MORPHOLOGICAL SHELL VARIATION OF *Zidona dufresnei* (CAENOGASTROPODA: VOLUTIDAE) FROM THE SOUTHWESTERN ATLANTIC OCEAN

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ABSTRACT. The volutid gastropod *Zidona dufresnei* is an important fishery resource from the Southwestern Atlantic Ocean. This species exhibits strong interpopulation differences in life history features, which lead to postulate the existence of two morphotype ('normal' and 'dwarf'). In this study, we combine and compare traditional and geometric morphometrics to capture shell shape variation of *Z. dufresnei* among three populations from Mar del Plata (37° S) to San Matías Gulf (42° S) to test the hypothesis that the phenotypic variation already described in the life cycle and size is also expressed in the shell shape. Significant differences in the shell morphology among these three populations were detected, mainly associated to the maximum size of individuals and shell shape. The Bahía San Antonio morphotype had shells with higher general roundness and weight compared to San Matías Gulf and Mar del Plata morphotypes, which were not differentiated. Our results support the hypothesis of Lahille (1895) who distinguished the morphotype of Bahía San Antonio ('dwarf' morphotype) as *Voluta angulata affinis*. The functional significance of the variability found is discussed in terms of the ecological and genetic effects on shape and size.

Key words: Marine gasteropod, shell variation, geometric morphometry, South Atlantic.

INTRODUCTION

Comparison of the anatomical characteristics of organisms has been a key point in biological research. Studies focused on taxonomic classification of organisms have mainly been on the characterization of body size and shape of individuals (Rohlf 1990; Adams et al. 2004). In the case of gastropods, shell morphology has been one of the most important features to identify species and to understand phenotypic variation within species (e.g. Trussell 2000; Hollander et al. 2006; Conde-Padín et al. 2007, 2009). Several methods have been used to analyze intra- and inter-specific shell variation in morphology, but traditional and geometric morphometrics have been the most frequent since the shell is rigid and characterized by noticeable anatomical points (Carvajal-Rodríguez et al. 2005; Marko 2005; Guerra-Varela et al. 2009; Avaca 2010; Valladares et al. 2010; Teso et al. 2011).

The volutid gastropod *Zidona dufresnei* (Donovan, 1823), known locally as 'caracol fino' (fine snail) or 'caracol atigrado' (tabby snail), is distributed on the western coast of the South Atlantic Ocean from Río de Janeiro, Brazil (22° S-42° W) to Patagonian waters of San Matías Gulf, Argentina (42° S-64° W) (Kaiser 1977; Rosenberg 2009). This species lives on sandy or muddy bottoms between the low intertidal zone and 200 m water depth and exhibits a patchy distribution pattern (Scarabino 1977; Pereyra et al. 2009; Medina et al. 2015, 2016).

Z. dufresnei is one of the most important gastropods which have been subject to fishing pressure in Argentina and Uruguay with annual landings ranging from 500 to 3,000 t (Fabiano et al. 2000; Giménez et al. 2005; Roche et al. 2013). Similar to other volutid gastropods, their life history parameters (large body size and somatic production, slow growth rate, late reproductive maturity and direct development) make this species extremely vulnerable to overexploitation (Giménez and Penchaszadeh 2002; Giménez et al. 2004; Medina et al. 2015, 2016). Further, the occurrence of direct (intracapsular) development and absence of a pelagic larval stage is usually recognized as a factor preventing gene flow and leading to genetic differentiation of allopatric populations (e.g. Scarabino 1977; Darragh et al. 1998; Pereyra et al. 2009). Several studies reported differences in the maximum size and weight of individuals of *Z. dufresnei* along the geographical distribution of the species, possibly due to different environmental conditions (Pereyra et al. 2009; Medina et al. 2015). Particularly, two different populations were described in San Matias Gulf based on the maximum size and weight of mature individuals (Medina et al. 2015, 2016). One of these populations, whose individuals reach 230 mm long and 831 g in weight, inhabits deep waters (between 35 and 130 m) inside the gulf. The other population, with individuals reaching 120 mm long and 113 g in weight is located in shallow waters of the gulf (less than 2 m depth) (Medina et al. 2015) (Figure 1). These differences led to postulate the existence of two morphotypes: a 'normal' (from relatively deep waters) and a 'dwarf' morphotype (from shallow waters) (Lahille 1895). Even Lahille (1895) referred to a small volutid identified as *Voluta angulata affinis*, which would be a specimen from the San Antonio Bay. Later, Clench and Turner (1964) based on morphological characters unified the variety *V. angulata affinis* with *Z. dufresnei* leading to potential taxonomic inconsistencies related to the issue of whether these morphotypes are subspecies or even separate (cryptic) species.

Considering the high degree of morphological variation reported for the species (Roche et al. 2013; Medina et al. 2015, 2016), *Z. dufresnei* offers the opportunity to investigate morphological pattern in heterogeneous environment. Despite that, no studies have been conducted to determine differences in shell shape since differences in shell size have already been investigated. In this context, the aim of this study was to analyze the differences in shape between morphotypes of *Z. dufresnei* using traditional and geometric morphometrics approaches among and within three populations distributed along the Argentine Sea. The results obtained by both methodologies were also compared. We tested the hypothesis that the isolation among populations favors a phenotypic variation expressed at the shell shape level. Overall, these results are expected to contribute to a better understanding of the taxonomic status of *Z. dufresnei*, and thus provide basic knowledge to achieve a sustainable management of this fishing resource by designing strategies that account for the variability between local taxonomic units.

