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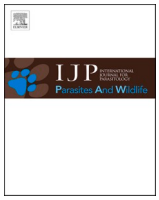

Prevalence, molecular characterisation and phylogenetic analyses of hydatid cysts and cysticercus tenuicollis isolates and first report of *E. canadensis* (G6/G7) in wild boars in Bingol province, Türkiye

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ABSTRACT

Wild boars can directly or indirectly transmit numerous zoonotic helminths to humans in rural communities as they serve as reservoir hosts. This study was conducted to determine the occurrence and molecular characterisation of cystic metacestode forms of helminth parasites in wild boar and to identify haplotypes. To this end, 23 wild boar carcasses shot by hunters during the 2023 hunting season were necropsied and all internal organs were examined. Cysticercus tenuicollis (n = 07) and hydatid cyst (n = 10) isolates were obtained from the examined boars. Species identification of Cysticercus and hydatid cyst isolates was performed by amplification of partial fragments of the *cox1* gene. According to BLAST search, all sequences of C. tenuicollis isolates were identified as *Taenia hydatigena*. Out of the hydatid cyst isolates, seven were classified as *Echinococcus granulosus sensu stricto* (G1/G3) and one sample was identified as *Echinococcus canadensis* (G6/G7). All isolates of *E. granulosus s.s.* (G1/G3) were re-amplified with the NADH dehydrogenase subunit 5 (*nad5*) in order to distinguish between G1 and G3 genotypes. Based on the sequence analysis, it was found that five of the *E. granulosus s.s.* isolates were classified as G1, while two were classified as G3. Based on the results of this study, it can be concluded that the G1 genotype is the most prevalent genetic variant among wild boar populations in Bingol province, Türkiye. In this study, a total of five novel haplotypes were identified. A previously unidentified haplotype was revealed through the haplotype analysis of *E. canadensis* (G6/G7). All isolates in the haplotype network of *T. hydatigena* were shown to exhibit distinct and geographically specific haplotypes. According to the findings of the study, wild boars include a substantial amount of genetic variety in *E. granulosus s.s.* And *T. hydatigena*.

1. Introduction

The wild boar (*Sus scrofa*) is a species of wild animal that is hunted for nourishment and physical activity worldwide (Sales and Kotrba, 2013). Nevertheless, engaging in hunting activities without implementing adequate hygiene protocols can potentially expose humans and other household animals to the transfer of pathogens. (De-la-Rosa-Arana et al., 2021). In Türkiye, the hunting of wild boars is carried out in a controlled manner within certain rules, as stipulated by the Land Hunting Law No. 4915 (KAK), which came into force in 2003 (Legal Gazette, 2003). There are wild boar groups in steppes and forests all over the world. The European wild boar species, *Sus scrofa*, is found all over Eurasia, including in Türkiye (Groves, 1981; Lahmar et al., 2019). Wild boars consistently engage in geographical expansion, demonstrating remarkable versatility in terms of dietary preferences and habitat suitability, in addition to their substantial reproductive capabilities. (Fredriksson-Ahomaa, 2019). According to Rossi et al. (2015), wild boars were responsible for 85% of agricultural damages between 2005 and 2009 and served as a reservoir of viruses, bacteria and parasites by hosting zoonotic diseases. Additionally, Jones et al. (2008) and Figueiredo et al. (2020) reported that 71.8% of current zoonotic diseases originate from wildlife, making them an important part of infectious disease surveillance. Wild boars have the potential to serve directly and/or indirectly as reservoir hosts for numerous zoonotic helminths that are independent of domestic cycles. People in rural areas may become infected with these parasites as a result of this. Therefore, disease monitoring and diagnostic studies carried out in wild species are essential for implementing health interventions for both humans and

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animals. (Vieira-Pinto et al., 2011). Studies are underway in numerous countries worldwide to assess the risk of pathogen transmission in both domestic and wild pig populations. The studies conducted by Fernandez-de-Mera et al. (2003), Fredriksson-Ahomaa (2019), Maleki et al. (2020), and Petersen et al. (2020) are relevant to this topic. Various stages of helminth development are hosted by wild boars, which are of significant importance in public health and veterinary matters (De-la-Rosa-Arana et al., 2021). Wild boars typically harbour adult specimens of *Ascaris suum, Trichuris suis,* and *Dicrocoelium dendriticum*, as well as larvae of species such as *Taenia solium*, *T. hydatigena*, *E. granulosus s.l., E. multilocularis*, and *Trichinella spiralis* (Yagoob et al., 2014; Enemark et al., 2015; De-la-Rosa-Arana et al., 2021). Previous studies have reported molecular evidence of *E. granulosus s.s.* (1/1) (G1/G3) (Kesik et al., 2021) and *E. multilocularis* (1/1) (Kesik et al., 2020) in wild boars in Elazig province, eastern Türkiye. Another study examined the helminth fauna in wild boars and reported a 4% (1/27) prevalence of *T. hydatigena* in Bursa province, Marmara region in Türkiye (Senlik et al., 2011).

