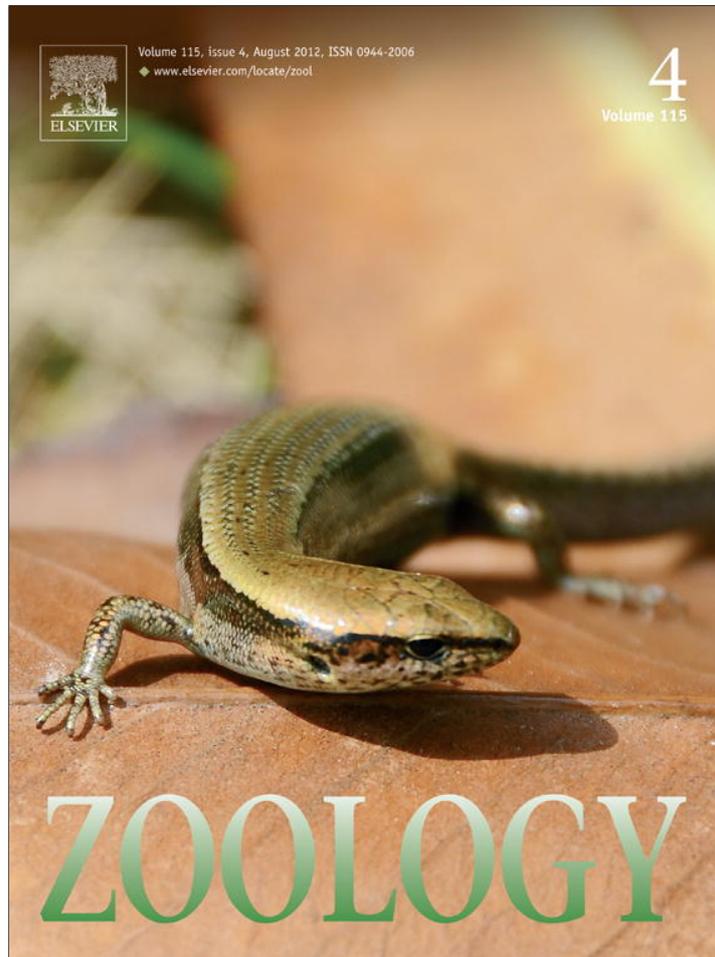


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Overlapping patterns of morphometric and genetic differentiation in the Mediterranean goby *Pomatoschistus tortonesei* Miller, 1968 (Perciformes, Gobiidae) in Tunisian lagoons

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ABSTRACT

The genetic and morphological variations of *Pomatoschistus tortonesei* Miller, 1968 were studied in samples collected from three Tunisian lagoons. The morphological analysis included 18 morphometric measurements and was based on linear discriminant analysis (LDA), whereas the genetic analysis was based on the 16S-rRNA and COI mitochondrial genes. Both analyses differentiated the populations and demonstrated consistently a well-supported differentiation between the western Mediterranean samples (Bizerta and Tunis South lagoons) and the eastern Mediterranean sample (El Bibane lagoon). The observed differentiation could be explained in terms of the geographic isolation of the various populations and the influence of environmental factors, which differ greatly between the different sites. The molecular results revealed that the populations are characterised by unique haplotypes which are well defined in relation to limited gene flow and restricted dispersal abilities. Additionally, it seems that local selective pressures have modelled biometrical variation. Morphological results can reflect a differential habitat use revealed in the cephalic features and a different response to hydrodynamic constraints developed in dissimilar dorsal and pelvic fin lengths.

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1. Introduction

Coastal lagoons are complex dynamic ecosystems, characterised by a constant fluctuation of environmental conditions. There are numerous lagoons throughout the Mediterranean area, the majority having appeared during the Holocene period. These lagoons are well recognised as being unique in terms of biodiversity, and are possibly capable of producing and protecting more or less distinctive evolutionary lineages (e.g. Porter et al., 2001; Trabelsi et al., 2004).

