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Allozyme electrophoretic studies in four species of groupers (Pisces: Serranidae) represented in the commercial fishery of Visakhapatnam - India

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Several species of groupers belonging to the genus *Epinephelus* have overlapping colour patterns. In the present study four species of groupers, *Epinephelus epistictus, E. latifasciatus, E. magniscuttis* and *E. radiatus,* that are represented in the catches of Visakhapatnam were analysed for allozyme variation. The eleven enzyme systems screened twenty-five scorable loci. Sixteen loci in *E. episticuts,* nineteen in *E. latifasciatus,* fifteen in *E. magniscuttis* and twenty-two loci in *E. radiatus* were found to be polymorphic at p = 0.95 level. Average heterozygosity ranged form 0.0582±0.063 to 0.0704±0.0442. Diagnostic alleles that help in clearly distinguishing the species were identified. UPGMA dendrogram revealed that *E. epistictus* is closely related to *E. magniscuttis* compared to other two species.

[Keywords: Groupers, allozyme loci, allele frequencies**]**

Introduction

Taxonomy is fundamental to conservation efforts of marine fish species, and the units on which conservation is based are determined ultimately by species. Generally groupers are identified by their color patterns or a suite of morphologic and characters like body configuration, size and by meristic characters. Though generally the colour patterns in medium-sized fishes are distinctive enough to identify different species, one need to be aware of intraspecific variations in color patterns of juveniles, which may be completely different from the adults of the same species[1]. Identification based purely on morphological features leads to confusion in closely resembling species[2]. Under such circumstances when morphological characters are unreliable, biochemical genetic methods have long been used to confirm the identity of species[3]. Molecular markers have become a major tool for systematic ichthyologists and are also useful to fishery biologists to solve taxonomic problems ranging between species and population[4].

Among different molecular markers, allozymes are proven tools to determine population structure and estimate inter and intra species gene flow in natural fish populations. These have been successfully used to determine stock structure of several fish species. The large biochemical differences between species, so readily resolved by allozyme electrophoresis make this a valuable technique for identifying members of different species and to investigate genetic variation among populations[5,6].

Allozyme electrophoresis is very much useful in defining genetic markers for stock identification on the basis of differences in allele frequencies between the stocks in many species. Previously these studies were carried out in the species of genus *Coreperca* from Korean waters that showed allozyme variations at 25 protein coding loci[7]. Karyological, allozyme and microsatellite survey was carried out in the dusky grouper, *Epinephelus marginatus,* of Mediterranean populations[8,9]. The electrophoretic analysis of 30 genetic loci of six protein systems was studied in six species of the genus *Hypoplectrus* that inhabited Cuban waters[10]. RAPD markers for specific identification of grouper *E. guaza* from Spanish waters[11] and analysis of genetic structure of *E. multinotatus* off north western Australian waters[12] were carried out. In India studies on taxonomic relationships using RAPD markers among seven species of groupers belonging to genus *Epinephelus*[13] were carried out. Thus a perusal of literature reveals that the taxonomic relationships among *E. epistictus, E. latifasciatus, E. radiatus* and *E. marginatus* were so far not studied.

A significant attempt has not been made to analyse the genetic structure of groupers of east coast of India,

especially off Visakhapatnam, north Andhra Pradesh. Understanding the present genetic make up of wild grouper populations has a significant practical value, particularly in fishery management and conservation of stocks. Moreover, there is no recorded information on genetic markers and distribution of genetic variation in the natural populations of the four closely related species of groupers of genus *Epinephelus: E. epistictus* (Temminck & Schlegel, 1842), *E. latifasciatus* (Temminck & Schlegel, 1842), *E. magniscuttis* Postal, Fourmanior & Guézé, 1963 and *E. radiatus* (Day, 1867) from waters of north Andhra Pradesh as these four species are often misidentified. Hence in the present study, allozyme based survey of genetic variation in the above four grouper species has been carried out.

Materials and Methods

A total of seventy-two fresh specimens of all size groups belonging to four species of groupers [*Epinephelus epistictus* (Fig. 1), *E. latifasciatus* (Fig. 2), *E. magniscuttis* (Fig. 3) and *E. radiatus* (Fig. 4)] were collected from traditional fish landing centers such as Bheemunipatnam, Rushikonda, Lawson's Bay, Pudimadaka and Visakhapatnam fishing harbour during December 2006 to March 2009. After identifying the species, total length, weight and sex of each specimen were noted and immediately muscle tissue was taken and stored at -20° C until further use.

