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Research Article **Araştırma Makalesi**

First Detection of *Eustrongylides excisus* in Mosquito Fish (*Gambusia holbrooki*) from Lake Eğirdir

Eğirdir Gölündeki Sivrisinek Balığı'nda (*Gambusia holbrooki*) *Eustrongylides excisus*'un İlk Tespiti

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Abstract: In this study, the presence of *Eustrongylides excisus*, one of the zoonotic nematodes, was determined for the first time in *Gambusia holbrooki* from Lake Eğirdir. 70 *Gambusia holbrooki* samples were examined macroscopically for *Eustrongylides excisus* infection. The four parasite samples were recorded in 4 fish, one in each fish. The parasitic species identification was performed using the classical method on *E. excisus* samples isolated from the host fish, then PCR was performed on 18S rDNA. As a result of molecular analyses, 18S rDNA loci fragment of *E. excisus* was determined to be 941 bases long and registered to the NCBI data system with accession number PP333225. The taxonomic position of the present isolates and other *Eustrongylides* isolates registered in NCBI was evaluated through a phylogenetic analysis. It was determined that the isolates in this study had similar sequences to the *Eustrongylides* samples previously reported from Türkiye. In addition, the isolates of this study were found to have close similarity to *Eustrongylides* isolates from countries geographically close to Türkiye, such as Iran and Italy, while the similarity rate with *Eustrongylides* isolates from countries such as China, Brazil and India was found to be low. As a result of this study, it was determined for the first time that *Gambusia holbrooki* plays a role as an intermediate host in the life cycle of *E. excisus*. In addition, the sequences belonging to the 18S rDNA locus of *E. excisus* samples from host fish were identified and contributed to the determination of the genetic characteristics of the parasite species.

Keywords
- DNA
- *Eustrongylides exicus*
- *Gambusia holbrooki*
- Lake Eğirdir

Özet: Bu çalışmada Eğirdir Gölü *Gambusia holbrooki*'sinde zoonotik nematodlardan biri olan *Eustrongylides excisus*'un varlığı ilk kez tespit edilmiştir. 70 *Gambusia holbrooki* örneği *Eustrongylides excisus* enfeksiyonu açısından makroskopik olarak incelendi. Her balıkta bir tane olmak üzere 4 balıkta dört parazit örneği kaydedilmiştir. Parazit tür teşhisi, konak balıktan izole edilen *E. excisus* örnekleri üzerinde klasik yöntem kullanılarak yapılmış, daha sonra 18S rDNA üzerinde PCR gerçekleştirilmiştir. Moleküler analizler sonucunda, *E. excisus*'un 18S rDNA lokus fragmanının 941 baz uzunluğunda olduğu belirlenmiş ve NCBI veri sistemine PP333225 erişim numarası ile kaydedilmiştir. Mevcut izolatların ve NCBI'da kayıtlı diğer *Eustrongylides* izolatlarının taksonomik konumu filogenetik bir analiz ile değerlendirilmiştir. Bu çalışmadaki izolatların daha önce Türkiye'den bildirilen *Eustrongylides* örnekleriyle benzer dizilere sahip olduğu belirlenmiştir. Ayrıca bu çalışmadaki izolatların İran ve İtalya gibi Türkiye'ye coğrafi olarak yakın ülkelerden elde edilen *Eustrongylides* izolatları ile yakın benzerlik gösterdiği, Çin, Brezilya ve Hindistan gibi ülkelerden elde edilen *Eustrongylides* izolatları ile benzerlik oranının ise düşük olduğu tespit edilmiştir. Bu çalışma sonucunda *Gambusia holbrooki*'nin *E. excisus*'un yaşam döngüsünde ara konak olarak rol oynadığı ilk kez tespit edilmiştir. Ayrıca, konak balıklardaki *E. excisus* örneklerinin 18S rDNA lokusuna ait sekanslar tanımlanmıştır.

Anahtar kelimeler
- DNA
- *Eustrongylides exicus*
- *Gambusia holbrooki*
- Eğirdir Gölü

1. INTRODUCTION

The mosquitofish, *G. holbrooki,* was globally introduced into freshwater ecosystems in the early 1920s for the purpose of biologically controlling malaria (Krumholz, 1948). Due to their high tolerance to environmental factors, they may thrive in many categories of water quality (Pyke, 2005). This species is extremely invasive and has been documented in the freshwater bodies of several regions. It has had significant detrimental impacts on ecosystems and the local fish populations (Pyke, 2008).

G. holbrooki was first introduced to Lake Amik (Hatay) in order to combat malaria in Türkiye, and then the species was inoculated into many water resources (Ekmekçi et al., 2013; İnnal, 2022). This fish generally leads a benthopelagic life, feeding on zooplankton, small insects and eggs and larvae of other fish (Alp et al., 1994; Balık et al., 2006).

The parasites which belong to genus *Eustrongylides* (Dioctophymatidae, Nematoda) have a heteroxenous life cycle. Fish-eating birds are the main hosts of the parasites and are known to harbor adult forms of the parasite. These birds spread the parasite eggs with their faeces into water sources. Oligochaetans eat these eggs and the eggs develop into the larval stages, L1 to L3. Second hosts are mostly fishes and rarely amphibians or reptiles. The hosts eat these infected Oligochaetes, *Lumbriculus variegatus, Tubifex tubifex* or *Limnodrilus* spp. Parasite larvae develop into L4 larval forms in the abdominal cavity, internal organs and muscle tissues of second hosts. When infected secondary intermediate hosts are ingested by aquatic birds, L4 stage parasites develop into mature stage in craw or intestine of the definitive hosts (Bjelić-Čabrilo et al., 2013; Coyner et al., 2002; Measures, 1988).