The organization of the haplotype network in ethnically varied groups is influenced by mutations that provide insights into demographic history and evolutionary mechanisms, including recombination, gene conversion, and selection. Single mutations and their density also answer how complex disease alleles depend on both evolutionary and demographic history (Tishkoff and Verrelli, 2003). Determining the genotypes and haplotypes of helminth larvae found in wildlife is crucial for understanding the evolutionary history of parasites. The current study aims to molecularly characterise and haplotype the cystic metacestode forms of some helminth parasites found in wild boar.

2. Materials and methods

2.1. Study area and sample collection

For this study, abdominal and thoracic necropsies of 23 wild boars were used that were killed by hunters in the countryside of Türkiye's Bingol province (38°27′–40°27′N, 41°20′–39°54′E) in 2023 hunting season (26 August- 3 March) were performed and examined for metacestodes of helminth parasites. Necropsies were performed on the wild boars' abdomens and chests at the hunting location, and a thorough examination of the internal organs was conducted to identify any metacestodes of helminth parasites. The detected cystic structures were collected by sectioning the relevant organ and transported to the laboratory in organ transport containers. The germinal membranes of the hydatid cysts and the whole cysticercus cysts were numbered and stored separately in falcon tubes containing 70% ethanol at −20 °C for molecular identification.

2.2. Molecular analyses

The total genomic DNA isolation was performed using the germinal layers of the hydatid cysts and the scoleces of cysticercus larva using with the PureLink™ Genomic DNA Mini Kit (Invitrogen™, Thermo Fisher Scientific, Missouri, TX, USA). The cyst materials were rinsed five times in sterile PBS (pH = 7.4) to eliminate any remaining ethanol. The 875 bp region of the *cox1* gene was amplified by the PCR technique using the primer set F/CO1 (5′-TTGAATTTGCCACGTTTG AATGC-3′) and R/CO1 (5′-GAACCTAACGACATAACATAATGA-3′), as previously reported by Nakao et al. (2000). To differentiate between G1/G3 strains of *Echinococcus granulosus s.s.*, we performed a PCR and subsequent sequence analysis utilizing EGnd5F1 (5′-GTTGTTGAAGTTGATTGTTTTGTTTG-3′) and EGnd5R1 (5′-GGAACAC CGGACAAACCAAGAA-3′) primers. The provided primers are designed to amplify a specific section of the mitochondrial *nad5* gene, which is 759 bp in length (Kinkar et al., 2018).

In order to characterise the molecular composition of C. tenuicollis isolates, an 866 bp region of the *cox1* gene was amplified utilizing the ThF (5′-TGCATTTAGCTGGTGCGTCA-3′) and ThR (5′-CCGGGGTAACC-CACAAG-3′) primer sets (Karakoc et al., 2024).

The PCR was performed using a 50 µL reaction mixture containing 5 µL of 10X PCR buffer, 5 µL of 25 mM MgCl2, 400 µM of each deoxyribonucleotide triphosphate (dNTP), 20 pmol of each primer, 0.2 µL of Taq DNA polymerase (1.25 IU) from Hibrigen in Türkiye, 28.8 µL of PCR-grade water, and 5 µL of the template genomic DNA (gDNA). The products were segregated using agarose gel (1.5%) electrophoresis. The PCR conditions for the *cox1* gene area of C. tenuicollis isolates are as follows: the first denaturation step at 94 °C for 5 min, afterwards 35 cycles of 30 s at 94 °C, 45 s at 60 °C, and 35 s at 72 °C. Following the previous cycle, it underwent a concluding elongation at a temperature of 72 °C for a duration of 10 min. The polymerase chain reaction (PCR) settings used for amplifying the *cox1* gene area of hydatid cyst isolates were as follows: an initial denaturation step at 94 °C for 10 min, followed by 30 cycles consisting of 30 s at 94 °C, 45 s at 52 °C, and 1 min at 72 °C. The last phase is carried out at a temperature of 72 °C for a duration of 10 min. The PCR methodology previously published by Kinkar et al. (2018) for the *nad5* gene area was optimized and employed. The procedure involves an initial denaturation step at a temperature of 95 °C for a duration of 5 min. This is followed by 35 cycles, each consisting of 25 s at 95 °C, 45 s at 55 °C, and 1 min at 68 °C. The last prolongation at a temperature of 68 °C for a duration of 6 min.

The gel was observed employing RedSafe (iNtRON Biotech, South Korea). The PCR products were purified and subjected to unidirectional sequence analysis using the sense primer set (BM Labosis, Ankara, Türkiye). The obtained findings were then submitted in the GenBank database.

2.3. Sequence, alignment and phylogenetic analysis

All the sequence results were analyzed using FinchTV 1.4.0 software (Geospiza Inc., Seattle Washington, USA). Subsequently, a BLAST search was conducted on the sequence results using the National Center for Biotechnology Information genome database (http://www.ncbi.nlm.nih.gov). The sequence data were then aligned using MEGA X (Kumar et al., 2018) with the Clustal W module for nucleotide sequence alignment. For the *T. hydatigena* phylogenetic tree module, previously published sequences of *T. solium*, *T. saginata*, and *E. granulosus* (Accession Numbers: AB271234, JN986693, and MW138947, respectively) were used as outgroups. In the phylogenetic tree created for the *E. granulosus* species, *T. solium* (Accession Number: AB524785) was used as an outgroup. The phylogenetic tree module of the *nad5* gene region was constructed using *E. multilocularis* (Accession Number: AB018440), *T. asiatica* (Accession Number: AF445798), and *T. saginata* (Accession Number: PP391461) as outgroups.