Muscle tissue was crushed and homogenized with extraction buffer (0.2 M Tris – EDTA buffer pH 7.0). Homogenates were centrifuged for 1 hour at 10,000 rpm at 4°C and the supernatant was recentrifuged for 30 minutes. The supernatant collected was used for futher analysis. Vertical polyacrylamide gel electrophoresis was used for separation of allozymes at different enzyme loci. Gels of 10 × 8 cm size were used. Electrophoresis was

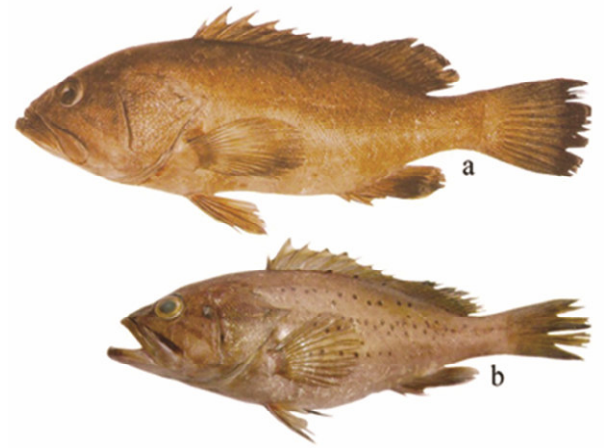

Fig. 1 – *Epinephelus epistictus* a 604 mm TL, b 267 mm TL

Fig. 3 – *Epinephelus magniscuttis* a 600 mm TL, b 214 mm TL

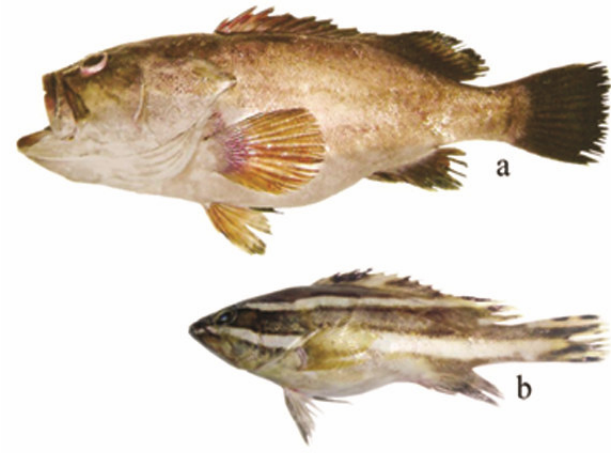

Fig. 2 – *Epinephelus latifasciatus* a 642 mm TL, b 94 mm TL

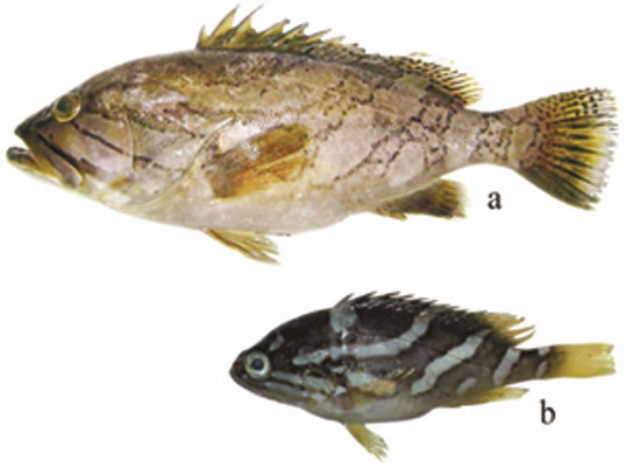

Fig. 4 – *Epinephelus radiatus* a 506 mm TL, b 72 mm TL

carried out at 100 V in cooling chamber at 4°C. The bands of each of eleven enzymes were visualized using specific histochemical staining methods[14], until sharp bands were visualized.

The locus and allele designations were followed according to the standardized genetic nomenclature for protein coding loci[15]. At all loci, most common allele was assigned as 100. Alternate alleles were designated as per their mobility, relative 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[16]. Cluster analysis was performed and dendrogram plotted on pairwise genetic distance estimated using the Unweighed Pair Group Method with arithmetic mean (UPGMA)[17] and parameters of genetic variation like number of polymorphic loci in each population and mean heterozygosities were calculated with POPGENE version 1.31[18] .

