

Molecular detection and COX1-based phylogenetic placement of *Dipylidium caninum* in stray dogs from Iğdır Province, eastern Türkiye

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Summary

Dipylidium caninum is a globally distributed zoonotic cestode commonly found in dogs and cats. Recent molecular studies have shown that the *D. caninum* complex includes largely host-associated canine and feline lineages, but molecular data from many endemic regions remain limited. In Türkiye, most prior reports have relied on conventional parasitological methods, and COX1-based data from dogs remain scarce. In this study, *D. caninum* from dogs in Iğdır Province, eastern Türkiye, was characterized using a partial mitochondrial COX1 marker.

Of 100 dog fecal samples examined, 8 were positive, yielding a molecular prevalence of 8 %. All positive samples shared the same COX1 haplotype, which was deposited in GenBank under accession number PZ299077.1. BLASTn analysis showed that *D. caninum* GenBank entries with identity values above 90 % were primarily dog-derived, whereas cat-associated sequences generally showed lower identity values. In the phylogenetic analysis, the Iğdır sequence was placed within the broader *D. caninum* assemblage. It showed affinity with dog-derived sequences from Türkiye, Ghana, the USA, Croatia and China, as well as bush dog-derived sequences from Colombia. However, dog- and cat-associated lineages were not completely separated in the analyzed dataset, and the placement was interpreted as molecular affinity rather than a definitive genotype-level assignment.

This study provides one of the first COX1-based molecular records of *D. caninum* from dogs in Iğdır Province and adds new mitochondrial data from an underrepresented region. Broader sampling and the inclusion of additional mitochondrial and nuclear markers will be important for clarifying the genetic diversity, transmission dynamics and zoonotic relevance of *D. caninum* in Türkiye.

Keywords: COX1; *Dipylidium caninum*; Phylogenetic analysis; Shepherd dogs; Türkiye

Introduction

Dipylidium caninum (Cestoda: Dipylidiidae) is a globally distributed tapeworm that inhabits the small intestine of domestic dogs and cats and may occasionally infect humans, particularly children (Bowman *et al.*, 2002; Jiang *et al.*, 2017). Given the document-

ed genetic heterogeneity and unresolved taxonomy within the *Dipylidium caninum* group, the lineage detected in the present study is hereafter referred to as *Dipylidium caninum* within the *D. caninum* species complex, unless otherwise specified. Transmission occurs through an indirect life cycle in which cysticercoids develop in flea intermediate hosts, mainly *Ctenocephalides felis*

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and *Ctenocephalides canis*, and definitive hosts become infected by ingesting infected fleas (Bowman *et al.*, 2002).

Although *D. caninum* is among the most frequently reported cestodes in companion animals, human dipylidiasis has traditionally been considered rare. However, confirmed human cases reported in Asia and Eastern Europe indicate that zoonotic transmission continues in regions with high flea exposure (Ramana *et al.*, 2011; Jiang *et al.*, 2017; Bronstein *et al.*, 2020). A recent global synthesis further supports the ongoing public health relevance of this parasite (Rousseau *et al.*, 2022).

Recent molecular studies have challenged the long-standing view of *D. caninum* as a genetically uniform species. Substantial mitochondrial divergence has been documented, and at least two host-associated genotypes, adapted to dogs and cats, respectively, have been proposed (Labuschagne *et al.*, 2018; Beugnet *et al.*, 2018). In addition, morphologically similar taxa such as *D. carracidoi* may have been historically misidentified as *D. caninum*, highlighting unresolved taxonomic complexity within Dipylidiidae (Millán & Casanova, 2009; Enítez-Bolívar *et al.*, 2022).

In Türkiye, numerous coprological surveys have reported *D. caninum* in both owned and shepherd dog populations across multiple provinces, with prevalence varying by geography, diagnostic approach and dog population structure (Yaman *et al.*, 2006; Orhun & Ayaz, 2006; Kozan *et al.*, 2007; Yıldırım *et al.*, 2007; Öter *et al.*, 2011; Işık *et al.*, 2014; Nas & Biçek, 2018; Karakuş & Denizhan, 2019; Ceylan *et al.*, 2024). These findings are consistent with earlier studies dating back to the late 1970s, indicating long-term endemic transmission in both rural and urban settings (Güralp *et al.*, 1977; Taşan, 1983; Tınar *et al.*, 1989; Doğanay & Öge, 1993; Şahin *et al.*, 1993; Aydenizöz, 1997; Ayçiçek *et al.*, 1998).

