Shell morphometric variation of *Ameghinomya antiqua* (Mollusca, Bivalvia) during the Late Quaternary reflects environmental changes in North Patagonia, Argentina

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ABSTRACT

Mollusk shells have shown to be useful as biological proxies for palaeo environmental reconstructions. In this study we perform an integral assessment of geometric morphometrics of *Ameghinomya antiqua* shells from San Matías Gulf (SMG) (Patagonia, Argentina) with special attention on the protected natural area Caleta de Los Loros. In order to relate morphological variation with environmental changes, we analyzed size and shape variation from Holocene and modern shells (temporal variation) and from within the gulf (spatial variation) by carrying out landmark and semilandmark morphometrics studies. Results showed that Holocene shells were rounder and larger than modern ones, with posterior muscle scar, cardinal teeth and ligament more developed. These features in Holocene shells from Caleta de Los Loros could be a response to a higher degree of mechanical stability in a high-energy habitat. Besides, morphometric differences in modern shells in SMG could be linked to variations in wave action, tidal influences, sea surface temperature substrate and/or predation pressure, which modify the shape and size of this species. This study allows highlighting the phenotypic plasticity of *A. antiqua* as a possible response to different environmental conditions making this species a good proxy when evaluating environmental changes.

1. Introduction

Morphometrics is the quantitative way of addressing the shape and size comparison of many biological structures (Bookstein, 1991). Bivalves shell morphology, given its calcareous nature and hardness; provides an opportunity for both spatial and through-time studies of the morphological variation considering solely the analysis of their shape profiles. Inter and intraspecific phenotypic variation in bivalve shells has been evaluated using conventional morphometric measurements (Renard et al., 2000; Baker et al., 2003; Barón et al., 2004; Lomovasky et al., 2008; Popa et al., 2010) based on discrete linear and angular measurements. However, the development of geometric morphometrics has revolutionized the study of shape changes offering the possibility of quantifying and visualizing morphological variations through graphical tools and powerful analytical methods (Bookstein, 1991; Zelditch et al., 2004; Márquez et al., 2010; Neubauer et al., 2013; Boretto et al., 2014,

among others).

The striped clam *Ameghinomya antiqua* (King, 1832) is a benthic species typical from the Magellanic region (Balech and Ehrlich, 2008) that inhabits the shallow sandy-soft bottom substratum, from intertidal until around 50 m deep, in temperate-cold waters (Rosenberg, 2009). In Argentinean Patagonia, *A. antiqua* shells are well preserved and distributed both on the present active beaches and on marine coastal deposits as (Holocene, Pleistocene) fossils (Feruglio, 1950; Escati-Peñaloza et al., 2010; Boretto et al., 2013; Boretto et al., 2014).

