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Morphometric analysis of three Mediterranean limpets *Patella caerulea* Linnaeus, 1758, *P. rustica* Linnaeus, 1758 and the endangered *P. ferruginea* Gmelin, 1791 (Gastropoda Patellidae) from the Algerian West Coast

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ABSTRACT The characterization of *Patella caerulea* Linnaeus, 1758, *P. rustica* Linnaeus, 1758 and *P. ferruginea* Gmelin, 1791 (Gastropoda Patellidae) has been a source of debate and controversy since they were proposed as species. This was due to the high shell variability, and some of them display intermediate characteristics. In the present work, samples of three species of Patella were examined and compared using several morphometric characters describing the shell and soft parts. 575 individuals of *P. caerulea*, 132 individuals of *P. rustica*, and 45 of *P. ferruginea* were sampled from Algerian West Coast. The biometry of the three species has been studied: length (L), width (Wi), height (H) of the shell and the weight (W) of every individual. A strong positive correlation was noted between these parameters for all three species. These parameters were used also for the Principal Component Analysis (PCA). The three *Patella* species showed substantial morphology variability. *P. rustica* was easily recognized due to small brown spots near the shell apex. *P. ferruginea (lamarckii* form, *rouxii* form) were well identified from the other *Patella* species but for *P. caerulea*, a high morphological variability was noted between us in studied localities.

KEY WORDS Morphometric analysis; Patellidae; endangered limpet; Western Mediterranean Sea; Algeria.

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INTRODUCTION

The family Patellidae or Limpets are the most dominant grazers of the benthic community in the rocky medio-littoral (Tlig-Zouari et al., 2011). It consists of 37 species, classified into four genera: *Patella* Linnaeus, 1758, *Cymbula* Adams, 1854, *Helicion* Montfort, 1810, and *Scutellastra* Adams, 1854 (Ridgway et al., 1998; Espinosa et al., 2010). Four species from *Patella* genus occur in the Mediterranean Sea (Bouzaza & Mezali, 2018; Meziane et al., 2020): *P. caerulea* Linnaeus, 1758, *P. rustica* Linnaeus, 1758 (=*P. lusitanica* Gmelin, 1791) that are quite common (Bresher et al., 2003; Petraccioli et al., 2009), *P. ferruginea* Gmelin, 1791 is highly endangered (Espinosa et al., 2007), and *P. ulyssiponensis* (Gmelin, 1791) that is less presented in the Mediterranean Sea (Beldi et al., 2012). The diversity of *Patella* species in an area is related to the number of species it contains (species richness), and the relative abundance of each species (relative abundance) (Withaningsih et al., 2022).

Patella rustica is characterized by a conic shell with brown spots near its apex that may make its identification easier (Petraccioli et al., 2009). *Patella caerulea* and *P. ulyssiponensis* present an overlapping shell morphology and color in several localities (Cretella et al., 1991). Their morphological variation makes them indistinguishable and a misidentification can take place. The apricot color of the foot for *P. ulyssiponensis* can be used as a tool to distinguish them (Mauro et al., 2003). *Patella ferruginea* presents a high morphometric plasticity (Laborel-Deguen & Laborel, 1991a), and two different forms (*rouxii* and *lamarckii*) (Espinosa et al., 2007). This species is on the verge of extinction, and especially has been during the 20th century. Strong human pressure - harbours activities, urban and industrialw astewater discharges - seems to be the cause (Kerfouf et al., 2022).

The morphological changes on the limpet shells are due to abiotic factors, but the reproduction, growth, mortality, density and distribution are dependent on a complex array of selective forces (abiotic and biotic factors) (Begon et al., 1996, Corte-Real et al., 1996, Vafidis et al., 2020). The biological traits (biotic factors) of limpets vary inter- and intraspecifically as a result of genetic differences and environmental influences (Bowman & Lewis, 1986, Griffin et al., 2008). The abiotic factors include type of substratum, which has great importance on the depth of fixation of the limpets (Pandita et al., 2013), wave exposure forms, steepness, tidal dynamics, thermal and desiccation stress (Williams & Morritt, 1995; Harley, 2008; Miller et al., 2009; Fraser et al., 2010; Christofoletti et al., 2011; Prusina et al., 2014).

