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RESEARCH PAPER

Morphometric Variation of Fishery-Exploited Muricidae (*Hexaplex trunculus)* in the Mediterranean Sea and the Northeastern Atlantic Ocean: Implications for Stock Identification

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Abstract

Morphological studies of species with wide distribution range and high commercial value, such as the banded murex *Hexaplex trunculus* (Linnaeus, 1758), provide information on stock structure, which is the basis for understanding fish population dynamics and enable resource assessment for fisheries management. In the present study, we examined morphological variation among Atlantic and Mediterranean populations of *H. trunculus* using multivariate analysis. Our results supported the existence of four distinguishable stocks (Atlantic, Alboran, Western Mediterranean and Eastern Mediterranean), correctly classified 71.7% of specimens, and indicated significant degrees of variation in morphometric characteristics between regions. Examination of the contribution of each morphometric variable to the principal components and canonical functions indicated that differences among samples seemed to be associated with the shell and aperture length and width. Samples from the Atlantic Ocean and the Alboran Sea had the largest shell size and the greatest morphometric divergence. This strong morphometric differentiation appears to be associated with local environmental factors (exposure on the rocky shores, food availability and predation) and oceanographic current barriers.

Key words: *Hexaplex trunculus*, morphometric variation, Atlantic, Mediterranean, stock identification.

Introduction

The banded murex, *H. trunculus*, is a common species of the family Muricidae and is distributed in the Mediterranean Sea, and adjacent coast of the Atlantic Ocean (from the Portuguese coast southward to Morocco and to the Madeira and Canary Archipelagos) (Poppe and Goto 1991; Houart 2001). This species occurs in intertidal and infralittoral zones between 1 m and 100 m depth (Chiavarini *et al.*, 2003). It inhabits both hard and soft substrates, from rocky shores to sandy–muddy. *H. trunculus* is regularly or occasionally fished for human consumption in several countries in the Mediterranean Sea (Poppe and Goto 1991; Houart 2001), namely in Italy, Cyprus, Turkey (Gaillard 1987), Croatia (Peharda and Morton 2006) and Tunisia (Gharsallah *et al.*, 2004). Its commercial value is relatively low in the southern Mediterranean costs (e.g., Tunisia 1–1.5€/kg; Lahbib *et al.*, 2010) compared to some European countries (e.g., Portugal 10–15€/kg; Vasconcelos *et al.*, 2004). During the last 5 years, the exploitation of this snail has become extensive as a result of its high commercial value (Vasconcelos *et al.*, 2004) and the drastic decrease of the harvest of the first fished clam, *Ruditapes decussatus*, which was affected in some sites by phycotoxins (Lahbib *et al.*, 2010).

Previous studies focusing on *H. trunculus* have been based on sexual abnormality known as imposex and reproductive biology (e.g., Axiak *et al.*, 1995; Gharsallah *et al.*, 2010; Terlizzi *et al.*, 1998; Lahbib *et al.*, 2008; Lahbib *et al.*, 2010). Nevertheless, the knowledge of fishing effort and stock structure of this species, which occurs in a large distribution range with different environmental conditions, is still very limited. The potential capacity of populations to adapt and evolve as independent biological entities in different environmental conditions is restricted by the exchange of individuals between populations. A sufficient degree of isolation may result in notable phenotypic and genetic differentiation among marine populations within a species, which may be recognizable as a basis for separation and management of distinct populations. Various methods have been proved to be powerful tools for studying stock structure, such as morphometric analysis, biochemical and molecular genetics techniques (Moritz *et al.*, 1987; Weber *et al.*, 1998; Tzeng and Yeh 1999). Morphometric characters, which are

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continuous characters describing aspects of body shape, have long been used to delineate stocks and continue to be used successfully (Villaluz and Maccrimmon 1988; Haddon and Willis 1995; Murta 2000; Silva 2003; Turan 2004; Turan *et al.*, 2006). Variation in such characters was assumed to be entirely under genetic control (McQuinn 1997), but is now recognized to have both environmental and genetic components (Foote *et al.*, 1989; Robinson and Wilson 1996; Cabral *et al.*, 2003). Studies of morphometric variation among populations continue to have an important role to play in stock identification and can be the first step in investigating the stock structure of species with large population sizes, despite the advent of biochemical and molecular genetics techniques which accumulate neutral genetic differences between groups (Swain and Foote 1999). In this context, the aim of the present study is to investigate the morphometric variability of *H. trunculus* populations using multivariate analysis to better understand the structure pattern of this species and to elucidate the existence of different stocks in the Northeastern Atlantic and Mediterranean Sea.