One of the most predominant fish taxa to be found in Mediterranean lagoons is the genus *Pomatoschistus* Gill, 1844 of the Gobiidae family. One of these gobiine species is *Pomatoschistus tortonesei* Miller, 1968, which is endemic to the Mediterranean Sea and listed as an endangered species (Abdul Malak et al., 2011). Despite its particular situation, there is scant knowledge regarding its spatial distribution and only sporadic records have been reported, e.g. in the Marsala lagoon (Sicily, Italy) (Miller, 1968), the Farawa lagoon (Libya) (Miller, 1982) and in various Tunisian lagoons (Mejri et al.,

2009a). *P. tortonesei* is a non-migratory, euryhaline species which spends its entire life in lagoons; benthic adults are considered poor swimmers because, as other gobies, their pelvic fins are fused in such a way as to form a suction disc (Miller, 1986).

Recent published results regarding the genetic structure of this species across its distribution range have revealed a significant divergence between the western and eastern Mediterranean populations, delimited by the Siculo-Tunisian Strait (STS) which acts as a breakpoint to gene flow in the Mediterranean Sea, and have shown a further degree of differentiation among western populations due to the hydrographic and ecological patterns of each locality (Mejri et al., 2009b). Despite such levels of genetic variation among populations, it is not yet known to what extent morphological and genetic differences correlate with each other.

It is known that morphological and genetic markers provide different but complementary information regarding population structure and this has been widely used in population differentiation studies and in stock assessments. Morphometric characters are partially genetically determined and they are strongly influenced by environmental conditions (Tudela, 1999; Turan, 2004). Thus, geographic isolation between populations is expected to promote the differentiation of both morphological and genetic characters, either due to drift or to different selective regimes related to

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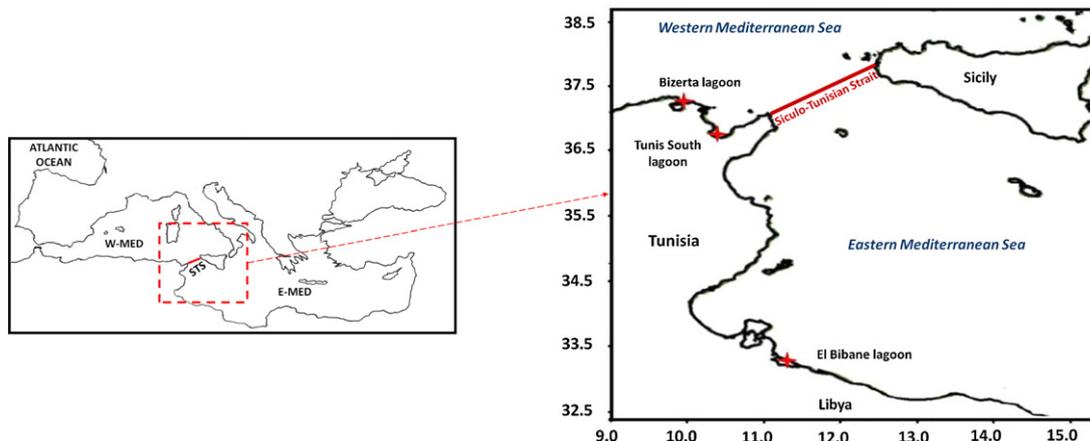


Fig. 1. Locations of the sampling sites in Tunisia: Bizerta lagoon (BIZ) and Tunis South lagoon (LST) in the western Mediterranean Sea (W-MED), El Bibane lagoon (BIB) in the eastern Mediterranean Sea (E-MED), divided by the Siculo-Tunisian Strait (STS).

environmental features (Slatkin, 1985). Consequently, population divergence may reflect insufficient gene flow and/or different selective pressures (Slatkin, 1985).

P. tortonesei is of particular interest in terms of evolution and adaptive radiation as it is well known that lagoons are characterised by wide variations in environmental parameters which may cause strong selective pressures on organisms. These factors, in association with geographic discontinuity, can play a key role in differentiating populations inhabiting these biotopes. In the light of these considerations, the available *mtDNA* data previously reported by Mejri et al. (2009b) were compared with the morphometric measurements of the present study with the aim of: (i) comparing morphological and genetic differentiation; (ii) identifying and quantifying phenotypic variability and the evolutionary role of lagoons; and (iii) inferring the importance of lagoon environmental parameters in population structuring.

