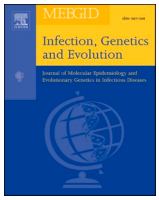
Infection, Genetics and Evolution 134 (2025) 105817

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid



Research paper

Metabarcoding characterization of gastrointestinal strongyle nematodes in captive Asian elephants (*Elephas maximus*) and white rhinoceroses (*Ceratotherium simum*) in a private zoo, Thailand

Mohamed H. Hamad [a,b,c], Witchuta Junsiri [a], Tanagrit Sumpanpae [d], Duriyang Narapakdeesakul [a], Piyanan Taweethavonsawat [a,e,*]

[a] Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand
[b] Infectious Diseases, Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt
[c] Department of Animal Diseases, Faculty of Veterinary Medicine, King Salman International University, Ras Sudr 46612, Egypt
[d] Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[e] Biomarkers in Animals Parasitology Research Unit, Chulalongkorn University, Bangkok, 10330, Thailand

ARTICLE INFO

Keywords:
Strongyle nematodes
ITS-2 rDNA metabarcoding
Elephas maximus
Ceratotherium simum
Parasite diversity
Captive wildlife

ABSTRACT

Gastrointestinal strongyle nematodes pose significant health risks to captive megaherbivores, including Asian elephants (*Elephas maximus*) and white rhinoceroses (*Ceratotherium simum*). Traditional diagnostic methods often fail to accurately identify species due to morphological similarities, limiting understanding of parasite diversity and host-specificity. This study is among the first in Southeast Asia to apply high-throughput internal transcribed spacer-2 (ITS-2) rDNA metabarcoding to characterize strongyle nematode communities in these endangered hosts. Fecal samples from six rhinoceroses and four elephants housed in a private zoo in Thailand were processed using flotation, larval culture, and DNA extraction protocols. Amplicon sequencing was conducted on the Illumina MiSeq platform, and taxonomic assignments were performed using the DADA2 pipeline and NCBI/GenBank databases. Our results revealed the presence of strongyle infections. *Murshidia* spp. were detected in both host species, while *Kilulumа ceratotherii* was found exclusively in rhinoceroses. Phylogenetic analysis based on ITS-2 rDNA sequences demonstrated clear host-associated clades and suggested potential cryptic species within *Kilulumа* and *Murshidia* lineages. These findings provide new genetic evidence of host specificity and evolutionary divergence among strongylid nematodes in captive wildlife. The study underscores the utility of DNA metabarcoding for non-invasive parasite surveillance and highlights the urgent need to expand molecular databases for better taxonomic resolution in wildlife parasitology.

1. Introduction

Elephants and rhinoceroses, two of the largest terrestrial mammals, play a vital role in maintaining the ecological balance of their natural environments. Elephants, classified under the order *Proboscidea*, include species such as the Asian elephant (*Elephas maximus*) and the African elephant (*Loxodonta africana*) (Fowler, 2006). In contrast, rhinoceroses belong to the order *Perissodactyla* and are represented by species such as the white rhinoceros (*Ceratotherium simum*) and the black rhinoceros (*Diceros bicornis*) (Geraads, 2020). Although taxonomically distinct, both species are megaherbivores with a primarily herbivorous diet and face comparable risks of gastrointestinal parasitic infections (Fynn and Provenza, 2023). These parasites pose serious threats to their health and well-being in both wild and captive environments, leading to poor body condition, population declines, and behavioral changes (Fowler, 2006; Obanda et al., 2011). Although available studies are limited, reports from Nepal indicate high prevalence rates in wild Asian elephants (95 %) and Indian rhinoceroses (82.5 %) (Shahi and Gairhe, 2019), while data from Sri Lanka show prevalence ranging from 38 % to 90 % in captive elephants under different management systems (Abeysinghe et al., 2017). Additionally, a study on captive elephants has found that parasite numbers vary widely among individuals, with certain animals

* Corresponding author at: Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.
E-mail address: Piyanan.T@chula.ac.th (P. Taweethavonsawat).

https://doi.org/10.1016/j.meegid.2025.105817
Received 21 June 2025; Received in revised form 23 August 2025; Accepted 27 August 2025
Available online 30 August 2025

harboring substantially higher loads than others (Chel et al., 2020).

In captive settings such as zoological parks and wildlife conservation centers, the risk of parasitic infections increases due to the proximity of different hosts, facilitating the transmission of gastrointestinal nematodes (Dibakou et al., 2021). Among these, strongyle nematodes from the family *Strongylidae* are commonly found in both elephants and rhinoceroses. Previous studies have identified multiple genera of strongyle nematodes, including *Kililuma, Quilonia, Paraquilonia, Murshidia,* and *Buissonia*, as commonly occurring parasites in these large herbivores (Witenberg, 1925; Gupta and Trivedi, 1984). While some species, such as those within the *Quilonia* and *Khalilia* genera, are shared between rhinos and elephants (Penzhorn et al., 1994; Qurratul-Saadah et al., 2023), others demonstrate host specificity. Most *Kililuma* species are parasitic in rhinoceroses, with one species reported in a tapir (Thapar, 1924, 1925; Beveridge and Jabbar, 2013; Beveridge, 2018). *Murshidia* species are predominantly found in elephants, though some have also been recorded in rhinoceroses and warthogs (Lichtenfels, 1980).