Distance-based analyses were performed using the MEGA X program, and the phylogenetic tree was created using the Maximum Likelihood algorithm. Bootstrap analyses were performed with 1000 repetitions.

2.4. Haplotype network and nucleotide sequence variation, diversity and neutrality indices

The data sets used in the phylogenetic analyses were also used for the haplotype network analysis. The DnaSP6 software was used to calculate haplotype numbers (h), nucleotide diversity (π), haplotype diversity (Hd), population diversity indices, and neutrality indices. (Rozas et al., 2017). A haplotype network was constructed using the isolates in this study and their sequences retrieved from the GenBank database. Sequences included in the haplotype analysis and Genbank accession numbers are provided in the Supplementary file. The network was generated with PopART-1.7 software using the Minimum Spanning Networks (MSN) method (Leigh et al., 2015).

3. Results

3.1. Molecular characterisation

During the examination of wild boars (n = 23), a total of nine hydatid cysts (Fig. 1A and B) and seven C. tenuicollis (Fig. 1C and D) were detected. The cysticercus cysts were located in the mesenterium while with five of the hydatid cyst isolates found in the lung and three in the liver. The PCR amplification of the retained part of the *cox1* gene for cysticercus isolates resulted in a DNA fragment of 866 bp in all samples and 875 bp in eight of the hydatid cyst isolates. Based on the BLAST search of cysticercus isolates, all sequences were identified as *T. hydatigena*. Out of the hydatid cyst isolates examined using BLAST, seven isolates were determined to be *E. granulosus s.s.* (G1/G3), one sample was identified as *E. canadensis* (G6/G7), and another one isolate was not able to be amplified by PCR. One sample (WB5-Ez) did not exhibit a band in the PCR analysis for the *cox1* gene region, whereas a band was observed in the PCR analysis for the *nad5* gene. The sequences of the isolates were taxonomically identified and submitted to the NCBI database (Table 1).

3.2. Sequence, alignment and phylogenetic analysis

The most appropriate model for the *T. hydatigena* dataset was found to be HKY + G. Supplementary figure-1 shows the phylogenetic tree topology of a total of 45 sequences, including the sequences of the six isolates in this study and the reference sequences taken from GenBank. *Taenia hydatigena* sequences were equalized to 744 bp for phylogenetic analyses. Based on the maximum similarity phylogeny obtained from *cox1* sequences, all *T. hydatigena* isolates were in the same cluster as the reference sequences, forming only sister clusters.

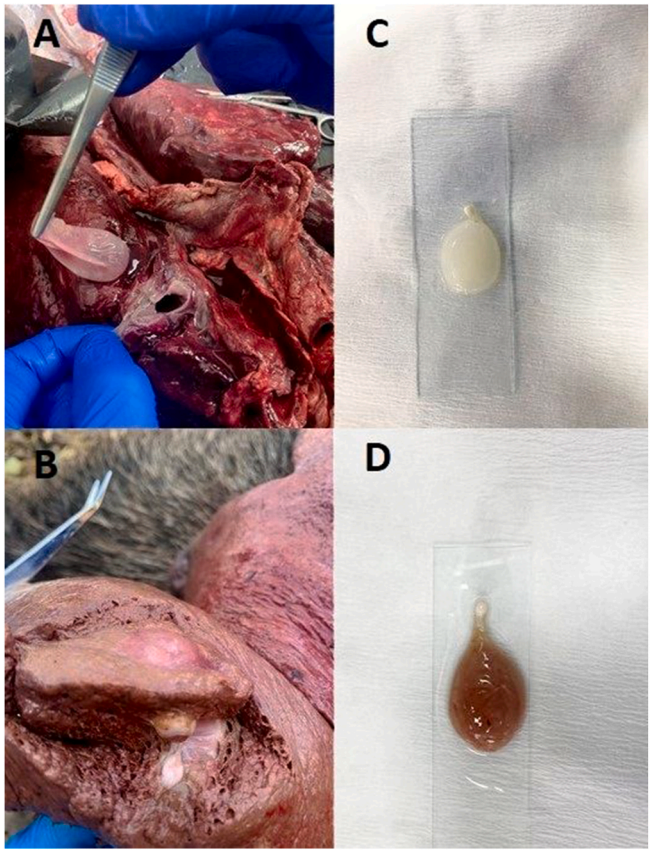

Fig. 1. Hydatid cyst image obtained from the lung (A) and liver (B) and C. tenuicollis (C,D) image obtained from its mesentery of wild boar.

Table 1
The organ localizations and Genbank accession numbers of the isolates obtained in this study.

