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INTRASPECIFIC SHELL-SHAPE VARIATION IN THE RAZOR CLAM *ENSIS MACHA* ALONG THE PATAGONIAN COAST

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ABSTRACT

Ensis macha has a wide distribution along the Pacific and Atlantic southern coasts of South America. The pattern of morphological shell variation of this razor clam was analysed in 12 populations sampled along its whole Atlantic distribution, covering a latitudinal range of 3,700 km. The southernmost extant population of *E. macha* was recorded in the Beagle Channel. Variation in shell outline was assessed using elliptic Fourier analysis of adult individuals followed by different multivariate analyses. Significant shell-shape variation was found along the latitudinal range. Elliptical Fourier analysis revealed that trends in shell slenderness and curvature were associated mainly with salinity and water depth of the localities. Each locality presented a distinct shell shape, different from all other localities. Nevertheless, the populations could be grouped into four large assemblages between which a latitudinal gradient of shell shape was observed. Phenotypic divergence in shell shape is suggestive of population differentiation, but assessing the underlying processes is not straightforward because of the complex interaction between genetic and environmental factors. The possible evolutionary processes that mediate the effects of environmental variables and geographical distances on the shell-shape variation are discussed.

INTRODUCTION

Morphological diversity is the product of genetic, developmental and environmental influences. Differences in shape may signal different functional roles, different responses to the same selective pressures (or differences in the selective pressures themselves) as well as differences in growth and morphogenesis. Shape analysis is one approach to understanding these diverse causes of variation (Zelditch *et al*., 2004).

In nature, organisms are exposed to various ecological challenges and phenotypes generally reflect the influence of multiple environmental variables, in addition to other evolutionary factors (Langerhans, Chapman & Dewitt, 2007; Costa *et al*., 2008; Zieritz & Aldridge, 2009). Intraspecific phenotypic variation is believed to arise from divergent selection pressures between different environments (Schluter, 2000 and references therein), from environment-independent phenotype generation, as well as from potentially nonadaptive effects of environment on phenotype (Schluter, 2000; West-Eberhard, 2003). Furthermore, the theory of genetic isolation by geographic distance predicts that increasing distance between populations – reflecting decreasing gene flow – should result in increasing phenotypic divergence (Haldane, 1948; Felsenstein, 1976; Slatkin, 1987; Hendry, Day & Taylor, 2001). In some bivalve species, stocks are structured as metapopulations, collections of populations of sedentary adults connected with each other by means of the dispersal of larvae (Orensanz & Jamieson, 1998). However, the connectivity among populations can be disrupted by biotic and abiotic barriers such as predation pressure, asynchronous reproduction and physical barriers such as tidal fronts, currents and freshwater discharge, among others. Thus the connectivity among marine populations is often lower than predicted by the dispersal capabilities of the larvae alone. Population connectivity requires more than just the simple exchange of settlers among populations; it also requires the successful establishment and reproduction of exogenous colonizers (Marshall *et al*., 2010). Consequently, the study of morphological variation could be helpful to understand the extent and connectivity of populations and hence to determine metapopulation structure.

The razor clam *Ensis macha* (Molina, 1782) is a burrowing bivalve that occurs from the low intertidal to depths of 55 m on sandy and muddy bottoms. This species offers a good opportunity to study the relative importance of the environment on the phenotype of marine clams since it presents a wide distributional range in Argentina, from the Magellan Strait (52°S) to north of San Matías Gulf (40°S) (Osorio, 2002; Lasta *et al*., 1998), covering 3,700 km of Atlantic coastline. As in the case of other bivalve species with a broad distributional range, the biogeographic distribution of morphological variation in *E. macha* has been little studied. The razor clam's Argentinean distribution encompasses two biogeographic provinces, the Argentinean Province (from southern Brazil to northern Argentinean Patagonia) and the Magellanic Province (Argentinean Patagonian coast and southern Chile) (Aguirre, Perez & Sirch, 2006). The most important inshore oceanographic features in this distributional range is a dominant north-northeastward flow, a product of the propagation of the semidiurnal tides as well as the effect of strong westerly and southwesterly wind forcing (Palma, Matano & Piola, 2004).

