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Genetic population structure in *Nacella magellanica*: Evidence of rapid range expansion throughout the entire species distribution on the Atlantic coast

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ABSTRACT

Nacella magellanica, the most abundant limpet in the Magellanic biogeographic Province of Argentina and Chile, is characterized by a noticeable variability in shell morphology and color patterns. Ecological as well as evolutionary features make this limpet an interesting species for evolutionary and population genetic studies. Here, arbitrary nuclear molecular markers, inter-simple sequence repeat-PCR, are used to analyze the population genetic structure in 14 localities of *N. magellanica* along 2900 km on the Atlantic coast of the Magellanic Province. We compare the present results, based on nuclear markers, with previous findings of a phylogeographic study that used the mitochondrial COI gene. All the results presented here suggest little genetic structure within *N. magellanica*, with moderate to high genetic connectivity among populations. The short time elapsed since the expansion of this species plus a possible long larval lifespan and the oceanographic and environmental conditions of the Magellanic Province might explain the lack of genetic structuring and the low levels of genetic differentiation in the species throughout its distribution range along the Argentine coast.

1. Introduction

Nacella magellanica belongs to a genus currently distributed in different biogeographic regions of Southeast Pacific, Southwest Atlantic and Southern Ocean (IBM-UNESCO, 1964), being the most abundant limpet in the Magellanic biogeographic Province of Argentina and Chile. It is widely distributed along the Atlantic coast from Río Negro (Argentina) southwards, and the Pacific coast from Valdivia (Chile) southwards, including the Staten and Malvinas Islands (Castellanos and Landoni, 1988; Valdovinos and Rüth, 2005). This limpet inhabits the rocky shores between 0 and 25 m in depth and is characterized by a noticeable variability in shell morphology and color patterns (Fig. 1; de Aranzamendi et al., 2009, 2011). In addition, *N. magellanica* has been a key trophic species in coastal populations of hunter-gatherers of the Patagonian and Fuegian coasts (Orquera, 1999; Zubimendi et al., 2007).

In benthic invertebrate species with low adult mobility such as limpets, pelagic larvae represent their primary dispersal stage (Thorson, 1946). Studies carried out in the sister genera *Patella* and *Cellana* showed that, in laboratory cultures, larvae can settle within 9 to 15 days after fertilization in *Patella vulgata* (Dodd, 1957), or 3 to 4 days after fertilization in *Cellana exarata*, *Cellana sandwicensis* and *Cellana talcosa*; however, *C. exarata* can delay settlement and remain at the pelagic veliger stage for at least 18 days (Bird et al., 2007; Corpuz, 1983). There is no information about the larval type of Magellanic *Nacella* species; there is only information for the Antarctic congener *Nacella concinna* which has planktotrophic larvae that could survive for periods of 1–2 months in the water column (Bowden et al., 2006). Several authors have suggested that egg size (an indicator of the amount of energy that can be used during larval development in the plankton) is a useful predictor of larval type in marine invertebrate species (Bhaud and Duchêne, 1996; Havenhand, 1995; Jaeckle, 1995). According to Morriconi (1999), *N. magellanica* has an abundance of small-sized eggs (150 μm), suggesting the presence of planktotrophic larvae in this species. The presence of the species in Malvinas Islands (Valdovinos and Rüth, 2005) and the asymmetric gene flow detected from Pacific Chilean Patagonia to these islands (González-Wevar et al., 2012) suggest that larval lifespan may be significantly long, as described for other marine molluscs inhabiting the Magellanic region (gastropod *Concholepas concholepas*, DiSalvo, 1988; bivalves *Zygochlamys patagonica* and *Hiatella meridionalis*, Schejter et al., 2010). If larvae of this limpet can stay in the water column for several weeks, being at the mercy of the ocean currents, the potential for dispersal would be much higher than if their larvae remained in the plankton for a shorter period, as in the case of lecithotrophic larvae (Avise, 2004; Kingsford et al., 2002; Pannacciulli et al., 2009; Scheltema, 1986; Thorson, 1950).

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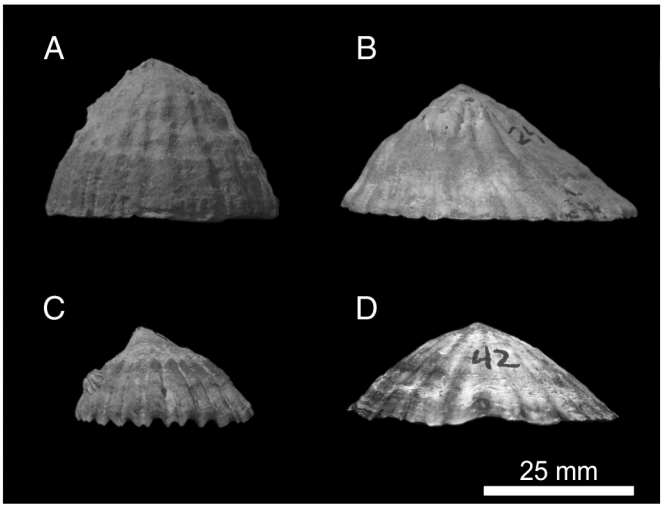

Fig. 1. Examples of shell morphology of *N. magellanica* (lateral view) from Patagonia and Tierra del Fuego (Argentina). A: Typical morphology with a central apex and high shell. B: Shell with a more anterior apex. C: Shell of irregular shape. D: Flattened shell.

