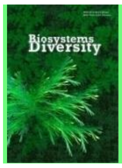

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Molecular identification and phylogenetic positioning of nematodes *Toxocara canis*, *T. cati* (Ascarididae) and *Toxascaris leonina* (Toxocaridae) from domestic and wild carnivores in the Fergana Valley, Uzbekistan

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Parasitic nematodes of the superfamily Ascaridoidea infect various vertebrate groups, with certain species, particularly *Toxocara*, presenting significant zoonotic risks. This study aims to identify and characterize ascarid species in wild canines and felines in Namangan region, Uzbekistan. Ascarid samples were collected from 18 stray dogs, 3 jackals, and 12 stray cats between February 2023 and March 2025. Adult roundworms were examined using light microscopy and subjected to molecular analysis through PCR amplification of the partial ITS1-5,8S-ITS2 region of ribosomal DNA. Morphological examination identified the nematode samples from dogs and jackals as *Toxocara canis* and from dogs as *Toxascaris leonina*, while the sample from the cat was identified as *Toxocara cati*. The nucleotide sequences of ITS were analyzed for similarity using BLAST, which showed that these parasites are 99–100% identical to the species *T. canis*, *T. cati* and *T. leonina*. Phylogenetic relationships were also assessed using maximum likelihood analysis with the inclusion of additional sequences from GenBank (NCBI). Phylogenetic analysis showed that *T. canis* clustered with sequences from wild canids, while *T. cati* was associated with felids. Notably, *T. leonina* was divided into subclades depending on the host origin, suggesting the presence of a complex associated with host infectivity. Further confirmation is needed by increasing sample sizes and comparing a wider range of morphological and genetic data. This study provides new molecular data on the identification and epidemiological significance of ascarids in wild and domestic dogs and cats in Uzbekistan.

Keywords: nematode; Ascaridoidea; wild animals; morphology; ribosomal DNA; phylogenetic relationship.

Introduction

Pets play an essential role in society worldwide. They are valuable companions that contribute to the physical, social, and emotional well-being of their owners, especially children (Safarov et al., 2023). Dogs are the most adapted and socialized canine species globally. Although they provide many benefits to numerous individuals, dogs can also pose a health risk, as several parasitic infections can be transmitted naturally from dogs to humans, either directly or indirectly through environmental factors (Safarov et al., 2025).

Parasitic worms are considered a global health threat with long-term consequences. For instance, ascariasis infects more than one billion people worldwide, leading to malnutrition, cognitive and physical impairments, and, in severe cases, death (Crompton, 2001). Published data indicate that the parasite *Toxocara canis* Stiles, 1905, has infected millions of people in impoverished areas of the United States. In humans, particularly in children, *T. canis* larvae invade various tissues and can cause visceral larva migrans, ocular larva migrans, neurotoxocariasis, or latent toxocariasis (Rubinsky-Elefant et al., 2010). Furthermore, clinical and experimental studies have shown an association between *T. canis* infection and allergic diseases such as asthma, chronic pruritus, and urticaria (Overgaauw & Knapen, 2013). Dogs serve as the primary hosts for several zoonotic intestinal parasites, with *Toxocara canis* recognized as the main zoonotic agent associated with canine disease (Gasser, 2013). This species has a complex life cycle that involves paratenic hosts, such as rodents. In adult dogs, infection can lead to diarrhea, while untreated infections in puppies may be fatal. Humans, along with other intermediate hosts, can become infected by ingesting *T. canis* eggs (Aklilu & Engdawor, 2024).

The ascaridoid nematodes from the genera *Toxocara* (Toxocaridae) and *Toxascaris* (Ascarididae) are the most common intestinal parasites in carnivores from the families Canidae and Felidae. Notably, species such as *T. canis*, *T. cati* (Schrank, 1788), and *Toxascaris leonina* (Linstow, 1902) are considered zoonotic or potentially zoonotic, posing a public health threat (Okulewicz et al., 2012; Gasser, 2013; Hajipour, 2019; Xie et al., 2020). Currently, 26 species of *Toxocara* have been described morphologically and four differentiated by molecular techniques, with the larvae of *T. canis* being the most widespread in humans (Ziegler & Macpherson, 2019). While *T. canis* and *T. leonina* have been relatively well studied clinically and epidemiologically, there remains a scarcity of global research on the molecular biology of these parasites (Zhu et al., 2007).