The San Matías Gulf (SMG) is located in northern Patagonia, and exhibits a multitude of geomorphological features and littoral deposits. The natural protected area Caleta de Los Loros (Río Negro province) is located in the north shore of SMG (Fig. 1), and has been the subject of several environmental changes (Del Río and Colado, 1999) likely triggered by shifts in climate regime (Sander et al., 2018). *A. antiqua* shells were previously studied in other sites in Patagonia to differentiate
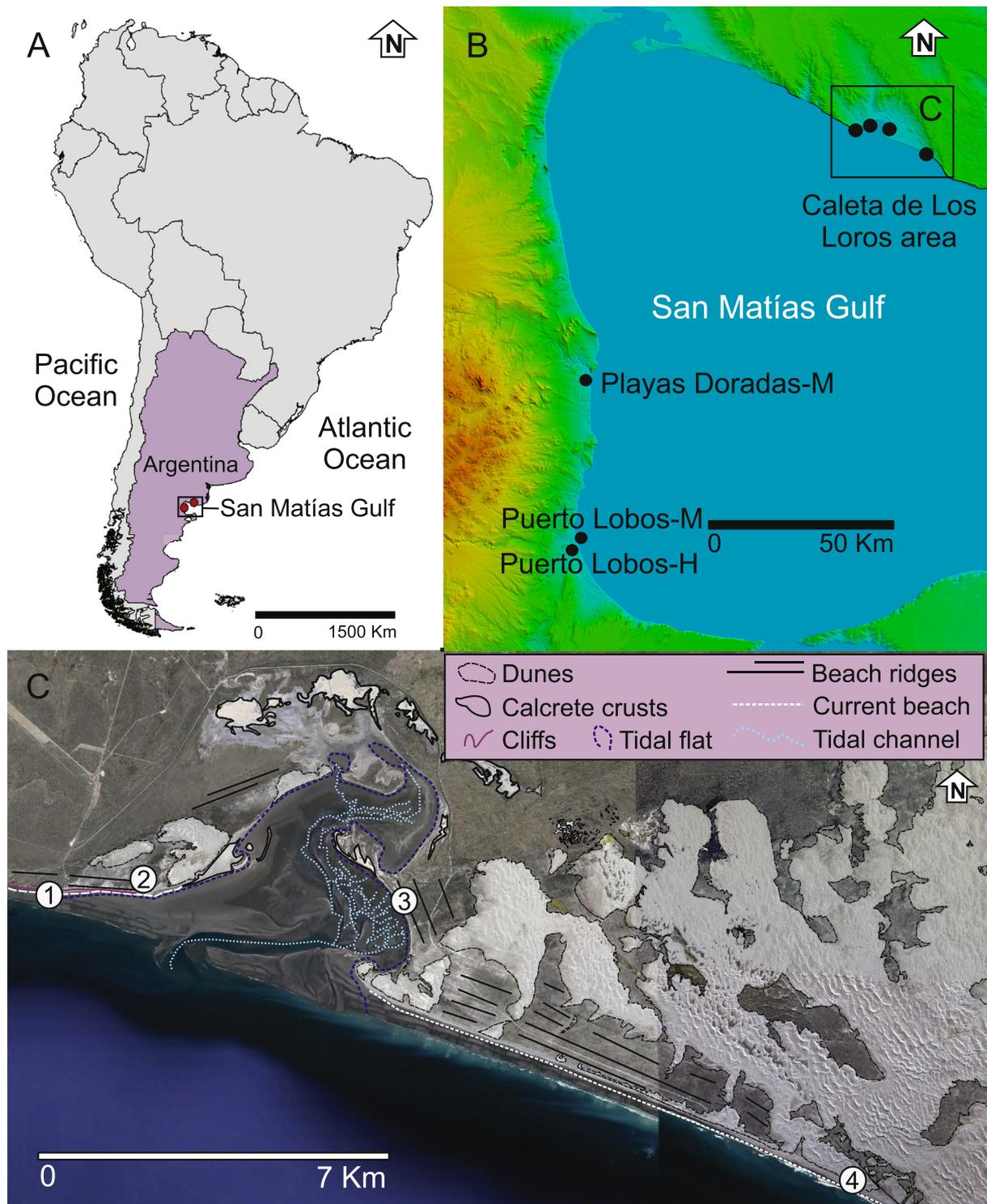
Fig. 1. (A) Regional localization of the study area. (B) San Matías Gulf and sites from where the samples were collected (Digital elevation model SRTM 90 m). (C). Caleta de Los Loros study area with classification of geomorphological units (Google Earth Pro image); site samples: 1. Caleta de Los Loros-M, 2. Caleta de Los Loros-H1, 3. Caleta de Los Loros-H2, 4. Bahía Creek-H.

both habitat (Márquez et al., 2010) and across-time morphological variability (Boretto et al., 2014). The aim of this study is to evaluate phenotypic variations of *A. antiqua* shells from SMG through time and space, with special attention on the protected natural area Caleta de Los Loros. This approach would allow us to identify environmental conditions in the area lending support to the utility of bivalves species as suitable proxies for evaluating environmental fluctuations. This integral analysis of shell morphology showed differences over time as possible

morphological responses to achieve mechanical stability or defense to predation, and geographical differences as well, probably related to changes in the oceanographic conditions present in SGM.

