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Shell-shape variation along the latitudinal range of the Chilean blue mussel *Mytilus chilensis* (Hupe 1854)

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Abstract

Shell-shape variation in the Chilean blue mussel *Mytilus chilensis* was examined in eight populations covering the totality of its distribution range, which represents over 1800 km of latitudinal gradient in the southeastern Pacific. The shell outline shapes were assessed using elliptic Fourier analysis followed by canonical variates analysis. Fourier coefficients showed a highly significant morphological variation between the populations studied. Canonical variates analysis showed a visual association of the First canonical with convexity of ventral edge and umbo shape, while the second axis was associated with shell elongation. Previously described genetic patterns were marginally congruent with our morphometric pattern, though geographic distance had a stronger effect on morphology. Shape change (elongation) was correlated with latitudinal clinal variation. The morphometrical analysis proved to be an important tool for evaluating the components of shell-shape variation in mussels and to document association patterns with geographical and ecological variables. Such patterns are useful to establish a wide field of work, including sexing, stock recognition, traceability studies and productive management in general.

Keywords: inter-group discrimination, image analysis, outlines, elliptic Fourier analysis, canonical variates analysis, biogeography, *Mytilus chilensis*, blue mussels

Introduction

The Chilean blue mussel *Mytilus chilensis* (Hupe 1854) is widely distributed on hard substrates from the lower intertidal zone to depths of 25 m along the Chilean coast (Brattström & Johanssen 1983). Its range on the SE Pacific covers over 40° of latitude, from Arica (18°S) to Cape Horn (56°S) (Lancellotti & Vásquez 2000), but actual natural banks are highly reduced north from Concepción (35°S) (Toro, Castro, Ojeda & Vergara 2006).

Genetic analyses using rapid amplification of polymorphic DNA (RAPD) and allozymes of *M. chilensis* have shown a low genetic differentiation between populations over the 1800 km of its range (35°S–53°S) (Toro, Ojeda & Vergara 2004; Toro *et al.* 2006). The differentiation pattern was correlated with geographical distance, suggesting a stepping-stone model of the population structure. This model was expected due to the large dispersal ability of long-lived planktotrophic larvae and the pattern of ocean currents along the Chilean coast (Toro *et al.* 2004, 2006). In addition, analysis of shell morphology using lineal morphometric variables on three populations within the southern Chilean range showed that the southern population (Punta Arenas) was the most divergent (Toro *et al.* 2004).

In the present study we examined shell morphology variation to see if it followed the same pattern of genetic markers along the 1800-km gradient. To date, most morphological analyses of *Mytilus sp.* shells have been based on shell linear morphometric

characters or their ratios (Seed 1968, 1992; Beaumont, Seed & García-Martínez 1989; McDonald, Seed & Koehn 1991; Gosling 1992; Karakousis & Skibinski 1992; Karakousis, Spandou, Sophronidis & Triantaphyllidis 1993; Mallet & Carver 1995; Gardner 1996). However, direct methods of shape analysis using elliptic Fourier algorithms on shell outlines, in addition to other geometric morphometrics methods, have been developed and successfully applied in molluscs, such as *Mytilus edulis* (Ferson, Rohlf & Koehn 1985; Innes & Bates 1999), *Mytilus trossulus* (Innes & Bates 1999), *Brachidontes purpuratus* (Aguirre, Perez & Sirch 2006), *Chamelea gallina* (Palmer, Pons & Linde 2004) and other marine species (Cadrin 2000; Quillevere, Debat & Auffray 2002). We used an elliptic Fourier analysis (EFA), a common geometric morphometrics (GM) approach, for four reasons. First, conventional analyses (linear morphometrics) are poor shape descriptors because linear measures are not size independent. Second, the GM methods are powerful techniques and provide crucial information about the direction of shape deformation. For round-typed objects such as mussels, outline methods are more useful than landmark-based GM approaches. Third, these methods have performed well or show high performance for detecting subtle shape changes even at the intra-specific level (Innes & Bates 1999; Palmer *et al.* 2004). Finally, a major advantage of GM methods is that they split the shell form into two components, shape and size, thus avoiding allometry confounding and allowing the direct visualization of shape differences within the body. (Ferson *et al.* 1985; Rohlf & Marcus 1993; Marcus, Corti, Loy, Naylor & Slice 1996; Lestrel 1997; Innes & Bates 1999; Cadrin 2000; Bertin, David, Cézilly & Alibert 2002; Palmer *et al.* 2004; Zelditch, Swiderski, Sheets & Fink 2004). In *M. edulis*, an association between genetic variation and salinity gradient has also been demonstrated (e.g. Koehn, Newell & Immermann 1980; Riginos & Cunningham 2005). In the Chilean coast, salinity increases between 35 °S and 45 °S decreases in southern latitudes, although temperature decreases with latitude (Dávila, Figueroa & Müller 2002).