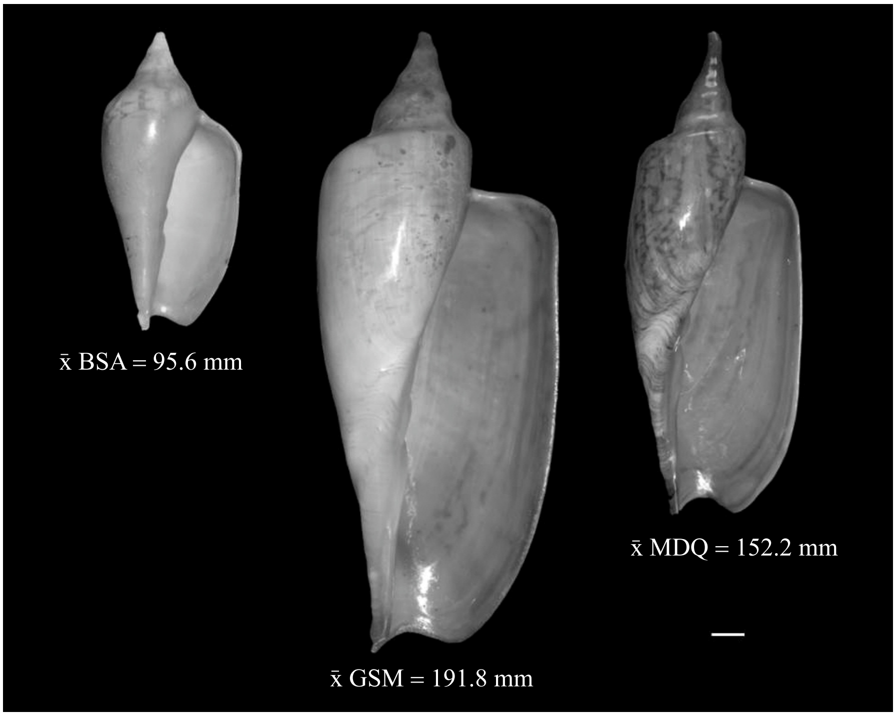

Figure 1. Representative specimens of mean total length of each studied population of *Zidona dufresnei*. BSA: San Antonio Bay, GSM: San Matías Gulf, MDQ: Mar del Plata. Scale bar = 10 mm.

MATERIALS AND METHODS

Study sites and samples collection

Individuals of *Z. dufresnei* were collected in three locations along the Argentine Sea: Mar del Plata (MDQ), San Antonio Bay (BSA) and San Matías Gulf (GSM) (Figure 2), from 2007 to 2011. These locations were selected since they are the only places where stable populations of this species in Argentine waters were properly described. At the same time, BSA population is the only 'dwarf' morphotype population of *Z. dufresnei* known so far. MDQ site was characterized by sandy bottom, mean salinity of 35, sea surface temperature (SST) range of 9-17 °C, and depth between 40 to 60 m (Guerrero et al. 1997). GSM is a semi-enclosed gulf with a surface of 19.700 km^2, characterized by a high rate of water retention due to its topography. Its maximum depth is 200 m in the center of the gulf and decreases up to 45 m in the mouth (Mazio and Vara 1983). The SST and salinity in the gulf vary between 11.3 and 13.5 °C and between 33.5 and 34.1, respectively (Williams et al. 2010). The seabed of the fishing zone consists mainly of a mixture of sand and mud. BSA is a shallow macrotidal system located in the northwestern region of GSM with tidal amplitudes of up to 9 m and strong tidal currents within their main channels. The dominant bottom type is sand, with variable content of interspersed pebble and cobble. Due to its narrow mouth (5 km long), the bay presents a low rate of water exchange with GSM. The water temperature in BSA oscillates between 6 and 28 °C throughout the year and salinity varies between 31.8 and 39.0 (Piola and Scasso 1988; Saad 2018 pers. comm.) but it could decrease to

