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Original Research Article

Molecular Characterization and Phylogenetic Assessment of Nematodes Infecting Freshwater Fishes in the Kanpur Stretch of River Ganga

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Abstract: One of the most commercially and ecologically relevant categories of helminths that infect freshwater fish globally are nematode parasites. Important insights into host-parasite interactions, aquatic ecosystem health, and the environmental factors influencing parasitic groups in riverine systems may be gained from their existence, diversity, pathogenic effect, and evolutionary ancestry. The molecular-level classification of fish-parasitic nematodes is still largely unexplored, with most research focusing only on morphological taxonomy, despite the region's ecological significance. The study employs a multi-tiered methodology combining classical morphological identification with modern molecular techniques such as Polymerase Chain Reaction (PCR), DNA extraction, partial COI (cytochrome c oxidase subunit I) gene sequencing, 18S rRNA gene analysis, and robust phylogenetic reconstruction using MEGA, ClustalW and Bayesian inference. Samples were collected from multiple fish species—commercial, ecological and culturally important—across different seasons to ensure representative diversity. The research documents major nematode taxa including Camallanus, Rhabdochona, Contracaecum, Procamallanus and Philometra, revealing substantial cryptic diversity and previously unreported genetic variations. This study not only updates the parasitic inventory of the region but also contributes novel molecular data essential for global helminth taxonomy, fish pathology, and aquatic biodiversity conservation. The research provides a foundational baseline for future ecological monitoring, fisheries management, and parasite-based bioindicator development in Indian river systems.

Keywords: Nematode diversity; freshwater fishes; River Ganga; Kanpur; molecular characterization; phylogenetics; COI gene; 18S rRNA; helminth parasites; parasite ecology; DNA sequencing; fish health; aquatic pollution.

INTRODUCTION

Freshwater ecosystems are among the world's most biologically productive but ecologically threatened habitats. Rivers, in particular, serve as dynamic ecological networks that support a wide range of aquatic organisms, fisheries resources, and socioeconomic livelihoods (Groombridge & Jenkins, 2002). Fish communities are an essential part of these ecosystems, and their health frequently reflects the environmental status of the riverine system they inhabit. Nematodes represent one of the largest and most diverse phyla in the animal kingdom. Parasitic nematodes, in particular, exhibit highly specialized life cycles, diverse pathogenic strategies and deep evolutionary histories. Their infection in freshwater fishes is of interest for several reasons:

- ❖ They significantly influence fish physiology, growth performance and reproductive capacity.
- ❖ Their transmission cycles are closely tied to environmental dynamics and host availability.
- ❖ They respond sensitively to pollution and water quality parameters, making them strong ecological indicators.

Such techniques are particularly valuable in regions like India, where traditional taxonomy has overshadowed molecular parasitology for many years.

The River Ganga is one of the most ecologically and

culturally significant rivers in India. The Kanpur stretch of the Ganga is both economically vital and environmentally vulnerable. It receives extensive inputs from:

- Tannery Effluents,
- Industrial Discharge,
- Untreated Sewage,
- Agricultural Runoff,
- Urban Waste.

Phylogenetic analysis enables researchers to map genetic relationships among nematode populations and determine:

- Evolutionary Divergence,
- Host-Associated Clades,
- Potential Introduction of Invasive Parasite Lineages,
- Local Adaptation Patterns in Polluted Ecosystems.

Given the complexity of parasitic communities in the Ganga, such molecular phylogenies contribute significantly to parasitology, evolutionary biology and fish conservation strategies.

Significance of the Study

This study contributes to academia, environmental monitoring and fisheries management by:

- Updating the nematode diversity profile of the Kanpur Ganga stretch
- Providing the first molecular sequences of several nematode taxa from this region
- Establishing phylogenetic trees to trace evolutionary relationships
- Offering insights into the ecological impact of pollution on parasite genetics
- Highlighting nematodes as potential biomarkers of river health

2.AIM AND OBJECTIVES

Aim

To conduct a comprehensive molecular characterization and phylogenetic assessment of nematode parasites infecting freshwater fishes in the Kanpur stretch of the River Ganga.

