RESEARCH ARTICLE

Molecular Characterization of *Trichuris globulosa* (Linstow, 1901) in Indian Gaur (*Bos gaurus*) from Tamil Nadu, India

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Abstract

An adult female Indian gaur (*Bos gaurus*) carcass presented for necropsy in the Udhagamandalam Division, Tamil Nadu was examined for parasitic infections. Adult worms recovered from the caecum were subjected to morphological and molecular characterization. Morphological examination revealed a whip-like appearance, trichuroid oesophagus, spicule sheath with spines in males and barrel-shaped eggs with bipolar plugs in females consistent with *Trichuris* spp. Genomic DNA extracted from adult worms was used to amplify 341 bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Sequence analysis revealed 100% identity with *Trichuris globulosa* reference sequences available in the GenBank database. Phylogenetic analysis based on COI sequences clustered the present isolate unequivocally within the *T. globulosa* clade. This study provides the first molecular confirmation of *Trichuris globulosa* in an Indian gaur from Tamil Nadu, highlighting the usage of molecular tools in wildlife parasitology and surveillance at the wildlife–livestock interface.

Key words: Indian Gaur, Nematode, Spill-over, *Trichuris*, Wildlife.

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Introduction

The Indian gaur (*Bos gaurus*), commonly known as the Indian bison is the largest wild cattle species native to the Indian subcontinent, classified under the order Artiodactyla and family Bovidae. It inhabits a range of forest ecosystems from the Western Ghats to Northeast India and is listed as Vulnerable on the IUCN Red List of Threatened Species, largely due to habitat loss, poaching and disease pressure (Duckworth *et al.,* 2016). The domesticated mithun (*Bos frontalis*) is believed to have evolved from Indian gaur populations in Northeast India, representing an important link in the evolutionary history of wild bovines and domestic stock. Free-ranging wild population like gaur remain poorly studied for health and disease and only limited data exist on their parasitic disease profiles (Balmik and Singh, 2025). Wild ungulates are increasingly being impacted by anthropogenic factors such as deforestation, urbanization and human-wildlife conflict, which not only fragment habitats but also lead to increased contacts at the wildlife-livestock interface and facilitate the transmission of pathogens, including gastro-intestinal parasites, between domestic ruminants and wild species like gaur that share grazing pathways and water sources in buffer zones adjacent to core forest areas (Allwin *et al.,* 2016). Parasites pose an insidious threat to host health, causing direct pathology and indirectly compromising host immunity (Thawait *et al.,* 2014). Gastro-intestinal parasitism can result in subclinical infections to severe morbidity or mortality, especially in nutritionally stressed or immunocompromised individuals (Sengar *et al.,* 2017). Among large intestinal nematodes, whipworms of the genus *Trichuris* are important parasites of ruminants. *Trichuris* species inhabit the cecum and colon, where heavy burdens can lead to enteritis, weight loss and reduced body condition (Bhaydiya *et al.,* 2021). Although *Trichuris* infections have been well documented in domestic livestock and several

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wild ruminants globally, molecular and epidemiological data specific to *Trichuris* in Indian gaur remain scarce. This study documents the first molecular characterization of *Trichuris globulosa* in an Indian gaur from Tamil Nadu, India.

Materials and Methods

An adult female Indian gaur carcass was presented by forest officials to the Cattle Breeding and Fodder Development Unit of the Animal Husbandry Department, Udhagamandalam Division, Tamil Nadu (India) for necropsy examination. A systematic necropsy was conducted following the standard procedures. During examination, adult worms were recovered from the caecum and collected for further identification. The recovered helminths were submitted to the Department of Veterinary Parasitology, Veterinary College and Research Institute, Orathanadu, TANUVAS, for morphological identification. The specimens were processed by dehydration through ascending grades of alcohol and subsequently cleared in lactophenol. Permanent mounts were prepared and the parasites were examined under light microscope following the standard morphological keys (Soulsby, 1982).

Genomic DNA was extracted from the worms using commercial DNA extraction kit (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions. The concentration and purity of the extracted DNA were assessed using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific™, USA). Polymerase chain reaction (PCR) was performed to amplify a fragment of the cytochrome c oxidase subunit-I gene using designed specific primers: forward primer 5'-GCATTTGGCGCGATTTTCAGA-3' and reverse primer 5'-AGGGATGCATTTGCCAGTGA-3' (Henedi *et al.,* 2024). PCR amplification was carried out in a thermal cycler (Genei, India) with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 61 °C for 30 s and extension at 72 °C for 30 s with a final extension at 72 °C for 5 min (Callejón *et al.,* 2013). The amplified PCR products were resolved in 1.5% agarose gel electrophoresis at 75 V. DNA 100 bp ladder (HiMedia, India) was used as a molecular size marker. The gel was visualized under ultraviolet illumination using a gel documentation system (Bio-Rad, USA). The PCR amplicons were purified and subjected to bidirectional sequencing using the same primers (Eurofins Genomics, Bengaluru, India). The obtained sequences were edited and assembled using BioEdit version 7.0. The consensus sequences were compared with available sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST). Phylogenetic relationship was inferred using MEGA X software based on the sequences retrieved from GenBank.

