



Morphological Characteristics and Molecular Identification of Cestode: *Taenia taeniaformis* in Domestic Cats in Vietnam

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ABSTRACT

Taenia taeniaformis is a unique tapeworm found in cats. However, limited information on this worm isolated from domestic cats has been documented, including morphological and molecular data on its adult stage. This study aimed to describe the morphological characteristics of *T. taeniaformis* and molecularly identify this tapeworm based on mitochondrial markers. Sixty-one intact adult worms were selected for measurement, specimen preparation, and subsequent observation of morphological characteristics of the scolex, neck, and proglottids. All tapeworms were then subjected to PCR assays to amplify partial *cox1* and 12S rRNA genes. Sequencing techniques were used to sequence the two PCR products amplified from each marker. In results, the mean length of adult tapeworms was 10.57cm, with widths ranging from 0.2 to 0.45cm. The scolex possessed a rostellum armed with double rows of hooks ranging from 32 to 48. The highest frequency was observed in tapeworms, with 38 hooks, constituting 32.79%. The neck was short, the immature proglottids were narrow and elongated, the testes were scattered throughout the proglottids, the ovary and vitelline gland were bilobed, the genital pores opened unilaterally on both sides, and the gravid proglottids consisted solely of a branched uterus. Molecular analysis classified this tapeworm as *T. taeniaformis*. This study represents the first examination of the morphology of adult *T. taeniaformis*, with particular emphasis on the variation in rostellum hook numbers (32-48). Further research is necessary to assess intraspecific variation in *T. taeniaformis* concerning this difference.

Key words: 12S rRNA, *cox1*, Domestic cat, Rostellum hooks, *Taenia taeniaformis*

INTRODUCTION

Taenia taeniaformis is a cyclophyllidean tapeworm (Cestode) that was initially discovered and described in 1786 and was identified as *H. taeniaformis* (Basch 1786). *T. taeniaformis* is widely distributed globally and is the sole tapeworm species in the genus *Taenia* that parasitizes cats. The life cycle of *T. taeniaformis* requires the involvement of a primary host, which is a predator (predominantly cats; additionally, lynx, raccoons and dogs) (Loos-Frank and Zeyhle 1982; Pfeiffer et al. 1997; Matoba et al. 2003; Lignon et al. 2024). In the definitive host, the tapeworm develops into an adult and parasitizes the small intestine. The intermediate hosts of this tapeworm include numerous species of rodents (voles, muskrats, squirrels, rabbits and rats) (Malsawmtluangi et al. 2011; Fuehrer et al. 2012; Ibrahim 2020; Alvi et al. 2021; Gupta et al. 2021; Schuster et al. 2021; Younis et al. 2021; Gomez-Puerta et al. 2021; Yamamoto et al. 2024). In intermediate hosts, larvae

develop into an infectious stage, termed as *Strobilocercus fasciolaris* and are frequently found in the liver. An experimental infection of *S. fasciolaris* in a laboratory rat demonstrated symptoms, including weight loss, lethargy, and a visibly poor coat condition. Gross pathological examination revealed a pale and soft cyst in the liver, measuring approximately 1.5cm in diameter (Venkatesan et al. 2024). Several studies have documented human infections caused by *S. fasciolaris* larvae in the liver and brain (Šterba et al. 1977; Garin et al. 2005). Notably, an unusual case of infection involving adult *T. taeniaformis* in humans was also recorded (Ekanayake et al. 1999).

Therefore, identification based on morphological characteristics is crucial for parasitology. For *T. taeniaformis*, there is a paucity of reports describing the morphological characteristics of adult tapeworms, particularly tapeworm samples collected from the primary host (cat), with the exception of a study by Al-Jashamy and Islam (2007) on the morphological characteristics of *T. taeniaformis*

tapeworm scolex collected from cats. Matoba et al. (2003) described certain morphological characteristics of *T. taeniaformis* collected from feral raccoons (*Procyon lotor*). Other studies have provided information on the metacystode larval stage (*Strobilocercus fasciolaris*) isolated from intermediate hosts (Malsawmtluangi et al. 2011; Fuehrer et al. 2012; Yamamoto et al. 2024).

