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# Discriminant-based study of the shell morphometric relationships of *Patella caerulea* (Gastropoda: Prosobranchia) of the western Mediterranean Sea

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**Abstract:** Our work is a contribution to the study of the morphometric dissimilarity of the limpet  $Patella\ caerulea\ (Linnaeus, 1758)$  inhabiting the seashore of the western Mediterranean Sea. For this study, 438 individuals of this species were sampled in 24 stations and separated into 2 groups of individuals: the first group (G1) originating from the upper infralittoral and the lower mediolittoral areas, and the second group (G2) originating from the upper mediolittoral area. The biometry of P caerulea has been studied by considering the total length (L), the total width (W), and the total height (H) of the shell. These parameters revealed a strong positive correlation, and were used for the principal component analysis (PCA). Other combined parameters (L/W, L/H, and W/H) were added for the discriminant function analysis (DFA). Both multivariate analyses showed that both groups (G1 and G2) were well separated. The morphometric survey based on the calculation of the geometric mean (GM) and the gibbosity index (GIB) revealed the existence of a morphological dissimilarity between the shells of groups G1 (flattened and stocky) and G2 (high and short). This morphological dissimilarity is discussed and compared with the results of other works on the same species and other related species.

Key words: Patella caerulea, morphometric, plasticity, morphological dissimilarity, western Mediterranean Sea

#### 1. Introduction

Because of their wide distribution in the world and their biological and ecological importance, marine gastropod molluscs have interested scientists for a long time. They are present in all marine habitats and are essential for maintaining trophic webs. They are situated at several levels in the food chain, which increases their determining role in the functioning of marine ecosystems (Hakenkamp and Morin, 2000). In addition, these marine invertebrates deeply affect the structure of communities by controlling the growth of marine algae (Branch, 1981).

The prosobranch limpets belonging to the genus *Patella* are very frequent on the rocky shores of the intertidal area (Ridgway et al., 1998). They are also present in the infralittoral fringe (Fekih and Gougerot, 1974). All existing species of the genus *Patella* are limited to the northeast Atlantic and the Mediterranean Sea, and constitute a monophyletic group (Ridgway et al., 1998; Koufopanou et al., 1999). Four species of limpets belonging to the genus *Patella* are present in the Mediterranean Sea: *Patella ferruginea* (Gmelin, 1791); *Patella rustica* (Linnaeus 1758); *Patella caerulea* (Linnaeus 1758); and *Patella ulyssiponensis* 

(Gmelin, 1791) (Cretella et al., 1994). Among these species, *Patella caerulea* is considered endemic to the Mediterranean Sea (Christiaens, 1973; Frenkiel, 1975). This limpet is present on the rocky shores, often in a calm zone, up to a few meters deep, preferring a location where humidity is almost permanent. The limpet must erode its shell to adapt to the irregularities of the substratum. This process allows this species to store water when emerged (Guelorget and Perthuisot, 1983).

Patella caerulea is a common grazer that feeds on microalgae (diatoms and Cyanophyceae) and other encrusting algae (e.g., Corallina elongata, Ulva lactuca) (Della Santina et al., 1993; Silva et al., 2008). Patella caerulea is a hermaphrodite protandric species. Spawning occurs from autumn to spring along the southwestern coast of Italy (Bacci, 1947).

The systematic distinction between all species of the genus *Patella* (including *P. caerulea*) is mainly based on the morphology of their shells. The great morphological plasticity of the individuals of each species, different evolutionary changes, and strong subjectivity has led to a wealth of named species and subspecies and caused

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disagreement on their morphological diversity and geographical distribution (Fischer-Piette and Gaillard, 1959). This morphological plasticity is very distinct in *P. caerulea*. Indeed, this species possesses adaptive strategies for important variations in ecological factors (Vermeij, 1973; Branch, 1981). This is why morphological analysis of the organisms is essential for highlighting evolutionary changes (Bachnou and Boughdiri, 2005). This kind of analysis can give us a better approach for the distinction of *P. caerulea* shells.

