Journal of the Marine Biological Association of the United Kingdom, 2012, 92(3), 553–563.
doi:10.1017/S0025315411001925

Shell morphology and relative growth variability of the invasive pearl oyster *Pinctada radiata* in coastal Tunisia

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The variability of shell morphology and relative growth of the invasive pearl oyster Pinctada radiata *was studied within and among ten populations from coastal Tunisia using discriminant tests. Therefore, 12 morphological characters were examined and 34 metric and weight ratios were defined. In addition to the classic morphological characters, populations were compared by the thickness of the nacreous layer. Results of Duncan's multiple comparison test showed that the most discriminative ratios were the width of nacreous layer of right valve to the inflation of shell, the hinge line length to the maximum width of shell and the nacre thickness to the maximum width of shell. The analysis of variance revealed an important inter-population morphological variability. Both multidimensional scaling analysis and the squared Mahalanobis distances (D^2) of metric ratios divided Tunisian* P. radiata *populations into four biogeographical groupings: the north coast (La Marsa); harbours (Hammamet, Monastir and Zarzis); the Gulf of Gabès (Sfax, Kerkennah Island, Maharès, Skhira and Djerba) and the intertidal area (Ajim). However, the Kerkennah Island population was discriminated by the squared Mahalanobis distances (D^2) of weight ratios in an isolated group suggesting particular trophic conditions in this area. The allometric study revealed high linear correlation between shell morphological characters and differences in allometric growth among* P. radiata *populations. Unlike the morphological discrimination, allometric differentiation shows no clear geographical distinction. This study revealed that the pearl oyster* P. radiata *exhibited considerable phenotypic plasticity related to differences of environmental and/or ecological conditions along Tunisian coasts and highlighted the discriminative character of the nacreous layer thickness parameter.*

Keywords: *Pinctada radiata*, shell morphometrics, relative growth, lessepsian migration, Tunisia

Submitted 30 April 2010; accepted 10 October 2011; first published online 9 December 2011

INTRODUCTION

Having an Indo-Pacific origin, the small pearl oyster *Pinctada radiata* (Leach, 1814) is described as the first exotic bivalve being introduced in the Mediterranean Sea by lessepsian migration (Monterosato, 1878) as *Meleagrina savignyi*. The lessepsian or erythrean migration phenomenon, is the most illustrative example of man-mediated, either intentionally or accidentally, environmental changes (Por, 1978, 1990). In fact, it has led to the introduction in the Mediterranean of hundreds of exotic species through the Suez Channel and has consequently caused significant ecological and economic impacts in this area (Galil, 2000; Zenetos *et al.*, 2004).

Advanced modes of introduction of the pearl oyster *P. radiata* in the Mediterranean Sea have been described in the literature: mariculture (intentional introduction); shipping which is considered to be the most likely introduction vector of non-indigenous species (Zibrowius, 1992); and transport by fouling on other migrants such as the turtle *Caretta caretta* (accidental introduction) (Oliverio *et al.*, 1992). Herdman & Hornell (1903) and Meng *et al.* (1996) speculated that *P. radiata* might be introduced in the Mediterranean through its larvae which have three weeks of planktonic life thus ensuring long-distance dispersal.

The pearl oyster *P. radiata* is the most important species used for producing cultured pearls (Gervis & Sims, 1992; Al-Matar *et al.*, 1993; Martinez-Fernandez *et al.*, 2003) and it is considered as a biomonitor for heavy metal levels in the marine environment (Al-Madfa *et al.*, 1998; De Mora *et al.*, 2004).

The taxonomic status of the pearl oyster *P. radiata* is still a subject of confusion. It has been described firstly as conspecific with *P. fucata* (Hynd, 1955) and later as its synonymy (Ranson, 1961). In Australia, it is named *P. imbricata* (Shirai, 1994; Colgan & Ponder, 2002), whereas in China, *P. radiata* is named *P. fucata*, *P. martensii* and *P. fucata martensii* (Yu & Chu, 2006). At present, the taxonomic name *Pinctada radiata*, given by Leach (1814), is the only name used for this small pearl oyster in the East Indies, Arabian Gulf and Mediterranean Sea, including Tunisian waters.

Called also rayed pearl oyster (Fisher *et al.*, 1987), *P. radiata* was first reported in Tunisia in 1891 in Djerba (Gulf of Gabès, southern coast) by Bouchon-Brandely & Berthoule (1891). *Pinctada radiata* specimens were recorded later in

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Sfax and Djerba (Vassel, 1899). By 1929, the occurrence of *P. radiata* was noted in the channel of Sidi Bou Harrouch in El Biban Lagoon (Seurat, 1929) and in the north-west of Bougrara lagoon (Zaouali, 1978), where it had disappeared after nearly sixty years (Zaouali & Beaten, 1985). In the early 1970s, the pearl oyster *P. radiata* began to extend its distribution to the northern and eastern coasts of Tunisia. Indeed, Ktari-Chakroun & Azouz (1971) reported that they had collected living individuals of *P. radiata* from Kelibia and Cani Islands located both in Cap Bon (north-east of Tunisia). A recent study revealed the dense presence of *P. radiata* on the northern and eastern coasts. At present, *P. radiata* colonizes most Tunisian coastal habitats from the south to the north but Bizerta lagoon represents the meridional limit of its distribution (Tlig-Zouari *et al.*, 2009).

Furthermore, morphometric characters of bivalve shells are prominent in intrapopulation and interpopulation differentiation, and reflect evidence of direct environmental influence on shell morphology that shows a high degree of phenotypic plasticity, particularly in oysters (Stenzel, 1971; Harry, 1985; Lawrence, 1995; Batista *et al.*, 2008), frequently being involved in the life history tactics (Madec & Daguzan, 1993). Besides conventional morphometric approaches, geometric morphometric methods, based on shell shape profile, were used. It has been shown that the elliptic Fourier analysis has a great power in discrimination between geographical groups (Ferson *et al.*, 1985; Palmer *et al.*, 2004; Allen, 2006) and between species with a high level of resemblance (Innes & Bates, 1999; Rufino *et al.*, 2006; Costa *et al.*, 2010).

