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Phylogenetic Relations And Electrophoretic Identification Of Allozyme In Four Species Of Snappers

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Abstract

In the present study, four species of snappers, Lutjanus: fulvus, lemniscatus, lutjanus and madras were embodying the catches of Visakhapatnam coast, analyzed for allozyme variations. The four enzyme systems screened five loci was 12 in fulvus, 3 in lemnsicatus, 29 in lutjanus and 26 in madras were found to be polymorphic at p = 0.95 level. Diagnostic alleles help in clear distinctive species had been identified. An unweighted pair-group method with arithmetic mean (UPGMA) method was used to construct the dendogram to know the genetic relationship between the four species of snappers Lutjanus: fulvus, lemniscatus, lutjanus and madras based on genetic distance revealed the presence of three clusters showing the percentage similarity between lutjanus and madras 0.961%, leminiscatus showed 0.918% similarity with above two species and fulvus showed 0.890% similarity with above three species.

Keywords: Allozyme; snapper; Phylogenesis; allele.

Introduction

Lutjanidae family is one of the prime in the order Perciformes and comprises 4 subfamilies, 17 genera and 110 species make the *lutjanids* are the most specious of the families Froese and Pauly (2016) and (Nelson, 2006). Snappers have been the focused of several allozyme studies. However, given the number of species in the family *lutjanids* have been under represented in such studies to date. Taxonomy is basic thing to conserve the efforts of marine fishery samples and the units on which conservation is based are determined ultimately by species. Snappers are well-known by their coloring patterns or a suite of morphologic and characteristics like body configuration, size and by meristic characters. Identification was based on morphological features leads to confusion in closely resembling species. Possibility of misidentification poses problems for management of fishery and stock structure analysis (Ward *et al*., 1995). Species identification Genetic levels of snappers also extremely valuable since morphological discrimination especially of early juveniles can be difficult. Therefore the combined efficacy of morphological analyses and biochemical phylogenetic methods have been followed here for providing a meaningful approach to solve taxonomic uncertainties concerning the status of species (Chow *et al*., 1993). Electrophoretic data in allozyme and isozyme form can be applied in systematic and taxonomic investigations. Under such circumstances

when morphological characters are unreliable, biochemical genetic methods have long been used to corroborate the species identity (Avise, 1975; Utter, 1991 and Chow *et al.,* 1993). In fisheries, these methods have been used to disclose cryptic species (Shaklee and Tamaru, 1981; Smith *et al.,* 1991; Grant and Utter. 1984) to resolve taxonomic problems (Smith and Robertson, 1981; Graves *et al.,* 1988). Nevertheless, *lutjanids* have contributed to our understanding of genetic variation in natural populations and an assortment of evolutionary process. Virtually that all previous applications to snappers systematics study should be reevaluated. Evaluation of these applications and recommendations for standard methods and future research are also discussed in present paper. Previously such studies were carried out by Ovenden *et al*., (2004) studied the genetic diversity of *Pristipomoides multidens* from East Indies triangle waters. Vasconcellos *et al*., (2008) studied nuclear and mitochondrial DNA sequence differences in between Brazilian and Caribbean population of yellow snapper, *Ocyurus chrysurus*. Sulaiman *et al*., (2003) investigate the genetic diversity of red snapper *Lutjanus malabaricus* in Brunei and Sabah using allozyme electrophoresis. So far no attempt has been made to analyse the genetic structure of *lutjanids* off Visakhapatnam coast. Understanding the present genetic makeup of wild *lutjanid* populations has a significant practical value particularly for fishery management and conservation of stocks. Hence in the present study, general proteins and allozyme based survey of genetic variation in the four species of snappers *Lutjanus fulvus, L. lemniscatus, L. lutjanus* and *L. madras* represented in the catches of Visakhapatnam, east coast of India. Govinda Rao *et al*., 2014 and Muddula Krishna *et al*., 2015 studied length weight relationship and length groups of two species of snappers, diversity of intertidal fish species works done from Visakhapatnam coastal waters, east coast of India.

