

Article

Integrative Morphological and Molecular Characterization of *Haemonchus contortus* and *Trichostrongylus axei* from a Goat in Mindanao, Philippines

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Abstract

Trichostrongylid nematodes are globally distributed gastrointestinal parasites of major veterinary and, in some cases, zoonotic importance. Accurate identification often requires an integrative approach because morphology alone may be compromised by specimen damage, cryptic diversity, and dimorphic species complexes. In the Philippines previous work has been largely limited to coproscopy, with no published DNA-based studies on adult trichostrongylids or scanning electron microscopy (SEM) documentation. Here, we provide an integrative characterization of *Haemonchus contortus* and *Trichostrongylus axei* recovered from a single goat in Mindanao, Philippines, in 2023. Identification was based on light microscopy, SEM, morphometry, and sequence data from the *cox1* and ITS regions. Phylogenetic analyses and a haplotype network for *H. contortus* placed the Philippine material within a widely distributed lineage complex with weak geographic structuring. Because the study is based on parasites from a single host, the findings should be interpreted as a taxonomic and faunistic record rather than evidence for broader epidemiological or population-level patterns. This study provides the first molecular record of trichostrongylid nematodes from the Philippines and the first SEM-based documentation of *H. contortus* in the country. Broader sampling is needed to assess parasite diversity, distribution, genetic variation, and potential veterinary and public health relevance nationwide.

Keywords: Iligan; small ruminants; trichostrongylids

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1. Introduction

Trichostrongylid nematodes are widely distributed parasites of domestic ruminants worldwide [1]. They are associated with substantial economic losses due to reduced weight gain, decreased milk production, poor body condition, and, in severe cases, mortality [2]. Their veterinary and epidemiological significance is further highlighted by the ability of some trichostrongylid species to infect humans [3,4], as well as numerous domestic and wild herbivorous mammals.

Among these nematodes, *Haemonchus contortus* is particularly important because of its high pathogenicity and blood-feeding behavior [5], whereas *Trichostrongylus axei* is a common and widespread parasite that may also contribute to production losses in in-

fectured animals [6]. Both species are not restricted to small ruminants and have been recorded from a broad range of domestic and wild ruminants, including Arctic reindeer (*Rangifer tarandus*) [7–9]. It suggests extensive opportunities for dispersal and complex distributional histories shaped by host movements [10,11].

Reliable identification of trichostrongylids is fundamental for parasite surveillance, epidemiology, and studies of geographic distribution. Morphological characters remain essential in nematode taxonomy. Scanning electron microscopy (SEM) can reveal subtle external morphological features that are difficult to distinguish under light microscopy (LM), thereby providing complementary characters for species identification and comparative morphological analyses. However, species identification may be difficult when specimens are damaged, incomplete, morphologically similar, or hybrids [12]. In addition, some trichostrongylid taxa belong to dimorphic species complexes expressed as major and minor morphs, which have historically been described as separate taxa [13,14]. Therefore, molecular markers are routinely used to complement morphology. The nuclear ribosomal internal transcribed spacer (ITS) region and the mitochondrial cytochrome c oxidase subunit I gene (*cox1*) are among the most widely used loci for species identification and comparative phylogenetic analysis in parasitic nematodes [15–17]. For species-level discrimination, the ITS region is particularly useful, whereas *cox1* often provides higher resolution for assessing intraspecific genetic variation.

The Philippines, as a tropical archipelago with extensive smallholder livestock production systems, provides favorable conditions for the persistence and transmission of gastrointestinal nematodes in ruminants [18]. Nevertheless, the available information on the gastrointestinal nematode fauna of small ruminants in the country remains limited and fragmented, particularly in Mindanao. Most published studies from the Philippines are based on coprological surveys [19–22]. In some cases, stool culture (coproculture) can provide additional taxonomic information by allowing the recovery of infective third-stage larvae from fecal samples, which may support the recognition of trichostrongylid genera in epidemiological and clinical investigations, although its utility is limited. Larval development is slow and may fail, especially in low-intensity infections. Morphological identification of L3 can also be unreliable because diagnostic features are not always clearly visible, and mixed cultures may contain taxa lacking larval descriptions. In addition, individual larvae provide very limited material for downstream molecular analysis, increasing the risk of failure with conventional sequencing approaches, while environmental DNA methods remain expensive [23,24]. For this reason, coproculture is valuable for surveillance and preliminary assessment, and although parasite molecular markers can also be successfully used in studies based on coproculture material [25], DNA extraction from larvae remains challenging. Records supported by the examination of adult worms and confirmed by both morphology and DNA sequence data were not found.

Integrative data on trichostrongylids of goats in the Philippines therefore remain insufficient, limiting reliable local species documentation and broader comparisons with parasite populations from other geographic regions. This limitation is especially relevant for a cosmopolitan parasite *H. contortus*. Its genetic lineages may have been extensively mixed through the long-term global movement of sheep and goats, while geographic structuring appears weak or inconsistent across different molecular markers and remains poorly characterized for many populations [10]. In view of this gap, we studied trichostrongylids recovered from a goat in Iligan, Mindanao. The aim of this work was to identify *H. contortus* and *T. axei* using morphological and molecular evidence, to provide additional scanning electron microscopic data for *H. contortus*, and to assess the phylogenetic affinities of the obtained ITS and *cox1* sequences with those available in GenBank.

2. Materials and Methods

2.1. Host Animal and Study Site

On 28 November 2023, three goats (*Capra hircus*; Figure 1A) were slaughtered for meat at a slaughterhouse in Iligan City, Mindanao, Philippines (8.23581 N, 124.26131 E; Figure 1B).

