



Resolving Species Delineation of Four Portunid Crab Species of the Gulf of Mannar using Truss Morphometry

Preetysh Nanda Patnaik¹, V.K. Venkataramani¹, N. Jayakumar¹, R. Durairaja¹, Adyasha Sahu¹ and C. Sudhan^{2*}

¹Department of Fisheries Biology and Resource Management, Fisheries College and Research Institute, Dr. J. Jayalalitha Fisheries University, Thoothukudi 628008, Tamil Nadu, India

²Department of Fisheries Resource, Harvest and Post-Harvest Division, ICAR-Central Institute of Fisheries Education, Mumbai, Maharashtra 400061, India

ABSTRACT

The present study used truss morphometry to investigate species delineation of four portunid crab species from the Gulf of Mannar coast. Four closely related commercially significant portunid crab species such as *Portunus pelagicus*, *Portunus sanguinolentus*, *Charybdis natator* and *Portunus gladiator* were selected based on body morphometry and morphology to delineate them by employing a truss network. With regard to conventional morphometry in portunid species, only few characters are seemed to be diagnostic and many characters are found to be overlapping. Hence, Truss morphometry was attempted. Eleven truss distance landmarks were fixed on the cephalothorax dorsal surface using tpsUtil and digitized using tpsDig computer software package. A set of multivariate methods (Principal component analysis and Discriminant function analysis) was performed to make detailed investigation on distinction and pattern of morphological variations among all the four studied species, which revealed that there is significant variation in morphometrics of the portunid species. The first two components of the principal component analysis explained 99.99% of the overall variation. The first principal component (PC1) explained 83.74% while second principal component (PC2) accounted for 16.24% of the total variation. Four distinct species were confirmed by the scatter-plot from PCA and DFA. Wilks' lambda tests of discriminant function analysis revealed highly significant ($P < 0.001$) differences in morphometric characters across all the species. The findings of this study justify the use of truss networks for diagnostic identification for several species of portunid crabs apart from conventional morphometry. The study will be of immense use particularly in providing intriguing insights for species identification and characterization, besides, this will also clearly reveal any ambiguity in taxonomical clarification.

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Authors' Contribution

PNP wrote the original draft, reviewed literature, generated and collected data. VKV presented the concept. RD, CS and VKV conducted the study. NJ and VKV supervised the study, and wrote and edited the manuscript. RD, AS and CS performed formal analysis. CS curated data AS wrote the manuscript. CS validated the results and study.

Key words

Portunid crabs, Truss network, tpsUtil, tpsDig, Multivariate techniques, Species identification

INTRODUCTION

Crabs constituted 9.6% of all crustacean landings in India from 1975 to 2020, making a significant resource in the country's maritime fisheries. The true crabs of the suborder Pleocyemata are one among the most well-known and intensively researched groups. Brachyuran crabs classified under order Decapoda, which are a diversified group

of living crustaceans. The world's oceans are home to 6,793 species that fall under 1,271 genera and 93 families of brachyuran crabs, according to Ng *et al.* (2008). They may be regarded as important groups in the marine benthos, both in terms of biomass and community structure, due to their vast abundance (Bertini *et al.*, 2004). In Indian waters, a significant portion of crustacean fisheries consists of brachyuran crabs with 705 species accommodated in 28 families and in 270 genera, inhabiting in both marine and estuarine environments (Venkataraman and Raghunathan, 2015).

* Corresponding author: sudhancphd@gmail.com
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Abbreviations

PCA, principal component analysis; PAST, paleontological statistics; DFA, discriminant function analysis; NMFDC, national marine fishery resources data centre; CL, carapace length; CH, carapace height; CW, carapace width; UCW, upper carapace width; LLLCW, lower left lateral carapace width; LRLCW, lower right lateral carapace width; ULLCW,

upper left lateral carapace width; URLCW, upper right lateral carapace width.

The national statistics on marine crab landings by species (2021) revealed that 38,780 tonnes of crabs were landed in Tamil Nadu, which is leading in crab production (CMFRI, 2022). Numerous crab species are being caught, mostly as bycatch in trawls and, in some places, as a targeted resource in gillnets along both the coasts of India.