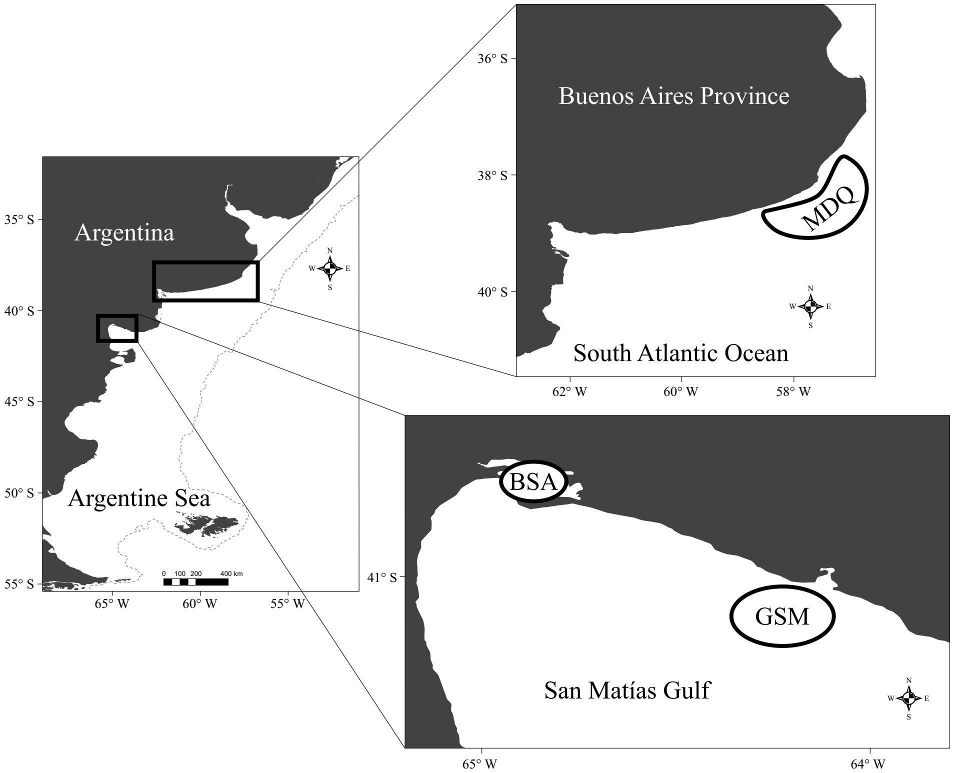

Figure 2. Collection site of *Zidona dufresnei*. MDQ: Mar del Plata, BSA: San Antonio Bay, GSM: San Matías Gulf.

29.0 with extreme rainfall (Salas 2019 pers. comm.). Contrary to MDQ and GSM sites, BSA area is a wave exposed intertidal environment.

In MDQ, individuals were obtained from the bottom trawl fishery that targets this species at 40-60 m depth. In this fishery, vessels are equipped with bottom nets of 42 mm mesh size. In GSM, individuals were collected from the bycatch of the bottom trawling fleet that targets the Argentine hake (*Merluccius hubbsi*). The depth at which the specimens of *Z. dufresnei* were obtained averaged 100 m. Mesh size used in this fishery ranges between 110 and 120 mm. In BSA, individuals were hand-collected by artisanal fishermen from the intertidal region (0-1 m depth), using an iron gaff. All specimens were sexually mature adults. Maximum size for each population was recorded (Lahille 1895; Clench and Turner 1964; Kaiser 1977; Scarabino 1977; Roche et al. 2013; Medina et al. 2015). Adult size was established separately for each population according to size at maturity described in the literature (Roche et al. 2015, Giménez and Penchaszadeh 2003). Although smallest individuals were not sampled in any of the populations we were able to compare among adults and maximum sizes.

Morphometric analysis

Both traditional and geometric morphometric methodologies were used to study shell shape variation as complementary analysis. The traditional morphometric analysis was conducted using 253 individuals (MDQ: 99, GSM: 78 and BSA: 76). These sample sizes were in concordance to the sample sizes estimated by power analysis method using G*power software (freeware, Faul et al. 2009). Power of the study was 95%. Only individuals in good enough condition to take the measurements were used for the traditional morphometric analysis (e.g. apically eroded specimens were discarded from the analysis). Animals were sexed based on the presence of the pedal gland in females and the presence of a penis in males. The following measures (mm) were taken for each individual shell using a digital caliper: total length (TL), total width (TW), aperture length (AL) and aperture width (AW) (Figure 3 A). Additionally, total weight (TW) and shell weight (SW) in grams were recorded. To analyze morphometric variations six indexes were used: general roundness (GR = TW/TL), relative length of the aperture (RLA = AL/TL), relative width of the aperture (RWA = AW/LT), relative shape of the aperture (RSA = AW/AL), relative expansion of the aperture (REA = AW/TW) and relative weight of the shell (RWS = SW/TW). Differences between populations were analyzed by Principal Component Analysis (PCA) and nonparametric tests using all indexes. Also, the following linear regressions were estimated and differences between sites and sexes were analyzed using ANCOVA for the relationships TL versus AL, TL versus AW, and SW versus TW. Before the analyses, data were tested for normality with the Shapiro-Wilk test and for homogeneity of variance with the Levene test.