Identifying strongyle infections in elephants and rhinoceroses and assessing their potential transmission is challenging due to the morphological similarity of their eggs and larvae in fecal samples. These eggs are often indistinguishable, and larval identification requires advanced expertise (McLean et al., 2012; Quintana et al., 2023). While adult worms exhibit species-specific traits, obtaining them typically requires host sacrifice, which is only possible post-mortem from natural causes or illness (Quintana et al., 2023). Even when adult worms are occasionally recovered from fecal samples (McLean et al., 2012; Chel et al., 2020), the reliance on morphology alone can limit accurate identification and hinder understanding of infection patterns (Beaumelle et al., 2021; Dibakou et al., 2021; Sargison et al., 2022). These challenges contribute to significant gaps in understanding parasite diversity, prevalence, and transmission dynamics.

To overcome these challenges, DNA metabarcoding, particularly targeting the internal transcribed spacer-2 (ITS-2) region, has become a powerful molecular tool for identifying complex nematode communities (Avramenko et al., 2015). This approach enables precise genetic differentiation of morphologically similar species, providing high-resolution insights into parasite diversity and host-parasite interactions. Its non-invasive sampling capabilities allow for effective monitoring of wildlife hosts and assessment of parasitic disease impacts across species (Miller et al., 2024). By generating extensive genetic data, DNA metabarcoding clarifies transmission pathways between cohabiting hosts and enhances understanding of parasite evolution, supporting conservation efforts and resolving taxonomic ambiguities (Nieberding et al., 2008; Hubert and Hanner, 2015).

This study aims to identify strongyle nematode species present in elephants and rhinoceroses at a private park in Thailand using ITS-2 DNA metabarcoding and to investigate the evolutionary relationships between these parasites and their hosts.

2. Materials and methods

2.1. Ethical approval and informed consent

This study adhered to the ethical guidelines of Chulalongkorn University, Thailand, and was approved under protocol number IBC 2531005.

2.2. Sample collection and processing

In April 2024, fecal boluses were collected from six white rhinoceroses (*Ceratotherium simum*) and four Asian elephants (*Elephas maximus*) residing in a private zoo in Khlong Sam Wa District, Bangkok, Thailand. Fresh fecal samples were gathered immediately after voiding to minimize contamination.

Fecal samples were analyzed for gastrointestinal nematodes using the simple flotation technique, as outlined by Ballweber et al. (2014). Samples testing positive for nematode eggs underwent individual larval cultures. Each culture was prepared in a wide-mouth jar and incubated at approximately 25 °C for seven days to facilitate the development of third-stage larvae (L3) (Abeysinghe et al., 2012). Around 2,000 L3 larvae were collected from each positive sample, preserved in 70 % ethanol, and stored at −20 °C for subsequent DNA analysis.

2.3. DNA isolation and molecular analysis

Genomic DNA was extracted from the L3 larvae using a NucleoSpin® Tissue DNA extraction kit (Macherey-Nagel, Germany) according to the manufacturer's protocol. The purity and concentration of the extracted DNA were assessed using a NanoDrop™ Lite Spectrophotometer (Thermo Fisher Scientific, USA). PCR amplification targeted the ITS-2 rDNA region using NC1 and NC2 primers, as designed by Gasser et al. (1993). Amplified PCR products were visualized on a 1.5 % agarose gel using gel electrophoresis to confirm successful amplification. The PCR products were subsequently purified using the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Germany). All sample examinations and processing were performed at the Parasitology Unit, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand.

Sequencing was performed on an Illumina MiSeq platform using a 500-cycle paired-end reagent kit (MiSeq Reagent Kits v2). For library preparation, purified PCR products from rhinoceros and elephant samples were pooled separately into two Eppendorf tubes, with each sample contributing approximately 40 ng of genomic DNA to its respective group. Library concentrations and fragment sizes were verified using a Qubit Flex Fluorometer (Invitrogen) and TapeStation 4200 (Agilent). Sequencing was conducted at U2bio Sequencing Service (https://www.u2bio.co.th), with raw Fastq files generated and provided for subsequent analysis.

2.4. Bioinformatics and taxonomic analysis

Raw ITS-2 amplicon sequencing data were processed using the Galaxy Europe web server (https://galaxyproject.org), accessed on 12 November 2024). Primer sequences (NC1 and NC2) were removed using the inbuilt Cutadapt tool, and the sequencing outputs were analyzed using the Divisive Amplicon Denoising Algorithm 2 (DADA2) pipeline to generate Amplicon Sequence Variants (ASVs). Quality control steps included checking quality profile plots to determine filtering parameters. Reads with base quality scores below 2, lengths under 200 base pairs, or estimated error rates exceeding 2 for forward reads or 4 for reverse reads were discarded using the Filter and Trim tool.

Error correction of the filtered reads was performed by training the DADA2 algorithm using the LearnErrors function, followed by modeling and applying the core dada algorithm. Forward and reverse reads were merged with a minimum overlap of 12 nucleotides, allowing no mismatches. The removeBimeraDenovo function was used to detect and eliminate chimeric sequences through a consensus-based method. Non-ITS-2 flanking regions were excluded using the ITSx tool.

Taxonomy was assigned using the assignTaxonomy function with a 50 % bootstrap cutoff to maximize species identification (Poissant et al., 2021; Hamad et al., 2024). The nematode ITS-2 database v1.6 (https://www.nemabiome.ca/its2-database, accessed on 23 November 2024) served as the reference. Following data processing, unclassified ASVs and those unrelated to gastrointestinal nematodes were omitted from the analysis as potential contaminants. To further reduce the influence of sequencing artifacts and low-abundance noise, ASVs representing less than 0.1 % of the total reads per sample were excluded from downstream analysis. Each ASV was further validated by performing a BLAST search against the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/BLAST/), retrieving the top matching species based on high-confidence alignments to ensure analytical precision (Zahid et al., 2024). These results, along with their associated data, are listed in

Supplementary Table S4. Species abundances were calculated as the percentage of reads assigned to each species relative to the total reads per sample, representing their distribution within the dataset.