Results and Discussion

In the eleven enzyme systems studied, 25 scorable loci were detected in the present study, two loci for the allozymes Acid Phosphatase (ACP-1*, ACP-2*), Aspartate AminoTransferase (AAT-1*, AAT-2*), Alcohol Dehydrogenase (ALD-1*, ALD-2*), Glutamate Dehydrogenase (GDH-1*, GDH-2*), Glucose 6 Phosphate Dehydrogenase (G6PDH-1*, G6PDH-2*), Lactate Dehydrogenase (LDH-1*, LDH-2*), Malate Dehydrogenase (MDH-1*, MDH-2*) and Phosphoglucomutase (PGM-1*, PGM-2*); three loci for allozyme SuperOxide Dismutase (SOD-1*, SOD-2*, SOD-3*); five loci for Esterase (EST-1*, EST-2*, EST-3*, EST-4*, EST-5*) and single locus for Glutamine Synthetase (GSN*) were detected. A total of 87 alleles were detected and their frequencies are given in Table 1.

In *E. epistictus* thirteen loci (ACP*-2, AAT*-1, ALD*-2, EST*-4, GDH*-1, G6PDH*-1, G6PDH*-2, GSN*, LDH*-1, LDH*-2, MDH*-1, PGM*-1, PGM*-2, SOD*-1 and SOD*-3) were found to be polymorphic, where the frequency of most common allele is <0.95. In *E. latifasciatus* eighteen loci (AAT*-1, ALD*-1, ALD*-2, EST*-1 to EST*-5, G6PDH*-1, GSN*, LDH*-1, LDH*-2, MDH*-1, MDH*-2, PGM*-1, PGM*-2, SOD*-1 and SOD*-2) were found to be polymorphic. In *E. radiatus* all the loci except three loci (ACP*-2, G6PDH*-2 and SOD*-1) are polymorphic and in *E. magniscuttis* fourteen loci (ACP*-1, AAT*-1, AAT*-2, ALD*-1, EST*-1, EST*-3, G6PDH*-2, GSN*, LDH*-1, LDH*-2, MDH*-1, PGM*-1, SOD*-1 and SOD*-2) are polymorphic.

Diagnostic locus where no alleles shared with any other species[19] for *E. radiatus* was observed at GDH-2*. Among the four species number of diagnostic alleles ranged between one and nine (Table 2). Locus GDH*-2 was expressed by two alleles, loci ACP*-1, AAT*-2, ALD*-2, EST*-2, EST*-4, GSN*, LDH*-2, MDH*-1, SOD*-1 and SOD*-3 were expressed by three alleles and remaining all loci were expressed by four alleles each. Parameters of genetic variation are given in Table 3. The mean number of alleles per locus was found to be highest in *E. radiatus.* The observed heterozygosity varied from 0.058 to 0.124. Probability test was performed to assess the conformity of allele frequencies to that expected under Hardy-Weinberg expectations. A significant deviation from HW expectation after sequential Bonferroni adjustment ($p<0.005$) of probability levels[20] was observed at loci G6PDH*-1 and PGM*-1 in *E. epistictus,* at AAT*-1 and EST*-1 in *E. latifasciatus,* at EST*-4 and SOD*-3 in *E. radiatus* and at locus SOD*-1 in *E. magniscuttis.* A perusal of literature reveals that the deviation from HW equilibrium indicates significant heterozygote deficiency. In the present study the exact reason for this deviation can possibly be given by carrying out further studies with samples from other areas of this region. Estimates of genetic identity and genetic distance between pairs of four species of groupers given in Table 4.

In the present study based on allozyme studies, UPGMA dendrogram (Fig. 5) has been constructed using POPGENE software version 1.31 which shows that *E. epistictus* and *E. magniscuttis* formed one cluster, *E. radiatus* formed a separate branch and the species *E. latifasciatus* radiated from the above three species forming a separate branch showing that *E. epistictus* and *E. magniscuttis* are closely related than the remaining two species. This study helps us to assess the genetic variation existing among different species and also derive diagnostic loci and alleles that are useful for identification of closely related species of this region.

Table 1—Allele frequencies of four species of groupers represented in the catches of Visakhapatnam

