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Morphometric Variation and Allozyme Electrophoretic Studies in Hind Grouper Species of Genus *Cephalopholis* (Epinephelidae) off Visakhapatnam, Central Eastern Coast of India

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Abstract: ***Most studies of groupers have focussed on species of commercially important genera. For smaller Epinephelines such as species of genus Cephalopholis studies are very little despite having commercial value as food in some regions and increasing inclusion in the aquarium trade. The present paper deals with morphometric and meristic characters used to identify six species of hind groupers of genus Cephalopholis that are represented in the coastal waters of Visakhapatnam . Multivariate analysis (Principal Component Analysis) has been carried out to study existing variation in biometric characters of two closely related species – C. nigripinnis (Valenciennes, 1828) and C . sonnerati (Valenciennes, 1828). Cephalopholis microprion (Bleeker, 1852) is new record from coastal waters of mainland India. The genetic variability in four species – C. formosa, C. miniata, C. nigripinnis and C. sonnerati was evidenced by allozyme electrophoretic studies carried out on eleven enzyme systems that revealed seventeen loci out of which ten are polymorphic. Species are easily distinguished and also diagnostic locus and alleles were identified that can aid in species identification. This data along with morphometric and meristic data has varied application in research on evolution, conservation and management of natural resources and genetic improvement programmes.***

Keywords: six hind grouper species, one new record, biometric characters, genetic variation

1. Introduction

Though the taxonomic ambiguities are clarified [1]-[4], still lot of confusion exist in the identification of the Epinepheline species in different regions of the world. More recently, these authors have revised the classification of groupers, particularly in relation to the number of genera within the Epinephelinae subfamily. Groupers range in size from around 20 cm (eg *Cephalopholis leopardus*) to more than 250 cm (eg *Epinephelus lanceolatus*). Most studies of groupers have focussed on species of commercially important genera *Epinephelus*, *Mycteroperca* and *Plectropomus*. For smaller Epinephelines such as species of genus *Cephalopholis* studies are very little despite having commercial value as food in some regions and increasing inclusion in the aquarium trade. Most species of *Cephalopholis* are secretive groupers seen hiding in or near coral reefs. Although some species like *C. boenak, C. formosa* and *C. microprion* are often seen in silty areas, most species prefer clear water environments, from tide pools out to depths of 200m [1].

Several authors reported new species of genus *Cephalopholis* from different regions of world waters; new record of species *C. xanthopterus, C. taeniops* (Valenciennes) [5], [6], a small grouper of the genus *Cephalopholis* caught from New Caledonia and identified it as a hybrid of species *aurantia* and *spiloparaea* [7]. Key to species of Red Sea groupers that include five species of genus *Cephalopholis* is given [8]. Some authors provided useful information on ecology, distribution and habitat use of *Cephalopholis* species in view of their ecological importance [9]-[11], RAPD PCR analysis was carried out to differentiate four species of this genus [12].

The fundamental information as is available on the systematics of grouper fishes from Visakhapatnam has been published previously, four *species* of genus *Cephalopholis* – *pachycentron* (Valenciennes, 1828) (= *boenak), sonnerati* (Valenciennes, 1828), *urodeta* (Schneider, 1801) *(= nigripinnis)* and *formosa* (Shaw and Nodder, 1801) were recorded and a key to species of this region is given [13], [14].

Unusually heavy landings of about 7.8 t and 3.2 t of *C. sonnerati* during the years 2008 and 2009 was reported at Chennai [15]. A new distribution record of *C. aurantia* Valenciennes, 1828 from Andaman and Nicobar Islands was given[16]. RAPD markers used to resolve taxonomic ambiguity in groupers that include seven species of genus *Epinephelus* and single species *formosa* of genus *Cephalopholis* [17]. Lot of confusion prevails in identification of *nigripinnis, urodeta* that closely resemble each other. They are often misidentified. Even DNA barcode generated for these two species showed 99% match there by proposed that these two may be synonymised [18].

Hind grouper of genus *Cephalopholis* (Bloch and Schneider,1801) is clearly separated from other genera of this family by the following combination of characters. Preopercle rounded with serrated edge without enlarged serrae at the corner. Opercular flap rounded present behind the middle spine. Snout distinctly longer than eye diameter. Opercular flap broad, obtuse with or without notch, preopercle angle

rounded. Proximal part of fins covered with scales. Brilliant coloured fishes.

Dorsal with nine spines; third dorsal spine longest. Soft dorsal and anal rounded posteriorly. Second anal spine stoutest. Pectoral, pelvic and caudal fins also rounded. Lateral line runs parallel to dorsal profile. Head covered with cycloid scales; body mostly covered with ctenoid scale; vertebrae 24 (10 precaudal + 14 postcaudal); pyloric caeca 7-12.

Of the 23 species of genus *Cephalopholis*, six species *boenak* (Bloch, 1790), *formosa* (Shaw, 1812), *microprion* (Bleeker, 1852), *miniata* (Forsskal, 1775), *nigripinnis* (Valenciennes in Cuv. and Val. 1828) and *sonnerati* (Valenciennes, 1828), represented in the catches of Visakhapatnam (lat 17˚44'N; long 83˚23'E). Among these six species the current distributional records [4], [19] are accompanied by a map showing distribution of only four species – *boenak, formosa, miniata* and *sonnerati* in Indian waters.

The species *nigripinnis* is included in IUCN Red List as Data Deficient (DD) while the other five species are included in IUCN Red List as Least Concerned (LC).