Material and Methods

A total of 70 fresh specimens of all size groups belonging to four species of snappers of genus *Lutjanus fulvus, lemnsicatus, lutjanus* and *madras* that were collected from traditional fish landing centers and Visakhapatnam fishing harbour, east coast of India (Fig.1) during 2010-2012 in the catches. After species identification, total length, weight and masculinity of each specimen were noted and immediately muscle tissue was taken and stored at -20^{0}C for further analysis. Muscle tissue crushed and homogenized with extraction buffer (0.2 M Tris EDTA buffer $_P$H 7.0). Homogenates were centrifuged for 1 hour at 10,000 rpm at 4^0 C and supernatant was recentrifuged for 30 minutes. The supernatant collected was used for further 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 carried out at 100 V in cooling chamber at 4^{0}C. The bands of each of four enzymes were visualized using specific histochemical staining methods (Whitmore, 1990), until sharp bands were visualized. Enzyme systems studied in allozyme analysis nomenclature of loci and alleles was followed as recommended by Shaklee *et al*., (1990). 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 (Raymond and Rousset, 1995a).

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Results

Allozyme banding pattern of 70 specimens belonging to four species of snappers of genus *Lutjanus: Lutjanus fulvus* (Fig.2); *L. lemniscatus* (Fig.3); *L. lutjanus* (Fig.4); *L. madras* (Fig.5) that were represented in the catches of Visakhapatnam, east coast of India was studied. The four enzyme systems were Alcohol Dehydrogenase (Fig.7), Lactate dehydrogenase (Fig. 8), Phosphoglucomutase (Fig. 9) and Super Oxide Dismutase (Fig. 10) in four species genus *Lutjanus: fulvus, lemnsicatus, lutjanus* and *madras* were studied. Of the four systems studied, eight loci were detected in four species of lutjanids. In the present study, single locus for the allozymes Alcohol Dehydrogenase (ADH*) and Super Oxide Dismutase (SOD*) and three loci each for Lactate Dehydrogenase (LDH-1*, LDH-2*, LDH-3*) and Phosphoglucomutase (PGM-1*, PGM-2*, PGM3*) were detected. A total of 21 alleles were detected and their frequencies were given in Table 1. The locus ADH* was expressed by four alleles, loci LDH-1* and SOD* PGM-1*, PGM-2*, PGM3* were expressed by three alleles and remaining all loci by two alleles each.

Discussion

Diagnostic locus where no alleles shared with any other species (Ward et al., 1995) for *lemniscatus* was observed at LDH-2* and for *lutjanus* at LDH-3*. Among the four species between one and four diagnostic alleles were detected (Table 2). In ADH* alleles *a* and *d* observed to be commonly found in all four species. In LDH-1* allele observed to be commonly shared by the four species. Locus LDH–2* is expressed only in *lemniscatus* and LDH-3* only in *lutjanus*. Thus they can be referred to as diagnostic loci.

From Indian waters, Menezes (1993, 1994) studied genetic relationship among three species of genus *Sardinella* and four Sciaenid species from Arabian Sea, genetic and morphometric differences between yellowtail snapper populations of tropical West Atlantic were studied by Vasconcellos (2008) using allozymes and mitochondrial DNA was observed that morphometric differences were not incompatible with the existence of discreet fish stocks. Miller and Cribb (2007a) studied mitochondrial DNA based phylogenetic relationships among Indo-Pacific snappers. In this study, the precise cause to the divergence could perhaps given by carrying out further studies with sample from other areas of this region. Estimates of genetic identification and genetic distance between pairs of four species of snappers given in Table 2. The present study based on allozyme, UPGMA dendrogram (Fig.6) has been constructed using POPGENE software version 3.4 which illustrated that *lutjanidae* species were closely related. This study assists us to evaluate the variation of phylogenetic existing among species variation and also derive diagnostic loci and alleles that are useful for evaluation of closely related species of this region.