Material and Methods

Sampling

A total of 526 specimens were collected from 17 localities across most of the distribution range of this species (Figure 1, Table 1). All specimens were collected between 0.5 and 30 m depth from January 2009 to October 2011. Following capture, samples were transported to the laboratory and kept in deep freezer (-20°C) for 3 hours. After that, the flesh was removed and shells were dried at 60 °C for 48 h.

Morphometrics

To select characters for use in morphometric analyses, we reviewed the available morphometric studies of marine and freshwater gastropods from the literature (Janson 1982; Janson and Sundberg 1983; Armbruster 1995; Chiu *et al.*, 2002) and adopted shell characters that appeared practical in identification. The results of morphometric analyses might directly reflect the importance and effect of each character. A total of 8 continuous shell measurements were recorded with a digital caliper from each individual to the nearest 0.01 mm (Figure 2). Shell length (SL) is the distance between the apex (a) and the bottom (h) of the shell; Shell width (SW) is the maximum width perpendicular to the shell length distance (b-d), Shell height (SH) was measured along an axis passing through (i) and (j), Aperture length (AL) is the length from the beginning of the 1st suture (b) to the bottom of the aperture (g), Aperture width (AW) is the maximum aperture width perpendicular to the aperture length (e-f), Siphonal canal length (SCL) is the length from the bottom of the aperture (g) to the bottom of the shell (h), Spire length (SPL) was measured from the beginning of the first suture (b) to the apex of the shell (a), Body whorl length (BWL) is the distance between the beginning of the first suture (b) and the bottom of the shell (h).

Multivariate Analysis

Patterns of morphometric relationships can be influenced by the effect of allometric growth and size in species of undetermined age. The influence of body size was removed by standardizing all morphometric characters according to the equation of Elliott *et al.*, (1995): $M_S = M_0(L_S/L_0)^b$.

