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Morphometric and meristic identification and differentiation between two sympatric *Macrobrachium* prawn species in the northwest Bangladesh

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Abstract

Species identification is becoming a difficult task when there is a very little difference among them. In crustaceans things are more complicated as because they are quiet similar in appearance. To identify and differentiate between two sympatric freshwater prawn *Macrobrachium lamarrei* and *M. lanchesteri* in the northwestern Bangladesh collection of experimental samples were done from three different rivers from three different districts. Morphometric and meristic characters were applied for species recognition and statistical analyses were conducted to differentiate the prawn species. ANOVA (One way analysis of variance) was conducted at 5% level of significance to compare the values of 22 morphometric and4 meristic characters. The ANOVA showed the difference significantly at p>0.001, 0.01 and 0.05 in 15 morphometric and 3 meristic characters. To understand variation PCA (Principal Component analysis) and DFA (Discriminant Function Analysis) were tested. PCA products produced three components describing 71.77% variation due to 15 of 22 morphometric characters. The discriminate scores separated and correctly classified the two species. Hierarchical cluster analysis also distinguished the two species into two separate clusters. However, reliable molecular study needed for more authentications to differentiate species due to morphological plasticity. This study, by differentiating and correctly identifying of these species, will be helpful to conduct research in future.

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Introduction

Prawns are the important fisheries commodities in Bangladesh having good market demand and many health benefits. Although these are one of the important export fisheries items but the numbers of species for export are quiet few There are 62 documented species of shrimp and prawn in the country of which 6 are extremely freshwater, 14 inhabits both in fresh and saltwater and rest are marine (Hossain, 2013). Among the freshwater *Macrobrachium* under the family Palaemonidae constitutes the large number of prawn.

Macrobrachium contains more than 200 described species in the world (Jayachandran, 2001; Short, 2004). Mar and Myint, 2014 documented eight and Arumugam, 2011 reported 24 palemonid prawns species from neighboring country Myanmar and Tamil Nadu, India respectively. In Bangladesh, there are as many as 24 freshwater prawns species along with *Macrobrachium* (Shampa *et al.,* 2017). *Macrobrachium* represented by 12 species (Hossain, 2013), occurring throughout the vast area of river, lake, beels, marsh, riceland and vegetated area etc. Ray *et al.,* 2020 described 6 *Macrobrachium* species from the northern part of Bangladesh.

Although several prawns are exportable many are still not in concern. Furthermore exportable freshwater prawn is not always available to rural subsistence people and also costly. They satisfied their desire by consuming smaller and non-commercial prawn. Hence these smaller prawn species draws attention to evaluate their stock structure and potentiality to introduce in the aquaculture of Bangladesh.

Fisheries science deployed many tools such as genetics and morphometric (Mir *et al*., 2013) to identify and differentiate various taxonomic groups (Reist, 1985) and determine relationships among them (Turan, 1999). Morphometrics is the measurement of quantitative morphological traits that express body form and is a powerful tool to measure differences and relatedness among stocks (Ihssen *et al*., 1981; Melvin *et al*., 1992; Digo *et al,*. 2015).

Precise identification of species is very challenging in assessment of distributions and population dynamics and it is very tough due to morphological similarity of the sympatric species.

Thus, this study highlighted the relationship of two sympatric *Macrobrachium* species, *M.lenchesteri* and *M. lamarrei* in morphology and habitat used. *M.lamarrei* is a benthic freshwater decapod, omnivorous in nature. They mostly found in big waterbodies like beels, swampy area, and river and like to stay in turbid water (De Grave and Fransen, 2011). *M. lenchesteri* is also benthic freshwater decapods, mostly gonochoric and usually found in mud, sand and shells (Hajisamae and Yeesin, 2014.)

Monod, 1980 cited the merus and carpus lengths were most discriminant characters of *Macrobrachium* that were undefined for the other species. This result difficulties in the identification of morphologically similar species and are generally occurs sympatry (Ville, 1970; Marioghae, 1982) in different waterbodies (Goore´ Bi, 1998; Le´veˆque *et al,*. 1983).

Therefore the prime focus of this study was to fix the morphological differences for quick identification of the two prawn species from northwest Bangladesh.

