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Research Article

"The Molecular Study of Fish Species from Kolar Dam of Bhopal District (Madhya Pradesh) Using COI Gene Sequencing"

Sonal Vermar1, Priyanka Tiwari1*, Deepak Bharti2
[1]Faculty of Sciences, SAM Global University,Raisen-464 551,Madhya Pradesh, India
[2] CMBR Biotech Pvt. Ltd., Bhopal-462022, Madhya Pradesh, India
*Corresponding Author Email: drpriyankatiwari6@gmail.com

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Abstract

Identifying fish species correctly is a key step in biodiversity assessment, fisheries management and conservation planning but traditional morphology-based methods have limitations due to phenotypic plasticity, ontogeny and the inability of taxonomists to describe all species. In the current internship work, a DNA barcode approach based on cytochrome c oxidase subunit I (COI) was employed to fish material samples collected from Kolar Dam (Bhopal district, Madhya Pradesh). We generally used muscle tissue from cyprinuscarpio (common carp) to optimize genomic DNA extraction, COI amplification and Sanger sequencing methods. In contrast, a high-quality genomic DNA was isolated using CTAB-phenol-chloroform extraction; however, a 655 bp fragment of the COI gene was desired and its successful amplification with universal fish primers and agarose gel visualization. The unsuccessfully amplified PCR product was purified and the sequencing of this product resulted in high quality chromatograms at 600 – 700 bp, which is suitable for downstream BLAST analysis (work that could not be completed during the internship period). Very few molecular studies have been conducted on the fishes from Kolar Dam till date, and theirs is the first study demonstrating that standard protocols to COI barcoding can be easily applied for fish from this site, while providing a fundamental molecular pipeline which not only can serve as a tool in their future studies but also can be extended to other species & populations.

Keywords: COI gene; DNA barcoding; Cyprinuscarpio; fish identification; Kolar Dam; Bhopal

1. Introduction

Fish represent the most diverse group of vertebrates, with more than 35,000 recognized species worldwide (Zhang &Hanner, 2012; Bingpeng et al., 2018). They play key roles in aquatic food webs and provide critical protein and micronutrients for human populations, especially in inland regions. However, the reliable identification of fish species using only external morphology and meristic characters can be difficult. Convergent body forms, ontogenetic

changes, phenotypic plasticity and the existence of cryptic species frequently lead to misidentification (Keskin&Atar, 2013; Bortolus, 2008). This problem is particularly acute in regions with high diversity and limited taxonomic capacity, such as many Indian river basins (Khedkar et al., 2014; Chakraborty&Ghosh, 2014). DNA barcoding based on a short standardized region in the mitochondrial cytochrome c oxidase I (COI) gene has become a promising tool for bolstering classical taxonomy at higher levels (Ward et al., 2005; Lakra et al., 2011) especially in fishes. COI is maternally inherited, it does not contain introns and has relatively high substitution rates with set priming sites that are conserved making this gene many (Li & He, 2015; Lukas et al., 2012), but suitable for species-level discrimination in most animal groups (Hoque et al., 2013; Wilson-Wilde et al. Great reference libraries with thousands of COI sequences for marine and freshwater fishes from most regions of the world including Indian waters are now available (Lakra et al., 2011; Khedkar et al.An example of one such is Kolar Dam over the Kolar River in Bhopal district, which supplies drinking water and inland fisheries. Although traditional surveys have reported the fish fauna in the Kolar (Shukla et al., 2023), molecular data remain scarce

The present internship work aimed to establish and test a basic COI-based identification pipeline using a locally available species, *Cyprinuscarpio*(common carp) , as a model. This is a common Indian major carp of high commercial importance and a good starting point for protocol optimization.

2. Materials and Methods

2.1 Sampling

Fresh muscle tissue was collected from morphologically identified *Cyprinuscarpio*(common carp) individuals obtained from Kolar Dam landings. Approx. 100 mg of dorsal muscle was excised with sterile tools, placed into pre-chilled microcentrifuge tubes and kept on ice during transport to the laboratory at CMBR Biotech, Bhopal. Samples were stored at -40 °C prior to DNA extraction.