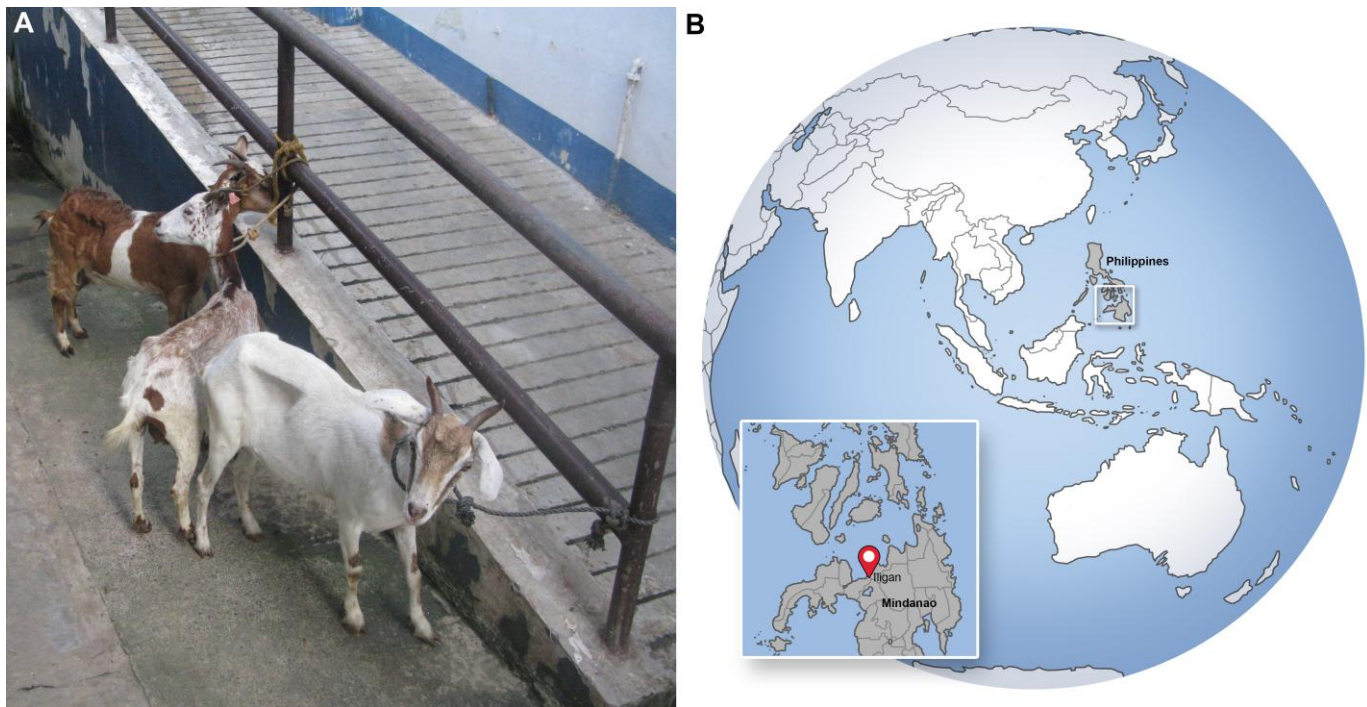


Figure 1. Collection site and host animals. (A) Goats brought to the slaughterhouse for meat production; (B) global location of the nematode collection site in the Philippines, with an inset showing Iligan City on Mindanao Island.

2.2. Collection, Fixation, and Preservation of Helminths

Following slaughter, two of the co-authors (N.H.S. and S.E.S.) were provided with the gastrointestinal tracts for parasitological examination. Upon opening the organs, they detected several dozen trichostrongylid nematodes in the abomasum of a light-colored 2-year-old goat (no nematodes were found in the other two goats). The abomasum was then separated from the alimentary tract and transported to the laboratory at the Iligan Institute of Technology, Mindanao State University. The nematodes were collected from the opened abomasum using a needle and were immediately fixed either in 6% buffered formalin for morphological examination or in 80% ethanol for DNA extraction.

2.3. Light Microscopy

The morphology of trichostrongylids was examined in male specimens using LM and SEM. Morphological examination focused on the overall body length and width of males, as well as the structure of the reproductive system, including the shape and dimensions of the spicules, the morphology of the copulatory bursa, and the configuration of the bursal rays. Cuticular features were also assessed, including ridges, striation, papillae, amphids, the synophe, and other relevant structures. For LM, permanent glycerol mounts were prepared following the method of Seinhorst [26]. Light microscopy was performed using Mikmed-6 microscope (LOMO-MA, Saint Petersburg, Russia) equipped with an SCMOS08300KPA digital camera (KORITCAM, Shenzhen, China). After preliminary sorting into two morphotypes based on overall body size and key male mor-

phological characters, including the presence or absence of cervical papillae, the development of the copulatory bursa, and spicule symmetry, morphometric measurements were obtained from a randomly selected subset of 10 intact male specimens of each morphotype. Morphometric measurements were obtained from the captured images using Fiji/ImageJ Version 1.2.4 software, RRID:SCR_003070 (National Institutes of Health, Bethesda, MD, USA).

2.4. Scanning Electron Microscopy

For SEM, the best-preserved male specimen of *H. contortus* was prepared by dehydration through a graded ethanol series, transfer to acetone, critical-point drying using a Leica EM CPD300 (Leica Microsystems Vertrieb GmbH, Vienna, Austria), and platinum sputter-coating using an S150A Sputter Coater (Quorum Technologies Ltd., Laughton, UK). SEM observations were performed with a MIRA 3 LMH scanning electron microscope (TESCAN, Brno, Czech Republic) in the Joint Usage Center “Instrumental Methods in Ecology” of the IEE RAS, Moscow, Russia.

2.5. Morphometric Analyses

Descriptive statistics were calculated in R Version 4.3 software, RRID:SCR_001905 (R Foundation for Statistical Computing, Vienna, Austria). Data are presented as mean $\pm$ SD, range, and sample size. Identifications were made with reference to standard taxonomic keys and relevant thematic guides [27–29].

2.6. DNA Analyses

To directly link morphology and molecular data, the same individual specimens were used for both analyses: after light-microscopic examination and identification, the anterior and posterior ends of each selected worm were retained as vouchers, while the middle part of the body was used for DNA extraction. Two individual specimens of each species were processed for amplification of the ITS rDNA region and the *cox1* gene. Thus, four specimens in total were examined molecularly. DNA was extracted from individual male specimens using the QIAamp DNA Micro Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s protocol. A partial ITS rDNA sequence was amplified using the primer pair TW81 (GTTTCCGTAGGTGAACCTGC) and AB28 (AT-ATGCTTAAGTTCAGCGGT) following Joyce et al. [30]. PCR was performed in a total reaction volume of 25 μ L using the Encyclo Plus PCR Kit (Evrogen, Moscow, Russia) according to the manufacturer’s instructions. The amplification profile consisted of an initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 45 s, 57 °C for 45 s, and 72 °C for 70 s, with a final extension at 72 °C for 5 min. A partial fragment of the *cox1* was amplified using the primer pair COI_F1 (5'-CCTACTATGATTGGTGGTTTGGTAATTG-3') and COI_R2 (5'-GTAGCAGCAGTAAAATAAGCACG-3') proposed by Kanzaki and Futai [31]. PCR was carried out under the following conditions an initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 45 °C for 15 s, and extension at 72 °C for 60 s, with a final extension at 72 °C for 5 min. Negative controls consisting of reaction mixtures containing all PCR reagents but no template DNA were included in each amplification run. PCR products were visualized on agarose gels, and the corresponding bands were excised and purified using the HiPure Gel DNA Mini Kit (Magen Biotechnology Co., Ltd., Guangzhou, China). Purified amplicons were sequenced directly with the same primers as those used for PCR by Genotech Ltd. (Moscow, Russia). Because direct sequencing of the ITS rDNA amplicons produced multiple peaks in the ITS1 region in the one specimen of *H. contortus*, its PCR products were ligated into the pGEM-T vector and cloned by transformation into JM109 High Efficiency