M_S is the standardized measurements, M_0 is the

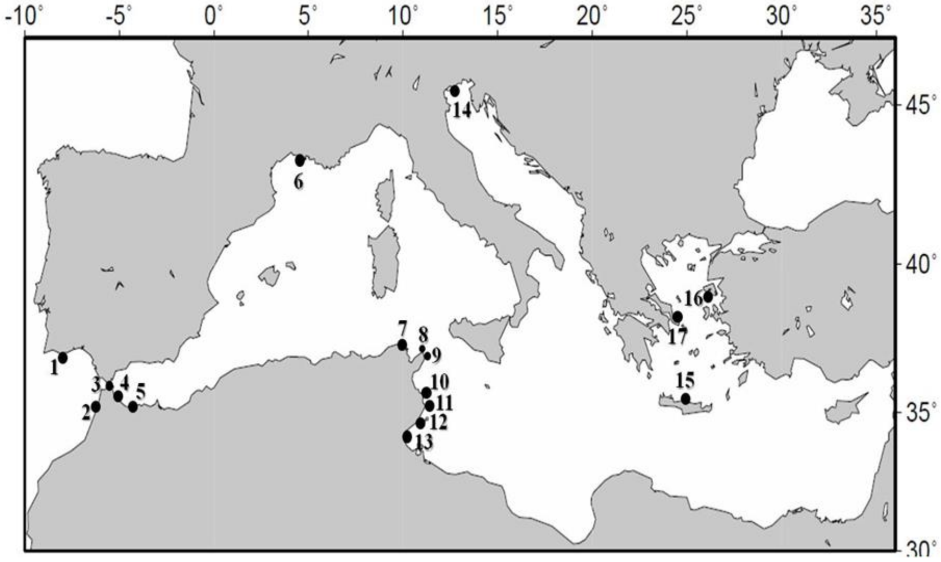

Figure 1. Map showing the collection localities of *H. trunculus.*

Table 1. Geographic origin of *H. trunculus* samples. *N*: Number of samples

Population Codes	Locality	Coordinates	N
1	Algarve, Portugal	37°03 N, 08°14 W	22
2	Assilah, Morocco	35°09 N, 06°02 W	18
	Atlantic		40
3	Tanger, Morocco	35°46 N, 05°47 W	25
4	M’diq, Morocco	35°41 N, 05°19 W	24
5	Al Hoceima, Morocco	35°17 N, 03°56 W	21
	Alboran		70
6	Marseille, France	43°12 N, 09°48 E	25
7	Manzel Bourguiba, Tunisia	37°09 N, 06°57 E	63
8	Haouaria, Tunisia	37°05 N, 11°02 E	50
9	Kelibia, Tunisia	36°50 N, 11°07 E	49
	Western Mediterranean Sea		187
10	Sayada, Tunisia	35°40 N, 10°54 E	43
11	Chebba, Tunisia	35°08 N, 11°06 E	37
12	Mahres, Tunisia	34°55 N, 10°27 E	20
13	Skhira, Tunisia	34°12 N, 10°03 E	35
14	Venice, Italy	45°21 N, 12°20 E	47
15	Crete, Greece	35°28 N, 24°48 E	12
16	Mytilene, Greece	38°57 N, 33°41 E	15
17	Oropos Attiki, Greece	38°31 N, 24°12 E	20
	Eastern Mediterranean Sea		229
Total			526

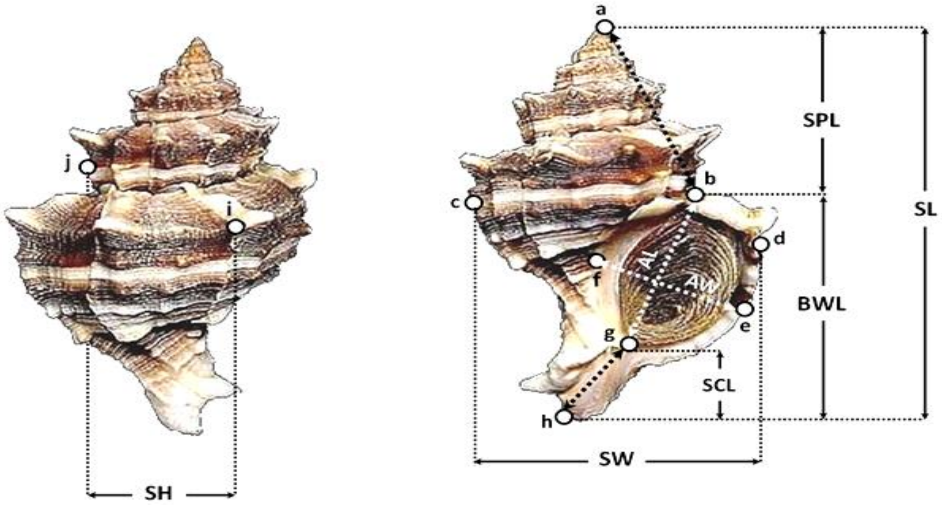

Figure 2. Diagram showing the morphometric variables measured in the specimens of *H. trunculus.*

length of measured character, L_S is the arithmetic mean of the standard length (Shell length in the present study) for all Murex from all samples in each analysis and L_0 is the standard length of each specimen. The value of the parameter (b) was estimated for each character from the observed data by allometric growth equation $M = aL^b$. Coefficient (b) was evaluated as the slope of regression of $\log_{10}M_0$ on $\log_{10}L_0$ using all Murex in each population. The efficiency of size adjustment transformations was assessed by testing the significance of correlations between transformed variables and standard length.

Normality of the data was determined using the *Shapiro-Wilk’s W-test* implemented in PAST version 3.01 (Hammer *et al.,* 2001). When non-normally distributed, data were log-transformed in order to meet the assumption of normality more closely. Three levels of statistical analyses were performed, first, analyses based on individual specimens (PCA and SIMPER), second, analyses based on populations (SIMPER, MSD, and HCA) and third, analyses based on groups of populations which were determined at the previous step (CDA). Principal component analysis (PCA) was applied to identify which variables explain the majority of the variance observed. The objective was to demonstrate the structure of our data, by explaining the observed variability with fewer variables or principal components. Similarity percentages (SIMPER) were calculated to determine which morphometric characters contributed most to the within-locality similarity and among-locality dissimilarity.

