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Genetic and morpho-physiological differentiation in a limpet population across an intertidal gradient

Jesús Darío Nuñez*, Pedro Fernández Iriarte, Emiliano Hernán Ocampo, Enrique Madrid and Maximiliano Cledón

Abstract

Marine organism adaptive capacity to different environmental conditions is a research priority to understand what conditions are important in structuring the spatial distribution of natural populations. In this context, this study evaluates whether potential differences in *Siphonaria lessonii* morphology (size and shell shape) and physiology (water loss regulation) at different shore heights are linked to genetically distinguishable individuals. To achieve this goal, we compared size-frequency distribution, morphometric, and genetic data (ISSR-PCR technique) of *S. lessonii* from the upper, middle and lower shore. We complemented these field samplings with laboratory experiments on water loss and mortality. Genetic analysis showed different genetic composition for individuals from the upper and lower shore. This pattern was accompanied by morpho-physiological variations: the upper shore had fewer small limpets, lower shell shape dispersion (with a morphotype characterized by a higher shell), and less water loss and mortality related to air exposure than the lower shore. The results reported herein support the idea that the extreme and unpredictable conditions of the upper shore may impose strong selection pressure on its inhabitants, leading to considerable morpho-physiological differentiation consistent with different genetic composition. This probably plays an important role in structuring the spatial distribution of natural *S. lessonii* populations with a possible effect on size-structure distribution.

Keywords: *Siphonaria lessonii*, Vertical distribution, Desiccation, Shell shape, ISSR

Background

Marine organism adaptive capacity to different environmental conditions is a research priority to understand what conditions are important in structuring the spatial distribution of natural populations [1, 2]. Intertidal species generally are subject to extreme environmental fluctuations that affect their desiccation rates, among other effects. To cope, they have developed a variety of adaptations for regulating body temperature and evaporation [3]. For example, they have developed behaviors that minimize stress [4–6], physiological regulations that modify their metabolic rates [7–10], and morphological changes, such as body size and shape, that reduce water loss [11]. In recent years, ecological researchers have integrated these adaptive changes with genetic studies, enabling the development of hypotheses with an eco-genetic context. These studies have found evidence of changes in the frequencies of some alternative alleles related to local adaptation across environmental gradients, such as in populations distributed along latitudinal clines [12, 13] or intertidal gradients [14–16]. In the latter case, some adaptive characteristics such as changes in body shape and color or physiological traits may result from the strong selection pressure on the inhabitants of the upper shore levels, leading to considerable physiological [17] and genetic differentiation [16, 18–20] even within a single species [14, 15]. In this context, and due to recent advances in the studies of species evolution, the

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specialists emphasize the importance of gene flow and divergent natural selection as key concepts in studies on the evolution of reproductive isolation (and subsequent speciation) [21].

In the southwest Atlantic, one of the most common limpet species is *Siphonaria lessonii* (Pulmonata, Siphonariidae) [22, 23]. Its distribution in South America extends from Peru in the Pacific Ocean to the north of Uruguay in the Atlantic Ocean [24]. The way in which some of the characteristics of this species vary according to the environmental conditions it lives in has been well studied. For example, the shape and size of the shell can change along the shore height depending on environmental humidity [25–29]. Its behavior patterns can also change with the environmental evaporation rates: in the lowest latitudes (such as 38°) of the Argentinean coast, the environmental evaporation is lower than middle latitude (40°), *S. lessonii* does not exhibit site fidelity [6, 30, 31], while in the mean latitudes of the Argentinean and Chilean coasts, it exhibits moderate site fidelity [6, 32]. Thus, we hypothesize that due to the highly stressful environment where *S. lessonii* lives, which is translated into morphological and physiological differences, individuals of the different intertidal heights will have genetic differences.

The aim of our study is to evaluate whether differences in *S. lessonii* morphology (size and shell shape) and physiology (water loss regulation) at the different shore heights are linked to genetically distinguishable individuals.

Methods

Study sites and sampling

This study was carried out in the Waikiki rocky shore, (hereinafter WRS) (38°04′50S, 57°30′08W), Buenos Aires, Argentina. Average WRS tidal amplitude is 0.80 m (maximum 1.69 m) and during low tide, the beach is 10–20 m wide.

Field sampling for this study was performed during April 2010 (mid-fall) at three selected shore heights. The lower shore was defined as the minimum sea level at spring tides, which was dominated by mussel beds. The middle shore was defined as the maximum sea level at neap tides, which was predominantly covered by diverse species of native algae. The upper shore was defined as the maximum sea level at spring tides, dominated by bare rock with mussels and barnacles in cracks and crevices [29]. During sample collection, patchy micro-habitats such as crevices were avoided in order to avoid confounding habitat variation with shore height variation.

