Quaternary International xxx (2014) 1–11

Contents lists available at ScienceDirect

Quaternary International

journal homepage: www.elsevier.com/locate/quaint

Shell morphometric variations in a Patagonian Argentina clam (*Ameghinomya antiqua*) from the Mid-Pleistocene (MIS 7) to the present

G.M. Boretto [a], [*], M.C. Baranzelli [b], S. Gordillo [a], I. Consoloni [c], G. Zanchetta [c], G. Morán [a]

[a] *Centro de Investigaciones en Ciencias de la Tierra, Consejo Nacional de Investigaciones Científicas y Técnicas (CICTERRA, CONICET-UNC), Av. Vélez Sarsfield 1611, X5016GCA Córdoba, Argentina*
[b] *Laboratorio de Ecología Evolutiva y Biología Floral, Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET–Universidad Nacional de Córdoba, Av. Vélez Sársfield 1611, X5016GCA Córdoba, Argentina*
[c] *Dipartimento di Scienze della Terra, University of Pisa, Via S. Maria 53, 56126 Pisa, Italy*

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Ameghinomya antiqua
Bivalvia
Pleistocene
Holocene
Geometric morphometrics
Patagonia

ABSTRACT

This study analyses the shell morphological variations in the venerid clam *Ameghinomya antiqua* recovered from Bustamante Bay, in Patagonia Argentina, from the Pleistocene (MIS 7–MIS 5) to the present. Fossil and modern *A. antiqua* shells were analyzed using the Elliptic Fourier Analysis (EFA) and landmark methods. Both methods were successful in separating the studied bivalves into four chronologic groups -MIS 7, MIS 5, Holocene and Modern-, showing significant shell shape and size variations throughout the considered period. The MIS 7 shells have an ovoid rounded shape, the MIS 5 shells exhibit an ovoid and marked prosogyrous condition, while Holocene and Modern shells are more rounded to subquadrangular. MIS 5 shells are also larger than the others groups. These morphological variations could be related to the phenotypic plasticity of *A. antiqua* as a response to different environmental conditions, representing the ability of this species to adapt to different sub-environments as an answer to substrate variations and the hydrological changes which apparently took place during the period.

1. Introduction

Geometric morphometric analyses are used to find variations in the shape and size of many biological structures in a wide spectrum of organisms (McGuire, 2010; Van Heteren et al., 2013; Taglioretti et al., 2014). In this sense, the shape in bivalves is a morphological key that reflects both phylogenetic history and life habits (Stanley, 1970; Crampton and Maxwell, 2000; Gordillo et al., 2010). However, although some studies have used mollusc shell shape to differentiate between species of a similar shape (Ferson et al., 1985; Innes and Bates, 1999; Dommergues et al., 2003; Rufino et al., 2006; Costa et al., 2008a, b) or to analyze intra-specific variation across a wide geographical range (Palmer et al., 2004; Krapivka et al., 2007; Márquez et al., 2010), there are few studies which analyse the variation in shell shape through the geological time and which address the factors that may affect the variation in morphological patterns. Ferson et al. (1985), Innes and Bates (1999), Palmer et al. (2004), Rufino et al. (2006), Krapivka et al. (2007); Costa et al. (2008a, b), Gordillo et al. (2010), Neubauer et al. (2013), have demonstrated that Elliptic Fourier Analysis (EFA) of mollusc shell outlines and landmark analyses can be very useful for defining specific shape and size features in fossil specimens.

In Patagonia Argentina, *Ameghinomya antiqua* shells are common and well preserved as fossil (Holocene and Pleistocene) specimens in the marine coastal deposits. Modern shells are abundant in the present active beach, making this species suitable for geometric morphometric analyses. The Quaternary deposits from this region preserve impressive traces of sea-level oscillations and are therefore of high scientific value, as they provide an insight into climatic changes and tectonic uplift history in this area from the Middle–Late Pleistocene to the present (Feruglio, 1950; Rutter et al., 1989, 1990; Codignotto et al., 1992; Rostami et al., 2000; Schellmann and Radtke, 2000, 2003, 2010; Pedoja et al., 2010, 2011; Isola et al., 2011; Ribolini et al., 2011). Bustamante Bay, the study area of this work, is dominated by Quaternary coastal marine outcrops, organized in series of beach ridges and marine terraces composed of sand, gravel and mollusc shells. Along the coastline, a

---

* Corresponding author.
E-mail addresses: gmboretto@yahoo.com.ar, gmboretto@gmail.com (G.M. Boretto).

http://dx.doi.org/10.1016/j.quaint.2014.09.033
1040-6182/© 2014 Elsevier Ltd and INQUA. All rights reserved.

Please cite this article in press as: Boretto, G.M., et al., Shell morphometric variations in a Patagonian Argentina clam (*Ameghinomya antiqua*) from the Mid-Pleistocene (MIS 7) to the present, Quaternary International (2014), http://dx.doi.org/10.1016/j.quaint.2014.09.033

sandy-gravel beach develops and then to the inland, Holocene landforms (3–10 m asl; Schellmann and Radtke, 2000, 2010) and several coastal Pleistocene deposits are extended. Those, belonging to Last Interglacial, Marine Isotopic Stage (MIS) 5 (10–19 m asl; Schellmann and Radtke, 2000, 2010) and those dated as Penultimate Interglacial, MIS 7 (15–23 m asl; Schellmann and Radtke, 2000, 2010) were considered.

In this context, the aims of this study are: (1) to determine whether *A. antiqua* shells from different Patagonian Quaternary coastal deposits located along the Bustamante Bay shoreline can be distinguished based on outline (EFA) and landmark analyses (taking shape and size into consideration); and (2) to evaluate whether the shape of fossil and modern *A. antiqua* shells from a single region display morphologic changes through time in order to use morphometrics as another environmental proxy when evaluating environmental changes in the region.

2. Geological and geographical setting

Bustamante Bay (Chubut Province) is located between 45°13′–44°54′S and 66°50′–65°48′W (Fig. 1). An outstanding succession of raised beach deposits is exposed over a total area of about 350 km^2, extending up to 10 km landward and reaching an elevation of 100 m (a.s.l.). Past landforms and deposits are particularly well preserved because weathering processes are very limited (Isola et al., 2011). The oldest rock unit, the Complejo Marifil (Marifil Complex), consists of Jurassic rhyolites, ignimbrites and volcanoclastic conglomerates. It is often covered by thin to very thick debris deposits and crops out at the base of present and fossil shore lines, islands and cliffs (Isola et al., 2011). Subordinate outcrops expose the Paleocene marine deposits of the Salamanca Formation and the Paleocene continental deposits of the Río Chico Formation (Sciutto et al., 2000; Lema et al., 2001). The landward part of the area is characterized by the outcrop of a pediment surface covered by rounded volcanic pebbles and gravel in a sandy matrix, occasionally strongly cemented by carbonate (Isola et al., 2011).

Due to its latitudinal location and to the presence of the Andean Cordillera, the Patagonian climate is characterized by two main meteorological features: very strong winds and low precipitation (Coronato et al., 2008). Constant dry winds blow strongly from the west, particularly in the summer months, and aeolian erosion shapes rocky outcrops and deflates loose sediments (Isola et al., 2011). The low amount of precipitation (<300 mm/y) and moderate thermal amplitude allow the growth of a sparse cover of grass and shrubby vegetation, consistent with the semiarid climate of Patagonia (Isola et al., 2011). Wave action is the most important morphoclimatic agent responsible for the past and present landscape. The area is characterized by a high-energy system with intense storms and a macrotidal regime. A tidal range of >4 m can be reasonably assumed for most of the Patagonian coast (Isola et al., 2011) (Fig. 1).

The landscape of Bustamante Bay is characterized by elevated littoral deposits along the Patagonian Atlantic coast, which contain pebbly gravel and sand with highly fossiliferous marine strata (Feruglio, 1950; Cionchi, 1988; Rutter et al., 1989, 1990; Schellmann and Radtke, 2000, 2010; Aguirre et al., 2005). Schellmann and Radtke (2000, 2010) discriminated the spatial distribution of these deposits, classified as beach ridge systems and littoral terraces based on features (morpho-pedo-stratigraphic differentiation) and their ages. Their elevation is probably a consequence of glacioeustatic sea-level fluctuations (Schellmann and Radtke, 2000) or of tectonic movements (Rostami et al., 2000; Pedoja et al., 2010, 2011). Schellmann and Radtke (2000) dated these quaternary deposits using different methods: electron spin resonance (ESR) and α-spectrometric ^{230}Thorium/^{234}Uranium for the

