



Multivariate morphometric differentiation of *Macrobrachium* species (Crustacea: Palaemonidae) along the northern rivers of Bangladesh

IMRAN HOSHAN, AKTHERA YESMIN¹, MADHAB RAY, FEE FAYSAL AHMED²
AND SAROWER MAHFUZ^{3*}

Department of Fisheries Biology and Genetics,

Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh

¹Department of Fisheries Technology, HMDSTU, Dinajpur 5200, Bangladesh

²Dept. of Mathematics, Jashore University of Science and Technology, Jashore 7408, Bangladesh

³Department of Fisheries and Marine Bioscience, Jashore 7408, Bangladesh

* Corresponding author's e-mail: sa.mahfuz@gmail.com

Abstract. Understanding interspecific morphometric variation is a prerequisite of species identification, evolutionary and taxonomic studies. The external features (i.e. morphometric and ratios) of five *Macrobrachium* species viz., *Macrobrachium rosenbergii*, *M. lanchesteri*, *M. dolichodactylus*, *M. idella* and *M. lamarrei* from three rivers in northern Bangladesh, were used to analyze interspecific morphometric variation. *Macrobrachium* samples were collected from Jamuneshwari river, Tangon river and Atrai river. Morphometric and ratios data were subjected to univariate ANOVA test. To assess variations among the species, a principal component analysis (PCA) and canonical variate analysis (CVA) were performed. Four PCA explained 95.31% of the variation in morphometric and ratios data. Four CVA were correctly classified as 46.53%, 30.02%, 18.10% and 5.35% respectively using morphometric and ratios data. Morphometric and ratio results from PCA formed a compact cluster among species while in CVA showed that species were completely formed a separate cluster. A dendrogram independently developed based on phenotypic traits among the individuals of the five *Macrobrachium* species confirmed the presence of phenotypic differentiation. The baseline evidence ensuing from the current study would be advantageous for geometric morphometric, genetic investigations and further *in situ* conservation of *Macrobrachium* species in Bangladesh.

Keywords: Morphometric characters, *Macrobrachium* species

Introduction

Freshwater crustaceans of the genus *Macrobrachium* are a varied group of decapod crustaceans comprising 246 species found in tropical and subtropical climates around the world (De Grave *et al.* 2009, Nogueira *et al.* 2019) and several of them have been commonly used in aquaculture. In most freshwater prawns, on the other hand, require brackishwater to complete their life cycle (Mariappan and Balasundaram 2004). The genus *Macrobrachium* is of interest to scientists not just from a biological standpoint but also from a social and economic perspective. There are 20 species of *Macrobrachium* in Bangladesh, including Palaemonid prawns, of which 11 are classified as Least Concern (LC) and 9 categorized as Data Deficient (DD) (IUCN 2015). The continued description of new *Macrobrachium* species is a clear indicator that the taxonomic spectrum of *Macrobrachium* in Bangladesh still has unexplored species richness. A comprehensive understanding of phenotypic divergence is required to differentiate the genus *Macrobrachium*. Nevertheless, because of their morphological versatility and redundant morphometric features, taxonomic identification of the genus *Macrobrachium* is incredibly challenging (Konan *et al.* 2010).

Macrobrachium rosenbergii, the world's largest palaemonid, is one of the most frequently crustacean species due to its fast growth and high market value. Due to the abundance of this economically significant species in natural waters in several Asian countries, a considerable

fishery industry has developed based on wild stocks of this species (de Bruyn *et al.* 2004). Nonetheless, due to over-harvesting, habitat loss, and rising pollution, wild populations of this species are fast declining (Moraes-Rioldades and Valenti 2004). Similarly, *Macrobrachium dolichodactylus*, globally known as Ghoda River Prawn and locally known as Ghoda Icha, is found throughout Bangladesh's river systems. *M. dolichodactylus* can be found in the Halda, Naaf, Meghna, Sangu, and Karnaphuli rivers of Bangladesh (Zafar *et al.* 2007). Correspondingly, *Macrobrachium lamarrei*, also known as Kuncho River Prawn globally and Kuncho Chingri regionally, is extensively spread in Bangladeshi water bodies and is thereby categorized as Least Concern (LC). In Bangladesh, little is known such as habitat requirements, population and risks of *M. idella*. The species is classified as Data Deficient based on the given characteristics (DD) (IUCN 2015). Correspondingly, *Macrobrachium lanchesteri*, globally termed as Rice Land Prawn and locally regarded as Dhanua Chingri. There is a scarcity of information on the abundance and distribution of *M. lanchesteri*. There appears to be insufficient data to provide a gross assessment of extinction and threats based on this species distribution status in Bangladesh. Therefore, *M. lanchesteri* is classified as Data Deficient (DD) based on the characteristics listed above.

The taxonomy of *Macrobrachium* species has been a topic of contention for several years owing to their considerable morphological closeness and deficiency of specific morphological distinguishing features. The reconsideration of morphological features by Keenan *et al.* (1998) effectively resolved the taxonomic identity of *Macrobrachium* species. Morphometric investigations are particularly important for determining the forms of varied populations and species across geographical arrays as well as for demonstrating regional disparities in crustaceans (Rufino *et al.* 2006, Konan *et al.* 2010). External morphological variances could potentially be linked to local environmental adaptations and lineage differentiation as a result of geographic isolation (Moraes *et al.* 2020).

