Protein profiling for phylogenetic relationship in snakehead species

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Abstract

Protein banding pattern of eight snakeheads – *Channa* species viz., *Channa striatus, Channa marulius, Channa punctatus, Channa diplogramme, Channa bleheri*, *Channa gachua, Channa stewartii* and *Channa aurantimaculata* collected from different regions of India were used to study the phylogenetic relationship among them. The banding pattern from muscle protein indicated a unique profile for each species and the electrophoregrams showed similarities among the species studied. In the SDS-PAGE, a maximum of 12 protein bands were obtained for *C. gachua* followed by 11 for *C. diplogramme* and 10 for *C. marulius* whereas less number of bands were recorded for the remaining species. Molecular weight of the protein bands varied from 16 kDa - 232 kDa. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram revealed that the phylogenetic relationship was very close among *C. aurantimaculata* and *C. bleheri* and also between *C. gachua* and *C. stewarti*.

Keywords: Snakehead, Phylogenetic relation, SDS- PAGE, Protein banding

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Introduction

Genetic diversity/variation has vital importance in the understanding and management of individuals and populations. Application of electrophoresis and chromosomal techniques have significantly increased to observe genetic variation and have for many years been considered as the standard tool in genetic studies of wild and cultured fish stocks. Different molecular tagging and biochemical methods for species identification have been widely applied in fish since 1960 (Saad *et al*., 2002; Na-Nacron *et al*., 2004; Yilmaz *et al*., 2007) and proteins/enzymes are used as genetic markers as direct products of gene action (Crick, 1963; Nirenberg *et al*., 1963). Gene controlled proteins form the structural basic source of genetic information at various levels of species organization. Due to the existence of morphological plasticity among individuals, conventional morphological characters are often found to be deceiving in the exact detection of a species (Menon, 1989). Electrophoretic techniques have been found to be useful in studying problems involving taxonomic ranks, and solving taxonomic ambiguity and synonymous confusion. No doubt, genetic markers are superior to artificial markers and tags as they are natural and can be applied to all stages of animals (Kapila and Kapila, 1996). In SDS-PAGE, protein is separated according to its molecular weight. Resolution of this technique is very high and therefore it could be used as a reliable tool for taxonomic purposes (Bartke *et al.*, 1966).

Snakeheads, commonly called as murrels are obligatory air-breathing and precious edible freshwater fish that reside in swamps, slow-flowing streams and in crevices near riverbanks. Regarding taxonomy, they belong to the family Ophiocephalidae/ Channidae (Qasim, 1966). Taxonomy of these fishes is in flux, but leading authorities on snakehead systematics currently recognized 26 species of Channa and 3 of Parachanna (Haniffa, 2010). Taxonomical mystery and synonymous confusion are more common in snakeheads rather than in other fishes. In this regard, Allen *et al.* (2011) solved the 146 year old taxonomic puzzle of *Channa diplogramme* and *C. micropeltes* and finally, the resurrection of *C. diplogramme* from the synonymy of *C. micropeltes* has been confirmed 146 years after its initial description and 134 years after it was synonymised. Within parts of their native ranges, some species of snakeheads are highly valued as food fishes (*C. striatus, C. marulius, C. punctatus* and *C. diplogramme*), whereas others are ornamental in Southeast Asia (*C. gachua, C. bleheri, C. stewarti* and *C. aurantimaculata*) because of their beautiful colouration.

In the present study, an attempt was made to detect the diversity of eight different snakehead species collected from different locations of India viz: *C. aurantimaculata* (Musikasinthorn, 2000), *C. bleheri* (Vierke, 1991), *C. gachua* (Hamilton, 1822), *C. marulius* (Hamilton, 1822), *C. diplogramme* (Cuvier, 1831), *C. punctatus* (Bloch, 1793), *C. stewartii* (Playfair, 1867) and *C. striatus* (Bloch, 1797) using electrophoresis technique– SDS-PAGE, as a basic tool for their protein identification.

Materials and methods

Preparation of snakehead fish muscle tissue

Live samples of snakeheads viz: *C.striatus* (river Periyar, 10º10' N, 76º13'E, Kerala), *C. punctatus* (river Tamirabarani, 80º44' N 77º44' E, Tamil Nadu), *C. marulius* (Bhavanisagar Dam, 11º58' N 77º58' E, Tamilnadu), *C. diplogramme (* river Pampa, 9º27' N 6º78' E, Kerala) and *C. aurantimaculata*, *C. bleheri* and *C. stewartii* (river Brahmaputra, 24º8' N 89º42' E, Assam) were collected from different geographical locations (Fig. 1). The muscle tissue of the fish was sliced into smaller pieces and kept at -20°C prior to freeze-drying. Freeze dried tissue was homogenized to a powder form. Extraction of protein from fish muscle tissue was carried out following Lay-Harn Gam *et al*. (2006). Briefly, 1.0 mg of powdered fish muscle was extracted using 1 ml of 40 mM Tris (pH 8.8), vortexed and centrifuged at 12,000 rpm for 30 minutes at 4ºC and the supernatant was recovered.