N. magellanica can be considered a key player in the response of shallow water benthic communities to the rapid warming after the Last Glacial Maximum (LGM) in the Magellanic Province. The continental shelf area where the species is currently distributed was largely affected by the Quaternary glacial cycles (Rabassa, 2008). Events such as ice sheets calving into the ocean, retraction of the coastline and decrease in marine water temperature during glacial periods (Clapperton, 1993; Rabassa, 2008) affected the habitat of the species and therefore, its population size and geographical structure (de Aranzamendi et al., 2011; González-Wevar et al., 2012). Demographic changes due to climatic cycles usually leave a genetic signature in populations or species (Avise, 2000). Two phylogeographic studies conducted in *N. magellanica* analyzed the mitochondrial cytochrome oxidase I (COI) gene with the aim of inferring the contribution of historical and contemporary processes to the genetic structure of this limpet (de Aranzamendi et al., 2011; González-Wevar et al., 2012). The authors suggested a recent geographic expansion in *N. magellanica* in the Atlantic and Pacific coasts, respectively. This hypothesis is supported in both studies by the absence of genetic structuring, a dominant haplotype along the sampled geographic range, the lack of correlation between geographic and genetic distances, high migration rates between localities and a recent demographic growth. It was argued that after the post-glacial recolonization of *N. magellanica*, larval dispersal following the major ocean current systems could favor the high genetic exchange observed among localities. These two works are the only studies that have investigated intraspecific genetic variability in species of the genus *Nacella* in the Magellanic Province. Both works involved relatively conservative DNA markers with limited ability to resolve genetic differentiation among populations (de Aranzamendi et al., 2011; González-Wevar et al., 2012).

In the present paper, we investigated whether anonymous DNA molecular markers considered highly polymorphic in most species, could detect population subdivisions undetected by the COI gene in *N. magellanica*, along a 2900 km latitudinal gradient on the Atlantic coast. We used inter-simple sequence repeat-PCR (ISSRs), a technique that proved effective in detecting genetic structure at the intra-specific level in different mollusk species (Casu et al., 2006; Hou et al., 2006; Varela et al., 2007). Using these markers, de Aranzamendi et al. (2008) demonstrated that the littoral and sublittoral morphotypes of an Antarctic limpet of the same genus, *N. concinna*, may represent two genetically distinct populations. In addition, at inter-specific level, ISSRs were suitable to discriminate between closely related species of *Nacella*, whereas mitochondrial genes were not (de Aranzamendi et al., 2009).

Based on this background, the aim of the present study was to evaluate the usefulness of ISSRs to depict the level of genetic variability in *N. magellanica* and to analyze the population genetic structure of this limpet throughout its entire geographic range on the Atlantic coast. We compare the present results obtained with ISSRs to published findings obtained using the COI gene (de Aranzamendi et al., 2011). This comparison is reinforced by the fact that the sampling area and most of the samples used are the same in both studies.

2. Materials and methods

2.1. Study area and sampling

The Magellanic Province is affected by cold Subantarctic waters flowing with south–north direction from the Drake Passage and two currents: the Patagonian Current affecting shallow areas of the southern Argentine shelf, and the Malvinas Current flowing mainly in deep areas and slope all along the Argentine shelf; these currents favor the dispersal of the Subantarctic fauna to the Argentine shelf (Fig. 2). The sampling sites show variation in the type and origin of the substrates, as well as in their state of fragmentation (Kühnemann, 1969; Olivier et al., 1966; Ringuelet et al., 1962). Hard solid substrates of volcanic origin are observed in some localities, such as the coastal area of Tierra del Fuego, whereas quartz porphyry formations can be observed in Cabo Blanco and Puerto Deseado (Santa Cruz). In the remaining sites, less indurated sedimentary rocks dominate the coast, as well as abrasion platforms that are at the foot of the typical Patagonian cliffs. The intertidal zone on the shores of the Beagle Channel (Tierra del Fuego) is quite restricted due to the limited tidal range. By contrast, most of the Patagonian coast has an extensive intertidal zone, because of tidal amplitude and the shallow slope of the coast. This feature makes it clearly distinguishable from the Pacific Magellanic Province coast. This Pacific coast presents a very narrow continental shelf and a steep slope, since it reaches high depth within small coastal distance.

A total of 251 individuals of *N. magellanica* were collected from the coastal zone of Río Negro, Chubut, Santa Cruz and Tierra del Fuego in Argentina (Fig. 2; Table 1). Limpet specimens were identified based on morphological and morphometric shell characteristics, following several authors (Castellanos and Landoni, 1988; Forcelli, 2000; Morriconi and Calvo, 1993; Otaegui, 1974; Powell, 1973; Valdovinos and Rüth, 2005). Individuals' samples covered most of the morphological variability present in the species. Samples were labeled and preserved in 80% ethanol until genetic analyses were conducted.

2.2. DNA extraction and ISSR-PCR amplification

Total genomic DNA was obtained from foot muscle tissue following phenol-chloroform extraction (Maniatis et al., 1982). Once extracted, DNA was stored in TE buffer at 4 °C.

Four anchored and one non-anchored ISSR primers that showed a high number of clear, polymorphic bands were selected for the analyses: $(GTG)_3GC$, $(AC)_8C$, $(AG)_8Y$, $(CA)_6RG$ and $(CCA)_5$. ISSR-PCR amplification reactions consisted of 15 ng of template DNA, 0.6 units of Taq polymerase (Fermentas, Brazil), 1 μl of 10× reaction buffer [75 mM Tris–HCl pH 8.8, 20 mM $(NH_4)SO_4$, 0.01% Tween-20], 2 mM $MgCl_2$, 62.5 μM each dNTP (dATP, dCTP, dGTP, dTTP), 0.2 μM of each primer and double-distilled water to a final volume of 25 μl. PCR amplification was performed in an Eppendorf® Mastercycler programmed for 1 cycle of 3 min at 94 °C, 34–37 cycles of denaturation at 93 °C for 1 min, annealing at 47–49 °C for 1 min, and an extension at 72 °C for 1 min 30 s (Table 2). A post-treatment of 5 min at 72 °C and a final cooling at 4 °C were performed. Negative controls were included in each reaction to detect possible contamination. Each individual was amplified at least twice to verify the reproducibility of each selected band. PCR products were run in 1.5% agarose gels using 0.5× TBE buffer. Five microliters of 1 kb ladder (Invitrogen, Carlsbad, USA) were run for

