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# MORPHOMETRICS AND TRUSS-NETWORKING DISTANCES AMONG THREE SPECIES OF CROAKER (*JOHNIUS BORNEENSIS*, *JOHNIUS ARGENTATUS* AND *JOHNIUS BELANGERII*) FROM BANGLADESH COAST OF THE BAY OF BENGAL

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**ABSTRACT:** Morphometric features are efficiently used for the differentiation of species and long-term management of fish populations. Truss analysis is a frequently used taxonomic method as well as a stock identification tool to dissimilitude the morphologically similar species. Using landmark-based truss networking techniques following 18 morphometric lengths, 10 meristic counts, and 23 truss-network distance, the morphological variation of three croaker species viz. *Johnius borneensis, Johnius argentatus*, and *Johnius belangerii* were examined from Bangladesh coast. Data were analyzed and visualized through univariate ANOVA, canonical discriminate function analysis (DFA), and principal component analysis (PCA). Results found that the first DFA was responsible for 80.6% & 81.1%, whereas the second DFA was responsible for 19.4% & 18.9% variations among three species for the morphometric and truss-distances, respectively. In addition, 14 out of 18 morphometric and 12 out of 23 truss-network measurements were significantly different ($p$ < 0.05) among means of three species. 1st PCA found 70.76% & 64.49% explained variations and 2nd PCA displayed 12.8% & 11.5% explained variations respectively for morphometric and truss distances. The findings of the study demonstrates that *J. borneensis* and *J. belangerii* are highly morphologically similar and *J. argentatus* is completely distinct which might be due to their physiological and genetic variations. The present study might be helpful in differentiating species and managing existing stocks of marine fish. Further studies regarding the molecular characterization of the Cytochrome c oxidase subunit I (COI) gene could confirm the genetic variations among these species.

***Key words:*** morphometric features, landmark based morphometry, croakers, principal component analysis, discriminant function analysis.

## INTRODUCTION

Stock identification is a key concept in fisheries science, which is concerned with the study of self-sustaining components within natural populations (Cadrin & Friedland 2005). Different stocks in a given ecosystem are

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constantly evolving as a result of ongoing evolution within an existing species or population and their interdependent reactions to the environment. As individuals from geographically distant populations routinely interbreed, frequent variations occur in body shape among stocks of the same species (Anvari Far *et al.* 2011; Sajina *et al.* 2011). Diverse rates of growth, development, and maturation in response to environmental factors help organisms eliminate or add different ontogenetic traits that give rise to novel traits or physiology (Cadrin 2000). Since each stock exhibits different traits than others, all emerging stocks must be identified and managed individually for improved yields and conservation. Moreover, inadequate knowledge about existing stocks can lead to overexploitation, poor management, reproductive, and conservation failure (Devaraj & Vivekanandan 1999). Therefore, proper identification of existing stocks should be developed for better stock management in fisheries.

Fish populations in aquatic ecosystems are dispersed over large geographic areas with oceanographic barriers like temperature, salinity, food and predation. These characteristics are closely related with the carrying capacity, productivity, and resilience factors of population dynamics (Cole 1954). For an understanding of population dynamics and an assessment of sustainable harvests, it is thought to be required to identify intraspecific groups with different life history traits. Knowledge of stock structure allows for the development of management techniques that will aid in the preservation of biodiversity associated with various species, subspecies, stocks, and races (Turan *et al.* 2005). By maintaining distinct stock management, determining the stock-wise population abundance, maintaining biologically viable levels of productivity, assessing the responses of each stock to fishing exploitation and by ecosystem modeling, the goal of fishery stock assessment is achieved. Since every stock responds to exploitation, groups with different growth or reproductive patterns should be addressed and treated separately for the purpose of stock assessment (Cadrin & Silva 2005). It is possible to separate and manage a distinct population on the basis of phenotypic and genotypic variation among fish populations within a species that emerged as a result of a sufficient level of isolation (Turan 2004). So, the whole impact of management efforts, including determining the stock complexity of a fish species, must be taken into account as a fundamental element of stock identification.

Numerous fish stocks have been effectively differentiated using conventional multivariate morphometric analysis, which takes into consideration both size and shape variation (Turan 1999). Meristic, morphometric, traditional tags, parasites as natural tags, otolith chemistry, and a number of molecular markers (such as protein allozymes, mitochondrial DNA, and microsatellite DNA) have all been proposed as methods for stock identification. However, morphometric traits have proven to be the most widely used and economically expedient approach (Sajina *et al.* 2011). Fish have more diverse morphological differences within and between populations than any other animals (Brraich & Akhter 2015). All measurable traits in fish species are known as morphological features. In addition, meristic measurement is a branch of ichthyology that studies the quantitative traits of fish, including the number of scales or fins. A fish species can be determined using a prominent countable attribute of that particular species. After all, meristic and morphology are still frequently employed to distinguish species and to assess the phenotypic characteristics. Several populations including *Trachurus mediterraneus* (Turan 2004), *Megalaspis cordyla* (Sajina *et al.* 2011), *Alosa pseudoharengus* (Cronin-Fine *et al.* 2013), and *Acanthocybium solandri* (Zischke *et al.* 2013) have been identified using morphometric analysis. To date, several methods of morphological analysis have been developed for differentiating stocks, including univariate comparison, bivariate growth pattern analysis, and multivariate analysis (Cadrin 2000). For specific acknowledgment of morphological features in stock identification, multivariate analysis using principal component analysis (PCA), discriminate function analysis (DFA), or canonical discriminate function analysis has recently gained popularity (Specziar *et al.* 2009; Yakubu & Okunsebor 2011; Cronin-Fine *et al.* 2013).

