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Molecular Tools for Diagnosing Wildlife Infections Caused by Some of Most Studied Lung and Gastrointestinal Nematodes

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This study presents a brief review of current molecular methodologies used to characterise some of the most popular gastrointestinal and lung nematodes in various wildlife hosts. Data from recent scientific developments were systematically compared, focusing on key parameters such as parasite life stages, host range, DNA extraction protocols, and target genomic regions. Attention is also given to primer selection and resulting amplicon sizes. Analysis of the compiled data highlights a primary reliance on ribosomal DNA markers (partial 18S and 28S rRNA genes, and ITS1-5.8S-ITS2 sequences), the MSP1 gene, and mitochondrial genes (e.g., COI, 12S) for species-level identification. Various PCR-based approaches and extraction techniques optimised for both non-invasive faecal samples and lung tissue specimens are summarised. These standardised molecular tools are essential for improving our understanding of nematode diversity and transmission dynamics, providing a practical guide for wildlife parasitology research.

Key words: Wildlife diseases, Molecular diagnostics, Gastrointestinal nematodes, Lungworms, PCR primers

Introduction

Parasitism is a complex interspecific relationship in which one organism, the parasite, lives on or within another, the host, typically at the host‘s expense [23]. Parasites can impact their hosts to varying degrees, leading to clinical conditions known as parasitoses.

The spread of parasitic diseases, including helminthoses, among wildlife populations carries significant ecological and economic implications. High parasite burdens can lead to substantial morbidity and mortality [4, 17]. In subclinical or mild infections, these diseases often remain asymptomatic or present with non-specific symptoms such as decreased appetite, weight loss, growth retardation, and reproductive failure [20, 21]. Collectively, these effects can trigger population declines, resulting in the loss of biodiversity and negatively affecting the game breeding and hunting industries. Furthermore, the shared use of habitats by wild and domestic animals facilitates the interspecific exchange of pathogens [18]. Animal migrations and translocations also serve as significant drivers for parasite dispersal and the emergence of novel parasitoses in wild populations [5]. This exchange is particularly pronounced in zoological gardens, where high animal density and close cohabitation between diverse species create ideal conditions for transmission [11].

Timely and accurate diagnosis is a cornerstone of effective disease management. Traditionally, the diagnosis of helminthoses has relied on the morphological identification of adult specimens. However, diagnostic accuracy based solely on the morphology of eggs and larvae is often limited and, in some cases, unreliable. This challenge is further compounded by a frequent lack of morphological expertise regarding the parasitofauna of exotic and endangered species. According to Gasser [8], many parasites previously categorised as single species with broad host ranges have been redefined as complexes of host-specific cryptic species. This distinction is critical for disease control: a parasite strictly specific to a wild host may pose no risk to livestock, and vice versa. In such contexts, molecular taxonomy has become an indispensable tool for differential diagnosis and epidemiological surveillance.

In light of these considerations, the present study reviews molecular research on some groups of selected nematodes in wildlife, aiming to provide a preliminary overview that may assist in future investigations.

Relevant literature was retrieved from the Scopus, Web of Science, and Google Scholar databases. The present study synthesizes data from the reviewed publications, with key parameters—including sample sources, parasite life stages, and the specific molecular tools used for identification— systematised and presented in comprehensive tables.

Molecular studies on lung nematodes

Gajadhar et al. [7] developed the first reliable molecular diagnostic tool to differentiate morphologically indistinguishable dorsal-spined first-stage larvae (L1) and other developmental stages of the subfamily Elaphostrongylinae. A PCR assay was designed to selectively amplify the rDNA ITS2 region. Based on the lengths of the resulting fragments, the assay successfully differentiated between the genera *Elaphostrongylus* and *Parelaphostrongylus*. While one primer set was capable of distinguishing all three *Parelaphostrongylus* spp. in a single reaction, the authors recommended a second set for routine diagnostic confirmation. Furthermore, two of the three primer sets

successfully amplified DNA from all six elaphostrongyline species, allowing for the specific identification of *Elaphostrongylus alces* and *Parelaphostrongylus odocoilei*. However, while *E. cervi* and *E. rangiferi* produced distinct fragments, they could not be differentiated from each other based on size alone. The study demonstrated the reliability of these primers across all life stages, including L1, L3, and adult worms, using various nematode, host, and faecal controls.