Population size structure

To determine field distribution patterns of limpets, we sampled all individuals present in ten (50×50 cm) quadrats randomly placed along the three shore heights. Size-frequency distributions were developed for each shore height after sorting measured limpets into 1 mm size classes. A permutational multivariate analysis of variance (PERMANOVA) [33, 34] was performed to determine differences in population size-structure among shore heights. Prior to PERMANOVA, a test of multivariate homogeneity of variances (PERMDISP) [35] was run to find differences in within-group dispersion based on the Bray–Curtis index applied to fourth-root transformed data. The significance of the factor shore heights in both analyses was tested with 999 permutations. An analysis of similarity percentages (SIMPER) [36] was performed for data recorded for each size class present at each shore height. The SIMPER analysis identified the size ranges that contribute most to the observed differences for shore height by the Bray–Curtis similarities between samples.

Morphometric analyses

To analyze shell morphology among different shore heights, a set of 20 *S. lessonii* adult individuals were randomly collected at each shore height. We used adult specimens (shell length > 6 mm) because smaller limpet shells are easily broken by manipulation.

The shells were photographed with a digital camera (Canon Power Shot A580) from the right side. Digital images were taken against a white illuminated background in order to maximize the contrast of shell outlines. All the images were binarized (i.e. transformed into white for the shell outline and black for the background, in pixels) so the outlines of each continuous contour (interface between the black and the white pixels) were automatically obtained and digitalized using the SHAPE software [37]. The shell shape of these limpets is rather simple with very few homologous points to be used as landmarks. Moreover, the landmarks are difficult to locate, being classified as type 2 landmarks (maximum curvature along the boundary or outline of the specimen) [38]. Shell shape variation among *S. lessonii* individuals from the three shore heights was therefore measured using outline analyses based on the Elliptic Fourier analysis on the outline coordinates [39]. Elliptic Fourier analysis are preferred over classical morphometric analyses in cases landmarks are difficult to determine, and this analysis have been used before in *S. lessonii* [25]. Elliptic Fourier coefficients were mathematically normalized in order to avoid biased outcomes resulting from different sizes, locations, rotations and starting position of shells [39]. The closed curve of each shell was broken down into 15 harmonically related ellipses. These 15 harmonics represent 99.99% of the total Fourier power spectrum [40].

A PERMANOVA test with shore height as fixed factor was used to analyze the main morphometric differences. Prior to PERMANOVA, a PERMDISP test was run to find differences in within-group dispersion based on Bray–Curtis distances applied to fourth-root transformed data. For both cases, the significance of the factor shore height was tested with 999 permutations. A posteriori Tukey pairwise comparisons were subsequently conducted. Non-metric multidimensional scaling (NMDS) was used to show graphically the morphometric dissimilarities among individuals across shore height based on Bray–Curtis dissimilarity.

Water loss regulation capacity and mortality

In order to take a representative sample of all sizes and to have replacements in case they died during transport and/or acclimatization, a total 424 individuals of all the sizes found in each shore height (numbers of individuals: upper = 140, middle = 145, lower = 139) were randomly collected. A total of 405 out of 424 individuals were used in laboratory experimentation (the 19 rest were released). To minimize stress, the collected individuals were transported to the laboratory in an icebox with rocks collected in the natural environment.

Prior to the measurements, the limpets were acclimated in aquaria with a continuous seawater flux for at least 7 days. The water flow simulated a waterfall with a seawater spray similar to what limpets are exposed to in nature. Limpets were periodically feed by daily addition of rocks with biofilm in the systems. Water temperature was the same as the ambient seawater temperature (typically between 10 and 18 °C).

Evaporative water loss (W_L) was measured according to McMahon and Britton [41] and Sokolova and Pörtner [42]. Prior to starts the experiment, limpets were removed from the aquaria and blotted with tissue paper to remove excess water from the shell surface. Shell length was measured to the nearest 1 mm to separate individuals into size groups. Individuals were weighed to the nearest 0.025 mg and placed in groups of 9 individuals (3 small: 4–7 mm, 3 medium: 8–11 mm, and 3 large: 12–15 mm) in 250 cm^3 plastic bowls. These plastic bowls were incubated in a thermostatic chamber (INGELAB, model I-209D) with controlled photoperiod cycle of 8 L: 16 D. The thermostatic chamber was set with the maximum range of seawater temperature registered in the field (around 18 ± 3 °C) for the sample period [43]. The thermostatic chamber generates extra humidity that may result in higher water condensation in the plastic bowls, so silica gel was added to the experimental chambers to prevent this water condensation. After exposure periods of 12, 24 or 48 h, five plastic bowls from each shore height were removed from the chambers, and the limpets were weighed (a total 45 plastic bowls). Then they were placed in seawater, left to recover for 6 h and scored for mortality. The number of individuals used in the final analysis of evaporative water loss varied depending on the number of dead limpets. In order to estimate survival during the experiment, we identified and recorded the number of dead limpets. To estimate all the parameters of water loss in the equation, live limpets used in laboratory experiment were sacrificed (by freezing) and placed in pre-weighed aluminum pans, dried for 12 h at 75 °C, and then weighed.