The aim of this study was to search for shell-shape patterns in *M. chilensis* and its relationships with latitude and genetic markers. This opens the gate for more detailed work, both ecological and experimental, on shape-determinant factors and differentiation methods for shell-shape populations, the latter having potential applications in aquaculture and stock-managing policies.

Material and methods

About 120 mussels per population and a total of 959 individuals were collected subtidally (approximately 2.5 m below the low tide) by dredging or by scuba diving at eight localities extending over 1800 km along the Chilean coast (covering the whole natural distribution range of this species) from Arauco (37°14′S; 73°19′W) to Punta Arenas (53°08′S; 70°55′W) (Fig. 1). Mussels with shell lengths from 45 to 82 mm were collected and immediately dissected. After removing soft tissues, the right valve of each mussel was placed (convex side upwards) over an image scanner (CanoScan D646U, Canon USA, Inc., Lake Success, NY, USA). Microsoft PHOTO EDITOR 3.0.2.3 was used for image treatment (background deleting). Outlines were digitalized clockwise, starting at the umbo and with 100 *xy* coordinates using TPSDIG (Rohlf 2001).

Shell area (cm^2) was estimated using TPSDIG (Rohlf 2001) from the outline and was considered as a proxy for shell size. For statistical analysis of the effect of localities on area, we used a one-way analysis of variance (ANOVA) using PROC GLM SS3 for unbalanced raw data. Further comparisons were carried out using the Student–Newman–Keuls test and the Tukey test adjusted for multiple comparisons (SAS Institute, Cary, NC, USA). When criteria of homoscedasticity and/or normality were not met, data were rank-transformed (Conover & Iman 1981). The Spearman rank correlation test was applied between latitude and various shell parameters to test for latitudinal trends in size and shape.

We applied EFA using the NTSYS-PC software (Numerical Taxonomy System, Version 2.2 for Windows XP, Rohlf 2005). Elliptic Fourier analysis is the most common outline method (Ferson *et al.* 1985; Lestrel 1997; Baylac & Frieβ 2005; McLellan & Endler 1998). This fits a Fourier series, which consists of a combination of sine and cosine functions, to the points defining the outline contour. Then it scales and aligns outline coordinates to estimate the Fourier coefficients by the progressive addition of harmonics. We found that ten harmonics were appropriate to describe mussel shell-shape, in accordance with other studies (Ferson *et al.* 1985; Palmer *et al.* 2004). The Fourier space was composed of 40 morphometric variables and was analysed using a one-way multivariate analysis of variance (MANOVA) to test for shape differences among localities (SAS Institute).

Between-group shell-shape differences were described using a canonical variants analysis (Zelditch *et al.* 2004). This allowed us to place the average shape

