



Stock Structure Analysis of Unicorn leatherjacket, *Aluterus monoceros* (Linnaeus, 1758) (Tetraodontiformes: Monacanthidae) from Indian Coastal Waters

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Abstract

Unicorn leatherjacket, *Aluterus monoceros* (Linnaeus, 1758), (Teleostei: Monacanthidae) is a marine demersal fish distributed throughout the Indo-Pacific waters and shared commercial landings along the coast of Indian peninsula from east to west. The present study was conducted to understand the stock structure of *A. monoceros* from Indian marine waters using truss-network and morphometric based multivariate analysis. Fish were randomly collected from major fish landing centres viz., Gujarat and Kerala along the West coast, and Odisha and Tamil Nadu from the East coast of India, to represent four zones of the Indian Exclusive Economic Zone (EEZ). A total of 17 landmarks were considered to form 28 truss distances, along with 15 morphometric data, to differentiate potential fish stocks of *A. monoceros*. Principal component analysis (PCA), linear discriminant analysis (LDA) and hierarchical clustering based on Euclidean distances of truss measurements were employed to identify stocks along the coastal waters. Univariate ANOVA showed that 22 out of 28 truss distances were significant ($p < 0.001$), while 9 out of 15 morphometric data were significant. The biplot of the first two principal components indicated intermixing of samples from all zones, suggesting absence of discrete stocks. Major body truss measurements of

the anterior and caudal peduncle regions contributed significantly to this. LDA functions also showed no evidence of stock separation. The cluster analysis dendrogram depicted that the stocks are distantly located, forming two main clusters among the four fishing locations considered. A uniform management plan is recommended for better harvest of this demersal resource, irrespective of its wide distribution in Indian waters.

Keywords: *Aluterus monoceros*, stock structure, fish truss-network analysis, multivariate analysis.

Introduction

Aluterus monoceros (Linnaeus, 1758), commonly known as the Unicorn Leatherjacket filefish, is a monacanthid fish belonging to the family Monacanthidae, a group of tropical and subtropical marine fishes under the order Tetraodontiformes. It is a reef-dwelling, subtropical species inhabiting the continental shelf at depths up to 80-meters and is popularly referred to as “filefishes” in the Northern hemisphere and “leatherjackets” in the Southern hemisphere. There are 28 genera and 107 species of Monacanthidae around the world (Ghosh, Hoshalli, Mamidi, Rohit, & Achamveetil, 2021), of which 14 genera and 22 species have been reported in Indian waters (Gopi & Mishra, 2015). The genus *Aluterus* consist of two species recorded in India—*Aluterus monoceros* (Linnaeus, 1758) and *Aluterus scriptus* (Osbeck, 1765) (Nair & Kumar, 2018)—out of four species reported globally (Matsuura, 2015). Prior to 2008, this species was considered as a bycatch in trawl fishery, with a meagre amount of landing. Later, between 2008–2011, extensive high quantities

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of landing were observed, causing a sudden emergence of fishery for *A. monoceros* at major fish landing centres along the Arabian sea and Bay of Bengal (Ghosh et al., 2011; Kanthan & Zacharia, 2011; Saleela, Anil, Jasmine, & Raju, 2011; Varghese, Thomas, Gandhi, & Sreekumar, 2011; Senthil et al., 2016).

Earlier studies on *A. monoceros* were primarily based on its sudden emergence along the Indian coast (Ghosh et al., 2011; Kanthan & Zacharia, 2011; Saleela et al., 2011; Varghese et al., 2011; Barik, Swain, Sahu, Tripathy, & Acharya, 2020). Tehseen, Desai, Khileri, and Temkar (2020) studied the spatial and temporal distribution of *A. monoceros* along the Arabian sea and identified a migration pattern, where abundance shifted from Gujarat coast during the post monsoon and winter periods, while along the Maharashtra coast during the pre-monsoon and summer seasons. Ghosh et al. (2021) & Ghosh, Satishkumar, Manas, Rohit, and Gopalakrishnan (2022) studied the biological aspects and its stock assessment along the Bay of Bengal and found its fecundity (33640-12,39,202 eggs), and feeding habits as omnivorous and opportunistic, with an ontogenetic shift in diet when it reaches a total length of 40 cm. The species exhibits multiple spawning behaviour, with major peak from October to February and minor peak from April to June.

Stock identification is a prerequisite for successfully assessing and managing exploited fisheries (Begg, Friedland, & Pearce, 1999). It is a fundamental approach to recognise self-sustaining stocks within a natural population (Cadrin & Silva, 2005). Stock structure refers to the representation of stock units that collectively embody the entire population, rather than focusing solely on the population's structure based on factors like length or size. Studies have demonstrated that stable differences in shape between groups of fish may reveal further differences in growth, mortality or reproductive rates, making them relevant for defining stocks (Swain & Foote, 1999; Cadrin, 2000). Knowledge of population structure is crucial for managing a fishery resource sustainably, rationally, and effectively, as each fish stock must be managed separately to optimise sustainable yields (Grimes, Johnson, & Fable, 1987; Cadrin, Karr, & Mariani, 2014; Siddik, Hanif, Chaklader, Nahar, & Fotedar, 2016). Population structures can be intricate, leading to discrepancies between a species' ecology or biology and the

actual management unit, which can introduce biases into stock assessments and hinder the achievement of sustainable fisheries management (Cadrin et al., 2014; Kerr et al., 2017). Therefore, analysing stock structure is essential before developing fishery management plans, to ascertain the current recruitment levels necessary for population replenishment (Cadrin et al., 2014).

A Stock is a part of a fish population, usually characterised by a particular migration pattern, specific spawning grounds, and subject to a distinct fishery (ICES, 2012). Several methods have been widely used for stock identification. These include conventional morphometrics (Sajina, Chakraborty, Jaiswar, & Sudheesan, 2013; Sreekanth et al., 2015), image based truss-network systems (Sen et al., 2011; Mandal et al., 2021), meristics (Sreekanth et al., 2013; Sajina et al., 2013), parasites (Mackenzie et al., 2008), otolith shape analysis (Deepa et al., 2019; Ferreira et al., 2019), tagging (Fritsch, Morizur, Lambert, Bonhomme, & Guinand, 2007), otolith microchemistry (Moreira et al., 2018; Biolé et al., 2019), fatty acid profiling (Sajina et al., 2015), mitochondrial markers (Vineesh et al., 2018; Mohindra et al., 2019; Sukumaran et al., 2020), and nuclear markers (Acharya et al., 2019; Mohitha et al., 2021) for characterising fish stocks.