Several studies have been carried out on the morphological diversity of genus Patella and other related species around the world such as the study by Tablado and Lopez-Gappa (2001) on the morphometric diversity of the pulmonate limpet Siphonaria lessoni in the province of Buenos Aires (Argentina), and the study by Jerardino and Navarro (2008) on the shell morphometrics of limpet species (genera Cymbula and Scutelastra) on the South African coasts. In the southwestern Mediterranean Sea, similar studies have been made on several species of the genus Patella (P. ferruginea, P. caerulea, P. rustica) and the genus Cymbula (C. nigra) (Espinosa and Ozawa, 2006; Mezali, 2007; Rivera-Ingraham et al., 2011; Belkhodja and Romdhane, 2012; Bouzaza, 2012). These studies have shown several morphotypes that may belong to the same species. This intraspecific polymorphism could be influenced by the type of substratum, which has great importance on the depth of fixation of the limpets (Tablado and Lopez-Gappa, 2001; Pandita et al., 2013), the immersion time of the limpet species, and the surrounding physicochemical factors (Jarne et al., 1988; Moreteau and Vicente, 1988; Belkhodja and Romdhane, 2012).

The aim of our study is to determine whether the external factors of 2 paralic environments could influence the morphology of *P. caerulea*.

#### 2. Materials and methods

#### 2.1. Study area

Our survey was conducted at 24 stations on the western Mediterranean coast (Figure 1, Table 1) using a random sampling of a batch of 438 individuals of *P. caerulea* between October 2014 and May 2015. The individuals were collected using a penknife, and then placed in plastic bags labeled and numbered to indicate their habitat (i.e., mediolittoral and infralittoral fringes).

The samples were separated into 2 groups of individuals. The first group (G1) includes 186 individuals originating from the upper infralittoral and the lower mediolittoral levels; the second group (G2) is composed of 252 individuals originating from the upper mediolittoral level (Table 1). All the stations considered in the present work have a hard substratum with variable slope. The difference in the type of substratum is not very marked

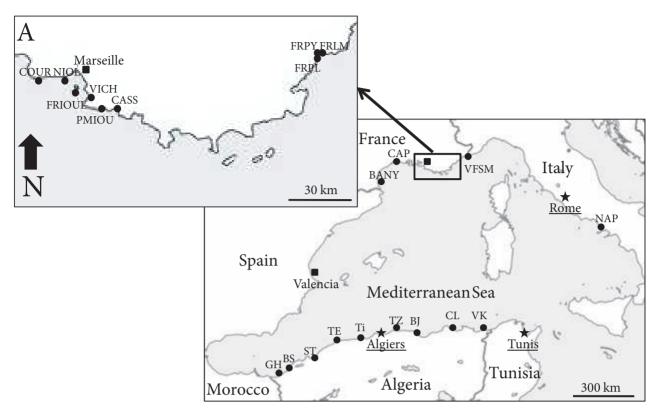
between the stations of group G1 (constantly immersed in water) and the stations of group G2 (immersed occasionally), since G1 is the continuity of G2 in depth. However, the fact that the G1 area is constantly submerged makes its substrate easier for the limpet to scrape in order to assimilate nutrients. All sampled individuals were taken from natural areas, except for the individuals originating from the harbor of La Vielle Chapel (VICH) station, which were collected from a dam constructed between 1964 and 1972 (Data from INSEE, France; Table 1).

# 2.2. Morphometric and statistical study

For each individual of P. caerulea, the biometric measurements (the shell length [L], the maximal width of the shell [W], and the maximal height of the shell [H]) were carried out using a caliper ( $\pm 0.01$  mm) (Tablado and Lopez-Gappa, 2001; Jerardino and Navarro, 2008; Rivera-Ingraham et al., 2011; Saucède et al., 2013). Other ratios such as L/W, L/H, and W/H were calculated (Jarne et al., 1988; Rivera-Ingraham et al., 2011) accordingly.