T. taeniaformis generally exhibits the typical morphological characteristics of the class Cestode, order Cyclophyllidea, which is divided into three distinct regions: the scolex, neck, and strobila. The scolex of the tapeworm is characterized by attachment organs, including four suckers surrounding the scolex. The rostellum, located at the apex of the scolex, was armed with two rows of hooks. The outer row of hooks is larger, measuring 0.36–0.44mm, while the inner row is smaller, ranging from 0.25–0.27mm (Matoba et al. 2003; Al-Jashamy and Islam 2007). The total number of hooks varies from 26 to 38 (Fuehrer et al. 2012) and 30 to 40 (Matoba et al. 2003; Al-Jashamy and Islam 2007). The neck is broad and nearly imperceptible, hence its alternative designation as "broad-necked tapeworm" (Al-Jashamy and Islam 2007). The body comprises three distinct proglottids: immature proglottids originating from the neck and mature and gravid proglottids. The proglottids exhibited rectangular shapes, with widths exceeding their lengths. Adult tapeworms can attain lengths up to 60cm (Al-Jashamy and Islam 2007).

In addition to morphology-based identification methods, molecular-based identification techniques, such as polymerase chain reaction (PCR) and gene sequencing, have been widely applied. Molecular markers from the mitochondrial genome, including *cox1* (cytochrome c oxidase subunit 1), ND1 (NADH dehydrogenase subunit 1), and 12S rRNA, as well as molecular markers from the nuclear genome, such as ITS1 (internal transcribed spacer 1) and ITS2 (internal transcribed spacer 2), serve as reliable tools for the identification and assessment of phylogenetic relationships in helminths in general, and cestodes in particular (Bowles et al. 1992; Bowles and McManus 1993). Notably, the mitochondrial genome has minimal non-coding DNA and no introns; thus, it has been utilized in taxonomic studies because of its rapidly evolving genome compared with the nuclear genome (McManus and Bowles 1996). Several studies have employed genes such as *cox1*, ND1, 12SrRNA, ITS1, and ITS2 to identify and assess the phylogenetic relationships in *T. taeniaformis* (Gasser et al. 1999; Matoba et al. 2003; Malsawmtluangi et al. 2011; Wang et al. 2022; Rojas et al. 2024).

To date, there has been no reports on the morphological and molecular characteristics of *T. taeniaformis* in Vietnam despite its high prevalence in cats. Therefore, this study was conducted to provide information on the morphological and molecular characteristics (based on partial sequences of the 12S rRNA and *cox1* genes) of adult *T. taeniaformis* collected from the small intestine of cats. The results of this study will serve as an important source of data for helminths and parasitic worms in cats.

MATERIALS AND METHODS

Collection of adult tapeworms from cats

Two hundred and fifty-one adult tapeworms were collected from the small intestines of cats in private cat

slaughterhouses in Nam Tu Liem District, Hanoi City, from November to December 2024. The tapeworms were thoroughly washed multiple times in saline solution (8.5%) to remove impurities. Subsequently, samples were preserved in 70% ethanol.

Morphological identification of tapeworms

Sixty-one intact tapeworms (with scolex) were selected for measurement of length, width, and proglottid count. Additionally, the scolex was placed under a stereomicroscope to enumerate hooks on the rostellum. Following macroscopic morphological observations, specimens were prepared for microscopic examination of their morphologies. Specimen preparation followed the protocol described by Upton (2005) with some modifications. The protocol was performed following five steps:

Step 1-Fixation: Initially, the tapeworm was sectioned into segments of approximately 3–5 cm in length. The specimens were then positioned between two glass slides, with a piece of filter paper measuring 2mm × 20mm serving as a spacer at the extremities of the slides. A cotton thread was used to secure the glass slides and fastened with a knot. The specimens were preserved in 70 percent ethanol for a minimum of 30 days.

Step 2-Staining: The Specimens were stained with 1% carmine. Initially, scissors were used to remove the cotton thread, and then forceps were used to gently extract the specimens from the glass slide and transfer them to a Petri dish containing the staining solution. The Petri dish was placed on an orbital shaker for 4h to facilitate dye absorption by the specimens (the staining duration was optimized during the staining process).

Step 3-Destain: A Destain solution was prepared (70% ethanol-hydrochloric acid (HCl 38%) = 1:1). During the destaining process, specimens were continuously observed under a stereomicroscope. The process was ceased when the desired color was achieved (destination time is approximately 5min; if the destaining solution darkens, replace it with fresh solution). Following the Destain process, specimen segments were compressed and fixed between two glass slides (Step 1).

