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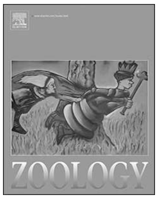

Island effect in the shell phenotypic plasticity of an intertidal gastropod

J. Vasconcelos[a,b,c,*], R. Sousa[d,e], V.M. Tuset[f], R. Riera[b]

[a] *Secretaria Regional de Educação, Avenida Zarco, Edifício do Governo Regional, 9004-528, Funchal, Madeira, Portugal*
[b] *Departamento de Ecología, Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Casilla 297, Concepción, Chile*
[c] *Centro de Ciências do Mar e do Ambiente (MARE), Quinta do Lorde Marina, Sítio da Piedade, 9200-044, Caniçal, Madeira, Portugal*
[d] *Direção de Serviços de Monitorização, Estudos e Investigação do Mar (DSEIMar), Direção Regional do Mar, Avenida do Mar e das Comunidades Madeirenses nº23, 1º andar, 9000-054, Funchal, Madeira, Portugal*
[e] *Observatório Oceânico da Madeira, Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação (OOM/ARDITI) – Edifício Madeira Tecnopolo, 9020-105, Funchal, Madeira, Portugal*
[f] *Department of Renewable Marine Resources, Institute of Marine Sciences (CSIC), Passeig Marítim 37-49, Barcelona 08003, Catalonia, Spain*

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ABSTRACT

The pattern of shell shape variation in populations of the top shell, *Phorcus sauciatus* from Madeira Island (NE Atlantic) was analysed as a function of substrate type and wave exposure, using geometric morphometrics. We hypothesized that *P. sauciatus* shows morphological variations to inhabit contrasting environments. Highly significant differences in shell shape were found depending on both substrate type and coastal exposure. The most marked differences were found between exposed and sheltered environments. Rounded shells in exposed environments may be explained by physiological reasons, since larger muscles are needed to ensure attachment to substrate in areas subject to harsh conditions. On the other side, conically-shaped shells may accommodate a larger body but with a smaller foot, an adaptation to sheltered environments. Slight shape variations were also observed among substrates, mostly in the degree of differentiation of some whorls. Differences could be related to a particular use of habitat and/or to the degree of exposure to water current. These results suggest that *P. sauciatus* is locally adapted to varying coastal hydrodynamics and may be considered as a good model in studies on adaptations of fauna to certain climate change effects.

1. Introduction

Intertidal environments exert a considerable stress on organisms inhabiting these heterogeneous habitats, both in terms of vertical zonation and across environmental gradients at biogeographic scales (Tomanek and Helmuth, 2002). These conditions trigger profound effects on rocky intertidal communities, thus promoting adaptations in terms of morphology, physiology and phenotypic plasticity (Helmuth et al., 2006). In particular, gastropods can be used for interpreting the drivers of morphological variation (e.g., Ramajo et al., 2013; Cazenave and Zanatta, 2016).

Phenotypic plasticity can largely affect individual fitness (Gotthard and Nylin, 1995; Van Buskirk et al., 1997) and population dynamics (Miner et al., 2005; Plaistow et al., 2006). This plasticity may be a benefit for individuals by 'fitting' their phenotype to the spatially or temporally heterogeneous environments (Van Tienderen, 1991; Hollander et al., 2006). Population's expansion into new environments may be facilitated by moderate levels of phenotypic plasticity, as the new feature may place the population on the slope of an adaptive peak (Price et al., 2003).

In fact, the diversity of gastropod shell shapes has been interpreted as a response to environmental conditions or phenotypic plasticity along environmental gradients (Caley et al., 1995; Trussell, 2000; Trussell and Smith, 2000). For instance, evolutionary history (genotype), environmental (i.e., sediment type, temperature, tidal and wave exposure, matrix stratification, thermal stress) and ecological (i.e., population density and predation) factors are known drivers of shell morphology (Trussell et al., 1993; DeWitt, 1998; Denny, 2000; Sokolova and Berger, 2000; Agrawal, 2001; Alunno-Bruscia et al., 2001; Beadman et al., 2003; Caro and Castilla, 2004; Briones and Guiñez, 2005; Funk and Reckendorfer, 2008; Harley et al., 2009). Within these, wave action has commonly been suggested as one of the most important environmental factors that influences phenotypic differentiation (Grahame and Mill, 1986; Johannesson, 2003). Understanding the direct and indirect impact of wave action on intertidal communities and how it influences the vulnerability, e.g. to dislodgement, are important

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* Corresponding author at: Secretaria Regional de Educação, Avenida Zarco, Edifício do Governo Regional, 9004-528, Funchal, Madeira, Portugal.

E-mail addresses: joana.pr.vasconcelos@madeira.gov.pt (J. Vasconcelos), ricardo.sousa@oom.arditi.pt (R. Sousa), vtuset@icm.csic.es (V.M. Tuset), rriera@ucsc.cl (R. Riera).

