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INVESTIGATION OF SOME FISH SPECIES OF SCOMBRIDAE FAMILY IN TERMS OF PARASITES

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ABSTRACT

The purpose of this investigation was to identify the presence of parasite infections and cystic formation in *Scomber japonicus*, *Scomber scombrus*, and *Thunnus thynnus*. *Euryphorus brachypterus* and *Clavellisa scombri* were identified from *Thunnus thynnus* and *Scomber scombrus*, respectively. *Anisakis pegreffii* was identified from *Scomber japonicus* and *Scomber scombrus* by using molecular methods. Additionally, *Anisakis typical* was determined from *Scomber japonicus*.

RÉSUMÉ: Étude de certaines espèces de poissons de la famille des Scombridae en termes de parasites.

Le but de cette enquête était d'identifier la présence d'infections parasitaires et les formations kystiques et les parasites du *Scomber japonicus*, du *Scomber scombrus* et du *Thunnus thynnus*, ont été examinés. *Euryphorus brachypterus* et *Clavellisa scombri* ont été identifiés respectivement sur le thon rouge et le maquereau. *Anisakis pegreffii* ont été signalés sur des maquereaux et des maquereaux par des méthodes moléculaires, *Anisakis typique* des *Scomber japonicus*.

REZUMAT: Investigarea unor specii de pești ale familiei Scombridae din punctul de vedere al paraziților.

Scopul acestei investigații a fost identificarea prezenței infecțiilor parazitare și a formațiunilor chistice și paraziților la *Scomber japonicus*, *Scomber scomber* și *Thunnus thynnus*. *Euryphorus brachypterus* și *Clavellisa scombri* au fost identificate la *Thunnus thynnus* și *Scomber scombrus*. *Anisakis pegreffii* au fost raportate de la *Scomber japonicus* și *Scomber scombrus* prin metode moleculare, *Anisakis typical* la *Scomber japonicus.*

INTRODUCTION

Diseases of different types seen in various fish species are known to be directly related to stress caused by environmental factors (Öktener and Bănăduc, 2023). In natural conditions, fish can migrate to better habitats when exposed to bad conditions, such as a decrease in oxygen level or an increase in ambient temperature. Diseased fish may move to warmer waters where they can increase their body temperature to enhance their inflammatory response.

The aquatic environment has some parameters that directly affect the maintenance of homeostatic balance, growth, and reproduction in fish. Changes in homeostatic balance may pave the way for various diseases or cause different conditions. Among these parameters, the most important ones for fish are temperature, light period, chemical and biological content of water, feed, and stock density (Pozio, 2015). Pathogenic microorganisms and parasites can spread rapidly in aquatic environments by means of contamination of the environment with feces or skin lesions of clinically symptomatic or latently infected fish and can be transmitted to other fish by mouth, gill, or other contact, causing epidemics in a short time, which in turn leads to important economic losses (Robert, 2012; Erer, 2020; Aydın and Pekmezci, 2023).

Türkiye is partly surrounded by the Black Sea, the Aegean Sea and the Mediterranean Sea, and has a rich aquaculture potential with a coastline of 8,333 km in length. Although it changes from year to year, the country's world ranking is in the thirties in terms of total aquaculture production in 2014 and is in the top five among EU countries (FAO, 2016). It is of great importance to gather a wide range of information about fish diseases (parasitic, bacterial, fungal, etc.) to increase the country's share in this ranking and to realize sustainable aquaculture production (Tayar, 2010, 2020).

Parasites, one of the disease factors, can continue their lives in the gill, under the gill cover, gill rakers muscle tissue, the body cavity, internal organs, and the digestive system, especially in the intestines or, in the gallbladder of some species (Garcia et al., 2011; Mladineo et al., 2011; Özak et al., 2012; Şahin and Sağlam, 2016; Fonkwa et al., 2021).

Anisakid nematodes, members of the Anisakidae family, are among the most significant parasitic organisms responsible for some human infections. The nematodes use fish and warm-blooded vertebrates as their hosts, thus have the potential to pass to human body after consumption of the infected organisms. Their final hosts are usually marine mammals. The symptoms in humans include stomach pain, nausea, vomiting, and various abdominal pains. Because the symptoms are similar to those of many other diseases, the diagnosis of the presence of these nematodes is not easy. *Anisakis simplex* has gained public health importance as it has caused an outbreak in humans in the Netherlands due to visceral larval migration. The mature parasites normally live as parasites in sharks and rays, and humans are infected by eating raw herring meat carrying the nematode larvae. Humans infected with this parasite experience severe pain and even death (Wekell, 1994).

