Morphological variations of the winged pearl oyster *Pteria penguin* (Roding, 1798) from South China Sea

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ABSTRACT

The present study was undertaken to evaluate the morphological variations of *Pteria penguin* (Roding, 1798) in wild as well as cultured populations from South China Sea. A total of 307 samples were collected from five different geographic locations comprising three wild (Pop. 1, 2 and 4) and two cultured (Pop. 3 and 5) populations and 10 measured traits were analysed. Principal component analysis (PCA), discriminant analysis and cluster analysis were used to evaluate the morphological variations in the collected samples. Results showed a low intra-population variation within the five sampled populations. Multivariate analyses indicated that the five populations were more or less differentiated on the basis of body characters, particularly those related to major dimensions of the shell outline and their ratios. Discriminant analysis revealed that the overall random assignment of individuals into their original groups was high (81.8%), indicating that these populations are highly divergent from each other. The proportion of individuals correctly classified into their original groups was 96.9, 76.7, 75.0, 78.0 and 82.0% for populations 1, 2, 3, 4 and 5 respectively. Cluster analysis showed highest morphological variation between Pop.1 and Pop. 2. All other populations (Pop.3, Pop.4 and Pop.5) were found to be morphologically similar and consistent with the respective geographical location. Results of the present study will be of use in resource assessment and management of *P. penguin* in the South China Sea.

Keywords: Geographical populations, Morphometric variation, Multivariate analysis, *Pteria penguin*

Introduction

Morphological variation is considered as one of the basic characteristics to assess the population differences in aquatic organisms. Measurement, description and analysis of the morphological variations are fundamental steps to answer questions of biological adaptability (Ge and Hong, 1995). Due to genetic differences or environmental factors, morphological characters of aquatic animals are often varying along with geographic gradient among wide-ranged coastal marine animals (Tzeng *et al.*, 2001; O'Reilly and Horn, 2004; Murta *et al.*, 2008). Intra-specific variation in animals is usually regarded as the adaptive mechanism to different environments (Orensanz *et al.*, 1991; Cadrin, 2000; Tzeng *et al.*, 2001; Marquez *et al.*, 2010). Studies on the morphological variations of target species can reveal the dynamics of populations and such information can be used to design management strategies (Orensanz *et al.*, 2005). As one of the solid evaluation parameters, morphological variation has been widely used in population studies (Murta, 2000; Tzeng *et al.*, 2001; Chen *et al.*, 2010), stock discrimination (Cadrin

et al., 2005; Turan *et al.*, 2006; Murta *et al.*, 2008) and bio-geographical evaluation (Von *et al.*, 2005). In molluscs, shell shape variation has been reported to distinguish species of similar shape (Innes and Bates, 1999; Aguirre *et al.*, 2006; Rufino *et al.*, 2006; Costa *et al.*, 2008, 2010) or to analyse intra-specific variation along a wide geographical range (Palmer *et al.*, 2004; Krapivka *et al.*, 2007; Marquez *et al.*, 2010).

The winged pearl oyster *Pteria penguin* (Roding, 1798) (family: Pteriidae), is widely distributed in Western Pacific Ocean and Indian Ocean (Southgate *et al.*, 2008). In South China, *P. penguin* is traditionally used for production of half-pearls ('mabé'). *P. penguin* is considered as an important pearl oyster species in Guangdong, Guangxi and Hainan provinces of China due to its high economic value (Qi *et al.*, 2011). Recently, as consequence of overfishing and habitat deterioration, the natural availability of *P. penguin* has reduced significantly. Moreover, the germplasm resources of *P. penguin* has changed considerably due to the long-term culture of the species without proper

germplasm management. Therefore, it is necessary to formulate effective germplasm management strategies for *P. penguin*. Recent studies on *P. penguin* focused on aspects like hatchery production (Teitelbaum *et al.*, 2008; Matthew and Southgate., 2012), biofouling (Guenther and De Nys, 2006), polychaete verminosis (Liang *et al.*, 2007), pearl production (Kripa *et al.*, 2008; Kishore *et al.*, 2013), pearl shell lectin (Naganuma *et al.*, 2006), gametogenic process (Arjarasirikoon *et al.*, 2004) and *Pteria* toxins (Takada *et al.*, 2001). Information on the morphological variations in *P. penguin* has not been reported so far. In the present study, 10 morphological characters of *P. penguin* within five established populations from the South China Sea were analysed with the objective of estimating morphometric variability among different populations.