Adult *Eustrongylides excisus* individuals can cause zoonosis in humans. They can become infected by eating raw or undercooked infected fish containing larvae of this species. The larvae can cause intestinal pain, inflammation, and even perforation of the intestinal wall (Guardone et al., 2021; Ljubojevic et al., 2015; Mazzone et al., 2019; Özmen et al., 2021).

Determining the diversity of host fishes of *Eustrongylides excisus* is of obvious importance for other organisms in the aquatic ecosystem and for humans. In addition, correct species identification of the larval parasite is also important. Identification of *Eustrongylides excisus* larvae based on their anatomical-morphological characteristics may be insufficient. In this case, molecular markers such as genetic characteristics of the larval nematode samples provide strong support (Guardone et al., 2021; Pekmezci and Bolukbas, 2021; Xiong et al., 2013; Youssefi et al., 2020). Identification of the genetic characteristics of this species also contributes to the understanding of biological characteristics and relatedness levels of populations in different localities too.

In this study, the host fish *Gobius holbrooki* from Lake Eğirdir was investigated for *Eustrongylides excisus* infection. The present research contributed to the host fish diversity of the parasite species, the zoonosis phenomenon and the identification of genetic characteristics.

2. MATERIAL AND METHODS

2.1. Study area

Lake Eğirdir, located between latitudes 35°37′41N and 38°16′55N and longitudes 30°44′39E and 30°57′43E, is the second largest freshwater lake in Türkiye (Alp et al., 1994; Geldiay and Balık 2009). Lake Eğirdir has an area of 482 km^2 with a maximum depth of 6-7 m and an average depth of 3.5-4 m (Dogan et al., 2025). (Figure 1).

2.2. Sampling

Within the scope of the research, *Gambusia holbrooki* samples were caught from Lake Eğirdir using special beach nets with a 5 mm mesh size. The beach nets had a wing length of 5 m, a wing height of 1.5 m and a bag mouth length of 5 m (Figure 2).

Before the parasitological examination of *G. holbrooki* samples, their lengths and weights were measured. Then, *G. holbrooki* samples were examined for *Eustrongylides excisus* macroscopically and with a stereo microscope. The anatomical and morphological features of the specimens were examined and identified according to Moravec (Moravec, 1994). Out of 70 samples, this parasite was detected in 4 samples and were fixed in 70% ethanol for molecular studies.

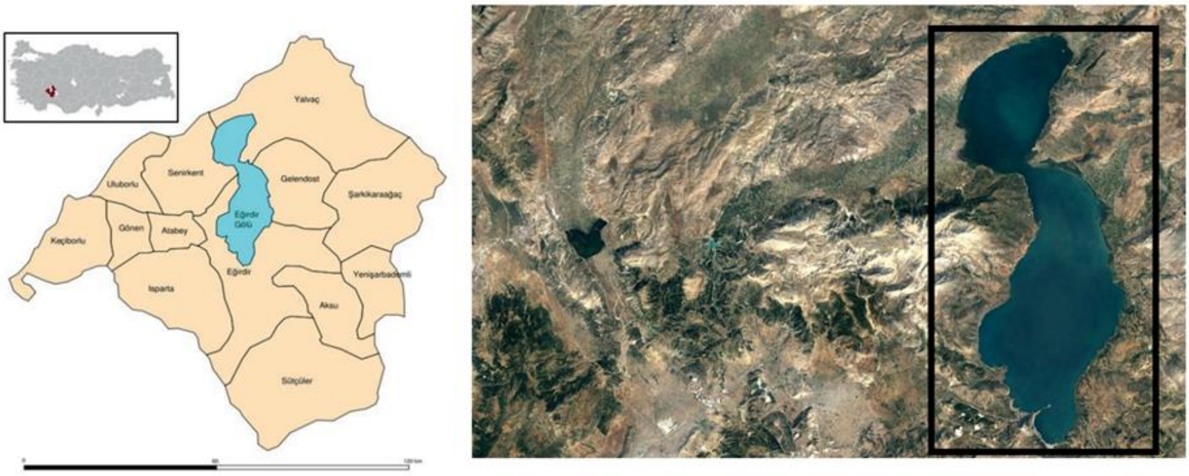

Figure 1. Location map of the Eğirdir Lake (from Google Earth).

Figure 2. Collection of *Gambusia holbrooki* samples (original).

2.3. DNA extraction, PCR assays and DNA sequencing

DNA extractions were performed with the High Pure PCR Template Preparation Kit (Roche Applied Science) following the manufacturer's instructions, quantified and visualized on 1,5 % agarose gel. The PCRs were performed using the 2x PCR Master Mix kit (Thermo Scientific, Carlsbad, California, USA). For the amplification of the nuclear ribosomal internal transcribed spacer (ITS) region gene, the primes 18SF (5' TTGGATGATTCGGTGAGGT 3') and 28SR (5' AACCGTTAGTAATATGCT 3'). PCR reactions were performed using 1-2 µl of genomic DNA, 12.5 µl of 2x PCR Master Mix (Thermo Scientifc, Carlsbad, California, USA), 1 µM of each specific primer, and nuclease-free water (Thermo Scientific, Carlsbad, California, USA), with a final volume of 25 µl. The PCR development conditions were 95 °C/5 min (95 °C/30 s, 53 °C/45 s, 72 °C/ 90 s, repeated 35×), 72 °C/10 min The PCR products were visualized on 1,5 % agarose gel with TBE buffer (0.045 M Tris-borate, 0.001 M EDTA pH 8.0). Sequenced in two directions with an automated sequencer and the same primers. The sequence of the sample has been registered in the GenBank database with accession number, PP333225.