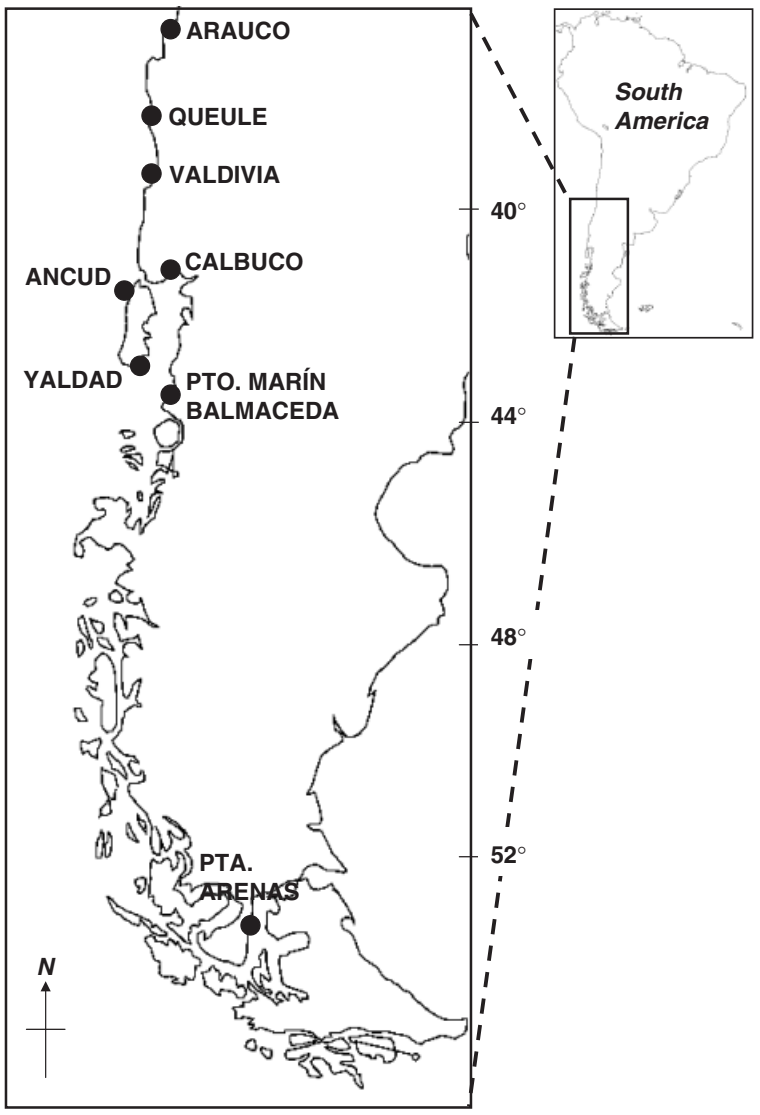

Figure 1 Location of the eight natural populations of the Chilean blue mussel (*Mytilus chilensis*) sampled along the Southern Chilean coast. Coastal mean salinities at 10 m depth from north to south location were 33.70, 33.48, 33.44, 33.30, 33.29, 33.22, 33.20 and 33.29 respectively (data from Dávila *et al.* 2002).

of each group in a canonical space. Each axis in this canonical space corresponded to a shape gradient between extreme configurations. The average shape for each locality was reconstructed from the mean values of Fourier coefficients using the inverse Fourier transformation (provided by NTSYS-PC). Shell area was correlated with the first canonical axes as a proxy for allometry. A high correlation implies that shape is affected by size, which restricts our conclusions and makes allometric studies necessary.

The morphometric generalized distance between populations was computed as the ordinary Cartesian distance from the matrix of canonical scores obtained from NTSYS-pc. For the genetic data, we preferred to use the information obtained from neutral markers such as DNA RAPD, because they allow analysis of a large number of loci, and thus provide a larger sample of the genome than other genetic markers such as allozymes. We used Nei genetic distances as estimated previously (Toro *et al.* 2004), based on RAPD developed from five primers and 54 loci. Geographical distances (km) among the sampled localities were estimated using official maps and tracing the shortest distance between pairs of locations along the coastal line.

To evaluate the relationships between morphological, genetic and geographical distances, we carried out simple and partial Mantel tests using ZT software and 100 000 permutations (Bonnet & Van de Peer 2002). Because the variables might be correlated, we

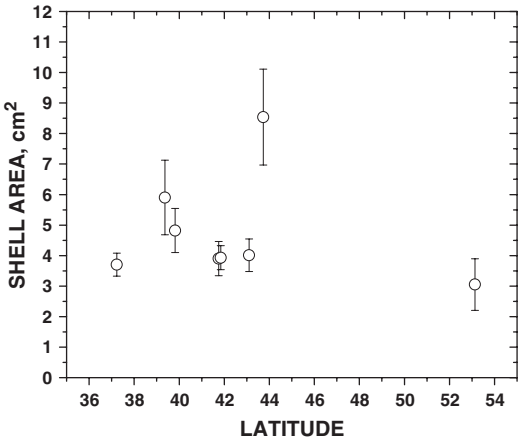

Figure 2 Relationship between mean shell area and latitude for the eight localities analysed. One SD unit is shown on mean values.

used partial Mantel tests in order to assess: (a) the association between morphological and genetic differentiation while setting geographic distance as a constant, and (b) the association between morphological differentiation and geography while setting genetic differentiation as a constant.

