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Morphometric Variation among Four Local *Ruditapes decussatus* Populations in Monastir Bay (Eastern Coast, Tunisia)

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HIGHLIGHTS

- Ruditapes decussatus from four sites belonging to Monastir bay (Tunisia) was first time analyzed based on morphometry and comparative statistics.
- The site of Ksiba was distinguishable from other sites by larger and heavier specimens.
- The sub-trapezoidal shape was dominant with small- size classes, in most sites.
- The principal component analysis (PCA) revealed different population groups, with two intrapopulation groups for Sokrine site.
- Allometric relationships showed highly significant correlation with different growth patterns (positive

allometric, negative allometric and isometric).

Abstract: In Tunisia, Monastir bay, extending for 38 km along the eastern coast of Tunisia, harbors different species of bivalves such as the clam *Ruditapes decussatus*, a species of major socio-economic interest. Despite its importance, no studies have been carried out about the morphology of this mollusc in Monastir bay. Thus, the current study aimed to analyze the shell morphological variations in the European clam *Ruditapes decussatus* from Monastir Bay coast. The comparison of the shell shape of individuals from

different populations was performed based on samples taken at four different sites (Khniss, Ksiba, Bkalta and Sokrine). Variance analysis of the morphometric indices (elongation, compactness, convexity and density) for the four populations indicated significant differences among sites with (p=0<0.05). Principal component analysis (PCA) revealed two intra-population groups for Sokrine site, supporting an intra-population variation in the shell morphology. Allometric relationships established between linear variables (shell length, height and width) and ponderal variables (total weight, shell weight) as well as the relative growth between variables (isometry vs. allometry) were analyzed. All morphometric relationships were highly significant (P<0.001) with high correlation coefficients (R²: 0.714-0.982), showing positive allometric growth for Bkalta and negative allometric growth for Sokrine. The data obtained will be useful in research studies and have practical application in diverse research fields such as biology, ecology, fisheries assessment and management.

Keywords: Monastir bay; *Ruditapes decussatus*; shell morphology; Multivariate analysis; Allometry.

INTRODUCTION

Bivalve molluscs are known for their remarkable morphological diversity. They are characterized by their solid, hard and stable shells, which performs the functions of support and protection [1-3]. Studies on mollusc shell morphology and its morphometric relationships are frequently used for diverse purposes, including size-based analyses of communities, analysis of sexual dimorphism and monitoring of stocks [4]. Knowledge of bivalve variability is important to the effective culture of these organisms. Several authors around the world have proved the intra- or inter-population variations in the morphology of the bivalves shell for different species such as mussels [5,6] and clams (*Ruditapes philippinarum; Ruditapes decussatus*) [7,8]. The European clam *Ruditapes decussatus* (Linnaeus, 1758) is widely distributed along the coastal and estuarine areas of Europe and Northern Africa and represents an important income resource due to its high commercial value [9,10]. In Tunisia, this venerid clam is particularly abundant along the southeastern coast of Tunisia (Gulf of Gabes), where 23% of people contributed to the collection of this clam. It represents an important economic endpoint since it is a natural resource and has a high commercial value [11]. Many studies have been conducted on *R. decussates* from different regions in Tunisia from the perspectives of biology [12,13], ecotoxicology [14-16] and stock assessment [17].

This carpet-shell clam is also available in some sites in Monastir bay (central eastern coast of Tunisia). Even with its low presence in the central eastern coast, *R. decussatus* populations constitute an additional income for local communities in this region. In addition, the adult clams have been shown to repopulate a harvested area in a relatively short period of time [18]. To ensure the conservation and the development of this stock, adequate morphological characterization is required. Therefore, the study of morphological variations could be considered in stock discrimination and could provide a useful tool to identify adequate management measures and strategies [19]. Such information would allow for comparison between *R. decussatus* from different geographical areas and could be useful as a reference for studies in marine invertebrates' biology and cultivation. Despite some few studies concerning aspects of bivalve populations from Monastir bay coast, the information available on morphometric relationships of these species remains scarce. In this context, the main objective of the present study was to understand the growth characteristics of *R. decussatus* from four different sites along Monastir bay coast, aiming to fill the information deficiency in the literature about biometric features of *R. decussatus* populations in this region, which may be considered as basis for further studies from other sites or ecological situations.

