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Truss based morphometric approach for the analysis of body shape in portunid crabs (*Charybdis feriatus, Portunus felagicus* and *P. Sanguinolentus*) along Ratnagiri coast, India

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Abstract
The present study was done on blue swimming crabs namely, *Portunus pelagicus, P. sanguinolentus* and *Charybdis feriatus* with a view to differentiate them on the basis of body shape using truss network. The truss distances between 14 landmarks on dorsal and 15 on ventral aspect of cephalothorax with 29 and 35 truss variables were measured. The Student's t-test significantly differentiated three species ($p<0.05$). A suit of multivariate techniques (PCA, FA and CVA) was performed to investigate distinction and pattern of morphological variation between species. PCA and FA performed on truss data yielded similar results where eigenvectors of first two components (PC1 & PC2) had explained 97.40% of variance for dorsal side while first component for ventral side data accounted 92.96% of the total variance. However, CVA strongly differentiated three species with a high magnitude of differences based on Hotelling's (Sequential Bonferroni significance) *p*-values ($p<0.0001$) on both the aspects. The findings support the use of truss network to unequivocal identification of numerous species, taxonomic clarification, morphological variation among species and it also provides interesting perspectives for the study of stock identification and geographic variation.

Keywords: blue swimming crabs; species differentiation; portunid crabs; truss morphometry

1. Introduction
Marine crabs with 705 species (Subphylum: Crustacea, Order: Decapoda, Infraorder Brachyura) is the important component of the crustacean fisheries of India lodging three principal families *viz.* Callipidae, Portunidae, Grapsidae of which family Portunidae is largest occupying marine and estuarine habitats [1]. Out of total, only few of them are commercially important and are used for human consumption like *Scylla serrate, S. Tranquebarica, Portunus pelagicus, P. sanguinolentus, Charybdis feriatus* etc. [2]. Understanding the origin, maintenance and consequences of inter-specific variation is the fundamental part of biological research and requires that the variation should be both precisely and accurately estimated [3]. This is true in case of the assessment and management of the marine fisheries resources [4]. One of the easiest ways of estimating variation within or between closely related species is to study the morphological variations between the species and this could be done by using conventional and truss morphometric approaches [5,6].
Conventional morphometrics involves measurements of linear distances (such as length, width and height) that are described by means of multivariate statistical tools to analyze the patterns of shape variation within and among groups [7] which further helps to describe allometric patterns in body shapes [8], growth pattern [9], predicting puberty moult [10], assessing geographic variation [11] and determining condition factors [12] in crustacean fisheries. With all advantages, conventional morphometrics has a drawback that it include the measurements taken from two different shapes could produce equal results because the data does not include location of where the measurements were taken relative to each other and the linear distance measurements are usually highly correlated which makes shape analysis difficult [8,10].
On the other hand, a new system of morphometric measurements, the truss network system (TNS), is increasingly being used for species and stock differentiation [13,14]. The truss network measurements have been defined as a series of measurements between landmarks that form a regular pattern of contiguous quadrilaterals or cells across the body form (Winans, 1984).

TNS covers the entire organism in a uniform network of lines (truss) and theoretically increases the likelihood of extracting morphometric differences between species backed by strong statistical computations [15]. Some of the advantages of using a truss network as described by Strauss and Bookstein (1982) include: systematic coverage across the form, in contrast to traditional characters which provide highly uneven coverage, thus enabling reconstruction of the original configuration of landmarks. The applications of statistical analyses such as analysis of variance (ANOVA) and multivariate analyses (Principal components analysis and Discriminant function analysis) could be correlated to differentiate among taxonomic groups and geometrical interpretations of studied organisms. However, morphological characters are prone to environmental influences and may not always corroborate with genetic variation of the species [13].

The marine catches of Ratnagiri are dominated by Portunid crabs. Despite of their prominence in local catches, the species have never been investigated or characterized on the basis of their morphometric variations. With this background, the present study was carried out with an objective to characterize morphometric variation of three blue swimming crabs *viz., Charybdis feriatus, Portunus pelagicus* and *Portunus sanguinolentus* along Ratnagiri coast, Maharashtra.