The correlation between L, W, and H of all P. caerulea individuals (G1 + G2) was realized using R 3.2.2 software. The Kolmogorov-Smirnov nonparametric test was applied to confirm the normality of our samples at each station. In order to see if the 2 groups (G1 and G2) had significantly different values from each other, a t-test was performed on each of the parameters (L, W, H, L/W, L/H, and W/H) using Xlstat (2009) software. The same software was used to achieve principal component analysis (PCA) of the studied variables and stations. To check the adherence of the individuals of each station to their respective group (G1 or G2), a discriminant function analysis (DFA) was realized. Thus, a Box's M-test was performed, and then the Wilk's Lambda parameter ( $\Delta$ -Wilk) was calculated with a Rao approximation (Saporta, 2006). To see if the stations were classified correctly into their groups, a classification was performed following the analyses. A one-dimensional test of class means was established in order to determine the most discriminative parameter. All of these analyses were performed using IBM SPSS 20.0 Statistics software.

Regarding the morphometric survey, we calculated the geometric mean (GM) according to the equation:  $GM = {}^{3}\sqrt{(L \times W \times H)}$  (Matousek and Nesetril, 2004), and the gibbosity index (GIB) according to the equation:  $GIB = H\sqrt{(L \times W)}$  (David and Fouray, 1984). The higher the GIB is, the higher and shorter the shell shape; the shell shape is stocky and flattened if the GIB is low (David and Fouray, 1984). A morphometric analysis of all sampled individuals was conducted by studying the regression between GM and the parameters L, W, H, and GIB using R 3.2.2 software. A Levene test was performed on the GIB index in order to test the homogeneity of the intragroup variances within G1 and G2. A t-test was carried out in order to determine the differences between G1 and G2 based on the parameters L, W, H, and GIB.



**Figure 1.** Study area showing the sampling stations (black dots). See Table 1 for abbreviations. A: Details of the *French Mediterranean coast*.

#### 3. Results

The biometric relationships established for the shell of *P. caerulea* showed a strong correlation between the length and the width (W–L), the height and the length (H–L), and the height and the width (H–W) (all P < 0.001; Figure 2). The mean length and the mean width of all individuals of group G1 are respectively higher than those of group G2 (31.183  $\pm$  7.960 > 27.496  $\pm$  5.765; 25.591  $\pm$  6.309 > 22.333  $\pm$  4.874, respectively). However, the mean height of all individuals of group G1 is lower than that of the individuals in group G2 (7.516  $\pm$  2.041 < 7.944  $\pm$  2.243).

The results of the t-test between the 2 groups (G1 and G2) established for the parameters L, W, H, L/H, and W/H are highly significant (P < 0.01). In contrast, the parameter L/W is not significant (P = 0.06). The mean value of L/W for G1 is almost equal to that of G2 (1.221 ~ 1.237, respectively). This shows that the L/W parameter remains more or less stable regardless of the depth of sampling for P. caerulea.

The PCA of the variables and individuals is represented in Figure 3A. Contributions of each variable (*L*, *W*, and *H*) to the PCA axes (F1 to F3) are shown in Table 2. The first and second axes together explain 96.38% of the total variance (F1: 80.55%, F2: 15.83%; Figure 3A); however, axis F3 explains only 3.62% of the variance, and shows no

correlation with any variables. The parameters L, W, and H have positive and very high contributions to F1 (which could be interpreted as a size factor; Table 2), and show highly significant correlation coefficients (0.97, 0.96, and 0.87, respectively; all P < 0.001). Moreover, L, W, and H are positively correlated with each other and considered as size variables (Nakhlé, 2003; Espinosa and Ozawa, 2006; Rivera-Ingraham et al., 2011; Belkhodja and Romdhane, 2012). The influence of the H parameter is fairly well marked on axis F2 with a positive correlation coefficient (r = 0.5; P = 0.02), while L and W show respectively a low and nonsignificant correlation with the same axis (-0.21, -0.22; all P > 0.05). In addition, the qualitative aspects of the 2 groups (G1 and G2) show a strong correlation on the F2 axis (r = 0.79; P < 0.001). As a result, the F2 axis may provide more information on the aspect of P. caerulea shell shape. The centroids of the 2 groups (G1 and G2) are well separated along the second axis F2 with the closeness of the station (VICH) to the centroid of G2 (Figure 3A), even if this station initially belonged to G1. After checking the homoscedasticity of the covariance matrices by Box's M-test (M = 140.08; P < 0.001), a DFA was achieved (Figure 3B). The obtained Wilk's  $\Delta$  value is equal to 0.151 (P < 0.001). This value indicates that there is good discrimination between G1 and G2 and that their

