

Research Article

Molecular Detection of *Taenia taeniaeformis* in Wild Rats and Its Definitive Hosts from Pulau Pinang, Malaysia

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ABSTRACT

Taenia taeniaeformis is a zoonotic tapeworm affecting cats and dogs as definitive hosts, rodents as intermediate hosts, and occasionally humans as accidental hosts. Infected animals may suffer from weight loss, vomiting, malnutrition, and intestinal obstruction, while their presence in urban settings raises public health concerns. This study aims to determine the occurrence and identification of *T. taeniaeformis* in wild rats, domestic cats, and dogs in several selected locations in Pulau Pinang, Malaysia. From October 2022 to March 2023, 146 wild rats were trapped on both the mainland and the island of Pulau Pinang, and faecal samples from cats and dogs were collected from public spaces, veterinary clinics, and shelters. A total of 162 faecal samples (107 cats, 55 dogs) were examined using Sheather's sugar flotation method. Only one cat (0.9%) tested positive for cyclophyllidean eggs, while none were detected in dogs. For molecular confirmation, 44 randomly selected samples (30 cats, 14 dogs) were examined, which yielded 20 (10 cats, 10 dogs) clear PCR bands, which were subjected to sequencing. Additionally, one cat faecal sample from Cat Beach Sanctuary that was examined positive for cyclophyllidean eggs was also sequenced. However, the resulting sequence did not match *T. taeniaeformis* sequences in the NCBI database. Based on morphological characterization, all the recovered larvae showed features consistent with *T. taeniaeformis*. Thus, three randomly selected larval isolates obtained from rats underwent molecular analysis. It was found that one sample (OR790451) yielded a clear PCR band, which showed high similarity with registered *T. taeniaeformis* sequences. Phylogenetic analysis of 12S rDNA revealed that this isolate clustered closely with a Chinese isolate from the leopard cat (*Prionailurus bengalensis*, ON055368), confirming its identity. The findings highlight wild rats as important reservoirs and emphasize the need for continued surveillance in companion animals.

Key words: Cats, dogs, genetic, tapeworm, wild rodent

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INTRODUCTION

Rodents serve as reservoir hosts and are known to transmit numerous diseases to humans, both directly and indirectly. They can carry various pathogens, including bacteria, helminths, protozoa, and viruses. Additionally, rodents play a crucial role as intermediate hosts for endoparasites of carnivores, such as *Toxoplasma*, *Echinococcus*, and *Toxocara* (Krücken *et al.*, 2017). The larvae of *T. taeniaeformis* (also known as *Cysticercus fasciolaris*) are commonly found in the livers of rodents, which act as their intermediate hosts (Singla *et al.*, 2008). This parasite is considered a zoonotic parasite, as it can infect both humans and animals (Taylor *et al.*, 2007; Bowman *et al.*, 2008). *T. taeniaeformis* belongs to the family Taeniidae, a group of parasitic flatworms. This tapeworm primarily infects felines. Its intermediate hosts are murid and cricetid rodents, in which the larval form develops within the liver. The definitive hosts – typically felines and occasionally

canines – become infected when they consume the liver of a rodent containing *C. fasciolaris*, the early larval stage of *T. taeniaeformis*. Notably, infected rodents usually show no clinical symptoms. In contrast, infected cats may exhibit signs such as weight loss and, in cases of heavy infection, intestinal obstruction (Bowman *et al.*, 2008). According to Mohd Zain & Sahimin (2010), the prevalence of *T. taeniaeformis* in free-ranging cats in Peninsular Malaysia was relatively low. However, a higher prevalence has been observed in wild rats, the intermediate hosts (Tijjani *et al.*, 2020). Studies conducted in the southern region of Malaysia reported prevalence rates of up to 30% in wild rats infected with *C. fasciolaris* (Paramasvaran *et al.*, 2009; Ibrahim, 2020; Tijjani *et al.*, 2020).

