Original Article

First Molecular Detection and Phylogeny of *Trichostrongylus axei* and *Spiculopteragia houdemeri* From Indonesian Deer

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How to Cite This Article Arif, R., Satrija, F., Tiuria, R., Ridwan, Y., & Nurhidayah, N. (2026). First Molecular Detection and Phylogeny of *Trichostrongylus axei* and *Spiculopteragia houdemeri* From Indonesian Deer. *Iranian Journal of Veterinary Medicine, 20*(1), 113-122. http://dx.doi.org/10.32598/ijvm.20.1.1005816

http://dx.doi.org/10.32598/ijvm.20.1.1005816

ABSTRACT

Background: Gastrointestinal nematode infections represent a major threat to the health of both wild and domestic animals, including captive deer. However, data on their prevalence and molecular characteristics in Indonesia remain limited. Understanding parasite diversity is crucial for establishing effective control and health management programs.

Objectives: This study aimed to detect, identify, and analyze the phylogenetic relationships of gastrointestinal nematodes infecting captive deer in West Java, Indonesia.

Methods: Fecal samples were obtained from 13 breeding centers for parasitological and molecular analyses. Parasitological analyses involved flotation and McMaster techniques to detect strongyle-type eggs, while polymerase chain reaction (PCR) targeting the ITS2 ribosomal DNA region was performed to amplify nematode DNA. Positive amplicons were sequenced; species identification was confirmed through BLAST analysis. Phylogenetic trees were constructed using maximum likelihood methods to assess the genetic relationships of the identified nematodes with reference sequences.

Results: The overall prevalence of gastrointestinal nematode infection was 18.1%, with egg counts consistently below 50 EPG, indicating mild infection levels. Molecular analysis identified two nematode species: *Trichostrongylus axei*, detected across multiple locations, indicating widespread presence, and *Spiculopteragia houdemeri*, found only in three specific sites. Phylogenetic analysis revealed high genetic similarity between Indonesian isolates and reference sequences from Europe and Asia, highlighting the potential global conservation of these species.

Conclusion: This study presents the first molecular detection of *T. axei* and *S. houdemeri* in captive deer populations in Indonesia, thereby providing critical baseline data to support future epidemiological surveillance and the development of health management strategies.

Keywords: Deer, Indonesia, Phylogenetic analysis, *Spiculopteragia houdemeri*, *Trichostrongylus axei*

Article info:
Received: 27 May 2025
Accepted: 30 Jul 2025
Publish: 01 Jan 2026

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Introduction

Gastrointestinal parasitic infections are a significant health concern affecting wild and domestic animals. Parasitic nematodes are common gut parasites in deer populations, contributing to clinical signs, such as weight loss, anemia, diarrhea, and, in severe cases, mortality (Modabbernia et al., 2021; Khattak et al., 2023; Yan et al., 2023). In addition to their direct effects on individual health, these parasites may also impact reproductive quality and population dynamics, especially in areas where deer are managed for conservation (Phetla et al., 2024). In Indonesia, studies on parasitic infections in deer remain limited, despite the country's rich biodiversity and the presence of both native and introduced deer species (Rahman et al., 2020).

Several species of gastrointestinal nematodes have been documented infecting deer. Common genera include *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*, *Oesophagostomum*, and *Spiculopteragia* (Davidson et al., 2014; Lyons et al., 2024). These parasites are transmitted through the faecal-oral route influenced by environmental conditions, host density, and management practices (Morgan et al., 2013; Roeber et al., 2013a). In Indonesia, although some studies have documented *Haemonchus contortus*, *Oesophagostomum* spp., *Trichostrongylus* spp., and *Fasciola* spp. infections in domestic and wild ruminants (Puspitasari et al., 2016; Purnama et al., 2021; Arif et al., 2024), specific information on nematode infections in deer remains rare.