Water loss (W_L) was determined sensu Sokolova and Pörtner [42] as a percentage of the total (corporeal + extracorporeal) body water:

$$W_L = \left(W_{en} - W_{exp}/W_{en} - W_{dry}\right) * 100$$

where W_L is water loss (%), W_{en}, W_{exp} and W_{dry} are initial weight, weight after a given exposure time, and final dry weight of a limpet (mg), respectively.

Data normality (Shapiro–Wilk test) and homogeneity of variances (Cochran's test) were tested and when necessary, data were transformed to meet statistical assumptions [44]. The slopes and elevations of the regressions of water loss W_L (dependent variable) were tested with analysis of covariance (ANCOVA) to assess the effect of shore height and time of exposure on the W_L using limpet shell length as the covariate. Two-way ANCOVA analyses can be used to compare elevations of regression lines if their slopes are not statistically different [43]. When slopes were different, we used Tukey multiple comparison tests [44] to determine which combinations of slopes differ. In these cases, we applied the Johnson–Neyman test [45] to identify the range of the covariate (i.e. shell length) where the elevations are not significantly different.

A generalized linear model (GLM) was used to evaluate whether the proportion of dead limpets (dependent variable) could be explained by shore height, time of exposure and their interaction using limpet shell length as the covariate (explanatory variables). The model was fitted using binomial distribution with logit link function [46]. When slopes were heterogeneous, interaction means comparison tests were used to determine which combinations of slopes differ for GLM [47].

Genetic ISSR analyses

To analyze the genetic diversity among shore heights, an extra set of 20 individuals of *S. lessonii* adult specimens (shell length > 6 mm) were randomly collected at each shore height. We used adults because small limpet shells are easily broken by manipulation and could be a contamination factor for genetic analysis. Genetic diversity was estimated using the ISSR-PCR technique. Inter simple sequence repeats (ISSR) provided a new

dominant genetic marker that amplifies nuclear non-coding DNA using arbitrary primers. Primers amplify DNA fragments between inverse-oriented microsatellite loci, with oligonucleotides anchored in the microsatellites themselves.

DNA was extracted from small pieces of mantle tissue using the Chelex 100 (Biorad) method sensu Walsh et al. [48]. The primers $(AG)_8Y$ and $(CT)_8GT$ were used due to the high polymorphic results yielded in previous tests. The amplification reaction was performed with 20 µl final volume including 2 µl of 10X buffer with $MgCl_2$ (1.5 mM), 1 µl of dNTPs (2.5 mM), 4 µl of each primer (2 mM), 1 µl of template and 0.08 units/ml AmplitaqTM (Sigma), completing the remaining volume with water. The PCR reaction included an initial denaturation cycle at 94 °C (2 min); followed by 5 cycles at 94 °C (30 s), 50 °C (45 s) and 72 °C (1 min) and then another 35 cycles at 94 °C (30 s), 40 °C (45 s) and 72 °C (1 min) and a final extension at 72 °C (2 min). PCR products were run in 1.5% agarose gels, stained by Ethidium Bromide, with a molecular weight marker (1 kb). ISSRs were visualized using a UV transilluminator and analyzed by digital photography. ISSR bands with high intensity were recorded as 1, while the absence of the band was recorded as 0.

To evaluate the importance of "between-groups" (among shore heights) differentiation relative to "within-group" (for each shore height) variation of the morphometric data, a multivariate analysis of variance (MANOVA) together with Principal Coordinates Analysis of distance matrix were performed with PAST [49]. A Wilk's Lambda test was carried out to detect if there were significant morphological differences between groups; and post hoc Hotelling pairwise comparisons (Bonferroni corrected and uncorrected) were conducted to detect significant differences using PAST.

Results

Size-structure

The analysis of the multivariate homogeneity of variances (PERMDISP) did not detect significant differences among shore heights ($F_{2,12}=1.40$, P-value = 0.283). The size-structureof *S. lessonii* was different among shore heights (PERMANOVA: $F_{2,12}=9.45$, P-value < 0.05) and subsequent pairwise test detected differences among all shore heights (P-value < 0.01). The SIMPER analysis showed that small shells (6–9 mm) largely contributed to the differences in size structure of the three heights (around 40% of contribution, see Table 1). There were fewer smaller individuals at the upper than at the lower and middle (Table 1). At the lower and middle heights, large shells (12–15 mm) also largely contributed to size structure differentiation (40% of contribution, see Table 1). There were fewer larger individuals in the middle than in the lower shore (Table 1).