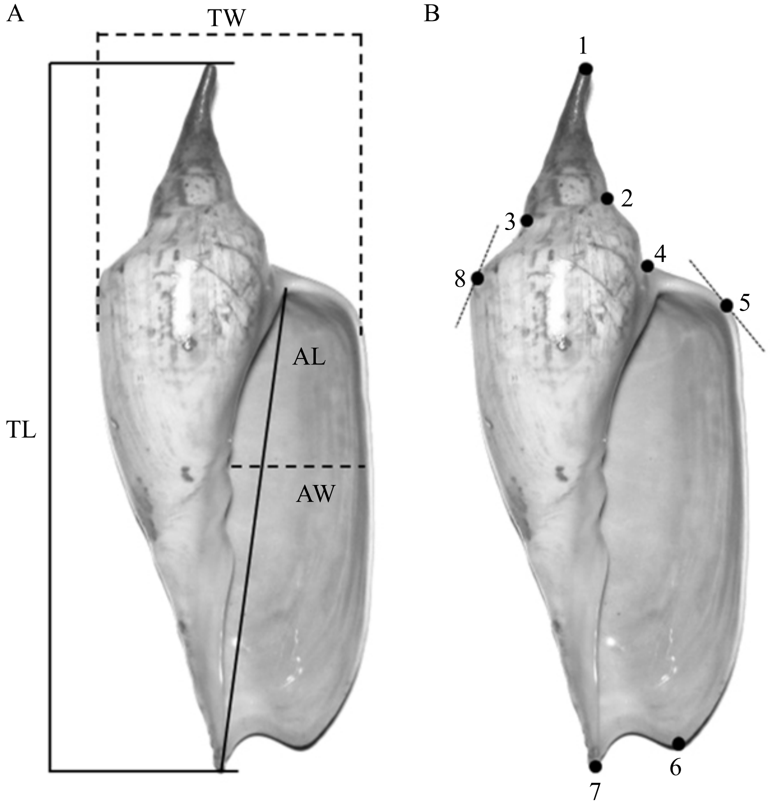

Figure 3. Diagram of the shell of *Zidona dufresnei*. A) Measurements used in the traditional morphometric analysis: total length (TL), total width (TW), aperture length (AL), aperture width (AW). B) The eight landmarks used in the geometric analysis. Landmark (L) 1: apex, L2: right border of the suture of the last anfract, L3: left border of the suture of the last anfract, L4: outer-end of the suture of the last right anfract, L5: posterior border of the outer lip, L6: right border of the siphonal channel, L7: left border of the siphonal channel, L8: end of the suture of the last left anfract.

Geometric morphometrics approach was conducted using a subset of 68 adult snails shells (MDQ: 19, GSM: 19, BSA: 30). We selected individuals with unbroken shells, which were difficult to obtain due to fishing procedures. In this way, the sample size was limited by the availability of samples in good condition. Shell photographs were taken with a digital camera (Nikon Coolpix P5100, 12.1 megapixels) mounted on a table top to ensure parallelism between the focal plane of the camera and frontal plane of individuals. All photographs were taken at the same resolution including a graded scale in each one as a reference. In order to reduce experimenter bias the photographic method was carried out by A.I.M. and repeatability was tested. Repeatability between sessions was high (t-test, $p > 0.90$ for all comparisons). Shells were placed with the aperture facing the plane of the camera and distance between shells and camera was large enough (respect to shell size) to minimize the error caused by the optical distortion of the lens (Zelditch et al. 2004). Eight landmarks to analyze shape variation were selected following the criteria of Conde-Padín et al. (2007) with slight modifications (Figure 3 B). Three landmarks (1, 6 and 7) were of type I (points where at least two distinct structures meet; i.e. the posterior tip of the body) and the remaining five landmarks of type II (points that are supported by geometric criteria; i.e. border of the suture of the last anfract) (Bookstein 1991). These landmarks are typically chosen to study shell variation in snails (Chiu et al. 2002; Cruz et al. 2012; Avaca et al. 2013; Vergara et al. 2016, Vaux et al. 2017; Amini-Yekta et al. 2019). Landmark coordinates were obtained by using TPSDig v.2 software (Rohlf 2001).

Translation, rotation and scale effects were removed by Generalized Procrustes Analysis (GPA) (Zelditch et al. 1998; Adams et al. 2004). In this method, landmark configurations are superimposed by least squares optimization and the process is iterated to compute the mean shape (Atchley and Hall 1991; Zelditch et al. 2004). After GPA, shape differences were analyzed by Procrustes distance differences. Centroid size (CS), which is calculated as the square root of the sum of the squared deviations of landmarks from a centroid (Bookstein 1991; Zelditch et al. 2004) for each specimen was used as a size proxy. The centroid size is a measure of size uncorrelated with all pure shape changes (Bookstein 1991). One-way ANOVA was used to compare the means of the centroid size between the three populations. Tukey test was used for *post-hoc* analyses.

The presence of allometry (changes in shape related to changes in size) was examined by a multivariate regression analysis between shape scores as a dependent variable (Procrustes coordinates) and centroid size (CS) as an independent variable. A canonical variation analysis (CVA) was performed, including the study site as a categorical variable, in order to obtain the Procrustes distances matrix. Subsequently, the main tendencies in shape variation between specimens within samples were summarized through PCA of the variance-covariance matrix of the Procrustes coordinates. All shape analyses were performed by using MorphoJ v1.05d (Klingenberg 2011). More details of the framework of geometric morphometrics using landmarks can be found in Zelditch et al. (2004).