The present work aims to study the morphology of three limpets species (*P. caerulea, P. rustica* and *P. ferruginea*) in order to explain the main factors which can affect their variability.

MATERIAL AND METHODS

Study area

The study was carried out along the Western Algerian Cost (6 stations) (Fig. 1, Table 1), during 2016–2019. 826 individuals of the genus *Patella* were measured. 575 individuals of *P. caerulea*, 132 individuals of *P. rustica*, and 45 of *P. ferruginea*. A 20-meter track was drawn on the foreshore (the

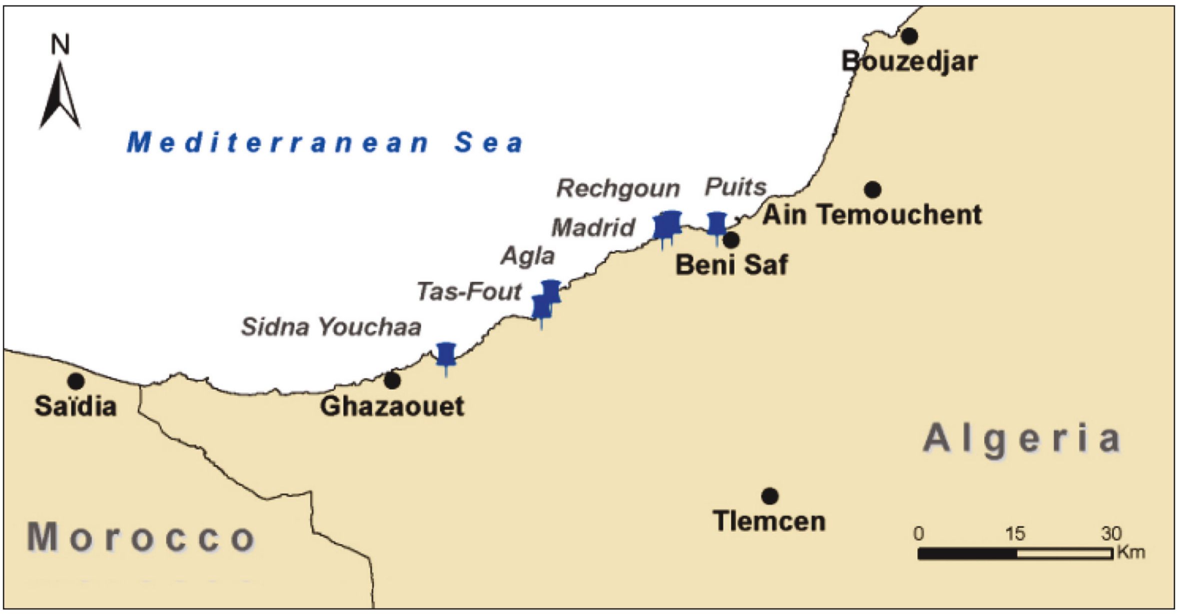


Figure 1. Geographic map of sampling stations for studied limpet species along the Algerian West Coast.

Stations and abbreviations	Coordinates		Sample size			
	Latitude	Longitude	*P. caerulea*	*P. rustica*	*P. ferruginea*	Total
Puits (PU)	35.301528	-1.402694	49	63	01	113
Rechgoun (RE)	35.297324	-1.479149	167	02	05	174
Madrid (MA)	35.304069	-1.466198	102	31	26	159
Agla (AG)	35.206758	-1.635769	117	27	07	151
Tas-Fout (TF)	35.185481	-1.648949	42	03	00	45
Sidna-Youchaa (SY)	35.118995	-1.782365	98	06	06	110
Total			575	132	45	752

Table 1. Abbreviation and geographical coordinates of sampling stations.