2.5. Phylogenetic tree construction

Phylogenetic analysis was performed using sequences derived from field samples, supplemented with those retrieved from the NCBI GenBank database, to confirm species identity and elucidate evolutionary relationships. These sequences were aligned using MAFFT version 7 (Kuraku et al., 2013; Katoh et al., 2019) with the Auto strategy, which selects the most appropriate alignment algorithm based on sequence characteristics, and manually inspected for accuracy in MSAViewer (Yachdav et al., 2016). The phylogenetic tree was inferred using the maximum likelihood (ML) method as implemented in the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at). Branch support for the inferred topology was assessed through 1000 bootstrap replicates. The evolutionary model was selected automatically using the default settings provided by IQ-TREE, with the HKY + F model identified as the best fit. Tree visualization and annotation were performed using iTOL (Interactive Tree of Life) (https://itol.embl.de), enabling clear representation of evolutionary relationships.

3. Results

3.1. Strongylid nematode detection and identification

Microscopic analysis confirmed the presence of strongyle nematode eggs in fecal samples from both elephants and rhinoceroses. Strongylid eggs were detected in five out of six rhinoceroses, while all four elephant samples tested positive. Representative strongyle nematode eggs at various developmental stages were observed in fecal samples from both species and are illustrated in Fig. 1.

Sequencing analysis generated 247,576 paired-end reads from the elephant sample and 178,412 from the rhinoceros sample. Following bioinformatic processing with the DADA2 pipeline, 191,355 and 147,067 high-quality reads were retained for the elephant and rhinoceros samples, respectively (Supplementary Table S1). In the elephant sample, 15 ASVs were identified at the species level. Of these, 11 corresponded to *Rhabditis blumi*, a free-living nematode commonly found in moist soil, and were therefore excluded from further analysis. The remaining four ASVs, representing only 2.69 % of the total reads, were classified within the family Strongylidae (Supplementary Table S2). Similarly, 18 ASVs were identified in the rhinoceros sample, all of which were classified within the Strongylidae family using a 50 % confidence threshold (Supplementary Table S3).

Species-level identification based on nemabiome metabarcoding revealed that the strongylid nematodes detected in the elephant sample were entirely composed of *Murshidia* species (100 %). The rhinoceros sample demonstrated a more diverse composition, with *Murshidia* species comprising 92.20 % and *Kililuma ceratotherii* representing 7.80 % of the overall species composition.

3.2. Species relationships and evolutionary insights

The ML phylogenetic tree, constructed using ITS-2 rDNA sequences from field-derived ASVs and reference sequences from the NCBI GenBank database (Fig. 2), identified two major clades representing the genera *Kililuma* and *Murshidia*, with 99 % and 89 % support, respectively. ASVs assigned to *Kililuma* species from white rhinoceroses formed two subclades, with bootstrap values of 73 and 94 %. The first subclade includes ASV3 and ASV16, which closely align with *K. ornata* isolate from captive rhinoceroses in Australia (GenBank accession number: JX982336.1). The second subclade comprises ASV18 and ASV11, which cluster with *K. ceratotherii* sequences (GenBank accession numbers: OR142650.1, OR142651.1, OR142652.1, OR142653.1) isolated from white rhinoceroses in the United States. Both subclades exhibit a high level of sequence identity with their respective reference sequences (Supplementary Table S4).

Within the Murshidia group, the ASVs identified as *Murshidia* sp. from elephants (ASV1, ASV2, ASV3, and ASV4) formed a well-supported clade, closely related to *M. linstowi* sequence (GenBank Accession: MK968095.1). Meanwhile, *M. linstowi* sequences retrieved from African elephants (GenBank Accessions: JN252662.1, JN252663.1, JN252664.1) clustered separately, forming a distinct clade. On the other hand, ASVs assigned to *Murshidia* sp. from white rhinoceroses displayed a more complex phylogenetic structure, clustering into multiple subclades with varying bootstrap support, suggesting potential intraspecific variation.

4. Discussion

Asian elephants and white rhinoceroses are iconic megaherbivores commonly maintained in captivity for conservation, educational, and recreational purposes. Despite their ecological significance, limited published data exist on gastrointestinal nematode (GIN) infections in these species, particularly in Thailand. Previous studies have largely relied on the morphological identification of eggs and larvae (Abhijith et al., 2018; Shahi and Gairhe, 2019; Dibakou et al., 2021), with relatively few utilizing molecular techniques to confirm species identity. Furthermore, these molecular studies have often been conducted in other regions and typically focus on a single host species (Chel et al.,