However, there is no information on stock structure of *A. monoceros* (Linnaeus, 1758) in Indian waters. The species is known to be distributed throughout the Indian EEZ and is landed in different maritime states of India, indicating its wide spatial distribution in Indian marine waters. In this background, the current study was conducted to investigate the stock structure of *A. monoceros* (Linnaeus, 1758) along the Indian coast.

Materials and Methods

The sampling sites for Unicorn Leatherjacket filefish were selected based on classification of the maritime states of India to four regional zones, north-west, south-west, south-east and north-east (Srinath, 2003). The sampling site along the east coast were Odisha-Puri and Tamil Nadu-Thoothukudi, representing the northeast and southeast zones respectively. Along the west coast, the sites were Gujarat-Veraval and Kerala-Munambam, representing the northwest and southwest zones respectively (Table 1). Fish identification was done following Fischer and Bianchi (1984).

The species is known to have the peak spawning period during October to February. In order to minimise the effects of migration or any other factors influencing the spatial distributions, and thereby sampling, landing centre collections were done during these months. Sampling took place during these months in the years of 2019 and 2020. Fish were collected from the commercial landings, especially from trawlers. Details of each location are listed in Table 1 and shown in Fig. 1. Samples were collected from single-day trawlers to make sure that fishing grounds were within the coastal waters of the selected states. Each sampling event was followed after the enquiry with stakeholders. The sample included a mixture of male and female fish; however, there was no external sexual dimorphism in the species and a total of 253 fishes were taken for the study.

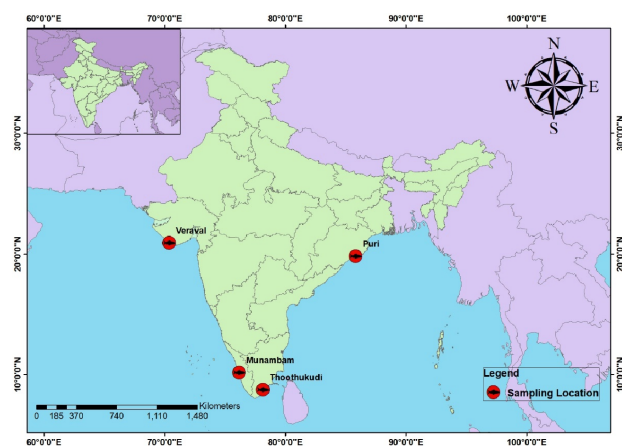


Fig. 1. Map showing locations from where samples were collected for the present study

The collected specimens were washed and placed on graph paper with 0.1 mm² units in the background on a flat surface and fins were erected. A unique code was tagged to each sample based on location. A Cyber-shot DSC-SX50HS digital camera (Canon) mounted on a levelling tripod was used to capture the digital images of each sample.

The truss protocol used in this study was based on seventeen landmarks, which were interconnected to forms 28 truss networks. The landmarks and the resulting truss network are shown in Fig. 2 and Table 2. The truss distances were extracted from each specimen's digital image by using a linear combination of two software, tpsDig2 V2.1 (Rohlf,

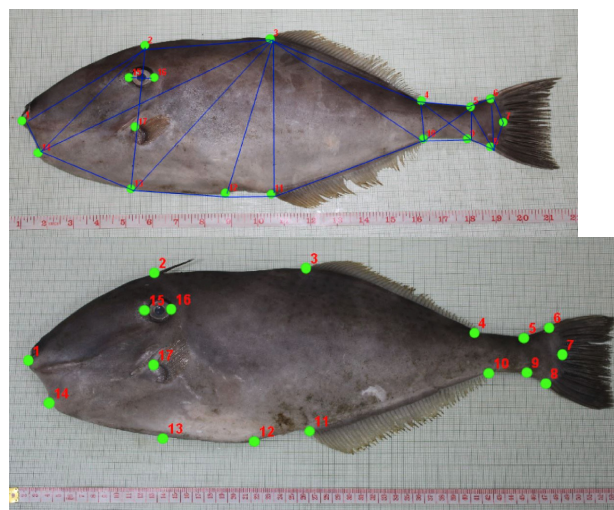


Fig. 2. *Aluterus monoceros* image showing seventeen landmarks of truss measurement, with the truss distances.

Table 1. Details of sample collection from different sampling locations.

Region	Coast-wise	GPS location	N	SL (Min.-Max. in cm)	Mean $\pm$ SD (cm)	Min.-Max. (gm)	Mean $\pm$ SD (gm)
North-west	Gujarat-Veraval	20°54' N, 70°22' E	65	25.10-49.30	38.90 $\pm$ 5.94	120-1900	800.76 $\pm$ 333.73
South-west	Kerala-Munambam	9°81' N, 75°61' E	65	39.10-63.40	53.81 $\pm$ 5.31	200-2700	1262.66 $\pm$ 396.18
North-East	Odisha-Puri	19°79' N, 85°73' E	73	24.40-52.60	41.21 $\pm$ 5.26	400-2100	1191.304 $\pm$ 236.23
South-east	Tamil Nadu – Thoothukudi	8°47' N, 78°09' E	78	16.40-30.50	23.08 $\pm$ 3.41	88-407	198.91 $\pm$ 87.51

Note: N= sample size; Min.=minimum; Max.= maximum; SD= standard deviation; SL = Standard Length.