Despite this extensive parasitological background, molecular data on *Dipylidium caninum* in dogs from Türkiye remain scarce. Although recent work has demonstrated the presence of *D. caninum* DNA in flea vectors (Özdemir *et al.*, 2025), molecular prevalence and sequence-based characterization studies in canine hosts are still limited, particularly at the regional level. In eastern Türkiye, previous studies have mainly focused on the molecular epidemiology of zoonotic and parasitic agents in animals and environmental samples, highlighting their prevalence and potential public health significance (Ertaş *et al.*, 2022; Ertaş & Ayan, 2022; Kılınç *et al.*, 2023; Koca *et al.*, 2023; Akkuş & Ertaş Oğuz, 2024; Ertaş Oğuz *et al.*, 2025). However, molecular information on Dipylidiidae cestodes in shepherd dog populations from this region remains limited.

Therefore, the present study aimed to investigate the molecular presence of Dipylidiidae cestodes in shepherd dogs from Iğdır Province, eastern Türkiye, using a COX1-based PCR approach and to characterize the detected lineage through partial COX1 gene sequencing. The resulting data provide new molecular evidence from an under-studied region and contribute to a better understanding of *Dipylidium* diversity and transmission dynamics in shepherd dog populations.

Materials and Methods

Sample Collection

In 2023, a total of 100 fecal samples were collected from shepherd dogs in Iğdır Province, eastern Türkiye. Before sampling, dog owners were informed about the study, and voluntary consent was obtained. During field visits, dogs were evaluated individually with assistance from their owners. Fresh fecal samples were collected non-invasively either directly from the ground when they could be attributed to the relevant dog or immediately after observed defecation. To prevent sample mixing, each sample was collected using disposable gloves, placed into a separate leak-proof container, and labeled with a unique sample code. Age, sex, and flock/farm information were also recorded. The samples were first transported to the laboratory of Iğdır University Animal Hospital and then transferred under cold-chain conditions to the Department of Genetics, Faculty of Veterinary Medicine, Van Yüzüncü Yıl University, for molecular analyses.

DNA extraction

Genomic DNA was extracted from approximately 200 mg of each fecal sample using the GeneMATRIX Stool DNA Purification Kit (Eurz, Poland), following the manufacturer's protocol. DNA concentration and purity were assessed spectrophotometrically, then all extracts were stored at -20 °C until PCR analysis.

PCR amplification

A 446-bp fragment of the mitochondrial cytochrome c oxidase subunit I (COX1) gene was amplified using the primer pair JB3 and JB4.5 described by Bowles *et al.* (1992). The primer sequences were as follows:

- JB3 (forward): 5'-TTTTTTGGGCATCCTGAGGTTTAT-3'
- JB4.5 (reverse): 5'-TAAAGAAAGAACATAATGAAATG-3'

PCR reactions were performed in a final volume of 25 µL containing 200 µM of each dNTP, 1.5 mM MgCl₂, 10 pmol of each primer, 1.25 U Taq DNA polymerase, 1× PCR buffer, nuclease-free water, and 2 µL of template DNA. Amplification was carried out in a Veriti™ Thermal Cycler (Applied Biosystems) with the following cycling conditions: initial denaturation at 95 °C for 15 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 45 °C for 1 min, and extension at 72 °C for 1 min, with a final extension step at 72 °C for 10 min. PCR products were resolved by electrophoresis on 1.5 % agarose gels stained with Safe-T-Stain, run at 90 V for 60 min, and visualized under UV light using a Syngene Bio-Imaging System.

Sequencing and sequence quality control

PCR amplicons that produced clear bands of the expected size were purified and bidirectionally sequenced by BM Labosis Laboratory (Ankara, Türkiye). Forward and reverse chromatograms (.ab1 files) were examined in MEGA11 (Tamura *et al.*, 2021), and base calls were manually inspected. Only sequences with clear

chromatogram peaks and agreement between forward and reverse reads at all variable positions were used for further analyses. A 393-bp consensus *COX1* sequence was generated from the forward and reverse reads.

BLAST analysis

The consensus *COX1* sequence was searched against the NCBI nucleotide database using BLASTn to assess molecular similarity with reference sequences. BLASTn hits were evaluated according to query coverage, percentage identity, E-value, accession number, host, country of origin, submission date, and reference/source information. The relevant BLASTn matches to *Dipylidium caninum* and related cestode sequences are presented in Supplementary Table 1.