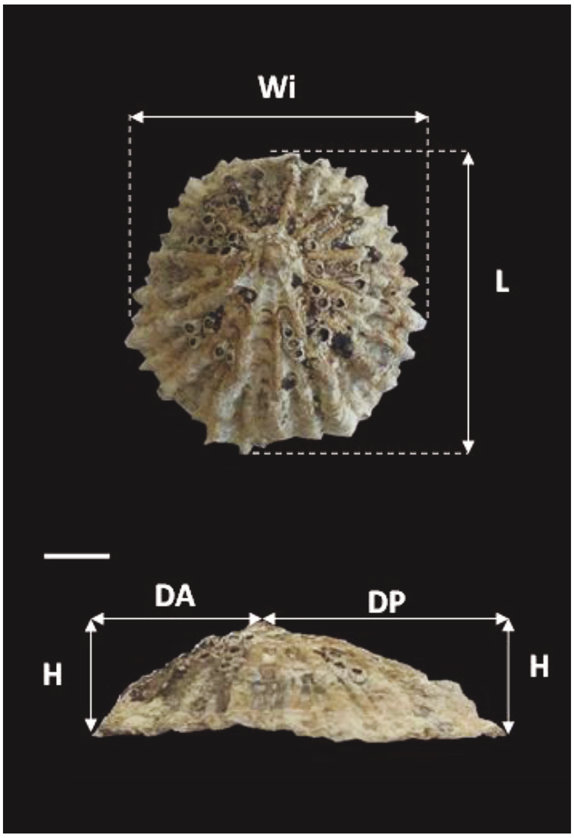


Figure 2. Shells measurements used in canonical analysis. Presented model: *P. ferruginea* (specimen from Agla (Honaine)). L: Length, Wi: width, H: Height, DA: distance between the apex and the anterior end, DP: Distance between the apex and the posterior end. Scal bar =1 cm (pictures were taken by Belmokhtar F.).

shoreline; long-shore) using a decameter. Three quadrats of 1m² were placed randomly on these lines at the level of the foreshore. The limpets which were at the level of these quadrats were counted then the biometric parameters were collected. Then they have been returned to their places, so that the stress induced on them was the least disturbing possible. This operation was repeated several times going eastward so as to cover a distance total of 200 m of coastline on each site.

Identification of limpets

The identification of the specimens was based on the morphology of the shell and color of foot (Fisher-Piette & Gaillard, 1959; Rolán & Otero-Schmitt, 1996; Cabral, 2003). Some shells of every form have been found in the coast and were then stripped of their flesh, washed, observed with the naked eye and/or under a binocular magnifier and photographed with a digital camera. The limpets were identified using specialized documentation (Parenzan, 1970; Poppe & Goto, 1991; Trigo et al., 2018) then counted by species.

Biometric measurements

Biometric measurements were taken on the alive limpet using a digital vernier caliper (Fig. 2). Shell length (L): greatest distance between the anterior and posterior ends; shell width (Wi): greatest distance between margins; shell height (H): highest vertical distance from apex to shell base; distance between the apex and the anterior end (DA); distance between the apex and the posterior end (DP) (Cabral, 2003). The total weight (W) of individual was measured using a digital balance.

Statistical analysis

The frequency of limpets was estimated by the dominance of each species in all studied stations, and each station.

The results of the morphometric measurements were presented in the form of a mean± standard deviation.

The correlation between L, Wi, and H of all individuals of studied species was realized. The ANOVA test was done to estimate similarities and differences between samples (ratio (L/Wi, L/H, and Wi/H), and one factor was considered (the station). Before ANOVA analysis, The Kolmogorov–Smirnov nonparametric test and Shapiro-Wilk test were applied to confirm the normality of our samples at each station. We used the Levene test to determine the homogeneity of variances. The principal component analysis (PCA) of the studied variables and stations was carried out (Fig. 4). All of these analyses were performed using IBM SPSS 23.0 Statistic Software.

RESULTS

Morphology of species

The shell's morphology of *Patella* can vary between sites due to the strong influence of environmental features, namely the degree of exposure to desiccation and the intensity of wave action. For our specimens we found these results:

Patella caerulea (Fig. 3): the shell was typically flat, although a few specimens were relatively high (ratio H/L: 0.28±0.37), the outline was ellipsoid, and serrated the length was between (13.8 mm- 67.3 mm), the apex was well below the middle of the shell. The external surface was striated with fine ribs. The inner face of the shell was sometimes iridescent blue, light grey. The color of the foot was gray to dark and sometimes abricot with blue inner face or cream.