Isolate Name	Species	Genbank accession no. for mt-CO1	Genbank accession no. for nad5	Localization
WB1	*E. granulosus s. s.* (G1)	PP508251	PP776586	Lung
WB2	*E. granulosus s. s.* (G1)	PP511906	PP776587	Lung
WB3	*E. granulosus s. s.* (G1)	PP511907	PP776588	Lung
WB4	*E. granulosus s. s.* (G3)	PP511909	PP776591	Lung
WB5	*E. granulosus s. s.* (G3)	PP511908	PP776592	Liver
WB5-Ez	*E. granulosus s. s.* (G1)	–	PP776590	Lung
WB6	*E. granulosus s. s.* (G1)	PP510457	PP776589	Liver
WB7	*E. canadensis* (G7)	PP510456		Liver
WB8	*T. hydatigena*	PP508240		Mesenterium
WB9	*T. hydatigena*	PP508241		Mesenterium
WB10	*T. hydatigena*	PP508242		Mesenterium
WB11	*T. hydatigena*	PP508193		Mesenterium
WB12	*T. hydatigena*	PP508243		Mesenterium
WB13	*T. hydatigena*	PP508244		Mesenterium
WB14	*T. hydatigena*	PP669791		Mesenterium

Echinococcus granulosus s.s. was analyzed using the HKY + I model, which was found to be the most appropriate for the *E. granulosus s.s.* (G1/G3) dataset. The phylogenetic tree topology was constructed using a total of 24 sequences, including seven isolates from this study and reference sequences from GenBank (Supplementary figure-2). *Echinococcus granulosus s.s.* And *E. canadensis* (G6/G7) sequences were equalized to 629 bp for phylogenetic analyses.

Conserved regions and positions of mutations were identified with reference to the sequence with accession number AB786664 reported by Nakao et al. (2013). A total of 14 polymorphic sites were present in 6 isolates obtained in this study. According to the analyses, 3 conserved regions were identified in the *cox1* sequence (466–500, 512–545, 571–613). The most common mutation was the T-G mutation and the positions were at nucleotides 429, 465, 672, and 675 in WB2 isolate. In WB3 and WB6, T-G mutation was observed at positions 844 and 982. WB6 isolate showed T-G mutation at positions 884, 970, and 979 different from the other sequences.

Echinococcus granulosus s.s. nad5 gene sequences were equalized to 628 bp for phylogenetic analyses. The results of the *nad5* gene data set analysis of the isolates obtained in this study indicate that three isolates (WB1, WB3, WB6) were found to be G1, while two isolates (WB4, WB5) were found to be G3. This was determined through BLAST analysis of the sequence results (Supplementary figure-3). The alignment of the *cox1* sequences of isolates WB4 and WB5 revealed the presence of a G3-specific C/T mutation at nucleotides 9863 and 10,054 (positions according to GenBank reference AB786664), as previously described by Kinkar et al. (2018). The maximum similarity phylogeny revealed that five of the *E. granulosus s.s.* isolates were grouped together with the reference sequences, while one isolate (WB2) formed a separate cluster in close proximity to the G3 cluster. The WB7 isolate obtained in this study exhibited identical sequence characteristics to those identified as *E. canadensis* (G6/G7) in Genbank references, clustering together.

3.3. Haplotype network

Taenia hydatigena sequences were equalized to 744 bp for haplotype analyses. In the 43 *T. hydatigena* sequences analyzed, 31 haplotypes were identified. The haplotype network exhibited a star-like expansion, with Hap08 as the central haplotype, despite its low population

frequency of 11.9%. The majority (80%) of the haplotype groups consisted of single haplotypes. The isolates obtained in this study formed seven distinct haplotype groups (Hap 1, Hap 2, Hap 3, Hap 4, Hap 5, Hap 6, Hap 7), which were independent of geographical location. Between the main haplotype and other haplotypes, there were 1–19 point mutations (Fig. 2).

Echinococcus granulosus s.s. Sequences were equalized to 629 bp for haplotpe analyses. A total of 52 haplotypes of *E. granulosus s.s.* were found in 122 isolates of the sequences. The network displayed a star-like expansion, with three main haplotypes (Hap02, Hap03, Hap 18) forming the center of the network (Supplementary figure-4).

The haplotype network of the *cox1* dataset revealed that the WB1 isolate obtained in this study formed a common haplogroup with G1 genotypes, as it was in Hap02, one of the main haplotypes. WB5 was found to be in a common group with G3 genotypes, along with Hap03. The remaining four isolates were found to form single haplotypes, each with geographically unique haplotypes. Between the primary haplotype and all the additional haplotypes, a range of one to seven point mutations were detected.

Echinococcus canadensis (G6/G7) sequences were equalized to 616 bp for haplotpe analyses. The sequence data of 43 isolates were processed in accordance with the *E. canadensis* (G6/G7) *cox1* dataset. The WB7 isolate obtained in this study exhibited a single point mutation that resulted in its divergence from the main haplotype Hap_04, thereby forming the geographically unique Hap_01 (Fig. 3).