As regards the pearl oyster *P. radiata*, morphometric characters have been used in the discrimination of populations from around Bahrain (Beaumont & Khamdan, 1991; Al-Sayed *et al.*, 1993) and in the differentiation of morphologically similar pearl oysters *Pinctada* species in eastern Australia (Colgan & Ponder, 2002). In Tunisia, previous studies focused on shell morphometry of *P. radiata* populations from the Kerkennah Islands (Tlig-Zouari & Zaouali, 1994) and northern and eastern coastal Tunisia (Tlig-Zouari *et al.*, 2009); there were, however, too few morphometric characters.

In the present study, we focused on the shell morphological and relative growth variability of *P. radiata* populations at ten sampling sites along coastal Tunisia using 12 morphometric characters. Obtained data provide further insights to the plasticity of *P. radiata* species, determine whether nacre thickness is a powerful morphometric character in *P. radiata* populations' differentiation and help in explaining the origin and the introduction mode of this species in Tunisian waters.

MATERIALS AND METHODS

Sampling

A total number of 693 individuals of the pearl oyster *Pinctada radiata* were collected randomly in ten sites along the Tunisian coastline (Figure 1; Table 1). With the exception of the intertidal specimens from Ajim, all other individuals were collected by SCUBA divers at 2–15 m depth. The total number of specimens sampled in each site is indicated in Table 1. Though Ajim and Djerba belong to the same location, the former is in-shore and the latter is off-shore.

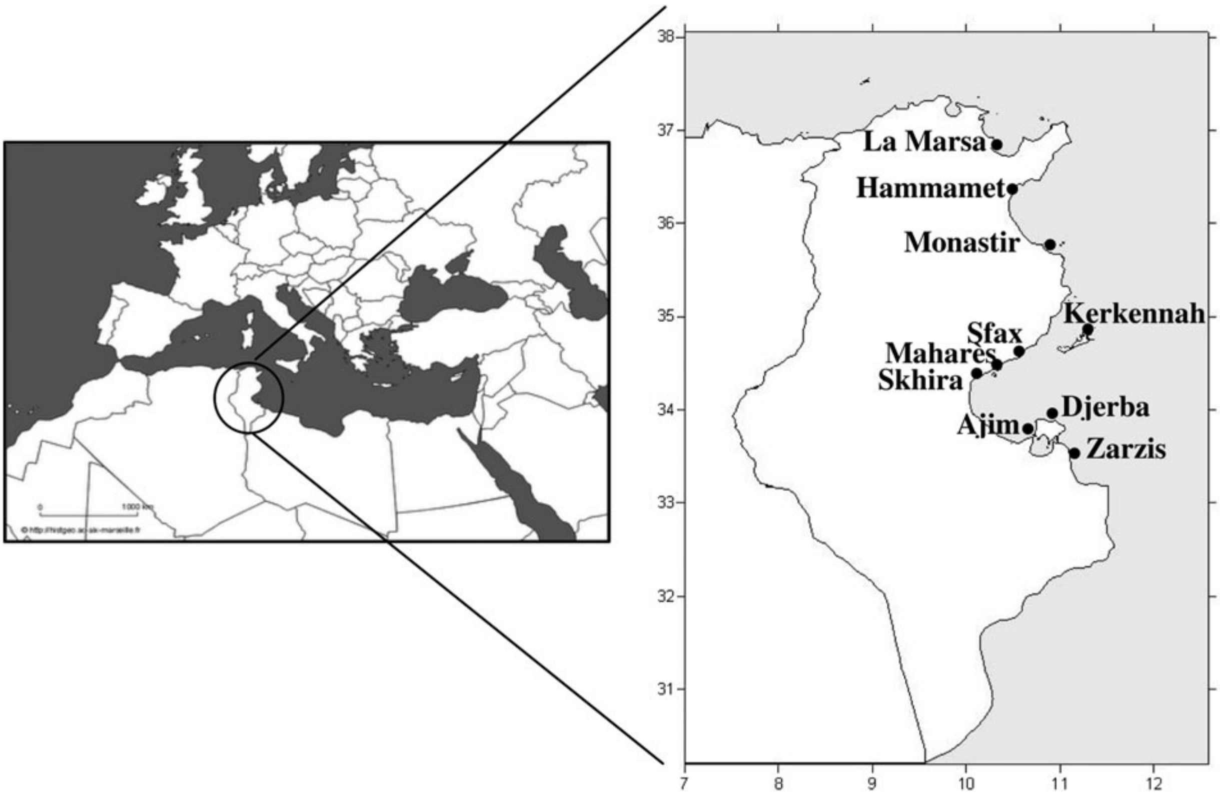

Fig. 1. Distribution map of sampling sites for the pearl oyster *Pinctada radiata* on coastal Tunisia: La Marsa, Hammamet, Monastir, Sfax, Kerkennah Island, Mahares, Skhira, Ajim, Djerba and Zarzis.

Table 1. *Pinctada radiata* populations from coastal Tunisia: sampling sites, total number of individuals, mean shell height and thickness of nacreous layer.