Patella rustica (Fig. 4): the shell was thick, conic and high (ratio H/L: 0.37±0.57), the length was between (15–40 mm). The outline was ellipsoid. The apex was slightly below the middle of the shell. The external surface showed brown spots near its apex. The interior surface was dark with double light rays. Color of foot was brown.

Patella ferruginea (Fig. 5): the length and height of the shell were varied: the *rouxii* form was taller and more conical and for the *lamarckii* form was shorter and flatter. The maximal length was 59.39 mm and the height was 22.69 mm. The external surface was well marked by a radial ribbed that extends beyond the edge of the shell. The foot was oval and its sole is apricot, yellow or cream, sometimes with a greyish edge; many species (epizoism) spread in the same eulittoral zone cover the shell, most notably the barnacle *Chthamalus stellatus* which can cover up to 100 percent of the shell's surface.

Dominance of species

In most of the studied stations, blue limpet *P. caerulea* was more abundant than the other limpets, followed by *P. rustica* than *P. ferruginea* (Table 2).

Some limpets were absent in few stations like, Station of Tas-Fout where just *P. caerulea* and *P. rustica* were present. *P. ferruginea* was present in stations but with a very low proportions.

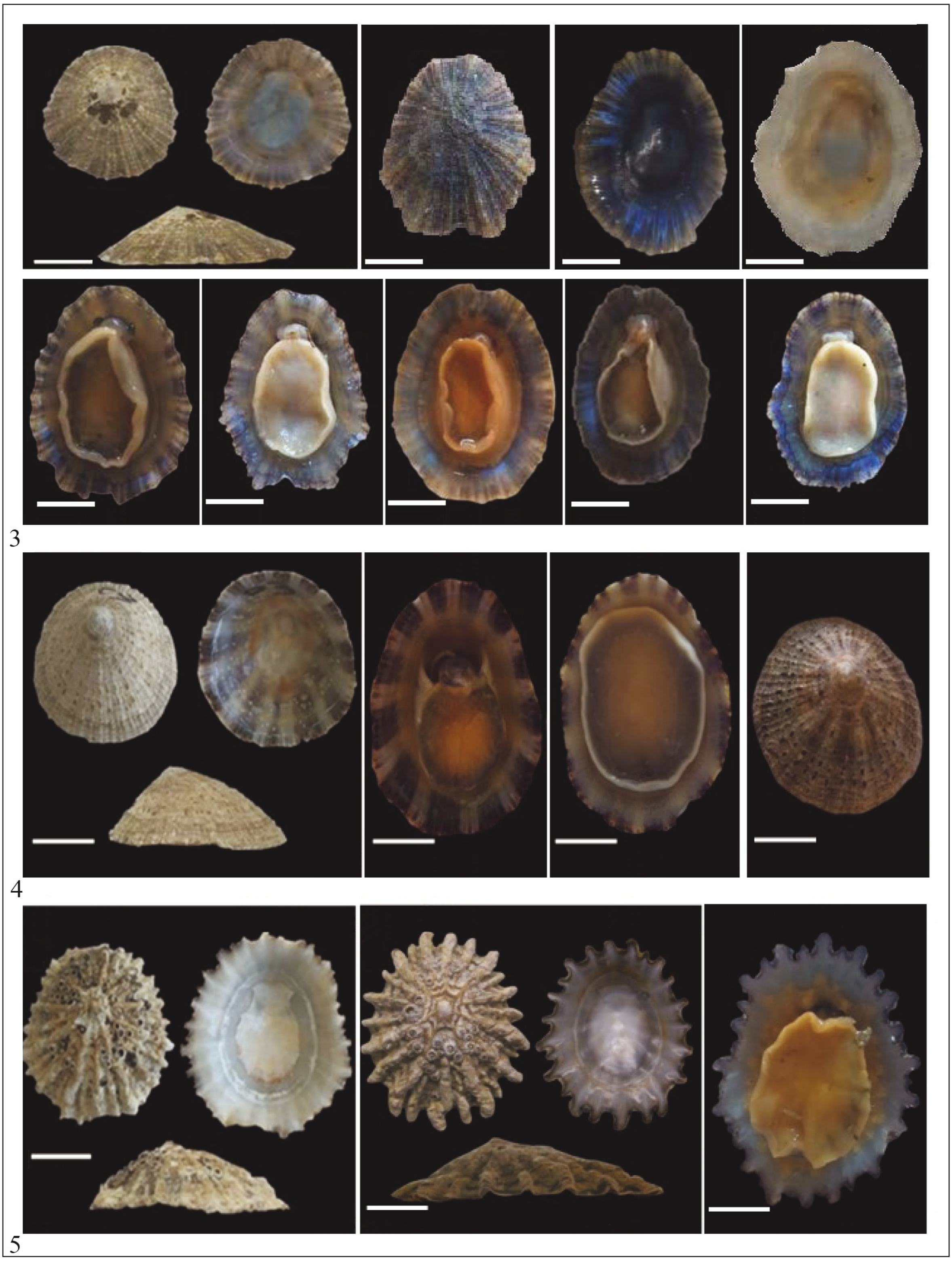
Biometric measurements

For mean biometric parameters, *P. ferruginea* presented the high values of length, width, height and weight than the others species *P. rustica* and *P. caerulea* (Table 3).

Individuals of *P. ferruginea* showed low values of H/L with higher values of DA/DP. This makes their shells lower with a more concentric top. H/L for *P. rustica* was higher than the other species (taller shells), while *P. caerulea* had the lowest H/L and DA/DP values (flattened shells) (Table 3).

The biometric relationship showed a strong correlation between the parameters (Length & width), (length & height), (width & height) for all studied species of *Patella* (Table 4, $P \leq 0.0001$).

For each limpet species, a linear regression model ($Y = ax + b$) was done. The coefficients of correlation for all morphometric equations were