Results and Discussion

Adult nematodes recovered from the caecum exhibited typical whip-like morphology characterized by a long, slender anterior portion and a comparatively short, stout posterior region. Male worms measured 45–70 mm in length, while females ranged from 45-60 mm (Fig. 1). The oesophagus was trichuroid type and consisted of a long tube surrounded by a single row of stichocytes forming a well-developed stichosome throughout the oesophagus (Fig. 2). In males, a single spicule measuring 5.0 mm in length was observed, enclosed within a spicule sheath bearing a distinct terminal spherical expansion. The spicule sheath was armed with spines, which were more prominent on the expanded distal portion than along the remaining sheath length (Fig. 3). Female worms showed a clear demarcation between the oesophagus and intestine, with the vulval opening located laterally on the body. The posterior extremity was blunt (Fig. 4) and the uterus contained numerous characteristic barrel-shaped eggs with bipolar plugs with parallel running intestine (Fig. 5). The eggs measured approximately 68 × 35 µm exhibited typical *Trichuris* morphology (Fig. 6). Based on these morphological features and morphometric measurements, the recovered nematodes were identified as *Trichuris* sp.

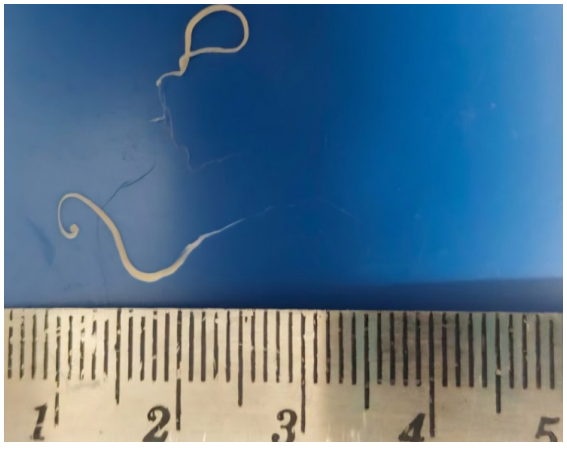

Fig. 1: Gross adult worms measuring 45mm

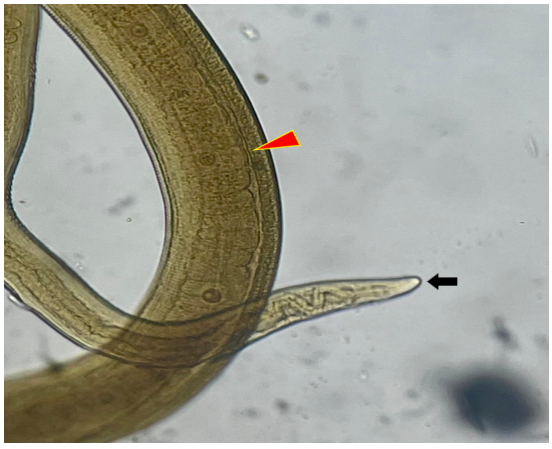

Fig. 2: Narrow anterior end (arrow); Oesophagus of the trichuroid type, consisting row of stichocytes (arrowhead) forming a well-developed stichosome – *T. globulosa* (x100)

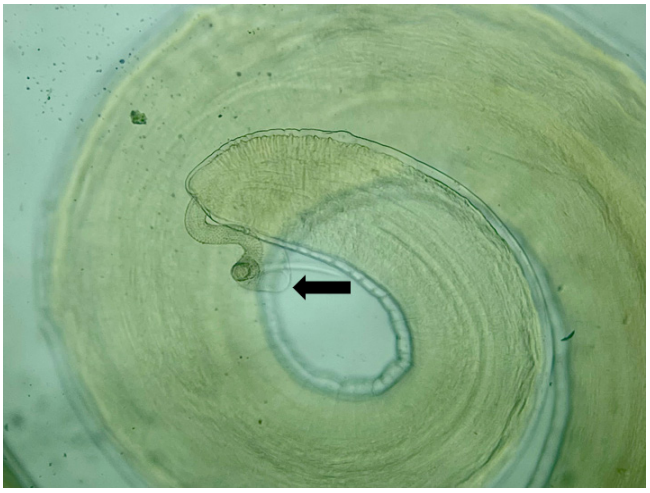

Fig. 3: Male posterior end showing a single spicule enclosed within a spicule sheath bearing a distinct terminal spherical expansion (arrow) – *T. globulosa*– Male (x400)