Spiculopteragia spp. are trichostrongyle nematodes that primarily inhabit the abomasum of cervids and other ruminants. Infections with *Spiculopteragia houdemeri* have been reported from several regions, including Austria, Japan, and France, mainly affecting sika deer (*Cervus nippon centralis*), red deer (*Cervus elaphus*), and roe deer (*Capreolus capreolus*) (Patrelle et al., 2014; Inoue et al., 2022). Similarly, *Trichostrongylus axei* is a cosmopolitan nematode that infects a broad range of hosts, such as cattle, sheep, goats, and various wildlife species, including deer (ten Doesschate et al., 2017; Halvarsson et al., 2022). Residing in the abomasum and sometimes the stomach, *T. axei* causes parasitic gastritis that may lead to decreased feed conversion efficiency and weight loss (Bhat et al., 2023). Although its presence is well-documented in parts of Europe, North America, and Asia (Davidson et al., 2014; Inoue et al., 2022), reports from Southeast Asia, particularly Indonesia, are still limited.

Molecular techniques have greatly enhanced the accuracy of parasite identification, particularly through the use of ribosomal DNA regions, such as internal transcribed spacer 2 (ITS2) (Meshgi et al., 2015; Workentine et al., 2020; Rojas et al., 2025). Phylogenetic analysis based on these markers can provide valuable insights into parasite diversity, evolutionary relationships, and biogeographical patterns (Kumar et al., 2021).

This study aimed to provide the first molecular identification and phylogenetic characterization of *T. axei* and *S. houdemeri* in Indonesian deer. Polymerase chain reaction (PCR) and ITS2 sequencing were used for species confirmation. Phylogenetic trees were constructed to examine the evolutionary relationships. This research is expected to improve understanding of parasitic nematode genetics and distribution in Southeast Asia and support future epidemiological studies.

Materials and Methods

Study area and sample collection

This study was conducted by collecting deer fecal samples from 13 breeding sites across West Java, Indonesia (Figure 1). Fecal samples were collected immediately after defecation to ensure sample freshness. The Timor deer (*Cervus timorensis*) is classified as vulnerable on the IUCN red list (IUCN, 2018) and is legally protected in Indonesia, while the spotted deer (*Axis axis*) is listed as least concern globally but also protected under national law (IUCN, 2025). Samples were transported in a cooling box to the Helminthology Laboratory, School of Veterinary Medicine and Biomedical Sciences, IPB University. The samples were kept in a refrigerator at 4 ˚C for further analysis.

Fecal examination and larval culture

Fecal samples were examined qualitatively using a flotation technique, which separates parasite eggs from fecal debris based on differences in specific gravity. This method allows clear visualization of eggs under a microscope for morphological identification. A quantitative McMaster technique was used to estimate egg counts per gram of feces (EPG), providing an assessment of infection intensity (Zajac et al., 2021). In addition, larval cultures were established using the Baermann technique and harvested on the seventh day post-culture. The harvested larvae were washed with distilled water, then transferred into 200 µL microtubes with 10 µL of distilled water allocated per larva. The tubes were stored at 1-4 ˚C and kept refrigerated until DNA extraction.

DNA extraction and molecular analysis (PCR and sequencing)

DNA extraction was performed using a lysis buffer composed of a direct PCR kit, 1 M dithiothreitol (DTT), and proteinase K, in a ratio of 25:1:1 per sample. A total of 10 µL of the lysis solution was added to each larval sample and incubated at 60 ˚C for 60 minutes, followed by 95 ˚C for 10 minutes.

PCR amplification targeted the ITS2 region using generic forward and reverse primers as described by Bisset et al.: Forward primer ITS2GF (5'-CACGAATTGCAGACGCTTAG-3') and reverse primer ITS2GR (5'-GCTAAATGATATGCTTAAGTTCAGC-3'), producing an expected product size of 370–398 bp. The PCR reaction was performed in a final volume of 30 µL, consisting of 15 µL GoTaq® Green Master Mix (Promega, Madison, WI, USA), 1.5 µL of 10 µM of each primer, 3 µL of DNA template, and 9 µL of nuclease-free water. The thermocycling conditions were as follows: Initial denaturation at 95 ˚C for 2 minutes, 40 cycles of denaturation at 95 ˚C for 15 seconds, annealing at 54 ˚C for 15 seconds, extension at 72 ˚C for 15 seconds, followed by a final extension at 72 ˚C for 7 minutes (Bisset et al., 2014) *Teladorsagia circumcincta*, *T. axei*, *Trichostrongylus colubriformis*, *Trichostrongylus vitrinus*, *Cooperia curticei*, *Cooperia oncophora*, *Nematodirus spathiger*, *Chabertia ovina*, and *Oesophagostomum venulosum*.

