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Morphological and molecular analyses of two Taenia species in wild canids in northern Iran

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ABSTRACT

Wild canids play a significant role in dissemination of parasitic helminth ova and may serve as a potential source of infection in humans and animals. Therefore, monitoring the helminth fauna of these species is essential for both veterinary and public health. The present cross-sectional study was conducted to assess cestode infection and to reveal the molecular characterization and phylogenetic status of large Taenia in wild canids in Iran. Descriptive statistics (frequencies and percentages) were used to summarize the data, and the Chi-square test was employed to evaluate differences in parasite distribution among different variables. For this aim, the intestines of 93 wild canids, including 69 jackals, 22 red foxes, and two wolves, were examined in northern, northeastern, and northwestern regions of Iran for tapeworms. Additionally, the identity of some helminths was confirmed using PCR and DNA sequencing. Based on our findings, 54.5% of red foxes and 47.8% (95% CI: 36.5-59.3%) of jackals were infected, among which single infections with *Mesocestoides* were highly prevalent (33.3% , 95% CI: 24.5-43.4%), followed by T. hydatigena (4.3%, 95% CI: 1.7-10.5%), *D. caninum* (2.2% 95%, CI: 0.6-7.5%%), and *T. polyacantha* (1.1%, 95% CI: 0.2-5.8%). Also, mixed infections were demonstrated in 13.6% of foxes (*Mesocestoides* + *T. polyacantha*) and 5.8% of jackals (*Mesocestoides* + *T. hydatigena*). Overall, cestode infection rate in wild carnivores was 48.4%. Molecular analysis with universal primers amplified a 450-bp fragment of the mitochondrial cox1 gene. Sequencing also demonstrated 100% and more than 99% identity between the sequences of *T. hydatigena* (n = 8) and *T. polyacantha* (n = 4) isolates and their corresponding GenBank reference sequences. This study represents the third report and the first molecularly confirmation of *T. polyacantha* in foxes in Iran. These findings emphasize the importance of monitoring cestode infections in wild canids for epidemiological and public health purposes.

Keywords

Cestode, Wild canids, Taenia polyacantha, Taenia hydatigena, Iran

Abbreviations

No Abbreviations

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Introduction

The Taenidae family comprises prevalent gut-dwelling helminths that develop from cystic stages, affecting various tissues of intermediate hosts and incurring financial costs for treatment in humans or organ/carcass condemnation in the livestock industry [1]. They are the most prevalent tapeworms in the small intestine of wild canines, such as dogs, jackals, wolves, and foxes [2]. Taenia species exhibit a predator-prey life cycle involving two mammals: one acts as the definitive host and the other as the intermediate host. Several Taenia species have previously been reported in Iran, including *Taenia hydatigena*, *Taenia pisiformis*, *Taenia polyacantha*, *Taenia multiceps*, *Taenia taeniaeformis*, *Taenia serialis*, and *Taenia ovis* [3, 4, 5, 6, 7, 8, 9, 10]. Among these, *T. hydatigena*, *T. pisiformis*, *T. polyacantha*, *T. multiceps*, *T. serialis*, and *T. ovis* are commonly found in the intestinal tract of canids [11, 12]. *T. hydatigena* is one of the most widespread taeniid cestodes infecting domestic and wild animals. Dogs, foxes, wolves, and cats act as definitive hosts. Metacestodes develop into large, pear-shaped cysticerci (*Cysticercus tenuicollis*) in the abdomen and organs of livestock, acting as intermediate hosts [13]. Another taeniid species, *T. polyacantha*, is found in canids across the Northern Hemisphere, especially in arctic foxes (e.g., *Alopex lagopus*) and red foxes, as well as dogs and wolves in southern regions. Rodents (e.g., *Microtus oeconomus*) serve as intermediate hosts, where the tapeworm resides in their peritoneal cavity or pleural space [14]. In *T. multiceps* infection, the larvae (*Coenurus cerebralis*) typically parasitize the central nervous system in livestock, causing neurological signs [15].

Apart from the genus *Taenia*, cestodes of the genera *Diplydium* and *Mesocestoides* are also common in wild canids. The flea tapeworm, *Diplydium caninum*, follows a heteroxenous cycle, involving definitive hosts (carnivores and sporadically humans) and intermediate hosts such as fleas and chewing lice of dogs and cats. Adult stages in wild and domestic carnivores may render symptoms, including diarrhea, scooting behavior, dullness, weight loss, anorexia, and poor hair coat [16]. Members of the genus *Mesocestoides*, possess a more complex life cycle involving multiple intermediate hosts, including rodents, amphibians, birds, and reptiles. Their larval stage (tetrathyridia), may lead to life-threatening peritonitis in animal hosts. Zoonotic infections in humans are usually caused by consuming raw/undercooked snake, chicken, or wild game viscera [17]. Although numerous Taenia species possess distinct morphological characteristics, several exhibit striking similarities, necessitating molecular analysis for effective differentiation [18, 19]. Various mitochondrial and nuclear genetic markers are used in the molecular analysis of cestodes, with a primary focus on diagnostic applications [20, 21]. Among the genes studied, mitochondrial genes (cox1 and nadh1) have proven valuable for genetic characterization of Taeniidae cestodes [18, 22].