Figures 3-5. External shell morphology of three species from the genus *Patella* presented in Western Cost of Algeria. Fig. 3: *P. caerulea*. Fig. 4: *P. rustica.* Fig. 5: *P. ferruginea* (left: *rouxii* form; right: *lamarckii* form). Scale bar = 1 cm (pictures were taken by Belmokhtar F.).

very high ($r \geq 0.64$, $p \leq 0.0001$) indicating their good predictive power.

Considering the six localities of the study (containing as well all studied species individuals), both normality (Shapiro-Wilk test, P>0.05 for small samples, and Kolmornow-Smiknow test for large samples, P>0.05) and homogeneity of variances (Levene test, P>0.05) were done. The assumption of all variables was satisfied.

Shell length, shell width and shell height of *P caerulea* and *P. rustica* were found considerably different between the six localities (ANOVA, p<0.0001, Table 5). For *P. ferruginea*, just the length showed a difference between studied localities.

The ratio L/H, Wi/H for *P. caerulea*, *P. rustica* was significantly different between all stations (ANOVA, p<0.001, Table 5). The ratio L/Wi presented a difference between stations just for *P. rustica* (ANOVA, p=0.002, Table 5).

The principal component analysis of the variables was shown in Figs. 6-8. In all cases, axis F2 explains only a very low percent of variation, while most of the variables (weight, length, width and height) were positively correlated with axis F1. Qualitative aspect of individuals from different stations was well projected on the two axes F1 and F2.

For *P. caerulea*, individuals from the Puits, Agla and Sidna-Youchaa station had a very significant mean weight compared to the other stations (too close to the F2 axis). At the same time, the individuals of Agla and Sidna Youchaa presented large sizes compared to the other stations (positively correlated with the F1 axis).

For *P. rustica*, individuals from these two stations (Tas-Fout and Madrid) showed less height and

Station / Species	PU (%)	RE (%)	MA (%)	AG (%)	TF (%)	SY (%)	All of stations (%)
P. caerulea	43.63	95.97	64.75	77.84	93.33	89.09	74.46
P. rustica	55.75	1.14	19.49	17.88	06.67	05.45	17.55
P. ferruginea	0.88	2.87	16.35	4.36	0	05.45	05.98

Table 2. Dominance (%) of the different limpets in all studied stations.