2.2 Genomic DNA extraction

Genomic DNA was isolated using a CTAB-phenol–chloroform protocol adapted for fish tissue. Briefly, frozen tissue (~100 mg) was ground in a chilled tube with a micro-pestle in 1 mL of digestion buffer (Tris-HCl, EDTA, NaCl, SDS and β-mercaptoethanol). After incubation at 50 °C for 30–60 min with occasional mixing, lysates were centrifuged, and the supernatant was extracted with phenol:chloroform:isoamyl alcohol (25:24:1). DNA was precipitated from the aqueous phase with 7.5 M ammonium acetate and cold 100% ethanol, pelleted by centrifugation, washed with 70% ethanol, air-dried and resuspended in TE buffer. DNA quantity and purity were checked by UV–VIS spectrophotometer, and integrity was examined on 0.8–1.0% agarose gel.

2.3 COI amplification by PCR

A fragment of the COI gene (~655 bp) was amplified using universal fish primers:

Forward:
5’-TCAACCAACCACAAAGACATTGGCAC-3’

Reverse:
5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′

Each 20 µL PCR reaction have template DNA (~1 µL), 2.0 µL 10× buffer, $MgCl_2$, dNTPs, 0.4 µL of each primer, Taq polymerase and nuclease-free water. Thermal cycling conditions used as initial denaturation at 95 °C for 8 min, 35 cycles of 95 °C for 30 s, 54 °C for 30 s, 72 °C for 30 s; and final extension at 72 °C for 10 min. PCR products checked on 1.0% agarose gel in 1× TAE buffer, stained with ethidium bromide. A 1 kb ladder used as a size marker.

2.4 Sequencing

Clear single bands of expected size were excised from the gel or directly purified using a standard PCR cleanup kit (outsourced step). Purified amplicons were subjected to Sanger sequencing in both directions using the same primers. The resulting chromatograms were visually inspected to confirm quality and read length.

3. Results and Discussion

The CTAB-phenol–chloroform protocol yielded intact, high-molecular-weight DNA from *Cyprinuscarpio*(common carp) muscle, as seen by clear, sharp bands on the agarose gel with very little smearing. The A260/280 ratios fell within the usual acceptable range for PCR work, so the DNA was clean enough for amplification. This shows that the “classical” extraction method, although a bit time-consuming and reagent-heavy, still works very well for fish tissue and does not necessarily require commercial kits or specialized equipment. When the universal fish COI primer set was used, a single strong band of about 650–700 bp was consistently amplified from C. carpio genomic DNA. This fragment size matches the expected COI barcode region reported in earlier fish barcoding studies (Ward et al., 2005; Lakra et al., 2011; Chu et al., 2019). The agarose gel did not show extra non-specific bands or heavy background, which suggests that both primer choice and PCR conditions were suitable for this species and template quality.