Echinococcus granulosus s.s. nad5 gene sequences were equalized to 628 bp for haplotype analyses. In the *nad5* dataset haplotype network, WB4 and WB5 co-occurred with the G3 genotypes, forming the geographically unique Hap05 and Hap06, respectively. The remaining isolates (WB1, WB2, WB3, WB5-Ez, WB6) were found to belong to the G1-specific haplogroup. Of these, WB3 and WB6 were in the main haplotype Hap03, while the others formed Hap01, Hap02 and Hap04, which were geographically clustered (Fig. 4).

3.4. Nucleotide sequence variation, diversity and neutrality indices

Population genetic markers were determined using nucleotide data from the *cox1* and *nad5* genes obtained from isolates in Türkiye and other countries (Table 2). A total of 57 polymorphic regions were detected in *T. hydatigena cox1* sequences, of which 22 (38.5%) were parsimony informative. For *E. granulosus s.s.* (G1/G3) *cox1* sequences, 56 polymorphic regions were detected, of which 21 (37.5%) were parsimony informative. However, the *cox1* sequences of *E. canadensis* (G6/G7) revealed the detection of 62 polymorphic regions, of which 8 (12.9%) were parsimony informative. The data set analyses of the *nad5* gene region revealed a total of 73 polymorphic regions, 30 (41.09%) of which were parsimony informative. All populations showed significantly negative Neutrality Indices as calculated by Tajima's D and Fu's Fs tests. Negative values of Tajima's D and Fu's Fs suggest the presence of numerous new mutations or population expansion. The significantly negative values of Fu and Li's D* and F* support the identification of a large number of rare haplotypes or a population expansion. The nucleotide diversity (Pi) and average number of nucleotide differences (K) were lowest (2.27) in *E. granulosus s.s.* (G1/G3), while the highest value (5.64) was found in *T. hydatigena* populations.

4. Discussion

This study analyzed *E. granulosus s.s.* (G1/G3) in a region with a high level of wildlife activity, specifically in a wild boar habitat. The study identified the occurrence of *E. canadensis* (G6/G7) and *T. hydatigena*, the former of which was identified molecularly for the first time in wild boars in Türkiye. The isolates obtained in this study were compared with other sequences from various geographical regions provided by

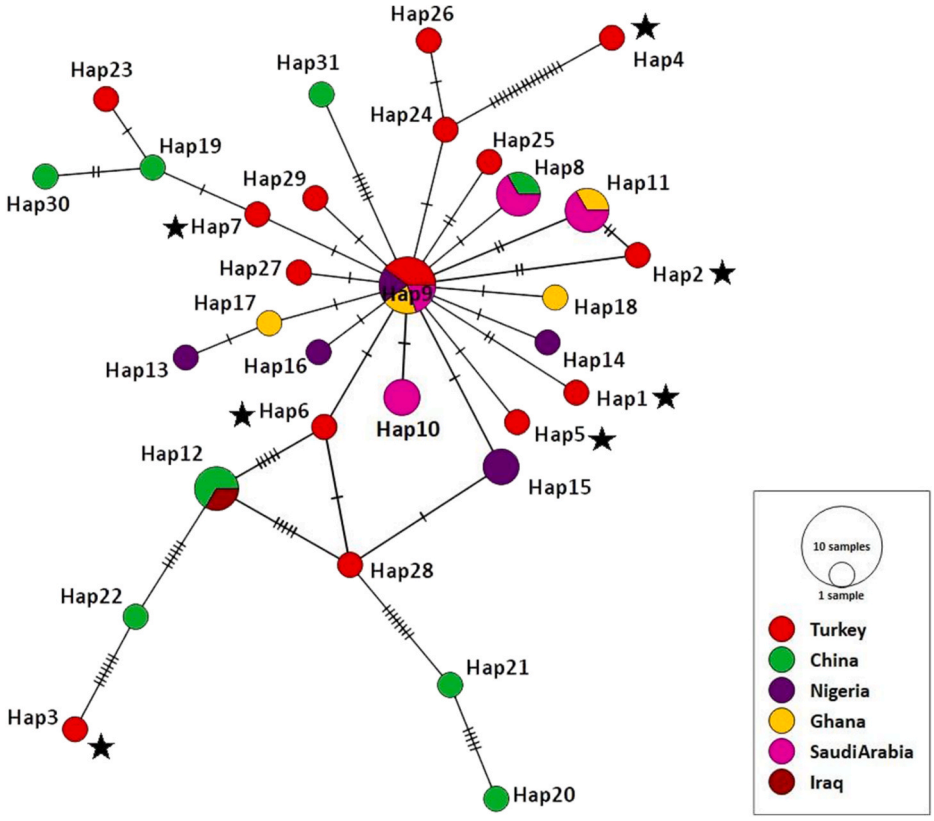

Fig. 2. Haplotype network constructed using *cox1* (744 bp) gene sequences of *T. hydatigena*. Seven haplotypes formed by the *T. hydatigena* isolates obtained in this study: (Hap 1-Hap 7). Circle size relative to haplotype data set frequency. Each hatch mark is representative of one nucleotide change. Haplotypes formed by the isolates obtained in this study are marked with an asterisk.