Larvae of Anisakid nematode species have been reported so far in different regions of the world (including Africa, Europe, South America, Australia, etc.) and in different fish species Anisakid larvae, particularly those of *Anisakis pegreffii* have been observed in various fish species, especially in Turkish seas (Ütük and Pişkin, 2012; Tepe and Oğuz, 2013; Pekmezci et al., 2014; Yardimci et al., 2014; Şahin and Sağlam, 2016; Becerik et al., 2020; Çelik et al., 2022; Aydın and Pekmezci, 2023).

In the present study, we studied cystic formations obtained from the gills, body cavity, and internal organs of some fish species belonging to the Scombridae family in order to determine the parasitic species in the specimens. It will show us the presence of disease agents that are difficult to prevent by examining the cystic stages of parasitic formations that affect fish health and can later turn into zoonotic structures.

MATERIAL AND METHODS

Study samples. The study was carried out on the parasites detected in chub mackerel (*Scomber japonicus*) and mackerel (*Scomber scombrus)* specimens belonging to the Scombridae family caught from Çanakkale Strait of the North Aegean Sea and in the dead bluefin tuna fish (*Thunnus thynnus*) taken aboard during the hunting season in the Mediterranean Sea. The fish specimens were obtained from the local fish market in Çanakkale, Türkiye during a six-month sampling period.

A total of 267 fish were sampled in the study. All samples were brought to the laboratory for parasitic investigations. Copepod parasites and *Didymozoon* parasites were observed under the gill cover, in the gill lamellae, tongue pad, and gill filaments of mackerel and chub mackerel samples. Only copepodite parasites under the gill covers of the bluefin tuna, and *Didymozoon* parasites in the gill lamellae were also observed. For further analysis, parasites were taken into cyro tubes containing absolute (97.99%) ethanol. The parasites were examined under stereo and light microscopes and photographed. Then, nematode parasites were sent to a diagnostic laboratory (Letgen Biotechnology Laboratory) in tubes containing ethanol for molecular analysis. Some copepod specimens were soaked in lactic acid for 24 hours and then dissected with dissecting needles under a Wild M5 stereomicroscope for morphological investigations. Each extremity of the dissected copepods was placed in glycerin-gelatin between a slide and a coverslip and the coverslip edges were covered with transparent nail polish to obtain permanent preparations.

Morphological analysis. The species determination of the parasitic nematodes was made by considering the anatomical and morphological features of the parasites such as size, general appearance, head and tail structures, oesophagus, and cecum lengths, using the relevant key studies (Setyobudi et al., 2011; Pekmezci et al., 2014; Serracca et al., 2014; Pozio, 2015; Mehlhorn, 2016; Şahin and Sağlam, 2016; Mladineo et al., 2017; Morsy et al., 2017; Pekmezci, 2019; Pekmezci et al., 2019; Pekmezci and Onuk, 2020; Pekmezci, 2021). In particular, the methods of Mladineo et al. (2011; 2017) and Kohn and Nascimento-Justo (2008) were used for the diagnosis of *Didymozoon* parasites.

Identifications and comparisons of the copepods were performed according to Kabata (1979; 2003) and Özak and Yanar (2016). Latin names and synonyms of the parasites and the hosts were checked from online databases (Fishbase, 2022; WoRMS, 2022). Nikon D 90 camera, CMOS (5MP) microscope camera, Zeiss Brand Light Microscope, Canon EOS 1100D connected to Olympus CH30 light microscope were used to obtain photographs of the investigated specimens.

Molecular analysis. COII, ITS region, and 12s rRNA gene regions were amplified using gene-specific primers; the primers 211F: 5'-TTTTCTAGTTATATAGATTGRTTYAT-3' and 210R: 5'-CACCAACTCTTAAAATTATC-3' were used for COII; the primers 5'-GTAGGTGAACCTGCGGAAGGATCATT-3' and NC2: 5'-TTAGTTTCTTTTCCTCCGCT-3' were used for ITS region; and the primers Anisakis-F: 5'-TGATTTTTAATTTTAGGGTGAAAT-3' and Anisakis-R: 5'-GGCAATTGATGGATGATTTG-3' were used for the rrnS gene region.

The NCBI blast program was used for identification of the nematode species (Mattiucci et al., 2011; Piras et al., 2014; Pekmezci, 2021). The reads obtained for the rrnS gene region were contiguous to form a consensus sequence. The Contig Assembly Program (CAP) was used in BioEdit software to perform this process.