2. Materials and methods

2.1 Sample collection

A total of 180 specimens (sixty specimens of each) representing three species of Portunid crabs viz., *C. feriatus*, *P. pelagicus* and *P. sanguinolentus* were collected from Ratnagiri coast (16° 59' 0" N and 73° 18' 0" E) by trawling off and were identified using FAO species identification sheets [16]. Before being digitized, only the intact specimens were cleaned, tagged and stored in 5% formalinized seawater.

2.2 Digitization of samples

Specimen digitization provides a complete archive of body shape and offers an opportunity for repeated measurements [17]. Samples to be digitized were placed on a flat platform with vertical and horizontal grids having an area of one centimetre square (cm^2) and were used in calibrating the coordinates of digital images. The pereiopods and the abdominal flaps were placed on the platform in such a way that make their origin and insertion points visible. Digitization was done with Nikon Coolpix L26 (ver1.0) digital camera (Nikon, Japan) by mounting on a levelling tripod with a bubble level as an indicator of the inclination. Images from both dorsal and ventral aspects of the cephalothorax of the three species were obtained and after digitization, the specimens were again returned to formalin for further analysis. All digital images were identifiable based on the tags attached.

2.3 Obtaining morphometric measurements

A series of software TPSUtil V1.38 [5], TPSDig2 V2.1 [5] and Paleontological Statistics (PAST) were used to extract morphometric data from each individual image. TPSUtil is a utility program basically used to convert all images from JPEG (*.jpeg) format to TPS (*.tps). TPSDig2 is a window-based programme for digitizing landmarks and outlines in the images of objects for geometric morphometric analyses. The landmarks were digitized on each image using the 'Digitize landmarks' mode of the software and the landmark data were encrypted into the TPS files as X-Y coordinates. PAST is a multivariate analysis software package which is designed particularly for statistical, plotting and modelling functions in palaeontology. However, it is indispensible in extracting the landmark distances from the digital images of objects for that the images need to be provided as landmark-fixed TPSDig2 images as input. The data encrypted TPS format image files were used as input source in PAST and the data on distances between the landmarks were extracted using the 'All distances from landmarks' and '2 dimensional' options under the 'Geomet' menu of PAST.

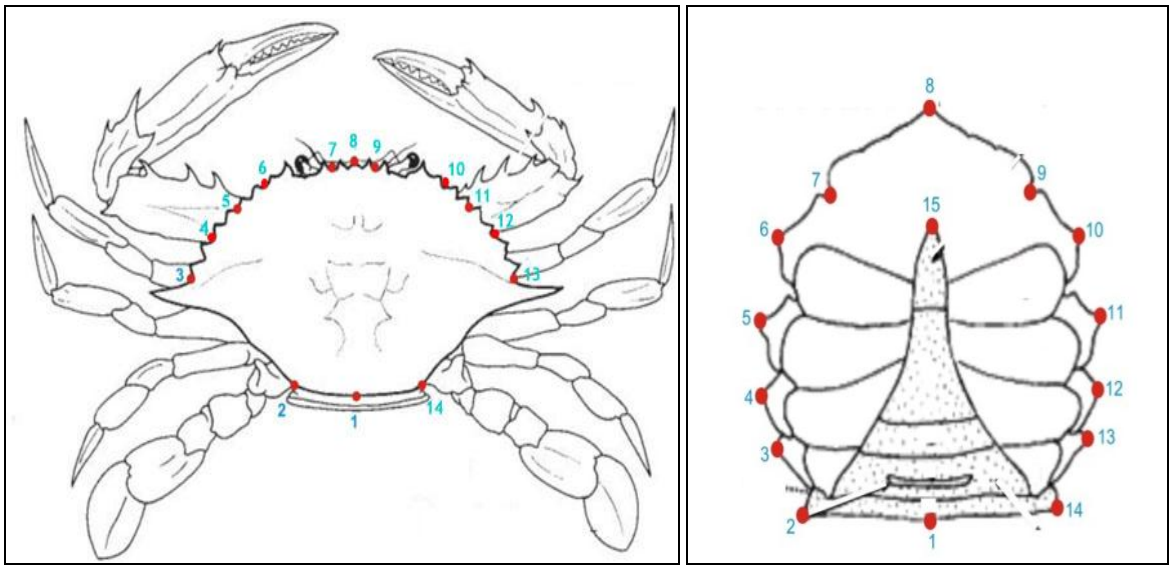

Fig 1: Schematic depiction of landmarks at dorsal and ventral aspect of the carapace