Materials and methods

A total 307 samples of *P. penguin* were collected from three wild populations, from Sanya Bay, Beihai Bay

and Lingao Bay (designated as Pop. 1, Pop. 2 and Pop. 4 respectively) and two cultured populations (from Xuwen Bay and Li'an designated as Pop. 3 and Pop. 5 respectively) in the South China Sea during October 2013 and June 2014 (Fig. 1, Table 1). All samples were transported live in cool moist condition to the Tropical Fisheries Research and Development Center, South China Sea Fisheries Research Institute, Hainan Province, P. R. China. The samples were scrubbed and washed thoroughly in seawater to remove the fouling organisms and silt, before taking morphometric measurements. Morphometric measurements were taken using a digital vernier caliper (Hangzhou) accurate to 0.01 mm. A total of 5 landmarks and 7 distances were measured (Fig. 2). These landmarks were selected based on the shell structure of *P. penguin*. Further, those landmarks which can be easily observed and assessed by naked eye were selected (Nie *et al.*, 2013). In addition to these, shell length (SL), shell height (SH) and shell width (SW) were also recorded. Linoy Libini *et al.* (2011) reported that the

Fig. 1. Map showing the location of study sites (●: sampling sites)

Table 1. Sampling details of *P. penguin* used in the study

Populations	Locality	Geographic coordinates	Sample size
Pop. 1	Sanya, Hainan Province, China	18°25'N, 109°39'E	65
Pop. 2	Beihai, Guangxi Province, China	21°07'N, 109°11'E	90
Pop. 3	Lingao, Hainan Province, China	20°04'N, 109°63'E	52
Pop. 4	Lingshui, Hainan Province, China	18°43'N, 110°06'E	50
Pop. 5	Xuwen, Guangdong Province, China	20°43'N, 109°94'E	50

Pop. = Population

Fig. 2. Profile of *P. penguin* showing details of morphometric measurements taken on each individual (right shell in the upper side)

SL: shell length; SH: shell height; SW: shell width; Locations of the other 8 landmarks for constructing the truss network are illustrated as numbers and morphometric distance measures between the dots as lines

hinge length in *P. penguin* becomes an unreliable measure, as the posterior ear tips are often broken during collection. Therefore, only samples with undamaged shell, except the posterior ear tips were included and analysed in this study.

Statistical analyses were performed with SPSS 13.0 software (SPSS Inc., Chicago, USA). To remove the effect of size and age, all morphometric characters were standardised according to the equation $L_s = L/SL$, where L_s = standardised measurement, L = measure traits and SL = shell length of each specimen (Wang *et al.*, 2009).

Descriptive statistics including mean ($\bar{X}$), standard deviation (SD) and coefficient of variation ($CV = SD/\bar{X}$) of each morphological character within and among populations were calculated using EXCEL 2003 (Microsoft Corporation, Redmond, WA, USA).

Principal component, discriminant factorial and hierarchical cluster analyses were used in this study. Principle component analysis (PCA) was used to reduce the amount of morphometric data and evaluate morphometric variation among specimens and to identify variables responsible for this (Veasey *et al.*, 2001; Samaee *et al.*, 2006, 2009). All PCAs with Eigen value >1 were considered as important (Chatfield and Collins, 1983). Use of the correlation matrix in PCA, in this study, allowed a direct interpretation of character loadings (>0.80) and a direct comparison between populations.