	P. caerulea	*P. rustica*	*P. ferruginea*
Width (mean±SD) (mm)	21.49±5.41	21.29±4.81	32.12±9.72
Length (mean± SD) (mm)	26.28±6.17	25.86±5.16	39.35±10.68
Height (mean±SD) (mm)	07.47±2.29	09.60±2.96	12.59±4.22
H/L(mm)	0.28±0.37	0.37±0.57	0.31±0.39
DA (mean±SD) (mm)	12.90±3.51	13.88±3.66	21.22±6.56
DP (mean±SD) (mm)	18.23±4.37	18.95±4.35	27.13±7.84
DA/DP(mm)	0.70±0.80	0.73±0.84	0.78±0.83
Weight (mean±SD) (g)	09.45±2.86	03.23±2.29	11.45±9.57

Table 3. Mean biometry (Length, Width and Height in millimeters and Weight in gram) of Limpets in all studied stations. DA: distance between the apex and the anterior end, DP: Distance between the apex and the posterior end: SD: Standard Deviation

Limpet	Y variable	X variable	a	b	r	P
P. caerulea	Width	Length	-0.73	0.84	0.92	≤ 0.0001
	Height	Length	-0.39	0.29	0.64	≤ 0.0001
	Height	Width	0.012	0.34	0.81	≤ 0.0001
P. rustica	Width	Length	-1.93	0.89	0.97	≤ 0.0001
	Height	Length	-3.21	0.49	0.90	≤ 0.0001
	Height	Width	-1.90	0.54	0.90	≤ 0.0001
P. ferruginea	Width	Length	-0.91	0.84	0.92	≤ 0.0001
	Height	Length	-1.51	0.35	0.90	≤ 0.0001
	Height	Width	0.86	0.36	0.70	≤ 0.0001

Table 4. Parameters of morphometric equations for the estimation of total limpet shell length, width, height from measurements on elements of the limpet shells. r: coefficient of correlation, Linear regression used: y= ax + b.

Variables	df	MS	F	P
P. caerulea				
L	5	419.18	12.053	<0.001
Wi	5	332.681	12.455	<0.001
H	5	71.654	15.229	<0.001
L/Wi	5	0.011	1.093	0.363
L/H	5	7.464	28.581	<0.001
Wi/H	5	4.772	29.991	<0.001
P. rustica				
L	5	260.435	15.007	<0.001
Wi	5	224.434	14.778	<0.001
H	5	94.259	17.527	<0.001
L/Wi	5	0.015	4.151	0.002
L/H	5	2.287	15.475	<0.001
Wi/H	5	1.121	11.612	<0.001
P. ferruginea				
L	4	273.361	2.775	0.039
Wi	4	195.196	2.309	0.075
H	4	25.001	1.459	0.233
L/Wi	4	0.011	0.933	0.455
L/H	4	0.053	0.225	0.923
Wi/H	4	0.126	0.656	0.626

Table 5. Analysis of variance (ANOVA) of considered metric variables between the localities of the study. L: Length, Wi: Width, H : Height, df: Degree of freedom, MS: medium square.