Truss system covers the entire organism in a uniform network of (truss) lines and theoretically increases the likelihood of extracting morphometric differences between species backed by strong statistical computations [15]. The truss network for the three species in the present study was based on 14 anatomical landmarks located on the dorsal side of the carapace and 15 anatomical landmarks located on ventral side were selected for truss morphometrics based on their capacity to capture overall body shape as depicted in Fig. 1. Truss analysis of the dorsal and ventral aspects included a total of 29 and 35 variables, respectively as described in Table 1.

Table 1: Landmarks and codes used for truss morphometrics

Dorsal Side										
Landmarks	1-8	3-13	4-12	5-11	6-10	7-9	1-7	2-13	3-14	4-13
Code	UP1	UP2	UP3	UP4	UP5	UP6	UP7	D1	D2	D3
Landmarks	3-12	5-12	4-11	6-11	5-10	7-10	6-9	2-3	13-14	3-4
Code	D4	D5	D6	D7	D8	D9	D10	L1	L2	L3
Landmarks	12-13	4-5	11-12	5-6	10-11	6-7	9-10	1-6	1-10	
Code	L4	L5	L6	L7	L8	L9	L10	L11	L12	
Ventral Side										
Landmarks	3-13	4-12	5-11	6-10	7-9	2-13	1-8	2-8	8-14	3-12
Code	UP1	UP2	UP3	UP4	UP5	UP6	UP7	T1	T2	D1
Landmarks	4-13	4-11	5-12	5-10	6-11	6-9	7-10	7-8	8-9	7-14
Code	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11
Landmarks	2-9	3-4	12-13	4-5	11-12	5-6	10-11	6-7	9-10	2-3
Code	D12	L1	L2	L3	L4	L5	L6	L7	L8	L9
Landmarks	13-14	2-7	9-14	1-7	1-9					
Code	L10	L11	L12	L13	L14					

2.1 Statistical analyses

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

3. Results

3.1 Correlation coefficient and Normality testing

All correlation coefficients were estimated to know the degree of association between the traits which showed positive and significant (p<0.05) correlations for all the three species (Table 2). The Student's t-test for the variables indicate that all the group means differed highly significantly (P<0.05) indicating that the species differed greatly from one another in terms of morphology (Table 3).

Table 2: Correlation matrix.

	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
								p<0.05	

Table 3: Comparison of data by Student's t-test

Parameter	**PS:PP**	**PP:CF**	**CF:PS**
UP1	0.00000159	0.001659	0.0000238
UP2	0.00013017	0.0013104	0.0000257
UP3	0.0000215	0.00000000947	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

3.2 Truss morphometrics

Truss data on the dorsal and ventral aspects of the carapace proper of the three species were subjected to a suit of multivariate techniques namely principal components analysis (PCA), factor analysis (FA) and canonical variate analysis (CVA).

3.2.1 Principal Component Analysis (PCA)

PCA is a variable reduction tool that reduces the variables by loading them on components (Principal component, 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 retained components [13].

PCA was performed and the eigenvalues and their proportions for both dorsal and ventral side data are given in Table 4. First two components were retained on dorsal side while only one component was retained on ventral (Fig. 2 and 3). Eigenvectors of the first two components (PC1 and PC2) for dorsal side data explained for 97.40% of the variance and first component (PC1) for ventral side data accounted for 92.96% variance (Table 5). PC1 in this case was interpreted as isometric size indicating the relative size of the specimens [14]. As against size variation that was accounted for by PC1, PC2 generally measures shape variation [17]. However, Marcus opined that beside size variation, the PC1 can also include significant amounts of shape variation in the first 'size' component and size variation in subsequent components [6]. In case of Catla, first component was contributed 91% of the body variability [18].

Out of 29 truss morphometric variables for dorsal side data, 21 were loaded equally on PC1, except 8 (paired landmarks) variables representing the lateral aspects of the upper

carapace, were discarded as they loaded nearly equally on both PC1 and PC2. In similar way, out of 35 variables, 33 variables loaded almost equally on PC1 for ventral side data while 2 variables namely D6 and L7 belonged to the group of paired variables were discarded since their loadings being meaningless.