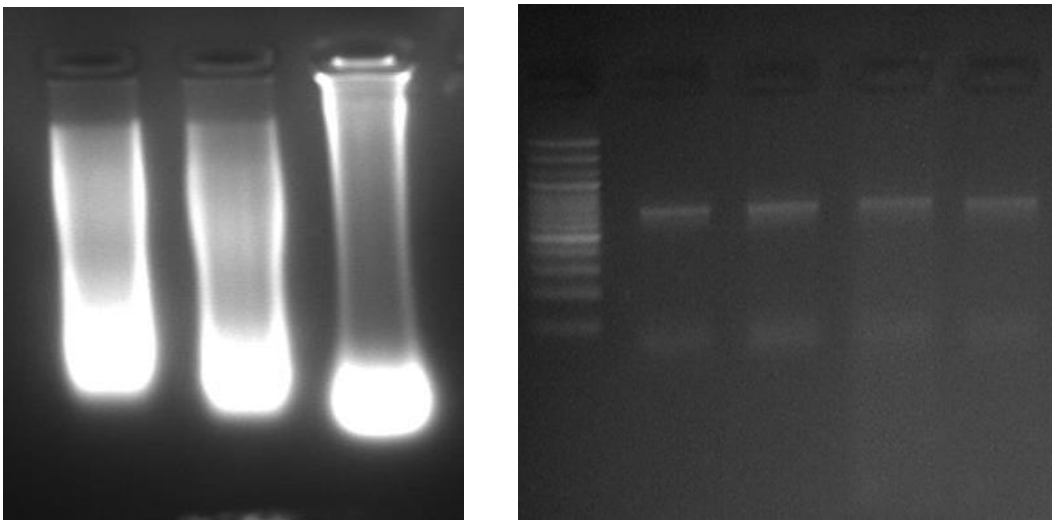

Figure 4.1: Agarose gel showing high-molecular-weight genomic DNA of *Cyprinuscarpio*(common carp)

and a single, specific PCR amplicon(655 bp)of the COI gene at the expected size.

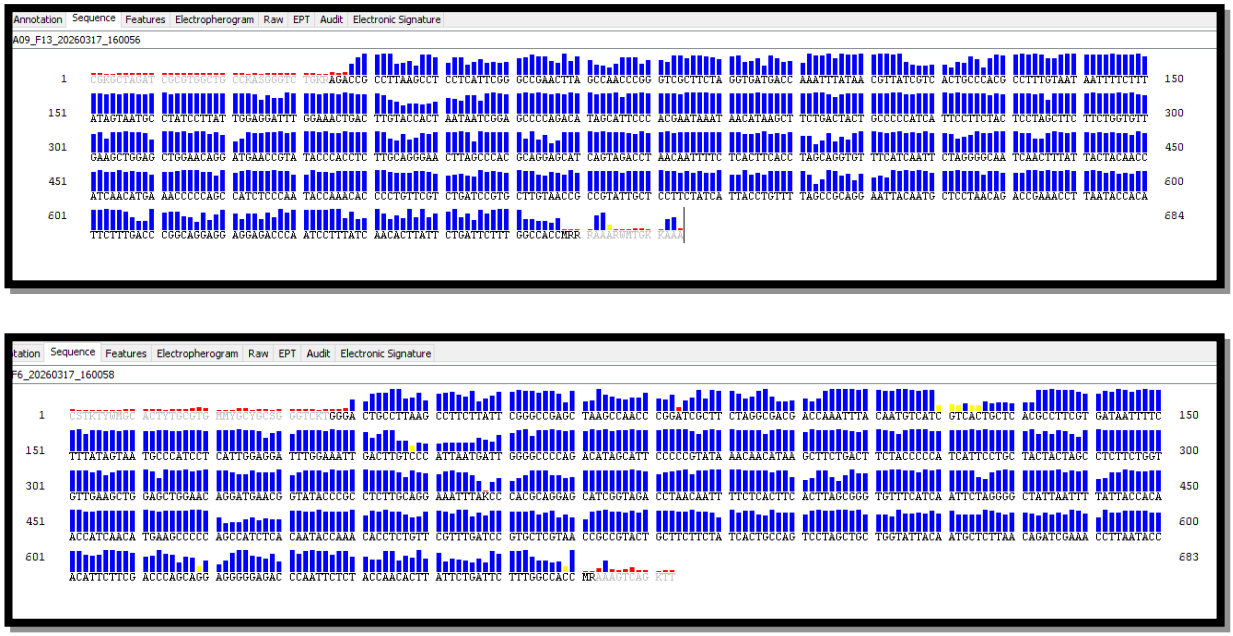

Figure 4.2: Sequencing result of the COI gene from *Cyprinuscarpio*(common carp) showing clear, high-quality base calls across the barcode region.

