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Use of shell shape variation as an assessment tool in the southernmost razor clam fishery

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ABSTRACT

Morphological variation provides a method for phenotypic stock differentiation at inter- and intra-specific levels. Various methods are used for the assessment of fishery stocks in mollusks; one of them is geometric morphometrics. We analyzed morphological variation in razor clams at ten fishing grounds, five from the Argentinean North Patagonian gulfs and five from Chile, and evaluated the occurrence of phenotypic stocks between Argentinean and Chilean fisheries, using geometric morphometrics methods. The Argentinean harvesting of *Ensis macha* is emerging, and represents a way to diversify the shell-fisheries in north Patagonia. Nevertheless, fishing and aquaculture play important roles for the Chilean economy. Various multivariate methods were applied to describe the differences among and between fishing grounds. We found significant differences in the average shell shape of individuals from either ocean, and these differences principally describe changes in the robustness of the shell. The average shell shapes differed among sites from the Pacific while those from the Atlantic Ocean did not show statistical differences. This study shows that geometric morphometric techniques are appropriate for the identification of phenotypic stocks in *E. macha*. Our results could be used for future resource management and to determine the origin of the product in razor clams from South America.

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1. Introduction

Several native bivalve species are of commercial interest in the North Patagonian gulfs, and are the basis of an important artisanal fishery. These include the Tehuelche scallop (*Aequipecten tehuelchus*), the purple clam (*Amiantis purpurata*), the mussel (*Mytilus sp.*) and the ribbed mussel (*Aulacomya atra*), which used to be captured in the 1970's using dredges, but are now fished mostly by diving (Orensanz et al., 2007). Recently, the razor clam (*Ensis macha*) represents an important alternative for artisanal fishermen divers to diversify the shellfish catches. Although the Patagonian region as a whole supports exploitation of fisheries such as deep-water hake and Patagonian red shrimp, artisanal exploitation of shellfish occurs primarily in San Matias and San Jose gulfs. In Argentina, a fishery for *E. macha* began at the start of 2000, but it has never been established as a regular fishing activity (Morsán and Ciocco, 2011). However, Chile is a country with important shell-fisheries along its highly productive coast, and one of the most important shellfish resources from the south-central harvesting areas is *E. macha* (SERNAPESCA, 2014). These geographical scenarios represent an optimal model to study "phenotypic" stocks (Booke, 1981). A phenotypic stock is a group of individuals with similar growth, mortality, and reproductive rates (Booke, 1981), which are exploited in a specific area. For fishery stock assessment, morphologically distinct populations should be modeled and managed as separate management units (Cadrin and Friedland, 1999, 2005; Cadrin, 2000, 2014). Therefore, establishing such units plays an important role in defining management measures for the shellfish resources at different geographical scales (intra-country and inter-country), but also would allow us to make a commercial tag with the origin denominations of the catches. The differentiation of food products by their particular qualities, such as their extraction/production and environmental features, is a useful tool to detect and avoid deliberate, as well as unintentional, substitution of different species and to reinforce labeling regulations (Fernández-Tajes et al., 2010). Knowing the origin of the catches would allow the industry to follow traceability, and facilitate commercial prod-