Sodium dodecyl sulphate-polyacrlyamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed as described by Laemmli *et al.* (1970) in 10% Polyacrylamide gel. Protein samples were then loaded and electrophoresis was performed at a constant voltage of 200 - 245 vs. The run was stopped when the dye front was 2 to 3 mm away from the bottom edge of the gel. On the completion of electrophoresis, the glass sandwich was disassembled. The stacking gel was discarded and the resolving gel was stained using Coomassie Blue. Molecular weights of the proteins were determined by comparing relative mobility of protein bands to the standard protein markers. The similarity index was calculated as 'Similarity Index (*S*) = Number of common bands×2/Total number of bands in both samples', according to Lamont *et al*. (1986). The NTSYS and TL 100 (Total Lab 100) software were used for the calculations and analysis. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram was constructed using the similarity coefficient.

Results

In the present study, protein isolated from the tissue of eight different *Channa* sp. was subjected to SDS-PAGE and analysed using TL 100 software (Fig. 2).

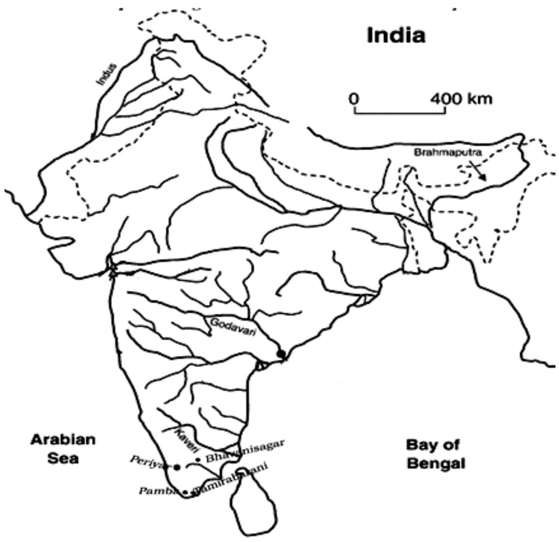

Figure 1: Map showing the collection sites of *Channa* sp.

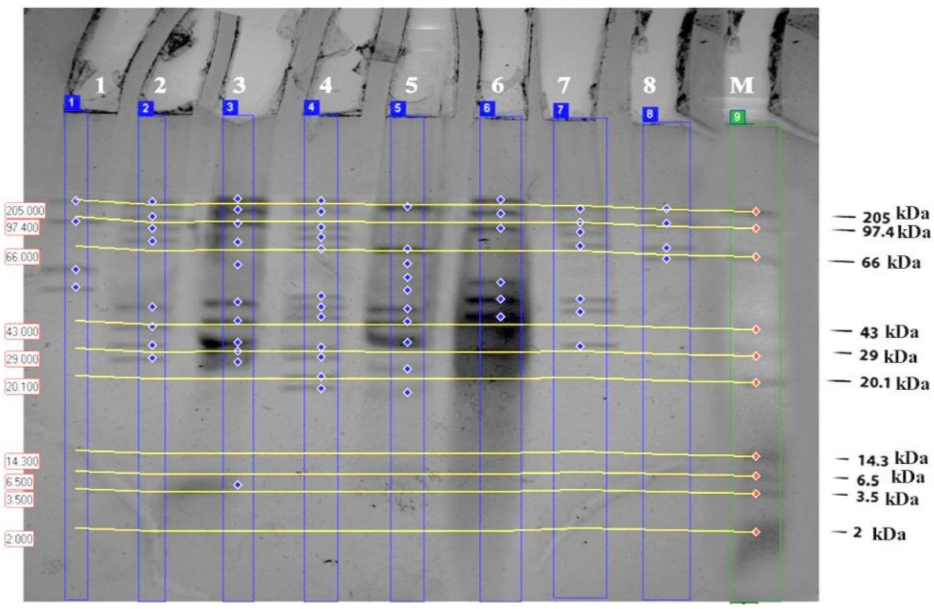

Figure 2: Protein profiling of muscle tissue of eight Indian *Channa* sp.
Note: 1. *Channa aurantimaculata,* 2. *Channa bleheri,* 3. *Channa diplogramme,* 4. *Channa gachua,* 5. *Channa marulius,* 6. *Channa punctatus,* 7. *Channa striatus,* 8. *Channa stewartii* and M- Protein Marker