2. Regional setting

Caleta de Los Loros located between 41°01′25′′S and 64°06′20′′W, is formed in a morphological depression within a stretch of cliffed

coastline (Del Río and Colado, 1999; Sander et al., 2015). The cliffs are formed by Cenozoic layers of sand and gravel, and defined a mature coastline to the east of the inlet. The landscape in the study area is characterized by different geomorphological units. The modern beach and Quaternary beach ridges are composed by sand, gravel and mollusk shells with different grades of preservation, and the crest systems extend preferentially to the west side of the lagoon. As a result of main wave and current direction, the system is markedly asymmetrical (Sander et al., 2015). The extension of the tidal plain is around 6 km, surrounded by tidal channels (Fig. 1C). A sticking out wedge of transgressive dunes moves in an ENE direction inland from the beaches and sand flats of Caleta de Los Loros (Fig. 1C). The local climate is characterized by two main meteorological features, as along the whole Patagonian coast: constant strong dry winds and low precipitation (< 300 mm/yr) (Coronato et al., 2005). Tides at Caleta de Los Loros are semidiurnal with mean amplitude of 6.04 m (SHN, 2013). Wave data for the Argentinean coast are generally scarce. Nevertheless, Reguero et al. (2013) indicated in their continental-scale modeling data an annual average wave height (Hs) of approx. 0.5 m for the northern coasts of SMG with a direction of mean energy flux from S to SE direction.

3. Materials and methods

3.1. Ameghinomya antiqua shells and study sites

A. antiqua fossils and modern adult specimens (length range 14–87 mm, following Escati-Peñaloza et al., 2010), were collected at four localities along the SMG: Puerto Lobos (PL), Playas Doradas (PD), Bahía Creek (BC) and Caleta de Los Loros (CL) (Table 1, Fig. 1).

Modern samples were taken randomly from the current active beaches (Fig. 1C) and most specimens had their ligaments preserved on different states of preservation. These death assemblages are formed by non-contemporaneous generation of organic remains, due to combined effects of time-averaging and spatial mixing. They preserve ecological fidelity (i.e., preservation of the biological signal that reaches shell beds after the influence of biological and physical agents and time averaging) which allows them to be used as proxies of living communities at regional spatial scale (Tomašových and Kidwell, 2009; Kidwell, 2013; Archuby et al., 2015). Fossil shells were taken randomly from the exposed marine deposits, which have been described and dated firstly by Bayarsky and Codignotto (1982) in PL and more recently by Sander et al. (2018) in CL as beach ridges formed during the Holocene. In total, 231 right valves were used in the geometric morphometric analyses.

3.2. Geometric morphometric methods

For geometric morphometric analyses, each right valve was photographed (Fig. 2) on the inner side. On each image, 12 landmark points were located on the position of internal features of the shells, similar to Boretto et al. (2014), 3 type-III-landmarks on the ends of shells, following Wrozyna et al. (2016), and 41 sliding semilandmarks on the shells outline were marked with tpsDig2 (Rohlf, 2005a) to quantify shape. Landmarks and sliding semilandmarks positions were rotated, scaled and translated through Generalized Procrustes Analysis using

Table 1
List of localities and material studied.

Locality	Age	Site	Code	N
Puerto Lobos	Modern	Puerto Lobos-M	PL-M	36
Puerto Lobos	Holocene	Puerto Lobos-H	PL-H	23
Playas Doradas	Modern	Playas Doradas-M	PD-M	25
Caleta de Los Loros	Modern	Caleta de Los Loros-M	CL-M	29
Caleta de Los Loros	Holocene	Caleta de Los Loros -H1	CL-H1	10
Caleta de Los Loros	Holocene	Caleta de Los Loros-H2	CL-H2	50
Bahía Creek	Holocene	Bahía Creek-H	BC-H	58

Fig. 2. Right valve of *Ameghinomya antiqua* with the location of the landmarks (empty dots, numbered from 1 to 15) used to define the shape of the right shell shape and sliding semilandmarks along the outline (dashed line). The landmarks were as following: (1) umbo, (2) the ventral tip of the posterior cardinal tooth, (3) the middle cardinal tooth, (4) the ventral tip of the anterior cardinal tooth, (5) the scar of the lunule, (6, 7) the tips of the anterior adductor muscle scar, (8) the lower tip of the pallial sinus, (9) the tip of the pallial sinus, (10,11) the tips of the posterior adductor muscle scar, (12) the tip of the posterior hinge ligament, (13) posterior end, (14) ventral end, (15) anterior end.

