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Phenotypic variation using truss network system in the deep-sea shrimp *Heterocarpus chani* Li, 2006 (Caridea: Pandalidae) off Arabian Sea and Bay of Bengal

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This study assesses the morphological differentiation of deepsea caridean shrimp, *Heterocarpus chani* Li, 2006 using a truss network system, to investigate different phenotypic stocks. Total, 1879 specimens were collected from Kalamuku (KAL), Sakthikulangara (SAK), and Colachel (COL) on the southwest coast of Arabian Sea (AS) and from Nagapattinam (NAG) and Tuticorin (TUT) on the southeast coast (BOB: Bay of Bengal) during 2013-2015. Multivariate analysis, such as Principal Component Analysis (PCA), Discriminant Factor Analysis (DFA), and Hierarchical Cluster Analysis (HCA) were used as statistical tools for differentiating the populations between the locations. Sex ratio was found to be 1:1. The coefficient of variation (CV) analysis was conducted for males and females using 39 morphometric truss variables which observed > 25 % variation. PCA indicates initial three components aggregately explained > 47 % of the total morphometric variation following the size effect correction. DFA demonstrated through the original and cross-validation 76.75 %, 72.26 % and 78.64 %, 74.81 % for male and female respectively, indicating significant variation in the first two canonical variables. DFA confirmed the presence of three distinct populations along the southern coasts of India. HCA also grouped the population into three major clusters specifically based on the 4th abdominal pleuron characters. The group-I included populations from NAG, group-II consisted of the TUT and group-III with SAK, KAL, and COL populations. Morphologically, the initial four abdominal pleuron characters were proved to be differentiating the population. The present study indicates the base study on morphological stock identification of *H. chani* indicating significant phenotypic heterogeneity between the populations of AS and BOB.

[**Keywords**: Deepsea shrimp, *Heterocarpus chani*, Multivariate analysis, Truss network]

Introduction

The deep-sea shrimp fishery in India from the late 1990's to the recent years is executed using conventional shrimp trawlers[1]. The peak landings were reported during the initial period (1985–2006) and subsequently, the trend in catch rates of deepsea shrimps showed significant decline[2-9]. The deepsea shrimp genus *Heterocarpus* Milne-Edwards (1881)[10] belongs to the family Pandalidae Haworth (1825), which is mainly distributed in tropical regions inhabiting deeper waters between the depth range from 73 to 2834 m[11]. Under this genera, only a few species are known for its commercial importance in world fisheries[12]. Among them, the species *Heterocarpus chani* Li, 2006[(ref. 13)] from Indian water forms the major commercial fishery along the southern region of India. In earlier records from Indian waters, *H. chani* was mistakenly reported as *H. gibbosus*[14]. Moreover, *H. gibbosus* species from India was included as species of interest in fisheries along the south west coast by Food and Agricultural Organization (FAO) catalogue[12]. However, taxonomically it was concluded that the previous records of *H. gibbosus* from Indian waters originally correspond to *H. chani*[15]. A few reports are present on the gut contents and reproductive biology of *H. gibbosus* (*H. chani*)[16]. The reproductive perspectives of sex proportion, egg counts and size of the egg in deepwater shrimps from Indian coast contributed to the deepsea resources[17-20]. However, there is no literature available on the stock characterization based on morphological shape of this highly commercially important species of *H. chani* from the Indian waters.

The morphometric variation among populations is appropriate for fisheries management and serves as a

basis for research in stock structure characterization. It is also appropriate to study the impact of environment in different populations[21-23]. There are multiple tools used for the aim of stock identification, such as molecular genetics, meristics and morphometrics and otolith chemistry. Comparatively morphometric analysis was found as one of the suitable cost-effective techniques. Fish stock identification based on size and shape, are considered to be an efficient morphometric methods that are commonly used in fisheries[24]. However, conventional methods has been improved by advanced image processing techniques and being finest appliance for stock identification[25,26]. Multivariate statistical methods (e.g., principal components analysis, canonical variant analysis, discriminant function analysis, or multivariate analysis of variance) have been effectively utilized in population, stock discrimination, and biodiversity studies[27]. Multivariate morphometric studies employing truss network system were considered to give meaningful results in the population studies of various species of marine origin[28]. The current research was undertaken to obtain the stock structure of the deepsea caridean shrimp, *H. chani*, using a multivariate truss network system along the southern coast of India.