Given the limited nationwide information on the molecular characterization of large *Taenia* spp. in wild carnivores in Iran, the present study was conducted to determine the morphometric and molecular analysis of large Taenia spp. in the small intestine of road-killed wild canids (jackal, fox, and wolf) along with the intestinal cestodes infection in the northern, northeastern, and northwestern regions of Iran.

Therefore, the aim of the present study was to investigate the occurrence, species diversity, and morphometric and molecular characteristics of intestinal cestodes in road-killed wild canids (jackals, foxes, and wolves) from northern, northeastern, and northwestern Iran. To the best of our knowledge, this study also reports the first molecular identification of Taenia polyacantha in wild canids in Iran.

Results

Epidemiological findings

Based on our results, 33 (47.8%) jackals and 12 (54.5%) foxes were infected with at least one tapeworm species. No infection was found in the two examined wolves. *Mesocestoides* spp. was the most prevalent cestode identified in both jackals and foxes. Among jackals, the overall prevalence of *Mesocestoides* spp. was 36.2%, including seven cases from northeastern Iran, seven from northwestern Iran, and fifteen from northern Iran. In foxes the overall prevalence of *Mesocestoides* spp. was 27.3%, including two cases from northeastern Iran, and seven from northwestern Iran. Other cestode species identified included: *T. hydatigena* (two foxes from northwestern Iran; and six jackals, two from northeastern Iran, and four from northwestern Iran), *T. polyacantha* (four foxes from northwestern Iran), and *Dipylidium* spp. (two jackals, one from northern Iran and one from northeastern Iran). Moreover, this study reports the first molecular identification of *T. polyacantha* in Iran. All four infected foxes were collected from Ardabil Province (Pars Abad, Qosabeh, and Meshgin Shahr cities) in northwestern Iran. Three of the four specimens (13.6%) were mixed infections with *Mesocestoides* spp. In addition, four jackal carcasses (5.8%) were found to be infected with *Mesocestoides* spp. and *T. hydatigena*. Among foxes, an equal number of males and females (n = 6) were infected with tapeworm species. In contrast, most infected jackals were female (n = 22; 41.5%). Most jackal and fox carcasses were collected from northernern (n = 35) and northwestern (n = 13) Iran, respectively. There was no statistically significant

association between the tapeworm infection and host species ($p = 0.09$), sex ($p = 0.32$), or geographical area ($p = 0.26$) (Tables 1 & 2). Microscopic observations of the parasites are illustrated in Figure 1.

Table 1.
Number and percentage of the wild canids infected with single or mixed cestode infections.

Type of animal	Type of Parasite Count (%)									
	Mesocestoides spp	*Taenia hydatigena*	*Taenia polyacantha*	*Dipylidium caninum*	*Mesocestoides spp + Taenia hydatigena*	*Mesocestoides spp + Taenia polyacantha*	Negative	Positive	Total	P-value
Fox	6 (27.3)	2 (9.1)	1 (4.5)	0 (0)	0 (0)	3 (13.6)	10 (45.5)	12 (54.5)	22 (100)	0.09
Wolf	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)	
Jackal	25 (36.2)	2 (2.9)	0 (0)	2 (2.9)	4 (5.8)	0 (0)	36 (52.2)	33 (47.8)	69 (100)	
Total	31 (33.3)	4 (4.3)	1 (1.1)	2 (2.2)	4 (4.3)	3 (3.2)	48 (51.6)	45 (48.4)	93 (100)	

Table 2.
Gender- and region-based prevalence of wild canids infected with tapeworms.

Parameters	Infection count (%)								
	Fox		Wolf		jackal		Positive	Total	P-value
Gender	Yes	No	Yes	No	Yes	No			
Male	6 (15)	4 (10)	0	1 (2.5)	11 (27.5)	18 (45)	17 (42.5)	40 (100)	0.32
Female	6 (11.3)	6 (11.3)	0	1 (1.9)	22 (41.5)	18 (34)	28 (52.8)	53 (100)	
Total	12 (12.9)	10 (10.8)	0	2 (2.1)	33 (35.5)	36 (38.7)	45 (48.4)	93 (100)	
Region									
North	0 (0)	0 (0)	0	0 (0)	16 (45.7)	19 (54.3)	16 (45.7)	35 (100)	0.26
Northeast	2 (7.1)	7 (25)	0	2 (7.1)	9 (32.2)	8 (28.6)	11 (39.3)	28 (100)	
Northwest	10 (33.3)	3(10)	0	0 (0)	8 (26.7)	9 (30)	18 (60)	30 (100)	
Total	12 (12.9)	10 (10.8)	0	2 (2.2)	33 (35.4)	36 (38.7)	45 (48.4)	93 (100)	