Morphometric analyses

Analysis of the multivariate homogeneity of variances among *S. lessonii* shell shapes at different shore heights was significant ($F_{2,52}=8.61$, P-value < 0.01) (Fig. 1a). Subsequent pairwise test showed that the upper shore was different from the middle and lower shores (P-value < 0.01). In general terms, the variation was explained mainly by shell height (Fig. 1b), with the upper shore showing less variable shell shape than the middle and lower shores.

Table 1 Average dissimilarity of size-structure of *Siphonaria lessonii* at different shore heights based on the similarity percentage (SIMPER) analysis for lower (L), middle (M) and upper (U) shore samples

Shore height	Average abundance			Contribution (%)		
	Upper (U)	Lower (L)	Middle (M)	U vs M	U vs L	M vs L
Range of size (mm)						
6–7	0	1.11	2.28	16.40	12.59	14.49
7–8	0.63	2.30	3.70	22.27	19.66	17.59
8–9	1.59	2.81	3.58	14.12	14.06	9.48
9–10	1.1	3.21	1.5	4.07	9.01	5.48
10–11	0.71	3.36	2.51	12.05	9.56	3.72
11–12	2.51	3.32	3.46	6.74	10.59	9.14
12–13	2.54	3.37	2.32	5.30	10.02	13.43
13–14	2.66	2.35	1.00	11.93	7.73	16.53
14–15	1.88	1.31	1.08	7.12	6.77	10.24

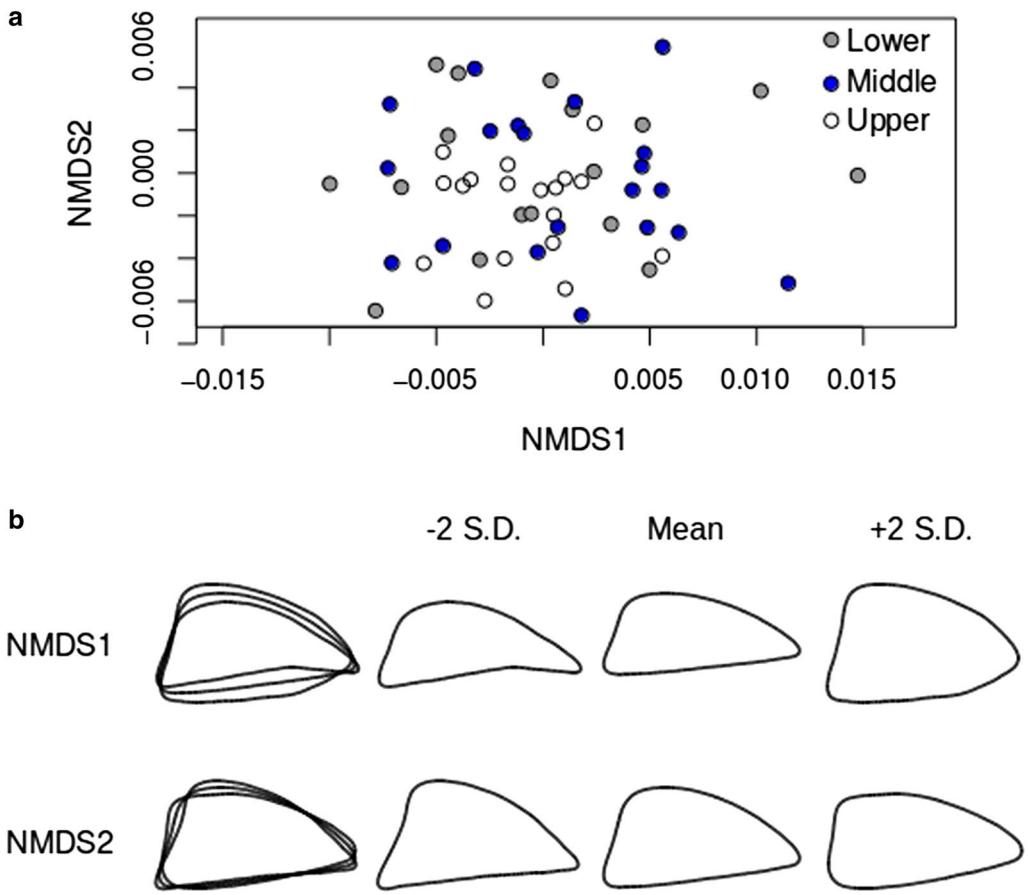

Fig. 1 **a** Nonmetric multidimensional scaling (NMDS) ordination plot of *Siphonaria lessonii* shell shape variation in two-dimensional space. Different colors indicate different shore heights. **b** Mean shape, +2 and −2 standard deviation (S.D.) along each principal component