The edible crabs caught in India occur in the Portunidae family, and three marine crab species accounted for around 60% of total portunid crab landings, which were *Portunus sanguinolentus* (28.2%), *Portunus pelagicus* (25%) and *Charybdis feriata* (7.7%). *Portunus gladiator*, *Charybdis annulata*, *Charybdis natator*, *Charybdis lucifera*, *Charybdis smithii*, *Podophthalmus vigil*, *Scylla serrata*, and *Scylla olivacea* were among the other notable edible species caught in significant quantities.

Species differentiation, stock identification, and spatial variations are critical for long-term resource management. Therefore, simple, trustworthy, yet modern methods must be established to make the identification process easier and more efficient for users from diverse disciplines, including physiologists, conservation biologists, and ecologists. Morphometric characterization techniques are a crucial tool for identifying stocks and assessing the structure of the population (Sajina *et al.*, 2011; Sen *et al.*, 2011), particularly with the usage of the truss networking system (Afifah *et al.*, 2020). As truss networks can exhibit enhanced ability to discern differences in the morphological shapes of each species, this delineation technique is more successful than manual distance measuring for regulation of fishery resources around the world (Mojekwu and Anumudu, 2015). Truss morphometry applied in several shrimp species (Marini *et al.*, 2017; Rebello *et al.*, 2013), and blue swimming crab (Afifah *et al.*, 2020) has been reported.

Truss network systems are constructed with the use of landmark points. As the arrangement of the constructed landmarks encompasses the complete body with no less information and is more sensitive to changes, it is possible to delineate various species. High levels of isolation can result in significant morphological, meristic, and shape differentiation between populations of a species, which makes it easier in identifying the stocks. The characteristics may be best suited for studying short-term, environmentally induced discrepancies, and the findings can be effectively used for improved fisheries management.

Portunid crabs dominate the marine catches near the Gulf of Mannar year-round. Various crab species have never been examined or identified based on their morphometric variances, despite their prevalence in local catches. With this background, the present investigation was

attempted using morphometric variations to characterize four commercially important portunid crab species viz., *Portunus pelagicus*, *Portunus sanguinolentus*, *Charybdis feriatus* and *Portunus gladiator* along the Gulf of Mannar coast, Tamil Nadu, south-east coast of India, based on their morphometric variations.

MATERIALS AND METHODS

Sample collection

A total of 240 specimens (60 specimens of each species) representing four species of crabs of the family Portunidae viz., *P. pelagicus*, *P. sanguinolentus*, *P. gladiator* and *C. natator* were collected from various landing stations (Fig. 1) of the Gulf of Mannar (9.13° 28' 0" N and 79° 01' 0" E) of Tamil Nadu from June 2021 to May 2022. The landing stations and the sampling details of the studied crab species are depicted in Table I. The sampling locations were selected based on the abundance of the portunid group. These species were collected and identified using FAO species identification sheets (Fransen, 2014). Only the intact specimens were cleaned, labelled, and preserved in 5% formalinized seawater prior to digitization.

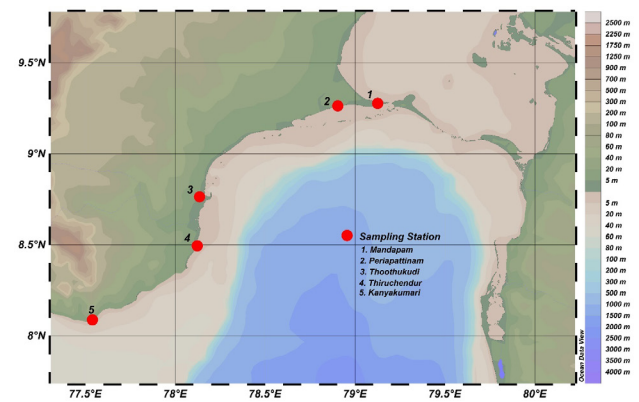


Fig. 1. Map demonstrating the sampling stations alongside the coast of Gulf of Mannar, Tamil Nadu (source: Ocean data view).

Sample digitization

The complete body shape record is provided by specimen digitalization, which also allows for repeated measurements (Cadrin and Friedland, 1999). Samples were first cleansed in running water, drained, and placed on a flat base with vertical and horizontal grids having an area of one cm², such as a graph sheet in the background, which was used in calibrating the coordinates of digital images. Then labelling was done with a specific code for identification for each individual specimen. The

pereiopods and the abdominal flaps were placed on the platform in such a way that make their origin points visible.

