Assessing Fish Invasions in the Ganges: Molecular Detection and Surveillance of Juvenile Invasive Species Using DNA Barcoding

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ABSTRACT

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| The Ganges River, the life line of India is facing ecological threats due to invasive fish species. Effective management and conservation of native biodiversity requires accurate identification of these non-native species especially at their early life stages. This study evaluates the potential of DNA barcoding as a molecular tool to detect and identify juvenile invasive fish in the Ganges ecosystem. The genetic analysis identified the invasive species namely *Oreochromis niloticus* (Nile tilapia) within the collected juvenile specimens. DNA barcoding proves essential for biodiversity preservation as well as controlling invasive species particularly when establishing identities of natural resources in their initial developmental phases. The identification process in traditional morpho-taxonomy needs entire developed specimens to achieve proper species determination because essential diagnostic traits appear fully during maturity. |

*Keywords: Molecular Surveillance, Fish Juveniles, Invasive species, DNA Barcoding, Ganges River*

1. INTRODUCTION

The introduction of invasive species creates multiple threats to native biodiversity and ecology which include resource competition, habitat destruction and functional process interference. These species cause native taxa populations to decrease or become extinct mostly in areas with high endemism levels such as islands and freshwater ecosystems which have low ecological resilience (Simberloff et al., 2013). The introduction of invasive plants results in changes to fire dynamics and nutrient processes whereas invasive predators along with herbivores tend to significantly diminish native fauna (Vilà et al., 2011). Invasive species management and associated damages from these species amount to billions of dollars every year worldwide according to Pimentel et al. (2005). Invasive species continue to expand because of global trade movements together with climate change effects and changes in land use which demand swift and sustained control measures (Ricciardi et al., 2017).

The Ganges River which supports millions of people and diverse aquatic biodiversity functions as a vital freshwater source yet faces growing ecological disturbances because non-native fish species continue to spread (Singh & Lakra, 2011). Non-native fishes seize valuable resources from native species while bringing negative effects on food webs which leads to decreasing native populations (Singh et al., 2010). Accurate detection of invasive species needs precise identification strategies particularly at their early developmental phase because morphological signs become unreliable for proper differentiation (Uh-Navarrete et al., 2021). Thus morpho-taxonomic examinations usually fall short when applied to juvenile specimens because key diagnostic traits have not yet fully developed. DNA barcoding represents an effective molecular identification system based on the COI gene region that provides swift and precise biological species recognition throughout developmental life stages (Ward et al., 2005). The identification of fish species diversity in Indian freshwater systems including the Ganges uses this method successfully for the detection of invasive species and makes decisions about management (Lakra et al., 2016).

Morphological identification techniques face challenges when determining species accurately because they work poorly with early life stages of organisms and cryptic species groups that show few morphological differences (Pfenninger & Schwenk, 2007). DNA barcoding represents a molecular identification method which uses the mitochondrial cytochrome c oxidase subunit I (COI) gene sequence at 655 base pairs for fast and accurate species recognition (Hebert et al., 2003). The identification approach of DNA barcoding serves as a vital tool for biodiversity assessment that helps discover unknown species and boosts species recognition in multiple organism groups (Hajibabaei et al., 2007). The identification of species and management of invasive species in the Ganges River requires DNA barcoding due to its high species diversity and dangerous invasive species threats. The study by Lakra et al (2016) used DNA barcoding to examine the fish diversity in the Ganges and identified both native and invasive species to enhance the observation of India's important river biodiversity and therefore early detection of such invasive species is crucial for implementing timely management strategies.

2. material and methods

**2.1. Sample collection**

Fish juveniles were collected from Gaighat, Patna, Bihar (25°39'11"N 85°05'43"E) on 24th November 2024 along the Ganges River using gill nets and cast nets. Specimens could not be morphologically identified up to species level due to its juvenile phase, hence preserved in 95% ethanol and stored at -20°C until DNA extraction.