The Truss network system is a landmark-based method of geo-morphometric, which has no restriction on the directions of variation and localization of shape changes and is very useful in capturing information about the shape of an organism (Cavalcanti *et al.* 1999). This technique has been utilized more and more to differentiate between population shape differences and within-population allometry (Tarun 1999). The truss system can be used to explore stock separation within a species with the same set of measurements, which enables, over time, a better and more direct comparison of the morphological history of stocks. To determine each unique shape for each fish species, landmarks—a randomly chosen group of places on the body—are used. Till now, Landmark-based truss network approach has been applied to differentiate among four different *Macrognathus aculeatus* fish population (Sarower-E-Mahfuj *et al.* 2021). In addition, a landmark-based truss network study also applied in differentiating stock and species of *Tenualosa ilisha* (Das *et al.* 2020), *Lepidocephalichthys guntea* (Sarower-E-Mahfuj *et al.* 2019), *Rhinomugil corsula* (Hossain *et al.* 2015), *Cobitis keyvani* and *Cobitis faridpaki* (Mousavi-Sabet & Anvarifar 2013), *Sillaginopsis panijus* (Siddik *et al.* 2016), *Labeo calbasu* (Hossain *et al.* 2010) and many other species.

Croakers, also known as Jewfish, are one of the most extensively fished species in the Bay of Bengal waters. They are members of the Sciaenidae family and are classified as Perciformes. Croakers have made a demandable market both locally and internationally, especially in Saudi Arabia, Kuwait, Qatar, and Middle-East countries in fresh and dry conditions. Due to the high demand for croakers and other fisheries, there is increased fishing pressure in the coastal waters of the Bay of Bengal. Additionally, the stock of both pelagic and demersal fisheries in this area is being hampered by the unselective operation of set bag nets (SBN) and other fishing gear in coastal seas. Because of heavy fishing pressure, the croaker has been overfished for many years. The Pama croaker (Poa) constitutes a major portion of the total catch, which is the 3rd most available fish species in the Bay of Bengal coast of Cox's Bazar, Bangladesh (DoF 2022). They are found mainly in the estuaries, and the Bay of Bengal and they enter rivers as far as the tide extends (Rahman 2005). Within several types of Jew fish, *Johnius borneensis, Johnius argentatus,* and *Johnius belangerii* are more available and demandable. However, there are just a few studies on the length-weight relationship, growth patterns, and mortality of a few croaker species, and there is no comprehensive data on the state of the croaker stock in the Bay of Bengal, Bangladesh. Although being commercially harvested off the coast of the Bay of Bengal, croakers are difficult to distinguish from one another. Therefore, the present investigation employs univariate analysis of morphometric, meristic, and landmark-based truss networking to examine the morphometric and form variations among three species of *Johnius* croaker (*Johnius borneensis, Johnius argentatus,* and *Johnius belangerii*).

## MATERIAL AND METHODS

*Ethical Statement:* The study was conducted in a field location that is not privately owned or protected. There was no endangered or protected species involved in the study. Thus, the ethical issue was not necessary for the described study in Bangladesh.

*Sample collection:* A total of 90 individuals of Jew fish *(Johnius borneensis, J. argentatus,* and *J. belangerii)* were collected from the marine fisheries landing center and different other fish market of Cox's Bazar, Bangladesh. Apparently healthy, fresh and disease-free specimens were collected from September to November, 2021 and were considered for the morphometric study. On-field identification of the target species was done by observing the external phenotypic traits. Visual assessment of genital organ and external sexual features was used to determine fish sex. Immediately after collection, the collected species were kept in ice boxes before being taken to the lab at Coastal Biodiversity, Marine Fisheries and Wildlife Research Center, Cox's Bazar of Chattogram Veterinary and Animal Sciences University (CVASU).

**Table 1. General Morphometric characteristics and their descriptions applied for the differentiating among three species of Jew fish**

| Character | Description |
|---|---|
| Total length (TL) | Distance from the tip of the snout of fish to the longest caudal fin ray |
| Standard length (SL) | Distance from the tip of the snout of fish to the end of the vertebral column |
| Head length (HL) | From tip of the snout to posterior margin of operculum |
| Pre-orbital length (PrOL) | From tip of the snout to anterior margin of eye |
| Eye diameter | Diameter of eye |
| Post orbital length (PoOL) | From posterior margin of eye to end of operculum |
| Highest body depth (HBD) | Vertical range of anterior part of first dorsal fin and ventral part of the fish body |
| Lowest body depth (LBD)) | Vertical distance at the end of vertebrae |
| Dorsal fin length (DFL) | From base of first dorsal spine to base of last dorsal ray |
| Pre-dorsal fin length (PrDFL) | From tip of the snout to origin of dorsal fin |
| Post–dorsal Fin Length (PoDFL) | End of dorsal fin to longest caudal fin ray |
| Pectoral fin length (PcFL) | Base length of pectoral fin |
| Pelvic fin length (PvFL) | Base length of pelvic fin |
| Pre-pelvic fin length (PrPvFL) | From tip of the snout to the origin of pelvic fin |
| Anal fin length (AFL) | Length of base of anal fin length |
| Pre-anal fin length (PrAFL) | From tip of the snout to origin of anal fin |
| Caudal peduncle length (CFL) | From base of anal fin to base of caudal fin |
| Caudal fin length (CFL) | Base length of caudal fin |