Water loss regulation capacity and mortality

The overall results show that the individual shell size has a positive association with the loss of water (see Fig. 2). The two-way ANCOVA showed that size had a significant effect on W_L. Larger individuals from all three shore heights lost water faster under desiccating conditions in air (Table 2). We found that the changes in water loss related to shore height was dependent on the time of exposure (Table 2). Multiple comparison of 12 h showed that the slopes and elevations among shore heights did not differ. At 24 h, comparisons showed that slopes of all combinations did not differ while the elevation for lower shore was higher than for middle and upper shores. At 48 h, comparisons showed that the slope of middle shore was lower than lower and upper shores. The Johnson–Neyman test detected no difference in the elevation of the lines depicting the relationship between W_L and shell length only in limpets of 1.5–2.1 (corresponding to 4.5–8.2 mm for non-transformed data) between lower and middle shores, and only in limpets 2.3–2.75 (corresponding to 10–16 mm for non-transformed data) between lower and upper shores (Fig. 2).

The overall results show that individual shell size has a logistic negative association with the rate of mortality (see Fig. 3). The GLM showed that smaller individuals were significantly more likely to die than larger individuals (Table 3, Fig. 3). We found that the changes in the proportion of dead of *S. lessonii* individuals at different shore heights were dependent on the time of exposure. Multiple comparison of 12 h showed that the slopes and elevations among shore heights did not differ (Table 3). At 24 h, comparisons showed that the slope of lower shore differed from the slopes of middle and upper shores, the main difference was that lower shore decreased with size more slowly than middle and upper shores (Fig. 3). At 48 h, comparisons showed that the slopes of all combinations differed significantly, the main difference was that the lower shore decreased with size more slowly than middle shore, and middle shore more slowly than upper shore (Fig. 3).

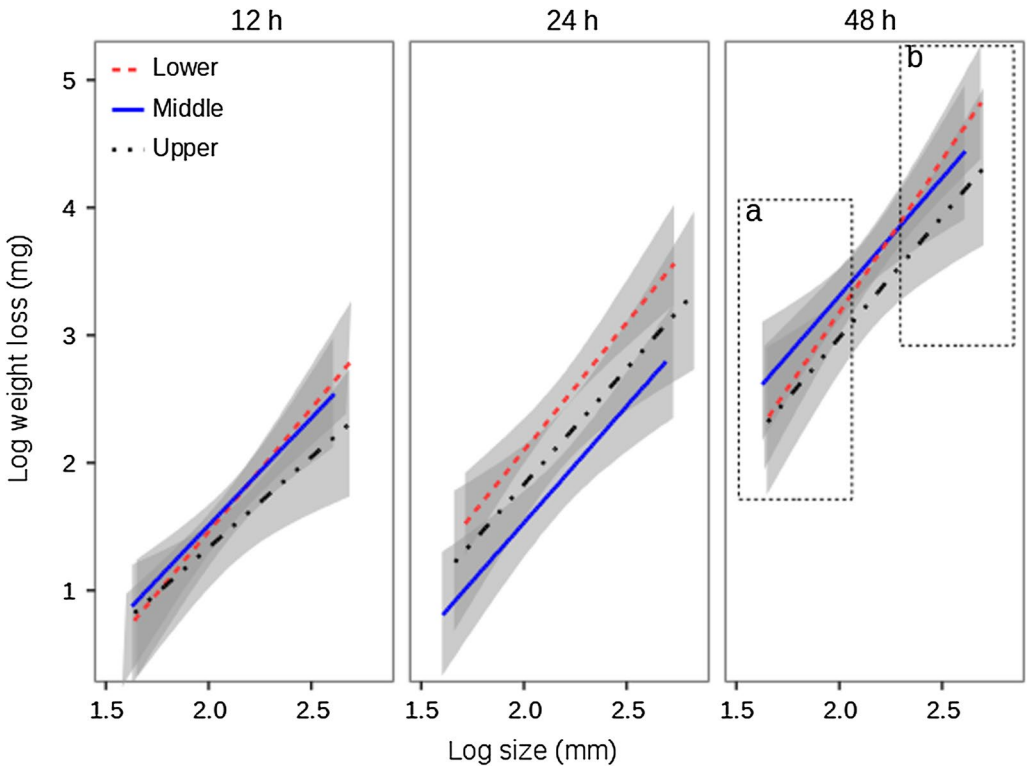

Fig. 2 Plot showing linear increase in water loss (W_L) of *Siphonaria lessonii* related to shore heights at different periods of exposure (12 h, 24 h and 48 h). The line corresponds to adjusted linear function. Different line types indicate different shore heights. For comparisons where slopes differ, the Johnson–Neyman test provides the size range in which there was a significant differential response (highlighted in dashed rectangles) over which the compared regression lines did not differ significantly in elevation. a Between lower vs middle and b between lower vs upper