PCR products were visualized by electrophoresis on a 1% agarose gel stained with BioSafe™ DNA Stain. A 100 bp DNA ladder was used to estimate product size. Positive PCR products were sent to Integrated DNA Technologies (IDT), Singapore, for nucleotide sequencing.

Phylogenetic analysis

All nucleotide sequences were analyzed using MEGA11 software and BLAST. Forward and reverse sequences were aligned, trimmed, and assembled into consensus sequences. These were compared against reference ITS2 sequences in GenBank using BLAST to identify the closest matches. The selected sequences were used to construct a phylogenetic tree using the neighbor-joining method and the Kimura 2-parameter model in MEGA11. Bootstrap analysis with 1000 replicates was performed to evaluate the robustness of the tree topology (Tamura et al., 2021).

Results

Sample location and prevalence

The study was conducted at 13 deer breeding sites located across various districts in West Java Province, Indonesia. The sites included Cariu (Bogor), Panisan (Subang), Ranca Upas and Bandung Zoo (Bandung), Tahura (Bandung), Vedca (Cianjur), Pangalengan and Soreang (Bandung), Kehati and Bumi Patra (Indramayu), Gedung Negara (Cirebon), Taman Satwa (Tangerang), and Mabda Islam (Sukabumi). Figure 1 illustrates the sampling locations, indicating the spatial distribution of the deer populations across the province.

A total of 248 deer were examined, comprising three species: *A. axis* (spotted deer), *C. timorensis* (Timor deer), and *Rusa unicolor* (sambar deer). Parasite infections were present in multiple locations, though the intensity of infection was consistently low (Table 1). The prevalence of helminth eggs among all deer sampled was 18.1%. The highest infection prevalence was recorded at the Cariu site in Bogor District, where both *C. timorensis* and *A. axis* demonstrated a 60.0% positivity rate. Ranca Upas in Bandung District also showed a high prevalence in *C. timorensis*, with 17 out of 37 individuals (45.95%) testing positive. Moderate levels of infection were observed in Bumi Patra, Indramayu (33.33%), and Tahura, Bandung District (28.57%). In contrast, several sites, such as the Bandung Zoo, Vedca (Cianjur), and Pangalengan (Bandung), showed no positive cases. Similarly, zero prevalence was recorded in *R. unicolor* at Bandung Zoo. In a few sites, such as Soreang (12.5%) and Gedung Negara (20.0%), the prevalence was relatively low, affecting a minority of the sampled individuals.

All positive samples showed an EPG value of less than 50, indicating a uniformly low worm burden across all infected deer. The presence of infection was limited to light intensities and did not exceed the 50 EPG threshold in any case. This pattern was consistent across all sites where infection was detected.

PCR identification

PCR amplification targeting the ITS2 region (~370 bp) was successfully conducted on 26 larval DNA samples as shown in Figure 2. Clear and specific bands around 370 bp were detected in samples from eight sampling sites: Tahura (lane 3), Kehati (lane 7), Bumi Patra (lane 8), Paniisan (lanes 11 and 12), Vedca (lanes 13 and 14), Cariu (lane 16), Soreang (lane 18), Mabda Islam (lane 24), and Taman Satwa (lanes 25 and 26). These results confirm the presence of *S. houdemeri* and/or *T. axei* DNA in these samples.