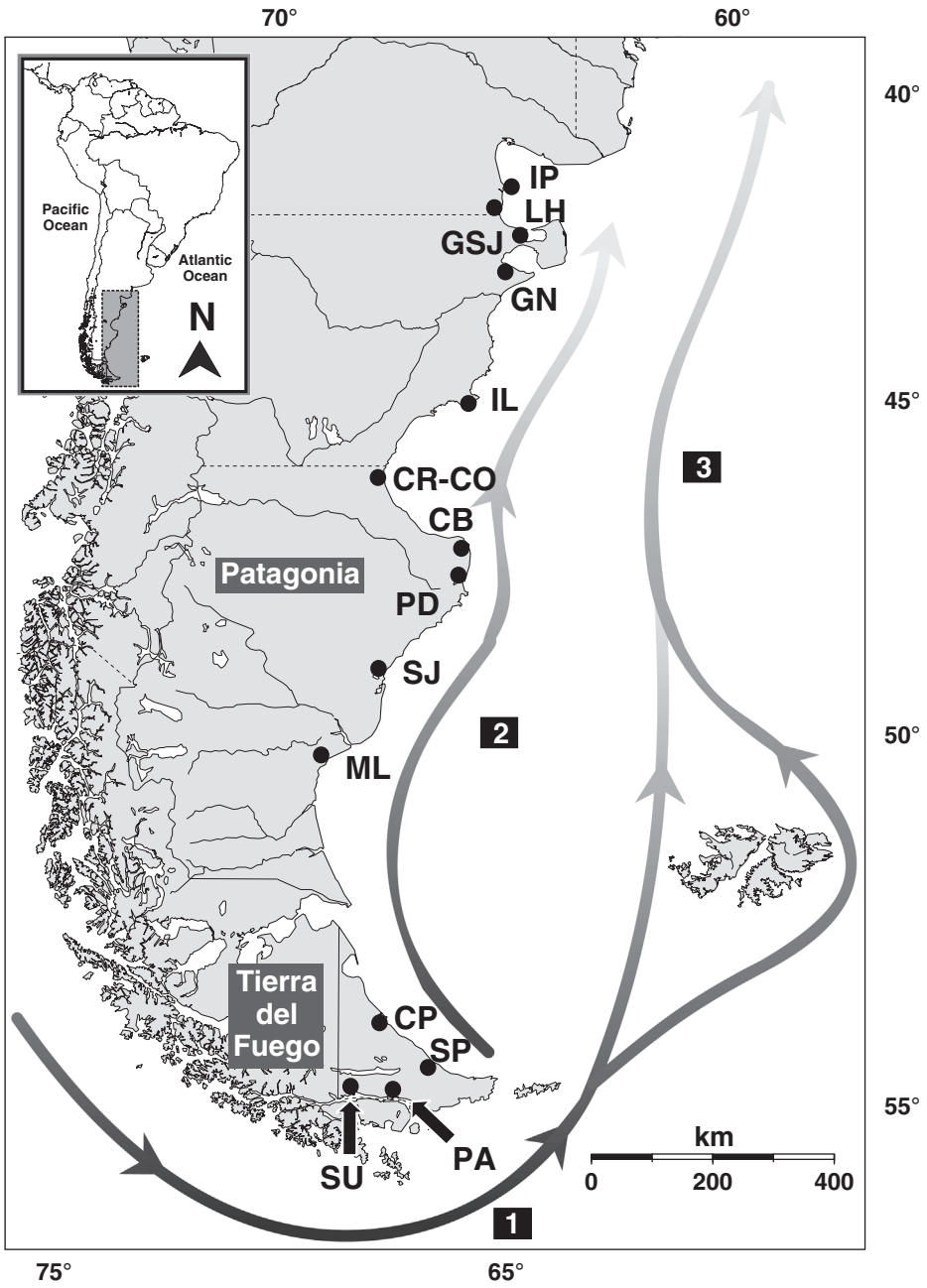

Fig. 2. Sampling sites of *N. magellanica* along the study area in Patagonia and Tierra del Fuego (Argentina). IP: Islote de los Pájaros (41° 26′ 59″ S, 65° 02′ 3″ W); LH: Los Hornillos (41° 51′ 02″ S, 65° 02′ 45″ W); GSJ: Golfo San José (42° 24′ 18″ S, 64° 18′ 34″ W); GN: Golfo Nuevo (42° 49′ 35″ S, 64° 52′ 58″ W); IL: Isla Leones (45° 03′ 18″ S, 65° 36′ 43″ W); CR–CO: Intertidal reef between Comodoro Rivadavia and Caleta Olivia (46° 4′ 02″ S, 67° 36′ 54″ W); CB: Cabo Blanco (47° 12′ 30″ S, 65° 44′ 28″ W); PD: Puerto Deseado (47° 45′ 12″ S, 65° 52′ 04″ W); SJ: San Julián (49° 14′ 50″ S, 67° 40′ 12″ W); ML: Monte León (50° 15′ 52″ S, 68° 40′ 31″ W); CP: Cabo Peñas (53° 50′ 43″ S, 67° 32′ 32″ W); SP: San Pablo (54° 26′ 59″ S, 66° 28′ 52″ W); PA: Puerto Almanza (54° 53′ 13″ S, 67° 40′ 59″ W); and SU: South of Ushuaia (54° 47′ 53″ S, 68° 14′ 5″ W). Arrows represent principal sea surface currents affecting the coasts in the study area: 1: Cape Horn Current, 2: Patagonian Current, and 3: Malvinas Current.

fragment size reference within each gel. Gels were run at 120 V for about 4 h and stained using a 0.005 $\mu g \cdot ml^{-1}$ ethidium bromide solution. ISSR banding patterns were visualized using a UV transilluminator and recorded by digital photography.