Existing studies on *Macrobrachium* species such as growth, mortality, and recruitment patterns have been undertaken all over the world (Torres *et al.* 2014). There have been no interspecific morphometric studies conducted on the five aforementioned species, but there have been some intra-species morphometric studies performed on *M. austral* (Zimmermann *et al.* 2012), *M. borellii* (Torres *et al.* 2014), *M. rosenbergii* (Munasinghe and Thushari 2010), *M. potiuna* (de Melo and Masunari 2017), *M. vollenhovenii* (Konan *et al.* 2010), *M. nipponense* (Chen *et al.* 2015). Furthermore, Konan *et al.* (2010) found inter-specific morphometric differences between *M. vollenhovenii* and *M. macrobrachion*. Morphometric investigations are effective for identifying different populations across a species' geographical range (Miller *et al.* 1988). Furthermore, physical differences across populations play a significant role in species differentiation (Swain and Foote 1999, Turan *et al.* 2006). Using data analyses from a set of phenotypic measures (conventional morphometric and ratio), the current study analyzes interspecific differences across five *Macrobrachium* species viz., *M. rosenbergii*, *M. lanchesteri*, *M. dolichodactylus*, *M. idella* and *M. lamarrei*. In essence, the study focused on morphometric features for species differentiation and to describe the interspecific morphometric variability amongst five *Macrobrachium* species.

Material and Methods

Fish sampling: A total of 150 wild *Macrobrachium* species were collected from three rivers viz., Atrai river, Jamuneshwari river and Tangon river (Fig.1 and Table I) from the northern regions of Bangladesh. To avoid seasonal fluctuations in their phenotypic features, the samples were collected during the pre-monsoon (April to May 2020) and monsoon (July to August 2020) periods. *Macrobrachium* samples were collected straight forwardly from local fishers at 11.00 to

14.00 hours using cast net (mesh size, 1.0 cm) and push net (mesh size, 1.0 cm). The sampled species were recognized based on external phenotypes and then the samples were preserved in ice and transferred to the laboratory of post graduate laboratory, Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh for further experimentation.

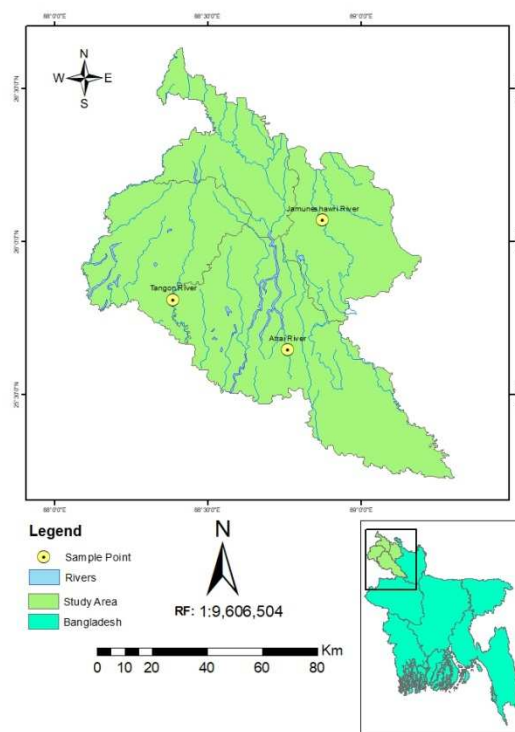


Fig. 1. Map representing the collection spots of *Macrobrachium* species from three rivers of Bangladesh.

Table I. Sampling details of *Macrobrachium* species collected for phenotypic differentiation

Species	Sampling Sources	Sample size	Sampling location	Date of collection	Total length (TL)		
					Min	Max	Mean (SD)
<i>Macrobrachium rosenbergii</i>	Jamuneshwari river	30	Nilphamari	7–9 April and 17–19 July	13.90	16.90	15.45 (0.72)
<i>Macrobrachium lanchesteri</i>	Tangon river	30	Thakurgaon	11–13 April and 6–8 August	3.52	5.61	4.46 (0.50)
<i>Macrobrachium dolichodactylus</i>	Atrai river	30	Dinajpur	11–13 April and 23–25 August	4.79	5.99	5.50 (0.39)
<i>Macrobrachium idella</i>	Tangon river	30	Thakurgaon	26–28 April and 6–8 August	3.52	5.61	5.12 (0.50)
<i>Macrobrachium lamarrei</i>	Jamuneshwari river	30	Nilphamari	21–24 May and 21–24 August	4.99	7.88	6.16 (0.59)

SD: Standard deviation

Measurement of conventional morphometrics and ratios: A Vernier caliper with a precision of 0.01 mm was used to perform traditional morphometric measurements over the crustacean body.

MULTIVARIATE MORPHOMETRIC DIFFERENTIATION OF *MACROBRACHIUM*

Morphometric characteristics were recorded from the left to right direction of each specimen. The eight morphometric characters that were measured and described in table II. Additionally, eight ratios were incorporated using the eight morphometric characters (TL_SL, SL_RL, SL_CL, SL_AL, AL_TeL, CL_RL, CL_CH, and TeL_TeW).