Multiple sequence alignment

Selected reference *COX1* sequences of *D. caninum* and related dipylidiid taxa were retrieved from GenBank. All sequences were combined into a single FASTA dataset. The sequences were first

checked, and multiple sequence alignment was then performed using MAFFT with the “adjust direction” option. Poorly aligned, ambiguous, and non-overlapping regions were removed to improve alignment quality, resulting in a final alignment of 358 bp.

The accession numbers and metadata of these sequences are listed in Supplementary Table 1. ON981095 (*Joyeuxiella pasqualei*) was used as the outgroup.

Phylogenetic analysis

Phylogenetic analysis was performed in IQ-TREE v3 using the Maximum Likelihood method. The best-fit nucleotide substitution model was selected with ModelFinder Plus, and the GTR+I+G model was used for tree construction. Branch support was assessed with 1,000 ultrafast bootstrap replicates and 1,000 SH-aLRT replicates. The resulting tree was rooted using *Joyeuxiella pasqualei* ON981095 as the outgroup and visualized in FigTree v1.4.5. Branch values are shown as SH-aLRT support (%) / ultrafast bootstrap support (%).

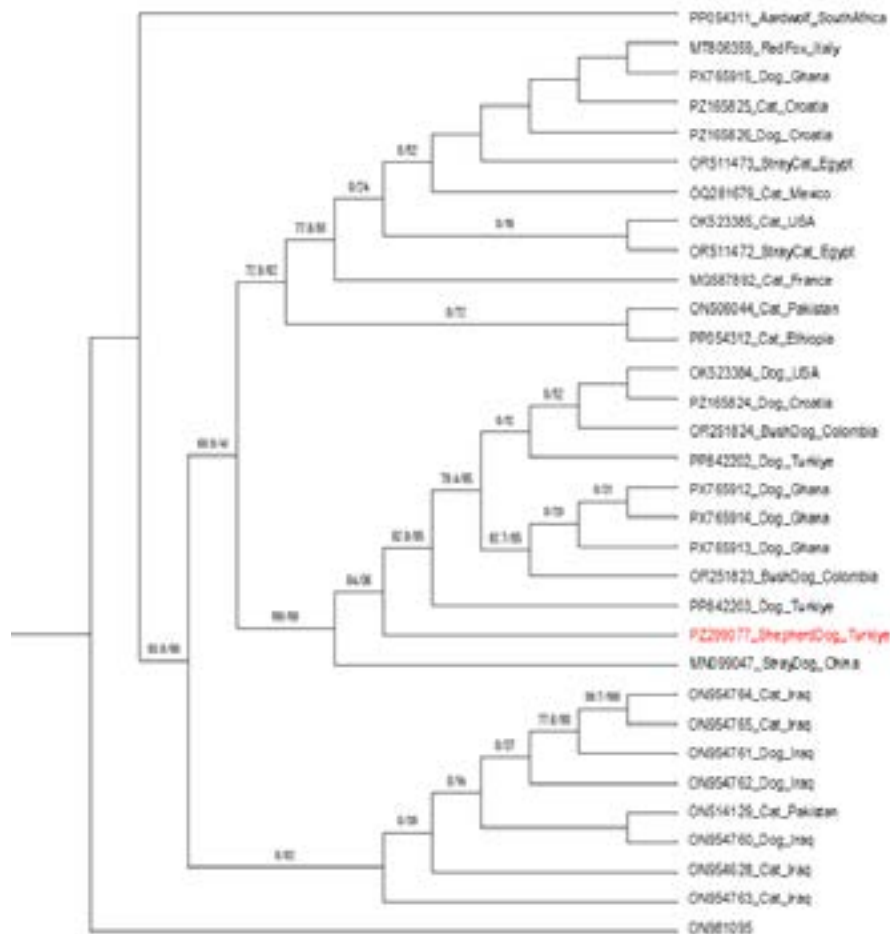


Fig. 1. Maximum Likelihood phylogenetic tree based on a 363-bp *COX1* alignment of *Dipylidium caninum* and related Dipylidiidae sequences.

The tree was reconstructed in IQ-TREE v3 using the GTR+I+G substitution model and rooted with *Joyeuxiella pasqualei* ON981095 as the outgroup. Branch values are shown as SH-aLRT support (%) / ultrafast bootstrap support (%) based on 1,000 replicates. The Turkish isolate generated in this study, PZ299077.1, is highlighted in red.