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**Table 1.** Abbreviations, geographical coordinates, and depths of sampling stations. (a) Lower mediolittoral and infralittoral area. (b) Upper mediolittoral area.

Station abbreviation	Coastal country	Geographical coordinates			D (1 ( )
		Latitude	Longitude	Group	Depth (m)
Ghazaouet (GH)	Algeria	35°6′1.25″N	1°52′13.87″E	G2	0-0.5
Beni Saf (BS)	Algeria	35°18′45.70″N	1°22′26.03″E	G1	1-8
Ténès (TE)	Algeria	36°31′9.03″N	1°18′32.54″E	G2	0-0.5
Tipaza (a) (Tia)	Algeria	36°36′37.78″N	2°24′52.09″E	G1	1-8
Tipaza (Tib)	Algeria	36°36′37.78″N	2°24′52.09″E	G2	0-0.5
Tigzirt (TZ)	Algeria	36°53′43.28″N	4°7′47.00″E	G2	0-0.5
Collo (CLa)	Algeria	37°0′51.40″N	6°33′56.59″E	G1	1-8
Collo (CLb)	Algeria	37°0′51.40″N	6°33′56.59″E	G2	0-0.5
Banyuls-sur-Mer* (BANY)	France	42°29′12.46″N	3°7′51.69″E	G2	0-0.5
Cap d'Agde* (CAP)	France	43°16′28.48″N	3°30′52.53″E	G2	0-0.5
Fréjus Plage* (FRPL)	France	43°33′17.03″N	4°4′30.08″E	G2	0-0.5
Fréjus Le Lion de Mer* (FRLM)	France	43°33′17.01″N	4°4′30.10″E	G1	1-8
Fréjus Les Pyramides* (FRPY)	France	43°33′17.03″N	4°4′30.10″E	G1	1-8
Couronne (COUR)	France	43°19′46.35″N	5°2′57.54″E	G2	0-0.5
Niolon (NIOL)	France	43°20′12.91″N	5°15′20.95″E	G1	1-8
Frioul (FRIOUL)	France	43°16′57.80″N	5°17′59.33″E	G2	0-0.5
La Vieille Chapelle (VICH)	France	43°14′56.86″N	5°22′20.93″E	G1	1-8
Port Miou (PMIOU)	France	43°12′8.75″N	5°30′35.81″E	G1	1-8
Cassis (CASS)	France	43°12′42.15″N	5°32′17.01″E	G2	0-0.5
Villefranche-sur-Mer* (VFSM)	France	43°42′17.88″N	7°19′5.04″E	G1	1-8
Naples* (NAP)	Italy	40°50′16.31″N	14°15′54.08″E	G1	1-8
Stidia (ST)	Algeria	35.83° N	0°	G1	1-8
Vielle Calle (VK)	Algeria	36.9° N	8.45° E	G1	1-8
Béjaïa (BJ)	Algeria	36.87° N	4.84° E	G1	1-8

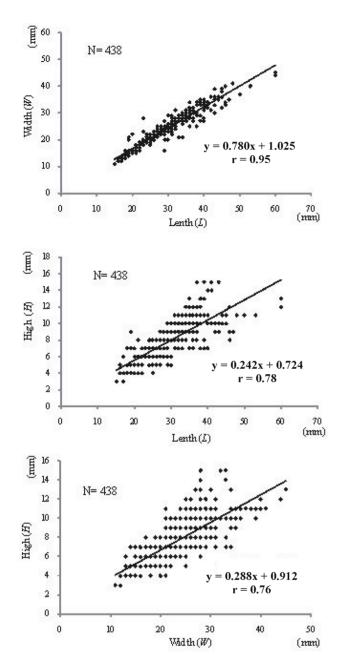
<sup>\*:</sup> The individuals from these stations were collected by the IMBE (Mediterranean Institute of Marine and Terrestrial Biodiversity, France).