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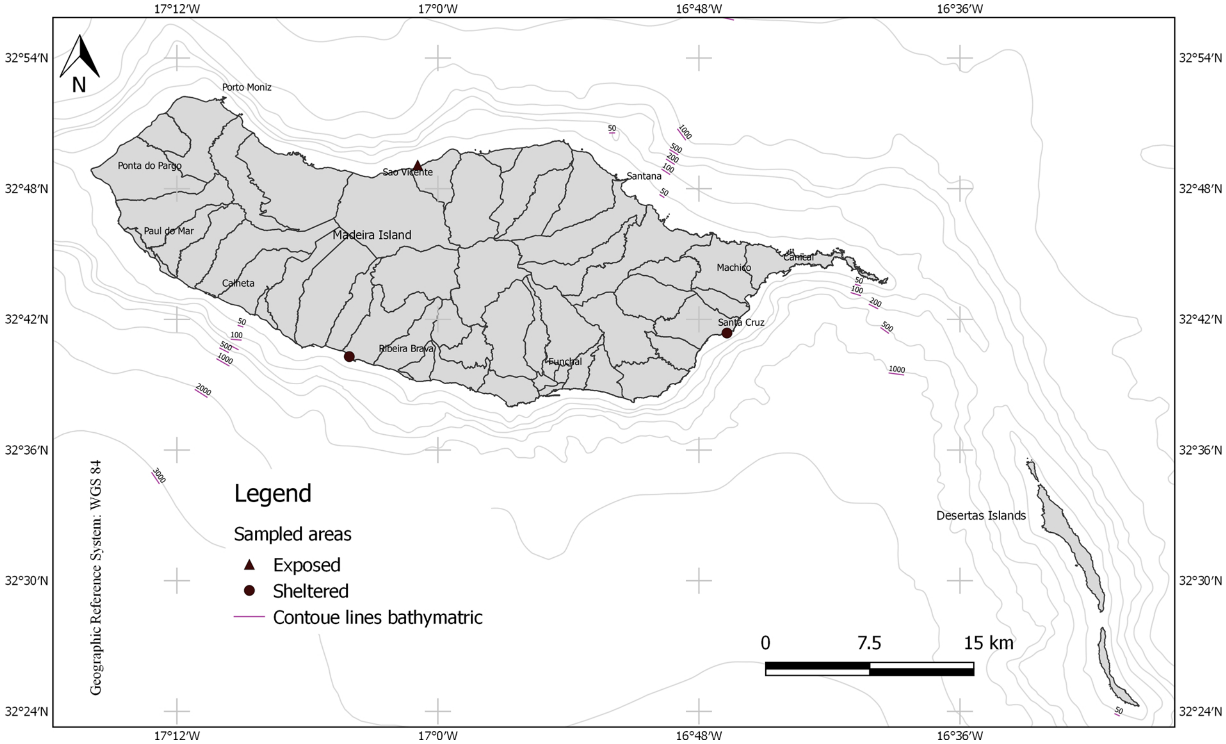

Fig. 1. Map showing the 3 coastal settlements where the marine top-shell *Phorcus sauciatus* were sampled in April 2018 for their study according to wave effects (exposed vs. sheltered sectors) and substrate type (slope, pebbles and horizontal substrate): one on the North coast of the Island (1 - São Vicente) and two on the South coast (2 - Santa Cruz and 3 - Ribeira Brava).

goals in marine ecology (Menge and Sutherland, 1987; Trussell et al., 1993). On bare and steep rocky shores under heavy wave exposure, flow velocities and related hydrodynamic forces are greater than on boulder beaches with shallow gradients (Denny, 1995). It has been shown that shell morphology of various mobile intertidal snails varies along a gradient of wave exposure (Stebbins, 1988; Denny, 1989; Trussell et al., 1993; Le Pennec et al., 2017) so that those with a shell shape that reduces drag can better resist hydrodynamic stress and dislodgement (Trussell et al., 1993; Le Pennec et al., 2017); hence geometric morphometric analysis can be used to describe the shape changes in snails according to different hydrodynamic exposures.

One of the most valuable invertebrates as a seafood resource in Madeira, an oceanic archipelago in the NE Atlantic Ocean, is the gastropod *Phorcus sauciatus* (Koch, 1845). It is a common subtropical grazer that inhabits rocky shore platforms along the East Atlantic including the Macaronesian archipelagos of Madeira, and the Canaries and reaching its northern boundary in the Iberian Peninsula (Rubal et al., 2014). In 2013, *P. sauciatus* was first reported on the south coast of Santa Maria Island in Azores, in the upper intertidal zone, in basalt crevices and in small intertidal pools (Ávila et al., 2015). Despite being highly exploited since the fifteenth century for human consumption in Madeira, the regional morphological variation of this species is unknown. Most of the studies concerning *Phorcus sauciatus* from Madeira (NE Atlantic Ocean) have focused on the biological parameters and exploitation rate (Sousa et al., 2019a) and in the effects of harvesting pressure on the size-structure and abundance of populations (Sousa et al., 2019b).

Just recently, plasticity has been referred to as an important mechanism for local adaptation (Crispo, 2007; Lande, 2009) and speciation (West-Eberhard, 2003; Crispo, 2007). Nevertheless, as mentioned before, variation in wave exposure has been identified as a pivotal factor associated with shell morphological adaptation in intertidal organisms. However, very few studies have focused on the variations of body shape related to island effect. In order to analyse the local adaptations to environmental characteristics (coastal exposure and substrate type), a comparative analysis was conducted on the shell shape of *P. sauciatus* from Madeira along three different habitats (horizontal substrate, cliffs and pebbles) and two degrees of wave exposure (exposed vs. sheltered). The present analysis was performed to determine if the variation can be explained by site origin and hence, to identify some ecological requirements of these species using their morphological attributes.

2. Materials and Methods

2.1. Collecting and sampling

The study was conducted on specimens of *P. sauciatus* collected randomly in April 2018 from three coastal settlements in Madeira, one on the North coast of the Island (São Vicente) and two on the South coast (Santa Cruz and Ribeira Brava) (Fig. 1), characterized by differences in exposure to wave action (wave-exposed shores vs. sheltered sectors, i.e., less exposed shores) and substrate type (slopes, pebbles and horizontal substrate): 180 specimens from sheltered zones in Ribeira Brava (pebbles = 60) and in Santa Cruz (horizontal substrate = 60; slope = 60) and 180 from exposed areas in São Vicente (horizontal substrate = 60; slope = 60; pebbles = 60). Each group of 60 individuals consisted of 20 juveniles (< 11.00 mm), 20 pre-adults (11.10-12.99 mm) and 20 adults (> 13.00 mm), according to size at first maturity (12.95 mm) of *P. sauciatus*, estimated for Madeira (Sousa et al., 2019a). Total shell width (mm) and shell height (mm) were measured using a digital Vernier caliper (± 0.01 mm).

Shells were stabilised on a piece of clay and then photographed in standardised and homologous lateral position using a Canon EOS 700D digital camera with a Canon EF S 18-55 mm lens placed on a firm support to maintain a right angle and adequate height to stabilize and avoid image distortion. The lateral view only shows the shell with the apex, the sutures of the whorl, and the attachment points of the aperture on the previous whorl (Fig. 2).