In order to determine the phylogenetic analyzes among the nematode species identified by the BLAST analysis of the COII gene region, it was aligned using the CLUSTALW V.1.6 parameter in the GENIOUS 9.0.5 program, and a phylogenetic tree was created in the MEGA 11 program. For this purpose, ITS-RNA, COII, and rrnS-12S gene region sequences of different *Anisakis pegreffii* (Campana-Rouget and Biocca, 1955) and *Anisakis typica* (Diesing, 1860) species were downloaded and recorded in FASTA format using the NCBI nucleotide database for *A. pegreffii* and *A. typica*. *Anisakis pegreffii* (COII: NC0340329.1; ITS: MH211473.1, MF539764.1, MF422217.1, MF803221.1, JQ934869.1, JQ934867.1, JQ934873.1; rrnS 12S: MT312518.1- MT484284.1- MF140359.1) and *A. typica* (AB517571.1) were used as references. In addition, to determine the distance between the species, *Contracaecum rudolphii* (Hartwich, 1964) (COII: EF122202.1; ITS: MH778113.1; rrnS 12S: NC_014870.1) and *Pseudoterranova decipiens* Sensu (Krabbe, 1878) (COII: NC031645.1; ITS: KM273087.1; NC_0316:45.1) were used as the outgroups.

The COII, rrnaS 12S, and ITS gene sequences of the identified nematode species were uploaded to the GenBank database on the NCBI database, and the gene bank accession numbers were obtained for the relevant gene regions.

RESULTS AND DISCUSSION

50 cystic and free nematode parasites, 50 *Didymozoon* cysts, and 80 (53 female and 27 male) parasitic copepods were isolated from the fish samples (Fig. 1). Parasitic copepods isolated from bluefin tuna fish were identified as *Euryphorus brachypterus* (Figs. 2-3), and those from mackerel were *Clavellisa scombri* (Fig. 4). Didymozoons were identified by classical methods, all were identified at genus level as *Didymozoon* sp. was found (Fig. 1c).

The nematodes sampled from chub mackerel (*Scomber japonicus*) were *A. typica* and *A. pegreffii*, and nematodes in mackerel (*Scomber scombrus*) were *A. pegreffii* (Fig. 1b).

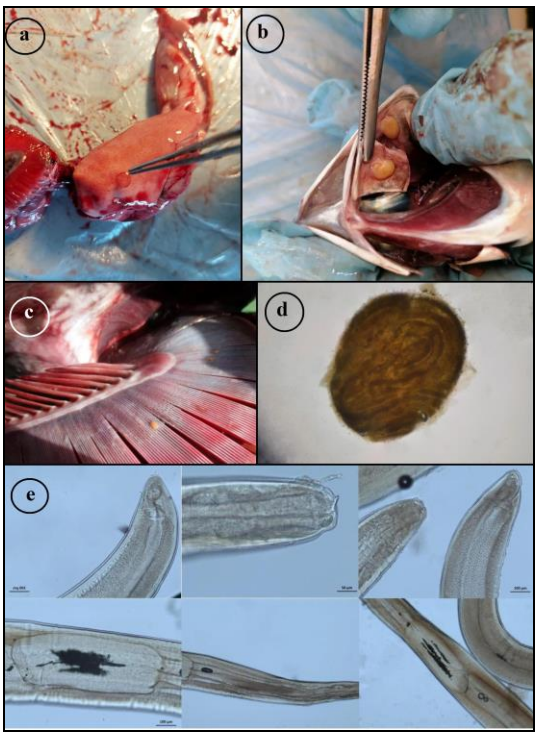


Figure 1: a. Nematode cyst in fish liver, *Didymozoon* parasite cyst on the inner wall of the gill cover, and the gill lamellar, b. Microscopic image of the nematode parasites, c. *Didymozoon*.