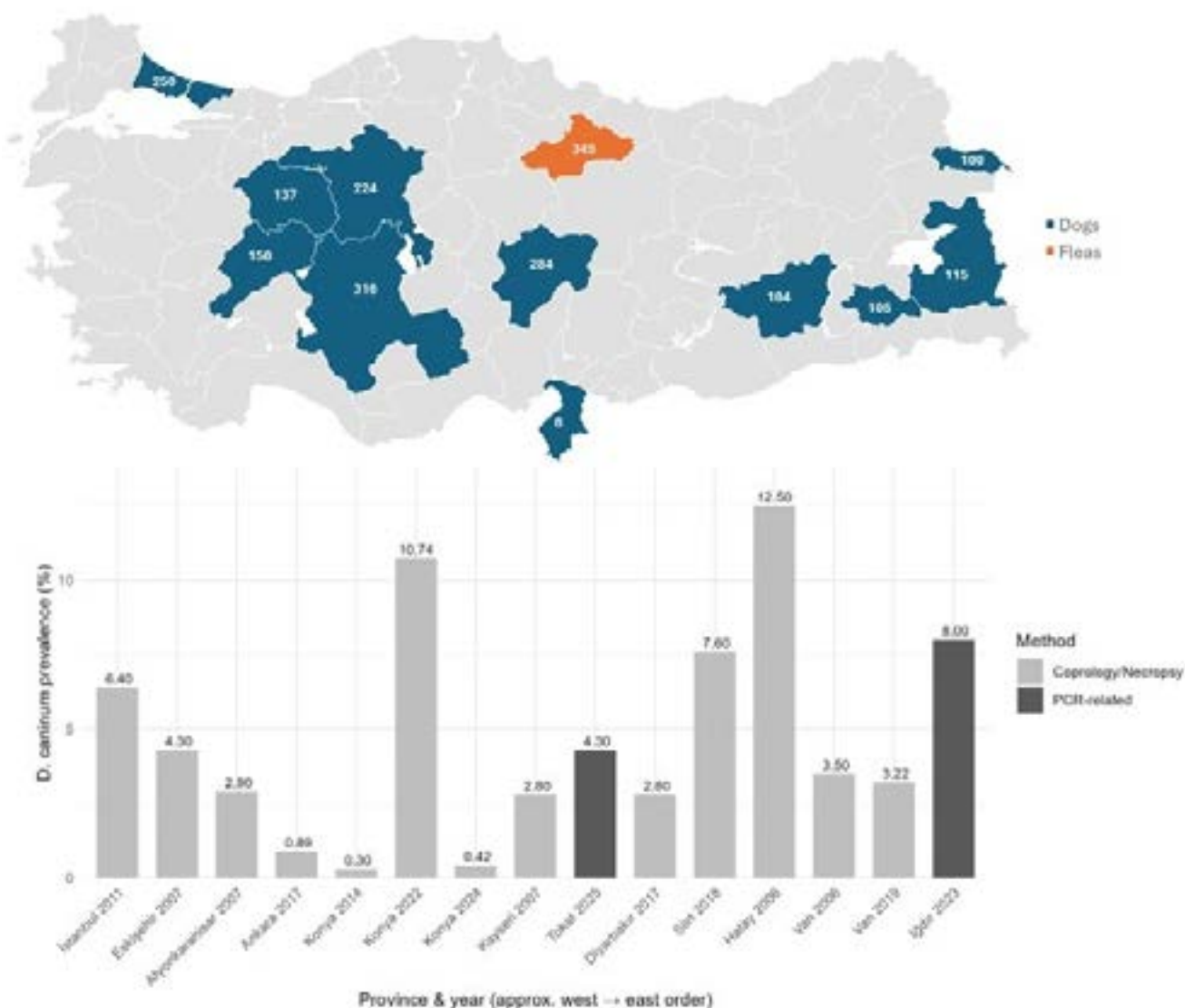


Fig. 2. Geographic and temporal distribution of *Dipylidium caninum* in Türkiye.

The map and accompanying bar plot summarize published prevalence data from 2006 to 2025 across different provinces of Türkiye. Coprological and necropsy-based surveys are shown in light shading, whereas PCR-based studies are shown in dark shading. The figure illustrates the long-term and widespread circulation of *D. caninum* in both western and eastern regions of the country.