Competent Cells (Promega, Leiden, The Netherlands) according to the manufacturer's instructions. Insert-containing clones were amplified and sequenced using the same primers as those employed for the primary amplification, TW81 and AB28. Three distinct clone sequences were identified for one specimen of *H. contortus*.

Sequence similarity values are presented as ranges derived from pairwise comparisons with representative GenBank sequences. For each locus, the range for the identified species was calculated from the closest non-redundant matches of the same species showing full overlap with our sequences, whereas the ranges for closely related species were calculated from representative sequences of the nearest congeners.

3. Results

3.1. Preliminary Morphological Identification of Helminths

Based on LM observations, the male trichostrongylids recovered in the present study were divided into two morphological groups. The morphological description provided below is based on the examination of ten individuals for each morphotype.

The larger males measured 20.4 ± 1.2 mm in length (18.9–22.1) and 0.35 ± 0.05 mm in width (0.25–0.42). Prominent cervical papillae were present at the anterior end. The body exhibited transverse striations and longitudinal ridges. The copulatory bursa consisted of two large lateral lobes and a small dorsal lobe directed to left. Their spicules were equal in length, measuring 0.50 ± 0.02 mm (0.47–0.53).

The smaller males had a total length of 3.95 ± 0.32 mm (3.4–4.4) and a width of 0.055 ± 0.005 mm (0.05–0.06). Cervical papillae were absent, and only the lateral lobes of the copulatory bursa were well developed. Their spicules were unequal. The longer spicule measured 0.118 ± 0.013 mm (0.10–0.14), whereas the shorter one measured 0.095 ± 0.005 mm (0.09–0.10).

Based on the host species (goat), the site of recovery (abomasum), and the morphological characteristics revealed by LM, male specimens of the first group were preliminarily assigned to *H. contortus*, whereas those of the second group were assigned to *T. axei*.

SEM revealed additional details of the male morphology of *H. contortus*. Characteristic species-specific features included a synlophe with approximately 30 ridges at mid-body (Figure 2A), robust cervical papillae (deirids) at the level of the nerve ring (Figure 2B), characteristic cuticular sculpture on both surfaces of the male bursa (Figure 2C), and cuticular annulations between the ridges (Figure 2D). The male bursa itself was the most distinctive feature, its dorsal lobe directed to left (Figure 2E), together with the typical barbed spicules (Figure 2F).

3.2. Molecular Confirmation of Preliminary Diagnoses

Standard Nucleotide BLAST (BLASTN; NCBI, Bethesda, MD, USA) comparison of the ITS and *cox1* nucleotide sequences obtained from trichostrongylids preliminarily identified as *H. contortus* and *T. axei* against GenBank records showed high sequence similarity, reaching up to 100% with reference sequences of these species. For *T. axei*, species assignment was based primarily on morphology and ITS, whereas *cox1* was used as a supplementary marker because its best GenBank match (90.86%) was insufficient, by itself, for confident species-level confirmation. Detailed results are presented in Table 1.

The sequences obtained for *H. contortus* and *T. axei* are summarized in Table 1 together with voucher information, sequence lengths, GenBank accession numbers, and similarity values to the identified species and closely related congeners. For *H. contortus*, both *cox1* and ITS region sequences showed the highest similarity to conspecific reference sequences and lower similarity to other *Haemonchus* species (*H. placei*, *H. longistipes*), in

agreement with the morphological identification. Similarly, the ITS region sequences of *T. axei* showed very high similarity to conspecific references and somewhat lower similarity to *T. colubriformis*. In contrast, the *T. axei* *cox1* sequences showed relatively low nucleotide similarity to currently available reference sequences. However, their translated amino acid sequences were highly conserved and differed by only a single amino acid residue from two reference sequences. Taken together, these data are consistent with, but do not by themselves conclusively establish, the assignment of these specimens to *T. axei*.