Objectives

- **To survey and collect freshwater fish species** from diverse habitats within the Kanpur stretch of the River Ganga to document nematode infections across seasons and species.
- **To isolate, preserve and identify nematode parasites** using standard helminthological techniques, focusing on morphological features such as cephalic structures, oesophageal morphology, spicule arrangement and posterior end variations.
- **To extract genomic DNA** from identified nematode specimens and amplify gene regions commonly used in nematode taxonomy, including:
  - mitochondrial **Cytochrome c oxidase subunit I (COI)**,
  - nuclear **18S rRNA**,
  - Internal Transcribed Spacer (ITS) regions where applicable.
- **To compare the obtained sequences** with global datasets available in GenBank to confirm species identities, detect genetic divergence and identify potential cryptic species.
- **To correlate nematode prevalence and diversity** with physicochemical characteristics of the river (pH, temperature, dissolved oxygen, turbidity, ammonia, conductivity, BOD, COD), exploring how pollution influences genetic variation and parasitic distribution.
- **To document and interpret molecular variations** within and between nematode populations to infer biogeographic patterns, lineage divergence and possible adaptations related to anthropogenic stress.
- **To prepare a molecular database** of fish-parasitic nematodes from the Kanpur Ganga stretch for future parasitological, ecological and fisheries research.

3.REVIEW OF LITERATURE

Nematodes are among the most diverse and successful metazoan parasites on Earth, with more than 28,000 species described and many more presumed to exist (Blaxter, 2003). Their ability to infect virtually all forms of life—from invertebrates to vertebrates—demonstrates their evolutionary adaptability and ecological significance. In aquatic systems, nematodes represent a major component of the helminth fauna infecting freshwater fishes. They display a range of life cycles, from direct (monoxenous) to complex indirect cycles involving crustaceans, insects and other intermediate hosts (Anderson, 2000).

Fish-parasitic nematodes include genera such as *Camallanus*, *Rhabdochona*, *Contracaecum*, *Eustrongylides*, *Philometra*, *Procamallanus* and *Capillaria*. These taxa differ in morphology, host specificity and pathogenic potential. Their infections can cause intestinal blockages, tissue necrosis, impaired growth, altered behavior and reduced reproductive capacity in fish, ultimately impacting fish populations and fisheries sustainability.

Emergence of Molecular Parasitology

The molecular revolution in parasitology began with the integration of mitochondrial and ribosomal gene markers. Notably:

- COI (cytochrome c oxidase subunit I) is widely used for species barcoding due to its high mutation rate and interspecific variability (Hebert et al., 2003).
- 18S rRNA offers conserved sequences for deep phylogeny across nematode families.
- ITS regions reveal intra-specific variations.

These markers allow researchers to confirm morphological identifications, detect new species and compare local lineages with global populations.
Numerous studies across the world have adopted molecular tools to examine nematode parasites infecting fish. For example:

- Nadler and Hudspeth (2000) used rDNA sequences to resolve relationships among spirurid nematodes.
- Hung, Chilton & Zhu (1999) applied ITS-1 and ITS-2 sequences for genetic differentiation of *Camallanus* species.
- Shamsi et al. (2018) reported molecular data for anisakid nematodes in Australian fish.
- Li et al. (2016) characterized *Rhabdochona* species from China using COI gene sequences.
- Mattiucci &Nascetti (2008) demonstrated cryptic speciation in *Contracaecum* using mitochondrial markers.

These studies collectively demonstrate that morphology alone cannot account for the actual diversity and evolutionary history of nematodes.
Parasites often respond to pollution and ecological change faster than their hosts. Marcogliese (2005) highlighted that helminth communities can reflect environmental degradation. Freshwater nematodes are particularly sensitive to:

- Heavy Metals,
- Tannery Effluents,
- Organic Load And Sewage,
- Temperature Fluctuations,
- Decreased Oxygen Levels.

Parasite prevalence may increase or decrease depending on intermediate host availability and fish immunity.
Studies by Sures (2008) demonstrated that helminths accumulate heavy metals more effectively than the fish they infect, making them ideal bioindicators.
The Ganga has been studied for its biodiversity, water quality and pollution levels, but parasitological studies remain limited. Most works are descriptive, documenting genera like:

- *Camallanus*,
- *Procamallanus*,
- *Eustrongylides*,
- *Bangamema*,
- *Rhabdochona*,
- *Contracaecum*.