In shell-shape studies the conventional morphometric approach, based on discrete linear and angular measurements, is less likely to be successful in cases where diversity of shapes is not large and the morphometric trends are subtle. In this case elliptical Fourier analysis (EFA) (Kuhl & Giardina, 1982) is more appropriate, since it captures the entire two-dimensional outline in a systematic and precise manner (Rohlf & Archie, 1984). Studies of shell morphological variation using EFA have

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been developed for several bivalve species (Ferson, Rohlf & Koehn, 1985; Crampton, 1995; Innes & Bates, 1999; Palmer, Pons & Linde, 2004; Krapivka *et al.*, 2007; Márquez *et al.*, 2010; Gordillo *et al.*, in press, among others). This technique captures the complete shell shape even when few or no landmarks are available (Rohlf & Archie, 1984).

In the present study we describe the morphological variation between *E. macha* populations, using EFA, and test if environmental factors and/or geographical distance are influencing the shell-shape variation. Specifically, we asked the following question: (1) do salinity, temperature and sediment composition influence shell shape? and (2) is shell-shape variation higher between more distant populations than between neighbouring populations?

MATERIAL AND METHODS

Localities, sampling and outline analysis

The study area covered the entire distribution of *Ensis macha* on the Argentinean coast. We collected specimens from 12 localities along *c.* 3,700 km of latitude, from El Sótano, in the north of San Matías gulf (41°34′S), to the Beagle Channel (54°52′S) (Fig. 1). A total of 1,200 adult individuals were collected by scuba divers using a water pump (92–189 mm shell length; *c.* 100 individuals from each locality). Soft parts were removed and images of the inner side of the left valves were obtained using a digital camera. Shell area (cm^2) was estimated using SHAPE – ChainCoder software (Iwata & Ukai, 2002) and was used as a proxy for shell size.

To analyse shell-shape variation, elliptic Fourier analysis was applied using the software package Shape v. 1.2 (Iwata & Ukai, 2002). The minimal number of harmonics (n) sufficient to explain shape variability was estimated following Crampton (1995). The Fourier series was truncated at $n = 15$ with an average cumulative power of 99.98% of the total average power. There are four elliptic Fourier coefficients for each harmonic and they were normalized for size, rotation and starting point of trace, which caused the degeneration of the first three coefficients to fixed values. Therefore, the shape of each shell was approximated to 57 coefficients of normalized elliptical Fourier descriptors (EFDs).

Environmental variables

The following environmental variables were measured at each locality: latitude, depth, sediment composition, salinity and sea surface temperature (SST). To describe the sediment composition, five sediment cores (6 cm diameter × 20 cm deep) were collected with a circular corer from each locality and stored for the analysis of grain-size distributions. Sediment composition was measured by standard dry-sieving of each sample: sediments were sieved through a tower of six sieves with a mesh size in the range 62–2,000 μm. The median grain-size diameter (the diameter corresponding to 50% mark on cumulative frequency curve expressed in phi) was calculated using linear interpolation by the graphical method of Folk & Ward (1957) in Gradistat v. 6.0 software. Median grain-size diameter was used in subsequent statistical analyses as a sediment variable for each locality. The salinity was obtained from published sources (Hernando *et al.*, 2006; Piola & Falabella, 2009). To estimate average temperatures for a 10-year period (1998–2008) in each location, we explored the SST time series using remotely sensed data. For all localities, we obtained a 10-year monthly average SST series, based on data downloaded from a 4 × 4 km spatial resolution satellite image database (NOAA/NASA, AVHRR Pathfinder 5, http://poet.jpl.nasa.gov/).

Statistical analysis

Principal component (PC) analysis of the variance–covariance matrix (Rohlf & Archie, 1984; Crampton, 1995) was used to