Materials and Methods

Sampling and morphometric assessment

The samples of *H. chani* were collected from deepsea trawl shrimp catches obtained from five major fishing harbours along the southern coast of India. The sampling sites are Kalamuku (KAL), Sakthikulangara (SAK), Colachel (COL) on the southwest coast and Tuticorin (TUT), and Nagapattinam (NAG) on the southeast coast (Fig. 1). Information on study sites, geographical coordinates, shrimp sex and the sample size from each location are represented in Table 1. The samples were acquired in peak breeding season to

Table 1 — Details of coast, sampling locality, geographical coordinates, sex and number of specimens collected

Coast	Locality	Latitude, Longitude	Sex	Sample size (n)
South west	Kalamuku (KAL)	9°59′01"N, 76°14'32"E	Male	216
			Female	207
	Sakthikulangara (SAK)	8°56′00"N, 76°32'33"E	Male	206
			Female	197
	Colachel (COL)	8°10′23"N, 77°15'03"E	Male	155
			Female	156
South east	Tuticorin (TUT)	8°47′40"N, 78°09'37"E	Male	155
			Female	180
	Nagapattinam (NAG)	10°45′38"N, 79°50'58"E	Male	252
			Female	155

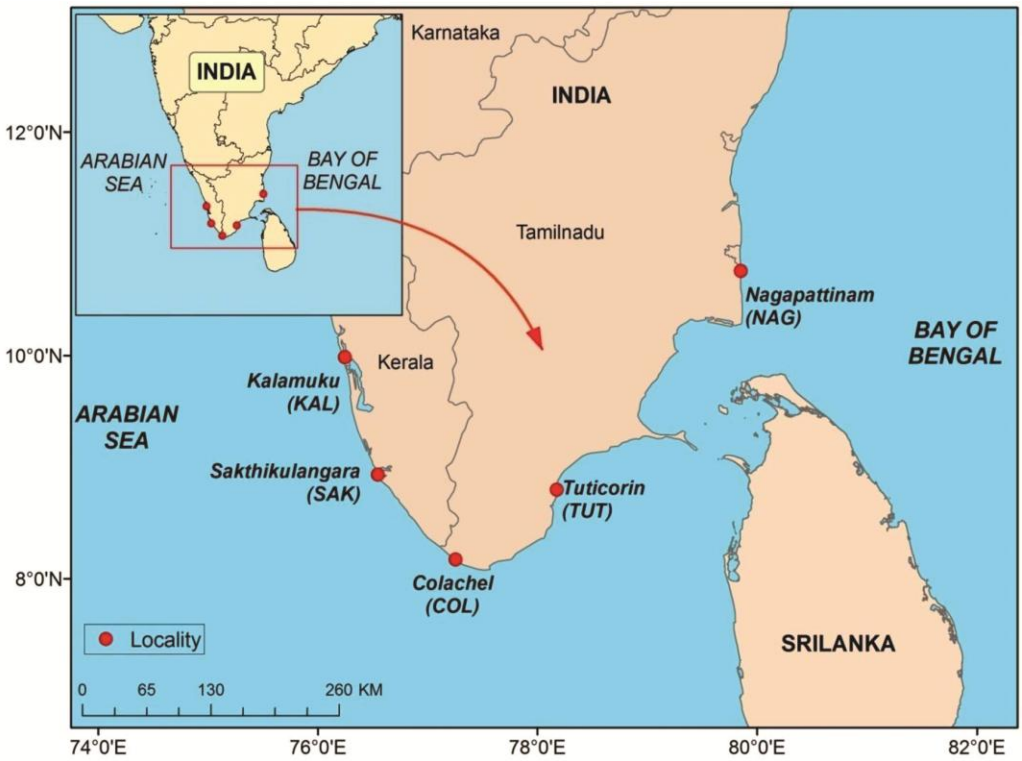

Fig. 1 — Sampling locations used for the collection of *H. chani* specimens

ascertain that they constitute to their parent population. The specimens from the southwest coast were obtained during November 2013 to December 2015 and from the southeast coast were obtained during January 2014 to January 2015. The collected shrimp samples were preserved on site in deep-frozen condition and brought to the laboratory. Segregation of specimens based on sex was done on the basis of presence (male) or absence (female) of appendix masculina on the second pleopods, while in females ovigerous and non ovigerous were identified by the presence of eggs on pleopods. The carapace length (CL) was measured from orbital notch to the posterior margin of carapace along the mid-dorsal line. Furthermore, the juveniles and the ovigerous females (limited number) were excluded from the morphological analysis and only the matured specimens (carapace length: male: > 14 cm; female: > 11 cm) were used in the present investigation.

Digitization of specimens and morphometric measurements