TpsRewl (Rohlf, 1999) minimizing the bending energy matrix (for more details on geometric morphometric methodologies using landmarks, see Bookstein, 1997; Adams et al., 2004; Zelditch et al., 2004) and MorphoJ software (Klingenberg, 2011).

In order to evaluate the differences in shell size, centroid size was calculated using MorphoJ. Centroid size is defined as the square root of the summed squared distances of specimen's landmarks from their geometric centroid, and was used as a proxy of valve size. The size differences among locations were examined by a Kruskal-Wallis test and *a posteriori* Mann-Withney test in order to identify the periods and localities in which shell size differences occurred. These analyses were performed using PAST software v3.6 (Hammer et al., 2001). The variance associated with sites as a grouping factor was estimated with linear models following Martínez et al. (2016) and Martínez and Gardenal (2016). Significance of regression parameters was assessed using a permutation test with 10,000 replicates.

To assess putative allometric effects, we computed the multivariate regression of shape (Procrustes coordinates used as dependent variables) on size (independent variable) using MorphoJ software (Klingenberg, 2011). Such regression is useful to detect and remove the effect of size before shape comparisons between groups (Klingenberg, 2008). To evaluate the independence between the shape and size variables, we carried out a permutation test with 10,000 rounds. The principal components of shape were calculated from the variance–covariance matrix of the Procrustes coordinates. Principal components were used for subsequent statistical analysis and for generating a graphical representation of the morphospace. A between-group PCA was performed using MorphoJ, which projects the data onto the principal components of the group means, to display the major features of shape variation along the axes (Mitteroecker and Bookstein, 2011). To test if assemblages are morphologically distinct and to observe the percentage of correctly classified individuals in each group, Discriminant Analysis was undertook. Rates of correct classification were estimated by comparing the *a priori* assignments with those based on the Canonical Variate Analysis (CVA). Each specimen was assigned to a given geological period based on the minimum Mahalanobis distance to the group means (Klingenberg, 2011). Group assignments were cross-validated by a Jackknife resampling routine in MorphoJ. We estimated the
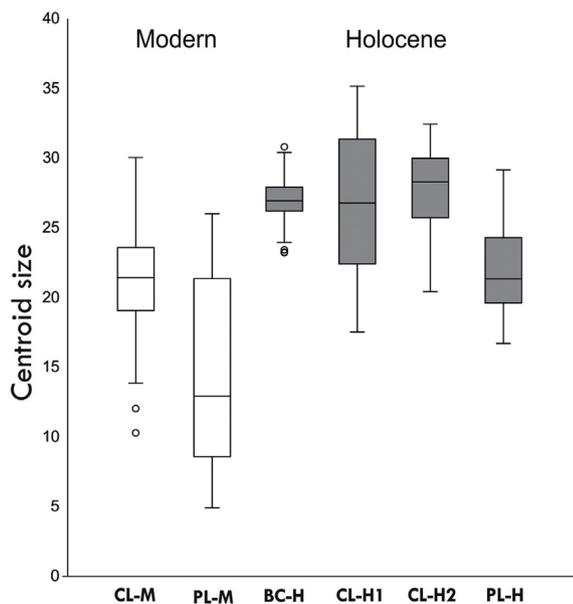
Fig. 3. Box plots of differences in centroid size between modern (white boxes) and Holocene (grey boxes) assemblages of *A. antiqua*. See Table 1 for abbreviations.

proportion of shape variance among sites relative to total variation. The estimation was performed with a linear model using sites as the grouping variable. The statistical significance was evaluated using 10,000 permutations. Procrustes distances between sites and associated *p*-values based on 10,000 permutations were estimated using MorphoJ software (Klingenberg, 2011).