Sampling sites	Population denomination	N	Shell height		Shell nacreous layer thickness	
			Mean (min–max)	±SE	Mean (min–max)	±SE
La Marsa, Gulf of Tunis	MAR	56	49.90 (21.08–72.16)	8.16	0.78 (0.32–1.12)	0.19
Hammamet harbour, Gulf of Hammamet	HAM	53	72.71 (13.37–104.37)	22.41	1.12 (0.51–1.50)	0.31
Stah Jaber harbour, Monastir	MON	80	56.43 (6.48–86.54)	20.90	0.98 (0.36–1.38)	0.18
Sfax	SFA	64	58.09 (18.06–84.10)	10.62	0.85 (0.49–1.14)	0.15
Kerkennah Island	KER	120	61.96 (12.45–82.90)	15.38	0.81 (0.41–1.34)	0.22
Maharès	MAH	52	69.87 (19.92–85.28)	12.01	1.34 (0.74–2.07)	0.24
Skhira	SKH	77	65.30 (12.52–81.12)	13.62	0.94 (0.33–1.91)	0.29
Ajim	AJI	80	59.62 (47.22–74.38)	6.12	0.69 (0.63–1.52)	0.23
Djerba	DJE	31	65.38 (20.14–85.32)	15.98	1.26 (0.68–1.93)	0.28
Zarzis harbour, Zarzis	ZAR	80	69.47 (14.16–89.36)	13.22	0.91 (0.53–1.27)	0.16

min, minimum; max, maximum.

Morphometric measurements

The following morphometric characters were recorded for each individual: maximum height (H) and width (W) of shell, inflation (R), hinge length (HL) and total weight of shell and soft body (TW). After opening specimens, tissues were removed and each valve was assigned a number and subjected to further morphological measurements: maximum height and width of nacreous layer of right (HNR; WNR) and left (HNL; WNL) valve, shell weight (SW) and weights of right (RW) and left (LW) valve. Length measurements were conducted using a digital caliper (precision of 0.01 mm) and weight measurements were made using an electrical digital balance (precision of 0.001 g).

Nacreous layer thickness measurements

On each shell valve of *Pinctada radiata* specimens, a section axis was traced through the nacre layer surface considering its growth lines according to Hynd (1955) and Anwar *et al.* (1990). The section axis was perpendicular to the hinge line and it started at the third part of the hinge line length (Figure 2A). Valves were then delicately sectioned using a diamond disc (HORICO, DIAFLEX) and the thickness of nacreous layer was measured at four points (A, B, C and D) under a stereozoom microscope (63×) equipped with a micrometer (Leica MZ8) (Figure 2B). The average thickness was calculated first for the right and left valves separately and then for both of them. Shell nacreous layer thickness (TN) was then obtained.

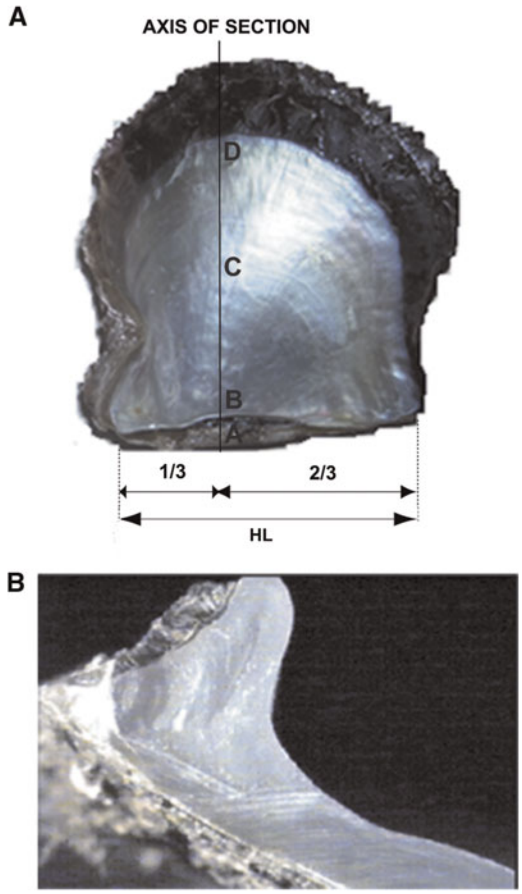

Fig. 2. (A) Shell valve of the pearl oyster *Pinctada radiata* showing the section axis through the nacre surface and the four points (A,B, C and D) at which nacre thickness was measured; (B) a stereomicrograph of sectioned valve showing thickness of nacreous layer of *P. radiata* (63 ×).

Relative growth

Allometric relationships between morphometric shell characters (shell height, shell width, shell inflation, total weight, shell weight and nacreous layer thickness) were established, through regression analysis, for all populations combined and separately. The linear least squares method for log-transformed data of each parameter was applied (Carino & Monteforte, 1995):

$$\log_e Y = \log_e a + b \times \log_e X (Y = aX^b)$$

where Y and X are dependent and independent morphometric parameters, respectively, «a» is constant and «b» is an exponent. The Student's *t*-test was carried out to determine the type of growth allometry.

Data analyses

All recorded morphometric characters were used to calculate 28 metric ratios (W/H, R/H, HL/H, HNR/H, HNL/H,

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WNR/H, WNL/H, TN/H, R/W, HL/W, HNR/W, HNL/W, WNR/W, WNL/W, TN/W, WNL/HNL, WNL/WNR, HL/R, HNR/R, HNL/R, WNR/R, WNL/R, TN/R, TN/HL, HNL/HNR, WNR/HNR, HNL/WNR and WNL/HNR) and 6 weight ratios (SW/TW, RW/TW, LW/TW, RW/SW, LW/SW and LW/RW). The squared Mahalanobis distances (D^2) between centroids based on the Bray–Curtis dissimilarity, analysis of variance (ANOVA), multidimentional scaling (MDS) and Duncan's multiple comparison test were applied to compare these ratios using the Statistical Analysis System (SAS) and Statistical Package for the Social Sciences (SPSS 6.1).