Identification of ascaridoids from the genera *Toxocara* and *Toxascaris* typically relies on traditional morphological characteristics, host species, and geographic distribution. However, these criteria often fall short in distinguishing closely related species, particularly during the larval and egg stages (Gasser, 2006; Okulewicz et al., 2012). Consequently, developing more efficient and reliable methods for species identification is essential for clinical diagnosis and epidemiological research, achievable primarily through molecular techniques (Li et al., 2006, 2016; Chen et al., 2012, 2022).

Numerous studies have demonstrated the efficacy of ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) genes in identifying *Toxocara* and *Toxascaris* species (Chen et al., 2012; Gasser, 2013; Fogt-Wyrwas et al., 2016, 2019; Jin et al., 2019). For instance, Jacobs et al. (1997) were the first to differentiate *T. canis*, *T. cati*, and *T. leonina* from foxes, cats, and dogs in Australia using the second internal transcribed spacer (ITS2) of rDNA. Similarly, Li et al. (2007)

developed a species-specific PCR tool based on ITS1+ITS2 sequences for diagnosing *T. canis*, *T. cati*, *T. leonina*, and *T. malaysiensis* (Gibbons et al., 2001) from dogs and cats across several countries. Another study utilized ITS1-based PCR for detecting *T. cati* in feral cats in Argentina (Vega et al., 2018). Molecular analyses based on ITS1 and ITS2 markers revealed that *Toxocara* sp. cf. *canis* from Malaysian cats represented a distinct species, named *Toxocara malaysiensis*, which is genetically closer to *T. cati* than to *T. canis* (Gibbons et al., 2001). Notably, mtDNA gene sequences are more variable within and between species compared to rDNA, making mtDNA a preferred choice for genetic analysis of *Toxocara* and *Toxascaris* (He et al., 2018; Jin et al., 2019; Gao et al., 2024). Genes such as cytochrome c oxidase subunits 1 and 2 (cox1 and cox2) and NADH dehydrogenase subunits 1 and 4 (nad1 and nad4) have proven useful in elucidating relationships among *Toxocara* and *Toxascaris* species in dogs, cats, and cattle (Li et al., 2008; Mikaeili et al., 2016; Fogt-Wyrwas et al., 2016, 2019; Jin et al., 2019). This mtDNA evidence corroborates the rDNA-based conclusion that *T. malaysiensis* is indeed a distinct ascarid species in cats (Li et al., 2006).

Several scientists in Uzbekistan, like their counterparts in other countries, have identified *T. canis*, *T. cati*, and *T. leonina* in nearly all carnivorous mammals (Yong et al., 2019; Berdibaev, 2021; Safarov, 2023; Turgunov et al., 2024). These researchers have primarily relied on morphological methods to confirm the presence of these parasitic helminths. However, molecular genetic analyses of *T. canis*, *T. cati*, and *T. leonina* have not yet been conducted in Namangan region, Uzbekistan. Our recent publication focused on the distribution, morphology and molecular characterization of the nematode *T. canis* in stray dogs from the Fergana Valley. For molecular identification we used the mtDNA *cox1* gene and the isolates were found to be 99% identical to *T. canis* isolates from Genebank (NCBI) (Sotiboldiyev et Kuchboev, 2025). This highlights the importance of integrating molecular identification alongside traditional morphological diagnosis. Comparing our findings with species identified in other countries is crucial for advancing research in Uzbekistan.

This study represents the first molecular characterization and phylogenetic analysis based on the ITS1-5.8S-ITS2 region of rDNA of *T. canis*, *T. cati*, and *T. leonina* found in the Canidae and Felidae families (dogs, jackals and cats) in Namangan region, Uzbekistan.

Materials and methods

The study was reviewed and approved by the center's ethics committee (approval code: No. 08/1 protocol on 29 March 2023).

Sample collection and processing. From February 2023 to March 2025, adult *Toxocara* and *Toxascaris* nematodes were collected from the intestines of 18 stray dogs (*Canis familiaris*), 3 jackals (*Canis aureus*), and 12 domestic cats (*Felis catus*) who had died in vehicle accidents in Namangan region, Uzbekistan (Fig. 1). The specimens were processed following the methods generally recognized for helminthological autopsies of animals. The nematodes were washed with a 0.9% NaCl solution and stored in 70% alcohol until DNA extraction. The nematode species were photographed using a ML5300L binocular microscope (Meiji Techno, Japan) coupled with a ToupCam camera and software. Each ascaridoid specimen was morphologically identified using established taxonomic keys, based on the presence of a post-oesophageal bulbus and the length and shape of the cervical alae (Taylor et al., 2007; Jenkins, 2020).

