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Hookworm infections in carnivores in Iran: a One Health concern

Meysam Sharifdini[1†], Aida Vafae Eslahi[2†], Majid Pirestani[3†], Ali Asghari[2], Giovanni Sgroi[4], Elham Hajialilo[5], Arian Ghannadi Karimi[6,7], Anastasia Diakou[8*] and Milad Badri[2*]

Abstract

Background Dogs and cats can harbor hookworms, which may contribute to zoonotic infections. This study investigates hookworm infections in carnivores from the Caspian Sea littoral region of northern Iran, focusing on molecular and morphological identification.

Methods A cross-sectional study was conducted between September 2015 and October 2024, involving 172 road-killed carnivores including 78 stray dogs (*Canis familiaris*), 62 golden jackals (*Canis aureus*), and 32 stray cats (*Felis catus*), all collected from the northern Iranian provinces of Guilan and Mazandaran.

Results Overall, 46 (26.74%) animals tested positive for hookworms. Among these, *Ancylostoma caninum* was found in dogs and golden jackals, *Ancylostoma tubaeforme* in cats, and *Uncinaria stenocephala* in dogs and golden jackals. Morphological analyses confirmed key differences between *A. caninum* and *A. tubaeforme*, including size, esophageal features, and bursal structure. Molecular identification was supported by 18S, ITS1-5.8S-ITS2 rDNA, 28S, and mitochondrial cytochrome c oxidase 1 (COX1) gene sequences, demonstrating high similarity with previously identified isolates in GenBank. Phylogenetic analysis of the ITS and COX1 sequences revealed distinct clades for each species, with *A. caninum* and *A. tubaeforme* clustering together in the *Ancylostoma* spp. group.

Conclusion These results provide important insights into hookworm diversity and highlight the zoonotic risks posed by these parasites.

Keywords *Ancylostoma*, *Uncinaria*, Road-killed carnivores, Zoonoses, Iran

†Meysam Sharifdini, Aida Vafae Eslahi and Majid Pirestani contributed equally to this work.

*Correspondence:
Anastasia Diakou
diakou@vet.auth.gr
Milad Badri
Badri22.milad@gmail.com
[1] Department of Medical Parasitology and Mycology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
[2] Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran
[3] Department of Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[4] Department of Animal Health, Experimental Zooprophylactic Institute of Southern Italy, Portici, Italy
[5] Department of Parasitology and Mycology, Qazvin University of Medical Sciences, Qazvin, Iran
[6] Student Research Committee, Qazvin University of Medical Sciences, Qazvin, Iran
[7] Cardiovascular Imaging Core Facility, Tehran University of Medical Sciences, Tehran, Iran
[8] Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

Background

Human activities such as environmental fragmentation, wildlife exploitation, tourism, land use changes, as well as industrialization and urbanization have increasingly encroached upon wild habitats. This has altered ecosystems, reshaped wildlife populations, and blurred the boundaries between domestic and wild environments, driving the emergence of zoonotic diseases and consequently increasing the prevalence of zoonoses. Consequently, the exchange of parasites between wild and domestic carnivores has heightened the public health significance of wild carnivores, impacting the epidemiology of numerous zoonotic parasitic diseases [1–3].

Approximately 43% of zoonotic infections in humans, including those caused by viruses, protozoa, bacteria, and fungi, are thought to have originated from carnivore hosts [4]. Carnivorans (members of the order Carnivora, hereafter referred to as carnivores) harbor 182 identified zoonotic parasite species, leading to 46 distinct zoonoses [5]. Hookworm infections, caused by blood-feeding nematodes of the Ancylostomatidae family, are associated with major yet often overlooked tropical diseases affecting both humans and animals globally, especially in tropical and subtropical regions with inadequate sanitation [6, 7].

The distribution and prevalence of hookworm species are influenced by local environmental factors such as humidity, temperature, and the density of host populations. These factors contribute to a dynamic pattern of infection across animals, humans, and wildlife [3].

Dogs and cats serve as hosts for hookworms, which can lead to zoonotic infections, with cutaneous larva migrans being one of the most notable conditions, highlighting a One Health concern [8]. Insufficient management of canine populations in developing nations has led to overpopulation, presenting a significant public health issue. Dogs can harbor infections caused by pathogens that may spread to humans, underscoring the importance of effective population control [9]. Hookworms in cats and dogs were recognized and documented as early as the 19th century, with descriptions provided by Zedler in 1800 and Ercolani in 1859 [10]. Canine hookworms include the species Ancylostoma caninum, Ancylostoma braziliense, Ancylostoma ceylanicum and Uncinaria stenocephala [8]. Feline hosts can be infected with Ancylostoma tubaeforme, A. braziliense, A. ceylanicum, A. caninum, and U. stenocephala [11].

Among these, A. caninum, A. braziliense, A. ceylanicum, and U. stenocephala are recognized as zoonotic, with A. tubaeforme also demonstrating zoonotic potential [12].

Canines and felines become infected when third-stage larvae are ingested (from the environment, prey, or, in the case of A. caninum, through milk during nursing) or penetrate the skin [13–15]. Ancylostoma caninum is a significant pathogen in dogs, leading to blood loss, anemia, and occasionally death, particularly in puppies [16]. It has been linked to eosinophilic enteritis and is also considered a potential cause of diffuse unilateral subacute neuroretinitis in humans [8].

In humans, infection primarily occurs through percutaneous penetration of larvae from contaminated soil or via zoonotic transmission from canine and feline hosts [17].

Without identifying the specific species of hookworms present or predominant in a region, it is difficult to determine whether morbidity or drug efficacy findings apply to all hookworm species or if differences between species account for varying results in different studies [18]. The prevalence of hookworms and the risk to canine, feline, and human populations in the Caspian Sea region is not well-documented. Evaluating prevalence is difficult due to several interrelated factors that impact infection rates. These include the management practices for owned dogs (whether they are free-roaming or confined), access to veterinary care, frequency of anthelmintic usage, the number of stray dogs and cats, and the removal of feces from the environment. Additionally, the high variability in the sensitivity of diagnostic methods available to detect infections further complicates the assessment [19, 20].

Understanding the molecular characteristics and phylogenetic relationships of hookworms in carnivores in the Caspian Sea littoral is essential for developing effective control programs to safeguard animals and public health, particularly given the growing concerns about developing anthelmintic resistance. The current study represents the first effort to identify and analyze the molecular profiles of hookworms in carnivores from the Caspian Sea littoral. By employing advanced molecular techniques, we aim to elucidate the phylogenetic profile of these parasites, providing insights into their diversity and potential public and animal health implications emphasizing the need for increased awareness and intervention measures in this region.