Discriminant function analysis (DFA) was conducted to test the effectiveness of the characters in predicting different group locations, which clarify

the relative importance of such traits as discriminators between *a priori* for groups, as performed by Kong *et al.* (2007) and Konan *et al.* (2010). A forward stepwise function analysis was carried out to reduce the number of variables (Jain *et al.*, 2000; Poulet *et al.*, 2004; 2005) and to identify the combinations of variables which separate groups (Hair *et al.*, 1996). The classification success rate was evaluated based on the percentage of individuals correctly assigned in to original sample (Silva, 2003; Marques *et al.*, 2006). The relative importance of meristic and morphometric characters in discriminating populations was assessed using the F-to-remove statistic (F-to-enter, 3.84; F-to-remove, 2.71) (Turan *et al.*, 2006). A complement to discriminant analysis *i.e.*, hierarchical cluster analysis (HCA) based on Euclidean nearest neighbour was used to test whether there was any correlation between morphological and geographical distances (Turan *et al.*, 2006; Ferrito *et al.*, 2007).

Results and discussion

The present study aimed to assess the morphological variations in different geographic populations of *P. penguin* in South China Sea. The parameters of measured traits are given in Table 2. The coefficients of variation (CVs) of most measured traits from Pop.1 were higher than the other four populations. The highest CVs of D (1-2) was obtained in Pop. 4 (19.82%), while the lowest value (4.18%) was recorded in D (1-3) from Pop. 3. The CVs of all measured traits from five different populations were relatively low ($CV < 20\%$, Table 2). Results from the present study showed that intra-population variation in morphometric characters were found to be low for the five geographic populations tested. This was proved by low values of coefficient of variation ($CV < 20\%$) for all measured variables and justified that a phenotypically homogeneous group was there within each population (Ferrito *et al.*, 2007; Konan *et al.*, 2010). Recent reports indicated that low values of CV may justify high inheritability (Mamuris *et al.*, 1998) and consequently limited influence of environmental variation on morphological variability (Soule and Cuzin-Roudy, 1982). Results of the present study indicated that all the five populations were with low variation and morphologically not much influenced by environmental conditions.

The results of PCA showed that a total of 68% of the total variation associated with the 10 morphometric characters were accounted for the first two principal components in the tested populations, which explain the variability manifested between individuals (Table 3). The characteristics with an Eigen vector of >0.80 were included and the others discarded. PC1 loadings explained 53.84% of the total variations, while only

Table 2. Descriptive statistics of various morphological characters within five populations of *P. penguin*