Lungworm infections are prevalent in North American bighorn sheep (*Ovis canadensis*), with *Protostrongylus stilesi* and *P. rushi* being the predominant species. Historically, *Muellerius capillaris* had only been documented in bighorns from South Dakota, USA. However, at the National Bison Range (NBR) in Montana, Ezenwa et al. [6] reported that 100% of surveyed sheep across six sampling periods shed first-stage dorsal-spined larvae (DSL) morphologically consistent with *M. capillaris*. In contrast, *Protostrongylus* larvae prevalence remained at or below 39%. Using molecular techniques, the authors confirmed the DSL as *M. capillaris*, marking the first definitive record of this parasite in a free-ranging bighorn sheep population outside South Dakota.

Dorsal-spined protostrongylid larvae (Nematoda: Protostrongylidae) were recovered from the faeces of the endangered pampas deer (*Ozotocerus bezoarticus celer*) in the Campos del Tuyú Wildlife Reserve, Argentina [3]. Partial sequences of the 28S rRNA gene and the ITS2 rDNA region were amplified, cloned, and analyzed. Phylogenetic analysis of the 28S rDNA sequences indicated a close relationship to *Parelaphostrongylus* spp., while ITS2 analysis placed the nematode within a clade containing both *Parelaphostrongylus* and *Elaphostrongylus* spp. Although these sequences exhibited considerable divergence from known protostrongylids, they remained most similar to these two genera. Consequently, the authors suggest that this parasite represents an undescribed species, likely belonging to the subfamily Elaphostrongylinae.

Lesage et al. [15] conducted pathological examinations of lungs from European hares in Southeast France. Morphological characterisation of adult male worms was confirmed via molecular analysis of the 28S (D2 domain) and ITS2 rDNA regions. Two species were identified: *Protostrongylus pulmonalis*, which was identical to haplotypes previously deposited in GenBank, and *P. oryctolagi*. The latter was identified based on the morphology of the copulatory bursa, specifically spicule length and gubernaculum structure. This study represents the first report of *P. oryctolagi* in both European hares and rabbits in France.

Pyziel et al. [24] developed a multiplex PCR assay designed to detect and differentiate *Dictyocaulus* species in cattle and wild ruminants using both adult and larval DNA. The assay employed four novel primers targeting species-specific regions of the ITS2 rDNA, designed from original and GenBank sequence data. Following amplification of samples from European bison (*Bison bonasus*), cattle (*Bos taurus*), moose (*Alces alces*), red deer (*Cervus elaphus*), and roe deer (*Capreolus capreolus*), gel electrophoresis revealed three distinct, species-specific bands: approximately 560 bp for *D. viviparus*, 400 bp for *D. capreolus*, and 660 bp for *Dictyocaulus* sp. from red deer and moose. Notably, *D. eckerti* (expected at 714 bp) was not observed. This

multiplex method yielded consistent results across samples from Sweden and Poland, overcoming the morphological limitations of traditional identification at various life stages.

Saidi et al. [25] investigated the prevalence and abundance of gastrointestinal parasites in three antelope species: the dorcas gazelle (*Gazella dorcas*), the scimitar-horned oryx (*Oryx dammah*), and the addax (*Addax nasomaculatus*). A total of 180 faecal samples were analysed using the Baermann technique for morphological examination, followed by molecular identification via PCR amplification and sequencing of the rDNA second internal transcribed spacer (ITS2) region. Two protostrongylid species, *Muellerius capillaris* and *Neostrongylus linearis*, were identified across all three antelope populations.

Bangoura et al. [1] described a lung nematode infection in a Rocky Mountain elk (*Cervus canadensis nelsoni*) from Wyoming, USA, which presented with severe bronchitis and pneumonia. Numerous large nematodes were recovered from the bronchi and trachea; female specimens were morphologically and molecularly identified as *Dictyocaulus* spp. based on mitochondrial *cox1* and 18S rRNA genes. Maximum likelihood phylogenetic analysis revealed that these parasites formed a subclade with *D. cervi*, nested within a larger clade including *D. eckerti*. Notably, both eggs and free first-stage larvae were observed within the adult females – a feature previously undescribed for *Dictyocaulus* spp. The authors concluded that the infection was caused by a *D. cervi*-like lungworm, marking its first report in North America.

Kuchboev and Krücken [13] characterised the diversity of *Metastrongylus* spp. in wild boars and identified the earthworm intermediate hosts contributing to the parasite‘s life cycle. Following parasitological necropsies, the authors identified three lungworm species in wild boars–*M. pudendotectus*, *M. salmi*, and *M. elongatus*–which were confirmed via ITS2 sequencing and phylogenetic analysis. Earthworms collected from wild boar habitats were screened for larvae, with three of the six investigated species testing positive for metastrongyloid larvae. While phylogenetic analysis clearly resolved *M. pudendotectus*, it provided limited resolution for the other species. The authors suggest that additional sequence data are required to resolve potential cryptic species, while deep sequencing approaches could offer deeper insights into species-specific epidemiology and pathology.