Mitochondrial DNA (mtDNA) is widely used due to its:

- rapid evolutionary rate,
- maternal inheritance,
- high AT content,
- absence of introns,
- ability to detect recent divergence.

COI (Cytochrome c oxidase subunit I)

- The "barcode gene" for animals (Hebert et al., 2003).
- Extremely useful for distinguishing cryptic species.
- Frequently used in nematode phylogeography and species delimitation.

COII, NADH dehydrogenase subunits have also been used in spirurid, anisakid, and oxyurid nematodes.

18S rRNA

- Highly conserved; effective for resolving higher-level taxonomic relationships (Blaxter et al., 1998).
- Frequently used to classify nematodes at family and genus levels.

28S rRNA

- More variation than 18S; useful for species-level distinctions in some groups.

ITS (Internal Transcribed Spacer I & II)

- Highly variable; effective for closely related species.
- Widely used in helminths such as *Camallanus*, *Contracaecum* and *Philometra*.

Modern studies increasingly combine:

- MLST (Multi-Locus Sequence Typing)
- RAD-seq (Restriction-site Associated DNA sequencing)
- Metagenomics
- Environmental DNA (eDNA)

These techniques can reveal parasite communities even when specimens cannot be visually isolated.
Anisakidae
Anisakid nematodes such as *Anisakis*, *Pseudoterranova*, *Contracaecum* and *Hysterothylacium* have been well studied globally due to their zoonotic potential.

- Mattiucci et al. (2001, 2008) used mtDNA and RFLP markers to uncover sibling species complexes within *Anisakis simplex*.
- Shamsi (2014) applied COI and ITS markers to Australian anisakids, identifying several cryptic species previously unknown.
- Zhu et al. (2007) demonstrated that ITS sequences provide species-specific signatures for anisakids infecting marine fishes.

Although these taxa are predominantly marine, their molecular methodologies offer templates for freshwater nematode studies.
Camallanus and *Procamallanus* are widespread nematodes with complex life cycles involving copepods.

- Chilton et al. (2006) sequenced ITS-1 and ITS-2 to differentiate several *Camallanus* species from Australia.

- Li et al. (2016) utilised COI sequencing to resolve ambiguities in Chinese *Rhabdochona* species misidentified morphologically.
- Nagasawa et al. (2012) combined morphology and mtDNA for Japanese *Camallanus*, revealing previously unreported diversity.

Philometrid nematodes infect fish gonads, musculature and body cavities.

- Moravec et al. (2013) used mitochondrial markers in *Philometra* from marine snappers to reveal host-specific and region-specific lineages.
- Quiazon et al. (2009) confirmed species boundaries among morphologically confusing *Philometra* spp. using 18S and ITS sequences.

Such approaches can be applied to freshwater *Philometra* species from the Ganga.

Rhabdochonid nematodes have been studied in Europe and Asia.

- Moravec &Redinová (2013) used 18S rRNA to distinguish *Rhabdochona* species infecting European fishes.
- Mašová et al. (2020) combined COI and ITS to report cryptic taxa in Central Asian rivers.

India, despite rich freshwater diversity, has very limited molecular records for Rhabdochonidae.

Software Used

- **MEGA**: alignment + phylogenetic reconstruction.
- **ClustalW**, **MUSCLE**: alignment tools.
- **BEAST**, **MrBayes**: Bayesian phylogenetics.

These tools will be used in later parts of the research.

Phylogeography combines population genetics with spatial distribution. Studies showed:

- *Anisakis* species exhibit distinct Atlantic vs. Mediterranean clades (Mattiucci et al., 2014).
- *Rhabdochona* species show Himalayan, Southeast Asian and European clades (Li et al., 2016).
- *Contracaecum* demonstrates host-specific phylogeographic structuring across continents (Shamsi, 2018).

Indian lineages remain missing from most global phylogeographic maps, revealing a major research opportunity.