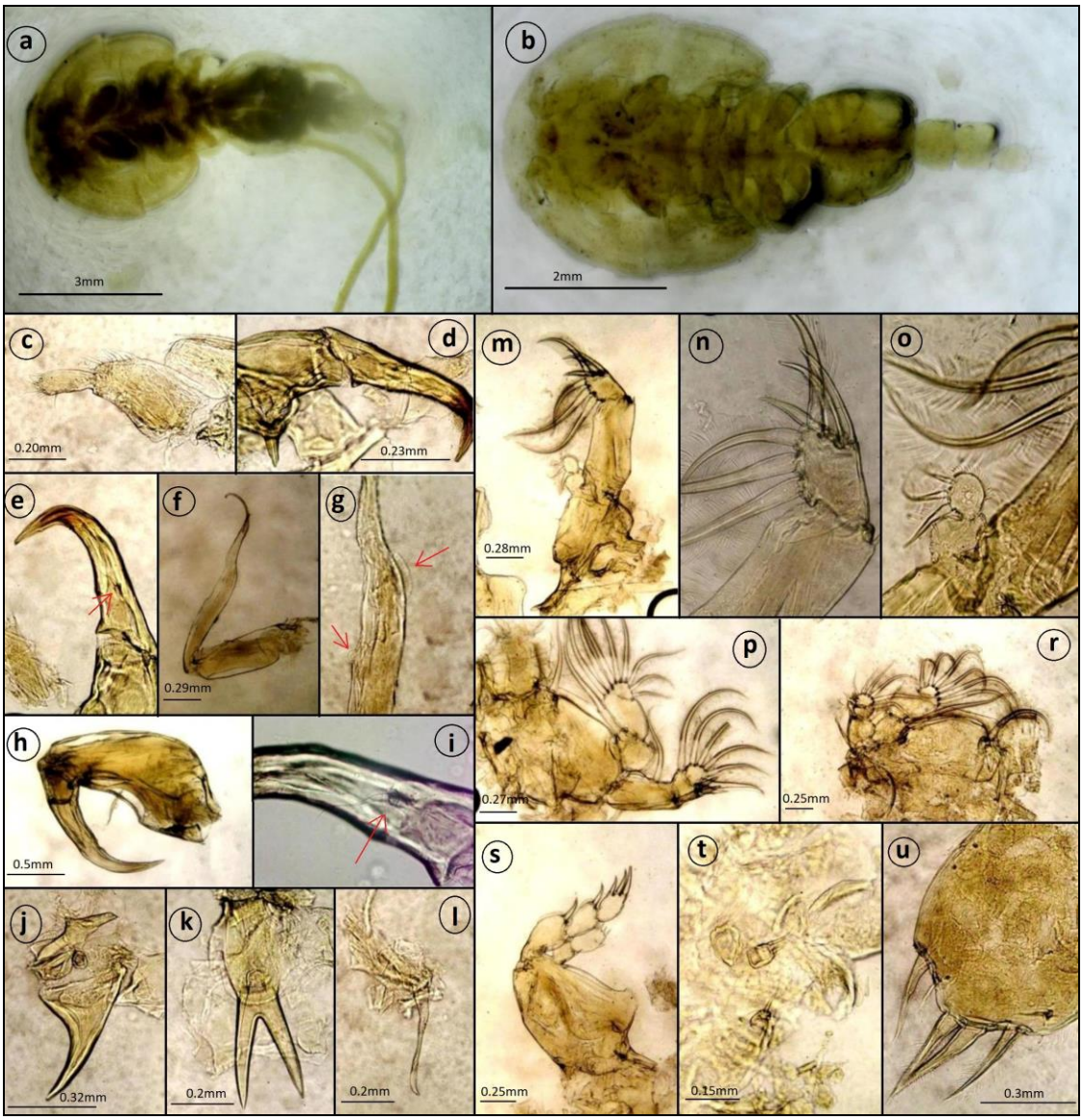


Figure 2: *Euryphorus brachypterus*, a. female, b. male, *Euryphorus brachypterus*, female, c. antennule, d. antenna, e. distal of antenna, f. maxilla, g. seta on maxilla, h. maxilliped, i. distal of maxilliped, j. maxillule, k. sternal furca, l. mandible, m. 1st leg, n. distal of 1st leg, o. distal of 1st leg, p. 2nd leg, r. 3rd leg, s. 4th leg, t. 5th leg, u. caudal ramus.

