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ANALYSIS OF MORPHOLOGICAL VARIATION OF FOUR POPULATIONS OF *MACROBRACHIUM ROSENBERGII* (DE MAN, 1879) (CRUSTACEA: DECAPODA) IN SRI LANKA

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ABSTRACT

Freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879) is one of the most economically important crustacean species cultured within its' natural distribution and beyond. In Sri Lanka, *Macrobrachium rosenbergii* has gained a value as an important organism in aquaculture. In culturing programs, brood stocks are collected from the wild populations. Thus, wild stocks are important as an immediate resource for addressing diversity problems in cultured stocks. This study analysed intraspecific variation among four populations of *M. rosenbergii* using morphological data. Samples were collected from four localities (populations): Negombo lagoon, Bolgoda estuary, Walawe River estuary and Nilwala River estuary. Total of 179 individuals were analysed using 10 morphometric and 2 meristic parameters. To determine the significant difference of size standerdized morphometric parameters among four populations, One way analysis of variance (ANOVA) was used. To distinguish four populations, Discriminant Function Analysis (DFA) was performed using morphometric parameters. First two discriminate functions totally accounted for 88.9% (69.3% and 19.6%) variables. First discriminate function by itself identified nine morphometric characters as contributors for this analysis. According to the results of ANOVA, all morphometric characters were variable among four populations which indicated the availability of adaptive traits within the species. However, Discriminant Function Analysis failed to distinguish geographically separated four *M. rosenbergii* populations. The importance of collecting information on population diversity levels of economically valuable aquatic species when constructing aquaculture and conservation programs are discussed.

Key words: aquaculture, adaptive traits, population variation

INTRODUCTION

Giant fresh water Prawn *Macrobrachium rosenbergii* (De Man, 1879) is the largest known palaemonid in the world. The natural distribution of *M. rosenbergii* extends from western Wallace line to the southern part of Asia. Among crustacean, *M. rosenbergii* attracted more attention in the recent years which causes to expand its distribution not only within its natural range, but even the beyond (Mather & de Bruyn, 2003). During past thirty years, the production of farmed *M. rosenbergii* was significantly increased and is expected that the production will be exceeded 400,000 tonnes by 2010 (FAO web site). Due to high abundance in natural water bodies in many countries of Asia, a significant fishery industry has been established based on the wild stocks of this economically important species (Mather & de Bruyn, 2003). However, it is reported that the wild stocks of this species are declining rapidly due to over-harvesting, habitat-loss and increased pollution (New, 2000; Ng, 1997).

To improve and expand the freshwater prawn fishery, National Aquaculture Development Authority (NAQDA) in Sri Lanka has launched a free stocking program of *M. rosenbergii* in selected medium irrigation tanks. Fingerlings for stocking programs are collected from hatcheries and the brood stocks are collected from the wild populations. Thus, wild stocks are important as an immediate resource for addressing genetic diversity problems in cultured stocks. Most genetic variations represent by phenotypic characteristics. Therefore, evaluation of diversity within and among populations could be initiated through morphological studies.

Long, stout and robust 2nd pair of periopod is a prominent feature of the genus *Macrobrachium*. Therefore, this character has

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been taken into account in many morphological studies recorded on this genus (Holthuis, 1950, Bruce and Chace 1993, Suzuki and Kusamura 1997). However, pereiopods of prawns are regenerated appendages, which are mainly used in reproductive and agonistic behaviours, thus are subjected to damage during their activities. More often they shed their periopods during trapping and collecting processes. If these appendages are in regenerating stage, the data collect at this stage could lead to erroneous results. Therefore, this study is designed to investigate the intra-specific variations among four populations of *M. rosenbergii* in Sri Lanka using morphological data other than periopods.