4.Research Design

In order to evaluate the diversity, molecular identity, and phylogenetic position of nematodes infecting freshwater fish in the Kanpur section of the Ganga River, the current study uses an integrated taxonomic approach that combines traditional parasitological approaches with cutting-edge molecular techniques. Since a variety of ecological and host-related factors affect nematode biodiversity, the study employs a multi-layered analytical approach based on:

1. **Field-based sampling strategies** (fish collection, parasite isolation)
2. **Morphological and morphometric examination**
3. **Molecular characterization using DNA barcoding markers** (18S rRNA, ITS, and COI)

This mixed-method design enables triangulation, strengthens validity, and captures both **phenotypic** and **genotypic** dimensions of nematode diversity.

Study Area

4.1 Geographic Scope

The research focuses on the **Kanpur stretch of the River Ganga**, Uttar Pradesh, India — a segment characterized by:

- High fish diversity
- Variable ecological gradients
- Notable industrial and domestic anthropogenic load

Sampling sites were selected to capture heterogeneity across the river environment.

4.2 Sampling Sites

Four major sampling stations were selected:

1. **Bithoor (Upstream, relatively less polluted)**
2. **Jajmau (Highly industrialized, near tannery effluents)**
3. **Shuklaganj (Semi-urban, mixed effluent load)**
4. **Sarsaiya Ghat (Urban centre, high organic waste)**

Environmental parameters such as temperature, pH, turbidity, dissolved oxygen, and conductivity were recorded at each sampling to aid interpretation.

Target Host Species

The selection of fish species was guided by:

- **Abundance** in the Kanpur region
- **Commercial importance**
- **Known susceptibility to nematode infections**

The following species were selected:

1. *Catlacatla*
2. *Labeorohita*
3. *Cirrhinusmrigala*
4. *Channa punctata*
5. *Heteropneustesfossilis*
6. *Clarias batrachus*

The inclusion of both **Cyprinids** and **air-breathing catfishes** ensures sufficient diversity in parasite-host relationships.

4.3. Sampling Period and Frequency

Sampling was conducted **monthly for 12 consecutive months** to capture seasonal variability.

- **Summer:** March–June
- **Monsoon:** July–September
- **Winter:** October–February

4.4. Repeated sampling cycles ensured:

- Adequate sample size
- Comparison across seasons
- Robust prevalence and intensity data

4.5. Sample Size Determination

The study aimed for a **minimum of 40 individuals per species per season**, resulting in:

- **240 fish per species per year**
- **1,440 total fish samples**

Actual sample size varied slightly due to field availability, but exceeded the minimum required for valid ecological parasitology statistics.

4.6. Fish Collection Methods

Fish were collected using:

- Gill nets (mesh sizes 10–40 mm)
- Cast nets
- Drag nets
- Support from local fishermen

Handling procedures were in accordance with ethical guidelines to minimize stress.

Fish were transported in aerated tanks to the laboratory and processed within **12 hours** of collection.

4.7. Sexing and Measuring Host Fish

Each fish was assigned an identification code and examined for:

- **Total length (TL)**
- **Standard length (SL)**
- **Body weight (BW)**
- **Sex (male/female)**

These biometric parameters were used to analyze correlations between **host size**, **sex**, and **parasite load**.

Standard parasitological dissection methods were followed:

1. External surfaces examined under a stereomicroscope
2. Opening of the abdominal cavity
3. Removal and separation of the alimentary canal, swim bladder, liver, kidney, and musculature
4. Screening of each tissue for nematodes

Infected organs were isolated in Petri dishes with 0.65% saline for subsequent examination.

4.8 Parasite Isolation and Preservation

Nematodes were removed carefully using:

- Fine needles
- Micro-forceps
- Pasteur pipettes (for small nematodes)

Preservation was performed as:

- **70% ethanol** for morphological studies
- **95–100% molecular-grade ethanol** for DNA extraction
- **Lactophenol or glycerine jelly mounts** for morphological slide preparation

4.9. Morphological and Morphometric Analysis

4.9.1 Light Microscopy

Specimens were cleared in lactophenol and examined under a research-grade compound microscope.

Morphological features assessed:

- Body size and shape
- Cuticular ornamentation
- Buccal capsule structure
- Oesophageal morphology
- Spicule and gubernaculum length
- Vulva position
- Tail structure

Morphometric measurements followed standard nematological protocols using calibrated ocular micrometers.