Literature data extraction

To place the findings from İğdır in a broader epidemiological context, published prevalence data for *Dipylidium caninum* infections in dogs were compiled from the literature. For each country, mean prevalence values were calculated across available studies irrespective of diagnostic method, and these country-level estimates were subsequently grouped to generate continent-level summaries. Although this approach does not constitute a formal meta-analysis, it provides a comparative framework for interpreting regional variation and situating the İğdır data within global transmission patterns. A descriptive overview of the global prevalence of *D. caninum* in dogs is presented in Supplementary Figure S1.

Data handling and visualization were performed using R statistical software (R Core Team, 2025).

Ethical Approval and/or Informed Consent

Permission was obtained from the animal owners for this study. Additionally, fecal samples were collected from the ground without coming into contact with the animals. According to Turkish HADYEK (Local Ethics Committee for Animal Research) regulation, fecal sample collection is not subject to ethics committee approval when no animal handling or intervention is performed. Therefore, an ethics committee approval number was not required for this study.

Results

Out of 100 screened fecal samples, eight (8 %) yielded a PCR product of the expected size for the COX1 gene fragment. All positive samples produced identical sequences across the analyzed region, indicating the presence of a single mitochondrial haplotype among the infected dogs.

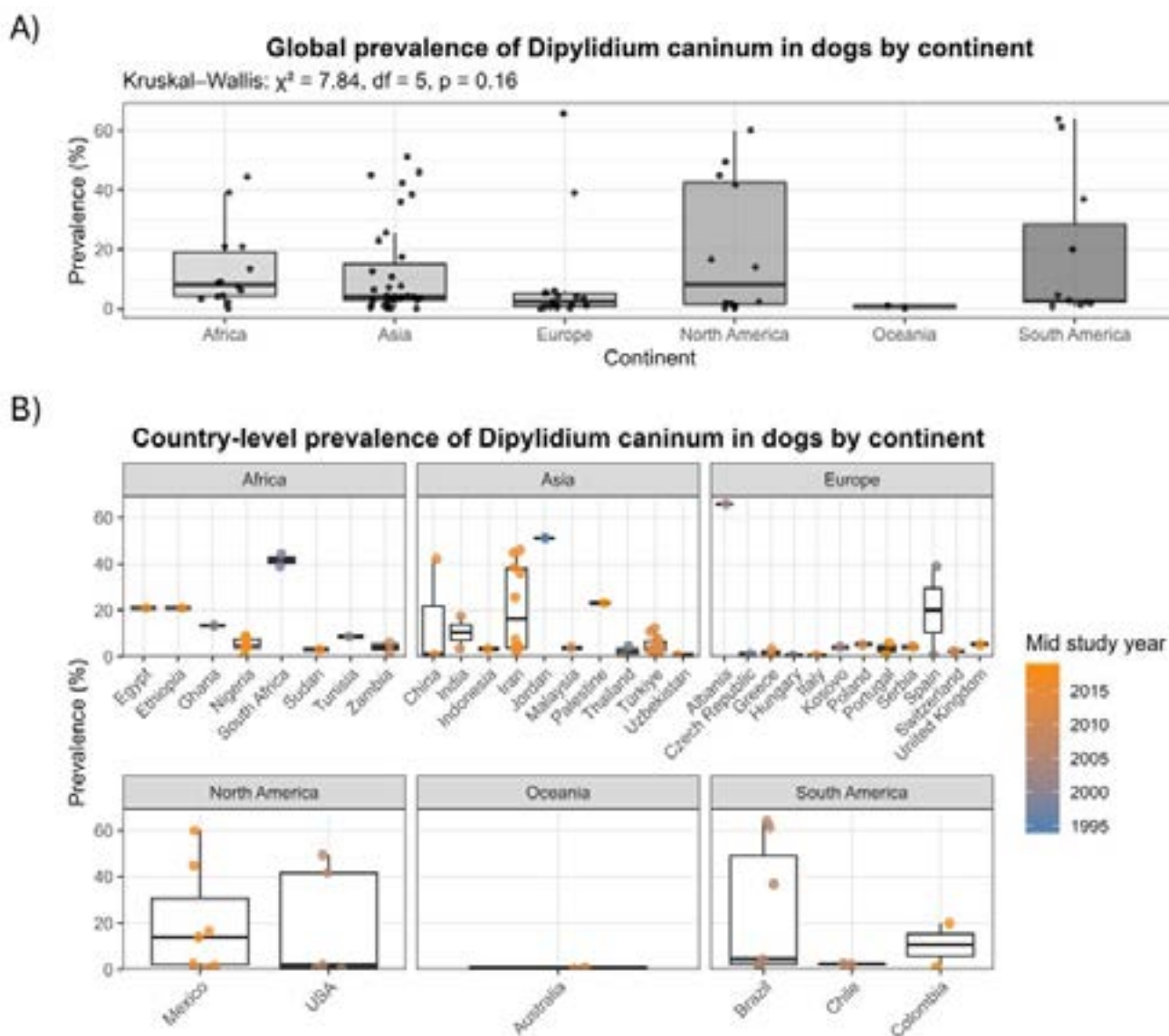
The obtained 396 bp partial mitochondrial COX1 sequence of *Dipylidium caninum* isolate Igdir_Dog1 from a shepherd dog in Igdir Province, Türkiye, was deposited in GenBank under accession number PZ299077.1.