Additionally, a multidimensional scaling analysis (MDS), which gives a general overview of the data and used for illuminating relationships among populations, was performed using Euclidean distances. PCA, MDS and SIMPER were carried in PAST 3.01. To investigate the phenotypic relationships between populations a Hierarchical cluster analysis (HCA) based on Mahalanobis distance matrices was carried out. The HCA procedure categorized homogeneous groups based on distances, using an algorithm that started with each case in a separate cluster and combined clusters until only one was left. The hierarchical clustering process was represented as a dendrogram. The minimum variance clustering method or Ward's method was used with the Euclidean distances. A multivariate canonical discriminant analysis (CDA), which provides maximum discrimination among groups using linear combinations of variables, was performed to discriminate among the *H. trunculus* groups previously determined by the MDS and the HCA. CDA quantifies characters of the shell and assesses how successful the characters are at allocating individuals to their a priori groups. The following specifications were used for the analysis: Forward Stepwise, Tolerance = 0.010, F to enter = 1.0, F to remove = 0.00 and *prior* probabilities were estimated to be proportional to population sizes. CDA was carried using the software package STATISTICA, version 8.0 (StatSoft Inc. 2007).

Results

The PCA showed that all morphometric variables differed significantly between the localities. PC1 accounts for more than 90% of variance and is therefore clearly the dominant pattern of morphometric divergence between *H. trunculus* populations. It was positively correlated with all characters studied. SL, SW, AL and AW were positively correlated with the PC2 (which explained 3.9% of variance) and showed the highest positive loading for PC1 (Table 2). Differences among samples seemed to be associated with the shell length and width and aperture length and width. The SIMPER confirmed this result, the 4 characters previously cited contributed to 78.8% of dissimilarity between studied populations. The SL presented the highest dissimilarity percentage (34.4%) (Table 2).

An ordination plot of MDS based on standardized morphometric measurements revealed separation between 4 geographic regions (Figure 3) and divided the Murex stock in 4 distinct groups. The first group (I) included Atlantic populations (Algarve and Assilah) and the second (II) included Alboran populations (Tanger, Mdiq and Mestassa). Manzel Bourguiba, Kelibia, Haouaria (Western of Tunisia) and Marseille populations were clustered in the third group (III) representing the Western Mediterranean basin (WMS). The fourth group included the other populations (Sayada, Chebba, Mahres, Skhira (Eastern of Tunisia), Crete, Oropos Attiki, Mytilene and Venice) belonging to the Eastern Mediterranean basin (EMS). These results were confirmed by the hierarchical cluster analysis, which revealed that the sampled populations were clustered into the same four groups determined by MDS analysis (Figure 4). The morphometric differentiation between *H. trunculus* populations followed a geographic gradient West-East. The average values, ranges and standard errors for all measured variables are given in Table 4. We found significant differences in shell size of the banded murex among the four geographical regions. Murexes in the Northeastern Atlantic Ocean were overall the largest (mean shell lengths around 6 cm). The smallest specimens were caught close to the Western Mediterranean region (WMS).

The discriminant analysis carried out considering the groups of *H. trunculus* indicates a clear separation between the four studied groups (Wilk's λ=0.194, P<0.0001) (Table 5). All variables discriminate *H. trunculus* groups. Correlations between morphometric characters and canonical roots (CR1 and CR2) show that AL, SL, SPL and SH were positively correlated with CR1 (90% of the total

Table 2. Principal component loadings for *H. trunculus* populations

	PC 1	*PC 2*	*PC 3*	*PC 4*	*PC 5*	*PC 6*	*PC 7*
SL	0.602	0.569	0.191	-0.075	-0.461	-0.151	-0.190
SW	0.449	0.463	-0.514	-0.554	-0.060	-0.079	-0.054
SH	0.283	-0.254	-0.041	0.616	0.260	-0.583	0.000
AL	0.396	0.520	0.635	0.053	-0.035	0.417	-0.247
AW	0.362	0.068	-0.471	0.498	-0.131	0.642	0.169
SCL	0.230	-0.003	0.269	-0.156	0.054	-0.007	0.921
SPL	0.285	-0.350	0.016	-0.183	0.834	0.211	-0.153
Eigenvalues	3.616	0.154	0.081	0.047	0.036	0.025	0.021
Variance (%)	90.9	3.9	2.0	1.2	0.9	0.6	0.5
Cumulative variance (%)	90.9	94.7	96.8	98.0	98.8	99.5	100.0

among-groups variance) and that SL represents the highest correlation score. Also, for CR2 (which explained 7% of variance), SL is the variable which contributes most to the overall among-groups differences (Table 6). The stepwise discriminant analysis developed for the samples correctly classified 71.7% of cases to geographical regions with *H. trunculus* from the Alboran Sea and the Atlantic being morphologically the most distinct forms. Individuals from the Alboran Sea and Atlantic were respectively, correctly assigned in 98.6% and 80% of the cases by the classification functions (Table 7). The canonical root 1 (CR1) and canonical root 2 (CR2) were plotted to allow visual examination of the distribution of each sample along the CR axis that showed a clear differentiation between the four studied groups of banded murex (Figure 5). In the discriminant space, the WMS and EMS groups are partially separated, showing some overlap in their morphology. Remarkably, individuals from the Alboran Sea appear as an intermediate form between the Atlantic and the other groups (WMS and EMS).