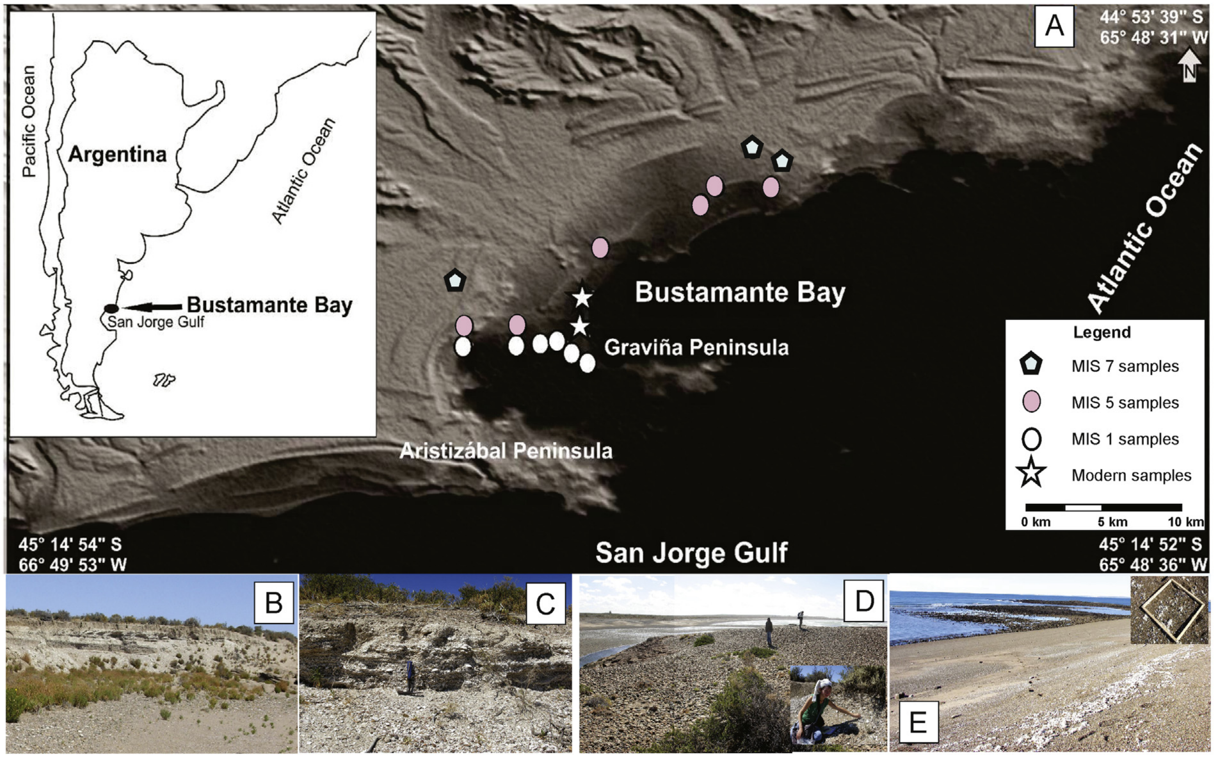

Fig. 1. A. Location of the study area. B. MIS 7 coastal deposits. C. MIS 5 coastal deposits. D. MIS 1 coastal deposits. E. Modern beach of Bustamante Bay and detail of the quadrate used for sampling modern specimens.

Please cite this article in press as: Boretto, G.M., et al., Shell morphometric variations in a Patagonian Argentina clam (*Ameghinomya antiqua*) from the Mid-Pleistocene (MIS 7) to the present, Quaternary International (2014), http://dx.doi.org/10.1016/j.quaint.2014.09.033

Pleistocene beach ridges and radiocarbon (^{14}C) for the Holocene deposits. These authors indicated these several beach ridge systems have been formed starting at around 400 ka and represent episodes equivalent to the maximum sea-level highstands of the last interglacials.

3. *Ameghinomya antiqua*

This species is a benthic bivalve typical of the Magellanic region (Balech and Ehrilch, 2008) extending along the Atlantic Ocean from 34°S to the Beagle Channel (54°S) and in along the Pacific Ocean as far as Callao (12°77S) in Perú (Castellanos, 1967; Márquez et al., 2010). It lives in the sandy soft bottom substrate, from the shallow intertidal up to 100 m deep in temperate-cold waters (Castellanos, 1967). In the study region the maximum depth from where this species was recovered alive is 60 m, and the absent at greater depths is probably associated with an increment of the fine sediment fraction (Zaixso, 1996). The *A. antiqua* well preserved shells are valuable for morphometric geometric analyses, as they are complete and retain the scars of their internal muscles, the most important conditions to apply these methods.

3.1. Feeding mode

A. antiqua is a filter species, strict suspension feeder, which does not extend the siphon beyond the surface sediment, so it must stay close to the surface to feed (Lardies et al., 2001). It is often found in regions of high primary productivity, combined with upwelling areas (Urban, 1996). In this species, as other suspension feeders, feeding behavior is strongly associated with the hydrodynamic conditions of the environment (Lardies et al., 2001; Escati Peñaloza, 2005).

3.2. Shell features

The shells of *A. antiqua* have an external aspect typically equivalve, with subcircular contorn, prosogyrous and sub-central umbos, and strong external ligament (Urban, 1994; Escati Peñaloza, 2005). The external sculpture consists of fine concentric lines, sometimes high, crossed by numerous radial grooves, wide and flattened giving a whole aspect of crosslinked (Escati Peñaloza, 2005). In juvenile specimens, concentric lines are delicate, high and at 90° to the surface of the shell, while in the larger bodies of the adults these sheets remained elevated in both anterior and posterior zones. The external color is white to cream (Escati Peñaloza, 2005).

3.3. Adult size

The adult stage of bivalves is related to the size achieved with the first sexual maturity (Calvo et al., 1998). *A. antiqua* reaches reproductive maturity before the first year of life (size around 20–24 mm of length) according to Verdinelli and Schuldt (1976), from samples collected in the Golfo Nuevo (Patagonia Argentina). Osorio et al. (1982) suggested the adult stage occurred when the bivalves were two years old, and Escati Peñaloza et al. (2010) indicated a size of sexual maturity between 13 to 22 mm, estimated around 2 years of age. Fertilization is external. The effort put in reproductive processes for this species would be carried out in the winter months, decreasing the energy invested in growth (Escati Peñaloza, 2005). The latter would generate a seasonal growth pattern causing marked annual growth rings in the winter (Verdinelli and Schuldt, 1976; Clasing et al., 1994).

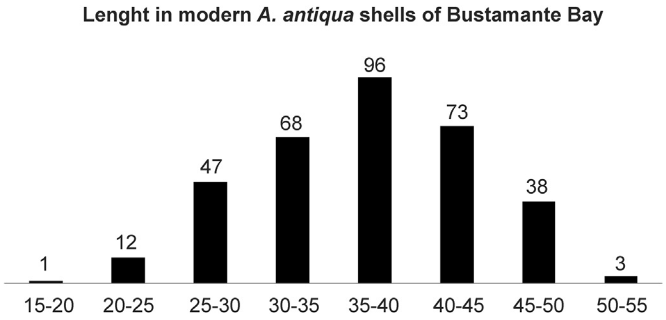

Fig. 2. Histogram showing the $N_M = 338$ and the size distribution of *A. antiqua* based on shell length in modern assemblages.

4. Materials and methods

4.1. Field sampling and shell selection

All adult specimens of *A. antiqua* were collected in April 2010 and in February 2011 along the area of Bustamante Bay (Fig. 1). Fossil specimens from the Holocene (MIS 1), MIS 5 and MIS 7 were taken from bulk samples (50 cm^3) randomly chosen considering the exposed marine deposits dated by Schellmann and Radtke (2000). For comparison with data on fossil clams, modern shells were randomly sampled using a square of 1 × 1 m (Fig. 1D) from the present active beach, which includes the material obtained in the surface from 5 squares placed along the high intertidal level to obtain a representative N. These modern samples preserved their hinges, which indicate that their death took place a short time before being collected.

The number (N) of the fossil samples obtained was: $N_H = 50$, $N_{M5} = 68$, $N_{M7} = 56$, minor quantities respect the N taken for the Modern shells, $N_M = 338$. Details of the length from these shells are showed in Fig. 2. To compare similar amounts of samples from each population, a sub-sampling in the Modern assemblages was done within the 5 quadrats, randomly getting 40 modern valves. The reduction in the number sample of the modern specimens is made mainly to avoid an imbalance between the factor levels to analyse (i.e. “assemblages from different times”) (Sokal and Rohlf, 1981).

In the laboratory, the samples, which were divided into four groups according to their age: MIS 7, MIS 5, Holocene (H) and Modern (M), were reselected considering arbitrarily the right valves, entire and with the internal muscle scar, in order to analyze the shape and size variability through the geometric morphometric methods. For these reason, the number of samples processed were $N_{MIS7} = 26$, $N_{MIS5} = 26$, $N_H = 30$, $N_M = 30$ (Table 1). After removing the sediment and cleaning the shells, all valves were digitized (concave side upwards) with a Nikon Coolpix P-100 digital camera. Shell shape was studied in terms of shape variables generated from two 2D geometric morphometric methods, i.e. outline and landmark analysis.

Table 1
List of samples collected. The sites sampled were defined stratigraphically and dated by *Schellmann and Radtke (2000): **ESR method, ***^{14}C method. The Pleistocene samples are correlated with the respective Marine Isotopic Stage (MIS).

Marine deposit stratigraphy*	Dated*	N° of shells	Length range (mm)	High range (mm)
MIS 7	~221 ± 27 ka**	26	46–59	41–52
MIS 5	~127 ± 15 ka**	30	49–63	42–56
Holocene	~4924 ± 80 BP***	30	34–58	38–55
Modern	Modern	30	21–50	18–45

Please cite this article in press as: Boretto, G.M., et al., Shell morphometric variations in a Patagonian Argentina clam (*Ameghinomya antiqua*) from the Mid-Pleistocene (MIS 7) to the present, Quaternary International (2014), http://dx.doi.org/10.1016/j.quaint.2014.09.033

Individual ontogenetic age of each specimen was calculated using the method employed by Escati Peñaloza (2005), which consists of recording the number of growth rings that develop along the growth axis in each shell. To facilitate the reading of the rings, the outer surfaces shells were polished, shifting the reticular sculpture, to recognize the clear growth marks. Escati Peñaloza (2005) considered those brands as annual, well-defined, sharply delineated, dark, and forming conspicuous concentric rings, easily identifiable, even to the naked eye. The individual ontogenetic ages were determined to calculate static allometry in the samples.