Variable		Pop. 1	Pop. 2	Pop. 3	Pop. 4	Pop. 5
SL	Mean $\pm$ SD	127.21 $\pm$ 14.44	120.11 $\pm$ 9.56	93.26 $\pm$ 6.59	89.57 $\pm$ 5.73	90.11 $\pm$ 5.34
	Range	82.24 - 162.60	91.16-145.5	73.52-126.21	74.42-104.41	74.31-104.46
	CV (%)	11.35	7.96	7.07	6.40	5.92
SH	Mean $\pm$ SD	175.04 $\pm$ 18.85	155.32 $\pm$ 12.38	114.48 $\pm$ 5.94	110.16 $\pm$ 6.81	113.05 $\pm$ 7.90
	Range	175.04 - 225.08	115.16-190.00	97.85-129.11	92.39-131.05	86.31-162.60
	CV (%)	10.77	7.97	5.19	6.18	6.99
SW	Mean $\pm$ SD	54.9 $\pm$ 5.79	62.09 $\pm$ 4.11	45.75 $\pm$ 2.98	49.62 $\pm$ 3.09	46.65 $\pm$ 2.77
	Range	23.37 - 71.23	51.32-77.22	37.98-51.89	36.30-57.34	39.43-56.12
	CV (%)	10.54	6.62	6.50	6.22	5.94
D (1-3)	Mean $\pm$ SD	231.86 $\pm$ 23.90	204.80 $\pm$ 15.73	142.60 $\pm$ 5.96	139.46 $\pm$ 7.82	140.95 $\pm$ 8.32
	Range	157.55 - 285.99	155.05-250.82	122.61-174.44	120.02-160.55	115.43-173.12
	CV (%)	10.31	7.68	4.18	5.60	5.90
D (1-4)	Mean $\pm$ SD	112.06 $\pm$ 9.56	105.32 $\pm$ 10.25	78.82 $\pm$ 5.16	84.57 $\pm$ 5.71	79.97 $\pm$ 4.09
	Range	88.00 - 133.31	81.45-238.40	66.23-121.52	69.88-101.60	70.29-90.48
	CV (%)	8.53	9.73	6.55	6.75	5.11
D (2-3)	Mean $\pm$ SD	250.29 $\pm$ 24.45	221.15 $\pm$ 16.33	153.53 $\pm$ 6.66	149.04 $\pm$ 9.25	149.28 $\pm$ 8.03
	Range	170.60 - 250.29	167.47-273.47	132.37-187.09	129.38-170.23	130.31-180.07
	CV (%)	9.77	7.38	4.34	6.21	5.38
D (2-4)	Mean $\pm$ SD	137.77 $\pm$ 12.08	124.26 $\pm$ 8.86	95.95 $\pm$ 6.90	97.02 $\pm$ 7.46	90.66 $\pm$ 4.30
	Range	105.97-168.38	94.24-146.99	77.64-150.31	80.51-122.07	79.83-103.71
	CV (%)	8.77	7.13	7.20	7.69	4.74
D (1-2)	Mean $\pm$ SD	30.42 $\pm$ 5.52	30.02 $\pm$ 3.96	19.38 $\pm$ 3.60	17.50 $\pm$ 3.47	14.12 $\pm$ 1.93
	Range	12.91-47.88	16.28-40.02	8.76-27.79	9.80-26.35	8.51-18.88
	CV (%)	18.13	13.20	18.59	19.82	13.68
D (1-5)	Mean $\pm$ SD	108.13 $\pm$ 9.39	107.46 $\pm$ 12.47	87.98 $\pm$ 7.46	81.33 $\pm$ 5.51	78.44 $\pm$ 4.51
	Range	84.12-131.73	81.12-149.54	40.09-109.74	68.15-99.84	63.20-89.82
	CV (%)	8.69	11.61	8.48	6.78	5.75
D (2-5)	Mean $\pm$ SD	136.76 $\pm$ 11.89	125.77 $\pm$ 9.4	84.87 $\pm$ 10.81	95.76 $\pm$ 7.39	88.61 $\pm$ 4.20
	Range	107.39-163.25	101.28-155.00	62.14-115.70	78.55-122.67	78.08-100.90
	CV (%)	8.70	7.47	12.74	7.71	4.74

D (number a - number b): Distance between two landmarks; SL: Shell length; SH: Shell height; SW: Shell width; SD: Standard deviation; CV: Coefficient of variation; Pop.: Population

Table 3. Contributive proportion and loading of principal components on the morphological characteristics of *P. penguin*

Characters	Principal components	
	1	2
SH/SL	0.394	0.070
SW/SL	0.480	0.604
D(1-3)/SL	0.764*	0.060
D(1-4)/SL	0.915*	-0.178
D(2-3)/SL	0.796*	-0.098
D(2-4)/SL	0.941*	0.036
D(1-2)/SL	0.151	0.871*
D(1-5)/SL	0.823*	-0.327
D(2-5)/SL	0.906*	-0.02
Eigen value	4.845	1.282
Variance (%)	53.837	14.239
Cumulative variance (%)	53.837	68.076

*Indicates that loading value exceeds 0.75; D (number a - number b): Distance between two landmarks; SL: Shell length; SH: Shell height; SW: Shell width.

14.24% of the total variation was explained by PC 2 (Table 3). PC 1 of the analysis was related to major dimensions of shell outline, including D(1-3)/SL, D(1-4)/SL, D(2-3)/SL, D(2-4)/SL and some traits of shell hinge, such as D(1-5)/SL and D(2-5)/SL. In PC 1, the most important character was D(2-4)/SL, with contribution of 94.1%, D(1-4)/SL followed with the value of 91.5%. The traits loading on PC 2 was only intensively related to the anterior ear tips and D (1-2)/SL had the maximum contribution (87.1%). Recent studies have reported that dorso-ventral traits are used as a measure of growth index for *P. penguin* (Linoy Libini *et al.*, 2011; Milione and Southgate, 2011, 2012), which indicated that the major morphological characters for the bivalves reflect both phylogenetic history and life habits (Gordillo *et al.*, 2014). The scores of visual examination of the plots through PC1 and PC2 are presented in Fig. 3. All populations were located in the middle group of the first PC and the second PC

(Fig. 3). The shell shape traits used in the present study have shown to provide an insight into the discrimination of different populations of *P. penguin*.