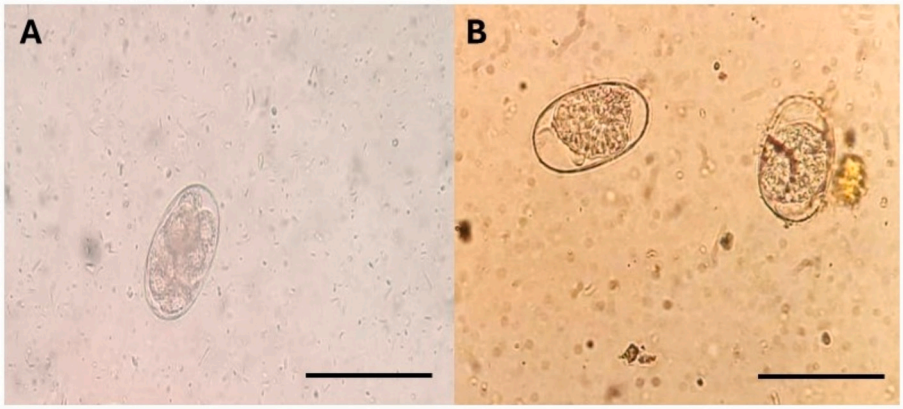


Fig. 1. Strongyle nematode eggs observed in Asian elephants (*Elephas maximus*) (A) and white rhinoceroses (*Ceratotherium simum*) (B) at Private Zoo Park. The eggs, typically measuring between 70 and 90 μm in length, are elongated with a multi-celled interior and a thin, translucent shell, as described by Thurber et al. (2011). Images were captured at 40× magnification, with a scale bar representing approximately 100 μm.

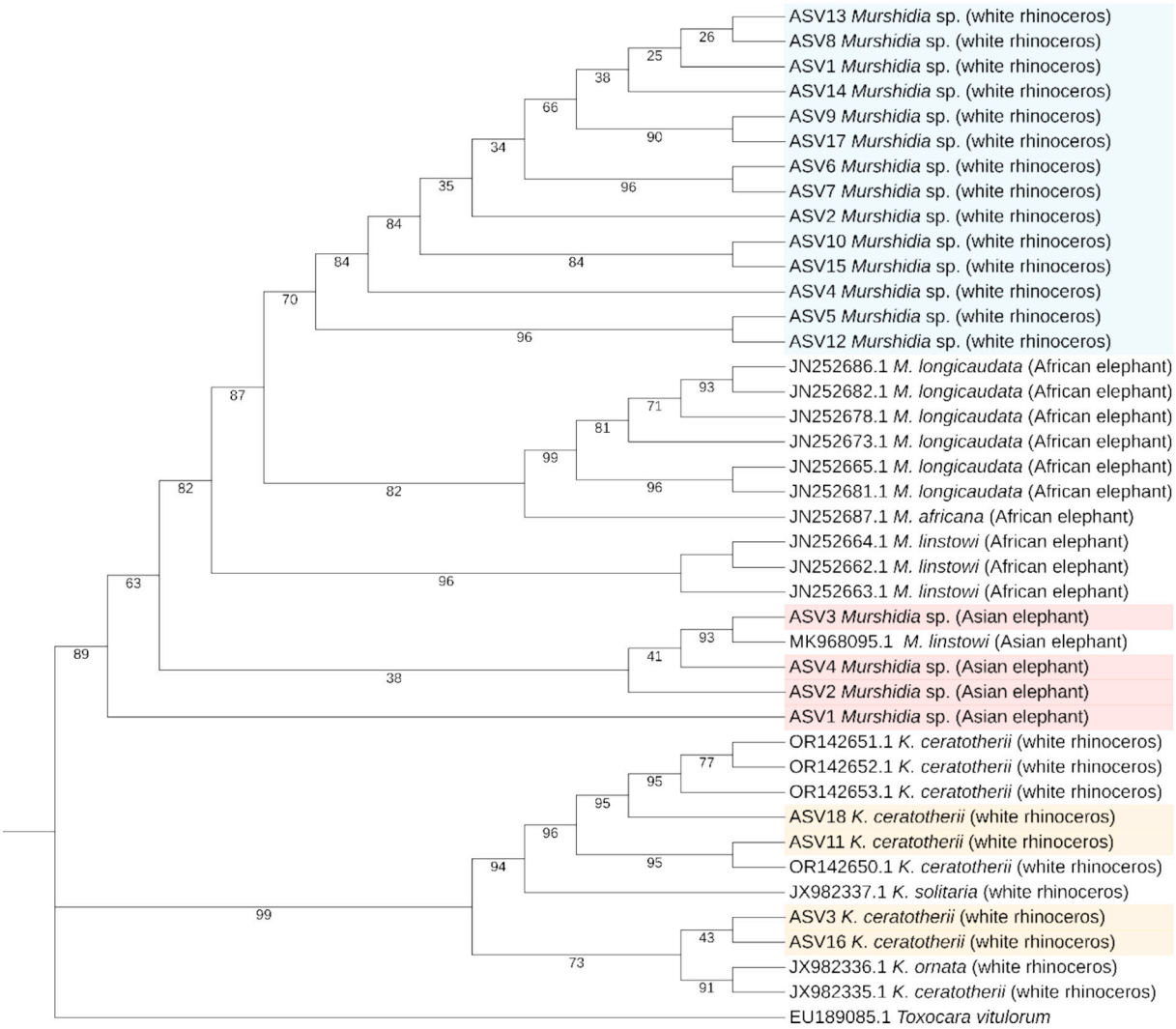


Fig. 2. Phylogenetic tree illustrating the relationships among Strongylid nematode sequences isolated from elephants and rhinoceroses in a private park in Thailand, constructed using the Maximum Likelihood method based on ITS-2 gene sequences. Sequences identified from field samples (denoted as ASVs) are shown alongside reference sequences from GenBank (indicated by accession numbers).

2020; Hota et al., 2020; Quintana et al., 2023). This study employs nemabiome metabarcoding of ITS-2 rDNA to provide a comprehensive and accurate characterization of strongylid nematode infections in these iconic animals.