Table I. Sampling details of the four portunid crab species along Gulf of Mannar

Sampling station/ Location	Sample size	Sampling date	Sex (M/F)	Size range (cm)							
				<i>P. pelagicus</i>		<i>P. sanguinolentus</i>		<i>C. natator</i>		<i>P. gladiator</i>	
				CL	CW	CL	CW	CL	CW	CL	CW
Station 1: Mandapam	48	15.06.21	26/22	3.5-6.8	5.4-13.4	3.0-6.6	4.1-12.6	4.1-7.8	5.9-12.0	3.0-5.7	4.2-8.7
Station 2: Periapattinam	54	15.06.21	28/26	3.1-7.6	4.7-14.6	2.7-6.9	3.8-14.3	3.8-8.4	5.2-11.4	3.2-6.1	4.4-9.1
Station 3: Thoothukudi	52	17.11.21	28/24	3.0-8.3	4.6-15.6	2.8-7.5	3.7-14.8	3.4-8.9	5.4-12.8	2.8-6.2	3.9-9.6
Station 4: Thiruchendur	46	02.02.22	25/21	5.8-7.7	6.7-12.4	2.9-6.1	5.1-12.3	4.2-8.3	5.6-11.7	3.0-5.1	4.7-7.0
Station 5: Kanyakumari	40	15.05.22	19/21	3.5-7.0	5.1-13.2	3.1-6.4	4.6-13.1	4.6-8.0	6.0-12.1	3.3-5.8	4.0-8.3

Note: CL, carapace length (cm); CW, carapace width (cm).

Digitization was done with digital camera and the images from the dorsal aspect of the cephalothorax of the four species were obtained for further analysis.

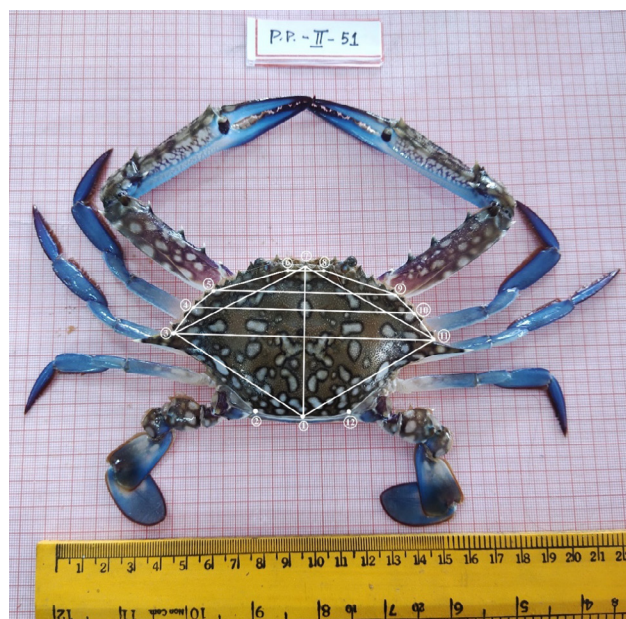


Fig. 2. Schematic depiction of 12 landmarks of carapace of crab species (*Portunus pelagicus*).

Obtaining morphometric measurements

The morphometric data were extracted from each individual image using a combination of softwares namely tpsUtil V1.46, tpsDig2 V2.16 and Paleontological Statistics (PAST). A utility program called tpsUtil is

primarily used to convert all photos from JPEG (*.jpeg) to TPS (*.tps), which is then utilised as input in tpsDig. A window-based application called tpsDig is used to digitise landmarks and outlines in specimen images for the analysis of the geometric morphometry. The multivariate analysis software package, that is the PAST (Hammer *et al.*, 2001) is designed specifically for statistical, graphing and modelling functions in palaeontological studies. The data encrypted TPS format image files were used as input source in PAST. The respective locations of landmarks to cover overall body form on the cephalothorax of crab using tpsDig V2.16 are shown in Fig. 2. Eleven morphometric distances representing the dorsal aspect were selected based on their capacity to capture overall body shape in the studied portunid crabs. Then, the morphometric data were extracted using paleontological statistics (PAST). The conventional morphometric landmarks, their codes and descriptions are given in Table II.