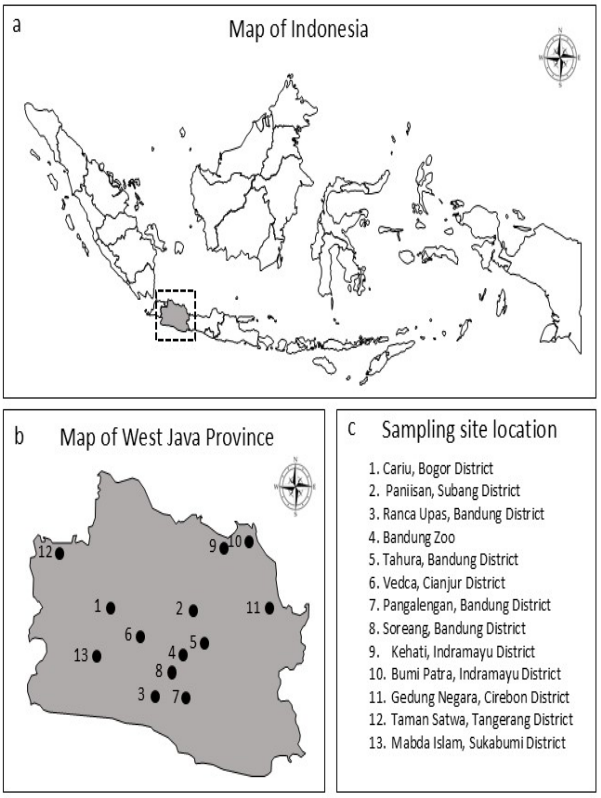

Figure 1. Sampling locations of captive deer surveyed for gastrointestinal nematodes in West Java, Indonesia

a) National map indicating West Java Province., b) Detailed map of West Java, showing sampling sites, c) List of corresponding sampling site locations

In contrast, samples from five other locations—Ranca Upas (lanes 2 and 10), Pangalengan (lane 5), Soreang (lanes 6, 17–20), and Gedung Negara (lanes 9, 21–22)—did not show visible PCR bands, indicating negative results for the targeted nematodes. For Cariu, although two samples were tested (lanes 15 and 16), only one sample (lane 16) yielded a positive band, suggesting intra-location variability in parasite detection. The gel electrophoresis data align with the fecal examination findings and reinforce the molecular confirmation of gastrointestinal nematode presence in deer populations across multiple breeding locations.

Sequencing and phylogenetic analysis

The sequencing of PCR-positive samples revealed the presence of two nematode species infecting captive deer in West Java: *T. axei* and *S. houdemeri*. BLAST comparisons against the NCBI database confirmed the identity of each sample. Samples identified as *T. axei* originated from Paniisan (Subang District), Vedca (Cianjur District), Taman Satwa (Tangerang District), and Mabda Islam (Sukabumi District). Meanwhile, *S. houdemeri* was detected in samples from Tahura (Bandung District), Kehati (Indramayu District), and Bumi Patra (Indramayu District).

The BLAST percent identity scores for *T. axei* ranged from 99.12% to 100%, with the highest match (100%) recorded in the sample from Paniisan (Subang District). For *S. houdemeri*, the percent identity ranged from 99.42% to 99.74%, with identical sequence similarity observed in samples from Kehati and Bumi Patra, both located in Indramayu District.

Further analysis was conducted using a phylogenetic tree to confirm the evolutionary relationship of the *T. axei* sample from Subang. As shown in Figure 3, the sample labeled "5242840 Subang 1 ITS F" clustered tightly with *T. axei* isolates from Egypt, Denmark, New Zealand, and Scotland. This clade demonstrated minimal branch distances, indicating a high level of genetic similarity. The Indonesian isolate clearly grouped within the *T. axei* lineage and was distinctly separated from other *Trichostrongylus* species, such as *T. vitrinus*, *T. retortaeformis*, and *T. colubriformis*. *H. contortus* from Uganda was used as an outgroup and formed a distant branch, supporting the overall phylogenetic topology and confirming the placement of the Indonesian isolate within the *T. axei* clade.

Table 1. Prevalence of gastrointestinal nematodes in captive deer across selected locations in West Java, Indonesia