The presence of strongyle nematode eggs in both elephant and rhinoceros fecal samples observed in this study highlights the widespread occurrence of GIN infections in these captive species, indicating a significant parasitic burden in the population. Moreover, the observation of eggs at different developmental stages suggests active shedding and the potential for environmental contamination (Nielsen et al., 2007; Shite et al., 2015). Similar studies conducted in zoos and wildlife parks have reported infection rates ranging from 61.1 % to 68.2 % (Abeysekara et al., 2018; Rahman et al., 2023). Strongyle nematodes are among the most commonly detected parasites, exhibiting high prevalence and intensity in elephants and other captive species (Abeysinghe et al., 2017; Abeysekara et al., 2018; Qurratul-Saadah et al., 2023; Ali et al., 2024). The detection of gastrointestinal parasites, even in the absence of clinical signs, indicates that subclinical infections are prevalent and underscores the importance of implementing regular monitoring and effective control measures to safeguard the health of captive wildlife populations (Rahman et al., 2023).

ITS-2 metabarcoding analysis provided a detailed characterization of the community composition within the studied population, offering comprehensive insights into the diversity and relative abundance of strongylid nematodes. The complete dominance of *Murshidia* species in the elephant samples indicates a high degree of host-parasite specificity. This finding aligns with previous studies that have reported its presence in both African and Asian elephants. For instance, research conducted in Amboseli National Park, Kenya, identified *M. linstowi* in wild African elephants (McLean et al., 2012). Additionally, a case in India documented the detection of *M. linstowi* in a free-ranging juvenile male elephant calf (Hota et al., 2020). The elephant population in the studied zoo harbored a lower parasite burden or a less diverse nematode community, potentially due to host-specific differences in susceptibility, immune responses (McRae et al., 2015), or prior anthelmintic treatment (Vercruysse and Claerebout, 2001). Alternatively, the reduced number of ASVs detected in the elephant samples may reflect differences in larval shedding rhythms or the timing of sample collection. It has been shown that parasite detection can be influenced by host defecation patterns and circadian variation in parasite release. For example, metastrongyloid nematodes in rats show nocturnal accumulation of infective larvae in feces, suggesting that samples collected outside these peak periods might underestimate actual diversity (de Azevedo et al., 2011). In contrast, the rhinoceros sample exhibited greater nematode diversity compared to the elephant sample. The exclusive detection of *K. ceratotherii* in rhinoceros samples, with no evidence of its presence in

elephant samples, is consistent with previous reports documenting this species in rhinoceroses across regions, including Australia (Beveridge and Jabbar, 2013) and the United States (Quintana et al., 2023). This finding supports the suggestion that *K. ceratotherii* is host-specific to rhinoceroses (Quintana et al., 2023). Although *Murshidia* species are commonly reported in elephants (McLean et al., 2012; Heinrich, 2016; Hota et al., 2020), their presence in rhinoceroses, as observed in this study, is not unprecedented. Previous records, such as those by Round (1968), have documented *Murshidia* species, including *M. aziza*, *M. bozasi*, and *M. memphisia*, in African rhinoceroses. Therefore, the detection of Murshidia in rhinoceroses in this study is consistent with historical data and emphasizes the need for further investigation into the host–parasite relationships and possible overlaps in strongyle communities between these megaherbivores.

The phylogenetic analysis provided insights into the genetic relationships and host specificity of the detected nematodes. The ITS-2 rDNA marker revealed a basal separation of two major clades corresponding to the genera *Murshidia* and *Kilulumа*, reflecting their divergent genetic lineages in elephants and rhinoceroses.

Our evolutionary analysis reveals a distinct separation of *K. ceratotherii* into two well-supported clades, indicating greater evolutionary divergence than previously recognized. This contrasts with the findings of Quintana et al. (2023), who reported *K. ceratotherii* forming a single clade alongside the *K. ceratotherii* sequence from Beveridge and Jabbar (2013), distinct from *K. ornata* (Beveridge, 2018).

In our study, ASV3 and ASV16 closely align with *K. ornata* (Beveridge, 2018), a species identified in captive rhinoceroses in Australia. This sequence was initially reported as *Kilulumа* sp. (JX982336.1) by Beveridge and Jabbar (2013) but remained unnamed due to insufficient material at the time. Meanwhile, ASV18 and ASV11 cluster with *K. ceratotherii* from white rhinoceroses in the United States (Quintana et al., 2023), forming a distinct lineage.

The presence of two genetically distinct clades raises the possibility of unrecognized species-level diversity, indicating that *K. ceratotherii*, as currently recognized, may encompass multiple genetically distinct lineages. Moreover, the lack of reference sequences for *Kilulumа* species in publicly available databases, such as nemabiome database or GenBank, represents a significant gap in the genetic characterization of this genus, highlighting the need for further molecular studies. This finding underscores the importance of morphological and genomic investigations, including whole-genome sequencing, to clarify species boundaries within the *K. ceratotherii* complex.

Regarding the phylogenetic analysis of *Murshidia* spp., the findings reveal a complex evolutionary structure. Sequences derived from Asian elephants show high similarity to a sequence previously identified in an Indian elephant by Hota et al. (2020) (GenBank: MK968805.1), labeled as *M. linstowi* in both GenBank and the nemabiome database. However, these sequences form a distinct clade, separate from *M. linstowi* sequences reported in African elephants by McLean et al. (2012), suggesting possible phylogenetic divergence. This may reflect interspecific variation or high inter-individual genetic diversity. Additionally, Chel et al. (2020) reported that although Asian and African elephants share the same genera of parasites, the species composition within these genera can differ.