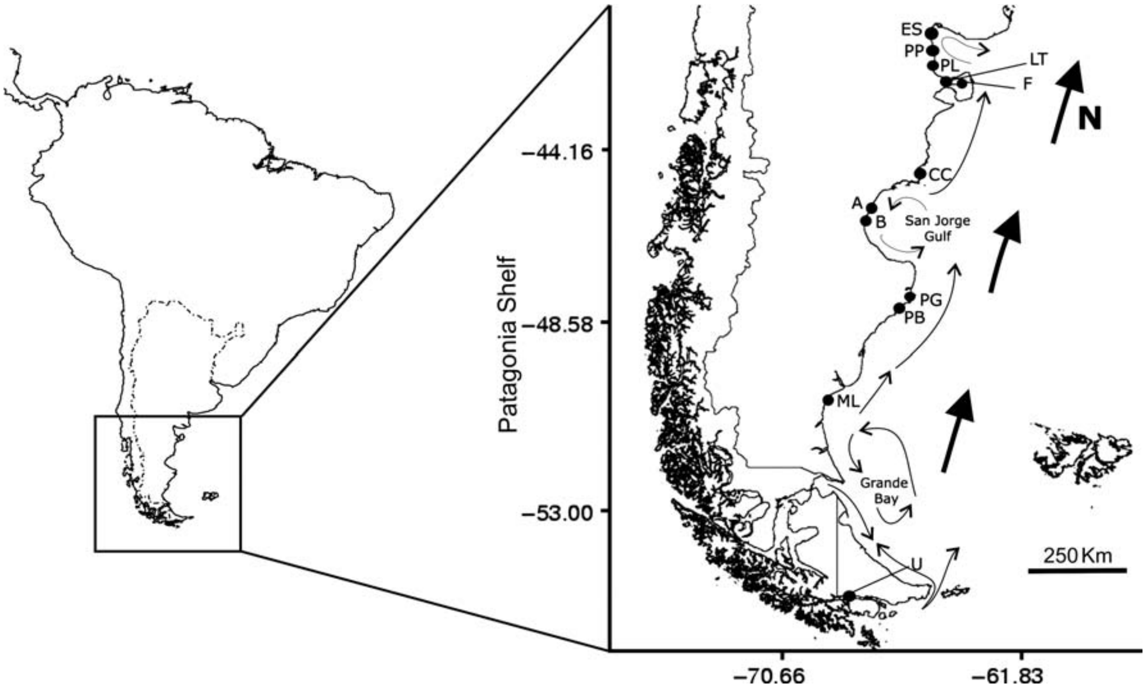

Figure 1. Map of the Patagonian shelf showing the 12 sampling localities of *Ensis macha*, and the schematic water circulation in the southwestern Atlantic (adapted from Palma *et al.*, 2004). Codes for localities: ES, El Sotano, 40°56′S; PP, Punta Pozo, 41°34′S; PL, Puerto Lobos, 42°00′S; LT, La Tapera, 42°21′S; F, Fracasso, 42°24′S; CC, Caleta Carolina, 44°54′S; B, Bonita, 46°00′S; A, Alsina, 46°03′S; PG, Punta Guanaco, 47°47′S; PB, Punta Buque, 48°02′S; ML, Monte León, 50°20′S; U, Beagle Channel, 54°52′S.

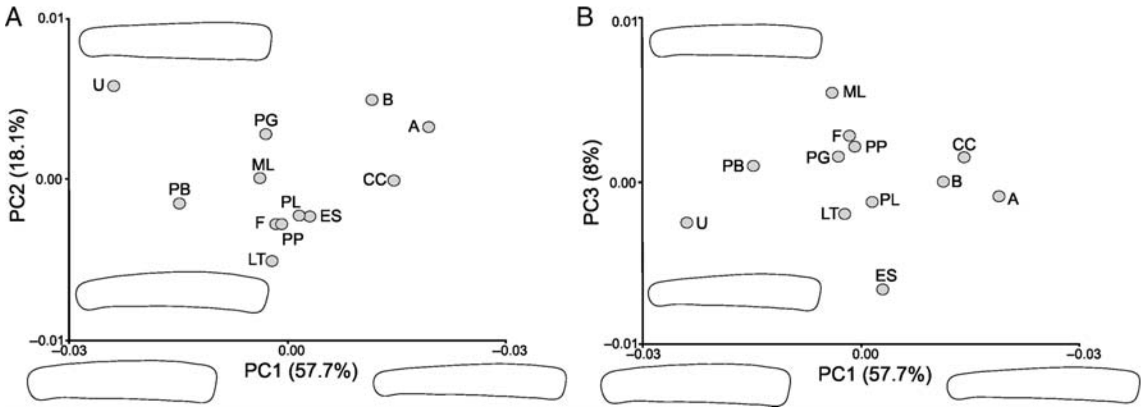

Figure 2. Plot of the PCs for *Ensis macha* localities based on 57 Fourier coefficients from shell outlines and diagrams of the reconstructed extremes of shell shape (mean − 2SD, and mean + 2SD). **A.** PC1 *vs* PC2. **B.** PC1 *vs* PC3. Percentages of explained variance for each axis are in parentheses. The circles indicate the averages for each population. See Figure 1 for locality abbreviations.

