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Morphometric identification of mud crabs of the genus *Scylla* available in Bangladesh waters

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Abstract. There has been a lot of ambiguity in taxonomy of mud crab species of the genus *Scylla*. In order to identify and ascertain the number of species of genus *Scylla* available in Bangladesh waters, a total of 84 specimens were collected from five different coastal locations. Based on the morphometric analysis, the specimens were divided into three Morphotypes. The average carapace width of Morphotype 1, Morphotype 2, Morphotype 3 were 100.7±30.4, 113.3±21.3 and 95.6±17.3, respectively. On the other hand, in case of morphometric measurements, except two measurements (PD/PL, PL/ICW), 25 measurements out of 27 measurements such as LSH/ICW, CW/8CW, CL/ICW, PWC/ICW, FW/ICW, PWC/FW, FMSH/FW, FMSH/DFMS, DFLS/FW, DFLS/FW, DFMS/DFLS, SW/ICW, AW/SW, DL/PL, PW/PL, (PW*PD*0.7854)/PL, IPS/PL, OPS/PL OPS/PL, IPS/OPS, ICS/PL, OCS/PL, ICS/OCS, ML/PL, 5PW/5PL and 3 PML/ICW ratios were found to be significant at various level ($p<0.001$, $p<0.01$, and $p<0.05$). The variables were obtained via discriminant function analysis. The first three principal components (PC) accounted for 55.865% of the total variance (29.712% for PCI, 14.764% for PCII and 11.390% for PCIII). Component I contained ML/PL, FMSH/DFMS, DFMS/FW, LSH/ICW, DFLS/FW, PW/PL, CW/8CW and (PW*PD*0.7854)/PL; component II contained FW/ICW and IPS/OPS; component III contained IPS/PL, OPS/PL and 5PW/5PL. Sample centroids of discriminant function scores 100% of Morphotype 1 sample, 100% of Morphotype 2 sample, 100% of Morphotype 3 sample, 100% of Morphotype 4 sample and 100% of Morphotype 5 sample were correctly classified. In conclusion,three morphotypes identified resembling to three mud crab species such as *S. serrate, S. olivacea and S. paramamosain*. However, reliable molecular analysis can confirm if they are different species or the morphotypes are due to morphological plasticity.

Key words: *Scylla;* Mud crab; PCA; DFA; Taxonomy.

Introduction

Mud crab genus *Scylla* including (*S. serrata, S. olivacea, S. tranquebarica* and *S. paramamosain*) represent important commodity of coastal fisheries resources and valuable aquaculture practices in many countries. Mud crab is a usual inhabitant of mangrove areas, brackish or coastal waters along the shorelines, ponds and intertidal swamps in the coastal regions (Hoq 2008). The taxonomic classification of genus *Scylla* has been controversial worldwide for long time. Estampador (1949) studied external morphology and gametogenesis of mud crabs collected from

Philippines and classified the specimens into three species and one subspecies, namely *Scylla serrata, S. oceanica, S. tranquebarica* and *S. serrata* var. *paramamosain*. Serene (1952) categorized mud crab into two species from four forms in Vietnam. Stephenson and Campbell (1960) suggested only a single species named *S. serrata*. Joel and Raj (1983), Kathirvel and Srinivasagam (1992) reported the existence of *S. serrata* and *S. tranquebarica* from India on the basis of morphological characters. Keenan *et al.* (1998) extensively revised the taxonomy of the genus *Scylla* considering morphology, morphometric ratios and allozyme variability and classified this genus into four distinct species; *S. serrata, S. olivacea, S. tranquebarica*and *S. paramamosain*.