DNA extraction. Each ascaridoid sample was thoroughly washed in saline. High molecular weight genomic DNA was isolated using the manufacturer's protocol with the GeneJET Genomic DNA Purification Kit (Thermo Fisher, USA). DNA concentration and purity were measured using a NanoDrop Lite micro spectrometer (Thermo Fisher Scientific, USA).

PCR amplification and sequencing. The nucleotide sequence of the ITS1-5.8S-ITS2 ribosomal DNA (rDNA) of the adult ascaridoid nematodes of *T. canis* from dogs, *T. cati* from cats, and *T. leonina* from dogs was amplified using forward primer TW81 (5'-GTTTCC GTAGGTGAACCTGC-3') and reverse primer AW28 (5'-ATATGC TTAAGTTCAGCGGGT-3') (Curran et al., 1994). PCR was conducted in a final volume of 25 µL, incorporating 1 µL of genomic DNA extract, 1 U of DreamTaq Polymerase (Thermo Scientific), 10X DreamTaq buffer, 0.2 mM of each dNTP, and 10 µM of each primer. Amplifications were performed in a ProFlex™ PCR System (Thermo Fisher, USA) with the following cycling conditions: initial denaturation at 94 °C for 5 min; denaturation at 95 °C for 45 s; primer annealing at 55 °C for 45 s; elongation at 72 °C for 1 min 40 s; and final elongation at 72 °C for 5 min. Stages 2 to 4 were repeated for a total of 35 cycles (Kuchboev & Krücken, 2022). The quality of DNA and PCR products was assessed by electrophoresis on a 1.5% agarose gel at 80 V. DNA extraction and cleanup were performed using a DNA Gel Extraction and Cleanup Kit (Thermo Fisher Scientific, USA) according to the provided instructions. Sequencing was carried out using the BigDye™ Terminator v. 3.1 reagent kit, and reaction products were analyzed on an ABI PRISM 3100-Avant automated sequencer.

Phylogenetic analysis. In this study, rDNA ITS1-5.8S-ITS2 sequences of each *T. canis*, *T. cati*, and *T. leonina* species were edited using the BioEdit program. Individual sequence similarities were assessed via BLAST (NCBI; http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the nearest phylogenetic neighbors, which were then aligned with ClustalX 1.83 (Thomson et al., 1994). For phylogenetic tree construction, FASTA sequences were aligned using the MAFFT web interface with default settings. The tree was built using IQ-TREE (Nguyen et al., 2015) on the program server, with bootstrap support calculated in Standard mode for 100 replications. Outgroup species included *Anisakis simplex* (AJ937670) and *Anisakis typica* (AM706345), while *Ascaris suum* (PP179022), *Parascaris equorum* (PP373800), and *Ascaris lumbricoides* (MF358961) from the GenBank database were used for comparative analysis (Table 1). Given recent findings on the genetic separation of *T. leonina* based on host origin, newly generated and previously published nuclear sequences of *T. leonina* were combined to test this hypothesis using the same phylogenetic methods. The resulting sequences were deposited in the GenBank database under accession numbers PV211271 for *T. leonina*, PV211272 for *T. cati*, PV216844 for *T. canis* (from dogs) and PX062270 for *T. canis* (from jackals) (ITS).

Results

Morphological identification of ascaridoid nematodes. Helminthological studies identified the presence of *Toxocara* spp. and *Toxascaris* spp. nematodes in the intestines of stray dogs in the Namangan region, Uzbekistan. Among the 18 stray dogs examined, ten (55.5%) were found to be infected with *Toxocara* and *Toxascaris* spp., and among three of the examined jackals one (33.3 %) was also infected (*Toxocara* spp.). Additionally, of the 12 stray cats examined, three (25%) was found to be infected with *Toxocara* spp., with infection of intensity from 1 to 4 copies.

A total 56 ascarid samples from dogs in this study were morphologically identified as *Toxocara* sp. (n = 28) or *Toxascaris* sp. (n = 15). They were identified by the presence of a post-oesophagial bulbus and the length and shape of the cervical alae (Taylor et al., 2007; Jenkins, 2020). According to the results of morphological examination, the nematode samples found in the dogs were attributed to the species *Toxocara canis* and *T. leonina*, while the nematode from the cat was attributed to *Toxocara cati* (n-8). The samples from the jackal were identified as *Toxocara canis* (n-5) (Fig. 2). The intensity of the infection of *T. canis* was 2–18 copies and that of *T. leonina* was 1–6 copies.

