



Genetic Relationship of Snappers (Family: Lutjanidae) from Indian Waters Using SDS-PAGE Technique

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Abstract

Snappers belonging to the family Lutjanidae, genus *Lutjanus*, *Pinjalo* and *Pristipomoides* have overlapping colour patterns. Twelve species as *L. argentimaculatus*, *L. fulviflamma*, *L. fulvus*, *L. johnii*, *L. lemniscatus*, *L. lutjanus*, *L. madras*, *L. quinquelineatus*, *L. rivulatus*, *L. russelli*, *Pinjalo pinjalo* and *Pristipomoides typus* were collected from the Visakhapatnam fishing harbour, India. The present study is an attempt to evaluate the genetic diversity in the species of snappers by using Sodium Dodecyl Sulfate Polyacrylamide (SDS-PAGE) gel electrophoresis. Proteins were extracted from muscle tissue. The molecular weights of protein bands were observed and its range was from 5 to 226 KDA. Relative mobility values range from 0.014 to 0.959 in twelve species. UPGMA dendrogram formed revealed two major clusters.

Keywords: Visakhapatnam Coast, India, snapper fisheries, UPGMA, genetic analysis.

Introduction

Fishes of the family Lutjanidae is one of the largest in the order perciformes and comprises 4 subfamilies, 17 genera and 112 species, mainly found on coral reefs in tropical and subtropical regions of the Atlantic and Indo-Pacific (Froese & Pauly 2016). The family is divided into four subfamilies. The largest subfamily is Lutjaninae with six genera *Hoplopagrus*, *Lutjanus*, *Macolor*, *Ocyurus*, *Pinjalo* and *Rhomoblites* with about 72 species. The genus *Lutjanus* has about 64 species (Nelson, 2006). Lutjanidae are found in tropical waters around the globe and are often associated with reef habitats (Kotthaus, 1974; Fischer & Bianchi, 1984; Allen, 1985; Randall, Allen, & Anderson, 1987). Most lutjanids live above 100 m near coral reefs, although some species are found in deeper waters to 500 m. Tropical snappers are active predators and feed mostly at night (Bray, 2012). Snappers are one of the most important groups of the tropical marine fishes for aquaculture, due to their fast growth and high market demand. Species like snappers and groupers are suitable for mariculture (James, Murthy, & Nammalvar, 1996). Taxonomy is the foundation of traditional conservation practices (Avisé, 1989; Brophy, 2004).

Protein electrophoresis is a technique used for the study of species genetic structure and the

determination of phylogenetic relationships (Focant, Jacob, & Huriaux, 1981 and Pineiro, Vazquez, Marina, Barros Velazquez, & Gallardo, 2001). Soluble proteins of muscle sarcoplasm are among the easiest to extract and highly a rich reservoir of species specific and biochemical genetic markers (Tsuyuki *et al.*, 1965, O'Rourke, 1974; Ryman, Utter, & Laikre, 1995; Ryman & Utter 1987; Buth and Murphy 1999). Innovative studies are necessary for further species-specific identification of early juvenile stages (Richards *et al.*, 1994). Gel electrophoresis has been used in defining genetic markers for closely related species based on the differences in allele frequencies between them (Lundstrom, 1980; An, Marshall, Otwel., & Wei, 1988) utilized various electrophoresis support matrixes to successfully spate fish muscle proteins for identification. Somatic chromosomes from gill epithelia of commercially important marine fish species and germinal chromosomes from testes of two of the have been studied (Choudhury, Prasad, & Das, 1979). Chow and Walsh (1992) proposed a relationship between *L. analis* and *L. vivanus* and the summary compilation by (Richards *et al.*, 1994) added three genera and four species to the list of known snappers. Lee and Cheng (1996), Ovenden, Salini, O'conor, and Street, (2004), Zhang *et al.*, (2004), Rosmilah, Shahnaz, Masita, and Noormalin, (2005), Jongjareonrak, Benjakul, Visessanguan, Nagai, and Tanaka, (2005), Guo, Wang, Liu, Liu, and Liu,

(2007), Yılmaz, Ramazan, and Ali (2007), Nirchio *et al.*, (2008) and Rocha and Molina (2008) have provided evidence of the sub-structuring of snappers. Vasconcellos, Viana, Paiva, Schama, and Sole-Cava (2008) based on morphometric, allozymes and mitochondrial DNA (Control region) analysis identified a single Brazilian stock, revealing significant levels of genetic sub-structuring between populations from Belize and Brazil. Sulaiman, Abdul Rahman, Ying, Taha, and Muhdtaha, (2008) investigate the *L. malabaricus* genetic population structure of red snapper and groupers species in Brunei. Klangnurak, Phinchongsakuldit, and James, (2012) demonstrate potential population genetics differences that may imply the existence of hitherto unsuspected barriers between the Gulf of Thailand and populations within the Andaman Sea, which has important consequences for stock management of a vital food fish. Govinda Rao, Krishna, and Sujatha (2014), Krishna, Rao, and Venu, (2015) and Govinda Rao, Krishna, and Sujatha (2017) studied length weight relationship of two snappers species, intertidal fish diversity of fish species as well as allozyme studies were done from Visakhapatnam coastal waters, east coast of India.