Historically, six *Murshidia* species have been reported in captive and zoo-housed Asian elephants: *M. elephasi, M. murshida, M. indica, M. falcifera, M. lanei,* and *M. neveulemairei* (Lane, 1914; Ware, 1924; Witenberg, 1925; Wu, 1934; Van Der Westhuysen, 1938; Gupta and Trivedi, 1984; Gupta and Jaiswal, 1984; Zhang and Xie, 1992). However, no ITS-2 sequence data are currently available for these species in nemabiome database and ultimately in GenBank, limiting the ability to accurately determine their genetic identity.

Furthermore, the detection of *Murshidia* spp. in white rhinoceroses revealed several subclades distinct from *M. linstowi* and *M. longicaudata*, which have been previously reported in African elephants (McLean et al., 2012). This suggests that the *Murshidia* spp. identified in Asian elephants and white rhinoceroses may belong to an Asian species within the Murshidia genus. However, the absence of ITS-2 reference sequences for many described *Murshidia* spp. limits accurate identification. This highlights the need for expanded genomic data, morphological reassessment, and multi-locus analyses to clarify taxonomy and host specificity, and to enhance nemabiome applications in wildlife parasitology. Importantly, understanding the diversity and host specificity of strongylid nematodes in these captive megaherbivores supports the development of targeted parasite control strategies, reduces the risk of cross-species transmission, and informs health monitoring and quarantine protocols. These data are essential for conservation initiatives, particularly in the context of translocation and reintroduction programs, where undetected parasitic infections may compromise individual health and population viability.

5. Conclusions

This study offers valuable insights into the genetic diversity and host specificity of strongylid nematodes infecting Asian elephants and white rhinoceroses. It underscores the complexity of host-parasite interactions and highlights the significance of molecular techniques in refining our understanding of nematode taxonomy and epidemiology. Future research should prioritize expanding geographic and host sampling while incorporating comparative genomic approaches to further unravel the evolutionary dynamics and host adaptation strategies of these parasitic nematodes.

CRediT authorship contribution statement

Mohamed H. Hamad: Writing – original draft, Methodology, Formal analysis, Data curation. **Witchuta Junsiri:** Writing – review & editing, Resources, Investigation, Conceptualization. **Tanagrit Sumpanpae:** Writing – review & editing, Resources, Investigation. **Duriyang Narapakdeesakul:** Writing – review & editing, Resources, Investigation. **Piyanan Taweethavonsawat:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

All methods were performed in accordance with the relevant guidelines and regulations.

Acknowledgements

This research received funding from the Thailand Science Research and Innovation Fund, Chulalongkorn University (FOODF68310009).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2025.105817.

Data availability

The nucleotide sequences generated in this research have been deposited in the GenBank database and can be accessed under the BioProject accession number PRJNA1217901.

References

Abeysekara, N., Rajapakse, R.P.V.J., Rajakaruna, R.S., 2018. Comparative cross-sectional survey on gastrointestinal parasites of captive, semi-captive, and wild elephants of Sri Lanka. J. Threat. Taxa 10, 11583–11594.

Abeysinghe, K.S., Perera, A., Fernando, P., 2012. Developing a practical and reliable protocol to assess nematode infection in Asian elephants. Gajah 37, 22–26.