Step 4-Dehydration: The specimens were sequentially immersed in ethanol solutions of increasing concentrations (70, 80, 90, 95, 99, and 99.5%), with each concentration maintained for half a day. Subsequently, the specimens were transferred to a 100% ethanol solution thrice, and each immersion lasted for two hours. Subsequently, the specimens were placed in a mixture of xylene and 100% ethanol (1:1) for 20 min. Finally, the specimens were immersed in xylene for 20 minutes.

Step 5-Permanent: The tapeworm specimens were sectioned into approximately 1cm segments, each containing at least one complete proglottid. Subsequently, the specimens were fixed between a glass slide and cover glass using Canada balsam. The specimens were maintained at room temperature until the mounting medium solidified and then stored in containers.

The morphological characteristics of tapeworm proglottids were observed under a stereomicroscope at a magnification ranging from 8 to 60X.

Molecular identification based on mitochondrial markers *cox1* (cytochrome C oxydase subunit 1) and 12S rRNA

DNA was extracted using an alkaline lysis method. The procedure was as follows: 50mg of each tapeworm specimen was excised from adult tapeworms using sterile surgical scalpels and transferred to 1.5mL Eppendorf tubes. Then, 180mL of 50mM NaOH was added and the samples were incubated at 95°C overnight. The subsequent steps were performed according to the protocol described by Nguyen et al. (2016).

PCR amplification Two mitochondrial molecular markers, 12S rRNA and cytochrome oxidase subunit 1 (*cox1*), were used for amplification. The primer sequences used for PCR amplification are listed in Table 1 (Table 1).

The PCR reaction components consisted of 15μL Master Mix 2X (Phusa Biochem, Vietnam), 0.75μL of each forward and reverse primer (final primer concentration of 0.4mM) (Phusa Biochem, Vietnam), 11.5μL distilled water, and 2μL template DNA. The PCR amplification conditions for the P60F-P375R primer pair were as follows: initial denaturation at 95°C for 5min, followed by 50 amplification cycles of denaturation at 93°C for 1min, annealing at 55°C for 1min 30sec, extension at 73°C for 2min, and a final extension at 72°C for 5min (Dinkel et al. 1998). For the JB3-JB4.5 primer pair: 94°C for 5min; 30 cycles of 94°C for 30sec, 55°C for 30sec, 70°C for 30sec; 72°C for 5min. The results of the gene amplification process were verified by electrophoresis on a 1% agarose gel (supplemented with DNA stain GelRed® (Biotium, Fremont, CA)) at 135V for 30min. The size of the PCR products was estimated using a 100 bp ladder (BioFact, Daejeon, Republic of Korea).

Sequencing technique and phylogenetic tree analysis

Based on the PCR results, the two sequences amplified by each selected primer pair were subjected to bidirectional sequencing using the same primer pairs employed in the PCR reaction. Sequencing was conducted using 1st BASE (Selangon, Malaysia). The obtained DNA sequences were processed using the BioEdit software and compared with sequences published in the GenBank database using the BLAST search tool. The phylogenetic tree was constructed using the maximum likelihood algorithm with 1000 bootstrap replicates using MEGA X software.

Data Access Statement: Research data supporting this publication are available from the NCBI (National Center for Biotechnology Information) repository at located at <https://blast.ncbi.nlm.nih.gov>.

Ethical approval

As no live animal was used in this study, ethical approval was not required from any of the participating institutions.

RESULTS

The morphological characteristics of tapeworms

A total of 61 adult tapeworms were measured and the hooks on their armed rostellum were enumerated. The length of adult tapeworms ranged from 6.5 to 20.6cm (mean length: 10.57cm), with a width of 0.2 to 0.45cm. The tapeworms examined in this study exhibited the typical morphological characteristics of the taeniid type. The scolex possessed crowns with a rostellum armed with double rows of hooks, ranging from 32 to 48. The highest frequency was observed for tapeworms, with 38 hooks (20/61) constituting 32.79% of the sample, followed by 36 and 40 hooks (14 and 16 occurrences, respectively). Tapeworms with 32, 44, 46, or 48 hooks were observed once each (Table 2). All the hooks exhibited a long, blunt handle with a sharp, pointed blade. The numbers of rostellum hooks and their respective frequencies are listed in Table 2.

The neck is a short and unsegmented region immediately posterior to the scolex from which immature proglottids are formed. These immature proglottids were narrow, elongated, and lacked reproductive organs. Mature proglottids contain both the male and female reproductive organs. The male reproductive system comprises multiple testes scattered throughout the proglottid, a vasa efferentia unit forming convoluted common vas deferens, and a cirrus enclosed within the sac. The female reproductive system consists of a bilobed ovary, a bilobed vitelline gland, and a branched uterus containing eggs. The genital atrium was opened unevenly on both sides. Gravid proglottids consist solely of branched uteri and other degenerated reproductive structures (Fig. 1).