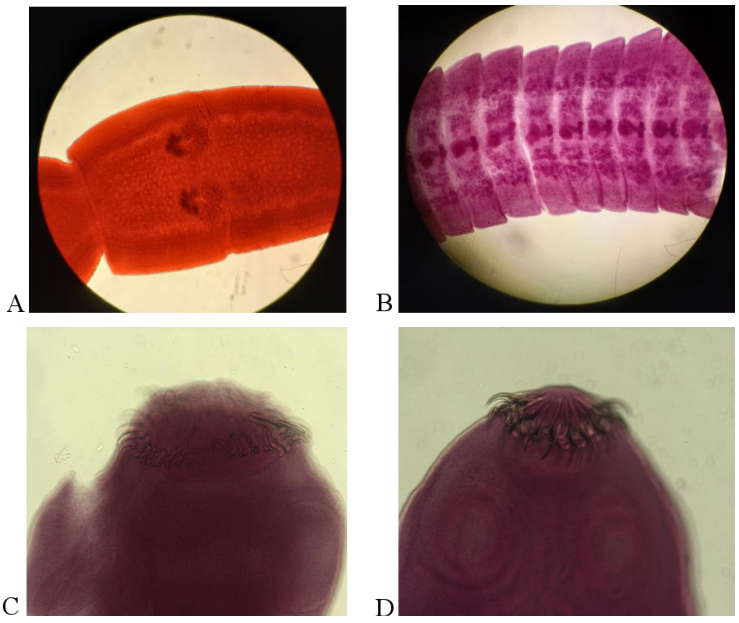

Figure 1.
Microscopic images of the parasites. (A) *Dipylidium caninum* (B) *Mesocestoides spp* (C) *Taenia polyacantha* (D) *Taenia hydatigena*.

Hook morphometry

The morphometry of small and large hooks was measured in *T. hydatigena* and *T. polyacantha*, which are primarily found in northwestern Iran. For *T. hydatigena*, the mean estimates of small hooks were 127.85 µm for TL, 55.62 µm for TW, and 58.5 µm for BL. The corresponding mean values for large hooks were 194.4 µm for TL, 59.28 µm for TW, and 90.52 µm for BL. In *T. polyacantha* the mean measurements of small hooks were 102.96 µm for TL, 43.19 µm for TW, and 65.03 µm for BL, whereas large hooks showed a mean of 182.33 µm for TL, 44.39 µm for TW, and 74.34 µm for BL. Among *T. hydatigena* isolated from a female fox, small hook estimates were higher than for the other isolates. Regarding large hooks, the highest TL (209.8 ± 4.88 µm) was recorded in *T. hydatigena* isolated from a male jackal, and the highest TW (66.77 ± 1.81 µm) was observed in *T. hydatigena* isolated from a female fox, both originating from the northwestern Iran. Also, the highest BL (111.3 ± 6.84 µm) was observed in a *T. hydatigena* specimen isolated from a male jackal in northeastern Iran. The highest TL (108.3 ± 1.35 µm) and TW (44.4 ± 3.14 µm) in the small hooks of *T. polyacantha* were recorded in a male fox, while the highest BL (66.38 ± 4.2 µm) was observed in another *T. polyacantha* specimen isolated from a different male fox. In addition, the highest TL (187 ± 1.22 µm) and TW (48.6 ± 4.34 µm) among the large hooks of *T. polyacantha* were recorded in a female fox, while the highest BL (75.15 ± 2.89 µm) was observed in a male fox. Other estimated hook lengths are tabulated in Table 3. Also, quantitative measurements of hooks dimensions and arrangement are provided in Figure 2.