Materials and methods

Specimen collection and preservation

The study was conducted from the month July, 2019 to June, 2020. Sixty prawn samples were collected from the fishermen of the three districts (Rangpur, Dinajpur and Thakurgaon) of northern region of Bangladesh from three rivers namely Jamuneshwari (25°40'34.2" North and 89°03'43.0" East), Atrai (25°36'46.4" North and 88°41'58.0" East), and Tangon (25°49'32.8" North and 88°23'07.5" East) (Fig. 1). After collection the samples were sorted, photographed and preserved in 70% Ethanol. Morphometric identification and alcohol preservation of prawns were done at the post graduate laboratory of the Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.

Table 1. Morphometric characters used to differentiate two sympatric prawn species

Abbr.	Measure character	Description of Character
Morphometric		
TL	Total length	Distance from the tip of rostrum to the tip of the straightened body telson
SL	Standard length	Distance from the base of eye to the uropode base
HL	Head Length	Distance from the rostrum tip to the carapace end
OL	Orbital Length	Length from upper to lower part of eye
RL	Rostrum length	Distance from the rostrum tip to the posterior part of eye
CL	Carapace length	Distance from the base of eye stalk to the end of carapace
CW	Carapace width	Distance from the left and right side of carapace
CH	Carapace height	Highest depth of the carapace
DaL	Dactylus length 1&2	Length of dactylus
PrL	Propodus length 1&2	Length of dactylus
CaL	Carpus length 1&2	Length of carpus
MeL	Merus length 1&2	Length of merus
IsL	Ischium length 1&2	Distance from proximal and distal margin of ischium
AL	Abdominal length	Distance from the first abdominal segment to the base of the uropode
SSH	Second abdominal segment height	Highest depth of the 2nd abdominal segment
SiSL	Sixth abdominal segment length	Length from the base of 6th abdominal segment to the the telson base
TeL	The length of the telson	Length from the base to the tip of the telson
TeW	The width of the telson	Most width point of the telson
Meristic		
URT	Rostrum dorsal teeth	No. of teeth on upper/ dorsal side of rostrum
LRT	Rostrum ventral teeth	No. of teeth on lower/ ventral side of rostrum
PrOT	Pre-orbital teeth	No. of rostrum teeth in front of orbit
PsOT	Post-orbital teeth	No. of rostrum teeth orbit

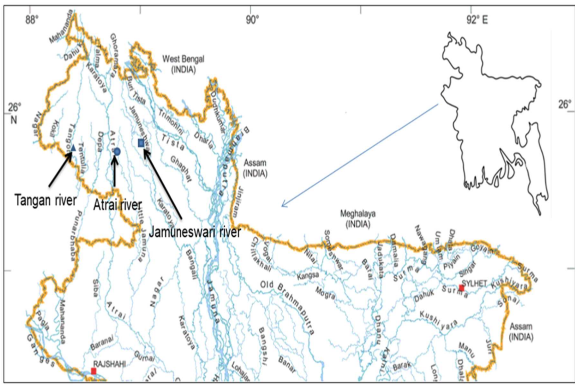

Fig. 1. Map showing the sample collection site

Morphological and morphometric observation

Morphometric and meristic characters were measured for all specimens. There were 4 meristic characters from rostrum and orbital point and 22 morphometric characters from body length, rostrum, walking legs, carapace, abdomen and telson of the specimen were selected (Table 1, Fig. 2). The selected characters were then counted and measured by using slide-calipers, normal centimeter scale, compus and forceps. The mophometric and morphological observation were performed on the account of Dineshbabu *et al.,* 2013, Konan *et al.,* 2008.