So far no attempt has been made to analyze the genetic structure of snappers from Indian waters. The present study investigate the feasibility of using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and analyses of protein profiles for identification of twelve snapper species presented in the catches of Visakhapatnam. Precise species determination of wild snappers has a significant value for sustainable management and conservation of its stocks.

Materials and Methods

Sample Collection

The total of 128 fresh specimens of all size groups of 12 species *Lutjanus*, *Pinjalo* and *Pristipomides* *L. argentimaculatus* (Forsskal, 1775), *L. fulviflamma* (Forsskal, 1775), *L. fulvus* (Forster, 1801), *L. johnii* (Bloch, 1792), *L. lemniscatus* (Valenciennes, 1828), *L. lutjanus* (Bloch, 1790), *L. madras* (Valenciennes, 1831), *L. quinquelineatus* (Bloch, 1790), *L. rivulatus* (Cuvier, 1828), *L. russelli* (Bleeker, 1849), *Pinjalo pinjalo* (Bleeker, 1850) and *Pristipomides typus* Bleeker, 1852 were collected from Visakhapatnam fishing harbour, east coast of India during January 2013 to December 2015. Morphological identification of specimens was done by taxonomic characters such as body and fins colour, the presence or absence of scales on the cheek, number of dorsal, anal, pelvic spines and soft rays of pectoral, dorsal, ventral and caudal fin (Allen, 1985). The length, weight and sex of each specimen were noted and immediately brought to the laboratory in an insulated ice box.

Protein Extraction and SDS-PAGE:

A piece of muscle tissue (125 mg) was homogenized with 1 ml of chilled extraction buffer, and the sample was centrifuged at 10,000 rpm at 4°C for one hour. Supernatant was collected and used as protein source. Methodology for protein extraction, casting of gel was performed according to (Laemmli, 1970). After running gel was stained and the position of the protein band in the gel was expressed to compare with standard protein markers with known molecular weight. The banding pattern obtained was subjected to cluster analysis using XLSTAT software. Dendrograms and similarity matrices were obtained by Unweighted Pair Group Method (UPGMA) method by using NTSYS pc software.

Results

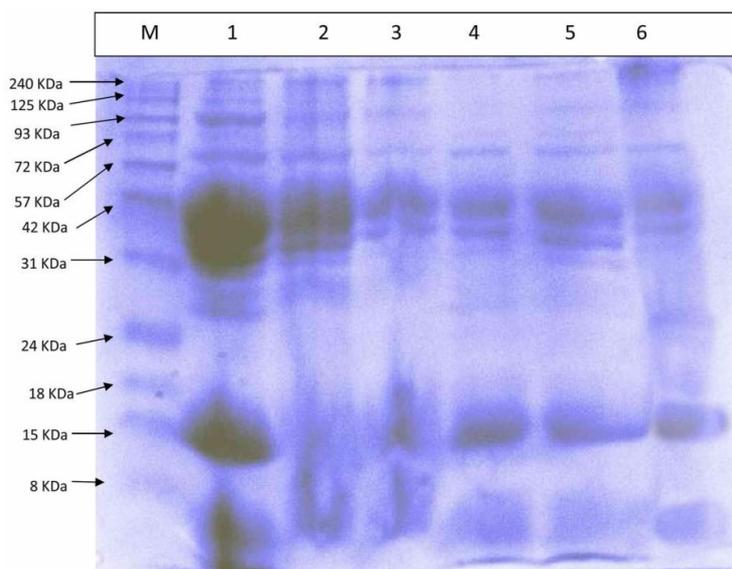
A total of 131 bands were observed, the highest number of (17) bands was observed in *L. argentimaculatus*, followed by 14 bands in *L. russellii*, 13 bands in *L. fulviflamma* and *L. lemniscatus*; 12 bands in *L. quinquelineatus* and *L. rivulatus*, 11 bands in *P. typus*, 10 bands found both in *L. Fulvus* and *L. madras*, 10 bands (10) in *P. pinjalo* while the least number bands (9) in *L. johnii* (Table 1).