BLASTn analysis showed that the sequence matched *D. caninum* entries deposited in GenBank, including partial COX1

sequences and complete mitochondrial genome records. The highest identity was observed with a dog-derived sequence from Ghana (PX765914.1; 92.12 % identity, 93 % query cover), followed by bush dog-derived Colombian sequences (OR251824.1 and OR251823.1), a canine complete mitogenome from the USA (OK523384.1), and a dog-derived Croatian sequence (PZ165824.1), each showing 91.92 % identity and 100 % query cover. Turkish dog-derived sequences from Elazığ (PP842203.1 and PP842202.1) showed 91.27 % identity with 95 % query cover. Cat-associated sequences were also included in the comparison and showed lower identity values, ranging approximately from 88.71 % to 89.80 %. The full BLASTn comparison is provided in Supplementary Table 1.

Supplementary Table S1. BLASTn comparison and phylogenetic dataset of *Dipylidium caninum* COX1 sequences

No.	Accession	Isolate / description	Host	Country	Identity (%)	Query cover	Sequence length (bp)
1	PZ299077.1	<i>D. caninum</i> isolate Igdir_Dog1	Shepherd dog / <i>Canis lupus familiaris</i>	Türkiye	100	100 %	396
2	PX765914.1	<i>D. caninum</i> isolate Hp03	Dog	Ghana	92.12	93 %	373
3	OR251824.1	<i>D. caninum</i> isolate Sv_PS_Amz_22	<i>Speothos venaticus</i> / bush dog	Colombia	91.92	100 %	1602
4	OR251823.1	<i>D. caninum</i> isolate Sv_PS_Amz_21	<i>Speothos venaticus</i> / bush dog	Colombia	91.92	100 %	1602
5	OK523384.1	<i>D. caninum</i> isolate Dcan_canine_FL1, complete mitogenome	<i>Canis lupus familiaris</i>	USA	91.92	100 %	14296
6	PZ165824.1	<i>D. caninum</i> isolate DICA	<i>Canis lupus familiaris</i>	Croatia	91.92	100 %	396
7	PX765913.1	<i>D. caninum</i> isolate Hp02	Dog	Ghana	91.85	93 %	373
8	PX765912.1	<i>D. caninum</i> isolate Hp01	Dog	Ghana	91.85	93 %	373
10	MN099047.1	<i>D. caninum</i> complete mitogenome	Stray dog	China	91.67	100 %	14226
11	PP842203.1	<i>D. caninum</i> isolate DogSRTDc2	Dog	Türkiye	91.27	95 %	383
12	PP842202.1	<i>D. caninum</i> isolate DogSRTDc1	Dog	Türkiye	91.27	95 %	383
13	MG587892.1	<i>D. caninum</i> isolate R166, complete mitogenome	Feline	South Africa	89.8	99 %	13598
14	OQ281679.1	<i>D. caninum</i> isolate VCG6	<i>Felis catus</i>	Mexico	89.8	99 %	416
15	OK523385.1	<i>D. caninum</i> isolate Dcan_feline_KS1, complete mitogenome	<i>Felis catus</i>	USA	89.8	99 %	13598
16	MT806359.1	<i>D. caninum</i> voucher 4245P18	<i>Vulpes vulpes</i> / red fox	Italy	89.8	99 %	411
17	PX765915.1	<i>D. caninum</i> isolate Hp04	Dog	Ghana	89.67	93 %	373
18	OR511473.1	<i>D. caninum</i> isolate Alex-2	Stray cat	Egypt	89.53	92 %	363
19	PZ165825.1	<i>D. caninum</i> isolate DICAB.1	<i>Felis catus</i>	Croatia	89.39	90 %	358
20	ON506044.1	<i>D. caninum</i> isolate lsb1	Cat	Pakistan	89.34	92 %	384
21	PP054312.1	<i>D. caninum</i> haplotype A	<i>Felis catus familiaris</i>	Ethiopia	89.27	96 %	963
22	OR511472.1	<i>D. caninum</i> isolate Alex-1	Stray cat	Egypt	88.71	92 %	363
23	ON954760.1	<i>D. caninum</i> isolate dog1	Dog	Iraq	88.01	92 %	384
24	ON954628.1	<i>D. caninum</i> isolate cat1	Cat	Iraq	88.01	92 %	384
25	ON514129.1	<i>D. caninum</i> isolate lsb2	Cat	Pakistan	88.01	92 %	384
26	ON954763.1	<i>D. caninum</i> isolate cat1	Cat	Iraq	87.91	92 %	383
27	ON954761.1	<i>D. caninum</i> isolate dog2	Dog	Iraq	86.58	92 %	384
28	PP054311.1	<i>Dipylidium</i> sp. 01-SA haplotype A	<i>Proteles cristata</i> / aardwolf	South Africa	85.86	100 %	1647
29	PZ165826.1	<i>D. caninum</i> isolate DICAB.2	<i>Canis lupus familiaris</i>	Croatia	—	—	358
30	ON954762.1	<i>D. caninum</i> isolate dog3	Dog	Iraq	—	—	384
31	ON954764.1	<i>D. caninum</i> isolate cat2	Cat	Iraq	—	—	384
32	ON954765.1	<i>D. caninum</i> isolate cat3	Cat	Iraq	—	—	384