Discriminant function analysis (DFA) could be a useful method to distinguish different stocks of the same species (Murta *et al.*, 2008). In the present study, the DFA of morphometric characters produced four functions, which explained 100% of the accumulated population variance (Table 4). It was found that 87.9% of the total variance was contributed by the first and second canonical variables (67.80% and 20.10% respectively) indicating that the greatest proportion of the total variance was due to the first two canonical variables (Table 4). The stepwise discriminant analysis retained seven variables

Fig. 3. Scatter plot of principal components from the principal components analysis of different populations of *P. penguin*

that are most discriminant among the different populations (Table 5). These characters of primary importance in distinguishing among the five populations where D(2-3) ($\lambda = 0.52$; $F=71.23$; $p<0.001$), SW ($\lambda=0.237$; $F=79.38$; $p<0.001$), D(1-2) ($\lambda=0.179$; $F=60.55$; $p<0.001$), D(2-5) ($\lambda = 0.128$; $F = 54.90$; $p<0.001$), D(1-5) ($\lambda= 0.085$; $F=54.37$; $p<0.001$), D(2-4) ($\lambda=0.070$; $F=49.25$; $p<0.001$) and SH ($\lambda=0.066$; $F=42.81$; $p<0.001$). Summary statistics indicated that the coefficients SW/SL (D (1-2)/SL and D (2-5)/SL) contributed the most to the first 2 discriminant functions, respectively (Table 6). Based on DFA evaluation, the overall random assignment of individuals into their original groups was 81.8% (Table 7), which indicate a high degree of inheritance between populations in shell morphology in the study area. The most well-defined population was found to be Pop.1 with classified individual percentage of 96.90% (Table 7), followed by Pop. 5 (82%) and Pop. 4 (78%). The incorrect percentages in five populations ranged from 2 to 18% and the majority of misclassification proportion (18%) of Pop. 4 has morphometric characters similar to Pop. 5 (Table 7). In addition, only a proportion of 3.1% of Pop.1 was allocated to Pop. 2 and only 4.4% of Pop. 2 was classified as Pop. 1. The confusion matrix ranged from 13.5-18% and the misclassification proportion among Pop. 3, Pop. 4 and Pop. 5 were high. The scatter plots of DFA scores for the study are showed in Fig. 4. Visual examination of the plots of DFA 1 and DFA 2 scores indicated that selected samples are grouped into five major respective areas. This explained the efficiency of discriminator power of morphometric characters used. Indeed, a strong discriminating power of the morphometric variables was found for comparison

Table 4. Statistical analysis of first three discriminant functions for the ratios of the populations of *P. penguin*

Function	Value	Variance (%)	Cumulative variance (%)	Chi Square	DF	p
1	3.450	67.80	67.8	813.56	28	<0.001
2	1.020	20.10	87.9	365.66	18	<0.001
3	0.497	9.80	97.7	154.67	10	<0.001
4	0.119	2.30	100	33.60	4	<0.001

DF: Degrees of freedom; p: Probability value

Table 5. Discriminatory power of morphometric characters of individuals of *P. penguin* retained by step discriminant analysis

Variables	Wilk Lambda (λ)	F to enter/remove	Probability	Tolerance
SH/SL	0.070	42.805	***	0.615
SW/SL	0.237	79.384	***	0.698
D(2-3) /SL	0.515	71.230	***	0.371
D(2-4) /SL	0.070	49.253	***	0.437
D(1-2) /SL	0.179	60.546	***	0.649
D(1-5) /SL	0.085	54.367	***	0.528
D(2-5) /SL	0.128	54.902	***	0.348