Molecular and phylogenetic tree analysis

Through PCR assays, two mitochondrial molecular markers, *cox1* and 12S rRNA, were successfully amplified with approximate sizes of 450 and 380bp, respectively (Fig. 2). The consensus nucleotide sequences obtained from the PCR sequencing were 435bp for *cox1* and 364bp for 12S rRNA. These sequences were subjected to BLAST analysis and compared with the sequences available in GenBank. BLAST results indicated that the tapeworm examined in this study was *T. taeniaformis*, as represented in the phylogenetic tree (Fig. 3A-B). For the 12S rRNA gene, sequence identity among the samples in this study, compared to reference sequences, ranged from 98.7 to 99.7%. Nucleotide identity between the two sequences in this study was 100%. The sequences obtained in this study were closely related to sequence AB031354, which originated from a metacestode larva. Regarding *cox1*, the similarity between the sequences obtained in this study and the reference sequences ranged from 99.06 to 99.53%, and the nucleotide identity between the two sequences in this study was 99.32%.

Table 1: Forward and reverse primers used in PCR assays

The name of genes	Primer names and sequences	Expected size (bp)	References
Cox 1	JB3: 5'-TTTTTTGGGCATCCTGAGGTTTAT-3'	~ 450	Bowles et al. (1992)
	JB4.5: 5'-TAAAGAAAGAACATAATGAAAATG-3'		
12S rRNA	P60F: 5'-TTAAGATATATGTGGTACAGGATTAGATACCC-3'	~ 380	Dinkel et al. (1998)
	P375R: 5'-AACCGAGGGTGACGGCGGTGTGTACC-3'		