Supplementary Fig. S1. Global prevalence of *Dipylidium caninum* in dogs.

Reported canine *D. caninum* prevalence data from previously published studies were descriptively summarized by continent and country to provide broad epidemiological context. (A) Prevalence values across continents. Box plots show the median, interquartile range, and individual study points. (B) Country-level prevalence grouped by continent. Colored points represent individual studies according to approximate mid-study year. No molecular sequence data were included in this overview. Because the included studies differed in sampling design, dog population, geographic setting, and diagnostic method, this figure should be interpreted only as descriptive context and not as a formal quantitative comparison among regions.

Discussion

The present study provides molecular evidence of *Dipylidium caninum* in dogs from Iğdır Province, eastern Türkiye, and represents one of the first COX1-based molecular characterizations of Dipylidiidae in this region. Although *D. caninum* has long been reported in Türkiye using conventional parasitological methods, molecular data remain limited. Two unpublished GenBank records from Elazığ (PP842202.1 and PP842203.1; Aslan Celik *et al.*, GenBank direct submissions) and the molecular detection of *D.*

caninum DNA in fleas from Tokat indicate that molecular evidence from Türkiye is gradually increasing (Özdemir *et al.*, 2025). In this context, the Iğdır sequence reported here adds new COX1 data from eastern Türkiye and helps expand the limited molecular dataset available for Türkiye.

In the phylogenetic tree, the analyzed *D. caninum* sequences were arranged into three broad groups, but the separation between dog- and cat-associated lineages was unclear (Fig. 1). The first group mainly contained cat-associated and mixed-host sequences from South Africa, Mexico, the USA, Italy, Egypt, Cro-

atia, Pakistan and Ethiopia (Labuschagne *et al.*, 2018; Jesudoss Chelladurai *et al.*, 2023; Camacho-Giles *et al.*, 2024; Citterio *et al.*, 2021; Satour *et al.*, 2026; Beck & Šikić, 2026; Alvi *et al.*, 2022; Dumendiak *et al.*, 2024). This group also included some non-cat hosts, suggesting that host origin alone may not fully explain the observed phylogenetic pattern. Dogs dominated the second group and other canine-derived sequences from Ghana, Colombia, the USA, Croatia, China and Türkiye (Xie *et al.*, 2019; Uribe *et al.*, 2023; Jesudoss Chelladurai *et al.*, 2023; Addy *et al.*, 2026; Beck & Šikić, 2026; Aslan Çelik *et al.*, 2024). The Iğdır isolate PZ299077.1 was positioned within this broad canine-derived group, together with the previously submitted Turkish dog sequences from Elazığ and several dog or wild-canid sequences from other regions. This placement indicates that the Iğdır sequence is not isolated from the global *D. caninum* dataset, but falls within a group largely represented by canine hosts. The third group included mainly Iraqi and Pakistani sequences obtained from both dog and cat hosts, forming a mixed regional cluster (Al-Ardi, 2022; Alvi *et al.*, 2022). This pattern may reflect regional sequence similarity rather than a strict host-associated structure. Overall, the phylogenetic pattern supports the identification of the Iğdır isolate *D. caninum* and shows that it contributes new eastern Turkish COX1 data to the limited molecular records available from Türkiye. However, because several branches showed low to moderate support values and the analyzed COX1 fragment was relatively short, the observed clustering should be interpreted cautiously rather than as definitive evidence of host-specific lineages.