**2.2. Genomic DNA Isolation**

Genomic DNA was extracted by using NucleoSpin® Tissue Kit (Macherey-Nagel) by following the manufactures protocols. The tissues were placed in a 1.5 ml microcentrifuge tube by adding 180 µl of T1 buffer together with 25 µl of proteinase K for an incubation at 56oC in a water bath until the tissue was fully lysed. The RNase A solution (5 µl of 100 mg/ml concentration) was added to the lysed mixture followed by a 5-minute incubation at room temperature. Subsequently, 200 µl of B3 buffer was added and heated at 70oC for 10 minutes. Then, 210 µl of 100% ethanol was added while thoroughly vortexing the mixture. The mixture was pipetted into NucleoSpin® Tissue column placed in a 2 ml collection tube and centrifuged at 11000 x g for 1 minute. The NucleoSpin® Tissue column was transferred to a new 2ml tube and washed with 500 µl of BW buffer. Wash step was repeated using 600 µl of B5 buffer. After washing the NucleoSpin® Tissue column was placed in a clean 1.5 ml tube and DNA was eluted out using 50 µl of BE buffer.

**2.3. Agarose Gel Electrophoresis for DNA Quality check**

The quality of the DNA isolated was checked using agarose gel electrophoresis. 1µl of 6X gel-loading buffer (0.25% bromophenol blue, 30% sucrose in TE buffer pH-8.0) was added to 5µl of DNA. The samples were loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was performed with 0.5X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (Genei) and the image was checked for DNA quality under UV light using Gel documentation system (Bio-Rad), before PCR.

**2.4. PCR & Sequencing using BigDye Terminator v3.1**

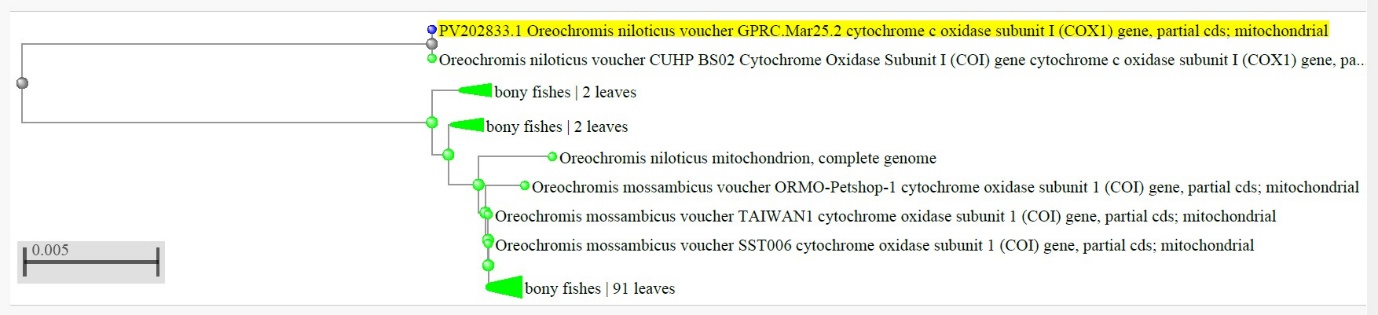
The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) and the COI gene was amplified using universal primers LCO1490 and HCO2198 (Folmer et al., 1994) with the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol and sequenced in ABI 3500 DNA Analyzer (Applied Biosystems). The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al., 2010). (Part of the above work was carried out at Rajiv Gandhi Centre for Biotechnology (RGCB), Ministry of Science and Technology (Department of Biotechnology), Government of India, Thiruvananthapuram, Kerala).