Results

Size differentiation

Shell-size variation between populations was highly significant (ANOVA, $F(7, 941) = 458.28$, $P < 0.0001$, Fig. 2) as well as the rank-transformed data (ANOVA, $F(7, 941) = 308.41$, $P < 0.0001$). However, latitude and mean shell size were not correlated (Spearman's correlation, $R^2 = 0.002$; $P = 0.91$).

Shape differentiation

The MANOVA analysis performed on the Fourier coefficients indicated a highly significant shell-shape differentiation between populations (Wilk's $\lambda = 0.0211$, $F = 16.68$, d.f. = 280 and 6241.7, $P < 0.001$). The first seven canonical axes were statistically significant. The first two axes explained 49.9% and 28.1% of the variance, respectively, while the percentage of variance explained by each successive axis was $< 9\%$. Shell area was not correlated with the two first canonical axes (Spearman's correlation; $P > 0.53$). Several groups (each defined by the mean value of all the individuals collected per locality) corresponding to different geographical locations were successfully discriminated using canonical variate analysis (Fig. 3a). The groups formed by the Chiloé localities (YAL, ANC, CAL) were close to each other, segregated from the northern ones (VAL, QUE, ARA), and largely diverged from Punta Arenas (PAR) and Puerto Marín Balmaceda (PMA). Shape changes among groups were associated with the first canonical axis. This variation mainly affected the anterior-ventral edge and the umbo shape. For positive loadings on the first axis, mussels appeared to be ventrally more convex with a rounded umbo, while for negative loadings, mussels were antero-ventrally concave with a pointed umbo (see YAL vs. PAR, Fig. 3b). The second canonical axis was associated with a gradient of relative round shell (dorsoventral expansion, Fig. 3b) and showed a negative correlation with latitude (Spearman's correlation, $R^2 = 0.66$, $P = 0.015$). Southern mussels (e.g. PMA) were more elongated with a larger length/width ratio than northern individuals (e.g. VAL, Fig. 3b). Punta Arenas shells are a special case showing a high differentiation in curvature (first axis) and an elongation (second axis) similar to the group formed by the Chiloé localities.

Morphological and geographical distances were positively correlated ($P = 0.014$; Table 1), while morphology and genetic distances were only marginally correlated ($P = 0.056$; Table 1). Genetic and geographical distances were significantly correlated (Mantel test; $R = 0.74$; $P < 0.001$). Partial Mantel tests showed that, even when genetic differentiation was taken into account, geographical proximity still had an impact on morphological differentiation ($P = 0.003$; Table 1). In addition, genetic differentiation did not carry significant additional information about morphological differentiation ($P = 0.5$; Table 1) when geographical proximity was set as a constant.

Discussion

Shell area of the *M. chilensis* populations did not show any differentiation and was unrelated to the shape or the latitude; thus there are no regular size patterns. Size and shape were independent as is required for a *real shape* study. These results support the large significant differentiation observed in shell-shape between populations. Our results are in agreement with former conclusions (Toro *et al.* 2004) in which discriminant functions to analyse *M. chilensis* populations from Yaldad, Puerto Marín Balmaceda and Punta Arenas were used. In these former studies