In the present study meristic and morphometric characters and colour pattern have been utilized to identify six species of hind groupers represented in the catches of Visakhapatnam. A proper knowledge of the genetic make-up and variability of the fish stocks will help in management and conservation of species. The biometric characters of closely related species *C. nigripinnis* and *C. sonnerati* analyzed were subjected to multivariate analyses (Principal Component Analysis) that have shown influential characters that aid in distinguishing these two species. Allozyme analysis have proved useful in studies of inter and intra-species relationships and genetic structures of natural populations of marine fish. Such methods are valuable in providing additional data which may be used to test hypotheses based on morphological criteria [20]. Hence in the present study, morphometric and meristic data was utilized to establish the normal range of variation among species. Thus variation in biometric characters among six species of genus *Cephalopholis* forms the basis of this study along with genetic variation existing among the four congeners based on allozyme electrophoretic studies.

2. Material and Methods

Biweekly random samples were collected from the Bheemunipatnam, Lawson's Bay, Pudimadaka landing centers, local markets and Visakhapatnam fishing harbour (Fig.1) during the period February 2008 to August 2011.

2.1. Methodology for taxonomy of *Cephalopholis* species

The colour of the specimens was noted in fresh condition and morphometric and meristic data were taken for fresh specimens. Methodology for taking morphometric and meristic data follows standard procedures [1]. In the present study species were identified based on the description of type species, previous literature and published keys to species of the family Serranidae.

Data on other characters like pyloric caeca are also taken to distinguish hind grouper species off Visakhapatnam. In the course of examination of the specimens of each species for the present taxonomic study, the sex of each specimen was noted and the contents of the stomach were examined, albeit in a general way. Morphometric and meristic data of specimens of all size represented in the catches is taken to study any existing variation in biometric characters.

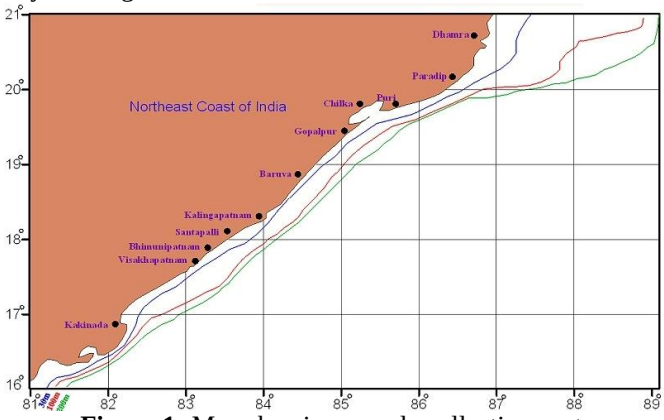

Figure 1: Map showing sample collection centers

To deal with more than two variables, multivariate data analysis like Principal Component Analysis (PCA) has been employed to closely related species [21]. In the present study PCA was carried out for two closely related species of genus *Cephalopholis – nigripinnis* and *sonnerati.* Before computation information from different characters were pooled into a comparable scale following standard procedure [22]. Due to size variation of fish, morphometric data was statistically adjusted to permit comparative analysis in terms of shape independent of size. Multivariate analysis was carried out using SPSS version 13.0 software. Tukey test was carried out for only those characters that became significantly different for the two species were considered.

2.2. Methodology for Allozyme electrophoretic studies in *Cephalopholis* species

Fresh specimens of all size groups belonging to four species genus *Cephalopholis: C. formosa*, *C. miniata*, *C. nigripinnis* and *C. sonnerati* were collected. After identification of species total length, weight and sex of each specimen is noted and muscle tissue was immediately brought to the laboratory in an insulated ice box with crushed ice in it. Methodology for allozyme extraction, casting of gels follows standard protocols [23], [24]. For separation of allozymes at different enzyme loci vertical native PAGE gels were used, electrophoresis was done at a constant voltage of 100 volts at 4°C. A total of eleven enzyme systems were examined. Visualization of different alleles of enzymes such as Acid phosphatase (ACP, E.C.no. 3.1.3.2), Aspartate aminotransferase (AAT, E.C.no.2.6.1.1), Alcohol dehydrogenase (ADH, E.C.no. 1.1.1.1), Esterase (EST, E.C.no. 3.1.1.1), Glucose -6-Phosphate dehydrogenase, (G6PDH, E.C. no. 1.1.1.49), Glutamate dehydrogenase (GDH E.C.no. 1.4.1.3), Glutamine synthetase (GLNS E.C.no. 6.3.1.2), Lactate dehydrogenase (LDH, E.C.no. 1.1.1.27), Malate dehydrogenase (MDH, E.C.no. 1.1.1.37), Phosphogluco Mutase (PGM, 5.4.2.2) and Superoxide dismutase (SOD, 1.15.1.1) was done by histochemical staining as per standard procedures [25]. Nomenclature of loci and alleles was followed as recommended

[26]. At all the loci, most common allele was designated as 100. Alternate alleles designated as per their mobility in relation to the most common allele. Calculation of allele frequencies and tests for conformity to Hardy – Weinberg expectations (probability test) were undertaken using GENEPOP version 3.4 software[27]. Cluster analysis was performed and dendrogram plotted based on pairwise genetic distance estimated using the Unweighted Pair Group Method with arithmetic mean (UPGMA) [28] modified from NEIGHBOUR procedure of PHYLIP version 3.5 using POPGENE version 1.31 (Yeh et.al., 1999) [29].