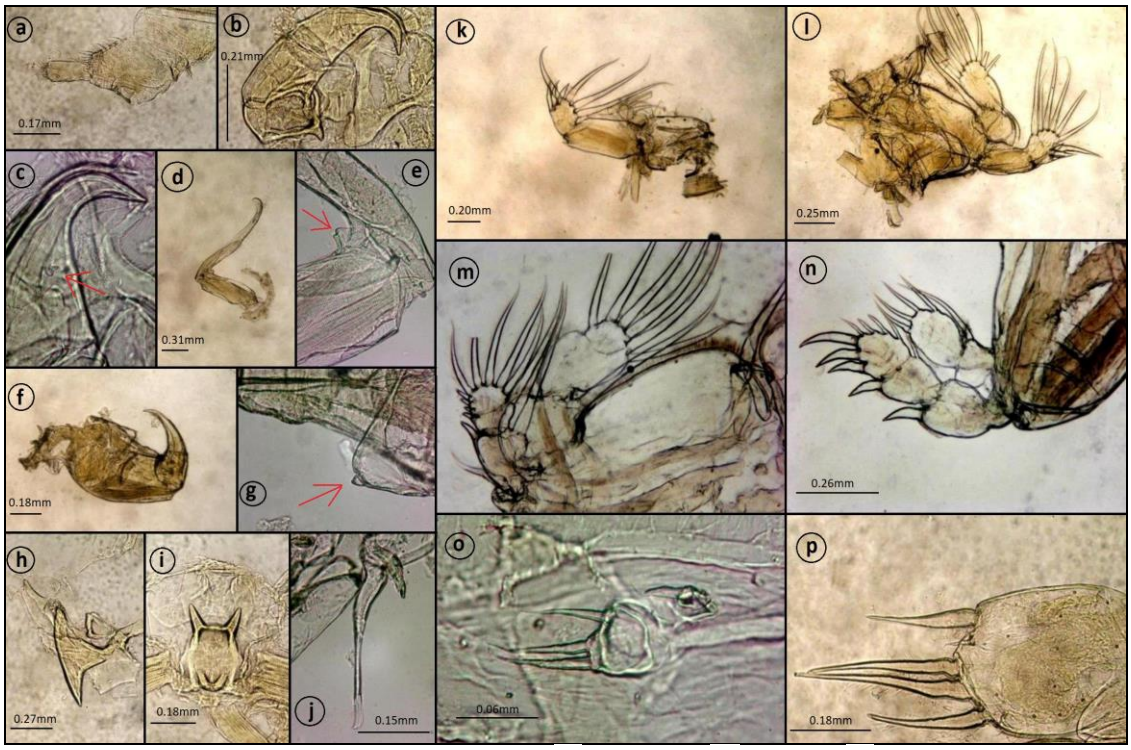


Figure 3: *Euryphorus brachypterus*, male, a. antennule, b. antenna, c. distal of antenna, d. maxilla, e. seta on maxilla, f. maxilliped, g. distal of maxilliped, h. maxillule, i. sternal furca, j. mandible, k. 1st leg, l. 2nd leg, m. 3rd leg, n. 4th leg, o. 5th leg, p. caudal ramus.



Figure 4: *Clavellisa scombri*, a. female, b.male (arrow).

The identified *A. typica* and *A. pegreffii* and the COII, ITS-rRNA and rrnS 12S rRNA sequences of the reference species from the database were aligned separately.

After the alignment, the Neighbor-Joining (NJ) tree was created. Bootstrap value above 50 are shown in the obtained NJ tree (Figs. 5-7). When the nucleotide sequences of the relevant gene region from the NCBI database were compared with the nucleotide sequences we obtained, the species were confirmed to be *A. typica* and *A. pegreffii*.

The GenBank accession numbers of the nematode species are as in the table 1.

Table 1: The GenBank accession numbers of nematode species.

Sampled name	Identified Nematode	Gen regions	Genbank number
Scomber japonicus 1	*A. typica*	COII	OP525256
S. japonicus 2	*A. pegreffii*	COII	OP525266
S. japonicus 3	*A. pegreffii*	COII	OP525267
S. japonicus 4	*A. pegreffii*	COII	OP525268
S. japonicus 5	*A. pegreffii*	ITS	OP176011
Scomber scombrus 1	*A. pegreffii*	rrnS 12S rRNA	OP179783
S. scombrus 2	*A. pegreffii*	COII	OP525261
S. scombrus 3	*A. pegreffii*	rrnS 12S rRNA	OP179784
S. scombrus 4	*A. pegreffii*	COII	OP525262