Statistical analyses

Descriptive statistics were obtained for all morphometric traits recorded. All the variations in size and shape between four species were analysed by principal components analysis (PCA) and discriminant function analysis (DFA). PCA is an effective technique for morphometric data reduction (Mir *et al.*, 2013) and independent variable extraction (Samaee *et al.*, 2009). DFA is a predictive model for group membership. Using the correlation matrix from PCA, eigenvalues, a percentage of variance, cumulative percentage, and cumulative eigenvalues were obtained. The software applications of STATISTICA and PAST 2.03 were used to conduct

statistical analyses.

Table II. Landmarks, codes and descriptions used for Truss morphometry.

Landmarks	Codes	Description
1-7	UP1	Carapace height (CH)
3-11	UP2	Carapace width (CW)
4-10	UP3	Upper carapace width (UCW3)
5-9	UP4	Upper carapace width (UCW4)
6-8	UP5	Upper carapace width (UCW5)
1-6	UP6	Midpoint of abdomen to left antennule
1-8	UP7	Midpoint of abdomen to right antennule
1-3	D7	Lower left lateral carapace width (LLLCW)
1-11	D8	Lower right lateral carapace width (LRLCW)
3-7	D9	Upper left lateral carapace width (ULLCW)
7-11	D10	Upper right lateral carapace width (URLCW)

RESULTS

Truss morphometrics

The dorsal features of the carapaces of the four species were studied using truss data, and a variety of multivariate approaches were used, including principal components analysis and discriminant function analysis.

Principal component analysis (PCA)

PCA performed on conventional morphometric traits is a variable reduction tool that reduces the variables by loading them on principal components (PC). Highly correlated variables 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 considered for by the other (Hidalgo and Goodman, 2013).

Principal component analysis of 11 morphometric measurements extracted two factors with eigen-values > 1, explaining 99.99% of the variance (Fig. 3). The first principal component (PC1) accounted for 83.74% of the variation and second (PC2) accounted for 16.24% (Table III).

In this analysis, the characteristics with an eigen-values exceeding one were included and others discarded. In this test, eigenvalues are plotted against the factors listed along the X-axis in descending order. The number of variables that are associated with each point in the function from the initial to the final bent-arm shape for the extraction of significant variables (Fig. 3). Its application to this data set led to the conclusion that the first two factors should

be accepted.

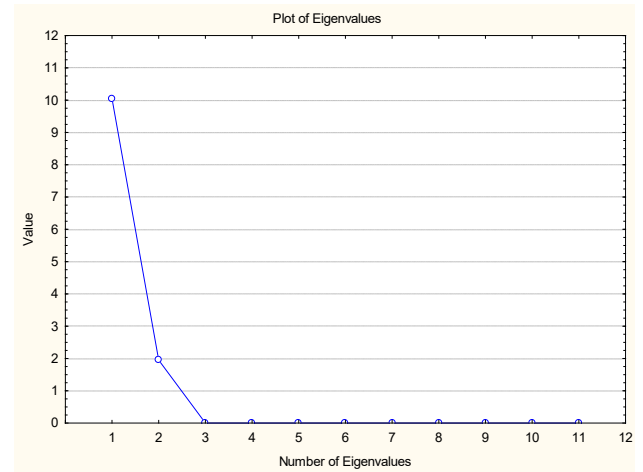


Fig. 3. Scree plot of the PCA.

Table III. Eigenvalues, percentage of cumulative variance and percentage of variance for the first two principal components according to PCA.

Factor	Eigen value	% Variance	Cumulative eigen-value	Cumulative %
PC 1	10.05	83.75	10.05	83.75
PC 2	1.95	16.25	11.95	99.99

It is vital to note that factor loading is regarded as significant when it exceeds 0.30, more important when it exceeds 0.40, and highly significant when it exceeds 0.50. Only those factors with loadings above 0.40 were deemed significant for parsimony in this study.

Eigenvectors of the two retained components (PC1 and PC2) showed that almost all conventional morphometric variables loaded approximately equally on PC1 except UP1, which indicates carapace height, was discarded from the analysis (Table IV).

Of the eleven variables assessed, ten variables (with the exception of UP1) loaded approximately equally on PC1. Therefore, here, PC1 can be seen as an isometric size that denotes the relative sizes of the specimens (Cadrin, 2005). The most significant loadings on PC1 were UP2, UP3, UP4, UP5, UP6, UP7, D7, D8, D9, D10. In case of PC2, within eleven characters that exhibited strong loadings, most were associated to carapace height variation that is UP1 (Table IV). In contrast to PC1's accounting for size variation, PC2 often measures shape variance (Cadrin, 2005).