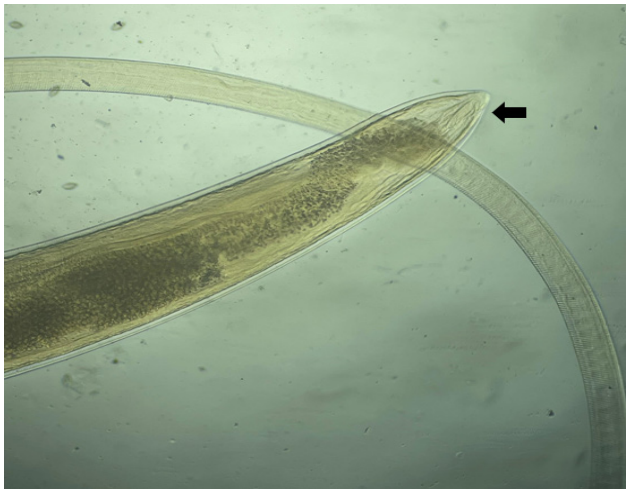

Fig. 4: Female worm showing the blunt posterior extremity - *T. globulosa* (x100)

Fig. 5: Female worm showing the uterus containing characteristic barrel-shaped eggs with bipolar plugs; the intestine runs parallel to the uterus – *T. globulosa* (x400)

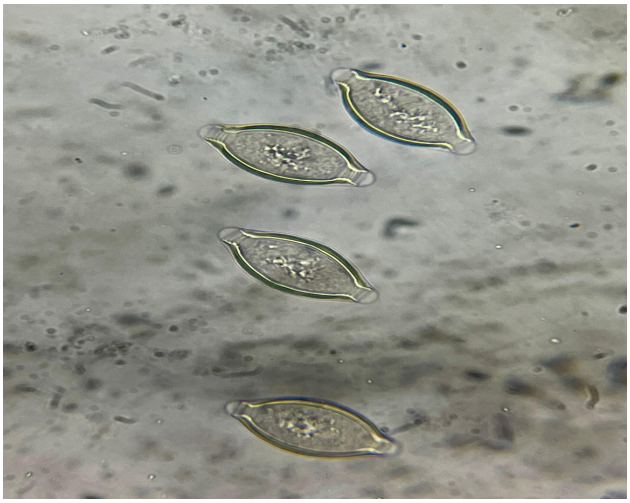

Fig. 6: Barrel-shaped eggs measuring 68 by 35µm size, with prominent bipolar plugs – *T. globulosa* (x400)

PCR amplification of parasite DNA yielded a 341 bp product (Fig. 7) corresponding to the cytochrome c oxidase subunit-I gene of *Trichuris globulosa,* which was subsequently sequenced and submitted to the NCBI database with accession number (PX864044.1). BLAST analysis revealed that the mitochondrial cytochrome c oxidase nucleotide sequence of *Trichuris globulosa* obtained in this study showed 100% identity with the reference sequences of *T. globulosa* available in the GenBank database (PP066032.1, OQ535017.1).

Molecular characterization of the parasite was further supported by phylogenetic analysis of the mitochondrial cytochrome c oxidase subunit I gene. The COI sequence generated in the present study clustered unequivocally within the *Trichuris globulosa* clade, along with previously reported *T. globulosa* isolates retrieved from the GenBank database. The present isolate (*T. globulosa*, Udagamandalam, Tamil Nadu) also grouped closely with *T. globulosa* sequences obtained from camels in Kuwait (Accession nos. OQ535020, OQ535022, OQ535023 and OQ535024) and an isolate from Thailand (OQ535017). This clustering was supported by high bootstrap values ranging from 98 to 100, indicating strong phylogenetic support for species-level identity. Sequences of *Trichuris suis* (PQ628226) formed a distinct lineage separate from the *T. globulosa* cluster, confirming interspecific divergence within the genus *Trichuris*. Additionally, *Moniezia expansa* sequences (OL689029 and PQ897960) were positioned as distant outgroups, which clearly separated from the *Trichuris* clade (Fig. 8).