MATERIAL AND METHODS

a) Study area and Sampling sites

Monastir bay (35°47'-35°37'N/10°45'E-11°50'E) is located in the southeastern Mediterranean Sea, more precisely on the eastern coast of Tunisia. It is a wide bay with a surface area of over 70 km² and is no more than 3 m deep up to 2 km from the shoreline. This semi enclosed lagoon is characterized by a weak hydrodynamic regime due to the submarine topography [20,21].

The specimens of *R. decussatus* were randomly collected from an intertidal zone sampling by handpicking between April 2017 and December 2017. Sampling was carried out at four stations (Figure 1), within which the clams were considered accessible and often collected by the villagers.

In this zone, the analysis of sediment [22] showed sediment transport along the coast; from north to south and vice versa, caused by alongshore drift and a rip current in the middle of the bay. The spatial distribution of mean grain size indicates that the coasts of Khniss and Ksiba were characterized by medium and thin sands and the southern coasts of Bkalta and Sokrine were characterized by very thin to thin sands.

Two hundred and thirty five (235) individuals of *Ruditapes decussatus*, were collected from the four mentioned sites (Khniss, Ksiba, Bkalta and Sokrine), in Monastir bay. The individuals collected in the field were stored in seawater inside isothermal boxes and were taken to morphometric measurements.



Figure 1. Study area and GPS location of sampling sites

b) Morphometric descriptors

To describe the morphology of the individuals, classical parameters were applied. In the laboratory, all shells were first cleaned and dried, then morphometric measures were taken on every individual using a dial caliper with a resolution of 0.01 mm. Bivalve morphometric analysis was based on the measurement of the three shell axes. This involves the measurement of shell length (L), shell height (H) and shell width (W).Shell length (L) was defined as the longest distance from front edge to back edge (mm) from the lateral view. Shell height (H) was defined as the height from the tip of umbo to the ventral margin from the lateral view. Finally, shell width (W) was defined as the longest distance of the valve in a lateral plane across the valve (mm) obtained from ventral view.

In addition, shell weight (SW, empty shell weight without organism), wet weight (WW, weight of the organism alone), total weight (TW, weight of shell and organism) and dry weight (DW, weight after flesh drying) of the specimens from the different sites were all determined using an electrical digital balance with 0.01 g accuracy. In order to further analyze and characterize bivalve morphology, diverse morphometric indices such as elongation index (Height/Length), compactness or roundness index (Width/Length) and convexity index (Width/Height) were determined for each individual.

Furthermore, three different ratios were defined involving the shell weight and successively the three dimensions (L, H, and W) with weight ratio 1 (SW/L), weight ratio 2 (SW/H), and weight ratio 3 (SW/W). The shape of the valve from lateral view was identified as follows: oval (L/H <1.5); elliptical (L/H = 1.5-2.0) and elongate or sub-trapezoidal (L/H \geq 2.0) [23].

c) Descriptive statistics

The descriptive analysis was carried out using SPSS software version 22. The average ratios H/L (elongation), W/L (compactness) and W/H (convexity) were compared according to the origin of the shell. The normality of the distribution of the shell dimensions was verified.

d) Analysis of variance

In order to compare the mean values, an univariate analysis was used. It consists of descriptive analysis of a single variable or several variables considered independently. An analysis of variance (ANOVA test) was used to analyze the variance of four morphometric indexes (Elongation index = H/L; Compactness index = W/L; Convexity index = W/H; weight ratio=SW/L) for the four populations studied with p<0.05.

e) Principal component analysis

Principal component analysis PCA is a data compression method allowing geometric representations of individuals and features. It builds artificial and graphical representations allowing visualizing relationships between variables as well as the possible existence of groups of individuals and groups of variables [24]. PCA was performed on six morphological variables (H/L, W/L, W/H, SW/L, SW/H, and SW/W) to find significant differences or similarities in shape of the specimens collected from the four studied sites.

f) Biometric relationships

The growth patterns of the studied populations (allometric or isometric) were analyzed through a curvefitting procedure. A linear regression of the log-transformed values of our measurements was used by fitting the points to the allometric equation:

 $Y = a X^b$

Where:

Y = body weight (mg).