Materials and methods

Study area

A cross-sectional study was carried out from September 2015 to October 2024 in the northern Iranian provinces of Guilan (37.2774°N, 49.5890°E) and Mazandaran (36.5656°N, 53.0588°E) (Fig. 1). These provinces are situated along the southern edge of the Caspian Sea, with their southern regions bordered by the Alborz Mountains. Covering an area of 38,544 km^2, these provinces have a humid subtropical climate, with Guilan Province

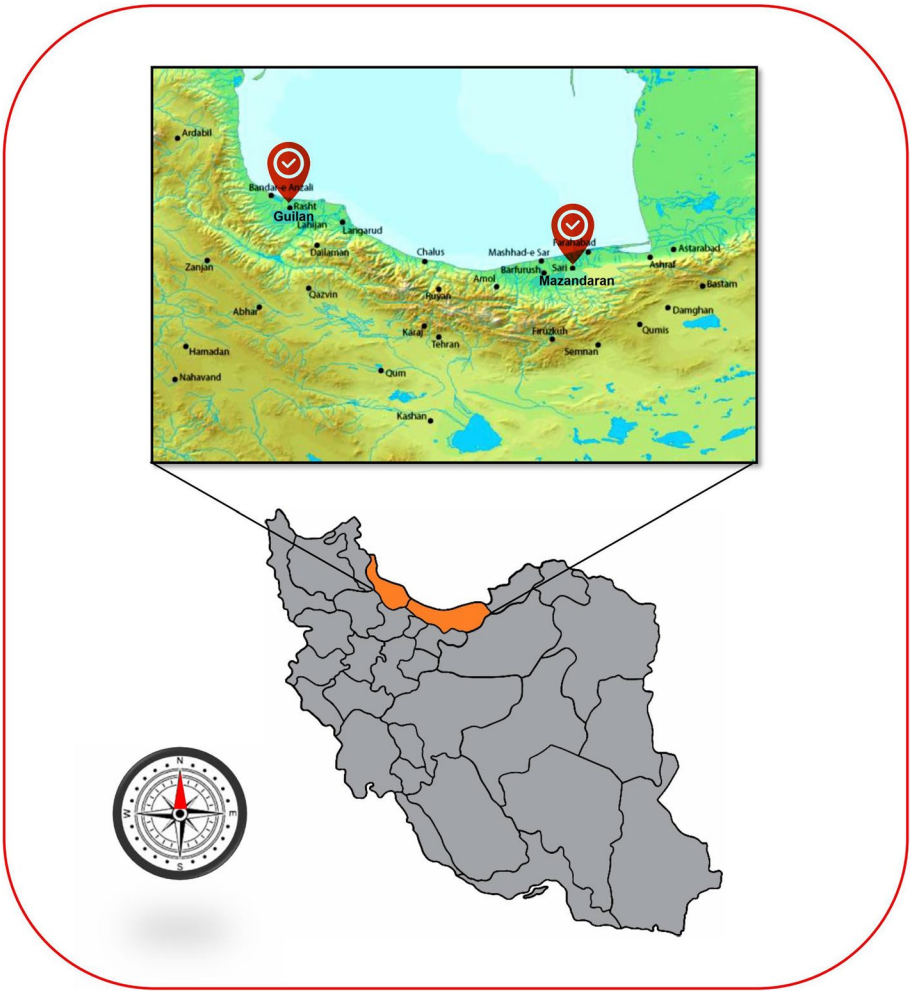

Fig. 1 Geographical location of Guilan and Mazandaran

receiving an average annual rainfall of 1,359 mm and Mazandaran Province around 1,000 mm. The average relative humidity is approximately 80%, with the highest levels occurring in autumn and winter, and lower levels in spring and summer [21, 22].

Sample collection

From September 2015 to October 2024, 172 road-killed carnivores including 78 stray dogs (*Canis familiaris*), 62 golden jackals (*Canis aureus*), and 32 stray cats (*Felis catus*) were collected from different locations in Rasht, Anzali, Rezvanshahr, Roudsar, Deylaman, Lahijan, Kuchesfahan, Roudbar, and Tonekabon, Nowshahr (Fig. 1).

Parasitological examination

Animal carcasses and hookworms were collected promptly to ensure the parasites remained intact, facilitating precise analysis. The digestive tract of the examined animals was excised, isolated, labeled, and transported to the laboratory for thorough dissection. In the laboratory, the large and small intestines were opened, their contents thoroughly screened with a 100-mesh sieve, and then carefully examined for parasites using a dissecting microscope. The hookworms were preserved in 70% ethanol with glycerin and subsequently mounted and cleared in lactophenol for identification and observed under a light microscope at magnifications between 10 × and 40 ×.

Accordingly, freshly collected hookworm specimens were preserved in a solution of 70% ethanol supplemented with 5% glycerin for a minimum of 24 h to maintain morphological integrity and prevent desiccation. After fixation, specimens were transferred to lactophenol solution for clearing. Specimens were soaked in lactophenol for 12–24 h, depending on size, until fully cleared [23, 24].

The helminths were identified following the identification guides published in the international literature [25, 26].

Molecular and phylogenetic analysis

Among 46 morphologically-confirmed cases, 14 were selected for molecular analysis. These include eight cases from stray dogs, four from golden jackals, and two from stray cats (Supplementary Table 1). For a selected number of hookworms, morphological identification was coupled with molecular identification (PCR). The DNA from the isolates was obtained using the FavorPrep kit (Tissue Genomic DNA Extraction Mini Kit, FAVORGEN), adhering to the manufacturer's guidelines. The extracted DNA was then preserved at − 20 °C for subsequent molecular analysis.

Molecular identification was carried out through a PCR test targeting ribosomal and mitochondrial genes. The primer sets used for amplification are presented in Table 1.

The PCR protocol began with an initial denaturation phase at 94°C for 5 min, followed by 40 cycles that included denaturation at 94°C for 30 s, annealing at varying temperatures—57°C for SSU, 50°C for the Internal Transcribed Spacer (ITS) region, 55°C for 28S region, and 53°C for mitochondrial cytochrome c oxidase 1 (COX1)—for 45 s, and extension at 72°C for 30 s. A final extension step at 72°C for 10 min concluded the program. Subsequently, the PCR products underwent gel electrophoresis on a 1% agarose gel, with electrical current of 80 amps applied for 50 min to facilitate separation.

The PCR products were purified using the Cleanup PCR kit (Expin™ Combo GP; Geneall, Korea) and sent to Pishgam Biotech Co. (https://www.pishgambc.com/) for bidirectional sequencing. The sequence data from each sample were validated using the Basic Local Alignment Search Tool (BLAST) found in the NCBI database (http://blast.ncbi.nlm.nih.gov/). This tool enables the comparison of the obtained sequences with established nucleotide sequences in the database for identification and analysis.