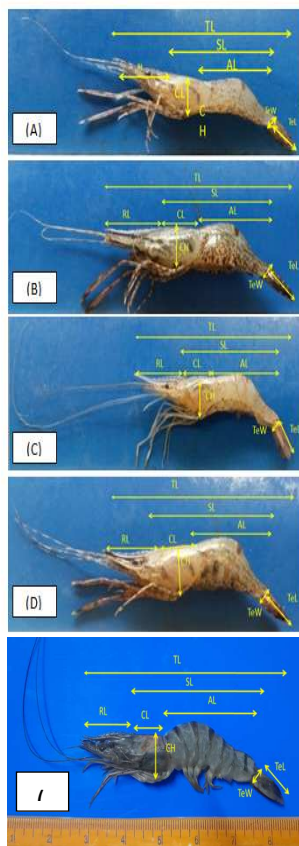


Fig. 2. Morphometric features of four *Macrobrachium* species (A) *Macrobrachium lamarrei*, (B) *M. dolichodactylus*, (C) *M. lanchasteri*, (D) *M. idella*, and (E) *M. rosenbergii* species collected from three rivers of Bangladesh.

Table II. Description of morphometric characters analyzed for *Macrobrachium* species differentiation

Characters	Description
Total length (TL)	The straight line between the rostrum's tip and the telson's distal tip
Standard length (SL)	The distance between the base of the uropod and the base of eye
Rostrum Length (RL)	The length between the base of the epigastric tooth and the rostrum's tip
Carapace Length (CL)	The distance between the left orbit's posterior margin and the cephalothorax's posterior margin
Abdominal Length (AL)	The distance between the anterior margin of the abdomen and the anterior margin of the telson
Carapace Height (CH)	The distance between the midpoints of the carapace's dorsal and ventral borders
Telson length (TeL)	The distance between the telson's base and tip
Telson width (TeW)	The broadest point of the telson

Statistical analyses: Prior to analyze all original morphometric and ratio data, all variables were subjected to check normality. An allometric model, $M_{adj} = M (L_s/L_o)^b$ developed by Elliott *et al.* (1995) which was used to eliminate the size effect. Based on the equation 'M' is the original measurement; M_{adj} is the size-adjusted measurement; L_o is the total length of prawn; L_s is the overall mean of total length for all prawn from all samples in each analysis. Parameter 'b' was estimated for each character from the observed data as the slope of the regression of $\log M$ on $\log L_o$, using all the prawn in all groups. To test the significance of morphological differences, a univariate ANOVA was carried out. The variables that were found to be insignificant in univariate ANOVA eliminated from further analysis.

To minimize the intensities of the adjusted morphometric measures and ratios jointly, a principal component analysis (PCA) was used, which might eliminate sample repetition. Multivariate models were developed using the PCA components and subsequent studies using the models such as canonical variate analysis (CVA) and cluster analysis (CA), were accomplished (Geladi *et al.* 2003). In many fish and crustacean morphology investigations, the use of a multivariate analysis can result in an inadequate sample size. Researchers of hypothetical works on PCA and CVA have recommended the ratio of the number of organisms observed (N) to the factors involved (P) in the analysis (Kocovsky *et al.* 2009). A small number of 'N' values may not adequately depict covariance or morphological variation, resulting in inaccurate group disparities (Kocovsky *et al.* 2009). All variables were kept constant in this analysis and the N/P proportion for both conventional and ratio data was 9.38. To investigate which morphometric and ratio effectively discriminate species, the contributions of the variables to PCA were explored. To validate the data's suitability for PCA, the Kaiser-Meyer-Olkin (KMO) metric and Bartlett's Test of Sphericity were utilized. Based on the KMO statistics the output ranges from '0' to '1'. The values are superior to 0.5, according to Kaiser *et al.* (1974), and are considered suitable. KMO statistical principles varied from 0.5 to 0.7, which is mediocre, to 0.7 to 0.8, which is good, and 0.8 to 0.9, which is exceptional. The obtained KMO value for the entire matrix in this study was 0.62, and Bartlett's Test of Sphericity was significant ($p < 0.05$), indicating that the data analyzed are suitable for a factor assessment approach. According to Jolliffe's criteria (eigen value ≥ 1.0) was utilized in PCA to select models and extract important factor contributions for further investigation. This analysis only focused at traits with eigenvalues greater than one, while others were excluded. The Scree test is one method for reducing the number of factors to a lower number than that found by the eigenvalue larger than unity criteria (Cattell 1966). In this test, the eigenvalues were calculated by graphing against the factors in decreasing order along the X-axis. To highlight the crucial aspects, a factor analysis (FA) was performed to compress a huge set of variables into a smaller group of latent variables. After that, the variables were subjected to a varimax-rotation process in order to obtain variables with high loadings. Factor loadings are more than 0.30 is deemed crucial, 0.40 is more significant and 0.50 or greater is considered extremely substantial (Nimalathasan 2009). Components with loadings were greater than 0.40 considered as significant. The CVA was utilized to check for correct identification of individuals within each species as well as to validate its accuracy. CA was applied to analyze multivariate data, and similar observations were collected to identify a common cluster. The unweighted pair group approach with arithmetic mean (UPGMA), which primarily focused on the Euclidean distance across species/groups, was employed to address the similarity of morphometric features. Data analyses were carried out using R-studio R.3.5.3 and SPSS 16.0 software. The significance level for all of the analyses was set at 5%.

Results

ANOVA demonstrated significant ($p < 0.05$) differences in all conventional morphometric measurements and ratios, excluding CL_CH character ($p > 0.05$) (Table III). Furthermore, in the multivariate analysis, the CL_CH character was finally curtailed. Four factors with eigenvalues ≥ 1 were found in a PCA of general morphometrics and ratios, accounting for 95.31% of the total variance. The first principal component (PC1) was responsible for 46.03% of the difference, while the second (PC2), third (PC3) and fourth (PC4) were responsible for 25.97%, 14.84% and 8.45% respectively (Table IV). The most substantial loadings on PC1 were SL, CL, CH, AL, TeL, TeW, and TL_SL (Table IV). Correspondingly, in PC2, the most crucial loadings were CL, CH, TeL, SL_CL, SL_AL and AL_TeL. The bi-plot revealed that all species were merged (Fig. 3).