2.3. ISSR-PCR analysis

Gels were scored manually. Each variable, clear and high-intensity band of the ISSR-PCR fingerprints was scored as present (1) or absent (0) in a data matrix that was used for calculations. Fragment length was estimated using the program DNAsize 1.0 (Raghava, 1994). For each ISSR primer, the Polymorphic Information Content (PIC) and Marker Index (MI) were obtained. The PIC value was calculated using the formula of Roldan-Ruiz et al. (2000): $PIC_i = 2f_i(1 - f_i)$, where f_i is the relative frequency of the amplified allele (band present) and $(1 - f_i)$ is the relative frequency of the null allele (band absent) of marker *i*. The MI was determined as the product of PIC and the number of polymorphic bands per primer (Powell et al., 1996).

The number of scored bands and the percentage of polymorphic loci per locality were obtained. Genetic diversity of each population was measured with the Shannon index (*I*) calculated using GENEALEX 6.5 (Peakall and Smouse, 2012). Since the data did not have a normal distribution, *I* values were compared among samples using the non-parametric Kruskal–Wallis test. The F_{ST} values were determined with the AFLP-

Table 1
Sampling localities and number of individuals analyzed of *N. magellanica*.

Locality	*N*	*N**	BS	P (%)	*I* (SD)	DW_7
IP	8	8	57	64.47	0.33 (0.27)	5.09
LH	19	19	67	78.95	0.34 (0.23)	4.41
GSJ	14	14	64	78.95	0.35 (0.24)	5.28
GN	8	6	52	55.26	0.29 (0.27)	4.50
IL	19	19	63	81.58	0.39 (0.23)	4.85
CR–CO	18	4	69	84.21	0.4 (0.23)	7.34
CB	24	16	68	86.84	0.39 (0.22)	6.80
PD	25	6	70	85.53	0.39 (0.22)	5.46
SJ	20	18	72	89.47	0.4 (0.23)	4.94
ML	25	15	71	86.84	0.38 (0.22)	4.68
CP	21	19	67	82.89	0.39 (0.23)	5.93
SP	19	19	70	82.89	0.36 (0.21)	5.98
PA	7	7	47	52.63	0.28 (0.28)	5.13
SU	24	11	73	88.16	0.36 (0.21)	5.62
Total	251	181				

N: total number of individual per locality; *N**: number of new individuals added in this study to the group of specimens used in de Aranzamendi et al. (2009); BS: number of scored bands; P: percentage of polymorphic loci; *I*: Shannon index; SD: Standard deviation; and DW_7: Frequency-down-weighted marker value, using seven randomly chosen individuals for calculation. Key to locality labels is given in Fig. 2.

SURV program (Vekemans, 2002) to measure genetic differentiation between locality pairs. Pairwise values were estimated through a bootstrap of 1000 replicates. Allele frequencies were estimated by implementing the method of Zhivotovsky (1999), assuming Hardy–Weinberg equilibrium. A Bonferroni correction for multiple comparisons was applied to F_{ST} values. Gene flow was estimated using Wright's formula (1943), $N_m = [(1 / F_{ST}) - 1] / 4$, where N_m is the number of effective migrants per generation.

A matrix of Nei and Li (1985) genetic distances (bootstrap = 500) between pairs of individuals was calculated from the matrix for the presence/absence of bands with RAPDPLOT software 3.0 (Black, 1995). A neighbor-joining tree (NJ) (Saitou and Nei, 1987) was constructed based on genetic distances using the programs NEIGHBOR and CONSENSE from the PHYLIP package version 3.66 (Felsenstein, 2004), to visualize the genetic relationships among individuals.

To examine the relationships among localities, a Principal Coordinate Analysis (PCo) was constructed using Nei and Li (1985) genetic distance values calculated between pairs of individuals with PAST (Hammer et al., 2001).

An additional measure of divergence among localities, the Frequency-down-weighted marker value (DW), was calculated. This index allows us to distinguish between populations with a long-term history of isolation and more recently founded ones (Schönswetter and Tribsch, 2005). The number of occurrences of each ISSR marker in each population was divided by the number of occurrences of that particular marker in the total dataset. Finally, these values were summed up. The DW value is expected to be high in long-term isolated populations, where rare markers should accumulate due to mutations, and low in newly established populations, therefore helping in the differentiation between old vicariance and recent dispersal. To even out the unequal sample size, the DW was calculated with only seven (including all localities) and 14 (not considering IP, GN and PA localities) randomly chosen individuals per locality. A non-parametric Kruskal–Wallis test was used to compare DW values.

To further infer the genetic structure of our dataset, the program STRUCTURE version 2.3.3 adapted for dominant markers was used (Falush et al., 2007; Pritchard et al., 2000). This program uses a Bayesian model-based clustering algorithm to estimate the likelihood of the model given the data and which individuals are most likely to belong to each cluster (the membership of each individual is estimated as *q*, which varies between 0 and 1 with the latter indicating full membership; Falush et al., 2003). We performed three independent runs for $K = 1$–14 for *N. magellanica* setting the correlated allele frequencies model and assuming admixture. Markov Chain Monte Carlo searches consisted of 50,000 burn-in steps, followed by 200,000 iterations; these lengths were found to be sufficient in preliminary runs, i.e., longer lengths did not change the results significantly. The posterior probability of the model was estimated using the maximal average value of Ln $P(D)$ as an ad hoc guidance.