summarize shape variation based on the EFDs for each shell of *E. macha*. Those PCs of which eigenvalues represented more than the 5% of the total variance were retained (Zelditch *et al.*, 2004). To appreciate the effects of each PC on the shell shape, the average ± 2 standard deviation (SD) shapes for each group were reconstructed from the mean values of EFDs using the inverse Fourier transformations (provided by SHAPE – PrinPrint software).

In order to assess allometry (association between size and shape), shell area was related with the PCs through a linear multiple regression (PCR method; De Maesschalck *et al.*, 1999; Costa *et al.*, 2008). To test shape differences between localities we used an extension to the multivariate case of a multiple-comparison method based on cluster analysis generated using an unweighted pair-group method with arithmetic mean (UPGMA-MDGC: Valdano & Di Rienzo, 2007; Márquez *et al.*, 2010). This new method is a hybrid technique that combines a hierarchical clustering based on Mahalanobis distances (Mahalanobis, 1948) for hypothesis testing in multivariate cases. This method is successful in determining the number of groups in hierarchical cluster analyses. The graphical output of MDGC test is a useful tool, since it shows a clear distinction between statistically different shell shapes from each locality as well as their relationships (Márquez *et al.*, 2010).

To evaluate the combined effects of the environmental variables (latitude, depth, grain size, salinity and SST) on principal shell-shape variables (PCs), stepwise multiple regression (PROC STEPWISE in Infostat software) was used, with $P = 0.15$ to enter or remove variables. We also calculated the Mahalanobis distance matrix based on the mean of the 57 EFD coefficients for each population and used these distances as pairwise population estimates of phenotypic distance. We used Mantel's simple test (Mantel, 1967) to measure the degree of association between the variations in shell shape with geographical distance (calculated as straight-line distances between localities using Google Earth software). Statistical analyses were run using Infostat software (Di Rienzo *et al.*, 2009).

RESULTS

Characterization of shell-shape variation

Only the first three PCs (summarizing 84% of EFA total variance) were used in the analyses. Negligible regressions were found between size (shell area) and shell shape ($R^2 = 0.1$). PCR on populations showed that only 10% of the variance in size can be explained by the first three PCs, thus we performed all subsequent analyses without allometric corrections. The reconstructed contours indicated that PC1, which accounted for 58% of the total shell-shape variation, was a good measure of shell slenderness. For negative loadings on the first axis, shells were more broad, while for positive loadings shells were slender. PC2 was associated with degree of curvature of the shell and accounted for 18% of the variance. On this second axis negative loadings corresponded to curved shells and positive ones to almost straight shells. PC3, which explained 8% of the variance, represented shape changes from more triangular (with the anterior edge narrower than the posterior one) in the negative loadings, to more rectangular (with both edges of the same width) in the positive loadings (Fig. 2).

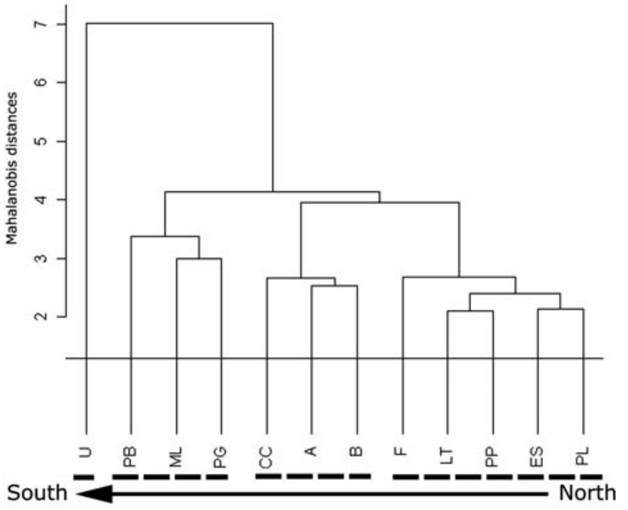

Figure 3. UPGMA dendrogram showing the relationships between the shell shapes of *Ensis macha* at the different localities. The cut-off criterion obtained with the MDGC test ($Q_{(12;57;1,200;0.99)} = 1.27$) is indicated with a horizontal line. All the localities have statistically different shell shapes. The four inferred geographical groups are shown with dashed lines. See Figure 1 for locality abbreviations.