A total of 62 bands were observed; the highest number of (12) bands was found in *C. gachua,* followed by 11 bands in *C. diplogramme* and 10 bands in *C. marulius,* while the least number of bands (4) was found both in *C. stewartii* and *C. aurantimaculata*. The total number of bands for each *Channa* species has been recorded in Table 1. Using Total Lab 100 protein analyzer software the molecular weight and the Rf value of each band were calculated. The molecular weights of proteins ranging from 16 kDa to 232 kDa were recorded in Table 1. The least molecular weight protein (16.64 kDa) was recorded in *C. diplogramme* with the highest Rf value 0.76 whereas, the highest molecular weight protein (232.65 kDa) was found in *C. punctatus* with the lowest Rf value of 0.17 (Table 1).

NTSYS pc software was used to analyze the phylogenetic relationship based on protein banding pattern. The similarity coefficient was calculated on the basis of presence and absence of bands which ranged from 0.25–0.80, and a UPGMA dendrogram was constructed using the similarity coefficient (Fig. 3). The clusters obtained from the dendrogram showed that the eight species of *Channa* were grouped into two clades C_1 and C_2 with a similarity coefficient of 35% holding *C. striatus* in a separate clade. C_1 were again divided into two sub-clades C_1S_1 and C_1S_2 with a similarity coefficient of 44%.The C_1S_1 clade holds *C. aurantimaculata* and *C. bleheri* with a similarity of 80%.

The C_1S_2 clade subdivides into C_1S_2a and C_1S_2b with a similarity of 56%. The C_1S_2a clade further divides into $C_1S_2a_1$ and $C_1S_2a_2$. The $C_1S_2a_1$ divides into two clades holding *C. gachua* and *C. stewarti* with a similarity of 80% in the upper clade and *C. diplogramme* with a similarity of 66% in the lower clade. Among the eight species, *C. aurantimaculata* and *C. bleheri* and *C. gachua* and *C. stewarti* shares more similarity between them.

Discussion

Different electrophoretic techniques have been conducted to identify the differences among fish species all over the world and muscle protein is commonly used to assess the polymorphism among fish species (Smith, 1990; Rashed *et al*., 2000). The electrophoretic techniques used for separation of proteins have their own limitations as well. However, biochemical as well as genetic studies has been carried out by many Indian scientists for the evaluation of genetic distances in varied fish species like mullet, *Mugil cephalus* (Vijayakumar, 1992); oil sardine, *Sardinella longiceps* (Venkita Krishnan, 1992) and *Lactarius lactarius.* Huang *et al.* (2006) used 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*). 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 of *Orthrias insignis euphyraticus* and *Cyprinion macrostomus* (Yilmaz *et al.*, 2005). Similarly, when the liver proteins of six species belonging to the Cyprinidae family, Acheilognathinae, Leuciscinae and Gobioninae subfamilies were separated using SDS-PAGE, *Cyprinus carpio* and *Pseudogobius esocinus* showed the smallest genetic distance.

Table 1: Molecular weights and Rf values of different *Channa* sp.

Ref Band No.	*C. aurantimaculata*		*C. bleheri*		*C. diplogramme*		*C. gachua*		*C. marulius*		*C. punctatus*		*C.striatus*		*C. stewarti*	
	MW (kDa)	Rf	MW (kDa)	Rf	MW (kDa)	Rf	MW (kDa)	Rf	MW (kDa)	Rf	MW (kDa)	Rf	MW (kDa)	Rf	MW (kDa)	Rf
1	191.16	0.18	217.96	0.17	232.76	0.17	218.04	0.17	185.45	0.18	232.65	0.17	179.10	0.18	201.06	0.18
2	-	-	-	-	163.26	0.19	153.27	0.19	-	-	148.56	0.20	-	-	-	-
3	-	-	137.44	0.20	107.71	0.22	-	-	-	-	-	-	116.17	0.21	125.68	0.21
4	100.18	0.22	95.80	0.23	-	-	95.72	0.23	-	-	95.80	0.23	85.88	0.23	-	-
5	-	-	66.71	0.25	63.23	0.26	72.22	0.25	-	-	-	-	59.08	0.26	61.52	0.26
6	-	-	-	-	-	-	53.68	0.27	55.08	0.27	-	-	-	-	46.78	0.28
7	-	-	-	-	37.70	0.30	-	-	39.29	0.30	-	-	-	-	-	-
8	31.36	0.32	-	-	-	-	-	-	30.71	0.33	28.43	0.34	-	-	-	-
9	24.56	0.36	-	-	22.25	0.38	23.57	0.37	25.53	0.35	23.10	0.37	22.78	0.37	-	-
10	-	-	21.60	0.39	-	-	21.42	0.39	21.26	0.39	-	-	20.57	0.40	-	-
11	-	-	19.07	0.43	19.56	0.42	20.00	0.41	19.55	0.42	20.11	0.41	-	-	-	-
12	-	-	17.89	0.47	18.00	0.46	17.78	0.47	18.03	0.46	-	-	17.81	0.47	-	-
13	-	-	-	-	17.63	0.48	-	-	-	-	-	-	-	-	-	-
14	-	-	17.42	0.49	17.31	0.50	17.44	0.49	17.17	0.52	-	-	-	-	-	-
15	-	-	-	-	-	-	17.04	0.53	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	16.90	0.56	16.85	0.57	-	-	-	-	-	-
17	-	-	-	-	16.64	0.76	-	-	-	-	-	-	-	-	-	-