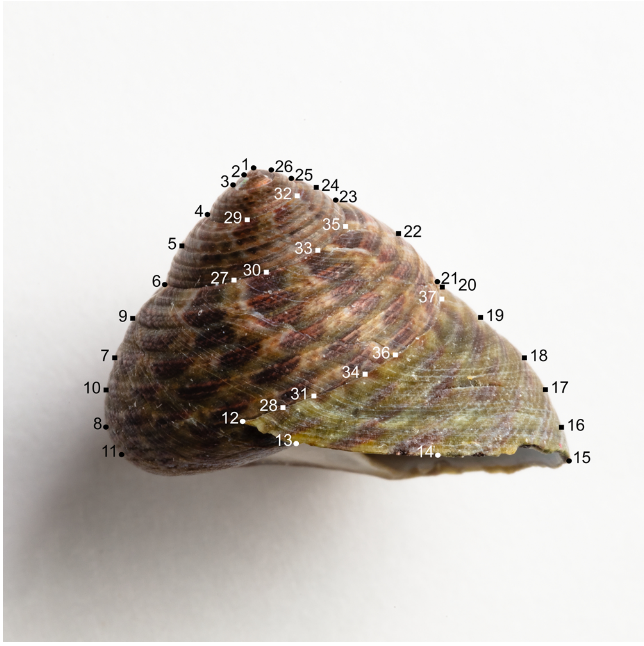

Fig. 2. Thirty-seven landmarks (circles) and semilandmarks (squares) (LM) placed on the shells of *Phorcus sauciatus*. LM1 – apex of the shell; LM2-4 and LM6 – sutures between major whorls on the left side; LM5 - Middle point between LM4 and LM6; LM7 - Middle point between LM6 and LM8; LM8 – outermost left point; LM9 – Middle point between LM6 and LM7; LM10 – Middle point between LM7 and LM8; LM11 – Last suture on the left side; LM12 – attachment point of the aperture lip; LM13 – crossing point of lip aperture and columella; LM14 – perpendicular to the last suture on the right side; LM15 – right most point on the aperture lip; LM16 – point opposite LM8 on the right profile; LM17 – point opposite LM10 on the right profile; LM18 – point opposite LM7 on the right profile; LM19 – point opposite LM9 on the right profile; LM20 – point opposite LM6 on the right profile; LM21 – right most point visible on the suture between the ultimate and penultimate whorl; LM22 – Middle point between LM21 and LM23; LM24 – Middle point between LM23 and LM25; LM 23, 25, 26 - sutures between major whorls on the right side; LM27 and LM28 - points on the first and second sutures in the line draw with the LM4 and LM13; LM29, LM30 and LM31 – points on the first, second and third sutures marked on the line draw with the reference point LM3 and perpendicular to the previous line; LM32, LM33 and LM34 – points on the first, second and third sutures marked on the line draw with the reference point LM25 and perpendicular to the previous line; LM35 and LM36 – points on the first and second sutures marked on the line draw with the reference point LM23 and perpendicular to the previous line; LM37– point on the first suture marked on the line draw with the reference point LM21 and perpendicular to the previous line.

2.2. Geometric morphometric analyses

Thirty-seven landmarks (homologous points called landmarks type I) and semilandmarks (non-homologous points called landmarks type III) (LM) (Bookstein, 1991) were placed on the studied shells (Fig. 2). The coordinates of these landmarks for each individual were acquired using the *tpsDig2* software (Rohlf, 2017). The landmark coordinates were superimposed using Generalized Procrustes Analysis (GPA) (Rohlf and Slice, 1990; Dryden and Mardia, 1998; Rohlf, 1999) to eliminate differences due to scale, position, and orientation, and to extract size variables (centroid size or Cs was defined as the square root of the summed squared inter-landmark distances and showed an effect on shape) and shape variables (Procrustes coordinates) from landmark data. Since a significant relationship was noted between shape and centroid size ($r = 0.717$, $P = 0.001$, *permutations* = 1 000), the residuals of this regression were used as 'size-free' Procrustes for the analyses (Klingenberg, 2016).

2.3. Statistical analysis

To assess whether shapes differ among ontogenetic stage (juveniles, pre-adults and adults) and factor environments, a nested (factor A = wave; factor B = substrate) 'size-free' Procrustes MANOVA with 1 000 random permutations of the residuals among groups for significance testing was applied.

A principal component analysis (PCA) on the covariance matrix of the 'size-free' Procrustes was conducted to quantify the variance of the warps explained by principal component (PC) axes (Rohlf and Marcus, 1993; Zelditch et al., 2004; Mitteroecker and Bookstein, 2011). Thin-plate spline deformation grids were employed for explaining the graphic visualization of changes through PC axes (Bookstein, 1991). We retained the PC's that cumulatively accounted for up to 95% of the total variation (Collar and Wainwright, 2006; Budinski et al., 2015).

Canonical variate analysis (CVA) was computed on the reduced PCA matrix to summarize the variation between factors maximizing their distances (Mardia et al., 1979; Linde et al., 2004; Tuset et al., 2016). To examine the mean variation in shapes associated with wave effect and substrate type, specimens were arranged according to their morphological similarity in a morphospace using the mean canonical scores of the two first CVA axes. The correct classification rate was quantified by cross-validation (leave-one-out procedure) (Lachenbruch and Mickey, 1968). The bias of the classification was determined with the Cohen-Kappa (κ) coefficient, which estimates the improvement over chance of corrected classification rates (Tuset et al., 2003). Values of κ range from 0 to 1, with zero indicating the discriminant analysis yielded no improvement over chance and 1 indicating perfect agreement (Titus et al., 1984). The κ values were scaled up to a percentage, and the prior probability of classification was equal for all groups (Vergara-Solana et al., 2014).

All analyses and graphical representation were carried out in the R package *Geomorph* v.3.3.2 (R Core Team, 2016; Adams et al., 2018), although the thin spline illustrations were obtained with PAST 3.12 (Hammer et al., 2001).

3. Results

The nested Procrustes MANOVA analysis showed significant differences in the shell shape depending on wave exposure ($F_{1,\ 358} = 29.248$, $P = 0.001$) and substrate type ($F_{4,\ 355} = 3.275$, $P = 0.001$), whereas it did not differ between ontogenetic stages ($F_{2,\ 357} = 1.413$, $P = 0.242$).