Loci	Alleles	*E. epistictus* (9)	*E. latifasciatus* (27)	*E. magniscuttis* (11)	*E. radiatus* (25)
ACP*-1	98(a)	0.333	-	0.182	-
	100(b)	0.667	0.91	0.727	0.84
	102(c)	-	0.09	0.091	0.16
ACP*-2	98(a)	0.84	0.333	-	-
	100(b)	0.16	-	-	0.909
	102(c)	-	0.667	-	0.091
AAT*-1	100(a)	0.667	0.111	0.182	0.760
	102(b)	0.222	0.111	0.091	0.080
	104(c)	0.111	-	0.727	-
	106(d)	-	0.778	-	0.160
AAT*-2	98(a)	-	-	-	0.88
	100(b)	-	-	0.909	0.12
	102(c)	-	-	0.091	-
ALD*-1	98(a)	-	-	0.273	-
	100(b)	-	0.185	0.727	0.12
	102(c)	0.333	-	-	0.88
	104(d)	0.667	0.815	-	-
ALD*-2	98(a)	0.778	-	-	0.12
	100(b)	0.111	0.074	-	0.88
	102(c)	0.111	0.926	-	-
EST*-1	98(a)	-	-	0.818	-
	100(b)	-	0.093	0.182	0.16
	102(c)	0.333	0.907	-	-
	104(d)	0.667	-	-	0.84
EST*-2	96(a)	-	0.296	-	-
	98(b)	-	-	-	0.182
	100(c)	-	0.704	-	0.818
EST*-3	96(a)	0.667	-	-	0.12
	98(b)	0.333	-	0.273	0.04
	100(c)	-	0.741	0.727	0.84
	102(d)	-	0.259	-	-
EST*-4	98(a)	0.778	0.148	-	-
	100(b)	0.222	0.852	-	0.7
	102(c)	-	-	-	0.3
EST*-5	98(a)	-	-	-	0.24
	100(b)	-	0.148	-	0.76
	102(c)	-	0.074	-	-
	104(d)	-	0.778	-	-
GDH*-1	100(a)	0.778	-	0.273	0.76
	102(b)	-	0.185	-	-
	104(c)	-	0.815	-	0.24
	106(d)	0.222	-	0.727	-
GDH*-2	100(a)	-	-	-	0.84
	102(b)	-	-	-	0.16
GSN*	96(a)	-	0.093	0.727	-
	98(b)	0.333	-	-	-
	100(c)	0.667	0.907	-	0.120
	102(d)	-	-	0.273	0.880
G6PDH*-1	98(a)	-	0.074	-	-
	100(b)	0.778	-	0.91	0.920
	102(c)	-	0.037	0.09	-
	104(d)	0.222	0.889	-	0.080
G6PDH*-2	98(a)	0.667	-	0.727	0.24
	100(b)	0.333	0.222	0.273	0.76
	102(c)	-	0.778	-	-

Contd—

Table 1—Allele frequencies of four species of groupers represented in the catches of Visakhapatnam—*Contd*.

Loci	Alleles	*E. epistictus* (9)	*E. latifasciatus* (27)	*E. magniscuttis* (11)	*E. radiatus* (25)
LDH*-1	100(a)	0.667	0.259	0.909	0.12
	102(b)	0.222	0.074	-	0.080
	104(c)	-	0.667	0.091	0.12
	106(d)	0.111	-	-	0.68
LDH*-2	98(a)	0.778	0.185	-	0.120
	100(b)	0.111	0.815	0.364	0.2
	102(c)	0.111	-	0.636	0.68
MDH*-1	100(a)	0.333	0.111	0.364	0.12
	102(b)	0.111	-	0.636	-
	104(c)	0.556	0.889	-	0.88
MDH*-2	96(a)	-	0.037	-	-
	98(b)	-	0.074	-	-
	100(c)	-	0.037	-	0.8
	102(d)	-	0.852	-	0.2
PGM*-1	100(a)	0.222	0.926	0.273	0.12
	102(b)	0.111	0.037	-	0.08
	104(c)	0.667	-	0.091	0.8
	106(d)	-	0.037	0.636	-
PGM*-2	100(a)	0.222	0.074	-	0.12
	102(b)	0.778	0.889	-	-
	104(c)	-	-	-	0.88
	106(d)	-	0.037	-	-
SOD*-1	98(a)	0.222	0.111	0.273	0.12
	100(b)	0.778	0.889	0.727	0.12
	102(c)	-	-	-	0.76
SOD*-2	98(a)	0.111	0.037	-	0.2
	100(b)	-	0.926	0.889	0.8
	102(c)	-	0.037	0.111	-
	104(d)	0.889	-	-	-
SOD*-3	98(a)	0.111	0.074	-	-
	100(b)	0.889	-	-	0.8
	102(c)	-	0.926	-	0.2

Table 2—Alleles present at each locus in the four species of groupers, diagnostic alleles are bold underlined.