Molecular techniques such as polymerase chain reaction (PCR) have become valuable tools for the detection and identification of various pathogens and parasites, including *Taenia taeniaeformis*. This parasite can be detected using various gene fragments. The

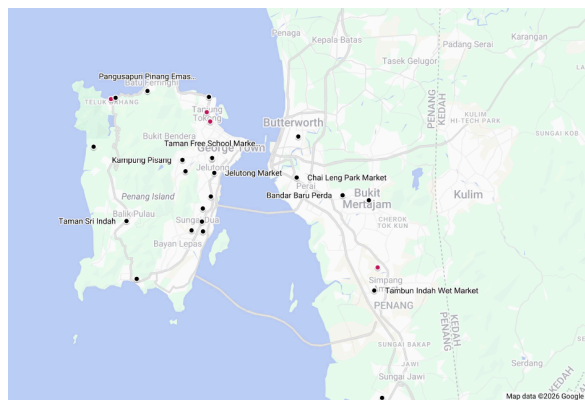


Fig. 1. The stray cats and dogs faecal sampling sites as indicated in red dots. The rat sampling sites are indicated in black dots (Google Map).

mitochondrial 12S rRNA gene and its DNA sequence (12S rDNA) have been widely used in molecular systematics and phylogenetic studies of *T. taeniaeformis* (Dinkel *et al.*, 1998; von Nickisch-Rosenegk *et al.*, 1999a, 1999b; Matoba *et al.*, 2003; Al-Sabi *et al.*, 2011; Banzai *et al.*, 2018; Al-Olayan *et al.*, 2020). There were several previous studies that contributed significantly to the understanding of the phylogeography, host-parasite interactions, and evolutionary history of *T. taeniaeformis* populations (Van Der Kuyl *et al.*, 1995; Dinkel *et al.*, 1998; von Nickisch-Rosenegk *et al.*, 1999a, 1999b; Al-Sabi *et al.*, 2011). In a recent study, 12S rRNA (12S rDNA) was used to identify *T. taeniaeformis* larvae from *Rattus rattus*, and the sequence showed a 99% similarity to the 12S rDNA sequence of *T. taeniaeformis* (adult) from a cat faecal sample in Osaka, Japan (Banzai *et al.*, 2018).

In Malaysia, the only molecular study to date that described the genetic characteristics of *T. taeniaeformis* larvae was conducted by Mohd-Saad *et al.* (2022). Therefore, this study provides the most recent insights into the genetic characteristics of *T. taeniaeformis* larvae in the Malaysian context. The molecular data generated and deposited from this study will serve as a valuable reference for future research and facilitate accurate identification of this parasite species.

MATERIALS AND METHODS

Stray cats and dogs faecal sampling

Faecal samples were collected from several locations in Penang, namely the Cat Beach Sanctuary, Teluk Bahang; Cuddles Clinic, Gurney; Cuddles Hospital, Tanjung Tokong; and the Seberang Perai City Council animal pound, Bukit Mertajam (Figure 1), as well as from the same sites where wild rats were sampled (indicated by black dots).

Collection of faecal samples

A total of 162 fresh faecal samples for cats ($n=107$) and dogs ($n=55$) were successfully collected from the ground as the animals were placed separately in individual cages. The samples from stray cats and dogs were stored in a wide-mouth screwcap container. Faecal sampling of cats and dogs was restricted to areas in which these animals co-occurred with the wild rats based on observation conducted during the wild rats sampling activities. Furthermore, sampling of cats and dogs was also conducted on animals from several shelters and veterinary clinics, which consisted of individuals that were formerly stray and captures from various locations throughout Pulau Pinang, providing a broader representation of the urban companion animal population.

Examination of *T. taeniaeformis* eggs

Faecal flotation was conducted to detect the presence of *T. taeniaeformis* eggs. Sheather's sugar flotation solution was prepared by following the protocol by Dial & Englar (2022), which consists of 454 g of granulated sugar, 355 ml of tap water, and 6 ml of

formaldehyde. The specific gravity of this solution is 1.27–1.33, in which the eggs of *Taenia* spp. can float, as the specific gravity of *Taenia* spp. is 1.2251 (Dial & Englar, 2022).