Sequence characterization and phylogenetic analysis. Nucleotide sequences representing the rDNA ITS1-5.8S-ITS2 region (614–675 bp) were obtained for three ascaridoid samples. BLAST searches revealed two distinct groups: the first group displayed a high nucleotide identity (91–100%) with *Toxocara* species from the Toxocaridae family, while the second group showed a 99–100% identity with *Toxascaris* sequences (Ascaridae). Following comparative identification, the aligned ITS sequences were extracted in FASTA format. Phylogenetic analysis of *Toxocara* and *Toxascaris* was performed by alignment of 37 of ITS domain sequences our dataset and others of the

GenBank database (Table 1). The resulting tree topologies are illustrated in Figure 3. Reference sequences from *Ascaris* species were included as outgroups for *T. leonina*. All data regarding the ascaridoid species in this study are summarized in Table 1. Genetic analysis revealed that *Toxocara* and *Toxascaris* species found in wild and domestic canines and felines are divided into three clades: *T. canis*, *T. cati*, and *T. leonina*. The phylogenetic tree showed robust resolution among all samples from the Toxocaridae family and *T. leonina* from the Ascarididae family. Additionally, samples from the genera *Ascaris* and *Parascaris* clustered within the Ascarididae family.

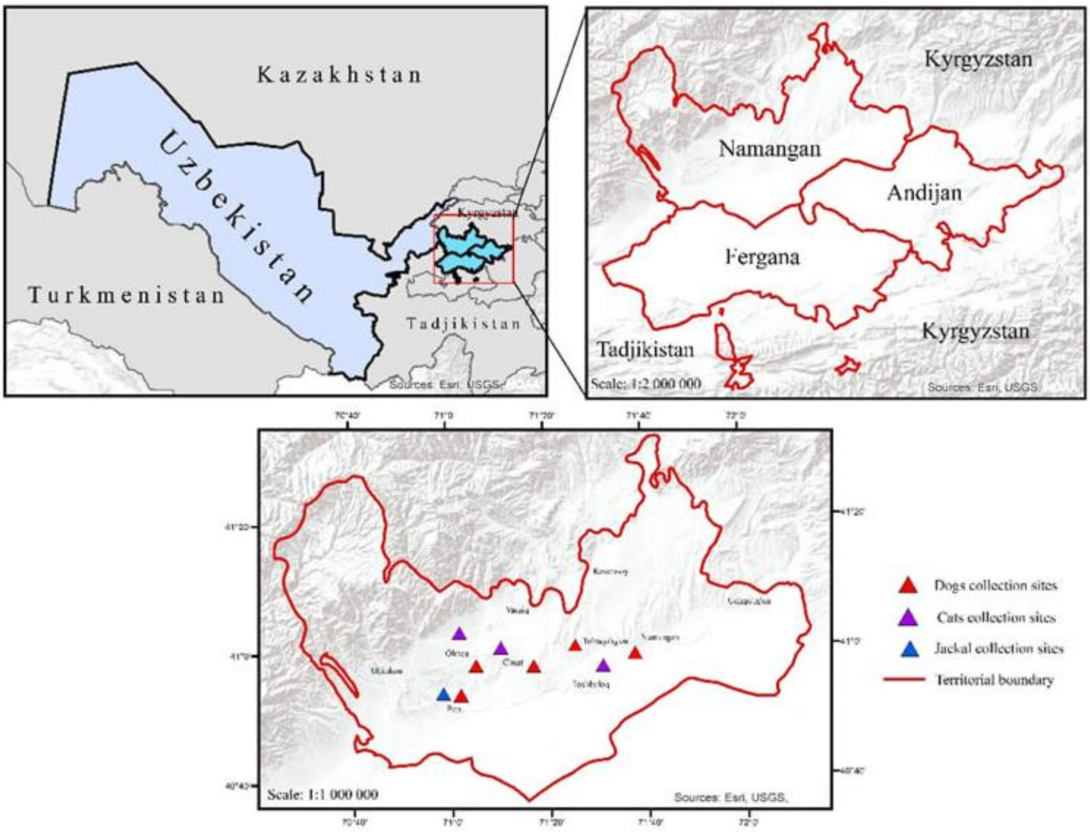

Fig. 1. Map of Namangan region, Republic of Uzbekistan showing the sampling sites