Phylogenetic analysis was conducted using newly obtained hookworm sequences alongside matching sequences of other Strongyloidea species available in GenBank. This approach allows for an in-depth comparison of genetic material across different species within the same taxonomic group. The resulting sequence data were aligned using the CLUSTALW algorithm, which is commonly used for multiple sequence alignment, ensuring that homologous nucleotides are correctly aligned to facilitate accurate comparisons. A phylogenetic tree was constructed using MEGA11® software, a widely used tool for phylogenetic analysis. Data analysis was performed employing the maximum likelihood (ML) method, which estimates the tree that is most likely to have produced the observed data. For the 18S rRNA sequences, the analysis was based on the Kimura 2-parameter model, which accounts for transitional and transversional substitutions. For the 28S rRNA, ITS regions, and COX1, the general time reversible (GTR) model was utilized, integrating a discrete gamma distribution with invariant sites (G + I) to model rate variation among different sites in the sequence data. The Bayesian information criterion (BIC) scores were used to inform the choice of the best-fitting model for the data being analyzed, helping to enhance the accuracy of the phylogenetic tree. To assess the robustness of the branches in the phylogenetic tree, bootstrap resampling was performed with 1000 replicates. This method generates a measure of confidence or support for each branch, allowing researchers to evaluate the reliability of the inferred relationships between the examined sequences.

Results

Parasitological findings

In total, 172 road-killed carnivores, i.e. 78 stray dogs (50 males and 28 females), 62 golden jackals (40 males and 22 females), and 32 stray cats (18 males and 14 females)

Table 1 Primers used for DNA amplification

Primer name	Gene target	Sequence (5'→ 3')	Product size (bp)	Reference
18S965 18S1573R	SSU	GGCGATCAGATACCGCCCTAGTT TACAAAGGGCAGGGACGTAAT	632	(Mullin et al., 2005)
BD1 BD2	ITS1 - 5.8S-ITS2	GTCGTAACAAGGTTTCCGTA TATGCTTAAATTCAGCGGGT	826–830	(Luton et al., 1992)
28S- 5' 28S- 3'	LSU	TACCCGCTGAACTTAAGCATAT CTCCTTGGTCCGTGTTTCAAGAC	651	(Zehnder and Mariaux, 1999)
JB3 JB4.5	COX1	TTTTTTGGGCATCCTGAGGTTTAT TAAAGA AAGAACATAATGAAAATG	434–441	(Bowles et al., 1992)

Table 2 Number of positive animals, number of hookworms based on gender, and prevalence of hookworm infections in road-killed carnivores in the northern part of Iran from 2015 until 2024

Year of sampling	Nº hookworms (M/F)[a]	Nº positive animals /Total	Prevalence % (95% CI)
2015	118/129	6/17	35.29 (17.31–58.70)
2016	79/101	5/21	23.80 (10.63–45.09)
2017	102/98	7/24	29.16 (14.91–49.17)
2018	99/82	6/14	42.86 (21.38–67.41)
2019	38/21	3/21	14.28 (04.98–34.64)
2020	95/71	5/16	31.25 (14.16–55.60)
2021	25/32	3/18	16.66 (05.83–39.22)
2022	22/12	2/9	22.22 (06.32–54.74)
2023	104/97	5/11	45.45 (21.27–71.99)
2024	51/66	4/21	19.04 (07.66–40.00)
Total	733/709	46/172	26.74 (20.69–33.81)

(M/F)[a] Male/Female

Table 3 Frequency of hookworms based on the morphometric features in surveyed carnivores

	Ancylostoma caninum	Ancylostoma tubaeforme	Uncinaria stenocephala
Host	Positive (%)	Positive (%)	Positive (%)
Dog	24 (30.76)	-	9 (11.53)
Golden jackal	11 (17.74)	-	6 (9.67)
Cat	-	2 (6.25)	-

were examined. Hookworms were found in 46 (26.74%) of the animals (Table 2).

According to the morphological identification, 24 (30.76%) of the dogs were infected with *A. caninum* and 9 (11.53%) with *U. stenocephala*, 11 (17.74%) golden jackals were infected with *A. caninum* and 6 (9.67%) with *U. stenocephala*, and 2 (6.25%) cats were infected with *A. tubaeforme* (Table 3).

Mixed infections with different hookworm species were not observed.

Based on morphometric features, *A. tubaeforme* was smaller in size than *A. caninum* (females: 12- 15 mm; males: 9.5–11.0 mm *vs* females: 14–16 mm; males: 10–12 mm, respectively), and males exhibited longer spicules (750–850 µm *vs* 650–750 µm, respectively), a smaller bursa compared to *A. caninum*, with lateral bursal rays arranged in a distinct pattern. The hookworms identified as *U. stenocephala* measured 5–12 mm (females: 7–12 mm, males: 5–8.5 mm), and had a buccal capsule featuring two cutting plates, one on each side of its ventral aspect, lacking any dorsal teeth [27] (Fig. 2 A-E).

In fourteen cases, including eight dogs, four golden jackals, and 2 cats, morphological identification of the parasites was confirmed by molecular methods (Table 4).

Molecular findings

The BLASTn search results (Table 4) revealed that the 18S gene sequences of the isolates in this study matched various species. Notably, perfect (100%) sequence identity was observed between some isolates and *A. caninum* and *U. stenocephala*. Interestingly, while the 18S sequence for *A. tubaeforme* was not available in the GenBank database, the isolates examined in this study showed complete (100%) sequence similarity to *A. caninum*. Similarly, analysis of the ITS1 -5.8S-ITS2 rDNA gene sequences showed high BLAST scores and minimal genetic divergence when compared to *A. caninum*, *A. tubaeforme*, and *U. stenocephala* (Table 4). For the 28S gene, all nucleotide sequences from *A. caninum* isolates in this study were identical, exhibiting 99–100% similarity with corresponding sequences available in GenBank. Despite the absence of large ribosomal subunit (28S) sequences for *A. tubaeforme* and *U. stenocephala* in the GenBank database, their similarities with *A. caninum* were 99.7% and 98.9%, respectively. Finally, sequencing of COX1 gene revealed that the isolates had a high degree of similarity (99–100%) to reference sequences available in GenBank.