*Measurement of morphometric and meristic characteristics:* Generally, morphological properties of fish were measured according to the standard procedures given by Hubbs (1958) (Fig 1 & Table 1). A vernier calipers and a metric scale were employed for measuring 18 morphological lengths in three different species of jewfish. Meristic features such as dorsal fin rays (DFR), pectoral fin rays (PcFR), pelvic fin rays (PvFR), anal fin rays (AFR), caudal fin rays (CFR), Branchiostegal rays, number and scales on the lateral line of each sample were counted (Table 2).

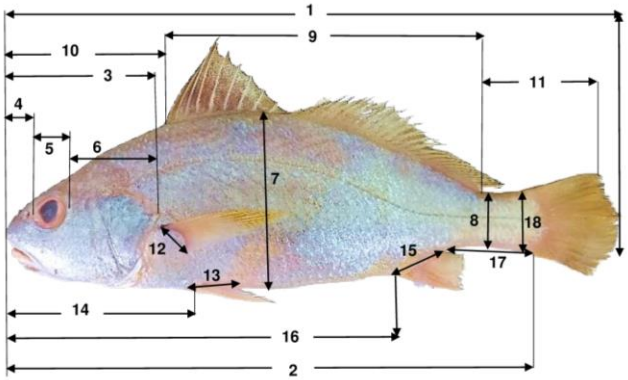

Fig. 1: Overview of different morphometric indices of pama croaker (1. TL= total length, 2. SL= standard length, 3. HL= head length, 4. PrOL= pre-orbital length, 5. ED= Eye diameter, 6. PoOL= post orbital length, 7. HBD= highest body depth, 8. LBD= lowest body depth, 9. DFL= dorsal fin length, 10. PrDL= pre dorsal length, 11. PoDL= post dorsal length, 12. PcFL= pectoral fin length, 13. PvFL= pelvic fin length, 14. PrPvFL= pre pelvic fin length, 15. AFL= anal fin length, 16. PrAFL= pre anal fin length, 17. CPL= caudal peduncle length, and 18. CFL= caudal fin length).

**Table 2. List of meristic characters used in this species to differentiate among three species of Jew fish**

| SL. No | Characters |
|---|---|
| 1 | Dorsal fin spine (DFS) |
| 2 | Dorsal fin soft rays (DFSR) |
| 3 | Anal fin spine (AFS) |
| 4 | Anal fin soft rays (AFSR) |
| 5 | Caudal fin rays (CFR) |
| 6 | Pectoral fin rays (PcFR) |
| 7 | Pelvic fin rays (PvFR) |
| 8 | Scales on the lateral line (LLS) |
| 9 | Scales on the lateral transverse (TrLS) |
| 10 | Number of branchiostegal rays |

**Table 3. Description of truss-network characters used for differentiating among three species of Jew fish**

| Character codes | Land-mark Points | Description of characters |
|---|---|---|
| A1 | 1–2 | Anterior tip of snout to the cranium |
| B1 | 2–3 | Cranium to the origin of dorsal fin base |
| C1 | 3–4 | Origin of dorsal fin base to end of spine dorsal fin |
| D1 | 4–5 | End of spine dorsal to lower end of soft dorsal fin |
| E1 | 5–6 | End of soft dorsal fin to end of the caudal fin base |
| F1 | 6–7 | Origin of upper caudal fin base to mid-point of caudal fin base |
| G1 | 7–8 | Mid-point of caudal fin to lower caudal fin base |
| H1 | 8–9 | Lower caudal fin to end of anal fin |
| J1 | 10–11 | Origin of anal fin base to mid-point of pelvic fin |
| K1 | 11–1 | Mid-point of pelvic fin to anterior tip of snout |
| A2 | 1–12 | Anterior tip of snout to mid-point of pectoral fin base |
| A3 | 1–3 | Anterior tip of snout to origin of dorsal fin base |
| C2 | 3–10 | Origin of dorsal fin base to origin of anal fin base |
| C3 | 3–11 | Origin of dorsal fin base to mid-point of pelvic fin |
| C4 | 3–12 | Origin of dorsal fin base to mid-point of pectoral fin base |
| D2 | 4–9 | End of spine dorsal fin to end of anal fin |
| D3 | 4–10 | End of spine dorsal fin to origin of anal fin base |
| D4 | 4–11 | End of spine dorsal fin to mid-point of pelvic fin |
| E2 | 5–7 | End of soft dorsal fin to mid-point of caudal fin base |
| E3 | 5–9 | End of soft dorsal fin to end of anal fin |
| F2 | 6–8 | Origin of upper caudal fin base to lower caudal fin base |
| B2 | 2–11 | Cranium to mid-point of pectoral fin base |
| K2 | 11–12 | Mid-point of pelvic fin to pectoral fin base |

*Measurements of land-mark distances:* At the frame of the fish, twelve (12) randomly selected land-marks points with twenty-three (23) land-mark distances were chosen and measured to achieve homogeneity of total body plane coverage among the three species of Jew fish (Fig 2 & Table 3) on the basis of Strauss & Bookstein (1982). To enable the accurate and consistent measurements, each landmark obtained by laying the fish sample on a square of paper and detecting the landmarks using a color pointer. Finally, the centimeter (cm) scale was used to calculate the distances.