The present study is an attempt to indicate the genetic structure of these four species using general proteins and allozymes as there were no previous studies on these species. In recent years there has been a worldwide diminishing the fishing stocks (Garcia and Grainger, 2005) overexploited, fully exploited and exhausted fisheries that were 69% in 1995 increased to 75% in 2002, only 1% of the stocks were in a state of retrieval (Ormerod, 2003).

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For this reason competent management policies based on unambiguous scientific data are necessary both to defend the fishing stocks and to maximize their exploitation without compromising their integrity. The correct stock as assessment and identification of species is essential to ascertain the maximum sustainable effort of a given marine resource (Ryman and Utter, 1987; Ryman, 1991). Many stock concepts can be found in the literature (Booke, 1981; Ovenden, 1990; Carvalho and Hauser,1995), but one of the most accepted and used is "a stockist an intraspecific group of randomly mating individuals with temporal and spatial integrity" (Ihssen *et al.*, 1981) which swathe most of the definitions given by other authors. The fish stock identification can be made efficiently by the use of highly polymorphic molecular markers (Blaber *et al.*, 2005; Caddy and Seijo, 2005).

Several species of snappers are morphologically similar and even the meristic counts are almost identical for these species. Identification based purely on morphological features leads to confusion in closely resembling species. Possibility of misidentification poses problems for management of fishery and stock structure analysis. As an aid to traditional taxonomic characters, biochemical genetic methods have been used in systematics to solve taxonomic ambiguities. Proteins are frequently used as biochemical genetic markers and the projected genetic variations are used to distinguish closely related species and natural populations of a given species. However, recent advancements in the field of molecular biology proved that allozymes are powerful biochemical genetic markers compared to proteins as they generate more reliable data. So far no attempt has been made to analyse the genetic structure of snappers of Indian waters as until recently they have not received due attention from geneticists given their commercial significance and need for information on stock structure to ensure sustainable management. As understanding the present genetic makeup of wild snappers population has a significant practical value particularly for fishery management and conservation of stocks.

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Table 1. Allele frequencies at nine loci in the four species of genus *Lutjanus* represented in the catches of Visakhapatnam

Locus	Allele	*fulvus*	*leminscatus*	*lutjanus*	*madras*
ADH*	100(a)	0.75	0.75	0.846	0.75
	102(b)	-	0.20	-	-
	104(c)	-	0.05	-	0.167
	106(d)	0.25	-	0.154	0.083
	n	12	3	29	26
LDH-1*	100(a)	0.927	0.75	0.769	0.875
	102(b)	0.073	-	0.231	0.125
	104(c)	-	0.25	-	-
	n	12	3	29	26
LDH-2*	100(a)	-	0.964	-	-
	102(b)	-	0.036	-	-
	n 2	12	3	29	26
LDH-3*	100 (a)	-	-	0.973	-
	102 (b)	-	-	0.027	-
	n	12	3	29	26
PGM-1*	100(a)	0.968	0.875	0.95	0.972
	102(b)	0.032	0.125	0.05	0.28
	n	12	3	29	26
PGM -2*	98(a)	0.075	0.025	0.168	0.005
	100(b)	0.925	0.975	0.832	0.930
	102(c)	-	-	-	0.065
	n	12	3	29	26
PGM -3*	98(a)	-	0.925	0.90	-
	100(b)	-	0.075	0.10	-
	n	12	3	29	26
SOD*	100(a)	0.908	0.875	0.925	0.918
	102(b)	-	0.024	0.075	0.082
	104(c)	0.092	0.101	-	-
	n	12	3	29	26

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Table 2. Alleles present at each locus, given as letters in alphabetic order according to their anodal mobility '*a*' representing the fastest migrating. Diagnostic alleles are underlined

