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Original Article

Molecular characterization based on cytochrome C oxidase I gene of the family Channidae from different riverine systems of Odisha, India

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Abstract
This study focuses on four *Channa* species from the northern part of the Eastern Ghats in India: *Channa punctata* (Bloch, 1793); *Channa striata* (Bloch, 1793); *Channa marulius* (Hamilton, 1822) and *Channa gachua* (Hamilton, 1822). The aim is to determine whether their molecular divergence aligns with their morphological distinctiveness. The molecular analysis based on mitochondrial COI gene sequences revealed distinct clusters for each species and genus, with K2P distances ranging from 17.28 – 27.96%. Notably, *C. marulius* was positioned in a distinct clade separate from the *C. punctata*, *C. striata*, and *C. gachua* groups. *Channa gachua* clustered with *Channa rara* and *Channa kelaartii*, suggesting a close evolutionary relationship. *Channa punctata* and *C. striata* also clustered together, reinforcing their status as sister species. Species delimitation using the Assemble Species by Automatic Partitioning method identified 10 partitions, with the most reliable delimitation showing a clear separation of *Channa* species of Odisha. These findings align with recent analyses and confirm that the molecular divergence among the four species is consistent with their morphological differences. The study underscores the importance of molecular methods in resolving taxonomic ambiguities and understanding species diversity. It provides a foundational molecular database for *Channa* species, supporting future research on genetic divergence and contributing to conservation and aquaculture efforts.

Keywords: biogeography; channids; Odisha; phylogeny; Teleostei

1 | INTRODUCTION

The family Channidae, consisting of snakeheads, is distributed across Africa and parts of Asia (Adamson and Britz 2018). The members of the family inhabit various freshwater environments and are of significant economic importance. They are consumed as food, kept as ornamental fish in aquariums, and even utilized for therapeutic purposes (Zhu *et al.* 2013). Currently, there are 59 recognized species within the Channidae family worldwide (van der Laan and Fricke 2024). The family Channidae encompasses species classified into two genera: *Channa* (Scopoli, 1777) and *Parachanna* (Teugels and Daget 1984). The *Channa*, with 56 recognized species predominantly distributed across Asia, spanning Southern Asia, Iran, and the Far East, constitutes the larger genus (Li *et al.* 2006; Britz *et al.* 2024; Fricke *et al.* 2024). In contrast,

Parachanna comprises only three valid species and is confined geographically to Central West Africa. All the Asiatic *Channa* species can be divided into eight distinct species groups viz. *C. argus*, *C. asiatica*, *C. gachua*, *C. lucius*, *C. marulius*, *C. micropeltes*, *C. punctata* and *C. striata* groups (Rüber *et al.* 2019). In India alone, there are reports of 26 valid species of the Channidae family (Froese and Pauly 2024; Fricke *et al.* 2024).

The taxonomy and nomenclature of the species within the genus *Channa* often pose challenges (Miyan *et al.* 2014; Rüber *et al.* 2019). This difficulty arises because these fish vary greatly in size, from the small *C. andrao* Britz, 2013 (about 10 cm) to the much larger species like *C. micropeltes* (Cuvier and Valenciennes, 1831) and *C. marulius* (Hamilton, 1822) (over 1 m). Additionally, their colours change as they grow and reach reproductive maturity, which can make it hard to distinguish between species and often leads to confusion, with juvenile and adult fish sometimes being mistakenly identified as different species (Rüber *et al.* 2019).

Molecular phylogenetic analyses, particularly those focusing on the NADH dehydrogenase subunit 2 (ND2) gene alongside its adjacent genes, including tRNAs and partial sequences of NADH dehydrogenase subunit 1 (ND1), spanning approximately 1.5 Kbp of mitochondrial DNA, reveal mutual monophyly of the Asian and African taxa within Channidae. Furthermore, the aforementioned study highlights numerous clades within the Asian lineage, aligning with their morphological distinctiveness (Li *et al.* 2006). Serrao *et al.* (2014) created a channid DNA barcode library with 25 species and 250 individuals. This study was recently re-examined by Conte-Grand *et al.* (2017) and expanded to include 35 species, seven undescribed species, and a total of 777 individuals. There are reports on the phylogenetic position of a few species of *Channa* from different riverine systems (Zhu *et al.* 2013; Ahmed *et al.* 2018; Praveenraj *et al.* 2018; Kamran *et al.* 2020; Liu *et al.* 2020). A recent assessment by Rüber *et al.* (2019) on the channid systematics and biogeography from Eastern Himalaya biodiversity hotspot based on two mitochondrial genes and one nuclear gene revealed a large intraspecific divergence in several *Channa* species that could represent additional undescribed species. The study also highlights the complex biogeographic patterns of Channids, with both vicariance and dispersal events potentially shaping their current distribution. Studies on genetic characterisation of *C. striata* from different habitats of Indonesia showed low genetic differences between the populations of the species from closed and open water bodies (Arisuryanti *et al.* 2020; Setyaningrum *et al.* 2022). A study on the species diversity and the genetic relationship of the genus from the North Eastern Hill region of India by Lakra *et al.* (2010) using mitochondrial genes showed two separate groups. These groups were genetically different from each other but had the same pattern in their evolutionary history.

