

# Use of shell-shape to discriminate between *Brachidontes rodriguezii* and *Brachidontes purpuratus* species (Mytilidae) in the transition zone of their distributions (south-western Atlantic)

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*Mussels are important components of rocky shore assemblages throughout the world. Several mytilid species are found as multilayered beds in the intertidal along the coasts of the south-western Atlantic. However, in contrast to the north communities in the Pacific and Atlantic, those of the south-western Atlantic are dominated by species of small size, locally named mejillines. These mid-intertidal beds attached to consolidated substrates are dominated by virtual monocultures of two small-sized species of Brachidontes: B. rodriguezii (d'Orbigny, 1842) and B. (Perumytilus) purpuratus Lamarck, 1819, respectively distributed in the warm and cold temperate sectors of the south-western Atlantic; both coexisting in the transition zone between 40° and 44°S latitude. Nevertheless, there has been some confusion about the separation of B. rodriguezii and B. purpuratus, as well as about the boundaries of their distribution ranges in the south-western Atlantic. Here, on the basis of a morphogeometric analysis of shell morphology, we describe a feasible way for the identification of both species.*

**Keywords:** *Brachidontes*, geometric morphometrics, mytilids, shell shape morphology, transition zone distribution

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## INTRODUCTION

Rocky shores mussels frequently occur as multilayered beds of considerable structural complexity (Guiñez & Castilla, 1999; Commito & Rusignuolo, 2000). In southern South America, the intertidal mussel beds are dominated by species of small size, mostly of the *Brachidontes* genus, however larger mussels (i.e. *Mytilus* sp.) are also present, but in low densities along the rocky shores (Penchaszadeh, 1973; López Gappa *et al.*, 1990; Adami *et al.*, 2004, 2008). The distribution pattern of *Brachidontes* intertidal mussel beds along the south-western Atlantic includes *Brachidontes solisianus* d'Orbigny, 1846 and *B. darwinianus* d'Orbigny, 1846 in south Brazil and Uruguay (Klappenbach, 1965; Rios, 1994; Scarabino *et al.*, 2006), *B. rodriguezii* from Uruguay to north Argentinean Patagonia (Castellanos, 1967; Scarabino *et al.*, 2006) and *B. purpuratus* Lamarck, 1819 on the Patagonian shore, extending towards the Pacific ocean along the Chilean coast to Ecuador (Bernard, 1983; Prado & Castilla, 2006). The latter species was frequently placed by several

authors under the genus *Perumytilus* Olsson, 1961 (Aguirre *et al.*, 2006).

Biogeographically, the *B. rodriguezii* distribution range is included in the Argentine province (warm-temperate), from the south of Brazil to the north of Patagonia, while *B. purpuratus* distribution is included in the Magellanic province (cold-temperate) from Las Grutas (40°50'S San Matías Gulf), to the Beagle Channel in the south-western Atlantic (Scarabino, 1977; Rios, 1994; Scarabino *et al.*, 2006; Adami *et al.*, 2007). The transition zone between these two biogeographical provinces has been shortly characterized and is located in the sector of the north Patagonian gulfs between 40°S and 44°S latitude (Balech & Ehrlich, 2008).

At present, the taxonomic status of these species is under discussion, and there are no simple tools to distinguish between them. *Brachidontes rodriguezii* and *B. purpuratus* shells have several particular features that allow to distinguish between them: the umbo is terminal and numerous strong and forked ridges run from the umbo out to the hind edge of the shell such as a fan in *B. purpuratus* while in *B. rodriguezii* ridges are fine and run parallel from the sub-terminal and rounded umbo (Penchaszadeh *et al.*, 2008). However, in certain intertidals the shells are very worn away and their differentiation based on these characteristics becomes virtually impossible. Phenotypic diversity provides valuable

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clues to the differentiation of species and subspecies, reflecting the historical processes that create taxonomic distinctions as well as plastic responses to the environment (Cardini & Elton, 2011). The possibility to discriminate between them is of great interest for ecological and biodiversity studies.