2. Materials and methods

2.1. Sample collection

Between April 2006 and March 2007 specimens of *P. tortonesei* were caught using a 10 m long purse seine with 0.5 mm mesh from three Tunisian coastal lagoons: the Bizerta lagoon (BIZ), the Tunis South lagoon (LST) and the El Bibane lagoon (BIB) (Fig. 1). The choice of these three sites was determined by ease of access and the abundance of the investigated species. The geographic coordinates of each mainland lagoon, their environmental features (Lemoalle, 1986; Sammari et al., 2006; Hlaili et al., 2008) and the numbers of individuals used in the present study are given in Table 1.

2.2. Morphological analysis

In total, 120 specimens of *P. tortonesei* were collected from the three lagoons (BIZ, LST and BIB). Eighteen morpho-anatomical

parameters, including horizontal as well as vertical dimensions of the body, were measured: standard length (LS); head length (H); right eye diameter (E); postorbital and interorbital length (PO and IO); cheek depth (CHd); body height (BH); first and second dorsal fin base lengths (D1 and D2); pectoral fin length (PI); anal fin length (A); pelvic fin length (Pv); distances from the snout to the origin of the first and second dorsal fins (SN/D1 and SN/D2); distance from the snout to the pectoral fin origin (SN/PI); distance from the snout to the anal fin origin (SN/A); distance from the snout to the pelvic disc origin (SN/Pv) and the minimum height of the caudal peduncle (CP) (Fig. 2). All morphometric measurements were taken by the same person on the left lateral aspect and measured to the nearest 0.01 mm.

For such analyses, it is important to eliminate any size effect especially when comparing fish of different sizes. Therefore, any size-dependent variation in morphometric characters was removed using the allometric approach by Reist (1985):

$$M_{\text{trans}} = \log M - \beta(\log SL - \log SL_{\text{mean}}),$$

where M_{trans} is the transformed measurement, M is the original measurement, β is the within-group slope regression of $\log M$ against $\log SL$, SL is the standard length of the fish and SL_{mean} is the overall mean of the standard length. Morphometric characters were analysed together and a multivariate analysis of variance (MANOVA) was performed to test the significance of differences among groups.

In order to obtain an optimal set of characters for evaluating the extent to which geographic entities with different morphometric features could be separated, a linear discriminant analysis (LDA) was performed on the 120 individuals from the three sites. This analysis obtains linear combinations of variables (discriminant functions) which can be used to provide the best separation of classes. Wilk's criterion values were estimated to test the significance of such a discrimination for a combination of variables, and discriminant functions were used to classify individuals into

Table 1
Sample locations of *Pomatoschistus tortonesei*, geographic coordinates, environmental features, number of individuals per analysis and mean standard lengths (MSL, average \pm s.d.).

Sampling locations	Geographic coordinates	Environmental features			Sample sizes		MSL (cm)
		Substrata	Vegetation encountered	Tide amplitude	Genetic study	Morphometric study	
Bizerta	37°13'N 9°51'E	Muddy + biodetritus	Algae	0.02–0.13 m	10	39 (4♂ 35♀)	2.90 \pm 0.31
Tunis South	36°47'N 10°14'E	Muddy	Algae	0.4 m	7	33 (2♂ 31♀)	2.45 \pm 0.17
El Bibane	33°16'N 11°17'E	Sandy + biodetritus	<i>Cymodocea nodosa</i>	1.8 m	9	48 (1♂ 47♀)	2.69 \pm 0.19