Table 2. Detail of Truss measurement

Sl.No	Truss distances	
1	T1 (1-7)	Standard length
2	T2 (1-2)	Distance between the tip of the snout to the dorsal spine origin
3	T3 (1-3)	Distance between the tip of the snout to the origin of the dorsal fin
4	T4 (1-4)	Distance between the tip of the snout to the posterior end of the dorsal fin base
5	T5 (1-10)	Distance between the tip of the snout to the origin of the anal fin base
6	T6 (1-11)	Distance between the tip of the snout to the origin of the dorsal fin base
7	T8 (1-15)	Distance between the tip of the snout to the origin of the eye
8	T9 (1-12)	Distance between the tip of the snout to the posterior tip of the urohyal bone
9	T10 (1-13)	Distance between the tip of the snout to the ventral point of the abdomen below the pectoral fin origin
10	T11 (1-14)	Distance between the tip of the snout to the anterior of the urohyal bone
11	T12 (2-3)	Distance between the dorsal spine to the origin of the dorsal fin base
12	T13 (2-10)	Distance between the origin of the dorsal spine to the posterior end of the anal fin base
13	T18 (3-4)	Length of dorsal fin base
14	T19 (3-10)	Distance between the origin of the dorsal fin base to the posterior end of the anal fin base
15	T20 (3-11)	Distance between the origin of the dorsal fin and to the origin of the anal fin
16	T21 (3-12)	Distance between the origin of the dorsal to the posterior tip of the urohyal bone
17	T22 (3-13)	Distance between the origin of the dorsal fin to the point below the pectoral fin origin
18	T24 (4-5)	Distance between the end of the dorsal fin base to the dorsal point of caudal peduncle depth.
19	T25 (4-10)	Distance between the posterior end of the dorsal fin base to the posterior end of the anal fin base
20	T26 (4-11)	Distance between the posterior end of the dorsal fin base to the origin of the anal fin base
21	T27 (5-9)	Caudal peduncle depth
22	T28 (5-6)	Distance between the dorsal point of caudal peduncle depth to the dorsal point of caudal fin base
23	T29 (7-17)	Distance between the 5 th of caudal fin rays to pectoral fin origin
24	T31 (9-10)	Distance between the ventral point caudal depth to the anterior anal fin
25	T32 (8-9)	Distance between the ventral point of the caudal fin base to the ventral point of the caudal peduncle depth
26	T33 (10-11)	Length of the anal fin base
27	T34 (11-12)	Distance between the origin of the anal fin base to the posterior-urohyal bones
28	T37 (13-14)	Distance between the ventral point of the abdomen below to the pectoral fin origin to the anterior tip urohyal bone

2005) and the Paleontological Statistics software package for education and data analysis (PAST) Version 4.13 (Hammer, Harper, & Ryan, 2001).

For the traditional morphometric data set, 15 body measurements including total and standard length were taken with vernier calliper (Fig. 3 and table 4).

The dataset was pre-processed to check for the presence of outliers. Detected outliers were removed, and the dataset was refined for further analysis. The effect of body size due to allometric growth was eliminated using the GroupStruct package (Chan & Grismer, 2022). Ontogenetic variations were adjusted using the following formula:

$$X_{adj} = \log_{10}(X) - B [\log_{10}(BL) - \log_{10}(BL_{mean})],$$

where;

X_{adj} = adjusted value for each independent truss measurement X ,

X = raw/unadjusted value for each independent truss measurement,

B = pooled regression coefficient (slope) of $\log_{10}(X)$ against $\log_{10}(BL)$

BL = observed value of the standard measure, which is taken as Standard-length SL in this case.

Linear correlation analysis between standard length was done for all the allometric size corrected measurements to know whether its effect persisted and to ensure that multicollinearity does not exist in the dataset after removing the allometric growth effect. Following this, the size-corrected measurements were subjected to univariate ANOVA to test the significance differences among the four locations. This was done after eliminating the standard length from the dataset, as it served as a basis for size-transformation. The significant ($p < 0.001$) factors of one-way ANOVA were selected for further data reduction techniques. Principal Component Analysis (PCA) was adopted as a dimensionality reduction procedure to understand the stock structure of *A. monoceros*. It is an appropriate technique for population or stock differentiation, as it reduces the number of morphometric variables (Vasey, Vencovsky, Martins, & Bandel, 2002), minimises redundancy among selected measurements (Samaee,

Table 3. Detail of traditional morphometric measurement

Sl.No		
1	Pre-Dorsal Spine Length	PDs
2	Pre-Dorsal Fin Length	PDF
3	Standard Length	SL
4	Pre-Anal Fin Length	PAF
5	Pre-Pectoral Length	PpL
6	Pre-Orbital Length	POL
7	Total Length	TL
8	Length Between Dorsal Spine and Dorsal Fin	LDsDF
9	Head Depth	HD
10	Dorsal Fin Base	DFB
11	Body Depth	BD
12	Caudal Peduncle Length	CDL
13	Caudal Depth	CD
14	Anal Fin Base	AFB
15	Pectoral Fin Base	PFB
16	Caudal Fin Base Length	CFBL
17	Eye Diameter	ED

Mojazi-Amiri, & Hosseini-Mazinani, 2006), and also reduces the number of independent variables (Samaee, Patzner, & Mansour, 2009). PCA was performed with the FactoMineR package (Lê, Josse, & Husson, 2008) and visualised with the factoextra package (Kassambara & Mundt, 2020). Bartlett's Test of Sphericity (Bartlett, 1951) was performed prior to PCA using the EFAtools package (Steiner & Grieder, 2020) to assess the suitability of the correlation matrix for PCA. This was done for both the truss network and morphometric data.

The recommended ratio of the number of fish samples (N) to the total number of selected variables or parameters (P) was also estimated to check the stability of the dataset for multivariate analysis (Kocovsky, Adams, & Bronte, 2009). Linear Discriminant Analysis (LDA) was performed using the MASS package (Venables & Ripley, 2002) to predict spatial differences among the sampling locations. LDA function was used to determine the accuracy of each individual *A. monoceros* being to its original coast-wise collection, applying the Jackknife cross-validation technique. The percentage of correctly classified individuals from the respective locations was considered for separating populations. Cluster

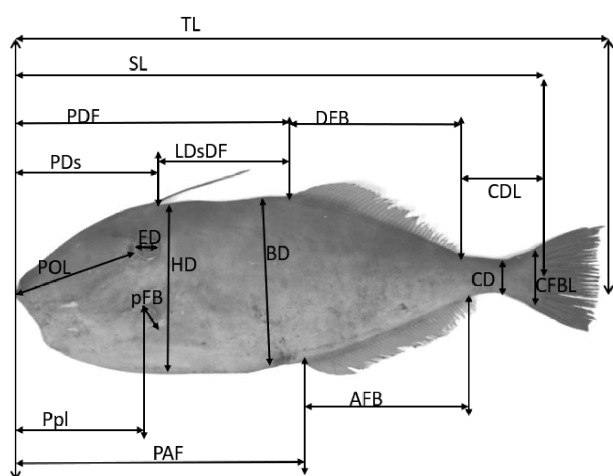


Fig. 3. Linear measurements taken for morphometric data of *A. monoceros*.

Analysis (CA) was done using the Unweighted Pair Group Method (UPGMA), and a dendrogram was constructed based on Euclidean distances (Sneath & Sokal, 1973). The dendrogram was visualised using the dendextend package (Galili, 2015). All the analyses were done in the R software package (R Core Team, 2021).