Table III. Univariate ANOVA of morphometric lengths and their ratios of five *Macrobrachium* species collected from the three rivers of Bangladesh

Characters	Wilks' Lambda	F	df1	df2	p
SL	0.721	14.031	4	145	0.000*
RL	0.759	11.537	4	145	0.000*
CL	0.665	18.282	4	145	0.000*
CH	0.646	19.831	4	145	0.000*
AL	0.743	12.561	4	145	0.000*
TeL	0.776	10.436	4	145	0.000*
TeW	0.555	29.093	4	145	0.000*
TL_SL	0.693	16.090	4	145	0.000*
SL_RL	0.363	63.579	4	145	0.000*
SL_CL	0.644	20.002	4	145	0.000*
SL_AL	0.720	14.067	4	145	0.000*
AL_TeL	0.474	40.306	4	145	0.000*
CL_RL	0.323	75.811	4	145	0.000*
TeL_TeW	0.472	40.630	4	145	0.000*

*significant value ($p < 0.05$)

Table IV. Factors loading of morphometric measurements and their ratios by using PCA among five species of *Macrobrachium* collected from three rivers of Bangladesh

Traits and ratios	PC1	PC2	PC3	PC4
SL	0.988	-0.173	0.186	-0.408
RL	0.379	-0.393	-0.848	-0.093
CL	0.834	-0.676	0.098	-0.320
CH	0.838	-0.409	0.065	-0.628
AL	0.890	0.242	0.229	-0.390
TeL	0.841	-0.533	-0.226	-0.324
TeW	0.684	-0.296	-0.078	-0.888
TL_SL	-0.984	0.183	-0.192	0.420

SL_RL	0.239	0.292	0.967	-0.118
SL_CL	-0.273	0.975	0.080	0.110
SL_AL	0.246	-0.946	-0.081	-0.031
AL_TeL	0.075	0.842	0.507	-0.070
CL_RL	0.359	-0.217	0.902	-0.149
TeL_TeW	-0.222	-0.091	-0.120	0.967
Eigenvalue	6.445	3.637	2.078	1.184
Variance%	46.034	25.976	14.844	8.454
Cumulative%	46.034	72.010	86.854	95.308

The first four principal components are shown here. The most important morphometric variables and ratios contributed to the PC1, PC2, PC3 and PC4 are marked as bold face.

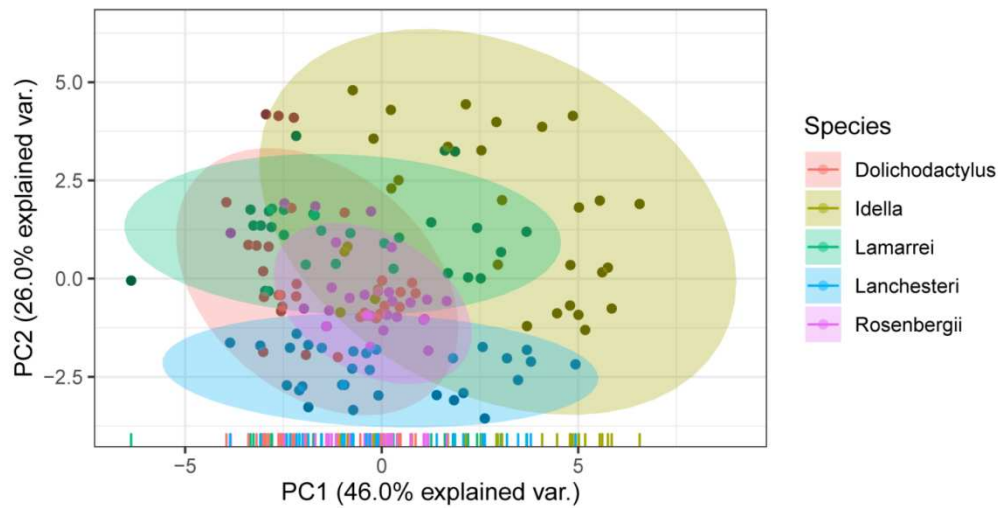


Fig. 3. Biplot of Principal component analysis (PCA) of morphometric measurements and ratios of *Macrobrachium* species obtained from three rivers of Bangladesh.

Four canonical varieties (CV) were formed using CVA, with CV1, CV2, CV3, and CV4 accounting for 46.53%, 30.02%, 18.10%, and 5.35% of the morphological variations, respectively (Table V). The first three CVAs were taken into consideration for further inferences. The following features *viz.*, CL_RL, TeW, CH, CL, TL_SL and SL were categorized into the first factor (CV1), which explained 46.53% of the total variances. SL_RL, TeL_TeW and RL were categorized in the second factor (CV2) that explained 30.02%, of total variances with meaningful loadings. Similarly, the third factor (CV3) explained 18.10% of total variances for which meaningful loadings were AL_TeL and TeL. The bi-plot (CV1 versus CV2) explained 76.55% of the total variances between the samples and revealed species isolated in *M. idella*, *M. lamarrei* and *M. dolichodactylus* and the species group of *M. rosenbergii* and *M. lamarrei* showed partially merged (Table V and Fig. 4).