Table 3.
Details of hook morphometry in isolated tapeworms from wild canids

Isolated Species	Host	Region	Small hooks						Large hooks					
			TL	ACL	PCL	BL	HL	TW	TL	ACL	PCL	BL	HL	TW
T. hydatigena	*Female fox*	*Northwest*	134.6 ± 8.57	70.85 ± 3.49	77.68 ± 9.23	63.85 ± 1.91	52.3 ± 9.63	65.85 ± 3.07	185.1 ± 2.99	93.0 ± 2.58	107.9 ± 2.3	81.57 ± 1.35	85.17 ± 2.49	66.77 ± 1.81
T. polyacantha	*Female fox*	*Northwest*	100.2 ± 1.24	65.2 ± 3.25	56.33 ± 3.85	62.53 ± 5.11	26.2 ± 3.2	42.37 ± 1.64	187 ± 1.22	90.27 ± 2.75	104.8 ± 1.5	75.1 ± 4.62	90.37 ± 6.32	48.6 ± 4.34
T. hydatigena	*Female jackal*	*Northwest*	121.1 ± 5.26	65.9 ± 2.73	67.33 ± 2.52	53.15 ± 2.62	51.4 ± 5.92	45.4 ± 3.64	188.5 ± 4.54	88.9 ± 5.76	107 ± 1.8	78.7 ± 3.01	89.93 ± 3.02	52.7 ± 6.29
T. hydatigena	*Male jackal*	*Northeast*	-	-	-	-	-	-	209.8 ± 4.88	117.5 ± 7.21	103.4 ± 2.36	111.3 ± 6.84	72.4 ± 5.85	58.37 ± 6.16
T. polyacantha	*Male fox*	*Northwest*	108.3 ± 1.35	74.4 ± 1.73	50.33 ± 2.3	66.2 ± 1.47	31.6 ± 4.09	44.4 ± 3.14	187 ± 0.21	91.05 ± 0.35	104.9 ± 0.35	75.15 ± 2.89	88.8 ± 5.09	44.65 ± 1.76
T. polyacantha	*Male fox*	*Northwest*	100.4 ± 0.88	72.58 ± 4.02	50.9 ± 2.84	66.38 ± 4.2	29.8 ± 2.82	42.8 ± 1.59	173 ± 8.31	85.87 ± 6.56	96.13 ± 5.83	72.77 ± 4.36	83.1 ± 5.4	39.93 ± 7.91

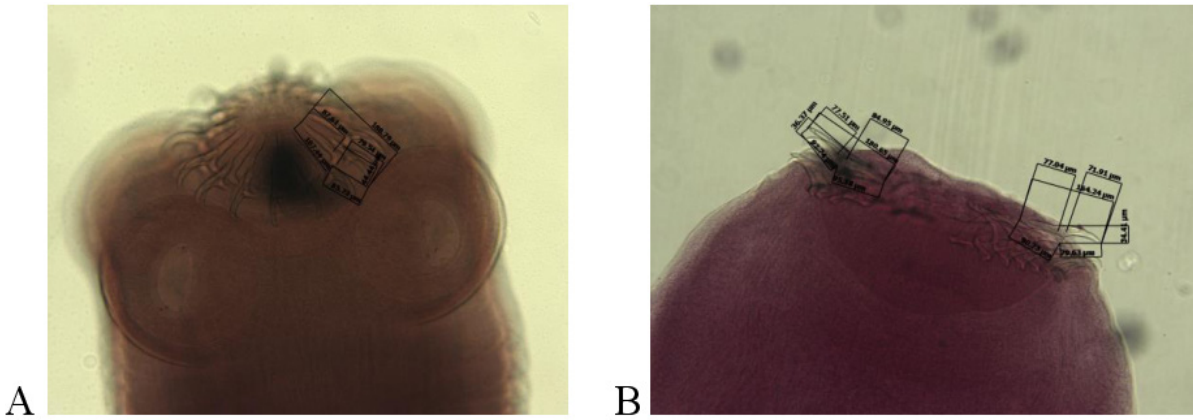

Figure 2.
Morphometric analysis of hooks. (A) *Taenia hydatigena* (B) *Taenia polyacantha*

A 450 bp fragment of mitochondrial COX1 was amplified (Figure 3), followed by DNA sequencing of Twelve isolates. Multiple sequence alignment analysis of *T. polyacantha* isolates with the standard isolate demonstrated a 100% nucleotide identity with the reference sequence available in GenBank (Accession No. EU544581.1). Similarly, more than 99% identity was obtained between *T. hydatigena* isolates in the present study with the registered GenBank sequence (Accession: MN478491.1); of note, single nucleotide substitutions were observed in position 261 (G → A) of *T. hydatigena* Ca13 and Ca14 isolates as well as in position 342 (T → C) of Ca6 isolate (Supplementary File). Phylogenetic analysis based on cox1 gene revealed that all four *T. polyacantha* isolates (isolates 6, 5, 13, and 14) were genotically identical to the Finnish genotype, as evidenced by multiple alignment. Also, they clustered within the other Finland isolates at the same branch. In contrast, *T. hydatigena* genotypes obtained in the present study (isolates 1, 2, 4, 7, 8, 9, 10, and 11) reported from wild canids clustered with reference sequences from Iraq (MZ313985), Slovakia (MZ345600, MZ298783), and Iran, Chabahar region (MN478491). More details on the phylogenetic relationship of the obtained isolates are illustrated in Figure 4. Deposition of DNA sequences for *T. polyacantha* isolates (Accession numbers: PP658436 (Vu1), PP658437 (Vu19), PP658443 (Vu4), PP658444 (Vu13)) and for *T. hydatigena* isolates (Accession numbers: PP658433 (Vu16), PP658434 (Ca2), PP658435 (Ca6), PP658438 (Ca10), PP658439 (Ca13), PP658440 (Ca14), PP658441 (Ca15w), PPP658442 (Ca15e)) was done in GenBank.