The first 11 PC components explained 95.4% of variance (see Table S1). The PC1 axis explained the 44.1% of total variance and revealed a high morphological heterogeneity, regardless the environmental factor considered, being related to the oblique angle of apex in relation to LM 13 (Fig. 3). In contrast, the negative records of PC2 axis (27.9% of total variance) represented shells with a rounded shape, typical of specimens inhabiting exposed coasts (São Vicente) (Fig. 4A). The positive values identified peaked shapes occurring in higher numbers at sheltered localities (Santa Cruz and Ribera Brava) (Fig. 4B).

Canonical variate analysis results of the mean shapes associated to wave effect (exposed vs. sheltered), the first CV axis explained 100% of variance and had an index of squared canonical correlation of 0.459, maximizing the differences between the PC2, PC4 and PC10 components (Fig. 5). The average classification by zone was similar 79.4%, showing a misclassification rate of 20.6%. The Cohen's kappa (κ) was 0.567, indicating that the classification efficiency was 56.7% better than would have occurred by chance alone (Table 1). In San Vicente, the MANOVA pointed out that significant differences were detected among substrates (Pillai test = 0.500, $F_{2,\ 175} = 5.094$, $P < 0.001$). The first function of CVA explained 56.3% of the variance with a canonical correlation index of 0.274; while the second function explained the rest and provided a canonical correlation value of 0.226. The overall classification accuracy was 63.4% and Cohen's κ indicates a classification

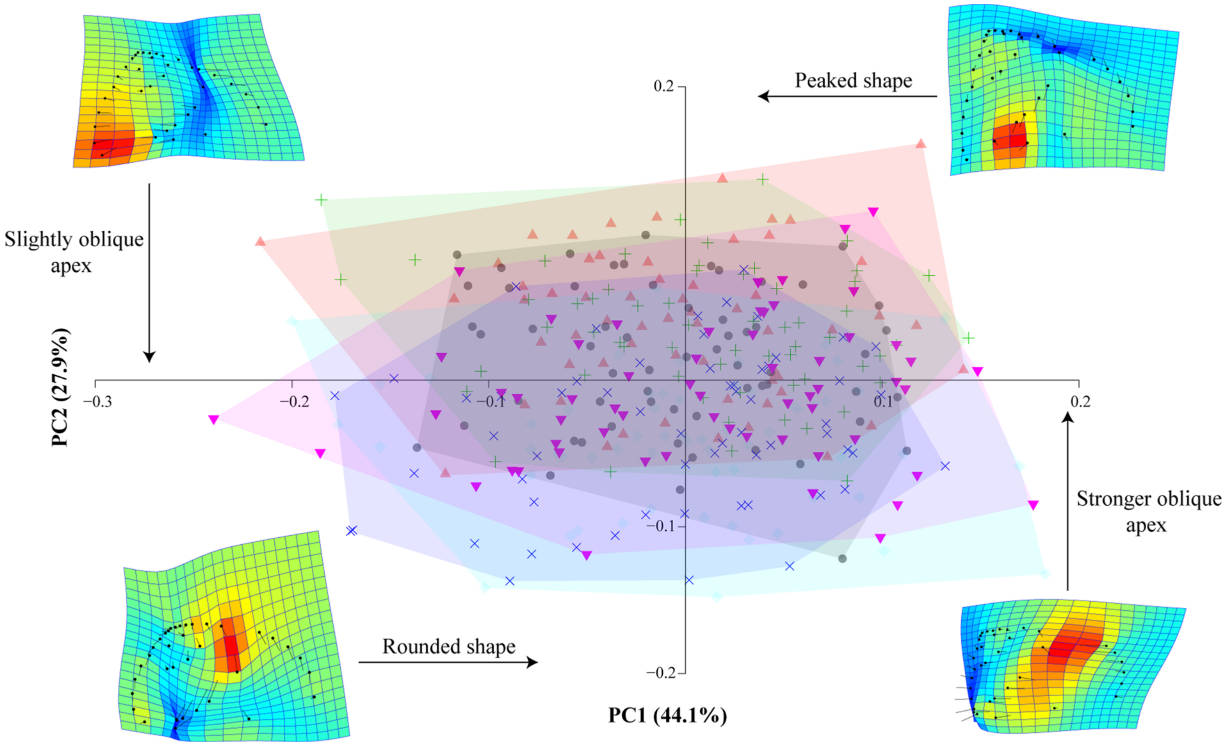

Fig. 3. Principal component analysis plot of PC1 vs. PC2 conducted on *Phorcus sauciatus* from exposed (dark blue multiplication: horizontal substrate of São Vicente; light blue diamond: pebbles of São Vicente; and pink inverted triangle: slope of São Vicente) and sheltered (black circles: pebbles of Ribeira Brava; red triangles: horizontal substrate of Santa Cruz; and green cross: slope of Santa Cruz) environments of Madeira Islands. Thin-plate spline deformation grids showing shape variation along PC axes are also represented.

efficiency of 45.0%. The successful percentage for each substrate type was similar: 61.7% for horizontal substrate and slope, and 66.7% for pebbles (Table 1). The individuals collected in pebbles displayed shells with first whorls well differentiated (Fig. 4C), which was related to PC5, PC6 and PC9 components (Fig. 6). The morphology of shells from horizontal substrate presented a more rounded shape and a more visible ultimate whorl (Fig. 4D), being defined by PC4, PC7 and PC11 components (Fig. 6). Finally, the specimens found in slope substrate showed a peaked shape (Fig. 4E) linked to PC2, PC3 and PC10 components (Fig. 6).

Finally, the comparison of shell shape between specimens collected in horizontal substrate and the slope off Santa Cruz also pointed out a significant effect of substrate type (Pillai test = 0.273, $F_{1,\ 117} = 3.694$, $P < 0.001$). However, the canonical correlation index was relatively lower ($R^2 = 0.274$), which it was indicative of a higher overlapping between phenotypes (Fig. 7). The variability was reported in the obliquity of whorls, affecting to ultimate or penultimate ones (Fig. 7). The total classification success was 68.3% and Cohen's κ indicates a classification efficiency of 36.7% (Table 1).