The reason for this asymmetry in the loadings for these paired variables is hypothesized to be related to the functional arrangement of the ventral aspects of the crabs. In other words, almost all functional appendages (mouth parts, chelae, abdominal flap, walking legs, swimming legs) are harbored by the ventral side of the crabs. Of these, the chelae are known to display size allometry in the same individual owing to handedness (e.g. right-handed of left-handed) of the crabs. Hartnoll suggest that high allometric growth in a particular organ has associated with it changes in the allometry of the adjacent structures [14]. The disparity associated with appendages functionally could be responsible for the unequal loadings observed in the paired variable.

Table 4: Eigenvalues of the correlation matrix associated with PCA

DORSAL; Total= 29; Average = 1				
	Eigenvalue	% variance	Proportion	Cumulative
PC 1	23.7528	81.906	0.81906	0.81906
PC 2	4.49098	15.486	0.15486	0.97392

VENTRAL; Total= 35; Average = 1				
	Eigenvalue	% variance	Proportion	Cumulative
PC 1	32.5367	92.962	0.92962	0.92962
PC 2	0.73582	2.1023	0.02102	0.95064

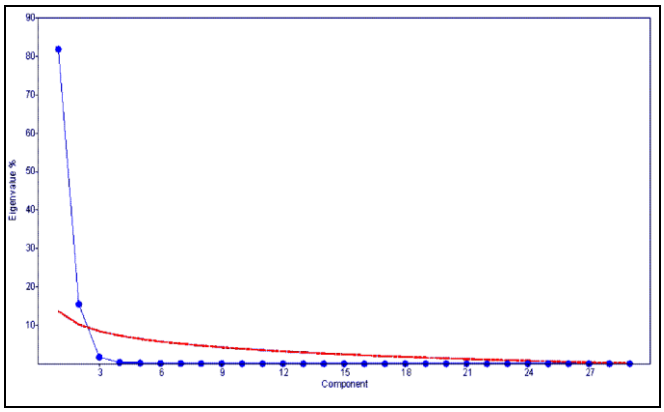

Fig 2: Scree plot for dorsal side

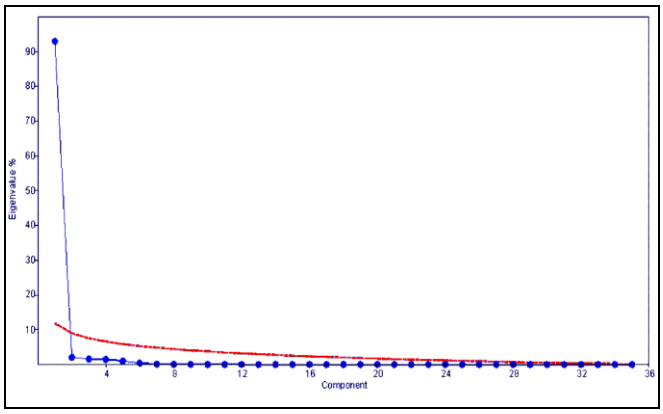

Fig 3: Scree plot for ventral side

Table 5: Eigenvectors for PCA on (a) dorsal and (b) ventral side truss data*

a) Dorsal side						b) Ventral side					
	PC 1	PC 2		PC 1	PC 2		PC 1	PC 2		PC 1	PC 2
UP1	0.978	-0.027	L2	0.953	0.178	UP1	0.997	-0.048	D12	0.998	-0.013
UP2	0.996	0.019	L3	0.699	0.527	UP2	0.997	-0.050	L1	0.997	-0.044
UP3	0.999	-0.023	L4	0.769	0.617	UP3	0.992	-0.018	L2	0.998	-0.043
UP4	0.979	-0.191	L5	0.818	0.549	UP4	0.991	-0.003	L3	0.995	-0.058
UP5	0.860	-0.510	L6	0.838	0.517	UP5	0.880	0.053	L4	0.997	-0.048
UP6	0.858	-0.386	L7	0.537	0.822	UP6	0.986	-0.073	L5	0.982	0.032
UP7	0.990	0.101	L8	0.565	0.805	UP7	0.996	-0.061	L6	0.994	-0.026
D2	0.997	0.059	L9	0.838	-0.536	D1	0.997	-0.045	L7	0.587	0.691
D1	0.995	0.059	L10	0.839	-0.536	D2	0.997	-0.044	L8	0.785	0.141
D3	1.000	0.017	L11	0.989	0.091	D3	0.997	-0.049	L9	0.928	0.074
D4	0.997	0.012	L12	0.950	0.293	D4	0.998	-0.032	L11	0.991	-0.011
D5	0.994	-0.097				D5	0.994	-0.059	L10	0.995	-0.012
D6	0.995	-0.091				D6	0.698	0.415	L12	0.993	-0.016
D7	0.935	-0.351				D7	0.991	-0.021	L13	0.989	-0.084
D8	0.936	-0.349				D8	0.891	0.073	L14	0.996	-0.059
D9	0.867	-0.491				D9	0.988	-0.033	T1	0.997	0.006
D10	0.868	-0.492				D10	0.991	-0.007	T2	0.998	-0.028
L1	0.966	0.180				D11	0.997	-0.044			

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

The scatter plots of the retained components showed only *C. feriatus* clearly discriminated from *P. pelagicus* and *P. sanguinolentus* by dorsal side data (Fig. 4), while the species differentiation was failed with ventral side data (Fig. 5). In similar way, Rebello and Barluenga were able to discriminate *Penaeus monodon* stocks from different parts of Kerala and two different species of sympatric Cichlids from Nicaraguan Crater Lake Apoyo respectively using PCA [19, 20]. Mangrove crabs (*Perisesarma guttatum*) from two different clades East African latitudinal gradient were significantly differentiated according to carapace shape [21]. Szlachciak differentiated among the four stocks of *Abramis brama* from four lakes of Poland on the basis of meristic and morphometric study following the truss network [22].