4.2. Morphometric geometric methods

4.2.1. Outline analysis

Variation of shell shape was studied applying Elliptic Fourier Analysis (EFA), which decomposes a curve into a sum of harmonically related ellipses (Lestrel, 1997). For each valve, images in internal view were used. The closed contours of each shell outline were obtained as chain-coded data from the digital images (Freeman, 1974). The sequences of points were then adjusted to a mathematical function, and the parameters of this function were used as valve shape variables. These parameters are called harmonics, and are sinusoidal curves which have increasing frequency and decreasing amplitude. These curves were added to the function in order to describe the outline. The number of harmonics was calculated following Crampton (1995) and the Fourier series was truncated at $n = 10$. The orientation, size and starting point of the different outlines were standardized (Kuhl and Giardina, 1982) so that three of the four elliptic Fourier coefficients describing the first harmonic ellipse were constant for all outlines. The software Shape v.1.3 (Iwata and Ukai, 2002) was used for all the analyses. Principal component analysis of the variance–covariance matrix (Rohlf and Archie, 1984; Rohlf and Marcus, 1993; Crampton, 1995) was applied to summarize shape variation based on harmonic coefficients for each shell. The principal components represent global changes in the shape of the structure studied. The first components describe the general outline and explain the greatest part of the shape variation, while the following components describe small scale outline variations.

4.2.2. Landmarks

The methodology described in Márquez et al. (2010) for *A. antiqua* was followed in order to select the 13 landmarks (Fig. 4), using the same shells as in the outline analysis. Landmark coordinates were captured using tpsDIG version 2.04 software (developed by F.J. Rohlf, Department of Ecology and Evolution, SUNY, Stony Brook, NY, USA). These coordinates were used to obtain average or consensus configurations in order to run a Generalized Procrustes Analysis. In a subsequent step, the partial warps were computed, which describe individual landmark configurations as deformations of the average or consensus configuration. Principal component analysis on partial warp scores was used to obtain $2p - 4$ orthogonal relative warps (RW; where p is the number of landmarks). RW summarize shape information and can be used in subsequent statistical analyses (Adams et al., 2004). Relative warps generated from the landmark analysis were considered to be invariant with respect to mathematical differences in translation, rotation and scale. This analysis was carried out using MorphoJ software (Klingenberg, 2011).

4.2.3. Shell size

To evaluate differences in shell size between specimens from the different Quaternary localities, landmark analyses were used to calculate the centroid size (CS), which represents the scalar value of the size of the structure analyzed (Zelditch et al., 2004).

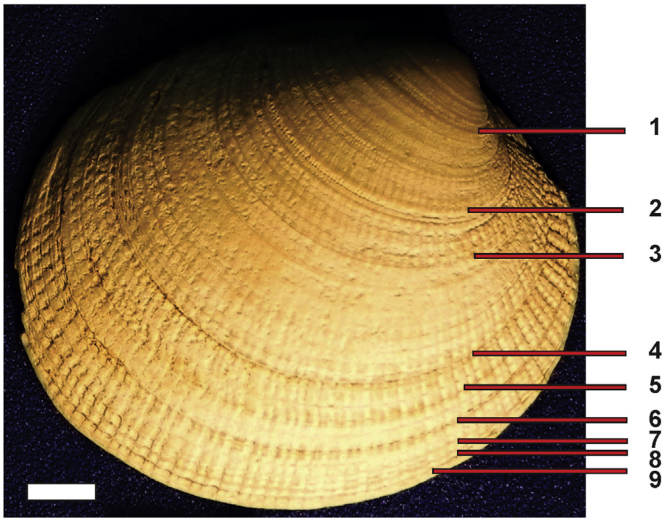

Fig. 3. External view of the superficial growth rings in *A. antiqua*. Scale bar = 10 mm. Each line indicates a year, considering the Escati Peñaloza (2005) method.

4.2.4. Statistical analyses

Different statistical tests and putative allometric controlling effects both related to the size allometry (effects of size/shape) and with age (effects of age/shape) were performed. For the landmarks analysis, we computed the multivariate regression of shape (RW, as dependent variables) on centroid size and age variables (independent variables). It has been suggested that such regression is useful to detect and remove the effect of size, age or environment before shape comparisons between groups (Klingenberg, 2008). Furthermore, this procedure was successful in assessing such allometric effects on these organisms (Márquez et al., 2010). For EFA analysis to evaluate the effect of ontogenetic allometry, simple linear

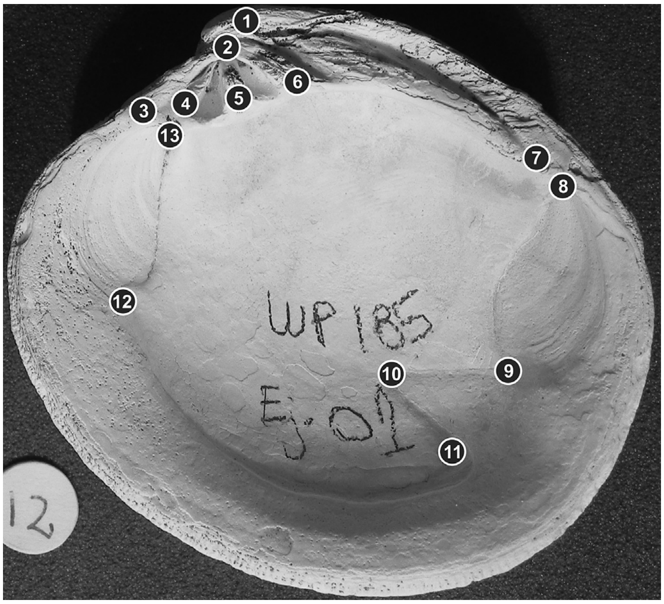

Fig. 4. Diagram showing the shell outline and the position of the 13 landmarks used to define the inner surface of the right shell shape. These landmarks are: (1) the tip of the dorsal hinge ligament, (2) the tip of the cardinal teeth, (3) the scar of the lunule, (4) the end of the anterior cardinal tooth, (5) the midline cardinal tooth, (6) the end of the posterior cardinal tooth, (7) the tip of the posterior hinge ligament, (8, 9) the anterior adductor muscle scar, (10) the tip of the pallial sinus, (11) the lower margin of the pallial sinus, (12, 13) the posterior adductor muscle scar. To improve the identification of points, all landmarks were painted with small dots of permanent marker.

Please cite this article in press as: Boretto, G.M., et al., Shell morphometric variations in a Patagonian Argentina clam (*Ameghinomya antiqua*) from the Mid-Pleistocene (MIS 7) to the present, Quaternary International (2014), http://dx.doi.org/10.1016/j.quaint.2014.09.033

regressions were performed between each harmonic and the individual shell age, as Claude (2008) recommended.

To describe the variation in shell shape in relation to age captured by the two analyses (EFA and landmark analysis), a canonical variate analysis (CVA) was performed using the sample lots as a criterion for classification. This type of analysis searches for those characteristics in a dataset, which are most appropriate to distinguish multiple groups. For that purpose, the Mahalanobis distance (MD) matrix for the shells was calculated and 10,000 permutations were performed to estimate the p value for significant differences between groups. Canonical variate analysis was performed using the *Candisc* package and permutation routine in R v.2.15.0 (The R Development Core Team, R Foundation for Statistical Computing, http://www.R-project.org/index.html). For the EFA analysis, shape differences along the first two CV axes were described, along with the average ±2 standard deviation shape of the main harmonics that were strongly correlated with them. For the landmark analysis, shape differences along the first two CV axes were described using thin-plate spline deformation grids, generated in MorphoJ.

To evaluate the differences in shell size, a one-way analysis of variance (One-way ANOVA) was performed using the temporal gradient as a factor and including the CS and area as response variables. An a posteriori Tukey test ($p < 0.05$) was also performed in order to identify the periods in which shell size differences occurred. These analyses were performed in R v.2.15.0.

Finally, to evaluate the relationships between different Quaternary groups, revealed by EFA, landmark analysis and shell size analysis, a cluster analysis was performed using an unweighted pair group method with Euclidean mean, including the variables from each analysis. This technique is based on the similarities or distances between multiple shape or size variables. This analysis was performed in InfoStat (Di Rienzo et al., 2009).

5. Results

In the EFA analyses, the first five harmonics explained 93.10% of the outline shape variation. The simple linear regressions between these harmonics and the individual age of the specimens were not significant ($p > 0.05$) for the 4 harmonics, and the 5 harmonic showed an allometric significant effect ($p = 0.021$; Appendix). Therefore, the following analyzes were performed using only the harmonics without allometric effect. The first canonical axis illustrates the degree of roundness associated with the antero-posterior elongation of the shells. The second canonical axis illustrates the changes related to the inflation of the umbo and corresponding lunule shape with respect to the dorsoventral axis (Fig. 5A). Shells from MIS 7, MIS 5 Holocene and modern coastal deposits were successfully distinguished applying the CVA, revealing overall differences in shell outline shape among groups. After the permutations, the Mahalanobis distance comparisons between groups were significant ($MD_{M\text{-}H} = 0.0240$; $MD_{M\text{-}MIS5} = 0.0340$; $MD_{M\text{-}MIS7} = 0.0261$; $MD_{H\text{-}MIS5} = 0.0365$; $MD_{MIS5\text{-}MIS7} = 0.0381$; $p < 0.05$ for all contrast), while the MIS 7 shells did not show significant difference respect the Holocene assemblage ($MD_{H\text{-}MIS7} = 0.0205$; $p = 0.835$). The Pleistocene specimens appear within the maximum values of the first axis. MIS 7 assemblages present an ovoid to rounded configuration and a less prosogyrous condition; they are extended along the second axis to positive values. The MIS 5 group is characterized to have ovoid shape and a marked prosogyrous condition. On the other hand, the Holocene and modern specimens are grouped on the minimum values of the first axis, defined by the opposite configuration, i.e. a largely circular shell shape (Fig. 5A). Holocene specimens are the most rounded and develop a prosogyrous condition. Modern specimens are grouped, mainly, within the maximum values of the second axis, and are differentiated by having higher inflation and a less prosogyrous umbo; their shell outline is slightly sub-quadrangular. Despite these differences, an overlap is observed between the polygons that defined the shape of each group. The dendrograms calculated on shape using the Euclidean distance matrix showed that the greatest distances occur between the MIS 5 group and the other assemblages, and the Holocene and MIS 7 assemblages seem to be most similar (Fig. 5B).

