Harrison 2004). Thus, the genetic analysis data in this work mirror the geologic history of southern South America and support the hypothesis raised, which predicts that if the glaciations isolated the limpets from both oceans, there would be a strong phylogeographic structure.

Species with presence of pelagic larvae commonly exhibit low levels of global population differentiation owing to high dispersal potential and apparent absence of physical barriers that limit gene flow (e.g., Hellberg et al. 2002; Carr et al. 2003; Palumbi 2003). Particularly, studies conducted on South American marine species support this hypothesis. For instance, Ocampo et al. (2013) did not observe a population structure between the Pacific–Atlantic populations in subtidal pea crab *Calyptraeotheres garthi*. For this species, the pelagic larval duration in the water column could be of about 1 month (Ocampo et al. 2011). Similar results were reported for the subtidal limpet *Nacella magellanica* from the Atlantic coast (de Aranzamendi et al. 2011). In this case, the pelagic larval duration

in the water column was also of about 1 month (de Aranzamendi et al. 2011). However, many recent studies have pointed out that the larval dispersal potential of marine species (pelagic larvae or direct development) from intertidal rocky sites is not the unique predictor of population connectivity level (e.g., Marko et al. 2010), and many authors postulate that other factors, such as oceanographic conditions (e.g., salinity or temperature), discontinuous distribution of suitable substrata and organism biology, could play a key role in migration regulation among populations (e.g., Riginos and Nachman 2001; Severance and Karl 2006; Becker et al. 2007; Sherman et al. 2008). Thus, while *S. lessoni* has a short planktonic larval phase, which probably contributed to the isolation, it is also worth noticing that the distribution of suitable substrata during the LGM, which was greater in the Pacific basin than in the Atlantic basin, probably contributed the most to establishing the phylogeographic pattern.

MAD (Puerto Madryn) in Golfo Nuevo in the Magellan province exhibited a higher genetic diversity index than other sites did since it was the only site that showed LI and LII lineages in sympatry. Two explanations are possible: (1) individuals from LI haplotypes migrate from the Pacific to MAD by gene flow or (2) incomplete lineage sorting following colonization. IMa analysis supported the first explanation by accepting a model of isolation that is in agreement with some "recent" migration to the Atlantic rather than to the Pacific. Migration between the Pacific and the Atlantic is unidirectional only when Madryn is considered in the IMA analysis. When MAD is not contemplated, the accepted models (data not shown) do not clearly establish a migration pattern. Since USH did not show individual LI haplotypes, the migration between the Pacific and the Atlantic population could have been along the Estrecho de Magallanes (EM) in northern Tierra del Fuego (Fig. 1). This possibility should be further explored in future works.

Finally, the results should be taken with caution for having been obtained with a single mitochondrial marker. In this sense, the inclusion of nuclear markers would contribute to a greater understanding of the complex demographic history of this beautiful species of rocky shores from South America.

Conclusions

This work reports unequivocal evidence of Pacific–Atlantic geographic isolation of a coastal species, which is probably related to the changes in the sea level and temperature after the glaciation periods that affected the region throughout the Pleistocene. The geographic range of *S. lessoni* was mostly affected by the LGM in the Magellan and Argentinean biogeographic provinces whose coasts had probably less rocky sites for limpet settlement.

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