DNA barcoding reveals distinct population of *Plotosus canius* (Siluriformes: Plotosidae) in Sundarbans waters

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**ABSTRACT**

The Gray eel-catfish, *Plotosus canius* is a widely distributed species in Indo-West Pacific region and predominantly found in marine and brackish water. Although the recent genetics study evidenced the different population of *P. canius* from Southeast Asia, sampling from Indian water is required to assess the ample population structure. Hence, the current study is aimed to generate the DNA sequences of *P. canius* from Sundarbans to recognize the genetic distinctiveness of the Indian population. Both *P. canius* and *P. lineatus* showed more than one clade in neighbor-joining (NJ) phylogeny. Further, the high genetic divergences in *P. canius* (5.2–11%) and *P. lineatus* (3.6–11%) depicted cryptic and diverse population. The median-joining network with 21 haplotypes also suggested unique haplogroup of *P. canius* from Sundarbans. However, more sampling of *P. canius* from diverse geographical locations and involving additional molecular markers would substantiate the conservation genetics and proper management.

**1. Introduction**

Fishes of the genus *Plotosus* (Family Plotosidae) are commonly known as the eel-tail catfish that are identified to be nine different species (Gomon and Taylor 1982). *Plotosus canius* and *P. lineatus* have cosmopolitan distribution, however, the other species have narrow distribution range. *Plotosus canius* is known from the Indo-West Pacific, coasts of India, Sri Lanka, Bangladesh and Myanmar, Indo-Australian Archipelago, Philippines, and Papua New Guinea. *Plotosus lineatus* is Indo-Pacific species, distributed in Red Sea, East Africa to Samoa, Japan, southern Korea, the Ogasawara Islands, Australia, Lord Howe Island, Palau and Yap in Micronesia, East Africa, and Madagascar (Eschmeyer 2012). *Plotosus limbatus* is distributed in the Indian Ocean, east coast of Africa, Knydna, South Africa. *Plotosus nkunga* is native to Western Indian Ocean, Kosi Bay to Knydna in South Africa, and possibly extending to Zanzibar, Tanzania. *Plotosus fissiona* is native to Western Indian Ocean, Madagascar; *P. papuensis* is native to Asia and Oceania, New Guinea; *P. abbreviates* is native to Western Pacific; *P. japonicas* is native to Japan, including the Ryukyu island; and *P. nhatrangensis* is native to the Western Pacific and Vietnam (Froese and Pauly 2018).

Besides the original description, *P. canius* has been exclusively discussed on their morphology (Kumar 2012; Usman et al. 2013), fecundity (Khan et al. 2002), feeding biology (Leh et al. 2012), biological properties (Prithiviraj 2014), and bioactive properties (Prithiviraj and Annadurai 2012). *Plotosus canius* is an amphidromous and demersal bony and venomous catfish that prefers marine and brackish water habitats and predominantly found in estuaries, rivers, lagoons, and shallow waters (Prithiviraj and Annadurai 2012). In the recent past, the phylogeny and population genetics of *P. canius* is largely attempted from the Malaysian coastal waters and evidenced the genetic variability within the population (Khallili Samani et al. 2016). However, the genetics of *P. canius* has never been discussed from the Indian waters. Nevertheless, it is reported that the population of many fish species were genetically isolated due to alterations of their native biogeography (Pauly et al. 2002, Thomsen et al. 2012). Hence, the combined study of morphology, ecology, and molecular studies are necessary to know the species precisely.

The morphology and DNA barcoding technique are largely used in biodiversity research and evidenced as an effective combined approach for species identification (Hebert et al. 2003; Tyagi et al. 2017; Kundu, Kumar, Tyagi, et al. 2018), evaluating the fish diversity (Abdullah and Rahbein 2016; Laskar et al. 2018a), resolving the taxonomic uncertainty (Kundu, Kumar, Laskar, et al. 2018; Laskar, Kumar, Kundu, Tyagi, et al. 2018), and estimating the population genetics (Khallili Samani et al. 2016). Thus, the present study aimed to generate the DNA barcode data of *P. canius* from Indian waters.
waters and genetically compared with the available database sequences generated from the different geographical locations. The estimation of genetic divergence, phylogeny, and haplotyping might resolve the status of _P. canius_ from Indian waters.