RESULTS

The analysis of the six morphometric indexes based on *Z. dufresnei* shell morphology showed significant differences between populations (Kruskal-Wallis, $p < 0.01$). General roundness (GR) and relative shape of the aperture (RSA) indexes were significantly higher in the individuals from San Antonio Bay (BSA) compared to the other populations (Table 1). Regarding relative length of the aperture (RLA) and relative expansion of the aperture (REA), individuals from Mar del Plata (MDQ) presented higher values than individuals from BSA and San Matias Gulf (GSM) (Table 1). Relative weight index of the shell (RWS) presented higher values in individuals from BSA.

Regressions between AL and TL were significant for the three populations studied (BSA $F_{1, 75} = 114.52$, IC β: 0.58 – 0.84; GSM $F_{1, 77} = 289.80$, IC β: 0.67 – 0.85; MDQ $F_{1, 98} = 361.16$, IC β: 0.68 – 0.84) (Table 2). Comparison of regression model between sexes was not significant for BSA and GSM (ANCOVA, $p > 0.05$), but was significant for MDQ. Regression between AW and TL was significant for the three populations (BSA $F_{1, 74} = 49.52$, IC β: 0.18 – 0.32; GSM $F_{1, 60} = 36.89$, IC β: 0.14 – 0.28; MDQ $F_{1, 81} = 148.60$, IC β: 0.24 – 0.33) (Table 2). There were not significant differences between sexes for the populations. Regressions between log(SW) and log(TW) were significant (BSA $F_{1, 52} = 216.04$, IC β: 0.90 – 1.18; GSM $F_{1, 53} = 18.17$, IC β: 0.24 – 0.67; MDQ $F_{1, 57} = 89.51$, IC β: 0.54 – 0.83) (Table 2). Comparison between sexes revealed no significant differences for the populations BSA and MDQ,

Table 1. Morphometric indexes for *Zidona dufresnei*. GR: general roundness, RLA: relative length of the aperture, RWA: relative width of the aperture, REA: relative expansion of the aperture, RSA: relative shape of the aperture, RWS: relative weight of the shell, BSA: San Antonio Bay, GSM: San Matías Gulf, MDQ: Mar del Plata.

		GR	RLA	RWA	REA	RSA	RWS
BSA	Mean ± SD	0.45 ± 0.05	0.73 ± 0.05	0.23 ± 0.02	0.52 ± 0.06	0.32 ± 0.04	0.61 ± 0.06
	Min-max	0.38-0.73	0.44-0.82	0.20-0.36	0.28-0.77	0.27-0.50	0.51-0.74
GSM	Mean ± SD	0.33 ± 0.03	0.74 ± 0.03	0.22 ± 0.02	0.65 ± 0.05	0.30 ± 0.03	0.16 ± 0.03
	Min-max	0.21-0.40	0.61-0.81	0.18-0.26	0.48-0.80	0.24-0.35	0.10-0.22
MDQ	Mean ± SD	0.34±0.04	0.76 ± 0.05	0.23 ± 0.02	0.66 ± 0.06	0.30 ± 0.03	0.25 ± 0.06
	Min-max	0.24-0.60	0.62-0.88	0.18-0.28	0.54-0.78	0.23-0.37	0.17-0.47

SD: standard deviation, min: minimum, max: maximum.

Table 2. Parameters of the relationships (linear regression analyses) obtained for *Zidona dufresnei*. TL: total length, AL: aperture length, AW: aperture width, SW: shell weight, TW: total weight, BSA: San Antonio Bay, GSM: San Matías Gulf, MDQ: Mar del Plata. R^2 values are expressed in parenthesis. All regressions were significant ($p < 0.01$).

	Females	Males	Total
BSA	AL = 0.7176TL + 2.2186 (0.68)	AL = 0.6918TL + 3.5171 (0.55)	AL = 0.7114TL + 2.2889 (0.60)
	AW = 0.2363TL – 0.9183 (0.39)	AW = 0.2782TL – 3,8232 (0.50)	AW = 0,2489TL – 1,6614 (0.46)
	$SW = 0.42TW^{1.09}$ (0.84)	$SW = 0.62TW^{0,996}$ (0.77)	$SW = 0.5085TW^{1,0423}$ (0.80)
GSM	AL = 0.7701TL – 4.6479 (0.82)	AL = 0.7407TL – 0.1231 (0.72)	AL = 0.7634TL – 3.8373 (0.79)
	AW = 0.2467TL – 4.1621 (0.46)	AW = 0.1739TL + 8.9763 (0.29)	AW = 0.2142TL + 1.6694 (0.38)
	$SW = 4.86TW^{0.47}$ (0.32)	$SW = 7.28TW^{0.44}$ (0.19)	$SW = 5.096TW^{0.4563}$ (0.26)
MDQ	AL = 0.7317TL + 6.1449 (0.81)	AL = 0.8004TL – 7.4892 (0.79)	AL = 0.7707TL – 1.3581 (0.78)
	AA = 0.289LT – 9.407 (0.69)	AA = 0.2759LT – 7.2562 (0.57)	AA = 0.2843LT – 8.6399 (0.65)
	$SW = 1.16TW^{0.76}$ (0.72)	$SW = 1.77TW^{0.62}$ (0.53)	$SW = 1.293TW^{0.6846}$ (0.61)

while for GSM females presented heavier shell than males (ANCOVA, $p < 0.01$). When comparing regression models between BSA and MDQ and between BSA and GSM, significant differences in the slope were observed (β BSA $\neq$ β GSM, $p < 0.05$; β BSA $\neq$ β MDQ, $p < 0.01$), while comparing GSM and MDQ no significant differences were observed.