In *Lutjanus argentimaculatus* the Rf values ranges from 0.014 to 0.959 and the molecular weight ranges from 5 to 226 KDa. 178 KDa MW band was unique to *L. argentimaculatus* (Figure 1). In *L. fulviflamma* the Rf values ranges from 0.014 to 0.893 and the molecular weight ranges from 7 to 226 KDa. The Rf values ranges from 0.014 to 0.893 and the molecular weight ranges from 7 to 226 KDa for in *L. fulvus*. A common 14 KDa molecular weight band was observed in *L. fulviflamma* and *L. fulvus* but it was not found in other species. In *L. johnii* the Rf values ranges from 0.130 to 0.893 and the molecular weight ranges from 7 to 64 KDa. In *L. lemniscatus* the Rf values ranges from 0.014 to 0.893 and the molecular weight ranges from 7 to 226 KDa. A species specific 178 KDa molecular weight band was observed in this species. In *L. lutjanus* the Rf values ranges from 0.014 to 0.893 and the molecular weight ranges from 7 to 226 KDa. In *L. madras* the Rf values ranges from 0.014 to 0.893 and the molecular weight ranges from 7 to 226 KDa (Figure 2). In *L. quinquelineatus* the Rf values ranges from 0.014 to 0.893 and the molecular weight ranges from 7 to 226 KDa. In *L. rivulatus*. The Rf values ranges from 0.014 to 0.893 and the molecular weight ranges from 7 to 226 KDa. In *L. russellii*. The Rf values ranges from 0.051 to 0.959 and the molecular weight ranges from 5 to 141 KDa. In *Pinjalo pinjalo* and *Pristipomides typus* all most all bands and banding pattern were same, the Rf values ranges from 0.13 to 0.893 and the molecular weight ranges from 7 to 64 KDa. High molecular weight bands were absent in *L. russellii*,

Table 1. General protein banding pattern based on Rf values and on molecular weights of snappers represented in the catches of Visakhapatnam

Band No	RF	MW	<i>L. argentimaculatus</i>	<i>L. fulviflamma</i>	<i>L. fulvus</i>	<i>L. johnii</i>	<i>L. lemniscatus</i>	<i>L. lutjanus</i>	<i>L. madras</i>	<i>L. quinquelineatus</i>	<i>L. rivulatus</i>	<i>L. russellii</i>	<i>P. pinjalo</i>	<i>P. typus</i>
1	0.014	226	+	+	+	-	+	+	+	+	+	-	-	-
2	0.032	178	+	-	-	-	-	-	-	-	-	-	-	-
3	0.051	141	+	+	+	-	+	+	+	+	+	+	-	-
4	0.089	98	+	-	-	-	+	-	-	-	+	-	-	-
5	0.13	64	+	+	+	+	+	+	+	+	+	+	+	+
6	0.165	60	+	+	+	+	+	+	+	+	+	+	+	+
7	0.224	55	+	+	+	+	+	+	+	+	+	+	+	+
8	0.262	44	+	+	+	+	+	+	+	+	+	+	+	+
9	0.303	40	+	+	-	-	+	+	+	+	+	+	+	+
10	0.349	38	+	-	-	-	-	-	-	-	-	+	-	+
11	0.397	35	+	+	-	+	+	+	+	+	+	+	+	+
12	0.441	33	+	+	-	+	+	+	+	+	+	+	+	+
13	0.566	31	-	-	-	-	-	+	-	-	-	-	-	-
14	0.595	30	+	+	+	+	+	-	-	+	-	+	+	+
15	0.670	28	+	+	+	+	+	-	-	+	+	+	+	+
16	0.711	26	+	-	-	-	-	-	-	-	-	+	-	-
17	0.759	14	-	+	+	-	-	-	-	-	-	-	-	-
18	0.893	7	+	+	+	+	+	+	+	+	+	+	+	+
19	0.959	5	+	-	-	-	-	-	-	-	-	+	-	-

* (Rf, + as presence, - as absence)

**Figure 1.** Protein profiling of muscle tissue snappers: 1. *Lutjanus argentimaculatus*, 2. *Lutjanus fulviflamma*, 3. *Lutjanus fulvus*, 4. *Lutjanus johnii*, 5. *Lutjanus lemniscatus* 6. *Lutjanus lutjanus* and M-marker

Pinjalo pinjalo and *Pristipomoides typus* species.