Existence of isolation by distance was assessed using the Mantel test (Mantel, 1967) between genetic distance and geographic distance matrices using 1000 permutations with PAST software (Hammer et al., 2001). Nei's genetic distances (Nei, 1978) were calculated with TFPGA and the coastline distances between locality pairs were obtained using Google Earth (Google Corporation, 2006). The coefficient of determination (R^2) indicating how well data points fit a linear regression model was calculated.

3. Results

Seventy-six polymorphic bands ranging from 480 to 1318 bp were selected (Table 2). The PIC values ranged from 0.215 to 0.288 (mean = 0.266). The MI values of individual primers ranged from 3.22 to 5.094 (mean = 4.255). The amplification patterns from each primer used showed no clear differences among localities (Supplementary material 1).

The number of scored bands per locality ranged from 47 in PA to 73 in SU (mean = 65, SD = 7.82; Table 1). The percentage of polymorphic loci per locality ranged from 52.63 in PA to 89.47 in SJ (mean = 78.48, SD = 12.06). The lowest values of scored bands and percentage of polymorphic loci per locality were observed in IP, GN and PA, which was consistent with the lower number of individuals analyzed at those sites than at the remaining sites. There were not exclusive bands for any of the 14 sample sites.

The Shannon index was not significantly different among localities (between 0.28 and 0.4; $p = 0.08$) (Table 1). Although most F_{ST} values between locality pairs were significant for $\alpha = 0.05$ (64.4% of the values; Table 3), only seven of those exceeded a value of $F_{ST} = 0.05$. The estimated values of gene flow between localities were greater than one in all cases. The NJ tree (Fig. 3) clearly shows that individuals belonging to the same locality were not grouped together in the same clade. In the PCo based on genetic distances between pairs of individuals, the first three coordinates explained a low percentage of the total variation (PCo 1: 5.2%; PCo 2: 4.7%; PCo 3: 4.5%). There were no groupings of individuals by location or by geographical proximity (Supplementary material 2). The first 17 coordinates account for a similar portion of the total variation and altogether represent a cumulative

Table 2
Primer sequences used in the inter simple sequence repeat (ISSR) analyses, annealing temperature, number of cycles, range of molecular weight in base pairs (bp) and number of polymorphic bands per primer amplified by ISSR-PCR for 251 individuals of *N. magellanica*.

Primer Sequence (5′–3′)	Annealing temperature (°C)	No. of cycles	Size-range of polymorphic bands (bp)	No. of polymorphic bands scored	PIC	MI
$(AC)_8C$	49	37	630–1318	17	0.283	4.811
$(AG)_8Y$	47	37	510–1306	12	0.215	5.094
$(GTG)_3GC$	48	37	480–1294	15	0.269	3.228
$(CA)_6RG$	49	38	662–1066	14	0.274	4.110
$(CCA)_5$	48	35	714–1155	18	0.288	4.032
			Total:	76	**0.266**	**4.255**

PIC: Polymorphic Information Content; MI: Marker Index. The mean values are shown in bold.

Table 3
Genetic distance (F_{ST}) and gene flow values (N_m) between pairs of populations from *N. magellanica*.

	IP	LH	GSJ	GN	IL	CR–CO	CB	PD	SJ	ML	CP	SP	PA	SU
IP	–	0.2797	0.0519	0.2178	0.0480	$p < 0.0001$	0.1349	0.0080	0.0120	0.0140	0.5704	0.0030	0.0120	0.0559
LH	0 (∞)	–	$p < 0.0001$	0.0480	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	0.0040
GSJ	0.012 (20.6)	0.031** (7.8)	–	0.5844	0.1229	0.0020	0.2607	0.1169	0.0589	0.0020	0.0050	0.0250	0.0100	$p < 0.0001$
GN	0.003 (83.1)	0.014* (17.6)	0 (∞)	–	0.0549	0.0090	0.0396	0.0050	$p < 0.0001$	0.1269	0.0170	0.0160	0.0120	0.0140
IL	0.017* (14.5)	0.038** (6.3)	0.006 (41.4)	0.012* (20.6)	–	0.0380	0.0450	0.0060	0.1129	0.0260	0.0280	0.2887	$p < 0.0001$	$p < 0.0001$
CR–CO	0.051** (4.7)	0.033** (7.3)	0.022* (11.1)	0.022* (11.1)	0.007* (35.5)	–	0.0020	$p < 0.0001$	0.0020	0.0090	0.0120	0.0070	$p < 0.0001$	$p < 0.0001$
CB	0.041 (5.8)	0.076** (3)	0 (∞)	0.015* (16.4)	0.008* (31)	0.032* (7.6)	–	0.8531	0.0370	0.0090	0.0090	0.0050	$p < 0.0001$	$p < 0.0001$
PD	0.038* (6.3)	0.064** (3.7)	0.002 (124.7)	0.009* (27.5)	0.014* (17.6)	0.021** (11.7)	0 (∞)	–	0.0240	0.2258	$p < 0.0001$	0.0030	$p < 0.0001$	0.0030
SJ	0.046* (5.2)	0.07** (3.3)	0.008 (31)	0.03** (8.1)	0.006 (41.4)	0.016* (15.4)	0.008* (31)	0.008* (31)	–	0.0060	$p < 0.0001$	0.0040	0.0020	$p < 0.0001$
ML	0.02* (12.2)	0.039** (6.1)	0.025** (9.8)	0.004 (62.2)	0.01* (24.8)	0.012* (20.6)	0.014* (17.6)	0 (∞)	0.017* (14.5)	–	0.0130	0.0110	$p < 0.0001$	0.0050
CP	0 (∞)	0.041** (5.8)	0.019* (12.9)	0.017* (14.5)	0.009 (27.5)	0.012* (20.6)	0.013* (19)	0.018** (13.6)	0.028** (8.7)	0.012* (20.6)	–	0.0210	$p < 0.0001$	$p < 0.0001$
SP	0.025* (9.7)	0.034** (7.1)	0.01* (24.8)	0.02* (12.3)	0 (∞)	0.015* (16.4)	0.018* (13.6)	0.015* (16.4)	0.019* (12.9)	0.012* (20.6)	0.01* (24.8)	–	$p < 0.0001$	$p < 0.0001$
PA	0.048* (4.9)	0.128** (1.7)	0.027* (9)	0.029* (8.4)	0.041** (5.8)	0.066** (3.5)	0.046** (5.2)	0.041** (5.8)	0.042* (5.7)	0.065** (3.6)	0.054** (4.4)	0.048** (4.9)	–	0.0060
SU	0.019 (12.9)	0.049* (4.9)	0.042** (5.7)	0.016* (15.4)	0.029** (8.4)	0.049** (4.9)	0.028** (8.7)	0.013* (19)	0.034** (7.1)	0.015* (16.4)	0.021** (11.6)	0.026** (9.4)	0.024* (10.2)	–