The conventional morphometric approach is less likely to be successful in cases where diversity of shapes is not large, and hence, the morphometric trends are apt to be rather subtle, as is the case of mussel shells. In this particular case, elliptical Fourier analysis (EFA) (Kuhl & Giardina, 1982) is more appropriate, since it allows researchers to capture the entire outline shape in a systematic manner and with a desired degree of precision (Rohlf & Archie, 1984). Previous studies have shown that EFA is effective in the analysis of bivalves shell shape variation when applied to distinguish similar species of bivalves (Rufino *et al.*, 2006; Costa *et al.*, 2010), between fossil and modern specimens (Gordillo *et al.*, 2011), and to analyse geographical variation in shape (Ferson *et al.*, 1985; Innes & Bates, 1999; Palmer *et al.*, 2004; Krapivka *et al.*, 2007; Costa *et al.*, 2008; Márquez *et al.*, 2010; Gordillo *et al.*, 2011; Márquez & Van der Molen, 2011; Rufino *et al.*, 2012). Furthermore this method lets researchers analyse complex outlines without losing information about shells' form (Rohlf & Archie, 1984).

The implementation of geometric morphometric tools to easily obtain the shell outlines and to reconstruct the shell contours of the analysed data will enable us to determine the main shell shape variation shown by these two species. In the present study we analyse the shell morphological pattern in the dominant mussel beds species, *B. rodriguezii* and *B. purpuratus*, along their Atlantic distribution range, including the transition zone, where both species coexist. The aim of this study is to discriminate shell-shape patterns between *B. rodriguezii* and *B. purpuratus* populations that could be a determinant factor to differentiate them when they coexist in the same rocky shore, since the environmental conditions are the same for both species, and to visually describe the differences to evaluate if it is possible to generate a key for an easy differentiation.

## MATERIALS AND METHODS

*Brachidontes rodriguezii* and *B. purpuratus* samples were collected from the two extremes of their distribution ranges in the south-western Atlantic, Punta del Diablo (34°02'S 53°32'W) and Ushuaia (54°49'S 68°11'W) respectively. In addition a third sample collection site was included in the transition zone: Punta Mejillón (41°01'S 64°08'W) in north Patagonia, which comprises the southern distribution location of *B. rodriguezii* and the northern extreme distribution range for *B. purpuratus* (Figure 1).

A total of 200 adult individuals were collected from mussel beds: 50 individuals from each sample site. The sample size-ranges were as follows: *B. rodriguezii* from Punta del Diablo: 127–226 mm; *B. rodriguezii* from Punta Mejillón: 157–228 mm; *B. purpuratus* from Punta Mejillón: 118–234 mm; and *B. purpuratus* from Ushuaia: 279–344 mm. Soft parts were removed and images of the inner side of the left valves were obtained using a digital camera. Shell area (cm<sup>2</sup>) was estimated using SHAPE-ChainCoder software (Iwata & Ukai, 2002) and it was used as a proxy for shell size.

To analyse the morphological variation between *B. rodriguezii* and *B. purpuratus*, we applied EFA using the software

package Shape v1.26 (Iwata & Ukai, 2002). An EFA method consists of decomposing a curve into a sum of harmonically related ellipses (Lestrel, 1997). The closed contours are obtained as chain-coded data from the digital images of the outlines (Freeman, 1974). The coefficients of the Fourier series (elliptic Fourier descriptors, (EFDs)), which are normalized to avoid variation related to the size, rotation and starting point of the contour traces, are then calculated from the chain-coded data using the procedure proposed by Kuhl & Giardina (1982). From a preliminary analysis, we concluded that 10 harmonics were sufficient to characterize mussels' shell shape in detail. By this procedure, the shape of each shell was approximated to 37 coefficients of normalized EFDs (four coefficients for each harmonic, excluding the first three that became constant after the normalizations).