METHODOLOGY

Giant freshwater prawn, *M. rosenbergii* samples were collected from four populations: Negombo lagoon (Ja Ela in figure 1), Bolgoda estuary, Walawe river estuary and Nilwala river estuary (Fig. 1). Sample collections were conducted from January to December 2008 with the help of fishermen. Fifty individuals were collected from each population and samples were preserved in 70% ethanol until the measurements were taken. Initially, twenty-four morphometric and two meristic parameters were measured and the data were examined for normality. All measurable characters were considered as morphometric parameters while teeth of the rostrum were counted as meristic parameters. Morphometric measurements were taken to the nearest 0.01 cm with the aid of rulers, vernier calliper and threads. Data for meristic characters were collected by observations and counting under naked eye.

Characters with missing data and data that skewed from normality test (even after transformed) were omitted from further analysis. Finally, data collected from ten morphometric and two meristic parameters (Fig. 2, Table 1) for 179 individuals from four populations were computed to perform Multivariate analysis.

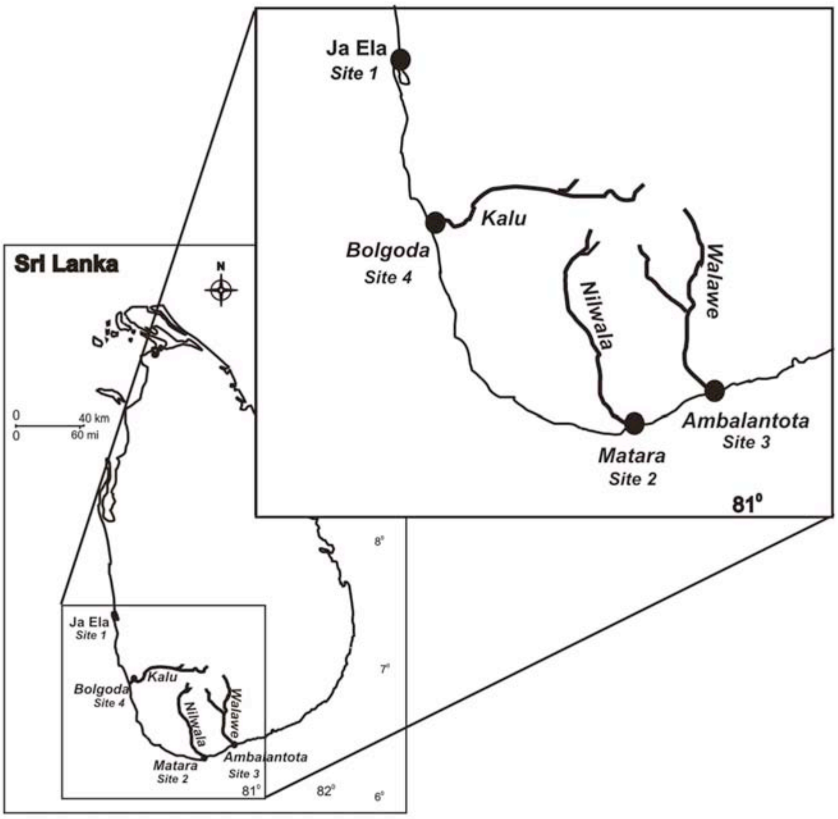

Figure 1. Map of Sri Lanka showing the sampling sites of *Macrobrachium rosenbergii.*