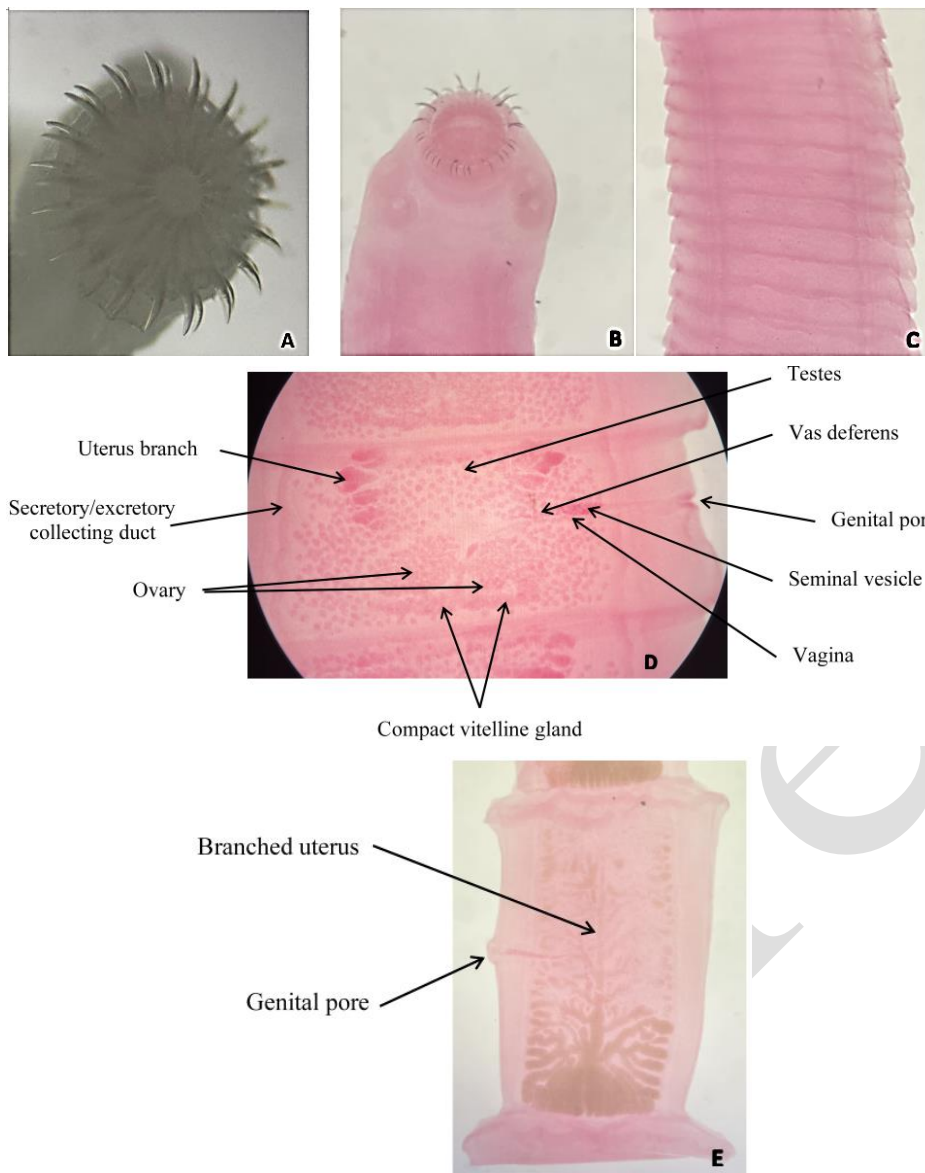


Fig. 1: The morphology of *T. taeniaformis* adult tapeworm isolated from domestic cats captured under stereomicroscopy (8-60X magnification). Unstained scolex (A), stained scolex (B), immature proglottid (C), mature proglottid (D), gravid proglottid (E).

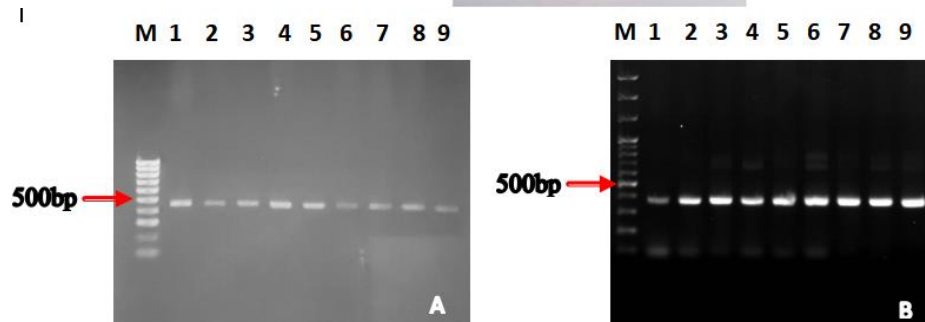


Fig. 2: The image of PCR products on agarose gel. (A) Estimated size of bands amplified from the partial cox1 gene; (B) estimated size of bands amplified from the partial 12S rRNA gene. M: 100bp ladder; lane 1-9: test samples. The red arrows show position of 500bp band.

Table 2: The number and frequency of rostellum hooks of *Taenia taeniaformis*

Number of rostellum hooks	Occurrence frequency of tapeworm	The rate of percentage (%)
32	1	1.64
34	2	3.28
36	14	22.94
38	20	32.79
40	16	26.23
42	5	8.20
44	1	1.64
46	1	1.64
48	1	1.64
Total	61	

DISCUSSION

The tapeworm *T. taeniaformis* is the most prevalent cestode of the genus *Taenia* that parasitizes felid animals (Bourgoin et al. 2022; Adhikari et al. 2023). The prevalence of *T. taeniaformis* in free-ranging cats is as high as 41.98% in Southern Poland (Wierzbowska et al., 2020). However, limited information, including morphological and molecular data, is available on this species. In general, the morphological characteristics of *T. taeniaformis* were fundamentally similar to those of other species in the *Taenia* genus, as described in the results. Morphological analysis in the present study indicated that the number of

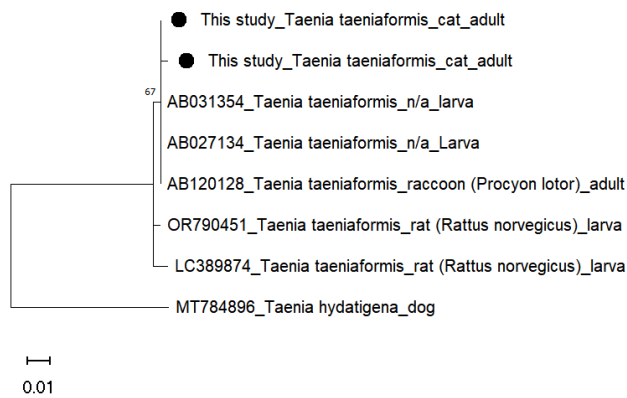


Fig. 3A: The phylogenetic tree relationship of *T. taeniaeformis*, constructed based on the partial 12S rRNA gene by Maximum likelihood algorithm with 1,000 bootstrap replicates on MEGA X. The *T. taeniaeformis* isolates in this study were indicated by solid black circles. “n/a” indicated no information provided for host species.