Locus	*E. epistictus*	*E. latifasciatus*	*E. magniscuttis*	*E. radiatus*
ACP-1	*ab*	*bc*	*ab*	*bc*
ACP-2	*ab*	*ac*	-	*bc*
AAT-1	*abc*	*ab*	*abc*	*ab*
AAT-2	-	-	***bc*** (0.091)	*ab*
ALD-1	*cd*	*bd*	*ab*(0.273)	*bc*
ALD-2	*abc*	*bc*	-	*ab*
EST-1	*cd*	*bc*	*ab*(0.818)	*bd*
EST-2	-	*ac*(0.296)	-	*bc*(0.182)
EST-3	*ab*	*cd*	*cd*	*abc*
EST-4	*ab*	*ab*	-	b***c***(0.3)
EST-5	-	***bcd***(0.074;0.778)	-	*ab*(0.24)
GDH-1	*ad*	*bc*(0.185)	*ad*	*ac*
GDH-2	-	-	-	*ab*(0.84,0.16)
GSN	*bc*(0.333)	*ac*	*ad*	*cd*
G6PDH-1	*bd*	*acd(0.074)*	*bc*	*bd*
G6PDH-2	*ab*	***bc***(0.778)	*ab*	*ab*
LDH-1	*abd*	*abc*	*ac*	*abcd*
LDH-2	*abc*	*ab*	*bc*	*abc*
MDH-1	*abc*	*ac*	*ab*	*ac*
MDH-2	-	***ab****cd(0.03, 0.074)*	-	*cd*
PGM-1	*abc*	*abd*	*cd*	*abc*
PGM-2	*ab*	*ab****d****(0.037)*	-	***ac****(0.88)*
SOD-1	*ab*	*ab*	*ab*	*ab****c****(0.76)*
SOD-2	*ad*	*abc*	*cd*	*ab****c***
SOD-3	*ab*	*ac*	-	*bc*

*Frequency of diagnostic alleles given in paranthesis

Table 3—Parameters of genetic variation at each allozyme locus in the four related species of grouper *E. latifasciatus* represented in the catches of Visakhapatnam

	E. epistictus	*E. latifasciatus*	*E. magniscuttis*	*E. radiates*
Sample size	9	27	11	25
No. of loci screened	25	25	25	25
No. of polymorphic loci	16	18	15	22
Mean heterozygosity	0.124 ± 0.098	0.090 ± 0.044	0.058 ± 0.064	*-0.061 ± 0.029

Table 4—Estimates of genetic identity (above diagonal) and genetic distance (below diagonal) (Nei, 1978) between pairs of four species of groupers represented in the catches of Visakhapatnam

	E. epistictus	*E. latifasciatus*	*E. radiatus*	*E. magniscuttis*
E. epistictus	*****	0.295	0.328	0.376
E. latifasciatus	1.220	*****	0.274	0.245
E. radiatus	1.114	1.296	*****	0.253
E. magniscuttis	0.978	1.406	1.376	*****

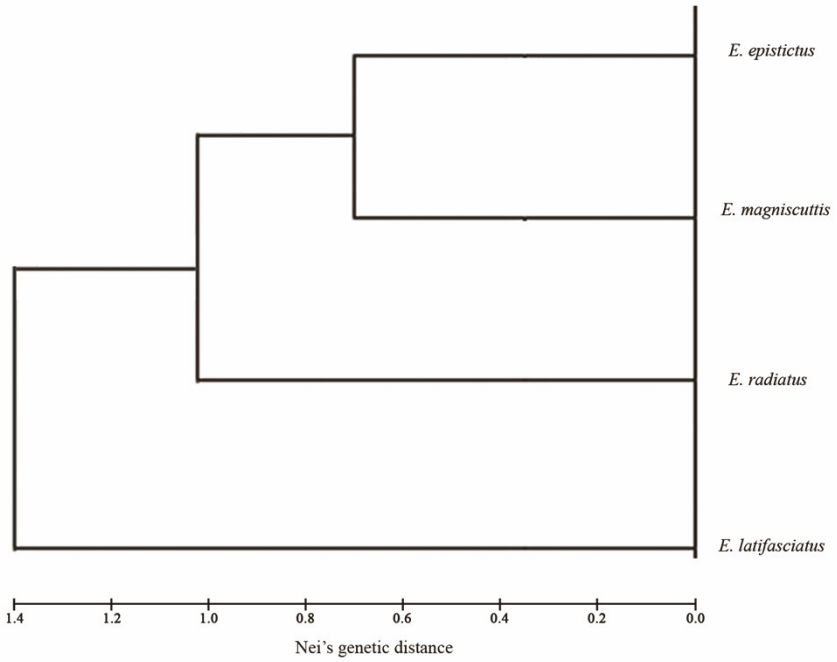

Fig. 5 – UPGMA Dendrogram showing hierarchic relationship among four related species of genus *Epinephelus*

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