*Data adjustment:* Since, differences in morphometric characters should not be correlated to the relative size of fish but rather to differences in body shapes (Reist 1985). So that, before the statistical analyses, the size effects from the data set were eliminated using a slightly modified version of the allometric formula provided by Elliott *et al.* (1995).

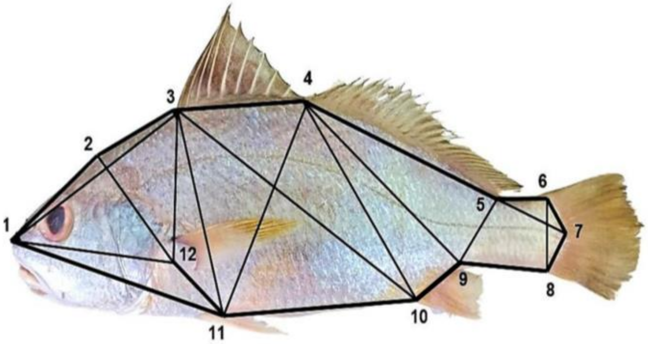

Fig. 2: Randomly selected landmark points and made a network on the fish body which was used in the study. The 12 landmark points refer to point 1: anterior tip snout of upper jaw, point-2: cranium, point-3: origin of first dorsal fin, point-4: end of spine dorsal fin, point-5: end of soft dorsal fin, point-6: origin of dorsal caudal fin, point-7: mid-point of caudal fin, point-8: origin of ventral caudal fin, point-9: end of anal fin, point-10: origin of anal fin, point-11: mid-point of pelvic fin, point-12: mid-point of pectoral fin.

$M_{adj} = M\ (Ls/Lo)^{b}$

Where, $M_{adj}$: size adjusted measurement,

M: original measurement,

Ls: overall mean of total length for all fish from all samples in each analysis

Lo: total length of fish

With all fish in all groups, the slope of the regression of log M on log Lo was used to estimate parameter "b" for each character from the observed data. TL and converted data were then connected with the effectiveness of the size adjusted values. To determine whether the size relations had been removed or not, the standardized data were evaluated using a bivariate plot in compared to the standard length.

*Statistical analysis:* The morphological variation among the collected samples were compared in the first stage of analysis of *Johnius borneensis, J. argentatus, and J. belangerii*. In the second level, the landmark distances among the *J. borneensis*, *J. argentatus*, and *J. belangerii* were compared. Kruskal-Wallis was calculated to compare means and medians among different species. Based on morphological data (size-adjusted) and landmark distance data, a univariate analysis of variance (ANOVA) was done to determine the significance of each morphological difference ($p < 0.01$). A Discriminant functional analysis (DFA) was applied to all morphological data (size-adjusted) and the data of landmark distances. Individual specimens were classified using functions generated from

DFA, and the success rate of DFA classification was determined based on the proportion of individuals that were correctly classified into the original samples. The principal component analysis (PCA) were applied to identify the morphometric or land-mark distance to successfully distinguish among three species of jewfish. A dendrogram was also constructed based on the Euclidean distances using Unweighted Pair Group Method (UPGMA) with arithmetical average cluster analysis (CA) for investigating the phenotypic relationships among populations, (Sneath & Sokal 1973). Microsoft Office Excel 2010, R programming, and statistical tools for social sciences (SPSS version 26.0) were used for all statistical analysis.

## RESULTS AND DISCUSSION

The descriptive data of length and weight of the collected fish species showing the mean values, minimum and maximum range, and standard deviations are presented in Table 4. In this study, no significant correlation ($p > 0.05$) were observed between standardize truss measurement and the standard length, indicating that the size effect was successfully removed with allometric transformations. The transformed data were then used for the further discriminant functions analysis (DFA) and principal component analysis (PCA).

**Table 4.** A descriptive data of three *Johnius* species

| Species name | No of Samples | Total Length (min-max) cm | Total length (mean ± SD) |
|---|---|---|---|
| *Johnius borneensis* | 30 | 20.5–25.3 | 22.5 ±1.15 |
| *Johnius argentatus* | 30 | 23.4–30.2 | 26.7 ±1.85 |
| *Johnius belangerii* | 30 | 20.2–25.7 | 23.3 ± 1.43 |