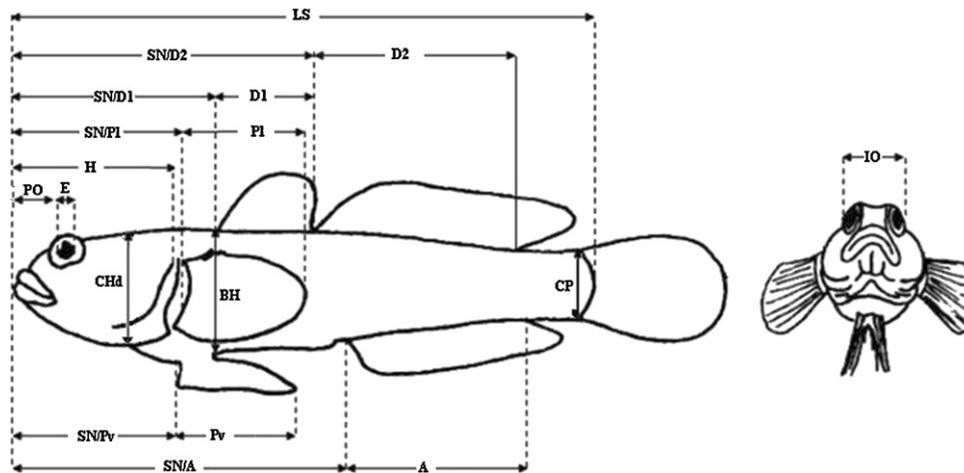


Fig. 2. Morphometric measurements (see text for details).

samples. The classification success rate (PCS) representing the percentage of individuals which were correctly assigned to the original sample was evaluated.

All calculations and graphs were made with Minitab 12.2 and R 2.8.1 software. The packages used were “ade4” (Chessel et al., 2004), Mass (Venables and Ripley, 2002), Stats (R Development Core Team, Vienna, and contributors worldwide) and Ape (Paradis et al., 2004).

2.3. Genetic analysis

For molecular analyses, 26 previously obtained sequences of *P. tortonesei* (Mejri et al., 2009b) were used. The data set contained samples from the three lagoons investigated in the present study. The procedure, which has already been reported in Mejri et al. (2009b), is briefly summarised here. Total DNA was extracted from analysed specimens and a fragment of both the mitochondrial 16S ribosomal RNA gene (16S-rDNA, 518 bp) and the cytochrome oxidase I gene (COI, 648 bp) were amplified. Levels of haplotypic diversity h (Nei, 1987) and nucleotide diversity π (Tajima, 1993) were calculated using DnaSP v. 4.50 (Rozas et al., 2003). Phylogeographic analyses were performed, using the UPGMA method, with the software MEGA v. 4 (Tamura et al., 2007). The tree was rooted with co-genus *Pomatoschistus marmoratus* haplotypes originating from Tunisia. Node robustness was estimated by running bootstrap tests with replicates encompassing 1000 data sets.

3. Results

3.1. Morphological analysis

The MANOVA for the 18 biometric characters revealed highly significant average differences ($p < 0.001$) among localities, leading to the rejection of the null hypothesis of ‘no heterogeneity’. The LDA of *P. tortonesei*’s log-transformed variables yielded two initial factorial axes, accounting for 86.49% and 13.51% of total variance, respectively. Hence, the two discriminant functions LD1 and LD2 were chosen for the analyses.

Plotting these two discriminant functions resulted in a distinction among the three studied lagoons (Fig. 3A). The first two groups were spread along LD2 and were composed of individuals originating from BIZ and LST from the north of Tunisia. The third group, which projected onto the positive side of LD1, represented the sample from BIB, which is located on the south coast. The significance of the inter-group variability between the lagoon samples was proven by Wilk’s criterion (Wilk’s $\lambda = 0.07$, $F = 16.80$, $p < 0.001$).

Table 2

Classification success rate (PCS) evaluated according to the percentage of *Pomatoschistus tortonesei* individuals correctly assigned to the original sample. BIZ: Bizerta lagoon; LST: Tunis South lagoon; BIB: El Bibane lagoon. N = sample size.

Sample	BIZ	LST	BIB
BIZ	35	4	0
LST	4	29	0
BIB	0	0	48
N total	39	33	48
N correct	35	29	48
Proportion	89.7	87.9	100

The most important discriminative characters in distinguishing between the groups were pelvic fin length (Pv) and the second dorsal fin length (D2) (Fig. 3C and D), both of which contributed to defining the first discriminating function, LD1. Cheek depth (CHd) and head length (H) (Fig. 3B), which characterise the cephalic region of the body, defined the second axis, LD2.

Using these morphometric characters, the discriminating analysis correctly classified 112 of the 120 fishes (i.e. 93.3%), while the cross-validation testing procedure correctly classified 105 of 120 fishes (i.e. 87.5%). The proportion of specimens correctly classified into their original group was highest (100%) for BIB (Table 2).