Table V. Contribution of morphometric measurements and ratios to the canonical variate scores in *Macrobrachium* species collected from three rivers in Bangladesh

Traits and ratios	CV1	CV2	CV3	CV4
CL_RL	-0.502*	-0.368	0.086	0.323
TeW	-0.296*	0.263	-0.004	0.159
CH	-0.292*	0.007	-0.109	0.145
CL	-0.278*	0.008	-0.128	0.084
TL_SL	0.261*	0.067	-0.052	0.182
SL	-0.241*	-0.076	0.057	-0.147
SL_RL	-0.366	-0.436*	0.309	0.028
TeL_TeW	0.277	-0.327*	-0.321	-0.217
RL	0.129	0.217*	-0.125	-0.048
AL_TeL	-0.046	-0.245	0.597*	-0.447
TeL	-0.141	0.033	-0.270*	0.039
SL_CL	0.192	-0.092	0.330	-0.403*
SL_AL ^a	-0.142	0.145	-0.262	0.287*
AL	-0.174	-0.135	0.174	-0.287*
Eigenvalue	6.017	3.860	2.254	0.322
Variance%	46.530	30.020	18.100	5.350
Cumulative%	46.530	76.550	94.650	100.000
Canonical Correlation	0.926	0.891	0.832	0.493

Variables ordered by absolute size of correlation within function.

* Largest absolute correlation between each variable and any Canonical variates.

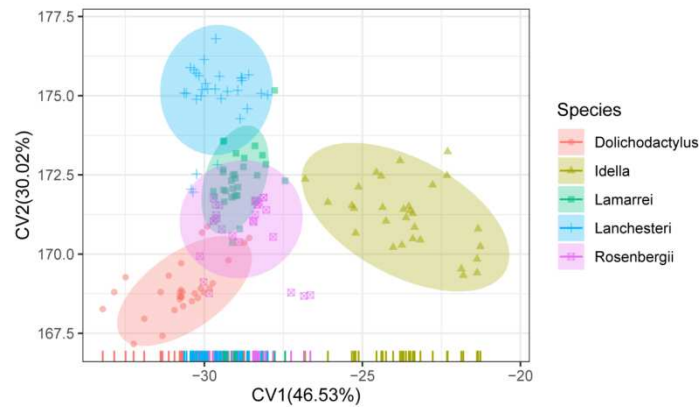


Fig. 5. Biplot of Canonical Variate Analysis (CVA) of morphometric measurements and ratios of *Macrobrachium* species obtained from the three rivers of Bangladesh.

CVA, using original and cross-validation classification, revealed that 90.7% and 88.7% of the individuals were classified as belonging to their original species, respectively. Original classifications were observed in all species, with the *M. idella* (93.3%) having the highest level of original classification, followed by the *M. lamarrei* (90.0%), *M. dolichodactylus* (90.0%), *M.*

rosenbergii (90.0%), and *M. lanchesteri* (90.0%). Furthermore, similar findings were found in the cross-validation results, with correct classification rates of 93.3% in the *M. idella*, followed by 90.0% in the *M. rosenbergii* and *M. lanchesteri*, 86.7% in the *M. dolichodactylus* and 83.3% in the *M. lamarrei* (Table VI).

Table VI. Classification of *Macrobrachium* species into their original stocks using classification matrix (original and cross-validated) of the CVA based on morphometric measurements and ratios

	Species	Predicted Group Membership					Total
		<i>M. lamarrei</i>	<i>M. idella</i>	<i>M. dolichodactylus</i>	<i>M. rosenbergii</i>	<i>M. lanchesteri</i>	
^b Original	<i>M. lamarrei</i>	27 (90.0)	0	0	3 (10.0)	0	30 (100.0)
	<i>M. idella</i>	0	28 (93.3)	0	2 (6.7)	0	30 (100.0)
	<i>M. dolichodactylus</i>	0	0	27 (90.0)	3 (10.0)	0	30 (100.0)
	<i>M. rosenbergii</i>	0	0	3 (10.0)	27 (90.0)	0	30 (100.0)
	<i>M. lanchesteri</i>	0	0	0	3 (10.0)	27 (90.0)	30 (100.0)
^{ac} Cross-validated	<i>M. lamarrei</i>	25 (83.3)	0	0	4 (13.3)	1 (3.3)	30 (100.0)
	<i>M. idella</i>	0	28 (93.3)	0	2 (6.7)	0	30 (100.0)
	<i>M. dolichodactylus</i>	0	0	26 (86.7)	4 (13.3)	0	30 (100.0)
	<i>M. rosenbergii</i>	0	0	3 (10.0)	27 (90.0)	0	30 (100.0)
	<i>M. lanchesteri</i>	0	0	0	3 (10.0)	27 (90.0)	30 (100.0)

^a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case. ^b 90.7 % of original grouped cases correctly classified. ^c 88.7 % of cross-validated grouped cases correctly classified.

Two clusters were mainly formed using general morphometrics and ratios (Fig. 6). The *M. idella* species were clustered in the first cluster, whereas the remaining four species *viz.*, *M. lamarrei*, *M. dolichodactylus*, *M. rosenbergii* and *M. lanchesteri* combinedly formed another cluster. Among the four species *viz.*, *M. lamarrei*, *M. dolichodactylus*, *M. rosenbergii* and *M. lanchesteri*, where, *M. lanchesteri* distinctly formed a sub-cluster and finally *M. lamarrei* also formed as sub-cluster against *M. dolichodactylus* and *M. rosenbergii*.