The multivariate regression of shape calculated with the landmarks method were not significant ($p = 0.06$), and the variation explained by the individual shell age was 4.27%. In the case of the

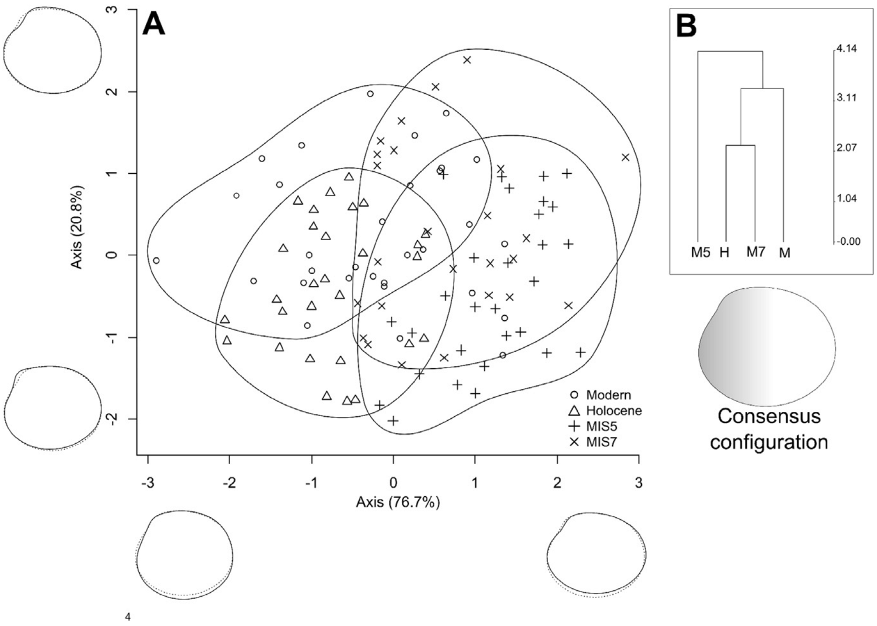

Fig. 5. Contour method. A. Analysis of shell shape variation along the first two canonical axes, plus diagrams of the reconstructed extreme configurations. The polygons represent confidence intervals involving >90% of the data for each period with respect to its average value. B. Cluster analysis showing the relationship of shell shapes between different ages.

Please cite this article in press as: Boretto, G.M., et al., Shell morphometric variations in a Patagonian Argentina clam (*Ameghinomya antiqua*) from the Mid-Pleistocene (MIS 7) to the present, Quaternary International (2014), http://dx.doi.org/10.1016/j.quaint.2014.09.033

multivariate regression of shape and centroid size was significant ($p < 0.01$). The allometric effect is given by the high degree of association between RW1 and shell size. Removing this RW, the correlation becomes nonsignificant ($p = 0.32$), in which the shell size explained a poor shape variation, around 2.37%. As RW1 cannot be discarded, because it explains the 19.72% of the shape variation, the subsequent multivariate analysis included the residual values obtained by the regression made between the RW1 and the centroid size, correcting the allometric effect. Shells of different ages are successfully identified applying CVA based on the total number of morphometric variables obtained from landmark analysis. The variation represented by the first CV axis is related to overall shell shape, particularly expressed in the landmarks 1, 3, 7, 8, 9, 11, 12 and 13, and the distance between landmarks 8 to 12. These differences include variation in the lunule region, cardinal teeth landmarks 2, 4, 5, 6 and posterior adductor muscle scar (Fig. 6A). As a result, the first canonical axis is associated with a gradient of dorsoventral expansion of the shell. The second CV axis is mostly associated with changes in the lunule region, anterior adductor muscle scar and pallial sinus, displayed in landmarks 1, 2, 3, 6, 8, 9, 10 and 11, and the distance between landmarks 4 to 13 (Fig. 6A). The CVA based on landmark method revealed differences in internal shell shape related to a temporal gradient. The Mahalanobis distances were significant: $_{\mathrm{MDM\text{-}H}} = 2.8051$; $\mathrm{MD}_{\mathrm{M\text{-}MIS5}} = 2.8013$; $\mathrm{MD}_{\mathrm{M\text{-}MIS7}} = 2.7798$; $\mathrm{MD}_{\mathrm{H\text{-}MIS5}} = 2.1273$; $\mathrm{MD}_{\mathrm{H\text{-}MIS7}} = 2.2262$; $p < 0.01$ for all contrast, but there was an exception between MIS 5 and MIS 7, as this value was nonsignificant, $\mathrm{MD}_{\mathrm{MIS5\text{-}MIS7}} = 1.6639$; $p = 0.4199$. In landmark analysis, differences between the assemblages were much greater than in the outline analysis. The Pleistocene shells present a similar landmarks configuration, but MIS 7 assemblages have more influence relate to the first CV axis, principal in landmarks 3-1-7-8-12, while MIS 5 shells are characterized by the landmarks 1-3, 2-4-5-6, 8-9-11. Holocene specimens are defined by the distances between landmarks 1-3 and 4-5-6, and the landmarks 9-11 indicate a more open pallial sinus than in the other groups. Modern shells are grouped along the second CV axis, the more significant points for their configuration between 1-3, 2-4-6, 7-8 and 4-13. The dendrogram calculated from landmark data showed that modern specimens are the most different, and the Pleistocene assemblages are the most similar (Fig. 6B).

Comparison of shell size showed clear differences between groups ($F = 7.57$ $p < 0.0001$), which are corroborated by an a posteriori Tukey test ($p < 0.05$) (Fig. 7A). The MIS 5 specimens are largest ($\mathrm{CS} = 7.41$), followed by the MIS 7 ($\mathrm{CS} = 6.99$), the Holocene ($\mathrm{CS} = 5.358$) and modern specimens ($\mathrm{CS} = 5.43$). The dendrogram based on the size parameter showed that modern shells are the most different from the other groups, while the Holocene assemblages are closest to MIS 7 (Fig. 7B).

6. Discussion

6.1. General discussion

The results have shown that based on shape and size, the EFA and landmark analyses successfully distinguished four (MIS 7, MIS 5, Holocene and Modern) assemblages of *A. antiqua*. The contour analysis essentially shows: (1) ovoid-rounded shaped and less prosogyrous shells for the MIS 7 assemblages; (2) ovoid, very prosogyrous and largest shells characterized the MIS 5 group; (3) circular and prosogyrous configuration shells defined the Holocene group, although smaller than the Pleistocene shells; and (4) the Modern assemblages are characterized to have the smallest size and a subquadrangular and less prosogyrous shape. The landmarks

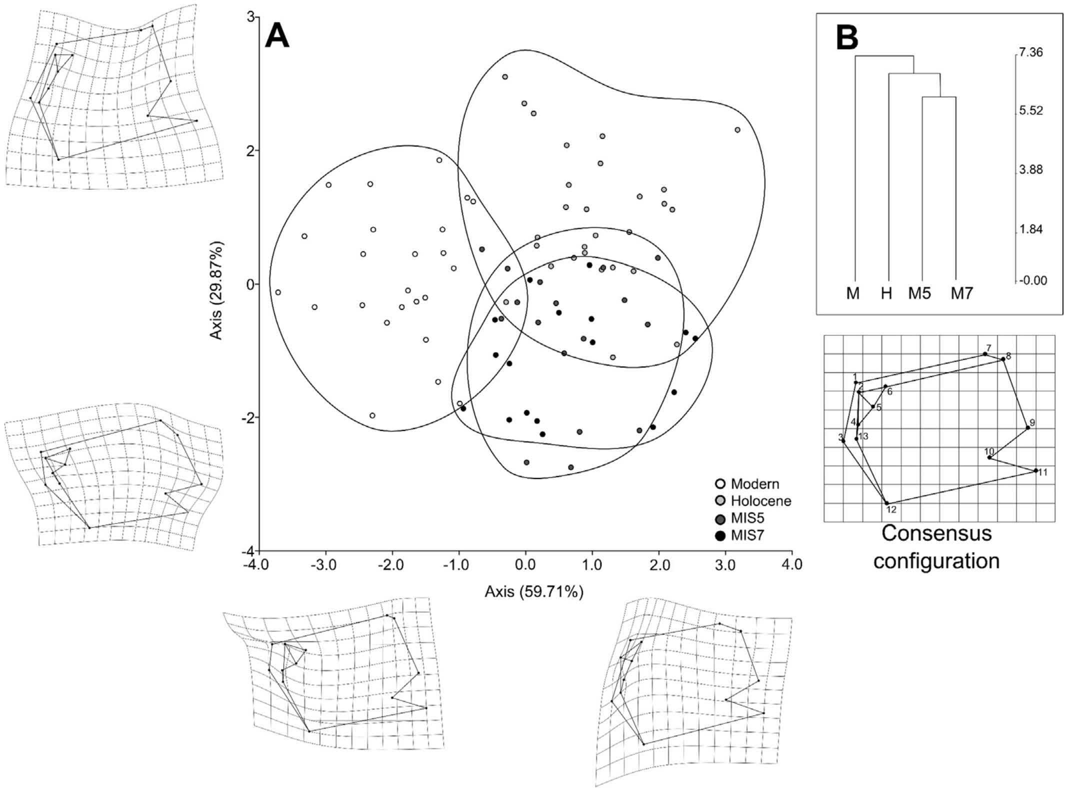

Fig. 6. Landmark method. A. Analysis of the overall shell shape variation along the first two canonical axes, and deformation grids along the first CV axis. The polygons represent confidence intervals involving >90% of the data for each period with respect to its average value. B. Cluster analysis showing the relationship of shell shapes between different ages.