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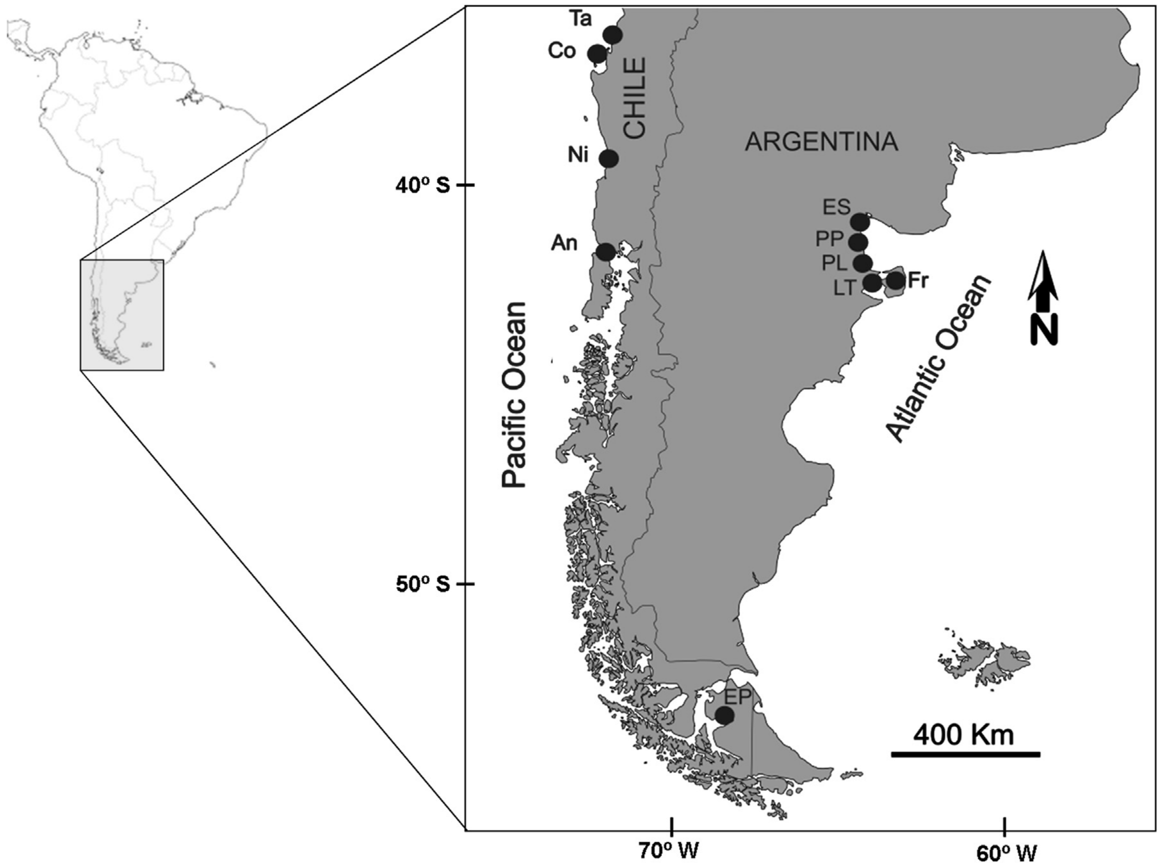

Fig. 1. Sample sites location. Sites codes from the Atlantic to Pacific oceans: ES: El Sotano; PP: Punta Pozos; PL: Puerto Lobos; LT: La Tapera; Fr: Fracaso; EP: El Porvenir; An: Ancud, Ni: Niebla; Co: Concepción and Ta: Talcahuano.

uct placement in new markets (Martínez Ruiz and Jiménez Zarco, 2006; CoFeCyT, 2008).

In addition to being an integral component of modern fisheries assessment (Begg et al., 1999) phenotypic stock identification is essential for the understanding the populations dynamics of a species in an ecological sense. Mollusks are an excellent target group for shell shape variation studies, since they have hard and stable shells (Rufino et al., 2006). The use of shell shape variables for phenotypic stock identification seems to be a realistic alternative for discrimination between groups since it corresponds to the most conspicuous portion of the body and presents high variability (Rufino et al., 2013; Márquez et al., 2010). Previous studies have reported that shell morphologic variation has been successfully used for phenotypic stock discrimination between marine bivalve species with similar shapes (Costa et al., 2008, 2010; Rufino et al., 2006), or between populations of the same species (Rufino et al., 2013; Márquez et al., 2010; Palmer et al., 2004). In the recent years, there has been an increasing interest in phenotypic stock studies using geometric morphometric methods (Cadrin, 2014). Geometric morphometrics (GM) is defined as the study of the shape variation and its covariation with other variables (Bookstein, 1991; Dryden and Mardia, 1998). GM techniques can be divided into two main groups, those that use Cartesian coordinates, in two or three dimensions, of homologous reference points (landmarks) and those based on object outlines. One of the main advantages of GM is that size and shape can be analyzed separately. Another advantage is that results of multivariate analysis can be visualized graphically (showing both the magnitude as well as the direction of change) since the implicit nature of geometric shape information is not lost during the analysis (Adams et al., 2004).

