



Research Article

Species differentiation of deep sea crabs (*Charybdis feriatus*, *Portunus pelagicus* and *P. sanguinolentus*) using conventional morphometric data off Ratnagiri Coast, India

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ABSTRACT

Deep sea crab biodiversity has been severely hampered by insufficient taxonomic knowledge. In present study, blue swimming crabs namely, *Portunus pelagicus*, *P. sanguinolentus* and *Charybdis feriatus*, off Ratnagiri coast, India, were investigated with a view to differentiate them based on conventional morphometry. The data was composed of nine variables on dorsal aspect of cephalothorax. The Student's t-test significantly differentiated three species ($p < 0.05$). Multivariate techniques, PCA and CVA, used for conventional data showed that PCA could only differentiate *Charybdis feriatus* as another genus; however, CVA set apart each species from others. The present investigation clearly differentiated the species investigated using efficient morphometric tool, which will be further used for stock identification, geographic, ecological and evolutionary variations.

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INTRODUCTION

Marine crabs (Subphylum: Crustacea, Order: Decapoda, Infraorder: Brachyura) with 705 species, forms an important component of the crustacean fisheries, inhabiting marine and estuarine environment (Venkataraman and Wafar, 2005 and Bal and Rao, 1990). Crustacean contributed on an average of 13% of total marine fish landings of India during 2013-2014. The major groups in the crustacean landings were non-penaeid shrimps (2.06%) followed by penaeid shrimps (1.83%), crabs (0.46%), stomatopods (0.24%) and lobsters (0.43%). Major group in the crab landing was *Charybdis spp.* (56%). *Charybdis feriata* contributed about 17% of the total catch followed by *Portunus pelagicus* (10%) and *Portunus sanguinolentus* (9%). Crabs were mainly exploited by multiday trawlers (72%) followed by gillnetters (10%), dolnetters (10%) and single-day trawlers (5%) (CMFRI, 2015).

Identification, differentiation and geographic variations of species are essential for sustainable management of these resources. Further, understanding of the extent of interspecific variation and its consequences on species adaptation are a fundamental part of biological research and requires that the variation in both precisely and accurately

estimated (Parsons, 2003). Morphological characters have been used to differentiate closely related species (Bookstein, 1991; Rohlf and Marcus, 1993; Marcus *et al.*, 1996). Shape differences related to patterns of fat reserve deposition and utilization showed significant variation in *Cichla temensis* (Cichlidae) using morphometric techniques (Reiss and Grothues, 2015; Paul and Thomas, 2015). *Barilius bendelisis* has significant phenotypic heterogeneity between the geographically isolated regions of Central Indian Himalaya (Mir *et al.*, 2015).

In spite of knowing the species on its external observable characteristics, morphology plays an important role in maintaining species integrity with respect to its existence in the respective environment (Mir and Chandra, 2013). The morphometric analysis offers more efficient and powerful tools to identify differences between fish populations, detecting differences among groups and to differentiate between species of similar shape (Mojekwu and Anumudu, 2015). Conventional morphometrics find diverse applications in fisheries such as, describing allometric patterns in body shapes, growth pattern, predicting puberty moult, assessing geographic variation and determining condition factors (Winans, 1984; Goes *et*

al., 1997; Chu, 1999; Palma and Andrade, 2002; Cardoso and Fransozo, 2004). Conventional morphometrics is also employed for delineation of species and/or stocks, describing the ontogenic changes in body forms and in phylogenetic relationships (Fransozo *et al.*, 2003). The main advantage of conventional morphometrics is that it has a very simple approach and the technique can be used by researchers to investigate within and between species variations, stock differentiation at both environmental and geographic variations (Lee, 1995). However, morphological characters are prone to environmental influences which can corroborate with genetic variation of the species with respect to mutation, migration and adaptation to sustain themselves in the environment (Merritt *et al.*, 1998; Pepe *et al.*, 2005; Hong *et al.*, 2012). Hence, in the present study three morph species of family Portunidae were investigated based on conventional morphometry.

MATERIALS AND METHODS

Sample Collection

A total of 180 specimens, representing sixty specimens of each species, of Portunid crabs viz., *Charybdis feriatius*, *Portunus pelagicus* and *P. sanguinolentus* of all size groups were collected off Ratnagiri coast (16°59'0"N and 73°18'0"E). Species were identified using FAO species identification sheets (FAO, 1983).