X = shell height or shell length or shell thickness (mm).

a = constant and equal to the intercept of the straight line with Y axis (initial growth coefficient)

b = the slope (relative growth rates of the variables)

This equation can be expressed in its linearized form: Log Y = Log a + b Log X. The association degree between the variables was calculated by the determination coefficient (R²). A t-test (Ho, b = 3) was performed with a confidence level of 95 % to check if the values of b obtained in the linear regressions were significantly similar to the isometric value (b = 3), expressed by the following equation: $t_s = (b - 3)/S_b$ [25], where t_s is t-test value, b is slope and S_b is the standard error of the slope (b). Statistical significance of b values indicates isometric form when b is equal to 3, negative allometric if (b) value < 3 and positive allometric if (b) value >3 [24].

RESULTS

Descriptive statistics

Descriptive statistics for each of the morphometric markers are given in Table 1. For the 235 individuals, the mean total length of *R. decussatus* ranged between 31.92- 44.55 mm for the four analyzed groups. The highest mean values for shell Length (L) and height (H) were recorded for clams collected from Ksiba (44.55 mm and 20.19 mm, respectively). The three other sites had almost close mean values (16-17 mm). Likewise, the bivalves of Ksiba had the highest mean value of width (31.06 mm). The total weight shellfish samples ranged between 6.17 g and 15.02 g and the highest shell weight was recorded for the samples collected from Ksiba. The measurement of shell and dry weights showed that the lowest mean value was obtained in Sokrine population (3.92 g and 0.27 g, respectively).

Morphometric descriptors	Parameters	s Sites			
		Khniss	Sokrine	Ksiba	Bkalta
L	Min-Max (mm)	26.88-52.65	25.82-39.12	35.77-54.05	30.64 - 44.54
	Mean ± SD	35.78 ±7.04	31.92±2.53	44.55±4.28	36.75±3.96
н	Min-Max (mm)	11.80-24.52	12.32-23.90	15.88-24.05	15.09-22.70
	Mean ± SD	16.05±3.80	16.99±3.22	20.19±2.03	17.76±2.23
W	Min-Max (mm)	18.74-37.15	17.48-31.98	25.10-37.55	23.19-32.60
	Mean ± SD	24.65±5.47	24.05±2.45	31.06±2.88	26.45±2.82
тw	Min-Max (g)	2.62-20.88	1.71-11.17	6.83-22.33	4.89-19.57
	Mean ± SD	7.75±5.73	6.17±2.09	15.02±4.18	8.77±3.86
SW	Min-Max (g)	1.69-14.90	1.94-6.79	1.90-15.39	3.68-16.75
	Mean ± SD	5.37±4.22	3.92±1.19	9.38±2.87	6.98±3.34
DW	Min-Max (g)	0.12-0.87	0.12-0.61	0.24-1.43	0.11-0.69
	Mean ± SD	0.37±0.20	0.27±0.09	0.56±0.21	0.32±0.13

Table 1. Morphometric shell parameters of *R. decussatus* sampled from four populations in Monastir Bay.

*L: shell length, H: shell height, W: length of hinge plate, TW: total weight, SW: shell weight, DW: dry weight.

The morphometry descriptors showed that the harvested clams presented various shapes. The clams specimens collected from the site of Khniss with sub-trapezoidal shape were more abundant (97.61%) than the clams with elliptic shape. The same remark was true for the populations of Ksiba and Bkalta, for which the majority of specimens had a sub-trapezoidal shape (97.29% and 86.66%, respectively).For the Sokrine population we have noted three distinct sub-populations with sub-trapezoidal shape (59.55%), oval shape (24.71%), and elliptical shape (15.73%), respectively.

The size class distribution of the collected clams showed a size range between 25 and 55 mm, defining six classes. The population structure of *R. decussates* for the four studied sites is shown in figure 2. A variation in the dominant size between sites was recorded, with the dominant class size [30-35 mm] at Sokrine (70.78%), Khniss (47.62%) and Bkalta (40%) sites. Conversely, larger clams [45-50 mm] were dominant only at Ksiba site (41.89%).