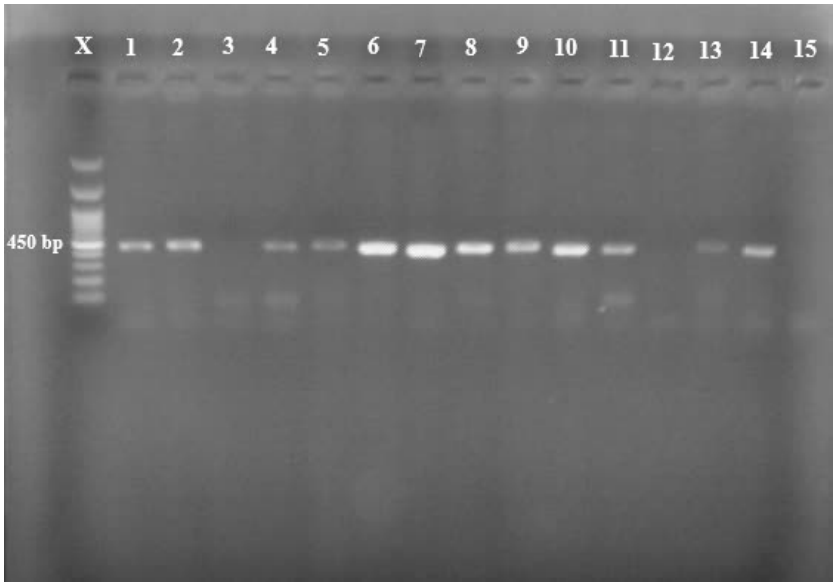

Figure 3.
X: DNA ladder 100bp, 1-15: Amplicon of mitochondrial cox1 gene (450 bp) using JB3 and JB4.5 primer.