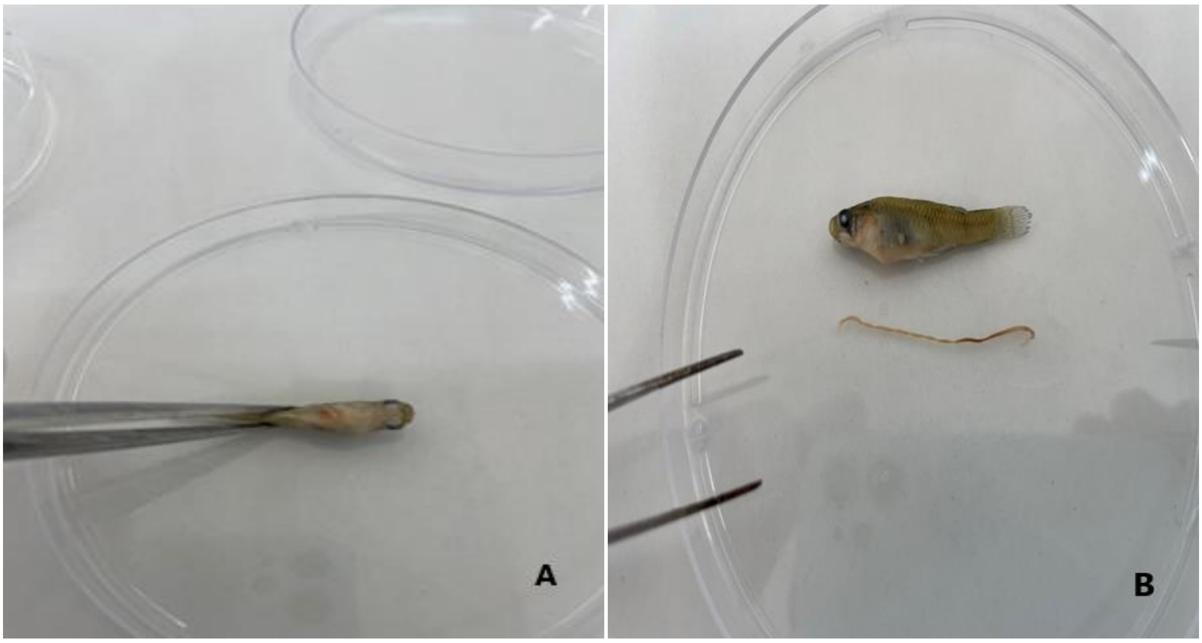

Figure 3. Parasitic examination at *Gambusia holbrooki* samples (original).

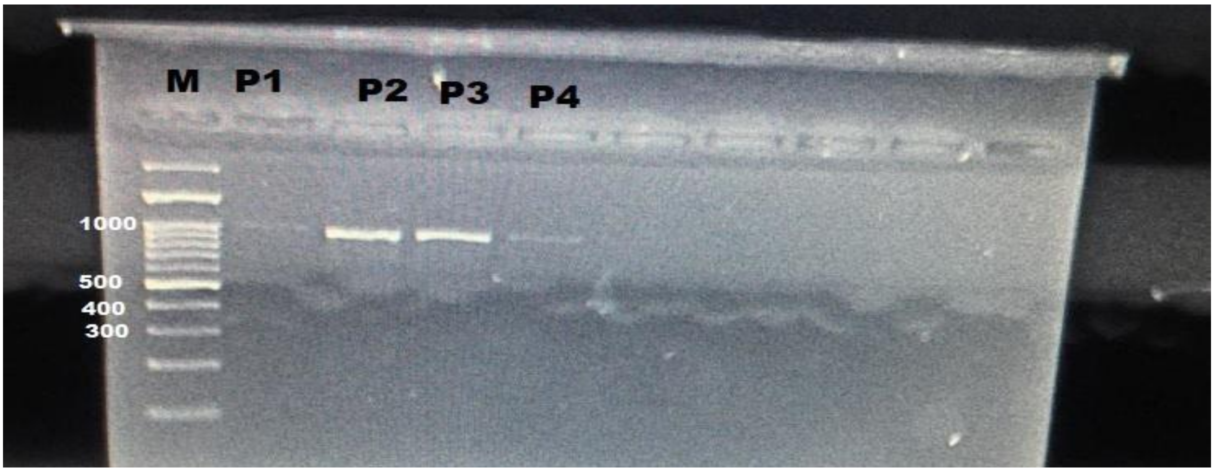

Figure 4. PCR gel image of the samples (original).

2.4. Phylogenetic analysis

The current sequence was edited using Bioedit (Hall, 2013). Data from the current study were matched with past 18S sequences of *Eustrongylides* spp. in GenBank using BLAST (Altschul et al., 1990). Using ClustalW in MEGA X, consensus sequences were aligned with other samples of *Eustrongylides* sequences (Kumar et al., 2018).

Phylogenetic analysis was performed using the Basic Local Alignment Search Tool (National Center for Biotechnology Information database) in comparison to GenBank sequences. Studies with *Eustrongylides* isolates in Türkiye, Italy, China, Japan, India, Iran, Japan, India, Iran and Brazil were constructed in the Bioedit programme to create a phylogenetic tree. The Mega-X was used to find the best model. And Maximum Likelihood method, and Tamura-Nei parameter method were determined (Kumar et al., 2018; Nei and Kumar, 2000). 1000 bootstrap re-saplings were used to produce a tree in MEGA-X. *Pellioditis morine* AJ867071(Derycke et al., 2005; Youssefi et al., 2020) and *Soboliphyme baturi* AY277895 (Rusin et al., 2003) were used as outgroups.