3.2. Genetic analysis

Values relating to indexes of general genetic diversity are reported in Table 3. It is noteworthy that the different populations under investigation presented private haplotypes which were only found in a single locality.

The UPGMA tree revealed a split between two highly divergent groups with no geographical overlap and a high bootstrap value (100%) (Fig. 4). The first clade was represented by the western Mediterranean samples from BIZ and LST, whereas the second clade was formed by the eastern Mediterranean sample from BIB. On a local scale, the neighbouring populations from BIZ and LST presented a further segregation within the major clade, appearing as separated sub-groups (Fig. 4). Thus, all haplotypes were related according to their geographic area of occurrence, and their topology was consistent with the degree of morphometric differentiation.

4. Discussion

The results from genetic and morphological analyses provided congruent evidence for a significant differentiation between all

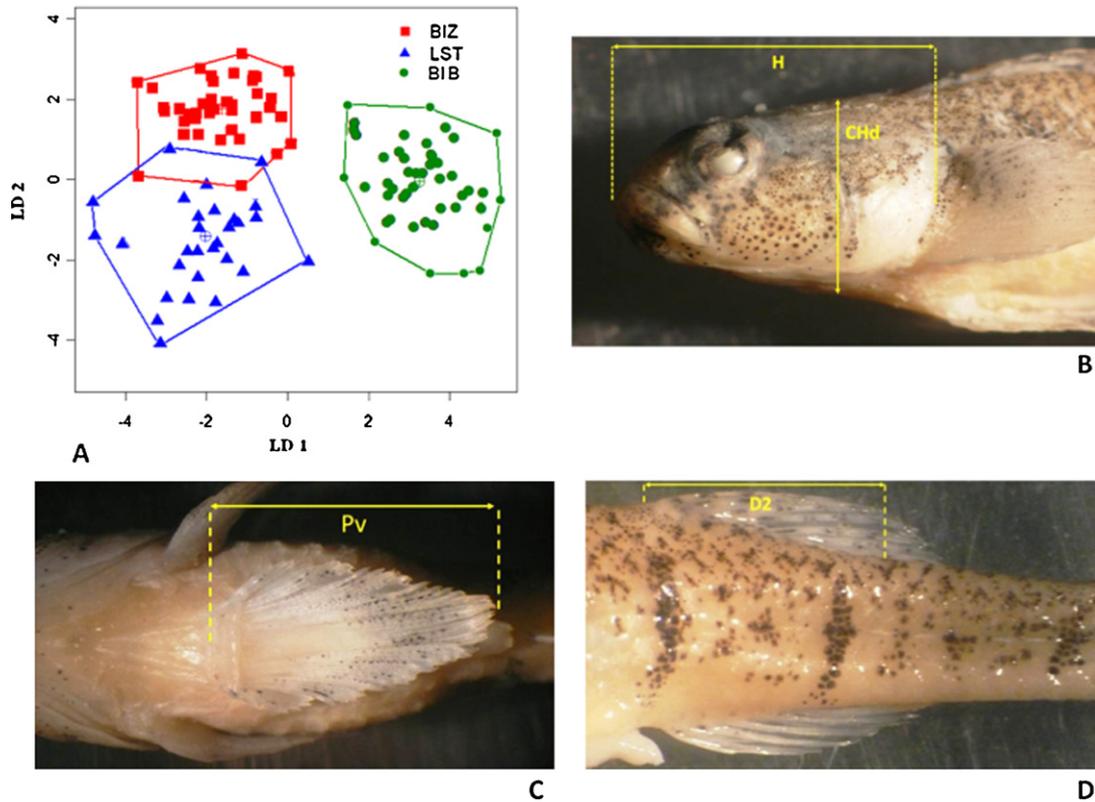


Fig. 3. (A) Plot of the linear discriminant analysis on the first plane (LD1, LD2) for the three lagoon samples: Bizerta lagoon (BIZ), Tunis South lagoon (LST) and El Bibane lagoon (BIB). (B) The parameters cheek depth (CHd) and head length (H), which contribute to LD2. (C and D) The parameters pelvic fin length (Pv) and second dorsal fin length (D2), which contribute to LD1.