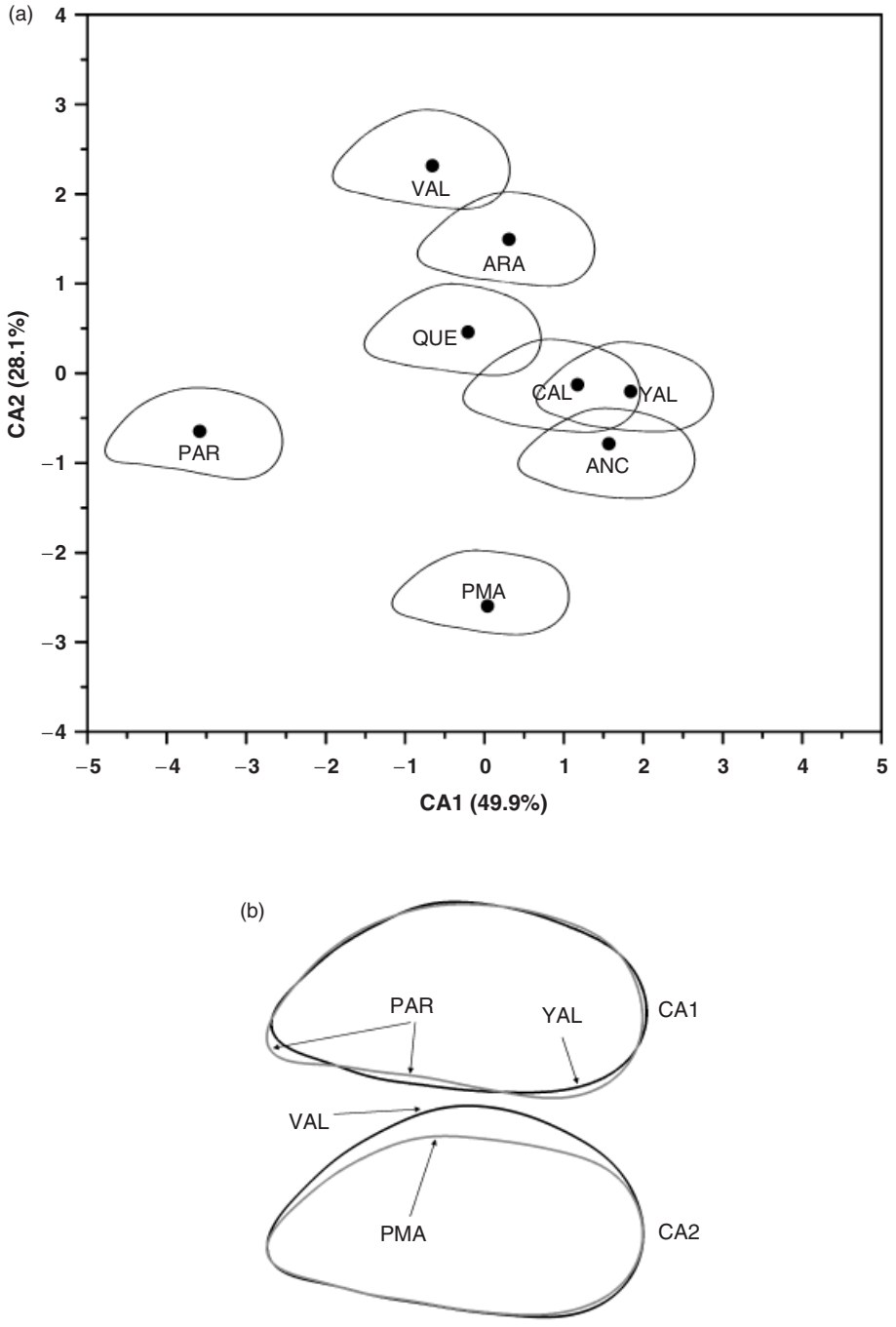

Figure 3 Canonical variates analysis of the Fourier coefficients. (a) First and second canonical axes (49.9% and 28.1% of total variance respectively). Dot indicates each population mean, and is surrounded by a visual representation of the 'averaged' shape of the population, constructed after inverse elliptic Fourier transformation. (b) *Mytilus chilensis* shell-shape variation illustrated by superimposing the outlines corresponding to the average shape of four populations. Calbuco (CAL), Queule (QUE), Valdivia (VAL), Calbuco (CAL), Ancúd (ANC), Yaldad (YAL), Puerto Marín Balmaceda (PMA) and Punta Arenas (PAR).

Table 1 Simple (R) and partial (R') correlations among morphological (Morpho), and genetic (Genet) and geographic (Geo) distances using Mantel tests

Simple Mantel test	***R***	***P***
Morpho-Geo	**0.66**	**0.014***
Morpho-Genet	0.49	0.056
Partial Mantel test	***R′***	***P***
Morpho-Geo (Genet)	**0.51**	**0.003****
Morpho-Genet (Geo)	− 0.01	0.500

Partial Mantel tests considered for the correspondence between the first two matrices while setting constant the third. All tests were based on 100 000 permutations. Significant correspondences are indicated in bold:
*$P < 0.05$.
**$P < 0.01$; one-tailed probability.

significant interpopulation morphological differences were observed for eight shell lineal characters. In this study as well as in Toro et al. (2004), the Punta Arenas population was the most divergent, which could be related to the influence of the West Wind Drift and the Cape Horn Current (Strub, Mesías, Montecinos, Rutlland & Salinas 1998). These currents could preclude larval transport, and thus gene flow, towards the northern mussel populations. However, the morphological differentiation of the Punta Arenas population could be due to differences in environmental conditions rather than genetic differences. (Toro *et al.* 2004, 2006). Altogether, previous and present morphometrical analyses have shown that Punta Arenas is a well-differentiated population from the rest of the species.