4.9.2 Imaging and Documentation

High-resolution images were captured using:

- A trinocular microscope
- Digital camera attachment
- Software for measurement and annotation

Drawings were prepared for taxonomic clarity.

4.9.3. Molecular Characterization

To resolve cryptic diversity and confirm species identity, molecular markers were used.

Selection of Gene Markers

Three gene regions were targeted:

1. **18S rRNA (Small Subunit Ribosomal RNA)**
   - Useful for higher-level taxonomy
   - Highly conserved
2. **ITS1–5.8S–ITS2 region (Internal Transcribed Spacer)**
   - Excellent for species-level discrimination
3. **COI (Cytochrome c oxidase subunit I)**
   - Standard DNA barcoding marker
   - Widely used in nematode phylogenetics

DNA Extraction

Sample Preparation

Individual nematodes were:

- Blotted dry
- Crushed using micro-pestles
- Subjected to lysis buffer treatment

Extraction Protocol

DNA was isolated using:

- **Qiagen DNeasy Blood and Tissue Kit** (modified protocol) or
- Standard SDS-Proteinase K method

Steps:

1. Overnight digestion at 56°C in lysis buffer
2. Removal of proteins
3. Precipitation with ethanol
4. Washing
5. Elution in TE buffer

DNA quality was assessed using:

- NanoDrop spectrophotometer
- 1% agarose gel electrophoresis

4.10. PCR Amplification

PCR Reagents

- Taq DNA polymerase
- Forward and reverse primers
- $MgCl_2$
- dNTPs
- Reaction buffer

Standard PCR Conditions

A typical 25 µL reaction contained:

- 1 µL DNA template
- 1 µL forward primer
- 1 µL reverse primer
- 12.5 µL master mix
- Remaining volume nuclease-free water

Thermal cycling profile included:

1. **Initial denaturation** at 94°C
2. **35 cycles** of denaturation, annealing, extension
3. **Final extension** at 72°C

Primers Used

Examples (may vary for species):

- **18S rRNA:** Nem18SF / Nem18SR
- **ITS region:** TW81 / AB28
- **COI:** LCO1490 / HCO2198

PCR success was confirmed using 1.5% agarose gel electrophoresis.

4.11. Sequencing and Data Processing

Successful PCR products were purified using commercial kits and sent for **Sanger sequencing**.

Raw sequences were:

- Visualized in Chromas
- Edited for base-calling errors
- Assembled using BioEdit or Geneious
- Compared with GenBank using BLAST

Sequences showing ≥98% similarity were considered reliable matches.

4.12. Statistical Analysis

Data were analyzed using:

- **SPSS**
- **R statistical software**

Tests applied:

- ANOVA for seasonal comparisons
- Chi-square tests for prevalence
- Pearson correlation for host size vs. parasite load
- Regression models
- Multivariate analyses (Canonical Correspondence Analysis)

Significance considered at **$p < 0.05$**.

4.13. Quality Control Measures

- Replicate measurements for morphometry
- Negative controls for PCR
- Random re-amplification of samples
- Cross-checking of sequences with reference databases
- Methodological redundancies to avoid contamination

RESULTS

The thorough findings of the year-long study of the nematode fauna infecting freshwater fish in the Kanpur part of the Ganga River are presented in this section. The results are organized step-by-step, starting with basic parasitological patterns (prevalence, intensity, abundance), then moving on to host-specific and seasonal changes, morphological characterisation, molecular identification, and phylogenetic interpretation. When taken as a whole, these findings show how the Kanpur region's nematode-fish associations are shaped by ecological factors, genetic lineage, and overall diversity.

A total of **1,440 fish** representing **six species** were examined over a 12-month period, out of which **728 specimens** were infected with one or more species of nematodes.

Prevalence Patterns Across Host Species

The overall prevalence (percentage of infected hosts) varied significantly across fish species:

Fish Species	No. Examined	No. Infected	Prevalence (%)
Catlacatla	240	94	39.17
Labeorohita	240	118	49.17
Cirrhinusmrigala	240	132	55.00
Channa punctata	240	156	65.00
Heteropneustesfossilis	240	132	55.00
Clarias batrachus	240	96	40.00

Interpretation:

- *Channa punctata* exhibited the **highest prevalence** (65%), indicating its high susceptibility and its ecological dominance in the sampling area.
- The lowest prevalence was recorded in *Catlacatla* (39.17%), possibly due to its feeding behaviour and habitat strata.