Table 3
Genetic variability of 16S-rRNA and COI sequences detected in *Pomatoschistus tortonesei* samples.

Locus	Bizerta lagoon		Tunis South lagoon		El Bibane lagoon	
	16S	COI	16S	COI	16S	COI
Sample size (N)	10	10	7	7	9	9
Segregating sites (SS)	2	1	2	1	2	4
Number of haplotypes (N _h)	3	2	3	2	3	4
Nucleotide diversity (π)	0.0010	0.0005	0.0014	0.0004	0.0008	0.0018
Haplotypic diversity (h)	0.5111	0.3555	0.6666	0.2857	0.4166	0.7500

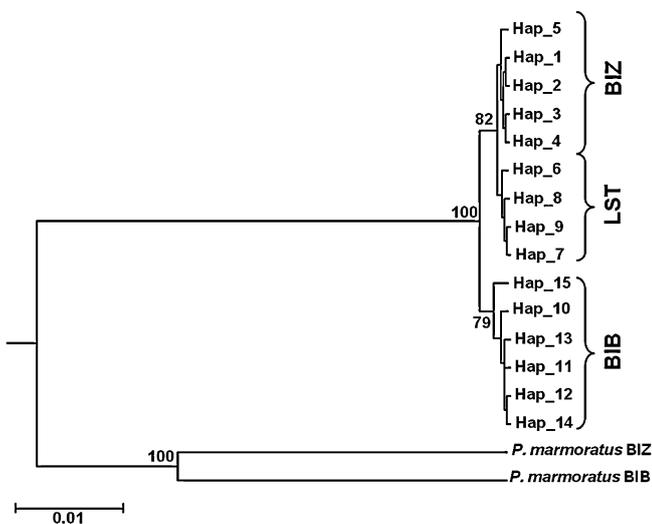


Fig. 4. Nucleotide divergence from 16S-rDNA + COI sequences, clustered by UPGMA (numbers indicate the percentage of 1000 bootstrap replicates that support each branch). BIZ: Bizerta lagoon; LST: Tunis South lagoon; BIB: El Bibane lagoon.

three samples, and, most predominantly, between the western vs. eastern Mediterranean areas, which correspond to the northern (BIZ and LST) and southern coasts of Tunisia (BIB), respectively. The role of the STS, which separates the gene pool of the western from that of the eastern Mediterranean samples, has been widely discussed in previous papers (Mejri et al., 2009b, 2011). Here, the dataset allows an additional differentiation with regard to morphometric data which is congruent with the genetic pattern.

A consideration of the various analysed metric parameters reveals the discriminating value of some of the samples. The causes of morphological differences between populations can often be quite difficult to explain. However, most authors agree that variations in environmental conditions play the largest part in determining morphological variation and phenotypic discreteness (Norton et al., 1995; Wainwright, 1996). Either these particular designs have been fostered by natural selection (because they are more appropriate than the alternatives), or they are particularly economical in terms of energy or materials (Alexander, 1988).

According to the LDA results (Fig. 3A), three phenotypically diverse groups could be distinguished, and a strong discrepancy between the *P. tortonesei* populations from the north of Tunisia (BIZ+LST) and the population from southern Tunisia (BIB) was

found. The whole LDA pattern is the result of the variation detected in the following characters: cheek depth (CHd) and head length (H), which have had the major weight in the discrimination on the LD2 axis, and dorsal (D2) and pelvic (Pv) fin lengths, which can be considered responsible for the discrimination on the LD1 axis (Fig. 3). According to the results, BIB specimens have the longest fins while the western populations, BIZ and LST, have the larger amplitude of variation in head shape.

Various authors have studied the intra-specific variation of head morphology and different trophic uses in marine fishes, such as carangids (Turan, 2004), labrids (Westneat, 1995; Wainwright et al., 2004) and sparids (Sarà et al., 1999; Hammami et al., 2011). These authors suggest that a detailed study of cephalic morphology and body shape could reveal different trophic groups. The morphological discrepancy in head traits may reflect a differential habitat use, especially regarding the exploitation of different ecological niches with variable diets (Hyndes et al., 1997; Delariva and Agostinho, 2001). In general, the link between morphology and diet in fish is provided by feeding performance (Norton, 1991; Wainwright, 1991; Motta and Kotschal, 1992). For example, head morphology is subject to various constraints determined by the feeding strategy and the type and size of ingested food (Gatz, 1979; Wainwright and Richard, 1995).