Table 2 Summary of the two-way ANCOVA analysis for water loss (W_L) in *Siphonaria lessonii* for each period of exposure (time) and factor (shore height) with limpet shell length as the covariate

Two way Ancova	df	*F*
Slope (shore:size)	2	3.029*
Slope (time:size)	2	11.260***
Slope (time:shore)	4	4.034*
Slope (time:size:shore)	4	0.917 n.s.
Elevation (shore)	2	27.624***
Elevation (time)	2	80.091***

The dependent variable and covariate are expressed as natural logarithms.
*p < 0.05; ***p < 0.001; n.s., not significant

Genetic ISSR analyses

ISSRs $(AG)_8Y$ and $(CT)_8GT$ showed 9 and 10 loci (different bands in the agarose gel), respectively. Total genetic diversity (H) was 0.4082 (sd = 0.027) while the diversity within groups (I) was 0.3758 (sd = 0.0072). The comparison among shore heights showed that the effective allele number, Shannon index and genetic diversity were higher for lower shore than for upper and middle shores (Table 4). Global genetic difference (G_{ST}) was 0.0795. The AMOVA indicated that 7% (P-value < 0.05) of the genetic variability among shore heights was explained by the population structure. The genetic differentiation PhiPT between shore heights sampled pairs was mainly ascribed to the difference between lower–middle (0.099, P-value < 0.05) and lower–upper shore (0.059, P-value < 0.05) and, to a lesser extent, between middle-upper shore (0.036, P-value < 0.05). Figure 4 shows the Principal Coordinates Analysis (PCoA) of the genetic distances among shore heights. This figure illustrates that individuals from the lower shore form a group which is slightly separated from the middle and upper shores.

Discussion

Genetic analysis using the ISSR-PCR technique showed different genetic composition for individuals from the upper and lower shores. This pattern was consistent with the fact that all the morpho-physiological variables studied also differed between the two shore heights: the upper shore had fewer small limpets, lower shell shape dispersion (with a morphotype characterized by a higher shell),

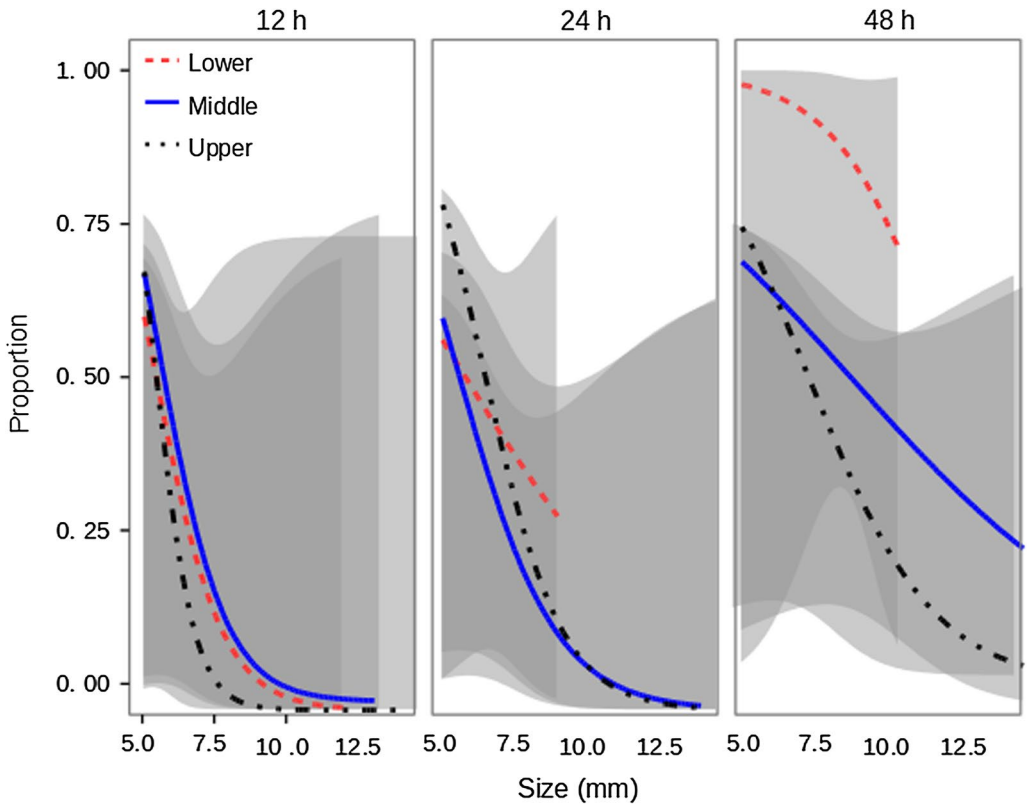

Fig. 3 Plot showing Logistic relation of proportion of mortality of *Siphonaria lessonii* related to shore heights at different periods of exposure (12 h, 24 h and 48 h). The line corresponds to adjusted logistic function. Different lines types indicate different shore heights