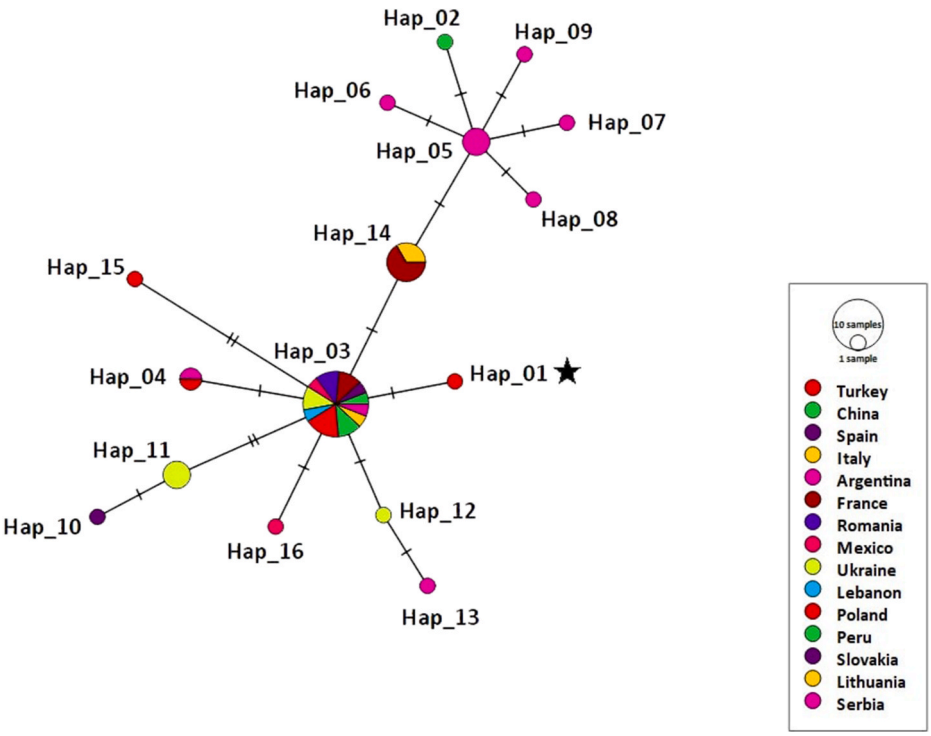

Fig. 3. Haplotype network for *E. canadensis* (G6/G7) using *cox1* gene (616 bp) sequences of different countries. The *E. canadensis* (G6/G7) isolate obtained in this investigation (Hap_01) and the sequences identified as G7 in the Genbank database were utilized. Circle size relative to haplotype data set frequency. Each hatch mark is representative of one nucleotide change. Haplotypes formed by the isolates obtained in this study are marked with an asterisk.