Locus	*fulvus*	*leminscatus*	*lutjanus*	*madras*
ADH*	*ad*	*abc*	*ad*	*acd*
LDH-1*	*ab*	*ac*	*ab*	*ab*
LDH-2*	-	*ab*	-	-
LDH-3*	-	-	*ab*	-
PGM -1*	*ab*	*ab*	*ab*	*ab*
PGM -2*	*ab*	*ab*	*ab*	*abc*
PGM -3*	-	*ab*	*ab*	-
SOD*	*ac*	*abc*	*ab*	*ab*

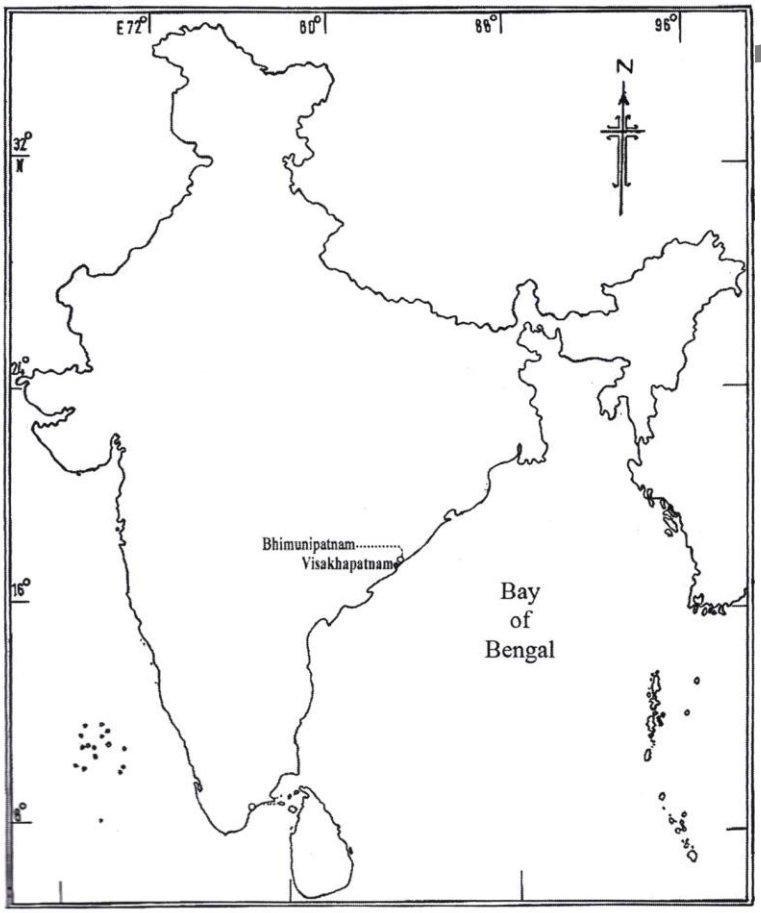

Figure 1. Outline map of India showing sampling station

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Figure 2. *Lutjanus fulvus* , 164 mm, TL.

Figure 3. *Lutjanus lemniscatus*, 296 mm TL.