For the present study, the nature of the substrata and their cover has been investigated at all sampling sites (Table 1). *P. tortonesei* from the northern Tunisian lagoons (BIZ and LST) were collected in muddy vegetated substrata where grasses abound, offering a good biotope for amphipods and ostracods, which are the preferred prey of this fish (Miller, 1982; personal observations). In the south (BIB), in contrast, this species was plentiful only in sandy areas lacking in vegetation and characterised by prey depletion. Specimens could have adapted their head size and shape according to food availability or density, and to the type and size of prey in relation to algal cover.

In addition to differences in cephalic features, we also observed morphological discrepancies in dorsal (D2) and pelvic (Pv) fin lengths. It is well known that soft dorsal fins act as a static keel or body stabiliser, generating thrust and lateral forces during locomotion. During turning manoeuvres, dorsal fins become a fixed pivot point for body rotation. The soft dorsal fins also play a significant role in steady propulsion and generate a large, lateral force (Drucker and Lauder, 2001, 2005). Along with the caudal and anal fins, the paired pelvic fins permit fish to swim steadily forwards with minimal roll and yaw (Drucker and Lauder, 2005). In gobiid species, the pelvic fins are fused into a suctorial disk which assists them in attaching themselves firmly to rocks, preventing them from being swept away by swift currents (Miller, 1986). Thus, various fins are used by fish to move and stabilise their bodies when suspended in turbulent flow. The range of stability conferred by the fins may cause body shape and habitat specialisation to play a lesser role in swimming in turbulence than in steady flow (Bioly and Magnan, 2002).

When considering the differences in hydrodynamic constraints (i.e. tide currents) in the lagoons under investigation (Table 1), we noticed that hydrographic currents are of great importance, participating actively in the circulation, turbulence flow and renewal of water masses, particularly in El Bibane lagoon (BIB). These conditions provide a stimulus for benthic species with a 'homing' behaviour (such as *P. tortonesei*) to develop and increase their fixation surface and stability organs in order to thwart drift through tidal currents and to be able to act in sudden perturbations. Indeed, fish subjected to complex flows normally demonstrate an ability to maintain stability, which can be achieved either passively by self-correcting mechanisms or actively through the powered movements of fins (Webb and Weihs, 1994; Jindrich and Full, 2002; Liao, 2002; Webb, 2004). The shape of the body and the posture

of fins and their intrinsic compliance can also contribute to passive mechanisms of stability (Liao, 2007).

However, the biometric differences among populations of *P. tortonesei* from the three different lagoons may reflect not only phenotypic plasticity but also the effect of geographic isolation. Molecular analyses confirmed such a scenario. Previous studies (Mejri et al., 2009b) have demonstrated that no haplotypes were shared between the three populations under investigation, implying that there was no gene flow between them (Fig. 4). The pattern described by the UPGMA tree is consistent with the degree of morphometric differentiation.

The current genetic structure of *P. tortonesei*, a species which exclusively inhabits lagoons, could be the product of an increasing divergence of lineages producing morphologically different forms. It can be assumed that a restricted gene flow between populations has constrained *P. tortonesei* populations to adapt and evolve as independent entities in different environmental conditions. Specifically, as *P. tortonesei* is strictly associated with lagoon habitats, which are transitional environments and characterised by an instability of their physico-chemical parameters (Sarà, 2009), it could have been exposed to selective pressures more frequently than marine species which inhabit open-sea habitats. Such selective pressures could have amplified pre-existent, morphological traits, especially since *P. tortonesei* is a benthic species with low dispersal abilities and short pelagic larval stages. Brackish water ecosystems are often exposed to wide variations in environmental parameters, a fact which may cause strong selective pressures on organisms (e.g. Trabelsi et al., 2004). In association with genetic and geographical discontinuity, these pressures can play an important role in separating species inhabiting these environments into different populations.

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