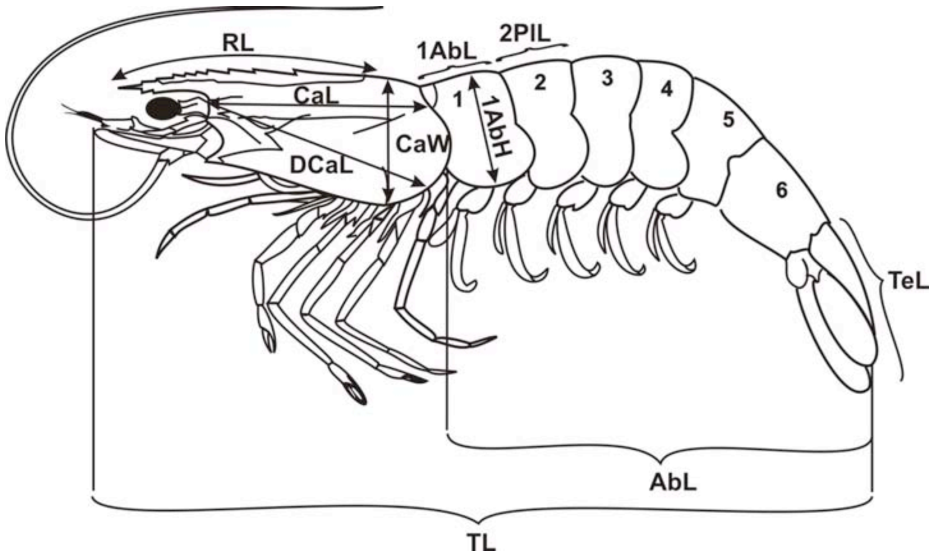

Figure 2. Schematic representation of *Macrobrachium rosenbergii* to show morphometric and meristic parameters used in the study. (Please refer Table 1 for abbreviations).

Table 1. Details of morphometric and meristic parameters of *Macrobrachium rosenbergii* used in the study.

Variable	Abbreviation	Measurements and Counts
Morphometric parameters		
Total Length	TL	The length from tip of the antennule's plate to end of the telson*
Abdominal Length	AbL	The length from the posterior end of the carapace to end of the telson*
Length of Telson	TeL	The maximum length of the telson*
Carapace Length	CaL	The length from the base of the eye stalk to posterior end of the Carapace*
Carapace Width	CaW	The length of the maximum depth of the carapace***
Diagonal Carapace Length	DCaL	The length from the base of the eye stalk to bottom of the posterior end of carapace**
First Abdominal Length	1AbL	The maximum length of the first abdominal segment*
First Abdominal Height	1AbH	The maximum height of the first abdominal segment***
Rostral Length	RL	The length from the tip to the end of the rostrum*
Second Abdominal Length	2PIL	The maximum length of second abdominal segment*
Meristic parameters		
Number of upper teeth of Rostrum	No.U	Total count of upper teeth of the Rostrum
Number of lower teeth of Rostrum	No.L	Total count of lower teeth of the Rostrum

* - Horizontal length measurements ** - Diagonal length measurements *** - Vertical length measurements

All morphometric measurements were size standardized using regression and residual analysis method (Thrope, 1976). The regression procedure uses the 'common' slope to estimate the relationship between the dependent variable (each morphometric parameter) and the independent /reference parameter (CaL). Therefore, any particular value of CaL (X_1), a value for any morphometric can be predicted (y_2) using this relationship. The estimated pooled mean value for the carapace length (CaL) was used to determine the predicted value for the dependent (Y_1) parameter. For a given specimen, the difference between this predicted and observed value (Y_1-Y_2) was entered for the analysis. Utility of size standardized data is important in this analysis as differences among groups for measured parameters may be biased due to growth stages and the sampling technique. One- way analysis of variance (ANOVA) was carried out to test the variability of morphometric characters among spatially separated four populations. This followed Turkey HSD multiple comparisons test for unequal sample size ($p<0.05$) (Zar, 1984). The significance of meristic characters among populations was estimated using Kruskal–Wallies nonparametric test. Discriminant Function Analysis (DFA) was performed to determine the most reliable morphological characters that are important to distinguish populations. Wilks' Lambda was used to test the significance of the discriminant function as a whole. Mahalanobis Squared Distance (D^2) was included in DFA and the distance between cases and the centroid for each group in attributed space was determined (Garson, 2008). Canonical scores derived from DFA analysis were plotted in two-dimensional spaces for visual detection of the separation of populations. All analysis was performed using SPSS (V. 16.0) or MINITAB (Version. 13.0) statistical packages.