3. RESULTS

3.1. The case of infection

Eustrongylides excisus specimens were found in 4 of 70 (5.7%) *Gambusia holbrooki*. The four parasite samples were recorded in 4 fish, one in each fish. The average weight of the 16 male host fish examined in the study was 0.47 g, while the average weight of the 54 females was 0.249 g. The parasitic samples were recorded in the abdominal cavity and anus region of infected host fish, Figure 3a. The parasitic samples were a thick filamentous shape, reddish color and approximately 25 mm long, Figure 3b.

3.2. Anatomical and morphological characteristic features of the parasitic species

The anatomical and morphological features of the parasite samples were recorded. Accordingly, the general body shape of the parasite samples was thick and filiform. The body was covered with a thick cuticle. The cuticle had transverse striations. Additionally, there were rows of papillae on both lateral sides of the body. Around the mouth, there were two rows of six cone-shaped papillae each. The inner papillae were larger than the outer ones. The nerve ganglion was located at the level below the papillae. The intestine had a straight cylindrical shape. In the caudal region, there was a deep longitudinal groove. This species is dioecious. The posterior end of male individuals was oval-shaped. The spicule and spicule sheath were straight and smooth. The posterior terminal of female individuals was blunt. The genital opening was located in the lateral area of the posterior terminal. The genital canal was straight and muscular. As a result, based on these characteristics, the parasite samples were determined to belong to the species *Eustrongylides excisus*.

3.3. Molecular characteristic features of the parasitic species

DNA extraction, A260/A280 and A260/230 ratios were measured between 1.8-2.0 in measurements made with nanodrop. After this process, the PCR gel image was shown in Figure 4. In the gel image, it is seen that the targeted region proliferated. The PCR product showed an expected fragment of nearly 1000 bp in length. After the sequencing, all sequences were multiple aligned and compared in the bioedit programme. They all had the same sequence, there were no variations. After trimming, 941 base length sequences were obtained. NCBI BLAST analysis of the sequence confirmed that it was *Eustrongylides excisus*. It was registered in the NCBI genebankunder accession number, PP333225.

3.4. Phylogram analysis

The taxonomic position of the present and other *Eustrongylides* isolates registered in NCBI was evaluated through a phylogram analysis using the Maximum Likelihood method, MEGA-X program. According to the obtained data, it was determined that the isolates in this study had similar sequences to the *Eustrongylides* samples previously reported from Türkiye. In addition, the isolates of this study, were found to have close similarity to *Eustrongylides* isolates from countries geographically close to Turkey, such as Iran and Italy, while the similarity rate with *Eustrongylides* isolates from countries such as China, Brazil and India was found to be low (Figure 5).

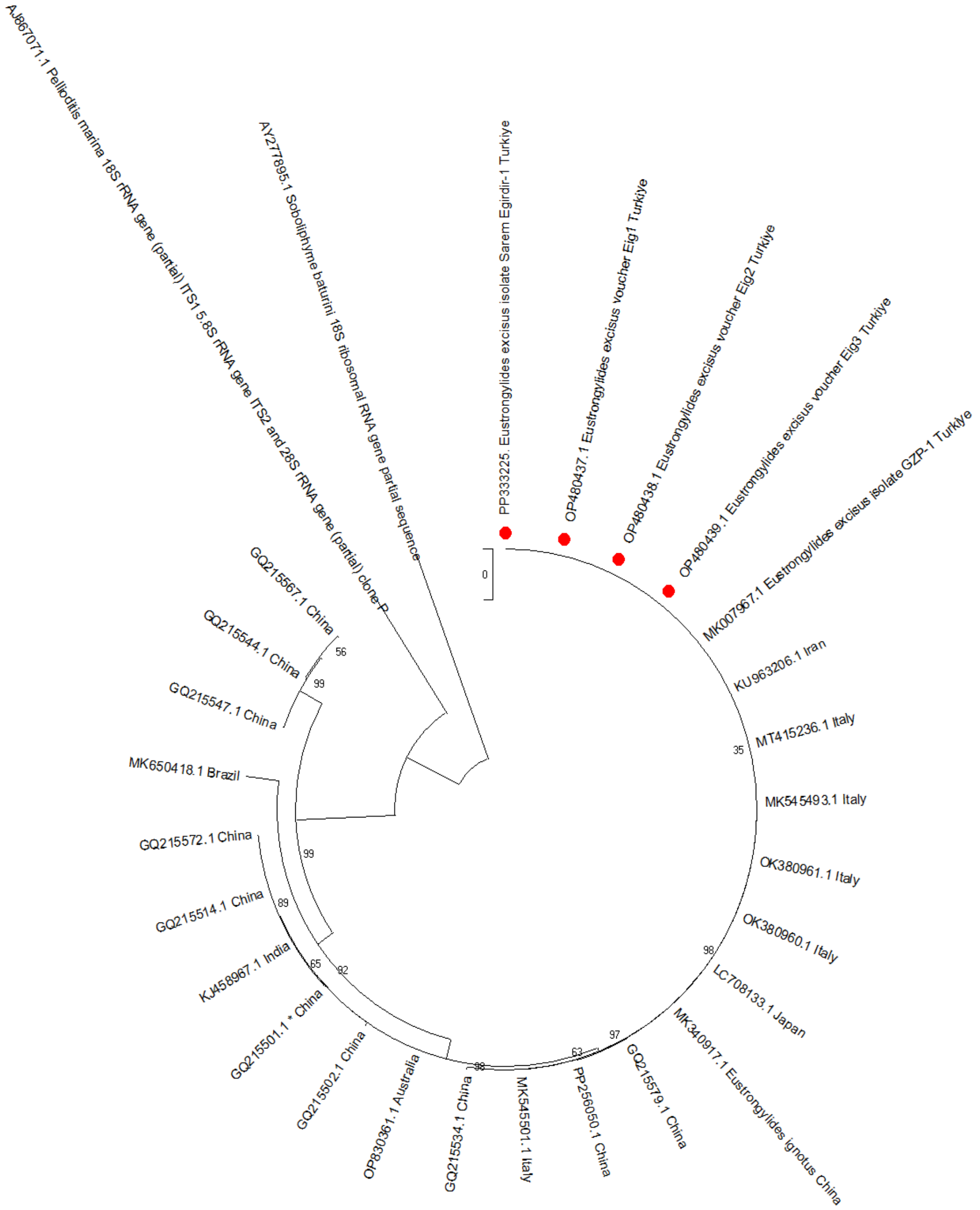

Figure 5. Phylogram tree based on 18S rDNA sequence data of *Eustrongylides* spp. from the present study and the GenBank database.