**Table 5. Counting of meristic characteristics of three species of the genus *Johnius* (*Johnius borneensis*, *Johnius argentatus* and *Johnius belangerii*)**

| Meristic characters | *Johnius borneensis* | *Johnius argentatus* | *Johnius belangerii* |
|---|---|---|---|
| Dorsal fin ray's spine | X-XI | X-XI | IX-XI |
| Dorsal fin rays soft | 26-28 | 26-29 | 26-28 |
| Caudal fin rays | 16-18 | 17-20 | 17-18 |
| Anal fin rays | I/7 | II/7 | II/7 |
| Pectoral fin rays | 17-19 | 17-20 | 17-18 |
| Pelvic fin rays | 6-7 | 6-7 | 6-7 |
| Lateral line scale | 52-54 | 57-61 | 49-53 |
| Transverse lateral scale | 7,11 | 7,11 | 6,10 |
| Branchiostegal rays | VII | VII | VII |

*Meristic counts:* Most of the meristic characters of *Johnius borneensis*, *Johnius argentatus* and *Johnius belangerii* were overlapped among the three species of the genus *Johnius* (Table 5). However, the major distinguishable meristic characters are- less number (one) of dorsal fin ray's spine in *J. belangerii*, one short anal fin rays in *J. borneensis* and highest number of scales on lateral line on *J. argentatus* in compared with the other species.

*Analysis of Morphometric Measurement:* Univariate analysis of variance (ANOVA) showed that, fifteen [Total length (TL), head length (HL), pre-orbital length (PreOL), eye diameter (ED), highest body depth (HBD), least body depth (LBD), dorsal fin length (DFL), pre-dorsal fin length (pre-DL), post-dorsal fin length (PoDFL), pectoral fin length (PcFL), pelvic fin length (PvFL), pre-pelvic fin length (pre-PvFL), anal fin length (AFL), caudal peduncle length (CPL), and caudal fin length (CFL)] out of nineteen morphometric measurements were significantly different ($p < 0.05$) among means of three different species (Table 6).

Discriminant function analysis (DFA) resolved that first and second DFA accounted for 80.6%, & 19.4% respectively among group variability and together they explained 100% of the total variability for morphometric measurements (Table 7). Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions revealed that among the nineteen morphometric measurements, twelve (TL, HL, PrOL, ED, HBD, LBD, PrDFL, PoDFL, PcFL, PrPvFL, PrAFL and CFL) were dominantly contributed to the first DF, while the rest seven were contributed to the second DF (Fig 3).

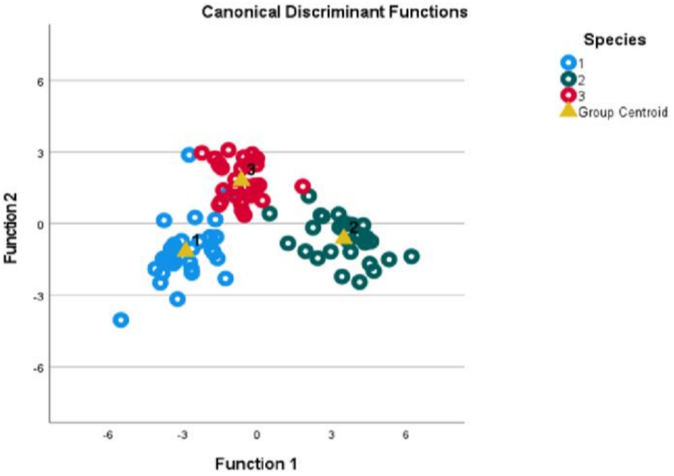

Fig. 3: Scatter plot of two Canonical Discriminate Function of morphometric characters for pama croaker (Where, sp1= *J. borneensis*, sp2= *J. argentatus*, sp3= *J. belangerii*).

PCA for the morphometric measurement of *J. borneensis, J. argentatus* and *J. belangerii* showed that, the value of KMO for the overall matrix was 0.821 and the Bartlett's Test of Sphericity was significant ($p < 0.01$). The result of KMO and

Bertlett's suggest that the sampled data was appropriate to proceed with a factor analysis procedure (Table 8).

**Table 6. Univariate statistics (ANOVA) among samples of *J. borneensis, J. argentatus* and *J. belangerii* from 19 morphometric measurements. Here, degree of significance was presented as *p < 0.05, **p < 0.01, ***p < 0.001**

| Tests of Equality of Group Means | | | | | |
|---|---|---|---|---|---|
| | **Wilks' Lambda** | **F** | **Df1** | **Df2** | **Sig.** |
| TL | 0.400 | 65.357 | 2 | 87 | <.001 |
| SL | 0.939 | 2.830 | 2 | 87 | .064 |
| HL | 0.824 | 9.320 | 2 | 87 | <.001 |
| PrOL | 0.556 | 34.672 | 2 | 87 | <.001 |
| ED | 0.273 | 116.082 | 2 | 87 | <.001 |
| PoOL | 0.935 | 2.999 | 2 | 87 | .055 |
| HBD | 0.588 | 30.419 | 2 | 87 | <.001 |
| LBD | 0.459 | 51.210 | 2 | 87 | <.001 |
| DFL | 0.885 | 5.657 | 2 | 87 | .005 |
| PrDL | 0.936 | 2.972 | 2 | 87 | .056 |
| PoDL | 0.688 | 19.728 | 2 | 87 | <.001 |
| PcFL | 0.853 | 7.508 | 2 | 87 | <.001 |
| PvFL | 0.776 | 12.568 | 2 | 87 | <.001 |
| PrPvFL | 0.638 | 24.649 | 2 | 87 | <.001 |
| AFL | 0.838 | 8.391 | 2 | 87 | <.001 |
| PrAFL | 0.859 | 7.143 | 2 | 87 | .001 |
| CPL | 0.894 | 5.185 | 2 | 87 | .007 |
| CFL | 0.251 | 129.729 | 2 | 87 | <.001 |
| MG | 0.939 | 2.805 | 2 | 87 | .066 |