No.	Location	Species	Population	Positive	EPG	Prevalence
1	Cariu, Bogor District	*C. timorensis*	5	3	<50	60
		A. axis	5	3	<50	60
2	Paniisan, Subang District	*C. timorensis*	26	6	<50	23.08
		A. axis	6	3	<50	50
3	Ranca Upas, Bandung District	*C. timorensis*	37	17	<50	45.95
4	Bandung Zoo	*C. timorensis*	6	0	-	0
		A. axis	6	0	-	0
		R. unicolor	1	0	-	0
5	Tahura, Bandung District	*A. axis*	5	0	-	0
		C. timorensis	28	8	<50	28.57
6	Vedca, Cianjur District	*A. axis*	10	0	-	0
		C. timorensis	27	1	<50	3.7
7	Pangalengan, Bandung District	*A. axis*	14	0	-	0
8	Soreang, Bandung District	*A. axis*	24	3	<50	12.5
9	Kehati, Indramayu District	*C. timorensis*	14	1	<50	7.14
10	Bumi Patra, Indramayu District	*C. timorensis*	9	3	<50	33.33
11	Gedung Negara, Cirebon District	*A. axis*	15	3	<50	20
12	Taman Satwa, Tangerang District	*A. axis*	5	1	<50	20
13	Mabda Islam, Sukabumni District	*A. axis*	5	1	<50	0

EPG: Egg counts per gram of feces.

The phylogenetic analysis of *Spiculopteragia* spp. is presented in Figure 4. Based on ITS region sequences, the positive samples identified from three locations—sample 3 (Tahura, Bandung District), sample 7 (Kehati, Indramayu District), and sample 8 (Bumi Patra, Indramayu District)—clustered within a monophyletic group alongside *S. houdemeri* sequences from Japan (LC628881.1 and AB682692.1). These sequences form a distinct clade with moderate to high bootstrap support (34–62), indicating a close evolutionary relationship among these Indonesian isolates and those from sika deer and wild ruminants in Japan.

Notably, the Indonesian samples are positioned closer to LC628881.1 (*S. houdemeri* from Sika deer, Japan), suggesting that the isolates found in Indonesia may share a more recent common ancestor with the East Asian strains. This close phylogenetic affinity reinforces the identification of these samples as *S. houdemeri*, supporting previous BLAST results with percent identity ranging from 99.42% to 99.74%. These findings indicate a potential geographical linkage or historical introduction event involving *Spiculopteragia* spp. among cervid hosts in East and Southeast Asia.

Discussion

This study provides a comprehensive insight into the prevalence and molecular identification of gastrointestinal nematodes infecting captive deer in West Java, Indonesia. Through an integrative approach combining field sampling, parasitological examinations, PCR amplification, DNA sequencing, and phylogenetic analysis, two nematode species, *T. axei* and *S. houdemeri*, were successfully detected across several deer breeding centers.

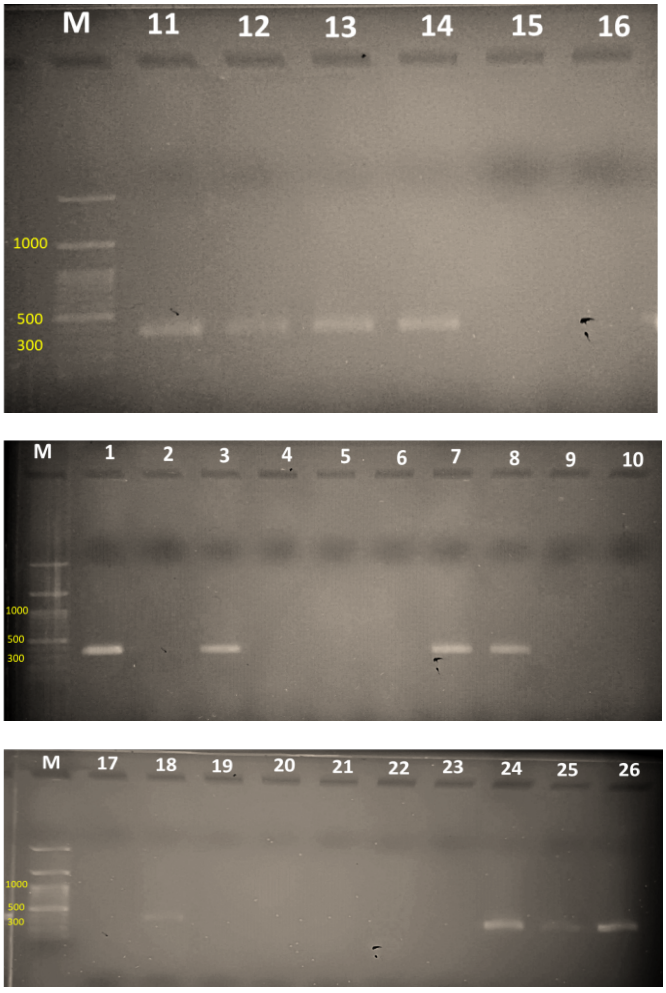

Figure 2. Gel electrophoresis of PCR products from different locations in the current study