Please cite this article in press as: Boretto, G.M., et al., Shell morphometric variations in a Patagonian Argentina clam (*Ameghinomya antiqua*) from the Mid-Pleistocene (MIS 7) to the present, Quaternary International (2014), http://dx.doi.org/10.1016/j.quaint.2014.09.033

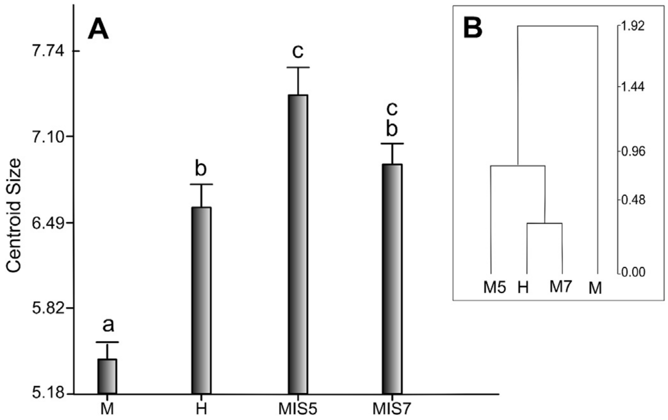

Fig. 7. A. Variation of shell size from different ages estimated through the centroid size value using the landmark method. Different letters indicate significant differences between groups. B. Cluster analysis showing the relationship of shell size between different ages.

analyses accentuate the shell internal features, and therefore these results are different from the EFA. The clusters from the outline method and the shell size produced a marked separation between the MIS 5 assemblages from the other groups. The clusters from landmarks indicated the most similar internal features are between MIS 7 and MIS 5 shells, while Holocene assemblages seems to represent a transition to the Modern group, which is isolated.

Previous work by Márquez et al. (2010) in living populations of *A. antiqua* from the San José Gulf (Patagonia, Argentina) found differences in shell morphology associated with environmental changes. These authors indicated that the specimens located in the eastern sector of the gulf are smaller and more rounded than the specimens found on the western side. Factors such as topography, substrate, bathymetry, current circulation and tides all distinguish the eastern side of the San José Gulf from the western side, and these environmental differences are reflected in the shell shape; the clam populations which live on the western side are affected by more dynamic environmental conditions, associated with more turbulent currents and marked seasonal changes in the sea surface temperature (SST) (Amoroso, 2004; Escati Peñaloza, 2005; Amoroso and Gagliardini, 2010). More recently, Neubauer et al. (2013), in other venerid species, considered the paleoenvironmental changes as the principal factor linked to the phenotypic evolution over time. These authors performed morphometric geometric analyses, landmarks and EFA, on bivalves of the late Middle Miocene from two stratigraphically successive biozones of the Central Paratethys Sea. Their studies indicated significant phenotypic differentiation between specimens from these biozones: the bivalves became distinctly larger and thicker over geological time and develop circular shells with stronger cardinal teeth and deeper pallial sinus. Neubauer et al. (2013) noted that data in the paleoenvironmental changes in this study area suggest the phenotypic shifts to be functional adaptations. The typical habits for the species *Polititapes* changed to extensive, very shallow shores exposed to high wave action and tidal activity. Due to the growing need for higher mechanical stability, the bivalves produced larger shells with stronger cardinal teeth. The deepening pallial sinus is related to a deeper burrowing habit, which is considered to impede being washed out in the new high-energy setting (Neubauer et al., 2013).

Therefore, given this background, we propose to associate the morphology variations in the *A. antiqua* shell from different Quaternary deposits of Bustamante Bay to the paleoenvironmental changes in the region. To provide a reliable explanation of this, an environmental characterization of the study area is necessary.

6.2. Paleoenvironmental interpretation of the study zone

6.2.1. Pleistocene interglacial: MIS 7 and MIS 5

Significant eustatic movements took place during the different interglacial/glacial cycles, with lower sea levels during colder periods of at least hundreds to tens of metres below present sea level (Rabassa et al., 2005; Rabassa, 2008). During these glacial periods, a large portion of the Patagonian continental shelf was exposed as a consequence of these glacioeustatic movements, and enormous plains developed along the Atlantic coast (Rabassa et al., 2005). The geographical configuration of Bustamante Bay during the Pleistocene would have been very different from the present day configuration, as the bay would not have been formed (Ponce et al., 2011) (Fig. 8). Taking into consideration the paleogeographical evolution model proposed by Ponce et al. (2011), the MIS 7 and the MIS 5 sample sites, the coastal margin during this time would have been linked to the open sea. Environmental factors such as the circulation of currents, tides, waves and the type of substrate sediments, among others (Schellmann and Radtke, 2000, 2010), would have been more dynamic than in a protected zone such as a bay. Under these conditions of high energy, the MIS 7 and MIS 5 specimens of *A. antiqua* would have been exposed to similar environmental requirements and selective pressures favouring a capacity of burial, as indicated by the similar landmarks configuration for both assemblages. The internal features emphasized are the overall shape configuration, umbo region, cardinal teeth and adductor muscles. Additionally, the sedimentological composition of the Pleistocene coastal deposits is dominated by medium-coarse sand to gravel (Cionchi, 1987, 1988; Schellmann and Radtke, 2000), thus indicating substrates that would have allowed a deeper range of movement during escape (Zwarts and Wanink, 1989). However, the main differences between MIS 7 to MIS 5 assemblages are the shape and size: the younger have an ovoid configuration, marked prosogyrous condition, and are larger. The deep burial requirements are associated with: (1) the ovoid shell shape (Stanley, 1975) and (2) the shape and dimensions of the pallial sinus, the scar linked to the length and maximum extensions of the siphons (Kondo, 1987, 1997; Zwarts and Wanink, 1989). In this context, MIS 5 shells could develop a deeper burial, associated with different environmental conditions which also affected the productivity. A glacial cycle (MIS 6) separated those interglacial cycles: we have no details about this in the study area, but it is possible that the environmental conditions from MIS 7 to MIS 5 would have been modified, with a similar coastal configuration link to an open sea. Regarding the shell size, the MIS 5 specimens are the largest in respect to the other assemblages. Body size is strongly correlated with physiological and ecological characteristics, as well as with life history, and some authors consider it an organism's most important attribute (McMahon and Bonner, 1983; Peters, 1983; McKinney, 1990; Blackburn and Gaston, 1994; West et al., 1997). In molluscs, the growth rate and size are generally associated with SST and with primary productivity (Vermeij, 1978; Harrington, 1987; Jones et al., 1989; Anderson, 2001). The size of these MIS 5 specimens could be related to the high paleoproductivity generated during glacial–interglacial cycles and upwelling processes that would have provided the system with a significant amount of organic matter (Schmiedl and Mackensen, 1997). Similar environmental conditions to those registered in the study region were recorded in the South Atlantic coast of Africa during the last interglacial by Schmiedl and Mackensen (1997). During MIS 5, the sea level reached a higher position than at present in the global scale; authors indicated a level of +6 m (Chappell and Shackleton, 1986), or +7 m (Bard et al.,

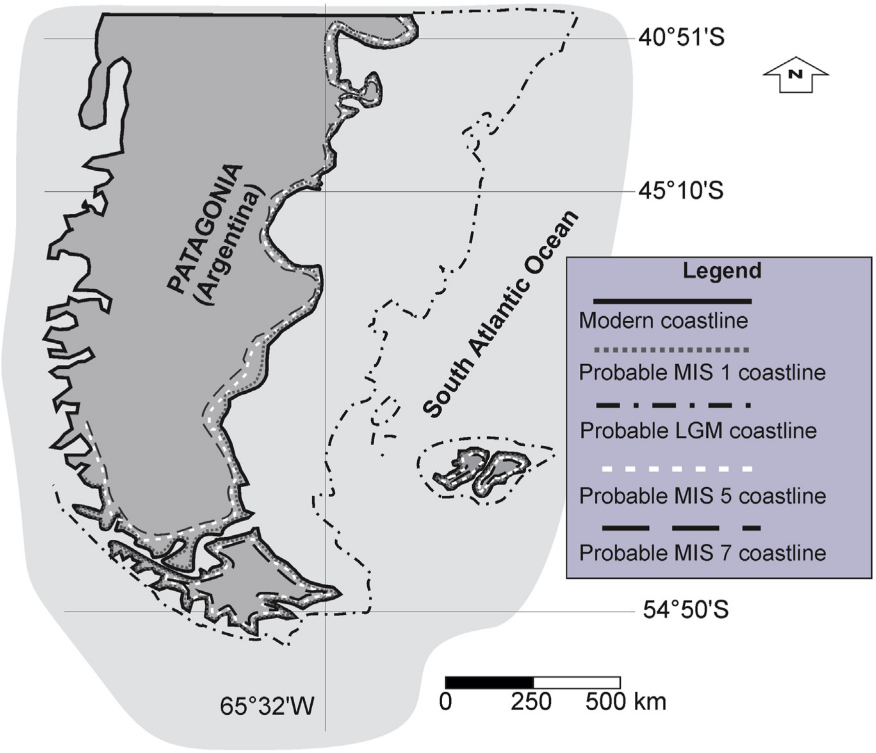

Fig. 8. Illustrative scheme showing the probable Patagonian coastal margin evolution. The reconstruction of the probable coastlines for the Pleistocene interglacial times (MIS 7, MIS 5) where based considering the altitude of the coastal marine deposits from Bustamante Bay measured and dated by Schellmann and Radtke (2000). The coastline obtained for the LGM was drawn taking account the paleogeographic evolution model by Ponce et al. (2011), and it is similar to the scheme obtained by Rostami et al. (2000) for the region.