Sample Digitization

Sample digitization provides a complete archive of body shape and offers an opportunity for repeated measurement (Cadrin and Friedland, 1999). Samples to be digitized were placed on a flat platform with vertical and horizontal grids having an area of one centimetre square (cm²) and were used in calibrating the coordinates of digital images. Digitization was done by mounting on a levelling tripod with a bubble level as an indicator of the inclination and the images were obtained.

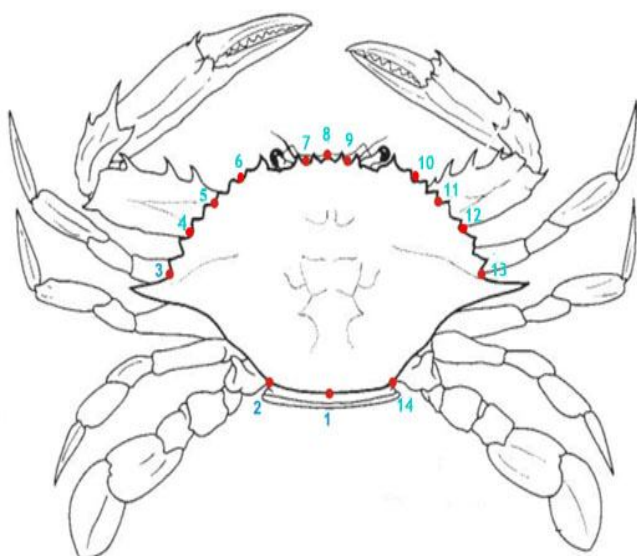


Fig 1. Schematic depiction of landmarks

Data Extraction

Utility program, TPSUtil V1.38 was used first to convert images from JPEG (*.jpeg) format to TPS (*.tps)

format. This is then followed by placing the respective positions of landmarks to cover overall body form on the cephalothorax of crab using TPSDig2 V2.1 as depicted in Fig. 1 (Rohlf, 2006). Nine morphometric distances representing the dorsal aspect were selected based on their capacity to capture overall body shape. Then, the morphometric data was extracted using Paleontological Statistics (PAST) (Hammer *et al.*, 2001). The nine conventional morphometric distances (landmarks), their codes and descriptions are given in Table 1.

Table 1: Landmarks, codes and description used for conventional morphometrics

Landmarks	Codes	Description
1-8	UP1	Carapace height (CH)
3-13	UP2	Carapace width (CW)
4-12	UP3	Upper carapace width (UCW3)
5-11	UP4	Upper carapace width (UCW4)
6-10	UP5	Upper carapace width (UCW5)
1-3	D7	Lateral carapace width lower left (LCWLL)
1-13	D8	Lateral carapace width lower right (LCWLR)
3-8	D9	Lateral carapace width upper left (LCWUL)
8-13	D10	Lateral carapace width upper right (LCWUR)

Statistical Analysis

Shapiro-Wilk's W-statistic used initially to test the data for normality of distribution followed by the estimation of coefficient of correlation. Descriptive statistics were obtained for all morphometric traits recorded. Further, Students't-test was done for between species comparison of means for conventional morphometric data. All the variations in size and shape between three species were analysed by Principal Components Analysis (PCA) and Canonical Variate Analysis (CVA). PCA was performed on SAS (9.3) whereas CVA was performed on PAST (V2.17).

RESULTS AND DISCUSSION

The means of conventional data were compared by Student's t-test, which yielded significant difference ($p < 0.05$) between the three species for means of all nine variables indicating that the species differed impressively from one another (Table 2). The correlation coefficients were estimated to the degree of association between the traits. All correlation coefficients were positive and significant for all the three species (Table 3).

Principal Component Analyses (PCA)

PCA performed on conventional morphometric traits is a variable reduction tool that reduces the variables by loading them on components (PC). Variables that are highly correlated are loaded on the same PC and therefore, the variables loading on different components are uncorrelated. Thus, each retained PC accounts for variation that is not accounted for by the other (Hidalgo, 2013). The computed eigenvalues and their proportions are given in Table 4.