Note: Lane 1: Positive control, Lane 2, 10: Ranca Upas, Lane 3, 4: Tahura, Lane 5: Pangalengan, Lane 6: Soreang, Lane 7: Kehati, Lane 8: Bumi Patra, Lane 9: Cirebon, Lane 11, 12: Paniisan, Lane 13, 14: Vedca, Lane 15,16: Cariu, Lane 17-20: Soreang, Lane 21,22: Gedung Negara, Lane 23, 24: Mabda Islam, Lane 25, 26: Taman Satwa.

The overall prevalence of gastrointestinal helminth infection was relatively low, with an average of 18.1%, and EPG values consistently below 50. This suggests a mild intensity of infection, aligning with findings from other wildlife studies indicating low parasite burdens, like in Europe (Barone et al., 2020; Brown and Morgan, 2024). Nevertheless, notable variation in prevalence was observed between breeding centers, with Cariu (Bogor District) recording the highest prevalence (60%), followed by Bumi Patra (Indramayu) and Ranca Upas (Bandung). These differences may reflect variations in management practices, environmental hygiene, enclosure density, and pasture contamination, factors previously reported as critical determinants of parasitic transmission (Kenyon et al., 2017; Vande Velde et al., 2018; Szewc et al., 2021).

Initial parasitological examination techniques revealed the presence of strongyle-type eggs. However, morphological similarities among strongyle nematode eggs limit precise identification of species (Seesao et al., 2017). Morphological identification of larvae is inherently limited in resolving species-level classification, as many closely related species share overlapping morphological traits, which can lead to less accurate results (Yan et al., 2023). Therefore, molecular identification targeting the ITS2 region of rDNA was employed, a method proven to offer high sensitivity and specificity for nematode diagnostics. The ITS2 region was selected for molecular analysis because it offers high interspecific variability with low intraspecific variation, enabling reliable species differentiation and facilitating comparisons with previous trichostrongylid studies (Roeber et al., 2013b; Sharifdini et al., 2017; Workentine et al., 2020). Electrophoresis results show successful amplification of the expected ~370 bp in samples from 9 out of 13 breeding locations.