3. results and discussion

**3.1. DNA polymorphism analysis**

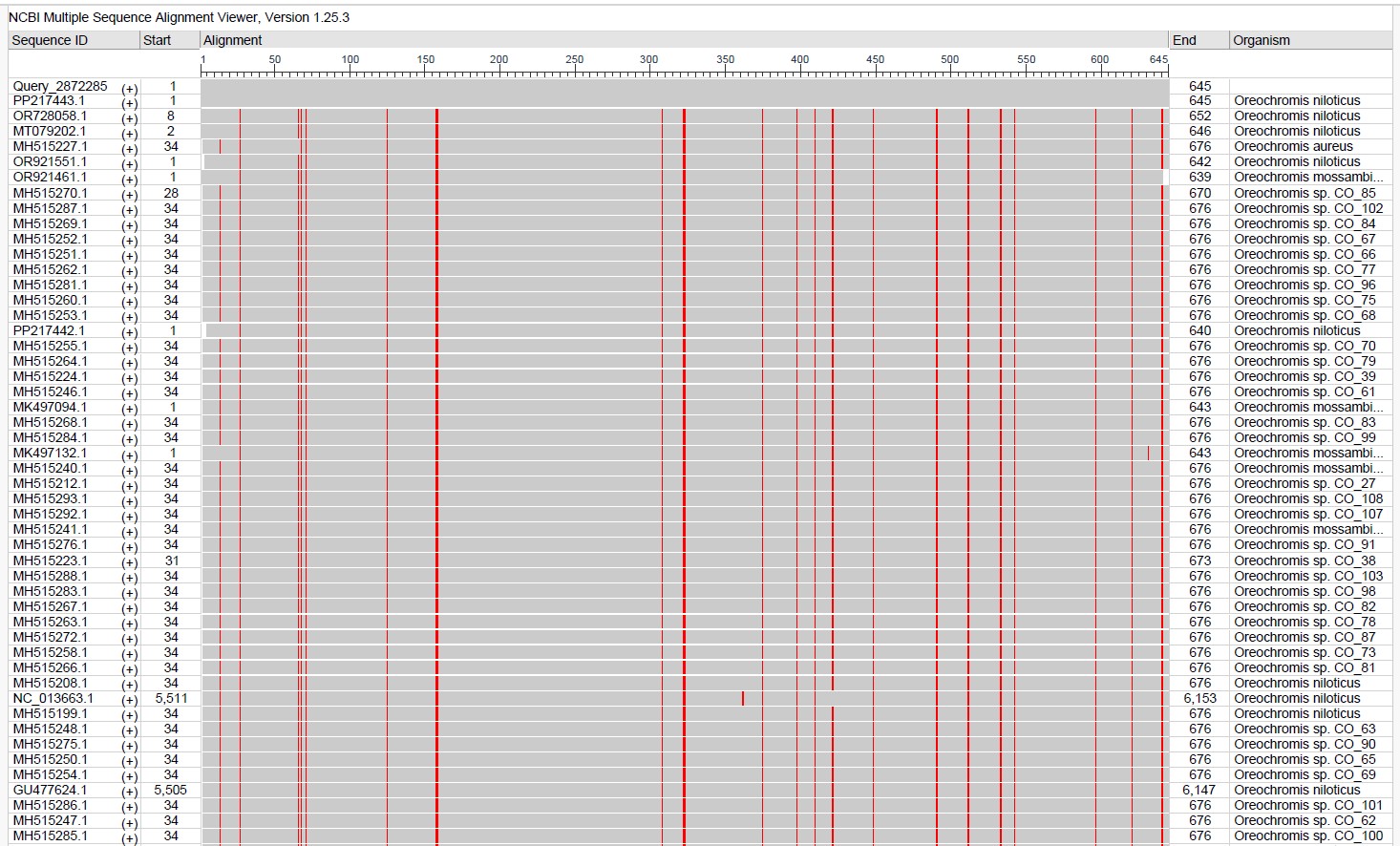
These sequences were aligned along with additional mitochondrial COI sequences retrieved from the NCBI, GenBank, and the sequence generated from the present study with latest Chromas version and MEGA (Koichiro Tamura, 2021). Based on similarity search the generated COI sequences showed similarity *Oreochromis niloticus* (Linnaeus, 1758) and were then deposited in NCBI GenBank database and accession number was obtained (PV202833). The identification of species was confirmed by using the BLAST program, NCBI (Zheng Zhang et al, 2000, 16. Aleksandr Morgulis et al 2008). The sequence was then used for polymorphism studies and further analysis with the COI sequences deposited from other countries etc., based on the geographical distribution and also as per available sequences from the NCBI nucleotide database.

The analysis of fish sequences used the Neighbor-Joining method to study the mitochondrial cytochrome c oxidase I (COI) gene which occurs frequently in DNA barcoding studies. Phylogenetic trees built using the Neighbor-Joining approach as one of the prevalent techniques allowed researchers to extract evolutionary relations from sequence genetic distances. The phylogenetic research demonstrated that *Oreochromis niloticus* belongs to the Cichlidae family with confirmed genetic proximity to other Oreochromis species. The phylogenetic tree obtained through this analysis displayed strong branches that exhibited proper clustering of sequences from *Oreochromis niloticus* together with their most related taxa (Figure.1 & 2). DNA barcoding studies supported by mitochondrial COI gene sequences proved effective in determining evolutionary relationships of Nile tilapia as well as taxonomic identification.