To explore evolutionary relationships, a phylogenetic tree was constructed using 18S rRNA sequences from *A. caninum*, *A. tubaeforme*, and *U. stenocephala*, along with other members of the Strongyloidea superfamily retrieved from GenBank (Fig. 3). This tree delineated a hierarchy consisting of ten distinct families: Crenosomatidae, Diaphanocephalidae, Filaroididae, Heligmosomidae, Heterorhabditidae, Metastrongylidae, Molineidae, Stephanuridae, Syngamidae, and Trichostrongylidae. The phylogenetic tree derived from ITS1 -5.8S-ITS2 rDNA sequences (Fig. 4) showed well-defined clades that illustrated the relationships among different families within the Strongyloidea superfamily. The isolates assigned to the Ancylostomatidae family formed a cohesive monophyletic group, which also included another genus from the same family. The phylogenetic analysis based on 28S revealed all sequences in this study clustered together within a strongly supported monophyletic clade (96% bootstrap support) using the Maximum Likelihood (ML) method (Fig. 5). The phylogenetic tree of the COX1 gene demonstrated that the analyzed sequences formed a monophyletic clade identified as the Ancylostomatidae family. Within this clade, *A. caninum* and *A. tubaeforme* sequences clustered together, distinct from the *U. stenocephala* sequences obtained from dogs and golden jackals (Fig. 6). This analysis shows that the *A. caninum* samples

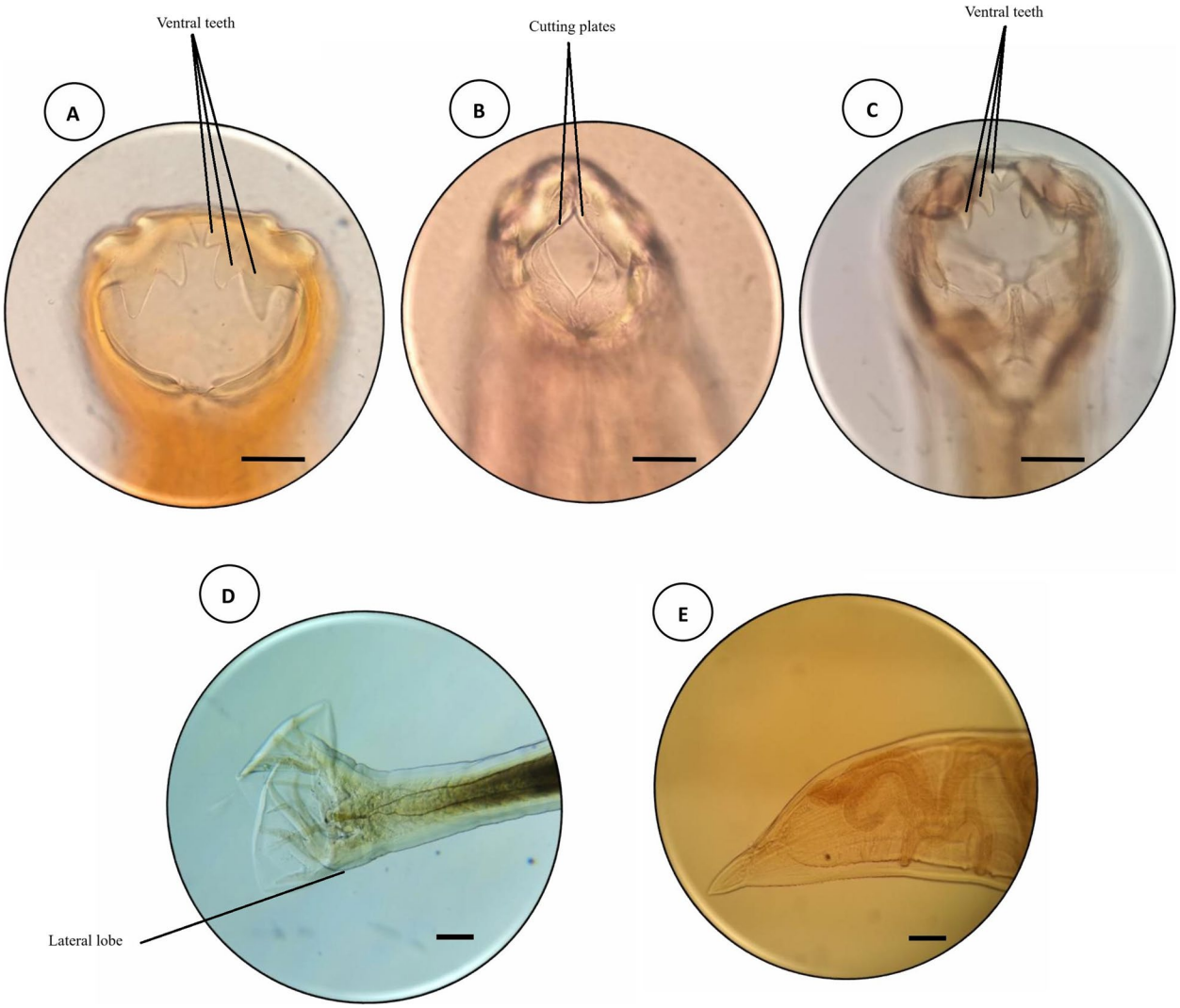

Fig. 2 Buccal capsule of *A. tubaeforme* showing three pairs of pointed teeth projecting from the dorsal margin (**A**); Buccal capsule of *U. stenocephala*, featuring two cutting plates, one on each side of its ventral aspect (**B**); Buccal capsule of *A. caninum* with teeth divided into three sets. Two ventral sets form a lower-jaw equivalent, while a further set projects from the dorsal side and loosely equates to an upper jaw (**C**); Copulatory bursa of *A. caninum* showing two lateral and one dorsal lobes (**D**); *A. caninum* female, with a terminal appendix of the tail (**E**). Scale bar: 25 µm

are grouped into two distinct clades with 100% statistical support. This separation may indicate significant genetic diversity within the studied populations. All sequences generated in this study have been deposited in GenBank (Table 4).

Discussion

This study provides the first molecular evidence of hookworm species infecting carnivores in northern Iran, identifying *A. caninum*, *A. tubaeforme*, and *U. stenocephala*. The prevalence rates of hookworms based on morphological features have been reported in various carnivores across Iran. In northern Iran, *U. stenocephala* (52.3%), *A. caninum* (41.2%) reported in dogs and golden jackals in Mazandaran [28], whereas *A. caninum* (14%) in dogs and golden jackals in Guilan [29]. In Northwestern Iran, hookworms were reported at 13.4% in red foxes (*Vulpes vulpes*), dogs and golden jackals [30]. Regarding *A. tubaeforme*, it was reported in domestic and stray cats in different parts of Iran ranging from 18 to 64% in northern Iran, 14% in Azarshahr, and 5.9% in Ahar (northwestern Iran) [29, 31–35]. It is also recorded in 8 wildcats (*F. chaus*) at 62.5% [36], and in a case report study in Persian leopard (*Panthera pardus saxicolor*) [37].