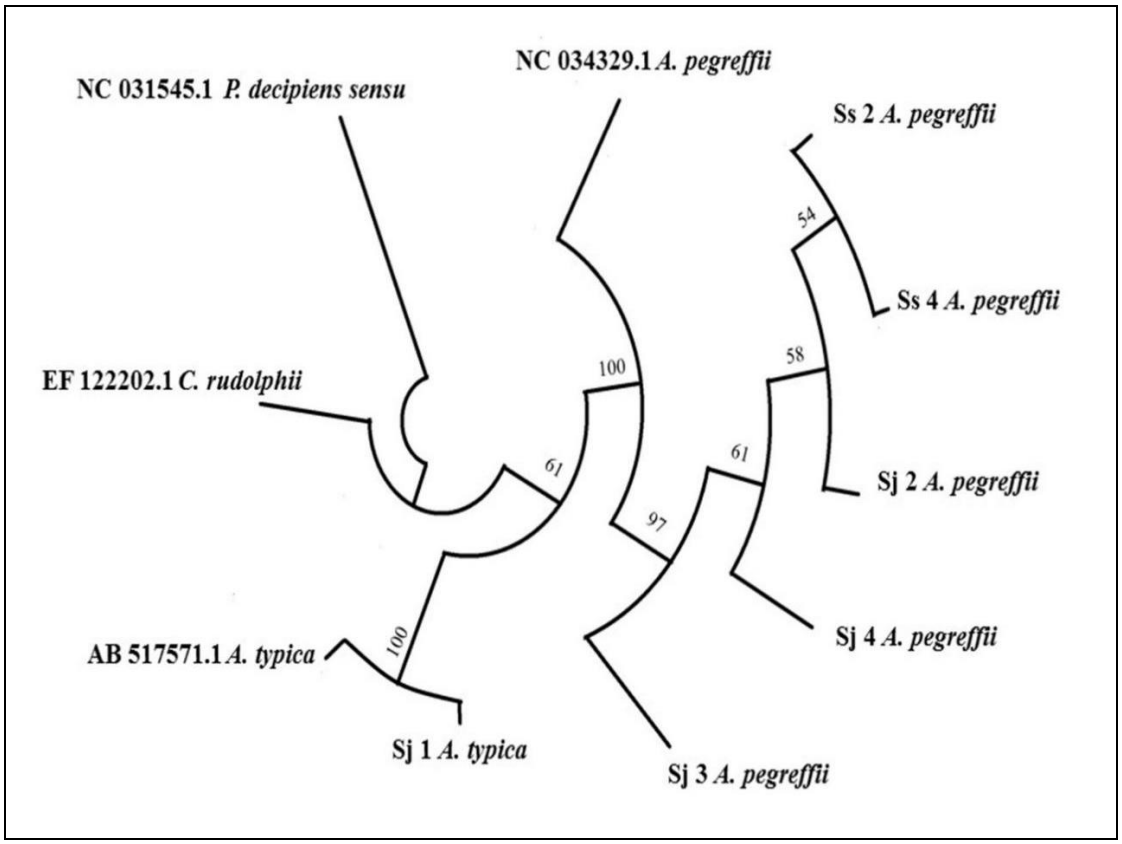


Figure 5: Neighbour-joining tree of COII gene regions of the identified nematode species (NCBI blast program and MEGA 11 program).

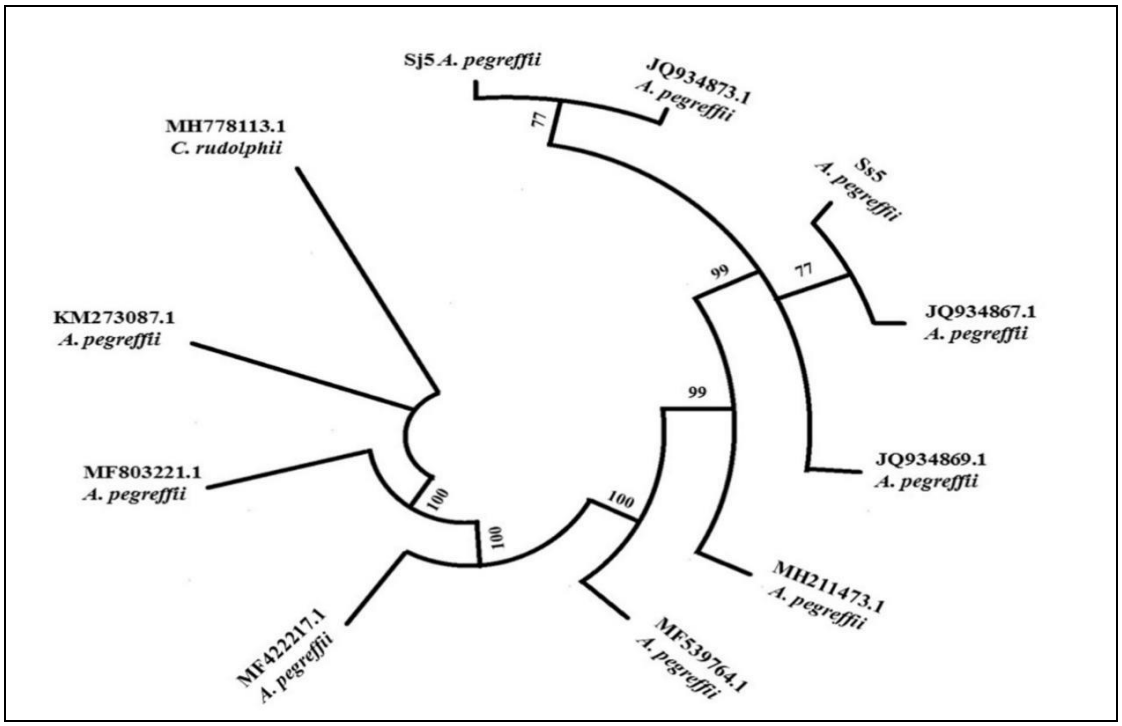


Figure 6: Neighbour-joining tree of ITS-rRNA gene regions of the identified nematode species (NCBI blast program and MEGA 11 program).