4. Results

Shell size comparison showed clear differences between sites (H = 152.9, *p* < 0.0001), being Holocene shells larger than modern ones, with the exception of shells from CL-M that presented an intermediate size (Fig. 3). Pairwise differences in size across sites are shown by *a posteriori* Mann-Withney test with Bonferroni correction (Table 2). Size differences between sites were highly significant (*p* = 0.0004); the percentage of size variance explained by sites differences was 10.7% of total centroid size variation. Allometric growth, in which shape varies with size, was negligible. The multivariate regression of shape on centroid size was statistically significant (permutation test with 10,000 random permutations, *p* < 0.0001) but only accounted for 4.14% of the total amount of shape variation.

The between-group PCA showed clear trends in shape change across Holocene and modern shells (Fig. 4). The first three principal components explained 44.05, 21.79 and 9.55% of shape between group variability, respectively. The variation across BG-PC1 indicates intraspecific shape variation, but also shows a trend to distinguish the shells from different sites: CL-H1, CL-H2 from those of BC-H, with the

Table 2
A posteriori Mann-Withney test for centroid size. Asterisks indicate significant statistical differences (*p* < 0.05). See Table 1 for abbreviations.

	CL-M	PL-M	PD-M	BC-H	PL-H	CL-H1	CL-H2
CL-M							
PL-M	*						
PD-M	*	–					
BC-H	*	*	*				
PL-H	–	*	*	*			
CL-H1	–	*	*	–	*		
CL-H2	*	*	*	–	*	–	

remaining sites being intermediate (Fig. 4). Shape variation along BG-PC1 mostly regards the relative positions of landmarks (LM) 7–12 and shell outline. LM 8–10 are located on the pallial sinus forming a triangle, which is more open and equilateral for BC-H than for CL-H1 and CL-H2, where the pallial sinus marks a narrowed triangle. On the other hand, the pair of LM 11 and 12 determines a longer posterior hinge ligament for CL-H1 and CL-H2 than for BC-H. Besides, semilandmarks configuration allows us to visualize that the outline in CL-H1 and CL-H2 is more oval (in the antero-posterior axis), forming an incipient posterior "rostrum", than in BC-H, where the contour is more circular. Shape variation along BG-PC2 corresponds to differences between the shells from Holocene deposits and modern shells. The shape reconstruction for BG-PC2 revealed that modern shells have a comparably larger and wider pallial sinus than fossil ones (LM 8-9-10), a smaller posterior muscle (LM 10-11), a shorter hinge teeth (LM 1-2-3-4) in the dorso-ventral axis, and a briefer hinge ligament (LM 12). In addition, the outline of modern shells is more oval (with the dorsal area flattened) than the Holocene ones, which are more circular.

Assignment of individuals (by means of Jaccknife leave-one-out validation) from different geological time intervals was correct in 44.6% (106 out of 231). For each site the percentage of correct classification was low (50% approximately or less) (Table 3 A-B). Shape differences among sites were highly significant (*p* < 0.0001). Sites explained 18.87% of total shell shape variation. The Procrustes distances comparisons between groups were significant (*p* < 0.05 for all the comparisons) except for PD-M and PL-M that does not show significant statistical differences (*p* > 0.05) (Table 4 A-B).

5. Discussion

5.1. Morphological variability across time

The analyses of shape and size variation performed in this study showed that shells of the Holocene assemblages were, in average, rounder and larger than modern ones, with posterior muscle scar, cardinal teeth and ligament more developed, and smaller pallial sinus. Moreover, modern assemblages showed ovoid and smaller shells, with a well-developed pallial sinus, and smaller posterior muscle scar, ligament and cardinal teeth. These morphological changes across time could be explained in function of the environmental trends. Here, we expose alternative models which might either individually or as a combination, explain morphological changes in *A. antiqua* in Patagonia, Argentina.