The Eastern Ghats is a discontinuous mountain range extending along the eastern coast of India, renowned for its unique flora and fauna. Studying DNA barcoding of *Channa* species from this region can offer valuable insights into their identification, evolutionary divergence and conservation. This research can also serve as a platform for sharing information and developing effective conservation strategies. The northern section of the Eastern Ghats spans primarily across Odisha and parts of Andhra Pradesh. In Odisha, there are 11 riverine basins, with the Mahanadi River basin being the largest (DWR 2021). There are reports of four known species of *Channa*: *C. punctata*; *C. striata*; *C. marulius* and *C. gachua*) from different riverine basins of Odisha. Mogalekar and Canciyal (2018) reviewed the freshwater fish species of Odisha and listed *C. orientalis* Bloch and Schneider, 1801 from Odisha based on previous literature. However, *C. orientalis* is endemic to southwestern Sri Lanka (Mishra *et al.* 2013; Ekanayake *et al.* 2021). The present study has been carried out to do the molecular characterisation of above mentioned four species of *Channa* in order to understand whether their morphological differences are in congruence with their molecular divergences based on the mtCOI gene sequences.

2 | METHODOLOGY

2.1 Sampling

A total of 46 *Channa* specimens belonging to different species were collected over a span of two years (2022 to 2024) across various major river systems of Odisha, including the Mahanadi, Budhabalanga, Rushikulya and Kolab rivers. Fishes were obtained from the wild populations with the aid of gill nets operated by the local fishermen. Sample collection was done using a random sampling method, ensuring no bias towards specific locations or species. Photographs of the fresh specimens were taken using a Nikon D5600 camera. The specimens were then preserved in 10% formaldehyde solution for further analysis. A portion of muscle tissue from each specimen was preserved in 70% ethanol for molecular analysis. Species identification was carried out using standard morphological references such as Talwar and Jhingran (1991), Jayaram (2010), and Mishra *et al.* (2013). Figure 1 represents the different *Channa* species collected during the present study.

2.2 DNA extraction and amplification

From the muscle tissue of the dead specimens preserved in 70% ethanol, total genomic DNA was isolated using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's instructions. The mitochondrial Cytochrome Oxidase I (COI) gene was selected and then amplified using the FishF1 and FishR1 primer pairs (Ward *et al.* 2005). PCR conditions followed those outlined by *Seth et al.*

(2023). Twenty-five gene sequences can be amplified out of the 46 isolated DNA. The low-quality sequences from the remaining 21 DNA samples were excluded from consideration due to poor template quality. Approximately 650bp of the mtCOI gene were then sequenced using the Sanger method at Barcode BIO-science Pvt. Ltd., Bangalore, India. The sequence assembly of the different species was conducted with BioEdit version 7.2, as detailed by Hall (1999). The resulting mtCOI gene sequences were submitted to the NCBI database to obtain the respective accession numbers. A detail of the accession numbers of the tissue samples from the different *Channa* species along with their respective sites of collection are represented in Table 1.