3. Results and Discussion

Descriptions are provided based on the examination of 90 specimens belonging to six species of the genus *Cephalopholis* (Table 1) that are represented in the catches of Visakhapatnam. Morphometric measurements for three species *boenak, formosa* and *microprion* given in Table 2 and remaining three species *miniata, nigripinnis* and *sonnerati* given in Table 3. A comparison of meristic characters of six species given in Table 4. Major distinguishing characters, colour pattern, coloured photographs are given for these species. This is followed by results of multivariate analysis i.e. principal component analysis that yielded diagnostic characters to distinguish two closely related species *nigripinnis* and *sonnerati*. Results of allozyme electrophoretic studies carried out for four species *formosa, miniata, nigripinnis* and *sonnerati* also given below that include allele frequency, diagnostic alleles and parameters of genetic variation.

3.1. Description of species

3.1.1. *Cephalopholis boenak* (Bloch, 1790) (Fig. 2)

Bodianus boenak Bloch, 1790: 43, pl. 226 (type locality: "Japan" [probably Java, as indicated by the native name that Bloch used for this species]; Spelt "boenack" on the plate).

(a) **Distinguishing characters:** Maxillary reaches far behind the posterior orbit of eye, its distal width almost equal to eye diameter. Three rows of caniniform teeth present on upper jaw, those on outer jaw being larger; two rows of caniniform teeth present on inner jaw, those on inner row being larger. Two rows of band shaped teeth present on palatines. Inter and suboperculum smooth; preopercular edge serrated. Third dorsal spine is longest. Maxillary scaly at anterior half and naked posteriorly. Body covered with ctenoid scales except dorsal fin base upto fourth dorsal spine and ventrally up to pelvic fin base- where cycloid scales present.

(b) **Colour:** Head and body chocolate brown in colour. Eight darker horizontal bands present on body, six of them are from dorsal origin to its end, one at caudal peduncle and one at caudal base, three dark brown bands radiating from eye. A dark colour spot present in between first and second opercular spine. Eyes greenish yellow, pupil with orange rim. Spinous dorsal paler with black edges, soft dorsal, anal, pectoral, pelvic and caudal fins are dark brown in colour with white edges. Caudal black with white margin on upper and lower corner.

Figure 2: *Cephalopholis boenak* – 196 mm TL

3.1.2. *Cephalopholis formosa* (Shaw, 1812) (Fig. 3,3a)

Sciaena formosa Shaw and Nodder, 1812; *Natur. Misc.* 23, pl. 1007 (type locality: Vizagapatnam, Coromandel coast of India, after "Rahtee Bontoo" of Russell, 1803: 22, pl. 129)

a. **Distinguishing characters:** Maxilla reaches below the posterior border of eye, its distal width is equal to eye diameter. Two rows of caniniform teeth present on upper and lower jaws, outer row being larger in upper jaw, in lower jaw inner row is larger. Preopercle rounded with serrated edge.

Opercular flap rounded. Central gill raker longer than gill filament. Pectoral reaches above upto anus. Second ana

Table 1: List of hind grouper species of genus *Cephalopholis* represented in the catches of Visakhapatnam

Species	Common name	Local Telugu name	n	Length range (mm TL)	Froese and Pauly (2014)
Genus *Cephalopholis*, Bloch and Schneider, 1801					
C. boenak (Bloch, 1790)	Chocolate hind	Bontoo	1	196	300
C. formosa (Shaw, 1812)	Bluelined hind	Rahtee-bontoo	53	135-334	340
C. microprion (Bleeker, 1852)	Freckled hind		1	202	250
C. miniata (Forsskål, 1775)	Coral hind		4	200-245	500
C. nigripinnis (Valenciennes, 1828)	Black fin grouper		5	140-242	280
C. sonnerati (Valenciennes, 1828)	Tomato hind, tomato seabass, tomato rock-cod,	Yerra Bontoo	26	202-502	570

spine stoutest than first and third spine and slightly shorter than third spine. Maxillary scaly at anterior half with six rows of scales and naked posteriorly. Body covered with ctenoid scales except spinous dorsal and pelvic bases.

(a) **Colour:** Head and body yellowish brown in colour with wavy blue bands. On head there are eight blue bands originating from orbit and directing downward towards opercle end. 2-3 blue bands originating behind the inter orbital and extends upto spinous dorsal fin end. Blue colour spots present on snout, maxilla, lower jaw. Fragmented bluish grey

band present on lower half of maxilla. On body 12-13 wavy blue bands present. 7 blue bands directed upwards upto soft dorsal fin edge and remaining blue bands extends upto caudal fin. All fins dark bluish black, anal fin with four wavy bands, first band parallel to anal base, second and third present middle of fin and fourth band present below the margin of fin. Five blue lines present on pelvic ventral side originating from the base extend upto edge. In specimens measuring 135 mm TL a black spot present in between middle and lower opercle spine. In male specimen blue colour spots present anterior to pelvic fin base and lower jaw, while such spots are absent in juvenile and female specimens.

3.1.3.*Cephalopholis microprion* (Bleeker, 1852) (Fig. 4)
Serranus microprion Bleeker, 1852b, *Nat. Tijd. Ned. Indie.*, 3: 552 (type localities: Ambon and Batavia)

Figure 3: *Cephalopholis formosa* – 334 mm TL

Figure 3a. *Cephalopholis formosa* – 109 mm TL

(a) **Distinguishing characters:** Maxilla reaches beyond the posterior border of eye, its distal width is less than the eye diameter. Two rows of caniniform teeth present on upper and lower jaws; in upper jaw outer row being larger and in lower jaw inner row being larger. Preopercle with serrated edge; inter and subopercle smooth covered with skin. Operculum with three spines, middle one larger nearer to the lower one. Central gill raker longer than gill filament. Interspinous membrane of dorsal fin incised. Pectoral length more than the postorbital part of head. Second anal spine slightly stouter than first and third spine and almost equal in length to third spine. Maxilla scaly posteriorly. Body covered with ctenoid scales except below spinous dorsal base and ventrally upto anal fin base where cycloid scales present.