Differences in shell-shape variation and relationships among localities

The dendrogram calculated on the Mahalanobis distance matrix showed that, even though each locality had a characteristic shell shape with statistically significant differences, similarities between sites grouped them in four large geographic areas: north, central, south and the Beagle Channel (Fig. 3). The north group (ES, PP, PL, LT, F) was closer to the central group (CC, B, A) than to the south one (PG, PB, ML); these three groups were more similar to one another than to the Beagle Channel locality (U).

Association between phenotypic variations, environmental variables and geographical distances

Sediment composition was similar at each locality (see Supplementary material). Water depths ranged from 2 m in the Beagle Channel (U) to 18 m at El Sótano (ES).

Stepwise linear regression analysis resulted in a bivariate model that explained 80% of the variation in PC1 by salinity and depth. PC2 was not significantly associated with any of the environmental variables; but PC3 was related to salinity resulting in a univariate model that explained 59% of the variation (Table 1).

The Mantel test showed a significant correlation between the Mahalanobis distance based on the 57 coefficients of the Fourier series and geographical distances ($R = 0.75$, $P = 0.001$; Fig. 4).

Table 1. Stepwise multiple linear regression analysis of principal shell-shape variables of *Ensis macha* as a function of latitude, depth of the bottom, median grain-size diameter, salinity and surface seawater temperature.

Variable	Parameter	Partial *T*	*P*
PC1			
Intercept	−0.05	−5.56	0.0005
Salinity	0.02	5.56	0.0005
Depth	−2.2E−03	−4.42	0.0022
PC3			
Intercept	0.11	3.82	0.0033
Salinity	−3.3E−03	−3.80	0.0035

Only variables with $P < 0.05$ were included.

DISCUSSION

We investigated morphological and environmental variation among 12 populations of *Ensis macha* along its entire Atlantic distribution in order to assess shell-shape variation along a latitudinal cline. The population of *E. macha* discovered in the Beagle Channel is the southernmost living record for this species (for environmental variables and waypoints, see Supplementary material). This is an important discovery since the Beagle Channel lies between the Atlantic and Pacific Oceans and is critical for the interpretation of faunal distribution patterns around the tip of South America.

The use of elliptic Fourier analysis allowed us to characterize, with high resolution, the shell morphology at each site and to suggest associations between them. As expected, the shell shape displays considerable variation along *c.* 3,700 km of Atlantic coastline. The major variations in shape, illustrated by the principal components, involve slenderness and curvature of the shell. There are several studies regarding the relationship between shell morphology and environmental factors such as latitude, currents, depth and type of sediment, among others. For example, Beukema & Meehan (1985) found a latitudinal cline of shell dimensions and growth in the tellinid *Macoma balthica*, Fuiman, Gage & Lamont (1999) suggested morphometric relationships between the shape of the deep-sea protobranch *Ledella pustulosa* and bottom currents, and Claxton *et al.* (1998) proposed that the quagga mussel *Dreissena bugensis* shows plasticity in shell morphology in relation to depth.

The sediment composition of *E. macha* habitats is similar between sites and does not show any relationship with the shell shape. Shape differences are related mainly to salinity and depth of the bottom. Our results are similar to those of Krapivka *et al.* (2007) and Aguirre *et al.* (2006) in the mytilids *Mytilus chilensis* and *Brachidontes* species, respectively, who showed a strong influence of salinity on shell shape along the clines. Salinity should be recognized as a potentially important influence on bivalve morphology.