From the scatter plot (Fig. 4), PCA differentiated *Charybdis natator* from *Portunus pelagicus*, *Portunus*

sanguinolentus and *Portunus gladiator* because of its different body shape which varies from other three species, which are having almost the same shape. Further, *P. sanguinolentus*, was found to be interspersed within *P. pelagicus* while *P. gladiator* forms a small cluster, separated from the other two species. Therefore, despite the fact that PC1 loaded the majority of the variables, they appear to represent differences in both size and shape rather than just size (Cadrin, 2005; Yakubu and Okunsebor, 2011). The scatter plot (Fig. 4) also showed that the truss landmarks which differentiated the species highly and positively were UP2, UP3, UP5, D7, D8 and D9.

Table IV. Eigenvectors (or loading values) for PCA on conventional data (Components with significant loadings are marked in bold).

Truss measurements	Components	
	PC 1	PC 2
UP1	-0.071287	-0.997456
UP2	0.997689	0.067932
UP3	0.997437	0.071537
UP4	0.997435	0.071563
UP5	0.997263	0.073774
UP6	0.997397	0.072002
UP7	0.997399	0.072058
D7	0.997505	0.070589
D8	0.997557	0.069551
D9	0.997139	0.074620
D10	0.997566	0.069289
Expl. Var	9.959005	2.040699
Prp. Totl	0.829917	0.170058

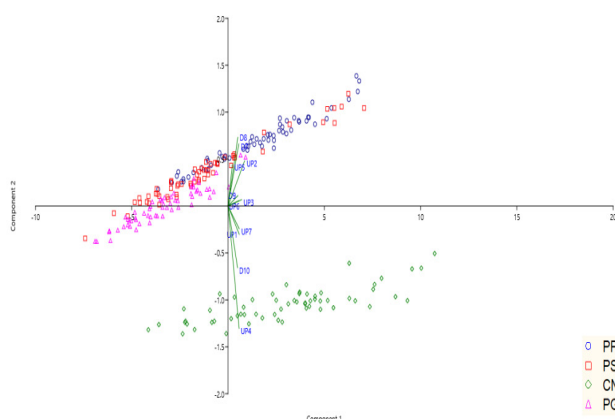


Fig. 4. Scatter plot of PCA (PC2 on PC1).

Discriminant function analysis (DFA)

Wilks' λ tests of discriminant function analysis

indicated that the variations in morphometric characters of all species were significant, which showed high discriminatory power (Table V). Here, 1 through 3 was highly significant that is $P < 0.001$ (99.9% level of significance), while 2 through 3 showed $P < 0.05$ (95% level of significance) and 3 showed $P > 0.05$ that is insignificant.

Table V. Wilks' λ test for verifying differences with morphometric measurements using Discriminant function analysis.

Functions	Wilks' lambda	Chi-square	df	P
1 through 3	0.00025	1921.04	30	0.00
2 through 3	0.0307	808.27	18	0.00
3	0.3632	234.99	8	0.00

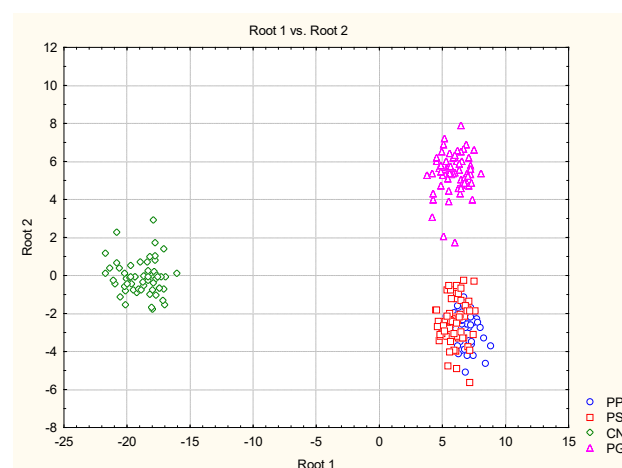


Fig. 5. Scatter plot of discriminant function analysis (DF2 on DF1).