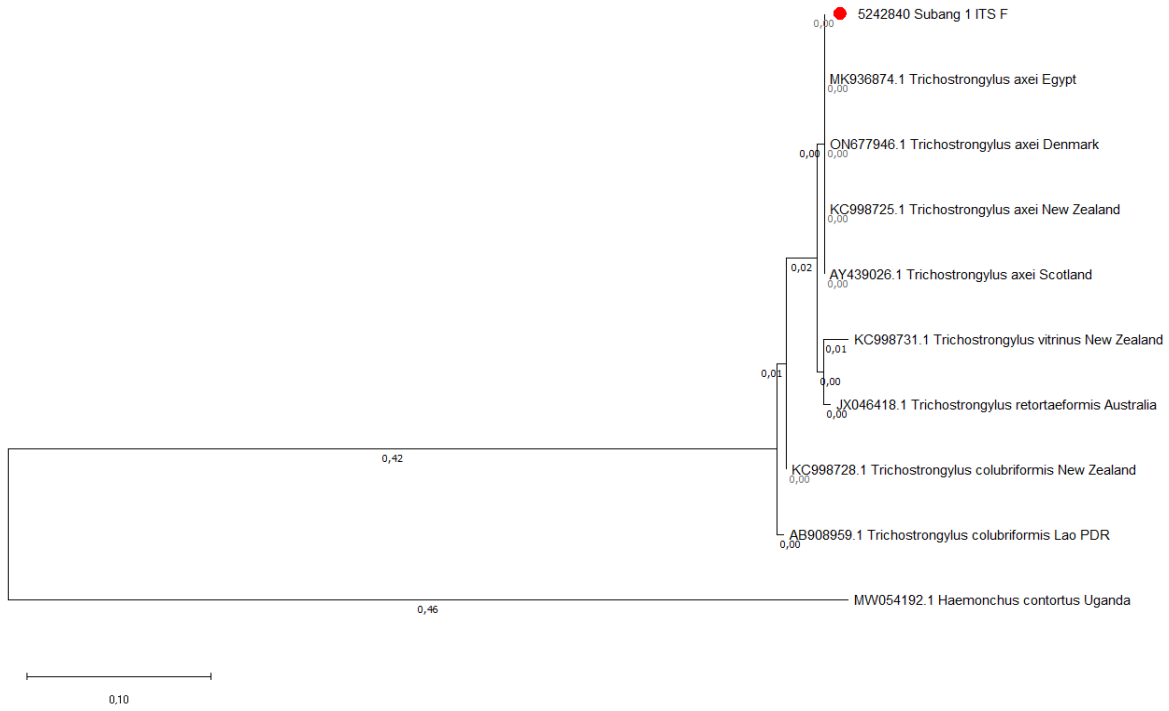

Figure 3. Phylogenetic tree based on ITS sequences illustrating the clustering of *T. axei* from Subang District, Indonesia, with reference isolates from Egypt, Denmark, New Zealand, and Scotland

Note: Other Trichostrongylus species form separate clades. *H. contortus* was used as the outgroup. Bootstrap values represent branch support.

DNA sequencing and subsequent BLAST analysis confirmed the presence of *T. axei* in samples from Panisan, Vedca, Taman Satwa, and Mabda Islam, with high sequence similarity (99.12%–100%). *T. axei* is known for its wide host range, infecting domestic ruminants and wildlife worldwide (Halvarsson et al., 2022; Brown & Morgan, 2024). Its detection across multiple locations may indicate environmental contamination via feed or fomites and anthropogenic movement between facilities (Roeber et al., 2013a; Rinaldi et al., 2022; Mohebati et al., 2024).

Meanwhile, *S. houdemeri* was detected in Tahura, Kehati, and Bumi Patra centers, with BLAST identity values ranging from 99.42% to 99.74%. This parasite, typically associated with cervids, has been reported from Europe and parts of Asia (Halvarsson et al., 2022; Inoue et al., 2022). Its identification in Indonesia suggests an underrecognized geographic distribution, reinforcing the need for expanded surveillance across Southeast Asia (Nazarbeigy et al., 2021; Yan et al., 2023; Taheri et al., 2024).

The phylogenetic trees (Figure 4) further corroborated species identifications. *T. axei* isolates from Indonesia clustered closely with reference isolates from Japan, Iran, and the United Kingdom, consistent with previous findings highlighting the genetic conservation of this species across continents (Kumar et al., 2021). Likewise, *S. houdemeri* sequences grouped with those from Russia and Germany, affirming its distinct phylogenetic lineage among trichostrongyles (Sultan et al., 2014).

Although *T. axei* and *S. houdemeri* are not considered major zoonotic threats, sporadic cases of *T. axei* infections in humans have been reported in Mazandaran Province, Iran, particularly under conditions of poor hygiene and close animal-human interactions (Sharifdini et al., 2017). This underlines the necessity of parasite monitoring not only for wildlife health but also for minimizing potential risks to public health and livestock biosecurity (Bautista-Garfias et al., 2022).