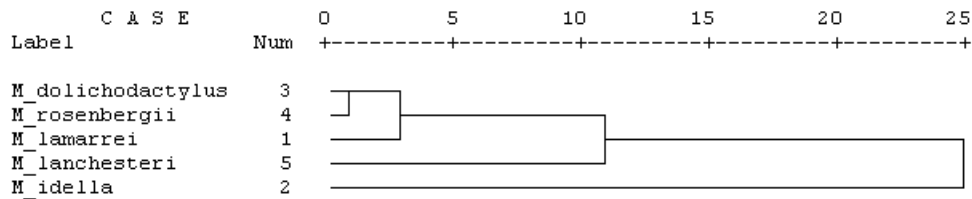


Fig. 6. Dendrogram from morphometric measurements and ratios derived from UPGMA cluster analysis based on the Euclidean distance between the species centroids. In the figure, *Macrobrachium* species areas are shortened as *M. lamarrei*, *M. idella*, *M. dolichodactylus*, *M. rosenbergii* and *M. lanchesteri*.

Discussion

Morphometric analyses are effective for identifying the phenotypes of different populations and species of crustaceans across large geographic areas (Torres *et al.* 2014). For controlling natural population groupings and assessing species structure in terms of phenotypes are a useful technique. Choosing a representative sample size is one of the most significant criteria in reaching a meaningful assertion utilizing a multivariate analysis. To escape incorrectness during examination, an N/P proportion must be obtained at least 3–3.5 (Nimalathasan *et al.* 2009). Numerous researches in this link studied an equivalent proportion of 14.50 in *Botiadarario* (Mahfuj *et al.* 2022), 5.89 in *Cirrhinus mrigala* (Dwivedi *et al.* 2021), 3.50 in *Chanda nama* (Azad *et al.* 2020). In a number of species, including *Nemipterusbi punctatus*, species distinction using traditional and ratio features was investigated (Vaisakh *et al.* 2019), *Cirrhinus cirrhosus* (Gain *et al.* 2017), *Macrognathus aculeatus* (Mahfuj *et al.* 2021), *Cirrhinus reba* (Mahfuj *et al.* 2021). Five *Macrobrachium* species demonstrated substantial morphological variability in this study, as shown by high PCA and CVA variances, and this degree of heterogeneity is linked to specimen size and morphology. However, analysis of conventional and ratio-based morphometric data revealed that *M. idella*, *M. lanchesteri* and *M. dolichodactylus* can be distinguished. Furthermore, based on conventional and ratio statistics, *M. rosenbergii* and *M. lamarrei* species partially showed amalgamate. To elucidate the characterizations, the following phenotypes were validated based on PCA analysis, including SL, CL, CH, AL, TeL, TeW, and TL_SL, as well as other noteworthy phenotypic characterizations were also tested based on CVA analysis, including CL_RL, TeW, CH, CL, TL_SL, and SL. Nevertheless, Konan *et al.* (2010) found that utilizing metric variables and ratios, they were able to clearly distinguish the two *Macrobrachium* species. There might have differences between the five *Macrobrachium* species in both sexes; however, we did not distinguish between sexes or seasons. Furthermore, univariate and multivariate analyses corroborated this phenotypic differentiation of five *Macrobrachium* species. Significant variations between group centroids (90.7% of original grouped data) were found, as well as high percentages of cross-validated categorization (88.7% of cross-validated classified data), and these findings are in accordance with Konan *et al.* (2010). This investigation suggested that morphometric parameters related to SL, CL, CH, AL, TeL, TeW, TL SL, and CL RL (PCA and CVA) have significant taxonomic power and can be used to quantify morphometric variations among the five *Macrobrachium* species. Undoubtedly, morphometrics are key ingredients in systematic research, according to Warheit (1992). Likewise, morphometric investigations have been frequently utilized for taxonomic considerations in the genus *Macrobrachium*. According to Jayachandran (1998) distinguished two closely related species, *M. malcolmsonii* and *M. birmanicum* based on the assumption of the proportions between merus and palm, carpus and chela, and dactylus. The carpus to propodus ratio was found to be a distinguishing feature in *M. nobilii* by Mariappan and Balasundaram (2004).

Parallel findings were obtained by Koshy (1973) and Nagamine and Knight (1980) in *M. dayanum* and *M. rosenbergii*, in which the propodus was larger than the carpus. Kuris *et al.* (1987) and Moraes-Riudades and Valenti (2004) distinguished male morphotypes in *M. rosenbergii* and *M. amazonicum*, respectively, based on the total length of the second pereopods. Nevertheless, in this work, we discovered that metric variables and ratios might be used to differentiate five *Macrobrachium* species phenotypically. Based on PCA and CVA, the key distinguishing features of both species were connected to SL, CL, CH, AL, TeL, TeW, TL_SL, and CL_RL. Since genetic changes cause the majority of phenotypic differences (Tzeng *et al.* 2001), combining morphometric and genetic analyses make it easier to recognize significantly different species.