ANOVA analysis

The variance analysis of the morphometric indices (elongation, compactness, convexity and density) for the four populations indicated significant differences among sites (p=0<0.05). For the 235 individuals, the mean values of elongation (H/L), compactness (W/L) and convexity (W/H) indices are given in table 2. Distributions of these morphometric parameters appeared to vary among sites. The comparison of the mean value of elongation index showed that the specimens from Khniss site had the lowest mean index (0.446 ± 0.028), which suggested that the shells were slenderer from a lateral view than those from Sokrine (0.536 ± 0.114 mean index). In terms of compactness, the specimens from Sokrine displayed the highest mean value of compactness index, compared to Bkalta, Ksiba and Khniss, suggesting that the dome of the valve is more curved from a ventral view in this site. In addition, individuals from Khniss and Ksiba sites had the highest mean convexity index (1.542 ± 0.077), confirming more convex shells from frontal view than specimens from Bkalta and Sokrine.

The mean weight ratios (SW/L; SW/H and SW/W) regarding weight and linear variables of shell valves, were different between sites and ranged from 0.123±0.037 to 0.460±0.119. The greatest mean ratios were recorded in Ksiba site followed by Bkalta, showing that the valves of Ksiba population were heavier than the valves of the other populations.

Parameters	Sites	Minimum	Mean	SD	Maximum	F	Р
Elongation index (H/L)	Khniss	0.398	0.446	0.028	0.503	22.475	0.00*
	Sokrine	0.369	0.536	0.114	0.763		
	Ksiba	0.364	0.453	0.025	0.516		
	Bkalta	0.442	0.482	0.023	0.535		
Compactness index (W/L)	Khniss	0.630	0.686	0.023	0.750	18.876	0.00*
	Sokrine	0.540	0.757	0.094	1.187		
	Ksiba	0.627	0.697	0.022	0.751		
	Bkalta	0.681	0.720	0.024	0.800		
Convexity index	Khniss	1.402	1.542	0.077	1.700	9.787	0.00*
(W/H)	Sokrine	0.802	1.444	0.177	1.920		
	Ksiba	1.311	1.542	0.098	2.035		
	Bkalta	1.353	1.493	0.049	1.588		
Weight ratio1 (SW/L)	Khniss	0.062	0.136	0.078	0.302	36.155	0.00*
,	Sokrine	0.070	0.123	0.037	0.206		
	Ksiba	0.039	0.207	0.051	0.332		
	Bkalta	0.111	0.184	0.067	0.376		
Weight ratio2	Khniss	0.143	0.299	0.159	0.629	64.958	0.00*
(SW/H)	Sokrine	0.159	0.228	0.040	0.378		
	Ksiba	0.088	0.460	0.119	0.742		
	Bkalta	0.236	0.378	0.128	0.753		
Weight ratio3	Khniss	0.090	0.196	0.107	0.434	49.731	0.00*
(SW/W)	Sokrine	0.096	0.161	0.039	0.333		
	Ksiba	0.055	0.298	0.074	0.465		
	Bkalta	0.158	0.255	0.093	0.514		

 Table 2. Variance analysis of morphometric index for the four studied populations of Ruditapes decussatus (n=235)

 Parameters
 Sites
 Minimum
 Mean
 SD
 Maximum
 F
 P

M - Mean, SD- Standard deviation, F - Fisher test, P - p-value, * - significant, p<0.05.

Principal component analysis

The principal component analysis (PCA) further evidenced morphological variability by providing a spatial visualization of the morphometric variables displayed by *R. decussatus* in the studied sites. The eigen values and their contribution to the total variance indicated a small number of components: 2 PC's axes explained about 84.278% of total variance. The first PC axis explained about 47.498% of the total variation while approximately 36.780% of total variance was explained by PC2 among the samples (Table 3).

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Component	Total	% of variance	% cumulative
1	3,325	47,498	47,498
2	2,575	36,780	84.278

Eigenvectors of the two retained components showed that elongation index, compactness and convexity were loaded on the axis 2. Weight linear ratios were loaded on axis 1 (Figure 3). The first component loadings were strongly positive for morphometric variables (weight ratios). The second axis was positively correlated with compactness and elongation index. In contrast, the convexity index was negatively correlated with this axis. These results indicated that, the PC2 was relative to the variation of slenderness and shape of valve (shell size) whereas the PC1 accounted for variation in heaviness.



Figure 3. Principal component plots of biological variables for the four populations of *R. decussatus*.