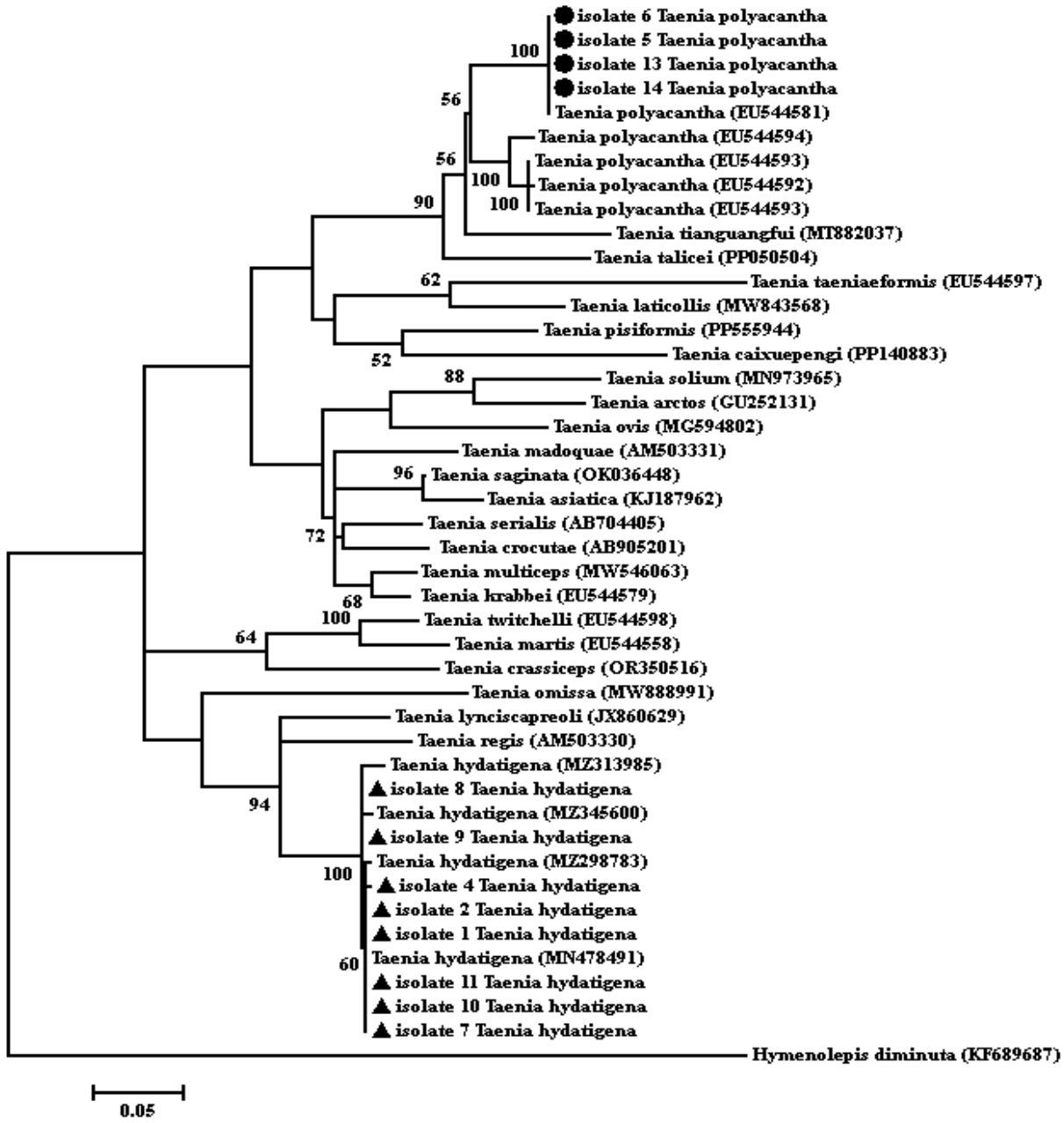

Figure 4.
Phylogenetic analysis of cestodes sequences obtained in the present study with those from other regions of the world. *Hymenolepis diminuta* was used as an outgroup. Iranian isolates of *T. polyacantha* (n = 4) clustered with Finland genotypes, while *T. hydatigena* isolates (n = 8) clustered with some genotypes from Iran, Iraq, and Slovakia in eastern Europe.

Discussion

Free-roaming jackals and red foxes can be a potential source of cestode infections, which are significant for both domestic animals and humans [23]. The present study assessed cestode infections in road-killed wild canids (n = 93, 69 jackals, 22 foxes, 2 wolves) from the northern territories of Iran. Our results determined four cestode species (*T. hydatigena*, *T. polyacantha*, *D. caninum*, and *Mesocestoides* sp.). The tolal prevalence of cestodes was 48.4%, with Mesosestoides spp representing the most common taxon (33.3%), followed by *T. hydatigena* (4.3%), *D. caninum* (2.2%), and *T. polyacantha* (1.1%). Although a higher prevalence rate was noted in foxes (54.5%) compared to jackals (47.8%), with *Mesocestoides* single infections predominating in jackals (36.2%) and foxes (27.3%). Intestinal helminths represent a significant portion of the worm burden in carnivore populations in Iran, accounting for 80.4% (95% confidence interval [CI]: 70.2% - 88.8%). Among these, jackals (97.32%) are the most heavily parasitized, followed by foxes (89.17%) and dogs (57.89%). Tapeworms are among the most common intestinal helminths found in domestic and wild canids in Iran, including *D. caninum* (20.45%),

T. hydatigena (15.28%), *M. lineatus* (11.83%), and *E. granulosus* (10%) [15]. Several studies have been conducted to document the cestode fauna in the canine population in Iran. In a nationwide investigation, red foxes and golden jackals from three different climatological zones in Iran were necropsied, representing a high predominance of *Mesocestoides lineatus* in jackals (15.2%-21.5%) and foxes (43.2% - 67.5%) [24]. In a focal study in the Moghan plain, northwestern Iran, of 85 necropsy specimens from wild canids, 96.47% were infected; among these, *Mesocestoides* (84.7%) was the most prevalent, followed by Joyuxiella (34.1%) and Taenia spp. (11.8%), and *D. caninum* (1.2%) [10].

In 63 intestinal specimens from dogs and jackals in Mazandaran province, northern Iran, a 95.2% prevalence of parasitic infection was observed, with 33.3% and 25.3% prevalence for *Mesocestoides* spp. and *D. caninum*, respectively [25]. In another study on 274 fecal samples from wild canids in this area, 85.2% were infected, and the ova of *D. caninum* was mostly found in 25.2% of dogs and 11.1% of jackals [26]. In a study on road-killed carnivores in the Caspian Sea littoral (2017), *D. caninum* (33.3% in dogs, 27.2% in jackals), *E. granulosus* (25.9% in dogs, 27.2% in jackals), and *T. hydatigena* (18.5% in dogs, 18.1% in jackals) were the most frequent isolated tapeworms; they also found less frequent cestodes, including *Mesocestoides, Joyuxiella,* and *Spirometra* (7.4%) [3]. In the present study, we also examined the small intestine of 2 wolves and it is noteworthy to say that they were without evidence of cestode infections. In Mazandaran, 5.2% of 19 wolves were infected with Taenia species [26]. Additionally, numerous studies worldwide have reported various cestode species in wolves [27, 28, 29].