Discussion

Both morphometric methods indicated significant phenotypic heterogeneity between Northeastern Atlantic and Mediterranean *H. trunculus* samples. The high a posteriori classification accuracy obtained by discriminant functions indicated a high degree of variation in morphology between populations. This explained the efficiency of discriminator power of morphometric characters used.

Hierarchical cluster analysis and MDS suggested

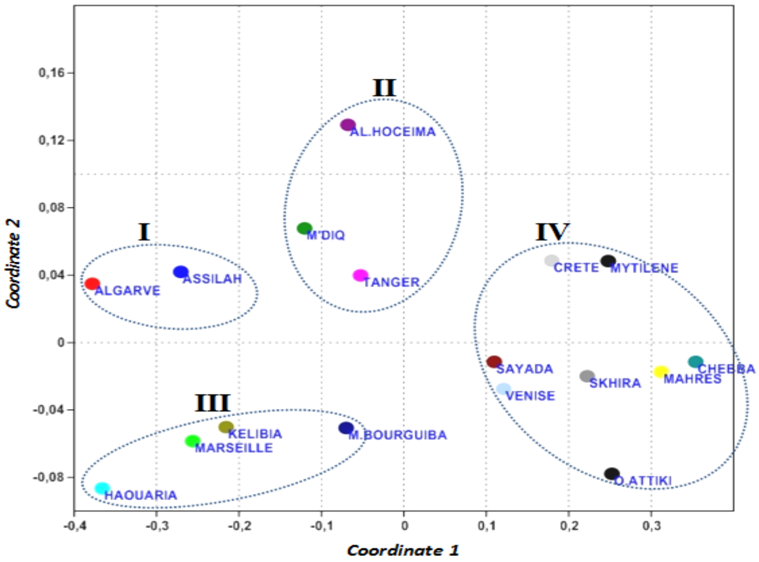

Figure 3. Scatterplot of multidimensional scaling for *H. trunculus* populations.

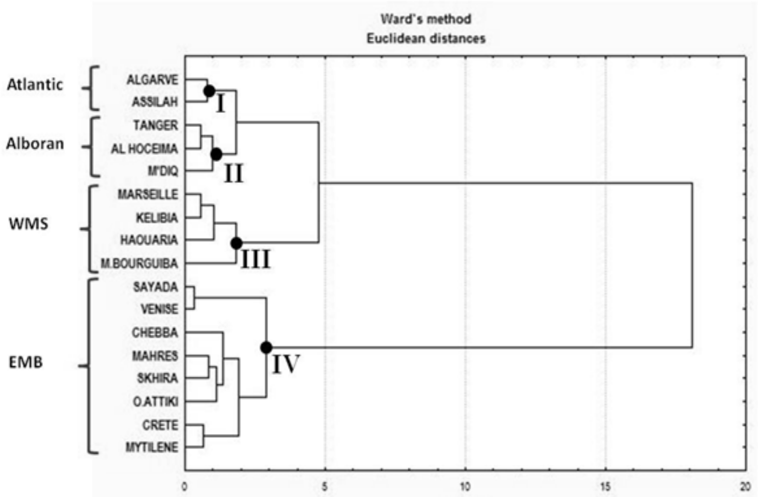

Figure 4. Dendrogram illustrating the patterns of morphometric similarity among *H. trunculus* populations (linkage rule: Ward's method; Euclidean distance measure).