Meristic counts were recorded from each individual fish, including the number of dorsal spines (DS), dorsal fin rays (DFR), pectoral fin rays (PFR), caudal fin rays (CFR), and anal fin rays (AFR). The Kruskal-Wallis test was performed to determine if any significant variations existed among the fish from different stocks.

Results and Discussion

The allometric size-corrected truss measurements showed a significant reduction in collinearity with standard length, as indicated by lower correlation coefficient values. This confirmed that the size effect had been removed and multicollinearity did not exist in the dataset. One-way ANOVA showed that 22 out of 27 truss distances were significantly ($p < 0.001$) different among the four sampling locations (Table 4). The N:P ratio, i.e., the ratio between the number of *A. monoceros* observed to the significant truss distances, was 11.5, indicating the dataset's stability for multivariate analysis for understanding shape variations and its use in population differentiation. Bartlett's test of sphericity was also significant ($p < 0.001$), confirming that the dataset after one-way ANOVA is suitable for data reduction procedures.

The PCA yielded a total of 22 principal components, of which the first six had contributed 80.14 % of the variance with eigenvalues more than 1 (Table 4), and components with eigenvalues less than 1 were discarded after that. The first PC contributed 33.9 % of the total variance, followed by PC2 (16.48%), PC3 (10.11 %), PC4 (7.90%), PC5 (7.11%) and PC6 (4.64%) (Table 4). The first two components together contributed maximum variance (50.37 %) (Table 5), as indicated by the elbow point on the scree plot (Fig.4). The biplot of PC1 and PC2 showed considerable mixing of populations, with no clear delineation of stocks observed (Fig. 5). Individuals were widely spread over both the negative and positive axes in all the quadrants, indicating that individuals of stocks are not delineated nor forming individual clusters or stocks, thereby showing weak separation. The ellipse covers 95% of the variability

Table 4. Results of univariate ANOVA of 27 truss distances, excluding standard length of *A. monoceros* sampled from different coasts of India. Truss variables found to be significant, with $p < 0.001$ (marked with *), are retained.

Variables	F value	P value
T2	27.0396	0.000*
T3	15.0363	0.000*
T4	8.1811	0.000*
T5	10.5127	0.000*
T6	8.1598	0.000*
T8	19.8793	0.000*
T9	20.3028	0.000*
T10	7.0950	0.000*
T11	4.8961	0.0025
T12	1.4376	0.2323
T13	4.3125	0.0055
T18	9.1563	0.000*
T19	202.7197	0.000*
T20	10.4542	0.000*
T21	6.8449	0.000*
T22	21.9810	0.000*
T24	3.0787	0.0281
T25	14.7690	0.000*
T26	8.1520	0.000*
T27	9.0605	0.000*
T28	10.5606	0.000*
T29	64.2730	0.000*
T31	10.5734	0.000*
T32	105.0012	0.000*
T33	12.9882	0.000*
T34	12.9882	0.000*
T37	4.2100	0.0063

of data, confirming the absence of individuals into separate stock in any ellipses. Among the variables, the heavy loadings on PC1 were by T2, T3, T6, T8, T9, T10, T20, T21, T22, T25, and T27, and all together the weighted loadings generated 33.89% of the total variance. On PC2, truss distances T4, T5, T18, T26 and T33 showed maximum loadings with high positive values and accounted for the second maximum loadings of 16.48%. The truss measurements aligned towards positive sides on both PC1 and PC2 contributing more to the overlap of stocks,

Table 5. Presented the eigenvalues, percentage of variance and cumulative percentage of variances of PCs of truss measurements PCA of *A. monoceros*.

PCs	Eigenvalue	Percentage of variance	Cumulative percentage of variance
comp 1	7.4565	33.8932	33.8932
comp 2	3.6254	16.4792	50.3725
comp 3	2.2248	10.1129	60.4854
comp 4	1.7388	7.9037	68.3891
comp 5	1.5655	7.1157	75.5049
comp 6	1.0203	4.6376	80.1425
comp 7	0.7697	3.4988	83.6413
comp 8	0.7543	3.4288	87.0701
comp 9	0.6600	2.9998	90.0699
comp 10	0.5023	2.2831	92.3530
comp 11	0.4527	2.0575	94.4106
comp 12	0.3261	1.4824	95.8930
comp 13	0.2365	1.0751	96.9681
comp 14	0.2036	0.9253	97.8934
comp 15	0.1435	0.6522	98.5456
comp 16	0.1212	0.5507	99.0963
comp 17	0.0856	0.3890	99.4853
comp 18	0.0634	0.2882	99.7736
comp 19	0.0404	0.1835	99.9570
comp 20	0.0072	0.0328	99.9898
comp 21	0.0019	0.0085	99.9983
comp 22	0.0004	0.0017	100

resulting in no separate structuring based on truss distances (Fig. 6). The truss measurements towards the horizontal positive side was T8, giving the maximum contribution among all 22 truss distances, followed by T3, T2, T22, T29 and T10. Truss measurements like T26, T33, T4, T31, T18 and T5 were oriented vertically along the positive axis. These truss measurements along positive directions are more influential in the non-separation of stocks of *A. monoceros* in Indian waters.

The variables with maximum PC loadings from the first two PCs (highlighted in Table 5) were sorted out based on their contribution and taken for further Linear Discriminant Analysis (LDA) and cluster analysis. LDA was done to produce a confusion matrix and reclassification matrix, and by using 15

Table 6. Contribution of truss measurements to PC components.

Variables	Dim.1	Dim.2
T2	0.85	0.01
T3	0.85	-0.05
T4	0.10	0.76
T5	0.17	0.64
T6	0.70	-0.19
T8	0.86	-0.01
T9	0.68	-0.07
T10	0.75	-0.20
T18	-0.45	0.65
T19	-0.41	0.18
T20	0.76	0.32
T21	0.71	0.28
T22	0.83	0.21
T25	0.50	0.04
T26	-0.06	0.87
T27	0.61	0.06
T28	0.15	-0.19
T29	-0.79	0.03
T31	-0.29	-0.66
T32	0.28	0.07
T33	-0.28	0.80
T34	-0.20	-0.06

truss measurements from the first two PCs to understand the percentage of intermixing of stocks (Table 7). The discriminant function analysis also revealed no discrimination among the four stocks in the Indian Exclusive Economic Zone. The fish from the Tamil Nadu coast showed less mixing with the others. The analysis extracted three variates, with LD1 having maximum morphological variation at 72%, followed by LD2 with 18% and LD3 with 10%. The scatterplot of LD1 and LD2 with percentage contribution also shows overlap of populations (Fig. 7). The scatterplot of LDA of these four stocks also suggest considering no stock structuring of *A. monoceros* in Indian waters.