centroids are well separated on axis F1, which contains more than 99.99% of the information (~100%; Figure 3B). The DFA shows that both groups (G1 and G2) are well separated and their centroids are very distant. The classification following the analyses (Table 3) indicates that the individuals originating from the VICH station are very close to the centroid of G2 and should be classified therein. In contrast, the individuals of the 2 stations FRPL and CASS were reclassified into group G1 (Table 3).

The confusion matrix shows a high classification rate (87.50%) for 1 station belonging to group G1 (VICH) which was supposed to be classified in G2, and 2 stations of group G2 (CASS and FRPL) that were supposed to be classified in G1 (Table 3). The one-dimensional equality

test of class means shows that the ratios L/H and W/H are the most discriminating parameters (P < 0.0001).

The morphometric results, based on the regression between the size (geometric mean [GM]), (length [L], width [W], height [H], and the gibbosity index [GIB]) are mentioned in Figure 4. A highly significant correlation is observed between L-GM, W-GM, and H-GM (all P < 0.001) (Figures 4A, 4B, and 4C, respectively). However, this relationship is very weak between GIB and GM (Figure 4D). These results indicate that the size of the shell increases with the parameters (L, W, and H). The Levene test performed on the GIB index within the 2 groups (G1 and G2) gives the following F-values:  $F_{G1} = 0.802$  and  $F_{G2} = 1.209$  (P > 0.05). As a result, no significant difference



**Figure 2.** Linear regression between length (L), width (W), and height (H) of P. caerulea. N: Number of individuals.

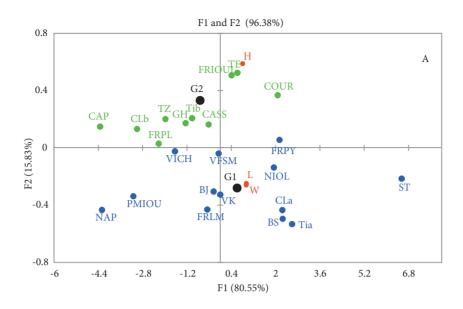
in variance was found between the groups. The mean gibbosity index (GIB) of all individuals of group G1 is lower than that of group G2 ( $0.270 \pm 0.041 < 0.319 \pm 0.045$ , respectively). The t-test established between the 2 groups (G1 and G2) on the variable GIB gives an observed value t = -11.188 (P < 0.001). This value indicates the existence of a "group effect" caused by the morphological dissimilarity of *P. caerulea* shells. Indeed, group G1 is characterized by individuals with flattened and stocky shells. In contrast,

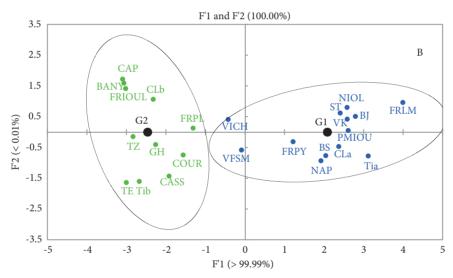
group G2 consists of individuals with high and less wide shells.

#### 4. Discussion

The parameters *L*, *W*, and *H* are often used in the biometric relationships of limpets since they evolve with their shell volume (Nakhlé, 2003; Espinosa et al., 2009; Rivera-Ingraham et al., 2011; Belkhodja and Romdhane, 2012). These parameters present a very strong correlation for *P. caerulea* species in our survey (Figure 2). This result corroborates with those obtained by Nakhlé (2003) and Belkhodja and Romdhane (2012) for the same species.