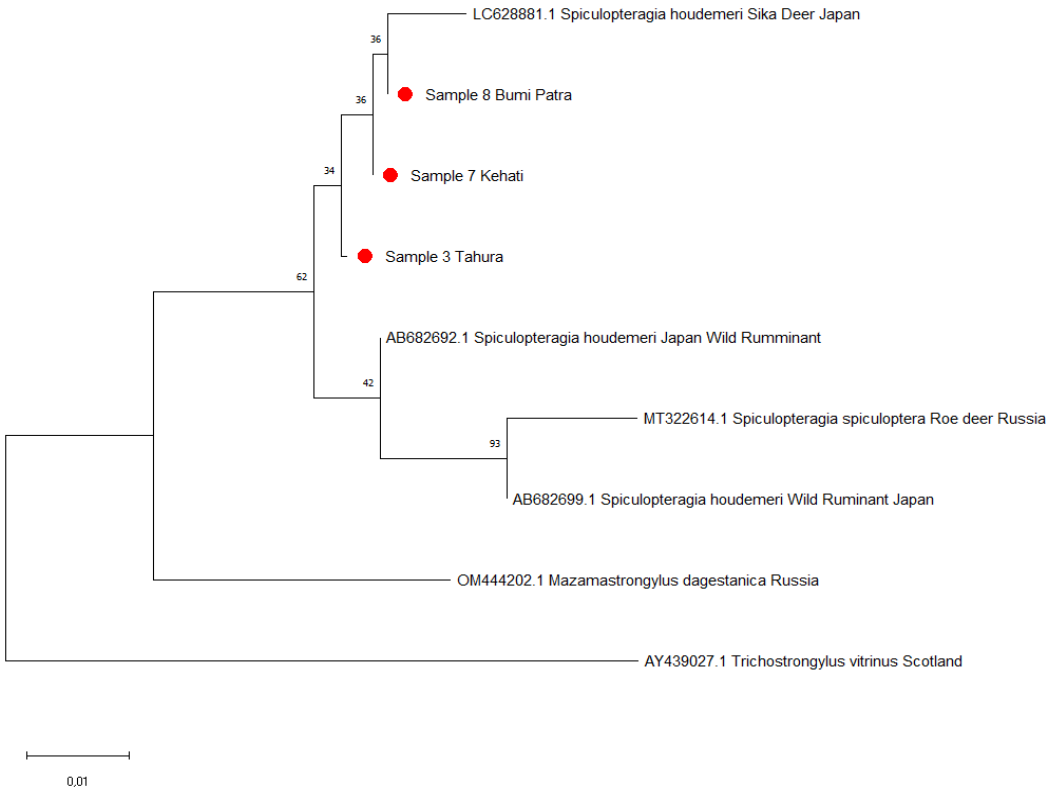

Figure 4. Phylogenetic tree based on ITS sequences showing the clustering of *S. houdemeri* isolates from Bumi Patra, Kehati, and Tahura (Indonesia) with reference sequences from Japan

Note: *Mazamastrongylus dagestanica* and *T. vitrinus* were used as outgroups. Bootstrap values are indicated at branch nodes.

Climate and environmental changes may also shift parasite transmission dynamics in the future. Increasing temperatures and humidity, as projected for Indonesia, could favor larval survival and enhance transmission cycles (Bautista-Garfias et al., 2022; Bhat et al., 2023; Miyankouh et al., 2025). Previous studies have shown that higher temperatures and humidity are positively correlated with the development, survival, and infectivity of gastrointestinal nematode larvae in the environment. Thus, proactive surveillance and management, including rotational grazing, strategic anthelmintic use, and improved sanitation, are recommended (Maqbool et al., 2017; Charlier et al., 2022).

Conclusion

In conclusion, this study provides foundational data on the molecular characterization and phylogenetic relationships of *T. axei* and *S. houdemeri* in Indonesian deer. These findings contribute to the baseline knowledge of gastrointestinal nematodes in wild ruminants and highlight the importance of continuous surveillance. Future research should explore seasonal patterns, geographical coverage, and the ecological factors influencing parasite distribution to better inform wildlife health management strategies.

Ethical Considerations

Compliance with ethical guidelines

This study was conducted in accordance with ethical guidelines and approved by the Institutional Animal Ethics Committee, IPB University, Bogor, Indonesia. (Code: 252/KEH/SKE/IX/2024). Additional research permissions were obtained from each deer breeding site through official faculty recommendation letters, with site management granting approval and facilitating sample collection with animal caretakers. Fecal collection was conducted without causing harm, and examination results were shared with the management to support routine health monitoring programs.

Funding

This study was conducted with financial support from the Ministry of Higher Education, Science, and Technology of the Republic of Indonesia, Jakarta, Indonesia (Grant No.: 22076/IT3.D10/PT.01.03/P/B/2024).

Authors' contributions

Study design, project administration and writing: Ridi Arif; Experiments: All authors.

Conflict of interest

The authors declared no conflict of interest.

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