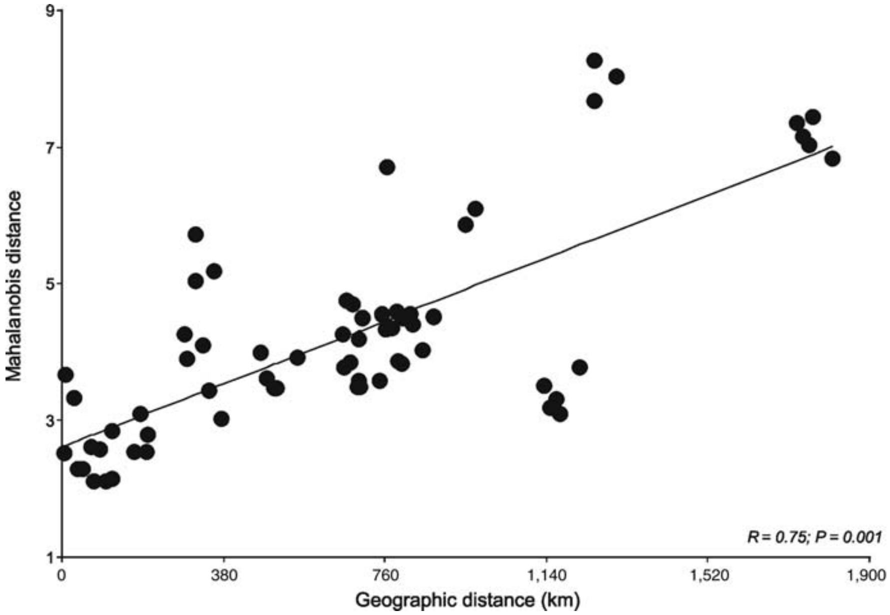

Figure 4. Relationship between the morphological Mahalanobis distances and geographical distances for *Ensis macha* populations.

In marine bivalves, the range of a species is generally characterized by a series of local populations that are connected by the exchange of planktonic larvae (Gaines *et al.*, 2007). High larval exchange among habitats produces genetically unstructured populations (Holt & Gomulkiewicz, 1996; Postma & Van Noordwijk, 2005). However, the connectivity between populations can be affected by both physical and biological barriers to larval dispersal. The principal factors determining transport of larvae are the direction and speed of ocean currents, distance between populations, maximum duration of larval development and larval behaviour (Scheltema, 1972, 1978; Burton & Feldman, 1982; Colebrook, 1982). The planktonic larval period of *E. macha* is *c.* 20 days (under laboratory conditions) until metamorphosis (Lépez Garcia, Arriagada Orregón & Véjar Durán, 2008). The pattern of shell-shape variation of *E. macha* is consistent with the likely seasonal retention zones of larvae on the Patagonian Shelf, produced by the offshore winds and tidal fronts. For example, the differences between the most divergent locality (U) and the others could have resulted from a barrier to larval dispersal caused by the tidal front in Grande Bay (Fig. 1). Another important tidal front is that at San Jorge Gulf, generating the central assemblage (localities A, B and CC). Furthermore, coastline indentations at both sites, San Jorge Gulf and Grande Bay, lead to the formation of quasi-stationary (counterclockwise) gyres and a northeastward boundary current is produced by offshore winds (Fig. 1), facilitating these possible retention zones.

The phenotype is a product of the interaction between genotype and environment and habitat heterogeneity is an important driver of biological adaptation (Ridley, 1996). Therefore, it is expected that adaptation to local environments generates shell-shape variation through selection, if gene flow between distant localities is low. Such adaptation is likely to occur among populations distributed along strong environmental gradients (clines), or when populations colonize new habitats, producing differentiated forms, ecotypes or even species. However, our results show significant shell-shape variation even between neighbouring localities; therefore we do not discount the influence of phenotypic plasticity on *E. macha* populations.

Thus, morphological variation within *E. macha* localities appears to be driven by two different phenomena depending on the spatial scale considered. Variation between neighbouring populations could be due to phenotypic plasticity, while between distant populations the restricted gene flow caused by physical and/or biological barriers could promote the adaptive divergence. To clarify the evolutionary mechanisms that underlie the shell-shape variation of *E. macha* molecular or quantitative genetic studies are required.

Despite the difficult sampling logistics (rapid burrowing and extreme environmental conditions), *E. macha* populations in the southwestern Atlantic provide an interesting model system for studying the mechanisms that originate and maintain shell-shape variations of subtidal infaunal species.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Molluscan Studies* online.

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