The resemblance factor was considered on the basis of presence and absence of bands which ranged from 0.014 to 0.959, and a UPGMA dendrogram was constructed using the similarity coefficient (Figure 3). The clusters obtained from the dendrogram revealed that the twelve species of snapper's genus *Lutjanus*, *Pristipomoides* and *Pinjalo* were grouped into two clades. In the dendrogram (Figure 3) 2 major clusters (Cluster 1 and 2) were formed (cut 2 classes). The Cluster 2 had only one species i.e. *L.fulvus* and it is distant from all other species. On the other hand

cluster 1 is subdivided into 2 clusters, Cluster A and Cluster B. The cluster B has two species i.e. *L. russellii* and *L. argentimaculatus*, where the distance value is 0.093, which infers least sequence similarity, hence the highest distance in the entire tree. Among cut 3 classes cluster A is sub divided into two sub clusters i.e. cluster A1 and A2, where cluster A2 is again bifurcated in to two with the distance value of 0.07. At 0.18 length point one of the branches of cluster A2 is further subdivided in to *P.pinjalo* and *P.typus* with the distance value 0.062. Cluster A1 after subdividing thrice finally ended with three sub

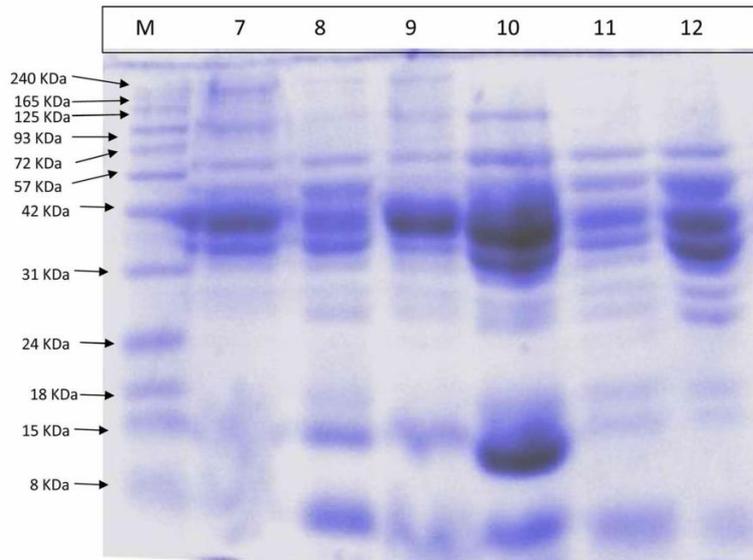


Figure 2. Protein profiling of muscle tissue snappers: 7. *Lutjanus madras*, 8. *Lutjanus quinquelineatus*, 9. *Lutjanus rivulatus*, 10. *Lutjanus russellii*, 11. *Pinjalo pinjalo* 12. *Pristipomoides typus* and M-marker

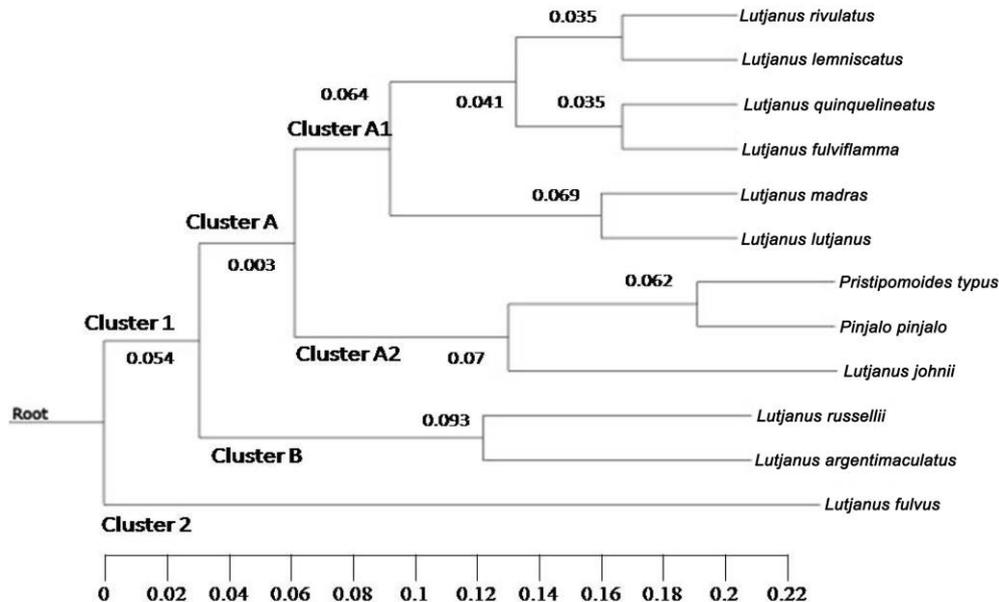


Figure 3. UPGMA dendrogram showing heirachic relationship between twelve species of snappers.

groups containing two species in each. The first including *L. rivulatus* and *L. lemniscatus* with 0.035 distance value, second including *L. quinquelineatus* and *L. fulviflamma* with 0.035 distance value and the third sub group contains *L. madras* and *L. lutjanus* with 0.069 distance value.