**Table 7. Summary of Canonical Discriminate Functions for morphometric analysis**

| Eigenvalues Morphometric Character | | | | |
|---|---|---|---|---|
| **Function** | **Eigenvalue** | **% of Variance** | **Cumulative %** | **Canonical correlation** |
| **1** | 7.152 | 80.6 | 80.6 | .937 |
| **2** | 1.721 | 19.4 | 100.0 | .795 |

**Table 8. KMO and Bartlett's Test of Morphometric Characters**

| KMO and Bartlett's Test | | |
|---|---|---|
| Kaiser-Meyer-Olkin Measure of Sampling Adequacy | | .821 |
| Bartlett's Test of Sphericity | Approx. Chi-square | 765.325 |
| | Df | 136 |
| | Sig. | <.001 |

The PCA based on 14 morphometric measurements retained five components with Eigen values >1, explaining 70.76% of the total variance. The first (PC1) and second (PC2) principal components accounted for 33.8% and 12.8% of total variance respectively. Scatter plots of the specimens relating to the first and second principal component analysis revealed a visual definition of the group as also revealed by the canonical discriminate function analysis (Fig 4). PCA dispersion showed a vast divergence in the SL, PoOL, PrDL, CPL, and MG in *J. argentatus* compared to the *J. borneensis* and *J. belangerii* indicating that *J. argentatus* is morphologically very dissimilar with other two species.

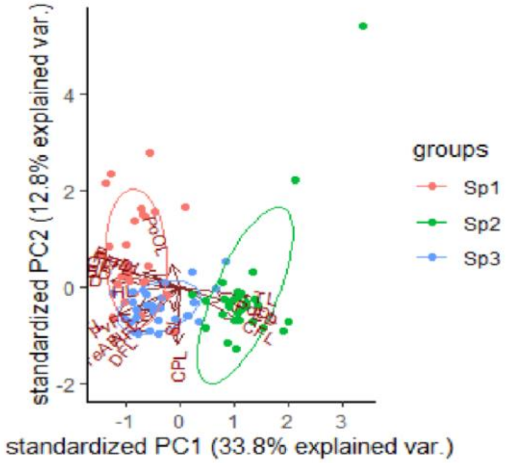

Fig. 4: Principal component analysis of morphometric characters for pama croaker (Where, sp1= *J. borneensis*, sp2= *J. argentatus*, sp3= *J. belangerii*).

**Table 9. Univariate statistics (ANOVA) showing the difference among measurement of 23 truss networking (*p < 0.05, **p < 0.01, and ***p < 0.001) of *J. borneensis, J. argentatus* and *J. belangerii***

| | **Tests of Equality of Group Means** | | | | |
|---|---|---|---|---|---|
| | **Wilk's Lambda** | **F** | **Df1** | **Df2** | **Sig.** |
| A1 | .981 | .838 | 2 | 87 | .436 |
| B1 | .998 | .104 | 2 | 87 | .901 |
| C1 | .943 | 2.651 | 2 | 87 | .076 |
| D1 | .860 | 7.101 | 2 | 87 | .001 |
| E1 | .926 | 3.476 | 2 | 87 | .035 |
| F1 | .968 | 1.441 | 2 | 87 | .242 |
| G1 | .990 | .427 | 2 | 87 | .654 |
| H1 | .990 | .440 | 2 | 87 | .654 |
| J1 | .990 | .458 | 2 | 87 | .634 |
| K1 | .989 | .481 | 2 | 87 | .620 |
| A2 | .988 | .511 | 2 | 87 | .602 |
| A3 | .956 | 1.999 | 2 | 87 | .142 |
| C2 | .957 | 1.966 | 2 | 87 | .146 |
| C3 | .856 | 7.306 | 2 | 87 | .001 |
| C4 | .909 | 4.372 | 2 | 87 | .016 |
| D2 | .588 | 30.505 | 2 | 87 | <.001 |
| D3 | .405 | 63.834 | 2 | 87 | <.001 |
| D4 | .724 | 16.543 | 2 | 87 | <.001 |
| E2 | .861 | 7.000 | 2 | 87 | .002 |
| E3 | .910 | 4.278 | 2 | 87 | .017 |
| F2 | .598 | 29.269 | 2 | 87 | <.001 |
| B2 | .631 | 25.406 | 2 | 87 | <.001 |
| K2 | .389 | 68.439 | 2 | 87 | <.001 |

*Analysis of Land-mark distance measurements:* Univariate analysis among the *J. borneensis, J. argentatus* and *J. belangerii* population using land-mark distances showed that, twelve distances (D1, E1, C3, C4, D2, D3, D4, E2, E3, F2, B2, K2) out of twenty-three truss measurements were significantly different among population in varying degrees ($p < 0.05$, $p < 0.01$, and/or $p < 0.001$) (Table 9).