5.1.1. From high-to low-energy habitats

Caleta de Los Loros evolved from an open coast subjected to the wave action to a more restricted system, leaving a deep, narrow open sector (Del Río and Colado, 1999; Favier and Scartascini, 2012). This change was likely initiated by the accumulation of older deposits as paleo-spits and coastal bars, limiting the entrance of the sea and generating a tidal inlet (Del Río and Colado, 1999). These environmental shifts have been reported as very young (between 2300 and 600-70 BP) (Sander et al., 2018).

During this stage, late Holocene bivalve assemblages were more exposed to wave action and the influence of tides. Such high-energy habitats required a higher degree of mechanical stability. It is well known that when storms occur, clams can be washed out from their semi-infaunal or infaunal life positions and transported and deposited outside the sediment (Neubauer et al., 2013). Under this situation, clams adopt a hydro-dynamically more stable horizontal orientation (Kondo, 1987; Gordillo and Archuby, 2012). In *A. antiqua*, both cardinal teeth and ligament are the main opponent to shearing stress, and muscles operate closing the two valves. In such settings, larger shells with a circular shape, strengthening of the cardinal teeth and bigger ligament and posterior muscle, as observed in Holocene shells from Caleta de Los Loros, are beneficial (Stanley, 1970; Neubauer et al.,
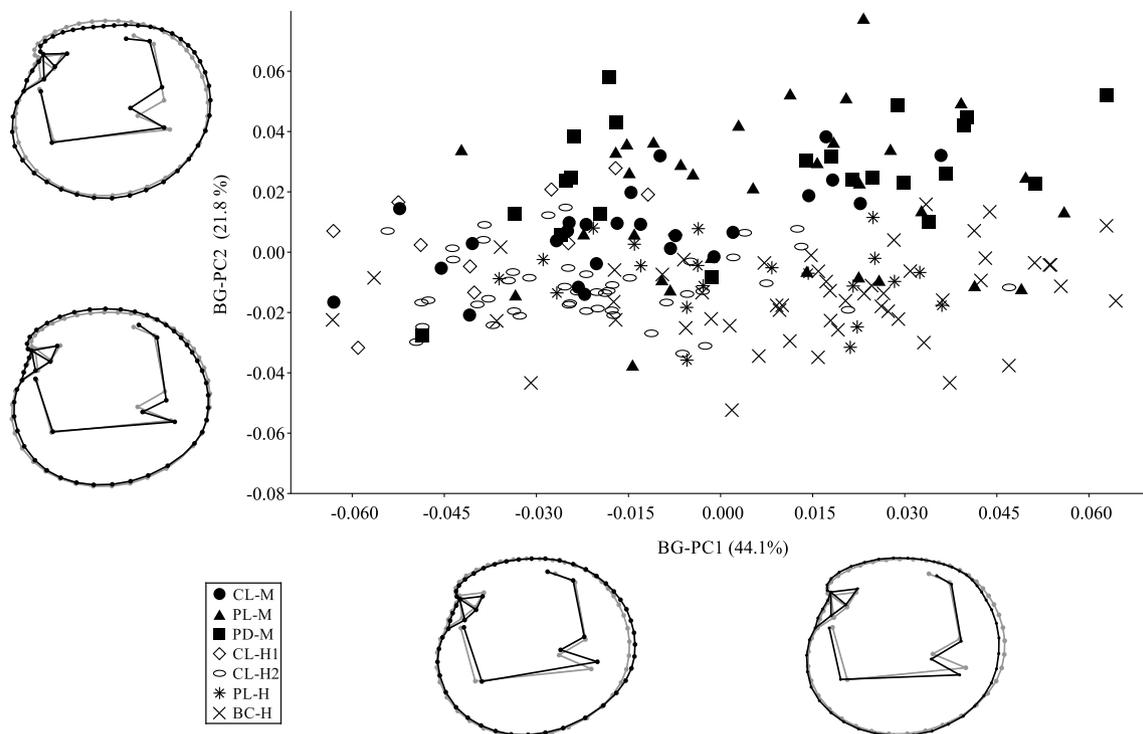
Fig. 4. First and second between-group principal components (BG-PC1 and BG-PC2, and respective percentage of the variance explained). Also the wireframe graphs of the reconstructed extreme configurations (black configuration) and consensus (grey configuration) are shown. See Table 1 for abbreviations.