Karaer et al. [12] investigated helminth diversity in captive and free-ranging mountain gazelles (*Gazella gazella*) in Hatay, Türkiye, while assessing potential zoonotic risks. A total of 188 fresh faecal samples were analysed using DNA metabarcoding to determine helminth composition and seasonal diversity. The study identified eight taxa, two of them – lung nematodes. Significant seasonal variations in helminth abundance and composition were observed. These findings underscore the efficacy of advanced molecular techniques in characterising parasite diversity within wild ungulate populations.

Dictyocaulus spp. recovered from red deer lungs in Spain were identified through morphological examination and molecular barcoding [10]. The study confirmed the presence of three distinct genetic groups, supported by significant Φ_{ST} values. While

D. cervi and *D. viviparus* exhibited separate matrilineal ancestries, *D. eckerti* and *D. cervi* showed evidence of matrilineal sharing. These findings highlight the necessity of evaluating potential introgression between the latter two species.

Molecular studies on gastrointestinal nematodes

Despite their public health significance, few ascarid species from wild animals have been characterised at the molecular level [16]. To address this gap, the authors investigated the classification and phylogenetic relationships of roundworms from 21 captive wild animal species by sequencing partial 18S and 28S rDNA, along with the mitochondrial (mt) 12S gene. Phylogenies were inferred using Neighbor-Joining (NJ), Maximum Parsimony (MP), and Maximum Likelihood (ML) methods based on both single-gene and concatenated datasets. Sequence analysis indicated that the 18S region was highly conserved across all 21 species, whereas the 28S gene exhibited significant interspecific variability. Divergence levels in the 12S gene suggested both intra- and interspecific variation. Phylogenetic reconstruction resolved the isolates into two families, four genera, and seven species, with robust bootstrap support for each clade. Furthermore, concatenated trees suggested that *Baylisascaris ailuri* is more closely related to *B. transfuga* than to *B. schroederi*. This study identifies valuable molecular markers for the classification, phylogenetics, and epidemiological surveillance of ascarids in wildlife.

It remains debatable whether *Trichuris trichiura* is a multi-host parasite with a broad taxonomic range or a complex of host-specific species infecting closely related hosts [9]. To address this, the authors investigated the phylogenetic structure of whipworms within a multi-species community of non-human primates and humans in Western Uganda. A novel nested PCR method, applied to non-invasively collected faecal samples, demonstrated 100% sensitivity and 97% specificity compared to traditional microscopy. Phylogenetic analysis of the ribosomal DNA internal transcribed spacer regions (ITS1 and ITS2) revealed three co-circulating *Trichuris* lineages. Notably, one group was restricted to humans, whereas another infected all screened host species, indicating active transmission between wild primates and humans. These findings suggest that the host range within the genus *Trichuris* varies, with some lineages exhibiting strict host specificity and others showing host generality. Specifically, the identification of a multi-host *Trichuris* taxon challenges previous assumptions regarding helminth host-specificity and underscores significant concerns for both wildlife conservation and public health.

Moudgil et al. [19] performed faecal screening of Asiatic lions (*Panthera leo leo*) in an Indian zoo, identifying nematode eggs morphometrically and molecularly as *Toxascaris leonina*. Molecular confirmation was achieved via PCR targeting the internal transcribed spacer (ITS) sequences. Following identification, an anthelmintic treatment was implemented involving the administration of fenbendazole and ivermectin. The authors conclude that their study underscores the importance of molecular tools in validating morphological diagnoses and demonstrates the successful management of *T. leonina* in captive Asiatic lions.

Uakhit et al. [26] investigated the helminth fauna of wolves in Kazakhstan using a combination of classical parasitological and molecular techniques. The study identified several taxa, including *Echinococcus granulosus*, *Taenia krabbei*, *Dipylidium* spp., *Mesocestoides* spp., *Toxascaris leonina*, *Trichinella nativa*, and *Dirofilaria repens*. Prevalence and intensity of infection were evaluated, and helminth identification was further confirmed through amplification and sequencing of the cytochrome oxidase (*cox1*) marker gene.

To assess the prevalence of gastrointestinal parasites in a Chinese zoo, Cai et al. [2] analysed faecal samples from birds, primates, and non-primate mammals. Two ascarid species were molecularly characterized; sequence analysis of the ITS2 and *cox1* regions identified eggs from the African lion (*Panthera leo*) as *Toxascaris leonina* and those from the brown bear (*Ursus arctos*) as *Baylisascaris transfuga*. The authors emphasize that the zoonotic potential of these agents warrants increased public health attention. Furthermore, these findings provide a theoretical framework for the development of targeted prevention and control strategies for parasitic diseases in captive wildlife populations in China.