(b) **Colour:** Head and body dark brown with bluish orange spots on head, chin and operculum. Small blue coloured spots, appear scattered (few number) on body. Dorsal fin darker in colour with small blue spots, spots present at base of dorsal larger than on fins. Anal fins darker with small spots. Dorsal and anal edges black. Spots present at base of pectoral. Pectoral and pelvic fin dark brown with no spots. Caudal fin dark brown with small blue colour spots and edges white in colour. A dark colour big spot present in between first and second operculum spine.

Figure 4: *Cephalopholis microprion* – 202 mm TL

3.1.3. Cephalopholis miniata (Forsskål, 1775) (Fig. 5, 5a)

Perca miniata Forsskål, 1775, *Descript. Animal.* Xii : 41 (type localities: Jeddah and Hudaydah, Red sea)

(a) **Distinguishing characters:** Maxilla reaching below the posterior border of eye, its distal width equal to eye diameter. Maxillary scaly with 17-19 rows of cycloid scales. Pelvic shorter than pectoral and not reaching to anus. Body covered with ctenoid scales except ventrally up to anal fin origin where cycloid scales are present. Morphometrics and meristics of two species of genus *Cephalopholis: argus* (n= 242) and *miniata* (n= 254) was studied [30]. The range and mean values of most of the morphometric characters for *C. miniata* are in agreement with the present study except for few characters like eye diameter and snout length. However sample size in present study is less (n = 4).

(b) **Colour:** Head and body reddish brown with small blue spots. On head blue spots are closely set. On head the background colour between the blue spot present a reticulate appearance. Pectoral orange red distally, bluish spots at base, and edges black. Soft dorsal, anal and caudal with blackish sub marginal lines. Pelvic bright red in colour with dark blackish grey edge. Blue colour spots present on soft dorsal, anal and caudal fins. Caudal fin rare edge white. A dark spot present in between middle and lower opercle spine.

Figure 5: *Cephalopholis miniata* – 245 mm TL

Figure 5a: *Cephalopholis miniata* – 200 mm TL

3.1.4. *Cephalopholis nigripinnis* (Valenciennes in Cuv. and Val. 1828) (Fig 6, 6a)

Serranus nigripinnis Cuvier and Valenciennes, Hist. Nat. Poiss., ii, 1828, p.339. *Cephalopholis nigripinnis* Bleeker, Atlas Ichth. Ind. Neerl., vii, 1876, Pl. Cclxxxiv, fig. 2 (plate 18).

(a) **Distinguishing characters:** Maxillary reaching beyond the posterior border of eye. Three rows of canine teeth present on upper jaw, those on outer row being larger; two rows of canine teeth present on lower jaw, those on inner row being larger. Operculum with three spines covered with skin and cycloid scales; upper and middle spine slightly longer than lower spine. Operculum flap small, pointed present just behind the middle opercular spine. Central gill raker longer than gill filament. Maxillary scaly posteriorly with 8-10 rows of cycloid scales. Pectoral reaching upto a level of anal fin origin and longer than pelvic fin. Second anal spine stouter than first and

Table 2: Comparison of morphometric data of three species of genus *Cephalopholis* represented in the catches of Visakhapatnam.

Standard length in mm SL	*formosa*, n=53		*boenak*, n=1	*C. microprion*, n=1
	110-278		160	164
	Min-Max	$\overline{X}$ ±SD		
As percentage of standard length:				
Total length	111.34-131.00	121.73±2.80	122.5	123.17
Body depth	31.81-43.86	37.02±3.14	37.50	37.80
Head length	35.56-45.16	39.02±1.98	38.75	39.63
Predorsal distance	32.98-45.31	40.68±2.55	41.87	39.63
Prepectoral distance	31.00-43.38	37.06±2.58	38.12	39.63
Prepelvic distance	32.55 -44.61	41.38±6.58	39.37	40.58
Preanal distance	63.83-75.82	68.68±6.60	73.12	67.68
Dorsal base	46.51-59.13	53.97±3.18	53.75	53.04
Anal base	12.40-23.19	17.03±2.74	18.12	19.51
First dorsal spine height	-	-	-	10.36
Third dorsal spine height	8.83-18.04	11.72±2.03	13.75	-
Soft dorsal height	11.48-21.13	14.70±2.33	16.87	18.29
Pectoral length	18.11-27.27	23.54±1.77	26.87	25.60
Pelvic length	12.90-23.76	20.72±1.64	20.00	21.95
Anal spine height	10.04-25.58	15.72±4.74	13.75	11.58
Soft anal height	13.76-21.83	18.65±1.77	21.87	20.73
As percentage of head length				
Head depth	75.86 -89.74	85.19±3.93	66.12	70.76
Head width	38.09-56.41	47.05±5.52	46.77	40.00
Preorbital	22.85-35.80	29.73±3.01	27.86	27.69
Postorbital	50.00-67.14	58.18±4.00	59.67	56.92
Upper jaw	40.17-48.27	44.28±2.02	50.00	46.15
Lower jaw	26.43-38.09	32.64±3.73	33.87	41.53
Snout length	21.68-29.76	24.59±2.39	19.35	18.46
Eye diameter	11.96-19.23	16.12±1.76	17.74	16.92
Interorbital width	10.00-16.21	12.32±1.24	14.51	13.84
Maxillary width	11.11-17.58	15.29±1.56	16.12	13.84

third spine, almost upto a level of anal fin origin and longer than pelvic fin. Second anal spine stouter than first and third spine, almost equal to third anal spine length; anal fin longest ray longer than longest anal spine. Body covered with ctenoid scales except first dorsal base and ventrally up to pelvic fin base where cycloid scales present.

b. Colour: Body orange red in colour. Soft dorsal ray, pectoral, pelvic and anal fins black in colour. Caudal fin black in colour with white edges. In the specimens measuring 140 mm TL, a black spot present in between first and second opercular spine. Orange red colour spots present on head and pale colour spots present on body.