The present study also shows a high interpopulation differentiation in shell traits (such as elongation, ventral concavity and umbo shape) throughout the whole range of *M. chilensis*; however, this between-group approach, based on true population criteria, does not account for the effect of the high intra-group variance restricting our conclusions to a large scale. This large-scale and regionally based morphometric differentiation could be due to two interacting effects. First, it may be related to phenotypic plasticity, and if that is the case, shell-shape provides no information about possible gene flow among populations because of the early fixation and adaptation of planktonic larvae to their respective latitudes. On the other hand, these shell traits could be under direct selective control at some developmental stage, so gene flow among localities could be at least theoretically detected using morphological markers.

Studies on bivalve shape variation associated with latitudinal ranges are scarce on the macro-and meso-geographical scale (> 300 km), and generally used only lineal characteristics (Beukema & Meehan 1985; Aguirre 1994; Steffani & Branch 2003; Aguirre *et al.* 2006). In this study, a significant latitudinal cline of shell elongation (second canonical axis) in *M. chilensis* was observed, with elongation increasing towards higher latitudes. Only one previous study has reported a latitudinal cline in shell-shape; latitudinal variation has been observed in the shell-shape of *Brachidontes* mussels from the American SW Atlantic (Aguirre *et al.* 2006). This study had a similar latitudinal range to our study in the SE Pacific. However, they found increased shell elongation towards the north. It has been suggested that the pattern was related to a decline in salinity towards the north due to the influence of the Río de La Plata and other smaller rivers (Aguirre *et al.* 2006). The salinity gradient in the SE Pacific decreases towards the south (Dávila *et al.* 2002), and we found a strong positive correlation between salinity at 10 m depth (Fig. 1; data from Dávila *et al.* 2002) and the second canonical axis in *M. chilensis* (Spearman's correlation, $R^2 = 0.70$, $P = 0.01$). This supports suggestions that the cline could be due to salinity or a related factor (Aguirre *et al.* 2006). Indeed, salinity has been recognized as a selective agent affecting the genetic structure of marine bivalves, their survival, shell size and thinning, growth rate, and susceptibility to sea stars predation (Westerbom, Kilpi & Mustonen 2002; Ridgway & Nævdal 2004; Riginos & Cunningham 2005). It has also been suggested that conflicting patterns of species segregation are, in part, caused by local adaptation to extremely low salinity (Riginos & Cunningham 2005; Braby & Somero 2006).

Shape variation in *M. chilensis* could also be due to the stochastic processes related to genetic drift and gene flow. In this case, shell-shape variation should be correlated with the pattern from molecular markers, especially those based on RAPDs, microsatellites and AFLP markers, which are considered to be non-coding and therefore selectively neutral (Liu & Cordes 2004). Our results showed that morphological variation and neutral genetic variation are only marginally correlated (Table 1); in fact, when the genetic effect was held constant in the partial Mantel tests, geographical distance was still correlated with morphological variation. Altogether these data suggest that there are additional environmental and/or

genetic factors influencing the morphological variation of *M. chilensis*. We suggest that shell-shape variation in *M. chilensis* is influenced by environmental factors (e.g. salinity or related factors), acting either as a selection force on additive genetic variation or a promoter of phenotypic plasticity (Lynch & Walsh 1998). This is not unexpected in marine invertebrates and can be due to plasticity and/or strong selective effects (e.g. Trussell 2000; Luttikhuizen, Drent & van Delden 2003). This hypothesis is currently being studied, regarding the heritability of shell-shape variation in *M. chilensis*.

In spite of the fact that shell-shape in mussels is an extremely variable parameter, both within and between populations, which depends on numerous environmental factors including salinity, depth, water turbidity, type of substrate, hydrodynamics and wave action, presence of predators, parasitic infestation and especially mussel density (Guiñez & Castilla 1999). This work shows interesting inter-population relations between shape, latitude, salinity and genetics on an unusually broad latitudinal scale (1800 km). For instance, Daguin, Bonhomme & Borsa 2001 studied a similar longitudinal scale in a genetic study Mytilus across all Europe. Our results may enrich the understanding on clinal variation in mussels and could be the basis for in-depth ecological studies, which include more ecological data, and for the design of management polices for aquaculture stocks and natural populations of mussels.

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