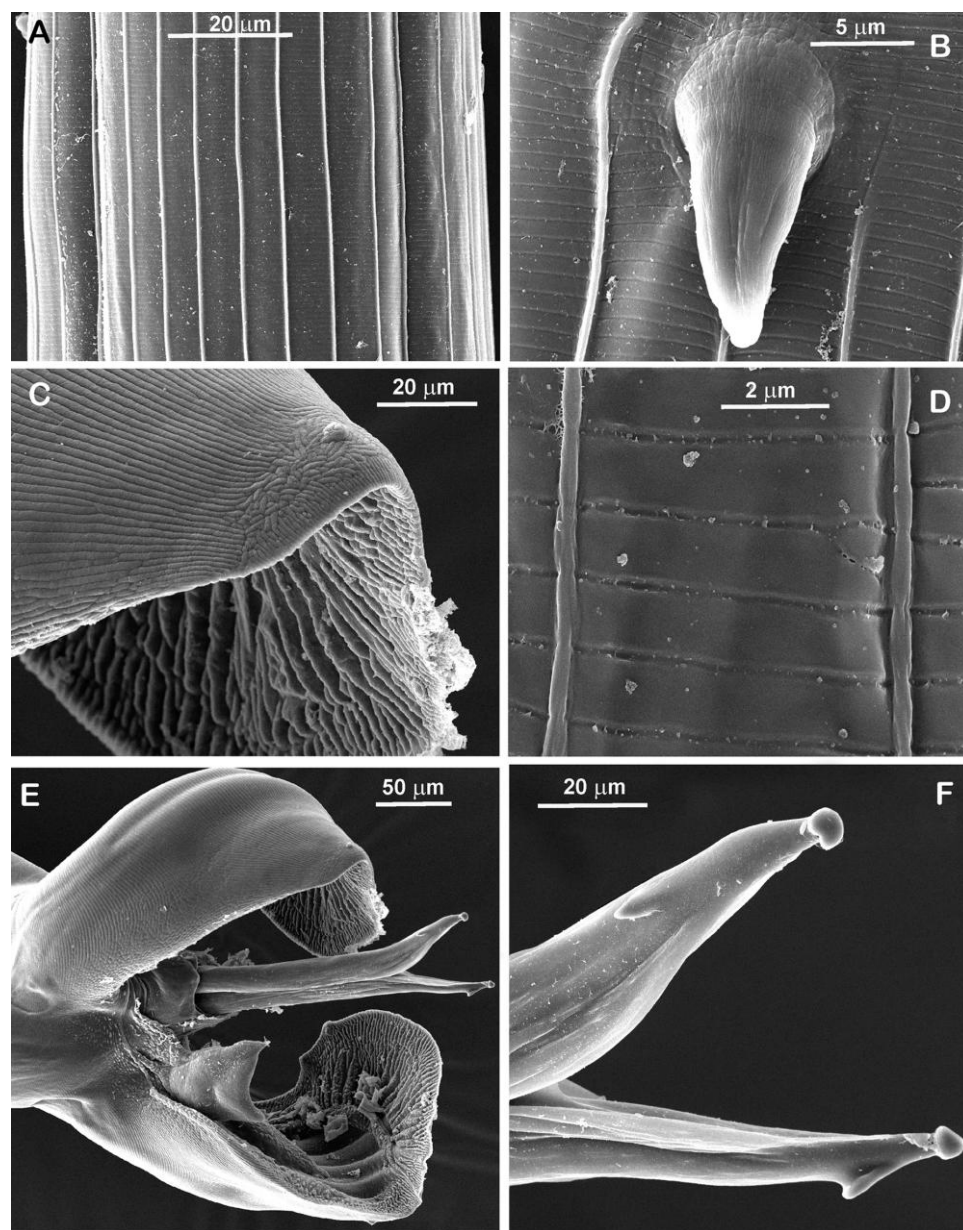


Figure 2. Scanning electron micrographs illustrating selected morphological features of the male *H. contortus* recovered from the abomasum of the goat in the Philippines. (A) Lateral view of the cuticular surface, showing the longitudinal ridges of the synlophe; (B) high-magnification view of a cervical papilla (deirid); (C) high-magnification view of a lateral lobe of the copulatory bursa; (D) high-magnification view of the cuticular surface, showing portions of two longitudinal synlophe ridges together with transverse cuticular annulations; (E) dorso-apical view of the copulatory bursa, showing the spicules, both lateral lobes, and a single asymmetrical dorsal lobe sharing a common base with the left lateral lobe; (F) high-magnification view of the spicules, showing the characteristic terminal inflation and a harpoon-like barb situated slightly proximally to the apex.

Table 1. BLAST comparison of ITS and *cox1* sequences of two trichostrongylids recovered from the abomasum of the goat in the Philippines with the GenBank sequences; query coverage are 100% for all comparisons. Similarity ranges were calculated based on pairwise comparisons with representative GenBank sequences of the identified species and closely related taxa selected from BLAST results.

Species	Voucher Name ¹	Loci	Length	GenBank Accession Number ²	Similarity to Species Identified, %	Similarity to Other Closely Related Species, %
<i>H. contortus</i>	14466	<i>cox1</i>	577	PZ122374	87.62–98.61	<i>H. placei</i> 87.52–88.87; <i>H. longistipes</i> 93.57
	14466	ITS region	583	PZ122197	95.21–98.30	<i>H. longistipes</i> 94.72–95.40
	14472	<i>cox1</i>	577	PZ754459	87.62–98.61	<i>H. placei</i> 87.52–88.87; <i>H. longistipes</i> 93.57
	14472	ITS region (clone 1)	583	PZ124337	95.21–98.30	<i>H. longistipes</i> 94.72–95.40
	14472	ITS region (clone 2)	586	PZ124338	95.21–98.64	<i>H. longistipes</i> 94.72–95.40
	14472	ITS region (clone 3)	586	PZ124339	95.21–99.15	<i>H. longistipes</i> 94.72–95.40
<i>T. axei</i>	14467	<i>cox1</i>	689	PZ122159	89.99–90.86	<i>T. colubriiformis</i> 88.68–88.82
	14467	ITS region	817	PZ124293	99.39–100	<i>T. colubriiformis</i> 97.43–97.68
	14561	<i>cox1</i>	689	PZ754458	89.99–90.86	<i>T. colubriiformis</i> 88.68–88.82
	14561	ITS region	817	PZ751039	99.39–100	<i>T. colubriiformis</i> 97.43–97.68

¹ In abbreviated form. Full voucher names are: IPEE_Parasites 14466, IPEE_Parasites 14467, IPEE_Parasites 14472, and IPEE_Parasites 14561. Voucher specimens with definitive identifications and accession numbers archived in the Museum of Helminthological Collections of the IEE RAS (Moscow, Russia). Voucher specimens consisted of the anterior and posterior ends of the same individuals from which the middle body portions were used for DNA extraction. ² GenBank accession numbers for sequences from the fragments of the adult males of representative species.

4. Discussion

The present study confirmed the occurrence of *H. contortus* and *T. axei* in the gastrointestinal tract of goat from the Philippines, based on the combined use of morphological and molecular approaches. Both taxa were detected in abomasum of one goat examined from Iligan, Mindanao, demonstrating that these economically important trichostrongylid nematodes are present in local small-ruminant populations [19–22]. The integration of conventional parasitological examination with LM, SEM, morphometric analysis, and *cox1* and ITS region sequencing increased confidence in species identification, particularly in the case of trichostrongylid nematodes recovered from the abomasum of goats, where closely related species may co-occur and where morphology alone may not always be sufficient for reliable discrimination.