The razor clam *E. macha* (Molina 1782) is a burrowing bivalve that inhabits sandy and muddy bottoms of shallow subtidals. This species offers a good opportunity to study the phenotypic stocks of marine clams since it has a wide distribution: from Caldera (27°) to Magellan Strait (55°) on the Chilean coast, reaching San Matías gulf (40°) on the Argentinean coast (Lasta et al., 1998; Osorio, 2002). However, fishing on this species in the Atlantic Ocean is recorded only in two gulfs (SMG and SJG). However, in the Pacific, the catches of *E. macha* is an important fishing activity and is concentrated in Corral, Golfo de Arauco and Magallanes region (Lepez Gárcia et al., 2011).

Patterns of morphological variation in *E. macha* shells were compared between fishing grounds in Argentina and Chile using geometric morphometric techniques to determine whether there are phenotypic stocks.

2. Methods

2.1. Sample processing

A total of 518 individuals (130–180 mm of shell length) were collected using scuba diving from ten populations: five from the Atlantic (Argentina) and five from the Pacific Ocean (Chile) (Fig. 1; Table 1). Once the soft parts had been removed, a photograph of the left shell was obtained together with a scale of 1 cm^2. Shells were placed with the concave side upwards on a plasticine base to prevent a pitching and/or rolling effect and to match the height of the scale (Zelditch et al., 2004). Pitching refers to the movement in the anterior-posterior direction along the transverse axis, rising and falling, and rolling refers to the rotation along the longitudinal axis (dorsal-ventral direction). It is important to check that these

Table 1
Study material of *Ensis macha* collected from Argentina and Chile fishing grounds. SL: shell length.

Ocean	Fishing ground	Code	Latitude	n	SL (mm)	Date of collection
Atlantic	El Sótano	ES	40° 56' 59,6"	49	16.45 (1.09)	Sept 2006; Oct 2007
Atlantic	Punta Pozos	PP	41° 34' 07,7"	51	15.71 (0.76)	Mar 2003; Jun 2007
Atlantic	Puerto Lobos	PL	42° 00' 29,5"	57	16.07 (1.38)	Jun 2006; Nov 2007
Atlantic	La Tapera	LT	42° 21' 05,4"	52	14.92 (2.01)	Jun 2005; Jun 2006
Atlantic	Fracaso	F	42° 24' 47,9"	54	14.7 (0.78)	Aug 2006; Jun 2007
Pacific	Talcahuano	T	36° 44' 07,14"	45	18 (1.09)	Nov 2008
Pacific	Concepción	Co	36° 57'30, 8"	49	16.3 (1.03)	Nov 2008; Aug 2009
Pacific	Niebla	N	39° 51' 00,04"	64	17.5 (2.23)	Nov 2008; Aug 2009
Pacific	Ancud	An	41° 52' 04,66"	46	16.3 (1.10)	Nov 2008
Pacific	El Porvenir	EP	53° 24' 23,24"	51	16.72 (1.18)	Apr 2009