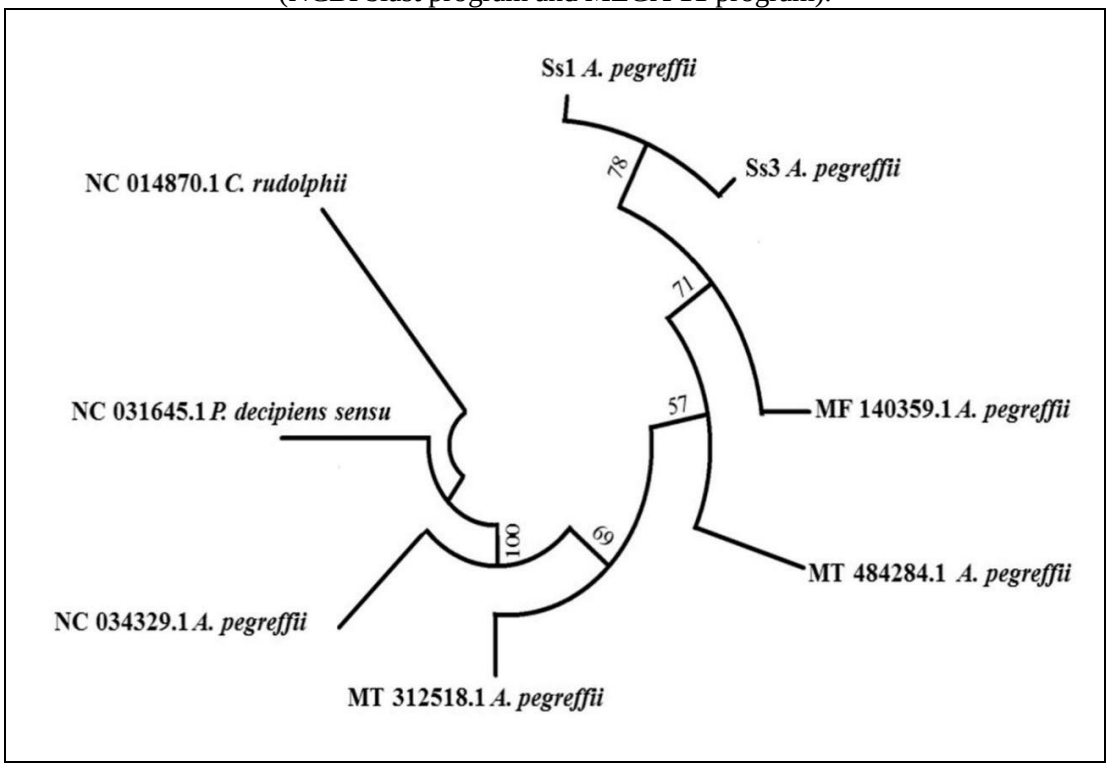


Figure 7: Neighbour-joining tree of the identified nematode species of rrnsS 12S gene regions (NCBI blast program and MEGA 11 program).

DISCUSSION

The study aims to parasitologically examine some fish species belonging to the Scombridae family, which were caught on the coasts of Çanakkale or the surrounding coasts, in terms of fish health. The cystic structures isolated from the gills of the fish were used for the determination of the parasites at the species level.

Cystic structures are one of the stages in which parasites maintain their life cycles by preserving their lives. If a parasite is still alive after the cystic stage, it will continue its life cycle by evolving within the host or moving to another host.

In a research on muscle parasites in marine fish, the molecular characteristics of didymozoids were studied. The differences in the last seven sequences in sequence analysis helped the diagnosis in the study. These differences helped in the molecular differentiation of didymozoids. The molecular separation of each parasite was made easier due to the clear sequence differences. It is the first molecular study to identify didymozoids (Abe et al., 2014).

A new host and a new geographical distribution for three *Didymocyctis* species of the Didymozoidae family were reported in a study on the tuna fish *Thunnus obesus* (Lowe, 1839) helminth parasites (Moreira-Silva et al., 2019). Scombrid fish, which is important in Brazil, may decrease in commercial value due to helminth parasites. Of the determined species, *Didymocystis alalongue* (Yamaguti, 1938) was isolated from the gill raker and operculum, *Didymocystis lamotheargumedoi* (Kohn and Nascimento-Justo, 2008) was isolated from the operculum in three mature forms and *Platocystis vivipara* (Yamaguti, 1970) was isolated from the skin of the dorsal part of the body. *Thunnus obesus* was reported as a new host for *D. alalongue* in the study. The study is also the first record of *Platocystis vivipara* and *D. lamotheargumedoi* in the South Atlantic (Moreira-Silva et al., 2019).