Table 3. Results of the SIMPER for morphometric characters studied

Parameters	Average dissimilarity	% of contribution	cumulative %
SL	2.891	34.4	34.4
AL	1.682	20.0	54.4
AW	1.204	14.3	68.8
SW	0.842	10.0	78.8
SH	0.716	8.5	87.3
SPL	0.612	7.3	94.6
SCL	0.453	5.4	100.0

Table 4. Descriptive statistics for *H. trunculus* groups (standardized measurements in cm)

Region	Statistics	SL	SW	SH	AL	AW	SCL	SpL
Atlantic	Mean	6.197	4.142	3.504	2.836	2.810	1.866	2.640
	SD of mean	0.033	0.043	0.038	0.024	0.026	0.019	0.035
	Minimum	5.358	3.877	1.920	1.561	1.959	1.452	1.888
	Maximum	6.727	4.673	3.970	3.212	3.355	2.579	3.018
Alboran	Mean	5.925	3.891	3.148	2.706	2.531	1.841	2.715
	SD of mean	0.024	0.028	0.021	0.018	0.023	0.012	0.019
	Minimum	5.477	3.377	2.677	1.805	2.151	1.566	2.068
	Maximum	6.389	4.580	3.541	3.337	2.973	2.113	3.031
WMS	Mean	5.573	3.770	3.077	2.592	3.037	1.829	2.498
	SD of mean	0.021	0.027	0.017	0.012	0.010	0.011	0.011
	Minimum	2.788	1.239	2.608	2.240	1.811	1.599	2.070
	Maximum	6.240	4.429	3.845	3.567	3.485	2.290	3.306
EMS	Mean	5.603	3.909	3.154	2.229	2.308	1.803	2.463
	SD of mean	0.025	0.021	0.016	0.016	0.015	0.013	0.013
	Minimum	2.484	2.862	2.570	1.824	1.792	1.475	1.892
	Maximum	7.368	5.853	4.157	3.990	3.327	2.420	3.124

Table 5. Chi-square tests with successive canonical removed roots for *H. trunculus* groups. ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

Removed roots	Eigen value	Canonical R	Wilk's lambda	Chi²	df	p-level
0	2.836	0.860	0.194	853.0	21	***
1	0.218	0.423	0.745	153.2	12	***
2	0.102	0.304	0.907	50.6	5	**

that populations of *H. trunculus* were clustered into four phenotypically distinct groups, demonstrating that there might be considerable divergence between four geographical regions. Although, if a stock is considered as an intra-specific group of individuals exhibiting unique phenotypic attributes, then, based on this result, the banded murex should have four separate stocks: Atlantic stock, Alboran stock and Western and Eastern Mediterranean stocks. Our MDS and HCA results are also compatible with other clustering schemes such as the one separating two main groups (Eastern Mediterranean populations on the one hand versus all the other populations) for instance, or the one with three groups (Atlantic and Alboran populations, western Mediterranean populations and eastern Mediterranean ones). There are also schemes with more than four clusters that appear compatible with both MDS and HCA, but such patterns of clustering appear less justified from a biogeographical and oceanological (e.g., Berline *et al.*, 2013; Rossi *et al.*, 2013) point of view (see below).

Table 6. Correlations of variables with canonical roots and cumulative proportion of the variance explained for *H. trunculus* groups

Variables	CR1	CR2
AW	-1.489	-0.833
AL	0.511	-0.781
SL	0.966	0.735
SW	-0.323	-0.093
SpL	0.384	-0.057
SCL	-0.159	0.219
SH	0.192	0.177
Eigen values	2.836	0.218
Variance (%)	90%	7%
Cumulative variance (%)	90%	97%

Table 7. Classification matrix for *H. trunculus* groups. Percent of correct assignment of the predicted (columns) versus the observed (rows) classifications

Populations	% Correct Assignment	Atlantic p=0.0739	Alboran p=0.1345	WMS p=0.3561	EMS p=0.4356
Atlantic	80.0	32	8	0	0
Alboran	98.6	0	69	0	1
WMS	68.4	0	0	128	59
EMS	64.8	0	24	57	149
% Total	71.7	32	101	185	209