Conclusions

In this study, the morphometric variations of five *Macrobrachium* species were assessed and compared using multivariate statistical techniques. These findings demonstrate the value of descriptive biology and raise the prospect of differentiating *Macrobrachium* species using descriptive morphological characteristics and their ratios (Hurlbut and Clay 1998). Based on PCA and CVA, the primary distinguishing characteristics of *Macrobrachium* species were connected to SL, CL, CH, AL, TeL, TeW, TL_SL, and CL_RL. In future investigations of these species, genetics and landmark morphometric analysis should be used to obtain precision of morphometric variability and differentiation. These various species could be classified as management plans and conservation activities addressed correspondingly.

Acknowledgement: The authors are highly acknowledged to Institute of Research and Training (IRT), Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for financial supports. The authors are sincerely thankful to the anonymous local fishers for cooperating fish sampling. Sincere thanks goes to Md Musfike Meraz, current student of Environmental Science Discipline, Khulna University, Bangladesh, for supporting to generate a map by using ARC-GIS software.

Literature Cited

- Azad, K.N. M.S. Mahfuj, T. Iqbal, K.N. Azad, M.A.I. Shafaq, 2020. Differentiation of intraspecific phenotypic plasticity of elongate glassy perchlet, *Chanda nama*: Insights into landmark-based truss morphometric and meristic variations. *J. Adv. Vet. Anim. Res.*, 7: 585-596.
- Azadi, M. A. and M. Arshad-ul-Alam, 2014. Biodiversity and conservation of fin and shellfishes of the River Sangu, Bangladesh. The IUCN Bangladesh-The Festschrift on the 50th Anniversary of the IUCN Red List of threatened Species. Dhaka, Bangladesh, 67-74.
- Cattell, R. B. 1966. The Scree test for the number of factors. *Multi. Behav. Res.*, 1(2): 245-276.
- Chen, P. C., T.D. Tzeng, C.H. Shih, T.J. Chu and Y.C. Lee, 2015. Morphometric variation of the oriental river prawn (*Macrobrachium nipponense*) in Taiwan. *Limnologica*, 52: 51-58.
- De Bruyn, M., J.A. Wilson and P.B. Mather, 2004. Huxley's line demarcates extensive genetic divergence between eastern and western forms of the giant freshwater prawn, *Macrobrachium rosenbergii*. *Mol. Phyl. Evol.*, 30(1): 251-257.
- De Grave, S., T.Y. Chan, K.H. Chu, C.H. Yang and J.M. Landeira, 2015. Phylogenetics reveals the crustacean order Amphionidacea to be larval shrimps (Decapoda: Caridea). *Sci. Rep.*, 5: 17464.
- deMelo, M.S. and S. Masunari, 2017. Sexual dimorphism in the carapace shape and length of the freshwater palaemonid shrimp *Macrobrachium potiuna* (Müller, 1880) (Decapoda: Caridea: Palaemonidae): geometric and traditional morphometric approaches. *Anim. Biol.*, 67(2): 93-103.
- Dwivedi, A.K., 2021. Implications of physical barriers on longitudinal connectivity in the Ganga river system through morphological assessment of *Cirrhinus mrigala* (Cyprinidae) Populations. *J. Ichthyol.*, 61: 270-279.
- Elliott, N.G., K. Haskard and J.A. Koslow, 1995. Morphometric analysis of orange roughy (*Hoplostethus atlanticus*) off the continental slope of southern Australia. *J. Fish Biol.*, 46(2): 202-220.
- FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in Action. Rome.
- Gain, D., M.S. Mahfuj, K.A. Huq, S.S. Islam, M.H. Minar, M.P. Goutham-Bharathi and S.K. Das, 2017. Landmark-based morphometric and meristic variations of endangered mrigal carp,