### 2. Materials and methods

#### 2.1. Sample collections and morphological identification

Five specimens in Sundarbans (PCS ZSI 01: 21.76 N 88.07 E, PCS ZSI 02: 21.86 N 88.17 E, PCS ZSI 03: 21.89 N 88.57 E, PCS ZSI 04: 22.11 N 88.78 E, PCS ZSI 05: 22.19 N 88.95 E) and preserved in 70% molecular grade ethanol with proper voucher IDs at the Centre for DNA Taxonomy, Zoological Survey of India (ZSI), Kolkata (Fig. 1). A meager amount of muscle tissue was collected from each sample by using a sterile surgical blade for downstream molecular experiments and stored at −80°C. The specimens were collected from outside the protected areas, thus no prior permission was required in this study. The morphological identification of the studied specimens was executed by following the previous literature and description (Gomon and Taylor 1982; Ng and Sparks 2002; Yoshino and Kishimoto 2008).

#### 2.2. DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted by using the QIAamp DNA Investigator Kit (QIAGEN Inc., Germantown, MD) as per the standard protocol mentioned by manufacturing company. Each genomic DNA was checked in 1% Agarose gel and quantification of the DNA was determined by using the Nanodrop (Eppendrof, Hamburg, Germany). The published primer pairs, FishF1–5′TCAACCAACCACAAAGACATTGCAC3′ and FishR1–5′TAGACTTCTGGGTGCAAGATAA3′ was used to amplify the partial cytochrome oxidase C subunit I (COI) segment of mitochondrial gene in a Veriti™ Thermal Cycler (Applied Biosystems, Foster City, CA) (Ward et al. 2005). The 25 μl PCR mixture contains 10 pmol of each primer, 20 ng of DNA template, 1X PCR buffer, 1.0–1.5 mM of MgCl2, 0.25 mM of each dNTPs, and 0.25 U of Platinum Taq DNA Polymerase High fidelity (Invitrogen, Life Science Technologies, Carlsbad, CA). The PCR products were checked in 1% agarose gel and purified using a QiagenQuickR Gel extraction kit (QIAGEN Inc., Germantown, MD). Bi-directional sequencing of each sample was carried out in 48 capillary array 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) following Sanger sequencing methods in ZSI, Kolkata.

#### 2.3. Sequence check and dataset preparation

The generated forward and reverse chromatograms of each sample were checked by Sequence Scanner software (Applied Biosystems Inc, Foster City, CA). Further, the consensus sequence of each sample was assembled by the forward sequences and ‘reverse complementary’ of the reverse sequences. The final sequences were checked through the online nucleotide BLAST program and ORF finder to examine the complete alignment, insertion-deletion, and start-stop codons (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Finally, the sequences were submitted in the GenBank database to acquire the specific accession number. The generated sequences were preliminarily identified through online identification system, in GenBank with Nucleotide BLAST search and in the BOLD database with identification engine. Further, 57 publicly available mtCOI sequences of same and related species (_P. canius_ = 29, _P. nkunga_ = 3, _P. lineatus_ = 20, _P. limbatus_ = 3, and _P. japonicas_ = 2) were acquired from the GenBank database after screening the collection localities. One sequence of _Cephaloscyllium silasi_ (family Scyliorhinidae) was incorporated in the dataset as an out-group. Total 63 sequences were aligned using ClustalX software (Thompson et al. 1997) to form a combined dataset (515 bp) and analyzed the genetic distance and clustering pattern through Kimura 2 parameter (K2P) and neighbor-joining (NJ) tree by using MEGA6 (Tamura et al. 2013). The number of haplotypes of _P. canius_ was estimated by using DnaSP 4.10.9 (Librado and Rozas 2009) and the identical sequences were considered as the same haplotype. Haplotype diversity (Hd) and nucleotide diversity (π) for different population of _P. canius_ were calculated through DnaSP 4.10.9. The median joining networks of all haplotypes were drawn in NETWORK 4.6.1 (Bandelt et al. 1999).