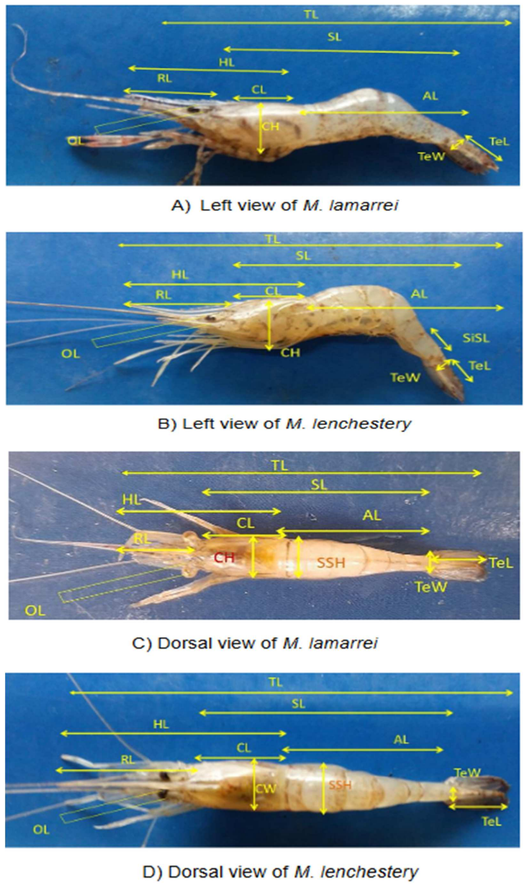

Fig. 2. Morphometric features of two *Macrobrachium* species

Statistical analysis of data

At first morphometric data were normalized with Shapiro-Wilk test. To minimize the size effect all the characters went to standardization with Elliot *et al.* (1995) formula before analysis.

$$M_{adj} = M_o (L_s / L_o)^b$$

Where, M_o is the initial measurement of the character; M_{adj} is the measurement after adjusting size; L_s is the total length of prawn and L_o is the mean of total length for all samples. Parameter b is the slope of regression of $\log M_o$ on $\log L_o$, using all prawn individuals.

To determine the correlation among the 22 morphometric characters Pearson's correlation coefficients were calculated. The comparison of different morphometrics values among the prawns were performed through one way analysis of variance (ANOVA). Morphometric measurements were analyzed with Principle Component Analysis (PCA), Discriminant Function Analysis (DFA) and Cluster Analysis (CA) to verify the classification in each group members.

As because morphological data has a tendency to correlate, we used principal component analysis (PCA) using Eigen value 1 and DFA was performed on the identified principal components to find out the unique dimension of morphological characters.

A Dendrogram with ward lingkage was constructed to assess the relationships prawn populations, proposed by Ferrito *et al.*, 2007 and Slabova and Frynta, 2007. The analysis of meristic characters was carried out with non-parametric (Kruskal-Wallis) test. All of the statistical interpretations were accomplished with SPSS-22 and Microsoft Excel-2007 at 95% confidence intervals.

Results

Statistical data analysis showed that there was great variability between the prawn species. The morphometric characters analyzed in the study of 60 prawn specimens separates into two morphotypes.

In the study there are 15 morphometric characters out of 22 (Table 2) were found to be significantly correlated and the significant difference ($p<0.05$) among the characters were displayed with ANOVA. The parameters SL, RL, DaL1, CaL1, SSH, SiSL and TeW were non-significant characters.

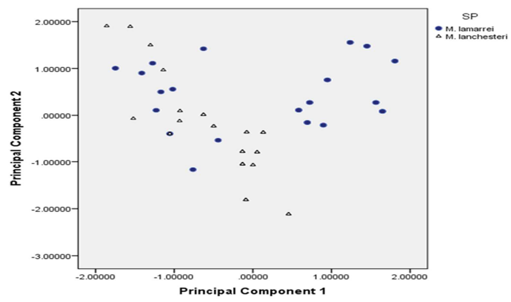

Fig. 3. Scatterplot of first two principal components from the principal components analysis

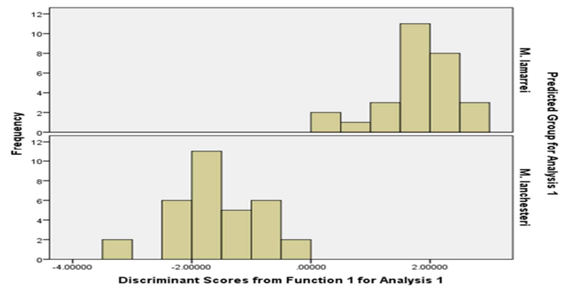

Fig. 4. Discriminant histogram of the two sympatric prawn

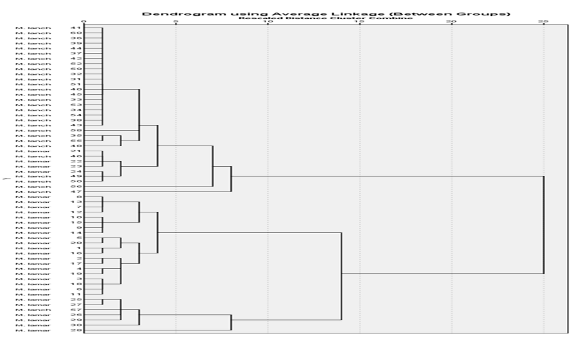

Fig. 5. Dendrogram of two prawn species

The differences in morphological traits between the two prawns were explained principal components analysis showed significance ($P<0.05$) with 0.773 KMO and Bartlett's Test. The PCA results three components, clarify 71.77% of the variation associated with the 15 of the 22 morphological traits studied (Table 3).