Based on the eigenvalue one criteria and the scree plot (Fig. 2), only two principal components together accounted for 98.74% of the total variance were used for interpretation. Eigenvectors of the two retained components (PC1 and PC2) (Table 5) showed that almost all conventional morphometric variables loaded nearly equally on PC1 except UP5, which indicates upper carapace width, was discarded from the analysis as it loaded equally on both PC1 and PC2.

Table 2: Comparison of conventional data by Student's t-test ($p < 0.005$)

Parameter	PS:PP	PP:CF	CF:PS
UP1	0.0000159	0.001659	0.0000238
UP2	0.00013017	0.0013104	0.0000257
UP3	0.0000215	0.0000000947	0.0033369
UP4	0.0000213	0.0000397	0.00000000000034
UP5	0.0016921	0.008567	0.00057462
D7	0.0000027	0.000000296	0.0003206
D8	0.00000942	0.00000055	0.000268
D9	0.0000182	0.000000909	0.0021818
D10	0.0000291	0.000000754	0.0032479

Table 3: Correlation matrix for conventional data

	UP1	UP2	UP3	UP4	UP5	D7	D8	D9	D10
UP1	0								
UP2	0.9229	0							
UP3	0.9517	0.9584	0						
UP4	0.8729	0.9659	0.9692	0					
UP5	0.7944	0.6256	0.6064	0.4692	0				
D7	0.9721	0.9498	0.9952	0.9472	0.6497	0			
D8	0.9738	0.9532	0.9953	0.9463	0.6629	0.9980	0		
D9	0.9751	0.9627	0.9943	0.9533	0.6778	0.9953	0.9961	0	
D10	0.9740	0.9642	0.9944	0.9549	0.6765	0.9943	0.9963	0.9986	0

Table 4: Eigenvalues of the correlation matrix associated with PCA

Conventional data; Total = 9; Average = 1				
Component	Eigenvalue	% variance	Proportion	Cumulative
PC 1	8.24351	91.595	0.916	0.916
PC 2	1.64263	7.1404	0.0714	0.9874

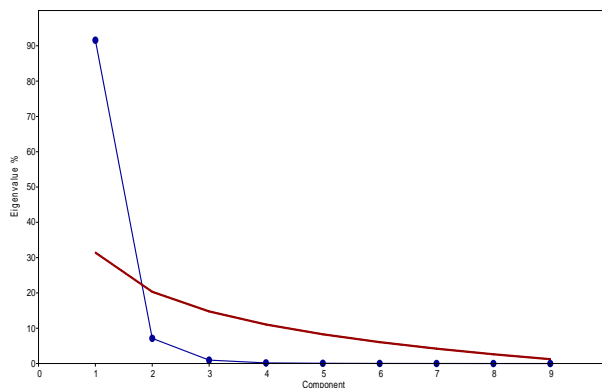


Fig. 2: Scree plot

Table 5: Eigenvectors (or variable loadings) for PCA on conventional data*

Traits	PC 1	PC 2
UP1	0.9806	0.1602
UP2	0.9697	-0.0952
UP3	0.9900	-0.1224
UP4	0.9482	-0.2907
UP5	0.7024	0.7078
D9	0.9982	-0.0309
D10	0.9982	-0.0334
D7	0.9930	-0.0581
D8	0.9950	-0.0434

* Highlighted variable(s) are discarded from the final analysis.

Of the nine variables assessed, eight variables (with the exception of UP5) loaded nearly equally on PC1. Therefore, PC1 in this case can be interpreted as isometric size indicating the relative size of the specimens (Cadrin, 2005). Consequently, PC2 in this context accounts for the maximum amount of the variation that is unaccounted for by PC1 (isometric size variance) (Cronin Fine *et al.*, 2013). As against size variation that is accounted for by PC1, PC2 generally measures shape variation (Cadrin, 2005). However, Marcus (1990) opined that beside size variation, the PC1 could also include significant amounts of shape variation in the first 'size' component and size variation in subsequent components.

From the scatter plot of PC2 on PC1, PCA differentiated (Fig. 3), *Charybdis feriatius* from *Portunus pelagicus* and *Portunus sanguinolentus* because of different body shape than other two species having almost same shape was not detected by PCA. Further *Portunus sanguinolentus*, which form a small cluster, was seen to be interspersed within *Portunus pelagicus*. Therefore, although most variables loaded on PC1, they seem to represent variations in both size and shape (Marcus, 1990) rather than only size (Cadrin, 2005; Yakubu and Okunsebor, 2011). Using conventional morphometric data, Rebello (2003) and Barluenga *et al.* (2006) were able to differentiate *Penaeus monodon* stocks from different parts of Kerala and sympatric Cichlids from Nicaraguan lake respectively using PCA.