The confusion matrix showed that the classification results of predicted groups ranged from 54.72% to 91.38% before cross-validation, and from 52.83% to 82.76% after cross-validation. About 76.68% of

original observations were correctly classified, and 73.12% of points were classified accurately after cross-validation. The original classification was found in all populations with varying percentages of LDA, with Tamil Nadu having highest accurate classification of 91.38%, followed by Kerala at 80%, Odisha at 77.61% and Gujarat at 54.72%. Similarly, after cross-validation, the percentage of accurate classifications was maximum for Tamil Nadu at 82.76%, followed by Kerala (78.67%), Odisha (74.63%) and Gujarat (52.83%). The highest percentage of misclassification was observed between the Gujarat population and those from Kerala and Odisha (22.64%), based on the original data points. After cross-validation, the populations of Gujarat and Kerala had 24.53% of misclassification.

Euclidean distances based on the similarity of truss measurements among the four stocks indicated that the four stocks were relatively distant from each other. The UPGMA method was adopted, and the resultant dendrogram showed that four stocks appeared in two major clusters (Fig. 8). The Kerala sample forms a distinct cluster, and the other three formed a single cluster; furthermore, two subclusters were formed where Tamil Nadu does not show any similarities with Odisha and Gujarat, resulting in a separate identical subcluster, while Gujarat and Odisha showed maximum similarity and formed close branches in the cluster analysis.

After size correction, multicollinear variables were absent in the dataset. One-way ANOVA results showed that 9 out of 15 variables were having significance ($p < 0.05$) (Table 7). Bartlett's test of sphericity was significant at the 0.05 alpha level,

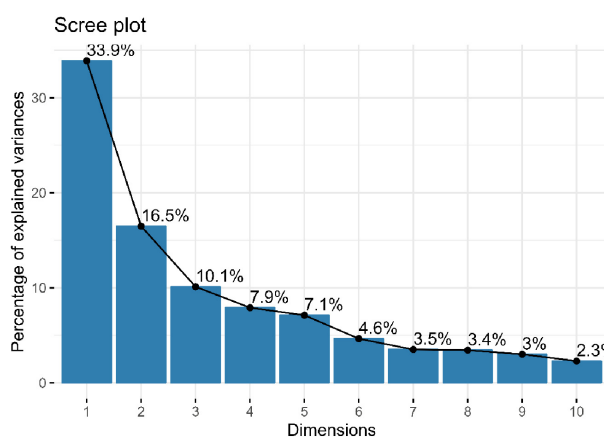


Fig. 4. Scree plot of PCs with the respective percentage of variance extracted

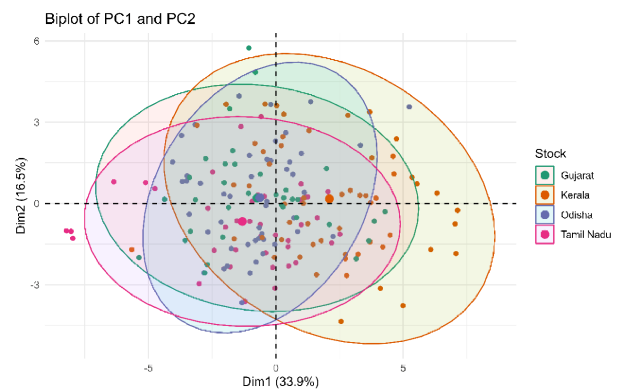


Fig. 5. Biplot of first two Principal Components. The ellipse covers 95% of the variability of data.

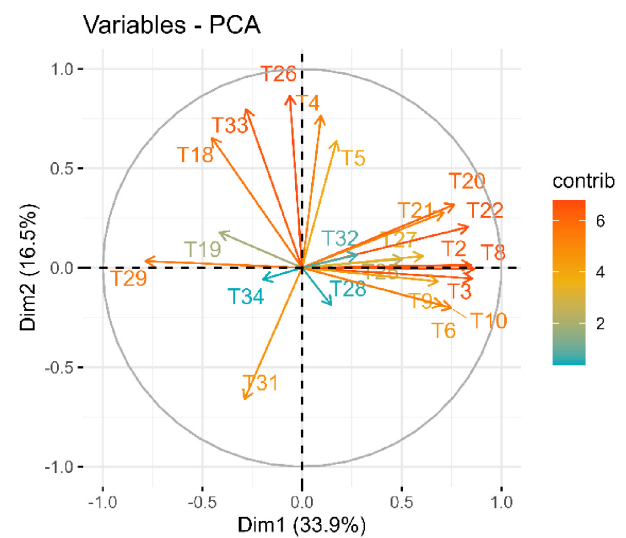


Fig. 6. Orientation of truss variables in the biplot of PC1 and PC2; the variables towards the positive axis are represented with orange colour.

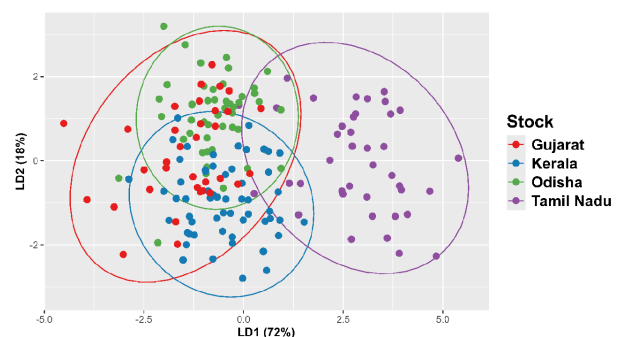


Fig. 7. Biplot of LD1 and LD2 from LDA of truss measurements of *A. monoceros* from different sampling locations

Table 7. Predicted group memberships in *A. monoceros* from four collection locations, original and cross-validated classifications are tabulated separately.