Loading plot derived from the PCA for the morphometric indices (Figure 4) revealed that Sokrine population was divided into two distinguishable sub-populations. Group 1 is well separated on the left. The group 2, including Bkalta, Ksiba and Khniss showed a significant overlapping on variate axis.



Figure 4. Loading plot derived from the PCA for the morphometric indices.

Biometric relationships

Allometric relationships established between weight (total and shell), shell length, width and height of *R. decussatus*, along the four sites, are given in table 4. The regression analysis showed highly significant correlations (p<0.001) between various correlates. All morphometric relationships displayed by bivalve from Bkalta site consistently presented positive allometric growth. Conversely, Sokrine site showed a negative allometry for all relationships established.

The obtained results revealed that the determination coefficients (R^2) were invariably higher for Khniss site than other sites. The lowest R^2 values were shown in Sokrine site especially in TW/W relationship (R^2 = 0.620). The relationship between total weight and shell length recorded a positive allometry for Khniss and Bkalta sites. No allometric relationship between weight and Length has been found for Sokrine population. In terms of TW/W, the morphometric relationships presented isometric growth for the sites of Khniss and Ksiba. Nevertheless. The relationship TW/H (range: 0.731–0.968) presented a negative allometry for the same sites (Khniss and Ksiba). Among the morphometric relationships, negative allometries (10 out of 22 ratios; 45.45%) and positive allometries (9 out of 22 ratios; 40.9%) were much more frequent than isometries (3 out of 22 ratios; 13.63%).

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Sites	Allometric	Regression equation	Determination	Type of growth
	relation		coefficient (R ²)	(t- test)
	TW/L	TW=0.000031 L ^{3.427}	0.963**	(+)Allometry
Khniss	SW/L	SW=0.000014 L ^{3.532}	0.937**	(+)Allometry
	TW/W	TW=0.000322W ^{3.097}	0.982**	Isometric
	SW/W	SW=0.000149W ^{3.216}	0.949**	(+)Allometry
	TW/H	TW=0.002329 H ^{2.866}	0.968**	(-)Allometry
	SW/H	SW= 0.001102H ^{2.955}	0.947**	Isometric
Sokrine	TW/W	TW=0.001715W ^{2.572}	0.620**	(-)Allometry
	TW/H	TW=0.066470H 1.589	0.814**	(-)Allometry
	SW/W	SW=0.000665W ^{2.716}	0.757**	(-) Allometry
	SW/H	SW=0.076153 H ^{1.384}	0.719**	(-) Allometry
Ksiba	TW/L	TW=0.00042 L ^{2.746}	0.747**	(-) Allometry
	SW/L	SW=0.000241 L ^{2.779}	0.771**	(-) Allometry
	TW/W	TW=0.000341 W ^{3.096}	0.804**	Isometric
	SW/W	SW=0.000599 W ^{2.808}	0.742**	(-) Allometry
	TW/H	TW=0.004649 H ^{2.675}	0.731**	(-) Allometry
	SW/H	SW=0.004521 H ^{2.536}	0.714**	(-) Allometry
Bkalta	TW/L	TW=0.000028 L ^{3.487}	0.837**	(+)Allometry
	SW/L	SW=0.000014 L ^{3.611}	0.813**	(+)Allometry
	TW/W	TW=0.000045 W ^{3.71}	0.910**	(+)Allometry
	SW/W	SW=0.000016 W ^{3.929}	0.930**	(+)Allometry
	TW/H	TW=0.000799 H ^{3.213}	0.922**	(+)Allometry
	SW/H	SW=0.000429 H ^{3.346}	0.924**	(+)Allometry

TW= Total Weight (g), SW= Shell weight (g), L = Shell Length (mm), W= Shell Width, H=Shell Height, (**) = p < 0.001.