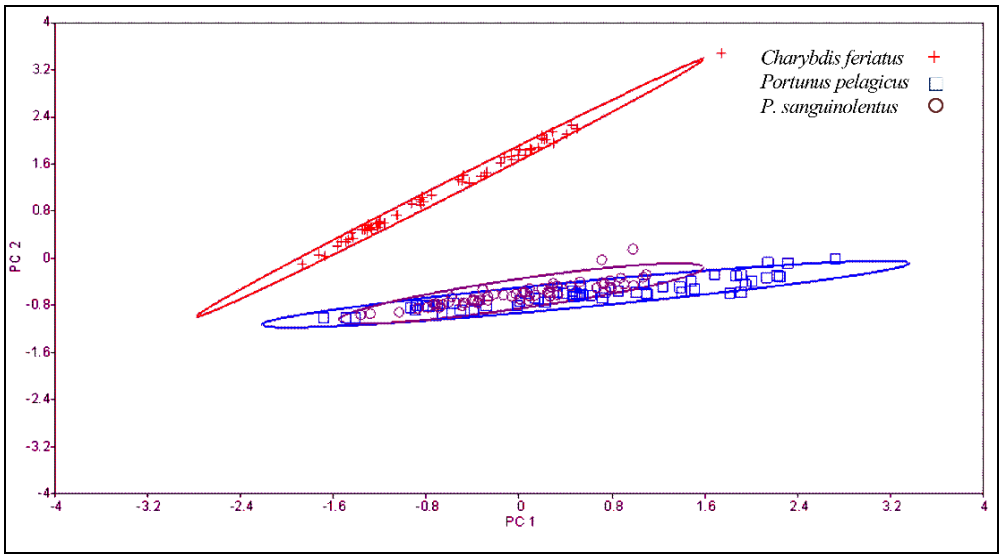

Fig 4: Scatter plot of PC2 on PC1 for dorsal side

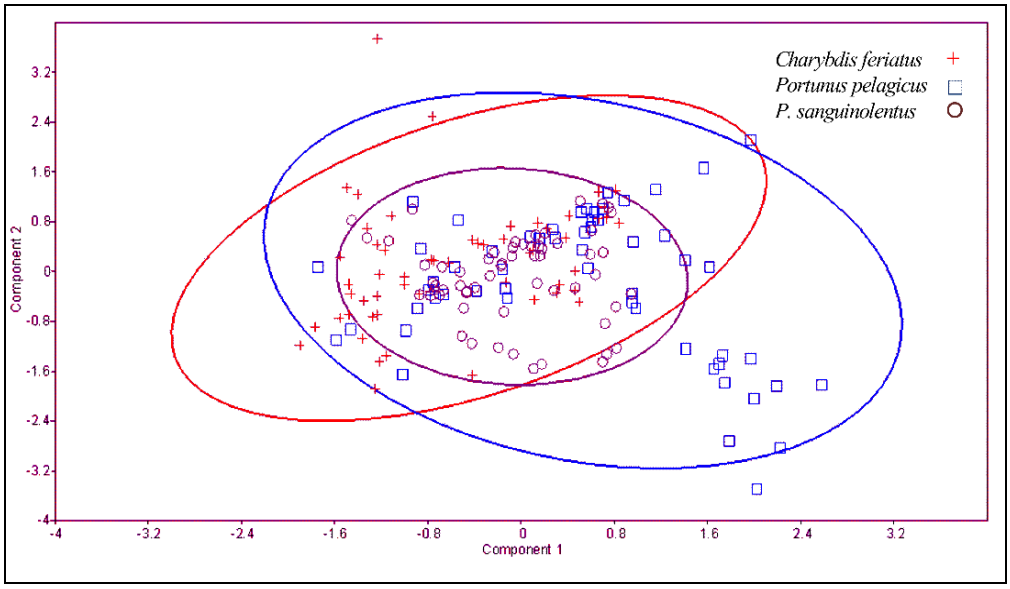

Fig 5: Scatter plot of PC2 on PC1 for ventral side

3.2.2 Factor Analysis

Factor analysis was performed using principal factor extraction technique and the eigenvalues and their proportions for both dorsal and ventral side data were obtained (Table 6). Only one factor was retained on dorsal and ventral side of *C. feriatus* and on the dorsal side of *P. pelagicus.* In *P. sanguinolentus* first two factors were retained on both sides and on the ventral side of *P. pelagicus*. In all the three species, factor loadings were almost similar with most variables contributing equally (> 0.90) to Factor 1. This observation was further supported by almost similar and high final communality estimates (> 0.80) for all variables of the three species for both dorsal and ventral side data excepting variables D6 in *P. sanguinolentus* on the ventral side, which had communality estimates of 0.22 (Fig. 6). However, variable D6 is a paired variable with D5. However, D5 had a very high (0.99) communality estimates.