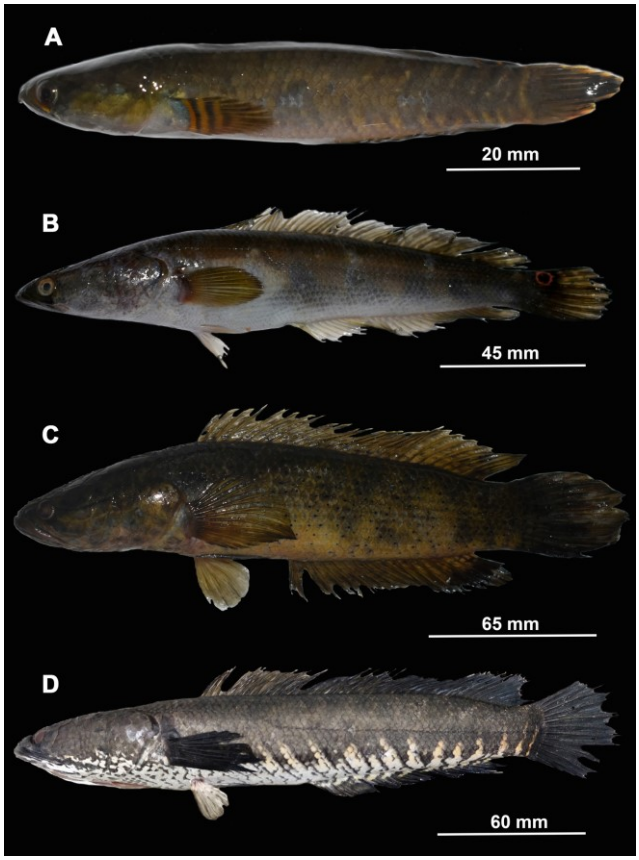

FIGURE 1 Species of the genus *Channa* collected during the present study. A, *Channa gachua*; B, *Channa marulius*; C, *Channa punctata*; D, *Channa striata*.

2.3 Sequence analysis

The mtCOI sequences of 25 individuals under the four species *viz. C. punctata*, *C. striata*, *C. marulius* and *C. gachua* were aligned to create the final alignment. Pairwise evolutionary distances between haplotypes were calculated using the Kimura 2-Parameter method (Kimura 1980) with MEGA X software (Kumar *et al.* 2018). The maximum likelihood (ML) tree was constructed using the best-fit model and validated with bootstrap analysis (1000 replications). Bootstrap analysis is crucial for assessing the reliability of a phylogenetic tree, as high bootstrap probabilities (Pb) for the interior branches indicate strong support for the inferred relationships (Katsura *et al.* 2017). MtCOI sequences of *Badis badis* and *Nandus nandus* retrieved from the NCBI database were used as outgroups. For species delimitation analysis, the online ASAP web browser was used to perform Assemble Species by Automatic Partitioning (ASAP) following the standard protocol and default settings as mentioned in Puillandre *et al.* (2021) and Seth and Barik (2021).

TABLE 1 Details of the accession numbers of tissue samples of *Channa* species along with their collection sites.

Location	Riverine systems	Voucher number	Accession number
Channa marulius			
Subarnapur	Mahanadi	MSEB102	OP349616.1
Cuttack	Mahanadi	MSEB472	PP291871.1
Channa gachua			
Subarnapur	Mahanadi	MSEB076	OP209956.1
Subarnapur	Mahanadi	MSEB103	OP355301.1
Subarnapur	Mahanadi	MSEB104	OP349651.1
Subarnapur	Mahanadi	MSEB105	OP355299.1
Haripur	Rushikulya	MSEB197	OQ118351.1
Baripada	Budhabalanga	MSEB403	OR554267.1
Channa punctata			
Berhampur	Rushikulya	MSEB072	OP209955.1
Berhampur	Rushikulya	MSEB073	OP208034.1
Subarnapur	Mahanadi	MSEB106	OP355433.1
Subarnapur	Mahanadi	MSEB107	OP355328.1
Koraput	Kolab	MSEB236	OQ194043.1
Koraput	Kolab	MSEB237	OQ195143.1
Balasore	Budhabalanga	MSEB258	OQ654047.1
Balasore	Budhabalanga	MSEB260	OQ654057.1
Baripada	Budhabalanga	MSEB261	OQ654069.1
Bahabalpur	Budhabalanga	MSEB285	OR138022.1
Bahabalpur	Budhabalanga	MSEB286	OR140820.1
Channa striata			
Berhampur	Rushikulya	MSEB051	OP168884.1
Subarnapur	Mahanadi	MSEB081	OP208042.1
Subarnapur	Mahanadi	MSEB109	OP355339.1
Subarnapur	Mahanadi	MSEB110	OP355443.1
Subarnapur	Mahanadi	MSEB111	OP355446.1
Baripada	Budhabalanga	MSEB262	OQ654107.1