Approximately 3 to 5 g of faecal samples was measured using a scale. A mixture of Sheather's sugar solution and the faecal sample was sieved into a beaker by a strainer to remove the debris from the sample. The sieved mixture was carefully poured into a tube. A cover slip was placed on the top of each tube for 20 min. The cover slip was later removed by lifting it straight up, together with a drop of fluid adhering to it, and placed on a microscope slide. The entire area under the cover slip was examined under the compound microscope for parasite eggs. After that, the image of the parasite eggs from the faeces of stray dogs and cats was obtained under a light microscope using Cell B Software.

Isolation of *T. taeniaeformis* larvae samples

Wild rats were sampled ($n=146$), and screening for *T. taeniaeformis* larvae was performed according to the methods in Yamamoto *et al.* (2024). Based on morphological characterization, all the recovered larvae showed typical and uniform features of *T. taeniaeformis*. Thus, three representative specimens were selected for molecular analysis in order to validate the morphological identification and to assess the evolutionary relatedness with other isolates reported from other countries.

Molecular technique

DNA extraction

For species confirmation, the DNA of three representative specimens of *T. taeniaeformis* larvae (*Cysticercus fasciolaris*) was extracted using the PrimeWay Genomic II DNA Extraction Kit (Apical Scientific), following the manufacturer's instructions. Meanwhile, a total of 44 faecal samples of companion animals ($n=30$ cats and $n=14$ dogs) were selected from areas exhibiting a high prevalence (50%) of *T. taeniaeformis* larvae in the sampled areas. DNA was extracted from these faecal samples using the NucleoSpin® DNA stool extraction kit (Takara Bio USA Inc). The purity of the extracted genomic DNA was assessed using a Q3000 UV spectrophotometer (Quawell Technology, Inc.), and the DNA samples were stored at -20°C until further use.

Polymerase Chain Reaction (PCR)

For *T. taeniaeformis*, there is no widely used universal specific primer. In this study, both eggs and larvae were amplified using a pair of primers: P60F (5'-TTAAGATATATGTGGTACAGGATTAGATACCC-3') and P375R (5'-AACCGAGGGTGACGGCGGTGTGTACC-3') targeting the mitochondrial 12S gene of taeniid cestodes as described by Dinkel *et al.* (1998). These primers target a 373 bp fragment of the mitochondrial 12S rRNA gene, which allows precise identification of *T. taeniaeformis* by comparison with reference sequences.

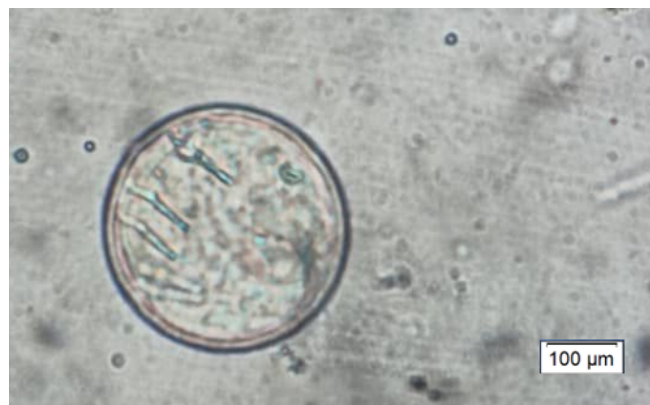


Fig. 2. The image of cyclophyllidean eggs under a light microscope.

The reaction mixture was optimized to a final volume of 25 μ L, consisting of 1 μ L of DNA template, 1 μ L of each primer (10 μ M), 9.5 μ L of ultra-pure water, and 12.5 μ L of NEXTM e PCR 2 $\times$ MasterMix (NEXTM Diagnostics, Korea). PCR amplification was carried out using a MyCyclerTM Thermal Cycler (Bio-Rad Laboratories, Inc.). Optimization of the PCR conditions was conducted by adjusting reagent concentrations and cycling parameters, based on the NEXTM e PCR 2 $\times$ MasterMix protocol and procedures described by Mohd-Saad *et al.* (2022), following general optimization guidelines (Lorenz, 2012).