A total of 1879 specimens of *H. chani* including 984 male and 895 female individuals were used in this study. Before digitization of the specimens, the frozen, preserved samples were kept for thawing under running tap water and later wiped with absorbent paper. The specimens were kept on graph paper over a thermocol sheet on a flat surface. The distances between the vertical and horizontal grids of graph paper were used in calibrating the coordinate's covering an area of 1 cm^2. Digital pictures of each specimen was captured using a camera (Canon G-15) fixed on a tripod stand over the specimen and the lens was altered with the margins of the graph paper and each specimen was given a unique code for easier recognition (Fig. 2a). The morphometric variables for truss network analysis were measured from the digital pictures of specimens using digitization softwares tpsUtil and tpsDig2 V2.1[29] and the data was extracted by using Paleontological Statistics (PAST) programming tool[30]. Every single image was procured with the ruler to obtain uniform standard measurements which were further scaled in tpsdig utilising the millimetre grid on graph paper. Truss network was constructed by interconnecting 18 landmarks retrieving 39 truss morphometric variables representing the entire specimen of deepsea caridean shrimp *H. chani*.

Statistical analysis

MANCOVA was performed in order to study the statistically significant differences between the sexes

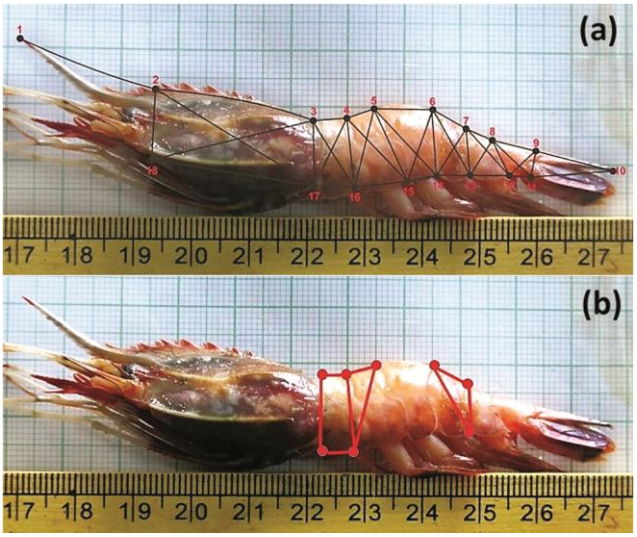

Fig. 2 — a) *H. chani* placed on the graph paper showing 18 landmarks and 39 truss distances; and b) Moprhometric truss variables with meaningful variations on first two canonical discrimination factors of truss network analysis of *H. chani* for both male and female

and geographical locality of the samples with log-transformed data and carapace length (CL) was included into the models as a covariate. Data sets (39 morphometric variables) were standardized by log transformation and tested for normality by SAS PROC UNIVARIATE procedure for removing outliers[31]. An allometric method was adopted to remove size-dependent variation in morphometric characters[32].

$$M_{trans} = logM - \beta\ (log\ CL - log\ CL\ mean)$$

Where, M_{trans} - final standardized measurement, logM - log-transformed of the original carapace length, CL - standard length of each specimen, CL mean - arithmetic mean of the carapace length and β - slope regressions of the logM against logCL. The mean ($\bar{x}$), and standard deviation (SD) was calculated for all the morphometric truss variables of each sample. The coefficient of variation (CV) percentage was processed as CV (%) = $100 \times SD/\bar{x}$ of morphometric truss variables for each population.