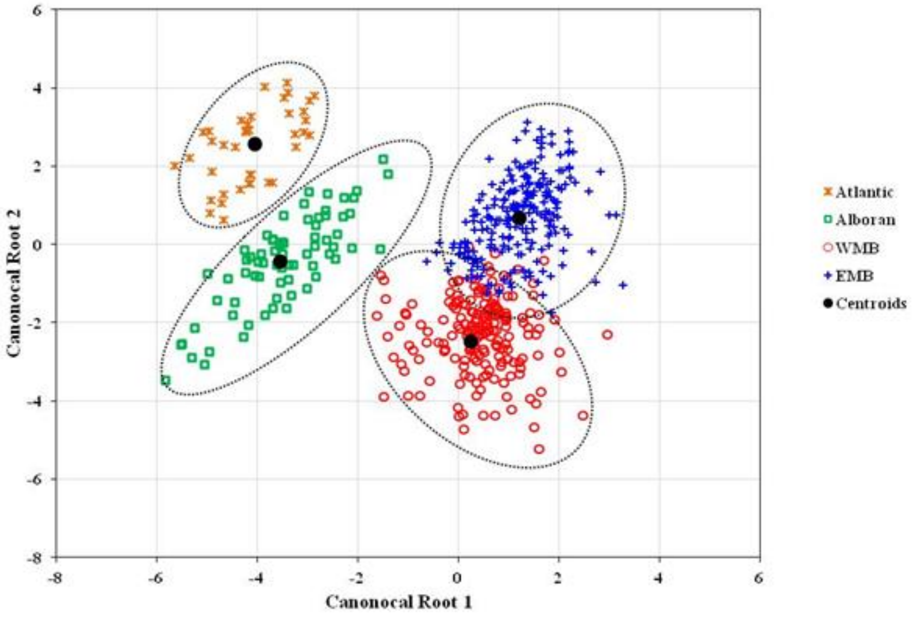

Figure 5. Individuals distributed in the Canonical Discriminant Space for *H. trunculus* groups. 95% confidence intervals are shown as ellipses.

In general, Variation in gastropod shell size amongst different geographical populations is attributed to different genetic structure of populations and/or different environmental conditions prevailing in each geographic region (Boulding and Hay 1993; Johannesson and Johannesson 1996; Carballo *et al.*, 2001). Adaptations to environmental and oceanographic conditions may play a crucial role in this morphometric differentiation. One preliminary hypothesis could be the oceanographic barriers which separate two marine biogeographical regions, the Mediterranean Sea and the Northeast Atlantic, and divided the Mediterranean Sea into different sub-basins. These barriers created environmental discreteness between different regions and accentuated the genetic and morphological divergence between marine species, especially species with direct intra-capsular development (i.e., lacking a planktonic larval stage) such as *H. truncului*. Literature data often rule out the Gibraltar strait as a zone of genetic and morphological discontinuity between Northeast Atlantic and Mediterranean Sea (e.g., Sanjuan *et al.,* 1994; Quesada *et al.,* 1995; Naciri *et al.,* 1999; Silva 2003; Duran *et al.,* 2004; Reuschel and Schubart 2006). Atlantic samples from Portugal (Algarve) and Morocco (Assilah) were similar to each other for morphometric characters. However, a discontinuity between banded murex populations across the Strait of Gibraltar supports the feature of the limit of the Atlantic stock which is characterized with large and robust shells. Previous morphometric study showed variances in body shape between Atlantic and Mediterranean populations of the sardine (*Sardina pilchardus*). Mediterranean sardine had a smaller shape than those from southern Iberia and northern Morocco. This variability was correlated to the current boundary splitting the two geographical regions (Silva 2003).

The differentiation pattern observed in the samples from the Alboran Sea is interesting (height percentage of correct classification ≈ 100%) considering its location related to the Mediterranean/Atlantic currents. The Alboran Sea is a mixture of Atlantic waters flowing into the Mediterranean basin from the North Atlantic and Mediterranean waters flowing westward (Peres 1967, Estrada *et al.,* 1985, Conde 1990). The present results indicate the Alboran sample as phenotypically intermediate between the Atlantic and the Mediterranean populations. This is exactly what has been postulated by Reuschel and Schubart (2006) concerning the morphological separation of *Xantho hydrophilus*. The authors suggested that population of this species from Ibiza (Spain) represents a transitional phenotypic form between the Atlantic and the Mediterranean Sea.

The Alboran stock tends to be closer to the Atlantic stock, and more distant from the Western and Eastern Mediterranean ones. The areas of Alboran Sea and the Western Mediterranean basin were separated by an oceanographic current front. The Almeria-Oran front, a strong large-scale ocean front between Almeria (Spain) and Oran (Algeria) (Tintore *et al.,* 1988; La Violette 1989), could be an important barrier to phenotypic and genetic divergences of the banded murex between the two sides of this front. These two adjacent areas have different biotic and abiotic conditions: the Mediterranean region has characteristically warm-temperate floral and fauna1 assemblages (Rodriguez 1982; Ben-Tuvia 1985; Ketchum 1985; Peres 1985; Ros *et al.,* 1985; Sara 1985). A similar mechanism has been suggested to account morphological and genetic differentiations of *Mytilus galloprovincialis* between Mediterranean and Alboran populations (Sanjuan *et al.,* 1994).