With regard to morphology, the examined specimens showed diagnostic characters consistent with *H. contortus* and *T. axei* as described in previous taxonomic accounts [27–29]. *Haemonchus contortus* was identified on the basis of large body size (20 mm), cervical papillae, synlophe/ridge pattern, asymmetrical dorsal lobe, barbed spicules, whereas *T. axei* was distinguished by five times shorter body, characters of the spicules, caudal bursa, esophageal region. A morphological comparison between the two species highlights clear differences in body size and form, and reproductive structures, reflecting their placement in different genera and their distinct taxonomic identities. In particular, cervical papillae and synlophe were present in *H. contortus* and absent in *T. axei*; dorsal lobe of caudal bursa was well developed and directed to left in *H. contortus*, whereas in *T. axei*, it was poorly developed and situated medially. These observations are important because trichostrongylid nematodes recovered from the gastrointestinal tract of goat may appear superficially similar, and careful examination of male copulatory structures and other diagnostic features remains essential for reliable discrimination.

The morphological findings in the present material are broadly congruent with earlier descriptions of *H. contortus* and *T. axei* from domestic ruminants in other regions [8,12]. However, some variation in morphometric values may occur among populations due to host-related factors, geographical origin, fixation quality, and intraspecific variability [32,33]. In the present material, males of *H. contortus* were generally larger than those reported from Malaysia [33], with a mean body length of about 20 mm versus 15 mm, and slightly longer spicules, approximately 0.5 mm compared with 0.4 mm in the Malaysian specimens. Therefore, these differences should be interpreted cautiously and are not sufficient alone to exclude conspecificity. In this context, the morphological data presented here are valuable not only for species confirmation but also for improving the comparative documentation of strongylid parasites from goat in the Philippines, where detailed illustrated or measured records remain limited.

The molecular results further supported the identification of the detected nematodes and complemented the morphological examination. The *cox1* and ITS sequences generated for *H. contortus* showed high similarity to reference sequences available in GenBank, supporting the taxonomic assignment of the Philippine specimens to this species. We identified three distinct *H. contortus* ITS variants by cloning. The ITS haplotypic diversity in *H. contortus* has previously been reported using the same cloning-based approach in isolates from Mongolia, in a study co-authored by S.E.S. [34].

Noteworthy is that the *cox1* result for *T. axei* requires cautious interpretation. Its highest similarity to a GenBank reference was only 90.86%, which is lower than would usually be expected for unambiguous species-level confirmation based on this marker alone. For this reason, we do not treat *cox1* as the primary evidence for species assignment in this case. Instead, identification of the specimen as *T. axei* is based on the combination of diagnostic morphology and the ITS sequence, the latter showing complete correspondence with reference *T. axei* sequences. The *cox1* sequence is therefore interpreted as additional mitochondrial evidence consistent with placement in *Trichostrongylus*, and as a useful sequence resource for future studies. The low identity value most likely reflects the limited availability and uneven taxonomic and geographic coverage of *Trichostrongylus cox1* sequences in GenBank, which currently constrains species-level comparison. These findings underscore the importance of expanding barcode datasets for trichostrongylid nematodes from Southeast Asia and of combining morphology with molecular data for robust species delimitation.

The sequences generated in this study should be interpreted cautiously. Their apparent distinctiveness may reflect local genetic variation but may also be an artefact of undersampling [35], especially given the lack of comparable molecular data from the Philippines. As the material was obtained from a single host, no inference is made regarding sequence frequency, parasite population structure, or transmission dynamics. This study is therefore presented as a taxonomic and molecular record only.

These limitations also define the next research priorities. Broader sampling across Philippine small ruminants is needed to determine the diversity, distribution, and host range of trichostrongylid nematodes, to assess the extent of mixed infections, and to test whether the haplotype detected here is locally restricted or more widespread. Future studies would benefit from combining integrative morphology with multilocus sequencing and denser geographic coverage across the archipelago [36,37].

In this respect, the present material constitutes a well-documented regional record and, to our knowledge, the first molecular characterization of trichostrongylids from the Philippines, as well as the first SEM-based documentation of *H. contortus* in the country.

From a veterinary standpoint, the detection of *H. contortus* is of particular importance because this species is one of the most pathogenic nematodes of small ruminants worldwide. As a hematophagous parasite of the abomasum, *H. contortus* can cause

anemia, hypoproteinemia, edema, weight loss, reduced productivity, and, in heavy infections, death, especially in lambs and nutritionally stressed animals [2,5]. Its presence in goat in the Philippines is therefore highly relevant for flock health and farm economics, particularly under tropical and subtropical conditions that favor year-round transmission and larval survival on pasture [28]. The finding of *T. axei* is also of veterinary significance. Although generally regarded as less pathogenic than *H. contortus*, *T. axei* may contribute to chronic gastritis, reduced feed efficiency, poor body condition, and production losses, especially in mixed infections. Co-occurrence of both species may aggravate the overall parasitic burden and complicate clinical presentation and control [38,39]. The recovery of *H. contortus* and *T. axei* from the same host is consistent with the well-recognized occurrence of mixed trichostrongylid infections in small ruminants [32,38,39]. Such co-infections are epidemiologically relevant because they can obscure species-specific diagnosis, complicate interpretation of clinical impact, and hinder targeted parasite control [40]. Although these nematodes are primarily of veterinary importance, their detection is also relevant from a One Health [41] perspective because parasite circulation in livestock systems may affect animal health, productivity, and host–parasite interactions within shared environments.

The ecological and epidemiological significance of these parasites should also be considered. Both *H. contortus* and *T. axei* have broad geographic distributions and can infect a range of domestic [7,34,38,39,42,43] and wild ruminant hosts [8,9,25,44]. Their detection in goat suggests the possibility of continuous transmission within grazing systems and potential exchange among sympatric livestock species such as goats and cattle where shared pastures or management systems occur. Such host overlap may facilitate maintenance of infection foci and hinder parasite control, particularly in regions with warm and humid climates. If sheep/cattle/cervids are present in the study area, they may serve as additional reservoirs contributing to environmental contamination with infective larvae [45–47].

Although the present study focused primarily on species identification, the results have practical implications for surveillance and parasite management in Philippine goat production. Accurate identification of gastrointestinal nematodes is essential for understanding local parasite fauna, assessing epidemiological risk, and informing evidence-based control measures. The occurrence of *H. contortus* in particular should prompt attention to targeted monitoring, strategic anthelmintic use, and integrated pasture management, given the well-documented global problem of anthelmintic resistance in this species [17,25]. Further studies based on larger sample sizes, wider geographic coverage, and additional genetic markers would help clarify the prevalence, population diversity, host range, and transmission patterns of *H. contortus* and *T. axei* in the Philippines.