Most people usually consider that commonly *S. serrata* is found in Bangladesh. When people discuss about mud crab, a common species i.e. *S. serrata* come into their mind and don't even think of other species names under the genus *Scylla*. Many authors (Islam 1976; Khan and Alam 1992; Azam 1998; Obayed 1998; Kamal 2002; Zafar 2003) used the name *S. serrata* and a few (Kamal 2004 and 2007; Ahmed 2005) used *S. olivacea* in their studies. Various taxonomic studies, however, revealed ambiguity in the taxonomy and identification of *Scylla* species around the world (Joel and Raj 1980; Keenan *et al.* 1998; Padate *et al.* 2013). Interestingly, most researchers used this name without prior identification of the specific species. Its taxonomic status has been confused for decades and a new classification has only recently been proposed. So, confusion rises in addressing the specific species under the genus *Scylla*. Various researches regarding mud crab had been carried out in Bangladesh. The researches include taxonomy (Shafi and Quddus 1982), stock assessment (Chantarasri 1994), growth, recruitment and economic performance (Zafar *et al.* 2006), fattening technology (Kamal *et al.* 2007), harvesting technique (Hussain and Ahmed 2006), production, marketing and transportation (Kamal *et al.* 2004), culture and bio-economics (Khan and Alam 1992; Ferdoushi and Xiang-Guo 2010) of mud crab. Detailed taxonomic study however, yet has not been conducted to identify and address the specific *Scylla* species available in Bangladesh. Present study therefore, aimed to identify the *Scylla* species available in Bangladesh through morphological and morphometric analysis.

Materials and Methods

Sample collection: Eighty four fresh mud crab (*Scylla* spp.) samples were collected from commercial collectors of five locations in coastal zones of Bangladesh (Table I).

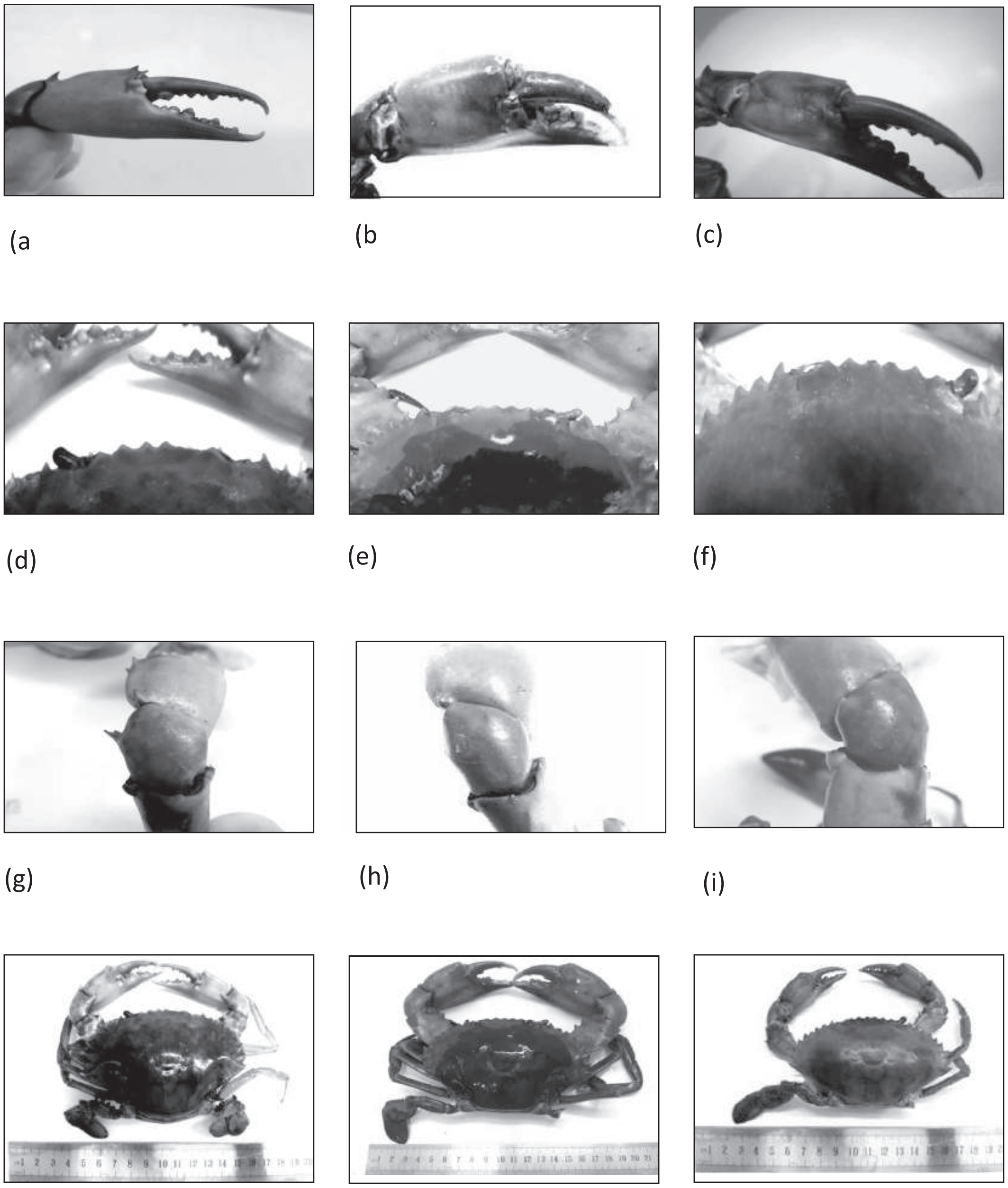
Table I. Sample sources, number of specimens and date of collection of *scylla* spp.