		Predicted group				Total
		Gujarat	Kerala	Odisha	Tamil Nadu	
Original (Numbers)	Gujarat	29	12	12	0	53
	Kerala	4	60	11	0	75
	Odisha	9	6	52	0	67
	Tamil Nadu	0	2	3	53	58
Original (Percentage)	Gujarat	54.72	22.64	22.64	0.00	100
	Kerala	5.33	80.00	14.67	0.00	100
	Odisha	13.43	8.96	77.61	0.00	100
	Tamil Nadu	0.00	3.45	5.17	91.38	100
Cross-validated (Number)	Gujarat	28	13	12	0	53
	Kerala	4	59	11	1	75
	Odisha	9	8	50	0	67
	Tamil Nadu	0	6	4	48	58
Cross-validated (Percentage)	Gujarat	52.83	24.53	22.64	0.00	100
	Kerala	5.33	78.67	14.67	1.33	100
	Odisha	13.43	11.94	74.63	0.00	100
Tamil Nadu	0.00	10.34	6.90	82.76	100	

indicating that the dataset is suitable for data reduction techniques. The estimated N:P ratio was 28.22, which also shows the dataset suitability for analyzing population differentiation using statistical methods.

First three principal components had eigen values greater than 1 and contributed to 64.87% of variance in the PCA of measurement data (Table 8). PC1 and PC2 had variance of 37.05% and 15.90%, totalling 52.95%. The scree plot also confirms that the first two components were having maximum contribution and the subsequent components showing much lower variations as indicated by the elbow point (Fig. 8). The PCA biplot shows the dispersion of individuals without any clear separation of identical stocks (Fig. 9). The biplot of first two PC components have the overlap and complete mixing of individuals regardless of their coast-wise distribution or if they were from east or west coast of India. The complete overlap of individuals and even spread indicates that there are no stock delineations based on the morphometric data (Fig. 9). This suggests that in Indian waters, *A. monoceros* has single, phenotypically homogenous population. Among the first PC, PDs, HD, and PpL contributed to maximum

variations, while AFB and CDL had maximum loadings along the second PC (Fig. 10).

The Linear Discriminant Analysis (LDA) of the multivariate dataset also resulted in similar findings as PCA. The LDA model fitted had 80% and 19% of variations along LD1 and LD2 respectively. The first two discriminant functions could explain maximum variations. The biplot of the model (Fig.

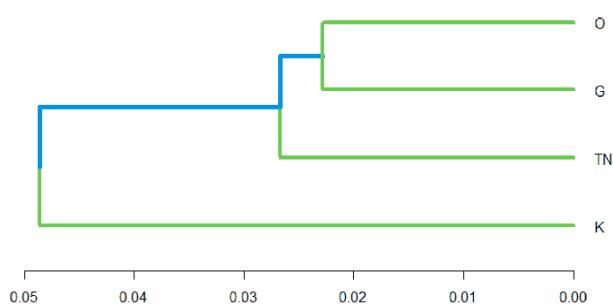


Fig. 8. Dendrogram from truss distances derived from UPGMA cluster analysis based on the Euclidean distances between the stock centroids from truss variables. Stock names are abbreviated: G, K, TN and O stand for Gujarat, Kerala, Tamil Nadu, and Odisha, respectively.

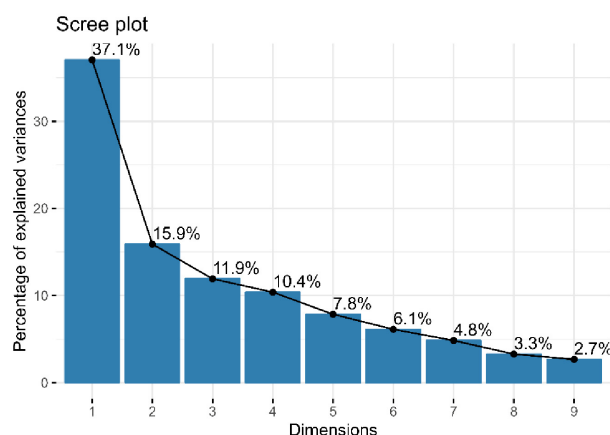


Fig. 9. Scree plot of PCs of measurements with the respective percentage of variance extracted.

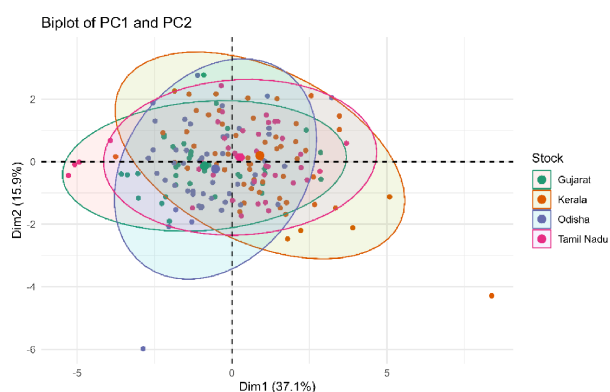


Fig. 10. PCA biplot of body measurements of *A. monoceros*, with ellipses covering 95% of variability.

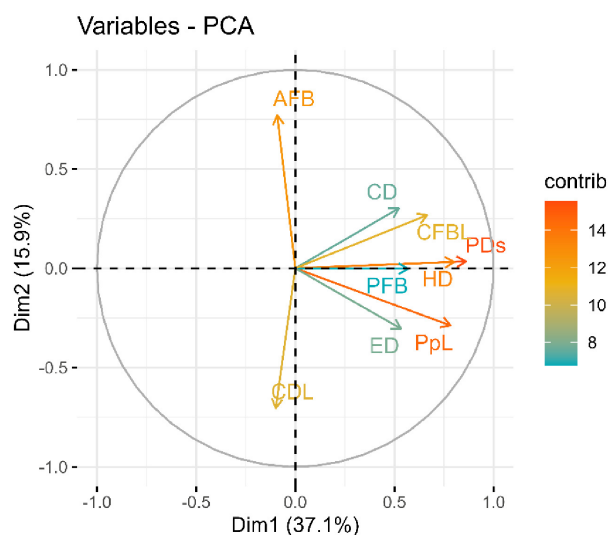


Fig. 11. Orientation of body measurements in the biplot of PC1 and PC2; the variables towards the positive axis are represented with orange colour.

Table 8. Results of univariate ANOVA of 15 body measurements, excluding standard length of *A. monoceros* sampled from different coasts of India. Truss variables found to be significant, with $p < 0.001$ (marked with *), are retained.