5. Conclusions

This study provides the first molecular record of trichostrongylid nematodes from the Philippines and the first SEM-based documentation of *H. contortus* in the country. Using an integrative approach, *H. contortus* and *T. axei* were reliably identified from a goat in Mindanao. The findings confirm the presence of economically important gastrointestinal nematodes in Philippine small ruminants and highlight the value of combining morphological and molecular methods for accurate diagnosis. However, given that the analyzed material was recovered from a single host individual, these findings should be regarded as preliminary until confirmed by broader sampling, necessary to clarify parasite diversity, distribution, and genetic variation in the country.

Author Contributions: Conceptualization, O.A.L. and S.E.S.; methodology, A.V.L.; software, S.E.S.; validation, O.A.L., A.V.L. and S.E.S.; formal analysis, O.A.L.; investigation, S.E.S. and N.H.N.S.; resources, N.H.N.S.; data curation, A.V.L.; writing—original draft preparation, O.A.L.; writing—review and editing, A.V.L., N.H.N.S., and S.E.S.; visualization, O.A.L. and S.E.S.; supervision, S.E.S.; project administration, N.H.N.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Ethical review and approval were waived for this study because the helminths were obtained during a routine veterinary and sanitary post-mortem inspection at a slaughterhouse. The goat was not killed for research purposes or for helminth recovery, but was slaughtered for meat production at the request of its Filipino owners.

Informed Consent Statement: Not applicable.

Data Availability Statement: ITS and *cox1* nucleotide sequences of *H. contortus* and *T. axei* generated in this study have been deposited in GenBank and are available under accession numbers (PZ122159, PZ122197, PZ122374, PZ124293, PZ124337, PZ124338, PZ124339, PZ751039, PZ754458, PZ754459). The corresponding voucher specimens are archived in the Museum of Helminthological Collections of the A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences (Moscow, Russia) and are available under accession numbers (IPEE_Parasites 14466, IPEE_Parasites 14467, IPEE_Parasites 14472, IPEE_Parasites 14561).

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

A	Adenine
BLAST	Basic Local Alignment Search Tool
bp	Base Pair
C	Cytosine
<i>cox1</i>	Cytochrome C Oxidase Subunit I Gene
DNA	Deoxyribonucleic Acid
E	East
G	Guanine
GPT	Generative Pre-trained Transformer
IEE RAS	Institute of Ecology and Evolution of the Russian Academy of Sciences
ITS	Internal Transcribed Spacer
LM	Light Microscopy
L3	Third-Stage Larva of Nematode
N	North
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction
SEM	Scanning Electron Microscopy
SD	Standard Deviation

T Thymine

References

1. Wei, W.; Gu, X.; Shi, R.; Li, Z.; Sa, R.; Jiao, D.; Bian, P.; Ma, C.; Li, J.; Bai, H.; et al. Global Prevalence and Risk Factors Associated With *Trichostrongylus* Infection in Humans, Ovines, and Bovines: A Systematic Review and Meta-Analysis. *Transbound. Emerg. Dis.* **2026**, *3544505*. <https://doi.org/10.1155/tbed/3544505>.
2. Strydom, T.; Lavan, R.P.; Torres, S.; Heaney, K. The Economic Impact of Parasitism from Nematodes, Trematodes and Ticks on Beef Cattle Production. *Animals* **2023**, *13*, 1599. <https://doi.org/10.3390/ani13101599>.
3. Ashrafi, K.; Sharifdini, M.; Heidari, Z.; Rahmati, B.; Kia, E.B. Zoonotic transmission of *Teladorsagia circumcincta* and *Trichostrongylus* species in Guilan province, northern Iran: Molecular and morphological characterizations. *BMC Infect. Dis.* **2020**, *20*, 28. <https://doi.org/10.1186/s12879-020-4762-0>.
4. Jongthawin, J.; Wangdi, K.; Mahittikorn, A.; Masangkay, F.R.; Kotepui, M. Global Burden of *Trichostrongylus* Infections in Humans: A Systematic Review and Meta-Analysis. *Medicina* **2026**, *62*, 408. <https://doi.org/10.3390/medicina62020408>.
5. Flay, K.J.; Hill, F.I.; Muguiro, D.H. A Review: *Haemonchus contortus* Infection in Pasture-Based Sheep Production Systems, with a Focus on the Pathogenesis of Anaemia and Changes in Haematological Parameters. *Animals* **2022**, *12*, 1238. <https://doi.org/10.3390/ani12101238>.
6. Laubinger, B.L.; Harvey, K.M.; Jumper, W.I. Life Stage-Specific Burdens and Impacts of Gastrointestinal Nematodes in Beef Cattle in the United States: A Review of Diagnostics, Impacts on Productivity, and Immune Response. *Vet. Sci.* **2026**, *13*, 210. <https://doi.org/10.3390/vetsci13030210>.
7. Isaitschikoff, I.M. The Sixth Russian Helminthological Expedition, organized in 1921 to the Arctic Ocean. *Veterinarnoye Delo.* **1922**, *2–3*, 36–49. (In Russian)
8. González, S.; del Rio, M.L.; Díez-Baños, N.; Martínez, A.; Hidalgo, M.d.R. Contribution to the Knowledge of Gastrointestinal Nematodes in Roe Deer (*Capreolus capreolus*) from the Province of León, Spain: An Epidemiological and Molecular Study. *Animals* **2023**, *13*, 3117. <https://doi.org/10.3390/ani13193117>.
9. Brown, T.L.; Morgan, E.R. Helminth Prevalence in European Deer with a Focus on Abomasal Nematodes and the Influence of Livestock Pasture Contact: A Meta-Analysis. *Pathogens* **2024**, *13*, 378. <https://doi.org/10.3390/pathogens13050378>.
10. Hoberg, E.P.; Zarlenga, D.S. Evolution and Biogeography of *Haemonchus contortus*: Linking Faunal Dynamics in Space and Time. *Adv. Parasitol.* **2016**, *93*, 1–30. <https://doi.org/10.1016/bs.apar.2016.02.021>.
11. Masuko, R.; Ayin; Masaoka, M.; Kawaguchi, F.; Sasazaki, S.; Dagong, M.I.A.; Bugiwati, S.R.A.; Masangkay, J.S.; Yonezawa, T.; Mannen, H. Maternal and Paternal Lineage Analysis of Island Southeast Asian Goats Reveals Continental Propagation Routes and Introgression Through the Indian Ocean. *Sci. Rep.* **2025**, *15*, 9411. <https://doi.org/10.1038/s41598-025-93651-9>.
12. Lichtenfels, J.R.; Pilit, P.A.; Hoberg, E.P. New Morphological Characters for Identifying Individual Specimens of *Haemonchus* spp. (Nematoda: Trichostrongyloidea) and a Key to Species in Ruminants of North America. *J. Parasitol.* **1994**, *80*, 107–119. <https://doi.org/10.2307/3283353>.
13. Drózd, J. Polymorphism in the Ostertagiinae Lopez-Neyra, 1947 and Comments on the Systematics of These Nematodes. *Syst. Parasitol.* **1995**, *32*, 91–99. <https://doi.org/10.1007/BF00009507>.
14. Hoberg, E.P.; Monsen, K.J.; Kutz, S.; Blouin, M.S. Structure, Biodiversity, and Historical Biogeography of Nematode Faunas in Holarctic Ruminants: Morphological and Molecular Diagnoses for *Teladorsagia boreoarcticus* n. sp. (Nematoda: Ostertagiinae), Dimorphic Cryptic Species in Muskoxen (*Ovibos moschatus*). *J. Parasitol.* **1999**, *85*, 910–934. <https://doi.org/10.2307/3285831>.
15. Blouin, M.S. Molecular Prospecting for Cryptic Species of Nematodes: Mitochondrial DNA versus Internal Transcribed Spacer. *Int. J. Parasitol.* **2002**, *32*, 527–531. [https://doi.org/10.1016/S0020-7519\(01\)00357-5](https://doi.org/10.1016/S0020-7519(01)00357-5).
16. Bogale, M.; Baniya, A.; DiGennaro, P. Nematode Identification Techniques and Recent Advances. *Plants* **2020**, *9*, 1260. <https://doi.org/10.3390/plants9101260>.
17. Maurizio, A.; Dotto, G.; Tessarin, C.; Beraldo, P.; Franzo, G.; Cassini, R. Use of a Novel Real-Time PCR to Investigate Anthelmintic Efficacy Against *Haemonchus contortus* in Sheep and Goat Farms. *Vet. Sci.* **2025**, *12*, 569. <https://doi.org/10.3390/vetsci12060569>.
18. Ortega, A.D.S.V.; Mujitaba, M.A.; Xayalath, S.; Gutierrez, W.; Soriano, A.C.; Szabó, C. Perspectives of the Livestock Sector in the Philippines: A Review. *Acta Agrar. Debreceniensis* **2021**, *1*, 175–188. <https://doi.org/10.34101/ACTAAGRAR/1/9101>.
19. Rupa, A.P.M.; Portugaliza, H.P. Prevalence and Risk Factors Associated with Gastrointestinal Nematode Infection in Goats Raised in Baybay City, Leyte, Philippines. *Vet. World* **2016**, *9*, 728–734. <https://doi.org/10.14202/vetworld.2016.728-734>.