4. DISCUSSION

The mosquito fish, *Gambusia* sp. one of the world's invasive fish taxa. The fish taxon was reported to exist in at least 89 countries (Kurtul et al., 2022). This species poses a danger to water resources due to its long life span, rapid growth and reproduction, high egg production, attacking large fish, wide distribution and being the dominant species in water resources where it is inoculated (Polat et al., 2011). Benthopelagic mosquitofish feed on zooplankton, small insects and eggs and larvae of other fish. Insecta, Crustacea, Arachnida, Mollusca (Gastropoda), Plants (especially filamentous algae) and Detritus were detected in the digestive tract of *Gambusia holbrooki* (Sac, 2023). The food materials in the digestive system of host fish in the present research area have similar characteristics.

The parasitic species, *Eustrongylides excisus* was detected in *Carassius gibelio*, *Cyprinus* *carpio*, *Sander lucioperca*, *Atherina boyeri*, *Pseudophoxinus egridiri*, *Aphanius iconii*, *Knipowitschia caucasica* in Lake Eğirdir (Akcimen et al., 2014; Akcimen et al., 2018; Innal et al., 2019; Metin et al., 2014; Ozturk et al., 2002; Özmen et al., 2021; Ozturk and Ozturk, 2023) and the other localities (Aydogdu et al., 2024; Aydogdu et al., 2011; Bjelić-Čabrilo et al., 2013; Guven and Ozturk, 2018; Juhásová et al., 2019; Menconi et al., 2021; Novakov et al., 2013; Ozturk et al., 2001; Pekmezci and Bolukbas, 2021; Rahmati-Holasoo et al., 2024; Sattari, 2004; Soylu, 2005). In this study, *Eustrongylides excisus* was reported for the first time from *Gambusia holbrooki* in Lake Eğirdir, Türkiye, and also in the world.

As known, the parasite species completes its life cycle on water birds. According to the Water Bird Census Report of the Turkish Ministry of Agriculture and Forestry, a total of 83,382 birds belonging to 32 different species were reported in Lake Eğirdir in 2024 (Kosks, 2024). The richness of the aquatic birds fauna of Lake Eğirdir may have supported the infection of *Gambusia holbrooki* by *E.excisus*. In addition, this first record data of *E. excisus* in the present study has provided support for the effect of local ecological characteristics on biodiversity and the difference in parasite-host diversity in different geographic regions.

In current taxonomic studies, in addition to identifying species based on their anatomical and morphological characteristics, using molecular markers has been considered a valid method. Genomic data provide reliable support for determining the taxonomic position of the same species, especially in different geographical regions and in different hosts, and for understanding similarities or differences between populations (Moravec and Justine, 2017).

This study presents the first record of *Eustrongylides excisus* from *Gambusia holbrooki* and its 18S rRNA sequence. The 18S rDNA locus of *E. excisus* was determined to be 941 bases long and registered to the NCBI data system with accession number PP333225. The taxonomic position of the present isolates and other *Eustrongylides* isolates registered in NCBI was evaluated through a phylogenetic analysis using the Maximum Likelihood method. It was determined that the isolates in this study had similar sequences to the *Eustrongylides* samples previously reported from Türkiye (Ozturk and Ozturk, 2023; Pekmezci and Bolukbas, 2021). The sequence of the present study was found to close similarity to *Eustrongylides* isolates from countries geographically close to Türkiye, such as Iran and Italy, while the similarity rate with *Eustrongylides* isolates from countries such as China, Brazil and India were found to be low (Guardone et al., 2021; Franceschini et al., 2022; Mazzone et al., 2019; Kuraiem et al., 2019; Shamsi et al., 2023; Xiong et al., 2013; Youssefi et al., 2020). The phylogram results show that the sequence variation among the isolates of different populations of the same species increases gradually with distance. These results supported the effect of the allopatric isolation mechanism in the formation of variations among populations. There was also a small evidence showing the effect of the isolation mechanism on the evolution of taxa.

Classical, traditional anthelmintic drugs have poor efficacy against *Eustrongylides* species, because these types of drugs suppress parasites that mostly live in the gastrointestinal tract. *Eustrongylides* species are located in the body cavity outside the stomach, in the internal organs and even in the muscle tissues of fish. Parasites in this group can only be removed from fish surgically, which is practically impossible. In order to completely stop the life cycle of this parasite and to eradicate the parasite occurrence, it is necessary to destroy all fish infected with the parasite(Coyner 2002). The parasitic *E. excisus* species was found to be a second intermediate host in various fish species in Lake Eğirdir and to cause infection in the host fishes (Akcimen et al., 2014; Akcimen et al., 2018; Innal et al., 2019; Metin et al., 2014; Ozmen et al., 2021; Ozturk and Ozturk, 2023). Lake Eğirdir is rich in both zooplankton such as *Tubifex* sp, which are the first intermediate hosts for *E. excisus*, and many aquatic birds species which are the final hosts for *E. excisus* (Apaydın-Yagcı et al., 2014; Apaydın-Yagcı et al., 2018; Kosks, 2024). So, *E. excisus* can easily continue its existence and life cycle in Lake Eğirdir. *E. excisus* is known to be an important zoonotic agent for humans (Guardone et al., 2021; Ljubojevic et al., 2015; Mazzone et al., 2019). Therefore, fish species infected with this parasite in Lake Eğirdir pose a significant zoonotic source risk for the local human population. As a preventive measure, the internal organs of fish caught in Lake Eğirdir should be removed, examined for *E. excisus* under purple light, and eaten after cooking at high temperatures, and never consumed raw. In addition, when cage fishing is planned in Lake Eğirdir in the future, it should be taken into consideration that *E. excisus* infection may have negative effects on the growth and meat quality of cultured fish.