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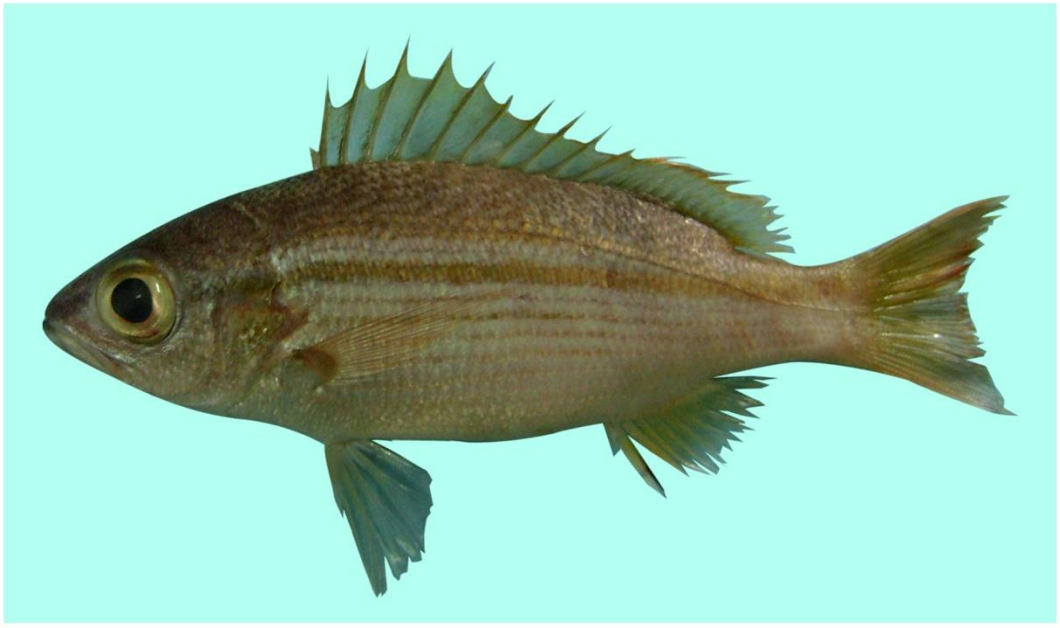

Figure 4. *Lutjanus lutjanus* , 203 mm, TL

Figure 5. *Lutjanus madras*, 211 mm, TL

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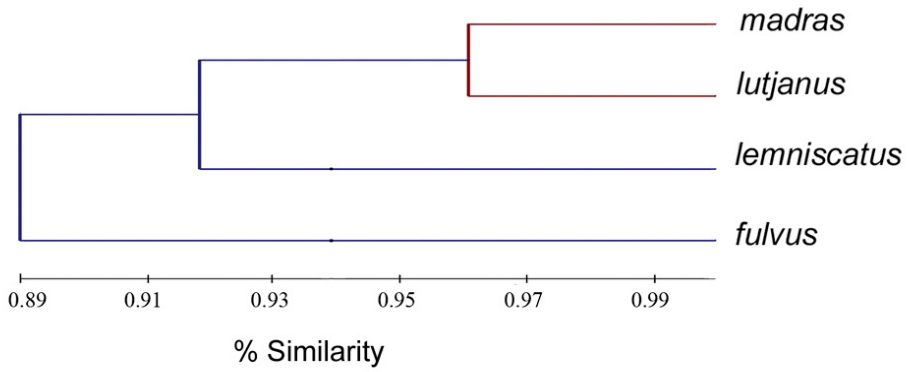

Figure 6. UPGMA dendrogram showing hierarchic relationship between four species of genus *Lutjanus*

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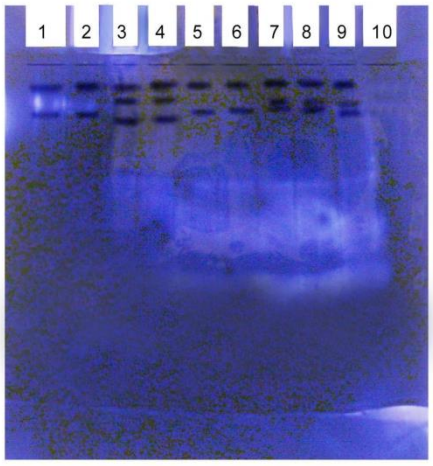