So far, there are no molecular-based studies on hookworms in carnivores in Iran. The present study

Table 4 The GC content percentage, GenBank accession numbers, reference sequences, and similarity percentage for the species isolated in this study are presented for the SSU, ITS, LSU, and COX1 genes

Genes												
Hookworm species	**SSU**			**ITS1 - 5.8S-ITS2**			**LSU**			**COX1**		
	GC (%)	**Accession no**	**Ref seq (% similarity)**	**GC (%)**	**Accession no**	**Ref seq (% similarity)**	**GC (%)**	**Accession no**	**Ref seq (% similarity)**	**GC (%)**	**Accession no**	**Ref seq (% similarity)**
A. caninum	49.37	PQ317213 - 21	AJ920347 (100)	47.17	PQ316544 - 52	LC036567 (99.6)	50.08	PQ317814 - 22	AM039739 (100)	32.26	PQ555168 - 76	AJ407966 (98.7)
A. tubaeforme	49.37	PQ317225 - 6	NR	46.97	PQ316556 - 7	JQ812691 (99.5)	50.08	PQ317826 - 7	NR	29.43	PQ555180 - 1	AJ407940 (99.8)
U. stenocephala	49.68	PQ317222 - 24	MN218457 (100)	48.07	PQ316553 - 5	AF194145 (99.4)	49.92	PQ317823 - 5	NR	30.61	PQ555177 - 9	MT361501 (100)

NR Not Reported

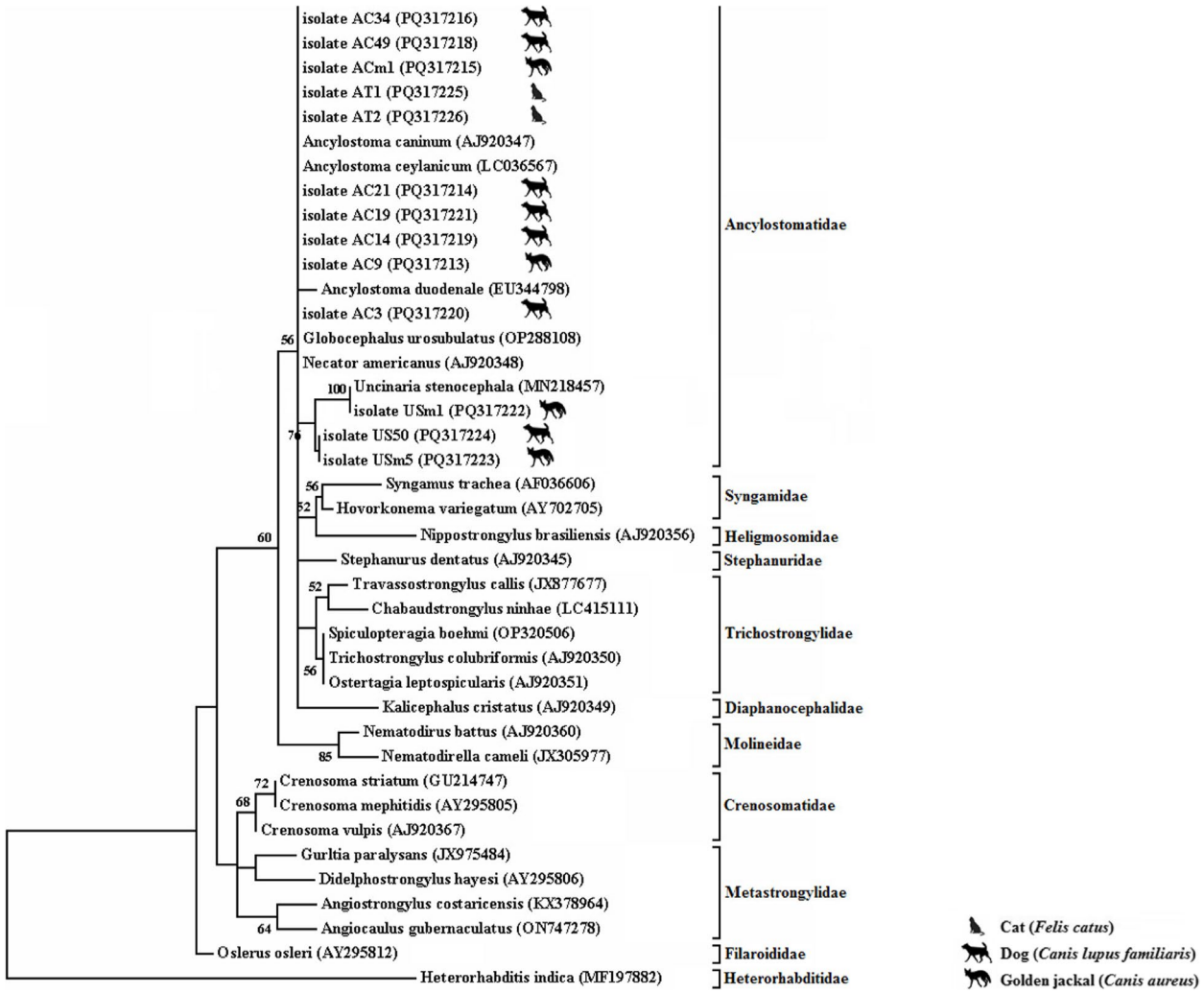

Fig. 3 The phylogenetic tree was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model for the 18S rRNA gene. The tree with the highest log likelihood of – 1322.57 is presented. A discrete Gamma distribution (5 categories, + G, parameter = 0.1000) modeled the evolutionary rate differences among sites, with 46.63% of sites being evolutionarily invariable (+ I). This analysis included 41 nucleotide sequences. The evolutionary analyses were performed using MEGA11

investigated the species and genetic diversity of hookworm infections in road-killed carnivores from northern Iran using molecular techniques. Specifically, we targeted a partial region of the 18S, 28S, and ITS1 - 5.8S-ITS2 regions of ribosomal RNA genes, along with the COX1 gene. The identified hookworm species in these carnivores were *A. caninum*, *A. tubaeforme*, and *U. stenocephala*. These findings aligned with previous studies employing molecular methods, which have reported *A. caninum*, *U. stenocephala* and *A. tubaeforme* in carnivores from various countries, including Mexico [38], the USA [39], Australia [40], China [11, 41, 42], Kenya [43], India [44], Malaysia [45], Cambodia [46], Spain [47], Thailand [48]. Phylogenetic analysis of the 18S sequences of *A. caninum*, *A. tubaeforme*, and *U. stenocephala* confirmed their identity, corresponding with previously reported sequences from the Ancylostomatidae family [49–51].