Table 2. Statistics of morphometric measurements of two sympatric prawns. Significant levels (P) derived from ANOVA

Parameters	*M. lamarrei*				*M. lanchesteri*				*F*-values	*P*-values
	Min	Max	Mean	Std. Dev	Min	Max	Mean	Std. Dev		
SL	3.22	3.92	3.6080	0.16764	2.98	3.95	3.5930	0.28709	0.062	0.805
HL	1.92	3.73	3.0418	0.44426	1.90	3.34	2.2311	0.25779	74.737	0.000***
RL	0.81	1.41	1.0308	0.16753	0.69	1.33	0.9621	0.15927	2.645	0.109
OL	0.16	0.24	0.1895	0.01916	0.14	0.25	0.1759	0.02182	6.595	0.013**
CL	0.91	1.21	1.1126	0.10569	0.54	1.09	1.0303	0.10470	9.163	0.004**
CW	0.52	0.71	0.6411	0.04895	0.51	0.65	0.5855	0.04327	21.727	0.000***
CH	0.60	0.79	0.7103	0.05891	0.59	0.72	0.6634	0.03660	13.667	0.000***
DaL1	0.10	0.92	0.1720	0.14428	0.10	0.19	0.1387	0.02129	1.561	0.217
PrL1	0.20	0.32	0.2672	0.03643	0.20	0.29	0.2494	0.02684	4.620	0.036**
CaL1	0.43	0.57	0.5298	0.03069	0.42	0.59	0.5159	0.04560	1.915	0.172
MeL1	0.38	0.48	0.4366	0.02728	0.36	0.48	0.4162	0.02933	7.823	0.007***
IsL1	0.18	0.36	0.2543	0.03733	0.21	0.39	0.3048	0.04754	20.976	0.000***
AL	1.54	2.13	1.9998	0.12002	1.75	2.42	2.1372	0.15471	14.786	0.000***
DaL2	0.16	0.56	0.3789	0.14591	0.17	0.30	0.2209	0.02562	34.104	0.000***
PrL2	0.35	1.21	0.8323	0.33604	0.33	0.57	0.5082	0.06499	26.896	0.000***
CaL2	0.44	0.97	0.7886	0.12594	0.62	0.79	0.6926	0.03957	15.877	0.000***
MeL2	0.42	0.76	0.6103	0.12131	0.44	0.58	0.4675	0.03408	38.560	0.000***
IsL2	0.33	0.57	0.4424	0.07417	0.37	0.65	0.5394	0.09121	20.438	0.000***
SSH	0.59	0.73	0.6503	0.03359	0.53	0.73	0.6629	0.04402	1.544	0.219
SiSL	0.43	0.60	0.4891	0.03468	0.42	0.60	0.5039	0.04802	1.883	0.175
TeL	0.47	0.76	0.6723	0.04953	0.60	0.73	0.6361	0.03507	10.676	0.002***
TeW	0.18	0.25	0.2064	0.01882	0.18	0.26	0.2093	0.02086	0.318	0.575

The PCA analysis indicated that 64.59% variation was due to the first two components (49.14% for PC1 and 15.45% for PC2). The principal component 1 (PC1) was represents by second pereiopods and carapace characters mainly: DaL2 with factorial loading, 0.95, PrL2 (0.93), CaL2 (0.82), MeL2 (0.92), Head length (HL: 0.7) and carapace length (CL: 0.71), carapace width (CW: 0.75) and carapace height (CH: 0.7). The second PC was highly connected with abdomen length (0.72) and orbital length (−0.67). The third component explained 7.18% of the total variance with a relatively small eigenvalue (1.08). Scatter plots of PC1 and PC2 for the prawns an obvious morphological variation in the traits (Fig. 3).

Table 3. Component scores of morphometric traits producing from a Principal Component Analysis (PCA).