20. Sabirin, A.-N.N.; Dominguez, J.M.D.; Alvia, E.T.; Moneva, C.S.O.; Kim, K.S. Single Nucleotide Polymorphism of Interferon Gamma (Ifn- γ) Gene and Its Association to Gastrointestinal Parasite Burden of Crossbred Anglo-Nubian Goats. *Philipp. J. Vet. Anim. Sci.* **2024**, *50*, 97–109.
21. Balbin, A.J.M.; Nayga, N.J.; Bacton, J.; Bautista, J.; Catalonia, H.M.D.; dela Cruz, K.; Fajardo, M.A.D.; Ignacio, A.D.; Quiming, L.; Sapaden, J. Gastrointestinal Parasitic Infections of Ruminants in Backyard Farms of Southern Isabela, Philippines. *Environ. Sustain. Anim. Ind.* **2025**, *191*, 1–9. <https://doi.org/10.1051/bioconf/202519100015>.
22. Sabirin, A.-N.N.; Dominguez, J.M.D.; Tabugo, S.R.M.; Sumaya, N.H.N.; Alvia, E.T.; Kim, K.S.; Moneva, C.S.O. MHC-II DRB Gene Polymorphism and its Association to Gastrointestinal Parasite Burden of Crossbred Anglo-Nubian Goats from a Single Animal Farm in Sultan Naga Dimaporo, Lanao del Norte, Philippines. *Philipp. J. Vet. Med.* **2025**, *62*, 65–77.
23. Mancilla-Montelongo, G.; Castañeda-Ramírez, G.S.; Can-Celis, A.; Chan-Pérez, J.I.; Sandoval-Castro, C.A.; de Jesús Torres-Acosta, J.F. Optimal age of *Trichostrongylus colubriformis* larvae (L3) for the in vitro larval exsheathment inhibition test under tropical conditions. *Vet. Parasitol.* **2020**, *278*, 109027. <https://doi.org/10.1016/j.vetpar.2020.109027>.
24. Patch, M.E.; Goodman, G.B.; Weinstein, S.B. Scoop That Poop: Optimising Faecal Sample Pre-Processing for Parasite Metabarcoding. *Mol. Ecol. Res.* **2026**, *2*, e70112. <https://doi.org/10.1111/1755-0998.70112>.
25. Garretson, P.D.; Hammond, E.E.; Craig, T.M.; Holman, P.J. Anthelmintic Resistant *Haemonchus contortus* in a Giraffe (*Giraffa camelopardalis*) in Florida. *J. Zoo. Wildl. Med.* **2009**, *40*, 131–139. <https://doi.org/10.1638/2007-0094.1>.
26. Seinhorst, J.W. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* **1959**, *4*, 67–69.
27. Skrjabin, K.I.; Shikhobalova, N.P.; Schulz, R.S.; Popova, T.I.; Boev, S.N.; Delamure, S.L. *Keys to Parasitic Nematodes: Strongylata*; Izdatelstvo Akademii Nauk SSSR: Moscow, Russia, 1952; Volume 3, p. 890. (In Russian)
28. Skrjabin, K.I.; Shikhobalova, N.P.; Schulz, R.S. *Fundamentals of Nematology: Trichostrongylids of Animals and Humans*; Izdatelstvo Akademii Nauk SSSR: Moscow, Russia, 1954; Volume 3, p. 684. (In Russian)
29. Ivashkin, V.M.; Oripov, A.O.; Sonin, M.D. *Keys to Helminths of Small Ruminants*; Nauka: Moscow, Russia, 1989; pp. 256. (In Russian)
30. Joyce, S.A.; Reid, A.; Driver, F.; Curran, J. Application of Polymerase Chain Reaction (PCR) Methods to the Identification of Entomopathogenic Nematodes. In *Genetics of Entomopathogenic Nematode–Bacterium Complexes*; Proceedings and National Reports 1990–1993; Publications Office of the European Union: Luxembourg, 1994; pp. 178–187.
31. Kanzaki, N.; Futai, K. A PCR primer set for determination of phylogenetic relationships of *Bursaphelenchus* species within the xylophilus group. *Nematology* **2002**, *4*, 35–41. <https://doi.org/10.1163/156854102760082186>.
32. Demissie, T.; Tesfaye, D.; Fekadu, A.; Asefa, I. Study on abomasal nematodes of sheep and goats: Comparison and characterization of vulvar morphology of *Haemonchus* in Hawassa, Ethiopia. *Afr. J. Agric. Res.* **2013**, *8*, 5181–5186. Available online: https://academicjournals.org/article/article1382107844_Demissie%20et%20al.pdf (accessed on 4 August 2026).
33. Gharamah, A.A.; Rahman, W.A.; Noor, S.A.M. Phenotypic differences of *Haemonchus contortus* from Sheep and goats in the States of Perak and Kelantan, Peninsular Malaysia. *Acta Parasitol.* **2011**, *56*, 412–417.
34. Aksenov, A.P.; Spiridonov, S.E. Diversity of the rDNA ITS Haplotypes of the Nematodes *Haemonchus contortus* (Trichostrongyloidea, Rhabditida) of the same host. *Biol. Bull.* **2013**, *40*, 36–44. <https://doi.org/10.1134/S1062359013010032>.
35. Gaytán, Á.; Bergsten, J.; Canelo, T.; Pérez-Izquierdo, C.; Santoro, M.; Bonal, R. DNA Barcoding and geographical scale effect: The problems of undersampling genetic diversity hotspots. *Ecol. Evol.* **2020**, *10*, 10754–10772. <https://doi.org/10.1002/ece3.6733>.
36. Lefoulon, E.; Bain, O.; Bourret, J.; Junker, K.; Guerrero, R.; Cañizales, I.; Kuzmin, Y.; Satoto, T.B.T.; Cardenas-Callirgos, J.M.; de Sousa Lima, S.; et al. Shaking the tree: Multi-locus sequence typing usurps current onchocercid (filarial nematode) phylogeny. *PLoS Neglected Trop. Dis.* **2015**, *9*, e0004233. <https://doi.org/10.1371/journal.pntd.0004233>.
37. Clavero-Camacho, I.; Palomares-Rius, J.E.; Cantalapiedra-Navarrete, C.; León-Ropero, G.; Martín-Barbarroja, J.; Archidona-Yuste, A.; Castillo, P. Integrative Taxonomy Reveals Hidden Cryptic Diversity within Pin Nematodes of the Genus *Paratylenchus* (Nematoda: Tylenchulidae). *Plants* **2021**, *10*, 1454. <https://doi.org/10.3390/plants10071454>.
38. Chartier, C.; Hoste, H. Response to challenge infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in dairy goats differences between high and low-producers. *Vet. Parasitol.* **1997**, *73*, 267–276. [https://doi.org/10.1016/S0304-4017\(97\)00131-3](https://doi.org/10.1016/S0304-4017(97)00131-3).
39. Tan, T.K.; Panchadcharam, C.; Low, V.L.; Lee, S.C.; Ngui, R.; Sharma, R.S.; Lim, Y.A. Co-infection of *Haemonchus contortus* and *Trichostrongylus* spp. among livestock in Malaysia as revealed by amplification and sequencing of the internal transcribed spacer II DNA region. *BMC Vet. Res.* **2014**, *10*, 38. <https://doi.org/10.1186/1746-6148-10-38>.
40. Roeber, F.; Jex, A.R.; Gasser, R.B. Advances in the diagnosis of key gastrointestinal nematode infections of livestock, with an emphasis on small ruminants. *Biotechnol. Adv.* **2013**, *31*, 1135–1152. <https://doi.org/10.1016/j.biotechadv.2013.01.008>.