Sl. No.	Sample sources	No. of specimens	Longitude and latitude	Date of collection
1	Paikgacha	20	22.1°N, 90.3°E	27 February, 2016
2	Chakoria	14	22.1°N, 92.0°E	04 March, 2016
3	Cox's Bazar	28	21.3°N, 92.0°E	05 March, 2016
4	Patuakhali	12	22.3°N, 89.2°E	07 May, 2016
5	Mongla	10	22.3°N, 89.3°E	08 May, 2016
	Total		84	

Morphological analysis: Propodus spines, frontal lobe spines, carpus spines, polygonal patterning and coloration of the carapace of the specimens were critically studied and used to distinguish the specimens into three Morphotypes (Fig. 1. and Table II).

Morphometric analysis: Twenty four morphometric characters (Table III) from carapace, frontal spines, chelipeds, pereopods and sternum were measured by using digital slide-calipers, screw-gauge, normal centimeter scale, divider and forceps. Subsequently, a total of 27 ratios, 13 related to carapace, 12 related to chelipeds and two related to pereopods were derived for morphometric analysisas described by Keenan *et al.* (1998).

Statistical analysis of morphometric data: The morphometric ratio data were transformed into square root format for improving the normality and ANOVA was conducted to compare morphometric ratios among the morphotypes (Zar 1984). A multivariate discriminant analysis of morphometric data was used to identify the combinations of variables that distinguished the morphotypes and identified the *Scylla* sp. Statistical analyses were performed with SPSS software (SPSS, v-20). For removing the size effect, all morphometric characters were standardized according to the equation $M_S = M_0(L_S/L_0)^b$ (Elliott *et al.* (1995). M_S is the standardized measurements, M_0 is the length of measured character, L_S is the arithmetic mean of the standard length (carapace width) for all crab from all samples in each analysis and L_0 is the standard length of each specimen. The value of the parameter *b* was estimated for each character from the observed data by allometric growth equation $M = aL^b$. Co-efficient *b* was evaluated as the slope of regression of $\log_{10}M_0$ on $\log_{10}L_0$ using the entire crab sample in each group. The degrees of similarity among samples in the overall analysis and relative importance of each measurement for group separation were assessed by discriminant function analysis (DFA) with cross validation. The morphometric values of five swimming crabs were also included in the analysis as an out group. The population centroids with 95% confidence interval derived from the DFA was used to visualize relationships among the individuals of groups.



(a (b (c)

(d) (e) (f)

(g) (h) (i)

(j) (k) (l)

Fig. 1. Photographs of different morphological features of the three morphoypes of *Scylla* sp. a-c: Propodus spines; d-f: Frontal lobe spines; g-i: Carpus spine; j-l: Polygonal patterning.

Table II. Distinguishing morphological features among Morphotype-1, Morphtype-2 and Morphtype-3.