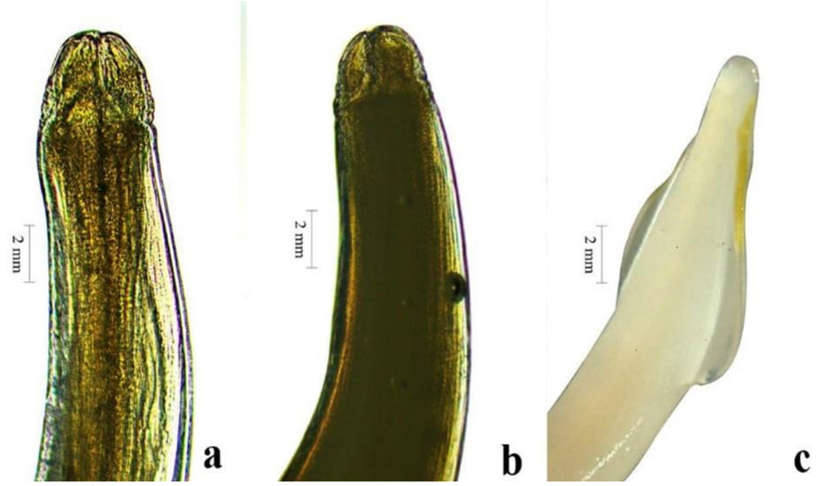

Fig. 2. Photographic evidence of detected *Toxascaris leonina*, *Toxocara canis*, and *Toxocara cati*: *a – T. canis*; *b – T. leonina*; *c – T. cati*

Three clades (IV, VI, VIII) encompassing all ascaridoid isolates identified in this study were formed, reflecting a range of definitive hosts. Notably, *T. canis* formed a separate clade (VI), with bootstrap values ranging from 90–100%, indicating strong support. This species was associated with wolves, red foxes, jackals, and domestic dogs. Clade VIII included *T. cati* isolates from lions, wildcats, and domestic cats, exhibiting high bootstrap support (93–100%). These two clades, *T. canis* and *T. cati*, represent related lineages (Fig. 3). Consequently, ascaridoids identified in wolves and dogs were classified as *T. canis*, while those in wild cats and lions were classified as *T. cati*. The reference species *T. vitulorum* (from European bisons) and *T. malaysiensis* (from cats) (VII) grouped closely with *T. cati*, indicating a sister group relationship within the Toxocaridae family. Additionally, *T. apodemi*, identified in mice from GenBank, formed a distinct fifth clade (V). Clade IV also encompassed *T. leonina* (bootstrap value 100%), containing samples from Eurasian lynx, lions, tigers, wolves, and dogs. This species is more closely related to Toxocaridae than to other Ascarididae species. Within the lineage of *T. leonina*, two subclades were identified based on definitive hosts: one containing *T. leonina* from wild felines and another from dogs and wolves (Fig. 3). The other ascaridoid species, *A. suum* (from horses), *P. equorum* (from pigs), and *A. lumbricoides* (MF358961) included in the analysis fell into three distinct clades (I, II, III) on the phylogenetic tree. The fact that the species *A. simplex* and *A. typica* (IX, X) (Anisakidae), are located in a distinct and basal position in the phylogenetic tree indicates that we used these specimens as an outgroup.

Table 1

Sources of specimens or sequences representing of *Toxocara/Toxascaris* used in comparisons and identification (rDNA ITS1-5,8S-ITS2)