Table 3
Classification matrix showing the number and percentage of individuals that were correctly classified (Jaccknife) by periods (A) and sites (B). See Table 1 for abbreviations.

A	Predicted membership		
	Modern	Holocene	Total
Modern	34	56	90
Holocene	72	69	141
Total	106	125	231

B	Predicted membership		
	Modern	Holocene	Correct percentage
CL-M	11	18	38.00
PL-M	15	21	42.00
PD-M	8	17	32.00
BC-H	31	27	47.00
PL-H	11	12	52.00
CL-H1	6	4	40.00
CL-H2	24	26	52.00

Table 4
(A) Procrustes distances between sites. (B) *p*-values for Procrustes distances among groups. See Table 1 for abbreviations.

(A) Procrustes distances between sites:

	CL-M	CL-H1	CL-H2	PD-M	PL-M	PL-H
CL-H1	0.0331					
CL-H2	0.0266	0.0283				
PD-M	0.0282	0.0484	0.0435			
PL-M	0.0272	0.0501	0.0441	0.0217		
PL-H	0.0327	0.0457	0.03	0.0362	0.0315	
BC-H	0.0384	0.0629	0.0427	0.0434	0.0388	0.0284

(B) p-values from permutation tests (10,000 permutation rounds) for Procrustes distances among groups:

	CL-M	CL-H1	CL-H2	PD-M	PL-M	PL-H
CL-H1	*					
CL-H2	*	*				
PD-M	*	*	*			
PL-M	*	*	*	–		
PL-H	*	*	*	*	*	
BC-H	*	*	*	*	*	*

2013). In line with this, Holocene assemblages from Bahía Creek (located on the open sea) showed these characteristics even more markedly than Holocene assemblages from Caleta de Los Loros, due to the bivalves were continually exposed to high wave action.

On the other hand, the observed differences between Holocene and modern assemblages from Puerto Lobos, located inland of the SMG, showed a similar morphological pattern to that observed in Caleta de Los Loros area. Boretto et al. (2013) reconstructed the Late Quaternary paleoenviromental evolution of this area through geomorphological and malacological analyses, concluding that the studied ridges were formed in a high-energy environment which does not correspond to nowadays environmental conditions. Also, they detected a malacofaunal shift as a consequence of a change in environmental conditions, i.e., in the present there is a greater proportion of species of the cold-

water Magellan Province typical (*Brachidontes purpuratus*, *Aulacomya atra*, among others), but in Holocene beach ridge the most typical species in the area (*Glycymeris longior*) was characteristic of northern Argentina (Argentinean Province). The Holocene samples from Puerto Lobos used in the present study were taken from the same ridges; therefore, the temporal morphological differences described in this area are possibly due to local environmental characteristics associated with temperature fluctuations that occurred during the Late Holocene.

5.1.2. Changes in primary productivity during the Holocene

A central topic of research in terms of body size evolution is Cope's rule, which states that a lineage increases in body size through time

(Jablonski, 1997; Gotanda et al., 2015; Heim et al., 2015). This could give advantages in defense against predation, predatory ability, and extended longevity, among others (Cope, 1896; Hone and Benton, 2005). In spite of this, most of explanations ignore the environmental context in which size evolution takes place (Hunt and Roy, 2006). Contrary to Cope's rule, our results demonstrate a considerably smaller size that distinguishes the modern assemblages from the fossil shells.

Growth rate and size in mollusks are generally associated with sea surface temperature and with primary productivity (Jones et al., 1989; Anderson, 2001). Particularly for *A. antiqua*, Escati-Peñaloza et al. (2010) showed that growth patterns are susceptible to productivity and sediments inside the San José Gulf (Patagonia, Argentina). Also, Boretto et al. (2014) found shell size differences between fossil and modern assemblages of *A. antiqua* from Bahía Bustamante (Patagonia, Argentina, Fig. 1), due to an increase in palaeo productivity and upwelling processes. Hence, it is plausible that the shell size differences across-time found in this study are down to differences in the primary productivity between the Holocene and the present.