1996; Sirocko et al., 2007). This interglacial cycle is characterized by widespread global warming, as reflected in the isotopic record, which led to a significant reduction of the ice cap of Antarctica and the disappearance of the Greenland ice sheet (Shackleton et al., 2003). Also, during MIS 5, warm substages are interspersed with cold substages, characterized by a substantial reduction in summer insolation due to a global cooling. The warmest substage is MIS 5e (Shackleton et al., 2003).

6.2.2. *Last Glacial Maximum*

During the Last Glacial Maximum (LGM; approximately 24,000 cal. BP; Rabassa, 2008) within the MIS 2, sea level became established at between 120 to 140 m below the present sea level (Fleming et al., 1998; Uriarte Cantolla, 2003). The shape and position of the Patagonian Atlantic coast during this last cold event would have been significantly different and the region's climate would have been much more continental than today (Ponce et al., 2011) (Fig. 8). The San Jorge Gulf would have appeared around 15,500–15,000 cal. BP, when sea level reached −90 m (Fig. 6). The origin of this gulf could have been associated with the sea flooding of a large, ancient endorheic basin (Ponce et al., 2011). The displacement of the coastal margin to the east and the exposure of the platform, compared to the coastal sector's geographical configuration during the Pleistocene (MIS 7, MIS 5), would have substantially modified the environmental conditions and the substrate in which the oldest assemblages developed.

6.2.3. *Holocene (~4924 ± 80 BP) and present configuration of Bustamante Bay*

Subsequently, the rapid transgression that occurred during the early Holocene (ca. 11,000 BP) would have caused sudden changes in the Patagonian flora and fauna, associated with the rapidly migrating coastal areas to the west (Ponce et al., 2011), and forcing environmental conditions which were very different from those in the Pleistocene. The melting of continental ice caps would have altered the chemical characteristics of the water masses, and also produced changes in the circulation of currents and the composition of bottom sediments. Taking into account the geographical configuration of Bustamante Bay, the Holocene specimens were collected in the area of the isthmus of Graviña peninsula (probably flooded during the Pleistocene) (Ponce et al., 2011), while the modern specimens were collected from the current beach (Fig. 1). The Quaternary coastal marine deposits show evidence of littoral margin evolution related to sea level variations (Scheffers et al., 2011; Tamura, 2012). Environmental conditions during the Holocene would therefore have been very different from those of the Pleistocene, and *A. antiqua* specimens would have gone through an adjustment process based on the new conditions in the bay. The cluster analyses performed associated the Holocene with the Modern assemblages. Based on the paleogeographical evolution model of Ponce et al. (2011), in general the characteristics of the coastal margin would have been preserved from the Holocene to the present (Fig. 8). The main differences observed in the shells from these two assemblages are based on the prosogyrous umbo condition, which is related to the speed of burial, as described by Stanley (1970, 1975).

The Holocene shells are more rounded and prosogyrous than the Modern samples, indicating faster burial with respect to the Modern and the Pleistocene assemblages. The pallial scar in the Holocene samples is more pronounced than in the Modern samples, thus indicating that the fossil assemblages had deeper burial. These shell features indicate either changes in the substrate or a relationship with predators that forced the Holocene clams to make a quick escape. A considerably smaller size is the feature that

Please cite this article in press as: Boretto, G.M., et al., Shell morphometric variations in a Patagonian Argentina clam (*Ameghinomya antiqua*) from the Mid-Pleistocene (MIS 7) to the present, Quaternary International (2014), http://dx.doi.org/10.1016/j.quaint.2014.09.033

distinguishes the Modern assemblages from the fossil shells. This peculiarity is linked to the lifestyle of the organism and local environmental conditions that affect their growth. Gordillo and Archuby (2014) highlighted the following issues in Modern specimens of *A. antiqua* from Bustamante Bay: (1) this clam spends most of the time in a semi-infaunal position (lying flat or in an upright position when partially buried) and therefore at risk of being attacked by predators, as witnessed by drilling marks found on the shells; (2) The seaweeds *Glacilaria* and *Enteromorpha* (Piriz and Casas, 1996; Boraso and Zaixso, 2007) are present in the bay, and these cause damaging chemical alterations in clams and can therefore affect the burial range, as was observed in the *Mya arenaria* bivalve from the Northern Hemisphere (Auffrey et al., 2004).

Therefore, our study on *A. antiqua* fossil and modern shells from Patagonia Argentina displays a multi-approach to the analysis of morphology. By including both a landmark and an outline morphometric methods, 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 Bustamante Bay during the Quaternary.

7. Final remarks

- Contour and landmark analysis are valuable tools for distinguishing samples of *A. antiqua* from Quaternary coastal deposits from MIS 7 to the present in Bustamante Bay and for determining the nature of subtle changes over time. The aspects of the shell variation along the time were captured and drawn, therefore the history of shape and size tendencies was detailed.
- Morphometric variability found in specimens from different time spans likely reflects environmental changes throughout the Quaternary in the coastal area of Bustamante Bay. Each assemblage reflects the coastal environment during the MIS 7, MIS 5, MIS 1 (Holocene) and the Modern littoral area. The environment conditions, substrates, SST, primary productivity and geographical area configuration have been very different especially after the LGM.
- Data about the geographic configuration of the bay suggest the morphological trends to be functional adaptations to a changing paleoenvironmental conditions. During MIS 7 and MIS 5, the assemblages were exposed to stronger wave action and a greater influence of the tides (open sea environment). These factors could generate the necessity for a better mechanical stability, especially observed in the MIS 5 shells, which are the largest, ovoid and prosogyrous, suggesting a deeper burial, thus reducing the chances of being removed in such high energy open sea conditions.
- Morphological analyses are useful for distinguishing adaptive differences, and this phenotypic variability reflects their history. The phenotypic plasticity observed in *A. antiqua* represents the ability of this species to adapt to different environments, including varied substrate, hydrological changes and probably predators, and these factors are seen as the main reason for morphological variability among the Quaternary assemblages.

Acknowledgments

The field-work was made possible thanks to the funding of the University of Pisa (Progetto Ateneo 2007, Leader G. Zanchetta) and MIUR (PRIN2008, Leader G. Zanchetta). Thanks are extended to Simon Schneider (University of Cambridge) for suggestions and comments on a first version of this manuscript, to Santiago Druetta (CICTERRA) for his collaboration during the images processing, and to two anonymous reviewers for their constructive comments and useful suggestions. This paper is dedicated to the memory of Aaron Swartz, a strong supporter of open access to academic journals, who died in the fight for making them freely available to the public. This study is a contribution to Gabriella M. Boretto's Ph.D project, and is supported by a fellowship obtained from the National Research Council, CONICET.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.quaint.2014.09.033.

References

Adams, D.C., Rohlf, F.J., Slice, D., 2004. Geometric morphometrics: ten years of progress following the “revolution”. Italian Journal of Zoology 71, 5–16.

Aguirre, M.L., Negro Sirch, Y., Richiano, S., 2005. Late Quaternary molluscan assemblages from Bahía Bustamante coastal area (Patagonia, Argentina). Journal of South American Earth Sciences 20, 13–32.

Amoroso, R.O., 2004. Heterogeneidad espacial en la dinámica de la metapoblación de vieira Tehuelche (*Aequipecten tehuelchus*) del golfo San José. Tesis de Licenciatura. Universidad Nacional de la Patagonia San Juan Bosco, Puerto Madryn, Chubut, 55 pp.

Amoroso, R.O., Gagliardini, D.A., 2010. Inferring complex hydrographic processes using remote-sensed images: turbulent fluxes in the patagonian gulfs and implications for scallop metapopulation dynamics. Journal of Coastal Research 26, 320–332.

Anderson, L.C., 2001. Temporal and geographic size trends in Neogene Corbulidae (Bivalvia) of tropical America: using environmental sensitivity to decipher causes of morphologic trends. Paleogeography, Paleoclimatology, Paleoecology 166, 101–120.

Auffrey, L.M., Robinson, S.M.C., Barbeau, M.A., 2004. Effect of green macroalgal mats on burial depth of soft-shelled clams *Mya arenaria*. Marine Ecology Progress Series 278, 193–203.

Balech, E., Ehrilch, M., 2008. Esquema biogeográfico del mar argentino. Revista de Investigación y Desarrollo Pesquero 19, 45–75.

Bard, E., Hamlin, B., Montaggioni, L., Cabioch, G., Faure, G., Rougerie, F., 1996. Deglacial sea level record from Tahiti corals and the timing of meltwater discharge. Nature 382, 241–244.