Two discriminant functions were produced by the DFA based on 11 truss measurements with highly significant ($p < 0.01$) differences among the species. To handle the problem of inequality of covariance matrices among groups in DFA, this approach is advised (Anderson and Bahadur, 1962). The first (DF1), second (DF2) and third (DF3) discriminant functions explained the total variance, in which the first two were considered for further interpretations. The DF1 and DF2, together amounted for 93% of the morphological variation, with DF1 and DF2 each confirming 65% and 28% of the total variance, respectively. The point clouds of species could be clearly distinguished from one another while little overlapping could be seen in the point clusters of *P. pelagicus* and *P. sanguinolentus* in the DF1 vs. DF2 scatter-plot (Fig. 5), which was in line with the clusters shown in the PCA

scatter-plot (Fig. 4). Eleven truss measurements with high correlations were found in the structure matrix (Table VI), which showed the intra-group correlations among each of the characters and the discriminant functions. The variables having considerable loadings on DF1 were UP3, UP5, UP7, D8, D9, D10 while on DF2 were UP2, D7 and on DF3 they were UP4, UP6; showing that these characters were mainly responsible for differences among the species. In DF1, all variables represent measurements covering the entire carapace of the crab; in contrast, the variable measurements were concentrated mostly in the posterior region (UP2 and D7) of the carapace in DF2.

Table VI. Structure matrix coefficients showing the intra-group correlations among each of the characters and the discriminant functions.

Character	Function		
	DF 1	DF 2	DF 3
UP2	-0.125773	0.818450 ^a	0.054444
UP3	-0.196984 ^a	-0.146060	-0.133438
UP4	0.069102	0.190525	-0.475498 ^a
UP5	0.339778 ^a	-0.264543	0.285291
UP6	-0.207687	-0.334631	0.363643 ^a
UP7	0.096559 ^a	-0.036361	-0.092419
D7	-0.084268	0.285937 ^a	-0.047769
D8	-0.665237 ^a	0.038238	-0.210042
D9	0.761247 ^a	0.104883	-0.168217
D10	-0.426909 ^a	-0.045988	0.312782

^a largest correlation between each variable and DF

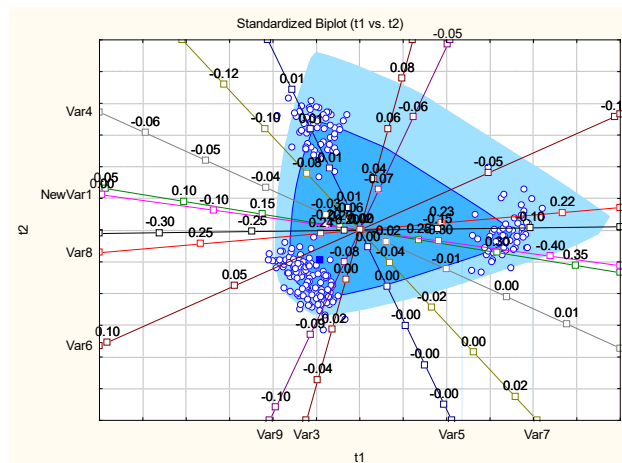


Fig. 6. Standardized biplot of the discriminant function analysis.

Another multivariate procedure for discrimination between the species is the Discriminant analysis biplot.

The results of the discriminant analysis are demonstrated in a standardized biplot (Fig. 6). This enables the cases to be presented in a two-dimensional projection, organized by groups together with the input variables. The variables are described by the course of the coloured axes in the standardized biplot, from which are derived the charges of the individual variables from the respective component axis t1 or t2. From the biplot (Fig. 6), it is evident that D7 (Var8) has a high charge towards component t1 since the variable axis is almost parallel to the corresponding component axis t1. In addition, the correlation of the variables among each other can be ascertained from the variable axes, such as, a very strong correlation between UP3 (Var3) and D8 (Var9) can be found in the biplot, which is shown by the very close neighbouring positions in the Figure 6.

DISCUSSION

The results of this study obtained from the truss-based morphometrics indicated that all the four commercially important portunid crab species (*P. pelagicus*, *P. sanguinolentus*, *P. gladiator* and *C. natator*) of the Gulf of Mannar coast, showed significant morphometric variability. Both multivariate analyses using PCA and DFA suggested four distinct species of portunid crabs. The segregation among species was confirmed by PCA and DFA scatter-plot based on scores for each sample that showed none, or slight, overlapping clusters of points for each species.