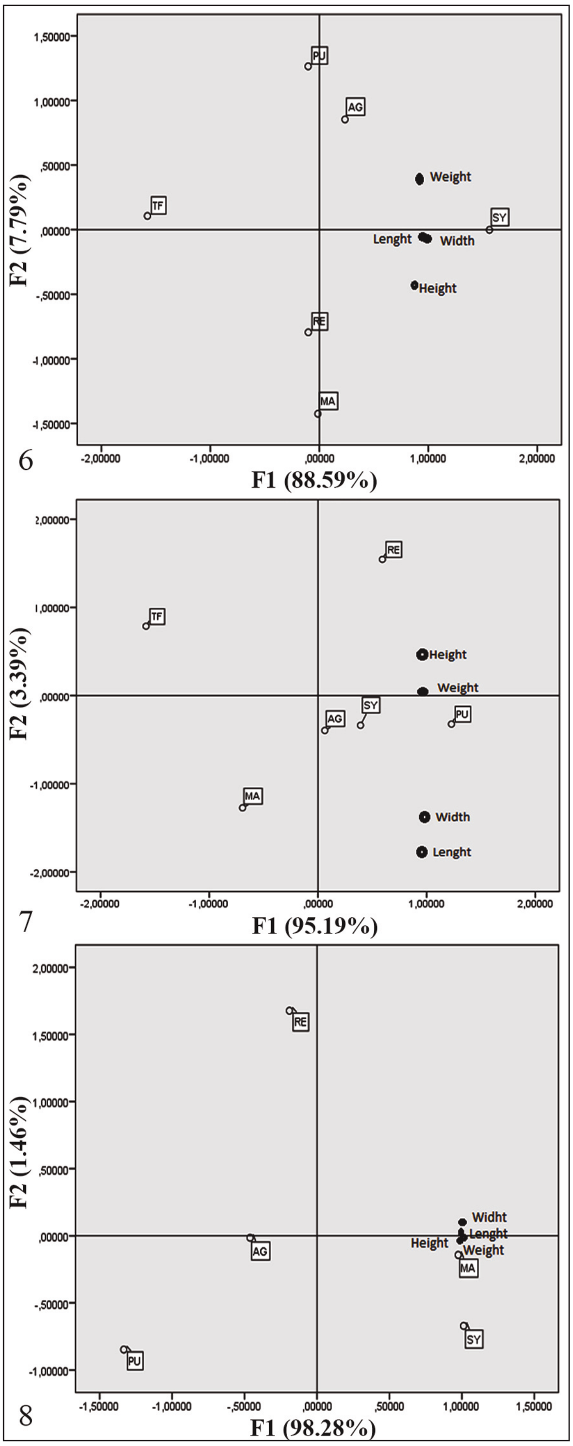
weight values compared to the other stations. While, for individuals from the stations of Tas-Fout and Rechgoun presented a high value of height, while the rest of the stations individuals had greater lengths and widths.

Relative to the point clouds, individuals from the two stations of Madrid and Sidna-Youchaa had the highest means of height and weight compared to the other stations. A positive correlation with the F1 axis was noted.

DISCUSSION

Our result suggested that *P. caerulea* was more abundant in all studied stations, followed by *P. rustica* and *P. ferruginea*. In fact, several studies presented the same results like Beldi et al., 2012, Kallouche, 2018 in the Coast of Oran, Battelli, 2017; Bouzaza & Mazali, 2018.

While *P. ferruginea*, is a patellid limpet species endemic in the Mediterranean Sea. It is considered to be at serious risk of extinction (Espinosa et al., 2009). In our case, 45 individuals (5.44%) of *P. ferruginea* were recorded in studied stations. In fact, Western Algerian Islands is a high ecological value given the number of endemic endangered marine species living there (Bachet et al., 2007). Instead, *P. ferruginea* have been recorded on Rachgoun (Frenkiel, 1975), Habibas Island (Boumaza & Semroud, 2001; Espinosa, 2009), Plane Island (Es-



Figures 6–8. Principal component analysis (PCA) established on individuals of limpets: (Fig. 6) *P. caerulea;* (Fig. 7) *P. rustica*; (Fig. 8) *P. ferruginea* using the mean values of the parameters (W: Weight, L; Length, Wi; Width, and H: Height). Projection of the parameters (W, L, Wi, and H) on the 2 most informative axes (F1 and F2) of the PCA. (See Table 1 for the abbreviation of stations).

pinosa, 2009), Oran and Ain Temouchent coasts (Kallouche, 2018), the Coast of Mostaganem (Salamandre, Stidia, Kharrouba) (Bouzaza, 2018) and Ghazaouet Coast (Benguedda et al., 2012). But in the Eastern Coast of Algeria, this species is absent (Beldi et al., 2012). This prosobranch gastropod is endemic to the western Mediterranean (De Beaufort & Lacaze, 1988; Laborel-Deguen & Laborel, 1990), some populations remain on Corsica (Laborel-Deguen & Laborel, 1991a), Sardinia (Porcheddu & Milella, 1991; Doneddu & Manunza, 1992) and the southernmost coast of Spain (Espinosa, 2006).