Blackburn, T.M., Gaston, K.J., 1994. Animal body size distributions: patterns, mechanisms and implications. Trends in Ecology and Evolution 9, 471–474.

Boraso, A.L., Zaixso, J.M., 2007. Algas marinas bentónicas. In: Boltovskoy, Demetrio (Ed.), Atlas de Sensibilidad ambiental del mar y de la costa. PNUD, 28 pp.

Calvo, J., Morriconi, E., Orler, P.M., 1998. Estrategias reproductivas de moluscos bivalvos y equinoideos. In: Boschi, E. (Ed.), El Mar Argentino y sus recursos pesqueros, vol. 2. Instituto Nacional de Investigación y Desarrollo Pesquero, Mar del Plata, 42 pp.

Castellanos, Z.J.A., 1967. Catálogo de los moluscos marinos bonaerenses. Anales de la Comisión de Investigación Científica de la Provincia de Buenos Aires (CIC) 8, 1–365.

Chappell, J., Shackleton, N.J., 1986. Oxygen isotopes and sea level. Nature 324, 137–140.

Cionchi, J.L., 1987. Depósitos marinos cuaternarios de Bahía Bustamante, provincia del Chubut. Revista de la Asociación Geológica Argentina 42, 61–72.

Cionchi, J.L., 1988. Geomorfología de Bahía Bustamante y zonas adyacentes, Chubut. Revista de la Asociación Geológica Argentina 43, 51–62.

Clasing, E., Brey, T., Stead, R., Navarro, J., Asencio, G., 1994. Population dynamics of *Venus antiqua* (Bivalvia: Veneracea) in the bahía Yaldad, Isla de Chiloé, Southern Chile. Journal of Experimental Marine Biology and Ecology 177, 171–186.

Claude, J., 2008. Morphometrics with R. Springer-Verlag, New York, 316 pp.

Codignotto, J.O., Kokot, R.R., Marcomini, S.C., 1992. Neotectonism and sea-level changes in the coastal zone of Argentina. Journal of Coastal Research 8, 125–133.

Coronato, A., Coronato, F., Mazzoni, E., Vázquez, M., 2008. The physical geography of Patagonia and Tierra del Fuego. In: Rabassa, J. (Ed.), The Late Cenozoic of Patagonia and Tierra del Fuego, pp. 13–55.

Costa, C., Aguzzi, J., Menesatti, P., Antonucci, F., Rimatori, V., Mattoccia, M., 2008a. Shape analysis of different populations of clams in relation to their geographical structure. Journal of Zoology 276, 71–80.

Costa, C., Menesatti, P., Aguzzi, J., D'Andrea, S., Antonucci, F., Rimatori, V., Pallottino, P., Mattoccia, M., 2008b. External shape differences between sympatric populations of commercial clams *Tapes decussatus* and *T. philippinarum*. Food and Bioprocess Technology 3, 43–48.

Crampton, J.S., 1995. Elliptic Fourier shape analysis of fossil bivalves: some practical considerations. Lethaia 28, 179–186.

Crampton, J.S., Maxwell, P.A., 2000. Size: all it' s shaped up to be? Evolution of shape through the lifespan of the Cenozoic bivalve *Spissatella* (Crassatellidae). In: Harper, E.M., Taylor, J.D., Crame, J.A. (Eds.), Evolutionary Biology of the Bivalvia, Geological Society of London Special Publication 177, pp. 399–423.

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2009. InfoStat Versión 2009. InfoStat Group, FCA. Universidad Nacional de Córdoba, Argentina.

Please cite this article in press as: Boretto, G.M., et al., Shell morphometric variations in a Patagonian Argentina clam (*Ameghinomya antiqua*) from the Mid-Pleistocene (MIS 7) to the present, Quaternary International (2014), http://dx.doi.org/10.1016/j.quaint.2014.09.033

Dommergues, E., Dommergues, J.L., Magniez, F., Neige, P., Verrecchia, E.P., 2003. Geometric measurements analysis versus Fourier series analysis for shape characterization using gastropod shell (*Trivia*) as an example. Mathematical Geology 35, 887–894.

Escati Peñaloza, G., 2005. Variación espacial en el crecimiento individual de la almeja rayada, *Ameghinomya antiqua*, en el golfo San José (Thesis). Universidad Nacional de la Patagonia "San Juan Bosco", Sede Pto. Madryn, Chubut, Argentina, 68 pp.

Escati Peñaloza, G., Parma, A.M., Orensanz, J.M., 2010. Analysis of longitudinal growth increment data using mixed-effects models: individual and spatial variability in a clam. Fisheries Research 105, 91–101.

Ferson, S., Rohlf, F.J., Koehn, R.K., 1985. Measuring shape variation of two dimensional outlines. Systematic Zoology 34, 59–68.

Feruglio, E., 1950. Descripción Geológica de La Patagonia, vol. 3. Dirección General de Y.P.F., Buenos Aires, p. 431.

Freeman, H., 1974. Computer processing of line drawing images. Computing Surveys (CSUR) 6, 57–97.

Fleming, K., Johnston, P., Zwartz, D., Yokoyama, Y., Lambeck, K., Chappell, J., 1998. Refining the eustatic sea level curve since the Last Glacial Maximum using far- and intermediate-field sites. Earth and Planetary Science Letters 163, 327–342.

Gordillo, S., Márquez, F., Cárdenas, J., Zubimendi, M.A., 2010. Shell variability in *Tawera gayi* (Veneridae) from southern South America: a morphometric approach based on contour analysis. Journal of the Marine Biological Association of the United Kingdom 91, 815–822.

Gordillo, S., Archuby, F., 2014. Live-live and live-dead interactions in marine death assemblages: the case of the Patagonian clam *Venus antiqua*. Acta Palaeontologica Polonica 59, 429–442.

Harrington, R.J., 1987. Skeletal growth histories of *Protothaca staminea* (Conrad) and *Protothaca grata* (Say) throughout their geographic ranges. Northeastern Pacific. Veliger 30, 148–158.

Innes, D.J., Bates, J.A., 1999. Morphological variation of *Mytilus edulis* and *Mytilus trossulus* in eastern Newfoundland. Marine Biology 133, 691–699.

Isola, I., Bini, M., Ribolini, A., Pappalardo, M., Consoloni, I., Fucks, E., Boretto, G., Ragaini, L., Zanchetta, G., 2011. Geomorphologic Map of Northeastern sector of San Jorge Gulf (Chubut, Argentina). Journal of Maps 7, 476–485.

Iwata, H., Ukai, Y., 2002. SHAPE: a computer program package for quantitative valuation of biological shapes based on elliptical Fourier descriptors. Journal of Heredity 93, 384–385.

Jones, D.S., Arthur, M.A., Allard, D.J., 1989. Sclerochronological records of temperature and growth from shells of *Mercenaria mercenaria* from Narragansett Bay Rhode Island. Marine Biology 102, 225–234.

Krapivka, S., Toro, J.E., Alcapán, A.C., Astorga, M., Presa, P., Pérez, M., Guiñez, R., 2007. Shell-shape variation along the latitudinal range of the Chilean blue mussel *Mytilus chilensis* (Hupé, 1854). Aquaculture Research 38, 1770–1777.

Klingenberg, C.P., 2008. Morphological integration and developmental modularity. Annual Review of Ecology, Evolution and Systematics 39, 115–132.

Klingenberg, C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources 11, 353–357.

Kuhl, F.P., Giardina, C.R., 1982. Elliptic Fourier features of a closed contour. Computer Graphics and Image Processing 18, 236–258.

Kondo, Y., 1987. Burrowing depth of infaunal bivalves – observation f living species and its relation to shell morphology. Transactions and proceedings of the Palaeontological Society of Japan, NS 148, 306–323.

Kondo, Y., 1997. Inferred bivalve response to rapid burial in a Pleistocene shallow-marine deposit from New Zealand. Paleogeography, Paleoclimatology, Paleoecology 128, 87–100.

Lardies, M.A., Clasing, E., Navarro, J.M., Stead, R.A., 2001. Effects of environmental variables on burial depth of two infaunal bivalves inhabiting a tidal flat in southern Chile. Journal of the Marine Biological Association of the United Kingdom 81, 809–816.

Lema, H., Busteros, A., Franchi, M., 2001. Hoja Geológica 4566-II y IV, Camarones (1: 250.000), Instituto de Geología y Recursos minerales. Boletín del Servicio Geológico Minero Argentino 261, 1–44.

Lestrel, P.E., 1997. Fourier Descriptors and Their Applications in Biology. Cambridge University Press, 466 pp.

Márquez, F., Robledo, J., Escati Peñaloza, G., Van der Molen, S., 2010. Use of different geometric morphometrics tools for the discrimination of phenotypic stocks of the striped clam *Ameghinomya antiqua* (Veneridae) in north Patagonia, Argentina. Fisheries Research 101, 127–131.

McGuire, J.L., 2010. Geometric morphometrics of vole (*Microtus californicus*) dentition as a new paleoclimate proxy: shape change along geographic and climatic clines. Quaternary International 212, 198–205.

McMahon, T.A., Bonner, J.T., 1983. On Size and Life. Scientific American Books, W. H. Freeman, New York, 255 pp.

McKinney, M.L., 1990. Classifying and analysing evolutionary trends. In: McNamara, K.J. (Ed.), Evolutionary Trends. Belhaven, London, pp. 28–58.