3.1.5. Cephalopholis sonnerati (Valenciennes, 1828) (Fig. 7, 7a)

Serranus sonnerati Valenciennes, 1828: *Hist. nat. poiss.*, 2: 299 (Type locality: Pondicherry; Ceylon)

Figure 6: *Cephalopholis nigripinnis* – 242 mm TL

Figure 6a: *Cephalopholis nigripinnis* – 200 mm TL

(a) **Distinguishing characters:** Maxilla reaches beyond the posterior border of eye, its distal width is slightly larger than eye diameter. Three rows of caniniform teeth present on upper and lower jaws, in upper jaw outer row are being larger and in lower jaw inner row being larger. Preopercle rounded with serrated edge, small notch on ventral side. Central gill raker slightly smaller than gill filament. Maxillary scaly posteriorly with 12-13 rows of cycloid scales. Interspinous membrane of dorsal fin incised. Pectoral fin symmetric, conical posteriorly, not reaching upto a level of pelvic fin end, its length equal to the post orbital distance of head. Pelvic reaches upto anus. Second anal spine stouter and shorter than third spine, soft anal fin. Body covered mostly with ctenoid scales except base of first dorsal and ventral side upto pelvic fin where the cycloid scales present. Previous study from Visakhapatnam [14] recorded the number of pectoral rays are 19 but in the present specimens it is 16-18.

(b) **Colour:** Head and body orange red in colour with purple colour net work (orange spots closely set and in between these spots, blue colour line forms which looks like blue colour network) on head, including maxilla, upper lips and lower lips.

Figure 7: *Cephalopholis sonnerati* – 342 mm TL

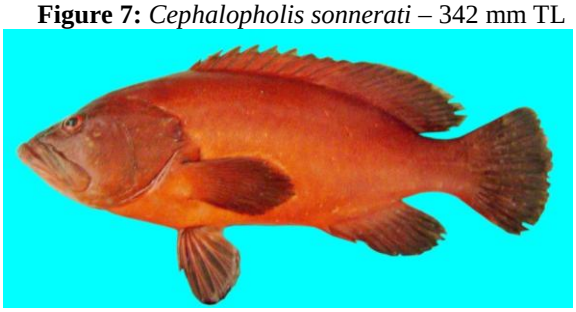

Figure 7a: *Cephalopholis sonnerati* – 202 mm TL

Dorsal fin orange red in colour, soft rays with black edges. White colour spot present on the soft rays of dorsal, anal and caudal fins; soft dorsal, pelvic, anal and caudal with black edges. Extreme edge of caudal is white in colour. No spots on pectoral and pelvic. In juveniles eight white colour spots present at the base of the first dorsal fin. In the specimen measuring 175 mm TL a black colour spot present in between middle and lower operculum spine

3.1.6. Result of Multivariate analysis

Principal Component Analysis of *Cephalopholis nigripinnis* and *C. sonnerati* was carried out and characters for which factor loadings are above 0.95 were considered significant. The variance explained by components was 84%. The first factor was mainly defined by the position of dorsal fin (Predorsal), size of fins (dorsal base, second dorsal height, pectoral length, anal length), by head length, eye diameter and maxillary length. The mean value of above characters in the two species of the genus *Cephalopholis* are given in the Table 3. Results of Tukey test ($p<0.05$) only for those characters that became significantly different for morphometric and meristic characters among the two species of genus *Cephalopholis* given in Table 5. Comparison of mean values revealed significant differences in the following five characters: predorsal distance, dorsal base, head length, eye diameter and maxillary length. These are the characters that became significantly different with PCA also, thus confirming that the above characters aid in differentiating the two species of genus *Cephalopholis*.

Cephalopholis formosa is represented in the catches throughout the year in this region and it is mainly caught by hook and line. Females were encountered in the catches during the months of February and August and males in February, March and August. Specimens of all length groups were encountered in the catches in the months of February and May. Gut contents observed in few specimens revealed that it feeds on small fish such as *Decapterus* sp, engraulids, penaeid prawns, *Ocypode* spp, brachyuran crabs, rock crab and ghost crab. It has commercial value as food fish and also valued as aquarium fish. As this species is highly valued in aquarium trade, now -a-days it is being increasingly exploited from this region

Table 3: Comparison of morphometric data of the three related species of genus *Cephalopholis* represented in the catches of Visakhapatnam.