Multivariate statistical analysis, such as principal component analysis (PCA), discriminant factor analysis (DFA) and hierarchical cluster analysis (HCA) were used for analysing the differences among the populations at various locations. PCA was performed for data reduction to assess morphometric variation among samples and recognize factors contributing considerably to that variation[33]. DFA is

commonly used to discriminate the effects of variables into known groups[34]. The percentage of similarity among the populations and its morphometric characters were taken into consideration for identification of different groups by DFA which is further validated by original and cross-validation test. Populations are classified into different groups in proportion to the level of correctly classified and misclassified samples. DFA provides the Mahalanobis distance between centroids and its probability was assessed. A scatter plot based on canonical scores was used for visual observation of groups and by cross-validation analysis which correctly classifies the samples into the original groups.

Hierarchical cluster analysis (HCA) in view of Mahalanobis distance separation matrices determined with DFA, was utilized to observe population relationships[35]. The entire analysis was accomplished by using statistical software (SAS 2014).

Results

The analysis of the MANCOVA using mean carapace length demonstrated no significant variation among males and females while significant variation in sex was noticed among locations (Table 2). Correlation coefficients among the morphometric truss variables was determined prior to and following the size effect removal (Figs. S1a, b). The results revealed highly significant coefficient values prior to the size effect removal compared to the values obtained after the size correction, which suggests the importance of using the log-transformation and standardization methods in dataset correction.

The coefficient of variation (CV) analysis was conducted for males and females using 39 morphometric truss variables. In males, the CV showed minimum values for the morphological landmark of 1-18 (7.35 %, 9.9 %, and 6.67 %) and maximum of 6-7 (24.16 %, 26.11 %, and 18.92 %) among the populations of KAL, SAK, and COL, respectively. While in the population of the TUT and NAG minimum values were noticed in the landmark 1-2 (7.75 %), 2-17 (7.77 %) and maximum in 11-12 (19.71 %) and 16-17 (18.83 %), respectively.

Table 2 — MANCOVA of sex and sampling locations of *H. chani based on 39 morphometric truss variables*

	Wilk's Lambda	F value	Hypothesis df	Sig
Sex	0.460	53.448[b]	39	0.00
Location	0.226	20.55	156	0.00
Sex * Location	0.464	9.66	156	0.00

Similarly, in females, the populations of KAL, SAK, COL, and TUT showed minimum values for the landmark of 1-18 (8.4 %, 8.13 %, 8.74 %, and 8.54 %) and maximum values in 6-7 (27.18 %, 30.31 %, 23.04 % and 19.74 %), respectively. However, in NAG minimum values were noticed in 1-18 (11.88 %) and maximum in 4-5 (24.45 %; see Table S1).

The interpretation of PCA showed that the first three components cumulatively explained > 47 % (male: 47.93 %; female: 47.25 %) of the total morphometric variation after the size effect correction Truss variables loaded heavily on PC1 (2-18, 3-17, 4-5, 4-16, 5-16, 6-14, 6-15, 7-13, and 8-13 for male and 2-18, 3-17, 4-16, 5-16, 6-14, 6-15, and 9-11 for female) which alone explained > 29 % of the entire variance. PC2 explained > 10 % variation in both the sexes and the variables loaded heavily on it are 6-7, 6-13, and 16-17; while PC3 explained > 7 % of the total variation (Tables S2a, b). The PCA with high loading landmarks represented the initial four abdominal parts of *H. chani* (Fig. 2b). PC1 & PC2, scatter plot revealed the clustering of specimens collected from KAL, COL and SAK clustered in one group and the samples collected from NAG, TUT forms a separate cluster with males whereas in case of females some of the specimens collected from the centres SAK, KAL gave scattered cluster with the specimens collected from NAG and TUT (Figs. 3a, b).