RESULTS

The summary of the statistical analysis of collected data from four populations for 10 morphometric and 2 meristic parameters are given in Table 2. According to the data, the length of the reference parameter (Carapace length) ranged from 0.39 to 1.04 and the highest value was recorded from the Negombo lagoon population. The statistical results of ANOVA revealed significant variability among the four populations for all morphometric parameters ($p<0.05$). According to the results of Kruskal–Wallies nonparametric test, a significant difference cannot be observed for the number of upper and lower teeth of the rostrum among Populations ($p>0.05$).

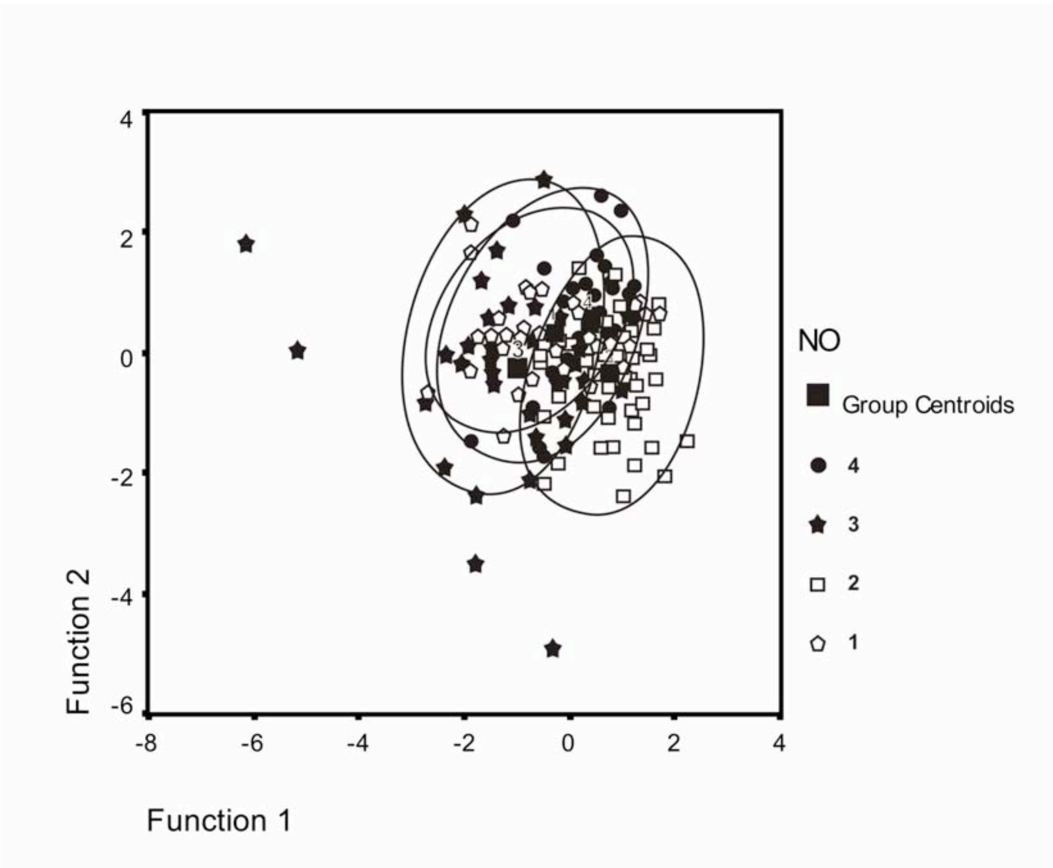

Figure 3. The Cluster Plot between 1st and 2nd Discriminant Functions for four populations of *Macrobrachium rosenbergii*.