Abeysinghe, K.S., Perera, A., Pastorini, J., Isler, K., Mammides, C., Fernando, P., 2017. Gastrointestinal strongyle infections in captive and wild elephants in Sri Lanka. Gajah 46, 21–27.
Abhijith, T.V., Ashokkumar, M., Dencin, R.T., George, C., 2018. Gastrointestinal parasites of Asian elephants (*Elephas maximus* L. 1798) in south Wayanad forest division, Kerala, India. J. Parasit. Dis. 42, 382–390.
Ali, W., Ali, S., Hussain, A., Haseeb, A., Ahmed, T., Shabeer, W., Anwer, M.W., Shaukat, M., Hani, U., Sadique, A., Hussain, M., 2024. Seasonal variation and prevalence of gastrointestinal parasites in captive mammals at Lahore zoo. Appl. Biol. Res. 26 (2), 309–320.
Avramenko, R.W., Redman, E.M., Lewis, R., Yazwinski, T.A., Wasmuth, J.D., Gilleard, J. S., 2015. Exploring the gastrointestinal "Nemabiome": deep amplicon sequencing to quantify the species composition of parasitic nematode communities. PLoS One 10, e0143559.
Ballweber, L., Beugnet, F., Marchiondo, A., Payne, P., 2014. American Association of Veterinary Parasitologists' review of veterinary fecal flotation methods and factors influencing their accuracy and use—is there really one best technique? Vet. Parasitol. 204, 73–80.
Beaumelle, C., Redman, E.M., de Rijke, J., Wit, J., Benabed, S., Debias, F., Duhayer, J., Pardonnet, S., Poirel, M.-T., Capron, G., 2021. Metabarcoding in two isolated populations of wild roe deer (*Capreolus capreolus*) reveals variation in gastrointestinal nematode community composition between regions and among age classes. Parasit. Vectors 14, 1–13.
Beveridge, I., 2018. A new species of *Kilulluma* Skrjabin, 1916 (Nematoda: Strongyloidea) from the white rhinoceros, *Ceratotherium simum* (Burchell) from South Africa. Syst. Parasitol. 95, 841–847.
Beveridge, I., Jabbar, A., 2013. New species of *Kilulluma* Skrjabin, 1916 (Nematoda: Strongylida) from the white rhinoceros *Ceratotherium simium* (Burchell), with a redescription of *K. Solitaria* Thapar, 1924. Syst. Parasitol. 85, 131–145.
Chel, H.M., Iwaki, T., Hmoon, M.M., Thaw, Y.N., Soe, N.C., Win, S.Y., Bawm, S., Htun, L. L., Win, M.M., Oo, Z.M., Masum, M.A., Ichii, O., Nakao, R., Nonaka, N., Katakura, K., 2020. Morphological and molecular identification of cyathostomine gastrointestinal nematodes of *Murshidia* and *Quilonia* species from Asian elephants in Myanmar. Int. J. Parasitol. Parasites Wildl. 11, 294–301.
de Azevedo, G.V., Rodriguez, R., Porto, S.M., Graeff-Teixeira, C., Fornari, F., 2011. Elimination of Angiostrongylus costaricensis larvae in feces from experimentally infected Swiss mice: circadian rhythm and correlation with survival. Parasitol. Res. 108, 537–540.
Dibakou, S.E., Maloueki, U., Ngoubangoye, B., Boundenga, L., Ntie, S., Tsoumbou, T.A., Moussadji, C., Zang, R.O., Kombila, D., Basset, D., 2021. Diversity of gastrointestinal parasites in sympatric mammals in Moukalaba-Doudou National Park. Gabon. Vet. World 14, 3149–3155.
Fowler, M.E., 2006. Parasitology. In: Biology, Medicine, and Surgery of Elephants. Blackwell Publishing, Oxford, UK, pp. 159–181.
Fynn, R.W., Provenza, F.D., 2023. Functional adaptive resources for large herbivores in African savannas: an ecological-gradient based framework. Front. Conserv. Sci. 4, 1133329.
Gasser, R.B., Chilton, N.B., Hoste, H., Beveridge, I., 1993. Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. Nucleic Acids Res. 21, 2525–2526.
Geraads, D., 2020. Perissodactyla (Rhinocerotidae and Equidae) from Kanapoi. J. Hum. Evol. 140, 102373.
Gupta, V., Jaiswal, R., 1984. On a new nematode, *Quilonia guptai* sp. nov.(Strongyloidea: Cyathostomidae Yamaguti, 1961) from an Indian elephant, *Elephas indicus* from Kanpur, UP. Indian J. Helminthol. 36, 138–142.
Gupta, S., Trivedi, K., 1984. Nematode parasites of vertebrates IV. A new nematode parasite *Quilonia simhai* sp. nov.(Strongylidea: Cyathostomidae) from an Indian elephant. Indian J. Helminthol. 36, 61–67.
Hamad, M.H., Islam, S.I., Jitsamai, W., Chinkangsadarn, T., Naraporn, D., Ouisuwan, S., Taweethavonsawat, P., 2024. Metabarcoding study to reveal the structural community of strongylid nematodes in domesticated horses in Thailand. BMC Vet. Res. 20, 70.
Heinrich, L., 2016. Prevalence and molecular identification of helminths in wild and captive Sri Lankan elephants, *Elephas maximus maximus*. Future 19, 21.
Hota, S.R., Sahoo, S., Dash, M., Pahari, A., Mohanty, B., Sahoo, N., 2020. Molecular detection of *Murshidia linstowi* in a free-ranging dead elephant calf. J. Threat. Taxa 12, 15359–15363.
Hubert, N., Hanner, R., 2015. DNA barcoding, species delineation and taxonomy: a historical perspective. DNA Barcodes 3 (1), 44–58.
Katoh, K., Rozewicki, J., Yamada, K.D., 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief. Bioinform. 20, 1160–1166.
Kuraku, S., Zmasek, C.M., Nishimura, O., Katoh, K., 2013. aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. Nucleic Acids Res. 41, W22–W28.
Lane, C., 1914. Bursate nematodes from the Indian elephant. Indian J. Med. Res. 3, 380–398.
Lichtenfels, J.R., 1980. Keys to genera of the superfamily Strongyloidea. In: Anderson, R. C., Chabaud, A.G., Willmott, S. (Eds.), CIH Keys to the Nematode Parasites of Vertebrates, vol. No. 7. Commonwealth Agricultural Bureaux, Farnham Royal, pp. 1–41.
McLean, E., Kinsella, J., Chiyo, P., Obanda, V., Moss, C., Archie, E., 2012. Genetic identification of five strongyle nematode parasites in wild African elephants (*Loxodonta africana*). J. Wildl. Dis. 48, 707–716.