4. Discussion

The findings of the present study demonstrate that specimens of *P. sauciatus* show a morphological variability depending on the substrate type and coastal exposure. The most marked differences in shell shape were found between exposed and sheltered environments that affect the whole shell configuration, being rounded in exposed areas and conical in sheltered ones. This phenotypic plasticity reflects, in some part, local adaptation to wave exposure. The presence of rounded shells and a wide aperture due to its oval shape in wave-exposed intertidal coastal areas may be explained physiologically, since larger muscles are needed to ensure attachment to the substrate in areas subject to harsh conditions, e.g. pronounced wave action and more turbulent and fast-flowing environments, as shown in *Elimia livescens* (Goodrich, 1934), *Acanthina monodon* (Wu, 1985; Reid and Osorio, 2000) and *Littorina obtusata* (Trussell, 1997). On the other side, conical shells may accommodate a larger body but with a smaller foot, an adaptation to shelter environments, as it has previously shown in the intertidal littorinid gastropods from Western Iberian Peninsula (Queiroga et al., 2011). A similar situation has been studied in *Littorina obtusata* and *L. saxatilis* in which it is demonstrated that individuals living at different wave-exposed conditions develop contrasting shells and body size (Trussell et al., 1993; Boulding et al., 1999; Trussell and Etter, 2001; Carvajal-Rodríguez et al., 2005; Conde-Padín et al., 2007), e.g. a "wave ecotype" is small, fragile and with a more globose shape confined to wave-swept shores and a "crab ecotype" is large, robust and more elongated and is found in less-exposed shores (Le Pennec et al., 2017).

Slight variations in shell shape were also observed among the studied substrates (pebbles, horizontal substrate and slope) that consist of differences in the degree of differentiation of first, penultimate or ultimate whorls. In sheltered areas, a high overlapping of phenotypes was observed since they are subject to lesser variation in water conditions (desiccation and wave action), and only slight differences were found in the shape of ultimate and penultimate whorls of the shell. The individuals collected in slopes and horizontal substrate at the sheltered location where at the mid intertidal level, thus occasionally submerged. Therefore, top-shells are less morphologically differentiated between substrates due to the high habitat homogenization. Moreover, differences in shell shape regarding last whorl, aperture and spire shapes were reported for the freshwater gastropods *Aylacostoma guaraniticum*, *A. chloroticum* and *A. stigmaticum* and these could be explained by a differential use of the habitat and/or by different degrees of exposure to water currents (Vogler et al., 2012). In the present study, the morphological variability in the shell shape of top-shells from different substrates is related to different degrees of wave exposure since the horizontal substrate and pebbles correspond to the intertidal being

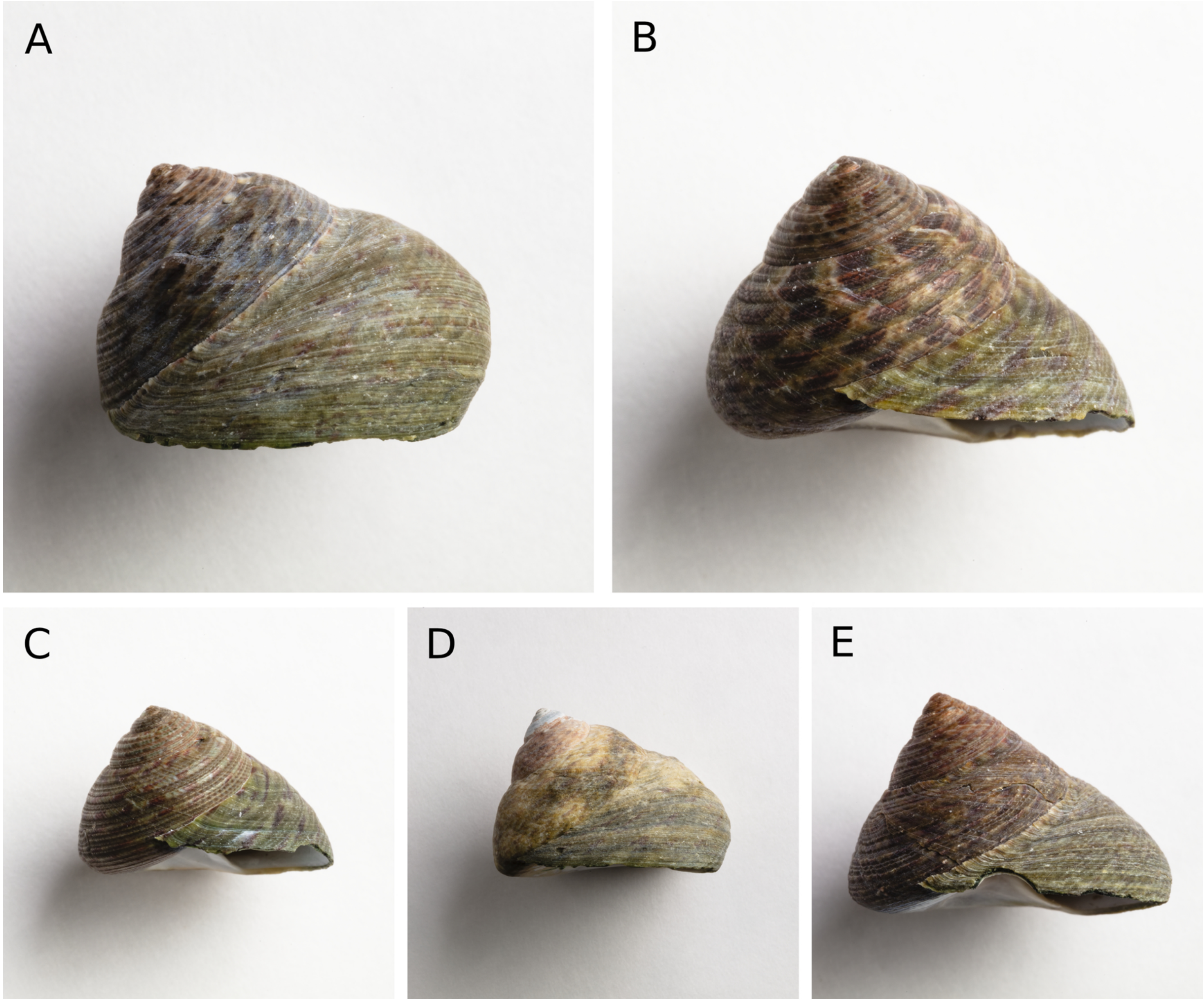

Fig. 4. Representative specimens of the marine top-shell *Phorcus sauciatus* according to wave effects (A - exposed and B - sheltered sectors) and substrate type (C - pebbles, D - horizontal substrate and E - slopes).