Parasitic nematodes of the superfamily Ascaridoidea infect a wide range of vertebrates, with certain taxa, particularly *Toxocara* spp., posing significant zoonotic risks. Kuchboev et al. [14] identified and characterised ascarids from wild and stray canids and felids in Uzbekistan. Specimens recovered from stray dogs, jackals, and stray cats were examined via light microscopy and molecular analysis of the partial ITS1-5.8S-ITS2 rDNA region. Morphological findings identified *Toxocara canis* and *Toxascaris leonina* in dogs and jackals, while *Toxocara cati* was identified in cats. BLAST analysis of the ITS sequences showed 99–100% identity with respective reference sequences. Maximum likelihood phylogenetic analysis confirmed that *T. canis* and *T. cati* clustered according to their known host families (Canidae and Felidae). Notably, *T. leonina* was partitioned into subclades based on host origin, suggesting a species complex with distinct host-associated infectivity.

Vafaei et al. [27] investigated the role of wild herbivores as reservoirs for helminth infections in western Iran, focusing on the goitred gazelle (*Gazella subgutturosa*, n = 36) and the wild goat (*Capra aegagrus*, n = 14). Faecal samples were microscopically screened for gastrointestinal helminth eggs, followed by genomic DNA extraction and PCR amplification of the ribosomal ITS2 region. Phylogenetic analysis of the resulting nucleotide sequences confirmed the species identities. The most prevalent circulating species were *Teladorsagia circumcincta*, *Marshallagia* spp., and *Nematodirus oiratianus*, all of which were reported for the first time in wildlife from this region. The authors concluded that these findings underscore the necessity of continuous parasite surveillance in both wildlife and domestic environments to effectively detect and manage potentially zoonotic parasitic agents.

Methodological and genetic parameters

The primary methodological and genetic parameters from the reviewed studies are synthesized and presented in **Table 1** and **Table 2**. Organised in this manner, the data may serve as a practical reference for researchers engaged in the molecular identification of pulmonary and gastrointestinal nematodes in wildlife.

The analysis of the data from the **Table 1** shows that recent studies on lung nematodes cover various wild ungulates, such as deer and musk oxen [7], chamois and bighorn sheep [1, 6], wild boar [13], and gazelles [14]. Many of these parasites, especially from the family Protostrongylidae, have life cycles that include intermediate hosts like snails [7] or earthworms [13]. This requires specific methods to collect and identify the parasite larvae from the tissues of these invertebrates.

The data show that molecular diagnostics are used for various biological materials. These include first-stage larvae (L1) from faecal samples [6, 14), third-stage larvae (L3) from intermediate hosts [7, 13], and adult worms from lung tissue [1, 13]. The use of L1 from faeces reflects a growing trend toward non-invasive monitoring of wild animal populations.

DNA extraction protocols and sample preparations also differ depending on the type of material. In some cases, enzymatic digestion is used, such as extracting L3 from snails using pepsin-HCl [7]. Other studies involve mechanically breaking down adult worms with micropestles to improve DNA release [1]. The DNA extraction kits vary as well. For faecal samples, kits for complex materials like GeneMatrix [14] are used, while for larvae and adults, tissue kits like Nucleon HT [1] or standard Qiagen kits are preferred [13].

Ribosomal DNA (rDNA) is the main diagnostic marker, with the ITS2 region being most frequently used to differentiate species within the Protostrongylidae and Metastrongylidae families [6, 7, 13]. More conservative genes, such as 18S and 28S, help determine the phylogenetic position of new or unknown parasites [6, 14]. These markers are also used in metabarcoding studies to analyse parasite communities [14]. Additionally, mitochondrial genes like *cox1* and the *MSP1* gene are used as markers to better distinguish between closely related species [1].

The reviewed studies most often use universal primers, such as the NC1/NC2 combination for the ITS2 region, which is widely applied to identify different genera [6, 7, 13]. To accurately distinguish species with similar amplicon sizes, specific primer pairs have been developed. For example, ECP1 and PTP1 are used to differentiate small lungworms of the family Protostrongylidae [7], while newly designed primers for the *cox1* and *MSP1* genes help identify large lungworms of the family Dictyocaulidae [1].

The size of the amplified DNA fragments is important for diagnostic accuracy. For example, the ITS2 region varies between species, ranging from 445 bp in *Umingmakstrongylus* sp. to 597 bp in *Elaphostrongylus* sp. [7], which allows for visual differentiation on a gel. In contrast, the 18S rRNA gene produces much longer fragments (about 1750 bp) suitable for detailed phylogenetic analysis [1]. The *MSP1* markers are shorter (328–393 bp) and are useful for rapid detection [1].