Principal component analysis (PCA) of the variance-covariance matrix (Rohlf & Archie, 1984; Crampton, 1995) was used to summarize shape variation based on the EFDs for each shell. To assess and control putative allometric effects, we computed a linear multiple regression (principal component regression method: De Maesschalck *et al.*, 1999; Costa *et al.*, 2008; Márquez & Van der Molen, 2011), where PCs were used as independent variables and shell area as dependent variable.

A discriminant analysis based on the shape (EFDs) of the two extreme populations (*B. purpuratus* from Ushuaia and *B. rodriguezii* from Punta del Diablo) was performed. Using 50% of the total sample (100 individuals from the two extreme populations *B. purpuratus* from Ushuaia and *B. rodriguezii* from Punta del Diablo) the discriminant function was formed. Then, this discriminant function previously defined was used to assign the individuals from the transition zone (Punta Mejillón) of both species, without group information, to one or other species. The average shell shape for each species was reconstructed from the normalized coefficients mean values of the EFDs using the inverse Fourier transformations (Iwata & Ukai, 2002).

Provided with the drawings of the shapes from the two species, previously achieved, seven collaborators classified the transition zone shells into two groups to determine if inter-specific differences in shell shape were distinguishable by visual observation. Next we calculated the percentage of individuals correctly assigned to each species. The proportion of correctly assigned individuals by visual observation was then compared with those correctly assigned by the discriminant function.

## RESULTS

Only the first three PCs (summarizing the 89% of the EFA total variance) were used in the analyses. Negligible regressions were found between size (shell area) and shell shape for PC1 and PC2 ( $R^2 < 0.003$ ), however the regression was significant for PC3 ( $R^2 = 0.51$ ). But given that the third axis explained only 8% of the total shape variance, only 8% of the shell shape variation is explained in 51% by size. Considering this, all the subsequent analyses were performed without allometric corrections.

The discriminant function successfully distinguished between the two species from the extreme locations (*B. rodriguezii* from Punta del Diablo and *B. purpuratus* from Ushuaia). There are a series of characters that allow