Below the diagonal: F_{ST} values with N_m in parenthesis; above diagonal: *p* values. Locality codes are shown in Fig. 2.
* $p < 0.05$.
** Considering Bonferroni correction ($p < 0.0011$).

variation value of 50.7%. The DW_{14} and DW_7 ranged from 5.59 in IL to 7.85 in CR–CO, and from 4.41 in LH to 7.34 in CR–CO, respectively (DW_{14} mean = 6.87, SD = 0.67; DW_7 mean = 5.43, SD = 0.85). None of them were significantly different among localities ($p_{DW_{14}} = 0.44$; $p_{DW_7} = 0.45$); only DW_7 values are shown in Table 1.

No population structure was detected by Bayesian clustering; the maximal average value of Ln $P(D)$ was obtained with $K = 1$. There was a weak but significant correlation between genetic and geographic distances ($r = 0.31$, $p = 2.7 \times 10^{-3}$); the linear regression model explained 9.64% of the variance between them ($R^2 = 0.096$; Fig. 4). Some localities were removed at random several times and the test was re-calculated. When the two localities situated in the Beagle Channel, PA and SU, were removed, the Mantel test did not show significant correlation ($r = 0.21$, $p = 0.9$).

4. Discussion

The mitochondrial DNA gene sequences, cytochrome oxidase I (COI) and cytochrome *b* (Cyt *b*), have been successfully used in different studies in the related genera *Patella* and *Cellana* to assess intraspecific phylogeographic patterns (Goldstien et al., 2006, 2009; Sá-Pinto et al., 2008). Nevertheless, a phylogeographic study using COI gene in the Antarctic limpet *N. concinna* did not detect genetic structuring in populations along the western Antarctic Peninsula (González-Wevar et al., 2011). Low levels of genetic differentiation were also found in *N. magellanica* using the same mitochondrial marker along its distribution range in Argentina and Chile (de Aranzamendi et al., 2011; González-Wevar et al., 2012). Beyond the results obtained, a number of disadvantages have been reported when working with mitochondrial genes. In recent years, in the field of molecular ecology and phylogeography there has been an increasing awareness that inferences based on this single molecule alone are not enough to answer all questions (Ballard and Whitlock, 2004; Knowles and Richards, 2005). In coding mitochondrial genes, mutation rates are relatively low and may be more affected by natural selection compared to other molecular markers, like arbitrary ones, questioning their relevance as witness of recent species and population history (Ballard and Whitlock, 2004; Galtier et al., 2009). Another problem is the potential bias caused by introgression (Ballard and Whitlock, 2004). If the evolutionary significance of past demographic and biogeographic events (in terms of their contribution to species divergence) is to be determined, it is necessary to consider many loci, including comparisons between nuclear and mitochondrial markers (Ballard and Whitlock, 2004; Knowles and Richards, 2005).

The nuclear molecular markers here employed, ISSRs, were used in many studies to evaluate the population structure of marine invertebrates, including limpets (Casu et al., 2005, 2006; Cossu et al., 2012; de Aranzamendi et al., 2008; Fernández et al., 2011; Hou et al., 2006; Maltagliati et al., 2004; Pannacciulli et al., 2009; Varela et al., 2007). Genetic differences among closely related species of *Nacella* were successfully revealed using ISSRs but not using mitochondrial coding genes COI and Cyt *b* (de Aranzamendi et al., 2009).

The Polymorphism Information Content (PIC) and its related measure, the Marker Index (MI), were used to assess the capacity of ISSR to detect polymorphic loci and their frequency in the data set. For dominant markers, the PIC, a measure based on the relative frequency of bands present and null alleles, has a maximum value of 0.5 (De Riek et al., 2001). In our study, almost all PIC values are above the mean value, evidencing that ISSR can be considered informative for this data set. The lack of PIC values close to 0.5 could be explained by the homogeneous distribution of genetic variation among the 14 geographic populations. In fact, no characteristic band pattern or exclusive bands were observed in any of the localities here analyzed.