RESULTS

Description of *Pinctada radiata* populations

Shell height frequency distribution of collected *Pinctada radiata* specimens, shown in Figure 3, revealed wide variability. The total average height of *P. radiata* individuals was 63.46 mm. The mean shell height varied considerably among *P. radiata* populations (Table 1). It ranged from 49.90 ± 8.16 mm in the population from La Marsa to 72.71 ± 22.41 mm in that from Hammamet. The largest *P. radiata* specimen recorded during this investigation was collected from Hammamet and measured 104.34 mm in shell height while the smallest one was spotted at Monastir harbour and measured 6.48 mm. We noticed that specimens larger than 70 mm were predominant in populations from Hammamet (77.36%), Maharès (59.62%), Skhira (44.15%) and Zarzis (47.5%). On the other hand, *P. radiata* juveniles having a shell length smaller than 15 mm were non-existent in La Marsa, Sfax, Maharès and Ajim populations and their frequency did not exceed 5.66% in the other prospected sites. We noted an important heterogeneity within all populations of *P. radiata* notably those from Monastir and Hammamet having standard deviations of 20.9 and 22.33, respectively.

Nacreous layer thickness analysis

Often, nacre thickness is considered as the main quantifiable characteristic for objective evaluation of pearl quality (Matlins, 1996). In this study, we used nacreous layer thickness, in addition to the conventional shell morphological characters, to reveal differences on shell growth and morphology among *P. radiata* populations. Collected *P. radiata* oysters ranged in nacreous layer thickness from 0.32 (La Marsa) to 2.07 mm (Maharès), with a mean value of 0.93 mm. Within populations, the average thickness of nacreous layer varied between 0.69 mm for the intertidal population of Ajim and 1.34 mm for that of Maharès. The populations of Hammamet, Maharès and Djerba showed higher mean nacreous layer thickness exceeding 1 mm. The greatest thickness recorded for *P. radiata* from coastal Tunisia (2.07 mm) was ten times smaller than that of the giant pearl oyster *P. maxima* (2 cm) (Milet *et al.*, 2004).

Relative shell growth analyses

In order to investigate the variability of shell growth in the pearl oyster *Pinctada radiata* from coastal Tunisia, six allometric relationships between morphometric shell characters were determined for all populations combined and separately. In this paper, only those of all combined populations are illustrated (Figure 4). Their linear equations and correlation coefficients are shown in Figure 4. Results showed that for the same allometric relationship, all three types of allometric growth (isometry and both positive and negative allometry) can be observed within populations. Therefore, for better interpretation of the data, populations will be classified according to the type of their allometric growth into groups arranged as follows: group (1) showing negative allometry; group (2) exhibiting isometric growth; and group (3) displaying positive allometry.

HEIGHT–WIDTH RELATIONSHIP

Assuming an allometric growth, a regression analysis showed a significant linear correlation between shell height and shell width of the pearl oyster *Pinctada radiata*. The correlation coefficient was 0.948 ($P < 0.05$) for the combined population (Figure 4A) and it ranged among populations from 0.90 (Ajim) to 0.98 (Hammamet). The b values fluctuated between 0.88 (Ajim) and 1.03 (Zarzis). For shell height and shell width relationship, group (1) was composed of populations from Ajim and Maharès and group (2) was made up of the other populations. The combined population of the pearl oyster *P. radiata* showed also an isometric growth.

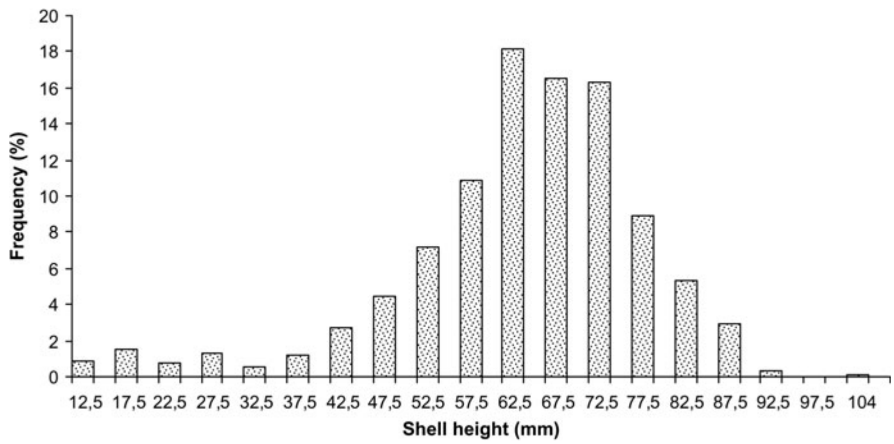

Fig. 3. Frequency distribution of shell height (H) of the pearl oyster *Pinctada radiata* from coastal Tunisia (N = 693).