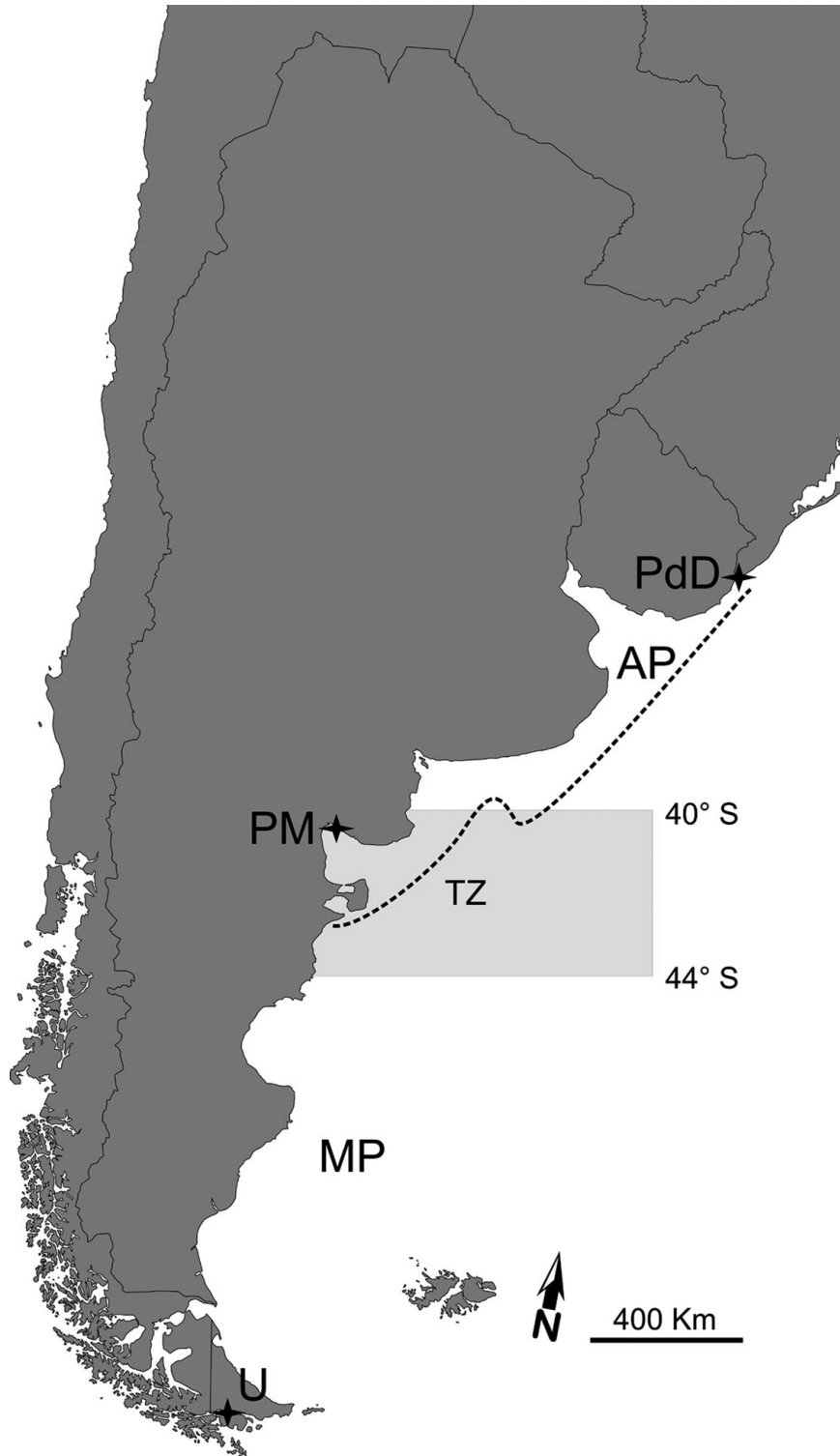


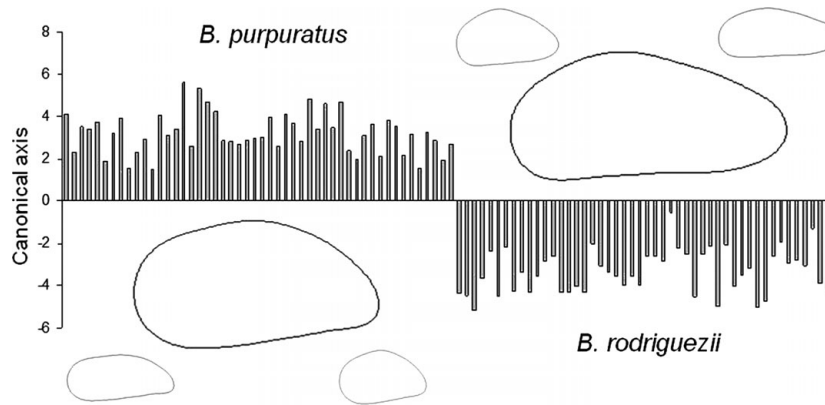
Fig. 1 - B/W online, B/W in print

**Fig. 1.** Map of the south-western Atlantic Ocean showing the sample collection sites. The broken lines separate the biogeographical provinces along the Atlantic coast: AP, Argentinean Province and MP, Magellanic Province. The grey clear box shows the transition zone (TZ). Codes of sample collection sites: PdD, Punta del Diablo; PM, Punta Mejillón; U, Usuahia.

their unequivocal separation: in *B. rodriguezii* valves of adult individuals are elongated and the position of the umbo is sub-terminal, while in *B. purpuratus* valves are globular and the position of the umbo is terminal (Figure 2). The proportion of correctly assigned individuals from the transition zone using the discriminant function obtained from the two

extreme populations was slightly different for each species. While *B. rodriguezii* shells were 100% correctly assigned, *B. purpuratus* shells were correctly assigned in 78.2%.