### 3. Results and discussion

The studied specimens were identified as _P. canius_ based on the dusky-brown coloration with a black dorsal fin tip and the long barbels on the nostrils that crosses the eyes (Fig. 1B–E). The generated sequences showed 97% similarity with the GenBank sequences of _P. canius_ (KX657716, MF588561, MF601472: collected from the Sundarbans, Bangladesh; MG495943: collected from the Sundarbans, India), _P. nkunga_ (KF511562, collected from the Hooghly River, Kolkata, India), and _Plotosus_ sp. (KJ956941, collected from the Hooghly River, Kolkata, India). Further, the generated sequences also showed 96.47% similarity with the BOLD system sequences of _P. nkunga_ (GDK288-13, Yanum Estuary, Puducherry). Thus, the sequence similarity search mislead the identification of the studied samples in both GenBank and BOLD system. Hence, the genetic divergence was calculated for the studied dataset to confirm the distinctiveness of _P. canius_ and _P. nkunga_. The overall mean genetic divergence was 12.8% observed in the studied dataset of five _Plotosus_ species. Three species, _P. japonicas_, _P. nkunga_, and _P. limbatus_ shows 0% intra-specific genetic divergence, while the other two species _P. canius_ and _P. lineatus_ shows 3.7 and 5.5% intra-specific genetic divergence, respectively. The inter-specific genetic distance was ranging from 5.1 to 22.7% in the studied dataset. The recently described _P. japonicas_ showed 5.1, 7.2, 19.6, and 22.7% inter-specific genetic distance with _P. lineatus_, _P. limbatus_, _P. canius_, and _P. nkunga_ respectively. Further, the high genetic divergence (13.9%) resulted in _P. canius_ and _P. nkunga_. The sequence of _P. nkunga_
KF511562 showed 13.6% genetic divergence with the three sequences of P. nkunga (JF494190, JF494191, JF494192) and 0.6–8.1% genetic divergence with 33 sequences of P. canius. Hence, considering the genetic divergence and distribution pattern of P. nkunga in South Africa, we argued the identification of the specimens (Accession no. KF511562 in GenBank and Sequence ID, GDK288-13 in BOLD system) and should be treated as P. canius.

The NJ phylogeny showed distinct clades of the studied species with high bootstrap support. Three distinct clades
were revealed in the phylogeny represented by *P. japonicas*, *P. nkunga*, and *P. limbatis* each. However, the mtCOI sequences of *P. canius* showed five clades (Malaysia + Indonesia + Java clade, Southern India clade, WB-India clade, Sundarbans-India clade, and Malaysia clade) in the studied dataset with >85 bootstrap supports (Fig. 1F). The *P. lineatus* also showed four clades (Indian clade, Mediterranean Sea clade, Lebanon-Mediterranean Sea clade, and India + South China Sea + Fujian + Beibu Gulf + Philippines + Malaysia clade) in the studied dataset with >99 bootstrap supports. The five different clades of *P. canius* showed 0–0.8% within-group genetic divergence in the studied dataset. The generated sequences of *P. canius* by Sundarbans-India clade represent 3.3% genetic divergence with the closest WB-India clade, however, showed 5.2, 5.8, and 11% genetic divergence with the Southern India clade, Malaysia + Indonesia + Java clade, and Malaysia clade, respectively. The four different clades of *P. lineatus* showed 0–0.2% within-group genetic divergence in the studied dataset. The Indian clade of *P. lineatus* showed 3.6% genetic divergence with Mediterranean Sea clade, 10.5% genetic divergence with Lebanon-Mediterranean Sea clade, and 11% genetic divergence with India + South China Sea + Fujian + Beibu Gulf + Philippines + Malaysia clade. In the recent past, the five populations of *P. canius* were demonstrated by mtCOI gene from Malaysian waters (Khalili Samani et al. 2016). Further, the high intraspecific genetic variability was also detected in *P. lineatus* (Bariche et al. 2015). In the present study with mtCOI gene also resulted in high-genetic variability in both *P. canius* and *P. lineatus*, which depicted cryptic diversity correlated with the different population from diverse geographical locations. The studied sequences of *P. canius* resulted in 21 haplotypes with 75 polymorphic sites, $H_d=0.95$ and $\pi=0.035$ in the studied dataset. The median-joining network depicted two distinct clustering of *P. canius* collected from Java + Indonesia + Malaysia and India. Further, the generated sequences of *P. canius* from Sundarbans, India resulted in shallow clustering discrepancy in the *P. canius* from Southern India and WB (West Bengal), India (Fig. 1G). Hence, the high-genetic variability, distinct clade in NJ phylogeny, haplotype, and clustering pattern in median-joining network evidenced the distinct population of *P. canius* in Sundarbans, eastern India.

The Sundarbans is the largest mangrove forest in the coastal region of the Bay of Bengal. This unique landscape is regarded as a UNESCO World Heritage site, shared by both India and Bangladesh. This region is well known for containing numerous endemic, ecologically, economically important flora and fauna and is an important habitat for many Endangered and distinct evolutionary significant unit of flora and fauna and is an important habitat for many Endangered and distinct evolutionary significant unit of biota. It is reported that the alteration of biogeography, directly influence the population structure through species dynamics, colonization, and isolation (Costello et al. 2003). Further, for acclimatization of any species in a foreign environment, the population accumulates genetic mutation (Charlesworth and Willis 2009). The resulted high-genetic variation in *P. canius* from the Sundarbans region offered a viable encouragement to monitor the local traits, genetic drift, and inbreeding effects (Tallmon et al. 2004; Duvernell et al. 2008; Leray and Knowlton 2015). In addition to this, the population structure of many other species could indicate the sustainable management and healthy environment of Sunderbans.

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S. Kundu et al.

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