**Figure.1. Molecular Phylogenetic analysis by Neighbour Joining method using mitochondrial cytochrome c oxidase 1 gene of Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758) through DNA Barcoding using juvenile sample.**

Additionally, NCBI MSA Viewer 1.25.0 is a powerful tool for analyzing multiple sequence alignments, facilitating detailed comparative analysis of genetic sequences. In the present study, the Nile tilapiasequence was identified using the BLAST (Basic Local Alignment Search Tool) algorithm, which compares the queried sequence against a database of known sequences to find regions of similarity. The MSA Viewer was then employed to visualize and interpret the multiple sequence alignment results. This tool employes to align the query sequence of Nile tilapia through DNA Barcoding with those of other related species, highlighting conserved regions, identifying genetic variations, and inferring evolutionary relationships. The visual representation provided by the MSA Viewer aids in the clear identification of sequence homologies and differences, making it an essential resource for molecular biologists studying the genetic makeup and evolutionary history of the Nile tilapia.



**Figure.2. NCBI Multiple Sequence Alignment Viewer 1.25.0 results showing query from the present study with the database of known sequences regions of similarity**

**3.2. Implications for Conservation and Management**

Nile tilapia (*Oreochromis niloticus*) originally from Africa introduced to India because of its quick growth rate and sturdy nature and high market value (FAO, 2020). The species continues to spread throughout Indian freshwater ecosystems through deliberate introductions and unintended escapes from aquaculture facilities which now affect sensitive ecological areas and biodiversity-rich zones (Kripal et al., 2021). Scientific research shows Nile tilapia invasions transform food chain dynamics through reduced ecological space for native herbivores and planktivores so these populations must change their feeding habits or experience population reductions (Canonico et al., 2005). The threat to native fish communities requires immediate monitoring and management strategies because Nile tilapia invasions damage native fish communities.

The introduction of Nile tilapia into other freshwater systems produces major threats to native and endemic fish species survival. The quick growth of Nile tilapia along with its adaptive behaviour leads to population competition which results in prey predation and habitat modifications that stress both broad-ranging fish species and those species with limited distribution areas. The species in Western Ghats biodiversity hotspot face high risk from these impacts because they occupy restricted ecological areas and possess poor dispersal abilities (Shuai F, Li J., 2022). Controlling aquaculture operations remains vital because it helps reduce these potential threats. A biosecurity framework with strict controls should be enforced to manage both non-native species movements and native fish farming of local-adapted species (Sugunan, 1995). The introduction of exotic species in aquaculture requires previous ecological risk assessments and local policies and monitoring systems to achieve balance between freshwater biodiversity conservation and aquaculture development (Gaupale, T. C., & Sontakke, G. K. (2023).

4. Conclusion

DNA barcoding tools enable early detection of invasive species which remains crucial for protecting native biodiversity and stabilizing ecosystems since biological invasions create significant threats. This research findings show the presence of juvenile invasive fish species within the Ganges. The combination of molecular tools and traditional ecological assessments enables to create effective methods which can protect the native aquatic biodiversity of the Ganges. Through DNA barcoding researchers can detect invasive species at their developmental stages before they become as an invader threat because of their resemblance to native species and lack of distinctions in early stages. Molecular identification systems provide faster and more exact species determinations compared to traditional morpho-taxonomic methods that need mature adult specimens for precise identification. DNA barcoding technology represents a major advancement in biosurveillance methods for detecting invasive species. High-throughput sequencing technologies enable taxonomic identification using reference libraries BOLD and GenBank to process minimum genetic materials including eDNA. DNA barcode technologies allow for finding invasive species at their earliest point of invasion thus enabling fast response measures. DNA barcoding operates as an essential bioinformatics-driven tool that protects Ganges River ecology through anthropogenic threat management. The advancement helps conservationists together with decision-makers to cope with the effects of biological invasions while safeguarding the Ganges ecosystem.

AcknowledgEments

The authors are grateful to the Director, Zoological Survey of India for the facilities provided help and encouragement.

**Disclaimer (Artificial intelligence)**

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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