Species	Parasite stage	Host range	Localization	Geographic distribution	GenBank accession ITS	References
Toxcara canis Stiles, 1905	adult	*Canis familiaris* Linnaeus, 1758	intestine	Namangan region, Uzbekistan	PV216844	this study
	adult	*Canis aureus* Linnaeus, 1758	intestine	Namangan region, Uzbekistan	PX062270	this study
	adult	*Canis familiaris*	intestine	Sri Lanka	FJ418788	Wickramasinghe et al., 2009
	egg	*Canis lupus* Linnaeus, 1758	from soil	Khuzestan, Iran	AB743615	Khademvatan et al., 2013
	adult	*Canis lupus*	unknown	Changsha, Hunan, China	OM876369	Unpublished
	adult	*Canis lupus*	unknown	HeiLongjiang, China	JN617989	Unpublished
	larvae	*Canis lupus*	unknown	Germany	LC762621	Unpublished
	adult	*Vulpes vulpes* Linnaeus, 1758	intestine	Guizhou, China	MK309928	Xie at al., 2020
	adult	*Canis familiaris*	intestine	Tamil Nadu, India	KJ777156	Unpublished
	adult	*Canis familiaris*	intestine	Qazvin, Iran	OP185363	Unpublished
Toxocara malaysienis (Gibbons et al., 2001)	adult	*Felis catus* Linnaeus, 1758	intestine	Guangzhou, China	AM231609	Li et al., 2008
Toxocara vitulorum (Goeze, 1782)	adult	*Bison bonasus* Linnaeus, 1758	intestine	Germany	KY442062	Unpublished
	egg	*Bos taurus* Linnaeus, 1758	faeces	Wales, United Kingdom	EU189085	Unpublished
Toxocara apodeme (Olsen 1957)	adult	*Mus musculus* Linnaeus, 1758	intestine	Zhejiang, China	OR231233	Gao et al., 2024
Toxacar cati (Schrank, 1788)	adult	*Felis catus*	intestine	Namangan region, Uzbekistan	PV211272	This study
	adult	*Felis catus*	intestine	Guangdong, China	KY003066	He et al., 2023
	adult	*Felis catus*	intestine	Kyoto, Japan	AB571303	Arizono et al., 2010
	adult	*Felis catus*	intestine	Arunachal, India	KJ777179	Unpublished
	adult	*Felis silvestris*	intestine	Germany	PP812131	Unpublished
	adult	*Felis catus*	intestine	North west Iran	MT939442	Unpublished
	adult	*Felis catus*	intestine	Fars, Iran	JX536258	Unpublished
	adult	*Panthera leo* Linnaeus, 1758	intestine	Bareilly, India	OK668295	Unpublished
	adult	*Felis catus*	intestine	Mexico	OQ256234	Unpublished
Toxascaris leonina (Linstow, 1902)	adult	*Canis familiaris*	intestine	Namangan region, Uzbekistan	PV211271	This study
	adult	*Canis lupus*	intestine	Sichuan, China	JF837174	Unpublished
	adult	*Canis lupus*	intestine	Sichuan, China	MK309916	Xie et al., 2020
	adult	*Canis familiaris*	intestine	Shanxi, China	PP456685	Unpublished
	adult	*Panthera tigris* Linnaeus, 1758	intestine	Sichuan, China	MK309890	Xie et al., 2020
	adult	*Panthera leo*	intestine	Sichuan, China	MK309908	Xie et al., 2020
	adult	*Lynx lynx* Linnaeus, 1758	intestine	Sichuan, China	JF837179	Xie et al., 2020
	adult	*Lynx lynx*	intestine	Sichuan, China	MK309903	Xie et al., 2020
Ascaris suum Goeze, 1782	adult	*Sus scrofa* Linnaeus, 1758	intestine	Kyoto, Japan	AB571302	Arizono et al., 2010
	adult	*Sus scrofa*	intestine	Fergana Valley, Uzbekistan	PP179022	Turgunov et al., 2024
Ascaris lumbricoides Linnaeus, 1758	egg	*Homo sapiens* Linnaeus, 1758	faeces	Bago, Myanmar	MF358961	Sadaow et al., 2018
Parascaris equorum Goeze, 1782	adult	*Equus caballus* Linnaeus, 1758	intestine	Namangan region, Uzbekistan	PP373800	Turgunov et al., 2024
Anisakis simplex (Rudolphi, 1809)	larvae	*Clupea harengus* Linnaeus, 1758	body cavity	Skagerrak, Sweden	AJ937670	Zhu et al., 2007
Anisakis typica Baylis, 1920	larvae	*Selar crumenophthalmus* (Bloch, 17	body cavity	Guangdong, China	AM706345	Zhu et al., 2007

Discussion

Today, parasites cause serious damage to the population size and productivity of predatory mammals – Mammalia: Carnivora (Canidae, Mustelidae, Felidae). The widespread distribution of helminthiasis, including nematodoses of zoonotic significance among predatory mammals leads to an expansion of epizootological and epidemiological risk. The appearance of these parasites among humans is the cause of dangerous situations. Accordingly, the definition of the species composition, the methods of morphology and molecular identification of nematodes of predatory mammals, the study of zoonotic species in natural and transformed areas and the improvement of methods for combating parasitic diseases of animals are of great scientific and practical importance.