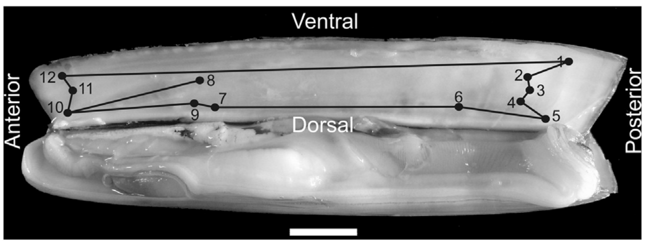

Fig. 2. Razor clam shell internal view showing the landmark configuration used. Landmarks are shown in black dots, each one with its respective number; (1) ventralmost point of the pallial sinus, (2) ending point of the ventral pallial sinus, (3) centralmost point (maximum curvature) of the pallial sinus, (4) ending of the dorsal of the pallial sinus, (5) dorsalmost point of the pallial sinus, (6) posteriormost point of the posterior adductor muscle of the foot, (7) posteriormost point of the anterior adductor muscle of the foot, (8) ventralmost point of the posterior limit of the anterior adductor muscle, (9) anteriormost point of the anterior adductor muscle of the foot, (10) dorsal anterior limit of the pallial line, (11) central point (maximum curvature) of the anterior pallial sinus, (12) ventral anterior limit of the pallial line. The landmarks configuration was modified from Rufino et al. (2013). Scale bar = 1 cm.

effects do not occur when the picture is taken because they cannot be mathematically standardized and they can influence the data set.

2.2. Geometric morphometrics

We used the landmark method to capture the Cartesian coordinates of a two dimensional configuration of 12 landmarks, using TpsDig2 software (Rohlf, 2004a) (Fig. 2). All landmarks were painted with small points of permanent marker to improve the identification of muscle scars on the inner surface of the valve (Signorelli et al., 2013). The landmark coordinates were superimposed using Procrustes analysis to remove rotation, translation and scale effects (Rohlf and Slice, 1990) in the TpsRelw software (Rohlf, 2004b). The Procrustes coordinates were then used as shape variables to perform the multivariate statistical analyses (see Section 2.3).

2.3. Shell shape variation among fishing grounds

The Centoid Size (CS) was used in GM as a proxy for size and it was calculated applying the square root of the sum of the square distances from the landmarks to the centroid which they define (Zelditch et al., 2004). Once the shape variables of all individuals were obtained, the presence of allometry was tested using a multivariate regression analysis (pooled within-fishing ground), between shape scores as a dependent variable (Procrustes coordinates) and size as an independent variable (CS), using MorphoJ v1.05d (Klingenberg, 2011). We applied a permutation test with 1000 replicates to evaluate the independence between the shape and size variables (Bookstein, 1991; Zelditch et al., 2004).

A principal component analysis (PCA) of the variance-covariance matrix (Zelditch et al., 2004) was conducted to study the magnitude and direction of shell shape variation. We used a multivariate analysis of variance (MANOVA) (Cuadras, 2008) to test for differences among fishing grounds. The maximum differences in shell shape separating fishing grounds were assessed using a Canonical Variate Analysis (CVA). Finally, to visualize and test the separation of shell shapes between Atlantic and Pacific fishing grounds, we performed a linear discriminant analysis (DA) using the leave-one-out cross validation procedure to estimate the reliability of the discrimination (Johnson and Wichern, 1998). Furthermore, to assess the Mahalanobis distances (shape distances) and identify different groups, we used the Multivariate Di Rienzo, Guzman and Casanoves (MDGC) method, which is an extension of the multivariate case of a multiple comparison method based on cluster analysis generated using an unweighed pair-group method with arithmetic mean (UPGMA; Valdano and Di Rienzo, 2007). This method is a hybrid between a hierarchical clustering method based on Mahalanobis distances and a statistical hypothesis test for a multivariate case, and is useful to solve, on the basis of inferential statistics, the problem of determining the number of groups in a hierarchical cluster analysis.

The MANOVA, CVA, DA and Cluster were performed using all PC scores (size corrected) as shape variables.

3. Results

3.1. Shell shape variation

Allometric regression between Procrustes coordinates and CS was significant ($p < 0.0001$) for the whole sample, and accounted for 2.5% of the total amount of shape variation. Thus, the PCA was performed on the residuals of the regression. The allometric-free shell shape variations are summarized in Fig. 3, which is a scatter plot of the first three principal component scores, which accounted for 77.21% of the total variation; the other PCs were not taken into account because their contribution was less than 5% (Zelditch et al., 2004). The first PC accounts for 61.95% of the variation and was associated with robust shells in the positive values, the shells being more anterior developed with an anterior-ventral expansion, posterior retraction, and an enlargement to the ventral part of the adductor muscle. Towards the negative values the shells are more triangular. The individuals located in the direction of the positive values of the PC2 (9.76%) presented an anterior expansion with posterior displacement of the anterior retractor muscle of the foot. Finally the PC3 (5.50% of the variation) was associated with ventral-dorsal broadening, the shells being more rectangular towards positive values (Fig. 3).