The phylogenetic tree analysis of 28S sequences revealed that *A. caninum* and *U. stenocephala* formed two distinct branches. Despite the absence of a reference sequence for *A. tubaeforme* in GenBank, the 98–99% sequence similarity between *A. tubaeforme* and *A. caninum* led to their classification within the same clade. Furthermore, the sequenced ITS1 - 5.8S-ITS2 regions displayed low intra-specific variation within *Ancylostoma* and *Uncinaria*. However, this analysis was based on sequences from the Strongyloidea superfamily, resulting

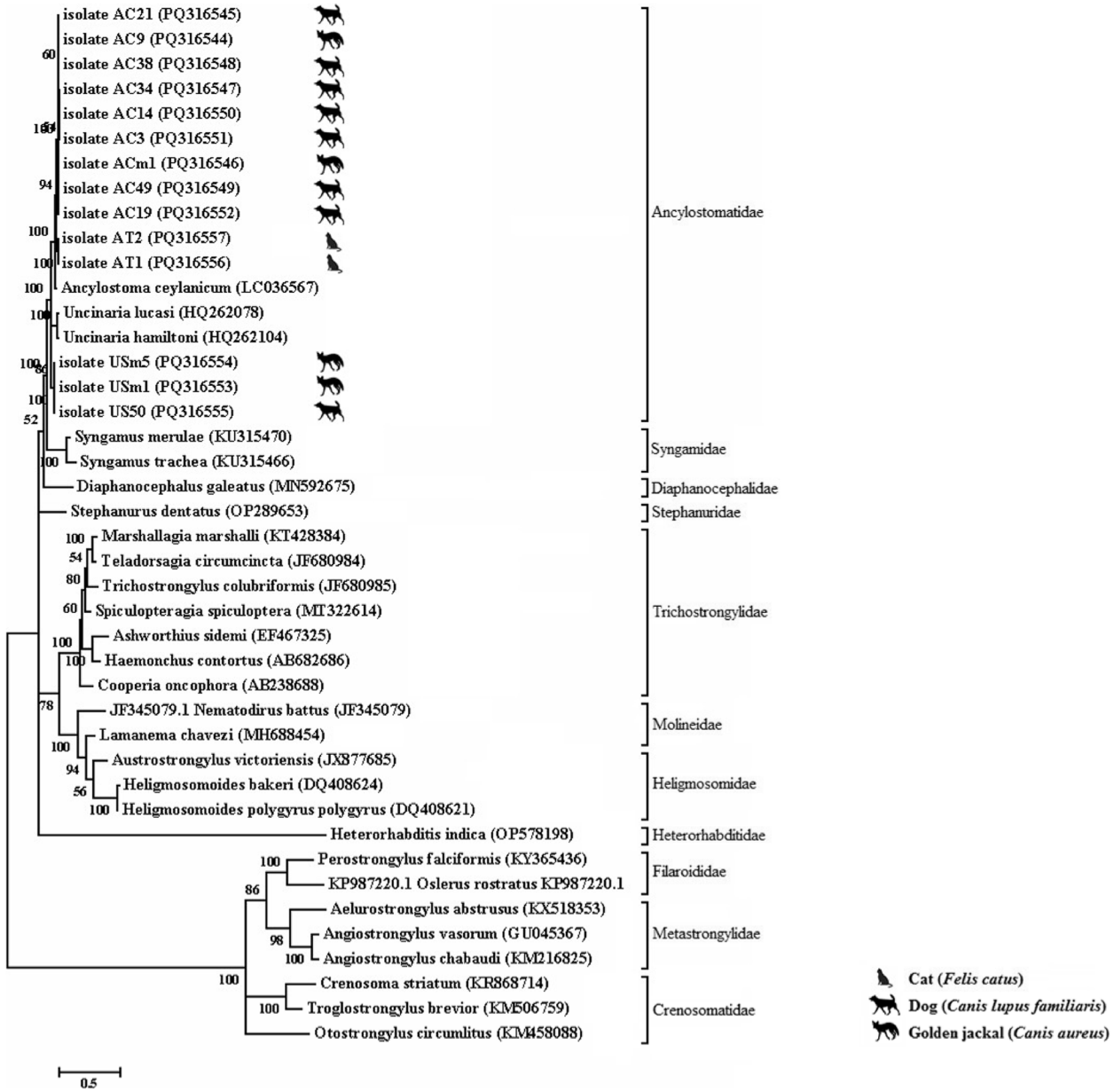

Fig. 4 The phylogenetic tree was inferred using the Maximum Likelihood method based on the General Time Reversible model for the ITS1 - 5.8S-ITS2 rRNA regions. The tree with the highest log likelihood of − 19,374.00 is shown. A discrete Gamma distribution (5 categories, + G, parameter = 1.0837) modeled the evolutionary rate differences, with 12.09% of sites being evolutionarily invariable (+ I). This analysis included 42 nucleotide sequences. The evolutionary analyses were performed using MEGA11

in certain nodes of the phylogenetic tree exhibiting high bootstrap support values due to significant mutational diversity within this region.

In contrast, the phylogenetic analysis of the COX1 region revealed three distinct branches within the Ancylostomatidae clade: *A. caninum*, *A. tubaeforme*, and *U. stenocephala*. Notably, the *A. caninum* isolates from our study were divided into two separate subclades. This divergence may be attributed to multiple factors. One possible explanation is the difference in evolutionary rates between mitochondrial and ribosomal genes. While ribosomal genes (18S, 28S, ITS) evolve at a slower rate due to their conserved cellular functions, mitochondrial genes such as COX1 exhibit higher mutation rates, making them more susceptible to genetic divergence. Additionally, selection pressures and environmental