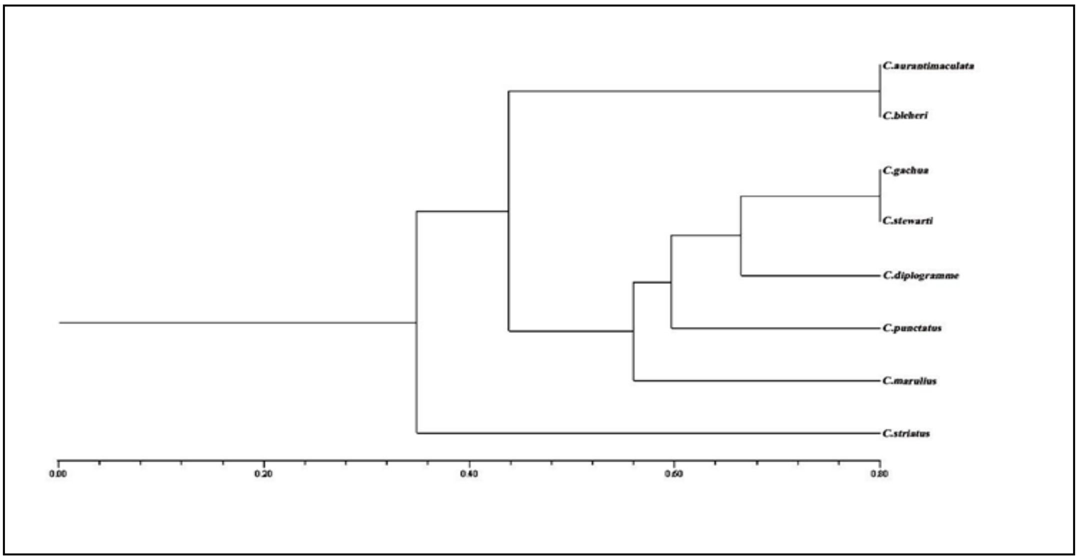

Figure 3: Dendrogram representing the inferred relationships of genetic similarity among the eight Indian *Channa* sp.

In another investigation, the serum proteins of the female *Cyprinus carpio* and male *Ctenopharyngodon idella* were analysed using SDS-PAGE and it was stated that there were differences in the electropherogram of each species (Li, 1991). In another research, the serum protein profiles of parr- smolt in masu salmon (*Oncorhynchus masou*) was analysed by two-dimensional SDS-PAGE which identified two proteins (43.80 kDa) as possible smolt-specific serum proteins that was confirmed using 2D SDS-PAGE (Ura *et al.*, 1994). UPGMA dendrogram generated from the RAPD data by Ajaz Ali *et al.* (2011)

stated that genetic relationship was very close between *C. aurantimaculata* and *C. bleheri, C. gachua* and *C. stewarti*, which was in accordance with our results. They also showed that, *C. marulius, C. striatus* and *C. diplogramme* as separate clades and were found to be genetically distant as reported in the present study.

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 and Principal Component Analysis (PCA) of morphometric and meristic characters. The genetic identity between the species ranged from 0.5526 to 0.7632 and the genetic distance ranged from 0.2703 to 0.5931. UPGMA dendrogram arrived by the morphological and molecular markers revealed the closeness between *C. striatus* and *C. marulius* among the five species whereas in the present study, both *C. striatus* and *C. marulius* were found in two different clades. Future studies using other biochemical as well as genetic markers like 2D gel electrophoresis and isoelectric focusing (IEF), hopefully will establish new ventures in the field of stock management and conservation of snakehead species.

In general, it can be concluded that all the eight species had varied differences in their protein banding pattern. Based on their protein banding pattern, the phylogenetic tree constructed shows that the species are more or less closely related to each other. Further analysis using molecular markers will help to understand the genetic variability of Channa species.

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