The results of the PCA and FA appeared to be similar, which can be attributed to communality values of same magnitude obtained in FA [23]. This reflects the homogeneity of the truss variables for all the three species thereby indicating strong correlation or the existence of isometry between the variables. This reflects the homogeneity of the truss variables for all the three species thereby indicating strong correlation or the existence of isometry between the variables.

Table 6: Eigenvalues of the correlation matrix associated with FA

Charybdis feriatus (DORSAL; Total = 29; Average = 1)				
	Eigenvalue	Difference	Proportion	Cumulative
Factor1	28.7488	28.6736	0.9913	0.9913
(VENTRAL; Total= 35 Average = 1)				
	Eigenvalue	Difference	Proportion	Cumulative
Factor 1	33.5821	32.9388	0.9595	0.9595
Portunus pelagicus (DORSAL; Total = 29; Average = 1)				
	Eigenvalue	Difference	Proportion	Cumulative
Factor 1	28.7118	28.6527	0.9901	0.9901

(VENTRAL; Total= 35 Average = 1)				
	Eigenvalue	Difference	Proportion	Cumulative
Factor 1	32.7359	31.6733	0.9353	0.9353
Factor 2	1.0626	0.4746	0.0304	0.9657
Portunus sanguinolentus (DORSAL; Total = 29; Average = 1)				
	Eigenvalue	Difference	Proportion	Cumulative
Factor 1	26.1253	23.6971	0.9009	0.9009
Factor 2	2.4282	2.2927	0.0837	0.9846
(VENTRAL; Total= 35 Average = 1)				
	Eigenvalue	Difference	Proportion	Cumulative
Factor 1	31.5993	30.5571	0.9028	0.9028
Factor 2	1.0422	0.1418	0.0298	0.9326