Standard length in mm SL	*miniata*, n=4		*nigripinnis*, n=5		*sonnerati*, n=26	
	160-202		109-200		142-410	
	Min-Max	$\overline{X}$ ±SD	Min-Max	$\overline{X}$ ±SD	Min-Max	$\overline{X}$ ±SD
As percentage of standard length:						
Total length	121.28-125.00	123.15±1.70	121.00-128.44	123.65±2.97	115.94-124.44	121.66±1.74
Body depth	35.62-38.41	37.38±1.35	33.50-36.76	35.32±1.73	31.42-44.01	36.86±2.56
Head length*	40.54-43.12	41.68±1.22	39.55-43.50	41.37±2.59	37.43-45.92	40.66±2.21
Predorsal distance*	36.58-43.75	39.97±3.08	40.50-44.03	42.05±1.31	35.47-48.83	40.79±3.28
Prepectoral distance	38.63-40.62	38.64±1.44	36.56-46.50	41.01±3.59	36.32-45.77	39.64±3.00
Prepelvic distance	40.00-42.07	41.21±0.89	37.31-50.50	42.61±4.88	39.39-48.88	43.03±2.99
Preanal distance	65.84-70.12	67.61±2.01	69.85-75.00	71.64±2.17	62.91-74.66	69.37±3.27
Dorsal base*	51.98-56.25	54.77±1.98	48.00-54.41	51.40±2.54	45.06-56.34	52.00±3.65
Anal base	15.85-19.88	17.26±1.65	17.50-21.10	19.65±1.57	14.13-23.25	18.25±1.76
1st dorsal spine height	11.38-12.50	12.02±0.57	-	-	-	-
3rddorsal spine height	-	-	11.00-18.34	15.42±1.08	8.33-14.78	11.20±1.44
2nd dorsal height*	15.00-19.51	16.78±2.00	13.00-18.34	15.42±2.05	12.85-20.93	16.84±2.00

Pectoral length*	25.00-26.73	25.99±0.73	22.00-29.35	25.24±2.66	18.11-28.83	24.19±2.40
Pelvic length	20.73-25.00	23.49±1.99	20.89-25.68	22.71±1.84	19.17-27.09	22.34±1.94
Anal spine height	11.36-12.19	11.81±0.40	10.00-15.59	12.69±2.25	7.97-12.87	10.68±1.26
Soft anal height*	20.73-25.00	23.49±2.17	18.50-21.10	20.27±1.08	13.76-22.53	18.21±2.13
As percentage of head length:						
Head depth	84.05-94.02	90.28±5.29	66.03-71.11	67.47±2.16	73.61-90.19	81.53±5.70
Head width	43.28-59.42	49.49±7.09	39.08-42.22	40.49±1.48	40.24-56.41	45.89±5.09
Preorbital	23.88-28.91	26.29±2.26	24.52-29.88	26.52±2.33	23.33-34.41	28.22±3.38
Postorbital	53.73-60.24	56.34±4.98	52.87-60.00	57.57±3.40	38.33-64.86	56.89±5.77
Maxillary length*	44.92-49.25	47.45±1.90	48.27-51.11	49.61±1.14	36.63-49.43	46.44±3.39
Lower jaw length	31.34-36.14	33.12±2.38	29.88-39.28	33.17±4.13	27.72-42.69	34.71±3.32
Snout length	16.41-22.89	20.34±2.83	15.55-20.75	19.28±2.22	11.11-27.27	22.15±3.50
Eye diameter*	15.66-16.41	16.00±0.54	13.79-18.86	16.62±2.15	11.96-18.33	15.19±1.72
Interorbital width	13.25-17.91	16.18±2.11	10.34-16.07	14.36±2.26	10.30-27.27	14.75±3.10
Maxillary width	14.92-15.94	15.51±0.42	12.64-16.07	14.36±1.46	11.11-17.56	14.76±1.78

*denotes significant characters of PCA for *nigripinnis* and *sonnerati*

Table 4: Comparison of meristic characters of six species of genus *Cephalopholis* compiled based on present study

Species	*boenak*	*formosa*	*microprion*	*miniata*	*nigripinnis*	*sonnerati*
Characters	n=1	n = 53	n = 1	n=4	n = 5	n = 26
Dorsal rays	15	15-17	15	14-15	15	15
Anal rays	8	8-9	9	8-9	8-9	8-9
Pectoral	15	14-17	16	16-18	17-18	16-18
Caudal	16	15-17	17	14-15	16-17	15-18
Ll scales	98	98-108	116	114-125	108-119	112-119
Ll pored scales	57	52-58	79	70-75	68-77	76-81
Ltr scales		19/44	19/42	14-22/30-45	17-19/50-51	19/44-52
Gill rakers	8+1+14 = 23	7-9+1+13-15 = 22-25	8+1+10=19	8+1+14=23	8-9+1+13-4=22-24	8-9+1+14=23-24
Pyloric caeca	-	7-11	-	11	10	9-12

Gut contents observed in few specimens revealed that it feeds on small fish such as *Decapterus* sp, engraulids, penaeid prawns, *Ocypode* spp, brachyuran crabs, rock crab and ghost crab. It has commercial value as food fish and also valued as aquarium fish. As this species is highly valued in aquarium trade, now -a-days it is being increasingly exploited from this region.

C. sonnerati is represented in the trawl catches throughout the year except in the months April, June and August. Common size in the catches is 200 to 340 mm TL.

Table 5: Results of Tukey test of *nigripinnis* and *sonnerati* represented in the catches of Visakhapatnam.

Characters	F value	p
Predorsal distance	7.161	.012
Dorsal base	7.092	.013
Pectoral length	7.183	.012
Head length	8.102	.008
Eye diameter	18.228	.000
Maxillary length	8.679	.006

Three mature females occurred in the catches, one specimen each in the month of January 2010, March and July of 2011 but males were not encountered in the catches during the entire study period of this region.