Fig. 7. Alcohol dehydrogenase

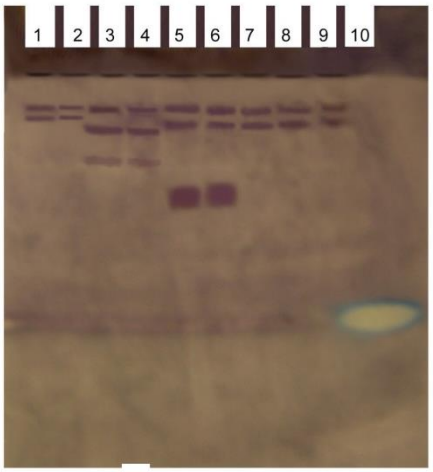

Fig. 8. Lactate dehydrogenase

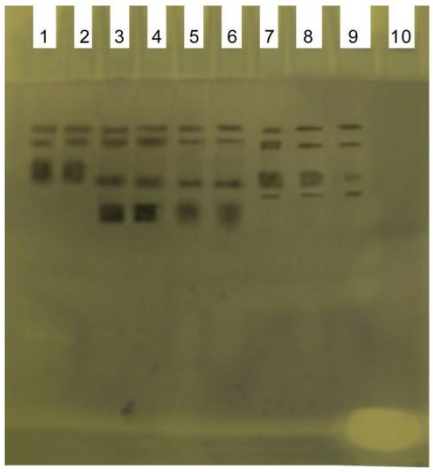

Fig. 9. Phosphoglucomutase

Fig. 10. Superoxide Dismutase

Fig. 7-10. Allozyme banding pattern in four species of genus *Lutjanus*

1, 2 - *fulvus*; 3, 4 - *lemniscatus*; 5, 6 - *lutjanus*; 7 to 9 -*madras*; 10 - tracking dye

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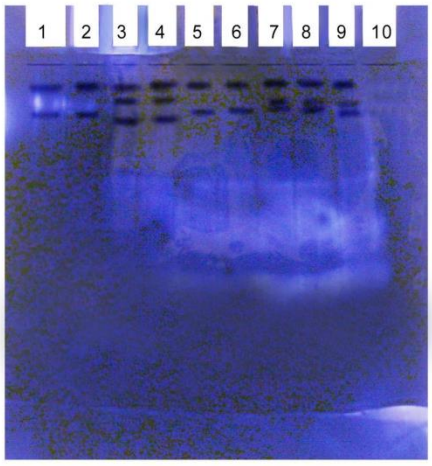

Fig. 7. Alcohol dehydrogenase

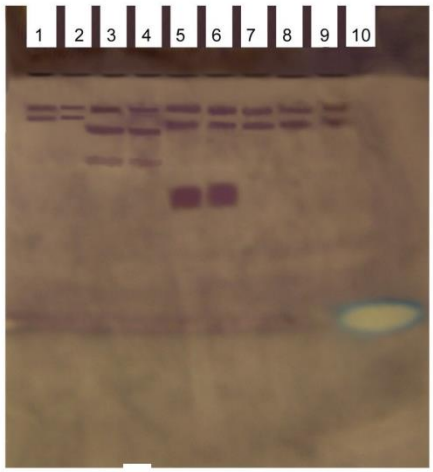

Fig. 8. Lactate dehydrogenase

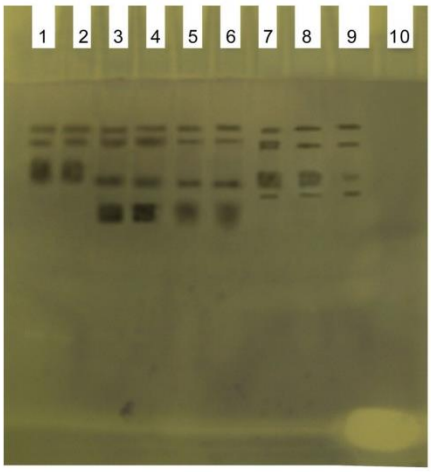

Fig. 9 . Phosphoglucomutase

Fig.10. Superoxide Dismutase

Fig. 7-10 . Allozyme banding pattern in four species of genus *Lutjanus*

1, 2 - *fulvus*; 3, 4 - *lemniscatus*; 5, 6 - *lutjanus*; 7 to 9 -*madras*; 10 - tracking dye