The MANOVA analysis indicated a highly significant shell shape differentiation among fishing grounds (Wilk's λ=0.02, $F = 14.86$, d.f.180 and 4052, $p < 0.0001$). The first three axes of the Canonical Variate Analysis (CVA) summarized 93.1% of the total variance. The most conspicuous trait separating shapes along the CV1 (69.68% of the variance) was the anterior expansion and a retraction of the pos-

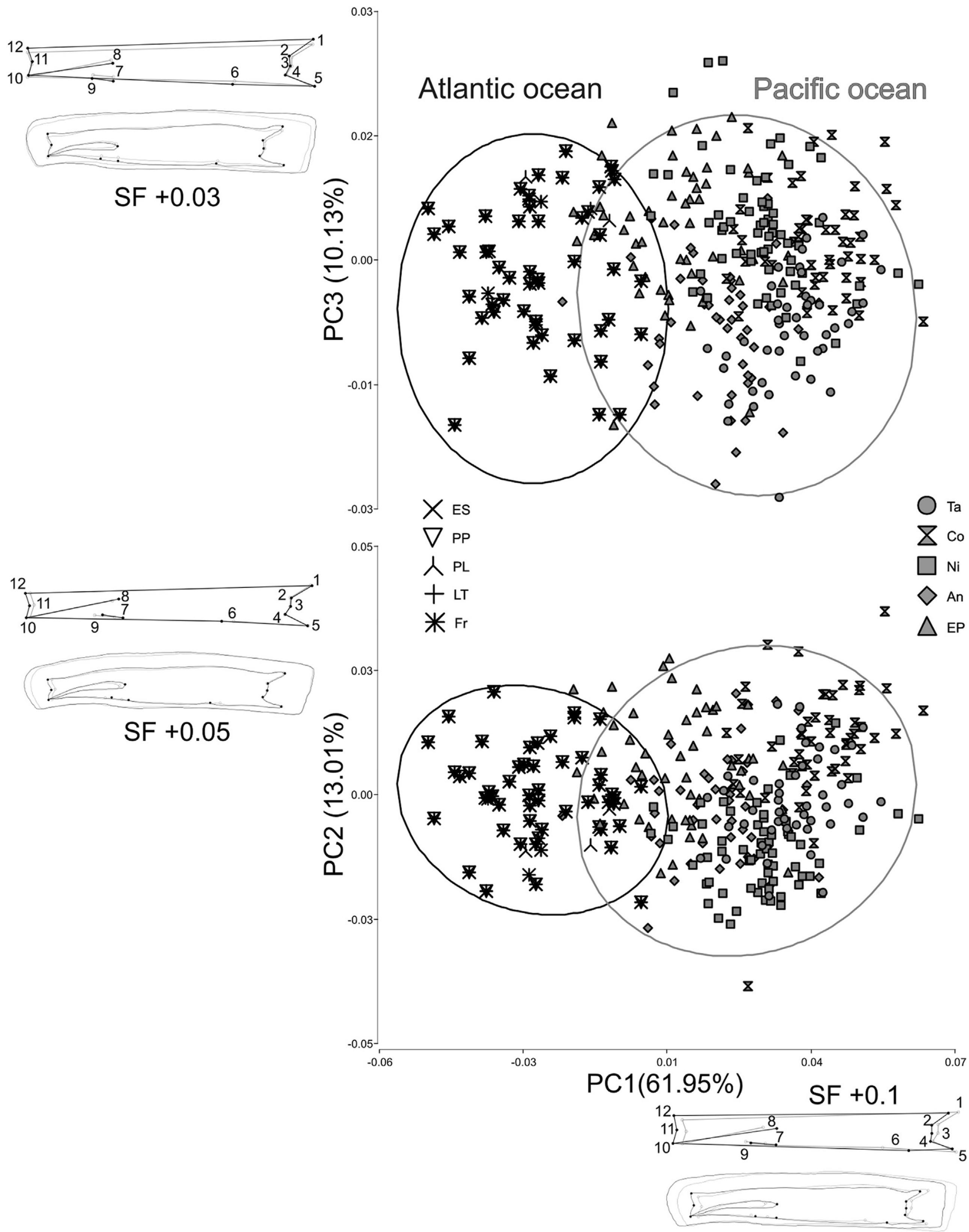

Fig. 3. Principal Component Analysis showing the total shell shape variation among all populations. The graphs outside each positive axis extreme represent the displacement vectors and drawn outlines from the overall mean shape (grey dot) to the positive extreme shapes (black dot, equal to + scale factor = SF) for PC1, PC2 and PC3. Up: PC1 vs. PC3. Bottom: PC1 vs. PC2. Between brackets is shown the variance explained by each axis. Population codes are the same as in Fig. 1.