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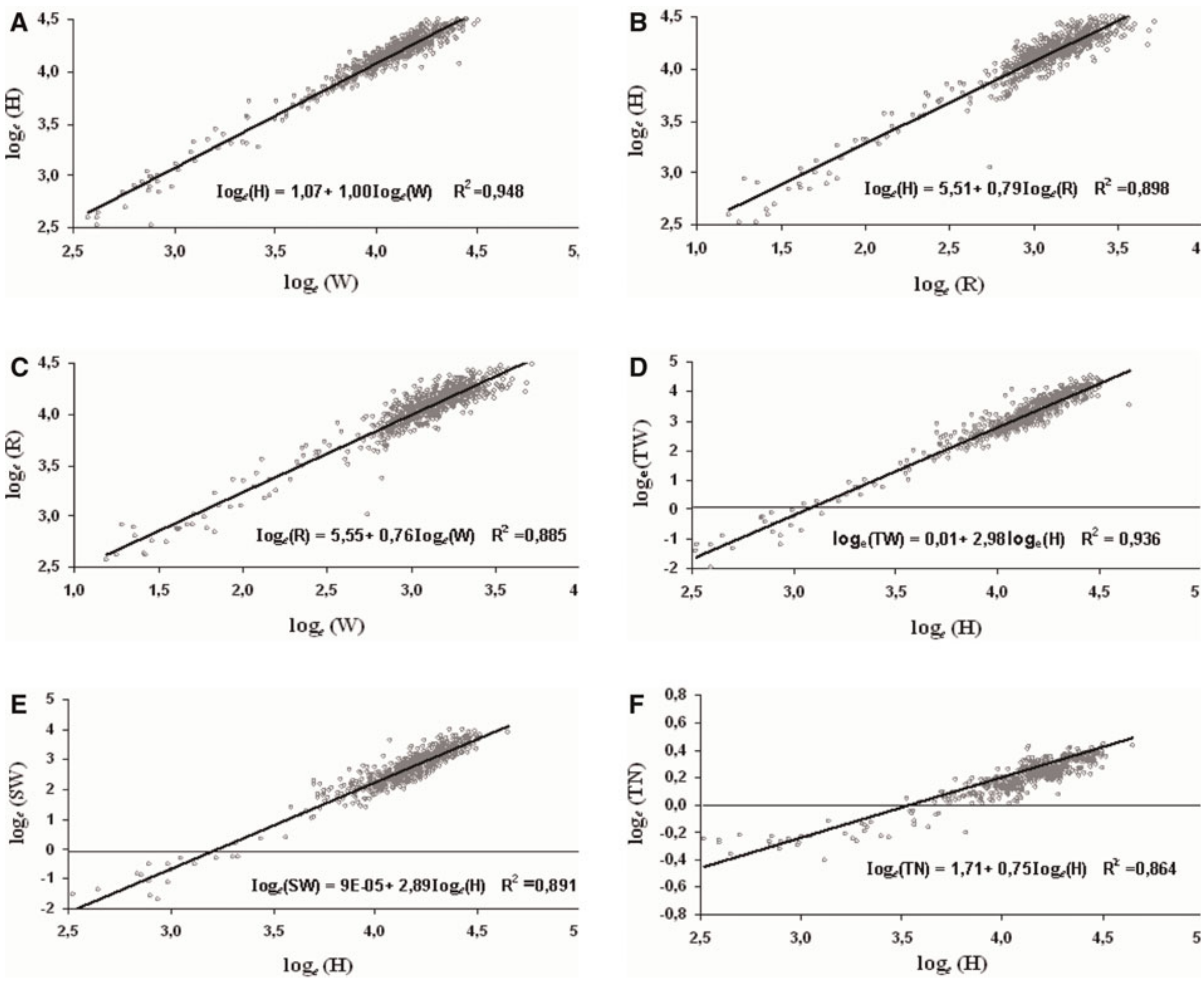

Fig. 4. Allometric relationships between shell morphometric characters of the pearl oyster *Pinctada radiata* from coastal Tunisia. (A) Shell height to shell width (H-W); (B) shell height to inflation (H-R); (C) inflation to shell width (R-W); (D) total weight to shell height (TW-H); (E) shell weight to shell height (SW-H); (F) nacreous layer thickness to shell height (TN-H).

HEIGHT–INFLATION RELATIONSHIP

A high correlation between shell height and shell inflation was detected in all populations combined ($R = 0.891$ at $P < 0.05$) (Figure 4B) and separately. Indeed, the correlation coefficient ranged between 0.77 (La Marsa) to 0.98 (Djerba and Hammamet). Populations from Maharès and Ajim displayed the highest (1.27) and the lowest (0.71) b values, respectively. Results revealed that the pearl oyster *Pinctada radiata* grew more slowly in shell height than in inflation. For this relationship, group (1) consisted of populations from Hammamet, Kerkennah Island, Skhira and Djerba, group (2) was composed of La Marsa, Monastir, Sfax, Ajim and Zarzis populations and group (3) was made up of the Maharès population. For the combined population, shell height and shell inflation was negatively allometric.

INFLATION–WIDTH RELATIONSHIP

For the combined population of *Pinctada radiata*, the pattern observed for this relationship was similar to that of the height–inflation relationship (Figure 4C), the regression analysis showed a positive correlation between shell inflation and shell width ($R = 0.885$ at $P < 0.05$) and the *t*-test calculated on the value of the exponent (0.76) indicated a negative allometric growth. Correlation between shell inflation and shell width was also obtained in the different studied populations. The lowest value (0.75) of correlation coefficient was detected in the La Marsa population whereas the highest one (0.98) characterized the Hammamet population. The b values varied between 0.71 in the Skhira population to 1.07 in that from La Marsa. Populations from Hammamet, Kerkennah Island, Maharès, Skhira, Djerba and Zarzis composed group (1) and group (2) consisted of the other populations.

TOTAL WEIGHT–HEIGHT RELATIONSHIP

The regression analysis showed a high correlation between total weight and shell height not only for the combined population of *Pinctada radiata* ($R = 0.936$ at $P < 0.05$) (Figure 4D) but also for each population. In fact, among populations, the correlation coefficient fluctuated between 0.829 (Ajim) and 0.989 (Djerba). The lowest b value (2.532) was detected in the La Marsa population whereas the Djerba population displayed the highest one (3.192). In the case of this relationship, populations from Hammamet and Skhira composed group (3), those from Kerkennah Island, Maharès and Djerba formed group (2) and group (1) was made up of the other populations. Analysis of the combined population revealed that the pearl oyster *Pinctada radiata* grew in total weight isometrically throughout the size-range examined (6.48–104.34 mm).

SHELL WEIGHT–HEIGHT RELATIONSHIP

For the combined population of *Pinctada radiata*, the pattern was similar to the previous relationship of total weight versus shell height (Figure 4E). As shown by the regression analysis, shell weight and shell height of the pearl oyster *Pinctada radiata* were highly correlated ($R = 0.891$ at $P < 0.05$). Unlike the precedent relationship, the *t*-test calculated on the value of the exponent (2.89) indicated a negative isometric growth pattern. A high correlation between shell weight and shell height was detected within all *P. radiata* populations. The Ajim population displayed the lowest correlation coefficient (0.803) whereas the population from Djerba exhibited the highest one (0.986). The b values ranged from 2.534 (La Marsa) to 3.267 (Kerkennah Island). For this relationship the three groups were distinguished as follows: group (1) consisting of populations from Kerkennah Island, Maharès and Skhira; group (2) was composed of Ajim and Djerba populations; and group (1) was made up of the other populations.