McRae, K.M., Stear, M.J., Good, B., Keane, O.M., 2015. The host immune response to gastrointestinal nematode infection in sheep. Parasite Immunol. 37, 605–613.
Miller, M.L., Rota, C., Welsh, A., 2024. Transforming gastrointestinal helminth parasite identification in vertebrate hosts with metabarcoding: a systematic review. Parasit. Vectors 17, 311.
Nieberding, C.M., Durette-Desset, M.-C., Vanderpoorten, A., Casanova, J.C., Ribas, A., Deffontaine, V., Feliu, C., Morand, S., Libois, R., Michaux, J.R., 2008. Geography and host biogeography matter for understanding the phylogeography of a parasite. Mol. Phylogenet. Evol. 47, 538–554.
Nielsen, M.K., Kaplan, R.M., Thamsborg, S.M., Monrad, J., Olsen, S.N., 2007. Climatic influences on development and survival of free-living stages of equine strongyles: implications for worm control strategies and managing anthelmintic resistance. Vet. J. 174, 23–32.
Obanda, V., Mutinda, N., Gakuya, F., Iwaki, T., 2011. Gastrointestinal parasites and associated pathological lesions in starving free-ranging African elephants. South Afr. J. Wildl. Res. 41, 167–172.
Penzhorn, B., Krecek, R., Horak, I., Verster, A., Walker, J., Boomker, J., Knapp, S., Quandt, S., 1994. Parasites of African Rhinos: a documentation. In: Proceedings of a symposium on rhinos as game ranch animals, South African Veterinary Association Wildlife Group, Onderstepoort, Republic of South Africa, pp. 9–10.
Poissant, J., Gavriliuc, S., Bellaw, J., Redman, E.M., Avramenko, R.W., Robinson, D., Workentine, M.L., Shury, T.K., Jenkins, E.J., McLoughlin, P.D., Nielsen, M.K., Gilleard, J.S., 2021. A repeatable and quantitative DNA metabarcoding assay to characterize mixed strongyle infections in horses. Int. J. Parasitol. 51, 183–192.
Quintana, T.A., Miller, K.R., Hill-Thimmesch, K.J., Schieferecke, G., Johnson, W.L., Herrin, B.H., Jesudoss Chelladurai, J.R., 2023. *Kilulluma ceratotherii* (Nematoda: Strongylida) in a white rhinoceros (*Ceratotherium simum*) from the United States: case report. Parasitologia 3, 241–247.
Qurratul-Saadah, Z., Che-Amat, A., Syed-Hussain, S.S., Kamaludden, J., Ariffin, S.M.Z., Basripuzi, N.H., Nor-Azlina, A.A., 2023. Gastrointestinal parasites in Asian and African elephants: a systematic review. Trop. Biomed. 40, 55–64.
Rahman, R., Nyema, J., Imranuzzaman, M., Banik, B., Pranto, P.S., Talukder, K., Sarkar, S.R., Nath, S.D., Islam, K.M., Nath, T.C., Islam, S., 2023. An update on gastrointestinal parasitic infection in captive wild animals in Bangladesh. J. Parasitol. Res. 2023, 3692471.
Round, M.C., 1968. Check List of the Helminth Parasites of African Mammals of the Orders Carnivora, Tubulidentata, Proboscidea, Hyra-Coidea, Artiodactyla and Perissodactyla. Technical communication no. 38. Commonwealth Bureau of Helminthology, the University of California, 252 p.
Sargison, N., Chambers, A., Chaudhry, U., Júnior, L.C., Doyle, S.R., Ehimiyein, A., Evans, M., Jennings, A., Kelly, R., Sargison, F., 2022. Faecal egg counts and nemabiome metabarcoding highlight the genomic complexity of equine cyathostomin communities and provide insight into their dynamics in a Scottish native pony herd. Int. J. Parasitol. 52, 763–774.
Shahi, M., Gairhe, K., 2019. Prevalence of helminths in wild Asian elephant and Indian rhinoceros in Chitwan and Bardia National Park, Nepal. Nepalese Vet. J. 36, 60–74.
Shite, A., Admassu, B., Abere, A., 2015. Large strongyle parastes in equine: a review. Adv. Biol. Res. 9, 247–252.
Thapar, G.S., 1924. On *Kilulluma* Skriabin, a genus of strongylid nematodes parasitic in the African rhinoceros. J. Helminthol. 2, 209–238.
Thapar, G.S., 1925. On some new members of the genus *Kilulluma* from the African Rhinoceros. J. Helminthol. 3, 63–80.
Thurber, M.I., O'Connell-Rodwell, C.E., Turner, W.C., Nambandi, K., Kinzley, C., Rodwell, T.C., Faulkner, C.T., Felt, S.A., Bouley, D.M., 2011. Effects of rainfall, host demography, and musth on strongyle fecal egg counts in African elephants (*Loxodonta africana*) in Namibia. J. Wildl. Dis. 47, 172–181.
Van Der Westhuysen, O., 1938. A monograph of the helminth parasites of the elephant. Onderstepoort J. Vet. Sci. Anim. Ind. 10, 49–190.
Vercruysse, J., Claerebout, E., 2001. Treatment vs non-treatment of helminth infections in cattle: defining the threshold. Vet. Parasitol. 98, 195–214.
Ware, F., 1924. Two bursate nematodes from the Indian elephant. J. Comp. Pathol. Ther. 37, 278–286.
Witenberg, G., 1925. Notes on Strongylidae of elephants. Parasitology 17, 284–294.
Wu, H., 1934. Notes on the parasitic nematodes from an Indian elephant. Sinensia 5, 512–533.
Yachdav, G., Wilzbach, S., Rauscher, B., Sheridan, R., Sillitoe, I., Procter, J., Lewis, S.E., Rost, B., Goldberg, T., 2016. MSAViewer: interactive JavaScript visualization of multiple sequence alignments. Bioinformatics 32, 3501–3503.
Zahid, O., Butler, M., Hopker, A., Freeman, E., Costa Júnior, L.M., Chaudhry, U., Sargison, N., 2024. Nemabiome metabarcoding shows a high prevalence of *Haemonchus contortus* and predominance of *Camelostrongylus mentulatus* in alpaca herds in the northern UK. Parasitol. Res. 123, 201.
Zhang, L., Xie, Q.-P., 1992. A new species of parasitic nematodes from Indian elephant (Strongylata: Trichonematidae). Acta Zootaxon. Sin. 17, 151–155 (in Chinese with English abstract).