Key points	Morphtype-1	Morphtype-2	Morphtype-3
Propodus spines	Pair of sharp spines on dorsal margin behind insertion of the dactyl (Fig. 1a)	Pair of blunt spines on dorsal margin behind insertion of the dactyl (Fig. 1b)	Pair of medium sharp spines on dorsal margin behind insertion of the dactyl (Fig. 1c)
Frontal lobe spines	Bluntly pointed with slightly concave margins and separated by inverted V shaped interspaces (Fig. 1d)	Rounded with rounded interspaces (Fig. 1e)	Typically triangular with angular interspaces (Fig. 1f)
Carpus spine	Sharp (Fig. 1g)	Blunt (Fig. 1h)	Not blunt nor sharp (Fig. 1i)
Polygonal patterning	Polygonalpattern on carapace and chelipeds (Fig. 1j)	Devoid of polygonal patterning (Fig. 1k)	Strong pattern on carapace and dactylus of last swimming legs (Fig. 1l)
Coloration of carapace	Varied from olive green to dark brown	Varied from greenish brown to dark brown	Light green

Results

***Morphological study*:** The morphological parameters analyzed in the present study grouped 84 mud crab specimens collected from the coastal regions of Bangladesh into three morphotypes. The key distinguishing features of the morphotypes are presented in Table II. The three morphotypes 1, 2 and 3 resembled three *Scylla* species- *Scylla serrata, S. olivacea* and S. *paramamosain* respectively based on taxonomic keys developed by Keenan *et al.* (1998).

Morphometric analysis: Mean ($\pm$SD) of two morphometric characters and 27 ratios are shown in Table III. A one way analysis of variance (ANOVA) was conducted to compare the values of different morphometric ratios among the three morphotypes. Efficiency of the allometric formula in removing size effects from the data was justified by using correlations among the adjusted characters. The ANOVA showed significant difference among all the morphotype ratios except PD/PL and PL/ICW (Table III).

The discriminate function analysis (DFA) revealed that nine morphometric measurements (PWC/FW, PW/PL, FMSH/DFMS, LSH/ICW, DFLS/FW, DFMS/FW, (PW*PD*0.7854)/PL, 5PW/5PL and ICS/OCS) contributed to DF1, nine measurements (CW/8CW, IPS/OPS, ICS/PL, FW/ICW, IPS/PL, OPS/PL, DFMS/DFLS, PWC/ICW and AW/SW) contributed to DF2, four measurements (ML/PL, OCS/PL, FMSH/FW and 3PML/ICW) contributed to DF3 while five measurements (CL/ICW, DL/PL, SW/ICW, PD/PL and PL/ICW) contributed to DF4 (Table IV). Among the four discriminate functions (DFs) formed, DF1 accounted for 64.9% of the total variation while DF2, DF3 and DF4 accounted for 21.7, 11.8 and 1.6%, of the total variation, respectively (Table IV). All the samples were separated from each other in the discriminate space (Fig. 2). Two morphometric ratios, FMSH/FW and FW/ICW of the three morphotypes were compared with those of *S. serrata, S. olivacea* and *S. paramamosain* provided by Keenan *et al.* (1998) and found to be very similar (Table V).

Table III. Mean, (±SD) of two morphometric characters and 27 ratios for three morphotypes of mud crab collected from throughout the coastal zone of Bangladesh