- ❖ Air-breathing catfishes showed moderate to high infection levels, consistent with their benthic habit where nematode larvae are abundant.

Mean Intensity and Mean Abundance

Fish Species	Mean Intensity	Mean Abundance
Catlacatla	3.15	1.23
Labeorohita	3.44	1.69
Cirrhinusmrigala	4.12	2.27
Channa punctata	5.28	3.43
Heteropneustesfossilis	4.91	2.70
Clarias batrachus	4.30	1.72

Interpretation:

- *Channa punctata* not only had the highest prevalence, but also the **highest mean intensity** (5.28), indicating heavy parasite burden.
- High intensity in *Heteropneustesfossilis* suggests it plays an important role as a reservoir host.
- Lower intensity in *Catlacatla* may be linked to its pelagic feeding habit.

Seasonal Variations in Infection

Seasonal Prevalence

Season	Prevalence (%)
Summer	48.20
Monsoon	66.30
Winter	41.10

Interpretation:

- ❖ **Monsoon season** showed the highest prevalence (66.30%), reflecting ideal environmental conditions for nematode larval development.
- ❖ Low winter prevalence is attributed to reduced metabolic and reproductive rates of parasites and hosts.
- ❖ Summer showed intermediate values but significant inter-site variation.

Seasonal Differences Across Host Species

- Cyprinids (*Catla, Rohu, Mrigala*) showed **peak infections in monsoon**.
- Air-breathing fishes maintained relatively high infections even in winter due to stable internal physiology.
- In *Channa punctata*, infection remained high through all seasons, confirming its role as the most reliable indicator species for parasitic nematode communities.

Diversity of Nematodes Identified

A total of **nine nematode species** were recovered and identified through morphological and molecular tools:

Gastrointestinal nematodes:

1. *Camallanusanabantis*
2. *Camallanuscarassii*
3. *Rhabdochonaconei*
4. *Rhabdochona magna*
5. *Procamallanus (Spirocamallanus) guttatus*

Tissue and swim bladder nematodes:

6. *Contracaecumrudolphii* (larvae)
7. *Eustrongylides sp.* (larvae)

Muscle-dwelling nematodes:

8. *Philometra ovata*

These findings corroborate earlier studies, but the molecular analysis revealed deeper insights into cryptic variability.

Morphological Characteristics

Key morphological findings include:

Camallanus spp.

- ❖ Cylindrical reddish body
- ❖ Buccal capsule strongly sclerotized
- ❖ Sexual dimorphism prominent
- ❖ Tail with characteristic bursal rays

Rhabdochona spp.

- Distinct prostomial tooth arrangement
- Elongated oesophagus
- Eggs with polar extensions
- Males with unequal spicules

Procamallanus spp.

- Spirally coiled male tail
- Well-developed esophageal bulb
- Cuticle with fine transverse striations

Larval Anisakids (*Contracaecum*, *Eustrongylides*)

- Large larvae encysted in mesenteries
- Anterior boring tooth
- Thick cuticle

These morphological results guided preliminary species assignments, later validated through molecular characterization.

Molecular Characterization Results

DNA Extraction and PCR Success Rates

- DNA extraction success rate: **92%** (184/200 specimens processed)
- PCR amplification success rates:
  - **18S rRNA:** 89%
  - **ITS region:** 81%
  - **COI:** 76%

Lower COI success is typical due to primer mismatch and degraded mtDNA.

BLAST Identification of Sequences
BLAST results revealed:

Nematode	Closest Match	% Identity
Camallanusanabantis	Malaysia isolate	98.2%
Camallanuscarassii	China isolate	97.6%
Rhabdochonaconei	Nepal isolate	99.1%
Rhabdochona magna	Assam isolate	98.7%
Procamallanus guttatus	Thailand isolate	97.4%
Philometra ovata	Vietnam isolate	99.3%
Contracaecumrudolphii L3	Iran isolate	100%
Eustrongylides sp.	Turkey isolate	96.8%

Discovery of a Possible Cryptic Species
One larval nematode originally mistaken as a **Neoechinorhynchus-like form** showed:

- Only **92–93% identity** with known database sequences
- Significant ITS divergence
- Distinct COI haplotype cluster

Phylogenetic Analysis
18S rRNA Phylogeny

- ❖ All *Camallanus* isolates clustered with Asian clades.
- ❖ *Rhabdochona* isolates formed a monophyletic group distinct from European and African lineages.
- ❖ *Contracaecum* larvae matched cosmopolitan avian anisakid clades, indicating birds as definitive hosts.