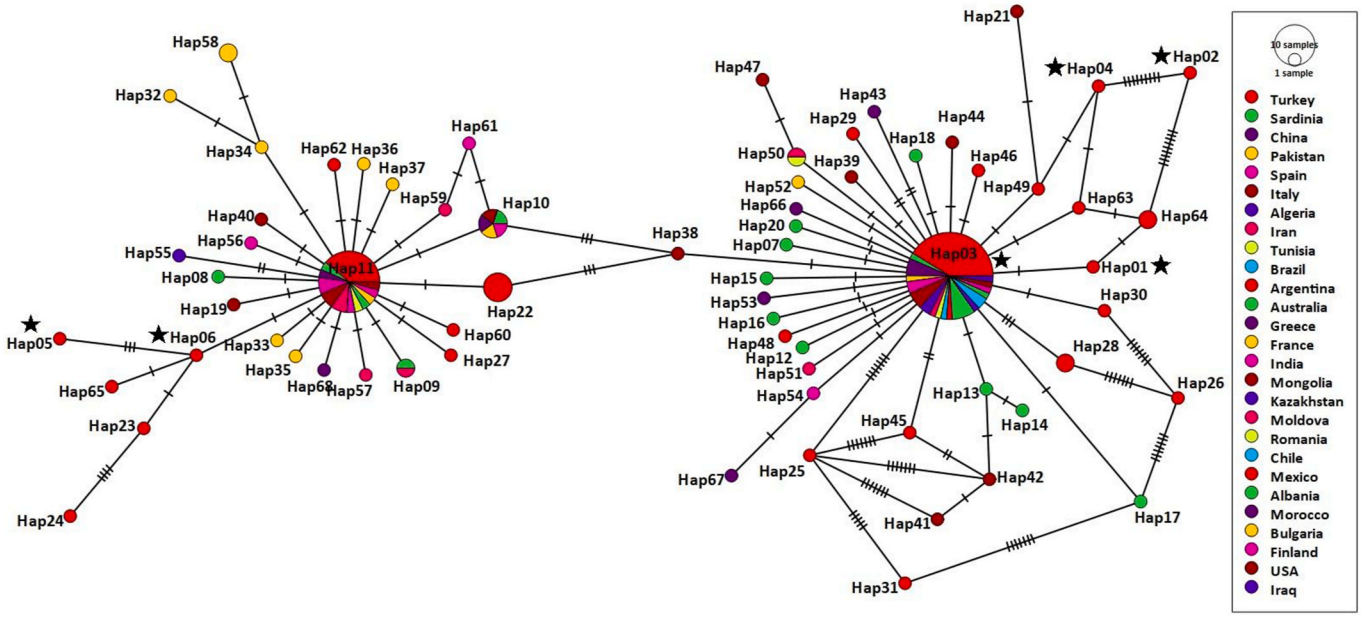

Fig. 4. Haplotype network of G1/G3 haplotypes identified on the basis of partial *nad5* gene (628 bp). The G1 isolates obtained in this study (Hap01-Hap04), G3 isolates (Hap05, Hap06). Hatch marks represent the number of mutations between the haplotypes and the size of circle corresponds to the frequency of each haplotype in the population. Haplotypes formed by the isolates obtained in this study are marked with an asterisk.

Genbank and their taxonomic data and population diversity were evaluated.

The larvae of *T. hydatigena,* known as C. tenuicollis, have been observed in ruminants and pigs in several countries. (De-La-Muela et al., 2001; Senlik et al., 2011; Mansouri et al., 2016; Paoletti et al., 2019). Paoletti et al. (2019) reported a 2.9% prevalence of *T. hydatigena* in 765 wild boars in Italy. In another study conducted in Italy, 229 (6.8%) of 3363 wild boars examined were found to be infected with C. tenuicollis (Sgroi et al., 2020). A study carried out on 100 wild boars in Estonia revealed a prevalence rate of 20% for *T. hydatigena* (Järvis et al., 2007). In this study, *T. hydatigena* larvae were detected in 7/23 (30%) wild boars.

According to studies, the prevalence of these active *T. hydatigena* metacestodes is similar to cystic echinococcosis. In their study, Paoletti

Table 2
Diversity and neutrality indices based on partial mitochondrial genes of *E. granulosus s.s.* (G1/G3), *E. canadensis* (G6/G7) and *T. hydatigena* isolates.

Species		Diversity					Neutrality			
		n	Hn	Hd ± S.D.	π± S.D.	(k)	Tajima's D	Fu's Fs	FLD	FLF
E. granulosus s.s (G1/G3)	mt-CO1	122	52	0,896 ± 0,022	0,00361 ± 0,00440	2274	−2,49517***	−71,475***	−5,59629**	−5,14798**
	mt-nad5	147	67	0.878 ± 0.022	0,00649 ± 0,0024	4078	−2,22761**	−76,646**	−5,94592**	−5,17003**
E. canadensis (G6/G7)	mt-CO1	43	17	0,825 ± 0,052	0,00669 ± 0,00715	4121	−2,57377***	−4507*	−5,34700**	−5,18803**
T. hydatigena	mt-CO1	46	30	0,976 ± 0,012	0,00759 ± 0,00553	5646	−2,17388*	−20,439**	−3,23210*	−3,39970**

Abbreviations are number of isolates (n), number of haplotypes (Hn), haplotype diversity (Hd), nucleotide diversity (π), Fu and Li's D* test statistic (FLD), Fu and Li's F* test statistic (FLF), average number of pairwise nucleotide differences (k), Standart deviation (S.D).Statistical significance:*, $P < 0.05$, **, $P < 0.02$, ***, $P < 0.001$.

et al. (2019) found that wild boars infected with cysticercosis and residing in regions polluted with *T. hydatigena* are more likely to be exposed to hydatid cysts than other species. *Taenia hydatigena* has a significant level of intraspecific diversity (Kedra et al., 2001). A study conducted in Poland reported that the genetic variations between adult *T. hydatigena* isolates and *T. hydatigena* metacestodes were found to be low (Filip et al., 2019). In this study, two of the six isolates obtained (Hap03 (WB10), Hap04 (WB11)) exhibited high variability in comparison to other isolates. The haplotypes (Hap01, Hap02, Hap05 and Hap06) formed by the *T. hydatigena* isolates obtained in this study are distinct from the main haplotype Hap08, which also includes sheep-goat isolates from Türkiye. The genetic similarity of metacestodes with other sequences from sheep and goats indicates that the domestic and wild cycles coexist in the region.

Echinococcus granulosus s.s. (G1/G3) which is commonly found in sheep and often associated with human cases, was identified based on its mitochondrial gene sequences. Additionally, the presence of *E. canadensis* (G6/G7) species, which are associated with pigs, was detected. Similar findings have been reported in previous studies that used the same molecular approach and identified the same genotypes (G1/G3, G7) in pigs in Peru and Brazil (Sánchez et al., 2012; Monteiro et al., 2014). These results indicate that wild boars in Türkiye may serve as hosts for various *Echinococcus* species.

A total of 2108 wild boars were tested and 93 of them tested positive for CE (4.4%) in Italy (Sgroi et al., 2019). The frequency of CE was 18.9% in 591 wild boars in Tunisia (Lahmar et al., 2019). In a study in France, 2527 pigs were examined and hydatid cysts were observed in 180 pigs (7%) (Umhang et al., 2014). In Romania, 33/267 (12.36%) boars were positive for hydatid cysts at necropsy (Onac et al., 2013). In this study, 8/23 (34%) wild boars were found to be hydatid cyst positive. Although the study had a small sample size, the frequency of CE was significantly higher compared to epidemiological studies conducted on wild boar. Based on the geographical region of the isolates, it appears that the final hosts often interact with the intermediate host, wild boar, due to the close proximity of water and food supplies.