5. CONCLUSION

As a result, *E. excisus* was identified for the first time in this study from *Gambusia holbrooki*. Thus, a new fish species was added to the host fish diversity of *E. excisus*. Also, it was noted that the parasite species may be a zoonotic threat to the local people. In addition, the sequences belonging to the 18S rDNA locus of *E. excisus* sample in the host fish was identified and contributed to the studies on determining the genetic characteristics of the parasite species.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

HE, CB and VY planned and sampled the fieldwork. HE and MMS carried out parasite detection and molecular studies. MOO contributed to the interpretation of the results and led the writing of the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

ETHICAL STATEMENT

Since the fish used in the study were obtained dead from nature, the Local Ethics Committee for Experimental Animals certificate is not required.

DATA AVAILABILITY STATEMENT

The data used in the present study are available upon request from the corresponding author. Data is not available to the public due to privacy or ethical restrictions.

REFERENCES

Akcimen, U., Emre, N., Bulut, C., et al. (2014). An investigation on *Eustrongylides excisus* (Jagerskiöld, 1909) parasite in fish of Lake Eğirdir. V. Eastern Anatolia Region Fisheries Symposium, May 31- June 02, Elazığ.

Akcimen, U., Apaydın-Yağcı, M., Yeğen, V., et al. (2018). First report of *Eustrongylides excisus* larvae and Lernaea cyprinacea on Eğirdir minnow (*Pseudophoxinus egridiri*). In Proceedings of the 13th International Symposium on Fisheries and Aquatic Sciences, Ankara, Turkiye.

Alp, A., Akyurek, M., Balık, S., et al. (1994). Akşehir Gölü'nün Bazı Biyoekolojik Özelliklerinin Tespiti Projesi Sonuç Raporu. Eğirdir Su Ürünleri Araştırma Enstitüsü Müdürlüğü, Eğirdir, Isparta.

Altschul, S. F., Gish, W., Miller, W., et al. (1990). Basic local alignment search tool. *J Mol Biol*, 215, 403–410.

Apaydın-Yagcı, M., Alp, A., Yagcı, A., et al. (2014). Diet and prey selection of pikeperch (*Sander lucioperca* Linnaeus,1758) population in Lake Egirdir (Turkey). *Arch Biol Sci*, 66, 1515–1527. https://doi.org/10.2298/ABS1404515Y

Apaydın-Yağcı, M., Alp, A., Yağcı, A., et al. (2018). Feeding ecology and prey selection of sand smelt, *Atherina boyeri* Risso, 1810 in Eğirdir Lake (Southern Anatolia, Turkey). *J Appl Ichthyol*, 34, 815–824. https://doi.org/10.1111/jai.13676

Aydogdu, N., Emre, N., Aco-Alburqueque, R., et al. (2024). Observations on Helminth Parasites in Endemic and Native Fish from Susurluk Basin, Türkiye Using a Genetic-Molecular Approach: With New Host and Geographical Records. *Journal of the Hellenic Veterinary Medical Society*, 75(2), 7281-7290. https://doi.org/10.12681/jhvms.32892

Aydoğdu, A., Emre, Y., Emre, N., et al. (2011). The occurrence of helminth parasites (Nemathelminthes) in some freshwater fish from streams discharging into Antalya Bay in Antalya, Turkey: Two new host records from Antalya. *Turkish Journal of Zoology*, 35(6), 859-864. https://doi.org/10.3906/zoo-0912-16

Balık, İ., Çubuk, H., Karasahin, B., et al. (2006). Food and Feeding Habits of the Pikeperch, *Sander lucioperca* (Linnaeus, 1758), Population from Lake Eğirdir (Turkey). *Turkish Journal of Zoology*, 30(1), 19-26.

Bjelić-Čabrilo, O., Novakov, N., Ćirković, M., et al. (2013). Research Note Title: The first determination of *Eustrongylides excisus* Jägerskiöld, 1909-larvae (Nematoda: Dioctophymatidae) in the pike-perch *Sander lucioperca* in Vojvodina (Serbia). *Helminthologia*, 50(4), 291-294. https://doi.org/10.2478/s11687-013-0143-1

Coyner, D. F., Spalding, M. G., Forrester, D. J. (2002). Epizootiology of *Eustrongylides ignotus* in Florida: Distribution, density, and natural infections in intermediate hosts. *Journal of wildlife Diseases*, 38(3), 483–499. https://doi.org/10.7589/0090-3558-38.3.4823

Derycke, S., Remerie, T., Vierstraete, A., Backeljau, T., Vanfleteren, J., Vincx, M., & Moens, T. (2005). Mitochondrial DNA variation and cryptic speciation within the free-living marine nematode Pellioditis marina. *Marine Ecology Progress Series*, *300*, 91-103.