The high loadings from PCA (2-18, 4-5, 4-16, 5-16, 6-13, 6-14, 6-15, 7-13, 8-13, 3-4, 16-17, 3-17 and 4-17) were subjected to Wilks' lambda tests which indicated significant differences among all the populations in both the sexes ($P < 0.001$; Table 3). DFA analysis which resulted through the original and cross-validation 76.75 %, 72.26 % and 78.64 %, 74.81 % for male and female respectively, indicating the highest level of deviation in first two canonical variables. The categorization of female and male individuals on the canonical factor I (male: 2-18, 4-5, 4-16, 5-16, 6-13, 6-14, 6-15, 7-13, and 8-13; female: 2-18, 4-16, 5-16, 6-14, and 6-15) and II (male: 3-4 and 16-17; female: 6-7, 14-15 and 16-17) showed good separation (Table 4) in the NAG and TUT population from the southwest coast population (Figs. 4a, b). Overall original and cross-validation analysis showed 76.75 % and 72.26 % of male and 78.64 % and 74.81 % of female individuals which were precisely categorized in their respective groups

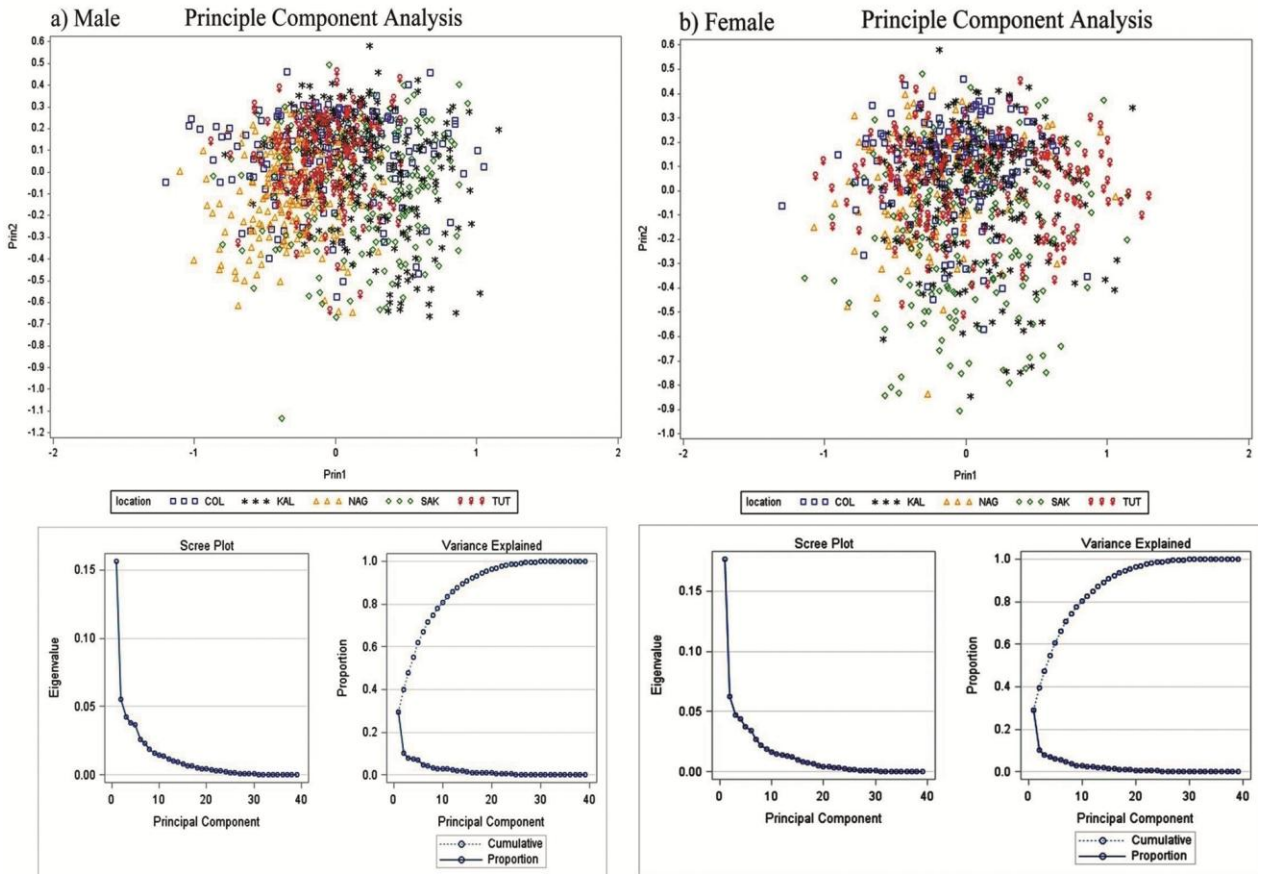

Fig. 3 — Principal components analysis: a) male; and b) female. Scatter plot with high loadings observed in PC 1 and PC2 component with the scree plot of the 39 morphometric truss variables