The thermal cycling conditions included an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 48°C for 30 sec, and extension at 72°C for 1 min. A final extension was performed at 72°C for 10 min. Ultra-pure water was used as a negative control in each PCR run to check for contamination (Mohd-Saad *et al.*, 2022).

Gel electrophoresis

The amplification products were then electrophoresed in 2% agarose gel (PROMEGA USA) and Tris-Borate-EDTA (TBE) buffer. Gels were stained with RedSafeTM Nucleic Acid Staining Solution (INTRON Biotechnology) and were run for about 35 min in 1 $\times$ TBE buffer at 80 V and 180 mA. Then, the gels were visualized under an ultraviolet (UV) gel documentation system (Syngene GeneFlash Network Bio Imaging system). PCR products were sent to the Centre of Chemical Biology (CCB), USM for purification and sequencing.

Sequencing and phylogenetic analysis

The sequence data were edited and arranged manually using the MEGA 11 software. Later, the nucleotide sequences of 12S rRNA genes obtained from this study were deposited in the National Center for Biotechnology Information (NCBI) GenBank. Sequence obtained from this study together with other Mitochondrial 12S rDNA gene of *T. taeniaeformis* from different regions were included for phylogenetic analysis (GenBank, NCBI) in which *T. pisiformis* (accession number MW350140) was used as the outgroup for the phylogenetic tree construction as it is the closest species to the ingroup and phylogenetic trees were constructed according to Okamoto *et al.* (1995), von Nickisch-Roseneck *et al.* (1999a) and von Nickisch-Roseneck *et al.* (1999b). Phylogenetic trees were then constructed using the MEGA 11 software using maximum-likelihood (ML) method analysis (Tamura *et al.*, 2021).

RESULTS

Occurrence of cyclophyllidean eggs in cats and dogs using the faecal flotation method

Out of 107 cat faecal samples, only one (0.9%) cat faecal sample from the Cat Beach Sanctuary was detected containing cyclophyllidean eggs (Figure 2), whereas none was detected from dogs out of 55 faecal samples that underwent the faecal flotation method.

Occurrence of cyclophyllidean eggs in cats and dogs using Polymerase Chain Reaction (PCR)

Out of 44 selected faecal samples collected from cats ($n=30$) and dogs ($n=14$), only 20 samples (10 cats & 10 dogs) yielded strong PCR amplification and were subsequently subjected to DNA sequencing. However, none of the sequenced samples matched the registered *T. taeniaeformis* sequences in the National Center for Biotechnology Information (NCBI) database.

Additionally, a faecal sample from the Cat Beach Sanctuary, which contained cyclophyllidean eggs, also showed no genetic match with *T. taeniaeformis* sequences in the NCBI database.

Species confirmation of *T. taeniaeformis* larvae (*C. fasciolaris*) from wild rats

Out of three representative specimens of *T. taeniaeformis* larvae (*C. fasciolaris*), only one sample showed a visible band under the UV illuminator machine. It was a relatively high match with the registered sequences of the *T. taeniaeformis* from the leopard cat (*Prionailurus bengalensis*) in China (ON055368) in the National Center for Biotechnology Information (NCBI) database. This sequence was submitted to NCBI GenBank, and the accession number was obtained (OR790451). A maximum likelihood tree was constructed with all highly matched sequences (above 90%) of 12S rDNA *Hy. taeniaeformis* (Synonym to *T. taeniaeformis*) samples that were annotated and deposited in GenBank (Figure 3).