Previous molecular and genomic studies have shown that *D. caninum* contains largely host-associated canine and feline lineages, with marked genetic differentiation between these groups (Labuschagne *et al.*, 2018; Rousseau *et al.*, 2022; Jesudoss Chelladurai *et al.*, 2023). However, host association is not strict, and dogs may carry parasites related to different genotype groups. Recent data from Ghana also reported both canine and feline-canine-associated genotypes, although most dog-derived isolates were assigned to the canine genotype (Addy *et al.*, 2026). Therefore, the placement of PZ299077.1 near Ghanaian dog-derived sequences and other canine-host records supports its affinity with the canine-derived group. Considering the known mitochondrial diversity within the *D. caninum* complex, the relatively low COX1 identity values observed here do not preclude this placement. However, because the analysis was based on a partial COX1 fragment, the result should be interpreted as molecular placement within the canine-derived group rather than as a final genotype-level conclusion.

Earlier parasitological surveys conducted in different provinces of Türkiye have repeatedly reported *D. caninum* in dogs using copromicroscopic examination or necropsy-based approaches, with prevalence values varying widely depending on geographic region, dog population structure, and diagnostic methodology (Güralp *et al.*, 1977; Taşan, 1983; Tınar *et al.*, 1989; Doğanay & Öge, 1993; Aydenizöz, 1997; Ayçiçek *et al.*, 1998; Yaman *et al.*,

2006; Kozan *et al.*, 2007; Yıldırım *et al.*, 2007; Öter *et al.*, 2011; Işık *et al.*, 2014; Nas & Biçek, 2018; Karakuş & Denizhan, 2019; Ceylan *et al.*, 2024). However, most of these investigations lacked molecular confirmation, leaving uncertainties regarding species identity and the extent of genetic diversity within *Dipylidiidae*. The national overview presented in Figure 2 supports this pattern. It shows that *D. caninum* has been reported from both the western and eastern regions of Türkiye, using both dog-based surveys and flea-based molecular detection. The 5 % molecular prevalence detected in Iğdır therefore adds PCR-based evidence from eastern Türkiye and supports the need for further molecular surveillance. Additionally, the Tokat flea-based PCR record was considered as supporting evidence for transmission rather than as directly comparable dog prevalence data (Özdemir *et al.*, 2025).

In conclusion, this study provides a new COX1-based molecular record of *D. caninum* in dogs from Iğdır Province, eastern Türkiye. It adds new mitochondrial data to the limited molecular dataset currently available from Türkiye. The analyzed sequence showed affinity with canine-derived *D. caninum* sequences in the phylogenetic analysis and contributes additional information to the growing body of evidence for genetic diversity within the *D. caninum* complex. In line with recent studies, the present findings also highlight the need for broader sampling and the inclusion of additional mitochondrial and nuclear markers from underrepresented regions to understand better the evolutionary structure and global diversity of this parasite. From a One Health perspective, continued molecular surveillance of dogs, together with monitoring of flea intermediate hosts, may contribute to an improved understanding of the transmission dynamics and zoonotic risk associated with canine dipylidiasis.

Author Contributions

Fatma Ertaş Oğuz: Study design, field sampling, laboratory work, and final review of the manuscript. Hasret Öztürk: GenBank data handling, data analysis, figure preparation, and manuscript writing and revision. Adnan Ayan: Laboratory work, data analysis, and final review of the manuscript.

Data Availability

The COX1 sequence generated in this study has been deposited in GenBank under accession number PZ299077.1.

Conflict of Interest

The authors declare no conflicts of interest.

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