Though taxonomic ambiguities have been clarified previously [13], [14], the present study further confirms with more number of samples from this region. *Serranus nigripinnis* of Valenciennes (1828) was synonymised with *Cephalopholis urodeta* (Schneider, 1801) [1]. *nigripinnis* was misidentified as *urodeta*.[14]. *C. nigripinnis* closely resembles *C. sonnerati* in external appearance especially in colour pattern which is often misleading in identification. Major distinguishing characters among these two species are:

- maxillary scales 8-10 in *C. nigripinnis* where as 12-13 in *C. sonnerati*
- lateral line scale series 108 – 114 in *C. nigripinnis* while 112 – 119 in *C. sonnerati*
- lateral line pored scales 68 – 77 in *C. nigripinnis* where as 76 – 81 in *C. sonnerati*
- three rows of caniniform teeth present on upper jaw and two rows on lower jaw
- in *C. nigripinnis* where as three rows present on both jaws in C. *sonnerati.*

Based on the results of PCA among morphometric characters important ones that help in distinguishing these two species are:

- eye diameter (16.62) vs (15.19) in *C. sonnerati*
- maxillary length (49.61) vs (46.44) in *C. sonnerati*
- head depth (67.47) vs (81.53) in *C. sonnerati*
- pectoral length (25.24) vs (24.19) in *C. sonnerati*
- dorsal base (51.40) vs (52.0) in *C. sonnerati*
- pre dorsal (42.05) vs (40.79) in *C. sonnerati*

3.2. Allozyme analysis of four species of genus *Cephalopholis* off Visakhapatnam:

Allozyme banding pattern in 57 specimens belonging to four species *formosa, miniata, nigripinnis* and *sonnerati* of genus *Cephalopholis* that were represented in the catches of Visakhapatnam was studied. Eleven enzyme systems: Aspartate amino transferase, Acid Phosphatase, Alcohol Dehydro-

genase, Esterases, Glucose 6 Phosphatase, Glutamate Dehydrogenase, Glutamine Synthetase, Lactate Dehydrogenase, Malate Dehydrogenase, Posphoglucomutase and Super Oxide Dismutase were screened to study genetic variation in these hind groupers. In the present study seventeen loci and a total of 40 alleles were detected and their frequencies given in Table 6. Diagnostic locus where no alleles shared with any other species for *C. sonnerati* observed at GDH-2*. Among the four species number of diagnostic alleles ranged from one to two (Table 7). The number of polymorphic loci exhibited by species in *Cephalopholis* group are in the range one to five (Table 8). The best estimate of genetic variation in natural population is the mean observed heterozygosity (Ho) per locus [31] which varies non randomly between loci, populations and species. Probability test was performed to assess the conformity of genotype numbers to that expected under Hardy-Weinberg Equilibrium. After sequential Bonferroni adjustment P value observed to be low (P<0.0001) at loci AAT*, ADH*, EST-1*, EST-2*, GDH-1*, G6PDH*, LDH-1* and MDH-2*. Estimates of genetic identity and genetic distance between pairs of species of *Cephalopholis* group based on results from seventeen loci given in Table 9. The overall relationships were apparent from UPGMA derived dendrogram (Fig. 8) that revealed two main branches where *Cephalopholis formosa*, *C. sonnerati* and *C. miniata* formed one cluster and *C. nigripinnis* formed a separate branch. Further two species *C. sonnerati* and *C. miniata* formed one cluster showing close similarities. Thus the results from allozyme analysis coincides with that of morphological studies where *C. sonnerati* and *C. miniata* showed close similarities in body shape and overlapping colour patterns where as *C. nigripinnis* showed striking differences with the remaining three species of genus *Cephalopholis.*

Table 6: Allele frequencies at seventeen loci in the four species of genus *Cephalopholis* represented in the catches of Visakhapatnam

Locus	Allele	*C. formosa*	*C. miniata*	*C. nigripinnis*	*C. sonnerati*
AAT*	100(a)	0.817	0.960	0.846	0.750
	102(b)	0.133	0.040	-	-
	104(c)	0.050	-	-	0.250
	106(d)	-	-	0.154	-
	n	30	5	4	18
ACP*	96(a)	0.067	0.800	0.192	0.250
	98(b)	0.183	0.200	0.808	0.125
	100(c)	0.750	-	-	0.625
	n	30	5	4	18
ADH*	98(a)	0.267	0.020	0.308	0.375
	100(b)	0.733	0.980	0.692	0.625
	n	30	5	4	18
EST-1*	100(a)	0.817	0.045	0.962	0.375
	102(b)	0.183	0.955	0.038	0.625
	n	30	5	4	18
EST-2*	100(a)	0.862	0.950	0.192	0.875
	102(b)	0.138	0.050	0.808	0.125
	n	30	5	4	18
GDH-1*	100(a)	0.862	0.965	0.846	0.750
	102(b)	0.138	0.035	0.154	0.250
	n	30	5	4	18
GDH-2*	100(a)	-	-	-	0.875
	102(b)	-	-	-	0.125
	n	30	5	4	18
G6PDH*	100(a)	0.967	0.200	0.077	0.250
	102(b)	0.033	0.800	0.846	0.750
	104(c)	-	-	0.077	-
	n	30	5	4	18
GSN-1*	100(a)	0.207	0.800	0.192	0.750
	102(b)	0.793	0.200	0.808	0.250
	n	30	5	4	18
GSN-2*	100(a)	0.867	-	0.846	0.875
	102(b)	0.133	-	0.154	0.125
	n	30	5	4	18
LDH-1*	100(a)	0.977	0.300	-	-
	102(b)	0.023	-	0.192	-
	104(c)	-	0.700	0.808	-
	n	30	5	4	18
LDH-2*	100(a)	0.224	0.700	-	0.875
	102(b)	0.776	0.300	-	0.125
	n	30	5	4	18
MDH-1*	100(a)	0.883	0.800	0.846	0.750
	102(b)	0.117	0.200	0.154	0.250
	n	30	5	4	18
MDH-2*	100(a)	0.982	-	0.154	-
	102(b)	0.018	-	0.846	-
	n	30	5	4	18
PGM*	100(a)	0.207	0.600	0.808	0.125
	102(b)	*0.690*	0.200	0.192	0.625
	104(c)	0.103	0.200	-	0.250
	n	30	5	4	18
SOD-1*	100(a)	0.800	0.800	0.154	0.750
	102(b)	0.200	0.200	0.846	0.250
	n	30	5	4	18
SOD-2*	100(a)	0.833	0.800	0.885	0.955
	102(b)	0.167	0.200	0.115	0.045
	n	30	5	4	18