Doğan, U., Şimşek, M., Akçay, İ., Koçyiğit, A. (2025). Eğirdir Gölü'nün düdenleri: Göl çukurluğu bir polye mi?. Jeomorfolojik Araştırmalar Dergisi(14), 31-47. https://doi.org/10.46453/jader.1605722

Ekmekci, F. G., Serife, G., Kırankaya, S. G., et al. (2013). Türkiye İçsularındaki İstilacı Balıkların Güncel Durumu ve İstilanın Etkilerinin Değerlendirilmesi. *Istanbul University Journal of Fisheries & Aquatic Sciences*, 28, 105-140.

Franceschini, R., Guardone, L., Armani, A., Ranucci, D., Roila, R., Valiani, A., ... & Branciari, R. (2022). Five-years management of an emerging parasite risk (Eustrongylides sp., Nematoda) in a fishery supply chain located on Trasimeno Lake (Italy). *Food Control*, *136*. https://doi.org/10.1016/j.foodcont.2020.107517

Geldiay, R., Balık, S. (2009). Türkiye Tatlısu Balıkları. Ege Üniv. Su Ürünleri Fakültesi Yayınları No:46, Bornova, İzmir.

Guardone, L., Ricci, E., Susini, F., et al. (2021). First detection of *E. excisus* (Nematoda: Dioctophymatidae) in big-scale sand smelt (*Atherina boyeri*) from the lake Massaciuccoli (Northwest Tuscany, Italy). *Parasitology Research*, 109, 769–780.

Guven, A., Ozturk, T. (2018). Metazoan parasite faunas of three Gobiid species (Actinopterygii: Gobiidae) inhabiting the lower Kızılırmak Delta in Samsun: A comparative study. *Türkiye Parazitol Derg*, 42(1), 33-38. https://doi.org/10.5152/tpd.2018.5635

Hall, T. (2013). BioEdit: Biological sequence alignment editor for Win95/98/NT/2K/XP, 95–98.

Innal, D., Yıldırım, M., Stavrescu-Bedivan, M. M., et al. (2019). Occurrence and infection dynamics of *Salsuginus* sp. (Monogenea, Ancyrocephalidae) and *Eustrongylides excisus* (Nematoda, Dioctophymatidae) in four endemic *Aphanius* (Cyprinodontidae) species: new host records for Turkish fauna. *Acta Biologica Turcica*, 32(2), 103- 109.

İnnal, D., (2022). Use of fishes as biological control agents for prevention of malaria in Turkey: A journey to history, *Acta Biologica Turcica*, 35(1), 36–45.

Juhásová, Ľ., Radačovská, A., Bazsalovicsova, E., et al. (2019). A study of the endohelminths of the European perch *Perca fluviatilis* L. from the central region of the Danube river basin in Slovakia. *ZooKeys*, 899, 47-58. https://doi.org/10.3897/zookeys.899.39638

Kosks. (2024). Ministry of Agriculture and Forestry. Mid-Winter Bird Count Report 2024. KOSKS_Raporu_2024.pdf Accessed, 13, 4.

Krumholz, L. A. (1948). Reproduction in the western mosquitofish *Gambusia affinis* and its use in mosquito control. *Ecological Monographs*, 18, 1-43.

Kumar, S., Stecher, G., Li, M., et al. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547-1549. https://doi.org/10.1093/molbev/msy096

Kuraiem, B. P., Knoff, M., Telleria, E. L., Fonseca, M. C. G. D., Machado, L. D. S., Cunha, N. C. D., ... & Clemente, S. C. D. S. (2019). Eustrongylides sp. (Nematoda, Dioctophymatoidea) parasitizing Hoplias malabaricus (Actinopterygii: Erythrinidae) collected from the state of Rio de Janeiro, Brazil. *Revista Brasileira de Parasitologia Veterinária*, *29*, e014519.

Kurtul, I., Tarkan, A. S., Sarı, H. M., et al. (2022). Climatic and geographic variation as a driver of phenotypic divergence in reproductive characters and body sizes of invasive *Gambusia holbrooki*. *Aquat Sci*, 84, 29. https://doi.org/10.1007/s00027-022-00862-7

Ljubojevic, D., Novakov, N., Djordjevic, V., et al. (2015). Potential parasitic hazards for humans in fish meat. *Procedia Food Science*, 5, 172-175. https://doi.org/10.1016/j.profoo.2015.09.049

Mazzone, A., Caffara, M., Gustinelli, A., et al. (2019). Morphological and molecular characterization of larval and adult stages of *Eustrongylides excisus* (Nematoda: Dioctophymatoidea) with histopathological observations. *J Parasitology*, 105(6), 882–889. https://doi.org/10.1645/19-44

Measures, L. N. (1988). Epizootiology, pathology, and description of *Eustrongylides tubifex* (Nematoda: Dioctophymatoidea) in fish. *Can J Zool*, 66, 2212–2222. https://doi.org/10.1139/z88-329

Menconi, V., Tedesco, P., Pastorino, P., et al. (2021). Could fish feeding behaviour and size explain prevalence differences of the nematode *Eustrongylides excisus* among species? The case study of Lake Garda. *Water*, 13(24), 3581. https://doi.org/10.3390/w13243581 1

Metin, S., Didinen, B. I., Boyacı, Y. Ö., et al. (2014). Occurrence of *Eustrongylides excisus* Jägerskiöld, 1909 larvae (Nematoda: Dioctophymatidae) in Pikeperch (*Sander lucioperca* L.) in Lake Eğirdir. *Eğirdir Su Ürünleri Fakültesi Dergisi*, 10(1), 20-24. https://doi.org/10.22392/egirdir.246371

Moravec, F. (1994). Parasitic nematodes of freshwater fishes of Europe. Kluwer Academic Publisher, Dordrecht.