Digenean trematodes belonging to the family Didymozoidae can reduce the commercial value of fish, especially when in muscle tissues (Mota et al., 2019). In this study, the didymozoids isolated from the muscles of chub mackerel were investigated. The study includes 64 chub mackerel, and parasites were detected by the utilization of stereomicroscopy and macroscopy techniques on the muscle tissues. The results showed that the prevalence and abundance of the detected infection were quite low and that didymozoids in muscles differ from those isolated from other organs. Concern has been envisaged that this parasite may cause softening of the muscles, thereby reducing its commercial value and meat quality.

Didymozoid was isolated from the muscle tissue infection of the fish in a study conducted on the Atlantic mackerel *Scomber scombrus*, and parasite-host interaction was studied. Multiple ribbon-like Didymozoid eggs were observed in the connective tissue of *S. scombrus*, mostly embedded between epoxy skeletal muscle fibers. These eggs are at different stages of development, from previtellogenic oocytes to fully embryonated eggs. As a result of molecular studies, it was determined that the eggs belonged to a didymozooid that has not yet been identified. It has been shown that eating mackerel meat will cause displeasure as concurrent severe infection causes muscle damage in the host. However, it has been reported that no situation will endanger public health (Pascual et al., 2006).

Various studies conducted in the coastal waters of Rio de Jeneiro, Brazil investigated the parasite species of different fish species within Didymozoidae family (Nascimento-Justo and Kohn, 2012a, b; Nascimento-Justo et al., 2013; Nascimento-Justo and Konhn, 2014). In these studies on three Scombrid species *T. atlanticus* (Lesson, 1831), *T. albacores* (Bonnaterre, 1788) and *Katsuwonus pelanis* (Linnaeus, 1758) *Didymocystis lamotheargumedoi* n.sp. was isolated, making the study the pioneer of other studies.

Costa et al. (2011) examined and interpreted the helminth parasites of the Atlantic chub mackerel associated with the Mid-North Atlantic, Canary Islands, and other Atlantic regions. In the study conducted on 68 chub mackerel, it was reported that they were infected with 11 different parasitic taxa. The most abundant parasites were the monogenean *Pseudokuhnia minor* (Goto, 1984) in the gills, larval anisakid nematodes in the body cavity, larval tetraphyllidean in the bile duct, and the didymozoid digeneans in the gills. *Didymozoon* cysts were also isolated from the gills of the chub mackerel included in our study. In addition, nematode parasites were also sampled from some chub mackerel. The molecular analysis confirmed that the nematodes were of the species *A. pegreffii* and *A. typica*. Since *Didymozoon* parasites could not be diagnosed on a molecular basis, the naming of parasites was left at the genus level as *Didymozoon* sp.

Şimşek et al. (2021) performed the molecular diagnosis of nematodes they isolated in *Mullus barbatus* (Linnaeus, 1758) in the Mediterranean Sea, using the rrnS gene, and revealed the presence of *Hysterothylacium fabri* (Rudolphi, 1819) larvae for the first time. In the present study, the rrnS gene was used and the nematodes in mackerel were identified as *A. pegreffii*.

Oğuz et al. (2000) isolated the nematode species *A. simplex* in their study on some teleost fish on the coast of Çanakkale. Our study is based on the timeline of parasitological examination of fish samples taken from the local fish market in Çanakkale. In our examination, we determined that there was predominant nematode contamination of *A. pegreffii*. Apart from that, *A. typica* nematode was also isolated.

Çelik et al. (2022) analyzed the nematode parasites isolated from the sea chub mackerel caught from Çanakkale Strait at a molecular level and reported the presence of *A. pegreffii*. In our study, we isolated the species *A. pegreffii* from chub mackerel and mackerel fishes caught from the Çanakkale Strait by using molecular techniques. During our study, we supported our results with both traditional techniques and molecular diagnostic techniques.

CONCLUSIONS

The study aims to parasitologically examine some fish species with high economic value belonging to the Scombridae family, which were caught on the coasts of Çanakkale or the surrounding coasts, in terms of fish health. The cystic structures isolated from the gills of the fish were used for determination of the parasites at species level. Cystic structures are one of the stages in which parasites maintain their life cycles by preserving their lives. If a parasite is still alive after the cystic stage, it will continue its life cycle by evolving within the host or moving to another host.

As a result, the study showed that, considering the previous samplings in the same season and the species, it is necessary to better investigate and consider the environmental and anthropogenic effects, interaction between fish, and internal factors of the host in parasitizing fish. In addition, the change times in water temperature with the effect of global warming showed that parasitic organisms were re-isolated at a time when they did not exist before. The presence of these parasites should play an important role in decrease in the commercial value of fish and once again show the importance of protection and control in our seas.

This study contribute to the identification of possible parasitic disease factors that may be encountered in marine fisheries and to take precautions against health problems that may occur in commercial fisheries.

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