Table 3 — Contribution of morphometric measurements to canonical discriminant functions of *H. chani* collected from five populations of the southern coast of India

Truss measurements	Canonical discriminant functions	
Male	Can 1 (68.6%)	Can 2 (15.3%)
2-18	0.303521	-0.1373
3-4	-0.225001	0.28909
3-17	0.1521	-0.0517
4-5	0.741837	-0.0801
4-16	0.392936	-0.2684
4-17	-0.025834	0.1996
5-16	0.555342	-0.1917
6-7	0.058789	0.06484
6-13	0.30997	-0.0439
6-14	0.439913	-0.2161
6-15	0.459806	-0.2418
7-13	0.420934	-0.1083
8-13	0.437914	-0.0623
16-17	-0.143756	0.30929
Female	Can 1 (43.6%)	Can 2 (25.7%)
2-18	0.32663	-0.1683
3-4	-0.216	-0.0033
3-17	0.14036	-0.1572
4-16	0.33983	-0.1408
5-16	0.43503	-0.2047
6-7	-0.1107	0.24066
6-13	0.07143	0.16015
6-14	0.27978	-0.0154
6-15	0.29509	0.12167
9-11	0.1133	0.06516
14-15	0.13764	0.34513
16-17	-0.0873	0.52518

(*Contd*.)

(Table 5). The highest proportion of grouping was recorded in NAG populations (male: 93.25 %, female: 81.29 %) and higher misclassifications were noted in males of KAL (10.32 %) followed by TUT (5.5 %). Cross-validation results conclude the clear differentiation among populations in the selected study sites.

The results of HCA showed three clear clusters from five populations of both sexes (Fig. 5). The group-I included populations from NAG, group-II consisted of the TUT and group-III with SAK, KAL,

Table 4 — Wilk's lambda test for verifying stocks of *H. chani* of discriminant function analysis indicated significant ($P < 0.001$)

Male				Female			
Variables	Wilks' Lambda	F value	Probability	Variables	Wilks' Lambda	F value	Probability
2-18	0.84	46.1	0.00	2-18	0.90	22.3	0.00
3-4	0.91	22.9	0.00	3-4	0.94	12.7	0.00
3-17	0.93	18.6	0.00	3-17	0.94	12.1	0.00
4-5	0.54	204.1	0.00	4-16	0.91	19.6	0.00
4-16	0.83	50.8	0.00	5-16	0.84	39.6	0.00
4-17	0.94	13.9	0.00	6-7	0.95	11.6	0.00
5-16	0.69	105.5	0.00	6-13	0.96	7.4	0.00
6-7	0.97	7.3	0.00	6-14	0.93	16.4	0.00
6-13	0.90	24.8	0.00	6-15	0.90	22.8	0.00
6-14	0.81	57.2	0.00	9-11	0.98	3.8	0.04
6-15	0.79	64.0	0.00	14-15	0.90	22.2	0.00
7-13	0.82	51.3	0.00	16-17	0.86	34.9	0.00
8-13	0.81	55.0	0.00				
16-17	0.89	28.9	0.00				