subject to greater wave action and the slope to the supratidal only being subject to occasional splashes during high tide. We herein observed that top-shells on horizontal substrates are more rounded while on slopes they show a more conical shape, slightly similar than shells from sheltered areas. Vogler et al. (2012) also observed more globose shells and more oval apertures in *Aylacostoma*, especially in *A. stigmaticum* and a lesser degree in *A. guaraniticum*, could be related to habitats and substrata, due to the highest water currents in the rapids. In contrast, *A. chloroticum* shows stylized shell related to the preference for more protected habitats (Ostrowski de Núñez and Quintana, 2008). Therefore, the more rounded shape is associated with substrate adherence, promoting a larger contact area between the top-shell and the substrate. This way, the top-shell reduces the likelihood of wave-induced dislodgement in highly-dynamic environments by having a shell morphology that reduces hydrodynamic forces (Branch and Marsh, 1978) and by increasing adherence to the substratum (Stebbins, 1988; Trussell et al., 1993) with a larger foot (Trussell et al., 1993; Greenwood and Thorp, 2001). In fact, greater forces are required to dislodge snails of a given size in wave-exposed environments due to their bigger foot area than that of protected snails (Etter, 1988; Trussell et al., 1993).

These shell shape variations are also believed to be responses to selective pressures of other factors such as desiccation and predation (Fletcher, 1995; Crothers, 2001; Sepúlveda et al., 2012). In fact, the effects of wave exposure, desiccation and predation are correlated to a large extent (Elner and Raffaelli, 1980; Palmer, 1990; Livore et al., 2018). Variation in aperture size and area affects susceptibility to desiccation and thermal stress, and differences in these characteristics may be related to different wave-exposure environments (Le Pennec et al., 2017). Wave-exposed shores have less predatory crabs and the risk of dislodgement is the main selective pressure (Boulding, 1993; Queiroga et al., 2011); this tends to produce globular shells, which reduces friction, and with a large opercular aperture to accommodate a large foot (Queiroga et al., 2011), allowing better adhesion to rocks, an essential feature for species inhabiting strong wave-exposure environments (De Wolf et al., 1997). In protected shores, the increase of predation pressure by shore crabs associated with an increase of heat stress and longer periods of desiccation (because of reduced wave splash) favours thicker shells, small apertures (Vermeij, 1973; Queiroga et al., 2011; Sepúlveda et al., 2012) and a more conical shape that decreases the contact area preventing water loss. This feature is useful, not only to escape predation, since it impedes the access of the crab claws, but also decreases heat stress, since contact with the substrate is reduced (De Wolf et al., 1997). When testing for differences in resistance to desiccation, and recovery from heat stress in individuals of the false limpet *Siphonaria lessonii* from two contrasting shore levels where limpets are abundant, the high (HT) and mid (MT) intertidal, 2 ecomorphs were defined using classic and geometric morphometric analysis (Livore et al., 2018). Individuals from the HT lost significantly less water under stressful heat conditions, showed higher recovery rates than those from the MT and a shell shape with a broad aperture, flatter lateral margin and larger size. In contrast to HT, shells from the MT morph presented a narrow aperture, arched lateral margin and smaller