*** $p<0.001$; D (number a - number b): Distance between two landmarks; SL: Shell length; SH: Shell height; SW: Sshell width

Table 6. Morphometric variables for *P. penguin* obtained using Wilk discriminant function

Characters	Function			
	1	2	3	4
SH	1.698	0.701	-4.976	-0.588
SW	-17.137	-7.373	13.948	-3.464
D23	7.316	-0.619	3.98	-4.613
D24	-2.524	3.311	-8.258	10.427
D12	8.126	15.454	14.527	10.708
D15	-3.673	8.476	-0.814	-1.23
D25	0.758	-14.697	-0.202	1.609
Constant	-3.203	4.57	-1.246	-3.131

Table 7. Results of the number of individuals classified and the percent in each group

Populations		Predicted group membership					
		Pop. 1	Pop. 2	Pop. 3	Pop. 4	Pop. 5	Total
Original grouping	Pop. 1	63	2	0	0	0	65
	Pop. 2	4	69	7	5	5	90
	Pop. 3	0	5	39	1	7	52
	Pop. 4	0	2	0	39	9	50
	Pop. 5	0	1	0	8	41	50
Percentage (%)	Pop. 1	96.9	3.1	0	0	0	100
	Pop. 2	4.4	76.7	7.8	5.6	5.6	100
	Pop. 3	0	9.6	75	1.9	13.5	100
	Pop. 4	0	4	0	78	18	100
	Pop. 5	0	2	0	16	82	100

A: 81.8% of original grouped cases correctly classified; Pop. : Population

Fig. 4. Scatter diagram for discriminant function 1 and discriminant function 2 of *P. penguin* from five different geographical populations

between populations (Murta, 2000; Garcia-Davila *et al.*, 2005; Ferrito *et al.*, 2007; Anastasiadou and Leonardos, 2008; Anastasiadou *et al.*, 2009). The differences found among the shape of shells could also be explained as the outcome of the phenotypic plasticity of the populations subjected to different environmental conditions (Marquez *et al.*, 2010).

Geographically, Beihai (Pop. 2), Lin'gao (Pop. 4) and Xunwen (Pop. 3) populations were found closer, while a shorter geographical distance was observed between Sanya (Pop. 1) and Lingshui (Pop. 5) (Fig. 1). Hierarchical cluster analysis revealed that the studied populations were clustered into two distinct groups (Fig. 5), demonstrating that there might be considerable morphological divergence among different geographical groups. This result indicated that Xunwen (Pop. 3), Lin'gao (Pop. 4) and Li'an (Pop. 5) populations are geographically close. The results speculated that the

Fig. 5. Dendrogram of *P. penguin* populations based on morphological characters

source of Li'an culture population might be Xunwen Bay. Sanya (Pop.1) and Beihai (Pop. 2) populations clustered into one group and the divergent distance between Pop.1 and Pop. 2 was significantly greater than the other populations (Fig. 5). The discriminant analysis also confirmed above results, which showed that the incorrect percentages between Pop.1 and Pop. 2 were <5% (Table 7). Recent study showed that the expression of phenotype is largely determined by the environment of the habitat (Brian *et al.*, 2006). But the causes of morphological differences among populations are often quite difficult to be explained (Poulet *et al.*, 2004). Variation in morphometric characters may be affected by genetic and environmental factors (Murta, 2000).

Although it is possible to suggest how variations in shell morphology of *P. penguin* could be adaptive in different environments, it is difficult to tease apart the possible explanations due to the complexity of the relationship between phenotype and the local physical and ecological conditions. Further verification of the stock structure may be essential by biological evidence, such as geometric morphometry and genomic analyses.

In this study, the shape analysis proved to be a powerful tool for stock discrimination of *P. penguin* in the South China Sea, representing this species with high probability of interbreeding. The present study demonstrates that five populations of *P. penguin* from different areas of the South China Sea are more or less discriminated on the basis of shape measurements related to D(1-4) and D(2-4). Results from the present study provide valuable information on the population identification of *P. penguin* in the South China Sea which can facilitate the development of management strategies, the design of biological sampling programs and strategies for conserving diversity of this species.

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