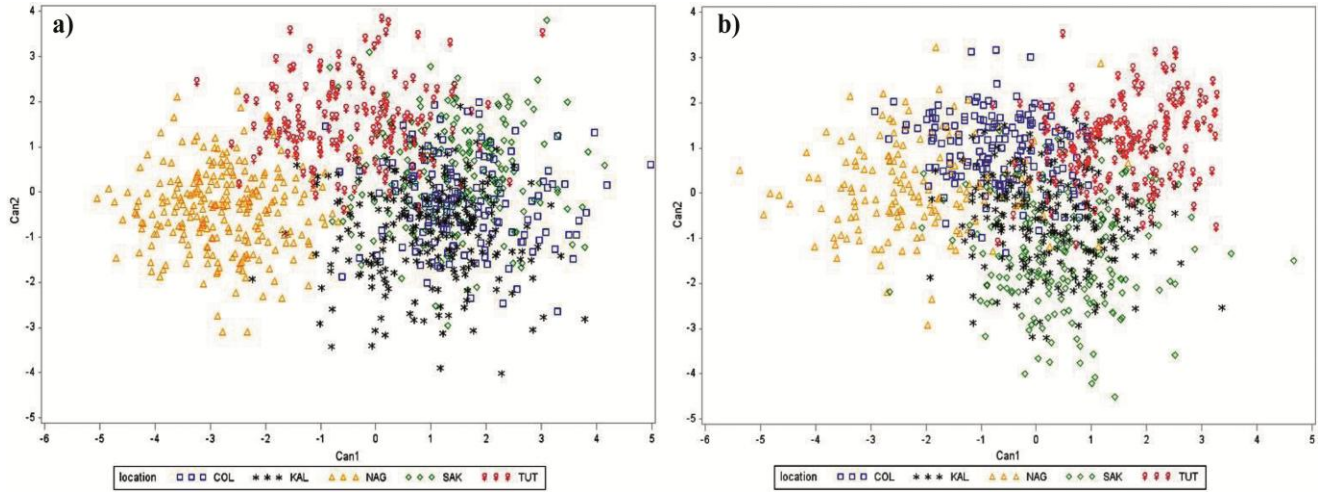

Fig. 4 — Canonical discriminant function analysis: a) male; and b) female. Scatter plot of high loadings from PCA of morphometric truss variables used for *H. chani*

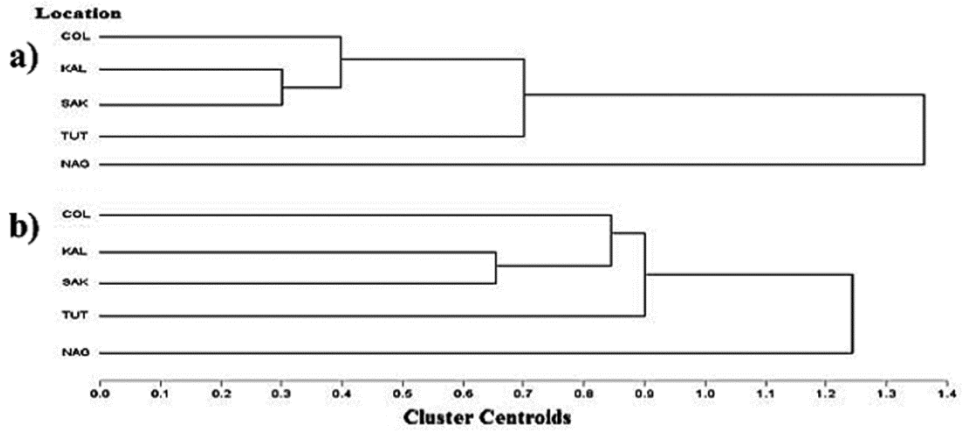

Fig. 5 — Dendrogram of *H. chani* populations based on morphometric truss variables (a: male; and b: female)

Table 5 — Classification of individuals into original and cross validated groups using classification matrix of the DFA based on morphometric measurements