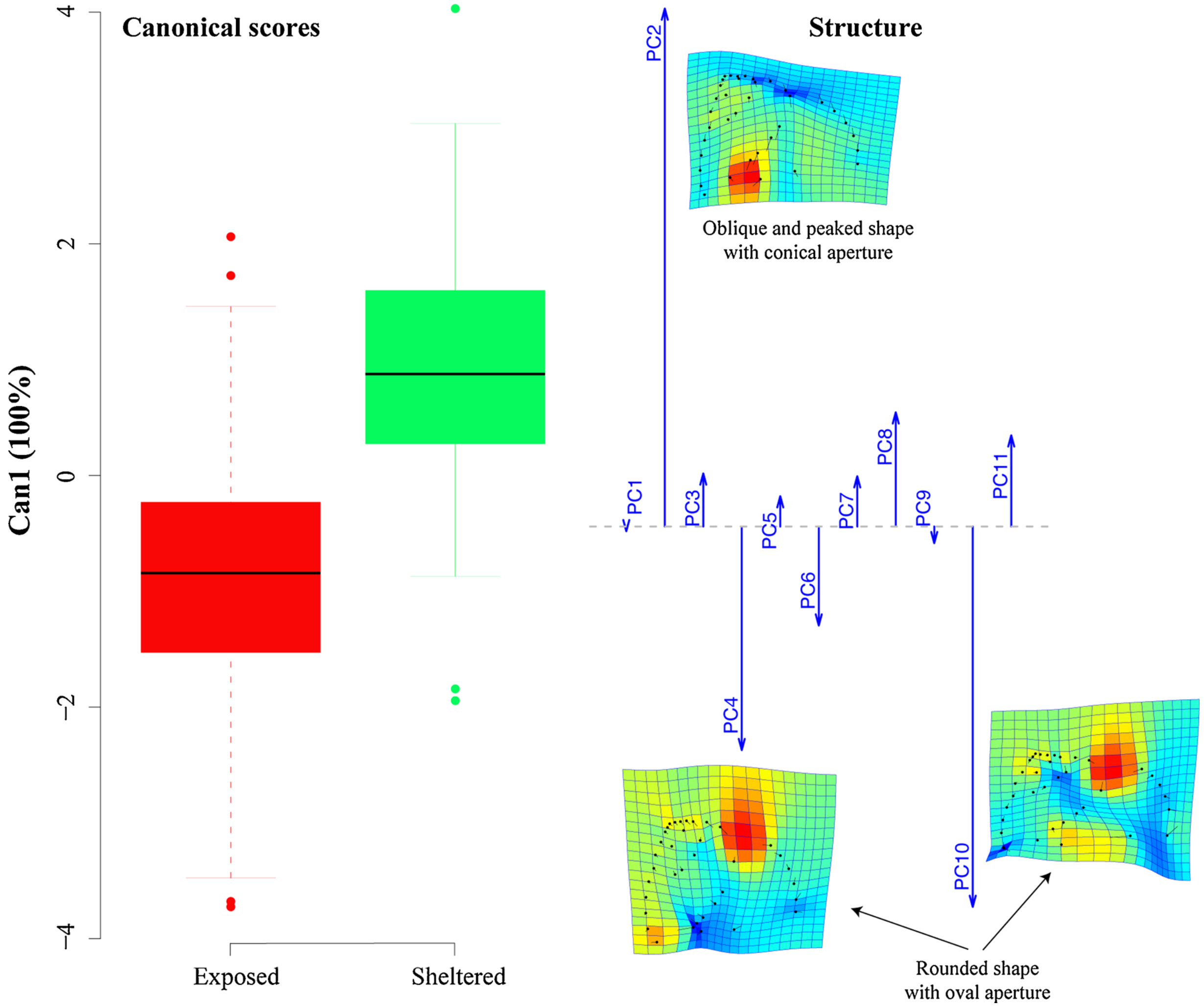

Fig. 5. Canonical variate analysis (CVA) results of the mean shapes associated to wave effect (exposed versus sheltered) conducted on specimens of *Phorcus sauciatus* from the Madeira Islands. Thin-plate spline deformation grids showing shape variation are also represented.

Table 1
Results of canonical variate analysis for *Phorcus sauciatus* shells depending on environmental factors. In bold, the assignation percentage of each actual group membership with its right predicted group. SC, Santa Cruz; SV, San Vicente.

Factor/Actual group	Predicted group membership (%)			Correct classification (%)	Cohen's kappa
	A	*B*	*C*		
Wave				79.4	0.567
Exposed (A)	**79.4**	20.6			
Sheltered (B)	20.6	**79.4**			
Substrate (SC)				68.3	0.367
Horizontal substrate (A)	**75.0**	25.0			
Slope (B)	38.3	**61.7**			
Substrate (SV)				63.4	0.450
Horizontal substrate (A)	**61.7**	16.7	21.7		
Pebbles(B)	20.0	**66.7**	13.3		
Slope (C)	18.3	20.0	**61.7**		

size (Livore et al., 2018). Together these results suggest the existence of 2 ecomorphs modulated by each contrasting habitat, providing ecological advantages by allowing the exploitation of resources from 2 different habitats (Livore et al., 2018).

Furthermore, predation and substrate affinity may play an important role regulating foot size and tenacity on protected environments (Trussell et al., 1993). In sheltered environments, well developed foots, i.e., large foot areas that extend outside the perimeter of the aperture makes the snail more vulnerable to predation (Trussell et al., 1993). As predation is an important selective factor favouring small foot areas in sheltered environments then greater tenacity compensates the smaller foot (Trussell et al., 1993). Lastly, a factor to take into account is concerning biogeographic features of the study area, i.e. an oceanic island distant to the nearest mainland. Insular morphological shifts have been shown to be adaptations to facilitate ecological niche expansion (Scott et al., 2003). Populations from islands conventionally experience weak interspecific competition and inhabit an impoverished environment relative to mainland assemblages (Whittaker, 1998). A thorough comparative study – mainland vs. oceanic islands - is needed to address the importance of biogeography as a driver on morphological diversity in intertidal systems and specifically on sea snails.

In conclusion, wave exposure is a determinant on morphological variation of the intertidal mollusk *Phorcus sauciatus* in Madeira. The phenotypical variability is mainly explained by body adaptations to harsh conditions in exposed coastal areas. Regardless of the coastal exposition, slight differences were observed concerning substrate types (pebbles, horizontal substrate and slope), mostly on the differentiation of the first and penultimate whorls and peaked shaped. These results support the hypothesis that this intertidal species is locally adapted to