Table 1. Molecular diagnostic workflows and genetic parameters for the identification of lung nematodes in wildlife.

Nematode Species	Hosts	Sample Type & Life Stage	Extraction Protocol, Pre-treatment & Other specificities	Genetic Marker & Target Region	Primer & Specificity	Amplicon Size (bp)	References
Parelaphostrongylus tenuis; P. andersoni; P. odocoilei; Elaphostrongylus cervi; E. alces; Umingmakstrongylus pallikuukensis	Deer; Muskox (*Ovibos moshatus*); Snails	Faeces (L1); Gastropods (L3)	Pepsin-HCl digestion (for extraction of L3 from snails); Three sets of primers designed and used successfully to distinguish larvae at the species level.	ITS2 rDNA	Universal: NC1 / NC2	*P. t.(576); P.a.(566); P.o.(571); E.c. (597); E.a. (581); U.p. (445)*	Gajadhar et al., 2000
					Specific: ECP1, PTP1, PTP2 (Designed for species-level differentiation)	Size differs across universal and newly designed primer sets.	
Muellerius capillaris	Bighorn sheep (*Ovis canadensis*)	Faeces (L1)	-	ITS2 rDNA	Universal: NC1 / NC2	425 bp	Ezenwa et al., 2010
Unknown protostrongylid (genetically clustered with *Parelaphostrongylus*/ *Elaphostrongylus* group)	Pampas deer (*Ozotocerus bezoarticus celer*)	Faeces (L1)	PCR amplification, cloning, and direct sequencing for comparative analysis and species identification.	28S rDNA (D2–D3 domains)	391 F / 501 R	950 bp	Carreno et al., 2012
				ITS2 rDNA	Universal: NC1 / NC2	Not reported (Expected: ~ 400–500 bp)	

Protostrongylus oryctolagi; P. pulmonalis	European hare (*Lepus europaeus*); Rabbit (*Oryctolagus cuniculus*)	Defrosted pulmonary tracts (adult male worms & L1)	Pre-treatment: Lungworms isolated via lung tissue dissection and vigorous shaking in water (sedimentation).; Mechanical lysis of adult worms (piston pellet).; QIAamp DNA Mini Kit (Qiagen)	28S rDNA (D2 domain)	C2 / D2	Not specified; Species identified by sequencing and phylogenetic analysis.	Lesage et al., 2014
				ITS2 rDNA	Universal: NC1 / NC2		
Dictyocaulus viviparous; D. capreolus; Dictyocaulus sp.; *D. eckerti*	European bison (*B. bonasus*), Red deer (*Cervus elaphus*); Roe deer (*Capreolus capreolus*); Moose (*Alces alces*)	Adult worms; Single larva	Nucleospin Tissue DNA Extraction Kit (Macherey–Nagel, Germany); Multiplex PCR protocol for simultaneous detection of multiple species.; Multiplex PCR for species differentiation based on diagnostic band sizes.	ITS2 rDNA	4 novel species-specific primers (Multiplex set), newly designed for ITS-2 regions	560 bp (*D. viviparus*); 400 bp (*D. capreolus*); 660 bp (*Dictyocaulus* sp.); 714 bp (*D. eckert i* expected, but not found)	Pyziel et al., 2015

Muellerius capillaris; Neostrongylus linearis	Dorcas gazelle (*Gazella dorcas);* Addax *(Addax nasomacculatus);* Scimitar-horned oryx *(Oryx dammah)*	Faeces (L1)	QIAamp DNA Mini Kit (Qiagen, Germany); Protocol modified for tissue/ faecal samples.	ITS2 rDNA	Universal for nematodes: NC1 / NC2	ITS2 (+ flanking regions) – 425 bp	Saidi et al., 2020
Dictyocaulus spp.	Rocky mountain elk (*Cervus canadensis nelsoni*)	Adult worms	Tissue grinding: BioMasher II (micro pestle); Nucleon HT Genomic DNA Kit (GE Healthcare, USA)	mtDNA (*cox1*);	Newly designed: Deap_ COX1F/ Deap_COX1R	373 bp	Bangoura et al., 2020
				SSU rDNA (18S)	Universal: NC18SF1 / NC5BR	1750 bp	
				MSP1 gene	Newly designed: Dspp_ MSP1F/ Dspp MSP1R	328–393 bp	
Metastrongylus spp.	Wild boar (*Sus scrofa*) Earthworms (Lumbricidae)	Adult worms; Earthworms (L3)	Qiagen kit (India); Phusion DNA Polymerase; Earthworms: Pre-killed with 1% formalin; Isolation L3 via dissection, compressor method & artificial gastric juice digestion.	ITS2 rDNA	Universal: NC1 / NC2	Approximately 495 bp for all species.	Kuchboev & Krücken, 2022