PCA conducted with morphometrical indexes explained 75.9% of total variation of data when the first two components were used (Figure 4). In this analysis, individuals from GSM and MDQ populations presented some degree of overlapping. *A posteriori* comparisons revealed significant differences between GSM and BSA and between MDQ and BSA, while no differences were detected between GSM and MDQ.

Geometric morphometric analyses showed a significant difference in centroid size (CS) among populations. Comparison of the centroid size (CS) between populations showed that individuals from GSM (CS: 2.99) were significantly larger than individuals of MDQ (CS: 2.82) and BSA (CS: 2.31) (ANOVA: $F_{2, 65} = 843$, $p < 0.01$). Multivariate regression of shape on CS was significant (permutation test with 10,000 random permutation, $p < 0.01$). Thus, subsequent analyses were performed with the residuals of the regression which are free of allometric effects. PCA explained 87.8% of total shape variation when the first four components were considered (PC1 61.1%, PC2 12.4%, PC3 9.8% and PC4 4.4%). Individuals from BSA were represented by positive values of PC1 which means a more rounded-shape shell

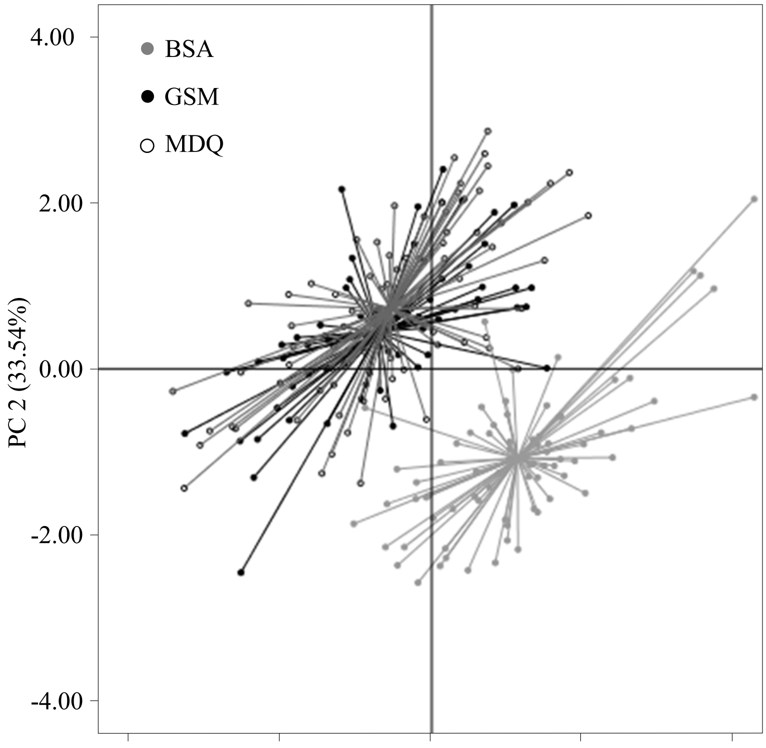

Figure 4. Principal Component Analysis (PCA) for shell shape variation of *Zidona dufresnei* with percentage of explained variance. GSM: San Matías Gulf, BSA: San Antonio Bay, MDQ: Mar del Plata.

than individuals from GSM and MDQ (Figure 5). Analysis of canonical components revealed a smaller distance between individuals from MDQ and GSM, and higher distance between individuals from BSA and MDQ (Procrustes distance: BSA-GSM: 0.0878; BSA-MDQ: 0.0954; GSM-MDQ: 0.0381, $p < 0.01$) (Figure 6).

DISCUSSION

Studying the adaptation of a population to a changing environment, whether modeled by selection, plasticity or the interaction of both, is an ongoing challenge in evolutionary studies (Reed et al. 2011; Grenier et al. 2016). These studies contributed to elucidate different local adaptive strategies to avoid predation or reduce intraspecific competition, among others (Trussell 1996; Marchinko 2003; Andrade and Solferini 2006; Hollander et al. 2006; Avaca et al. 2013).