DISCUSSION

The bivalves exhibit a wide morphological variation, which is intimately linked to their life cycle and ecology. The current study provides for the first time a wide informative groundwork on the morphometric relationships of the *R. decussatus* from Monastir bay sites. Four populations collected along Monastir bay coast were analyzed using statistical and analysis techniques on shape and size growth parameters. The comparison of the morphometric traits of *R. decussatus* revealed morphometric variation among the four populations. The shape analysis of the harvested clams showed the dominance of the sub-trapezoidal shape. Similar studies for the species Ruditapes philippinarum showed different results with oval shell [23] or round and globular predominant shapes [7]. Therefore, shape differences found could be explained as the outcome of the phenotypic plasticity of the populations subjected to different environmental conditions [26]. Population structure of the clams Ruditapes decussatus revealed that the size class [30-35] mm was dominant at three sites: Khniss, Sokrine and Bkalta, suggesting that they are made of a relatively young population, which has only recently established itself in these sites. A similar study conducted by Derbali and coauthors [17] showed that the majority of specimens collected along Sfax coasts (South of Tunisia) belonged to size classes [15-31] mm, which represented 79.15% of total samples. The dominance of larger clams for Ksiba population could be explained by the difference in fishing frequency in addition to the pollution effect and the presence of predators (crabs) in this site. These factors would have led to the extinction of the youngest individuals at this site. The similarity in mean values of elongation, convexity and compactness indices for the two sampled sites Khniss and Ksiba could be explained in terms of the geographical proximity of the two populations, which lie just a few kilometers apart. In fact, the two sites have been shown to have the same type of sediment (medium and fine sands) [22]. The comparison of the same indices revealed a considerable inter-population variability between Khniss and Sokrine populations. Significant heterogeneity was revealed between the studied populations when we compared the variances of the four-morphometric indices using Fisher test. Amane and coauthors [8] pointed out similar results for the same species R. decussatus from Morocco coastal region.

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To better understand the growth of bivalve species, the knowledge of allometry in shell and soft body characters is essential [27]. Studies were undertaken for morphometric relationship for different bivalve species in Tunisian coastal areas [28,29] but rare morphometric studies were established for R. decussates in central eastern coast. As revealed by the present study, high correlation coefficients were registered in the morphometric relationships established between linear and ponderal variables. This result indicated that the measurement of total or shell weight is an adequate predictor of shell length, shell height and shell width. Moreover, the highest correlation of morphometric relationships at Khniss and Bkalta sites, indicating lower variability in shell shape in terms of shell and total weight. The TW/L positive allometric relationship obtained for Khniss and Bkalta sites was in agreement with findings reported by Tenging [24] for the wedge clams Donax faba at the southwest coast of India. In contrast, for Ksiba population the length-weight relationship showing negative allometric growth patterns denotes that weight-gaining rates are relatively higher than the length increment rate. Similar findings were recorded by previous studies conducted for some Egyptian clams, namely Venerupis aurea, Ruditapes decussates [18] and Callista chione [30]. In Sokrine site, R. decussatus exhibited also negative allometric growth in all established morphometric relationships. Similar results were also obtained for three populations of Marcia opima collected from Mannar lagoon in Sri Lanka [31]. According to Vasconcelos and coauthors [32], the prevalence of negative allometries can reflected elongated, narrow and light shells, which is probably related to their active burrowing behavior into sandy sediments. Therefore, the morphometric relationships established between linear and ponderal variables of *R. decussatus* constitute accurate tools for mutual conversion between those linear and ponderal variables. The application of the principal component analysis (PCA) overview allowed for separating the populations in groups with more dissimilar shell shapes as a function of their morphometric characteristics. We have recorded two groups for Sokrine population, which indicated morphological variability among the same population. The PCA differentiated Sokrine group1 from the other groups, which was distinguished by thinner shell with less convex shape. This is reflecting the different specific morphological adaptations of these clams for coping with particular environmental conditions.

CONCLUSION

This is the first morphometric study of the European clam *R. decussates* in central eastern Tunisian coastal area. The analysis of morphometric indices showed morphological differences among sites and, in some cases, within the same site. The sub-trapezoidal shape was dominant with small size class in most sites. Allometric relationships showed highly significant correlation with different growth patterns (positive allometric, negative allometric, isometric) depending on morphometric variables and studied sites. Our results provide baseline data on the morphological variations and can be useful for adequate stock management in aquaculture fields. Indeed, recognition of bivalve variability is important for the effective culture of these organisms in this area. The presence of this species in this zone can be promoted through creating opportunities to open new aquaculture projects. Finally, further morphometric analysis tools and molecular genetic markers can be proposed to confirm phenotypic variation of this species.

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