Regarding the classification by the direct observation method, the proportion of correctly assigned shells varied between 34 and 94%, with an average of 66%.



**Fig. 2.** Discriminant analysis between the extreme populations of two species of the *Brachidontes* genus. Outline diagrams show the mean (middle),  $-2$  standard deviation (SD) (left) and  $+2$ SD (right) shell shape of each of them.

## DISCUSSION

*Brachidontes* genus includes species which often vary intra- and inter-specifically in response to environmental conditions as a result of ecophenotypic plasticity (e.g. Nalesso & Duarte, 1992; Tanaka & Magalhães, 1999). The most accepted hypothesis is that there is a morphological sequence of ecomorphs which respond to different environmental controls such as latitude, depth, substrate type and water energy (Aguirre *et al.*, 2006). Some biodiversity and palaeobiogeographical studies illustrate that the differentiation between *Brachidontes* species from the transition zone is difficult (Aguirre *et al.*, 2006; Cuevas *et al.*, 2006), since most morphological differences are only identifiable by experienced taxonomists. Therefore, we analysed the inter-specific shell shape variations, with special emphasis on individuals from the transition zone. The strength of this study is that the analysis was based on individuals that inhabit the same patch in the middle intertidal rocky shore, and so the shape variation that could be caused by local ecological conditions is almost completely reduced.

The *Brachidontes* mussel inhabits intertidal rocky shores where they form complex multilayered matrices or beds. These mussels have been defined as ecosystem bio-engineers and many taxa are associated with them on the rocky intertidals (Pérez *et al.*, 2008), and thus they are important for the regional biodiversity. Quantifying biodiversity is a crucial aspect of conservation, and molecular analyses have become the primary source for identification of biological diversity. However numerous studies have highlighted the risk of underestimating diversity by focusing on a single point of comparison (Cardini & Elton, 2011). In a phylogenetic reconstruction, Trovant *et al.* (2011) explain that *B. rodriguezii* and *B. purpuratus* from the south-western Atlantic, which distribution overlaps between  $40^{\circ}$  and  $44^{\circ}$ S show different biogeographical and phylogenetic affinities. In the present study we clearly distinguish both *Brachidontes* species inhabiting the transition zone by means of morphometric tools, showing that both phenotypic and genotypic studies are in agreement. Given this, the study of morphological differences provides important complementary perspectives on biodiversity (Cardini & Elton, 2011).

The EFA provided better results on the classification of individuals pertaining to different species from the transition zone than the results from direct observations. The principal advantage of EFA for visual observations is that EFA is not

a subjective method. So, the analysis of shell shape variation through EFA decomposition provides an objective tool to discriminate between *B. purpuratus* and *B. rodriguezii*, allowing simple and complete visualizations of specimens' means (Ferson *et al.*, 1985; Costa *et al.*, 2010). Furthermore, these mussels form dense multilayered beds and the time needed for manual classification is elevated, though using the automated procedures described in this work reduces the processing speed and also diminishes the chance of human error. Because only the experienced taxonomists can correctly classify individuals of both species based on their internal and external morphology, our results suggest that the use of the discriminant function from the EFA method could be easily used by non-taxonomist specialist observers to differentiate between species of *Brachidontes* genus of the transitions zone in future ecological and biodiversity research.

In the future, the implementation of this tool would enable development of simple user-friendly software that facilitates the determination of species or populations with less assignment error. Finally, the methodology presented here brings some promising insights concerning the inter-specific mussel identification: this new approach yielded the clearest results in the study of species identification improving, in this way, biodiversity studies.

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