Parasitic nematodes of the superfamily Ascaridoidea infect all major vertebrate groups, with several genera and species holding significant medical and veterinary importance. The genus *Toxocara* is particularly notable, as some species can be transmitted from animals to humans, resulting in serious zoonotic diseases (Pawlowski, 2001; Despommier, 2003; Fisher, 2003). Among these, *T. canis* serves as a definitive host for canids, including domestic dogs, jackals, dingoes (*C. dingo*), wolves (*C. lupus*), coyotes (*C. latrans*), foxes (*Vulpes vulpes*), arctic foxes (*V. lagopus*), and fennec foxes (*Megalotis zerda*), with rare cases observed in felines (Safarov, 2024). Conversely, felids can be infected with *T. cati*, which has been reported in various species, including domestic cats, feral cats (*F. silvestris*), servals (*F. serval*), jungle cats (*F. chaus*), lynxes (*Lynx lynx*), cheetahs (*Acinonyx jubatus*), pumas (*Puma concolor*), lions (*Panthera leo*), jaguars (*P. onca*), and tigers (*P. tigris*) (Bonilla-Aldana et al., 2024).

In contrast, *T. leonina* is a non-zoonotic species that can infect both canids and felids. Commonly found in the intestines of domestic animals and wild carnivores, *T. leonina* has a life cycle characterized by direct transmission via resistant eggs. While typically associated with milder symptoms, it can lead to nutritional deficiencies, particularly in young animals. Its potential for coinfection with other *Toxocara* species complicates clinical diagnosis and treatment, emphasizing the need for further epidemiological studies. Additionally, within the genus *Toxocara*, *T. apodemi* and *T. mackerras* are host-specific, non-zoonotic parasites primarily associated with the family Muridae (Asakawa et al., 1994).

Athough morphological characteristics and host species dependence can aid in species identification, existing keys and descriptions are often inadequate, particularly for differentiating *Toxocara* spp. and *Toxascaris* spp. This issue is especially pronounced in the larval and egg stages (Okulewicz et al., 2012; Fort-Wyrwas et al., 2019). Similar challenges arise in the species-specific identification of certain taxa obtained from wild animals. Recent advances in molecular diagnostics present significant opportunities to overcome these limitations. PCR-based approaches utilizing rDNA and mtDNA genetic markers have proven effective and have been employed in large-scale studies of parasitic nematodes (Khademvatan et al., 2013; Kuchboev et al., 2015, 2020; Sobirov et al., 2025), including ascaridoids at the species level (Kutz et al., 2007; Gasser, 2015; Sadaow et al., 2018; Safarov et al., 2025).

Considering the importance of domestic and wild carnivores in the dissemination of pathogens of serious helminthiasis of agricultural, game and commercial animals, as well as humans, studies of wild predators were conducted in the Republic of Uzbekistan by Berdibaev (2021), Safarov (2023) and Turgunov (2022). The authors provide data on the infection of dogs and jackals with toxocariasis (*T. canis*) in the North-West and East regions of Uzbekistan. In the current study, ascarids were isolated from five stray dogs, one jackal, and one cat, and subsequently amplified and characterized based on rDNA ITS1-5.8S-ITS2 genes. The 56 ascarid samples were morphologically identified as *T. canis* (from stray dogs and a jackal), *T. leonina* (from a stray dog), and *T. cati* (from a cat). These findings suggest the presence of three ascarid species: *T. canis*, *T. cati*, and *T. leonina*. To confirm this hypothesis, phylogenetic analyses (ML) based on ITS sequences were performed. The results indicated that *T. canis* was closely related to samples from the African lion and other wild canids, while *T. leonina* clustered with samples from wolf, African lion, Bengal tiger, and Eurasian lynx, showing high bootstrap values (Fig. 3). This supports the notion that *T. canis* and *T. leonina* can coexist in wild canids, and *T. cati* and *T. leonina* can coexist in wild felines (Smith, 2003; Popiolek et al., 2007; Xie et al., 2020).