Table 3 Summary of the analysis of deviance for the generalized linear model of *Siphonaria lessonii*, fitting the proportion of mortalityin each period of exposure (time) and factor (shore height) with limpet shell length as the covariate

Analysis of deviance	df	Deviance
Slope (shore:size)	2	5.983*
Slope (time:size)	2	10.435***
Slope (shore:time)	4	16.978***
Slope (shore:time:size)	4	0.514 n.s.
Elevation (shore)	2	45.001***
Elevation (time)	2	86.638***

GLM was fit under binomial distribution and logit link function. *$p < 0.05$; ***$p < 0.001$; n.s., not significant

and less water loss and mortality related to air exposure time than the lower shore. The genetic and physiological variables studied for the middle shore showed differences with respect to the lower shore but not to the upper shore. The middle shore had fewer medium-sized limpets in the size frequency distribution, and in general terms, less water loss and mortality related to air exposure time than the lower shore.

In opposition to what has been reported to date for *Siphonaria* species [50], we found genetic differentiation among individuals sampled from the different shore heights. However, genetic substructuring has been observed for mollusks with different morphotypes inhabiting different shore heights [51, 52]. For example, the marine gastropod *Littorina fabalis* has a small and a large morph in microhabitats with different wave exposure, but their distribution overlaps where wave exposure is intermediate. These morphotypes differed genetically [51]. Similarly, *Littorina picta* (currently *Echinolittorina hawaiensis*) and *Littorina saxatilis* show different shell morphs, representing ecotypes adapted to distinct ecological conditions such as different wave exposure and presence of predators, also with substantial genetic differentiation linked to morphs [52]. The limpet *Nacella concinna* has two morphotypes in Argentina, one inhabiting the intertidal (during summer) and the other inhabiting the subtidal (during fall and winter). The genetic differences detected using ISSRs indicate that the two forms can be considered as genetically distinct

Table 4 Effective number of alleles Ne, Shannon (I) and diversity (H) indexesof *Siphonaria lessonii* for lower (L), middle (M) and upper (U) shore samples

Shore heights	Ne	I	H
Lower	1.747 (0.065)	0.592 (0.032)	0.409 (0.027)
Middle	1.555 (0.073)	0.501 (0.038)	0.330 (0.032)
Upper	1.675 (0.058)	0.570 (0.029)	0.388 (0.025)
Total	1.659 (0.039)	0.554 (0.02)	0.376 (0.017)

Standard deviations are shown between parentheses

populations maintaining low levels of gene flow [15]. The general conclusion of these studies is that there may be independent parallel evolution of ecotypes adapted to the different habitats studied [14, 52]. Natural selection may act mainly by direct action on the additive genetic component of quantitative variation and the mechanism of phenotypic plasticity [53–55]. There are evidences that widespread ectothermic species reflect the intraspecific (within-population) genetic adaptation to local conditions [56, 57]. Our results showed less genetic diversity and dispersion of *S. lessonii* shell shape (with higher shells) and size in the upper shore than the lower shore.

There is evidence that the size, shape, and architectural and texture features of the shell surface of patelliform mollusks are related to causative environmental factors such as resistance to desiccation, thermal stress and wave impact [58–60]. The evaporative rate has been recognized as a common mechanism of thermoregulation in gastropods [17]. In this context, there are two mutually exclusive ways that could explain the difference in water loss along shore height and shell shape. If the water loss is the product of "thermoregulation response", the individuals in the higher shore should have higher evaporation rates (due to their need for greater regulation), and this should be independent of size and shape of the shell. However, if evaporation is the "desiccation exposure–response" (is simply a loss of water), which cannot be avoided when the individual is emerged, we would expect the individuals from the higher shore to be more capable of retaining the water, and this could be related to particular morphotypes (variation in size and shape of the shell). According to our results, the second hypothesis may be the most plausible, because we found that the individuals inhabiting higher shore are more capable of retaining water and also show less variation in shell shape (distinguishable morphotypes from the other shore heights). This is probably because larger shell size and higher shell shape (our results for the upper shore) help to reduce water loss by providing a more circular aperture and a higher spire [11, 60]. In this line, the size distribution structure also showed a pattern related to high shore, in which the upper shore had fewer small limpets and middle shore had fewer medium-sized limpets than the lower shore. Thus, and in agreement with Nuñez et al. [6] and Tablado and López Gappa [27], who studied nearby populations, our results suggest that the distribution along the shore heights is probably related to the individual response to environmental humidity and