NACRE THICKNESS–HEIGHT RELATIONSHIP

A relatively low correlation was derived from the analysis between nacre thickness and shell height ($R = 0.864$ at $P < 0.05$) (Figure 4F). The *t*-test calculated on the value of the exponent (0.75) indicated a negative allometric growth. Therefore, the pearl oyster *Pinctada radiata* grew faster in height than in thickness of the nacreous layer. Only two groups were distinguished. Group (1) was made up of *P. radiata* species from La Marsa and Ajim and the others populations composed group (2).

Comparative analyses of morphometric shell characters

Different discriminant analyses were performed to study the variability of morphometric shell characters of *Pinctada radiata* from coastal Tunisia. As shown in Table 2, the values of F calculated for all ratios ranged between 2.86 and 29.49 and were significantly greater than their respective theoretical F (approximately 2, $P < 0.05$). The ANOVA test carried out on morphometric ratios showed significant differences in *P. radiata* shell morphology. This result revealed an important phenotypical variability between the examined populations. The Duncan's multiple comparison test showed that the most discriminative ratios were WNR/R, HL/W and TN/W since they provided three non-overlapped groups of *P. radiata* populations. The squared Mahalanobis distances (D^2) of metric ratios revealed significant differences in the shell morphology between *P. radiata* populations from different sites. Four distinct biogeographical groupings could be then discriminated (Figure 5A): an isolated group represented by the population from La Marsa; a second group consisting of populations collected from harbours (Hammamet, Monastir and Zarzis); a third group composed of Sfax, Kerkennah Island, Maharès, Skhira and Djerba populations; and the last one made up of the population collected from the intertidal area, Ajim. However, the D^2 of weight ratios differentiated only three biogeographical groupings (Figure 5B). The discrimination of the Kerkennah Island population in an isolated group suggested particular trophic conditions in this area. The

Table 2. The mean values of morphometric ratios for the pearl oyster *Pinctada radiata* from coastal Tunisia. The F statistic contrasting the ratios and the correlation between sites are significant ($P < 0.05$).

Ratios	W/H	R/H	HL/H	HNR/H	HNL/H	WNR/H	WNL/H	TN/H	R/W	HL/W	HNR/W	HNL/W	WNR/W	WNL/W	TN/W	WNL/HNL	WNL/WNR
Average	0.93	0.35	0.81	0.69	0.76	0.63	0.32	0.013	0.38	0.87	0.74	0.82	0.68	0.74	0.014	0.91	0.94
F statistic	10.7	17.58	8.66	7.16	14.26	29.49	15.93	3.59	12.58	15.78	2.86	3.64	12.9	21.2	15.39	15.98	8.96
Ratios	**HL/R**	**HNR/R**	**HNL/R**	**WNR/R**	**WNL/R**	**TN/R**	**TN/HL**	**HNL/HNR**	**WNR/HNR**	**HNL/WNR**	**WNL/HNR**	**SW/TW**	**RW/TW**	**LW/TW**	**RW/SW**	**LW/SW**	**LW/RW**
Average	2.32	1.98	2.19	1.81	1.98	0.010	0.012	0.91	0.91	0.82	0.98	0.57	0.26	0.32	0.46	0.53	1.24
F statistic	6.69	5.35	8.79	9.43	7.98	7.85	16.45	3.92	15.99	16.47	13.49	19.39	19.54	16.89	4.3	6.46	4.59

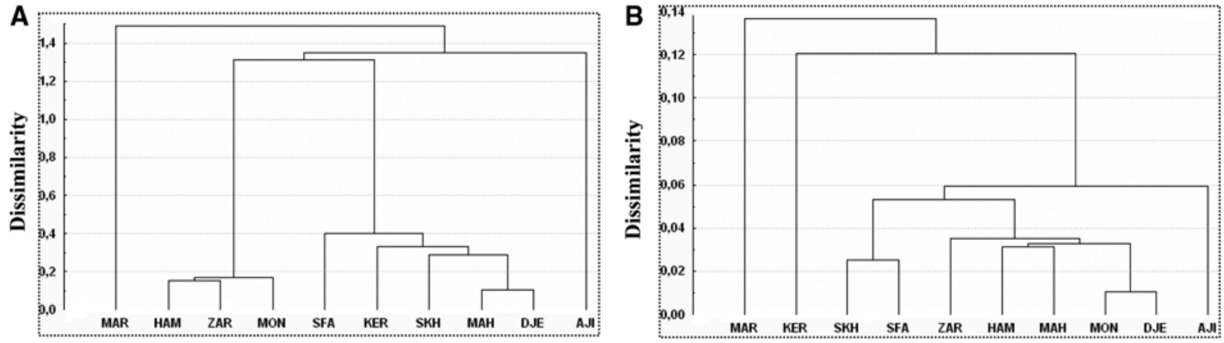

Fig. 5. Dendrograms of Mahalanobis distances using metric (A) and weight ratios (B) for *Pinctada radiata* populations from coastal Tunisia.

population clusters provided by the squared Mahalanobis distances (D^2) of both metric and weight ratios were discriminated following the geographical distribution of their sampling sites as well as their environmental condition changes. The MDS analyses of both metric (Figure 6A) and weight ratios (Figure 6B) perfectly corroborated the results of the squared Mahalanobis distances (D^2).

DISCUSSION

Due to the plasticity of shell morphology in pearl oysters, shell morphometry was widely used in both species identification and population differentiation. In this study, the variability of shell morphometrics and growth of *Pinctada radiata* populations at ten sampling sites along coastal Tunisia were investigated.