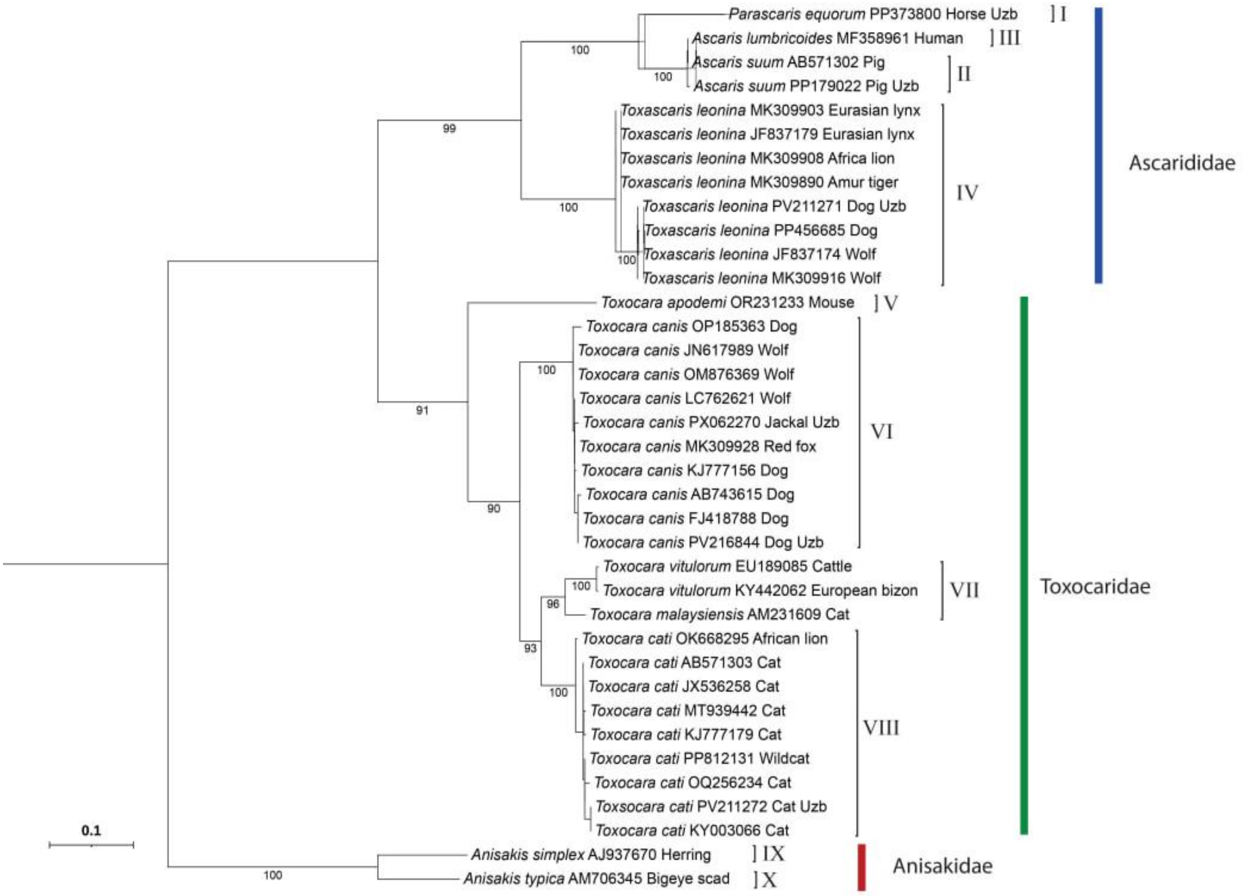

Fig. 3. Phylogenetic relationships of *Toxocara*, *Toxascaris*, and other ascaridoid species in wild and domestic dogs, feral cats, and cats based on rDNA ITS: the tree was built using IQ-TREE on the program server using default settings, bootstrap support was calculated in Standard mode for 100 replications

Conclusion

This study identified traditional morphological and mainly molecular characteristics of three genetically distinct ascarid species parasitizing wild canines and felines based on nuclear DNA analysis. A total of 56 ascarid samples were identified, revealing three distinct species: *Toxocara canis* (from stray dogs and a jackal), *T. cati* (from a cat), and *Toxascaris leonina* (from a stray dog). Phylogenetic analyses indicated that *T. canis* clustered with sequences from wild canids, while *T. cati* was associated with felids. Notably, *T. leonina* diverged into subclades based on host origin, suggesting a complex related to host infectivity. This study provides new molecular insights into the classification and epidemiological significance of ascarids in wild canines and felines. Phylogenetic analysis indicated that *T. leonina* diverged based on host specificity, with nucleotide sequences from wild feline isolates forming one subclade, while those from dogs, wolves, and foxes formed another. This suggests a potential *T. leonina* complex related to host infectivity. Further confirmation is warranted through larger sample sizes and comparisons of a broader range of morphological and genetic data. The findings of our research can provide accurate identification of these nematodes, and inform preventive measures and control strategies for these parasitic infections in the Uzbekistan.

The authors declare no competing interests.

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