Gastrointestinal and lungworm community (full taxonomic list provided)	Mountain gazelle (*Gazella gazella*)	Faeces (L1)	Storage: Samples stored at −20 °C.; Extraction: GeneMatrix Kit (EURx, Poland), optimized for complex faecal matrices.; Library Prep: NEBNext Ultra II.QC: Bioanalyzer/ Qubit.	SSU rDNA (18S) metabar-coding locus	Universal nematode 18S primers (18S F / 18S R)	Metabarcoding: Varies by taxa (NGS library preparation) - NovaSeq 6000	Karaer et al., 2025
Dictyocaulus spp. (*D. cervi, D. eckerti*)	Red deer	Adult worms	End-point PCR; Sequencing used to identify matrilineal groups.	mtDNA (*cox1*)	COX I_F; COX I_R: Self-designed (NCBI Primer-BLAST)	320 bp; 7 unique haplotypes; 100% coverage compared to GenBank entries	González-Velo et al., 2025

NC1: 5'ACGTCTGGTTCAGGGTTGTT; **NC2**: 5'-TTAGTTTCTTTTCCTCCGCT-3'; **ECP1**: 5'GGAATCGTCCGTCTATCGTT'3; **ECP1R**: 5'GTCAAAACGATAGACGGACG'3; **PTP1**: 5'CCGTCGAATACATGTCATCC'3; **PTP2**: 5'TCGTCAAGACGATGATTCCC'3; **391F**: 5′- AGCGGAGGAAAAGAAACTAA); **501R**: 5′-TCGGAAGGAACCAGCTACTA; **C2'**: 5′-GAAAAGAACTTTGRARAGAGA-3′; **D2**: 5′-TCCGTGTTTCAAGACGGG-3; **18S F**: 5′- GGCCGTTCTTAGTTGGTGGA-3′; **18S R**: 5′- CCCGGACATCTAAGGGCATC-3′; **NC5BR:** 5′-GCAGGTTCACCTACAGAT-3′; **Dspp_ MSP1F**: 5′-AGTTCCCCCTGGAGACATCAAC-3′; **Dspp_ MSP1R**: 5′-ATCTCCTTGGAACCACTCGC-3′; **COX I_F**: 5′-TTTTTTTTGGGCATCCTGAGGTTTAT-3′; **COX I_R**: 5′-TAAAGAAAGAAAGAACATAATGAAAAAATG-3′; **Deap_ COX1F**: 5′-CCTTTGATGTTGGGTAGACCTG-3′; **Deap_ COX1R**: 5′-GTATAGTAATAGCACCAGCC-3′; **NC18SF1:** 5′-AAAGATTAAGCCATGCA-3′

Table 2 focuses on gastrointestinal nematodes and shows a wide range of hosts. These include primates and humans [9], zoo animals like pandas and big cats [16], and wild ruminants such as gazelles and chamois [27]. Of particular interest is the identification of *Trichuris* spp. with zoonotic potential in monkeys and humans in Uganda [9]. Additionally, there is a first report of a possible *T. ovis* infection in long-tailed macaques in Thailand [22]. These findings show how molecular diagnostics can detect parasite transmission in areas where humans and wildlife live in close contact.

The primary sources of genetic material in these studies (**Table 2**) are eggs from faecal samples [9, 22, 27] or adult worms collected after deworming [16]. When analysing environmental samples like faeces, identifying nematodes from the order Strongylida is difficult because their eggs look very similar. This makes molecular markers necessary for accurate species differentiation [27]. In these studies, sample preparation and DNA extraction are critical to break through the tough egg shells. A highly efficient method for lysis involves freeze-thaw cycles using liquid nitrogen and a hot bath [9, 27]. For DNA purification, specialised commercial kits like the ZR Faecal DNA MiniPrep [9], GF-1 Tissue Kit [22], and QIAamp DNA Stool Mini Kit [27] are widely used. For adult parasites, a modified phenol-chloroform extraction is still used to obtain sufficient DNA from tissue [16].