The allometric study indicated that *P. radiata* from coastal Tunisia exhibited isometric growth for the relationship total weight versus shell height, similar to that from the Red Sea (Yassien, 1998), the Mediterranean Sea (Yassien *et al.*, 2000) and the Arabian Gulf (Mohammed & Yassien, 2003). Moreover, the values of coefficient b for total weight to shell height (2.98) and for shell weight to shell height (2.89) revealed clearly the significant increase of *P. radiata* growth in weight. Since it is generally assumed that the relative growth as regards the total weight and shell height reflects the influence of the ecological factors on animals' growth, this study indicated that the environmental conditions were favourable to the growth and the development of *P. radiata* species and corroborated its being well-established in coastal Tunisia. Recent studies on the distribution and densities of *P. radiata* in northern and eastern (Tlig-Zouari *et al.*, 2009) and in southern (Derbali *et al.*, 2011) coastal Tunisia have also showed that this species adapted well to coastal Tunisia.

The most discriminative allometric relationships were total weight to shell height and shell height to inflation since they provided three groupings of populations according to their allometric growth types. While, the allometric relationship involving the nacreous layer thickness, contrary to all expectations, showed any particular distinction within populations. However, the Duncan's multiple comparison test revealed clearly the discriminative character of nacreous layer thickness in *P. radiata* populations from coastal Tunisia and highlighted, thus, its use as a morphometric character in differentiation between pearl oysters populations.

A large variability in allometric growth among *P. radiata* populations was detected, with high correlation between

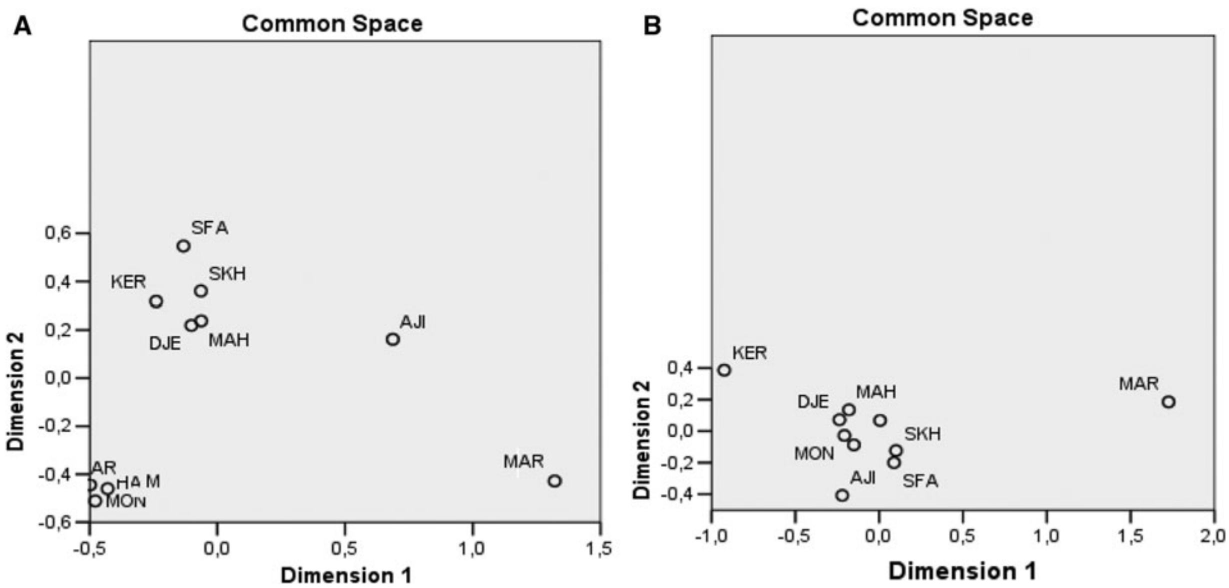

Fig. 6. Multidimensional scaling plots of metric (A) and weight ratios (B) for *Pinctada radiata* populations from coastal Tunisia.

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linear shell characters and between linear and ponderal ones. However, the allometric distinction of *P. radiata* populations shows no clear relatedness to their geographical distributions. On the other hand, the detection of the lowest values of correlation coefficient, in all cases, in populations from Ajim and La Marsa suggest their distinction from the other populations.

The morphological analysis revealed an important heterogeneity within *P. radiata* populations. In fact, most of them consisted of large-sized and juvenile individuals indicating that this pearl oyster had found an adequate environment for both growth and reproduction. The results of our study provided further evidence that the pearl oyster *P. radiata* exhibited a shell morphological variability, which is consistent with the findings of Beaumont & Khamdan (1991) and of Al-Sayed *et al.* (1993). The observed variability in shell morphology and allometric growth among *P. radiata* populations may have arisen due to differences of the local conditions and the environmental factors between sites, notably salinity, wave action and temperature which affected molluscs development and survival (Parache & Massé, 1987; O'Connor & Lawler, 2004). It may be also associated with population density and availability of food resources which affect the growth rate. In fact, numerous investigations have concluded that growth of low density populations is more important than that of high density populations characterized by more competition for food (Zanette & Deslous-Paoli, 1983; Brown & Hartwick, 1988; White *et al.*, 1990). Moreover, it has been demonstrated that the type of sediment (Newell & Hidou, 1982) and bottom (Claxton *et al.*, 1998) as well as burrowing behaviour ability and efficiency influence shell morphology and relative growth of many bivalve species (Eagar, 1978; Seed, 1980; Costa *et al.*, 2010).

The present study revealed that the morphological discrimination of *P. radiata* populations matches with their geographical distribution. This finding could be related to the environmental and ecological conditions of stations. In fact, the morphological distinction of the *P. radiata* population from Ajim (south of Tunisia) by the D^2 and the MDS analyses of shell metric (Figures 5A & 6A) and weight (Figures 5B & 6B) characters could be due to the particular environment of the Ajim site as an intertidal zone. This result supports the allometric distinction of the Ajim population. In addition, all sampled *P. radiata* specimens along coastal Tunisia had similar shell shape and colour, except those from the Ajim population which were less dark than the others. Several studies suggest that the variation of shell shape and colour in oysters is genetically controlled (Imai & Sakai, 1961; Wada, 1994; Brake *et al.*, 2004; Batista *et al.*, 2008). In the case of the Ajim population, the high and low tide marks might affect the morphology of *P. radiata* individuals; an ecomorphosis of adaptation to environmental stress can, thus, occur.