**Table 10. Summary of Canonical Discriminant Functions for truss networking**

| Eigen values | | | | |
|---|---|---|---|---|
| **Function** | **Eigen value** | **% of Variance** | **Cumulative** | **Canonical Correlation** |
| 1 | 4.204 | 81.1 | 81.1 | .899 |
| 2 | .977 | 18.9 | 100.0 | .703 |

**Table 11. KMO and Bartlett's Test of Truss Networking**

| KMO and Bartlett's Test | | |
|---|---|---|
| Kaiser-Meyer-Olkin Measure of Sampling Adequacy | | .857 |
| Bartlett's Test of Sphericity | Approx. Chi-square | 516.482 |
| | Df | 66 |
| | Sig. | <.001 |

The first DFA resolved 81.1% and the second DFA accounted for 18.9% respectively of among group variability and together they explained 100% of the total variability for landmark measurements (Table 10). Among the twenty-three measurements, nine measurements (D1, E3, C3, A1, A2, K1, J1, A1, and G1) dominantly contributed to the second DF, while the remaining fourteen measurements contributed to the first DF. By summary of the canonical discriminate function analysis, it can easily identify that, *J. borneensis* and *J. belangerii* were remained closely compared to the *J. argentatus* (Fig 5).

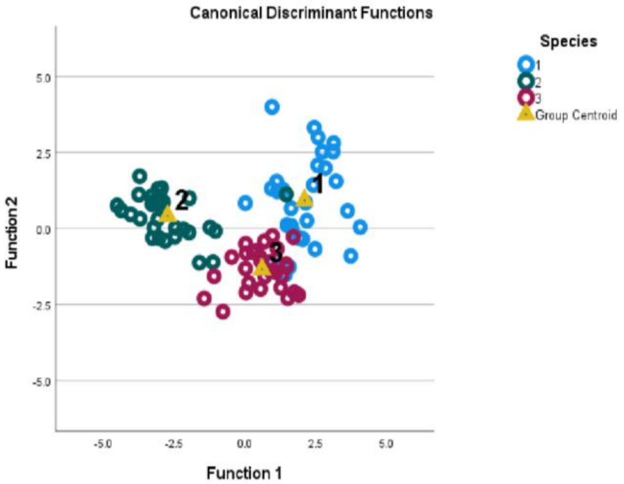

Fig. 5: Scatter plot of two Canonical discriminant functions of truss networking for pama croaker (Where, sp1= *J. borneensis*, sp2= *J. argentatus*, sp3= *J. belangerii*).

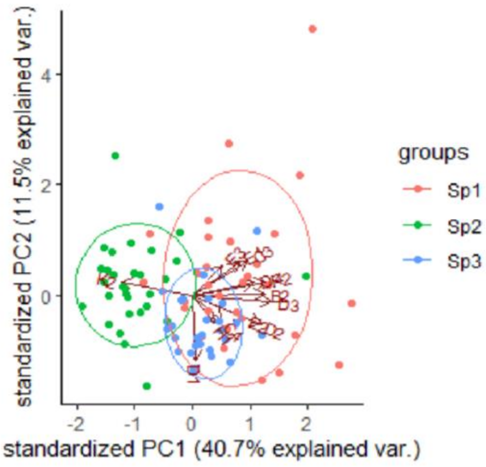

Fig. 6: Principal component analysis of truss network for pama croaker (Where, sp1= *J. borneensis*, sp2= *J. argentatus*, sp3= *J. belangerii*).

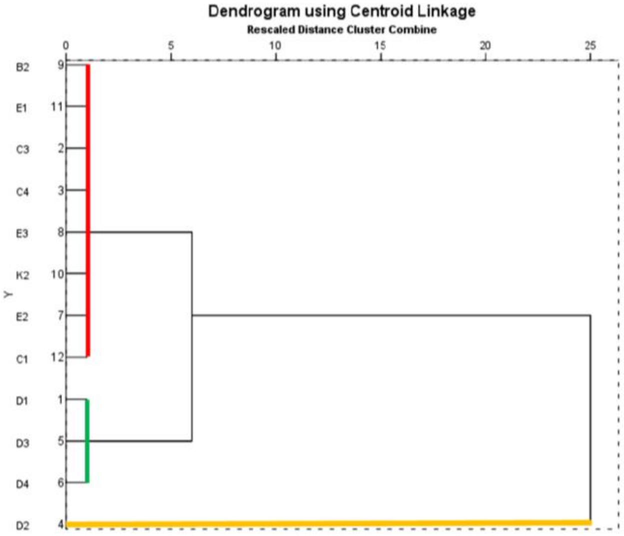

Fig. 7: Dendrogram presents the dissimilarity of landmark distances of pama croakers through three clusters

PCA was performed on the relevant attribute identified by univariate analysis (twelve truss network measurement). The truss distances having an Eigen value greater than 1 were included for PCA. Truss distances among the *J. borneensis, J. argentatus* and *J. belangerii* showed that, the value of KMO for overall matrix is 0.857 and the Bartlett's Test of Sphericity is significant ($p < 0.01$). The result of KMO and Bertlett's suggest that the sampled data was appropriate to proceed with a factor analysis procedure (Table 11).