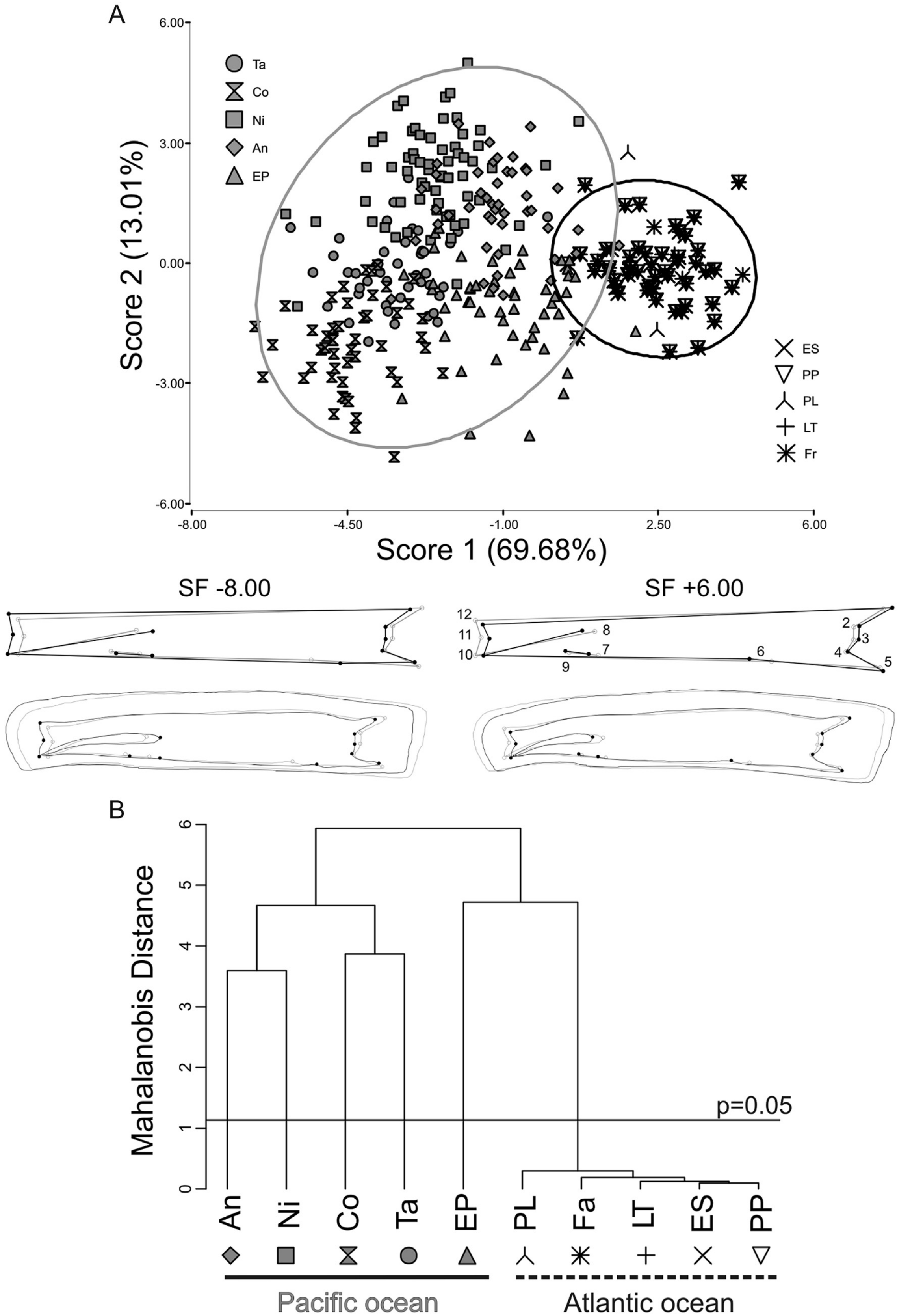

Fig. 4. (a) Canonic Variate Analysis on the shell variation of *Ensis macha* among fishing zones. The graphs below score 1 represent the displacement vectors, and outlines drawing from the overall mean shape (grey dot) to the positive and negative extreme shapes (black dot, equal to+- scale factor = SF). (b) Hierarchical clustering UPGMA showing the relationships between the mean shell shapes of the different fishing grounds. The cut criteria obtained by the MDGC test is represented by a horizontal line ($p < 0.05$). Population codes are the same as in Fig. 1.

Table 2
Classification matrix showing the cross-validated classification of each fishing ground and ocean. Shaded rows correspond to Pacific Ocean.

Fishing ground	ES	PP	PL	LT	Fr	Ta	Co	Ni	An	EP	Total	Percentage correct
ES	17	5	18	2	7	0	0	0	0	0	49	34.69
PP	17	5	20	2	7	0	0	0	0	0	51	9.8
PL	17	5	26	2	7	0	0	0	0	0	57	45.61
LT	17	5	21	2	7	0	0	0	0	0	52	3.85
Fr	17	5	23	2	7	0	0	0	0	0	54	12.96
Ta	0	0	0	0	0	40	4	0	0	1	45	88.99
Co	0	0	0	0	0	1	48	0	0	0	49	97.96
Ni	0	0	0	0	0	1	0	59	2	2	64	92.19
An	3	0	0	0	0	0	1	2	40	0	46	86.96
EP	0	3	0	0	0	0	2	1	1	44	51	86.27
Ocean	Atlantic					Pacific						
Atlantic	258					5					263	98.1
Pacific	12					243					255	95.3

terior part with an enlargement of the scars of the anterior adductor muscles and the anterior and posterior retractor muscle of the foot towards the positive values. The CV1 represents the main shape shell differences between Argentinean and Chilean fishing grounds. The positive values of CV2 were mostly associated with a thinning in the foot area, and the retraction of the exhalant and inhalant siphons (Fig. 4a). The hierarchical clustering indicated six groups (cut criteria is represented by a vertical line at $p < 0.05$ – Fig. 3b); Mahalanobis distances indicated that the greatest similarities were among Atlantic fishing grounds (which are clustered together and were not statistically significant, $p > 0.05$), the fishing grounds of Pacific differed among themselves (Fig. 4b). The cross-validated classification results (Table 2) showed that the accuracy of shell shape in predicting fishing grounds is better than an 86.3% random chance for the Pacific but only 46% in the Atlantic. The discriminant analysis (DA) successfully distinguished between Atlantic and Pacific stocks. The proportions of correctly assigned individuals were 98.1% and 95.3% for the Atlantic and Pacific fishing grounds, respectively.