Sex		Populations		KAL	COL	NAG	SAK	TUT	Total
M	Original	KAL	Count	154	16	2	30	14	216
			%	71.3	7.41	0.93	13.89	6.48	100
		COL	Count	11	127	0	13	4	155
			%	7.1	81.94	0	8.39	2.58	100
		NAG	Count	5	0	235	1	11	252
			%	1.98	0	93.25	0.4	4.37	100
		SAK	Count	41	30	0	118	17	206
			%	19.9	14.56	0	57.28	8.25	100
		TUT	Count	5	6	11	9	124	155
			%	3.23	3.87	7.1	5.81	80	100
	Cross validation	KAL	Count	140	24	4	32	16	216
			%	64.81	11.11	1.85	14.81	7.41	100
		COL	Count	14	114	1	19	7	155
			%	9.03	73.55	0.65	12.26	4.52	100
		NAG	Count	5	0	231	1	15	252
			%	1.98	0	91.67	0.4	5.95	100
		SAK	Count	42	34	0	111	19	206
			%	20.39	16.5	0	53.88	9.22	100
		TUT	Count	5	6	13	11	120	155
			%	3.23	3.87	8.39	7.1	77.42	100
F	Original	KAL	Count	156	15	8	16	12	207
			%	75.36	7.25	3.86	7.73	5.8	100
		COL	Count	10	133	6	5	2	156
			%	6.41	85.26	3.85	3.21	1.28	100
		NAG	Count	16	7	126	4	2	155
			%	10.32	4.52	81.29	2.58	1.29	100
		SAK	Count	17	20	5	135	20	197
			%	8.63	10.15	2.54	68.53	10.15	100
		TUT	Count	13	13	1	4	149	180
			%	7.22	7.22	0.56	2.22	82.78	100
	Cross validation	KAL	Count	144	15	9	24	15	207
			%	69.57	7.25	4.35	11.59	7.25	100
		COL	Count	12	123	10	8	3	156
			%	7.69	78.85	6.41	5.13	1.92	100
		NAG	Count	19	8	121	5	2	155
			%	12.26	5.16	78.06	3.23	1.29	100
		SAK	Count	20	20	6	131	20	197
			%	10.15	10.15	3.05	66.5	10.15	100
		TUT	Count	15	13	1	5	146	180
			%	8.33	7.22	0.56	2.78	81.11	100

and COL populations. The interpretation of results indicated that the samples obtained from the locations NAG and TUT represented a phenotypically distinct population while the morphometric resemblance between SAK, KAL, and COL stocks were observed to be high.

Discussion

Based on the multivariate statistical analysis, DFA confirms the presence of three distinct populations along the southern coasts of India. Morphologically, the initial four abdominal pleuron characters were proved to be differentiating characters for the populations. The separation might be due to physical and biological factors such as geographic variation, ecological factors, temperature, salinity, essential food availability, and fishing intensity[36]. The results of descriptive statistics inferred that females were larger (CL) than the males among all the populations. Ovigerous females were not included in the present investigation due to their smaller sample size, which

may cease to capture the covariance and morphological variation leading to erroneous results on population differences[37]. The higher values of CV (> 25) indicated differences in the populations pointing towards low inheritability and influence of environmental factors in causing morphological dissimilarity resulting in higher variations in the intraspecific populations from all the locations. The heavily loaded PCA variables represented the initial four abdominal lengths, height and rostral variation in both the sexes and in particular 6-7, 3-4 in males and 6-7, 14-15 in females showed high variation compared with other morphometric characters. This high variance describes the differences existing between the individuals[38]. The stocks differentiated based on the reproductive potential of fishes may be assigned to divergent spawning areas[39] or to the hydrographical conditions further preventing or reducing migration of the species[40]. However, in-depth study is required to conclude whether it is due to the interaction between the genotype and the environment or purely environmental factors. DFA, overall random assignment of individuals in their original and cross-validation group was 76.75 %, 72.26 % (males) and 78.64 %, 74.81 % (females), indicating high differentiation between populations further suggesting that the males were better discriminated by the canonical functions compared to females. The current pattern of BOB and AS was also found to modify the morphometrics of *Megalaspis cordyla* fish in India[41]. The uncommon hydrological conditions and geographic barrier also play an important role between populations responsible in differentiation among the individuals[41-44]. HCA was utilized to assess the genetic variation and categorize accessions into their relative groups. The current study demonstrated the intimate relationship among SAK, KAL, COL and NAG, TUT populations, revealing three distinct groups of populations in *H. chani*. The observed morphological variations in this species will aid in future research on fisheries management, reasonable improvement and separate management strategies for resource sustainability. Future examinations utilizing genetic markers and biochemical techniques are suggested to validate the discoveries from this investigation.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at http://nopr.niscair.res.in/jinfo/ijms/IJMS_49(12)1839-1847_SupplData.pdf

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Conflict of Interest

The authors are declaring that they have no conflict of interest.

Author Contributions

GK: Methodology, software, collection of sample, and writing - original draft; RDC: Concept development, project acquisition, planning, draft editing, investigation, and supervision; PP: Collection of sample. GK & RDC have equally contributed.

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