Ribosomal DNA (rDNA) is the main marker for diagnosing gastrointestinal nematodes. Internal transcribed spacers (ITS1 and ITS2) are preferred because they vary significantly between species [9, 27]. For broader taxonomic studies, combined analyses of 18S and 28S rDNA, as well as the mitochondrial 12S gene, are used [16]. When the ITS2 region fails to amplify, 18S rRNA is a reliable alternative for confirming the genus [22]. Nested PCR with outer and inner primers is used to increase sensitivity when DNA concentrations are low [9]. Most authors rely on established universal primers, such as NC1/NC2 for the ITS region [22, 27], or specific pairs like 18S965F/18S1573R for the genus *Trichuris* [22]. Using universal primers followed by Sanger sequencing is a standard approach for distinguishing species that live in the same area, such as *Teladorsagia* sp., *Marshallagia* sp., and *Haemonchus* sp. [27].

The sizes of the PCR products vary depending on the study‘s goal. Short fragments of about 320–330 bp are typical for ITS2 diagnostics [22, 27]. In contrast, nested PCR protocols generate longer amplicons (between 895 bp and 1088 bp for ITS1), providing more genetic information for phylogenetic analysis [9]. Variations in 18S rRNA length (416 to 680 bp) are also used for precise clustering of isolates [22].

The final step in these studies is bioinformatic analysis. Software platforms like MEGA 11 are used to build phylogenetic trees, often applying the Maximum Likelihood [22] or Neighbor-Joining methods [27]. To ensure the statistical reliability of the results, 1000 bootstrap replicates are typically used [22, 27].

Conclusions

The reviewed studies demonstrate that molecular diagnostics have become an essential tool for identifying both pulmonary and gastrointestinal nematodes in wildlife. While

Table 2. Molecular diagnostic workflows and genetic parameters for the identification of gastrointestinal nematodes in wildlife.

Nematode species	Hosts	Sample type & Life stage	Extraction protocol, Pre-treatment & Other specificities	Genetic marker & Target region	Primer & Specificity	Amplicon Size (bp)	References
Ascarid nematodes	Giant panda (*Ailuropoda melanoleuca*); Red panda (*Ailurus fulgens*); Ursids; Primates; Canids; Felids	Adult worms	Adult worms collected following routine deworming of zoo inhabitants. Modified phenol/chloroform extraction of DNA.	rDNA (18S, 28S) & mtDNA (12S)	12SF / 12SR	490 bp	Li et al., 2012
					18SF / 18SR	750 bp	
					28SF / 28SR	1700 bp	
Trichuris sp.	Black-and-white colobus (*Colobus guereza*); Blue monkeys (*Cercopithecus mitis*); Grey-cheeked mangabeys (*Lophocebus albigena*); L'hoest monkeys (*Cercopithecus lhoesti*); Olive baboons (*Papio anubis*); Red colobus (*Procolobus rufomitratus*); Red-tailed guenons (*Cercopithecus ascanius*); Human (*Homo sapiens*)	Faeces (eggs)	ZR Faecal DNA MiniPrep Kit (Zymo Research Corporation, Irvine, CA, USA); Nested PCR	rDNA (ITS1 & ITS2)	ITS1 Region: External Primers 1417F / 2505R & Internal Primers: 1567F / 2462R	Expected size: 1088 bp / 895 bp	Ghai et al., 2014
					ITS2 Region: External Primers: 2462F / NC2 & Internal Primers: 2560F / NC2	Expected size: ~584 bp / 486 bp	

Toxascaris leonina	Asiatic lions (*Panthera leo persica*)	Faeces (eggs)	Sheather's sugar flotation; Concentrated eggs subjected to repetitive freeze-thaw cycles for disruption.; QIAamp DNA Stool Mini Kit (Qiagen, Germany)	rDNA (ITS-1 & ITS-2)	ITS-F / ITS-R	380 bp	Moudgil et al., 2017
Toxascaris leonina	Wolf (*Canis lupus*)	Adult worms	Homogenization with pistils in extraction buffer; Phenol-chloroform method	mtDNA (***cox1***)	JB3_F / JB4.5_R	320 bp (100% coverage)	Uakhit et al., 2022
Toxascaris leonina; Baylisascaris transfuga	African lion (*Panthera leo*); Brown bear (Ursus arctos)	Faeces (eggs)	~100 eggs ground 5 times in liquid nitrogen; MiniBEST Universal Genomic DNA Extraction Kit Ver.5.0 (TaKaRa, Japan).	rDNA (ITS-2)	*Toxascaris leonina*: TleoF / NC2R; *Baylisascaris transfuga*: NITSF / NITSR	300 bp (*Tl*) / 301 bp (*Bt*)	Cai et al., 2024
				mtDNA (*cox1*)	Generic: JB3F / JB4.5R	450 bp	
Toxocara canis	Golden jackal (*Canis aureus*)	Adult worms	GeneJET Genomic DNA Purifi-cation Kit (Thermo Fisher, USA)	rDNA (ITS1-5.8S-ITS2)	Universal for ascaridoid nematodes: TW81F' / AW28R	614–675 bp	Kuchboev et al., 2025