Gastro-intestinal parasites represent an invisible but significant threat to the health and survival of wild ruminants, especially in biodiverse tropical forests where

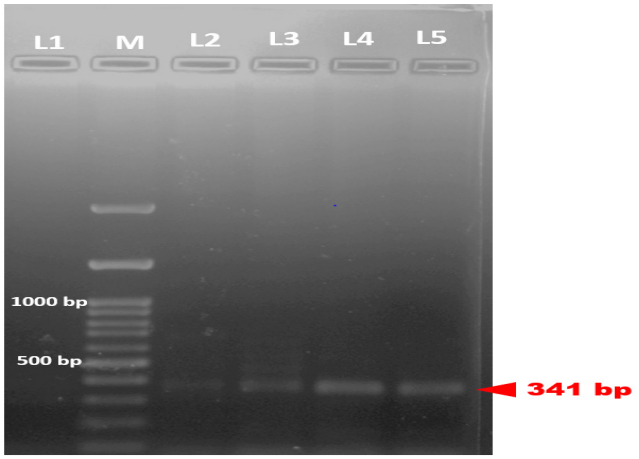

Fig. 7: Agarose gel (1.5%) electrophoresis showing amplification of cytochrome c oxidase subunit I (COI) gene of *Trichuris globulosa*. Lane M: 100 bp DNA ladder; Lane 1: Negative control (NC); Lanes 2-5: positive DNA samples showing the expected amplicon of 341 bp of *T. globulosa*

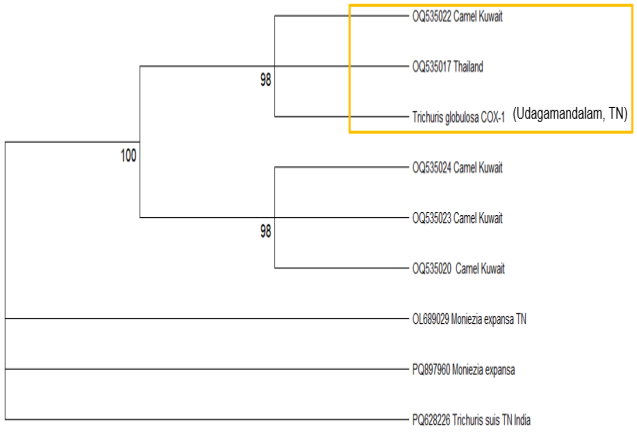

Fig. 8: Maximum Likelihood phylogenetic tree based on cytochrome c oxidase (COI) gene showing the relationship of *Trichuris globulosa* from Tamil Nadu, India with reference *T. globulosa* sequences from camels (Kuwait) and Thailand. (Bootstrap support values (≥70%) are indicated at the nodes)

multiple species share habitat and resources. In temperate and tropical regions such as India, the incidence of GI parasitic infection typically increases during the rainy season due to favourable conditions for the development and survival of infective stages in the environment (Gautam *et al.,* 2019). Parasites such as *Trichostrongylus* spp., *Strongyloides* spp., *Trichuris* spp., *Toxocara* spp., Amphistome, *Fasciola* sp., *Moniezia* spp. and *Eimeria* spp. have been reported in Indian gaur (*Bos gaurus*) indicating the broad diversity of GI parasites encountered in wild bovids (Balmik and Singh, 2025). These findings aligned with global observations that wild ruminants harbour a rich helminth fauna, often in mixed infections (Achhami *et al.,* 2016; Maharjan *et al.,* 2025). *Trichuris* worms are euryxenous nematodes found in a wide range of hosts including ruminants, marsupials, rodents and primates and are predominantly localized in the caecum and colon (Cavallero *et al.,* 2021; Soulsby, 1982). The morphological features observed in this study are in consistent with previous findings (Soulsby, 1982). Heavy infection can cause damage to the intestinal mucosa, inflammatory responses and reduced nutrient absorption, which may result in weight loss, anaemia, diarrhoea and mortality in severe cases (Tahmina and Khan, 2016; Bulbul *et al.,* 2020). These pathological effects reflect patterns seen globally in wild ungulates, where GI helminths have been linked with reduced body mass, poor condition and negative effects on reproductive potential and survival rates (Stein *et al.,* 2002). Wild ruminants such as gaur and deer share grazing areas and water sources with domestic animals, creating interfaces for parasite transmission. Environmental contamination of pastures allows gastrointestinal nematodes to infect multiple host species. Wild ruminants can act as reservoirs, enabling cross-species transmission and spill-over that affects both wildlife conservation and livestock health. Interactions at forest edges and buffer zones further facilitate this exchange, including the spread of anthelmintic-resistant parasite strains (Barone *et al.,* 2020; Brown *et al.,* 2022).

Conclusion

Given that information on parasites of wild animals remains limited, especially in India, non-invasive sampling strategies such as faecal collection provide valuable opportunities to explore parasite diversity and assess health status of wild hosts without disturbing them. Such epidemiological studies can help fill knowledge gaps in wildlife parasite ecology, inform conservation efforts and guide interventions to manage parasitic infections in both wild and domestic animal population.

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