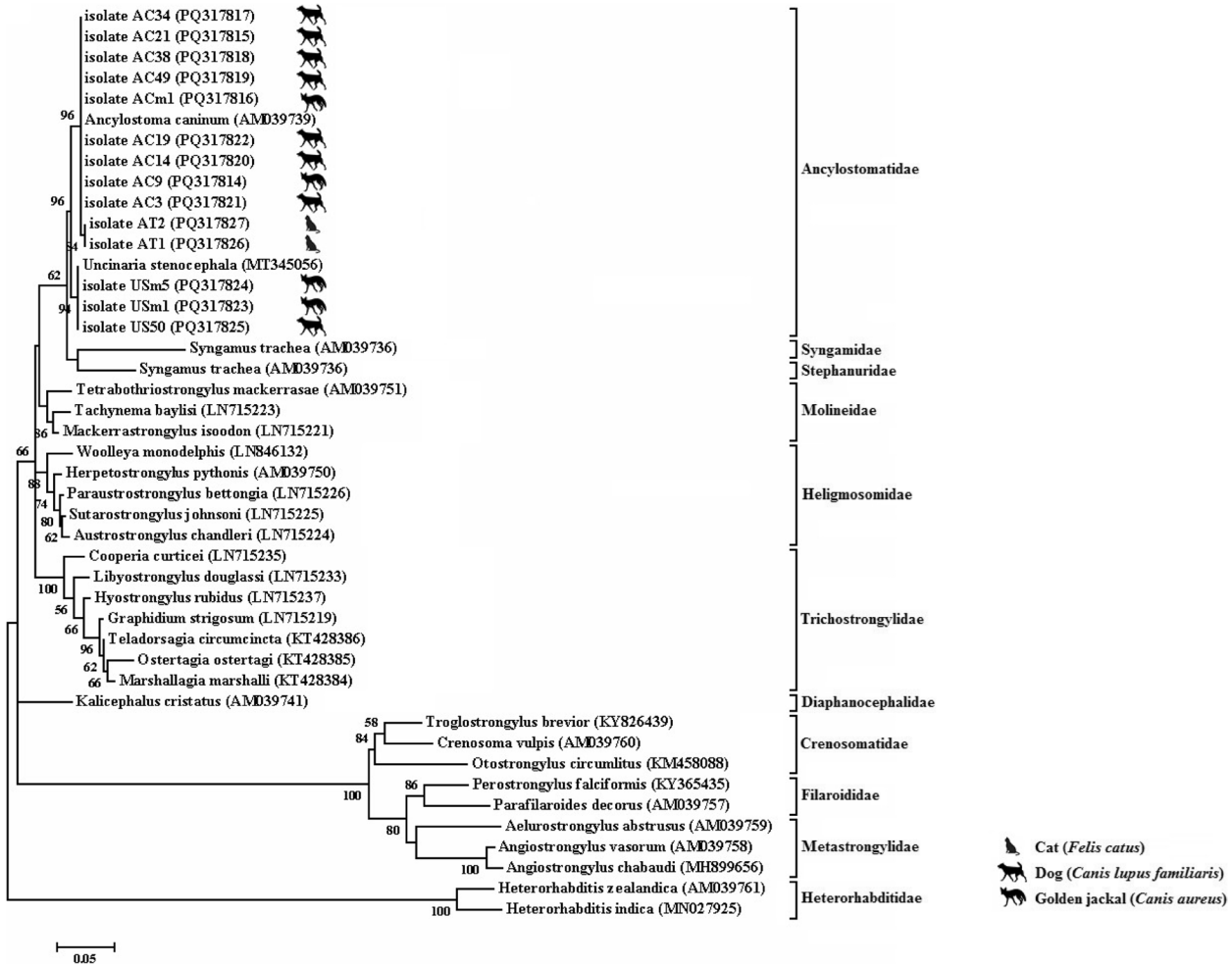

Fig. 5 The phylogenetic tree was inferred using the Maximum Likelihood method based on the General Time Reversible model for the 28S rRNA gene. The tree with the highest log likelihood of – 4388.92 is shown. A discrete Gamma distribution (5 categories, + G, parameter = 0.4143) modeled the evolutionary rate differences, with 28.83% of sites being evolutionarily invariable (+ I). This analysis included 44 nucleotide sequences. The evolutionary analyses were performed using MEGA11

adaptations could have played a role in shaping this divergence. Since the COX1 gene is involved in oxidative phosphorylation, variations in environmental conditions or host metabolism may drive adaptive changes in mitochondrial DNA, leading to distinct phylogenetic clustering [52].

Population structure and genetic drift may have also contributed to the observed divergence [53]. The presence of two distinct subclades within *A. caninum* could indicate historical lineage separation, restricted gene flow, or population bottlenecks that have shaped mitochondrial diversity over time [54]. Furthermore, the genetic divergence in the COX1 sequences may suggest the existence of cryptic diversity within *A. caninum*, which is not apparent in ribosomal markers [55]. This raises the possibility that some of the isolates studied belong to previously unrecognized evolutionary lineages, highlighting the need for further multilocus analyses, including nuclear genes, to confirm these findings [56].

Another potential factor influencing genetic divergence is temporal and host-associated variability [57]. Given the 10-year collection period, temporal changes in the parasite population, along with potential host-specific selective pressures, could have contributed to the genetic differentiation within *A. caninum*. Over time, shifts in host availability, environmental conditions, or migration patterns of the parasite populations may have led to the formation of distinct mitochondrial lineages [58].

Overall, while ribosomal genes provide a conserved framework for species identification, mitochondrial markers like COX1 offer higher resolution in detecting intra-species variation and evolutionary dynamics. The high mutation rate of COX1 compared to ribosomal genes could have played a significant role in the observed