The genetic diversity of *N. magellanica* in each locality was relatively high and did not vary considerably among sampling sites (mean P% = 78.48; mean $I = 0.36$). The Shannon's diversity index values are comparable to the ones obtained in two Portuguese localities of the

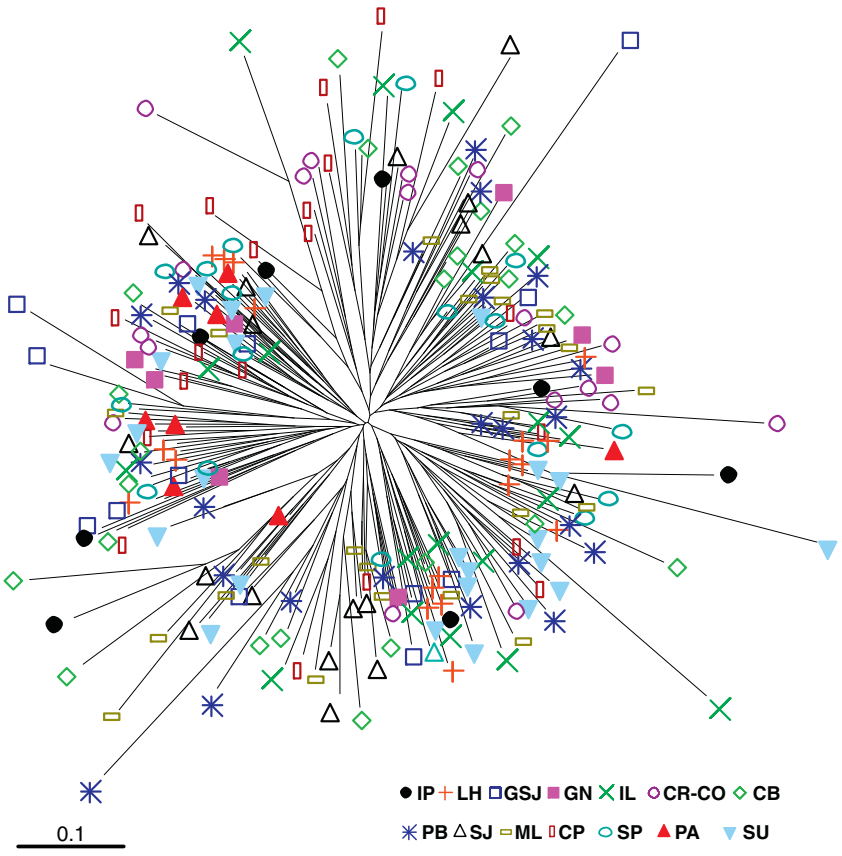

Fig. 3. Neighbor-joining tree based on Nei and Li (1985) genetic distance between pairwise individuals of *N. magellanica*. Symbols represent the individuals according to their localities of origin. Locality codes are indicated in Fig. 2. (Reproduce in color on the Web and in black-and-white in print).

commercial pullet carpet shell *Venerupis senegalensis* (I = 0.339–0.361; Joaquim et al., 2010) using RAPD dominant markers and are much higher than those reported for two barnacle species using ISSRs (I of *Chthamalus stellatus* = 0.147–0.222; I of *Tesseropora atlantica* = 0.111–0.190; Pannacciulli et al., 2009). The authors discussed these genetic variability values in relation to the population size and lifespan of the larval stage, being higher in large populations with extensive larval dispersal than in small populations with short larval lifespan.

Of the pairwise F_{ST} values obtained for *N. magellanica*, 64.4% were significant, suggesting incipient levels of differentiation. However, only seven of those comparisons had a $F_{ST} > 0.05$. According to the criteria established by Hartl and Clark (1997) to classify the degree of genetic differentiation with this statistic, most of these values would indicate low genetic differentiation between locality pairs (pairwise F_{ST} varying between 0 and 0.05); there were no values showing high genetic differentiation between all pair of localities (i.e. with F_{ST} between 0.15 and 1). F_{ST} values obtained in this limpet are similar to or even lower than those reported in *Tesseropora atlantica*, a crustacean with planktotrophic larval development, using the same molecular markers (F_{ST} between 0.014 and 0.041; Pannacciulli et al., 2009). Most gene flow values in *N. magellanica* were higher than three individuals per generation. The only exception was between PA and LH, with an N_m = 1.7. It is well known that an N_m = 1 is enough to prevent differentiation due to genetic drift (Slatkin, 1987). The estimated values of gene flow between *N. magellanica* population pairs therefore suggest moderate to high genetic connectivity. Although the statistical capacity to estimate population differentiation and levels of gene flow from F_{ST} values have been criticized (Hedrick, 2005; Jost, 2008; Whitlock and McCauley, 1999), these values can be informative for comparison among species or populations with similar evolutionary histories. In a recent phylogenetic study using COI as molecular marker, González-Wevar et al. (2012) reported that only 16.4% of population pairs showed low G_{ST} (equivalent to F_{ST}) values, although significantly different from zero, among 12 localities of *N. magellanica* along the Pacific coast of the Magellanic region in Chile. Although different markers were used in both studies, González-Wevar et al. (2012) and the present results suggest a similar scenario in the whole Magellanic region.

The NJ tree and the Principal Coordinate Analysis revealed intermixed individuals, which did not form groups by geographical origin or proximity. Furthermore, the Bayesian assignment analysis showed K = 1 as the most likely number of genetic entities in the sample and the individuals were not significantly assigned to any genetic group.