rostellum hooks ranged from 32 to 48. In contrast, previous studies reported that these hooks ranged from 26 to 38 (Fuehrer et al. 2012) or from 30 to 40 (Matoba et al. 2003; Al-Jashamy and Islam 2007). Consequently, the number of *T. taeniaeformis* hooks should be redefined to range from 26 to 48. Several reports have described the morphology of *T. taeniaeformis* (Matoba et al. 2003; Al-Jashamy and Islam 2007; Fuehrer et al. 2012); however, this is the first study to describe intact adult cestodes isolated from definitive hosts, such as domestic cats.

Molecular analysis has demonstrated significant advantages, particularly in terms of efficiency, owing to the morphological similarities between the *Taenia* genus, particularly in case of *T. taeniaeformis* species complex (Wang et al. 2022; Miljević et al. 2023; Addy et al. 2024; Rojas et al. 2024). In this study, two reliable molecular mitochondrial markers were used to identify cestode taxonomy, and the adult tapeworms were classified as *T. taeniaeformis*. The variation in hook numbers on the rostellum raises questions regarding potential intraspecific variation. Although differences in hook numbers exist, as observed in *T. taeniaeformis*, *T. lynciscapreoli* tapeworms isolated from different hosts and geographical areas were found to be identical (Myczkaa et al. 2020). However, a recent study revealed a high level of intraspecific variation among *T. taeniaeformis* species in different geographical areas. The percentage of mismatch reached 6.4% for ITS2, 14.3% for ITS1 and 2.1% for COI genes (Malsawmtluangi et al. 2011). Other tapeworms belonging to the genus *Taenia*, such as *T. solium* and *T. saginata*, also exhibit genetic diversity (Campbell et al. 2006; Rostami et al. 2015). Therefore, further research is necessary to evaluate the intraspecific variation in *T. taeniaeformis* with differences in rostellum hook numbers.

Although *T. taeniaeformis* has limited significance in human medicine, several sporadic human cases of infection by *Strobilocercus* larvae have been reported. Metacystode larvae have been identified in visceral organs such as the human liver and brain (Šterba et al. 1977; Garin et al. 2005), even it could develop into the adult stage (Ekanayake et al. 1999). Furthermore, numerous rodent

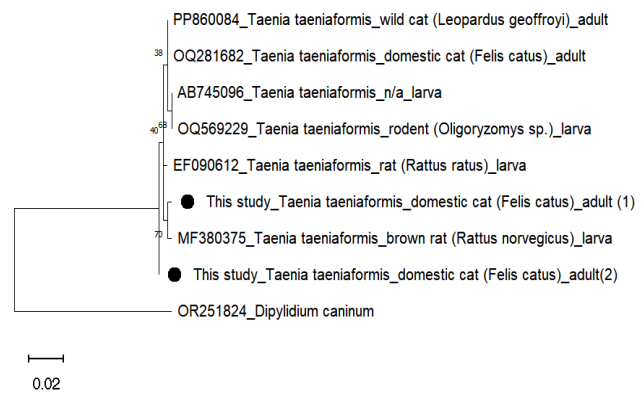


Fig. 3B: The phylogenetic tree relationship of *T. taeniaeformis*, constructed based on the partial cox1 gene by Maximum likelihood algorithm with 1,000 bootstrap replicates on MEGA X. The *T. taeniaeformis* isolates in this study were indicated by solid black circles. “n/a” indicated no information provided for host species.

species such as house rats and voles serve as intermediate hosts and reservoirs for this tapeworm (Singla et al. 2008; Alvi et al. 2021; Gomez-Puerta et al. 2021; Schuster et al. 2021; Younis et al. 2021; Alvi et al. 2023; Echchakery et al. 2024; Thaikoed et al. 2024). Thus, it poses a potential risk to the human transmission of this tapeworm. Consequently, it is imperative to establish preventive strategies against *T. taeniaeformis* infection to improve public health.

Conclusion

This study represents the first comprehensive examination of the morphology of adult *T. taeniaeformis*, both domestically and internationally, with particular emphasis on the variation in rostellum hook numbers (32–48) and the characteristics of mature proglottids. However, further research is required to assess the intraspecific variation in *T. taeniaeformis* with respect to differences in rostellum hook numbers.

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