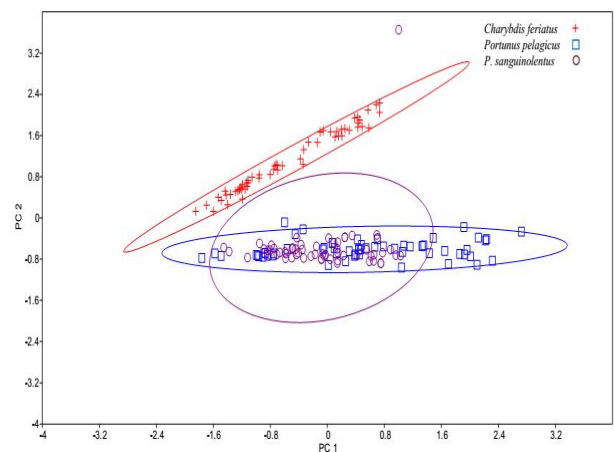


Fig. 3: Scatter plot of PC2 on PC1

Canonical Variate Analysis (CVA)

CVA is a supervised classification technique wherein data is classified a priori. CVA produces weightings that allow identifying those variables that are the most different between groups and discard the ones that are the same. CVA inferences were based on Hotelling's p -values (Sequential Bonferroni significance) (Table 6).

Table 6: Hotelling's p -values (Sequential Bonferroni corrected)

	CF	PP	PS
CF	0		
PP	3.32395E-64	0	
PS	1.72561E-61	2.21842E-26	0

When compared to the results of CVA with PCA, CVA was able to differentiate the three species as clearly indicate the existence of the different species (groups) (Fig. 4).

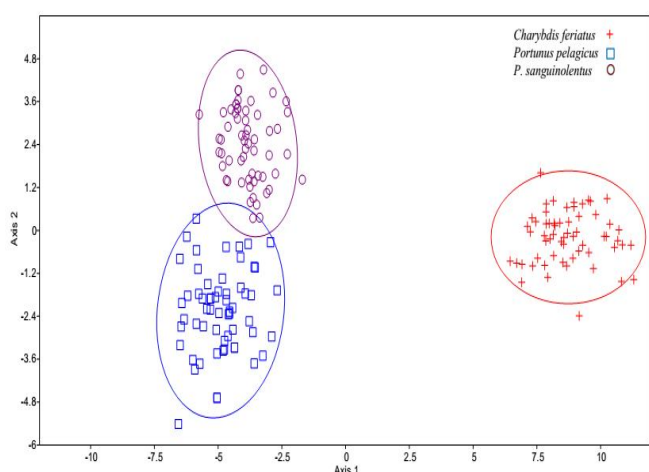


Fig. 4: Scatter plot for dorsal side

Actually, a high magnitude of differences was evident between the species based on Hotelling's (Sequential Bonferroni significance) p -values ($p < 0.0001$). Accordingly, CVA, Bagherian and Rehmani (2009) were able to differentiate between two populations of Shemaya, *Chalcalburnuschalcoides* from the estuaries of Haraz and Shirud rivers, Azerbaidzhan and Cavalcanti *et al.* (1999) between Serranid fishes from Brazil.

CONCLUSION

Data collected on nine variables subjected to Student's t -test showed significant differences ($p < 0.05$) indicating that the species differed greatly from one another morphologically. However, PCA could not differentiate between *Portunuspelagicus* and *Portunussanguinolentus*. Further, irrespective of the number of components retained (PCA) are almost loaded positively and heavily of first component. The reason for the similar loadings on a single axis could be attributed due to highly significant positive correlations ($p < 0.0001$) shared by all the variables investigated. As against PCA, CVA results showed the existence of three separate species with the differences between them being very highly significant ($p < 0.0001$). The morphometric tools used to differentiate the three

species were best suited based on their accuracy. The achieved taxonomic clarification leads to an improved basis for constructing identification keys of the three-species including morphological data in the future and also considered for stock identification and geographic variation.

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