Data collected on eleven variables subjected to Wilks' lambda test showed significant differences ($p < 0.01$) indicating that the species differed greatly from one another morphologically. However, PCA was not able to differentiate between *Portunus pelagicus* and *Portunus sanguinolentus*, regardless of the number of components retained (which almost loaded positively and heavily on the first component). The reason for the similar loadings on a single axis could be due to highly significant positive correlations ($p < 0.01$) shared by all the variables investigated. Truss measurements that were significantly different ($p < 0.01$) were further subjected to DFA with a distinct covariance matrix. An effective technique for distinguishing various population stocks within the same species is through DFA (Karakousis *et al.*, 1991). In the present investigation, DFA revealed that the existence of four separate species along the study area ($p < 0.01$). The morphometric tools used to differentiate the four species were best suited based on their accuracy. The acquired taxonomic clarity provides a better foundation for creating identification keys for the four species including morphological data in the future and also considered for

stock identification and geographic variation.

Affah *et al.* (2020) identified blue swimming crab stocks along the Indonesian Fisheries Management Area 712 using truss network analysis based on 29 morphometric characters, where the discriminant function analysis showed three groups of populations and the morphometric characters showed a significant difference of $p < 0.05$, which were in line with this present study. Apart from this, various truss networking works have also been done on fish species for species delineation, stock differentiation, phenotypic variation, morphological analysis. The present study is in conformity with the study of Sarower *et al.* (2021), which reported that the Truss networking system based morphometrics did not find significant morphological variations between the wild and hatchery-produced mud crab, *Scylla olivacea*, thereby removing the misconception of the crab farmers that the crablets from hatcheries are weak in terms of growth, survivability, and strength.

Colihueque *et al.* (2017) reported significant morphological variability of three populations of *Trichomycterus areolatus*, collected from two rivers of southern Chile, using multivariate analyses, including PCA (ANOSIM test, $r = 0.552$, $p < 0.0001$) and DFA (Wilks's $\lambda = 0.036$, $p < 0.01$), where PC1 and PC2 explained 34.72 and 21.44% of the overall variation, respectively and scatter-plot of DFA (DF1 on DF2) also validated the existence of three distinct populations. Mir *et al.* (2013) investigated the intraspecific variations of *Schizothorax richardsonii*, snowtrout, using truss morphometry based on 14 landmarks and showed that DF1 and DF2 accounted for 90.2% and 6.8% of the between groups variability, demonstrating 97% of the total between groups variability, indicating high variation between two stocks. Hanif *et al.* (2019) investigated the phenotypic structure of *Anodontostoma chacunda*, gizzard shad, using truss network model from four rivers of Bay of Bengal, Bangladesh and showed that the PCA analysis of two factors (whose eigenvalues accounted 16.41 and 2.09, respectively) justified 66.05% of the overall morphometric variation while the DFA analysis showed that DF1 and DF2 contributed to 98.58% of total variance demonstrating effective phenotypic discriminating between the populations under study.

Stock separation within a species can be successfully investigated using truss morphometry, as reported for other species in freshwater and marine habitats. The truss procedure used in this work demonstrated a significant distinction between the four portunid crab species along the Gulf of Mannar, indicating the need for separate management approaches to preserve the resources for future use. Molecular and biological techniques could be

employed to further confirm the outcomes of the current study. The use of molecular genetic markers such as mitochondrial DNA, besides truss morphometry will not only help as accurate identification tools but also will help in deducing the possibilities of cryptic species and colour variants among the same species. By such integrated approach, a revised identification is highly possible for various species portunid crabs.

CONCLUSION

The truss-based morphometrics are a set of measurements that improve group differentiation by systematically identifying variations in body shape in both horizontal and vertical directions (Strauss and Bookstein, 1982). Despite its potential to simplify the interpretation of morphological variation using multivariate analysis, such as PCA, this class of morphometric analysis has not been extensively studied in crabs.

The present work confirms that the Truss morphometry can be considered as inter-dependent and their application in combination with molecular or biochemical analysis provides a powerful tool for an accurate identification of the portunid crab species. The resolved taxonomic clarification for the four species using truss morphometry inclusive of morphological data as well as molecular studies will help in species identification and delineation along with geographic variations, if any, as all the studied portunid crabs are of high commercial importance from the point of its resource assessment.

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Ethical Statement:

No live specimens were used in the present investigation.

IRB approval

The Institutional Review Board have given approval for the article (Lr.No.326/Edn./Course work&Research work/2020, dated.16.09.2021).

Statement of conflict of interest

The authors have declared no conflict of interest.

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