Neubauer, T.A., Harzhauser, M., Mandic, O., 2013. Phenotypic evolution in a venerid bivalve species lineage from the late Middle Miocene Central Paratethys Sea: a multi-approach morphometric analysis. Biological Journal of the Linnean Society 110, 320–334.

Osorio, C., Lozada, E., Bustos, E., Valenzuela, E., Frassineti, D., y Guarda, S., 1982. La biología de *Venus antiqua* King & Broderip 1835 en Chile. Atlántica 5, 88–98.

Palmer, M., Pons, G.X., Linde, M., 2004. Discriminating between geographical groups of a Mediterranean commercial clam (*Chamelea gallina* (L.): Veneridae) by shape analysis. Fisheries Research 67, 93–98.

Pedoja, K., Regard, V., Husson, L., Martinod, J., Guillaume, B., Fucks, E., Iglesias, M., Weill, P., 2010. Uplift of Quaternary shorelines in eastern Patagonia: Darwin revisited. Geomorphology 127, 121–142.

Pedoja, K., Husson, L., Regard, V., Cobbold, P.R., Ostanciaux, E., Johnson, M.E., Delcaillau, B., 2011. Relative sea-level fall since the last interglacial stage: are coasts uplifting worldwide? Earth-Science Reviews 108, 1–15.

Peters, R.H., 1983. The Ecological Implications of Body Size. Cambridge University Press, Cambridge, 329 pp.

Piriz, M.L., Casas, G., 1996. Macroalgas de interés comercial en las costas del sur de Chubut y norte de Santa Cruz. Informe Técnico N 26 del Plan de Manejo Integrado de la Zona Costera Patagónica. GEF/PNUD/WCS/FPN (ISSN N 0328–462X). CENPAT, CONICET. Puerto Madryn. 36 pp.

Ponce, J.F., Rabassa, J., Coronato, A., Borromei, A.M., 2011. Palaeogeographical evolution of the Atlantic coast of Pampa and Patagonia from the last glacial maximum to the Middle Holocene. Biological Journal of the Linean Society 103, 363–379.

Rabassa, J., Coronato, A.M.J., Salemme, M., 2005. Chronology of the Late Cenozoic Patagonian glaciations and their correlation with biostratigraphic units of the Pampean region (Argentina). Journal of South American Earth Sciences 20, 81–103.

Rabassa, J., 2008. Late Cenozoic glaciations in Patagonia and Tierra del Fuego. In: Rabassa, J. (Ed.), The Late Cenozoic of Patagonia and Tierra del Fuego, Developments in Quaternary Science 11, pp. 151–204.

Ribolini, A., Aguirre, M., Baneschi, I., Consoloni, I., Fucks, E., Isola, I., Mazzarini, F., Pappalardo, M., Zanchetta, G., Bini, M., 2011. Holocene beach ridges and coastal evolution in the Cabo Raso Bay (Atlantic Patagonian Coast, Argentina). Journal of Coastal Research 27, 973–983.

Rohlf, F.J., Archie, J.W., 1984. A comparison of Fourier methods for description of wing shape in mosquitos (Diptera: Culicidae). Systematic Zoology 33, 302–317.

Rohlf, F.J., Marcus, L.F., 1993. A revolution in morphometrics. Trends in Ecology and Evolution 8, 129–132.

Rostami, K., Peltier, W.R., Mangini, A., 2000. Quaternary marine terraces, sea-level changes and uplift history of Patagonia, Argentina: comparisons with predictions of the ICE-4G (VM2) model of the global process of glacial isostatic adjustment. Quaternary Science Reviews 19, 1495–1525.

Rufino, M.M., Gaspar, M.B., Pereira, A.M., Vasconcelos, P., 2006. Use of shape to distinguish *Chamelea gallina* and *Chamelea striatula* (Bivalvia: Veneridae): linear and geometric morphometric methods. Journal of Morphology 267, 1433–1440.

Rutter, N., Schnack, E., Del Rio, L., Fasano, J., Isla, F., Radtke, U., 1989. Correlation and dating of Quaternary littoral zones along the Patagonian coast, Argentina. Quaternary Science Reviews 8, 213–234.

Rutter, N., Radtke, U., Schnack, J., 1990. Comparison of ESR and Amino Acid Data in correlating and dating Quaternary shorelines along the Patagonian Coast, Argentina. Journal of Coastal Research 6, 391–411.

Sciutto, J.C., Césari, O., Escribano, V., Pezzuchi, H., 2000. Hoja Geológica 4566-III. Comodoro Rivadavia, Provincia de Chubut, Istituto de Geológia y Recursos Minerales. Boletín del Servicio Geológico Minero Argentino 244, 1–53.

Scheffers, A., Engel, M., Scheffers, S., Squire, P., Dieter, K., 2011. Beach ridge systems archives for Holocene coastal events? Physical Geography 36, 5–37.

Schellmann, G., Radtke, U., 2000. ESR dating of stratigraphically well-constrained marine terraces along the Patagonian Atlantic coast (Argentina). Quaternary International 68, 261–273.

Schellmann, G., Radtke, U., 2003. Coastal terraces and Holocene sea-level changes along the Patagonian Atlantic coast. Journal of Coastal Research 19, 963–996.

Schellmann, G., Radtke, U., 2010. Timing and magnitude of Holocene sea-level changes along the middle and south Patagonian Atlantic coast derived from beach ridge systems, littoral terraces and valley-mouth terraces. Earth-Science Reviews 103, 1–30.

Schmiedl, G., Mackensen, A., 1997. Late Quaternary paleoproductivity and deep water circulation in the eastern South Atlantic Ocean: evidence from benthic foraminifera. Palaeogeography, Palaeoclimatology, Palaeoecology 130, 43–80.

Shackleton, N., Sánchez-Goñi, M.A., Paillerc, D., Lancelot, Y., 2003. Marine Isotope Substage 5e and the Eemian Interglacial. Global and Planetary Change 36, 151–155.

Sirocko, F., Clausen, M., Sánchez Goñi, M.F., Litt, T., 2007. The climate of past interglacials. Developments in Quaternary Science 7. Elsevier, Amsterdam, 622 pp.

Sokal, R.R., Rohlf, F.J., 1981. Taxonomic congruence in the *Leptopodomorpha* reexamined. Systematic Zoology 30, 309–325.

Stanley, S.M., 1970. Relation of shell form to life habits of the Bivalvia (Mollusca). The Geological Society of America Memoir 125, 1–296.

Stanley, S.M., 1975. Why clams have the shape they have: an experimental analysis of burrowing bivalves. Paleobiology 1, 48–58.

Taglioretti, V., Sardella, N.H., Fugassa, M.H., 2014. Morphometric analysis of modern faeces as a tool to identify artiodactyls' coprolites. Quaternary International. http://dx.doi.org/10.1016/j.quaint.2013.12.055 (in press).

Tamura, T., 2012. Beach ridges and prograded beach deposits as palaeoenvironment records. Earth-Science Reviews 114, 279–297.

Urban, J.H., 1994. Adaptations of six infaunal bivalve species of Chile: coexistence resulting from differences in morphology, burrowing depth and substrate preference. Archive of Fishery and Marine Research 42, 183–193.

Please cite this article in press as: Boretto, G.M., et al., Shell morphometric variations in a Patagonian Argentina clam (*Ameghinomya antiqua*) from the Mid-Pleistocene (MIS 7) to the present, Quaternary International (2014), http://dx.doi.org/10.1016/j.quaint.2014.09.033

Urban, J.H., 1996. Population dynamics of the bivalves *Venus antiqua, Tagelus dombeii* and *Ensis macha* from Chile at 36°S. Journal of Shellfish Research 15, 719–727.

Uriarte Cantolla, A., 2003. Historia del clima de la tierra. Servicio Central de Publicaciones del Gobierno Vasco, Victoria-Gasteiz, 306 pp.

Van Heteren, A.H., MacLarnon, A., Soligo, C., Rae, T.C., 2013. Functional morphology of the cave bear (*Ursus spelaeus*) cranium: a three-dimensional geometric morphometric analysis. Quaternary International 339–340, 209–216.

Verdinelli, M.A., Schuldt, M., 1976. Consideraciones preliminares sobre aspectos de la dinámica poblacional y reproductiva de la almeja rayada (*Ameghinomya antiqua, ing-Chionidae*) en Punta Loma, golfo Nuevo-Chubut. Universidad Nacional de la Plata, Facultad de Ciencias Naturales y Museo. Revista del Museo de La Plata XII sección Zoología 119, 183–202.

Vermeij, G., 1978. Biogeography and Adaptation: Patterns of Marine Life. Harvard University Press, Cambridge, 332 pp.

West, G.B., Brown, J.H., Enquist, B.J., 1997. A general model for the origin of allometric scaling laws in biology. Science 276, 122–126.

Zaixso, H.E., 1996. Asociaciones de moluscos bentónicos submareales del golfo San José y sur del golfo San Matías (Chubut, Argentina). Physis (Buenos Aires), Sección A 54, 1–21.

Zelditch, M.L., Swiderski, D.L., Sheets, H.D., Fink, W.L., 2004. Geometric Morphometrics for Biologists: a Primer. Elsevier, New York, 471 pp.

Zwarts, L., Wanink, J.H., 1989. Siphon size and burying depth in deposit- and suspension-feeding bivalves. Marine Biology 100, 227–240.

Please cite this article in press as: Boretto, G.M., et al., Shell morphometric variations in a Patagonian Argentina clam (*Ameghinomya antiqua*) from the Mid-Pleistocene (MIS 7) to the present, Quaternary International (2014), http://dx.doi.org/10.1016/j.quaint.2014.09.033