- Cirrhinus cirrhosus* (Bloch 1795) from wild and hatchery stocks. *Sains Malay.*, 46(5): 695-702.
- Geladi, P., M. Manley and T. Lestander, 2003. Scatter plotting in multivariate data analysis. *J. Chemometrics*, 17(8-9): 503-511.
- Holthuis, L.B., 1980. FAO species catalogue. Shrimps and prawn of the world. An annotated catalogue of species of interest to fisheries. FAO Fisheries Synopsis, 125(1): 261.
- Hurlbut, T. and D. Clay, 1998. Morphometric and meristic differences between shallow and deep-water populations of white hake (*Urophycis tenuis*) in the southern Gulf of St Lawrence. *Can. J. Fish. Aquat. Sci.*, 55: 2274-2282.
- IUCN Bangladesh, 2015. Red List of Bangladesh Volume 6: Crustaceans. IUCN, International Union for Conservation of Nature, Bangladesh Country Office, Dhaka, Bangladesh. 256p.
- Jayachandran, K.V., 1998. The taxonomic status of *Macrobrachium birmanicum* (Schenkel) and *M. choprai* (Tiwari) with a note on closely related species. *Ind. J. Fish.*, 45(3): 345-348.
- Kaiser, H. F. 1974. An index of factorial simplicity. *Psychometrika*, 39(1): 31-36.
- Keenan, C.P., P.J.F. Davie and D.L. Mann, 1998. A revision of the genus *Scylla* de Haan, 1833 (Crustacea: Decapoda: Brachyura: Portunidae). *Raff. Bull. Zool.*, 46(1): 217-146.
- Kocovsky, P.M., J.V. Adams and C.R. Bronte, 2009. The effect of sample size on the stability of principal components analysis of truss-based fish morphometrics. *Trans. Am. Fish. Soc.*, 138(3): 487-496.
- Konan, K.M., A.B. Adépo-Gourène, A. Ouattara, W.D. Nyinying and G. Gourène, 2010. Morphometric variation among male populations of freshwater shrimp *Macrobrachium vollenhovenii* Herklots, 1851 from Côte d'Ivoire Rivers. *Fish. Res.*, 103(1-3): 1-8.
- Koshy, M., 1973. Studies on the sexual dimorphism in the freshwater prawn *Macrobrachium dayanum* (Henderson, 1893) (Decapoda, Caridea), II. *Crustaceana*, 24: 110-118.
- Kuris, A.M., Z. Ra'anan, A. Sagi and D. Cohen, 1987. Morphotypic differentiation of male Malaysian giant prawns, *Macrobrachium rosenbergii*. *J. Crust. Biol.*, 7: 219-237.
- Mahfuj, M.S., S.K. Das, K.N. Azad, A.K. Paul, I. Hoshan, S. Sultana and M. Biswas, 2021. Truss network based morphometric and meristic variations among south-western populations of *Macrognathus aculeatus* in Bangladesh. *J. Env. Biol.*, 42: 887-894.
- Mahfuj, M.S., F.F. Ahmed, M.F. Hossain, S.I. Islam, M.J. Islam, M.A. Alam, I. Hoshan and Z.M. Nadia, 2022. Stock structure analysis of the endangered queen loach, *Botia dario* (Hamilton 1822) from five rivers of Northern Bangladesh by using morphometrics: Implications for conservation. *Fishes*, 7(1): 41-51.
- Mahfuj, M.S., S.I. Islam, A. Hanif and S. Mondal, 2021. Stock identification of the Reba carp, *Cirrhinus reba*: Insight into conventional and truss network-based morphometrics. *Egy. J. Aqua. Biol. Fish.*, 25(5): 463-478.
- Mariappan, P. and C. Balasundaram, 2004. Studies on the morphometry of *Macrobrachium nobilii* (Decapoda, Palaemonidae). *Brazil. Archi. Biol. Tech.*, 47(3): 441-449.
- Miller, A.D., N.P. Murphy, C.P. Burrige and C.M. Austin, 2005. Complete mitochondrial DNA sequences of the decapod crustaceans *Pseudocarcinus gigas* (Menippidae) and *Macrobrachium rosenbergii* (Palaemonidae). *Mari. Bio.*, 7(4): 339-349.
- Moraes, S.A., C.E. Alencar, S.M. Lima and F.A. Freire, 2020. Shape variation of the prawn *Macrobrachium jelskii* (Palaemonidae: Decapoda) in the Neotropical semiarid drainages: an intra-and inter-basin investigation. *Mari. Fresh. Res.*, 72(1): 84-98.
- Moraes-Riudades, P.M.C. and W.C. Valenti, 2004. Morphotype males in male Amazon River prawns, *Macrobrachium amazonicum*. *Aquaculture*, 236: 297-307.

- Munasinghe, D.H.N. and G.G.N. Thushari, 2010. Analysis of morphological variation of four populations of *Macrobrachium rosenbergii* (de man, 1879) (Crustacea: Decapoda) in Sri Lanka. *Ceyl. J. Sci.*, 39(1):
- Nagamine, C. and A.W. Knight, 1980. Development, maturation and function of some sexually dimorphic structures of the Malaysian prawn, *Macrobrachium rosenbergii* (de Man) (Decapoda, Palaemonidae). *Crustaceana*, 39: 141–152.
- Nimalathasan, B., 2009. Determinants of key performance indicators (KPIs) of private sector banks in Sri Lanka: An application of exploratory factor analysis. *Ann. Stefan. Cel. Mare. Univ. Suceava. Fac. Econ. Publ. Admin.*, 9: 9–17.
- Rufino M.M., P. Abello and A.B. Yule, 2006. Geographic and gender shape differences in the carapace of *Liocarcinus depurator* (Brachyura: Portunidae) using geometric morphometrics and the influence of a digitizing method. *J. Zool.*, 269: 458-465.
- Swain, D.P. and C.J. Foote, 1999. Stocks and chameleons: the use of phenotypic variation in stock identification. *Fish. Res.*, 43(1-3): 113-128.
- Torres, M.V., F. Giri and P.A. Collins, 2014. Geometric morphometric analysis of the freshwater prawn *Macrobrachium borellii* (Decapoda: Palaemonidae) at a microgeographical scale in a floodplain system. *Ecol. Res.*, 29(5): 959-968.
- Turan, C., 2006. The use of otolith shape and chemistry to determine stock structure of Mediterranean horse mackerel *Trachurus mediterraneus* (Steindachner). *J. Fish Biol.*, 69: 165-180.
- Tzeng, T.D., C.S. Chiu and S.Y. Yeh, 2001. Morphometric variation in red-spot prawn (*Metapenaeopsis barbata*) in different geographic waters of Taiwan. *Fish. Res.*, 53: 211–217.
- Warheit, K.I., 1992. The role of morphometrics and cladistics in the taxonomy of fossils: a paleornithological example. *Syst. Biol.*, 41(3): 345–369.
- Zafar, M.S., M.N. Amin, and M.J. Iqbal, 2007. Biodiversity of fisheries organisms in the Pagla river of Bangladesh. *Bangl. J. Fish.*, 30: 165-175.
- Zimmermann, G., P. Bosc, P. Valade, R. Cornette, N. Améziane and V. Debat, 2012. Geometric morphometrics of carapace of *Macrobrachium australe* (Crustacea: Palaemonidae) from Reunion Island. *Acta Zool.*, 93(4): 492-500.

(Manuscript Received: 16 June 2022)