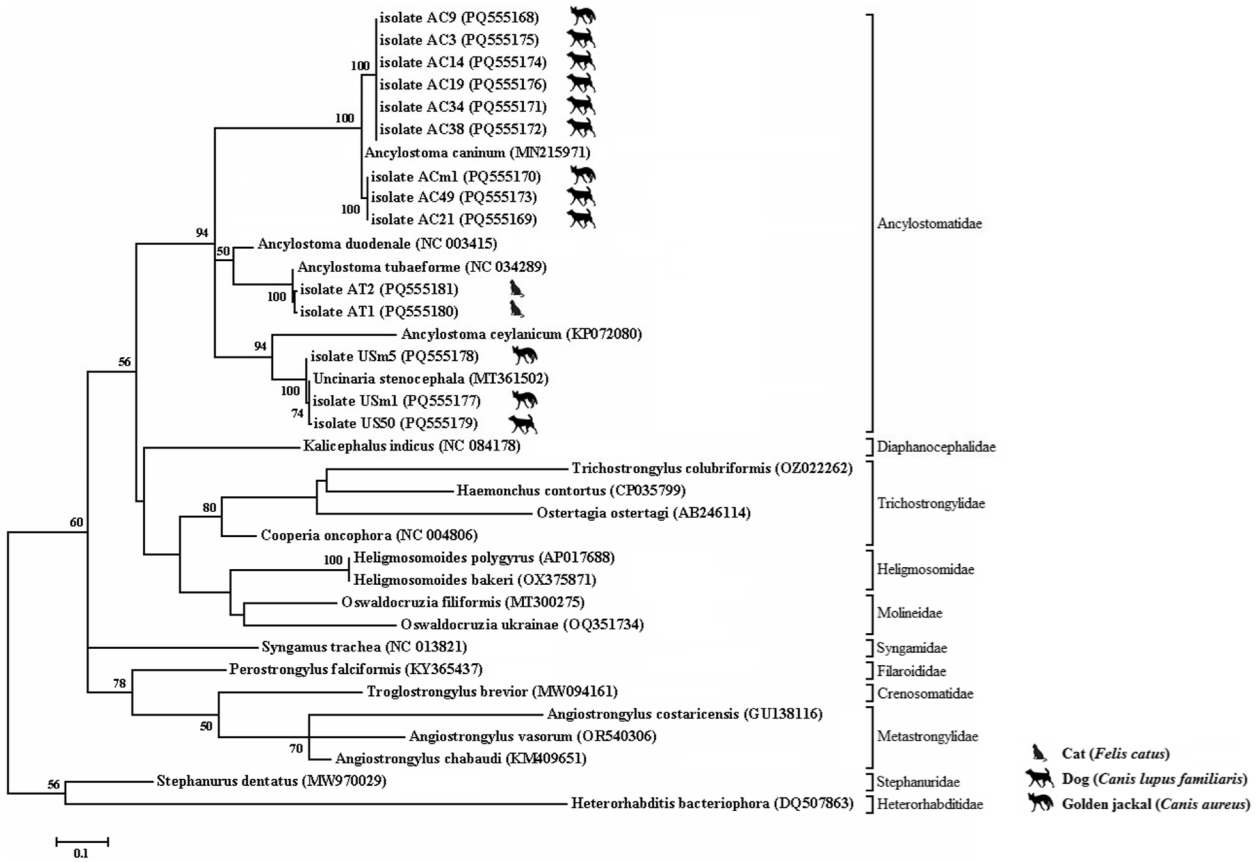

Fig. 6 The phylogenetic tree was inferred using the Maximum Likelihood method based on the General Time Reversible model for the COX1 gene. The tree with the highest log likelihood of − 3346.80 is presented. A discrete Gamma distribution (5 categories, + G, parameter = 0.3714) modeled the evolutionary rate differences, with 32.77% of sites being evolutionarily invariable (+ I). This analysis included 36 nucleotide sequences. The evolutionary analyses were performed using MEGA11

divergence. Future studies incorporating broader temporal sampling, additional nuclear markers, and whole-genome analyses will be valuable in further elucidating the evolutionary history of *A. caninum*.

The prevalence of hookworm infections in carnivores has been documented in numerous morphology-based global studies. Studies relying on morphological identification have reported infection rates of 67.9% in Spain (shelter and hunting dogs), 45% in Brazil (dogs and cats), 28.4% in Portugal (domestic dogs), 16.5% in Poland (red foxes), and 6.8% in Italy (stray and privately owned dogs) [59–63]. Morphological surveys conducted in Mexico (dogs) and Cuba (stray and domestic dogs) identified *A. caninum* infection rates of 88.1% and 21.6%, respectively [64, 65]. In Canada, a study reported a prevalence of 3.1% for *A. caninum* and 2.9% for *U. stenocephala* in shelter dogs, while *A. tubaeforme* was identified in 2% of shelter cats [66]. In Africa, the prevalence of *Ancylostoma* species has been reported at 55.7% in stray dogs in Zimbabwe, 40.1% in dogs in Ethiopia, and 33.2% in dogs in Nigeria [67–69]. A review and meta-analysis of studies from Asia revealed that golden jackals had the highest rate of infection among carnivorous animals, with a prevalence of 48%. This was followed by dogs at 41%, cats at 26%, and red foxes at 19% [70]. In Iran, domestic and stray dogs and cats are the primary carriers of zoonotic parasites. Additionally, foxes and golden jackals are recognized in many parts of the country as potential sources of human infection. While these animals usually reside in forests and mountainous regions, they have been reported near human communities. Various anthropogenic factors are expected to further amplify this behavior in the future [1]. Consequently, foxes and golden jackals should be considered in surveillance efforts as potential zoonotic threats due to their interactions with human-inhabited areas [71].

Climate and soil composition are key factors influencing the prevalence of hookworms. These parasites prosper in tropical and subtropical zones where the warm, moist conditions are perfect for the larvae to develop outside the host. The interaction of these environmental factors creates an ideal habitat for hookworms, leading

to their widespread distribution in these regions [7]. The Caspian Sea littoral region, a major tourist destination, is bordered by the Alborz mountains to the north and is covered by dense forests in Guilan and Mazandaran. At the same time, this region poses unique ecological and climatic conditions, providing an ideal habitat for different wildlife species, including carnivores [72], and supports the life cycles of several zoonotic helminths [73]. The presence of these parasites in carnivores, particularly dogs, cats, and golden jackals, highlights their role in the transmission cycle of zoonotic infections. Given the tourism significance of Guilan and Mazandaran Provinces and the continuous movement of people, the risk of transmission to both residents and visitors increases [73, 74].

The fragmentation of natural habitats has intensified interactions among wildlife, domestic animals, and humans, thereby elevating the risk of pathogen accumulation and transmission. This development has resulted in a surge of parasitic diseases within wildlife populations, raising significant concerns under the One Health approach [75–77].

Human infections with zoonotic helminths are often underdiagnosed, leaving the full extent of their zoonotic potential unclear. Addressing parasitic diseases in wildlife requires a One Health approach, emphasizing collaboration among healthcare institutions, veterinarians, and parasitologists to effectively diagnose helminth infections across wild mammals, companion animals, and humans. Integrating surveillance efforts for humans, domestic animals, and wildlife enables early detection of disease outbreaks, tracking of their spread, and identification of potential reservoirs and vectors [78].

Conclusions

This study presents the first molecular identification and phylogenetic analysis of hookworm species in carnivores from the Caspian Sea littoral region of northern Iran. Molecular analyses, including sequencing of the 18S, ITS1 -5.8S-ITS2 rDNA, 28S, and mitochondrial COX1 genes, not only confirmed morphological species identification but also provided valuable insights into the phylogenetic relationships within the Strongyloidea superfamily. These findings underscore the importance of continuous surveillance in both wild and domestic carnivore populations. Moreover, further molecular studies are essential to gain a comprehensive understanding of the distribution and transmission dynamics of these infections. Considering the potential public health implications, the development of targeted control strategies and parasite management programs is crucial, particularly in areas with high human and animal population density. Enhancing public awareness and improving knowledge about the complex interactions between humans, animals, and the environment within the One Health framework can play a key role in mitigating the risks associated with these infections. The findings of this study contribute to the understanding of the genetic diversity and transmission dynamics of hookworms, laying the groundwork for future public health strategies in the region.

Abbreviations

PCR	Polymerase Chain Reaction
BLAST	Basic Local Alignment Search Tool
ML	Maximum likelihood
ITS	Internal Transcribed Spacer
GTR	General time reversible
COX1	Mitochondrial Cytochrome c Oxidase 1

Supplementary Information

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Suppleemntary Material 1.

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Authors' contributions

Conceptualization; AVE, AD, MS and MB. Project administration; AA, MS and MB. Methodology; MP, AVE and MB. Data curation; EH, AGK, GS and AA. Formal analysis; MB, EH, AVE, and MP. Software; MB, AVE, GS and MP. Investigation; MB, AVE, and MS; Visualization; AA, MP, and, MB. Writing – original draft; MB, AD, MP and AVE. Writing – review and editing: MB, AVE, and AD. Supervision; AD, MP and MB. All the authors commented on the drafts of the manuscript and approved the final version of the article.

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Data availability

The data generated during and analyzed during the study are available from the corresponding author on reasonable request

Declarations

Ethics approval and consent to participate

The ethical approval was required and provided for this study, as stated by Vice-Chancellor for Research and Technology Affairs (Approval number: IR.QUMS.REC.1400.348).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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