Parameters	Morphotype-1 Mean ± SD	Morphotype-2 Mean ± SD	Morphotype-3 Mean ± SD	P-values
CW(mm)	100.7±30.4	113.3±21.3	95.6±17.3	
ICW(mm)	95.7±16.3	109.1±32.4	90.6±23.2	
LSH/ICW	0.030±0.009	0.017±0.005	0.039±0.009	0.000***
CW/8CW	1.009±0.017	0.890±0.068	1.038±0.025	0.000***
CL/ICW	0.696±0.037	0.703±0.054	0.678±0.021	0.000***
PWC/ICW	0.329±0.063	0.292±0.026	0.348±0.019	0.008**
FW/ICW	0.357±0.024	0.437±0.028	0.378±0.031	0.000***
PWC/FW	0.841±0.062	0.763±0.058	1.021±0.041	0.000***
FMSH/FW	0.067±0.023	0.022±0.005	0.045±0.012	0.000***
FMSH/DFMS	0.410±0.077	0.249±0.039	0.389±0.075	0.000***
DFMS/FW	0.143±0.037	0.121±0.011	0.152±0.027	0.000***
DFLS/FW	0.133±0.024	0.135±0.017	0.147±0.028	0.000***
DFMS/DFLS	1.070±0.076	0.909±0.072	1.057±0.194	0.013**
SW/ICW	0.523±0.028	0.539±0.029	0.529±0.025	0.006**
AW/SW	0.712±0.139	0.587±0.049	0.705±0.120	0.006**
PL/ICW	0.707±0.094	0.773±0.055	0.743±0.074	0.101ns
DL/PL	0.445±0.034	0.462±0.029	0.476±0.034	0.001**
PW/PL	0.417±0.026	0.436±0.050	0.456±0.046	0.000***
PD/PL	0.268±0.042	0.281±0.059	0.274±0.040	0.476ns
(PW*PD*0.7854)/PL	8.824±2.329	8.241±1.242	8.270±1.369	0.000***
IPS/PL	0.032±0.010	0.017±0.005	0.041±0.009	0.000***
OPS/PL	0.016±0.007	0.007±0.007	0.021±0.007	0.000***
IPS/OPS	1.866±0.661	5.503±1.389	2.883±0.602	0.000***
ICS/PL	0.005±0.002	0.001±0.001	0.005±0.001	0.000***
OCS/PL	0.029±0.009	0.007±0.007	0.017±0.005	0.000***
ICS/OCS	0.005±0.002	0.004±0.003	0.005±0.002	0.000***
ML/PL	0.446±0.075	0.471±0.018	0.529±0.027	0.000***
5PW/5PL	0.622±0.114	0.650±.0353	0.545±0.022	0.000***
3PML/ICW	0.357±0.023	0.442±0.057	0.405±0.050	0.000***

$**p < 0.01$ and $***p < 0.001$

Table IV. Pooled within-groups correlations between discriminating variables and discriminate functions

(Variables ordered by size of correlation within function, *denotes the largest correlation between each variable and discriminate functions).

Characters	Functions			
	DF 1	DF 2	DF 3	DF 4
WC/FW	0.385*	0.274	0.290	0.116
PW/PL	0.311*	-0.005	0.017	0.078
FMSH/DFMS	-0.298*	0.190	0.062	-0.124
LSH/ICW	-0.291*	0.153	0.238	0.168
DFLS/FW	0.208*	0.015	-0.008	0.003
DFMS/FW	0.166*	0.067	-0.051	-0.006
(PW*PD*0.7854)/PL	0.108*	0.005	-0.105	0.046
5PW/5PL	-0.093*	-0.065	-0.089	0.077
ICS/OCS	0.076*	0.033	-0.048	-0.006
CW/8CW	-0.294	0.375*	0.209	0.068
IPS/OPS	0.049	-0.308*	0.249	-0.278
ICS/PL	0.120	0.261*	-0.138	-0.021
FW/ICW	0.100	-0.213*	0.121	0.139
IPS/PL	0.137	0.182*	0.056	0.014
OPS/PL	0.089	0.143*	0.045	0.020
DFMS/DFLS	0.002	0.103*	-0.040	-0.020
PWC/ICW	-0.027	0.089*	0.061	0.074
AW/SW	-0.044	0.086*	-0.007	0.044
ML/PL	-0.181	0.048	0.320*	0.202
OCS/PL	0.075	0.113	-0.298*	-0.041
FMSH/FW	0.080	0.143	-0.273*	-0.050
3PML/ICW	0.102	-0.125	0.180*	-0.134
CL/ICW	-0.014	-0.041	-0.077	0.452*
DL/PL	-0.003	0.006	0.145	0.269*
SW/ICW	0.051	-0.040	0.009	0.224*
PD/PL	-0.005	-0.018	0.029	0.170*
PL/ICW	0.001	-0.045	0.084	-0.113*
Eigen value	44.74	14.93	8.16	1.113
% of Variance	64.90	21.70	11.80	1.600