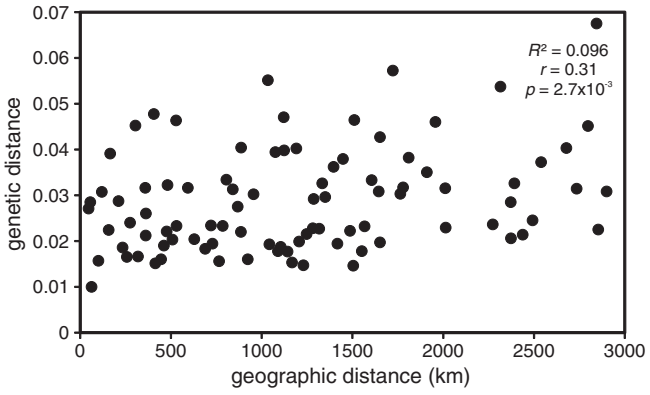

Fig. 4. Plot of the Nei's genetic distance (Nei, 1978) values and corresponding geographical coastline distance from pairwise population comparisons. R^2: coefficient of determination for the linear regression model; r: Pearson's correlation value; p: Pearson's correlation p value.

Since data on genetic vs. geographic distances show a very low coefficient of regression (R^2), isolation by distance model is unlikely. The significant but weak correlation observed could be the result of the high values of genetic and geographic distances between each of the two localities within the Beagle Channel and the rest; when these two localities were excluded from the analysis, the correlation lost significance. The pattern obtained would reflect that gene flow is much more influential than genetic drift, or that colonization from an ancestral population is very recent (Hutchison and Templeton, 1999). The lack of exclusive alleles and the nonsignificant differences among DW values support the idea that the sampled localities have a shared history and/or high levels of gene flow among them.

The high genetic differentiation between PA and SU from all the other localities is remarkable, especially from those also situated in Tierra del Fuego, but on the Atlantic coast. Since the two former sampling sites are located inside the Beagle Channel, which is characterized by many neighboring islands and broad channel systems, restricted gene flow between those two localities and the rest could be favored. This idea is in line with the lack of genetic differentiation between PA and SU and between SP or CP and most of the Patagonian localities.

All the results obtained in the present study indicate a low genetic structuring of *N. magellanica* along the Magellanic Province of the Argentine coast, which could be explained by at least three hypotheses: i) ISSRs might be actually too variable so that random genetic variability due to homoplasy is covering any signal of genetic structuring, ii) recent range expansion and/or iii) high gene flow mediated by marine currents.

Even though a potential problem with ISSR technique is the lack of homology of bands with the same electrophoretic mobility, known as fragment-size homoplasy, the first hypothesis does not appear as the most likely interpretation of our results. The PIC and MI obtained are relatively low, showing moderate levels of polymorphism revealed by the used ISSR markers, which do not support the idea of significant homoplasy masking some genetic structuring. Besides, all our present results are in agreement with those obtained in previous phylogeographic studies in *N. magellanica* using conservative markers (de Aranzamendi et al., 2011; González-Wevar et al., 2012).

Regarding the second hypothesis, since the population expansions reported in this limpet would be very recent in the Chilean (~9000 years ago; González-Wevar et al., 2012) and the Argentine coasts (~11,000 years ago; de Aranzamendi et al., 2011), it could be argued that the genetic divergence is incipient due to the short time elapsed since the expansion of this species, i.e. the genetic drift did not have enough time to clearly differentiate their populations.

On the other hand, it is known that in benthic organisms patterns of genetic structure and levels of gene flow among populations can be determined by the interaction of larval development, the dispersal potential and local recruitment, along with oceanographic features (marine shallow currents, tidal currents, ocean fronts, eddies, etc.) and water physicochemical parameters, such as temperature and salinity (Avise, 2004; Grosberg and Cunningham, 2001; Palumbi, 1994; Wares et al., 2001). Pannacciulli et al. (2009) studied the population structure of two species of cirriped crustaceans that differ in the time their larvae spend in the plankton, using ISSR-PCR as molecular markers. The species that has a pelagic larval development of 22 days showed very low levels of genetic structuring among populations, whereas the species whose larvae stay 24 h in plankton exhibited restricted gene flow levels with high genetic differentiation among localities. These results confirm the importance of pelagic stage duration to determine the level of genetic structure in marine invertebrates. A study using other dominant markers (AFLP) found very low genetic structuring in the Antarctic species *N. concinna* (considering individuals from intertidal and subtidal parts of the same group) along the Antarctic Peninsula, which could be explained by the high dispersal potential of the planktotrophic larvae of this species (Hoffman et al., 2010). Nevertheless, Shanks (2009) argues that not only larval duration in the water column but also larval behavior can play a key role in determining dispersal. Larval behavior can slow dispersal resulting in a dispersal distance up to 10,000 times smaller than that expected for passive particles in any kind of currents. Some types of larvae can remain close to the bottom or vertically migrate in the water column, where deeper currents are usually slower and frequently flow in a different direction than surface currents. The larval biology of *N. magellanica* is unknown, but egg size suggests the presence of larvae with a prolonged lifespan in the plankton (Morriconi, 1999; see Introduction). Furthermore, the effect of the main surface ocean currents that present a south–north direction (Fig. 2) and homogeneous environmental conditions on the Argentine Magellanic Province coast could favor high levels of gene flow during larval stage. Anyway, hypotheses ii) and iii) are not mutually exclusive.

In summary, our present results obtained using nuclear markers confirm previous findings involving the use of COI mitochondrial gene, suggesting a recent range of demographic and geographic expansion to explain the lack of genetic structuring and the incipient levels of genetic differentiation in *N. magellanica* along its entire distribution range on the Argentine coast. Nevertheless, the alternative and not mutually exclusive scenario of high gene flow mediated by marine currents cannot be discarded. Further studies about larval life-history traits of the species are necessary to distinguish the relative contribution of these two processes to the species' genetic structure patterns.

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Author contributions

MCdA designed the study, collected and analyzed the genetic data and wrote the manuscript.

RB designed the study, collected samples in the field and contributed to the interpretation of results and writing.

CNG contributed to data analyses and interpretation of results, and to the writing of the manuscript.

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