The mean values obtained for L, W, and H in the sampled individuals belonging to both groups (G1 and G2) (Table 1) showed that the shells of group G1 are more flattened than the shells of group G2, which are high and stocky. Our survey revealed that the shell sizes of the individuals of group G1 is higher than those of group G2 (i.e., the westernmost Algerian station BS [Table 1], which presented specimens having the most imposing sizes). This result coincides with the model of Vermeij (1972). This model indicates that the marine species living in the lowest levels of the intertidal areas (such as P. caerulea) possess imposing shell sizes, larger and lower in form than the individuals living in the highest intertidal areas. In addition, Rivera-Ingraham et al. (2011) showed that shell lengths of the limpet C. nigra of Ceuta (northwest coast of Africa) were negatively correlated with shell heights in upper sea levels. The same authors noted that shell heights decreased gradually towards the lower intertidal areas. According to Vermeij model (1972), the difference in the limpet shell sizes (larger in the lower benthic zone than in the upper benthic zone) is due to a downward vertical migration. Indeed, in the highest inshore areas, we found juveniles recruited by their own larvae. In contrast, individuals with large shell sizes were found in the lowest inshore areas (e.g., 3 sampling stations from the Algerian coast: BS, CLa, and ST [Table 1]). This migration could be horizontal for the most part at the other studied stations, such as for individuals belonging to the French coastal station VICH (Table 1). Other studies concerning other limpet species inhabiting the lower levels of the intertidal areas, such as Patella argenvillei (Stephenson et al., 1940) or P. intermedia (Vermeij, 1972), and of the species of acmeides such as Acmaeapelta (Shotwell, 1950) or A. testudinalis testudinalis (Stephenson and Stephenson, 1954) showed that these species could migrate horizontally. According to these authors, this suggests that these species could develop a preference for a specific type of substratum by a competition phenomenon. This hypothesis is shared by Tablado and Lopez-Gappa (2001), who showed that shell size differences of the pulmonate limpet Siphonaria lessoni were influenced by intraspecific competition and was closely linked to its habitat.





**Figure 3.** Multivariate analysis (A: principal component analysis [PCA]; B: discriminant function analysis [DFA]) established on all sampled individuals of *P. caerulea* using the mean values of the parameters ([*L*, *W* and *H*] for the PCA and [*L*, *W*, *H*, *L/W*, *L/H* and *W/H*] for the DFA). • Individuals of group G1. • Individuals of group G2 (see Table 1 for the abbreviations). • Centroids of both groups (G1 and G2). • Projection of the parameters (*L*, *W*, and *H*) on the 2 most informative axes (F1 and F2) of the PCA.

Our study also revealed that the ratios *L/H* and *W/H* are the most discriminating and seem to be independent of the age of the limpet, as indicated by Jarne et al. (1988) in their study on the morphometric of some Mediterranean clams. These authors showed that there is a real morphometric dissimilarity between these species. In addition, these ratios have been used by Espinosa and Ozawa (2006) and Mezali (2007) to distinguish between the *Rouxii* and *Lamarkii* forms of *Patella ferruginea* (Gmelin, 1791). Discriminant function analysis (Figure 3B) showed that there is clear discrimination between groups G1 and G2

with good classification (Table 3), except for the individuals of VICH station which might belong to group G2, and the individuals of 2 stations (CASS and FRPL) which could be classified in group G1 (Tables 1 and 3). This could be explained by a homing phenomenon that limpets usually practice (Breen, 1971). Indeed, these marine gastropods can move 10 cm/day in all directions in order to graze for a few hours around their resting points (Little and Kitching, 1996; Santini et al., 2004, Silva et al., 2008).

The morphometric survey revealed the presence of 2 different morphotypes of *P. caerulea* in 2 different ecotones.