In a similar study in Turkey (2021), *Mesocestoides* sp. and *D. caninum* were the most and least prevalent cestode species isolated from foxes [30]. Consistent with our findings, the pooled prevalence of *Mesocestoides* infection in terrestrial carnivores was estimated to be 21.72% (95% CI: 18.49% - 25.14%) [17]. In comparison, the North American dogs had the lowest rates (0.054%) [31]. Infection with adult worms was more pronounced in foxes [35.97% (95% CI: 29.54% - 42.66%)] and other wild canids [21.39% (95% CI: 15.25% - 28.24%)] than in domestic carnivores such as dogs and cats, highlighting the role of feeding behavior in the maintenance of parasite population; wild canids have free access to potentially infected intermediate hosts (e.g., rodents, birds, snakes, amphibians) harboring tetrathyridia. The authors also remarked a 28% (95% CI: 19.83% - 36.97%) prevalence of *Mesocestoides* infection in Iran [17]. Our finding regarding the dominance of *Mesocestoides* in the canine population in Iran overlaps with previous studies, whereas it contrasts with the Eslahi et al. study, which demonstrated *D. caninum* as the most prevalent tapeworm in road-killed carnivores in northern Iran [3]. We also demonstrated mixed infections of *Mesocestoides* sp. with *T. hydatigena* (5.8%) in jackals and with *T. polyacantha* (13.6%) in foxes.

The significant findings of the present study was the isolation and of *T. polyacantha* from fox populations, and the molecular confirmation of this species in Iran for the first time. Although, this is the third report of *T. polyacantha* in Iran, while in the previous studies in Iran molecular identification was not performed; the first documentation dates back to 1973, when Mobedi et al. isolated a *T. polyacantha*-like worm from 22.5% and 2.5% of red foxes in Kalibar and Moghan regions, northwestern Iran [5]. The second report was by Zare-Bidaki (2010) again in red foxes in the Moghan plain (6 recovered worms) [10]. Based on the reports above, it can be concluded that the Moghan Plain, along with the semi-arid mountainous areas of northwestern Iran with well-developed vegetation, represents favorable ecological niches for the development and propagation of *T. polyacantha* cestodes in red foxes, and potentially in their respective intermediate hosts. Various fox species (e.g., Vulpes and Alopex) are recognized as definitive hosts for adult *T. polyacantha*, acquiring infection through predation on infected small mammals, such as lagomorphs and voles [32] as a substantial part of red fox diets [33] The metacestodes cause fibrosarcoma, hepatitis, and peritonitis in affected hosts. Generally, canids in northern Eurasia and southern tundra ecosystems may harbor this taeniid species. Nevertheless, dogs and wolves in more southern areas may also harbor this tapeworm [34, 35, 36]. In the present study, *T. polyacantha* was found in 1/22 examined foxes (2.2%); mixed infections with *Mesocestoides* sp. were observed in 3/22 (13.6%) of foxes. This finding is consistent with that of Bouchard and colleagues (2021), who documented *T. polyacantha* in 3/176 (2%) of foxes in Canada [37]. Also, a similar trend was observed in 197 red foxes in mid-Wales, with a 4% prevalence of *T. polyacantha*. In contrast, this cestode species was found in 166/269 red foxes (61.7%) during post-mortem examination in Lithuania, eastern Europe [38]. In the neighboring country, Turkey, *T. polyacantha* has been reported to occur at a prevalence of 13.6%-15.53% [30]. However, a highly variable prevalence rate of 1.1% to 70.4% has been reported for *T. polyacantha* globally [39, 40, 41]. The adult worm is equipped with 44-68 rostellar hooks; the TL of the large hooks discovered in the present study was within the range of previous iso-

lates (178-234 µm), whereas our isolates demonstrated shorter small hooks than previous ones (120-182 µm). Hook length is not reliable in differentiating *T. polyacantha* from other sympatric species (*T. hydatigena* and *T. crassiceps*); however, the former has more hooks with somewhat distinctive shapes [14].

Another finding of the present study was the isolation and molecular characterization of *T. hydatigena* in both foxes (n = 2; 9.1%) [34] and jackals (n = 2; 2.9%). The low prevalence observed in the present study is consistent with the study done by Meshgi et al. (2010) in various regions in Iran [24] and with the study done by Bouchard et al. (2021) in wild canids in Canada [37]. Nevertheless, this taeniid species has previously shown moderately high prevalence rates in Iran, such as northern areas (18.5% Guilan [3], ~30% Mazandaran [42]) and eastern parts (45% Sistan region [43], 43% Mashahd [44]) as well as in other countries such as Uzbekistan (57.4% in dogs) [45] and Kazakhstan (21.7% in red foxes) [46]. Definitive hosts acquire this cosmopolitan infection through ingestion of infected domestic livestock, particularly sheep carcasses harboring *C. tenuicolis* metacestodes. This metacestode can render mortality in young animals and cause significant organ damage, mainly by affecting the liver, omentum, mesentery, peritoneum, pleura, and other internal viscera [47, 48].

Since taeniid species possess homologous adult and egg morphological characteristics, they should be differentiated using molecular techniques. In this sense, different PCR-based and sequencing approaches have been developed. Mitochondrial DNA is particularly valuable because of their rapid evolutionary rate and high copy number, and it is of particular interest for the molecular identification of closely related organisms [49]. The mitochondrial cox1 gene is one of the well-known genetic markers for cestode differentiation [50]. In the present study, a 450 bp cox1 fragment of this marker was amplified using universal primers (JB3, JB4.5), followed by sequencing of four *T. polyacantha* and eight *T. hydatigena*. Of note, three specimens isolated from northwestern Iran were negative in repeated PCR analyses; this could be due to degraded DNA molecules during extraction. Our results indicated 100% and more than 99% identity in multiple alignments of the obtained partial cox1 gene sequences with the standard sequences previously deposited in GenBank.