DISCUSSION

Taenia taeniaeformis larvae (*C. fasciolaris*), commonly known as the cat tapeworm, are typically found in wild rats and can also infect cats and dogs. In the study by Yamamoto *et al.* (2024), although a high prevalence of *T. taeniaeformis* larvae was detected in wild rats, the adult stage of the parasite was not found in definitive hosts (cats & dogs). This suggests that while wild rats serve as intermediate hosts, the transmission to definitive hosts requires ingestion of infected tissue, as the larvae encyst in the liver and can only develop into adults after being consumed (Lee *et al.*, 2016). Mohd Zain *et al.* (2013) reported a very low occurrence (2.7%) of adult *T. taeniaeformis* in definitive hosts such as cats and dogs, based on necropsy findings from stray cats. This low prevalence may be attributed to a reduced predatory behavior among free-roaming domestic cats. In urban settings, where food sources such as kitchen scraps and leftovers are readily available, cats have less incentive to hunt. Additionally, the presence of cat feeders – individuals who regularly provide food – further reduces the need for cats to prey on intermediate hosts like rodents. Natoli *et al.* (1999) found that cat feeders often provided food quantities exceeding the cats' physiological needs, driven by the perception that the animals were underfed. As a result, the reduced need to hunt likely contributes to the lower rates of *T. taeniaeformis* adult infections observed in these cats.

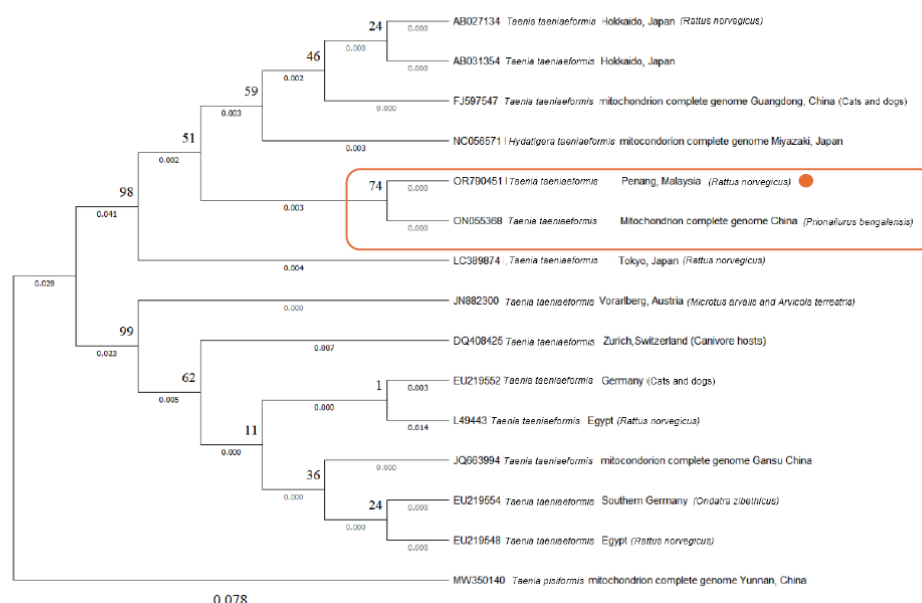


Fig. 3. Phylogenetic tree of the *T. taeniaeformis* sequences from Pulau Pinang, Malaysia (OR790451) and reference 12SrDNA gene sequences from GenBank. The tree was rooted with *Taenia pisiformis* as the outgroup. The evolutionary history was inferred using the Maximum Likelihood method and the Kimura 2-parameter model. The tree with the highest log likelihood (-734.49) was shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with the superior log likelihood value. This analysis involved 15 nucleotide sequences.

Although the prevalence of adult *T. taeniaeformis* in definitive hosts such as cats and dogs is low, the parasite appears capable of maintaining its life cycle with the production of relatively few eggs (Lee *et al.*, 2016). *Taenia* spp. eggs are highly resilient and can persist in the environment long enough to infect suitable intermediate hosts under favorable conditions. In field settings, these eggs have been reported to survive for up to one year. According to Jansen *et al.* (2021), *Taenia* spp. eggs prefer humid environments and temperatures between 0 and 20°C, with humidity playing a more critical role in egg viability than temperature. Their survival significantly decreases at higher temperatures (above 25°C) and under freezing conditions.