Table 7: Alleles present at each locus, given as letters in alphabetic order according to their anodal mobility '*a*' representing the fastest migrating. Diagnostic alleles of *Cephalopholis* group are underlined

Locus	*C. formosa*	*C. miniata*	*C. nigripinnis*	*C. sonnerati*
AAT*	*abc*	*ab*	*ad(0.154)*	*ac*
ACP*	*abc*	*ab*	*ab*	*abc*
ADH*	*ab*	*ab*	*ab*	*ab*
EST-1*	*ab*	*ab*	*ab*	*ab*
EST-2*	*ab*	*ab*	*ab*	*ab*
GDH-1*	*ab*	*ab*	*ab*	*ab*
GDH-2*	-	-	-	*ab*
G6PDH*	*ab*	*ab*	*abc(0.077)*	*ab*
GSN-1*	*ab*	*ab*	*ab*	*ab*
GSN-2*	*ab*	-	*ab*	*ab*
LDH-1*	*ab*	*ac*	*ac*	-
LDH-2*	*ab*	*ab*	-	*ab*
MDH-1*	*ab*	*ab*	*ab*	*ab*
MDH-2*	*ab*	-	*ab*	-
PGM*	*abc*	*abc*	*ab*	*abc*
SOD-1*	*ab*	*ab*	*ab*	*ab*
SOD-2*	*ab*	*ab*	*ab*	*ab*

Table 8: Parameters of genetic variation at each allozyme locus in the four related species of *Cephalopholis* group

Parameter/Species	*C. formosa*	*C. miniata*	*C. nigripinnis*	*C. sonnerati*
Sample size	30	5	4	18
No. of loci screened	17	17	17	17
No. of polymorphic loci	3	5	1	1
Mean heterozygosity	0.054±0.013	0.0232±0.152	0.0473±0.034	0.177±0.056

Table 9: Estimates of genetic identity (above diagonal) and genetic distance (below diagonal) (Nei, 1978) between pairs of four related species of *Cephalopholis* group represented in the catches of Visakhapatnam

Species	*C. formosa*	*C. sonnerati*	*C. nigripinnis*	*C. miniata*
C. Formosa	****	0.6589	0.6411	0.6342
C. sonnerati	0.4172	****	0.5339	0.3408
C. nigripinnis	0.4446	0.6276	****	0.6082
C. miniata	0.4554	0.3000	0.4973	****

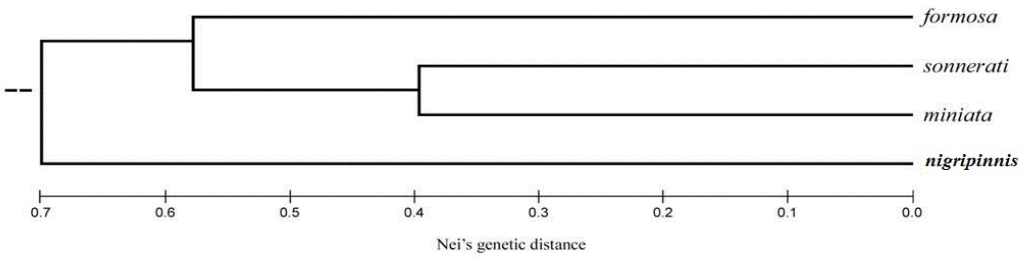

Figure 8: UPGMA Dendrogram showing hierarchic relationship among four species of genus *Cephalopholis*

4. Conclusion

Of the six species of genus *Cephalopholis* – *boenak, microprion, miniata* and *nigripinnis* are very rare in Visakhapatnam waters. During the present study a single specimen of *Cephalopholis boenak* measuring 196 mm TL and *C. microprion* measuring 202 mm TL were encountered in the trawl catches of this region. *C. microprion* is the first record from coastal waters of mainland India. *Cephalopholis miniata* is the first record from Visakhapatnam waters, towards north of Chennai. *C. formosa* and *C. microprion* are more suitable candidate species for keeping in aquarium. *C. boenak* is described first time as *pachycentron* (Valenciennes, 1828) [14]. The age of *C. boenak* of 231 mm TL (188mm SL) as 11 years from Hongkong [32]. In Indian waters *Cephalopholis microprion* is previously recorded from Andaman and Nicobar Islands by Rao et al. (2000), Rajan (2002) and Rao (2004) [33]-[35].

Morphometric and meristic characters are used to illustrate intra and inter specific variations. Analysis of these variations isolates specific morphometric indices and variants which have taxonomic potentials and discriminating powers away of the environment and geographical influences [30]. In the present study morphometric, meristic characters and colour patterns were used to identify six species of genus *Cephalopholis.* Among six species, *C. nigripinnis* and *C. sonnerati* closely resemble each other. Based on Principal Component Analysis and Tukey test, predorsal distance, dorsal base, pectoral length, head length, eye diameter and maxillary length were identified as significant characters that aid in distinguishing these two species.

The genetic variability in four species was evidenced by seventeen loci out of which ten are polymorphic. The mean heterozygosity value in the present study falls within range reported for marine fishes [36]. By employing allozymes, in the present study species are easily distinguished and also diagnostic locus and alleles that can discriminate these species are identified. This data along with morphometric and meristic data has varied application in research on evolution, conservation and management of natural resources and genetic improvement programmes.

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