Sequencing of the purified COI PCR products gave clean chromatograms, roughly 600–700 bp in length, without major overlapping peaks. Because of the limited duration of the internship, there was not enough time to run a full BLAST or BOLD search and formally confirm the identity against global databases. However, the quality of the sequences indicates that the overall pipeline from tissue sampling and DNA extraction through PCR amplification to Sanger sequencing functions as intended. Once these sequences are uploaded and compared to reference data, they are expected to show a high percentage match to existing *Cyprinuscarpio*barcodes, similar to what has been seen for other Indian freshwater fishes (Khedkar et al., 2014; Chakraborty&Ghosh, 2014; Kundu et al., 2019).

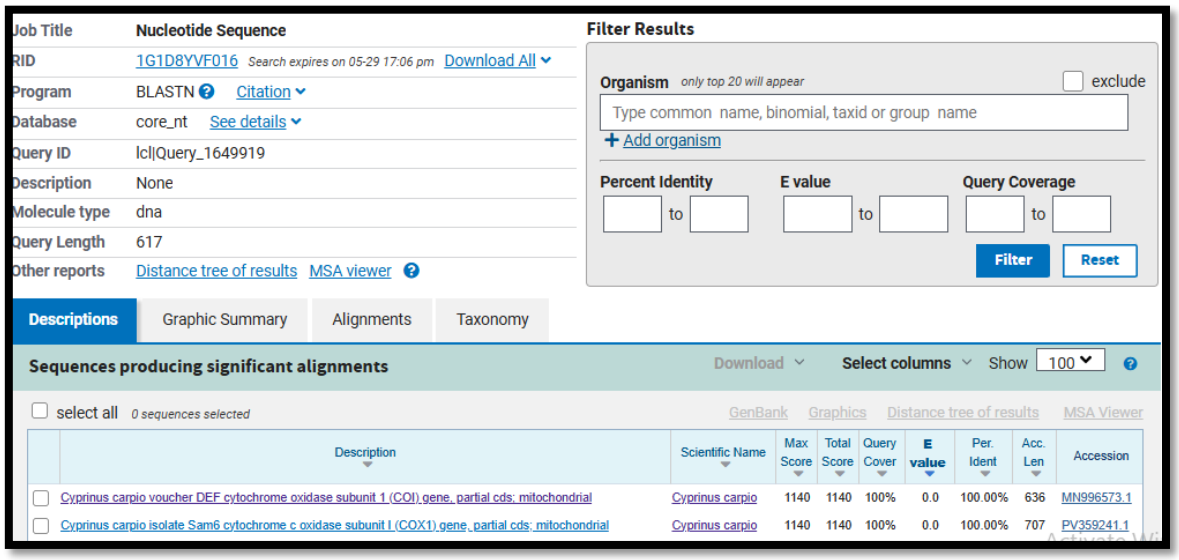

Figure 4.8. NCBI BLAST output confirming the molecular identification of *Cyprinuscarpio(common carp)*

The main contribution of this small study is thus methodological rather than taxonomic. It provides a tested, step-by-step protocol that can now be applied to multiple species from Kolar Dam and nearby water bodies. As more local fishes are processed using the same COI barcoding approach, a regional reference library can be established. Such a library would help reduce misidentifications based only on external morphology, especially in closely related cyprinids, and would allow larval and juvenile stages to be matched more reliably to adults. It would also increase the chance of detecting cryptic species, non-native introductions or early stages of invasion that might be missed in routine visual surveys (Steinke et al., 2009; Aquilino et al., 2011). In the long term, combining DNA barcoding data with classical faunistic and ecological surveys (e.g. Shukla et al., 2023; Khedkar et al., 2014) would give a stronger, more objective basis for fisheries management and conservation planning in the Kolar -Narmada region.

Conclusion

Within the limited period of this internship, protocols for genomic DNA extraction, COI PCR amplification and Sanger sequencing were successfully optimized for *Cyprinuscarpio*(common carp) collected from Kolar Dam. The workflow produced high-quality COI sequences suitable for DNA barcoding and future database submission. These results show that standard COI barcoding methods can be effectively implemented in a local laboratory setting and provide a starting point for a broader molecular survey of fish diversity in Kolar Dam and other central Indian freshwater systems.

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