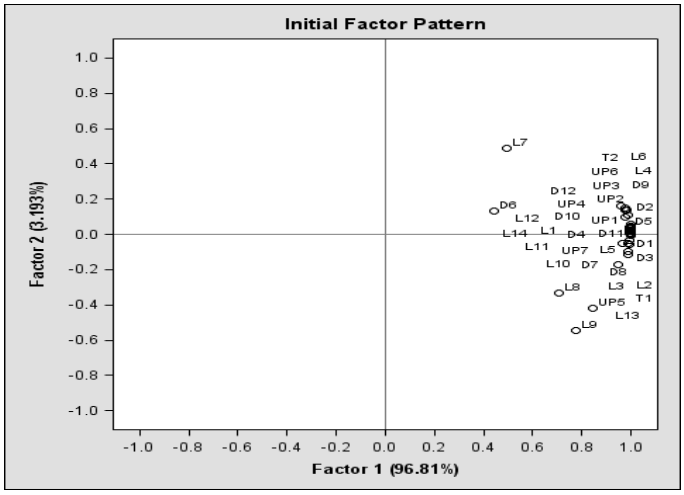

Fig 6: Initial factor pattern of *P. sanguinolentus*

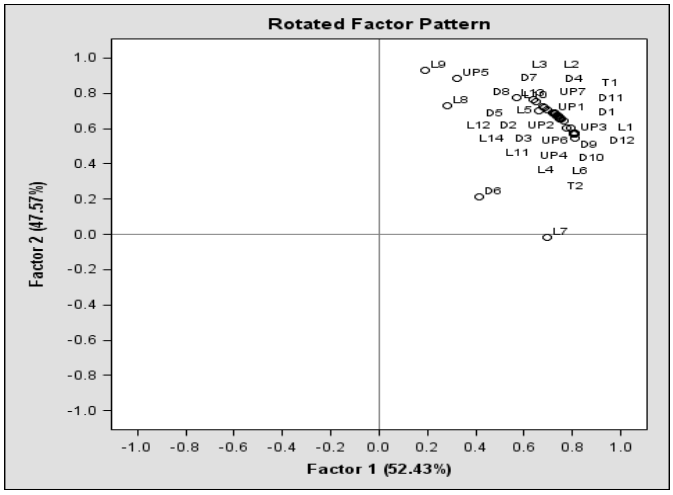

Fig 7: Rotated factor pattern

3.3.3 Canonical Variate Analysis

Canonical variate analysis (CVA) inferences were based on Hotelings p-values (Sequential Bonferroni significance). The three species were well differentiated based on truss morphometry analysis based on both dorsal and ventral side data (Fig. 8; 9).

In similar way, two populations of shemaya, *Chalcalburnus chalcoides* form the estuaries of the Haraz and Shirud Rivers were clearly discriminated on the basis of truss data [24]. Cavalcanti also discriminated Serranid fishes from Brazil using truss network [25].

Table 7: Hotelings *p*-values (Sequential Bonferroni corrected)

DORSAL			
	CF	**PP**	**PS**
CF	0		
PP	3.32395E-64	0	
PS	1.72561E-61	2.21842E-26	0
VENTRAL			
	CF	PP	PS
CF	0		
PP	5.98137E-41	0	
PS	4.80696E-41	2.0869E-16	0