ITS Sequence Phylogeny
The ITS tree revealed:

- **Strong species-level differentiation**
- Separation of morphologically similar *Rhabdochona* species
- Deep branching between *Procamallanus* and *Camallanus* species

These results confirm ITS as the most informative marker for species delimitation.
COI Phylogeny
COI resolved:

- Two **cryptic haplogroups** within *Camallanusanabantis*
- Distinct Indian lineage of *Philometra ovata*
- High divergence in the unidentified larval nematode

Bootstraps exceeded 90%, confirming robustness of the nodes.
Major Findings

- ❖ Infection patterns varied across fish species, seasons, and sampling locations.
- ❖ Molecular analysis validated morphological identifications and revealed **cryptic diversity**.
- ❖ Phylogenetic trees placed Kanpur isolates within established global clades but with Indian-specific divergences.
- ❖ Evidence for a **possibly undescribed nematode species** was found.

DISCUSSION

Kanpur Stretch of River Ganga provides an integrative understanding of nematode diversity, host–parasite interactions, and evolutionary relationships in a highly anthropogenically stressed freshwater system. The findings reaffirm that riverine ecosystems undergoing ecological pressure are often hotspots of parasitic diversity, with nematodes representing one of the most sensitive and informative biological indicators.Nematode parasitism in freshwater fishes reflects ecological conditions, availability of intermediate hosts, and the health status of fish populations. The detection of species belonging to genera such as Camallanus, Eustrongylides, Rhabdochona, and Contracaecum aligns with previously established patterns in polluted tropical river systems. However, the molecular approach adopted in the present study revealed nuances often masked by strict morphological identification.The integration of COI, ITS-1, ITS-2, and 18S rRNA markers provided a much-needed resolution in separating closely related nematode clusters.

The phylogenetic trees constructed during analysis consistently supported the monophyletic grouping of detected nematode species. For Camallanus, molecular clustering showed a clear distinction between C. anabantis and C. carangis, species previously considered morphologically ambiguous in Indian waters. In the case of Eustrongylides, the mitochondrial markers revealed a closer affinity with East-Asian lineages than with Indo-Gangetic species reported a decade earlier. This indicates potential

introduction events or the expansion of previously under-reported populations due to environmental shifts.The host species examined—such as Catlacatla, Labeorohita, Channa punctata, and Heteropneustesfossilis—are among the most commonly consumed and ecologically dominant fishes in the region. The study noted that omnivorous and benthic feeders, particularly Channa and Heteropneustes, exhibited higher nematode loads.

The molecular diversity observed, particularly the multiple haplotypes of Camallanus species, may reflect micro-evolutionary responses to environmental stress. High mutation rates detected in mitochondrial genes are consistent with findings from other polluted rivers across Asia and Africa.Molecular sequencing now allows species-level resolution, improving ecological and epidemiological interpretation.Phylogenetic clustering showed that several sequences isolated from Kanpur fish populations grouped with Southeast Asian and Middle-Eastern lineages. Such affinities raise questions regarding:

Migratory Bird Involvement
Movement of Aquatic Livestock
Climatic Influences On Intermediate Host Distribution
The Ganga Basin is a complex migratory corridor, and several piscivorous birds known to carry nematode eggs visit the region seasonally. Thus, the phylogenetic structure revealed here supports the hypothesis of trans-boundary parasite flow.

CONCLUSION

By integrating classical taxonomy with DNA barcoding and phylogenetic assessment, the research successfully identified multiple nematode species, clarified ambiguous taxa, and established evolutionary relationships previously undocumented in this region.

The results highlight:
High prevalence of zoonotically relevant species
Strong correlations between host feeding habits and parasitic load
Phylogenetic linkages extending beyond the Indian subcontinent

DECLARATIONS

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