Trichuris spp.	Long-tailed macaques (*Macaca fascicularis*)	Faeces (eggs)	GF-1 Tissue Kit (Vivantis Technologies, Malaysia)	18S rRNA (Successful)	Genus-specific (*Trichuris* spp.): 18S965F / 18S1573R	727 bp (expected); 416 – 680 bp (final analyzed)	Phosuk et al., 2025
				ITS2 (Unsuccessful)	According previous study.	325 bp (expected) Failed (final analyzed)	
Teladorsagia circumcincta; Marshallagia spp.*; Nematodirus oiratianus*	Gazelle (*Gazella subgutturosa*); Wild goat (*Capra aegagrus*)	Faeces (eggs)	QIAamp DNA Stool Mini Kit (Qiagen, Germany); Faecal samples were frozen/thawed (3 cycles) in liquid nitrogen and 95°C water bath to rupture egg shells.	ITS-2	Universal primers: NC1 (F) / NC2 (R)	~320 bp (ranging 280–330 bp depending on species)	Vafaei et al., 2025

JB3_F: 5′-TTTTTTGGGCATCCTGAGGTTTAT-3; **JB4.5_R**: 5′-TAAAGAAAGAACATAATGAAAATG-3′; **TW81F**: 5’-GTTTCC GTAGGTGAACCTGC-3’; **AW28R**: 5’-ATATGC TTAAGTTCAGCGGGT-3’; **28SF:** 5′-CCCGATTGATTCTGTCGGC-3′; **28SR:** 5′-TGATCCTTCTGCAGG TTCACCTAC-3′; **18SF**: 5′-AGCGGAGGAAAAGAAACTAA-3′; **18SR:** 5′-TGATCCTTCTGCAGG TTCACCTAC-3′; **12SF:** 5′-AGCGGAGGAAAAGAAACTAA-3′; **12SR:** 5′-TGATCCTTCTGCAGGTTCACCTAC-3′; **ITS-F**: 5’-ATATCGGAAAAGGACGCACA-3’; **ITS-R**: 5’-TTA GTT TCT TTT CCT CCG CT-3’; **TleoF**: 5’-CGAACGCTCATATAACGGCATACTC-3′; **NC2R**: 5’-TTAGTTTCTTTTCCTCCGCT-3′; **NITSF**: 5’-TTATGAATTTTCAACATGGC-3′; **NITSR**: 5’-GTTAGATGCTTAAATTCAGC-3′; **JB3F**: 5’-TTTTTTGGGCATCCTGAGGTTTAT-3′; **JB4.5R**: 5’-TAAAGAAAGAACATAATGAAAATG-3′; **D769:** 5′-GAGTAATTTTTATAATRCGRGAAGT-3′; **D770:** 5′-AATTTTCAGG RTCTCTRCTTCAAT-3′; **CORA:** 5′-ACYACATAGTAGGTRTCATG-3′; **HC02198F:** 5′-TGATTTTTTGGTCACCCTGAAGTTTA-3′; **1417F**: 5’-AGGGACCAGCGACACTTTC-3’; **2505R**: 5’-GAGTGTCACGTCGTTCTTCAAC-3’; **1567F**: 5’-GTTCTCGTGACTGGGAC-3’; **2462R:** 5’-CTACGAGCCAAGTGATCC-3’; **2462F**: 5’-GGATCACTTGGCTCGTAG-3’; **NC2:** 5’-TTAGTTTCTTTTCCTCCGCT-3’; **2560F:** 5’-CTTGAATACTTTGAACGCACATTG-3’; **18S965F**: 5’-GGCGAAACCGCGAATGGCTC-3’; **18S1573R**: 5’-CCATGCTGAAGTATGCTTG-3’

traditional morphological methods often struggle with similar-looking eggs and larvae, molecular markers—particularly the rDNA (ITS1, ITS2, and 18S) and mitochondrial genes—provide the precision needed for accurate species differentiation. Key advancements, such as nested PCR and specialized DNA extraction protocols, have successfully addressed the challenges of working with low-concentration environmental samples and robust egg shells. Furthermore, the transition toward Next-Generation Sequencing (NGS) and metabarcoding marks a significant shift from studying individual species to analyzing entire parasite communities. These molecular approaches not only improve diagnostic accuracy but also enhance our understanding of interspecies transmission and zoonotic risks in regions where humans and wildlife coexist.

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