Variables	F value	P value
PDs	8.9050	0.0000*
PDF	2.7762	0.0418*
PAF	1.3109	0.2713
PpL	1.2738	0.2838
POL	6.0908	0.0005*
LDsDF	0.2995	0.8257
HD	1.6905	0.1695
DFB	4.6573	0.0034*
BD	0.9565	0.4139
CDL	0.0469	0.9864
CD	18.9040	4.4078
AFB	3.6411	0.0133
PFB	15.6406	2.3589
CFBL	13.4098	3.8304
ED	18.1171	1.1388

11) with first two discriminant functions also shows the complete mixing of fishes collected from all four locations, indicating the absence of identical discrete stocks exist in Indian marine waters. The overall classification accuracy of the model before cross validation was 53.15% in 95% confidence interval (0.4681, 0.5941) with maximum accurate classification for fish from the Kerala coast (70.67%) and the maximum intermixing individuals were from Tamil Nadu coast, and hence showed the lowest accuracy in confusion matrix generated from LDA model (Table 11). The classification accuracy after cross-validation of the model was 48.82%, and Kerala stock (62.67) showed maximum accurate classification of population and the least accurately classified fish were from Tamil Nadu coast (15.52 %).

The meristic counts observed from the different stocks are tabulated in Table 12. Meristic counts that showed variations such as DFR and AFR were subjected to Kruskal Wallis test. The DFR did not show difference between and among the populations ($p > 0.05$). However, AFR showed significant variation and hence a Post-hoc Dunn test was also performed to derive a pair-wise comparisons between the locations.

The pair-wise comparison of AFR following Dunn test indicates that AFR of Kerala fishes differs significantly ($p < 0.05$) from all the other three stocks in consideration.

The present study focused on the stock structure analysis of *A. monoceros* along the Indian waters based on its morphometric studies (truss image and traditional), and meristic counts. Morphometric studies have the potential to portray the differences between fish populations and is regarded as a reliable tool for fish population discrimination (Palma & Andrade, 2002). The morphometric studies indicated an intermixing population regardless of all sampling locations, suggesting the absence of discrete stock of structure of *A. monoceros* populations.

The PCA results from truss measurement showed that out of 27 truss distances, 16 exhibited slight variations, involving the head, body, and caudal regions. Specifically, PC1 included T2, T3, T6, T8, T9, T10, T20, T21, T22, T25, and T27, while for PC2 involved T4, T5, T18, T26 and T33. In comparison, the traditional morphometric studies show slight variation in the head region that is PDs, HD, and Ppl for PC1 as well as in the caudal region which is AFB and CDL which is PC2 out of the 15 morphometric measurements taken. The difference between the traditional and Truss morphometric is because of the truss network system (Strauss & Bookstein, 1982). It is a landmark-based technique that uses a uniform network to cover the entire fish body without limits on the direction of variation or

Table 9. Eigenvalues, percentage of variance and cumulative percentage of variances of PCs of converted measurements PCA of *A. monoceros*.

PCs	Eigenvalue	Percentage of variance	Cumulative percentage of variance
comp 1	3.3347	37.0518	37.0518
comp 2	1.4311	15.9012	52.9529
comp 3	1.0726	11.9173	64.8702
comp 4	0.9345	10.3833	75.2535
comp 5	0.7059	7.8434	83.0969
comp 6	0.5508	6.1203	89.2172
comp 7	0.4352	4.8360	94.0531
comp 8	0.2951	3.2791	97.3322
comp 9	0.2401	2.6678	100.0000

the localisation of shape changes (Rawat et al., 2017), where as traditional morphometric is only based on its length data.

The PCA results based on both truss and traditional morphometric analyses indicated variations in the head, body and caudal regions. Similar patterns have been observed in various fish resources studied along the Indian coastal areas, *Megalapsis cordyla* (Sajina et al., 2011), *Nemipterus japonicus* (Sreekanth et al., 2015), *Nemipterus bipunctatus* (Vaisakh et al., 2019), *Eubleekeria splendens* (Rawat et al., 2019), *Nemipterus randalli* (Srihari, Bhushan, Nayak, Pavan-Kumar, & Abidi, 2021) and *Siganus canaliculatus* (Rasheeq et al., 2023). Palma and Andrade (2002) suggested that variation in head region can be attributed to diverse ecological condition in which the population was thriving, demonstrating the environmental differences of the study locations, viz., the Arabian sea on the west coast and the Bay of Bengal on the east coast of India respectively. Notably, the east coast of India (Bay of Bengal Sea) features distinct environmental features, such as lower salinity, higher turbulence, and very strong water currents as compared to Arabian sea (Kumar et al., 2010). Variations in caudal peduncle region have also been reported in *Decapterus russelli* (Sen et al., 2011), *M. Cordyla* (Sajina et al., 2011), *Harpodon neherus* (Pazhayamadam et al., 2015), *Nemipterus japonicus* (Sreekanth et al., 2015), and *Piracanthus hamurus* (Mallik, Bhushan, Chakraborty, Jaiswar, & Ramasubramanian, 2020). Similar findings were observed in this study as well, however, those variations have no effect on stock discrimination. Imre, McLaughlin, and Noakes (2002) concluded that variations in water velocity could be the cause of the morphological dissimilarity of the caudal peduncle area of Brook charr. Blake (2004) stated

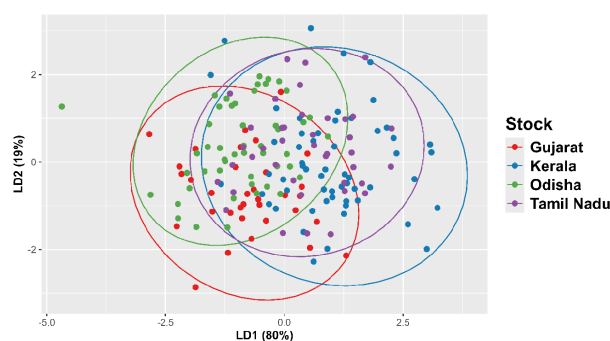


Fig. 12. Biplot of LD1 and LD2 from LDA of traditional morphometric traits of *Aluterus monoceros* from different sampling locations

Table 11. Predicted group memberships in *A. monoceros* from four collection locations, original and cross-validated classifications are tabulated separately, based on body measurements dataset.