Moravec, F., Justine, J. L. (2017). Two new species of nematode parasites, *Cucullanus epinepheli* sp. n. (Cucullanidae) and *Procamallanus* (*Spirocamallanus*) *sinespinis* sp. n. (Camallanidae), from marine serranid and haemulid fishes off New Caledonia. *Folia Parasitol*, 64,1–10. https://doi.org/10.1007/s00027-022-00862-7

Nei, M., Kumar, S. (2000). Molecular evolution and phylogenetics. *Heredity*, 86, 385–385.

Novakov, N., Bjelic-Cabrilo, O., Cirkovic, M., et al. (2013). Eustrongylidosis of European catfish (*Siluris glanis*). *Bulgarian Journal of Agricultural Science*, 19(1), 72-76.

Novakov, N., Radosavljević, V., Cirković, M. (2015). Diseases of freshwater fish. Novi Sad: Faculty of Agriculture, Novi Sad, Feljton.

Ozturk, M. O., Aydogdu, A., Dogan, I. (2002). The occurrence of Helminth fauna in sand goby (*Gobius fluviatilis*, Pallas, 1811) from lake Uluabat, Turkey. *Acta Veterinaria*, 52(5-6), 381-391. https://doi.org/10.2298/AVB0206381O

Ozmen, Ö., Stavrescu-Bedivan, M. M., Innal, D. (2021). Histopathological aspects caused by nematode parasite *Eustrongylides excisus* observed in some fish species of Eğirdir Lake, Turkey. *Agro Life Scientific Journal*, 10(1), 172-178. https://doi.org/10.17930/AGL2021119

Ozturk, K., Ozturk, M. O. (2023). DNA Sequence Based Molecular Identification of *Eustrongylides excisus* Larvas (Nematoda) in *Sander Lucioperca* from Lake Eğirdir. *Mehmet Akif Ersoy Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 14(2), 346-354. https://doi.org/10.29048/makufebed.1379991

Ozturk, M. O., Oğuz, M. C., Altunel, F. N. (2001). Research on the metazoan parasites of the sand goby (*Gobius fluviatilis* L.) from the Manyas Lake and a first record of two helminths in Turkey. *Türkiye Parazitol Derg*, 25(1), 88-93.

Pekmezci, G.Z., Bolukbas, C. S. (2021). Morphological and molecular characterization of *Eustrongylides excisus* larvae (Nematoda: Dioctophymatidae) in *Sander lucioperca* (L.) from Northern Turkey. *Parasitology Research*, 120, 2269–2274. https://doi.org/10.1007/s00436-021-07187-8

Polat, N., Zengin, M., Gümüş, A. (2011). İstilacı Balık Türleri ve Hayat Stratejileri. *The Black Sea Journal of Sciences*, 4(1), 63-86.

Pyke, G.H. (2005). A review of the biology of *Gambusia affinis* and *Gambusia holbrooki*. *Reviews in Fish Biology and Fisheries*, 15, 339-365. https://doi.org/10.1007/s11160-006-6394-x

Pyke, G. H. (2008). Plague minnow or Mosquitofish? A review of the biology and impacts of introduced Gambusia species. *Annual Review of Ecology, Evolution, and Systematics*, 39, 171-191. https://doi.org/10.1146/annurev.ecolsys.39.110707.173451

Rahmati-Holasoo, H., Azizzadeh, M., Ebrahimzadeh-Mousavi, H., et al. (2024). Histopathological, morphological, and molecular characterization of fish-borne zoonotic parasite *Eustrongylides excisus* infecting Northern pike (*Esox lucius*) in Iran. *BMC Veterinary Research*, 20(1), 291. https://doi.org/10.1186/s12917-024-04146-0

Rusin, L., Aleshin, V., Tchesunov, A., & Atrashkevich, G. (2003). The 18S ribosomal RNA gene of Soboliphyme baturini Petrow, 1930 (Nematoda: Dioctophymida) and its implications for phylogenetic relationships within Dorylaimia. *Nematology*, *5*(4), 615-628.

Saç, G. (2023). Diet and Feeding Ecology of the Invasive *Gambusia holbrooki* (Teleostei: Poeciliidae) in Lotic and Lentic Habitats (Northwestern Part of Turkey). *Inland Water Biol*, 16, 330–340. https://doi.org/10.1134/S1995082923020086

Sattari, M. (2004). The occurrence and intensity of *Eustrongylides excisus* (Nematoda: Dioctophymidae) in some bony fish species of the Caspian Sea and its basin. *Caspian Journal of Environmental Sciences*, 2(1), 9-12. https://doi.org/10.22124/cjes.2004.3830

Shamsi, S., Francis, N., Masiga, J., Barton, D. P., Zhu, X., Pearce, L., & McLellan, M. (2023). Occurrence and characterisation of Eustrongylides species in Australian native birds and fish. *Food and Waterborne Parasitology*, *30*, e00189.

Soylu, E. (2005). Metazoan Parasites of Catfish (*Silurus glanis*, Linnaeus, 1758) from Durusu (Terkos) Lake. *Journal of Black Sea/Mediterranean Environment*, 11(2), 225-237.

Xiong, F., Li, W. X., Wu, S. G., et al. (2013). Molecular phylogeny and host specificity of the larval *Eustrongylides* (Nematoda: Dioctophmidae) from freshwater fish in China. *J Parasitol*, 99, 137–144. https://doi.org/10.1645/GE-3163.1

Youssefi, M. R., Tabaripour, R., Hosseini, M. (2020). Molecular characterisation and histopathological study of *Eustrongylides excisus* nematode in the northern pike (*Esox lucius*). *Bulgarian Journal of Veterinary Medicine*, 26(1), 81-88. https://doi.org/10.15547/bjvm.2392