4. Discussion

This study supports the findings of much previous work in this field. The use of GM analysis allowed us to characterize, with high resolution, subtle changes in *Ensis macha* shell shape variation. Significant differences were found in the average shell shape between Atlantic and Pacific fishing areas, and the major changes in the shell shape of this species were described. We suggest that GM can be applied to differentiate individuals from Argentinean and Chilean fishing grounds. Partial isolation is enough for geographic differences to persist in spite of the fact that the phenotypic stock definition is less conservative than the genetic stock definition, (Cadrin, 2014). Thus this method can be used to promote the consumption of Argentinean razor clams and to avoid fraud through the implementation of source labeling of products for sale (Palmer et al., 2004). Therefore, our results could be used in a pragmatic mode for future resource management and to determine the origin of the product in *E. macha*.

When comparing the shell shape differences among Argentina and Chile fishing grounds, the PCA principally describes changes in the robustness of the shell. Often morphometric differences are assumed to reflect genetic divergence in response to local selection pressures, but sometimes they can be fully induced by the environment (Swain and Foote, 1999). At intraspecific level, it has been shown that different physical variables such as latitude (Márquez and Van der Molen, 2011; Costa et al., 2008; Krapivka et al., 2007; Aguirre et al., 2006; Beukema and Meehan, 1985) modify the shell morphology in various species of bivalves. Also, as in another mollusks, razor clams shell features could be affected by many factors (biotic and abiotic) including crowding, trophic conditions, water depth, wave impact and predators presence (e.g., Márquez et al., 2015; Valladares et al., 2010; Krapivka et al., 2007; Kirk et al., 2007; Beadman et al., 2003; Alumno-Bruscia et al., 2001; Akester and Martel, 2000; Reimer et al., 1995; Raubenheimer and Cook, 1990; Gardner and Thomas, 1987).

The average shell shapes in the Atlantic Ocean do not show statistical differences among fishing grounds, whereas the average shell shapes differed among sites in the Pacific. A possible explanation for this is that *E. macha* is exploited only in a small area of its distribution in the Atlantic (two degrees of latitude) compared to the Chilean fisheries which extend along 17° of latitude. Therefore, the variation found in the shell shape of *E. macha* can be attributed to an environmental gradient (Márquez and Van der Molen, 2011). This capacity of differential response to distinct environments by the bivalves in general and by *E. macha* in particular can be used to identify individuals/stocks/groups between and within the fisheries of different countries. The graphical output of the hierarchical cluster test (UPGMA-MDGC) not only shows a clear distinction between shell shapes for each location of Chile, but also states that the Argentinean fishing grounds do not present differences in shell shape. This was expected *a priori*, since the bivalves from the north Patagonian Gulfs are structured as metapopulations (Orensanz et al., 2006; Orensanz and Jamieson, 1998).

Morphometric variation has been used as a method for stock identification for various fishing resources (Cadrin, 2000, 2014) as geographic patterns in the individual shape are essential to identify discrete phenotypic stocks (Booke, 1981). The characterization of shape variation of the razor clam between the principal fishing areas of the two countries can be used as a fishing control measure to determine provenance of catches and/or to differentiate fishing grounds. In summary, the use of GM techniques is an appropriate way to identify potential fishing stocks in this species. Furthermore, the application of this type of methodology could be used for the design of resource management policies. Argentinean artisanal fishermen have the initiative to give value to their capture differentiating their products by developing a brand, origin designation or certifications (CoFeCyT, 2008). Artisanal fisheries display higher quality products than dredge fisheries, particularly those developed in the Argentine Patagonia gulfs which have high water quality, free from industrial pollution, urban effluents and TBT, because of the reduced sea traffic (Bigatti et al., 2009). These certifications have a significant dimension for the sector as this will allow the fishery industry to explore new markets that until now have been elusive because of the lack of an accreditation which gives them a quality label to their products (SENASA 31/03/08.). For all of this, it would be crucial to design a set of brands that identify and highlight the social, environmental and quality attributes of Argentinean artisanal fishing products.

5. Conclusion

This study suggests that there are different phenotypic stocks of *Ensis macha* present between Argentinean and Chilean fishing grounds. Another important practical implication is that geometric morphometrics results can be used as a fishing control measure to determine the provenance of catches and/or to differentiate fishing grounds.

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