*$p < 0.05$

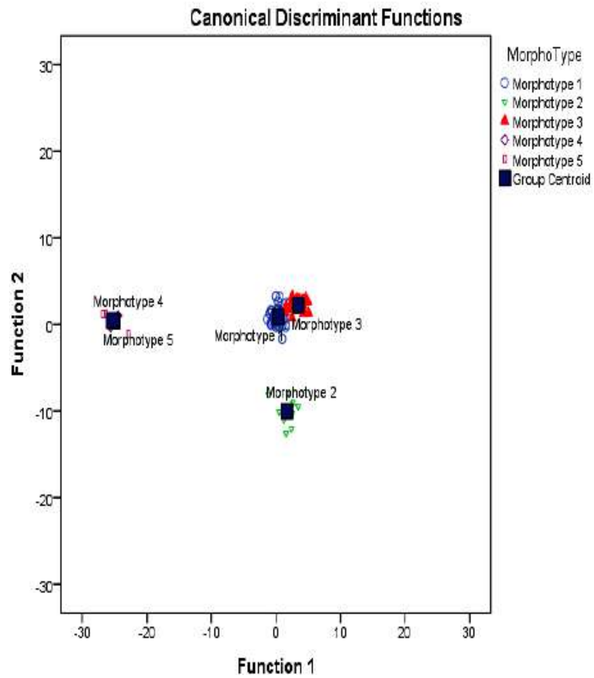


Fig. 2. Sample centroids of discriminate function scores based on truss measurement. Samples referred to- Morphotype-1, Morphotype-2, Morphotype-3, Morphotype-4 and Morphotype-5.

Table V. Comparisons of FMSH/FW and FW/ICW ratio obtained in this study and those reported by Keenan *et al.* (1998)

Present study			Keenan *et al*. (1998)		
	FMSH/FW	FW/ICW	Species	FMSH/FW	FW/ICW
Morphotype-1 (N=42)	0.067±0.023	0.356±0.023	*S. serrata*	0.061±0.010	0.371±0.016
Morphotype-2 (N=10)	0.021±0.004	0.436±0.027	*S. olivacea*	0.029±0.005	0.415±0.017
Morphotype-3 (N=27)	0.044±0.011	0.378±0.031	*S. paramamosain*	0.058±0.012	0.377±0.007

Discussion

Attempts to identify *Scylla* species have led to much confusion because of the subtle morphological differences among the species and plasticity of variation within species. Initially, mud crabs were assigned into three species namely, *S. serrata*, *S. oceanica*, and *S. tranquebarica* and one variety *S. serrata* var. *paramamosain,* based on its external morphology (Estampador 1949). Keenan *et al*. (1998) made a thorough revision of the genus *Scylla* using specimens collected from the Red Sea and throughout the Indo-Pacific region and concluded that there were four mud crab species namely *S. olivacea*, *S. serrata*, *S. paramamosain*, and *S. tranquebarica*.

Among the four mud crab species *S. serrata* and *S. olivacea* have been reported from India (Trivedi and Vachhrajani 2013; Mandal *et al.* 2014; Devi and Joseph 2015) and Thailand (Jirapunpipat *et al.* 2008). Hence this study was attempted to focus on *S. serrata* and *S. olivacea* considering the geographical proximity of *Scylla* species distribution. In the present study, three morphotypes (Morphotype-1, Morphotype-2 and Morphotype-3) were observed among the *Scylla* specimens resembling *S. serrata*, *S. olivacea*and *S. paramamosain,* respectively based on the morphological details provided in the identification key of Keenan *et al.* (1998). A recent study, however, claimed the occurrence of only one mud crab species namely, *S. olivacea* from Bangladesh coastal waters (Sarower *et al*. 2016). The *Scylla* species are differentiated mainly based on their carpus spine, frontal spine, polygonal pattern, propodus spines and coloration. The numbers of carpus spine and propodus spines were the same but the spines were sharp in Morphotype-1, blunt in Morphotype-2 and medium sharp in Morphotype-3. In Morphtype-1, coloration of carapace varied from olive green to dark brown which is the feature of *S. serrata*. In Morphotype-2, coloration of carapace varied from greenish brown to dark brown as is found in *S. olivacea*. In Morphtype-3, it was light green similar to *S. paramamosain*. The frontal lobe spines were steep, with 'V' shaped interspaces in Morphotype-1 whereas the spines rounded with rounded interspaces in Morphotype-2. Polygonal patterning on chelipeds and pereopods were very noteworthy distinguishing characters. Morphotype1 showed polygonalpattern on carapace and chelipeds (Fig. 1j), whereas Morphotype-2 was devoid of such patterning (Fig. 1k); strong pattern on carapace and dactylus of last swimming legs were the features of Morphotype-3 (Fig. 1l). Joel and Raj (1980) suggested that the color of carapace, chelae and leg, spine on finger bases and ploygonal pigment areas could be the critical characters in differentiating the species of the genus *Scylla*.

