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Morphometric, meristic and ISSR marker systems for species identification and evolutionary analysis in five Indian Channids

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ABSTRACT

Snakehead species belonging to Channidae are primary group of freshwater air breathing fishes having their confined distribution in African and Asian continents. ISSR – PCR was used to investigate the phylogenetic relationship among five Channidae species viz. *Channa striatus*, *Channa marulius*, *Channa punctatus*, *Channa diplogramme* and *Channa gachua*. In addition, morphometric and meristic characters were subjected to principal component analysis (PCA) and the bootstrap values within the species were also calculated. The genetic identity between the species ranged from 0.5526 to 0.7632 and the genetic distance ranged from 0.2703 to 0.5931. The Nei's gene diversity (*H*) was calculated as 0.2653 and the Shannon's information index (*I*) was 0.3842. UPGMA dendrogram arrived by the morphological and molecular markers revealed the closeness between *C. striatus* and *C. marulius* among the five species.

1. Introduction

Snakeheads commonly called murrels belong to the genus *Channa* and family Channidae. These are economically important air breathing freshwater food fishes. They have flattened heads; possess large scales on their heads and eyes being located in the dorso-ventral position on the anterior part of the head, resembling snake. The peculiar morphological features are elongated cylindrical body with dorsal and anal fins long and entirely soft-rayed, a large mouth with well-developed teeth on both upper and lower jaws, and suprabranchial organ, an accessory air-breathing apparatus. Channidae consists of two genera viz., *Channa* and *Parachanna*; *Channa* comprises more than 28-30 species in the global scenario but with taxonomic ambiguity/mystery as well as synonyms confusion. About 10 species of *Channa* have been reported in India based on morphometric and meristic characteristics (Haniffa, 2010).

Morphometric variation is being primarily used to differentiate fish species (Cadrin, 2000). Geometric morphometrics and molecular techniques have become major tools for systematic ichthyologists and fish biologists for ratification of taxonomic

Abbreviation: DNA, Deoxyribo Nucleic Acid; ISSR, Inter-Simple Sequence Repeats; PCA, Principal Components Analysis; PCR, Polymerase Chain Reaction; PAST, Paleontological Statistics Software; RAPD, Randomly Amplified Polymorphic DNA; UPGMA, Unweighed Pair Group Method with Arithmetic Average.

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problems at species and population levels (Ciftci and Okumus, 2002; Povh et al., 2008). Inter-Simple Sequence Repeat (ISSR) is a Polymerase Chain Reaction (PCR) based technique that amplifies sequences between adjacent, inversely oriented microsatellites using an ISSR-containing primer. It has been widely used for studying the genetic background of plant species. However, reports concerning usage of ISSR markers in fish studies are rare.

So far snakeheads have been identified conventionally based on morphological and anatomical characters. However, there are ambiguities due to morphological closeness and changing color patterns from juvenile to adult stage. In spite of their economic and scientific importance to date, only limited information is available on the extent of molecular genetic variation among these species (Ajaz et al., 2011), and therefore, it is important to discriminate the snakehead species by molecular techniques especially by employing genetic markers along with the available morphological tools (Adamson et al., 2010; Allen et al., 2011; Xia et al., 2006). Population genetic structure of snakeheads was studied using morphometric, meristic and molecular analyses earlier (Xia et al., 2006; Adamson et al., 2010; Ajaz et al., 2011; Allen et al., 2011; Jamaluddin et al., 2011; Song et al., 2013) showing congruence and variability of genetic data. The present study was attempted to investigate and compare the morphological and genetic identity and variation among five channid species, *Channa striatus*, *Channa marulius*, *Channa punctatus*, *Channa diplogramme* and *Channa gachua* using ISSR markers.

2. Materials and methods

2.1. Sample collection

Live snakehead samples of *C. striatus*, *C. punctatus* and *C. gachua* were collected from the river Tamiraparani, Tamil Nadu (10°0.10′N, 76°0.13′E). *C. marulius* was collected from Bhavanisagar Dam (80°0.44′N 77°0.58′E) Tamil Nadu and *C. diplogramme* was collected from river Pampa (9°0.27′N 76°0.78′E) Kerala and transferred to CARE Aquafarm, Tamil Nadu.

2.2. Morphometric study

Fishes were identified in the field and then preserved in 10% formalin for morphometric examination. Larger specimens were injected with 15% formalin carefully through the vent prior to preservation. The meristic and morphometric parameters were calculated following Howes and Teugles (1989). The morphometric measurements were taken using an electronic caliper and calculated using TPS software as described by Rohlf (1989). The Specimen was placed on a clean white sheet, and a camera, connected to a monitor, to capture and store the image on monitor screen for viewing inter landmark distances, following the method of Gatz (1979) and Hubbs and Lagler (1958).

For morphological study, a total of 41 morphometric and meristic characters were taken (data not shown), of these 19 measurements were made using digital caliper (nearest to 0.1 mm) and eight characters were taken by truss network method where each character measures the distance between the selected landmarks or coordinates. All geometric measurements were transferred to an excel spread sheet file and each specimen, the *x* and *y* uniform components were computed using TPS (Rohlf and Marcus, 1993). The uniform component refers as affine expressing the shape variation. The first uniform component accounts to stretching along *x* axis of the configuration whereas the second uniform component indicates dilations or compressions along *y* axis. In the present study, *x* axis corresponds to the anterio-posterior axis, and *y* axis corresponds to the dorso-ventral axis of the fish bodies.

2.3. DNA extraction

Genomic DNA was extracted from fin clips using the modified method of Miller et al. (1988). Quantification of isolated DNA was done spectrophotometrically and the quality was checked using electrophoresis on 1% agarose gel. The isolated genomic DNA was diluted to 30 ng/ml and stored at −20 °C for further use.

2.4. ISSR analysis

PCR-amplification was carried out in reaction mixture containing 2.5 μl of 10× PCR buffer, 1.5 μl of 25 mM $MgCl_2$, 4 μl of 10 mM dNTP, 0.1 μl Taq DNA polymerase (Bangalore Genie, India), 0.5 μl primer, 0.5 μl genomic DNA and 15.9 μl Distilled water. The reaction mixture was amplified in an Eppendorf PCR system (Model No.5341, Germany make). Amplification process included, initial denaturation of DNA at 94 °C for 2 min, denaturation at 94 °C for 30 s, annealing at 44 °C for 45 s and extension at 72 °C for 1 min and 30 s followed by thirty five cycles and final extension at 72 °C for 20 min, and was stored at 4 °C till further use. The amplification products were resolved by electrophoresis on 1.5% agarose gel containing ethidium bromide along with 1 Kb ladder DNA as a standard molecular weight size marker.

2.5. Data analysis

For the comparison of morphological characters, the measurements were expressed in percentage (data not shown). To find out the differences among the morphological and meristic characters between different species, Principal Component