Characters	PC 1	PC 2	PC 3
Head length	0.704	-0.248	-0.057
Orbital Length	0.269	-0.668	-0.188
Carapace Length	0.710	0.322	0.381
Carapace Width	0.752	0.248	0.094
Carapace Height	0.708	0.294	0.023
Propodus Length 1	0.610	0.562	0.046
Merus Length 1	0.613	-0.339	0.251
Ischium Length 1	-0.638	0.312	0.507
Abdomen Length	-0.315	0.715	-0.421
Dactylus Length 2	0.952	0.179	-0.067
Propodus Length 2	0.930	0.309	-0.006
Carpus Length 2	0.825	0.078	0.005
Merus Length 2	0.932	-0.018	-0.183
Ischium Length 2	-0.613	0.345	0.407
Telson Length	0.531	-0.516	0.426
Total Variance	7.370	2.318	1.077
% of Variance	49.136	15.451	7.182
Cumulative %	49.136	64.587	71.769

Table 4. Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions

	Function
	1
Head length	0.731
Merus Length 2	0.525
Dactylus Length 2	0.494
Propodus Length 2	0.438
Carapace Width	0.394
Ischium Length 1	-0.387
Ischium Length 2	-0.382
Carpus Length 2	0.337
Abdomen Length	-0.325
Carapace Height	0.312
Telson Length	0.276
Carapace Length	0.256
Merus Length 1	0.236
Orbital Length	0.217
Propodus Length 1	0.182

Variables ordered by absolute size of correlation within function.

Table 5. Classification Results of discriminant analysis of the morphometric characters of two prawn population

		Species	Predicted group membership		Total
			M. lamarrei	*M. lanchesteri*	
Original	Count	*M. lamarrei*	26	4	30
		M. lanchesteri	2	28	30
	%	*M. lamarrei*	86.7	13.3	100.0
		M. lanchesteri	6.7	93.3	100.0
Cross-validated[b]	Count	*M. lamarrei*	25	5	30
		M. lanchesteri	4	26	30
	%	*M. lamarrei*	83.3	16.7	100.0
		M. lanchesteri	13.3	86.7	100.0

a. 90.0% of original grouped cases correctly classified. b. 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. c. 85.0% of cross-validated grouped cases correctly classified.

Table 6. Kruskal-Wallis test for meristic counts of two prawn population

Meristic characters	Descriptive statistics of two prawn species(Minimum–Maximum)		Kruskal–Wallis Test (H-value)	Significance
	M. lamarrei	*M. lanchesteri*		
URT	7 (8-6)	7.07(8-6	0.246	0.620
LRT	6.67(8-3)	3.6(7-3)	34.394	0.000***
PrOT	4(5-3)	4.7(5-3)	21.758	0.000***
PsOT	3(3-3)	2.27(3-2)	34.158	0.000***

The Wilks' λ statistic of discriminant function was found significant (λ=0.293, χ^2 = 62.01, P<0.05), indicating a relatively considerable degree of interspecies variance. The DFA sort out HL (0.731) and MeL2 (0.525) as vital characters for discrimination. Furthermore, DFA also picks out DaL2 (0.494) and PrL2 (0.438) as discriminating character (Table 4). The discriminant function correctly assigned 90.0% of the prawn species and 85% after cross-validation provides support for the differences in morphometric between the species. (Table 5, Fig. 4).

Hierachial cluster analysis showed two main cluster differentiating two prawn species shown in the form of a dendrogram (Fig. 5). Kruskal-Wallis analysis revealed the statistics between the two prawn species in terms of meristic counts were significantly different (P >0.05) (Table 6).

Discussion

The differentiation and identification of *Macrobrachium* prawn population from three different geographic areas in the northwest Bangladesh was designed using morphometric variations. Attempts to promptly differentiate two *Macrobrachiums* species have led to much confusion because of the slight morphological differences among the species. Initially, prawns were assigned into two species namely *M. lanchesteri* and *M. lamarrei* based on morphological characters explained by (Cai, 2006; Raghunathan and Valarmathi, 2007; Mar and Myint, 2014, Ray *et al.,* 2020). Morphological characteristics showed significant heterogeneity between *M. lanchesteri*and *M. lamarrei.* In the present study, one way ANOVA of morphometric characters of *Macrobrachium* prawn revealed significant differences between the two different freshwater prawn species.

Multivariate analysis is a fruitful technique in discriminating various animal species with morphometric characters. It is used to distinguished several crustacean species as the hard, well-detached crustaceans body parts simplifies accurate data collection (Overton *et al.,* 1997). All the performed analysis in the present study showed morphological variations between the two prawn types.

The unique disparity between the two species was mainly in the cheliped and in the relative joints between pereiopod among the groups.

Konan *et al,*. 2008 performed morphometric study on 33 morphometric and seven meristic characters to compare two species of shrimps, *Macrobrachium.* They differentiate two Macrobrachium species by the morphology and the size of second pereiopods which justify the current differentiation of two prawn species by head length and periopods length. Jayachandran (1998) distinguished two *Macrobrachium* species based on the relation of carpus and palm of the second periopodes also shows the reliability of the current findings.