**Table 2.** Contribution of each variable (L, W, and H) to the 3 PCA axes.

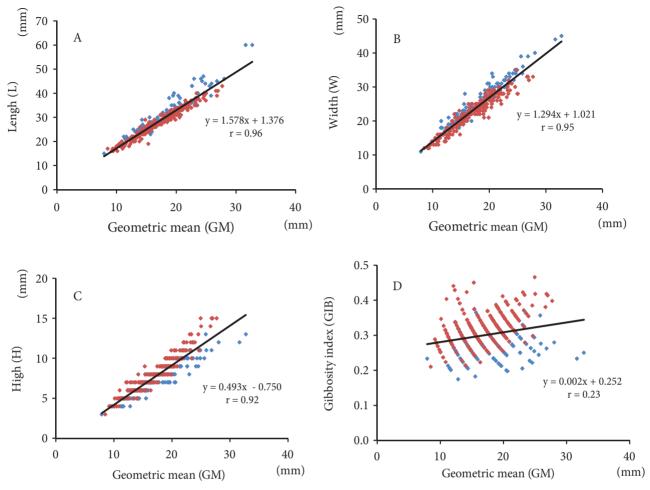
	F1	F2	F3
L	0.940	-0.248	0.234
W	0.938	-0.259	-0.232
Н	0.808	0.589	-0.004

This morphological difference is relative to the period of immersion of the individuals of *P. caerulea*, the nature of the substratum, and surrounding physical factors (Gérard, 1978; Moreteau and Vicente, 1988; Gray and Hodgson, 2003; Espinosa et al., 2009; Boukhicha et al., 2010). Group G1 includes individuals whose shells are more flattened and

lower in height than the shells of individuals of group G2, which are higher and shorter. The structure of the shells of group G2 (higher and shorter) allows them to store more water and assimilate a considerable quantity of oxygen in these paralytic areas (Guelorget and Perthuisot, 1983). This hypothesis is supported by Belkhodja and Romdhane (2012), who consider it an adaptive response to physical and ecological environmental conditions. Moreover, Jarne et al. (1988) demonstrated with populations of the Mediterranean clams Ruditapeses decussatus that the circumstances of growth of their populations have an influence on the shapes of their shells. In addition, it has been demonstrated that the morphology of the limpet shell is strongly influenced by the latitudinal position and the vertical distribution of individuals (Balaparameswara Rao and Ganapati, 1971; Vermeij, 1973; Branch, 1981; 1985).

**Table 3.** Classification of the sampling stations with membership probability for groups G1 and G2 (see Table 1 for the abbreviations).

Station			Membership probability		
	Initial classification	Post classification	G1	G2	
FRPY	G1	G1	1.000	0.000	
FRLM	G1	G1	1.000	0.000	
NIOL	G1	G1	1.000	0.000	
PMIOU	G1	G1	1.000	0.000	
VFSM	G1	G1	1.000	0.000	
VICH	G1	G2	0.000	1.000	
NAP	G1	G1	1.000	0.000	
BS	G1	G1	1.000	0.000	
FRPL	G2	G1	0.855	0.145	
FRIOUL	G2	G2	0.000	1.000	
COUR	G2	G2	0.000	1.000	
CASS	G2	G1	0.856	0.144	
CAP	G2	G2	0.000	1.000	
BANY	G2	G2	0.000	1.000	
TZ	G2	G2	0.000	1.000	
CLa	G1	G1	1.000	0.000	
CLb	G2	G2	0.000	1.000	
GH	G2	G2	0.000	1.000	
TE	G2	G2	0.000	1.000	
Tib	G2	G2	0.000	1.000	
BJ	G1	G1	1.000	0.000	
Tia	G1	G1	1.000	0.000	
VK	G1	G1	1.000	0.000	
ST	G1	G1	1.000	0.000	



**Figure 4.** Linear regression between the parameters (A: *L*–*GM*, B: *W*–*GM*, C: *H*–*GM*, and D: *GIB*–*GM*) of all measured individuals of *P. caerulea*. • Individuals of group G1. • Individuals of group G2.

It seems that the shell of *P. caerulea* takes 2 different forms related to a specific habitat: one associated with submerged areas (lower mediolittoral and infralittoral), and the other with drier areas (upper mediolittoral). This morphological plasticity allows this limpet to adapt to different ecotones and resist difficult environmental conditions that can affect its habitat.

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