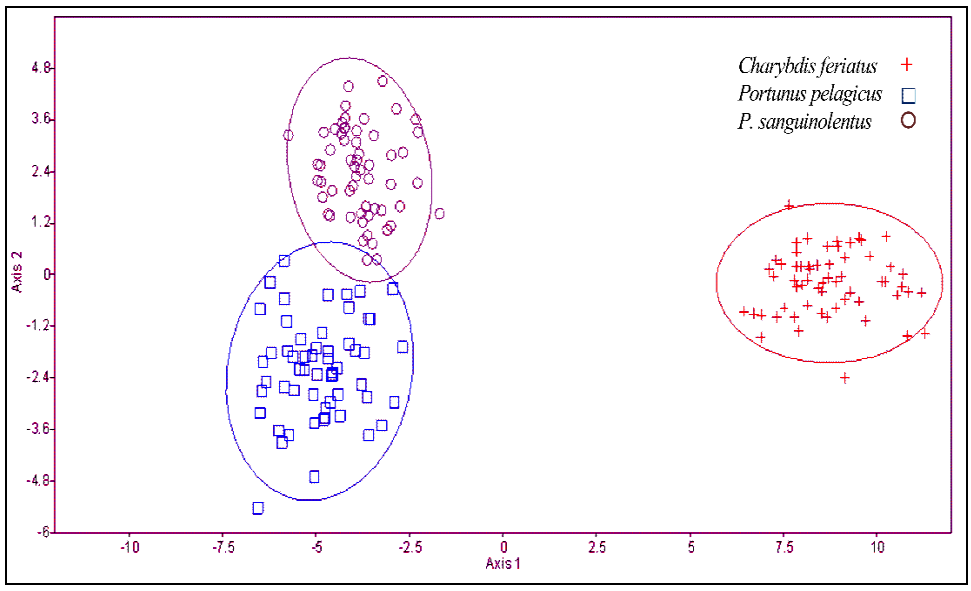

Fig 8: CVA scatter plot for dorsal side

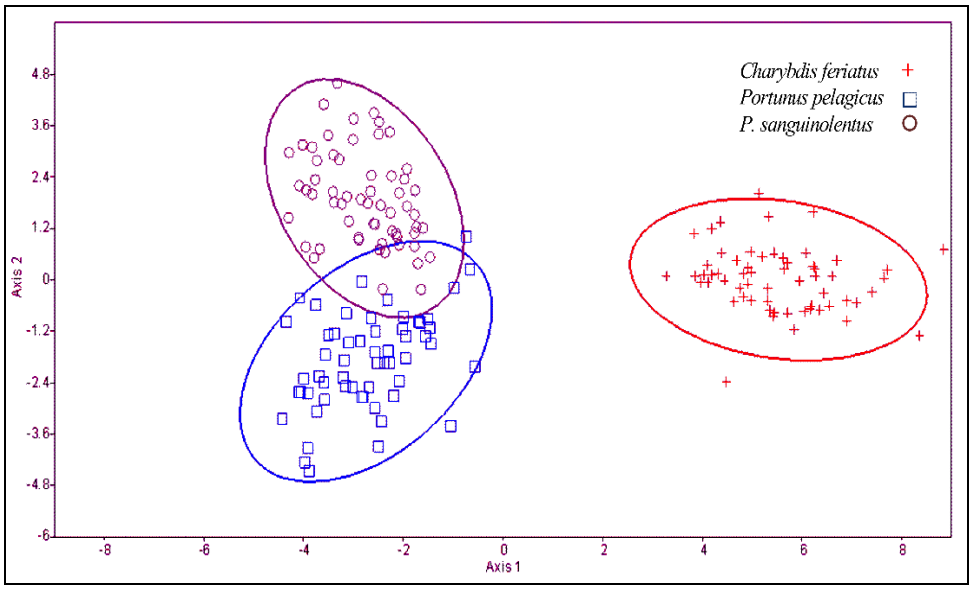

Fig 9: CVA scatter plot for ventral side

4. Conclusion

In summarizing, the t-test based on conventional data showed significant differences (p<0.05) between the nine variables used. However, PCA and FA could not differentiate between *P. pelagicus* and *P. sanguinolentus. C. feriatus* could be well differentiated from the other two species by both PCA and FA. Further, irrespective of the number of components retained (PCA) or the number of factors retained (FA) almost all variables loaded positively and heavily of first component. Overall, PCA and FA resulted into one component or one factored solution. The reason for the similar and high factor loadings on a single axis could be attributed to the highly significant positive correlations (p<0.0001) shared by all the variables investigated under truss morphometry. As against PCA and FA, CVA results showed the existence of three separate species with the differences between them being very highly significant (p<0.0001). In general, it can be stated that the truss morphometry appears to be a more appropriate tool for use in species discrimination than the conventional morphometry. The reason has still to be clarified, but it may be assumed that the truss data can clearly differentiate the species on their body form. The results were highly satisfactory as all morphometric tools used to discriminate the three species were best suited.

The present study confirms that the morphological tools can be regarded as inter-dependent and their application in combination provide a most powerful tool for an unequivocal identification of the numerous species. 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.

5. Acknowledgement

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