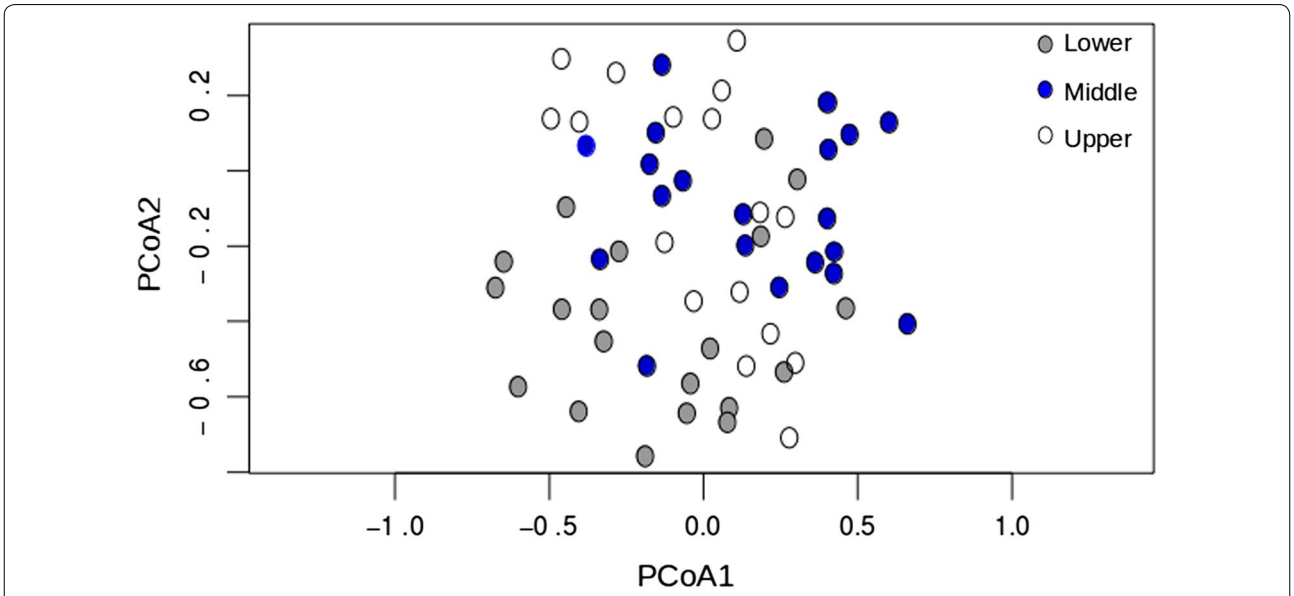

Fig. 4 Principal coordinates of the ISSR genetic distance of *Siphonaria lessonii* among shore heights. Different colored dots indicate different shore heights

is translated into changes in shell shape and size (mirrored by the size structure distribution along shore height) together with genetic differentiation. Although the genetic evidence shows the opposite, we cannot rule out the possibility that the lack of small limpets in the upper shore could be a result of this species' capacity to migrate along the shore (since individuals can travel up to 2 m along the shore [27]). Thus, further experiments are needed to unravel the role of the different environmental factors involved and understand more precisely which variables affect the variability in *S. lessonii* size distributions along of shore heigth.

The results reported herein support the idea that the extreme and unpredictable conditions of the upper shore height may impose strong selection pressure on its inhabitants, leading to considerable morpho-physiological differentiation consistent with different genetic composition. This probably plays an important role in structuring the spatial distribution of natural *S. lessonii* populations with a possible effect on size-structure distribution.

Abbreviations

U: upper shore; M: middle shore; L: lower shore; MDQ: Mar del Plata; S: sur; W: west; W_L: water loss; W_{in}: initial weight; W_{exp}: weight after a given exposure time; W_{dry}: final dry weight; GLM: generalized linear model; EFA: elliptic Fourier analysis; mg: miligram; m: meter; h: hour; NMDS: nonmetric multidimensional scaling; ANCOVA: analysis of covariance; PERMANOVA: permutational multivariate analysis of variance; MANOVA: multivariate analysis of variance; SD: standard deviation; PCA: principal component; ISSR: inter simple sequence repeats; PcoA: Principal coordinates; Ne: effective allele's number; I: diversity; H: genetic diversity; df: degrees of freedom; F: F value; P: p value; PhiPT: PhiPT value.

Authors' contributions

This study was part of the Ph. D of the first author, who collected the samples, conducted the analyses, interpreted the data and wrote the manuscript. PFI and EM assisted with the genetical sampled analyzes, figures and the writing of the manuscript. EO and MC with the figures and the writing of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Not applicable. All the data available is presented in tables and figures.

Consent for publication

The authors declare that they agree to publish in this journal.

Ethics approval and consent to participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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