3 | RESULTS

The phylogenetic study revealed that all individuals within each species and genus formed cohesive clusters. The K2P distances among the four species ranged from 17.28 to 27.96%. The highest nucleotide substitution was observed between *C. striata* and *C. gachua* (21.60 – 27.96%), while the lowest substitution was between *C. punctata* and *C. striata* (17.28 – 23.86%). The divergence range of K2P distances for the four species studied is summarised in Table 2. The maximum likelihood (ML) tree illustrates the

phylogenetic relationships among these four *Channa* species, as well as other members distributed in central and peninsular India (Figure 2). The ML tree analysis shows that *C. punctata* and *C. striata* are forming a distinct cluster; whereas *C. gachua*, *C. rara* and *C. kelaartii* form a separate cluster. *Channa marulius* is positioned in a separate clade with *C. pseudomarulius*. The species delimitation analysis using the ASAP method resulted in 10 partitions with ASAP scores ranging from 1.5 to 13.5. The partition with the lowest ASAP score (1.5) best represents species delimitation. All *Channa* species from Odisha show distinct separation in this partition, which includes nine nominal species and holds the highest rank (Figure 3).

TABLE 2 Divergence range of K2P distance in percentage of different species of the genus *Channa* from Odisha.

Species	*C. marulius*	*C. gachua*	*C. punctata*	*C. striata*
C. marulius	0			
C. gachua	20.68–25.62	0		
C. punctata	17.41–25.88	18.73–25.92	0	
C. striata	18.53–24.28	21.60–27.96	17.28–23.86	0

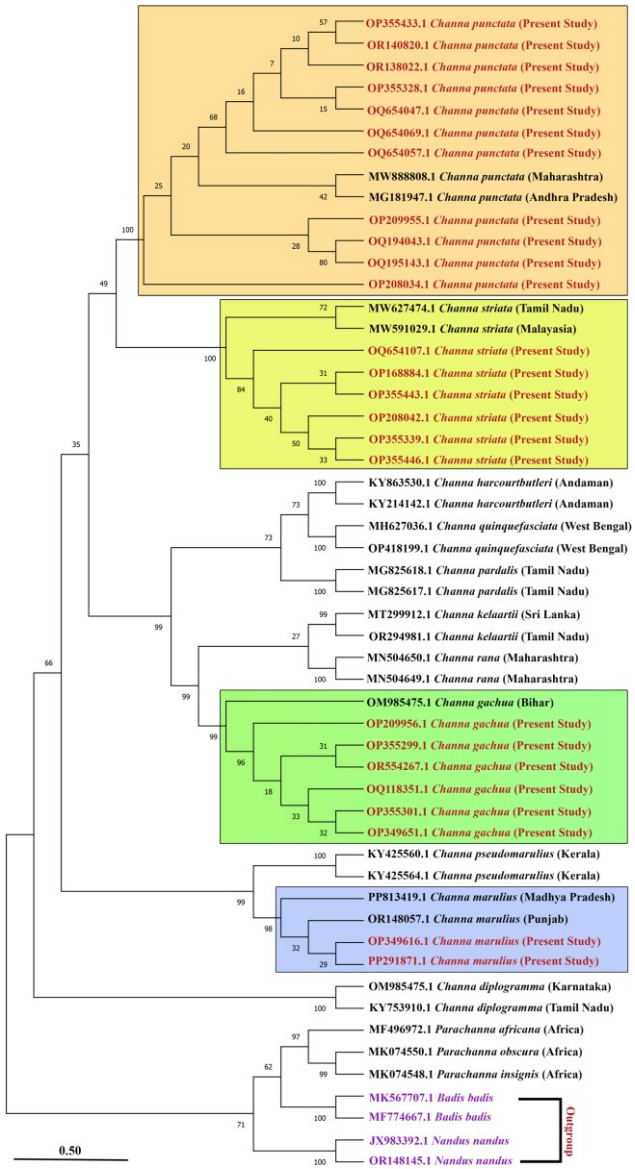

FIGURE 2 Maximum likelihood (ML) tree showing the relationship of different species of genus *Channa* based on mtCOI sequences. Different coloured boxes represent distinct lineages. The number shown in each node in the ML tree is the bootstrap value.