Table 2. Summary of the statistical analysis of morphometric (1-10) and meristic (I-II) parameters *of Macrobrachium rosenbergii*
(N= Number of samples used in each population)

	Negombo Lagoon (Site 01)(N=46)			Nilwala River (Site 02)(N=45)			Walawe River (Site 03)(N=45)			Bolgoda Estuary (Site 04)(N=43)		
Variable	Mean± SD	Maxim.	Minim.	Mean ± SD	Maxim.	Minim.	Mean ± SD	Maxim.	Minim.	Mean ± SD	Maxim.	Minim.
1. Lg TL	1.3651 ± 0.1169	1.5798	1.1673	1.4803 ± 0.0631	1.5855	1.3522	1.3362 ± 0.1507	1.5729	0.9777	1.4352 ± 0.0654	1.5717	1.3324
2. Lg AbL	1.1394 ± 0.1080	1.3345	0.9395	1.2404 ± 0.0525	1.3424	1.1239	1.0959 ± 0.1279	1.2833	0.7243	1.2012 ± 0.0625	1.3424	1.0607
3. Lg TeL	0.4304 ± 0.0973	0.6532	0.2788	0.5093 ± 0.0813	0.6435	1.5000	0.4016 ± 0.1430	0.6532	0.1461	0.47449 ± 0.06199	0.60206	0.34242
4. Lg CaL	0.7464 ± 0.1495	1.0414	0.4914	0.8775 ± 0.0879	1.0212	0.7243	0.7298 ± 0.1930	1.0212	0.3979	0.8464 ± 0.0749	1.0000	0.7404
5Lg CaW	0.6571 ± 0.1386	0.9395	0.4150	0.7869 ± 0.0877	0.9294	0.6335	0.6358 ± 0.1899	0.9494	0.3010	0.7517 ± 0.0789	0.8865	0.6128
6. Lg DCaL	0.8032 ± 0.1452	1.1038	0.5798	0.9311 ± 0.0984	1.0864	0.7782	0.7879 ± 0.1962	1.0899	0.4771	0.8954 ± 0.0913	1.0645	0.7404
7. Lg 1AbL	1.0556 ± 0.0945	1.2844	0.8751	1.1561 ± 0.0585	1.3010	1.0414	1.0545 ± 0.1578	1.2730	0.6990	1.1146 ± 0.0592	1.2175	0.9542
8. Lg 1AbH	1.6190 ± 0.0919	1.7800	1.4624	1.7197 ± 0.0442	1.7976	1.6101	1.5784 ± 0.1711	1.7462	0.6435	1.6563 ± 0.0867	1.7672	1.2175
9. Lg RL	0.9165 ± 0.1010	1.0960	0.7160	0.9982 ± 0.1094	1.4548	0.6990	0.8938 ± 0.1497	1.1239	0.6021	0.9648 ± 0.0736	1.0969	0.7924
10. Lg 2PIL	1.3651 ± 0.1398	1.5711	1.0000	1.4701 ± 0.0540	1.5711	1.3170	1.3430 ± 0.1394	1.6048	1.0414	1.3487 ± 0.0675	1.5502	1.2109
I. No .U	13.0 ± 1.183	15.0	9.0	12.0 ± 2.167	16.0	7.0	13.0 ± 1.534	16.0	8.0	13.0 ± 1.340	15.0	8.0
II. No. L	12.0 ± 1.414	7.00	14.0	11.0 ± 2.316	14.0	6.0	12.0 ± 1.886	15.0	3.0	12.0 ± 1.922	14.0	6.0

Three discriminant functions were revealed according to the canonical scores by DFA analysis. According to the respective Eigen-values, the first and the second functions accounted for 69.3% and 19.6% respectively. As the third function accounted for very low value (11.1%), only first two functions were taken into consideration. The highest discrimination is given by the first discriminate function by itself, which identified nine size corrected morphometric characters as significant contributors for the separation of the populations (Table 3). Among them, the highest correlation is recorded for the Abdominal Length (AbL) (0.892) while the lowest is recorded for the Rostral Length (RL) (0.527). According to the basic classification given by the DFA, the highest value can be observed for Nilwala River estuary population (Site 02) (77.4%) while the lowest value for Bolgoda estuary population (Site 04) (31.6%) whereas those for the Ja-Ela population (Site 01) and Walawe river estuary population (Site 03) were scored 56.5% and 56.1% respectively. Derived discriminant functions using size-corrected morphometric parameters identified nine parameters that can be considered as significant contributors (Wilk′s Lamda = 0.542, P = 0.000). Mahalanobis Squared Distances indicated that two third of the cases in each group have less than 0.05 chance of gathering around the relative centroids of each group ($D^2 > 1.96$).