The PCA based on 12 morphometric measurements retained three components with Eigen value >1 explaining 64.49% of total variance. The first (PC1) and second (PC2) principal component accounted for 40.7% and 11.5 % of total variance respectively. By summary of PCA it can accomplish that *J. borneensis* and *J. belangerii* are closely related species and *J. argentatus* is distinct species (Fig 6).

*Hierarchical cluster analysis:* Using the centroid cluster approach, cluster analysis consolidation steps were carried out based on landmark distances. A dendrogram was build consisting of different truss networking distances and found three or two different clusters based on Squared Euclidean distance measurement indicating that all three species are morphologically dissimilar. Cluster distances are rescaled so that they range from 0 to 25 in this plot (Fig 7). Here, in cluster-1 (Green), show similarity within the cases (D1, D3, and D4) and in cluster-2 (Red), the cases (B2, E1, C3, C4, E3, K2, E2, and C1) refer resemblance within the cases. Cluster-3 (Yellow), D2 indicates dissimilarity. The clusters are different from each other based on its cases. So, it can conclude that, the three clusters separated the three species of fish.

In the present study, morphometric differences among three species of croaker (*J. borneensis, J. argentatus*, and *J. belangerii*) were studied using land-mark-based truss-networking analysis from Bangladesh coast of the Bay of Bengal. The findings of the present study indicate that there is a high degree of similarity between *Johnius bornensis* and *J. belangerii* while *J. argentatus* is morphologically different. This study is the first report regarding the morphometric variations among three species of croaker from the Bay of Bengal, which may further help in stock identification, conservation, and management plan for these species.

Morphometric and meristic characters are found to be useful for identifying fish populations having higher phenotypic plasticity (Hossain *et al.* 2010; Ihssen *et al.* 1981). In the present study, the potential differentiation among the *J. borneensis, J. argentatus*, and *J. belangerii* populations from the Bay of Bengal, Bangladesh, has been examined using morphometric and meristic features with land-mark-based techniques. Bhakta *et al.* (2020) reported that the number of scales in the lateral line in *J. argentatus* was 48−52, but Talwar (1995) reported 44−48 in the lateral line. In the present study, the maximum number of meristic characters was found similar to the earlier studies with little difference in the number of gills rakers and the total number of lateral lines. Environmental factors like temperature, salinity, oxygen, pH, food availability, and growth rate may all play a role in these variations.

The truss networking system is a more feasible and effective strategy for explaining shapes, provides a better method of data collection, and allows the data to be used in a variety of ways of analysis to differentiate between phenotypic stocks than the traditional morphometric method. This is because of the set of the created landmarks encloses the entire fish body without affecting the detailed body plan (Dwivedi & Dubey 2013). Previous studies on the horse mackerel *Trachurus trachurus* (Murta *et al.,* 2008); Indian main carps (Hossain *et al.* 2010); mullet (Hossain *et al.* 2015); and catfish (Parvej *et al.* 2014, Rahman *et al.* 2014); have all been effectively differentiated and identified using ANOVA (Analysis of variance), DFA (Discriminant function analysis) and PCA (Principal component analysis) (Mousavi-Sabet & Anvarifer 2013). PCA based on morphometric measurements of the three experimental species revealed that *J. borneensis,* and *J. belangerii* species are closely related and *J. argentatus* is different from them. In the case of meristic counts, most of the characters were similar, but during taking the morphometric measurements, relatively significant variances were found. Out of nineteen morphometric characters, seventeen characters showed significant differences in univariate analysis among the population *of J. borneensis, J. argentatus,* and *J. belangerii* in varying degrees ($p < 0.05$ or $p < 0.01$, $p < 0.001$). It was described in the (Hanafi *et al.* 2022) research that *Johnius* is the most diversified in Sciaenid genus with verified monophyly. However, because of their external morphological similarities and overlapping meristic counts, accurate identification is difficult. Hence, *J. borneensis* and *J. belangerii* resolved as polyphyletic groups here, they represent the two more polyphylatic species within the genus.

In the present study, univariate analysis among the *J. borneensis, J. argentatus,* and *J. belangerii* populations using land-mark distances showed that, twelve out of twenty-three characters in various degrees were significantly distinct ($p < 0.05$ or $p < 0.01$, $p < 0.001$). The DFA segregation was partly confirmed by PCA, where according to the graphs of PCA1 and PCA2 scores for each sample used in the morphometric analysis, there was higher overlap between species *J. borneensis* and *J. belangerii.* Similarly, in the case of truss networking more overlapping was observed between *J. borneensis* and *J. belangerii*, and slight interlinking points were overlapped between *J. argentatus* and other two *Johnius* species. Molecular characterization of the cytochrome c oxidase subunit I (COI) gene could finally determine the genetic variations among these three species from other croakers in the Bay of Bengal.

## CONCLUSIONS

The marine fisheries sector of Bangladesh is under threat from overfishing, pollution, habitat destruction, improper use of agrochemicals, a lack of favorable habitat, low fertility, and other factors. The stock, meanwhile, is progressively decreasing. The findings of the present study can be used as primary data for stock management, allowing for efficient management techniques for the various fish stocks with a view to making sustainable fisheries management and adequate conservation programs in the near future.

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