Our results seem to indicate the existence of two varieties of *P. ferruginea* (*Lamarckii form, Rouxii form).* In fact, *Patella ferruginea* is known by its high morphometric plasticity (Laborel-Deguen & Laborel, 1991a). Its shell is present in two different forms (*rouxii* and *lamarckii*) (Tlig-Zouhari et al., 2011). The *rouxii* form has a high conical shell, blunt top, low-radiating ribs strongly tight and a contour which is more or less regular. As for *lamarckii* form, it is characterized by a flattened shell, top tip and strong ribs with a star contour (Laborel-Deguen & Laborel, 1990; Porcheddu & Milella, 1991; Espinosa & Ozawa, 2006).

Patella caerulea display wide shell morphological and colour variations in several localities (Cretella et al., 1991). Also, *P. rustica* showed a shell with brown spots near its apex that may make its identification easier: however, in some localities *P. caerulea* also showed brown spots on its shell, but with a lower apex than *P. rustica* (Petraccioli et al., 2010).

Cretella et al. (1990) reported that the principal differences among *P. caerulea* and *P. aspera* concern the colour of the sole; in *P. caerulea* it was dark grey with edge and center cream whereas in *P. aspera* it was yellow or cream.

The morphometric analysis of limpets in this study showed wide and significant morphological variations within species (shells morphology, color of interne surface of the shells, dimensions and foot color). This is due to the strong influence of environmental features, namely the degree of exposure to desiccation and the intensity of wave action (Cabral & Coelho, 2003).

In fact, from the sublittoral to low shore level, then to high shore, limpet shell becomes progressively taller (Davies, 1969), and from level to level the degree of exposure to desiccation increase (Powell, 1973), and the shell becomes higher. This is why limpets presented an increased height; it occurs between low spring up to high levels in shore like *P. rustica* and *P. ferruginea*. These species are more resistant to desiccation than those living in exposed situations in lower intertidal zone (Sella et al., 1993). The shell becomes flat, so that it could withstand wave stress, and the apex of the shell is located nearer to the anterior end (Cabral & Coelho, 2003). This is the case of *P. caerulea* and *P. ulyssiponensis*. These conclusions are similar with our results, as *P. rustica* shell presents an increased height, after *P. ferruginea* and *P. caerulea*.

Several studies confirm that when the limpet increase in length, the relative height of the shell increase, like the study of Davies (1969) for *P. ulyssiponensis* and *P. vulgata*, Muñoz & Acuña (1994) for british *P. caerulea*, Bouzaza & Mazeli, (2018) on the same species "*P. caerulea*" from Algeria, Cabral & Coelho (2003) on *P. intermedia*, *P. ulyssiponensis* and *P. vulgata* from Portugal.

The same results for *P. ferruginea* in the study of Tlig Zouhari et al. (2011) in Tunisia and in Italy for the same species (Coppa et al., 2012). The study of Ait Mohamed Amer et al. (2018) on *P. rustica* showed the same positive correlation, but their results revealed that the development of the shell is first in the height, followed by length.

For *P. caerulea*, the ratios L/H and Wi/H are the most discriminating as indicated by Bouzaza & Mezali, 2018. These authors showed that there is a real morphometric dissimilarity between these species: flattened and stocky or high and short. In addition, these ratios have been used by Espinosa & Ozawa (2006) to distinguish between the *rouxii* and *lamarkii* forms of *P. ferruginea*.

CONCLUSIONS

This study, based on the morphometric analysis of three species of *Patella*, was realized in order to evaluate the main morphological characteristics in the first, and to study the correlation of biometric parameters in each species in order to specify the environmental conditions could affect this relationship between all parameters.

Our data confirm the fact that *Patella* species show substantial morphology variability, which is probably due to environmental variation. *P. rustica* was easily recognised due to small brown spots near the shell apex but for *P. caerulea*, the shell morphology was too varied between individuals of this species in Mediterranean Sea. For *P. ferruginea*, two forms were noted: *rouxii* and *lamarckii*.

The biometric relationship showed a strong correlation between the parameters (length & width), (length & height), (width & height) for all studied species of *Patella*, and was significantly different between all stations. Finally, it would be interesting to enlarge the spectrum to several other sampling points for a wider and complementary study.

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