Multivariate analysis of morphometric characters has been found to be an effective technique in discriminating many animal species, including various types of crustaceans; moreover, the hard, well-defined body parts of crustaceans facilitate the collection of accurate data (Overton *et al.* 1997). In the present study, the multivariate analysis of morphometric characters of mud crabs of the genus *Scylla* also revealed significant differences among the three morphotypes. Out of the morphometric ratios analyzed, FMSH/FW and FW/ICW were found to be most contrasting among the morphotypes and similar to those of *S. serrata, S. olivacea* and *S. paramamosain* provided by Keenan *et al.* (1998) (Table V).

We have conducted the multivariate analysis of 27 morphometric ratios to discriminate among the *Scylla* morphotypes and found that 13 ratios such as ML/PL, FMSH/DFMS, DFMS/FW, LSH/ICW, DFLS/FW, PW/PL, CW/8CW,

(PW*PD*0.7854)/PL, IPS/OPS, FW/ICW, 5PW/5PL, OPS/PL and IPS/PL mostly contributed to discriminate the species. The first three principal components (PC) accounted for 55.865% (Table IV) of the total variance. Keenan *et al.* (1998) also observed seven characters including ICS/OCS, FMSH/FW, FW/ICW, ML/PL, AW/SW, PL/ICW and IPS/PL that could distinguish between the *Scylla* species. Sangthong and Jondueung (2006) noted nine morphometric characters including FMSH, DFMS, External carapace width (ECW), Frontal margin (FM), Right carpus length (RCL), Right dactyl length (RDL), Right propodus width (RPW), Upper paddle length (UPL) and Body depth (BD) which exhibited distinctive differences among *Scylla* species as well as *Scylla* population. Mandal *et al.* (2014) claimed that three ratios *viz.,* ICS/OCS, FMSH/FW and FW/ICW could be considered to discriminate between *S. serrata* and *S. olivacea.*

Discriminant Function Analysis (DFA) is a statistical tool, which is used to discriminate between two naturally occurring groups. Jaiswara *et al.* (2013) examined the effectiveness of DFA in the cluster analysis of male field crickets and claimed that the accuracy of discrimination using DFA was high and was not influenced by the number of taxa used. In the present study, when the scattering pattern was taken into account, the group centroid reveals that Morphotype-2 (*S. olivacea*) is entirely different from Morphotype-3 (*S. paramamosain)* and Morphotype-1 (*S. serrata)*. A mixing between the Morphotype-1 (*S. serrata*) and morphotype-3 (*S. paramamosain*) is clearly visible in the above analysis. Morphotypes-4 and -5 were used as out groups (swimming crab) and after DFA it was confirmed that Morphotypes-4 and -5 were similar and different from Morphtypes-1, -2 and -3. Therefore, from the morphometric study, it can be concluded that three morpho types of mud crabs are available in coastal waters of Bangladesh that are similar to *S. serrata, S. olivacea* and *S. paramamosain*. Jirapunpipat *et al.* (2008) conducted a similar morphometric analysis to discriminate between the Thai mud crab populations, but came out to be unsuccessful. They found an overlap of *S. serrata* and *S. tranquebarica* although these two species were clearly distinguishable from *S. olivacea* and *S. paramamosain* in the Discriminate Function Analysis.

In conclusion, we have identified three morphotypes resembling three mud crab species such as *S. serrate, S. olivacea and S. paramamosain*. However, reliable molecular analysis can confirm if they are different species or the morphotypes are due to morphological plasticity.

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