Morphometric techniques, both traditional and geometric, have been widely used in ecological and evolutionary studies (Carvajal-Rodríguez et al. 2005; Fedosov et al. 2011; Epherra et al. 2015). Shape variation of body structures, such as shells in gastropods, has a genetic basis but is also influenced by environmental and epigenetic processes (Atchley and Hall 1991; Valentin et al. 2002; Rufino et al. 2006; Amini-Yekta et al. 2019). Therefore, to fully understand factors that determine shape it is necessary to consider the ontogenetic development and also adaptations to

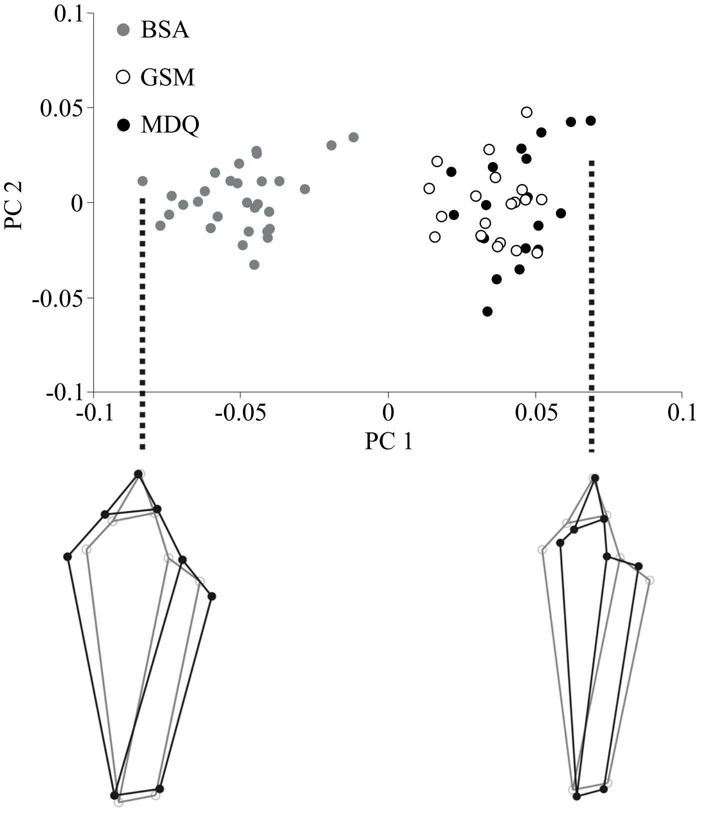

Figure 5. Principal Component Analysis (PCA) of Procrustes coordinates for *Zidona dufresnei* that explains 87.8% of total variation of data. GSM: San Matías Gulf, BSA: San Antonio Bay, MDQ: Mar del Plata.

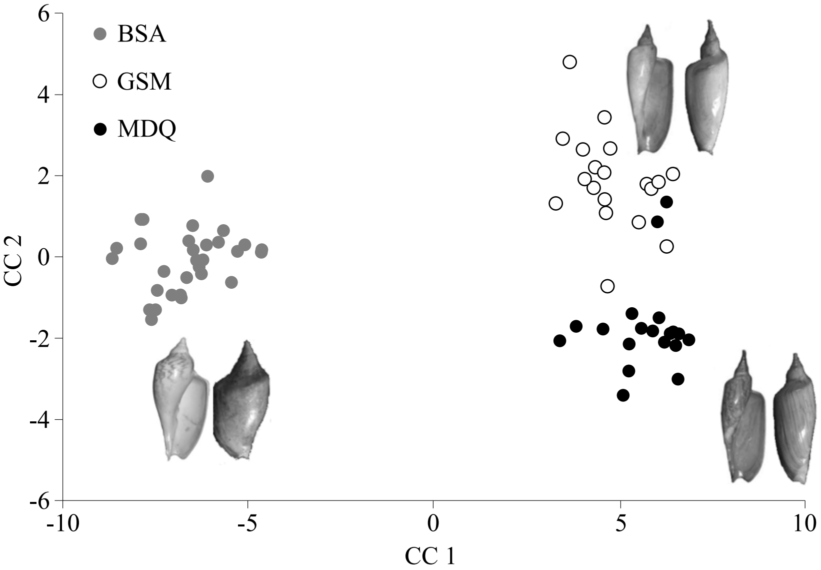

Figure 6. Canonical analysis of Procrustes coordinates of the shell of *Zidona dufresnei*. GSM: San Matías Gulf, BSA: San Antonio Bay, MDQ: Mar del Plata, CC: canonic components.

environment besides genetics (Hanken and Wake 1991; Lombard 1991; Müller 1991). In this paper, we combined traditional and geometric morphometric tools to analyze at the first time the shell morphology variation of *Z. dufresnei* in different locations along the geographical distribution of the species. According to these results, the three populations presented significant differences in size, but also in shell shape, showing allometric effects between populations. The analysis of morphological indexes showed that the shell of the individuals from BSA presented a higher value of general roundness compared to individuals from GSM and MDQ. In the case of relative length and width of the aperture, individuals from MDQ presented the highest values. The relative weight of the shell was higher in individuals from BSA. In general terms, individuals from BSA presented a shell characterized by a higher general roundness and relative weight, and lower relative aperture compared to individuals from GSM and MDQ. This was reflected in the multivariate analysis where individuals from BSA were notably different from individuals of MDQ and GSM.