The morphological homogeneity and heterogeneity was apparent in all populations of *Macrobrachium* prawn and DFA tool was used to discriminate between the two species. Significant differences between group centroids and high percentages of correct classification were obtained in the study. This accuracy of classification informed that the second pereiopod morphology is taxonomically very much useful and powerful are indicator to test the variation in morphometric group. Again, morphometrics are extremely important tools in systematic studies (Warheit, 1992). Furthermore, in genus *Macrobrachium*, morphometrics have been regularly applied for taxonomic research.

The rostrum armature and rostrum teeth, proportional size of second periopods and lenth and width of telson are the key characters to identify the freshwater prawn species (Win Mar, 2007). In Jayachandran,1998 isolated two morphologically similar species *M. birmanicum* (Schenkel, 1902) and *M. malcolmsonii* (Milne-Edwards, 1844) by the correlations between merus and palm, chela and carpus, palm and dactylus. Nagamine and Knight, 1980 and Kosh,y 1973 conclude with same decisions in *M. rosenbergii* (De Man, 1879) and *M. dayanum* (Henderson, 1893), respectively, showing the longer propodus length than the carpus.

In the two discussed species, there are visible differences was in the shape of rostrum, carpus and merus; the second pereiopod carpus is longer than chela merus and ischium but shorter than propodus in *M. lamarrei* but in *M. lanchesteri,* there are evident of longer second pereiopod carpus than chela, propodus, merus and ischium. There are also significant differences in rostrum size, rostrum teeth.

Current study in separating two species forms two distinct clusters of prawn. This clustering was may be attributable to different types of habitats (Corpuz *et al.,* 2013). Meristic characteristic in the current revision for all trials varied from 6-8 for teeth on dorsal margin of rostrum, 3-8 for teeth on ventral margin, 3-5 teeth for pre-orbital and 2-5 teeth for pos-torbital. These findings are quiet same to those described by Ray *et al.,* 2020 for *Macrobrachium* species.

Kruskal-Wallis test indicates the H-value of meristic characters significantly differed in three out of four characters among all stocks. Jayachandran, 1998 found 10-11 upper rostrum teeth and 2 post orbital teeth in *M. malcomsoni* to separate *M. biraminous* with 8-10 URT and 3 PsOT which was quiet alike with the present findings.

Species distinction

Although displaying variations, the morphological differences between the two prawn species are little and more research specifically on DNA analysis is strongly advised for ultimate identification.

The most distinctive characters between the two species were given below:

Macrobrachium lamarrei (Miline Edwards, 1837)

This species is identified by the following characters:

Rostrum: Elongated and upward curved. Dorsal margin contained 6- 8 teeth; ventral with 3-8 teeth; 4 pre-orbital and 3 post-orbital teeth.

Rostral formula: 3-5(4) +3/3-8(6.7)

Carapace: length 1.6 times > height; length>1.7 times than width.

Second periopode:

Dactylus< Propodus> Carpus> Merus> Ischium

Propodus is longer 1.1 times than carpus and 1.4 times than merus. But ischium 1.4 times shorter than merus

Macrobrachium lanchesteri (De Man, 1911)

This species is distinguished by the following characters:

Rostrum: Straight, long. Dorsal margin with 6-8 teeth; ventral with 3-7 teeth; 4 pre-orbital and 2 post-orbital teeth.

Rostral Formula: 3-5(4.7) + 2-3(2.3)/3-7(3.6)

Carapace: length 1.6 times > height; length>1.8 times than width.

Second periopode:

Dactylus<Propodus< Carpus>Merus<Ischium

Propodus is 1.4 times shorter than carpus but longer than merus 1.1 times. Here, the ischium 1.2 times longer than merus.

The present study assures that the variation between two species reveals the existence of high level of genetic variability among the different prawn populations. Hence, it is recommend more research especially molecular studies to flourish the argument of having genetic variation in the two sympatric prawn species from Northwestern Bangladesh.

Conclusion

The present study differentiated and identified two different species *M. lamarrei* and *M. lanchesteri*. The rostrum teeth and second pereiopods were observed the main distinguishing characters of the two species. Geometric morphometric and molecular analysis is recommended in future studies to get an accuracy of morphometric variability and differentiation between these prawn species.

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