		Predicted group				Total
		Gujarat	Kerala	Odisha	Tamil Nadu	
Original (Numbers)	Gujarat	26	9	15	3	53
	Kerala	8	53	6	8	75
	Odisha	14	3	44	7	68
	Tamil Nadu	4	28	14	12	58
Original (Percentage)	Gujarat	49.06	16.98	28.30	5.66	100.00
	Kerala	10.67	70.67	8.00	10.67	100.00
	Odisha	20.59	4.41	64.71	10.29	100.00
	Tamil Nadu	6.90	48.28	24.14	20.69	100.00
Cross-validated (Numbers)	Gujarat	25	9	16	3	53
	Kerala	10	47	6	12	75
	Odisha	15	3	43	7	68
	Tamil Nadu	4	28	17	9	58
Cross-validated (Percentage)	Gujarat	47.17	16.98	30.19	5.66	100
	Kerala	13.33	62.67	8.00	16.00	100
	Odisha	22.06	4.41	63.24	10.29	100
	Tamil Nadu	6.90	48.28	29.31	15.52	100

that the wide and lengthened caudal peduncle regions provide better thrust for fishes in turbulent water and control their swimming behaviour. Since the Arabian sea has lower water turbulence than the Bay of Bengal (Kolla, Henderson, & Biscaye, 1976; Chamarthi, Ram, & Josyula, 2008), this may explain the caudal peduncle's phenotypic variance observed in the present study. Increased turbulence resists and exerts more drag or frictional force against the fish when swimming, resulting in a slender body shape, especially in the caudal region.

Since the truss cover the body shape of the fish, the PCA result from truss also shows variation in mid-body region in addition to its head and caudal region. Sajina et al. (2011); Sen et al. (2011) found that the calm ambient conditions of the water support a wider fish body shape. Moreover, the variations in body regions may result from the synergistic effect of significant morphometric variations in both the head and caudal peduncle region, as both are vital for foraging the prey, directly affecting the fish's growth and body mass development.

The LDA biplot of the truss data shows a very slight divergence of Tamil Nadu stock from the mixing of other stocks. However, the ordination of Tamil Nadu fish truss data points over the LDA biplot in this form is not enough to substantiate stock discretion. Also, with traditional morphometric data LDA biplot shows a complete mixing among the stocks with no discrimination, which also supports the LDA findings of truss data. Similar findings were also observed in *Chanos chanos* (Hari et al., 2019) and *Lates calcarifer* (Gopikrishna, Sarada, & Sathianandan, 2006), attributed to the availability of coral reef ecosystems along the Tamil Nadu coast (Sajina et al., 2011). The higher productivity in the coral reef areas, the availability of Gulfs and calm water resulted in the morphological differences of Tamil Nadu fish compared with the other regions, as those ambient conditions are superior for fish habitat. Several studies have also noted considerable ecological variations between the northern and southern regions of the Arabian sea (Sreekanth et al., 2015), and the unique ecological conditions of Tamil Nadu were highlighted by many, as the Gulf of

Table 12. Meristic counts and the results of Kruskal-Wallis test.

Sl No.	Meristic count	Gujarat	Kerala	Odisha	Tamil Nadu	Kruskal-Wallis chi-squared	p-value
1.	Dorsal Spine	2	2	2	2	-	-
2.	Dorsal Fin Rays	43-50	44-54	44-50	43-50	0.5027	0.9183
3.	Pectoral Fin Rays	14	14	14	14	-	-
4.	Caudal Fin Rays	10	10	10	10	-	-
5.	Anal Fin Rays	42-52	46-55	45-52	42-52	15.641	0.0013*

*mark indicates the significance ($p < 0.05$).

Mannar, located between the Arabian sea and the Palk Bay of Tamil Nadu coast is an intermediate zone with a distinct mixture of oceanic and coastal conditions. Rao, Rao, Iyer, and Chittibabu (2008) also reported the ecology differences between Mandapam (Tamil Nadu) and Digha (West Bengal) in their study. Therefore, the reason for displaying a slight variation in the stock of Tamil Nadu in the present study may be attributed to its unique ecological and geographical location.

The LDA produced a confusion matrix from the traditional morphometric and truss image morphometric both showing intermixing within Tamil Nadu and Kerala stocks. The confusion matrix revealed 53.15% and 76.68% of original observations were correctly classified, while 48.82% and 73.12% of points were correctly classified after cross-validation for traditional and truss data respectively. Even though the sample were collected during its peak spawning season from all coasts, which coincided with the occurrence of maximum phenotypic difference amongst the stocks, intermixing stock of *A. monoceros* was present along the Indian coast (Cadrin, 2000). This may be attributed its pelagic larval stage, like the coral reef fishes, the larvae tend to have well-developed sensory abilities which help them in locating reefs and actively look for suitable settlement sites to thrive (Fisher, 2005). Palumbi (1992) also stated that marine organisms often have large population sizes, significant dispersal capabilities in their pelagic larval stages, and wide-ranging biogeographical distribution. The lack of population subdivision observed in Unicorn-Leatherjacket fish samples may also be linked to the seasonal variations in water circulation influenced by monsoon currents in the Indian Ocean. In the northeast monsoon, the upper ocean flow moves westward from the vicinity of the Indonesian Archipelago to the Arabian sea. Conversely, during the southwest

monsoon, the flow direction reverses, extending eastward from Somalia into the Bay of Bengal (Schott & McCreary, 2001). These seasonally reversing monsoon currents may be leading to the mixing of unicorn-leatherjacket populations from various spawning grounds in Indian waters, as the reversing monsoons, current reversal and spawning season coincides (Hastenrath & Greischar, 1991).

It can also be stated that the intermixing of the stock may be relevant to its movement pattern as stated by Tehseen et al. (2020) where the fish's abundances shifted between Gujarat and Maharashtra coasts. Guallart and Vicent (2009) reported the first occurrence of *A. monoceros* in Mediterranean Sea and reported that out of 108 collected 65 are considered to have migrated from the Red sea through Suez Canal and some are of Atlantic origin. These findings suggest that a migration pattern of fish in search of ambient environment for its survival exists.

Aluterus monoceros is widely found along the tropical and sub-tropical region and its abundance in fishery was first observed during the year 2011 along the Indian coasts. The present study is the first attempt conducted on *A. monoceros* to understand the stock along the Indian coastal waters based on truss network analysis and morphometric analysis. The results shows that the species is found to have single stock in the Indian marine waters, and the significant difference in the ecological conditions along the Tamil Nadu coast resulted in slight variations in body shape, resulting in slight differences in population along south east coast in comparison to the stock of rest of the studied geographical locations. This study suggests the requirement of strategic assessment and management of unicorn-leather jacket stock to be same along the coastal zone of India for sustainably in the future.

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Ethical statement

No separate approval of ethical committee is required as the fish samples for the study were collected from fish landing centres where commercial catches are landed.

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