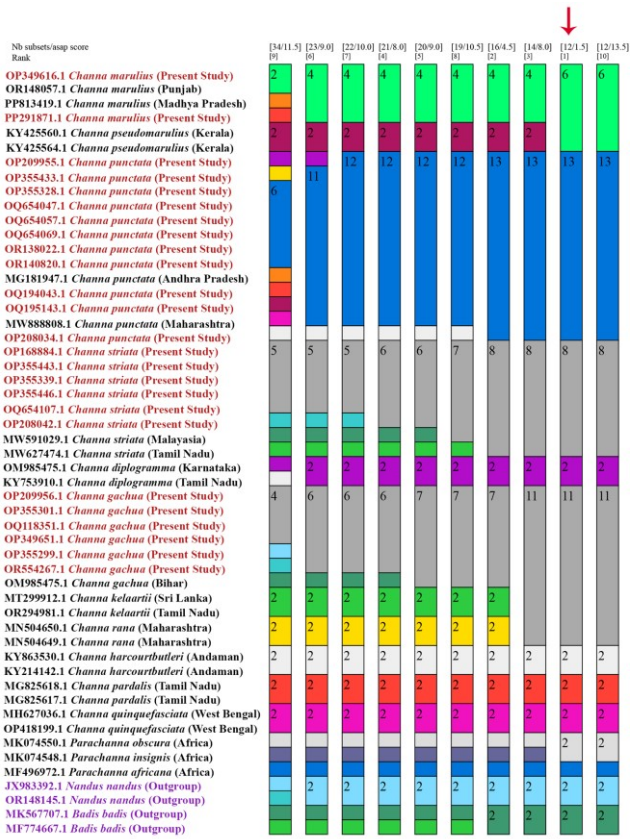

FIGURE 3 Box Species graph obtained after Assemble Species by Automatic Partitioning (ASAP) analysis of *Channa* species. (Note: Nb groups: number of species identified by ASAP in the corresponding partition, ASAP score).

4 | DISCUSSION

Historically, fish phylogenetic relationships and speciation were inferred using only morphometric and meristic characteristics (Musikasinthorn 2000). Identifying Channidae species has been challenging due to their overlapping morphological features at different developmental stages. This has led to numerous instances of mislabelling Channidae species globally (Nagalakshmi *et al.* 2016; Yan

et al. 2016). Consequently, relying solely on morphological traits for constructing phylogenetic trees is problematic because of the complex evolutionary changes in their physical and biological features.

In this study, molecular analysis based on COI gene sequences from four species in different riverine systems of Odisha enhances species identification and understanding of evolutionary divergence patterns. During the study, ML tree analysis helped us understand the evolutionary relationships among the species, while ASAP analysis facilitated rapid species partitioning from large barcode datasets, allowing us to investigate species boundaries effectively. Maximum likelihood tree analysis revealed that *C. marulius* forms a distinct clade separate from the *C. punctata*, *C. striata* and *C. gachua* groups. This finding aligns with recent analyses by Ahmed *et al.* (2018) and Rüber *et al.* (2019) on *Channa* evolutionary divergence. *Channa gachua* clusters with *C. rara* (Western Ghats) and *C. kelaartii* (endemic to Sri Lanka and southern Peninsular India), suggesting they are sister species, with a smaller number of nucleotide substitutions compared to other species which supports their morphological distinctiveness, due to banding on pectoral fins (Rüber *et al.* 2019). *Channa punctata* and *C. striata* also cluster together, reinforcing their status as sister species. The ASAP analysis indicates that *C. marulius* and *C. pseudomarulius*, although morphologically distinct, belong to the same cluster, likely due to recent evolutionary divergence. The same observation applies to *C. gachua*, *C. kelaartii* and *C. rara*. To gain a clearer understanding of evolutionary divergence and genetic distinctiveness, a long-term study of the family Channidae is essential. Overall, the genetic relationships observed in this study are consistent with previous findings (Conte-Grand *et al.* 2017; Barman *et al.* 2018).

This report presents the first DNA barcoding analysis of *Channa* species based on Maximum Likelihood (ML) trees and ASAP analysis for the region. It serves as baseline data to elucidate genetic divergence patterns across various riverine systems in Odisha. This information will be instrumental in enhancing our understanding of population ecology, management strategies, and ecosystem assessments over the long term.

5 | CONCLUSION

This study aims to create a comprehensive molecular database detailing the genetic characteristics of *Channa* species found in Odisha. The molecular divergence observed in the four species analysed aligns with their morphological differences. These molecular sequences will be valuable for future research on the genetic divergence of *Channa* species across different regions. Ultimately, molecular identification will enhance our understanding of the adaptive radiation of these species and may facilitate the aquaculture industries in future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

SR, SS and DP: Equal right, Data preparation and molecular analysis; PP and MM: Sampling and DNA isolation; AKM: Critical analysis and updating of the manuscript; JKS: Fund acquisition, conceptualization, critical analysis and important input to the manuscript. All authors read and approved the final manuscript.

ETHICAL APPROVAL STATEMENT

The species under the study are not included in any schedule list/protection categories of the Wild Life (Protection) Act, 1972 of India. The DNA isolation was carried out from the muscle of dead specimens. So, the ethical clearance certification is not required.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on reasonable request from the corresponding author.

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