In urban areas such as Pulau Pinang, the abundance of buildings and drainage systems may create shaded, humid microhabitats that support egg survival, particularly in commercial and residential zones. The observed seasonal patterns of infection suggest that transmission could also involve external sources, possibly including alternative definitive hosts. However, it remains unclear whether reduced egg viability during the dry season or the lack of suitable intermediate hosts contributes more significantly to the seasonal variation in transmission (Mohd Zain *et al.*, 2012).

There were several methods that can be used for the detection of cyclophyllidean eggs, including faecal flotation, sedimentation, or a combination of both flotation and sedimentation methods. However, Sheather's sugar flotation is commonly preferred for detection of cestode eggs due to its high specific gravity (SG 1.27–1.33), which allows efficient recovery of *Taenia* eggs in this study. In contrast, the commonly used saturated salt flotation method has a lower specific gravity, which is not suitable for the detection of *Taenia* eggs with the specific gravity of 1.2251 (Dial & Englar, 2022). Nevertheless, only one cat faecal sample was found positive for cyclophyllidean eggs by using this method, suggesting a low occurrence of infection of cestodes within the sampled population. It is well established that cestodes have a complex life cycle

which involves multiple developmental stages and hosts. Similarly, *T. taeniaeformis*, which exhibits a complex life cycle with larvae developing in rodents and adults in cats, particularly.

Also, findings from PCR amplification of 44 selected faecal samples of companion animals produced several clear bands from 20 faecal samples. Unfortunately, subsequent sequencing did not match with *T. taeniaeformis* sequences available in the NCBI database. This discrepancy could be due to amplification of other taeniid cestodes rather than *T. taeniaeformis*, as the primer used is a genus-level primer.

Interestingly, amplification of one larval sample of *T. taeniaeformis* (OR790451) showed the closest genetic relationship to an isolate from the leopard cat (*Prionailurus bengalensis*) in China (ON055368), based on the 12S rDNA genetic marker (NCBI, 2023). The availability of reference sequences for *T. taeniaeformis* larvae is limited, particularly in the Southeast Asian region (Catalano *et al.*, 2019; Alvi *et al.*, 2021) and in Malaysia (Okamoto *et al.*, 1995; Lavikainen *et al.*, 2016; Mohd-Saad *et al.*, 2022), which restricts broader phylogenetic comparisons. Despite this, the phylogenetic tree remains a valuable tool for understanding the genetic diversity of *T. taeniaeformis*.

Mello *et al.* (2018) reported that molecular analyses of *T. taeniaeformis sensu lato* (s.l.) across Asia, Europe, and Africa revealed the presence of three cryptic species within the genus, indicating a species complex. These have been categorized into: (1) Clade A – *Hydatigera taeniaeformis sensu stricto* (s.s.), commonly associated with rodents; (2) Clade B – *Hydatigera kamiyai* Iwaki, typically found in hosts from the Cricetidae family; and (3) Clade C – an undescribed *Hydatigera* species, which may represent an intraspecific variant or a novel species (Lavikainen *et al.*, 2016).

Phylogenetic differentiation in *T. taeniaeformis* has been associated with three main factors: morphological variation (Mello *et al.*, 2018), host specificity—both definitive and intermediate (Lavikainen *et al.*,

2016; Wang *et al.*, 2022), and geographic distribution influenced by human-mediated movements (Lavikainen *et al.*, 2016; Mello *et al.*, 2018; Wang *et al.*, 2022). These findings highlight the complexity and hidden diversity within *T. taeniaeformis* and emphasize the importance of molecular tools for accurate taxonomic and epidemiological assessments.

CONCLUSION

This study provided the most recent data on genetic characteristics of *T. taeniaeformis* larvae from wild rodents in Peninsular Malaysia. Meanwhile, the accumulated and deposited data on the morphology and molecular characteristics from this study will be a useful reference for parasite identification in the future.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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