On the other hand, morphometric divergence of *H. trunculus* populations was, also, observed between the two basins of the Mediterranean Sea. Specimens from the Eastern basin were longer and broader comparatively to those of the Western basin. The Siculo-Tunisian Strait has been proposed to be the divide between the two Mediterranean basins and created different hydrographic regimes. The water circulation of the Western basin has being more uniform than the Eastern one (Pinardi *et al.,* 1997). Until now, studies which have shown such a Western and Eastern Mediterranean subdivision for marine species have often been conclusive (e.g. bivalves, Quesada *et al.,* 1995; Nikula and Vainola 2003; Sromek *et al.,* submitted, prawns, Zitari-Chatti *et al., 2009*; brittlestars, Boissin *et al.,* 2011; several fish species, Borsa *et al.,* 1997; seabass, Bahri-Sfar *et al.,* 2000 and seagrass, Arnaud-Haond *et al.*, 2007).

The water circulation is not very active in the Adriatic and Aegean Seas, which are under the influence of cool and low salinity waters, showing cyclonic circulation causing isolation of their northern parts (Pinardi *et al.,* 1997). However, no significant morphological variation was observed between samples of the Eastern of Tunisia, Adriatic and Aegean Seas. These populations are closely grouped in the HCA tree. This result should be interpreted with caution, since marine populations tend to fluctuate naturally. Additional samples from the Eastern Mediterranean basin are already needed to confirm our results. It is also possible that differentiation (or similarities) in morphology reflect the influence of environmental factors, via phenotypic plasticity. Thus, morphological variation may not conform to hydrological barriers when populations are compared within one of the four major groups identified in the global distribution area.

The degrees of exposure on the rocky shores were one of environmental factors that may influence morphological differentiation; the rocky shore shows strong geographical and vertical environmental gradients (Raffaelli and Hawkins 1996) and this may influence shell morphology. Morphological differentiation due to the exposure on the rocky shores have been reported for different gastropods species, *Nucella lapillus* (Day 1990; Dixon *et al.,* 1994; Kirby 2000; Rolán *et al.*, 2003), Littorina saxatilis (Johannesson *et al*., 1993; Reid 1993; Rolán-Alvarez *et al.,* 1996) and *Ocenebra erinacea* (Rolán 1983). In the present study, the samples from Marseille and Western Tunisia were collected on upper and mid rocky shores. These populations are characterized by thinner shells and larger apertures than the other samples. Functionally, changes in aperture shape also have many repercussions in the life of gastropods because aperture morphology plays an essential role in determining the type of prey consumed, and morphological variations can lead to changes in predation ability. Thus, the observed changes in aperture length and width may be related to changes in the diet of banded murex. In general, their diet consists of bivalves, especially the black mussel *Mytilus galloprovincialis* (Perhada and Morton 2004). *H. trunculus* may respond in a plastic manner to the presence and/or the geographic variation of the black mussel *M. galloprovincialis* by modifying the shape of their apertures. According to Rodrigez *et al.*, (2011), changes in mouth shape of the bluemouth *Helicolenus dactylopterus* (Scorpaenidae) seemed to be related to the changing in ontogenetic diet of the species.

Furthermore, occurrence of predators in the area has been shown to influence shell shape variation in marine gastropods (Johannesson 1986; Wilding *et al.*, 2001; Grahame *et al.*, 2006). This hypothesis can be adopted to explain why the Atlantic and the Alboran samples have more globose shells. This may be due to the need for more robust shells in order to resist predator attacks, especially crabs.

Conclusion

In conclusion, based on the observed high phenotypic discreteness in relation to geography, the Northeastern Atlantic, the Alboran Sea, the Western Mediterranean and the Eastern Mediterranean basins may be considered four distinct stocks. Although the environmental and hydrographical factors may be governing to some degree the potential phenotypic discreteness of *H. trunculus* aggregations, the detected pattern of differences at least show that there is restriction to intermingling between populations. Application of geometric morphometry and molecular genetic markers would be effective methods to confirm this phenotypic divergence, to examine the genetic component of phenotypic discreteness between geographic regions and to facilitate the development of management recommendations.

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