Discussion

As an aid to traditional taxonomic characters, biochemical methods have been used in systematics. In fisheries, these methods have been used to reveal cryptic species (Shaklee & Tamaru 1981; Smith, Roberts, & Hurst, 1981; Grant, Cherry, & Lombard,

1984) to resolve taxonomic problems (Smith & Robertson 1981; Waples, 1981; Graves, Simovich, & Schaefer, 1988 and Masuda, Ozawa, & Enami, 1989). The application of separation and structural studies of proteins to solve taxonomic problems has been discussed by (Alston & Turner 1963; Tsuyuki, Roberts, & Vanstone, 1965) in biochemical systematics. Studies on genetic variation at protein level led to major contributions in diverse arrays of biologically oriented disciplines (Utter, 1991). Proteins are considered as gene products and electrophoretic mobilities of different proteins in closely related species or in different populations can be genetically interpreted (Byer & Ponnaiah 1983).

Different electrophoretic techniques have been used to identify the differences among fish species and muscle protein is commonly used to assess the polymorphism among fish species (Haniffa *et al.*, 2017; Smith, 1990; Rashed *et al.*, 2000). The number of protein bands of these twelve species of lutjanids from 1 to 19 is investigated and results compared with the similar studies conducted by several researchers (Ramaseshiah & Dutt 1984; Huang, Marshall, & Wei, 1995; Rajagopalan, Abinaya, & Balasubramanian, 2013) and Govinda Rao *et al.*, 2017). The protein banding pattern in three genera *Lutjanus* and *Pinjalo*, *Pristipomoides*, shown much variation, but overall of the three genera appear to exhibit similar protein banding in the present study. Biochemical as well as genetic studies has been carried out by many scientists for the evaluation of genetic distances in varied fish species like *Sardinella* and four *Sciaenid* species from Arabian Sea (Huang *et al.*, 1995; Sarver, Freshwater, & Walsh, 1996; Zhang, Huang, & Huo, 2004; Jongjareonrak *et al.*, 2005; Rosmila *et al.*, 2005; Miller & Cribb, 2007). Isoelectric focusing (IEF), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and two dimensional (2-D) gel electrophoresis for species identification of red snapper (*Lutjanus campechanus*) was reported by Huang *et al.*, (2006). Genetic differences between yellowtail snapper populations of tropical West Atlantic using allozymes were studied by Vasconcellos (2008). Sivie, Retnoningrum, and Suhartono, (2015) and Varghese and Jayasankari (1999) studied the muscle myogen patterns of four carangids species using horizontal slab polyacrylamide gel electrophoresis. The electropherogram generated by SDS-PAGE showed difference both in the number of bands and the molecular weight of the sarcoplasmic proteins between two species *Orthiasinsignis euphraticus* and *Cyprinus macrostomus* (Yilmaz *et al.*, 2005). When the liver proteins of six species belonging to the family Cyprinidae were separated using SDS-PAGE, the smallest genetic distance between *Cyprinus carpio* and *Pseudogobius esocinus* was found. Protein differences between species are specific for individuals representing a group. This can elucidate taxonomic problems in the case of disputed species (Smith, Wood, & Benson, 1979). UPGMA dendrogram arrived by the biochemical markers revealed the closeness between the species. Based on the results *L. madras*, *L. lutjanus*, *L. quinquelineatus* and *L. fulviflamma*, *L. rivulatus* and *L. lemniscatus* are very closely related compared to other species. Another cluster *L. russeli* and *L. argentimaculatus* are closely related and then *P. typus* and *P. pinjalo* species are very closely related. *L. fulvis* distant from all other species. The result was in congruence with the opinion of Huang *et al.*, (2006) and Haniffa *et al.* (2014) investigated the phylogenetic relationship among five Channids namely *C. striatus*, *C. marulius*, *C. punctatus*, *C. diplogramme* and *C.*

gachua using ISSR-PCR marker system.

Conclusion

The present study is the first attempt to study the genetic relationships of twelve species using general muscle proteins. Based on the protein band pattern phylogenetic tree shows that the species analyzed are more or less closely related to each other. Future studies using biochemical-genetic markers and DNA barcoding hopefully will establish new ventures in the field of stock management and conservation of snappers.

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