5.1.3. Changes in burrowing behavior

The size of the pallial sinus is usually associated with burrowing depth in infaunal bivalves (Kondo, 1987). A bigger pallial sinus is beneficial to accommodate longer siphons that allow maintaining a connection with bottom waters (Stanley, 1970; Kondo, 1987; Neubauer et al., 2013). Besides, larger and rounder shells experience higher resistance within the sediment (Stanley, 1970; McLachlan et al., 1995; Neubauer et al., 2013). Consequently, these three features (bigger pallial sinus, larger and rounder shells) present in modern assemblages might be a response to a deeper and faster burial compared to those from the Holocene associated with changes in the substrate or a relationship with predators that forced the modern clams to make a quick escape (Gordillo and Archuby, 2012).

5.2. Spatial differences in morphological variation

Our results also showed spatial variability in the striped clam shell shape. Modern assemblages from Playas Doradas and Puerto Lobos showed clear differences with modern shells from Caleta de Los Loros: bivalves from the first two assemblages were smaller, more oval and had a more developed pallial sinus. These differences might be explained by phenotypic plasticity as a result of the different oceanographic conditions that exist nowadays between sites. SMG presents two well-defined areas separated by a frontal system, caused by a combination of circulation, tidal currents, coastal topography, and bathymetry (Amoroso and Gagliardini, 2010). North and west of the front, waters are comparatively warmer, saltier and nutrient-poorer, which limits phytoplankton growth. Furthermore, a marked thermocline occurs during most of the year. South and east of the front, waters are cooler, less salty, richer in nitrates, and vertically more homogeneous (Carreto and Verona, 1974; Piola and Scasso, 1988). Average difference in sea surface temperature between the two regions varies from 1 to 3 °C, except during winter when the thermal front vanishes (Gagliardini and Rivas, 2004). Modern shells from Playas Doradas and Puerto Lobos correspond to colder and nutrient-richer oceanographic conditions than those corresponding to Caleta de Los Loros. At first sight, it would seem that our results are contradictory because our assessments show that modern shells from Playas Doradas and Puerto Lobos are smaller than those from Caleta de Los Loros. Nevertheless, a phenomenon of upwelling, conditioned by winds, observed through satellite remote sensing was recognized in SMG (Pisoni et al., 2014). Upwelling processes transport deep, nutrient-rich water to surface levels leading to high biological productivity in the area adjacent to Caleta de Los Loros (Pisoni et al., 2014), which might explain the large shell size of *A. antiqua* in the area.

6. Concluding remarks

In this study we considered both modern and fossil *A. antiqua* shells in order to improve the use of this species in palaeo environmental reconstructions. Therefore, our study displays an integral analysis of morphology on fossil and modern shells of *A. antiqua* from Argentinean Patagonia. By including both a landmark and a semilandmarks morphometric method, various aspects of the shell can be captured and a detailed picture of the shell shape and size changes can be drawn and associate with the paleoenvironmental evolution of the natural protected area Caleta de Los Loros and San Matías Gulf during the Late Quaternary.

The main points are:

- Holocene assemblages from Caleta de Los Loros and Bahía Creek were more exposed to wave action and the influence of the tides, hence, rounder and larger shells, developed posterior muscle scar, cardinal teeth and ligament might be an adaptation to this high-energy habitat by increasing mechanical stability.
- The morphological differences through time described for Puerto Lobos assemblages are possibly due to local environmental characteristics associated with temperature fluctuations that occurred during the Holocene in that area.
- Three features presented in modern assemblages (small size, ovoid-shaped and small pallial sinus) might be responses to a deeper and faster burial than in Holocene specimens; probably associated with changes in substrate or predation pressure.
- Spatial differences in morphological variation found in modern assemblages might be explained by the different oceanographic conditions that exist in SMG at the present due to two well-defined areas separated by a frontal system.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.quaint.2018.05.027.

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