This study confirms that *T. polyacantha* uses the red fox as its definitive host in Iran.

Phylogenetic analysis demonstrated that the Iranian isolates of *T. polyacantha* (n = 4) clustered with Finnish genotypes, while *T. hydatigena* isolates (n = 8) clustered with some genotypes from Iran, Iraq, and Slovakia in eastern Europe

Phylogenetic analysis indicated that *T. polyacantha* is closely related to *T. talicei* and more distantly related to *T. hydatigena*. These conclusions are corroborated by Gomez-Puerta [18]. Furthermore, *T. hydatigena* demonstrates a closer affinity to *T. lyncis-capreoli*, consistent with previous studies [51, 52].

Conclusion

The present study provides new insight into the molecular identification and phylogeny of Taenia species infecting wild canids in Iran. Most notably, it represents the first molecularly confirmation of *T. polyacantha* in Iranian foxes, indicating potential hotspots for the sylvatic circulation of this Taenia species.

Materials and Methods

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that no generative AI or AI-assisted technologies were used in the writing of this manuscript.

Ethical Considerations

During the study, proper precautions were taken to safeguard the researchers' health and safety. All procedures were conducted in accordance with the code of ethics (Ethical code: IR.UM.REC.1400.270).

Sample collection

This cross-sectional study involved necropsies of 93 road-killed wild canids collected between 2022-2024 in the country's northern, northeastern, and northwestern regions of Iran, including 69 jackals, 22 foxes, and 2 wolves. General characteristics such as location, date, animal species, sex, and age were documented for each animal.

Morphology and morphometry evaluation

Staining with acid carmine was performed after isolating the tapeworms from the wild canids' intestines [53, 54]. Species identification of the isolated cestodes was performed using established taxonomic key based on morphological and morphometric parameters, including the number and size of the hooks and the number of lateral uterus branches in the fertile proglottid. The following hook measurements were recorded in micrometers (µm): including total length (TL), anterior chord length (ACL), posterior chord length (PCL), blade length (BL), handle length (HL), and total width (TW). Morphometric analyses were conducted using AxioVision LE software. Measurements are presented as mean ± standard deviation (SD) [55, 56].

Molecular and phylogenetic analysis

DNA extraction was performed using a commercial DNA

extraction kit (MBST, Iran) to characterize Taenia species in accordance with the manufacturer's protocol. DNA concentration and purity were assessed using a NanoDrop spectrophotometer, and extracted DNA samples were stored at -20 °C until analysis. The universal primers (JB3, JB4.5) amplifying the 450 base pair (bp) fragment of the Mitochondrial Cytochrome C Oxidase Subunit 1 gene (cox1) of the helminths were used, as follows: JB3: 5'-TTTTTTGGGCATCCTGAGGTTTAT-3' and JB4.5: 5'-TAAAGAAAGAACATAATGAAAATG-3' [57, 58]. Polymerase chain reaction (PCR) mixture was prepared in a 25 µl volume, containing 12.5 µl of MasterMix, 2 µl of each primer (10 pmol), 1 µl of extracted DNA sample, and 9.5 µl of distilled water (DW). The PCR reaction was done in a Bio-Rad thermal cycler device, as follows: an initial denaturation step at 94 oC for 5 min; 40 cycles of denaturation at 94 oC for 30s; annealing at 52 oC for 45s); and extension at 72 oC for 35s); followed by a final extension at 72 °C for 10 min. PCR products were separated by electrophoresis on 1.5% agarose, and 450 bp bands were visualized under ultraviolet (UV) illuminator. The obtained amplicons were sequenced using the Applied Biosystems™ 3500 (Life Technologies) The ClustalW program was used to edit and align the sequences, while BLAST algorithms and the National Center for Biotechnology Information databases were utilized for sequence analysis. The MEGA v6.03 software generated a maximum-likelihood phylogeny, with Hymenolepis diminuta as the outgroup (Accession No.: KF689687).

Statistical analysis

Statistical analyses were performed using IBM® SPSS® software (Version 21, Armonk, NY USA). The Chi-square test was used to assess parasite distribution by gender, geographic area, and examined canine species. Statistical significance was set at $p < 0.05$.

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Authors' Contributions

Elahe Ebrahimzadeh conceived and planned the experiments. Zahra Sharifi Darvazeh carried out the experiments. Moein Abolhasani Darounkola contributed to sample preparation. Hassan Borji contributed to the interpretation of the results. Zahra Sharifi Darvazeh took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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Competing Interests

The authors declare that there is no conflict of interest.

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