The discrimination of the *P. radiata* population from La Marsa by the D^2 and the MDS analyses of shell metric (Figures 5A & 6A) and weight (Figures 5B & 6B) characters can be explained by the fact that La Marsa is the only site among all those sampled which is located on the north coast of Tunisia and, therefore, has particular environmental conditions. This finding supports the discrimination of the La Marsa population pointed out by the allometric growth study.

The grouping of *P. radiata* populations from Kerkennah Island, Sfax, Maharès, Skhira and Djerba by the squared D^2 and the MDS analysis of shell metric parameters (Figures 5A & 6A) could be due to the geographical relatedness of these sites. In fact, all of them are located in the Gulf of Gabès, south-eastern Tunisia. This result suggests that these populations have the same origin. Taking in consideration that the Gulf of Gabès is located in the south-eastern part of the Mediterranean Sea, the grouping of the Gulf of Gabès populations is in agreement with Zenetos *et al.* (2004), who reported that *P. radiata* was described as one of the very first exotic mollusc species in the Mediterranean Sea which it entered via the Suez Canal thus colonizing its eastern part. Besides, *Pinctada radiata* is abundant in the Levantine Basin (Barash & Danin, 1992) and has spread as far west as the Tyrrhenian Sea, as well as off Sicily, Malta, Pantellaria Island and France (Tomlin, 1927; Sabelli, 1969; Zibrowius, 1992; Di Natale, 1982; Ricordi, 1993). However, Monterosato (1899) reported a century ago that the thriving establishment of *P. radiata* in the Gulf of Gabès, where it formed large banks, made him cast doubts about the idea of its progressive penetration through the Suez Canal suggesting therefore that the *P. radiata* species was indigenous to the Mediterranean Sea. On the other hand, Galil & Zenetos (2002) reported that the lessepsian invaders exhibited a rapid geographical spread and successful establishment. In this regard, Oliverio (1995) concluded that the term lessepsian migrant should not be used indiscriminately for any species of Indo-Pacific origin found in the Mediterranean Sea.

The distinction of the *P. radiata* population of Kerkennah Island from the Gulf of Gabès grouping by the squared Mahalanobis distances (D^2) and the MDS analyses of shell weight parameters (Figures 5B & 6B) proves that Kerkennah Island have particular abiotic and trophic conditions. Indeed, many deep channels bordering the high depth of Kerkennah Island may contribute to its nutritive enrichment.

The grouping of *P. radiata* populations from the three sampled harbours (Hammamet, Monastir and Zarzis) by both D^2 and MDS analyses of morphometric characters could be obvious since the harbours have almost the same ecological conditions but not necessarily the same environmental ones. This result indicates that these populations might have the same origin and suggests that shipping is a vector for the introduction of *P. radiata* in Tunisian waters. Ship-related introductions included ballast waters, solid ballast and fouling. Similarly, Zenetos *et al.* (2004) reported that the recent finding of the Indo-Pacific bivalve *Fulvia fragilis* in Saronikos Gulf and in particular in Peiraias port suggested its transport via shipping although the species seemed to be lessepsian migrant. Furthermore, the *P. radiata* population from Hammamet harbour seemed to be particular for numerous reasons. Firstly, it represented an illustrative example of heterogeneity among all sampled populations, exhibiting the high frequencies of both larger sized (77.36%) and juveniles (5.66%) specimens. Secondly, the *P. radiata* population from Hammamet had the highest mean size in shell length (72.71 mm). Finally, the largest size specimen of *P. radiata* recorded at least in Tunisia and elsewhere was found at Hammamet harbour and was about 104.37 mm shell length which was larger than those observed for *P. radiata* populations from El Biban lagoon (85 mm) (Seurat, 1929), Kerkennah Island (74 mm) (Tlig-Zouari & Zaouali, 1998), Bizerta lagoon (Njila) (100.51 mm) (Tlig-Zouari *et al.*, 2009), the eastern Mediterranean (64 mm) and the Red Sea (93.2 mm) (Yassien, 1998; Yassien

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et al., 2000). These findings suggest that even if shipping is not the only vector for introduction of *P. radiata* species in Hammamet harbour, it may be involved in combination with lessepsian migration in the settlement of the Hammamet population. According to Galil & Zenetos (2002) shipping and then aquaculture are the next major introduction vectors for exotic marine biota in the eastern Mediterranean besides the introduction through the Suez Canal. Moreover, a combination of two modes of *P. radiata* introduction was assumed in the Mediterranean Sea particularly in Greek waters where, despite the *P. radiata* intentional introduction for aquaculture, the lessepsian mode of its introduction was not excluded (Zenetos *et al.*, 2004).

In conclusion, the conventional morphometric methods and the statistical analyses used in this investigation reveal that the invasive pearl oyster *P. radiata* from coastal Tunisia displayed an important variability in shell morphology and growth. Unlike the allometric growth differentiation, morphological discrimination of *P. radiata* populations shows geographical distinction. Two hypotheses can explain the recorded variability: (1) a plastic response of *P. radiata* species to different environmental and/or ecological conditions; and (2) a difference in origins of *P. radiata* populations. Further genetic studies are required to understand the cause of morphometric differences within and among *P. radiata* populations from coastal Tunisia.

ACKNOWLEDGEMENTS

This work was funded by grants from the «Ministère de l'Enseignement Supérieur, de la Recherche Scientifique et de la Technologie de la Tunisie». It was supported by the project VRR Nacre (VRROI-05). We thank the anonymous referees for their valuable remarks.

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https://doi.org/10.1017/S0025315411001925 Published online by Cambridge University Press