Molecular analyses conducted by researchers in Eastern European countries, including Poland, Slovakia, and Ukraine, have revealed the presence of *E. canadensis* (G6/G7) in pigs (Kedra et al., 1999; Turčeková et al., 2003; Bart et al., 2006; Onac et al., 2013). Various authors from different countries have reported *E. canadensis* (G6/G7) as the dominant species in pigs (Casulli et al., 2022). This species has also been found in humans, sheep, and cattle in Elazig province where the eastern site of Türkiye. Previous reports by Pawłowski and Stefaniak (2003) have shown the widespread of this species in humans. Furthermore, reports indicate the presence of *E. canadensis* (G6/G7) in cattle in Brazil and sheep in Türkiye, suggesting that there may be alternative hosts (Badaraco et al., 2008, 2008nábel et al., 2009). Unlike other studies that report the prevalence of *E. canadensis* (G6/G7) in wild boars, this study identified *E. granulosus s.s.* (G1/G3) as the most prevalent species. In Türkiye, it is known that *E. granulosus s.s.* (G1/G3) is the common species found in both animal and human hydatid cyst isolates (Utuk et al., 2008; Sarkari et al., 2019; Beyhan et al., 2020).

Of the seven *E. granulosus s.s.* (G1/G3) isolates identified in the study, two were found to be in the same ancestral haplotype as the reference sequences of the G3 genotype (Hap03, Hap 14). The remaining five isolates formed a cluster with the main haplotype of the G1 genotype and its branches. A study conducted on human, cattle and sheep isolates in Türkiye found that the isolates of *E. granulosus s.s.* (G1/G3) were identified as G1 (n = 61) and G3 (n = 10). In total, 23 different haplotypes were recovered from the 71 isolates (Celik et al., 2024). In a study conducted on isolates obtained from three different regions of Türkiye, the G3 genotype was detected in three cattle and 11 sheep among 47 sheep and cattle isolates (Cengiz and Gonenc, 2020). To date, the G3 genotype has only been recorded in wild boars in Italy (Laurimäe et al., 2019).

The mitochondrial gene sequences revealed that Hap_01, obtained in the haplotype network of the *E. canadensis* (G6/G7) isolate, differed from the main haplotype by a single point mutation and originated in Türkiye. The mutations were not found to be compatible with any other geographical location in the BLAST search.

This study provides the initial comprehensive evaluation on the molecular epidemiology of CE and *T. hydatigena* in wild boars in Türkiye. The findings of this study highlight the genetic diversity and phylogeographic features of *E. granulosus s.s.* (G1/G3), *E. canadensis* (G6/G7) and *T. hydatigena* in wild boars. The results of the current investigation suggest that the wild boars in question were discovered in regions frequented by domestic and wild carnivores (e.g., wolves and sheepdogs) that obtain unprocessed meat from infected sheep and/or other animals. Besides, the fact that *E. canadensis* (G6/7) was previously reported in a wolf in Bingol province (Kilinc et al., 2023) can be considered as evidence of a sylvatic life cycle between wolves and wild boars. The observation that *E. granulosus s.s.* (G1/G3) is more commonly detected in wild boars than *E. canadensis* (G6/G7) and that *E. granulosus s.s.* (G1/G3) is the dominant species responsible for human and farm animal CE cases in Türkiye indicates that wild boars are intertwined with domestic life.

5. Conclusion

This study revealed significant genetic diversity and prevalance in both *E. granulosus s.s.* (G1/G3) and C. tenuicollis isolates, including several previously uncharacterised haplotypes. These findings indicate that the population has expanded rapidly from a small size, as evidenced by the low nucleotide and high haplotype diversity observed. The distribution pattern of the isolates collected in this study within the haplotype network suggests that the haplotypes observed are not ancient variants of the parasite, but rather an emerging haplotype. The study found a great level of genetic diversity in *E. granulosus s.s.* (G1/G3) populations, which is confirmed by the demographic expansion observed. This is evident from the negative network structure in all populations and the highly significant overall diversity indices. The fact that the cestode larvae detected in this study were more common than expected indicates that these parasites circulate effectively in the wild cycle. Consequently, this study has highlighted the potential significance of the genetic diversity and epidemiology of important cestode larvae in wildlife, particularly in the eastern regions of Türkiye.

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Authors statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of this manuscript.

Data availability

Data will be made available on request.

Ethical statement

For this work, ethics committee approval was obtained from Bingol University Animal Experiments Local Ethics Committee, numbered E-85680299-020-150247 and dated April 04, 2024.

CRediT authorship contribution statement

Seyma Gunyakti Kilinc: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Figen Celik:** Writing – original draft, Visualization, Validation, Software, Resources, Investigation. **Harun Kaya Kesik:** Software, Resources, Investigation, Formal analysis. **Sami Simsek:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declares no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2024.100960

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