Mean values of shell length and width were significantly different between individuals of the three populations studied. Comparisons between regression models showed that main differences between BSA and GSM were related to the size of individuals since the relationships between length and width with total length were represented by the same model. When MDQ and GSM populations were compared, individuals from GSM presented larger shells than individuals from MDQ. The differences found in morphological variables in the present study may be related to differences in individual growth of each population (Giménez et al. 2004), and to particular environmental conditions at each location. The observed differences in maximum size between MDQ and GSM individuals could be also related to a long-term anthropogenic selection pressure by fishing which decreases the relative frequency of individuals with large body sizes. MDQ population has been directly exploited by the Argentinean and Uruguayan fleets for the last 40 years and seems to be in the over-exploitation phase (Giménez et al. 2005) while GSM population is not under direct fishing pressure and only sporadically caught as bycatch of demersal trawling fleet.

Previous studies pointed out that the aperture of the shell is a highly variable area where sexual dimorphism is expressed. For example, Family Bursidae is characterized by differences in the aperture borders between sexes (Beu 1998). For genera *Buccinum* and *Buccinanops,* differences in size of the aperture were reported between sexes with higher apertures in males than in females (Hallers-Tjabbes 1979; Avaca 2010; Avaca et al. 2013). However, our results did not reveal differences in aperture length and width between sexes, suggesting that such responses may vary according to the family under analysis.

Geometric morphometrics analysis allowed us to separate the individuals from the three populations, being GSM and MDQ the most similar. Main variations were observed in the size and volume of individuals. This result is in agreement with those obtained by traditional morphometric analysis. In general, size was the variable that explained the highest variation (70% of the total variation). When the effects of size and allometry were removed and only shape variation was considered for comparisons, a separation of populations through the principal axis of shape variation was clearly evident. GSM and MDQ showed similar shell shape morphology compared to BSA.

Comparisons between individuals of the same species from different sites or under different environmental conditions, using the combined approach of traditional and geometric morphometrics have been conducted in previous studies. Bigatti and Carranza (2007), studying the effect of the occurrence of imposex in *Odontocymbiola magellanica* from Patagonian waters detected some differences in shell shape and body using both univariate and multivariate approaches. Additionally, shape variations were determined for *Buccinanops deformis* in three populations of Patagonia (Argentina) using both techniques (Avaca 2010). Differences in shell shape were detected using geometric morphometrics that remained undetected by traditional morphometrics in two sympatric ecotypes of *Littorina saxatilis* (Carvajal-Rodríguez et al. 2005). This species also showed a larger aperture on exposed shores and a smaller aperture on sheltered shores in response to predation (Conde-Padín et al. 2009). In the case of *Z. dufresnei*, traditional and geometric morphometrics were useful both to describe and to quantify the shell shape variation observed between populations. These methods were reliable for distinguishing individuals from different locations based solely on their shell shape. Although the two morphotypes were much better separated by geometric morphometrics approach, traditional morphometrics were useful as a complementary technique since it allowed working with a larger number of samples. The number of samples available for geometric morphometrics was limited because it was difficult to access to individuals in good shape condition since samples from MDQ and GSM belonged to fisheries catches.

Our results support the hypothesis of Lahille (1895) who classified the individuals from BSA as a 'dwarf' morphotype based on shell morphology, highlighting the need to revise the taxonomic status of *Zidona*. Unfortunately, there are not published genetic data to validate the two species hypothesis from a molecular approach. The marked shell variations detected among populations of *Z. dufresnei* may be driven by several ecological factors other than growth pattern, such as changes in prey availability, presence of predators, and temperature (e.g. Dalziel and Boulding 2005; Doyle et al. 2010). BSA corresponds to an intertidal zone where snails are exposed to highly variable environmental conditions with clines of food availability, wave exposure, desiccation and presence of predators, contrasted with GSM and MDQ (Roche et al. 2011). These environmental pressures (Raffaelli and Hawkins 1999; Chapman 2000) may favor smaller size (i.e. the occurrence of a 'dwarf' morphotype population), higher general roundness and relative weight, and also smaller relative aperture in the individuals from BSA.

At the same time, certain characteristics of life history of *Z. dufresnei*, such as direct intracapsular development (Penchaszadeh and De Mahieu 1976; Giménez and Penchaszadeh 2002) and restricted range of spatial dispersion (Penchaszadeh et al. 1999; Pereyra et al. 2009; Roche et al. 2011, 2013) may have resulted in a reduction of gene flow among populations leading to such adaptations to local conditions. Considering that GSM and MDQ individuals were similar in size and shell shape morphology but showed the longest distance between them, ecotypes adapted to different conditions should be maintained as the most probable explanation for the variation between dwarf and normal morphotypes unless new data contradict this. In summary, issues affecting size and shell shape variation in *Z. dufresnei* are multiple and not mutually exclusive. Additional experimental studies are needed to sort out the role of the physical and ecological factors on the shell shape and to test whether this variation has an adaptive value. On the other hand, further investigation is needed to better understand if the phenotypic variation observed in shell morphology is also expressed at genetic level. This is also highlighted in the case of *Z. dufresnei* which is under an increasing fishing pressure.

The authors declare that they have no conflict of interest. No animal testing was performed during this study. All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

The study material is cataloged in the collection deposited at the Laboratory of Benthic Resources. Center for Applied Research and Technology Transfer in Marine Resources Almirante Storni (CIMAS). The data sets generated during and/or analyzed during the current study are available from the corresponding author at reasonable request.

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