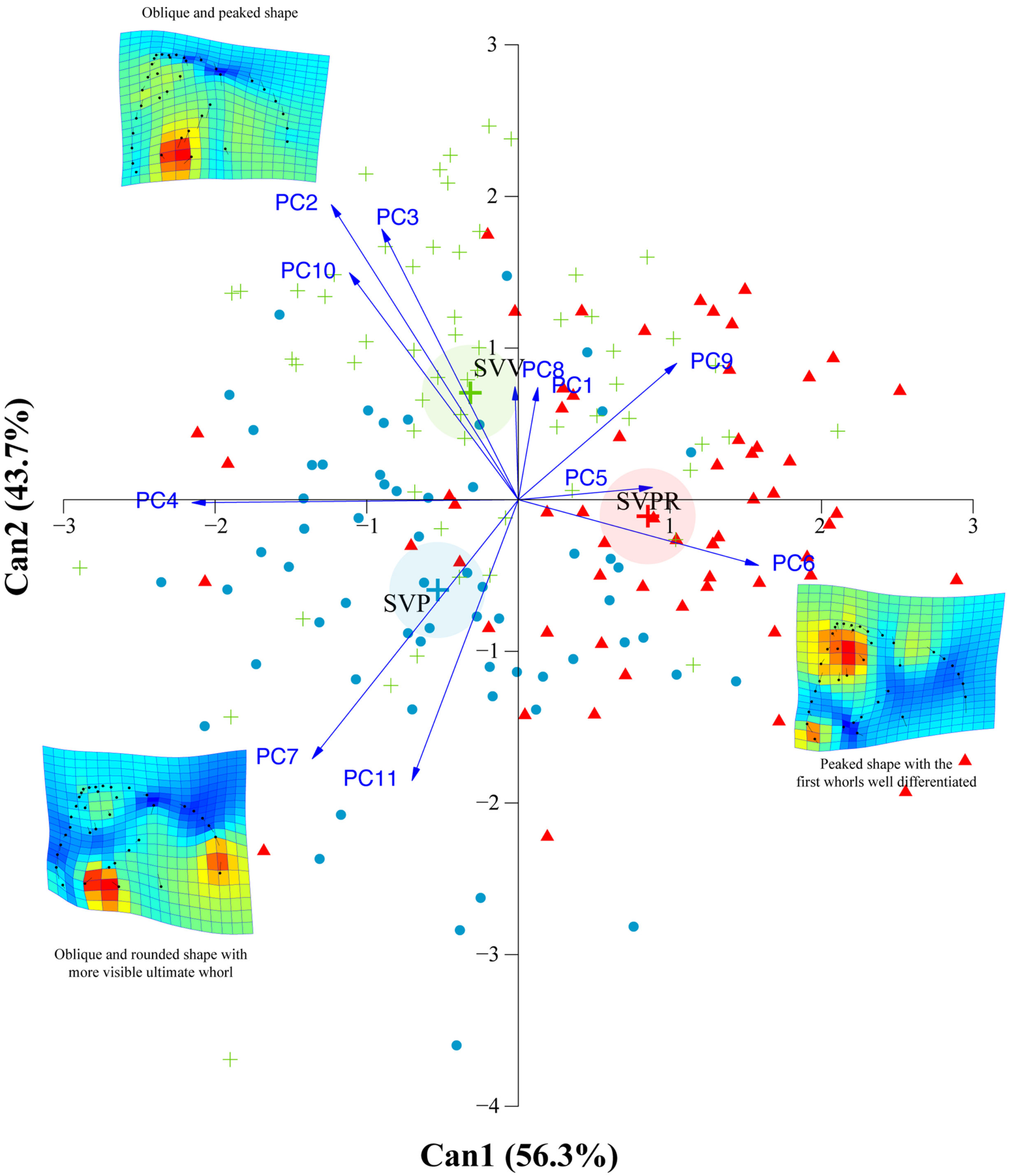

Fig. 6. Canonical variate analysis (CVA) results from shell shape comparison between specimens of *Phorcus sauciatus* collected on substrate types horizontal substrate (SVP, blue circles), pebbles (SVPR, red triangles) and slope (SVV, green crosses) off São Vicente. Thin-plate spline deformation grids showing shape variation are also represented. Bold crosses indicate class centroids per sampled area (each individual was allocated to the group with the nearest centroid). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

varying coastal hydrodynamics that may lead to spatial segregation, providing an ecological advantage by allowing it to use a range of habitats and resources thus reducing intraspecific competition. It may also be considered as a good model to develop studies on the adaptations of fauna to certain effects of climate change scenarios such as, the increase of periods of rough seas and increase of water forces as a consequence of increased seawater level worldwide.

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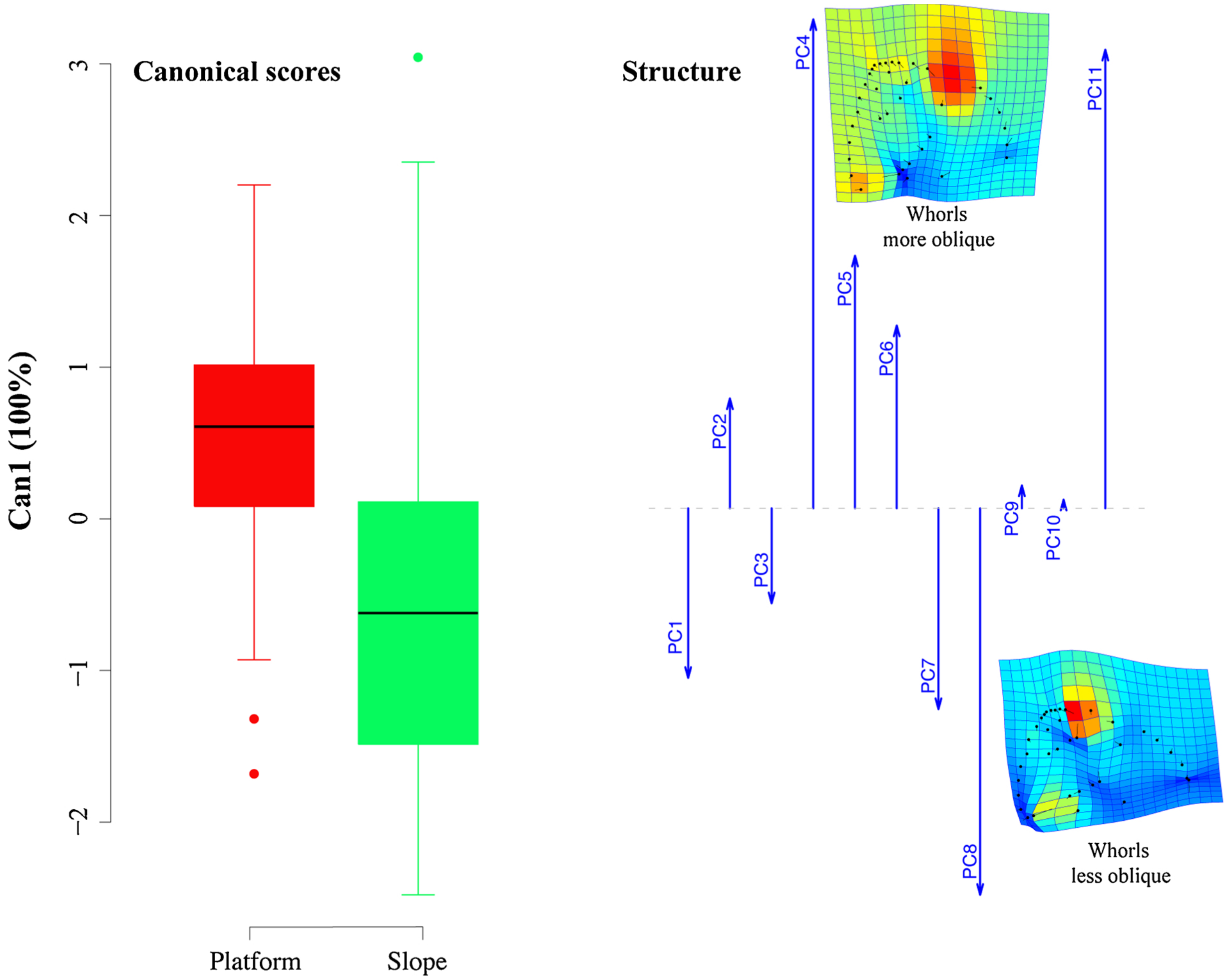

Fig. 7. Canonical variate analysis (CVA) results from shell shape comparison between substrate types horizontal substrate (platform) and slope of specimens *Phorcus sauciatus* collected in Santa Cruz, Madeira Islands. Thin-plate spline deformation grids showing shape variation are also represented.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.zool.2020.125802.

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