41. Mackenzie, J.S.; Jeggo, M. The One Health Approach—Why Is It So Important? *Trop. Med. Infect. Dis.* **2019**, *4*, 88. <https://doi.org/10.3390/tropicalmed4020088>.
42. Jex, A.R.; Hall, R.S.; Littlewood, D.T.J.; Gasser, R.B. An Integrated Pipeline for Next-Generation Sequencing and Annotation of Mitochondrial Genomes. *Nucleic Acids Res.* **2010**, *38*, 522–533. <https://doi.org/10.1093/nar/gkp883>.
43. Nabavi, R.; Conneely, B.; McCarty, E.; Good, B.; Shayan, P.; de Waal, T. Comparison of Internal Transcribed Spacers and Intergenic Spacer Regions of Five Common Iranian Sheep Bursate Nematodes. *Iran. J. Parasitol.* **2014**, *9*, 350–357.
44. Buchmann, K.; Christiansen, L.-L.; Kania, P.W.; Thamsborg, S.M. Introduced European Bison (*Bison bonasus*) in a Confined Forest District: A Ten Year Parasitological Survey. *Int. J. Parasitol. Parasites Wildl.* **2022**, *18*, 292–299. <https://doi.org/10.1016/j.ijppaw.2022.07.005>.
45. Manninen, S.M.; Thamsborg, S.M.; Laaksonen, S.; Oksanen, A. The reindeer abomasal nematode (*Ostertagia gruehneri*) is naturally transmitted to sheep when sharing pastures. *Parasitol. Res.* **2014**, *113*, 4033–4038. <https://doi.org/10.1007/s00436-014-4071-x>.
46. Robertsen, P.A. Gastrointestinal Parasites in Sympatric Reindeer (*Rangifer tarandus*) and Sheep (*Ovis aries*)-Evidence of Spillover and Consequences Thereof. Master's Thesis, Inland University, Hamar, Norway, 2020.
47. Utaaker, K.S.; Ytrehus, B.; Davey, M.L.; Fossøy, F.; Davidson, R.K.; Miller, A.L.; Robertsen, P.-A.; Strand, O.; Rauset, G.R. Parasite Spillover from Domestic Sheep to Wild Reindeer—The Role of Salt Licks. *Pathogens* **2023**, *12*, 186. <https://doi.org/10.3390/pathogens12020186>.

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