Table 3. Correlations between discriminating variables (parameters) and standardized canonical discriminant functions of *Macrobrachium rosenbergii*

Parameter	Functions		
	1	2	3
AbL	0.892*	-0.120	0.133
TL	0.784*	-0.209	0.247
1AbH	0.701*	-0.301	-0.073
2PIL	0.711*	-0.148	0.376
CaW	0.680*	-0.123	0.381
DCaL	0.608*	- 0.136	0.372
1AbL	0.579*	-0.317	0.344
TeL	0. 595*	-0.133	0.091
RL	0.527*	-0.146	0.138

* Largest absolute correlation between each variable and any discriminant function

The scatter-plot derived after plotting, 1st and 2nd Discriminant Functions for four populations of *M. rosenbergii* are given in Fig. 3. However, the plot indicated the overlapping of the populations and thus failed to distinguish the four geographically separated *M. rosenbergii* populations.

DISCUSSION

Morphometric variability among different geographical populations may be attributed due to distinct genetic structure and environmental conditions (Waldman *et al.*, 1988). Therefore, animals with the same morphometric characters are often assured to constitute a stock and that has been utilized widely in stock differentiation in fisheries industry (Avsar, 1994). For the genus *Macrobrachium*, morphological diverse geographical groups are reported in the literature (Carini and Hughes, 2004; Short, 2004, Wowor and Ng, 2007).

The current study indicates that meristic characters do not favour in intra-specific population studies of *M. rosenbegii*. Wowor and Ng (2007) also failed to distinguish closely related *M. rosenbergii* and *M. dacqueti* species using meristic characters. However, in previous studies, Cowles (1914) and Johnson (1973) indicated the usefulness of rostral teeth to distinguish the above two species. According to the results, the discriminant functions that were based on canonical scores failed to distinguish the four geographically separated *M. rosenbergii* populations. Although, the centroids for the four populations were clearly separated, the scatter plots for the four populations overlapped each other (Fig. 3). This was further confirmed by the results of Mahalanobis Squared Distances, which indicated the deviation of cases from centroids in each group ($D^2 > 1.96$). However, the observed morphometric differences indicated significant variability among the four populations. This may be due to local adaptations within populations as a result of environmental variability in different localities. Therefore, these populations could be considered as adaptive traits within the species and could be consider when applying conservation measures.

There is a possibility to some extent to mix populations of *M. rosenbergii* by sea currents as adult females arrive to spawn in estuaries. Especially, this can be occurred among the four sites due to their close proximity. However, genetic data are essential for clear understanding of the degree of variability among biogeographically separated *M. rosenbergii* populations. Past studies indicated that molecular genetic data are more reliable in estimating gene flow among populations and thus are important in identifying geographic variability among freshwater prawn populations (de Bruyn *et al*., 2004a, 2004b; Tong *et al*., 2000).

As *Macrobrachium* populations are declining, collection of information and conduction of this type of studies are important from a conservation point of view. In culturing programs, brood stocks are selected from wild populations, thus identification of geographically diverse *M. rosenbergii* populations are important. In the above cases morphological analyses are the fundamental approaches that have to be undertaken a priori. Most phenotypic variations arise due to genetic variations (Tzeng *et al*., 2001) and therefore, the combined approaches of morphometric and genetic analysis facilitate to recognize significantly divergent populations. These diverse populations could be treated as Management Units (MU) which can then be prioritized in conservation efforts.

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