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Digenean Metacercariae Parasitic in Fishes in Sarıkum Lagoon Lake, Sinop, Turkish Black Sea Coast: Species Diversity, Seasonal Occurrence and Histopathological Effects

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Abstract: The study aimed to identify the digenean metacercariae parasitic in fishes in the Sarıkum Lagoon Lake located at the Black Sea coast of Turkey. A total of 906 fish specimens belonging to 6 fish species (*Aphanius danfordii*, *Neogobius melanostamus, Proterorhinus marmoratus*, *Gasterosteus aculeatus*, *Platichthys flesus* and *Chelon auratus*) were examined monthly during a period of one year. Four digenean species recorded as encysted metacercariae were identified; these were three species belonging to Heterophyidae, i.e. *Ascocotyle coleostoma, Pygidiopsis genata* and *Cryptocotyle concava*, and one species of the family Diplostomatidae, i.e. *Posthodiplostomum* sp. *Ascocotyle coleostoma* was found to be the dominant species and detected in all examined fish species. *Posthodiplostomum* sp. was detected only in *A. danfordii*. *Cryptocotyle concava* was detected in *N. melanostamus* and *P. marmoratus*. Infection prevalence and intensity were calculated and discussed for each digenean species recorded as metacercariae in relation to season. The eye, heart and gill tissues of infected fish with parasites were examined histologically. The histological evaluation indicated that the histological lesions were more severe in gills.

Key words: Trematoda; encysted metacercariae; prevalence; morphology; histopathology

Introduction

Digenean trematodes are common and widely distributed parasites of fish-eating birds and mammals. They are also important fish parasites and fish may serve as both intermediate and final hosts. These parasites have complex life cycles, with sporocysts, rediae and cercariae developing in first intermediate (mollusc) hosts, metacercariae in second intermediate hosts and adults in final hosts. The adult digeneans are frequent internal (most frequently intestinal) parasites of piscivorous (or fish-eating) birds and mammals. Their encysted metacercariae are frequently found in different organs of fish and most freshwater and estuarine fish are potential hosts; moreover, juvenile fish, bottom dwellers and shallow water inhabitants are most vulnerable (Scholz et al. 2001, Hicks & Steele 2003). Several studies have been reported on the diversity of adult digeneans in marine fishes studied off Turkish coasts, including those of Black Sea, Sea of Marmara and Aegean Sea (Oguz & Bray 2006, Akmirza 2013, Özer et al. 2013, Tepe et al. 2014, Öztürk & Özer 2016). Only a few studies have been related to metacercarial stages of digeneans (Öztürk et al. 2011, Gül et al. 2014), probably because it is more difficult to locate, recognize and identify of metacercarial stages than adult worms. However, studies on digenean metacercariae are necessary to understand the presence of digeneans in the ecosystem, their effects on the hosts and the ecological relationships between digeneans and hosts.

Sarıkum Lagoon Lake and the Sarıkum Region are protected as a natural conservation area, mostly due to its significance for wildlife diversity, especially for hundreds of migratory aquatic bird spe-

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cies to rest in the region, particularly during winter. The aim of this research study is to investigate the digenean parasites (metacercariae) at this peculiar part of Turkey. This paper also makes some further contributions to our knowledge of parasite-host relationships, such as the seasonal diversity of digenean metacercariae in fish species as well as their prevalence and abundance. In addition, we provide information on pathological effects of *Ascocotyle coleostoma* and *Posthodiplostomum* sp. metacercariae on the second intermediate hosts.

Fig. 1. Map of Sarıkum Lake showing the sampling area (*).

Materials and Methods

Study site: The study area is the Sarıkum Lagoon Lake (42°00'N, 34°54'E), which is connected to the Black Sea (Fig. 1). The total lake surface area is 184 ha, with a watery area of 102 ha and a marshy area of 82 ha. The mean depth of the lake is approximately 1–1.6 m, with silt, muddy and sandy substrate. The lake is brackish, with a salinity 1–5‰. It is a lagoon with eutrophic waters.

Fish specimens: Sampling was carried out on a monthly basis during one year. A total of 906 specimens of six fish species were examined. The samples included *Aphanius danfordii* (Boulenger, 1890) (210 individuals), *Neogobius melanostomus* (Pallas, 1814) (46), *Proterorhinus marmoratus* (Pallas, 1814) (50), *Gasterosteus aculeatus* Linnaeus, 1758 (85), juvenile *Platichthys flesus* (Linnaeus, 1758) (296) and juvenile *Chelon auratus* (Risso, 1810) (219) were collected by net fishing, fixed in 4% formalin (except for *P. flesus* and *A. danfordii*) and transferred to the laboratory. *Platichthys flesus* and *A. danfordii* were transported alive in local water directly to laboratory. For parasitological examination, the gills, hearts, livers, intestine and spleen of these fish individuals were examined under a dissecting microscope for the presence of encysted metacercariae.

Isolation and identification of encysted metacercariae parasites: Encysted metacercariae were recovered from the gill tissue, heart, liver, kidney, intestine and body cavity of infected fish. They were isolated using fine needle forceps under a dissecting microscope. While some metacercariae were studied live and unstained, the digenean metacercariae removed from the fixed fish were washed several times with 10% neutral buffered formalin, stained with Semichon's acetocarmine and mounted in permanent slides. The slides of digenean metacercariae were examined under a light microscope. The morphological characteristics such as shape, size and cyst wall structure were used for identification of the encysted metacercariae. The digenean metacercarial parasites were identified according to Ransom (1921), Niewiadomska (2002) and Pearson (2008).

Infection indices and statistical analysis: The infection prevalence (%), mean intensity (MI), mean abundance (MA) and intensity range (IR) values were calculated in accordance with Bush et al. (1997). The Kruskal–Wallis test (Nonparametric ANOVA) was used to determine significant differences in the mean intensity of digenean metacercarial infections in different seasons and in hosts. P value of <0.05 was considered significant. The analyses were carried out using the computer programmes GraphPad Instat 3.0 and SPSS 9.0.

Histological studies: To determine the host tissue response to the digenean metacercariae, the intensely infected tissue and organs were used. The intensely infected gills, hearts and eyes were fixed in 10% buffered neutral formalin. The tissues were dehydrated through ascending series of ethanol (70%, 85%, 95% and absolute) and xylene, embedded in paraffin and sectioned at 5 µm thickness. The sections were stained with haematoxylin and eosin (H&E) and mounted with Canada Balsam. The prepared sections were examined under a light microscope.

Results

Four digenean species belonging to Heterophyidae (*Ascocotyle coleostoma, Pygidiopsis genata* and *Cryptocotyle concava*) and Diplostomidae (*Posthodiplostomum* sp.) were identified. Infection prevalence, mean intensity, mean abundance and intensity range were determined for each digenean species (Table 1). *Ascocotyle coleostoma* were *Pygidiopsis genata* were found to be dominant species; the former species had its highest prevalence in *A. danfordii* and *P. marmoratus* while the latter species had its highest prevalence (80.9%) in *A. danfordii.* Digenean metacercariae were determined to be either host specific or generalist. Some digeneans were found to be fish specific, especially *C. concava* for gobiid fish, *Posthodiplostomum* sp. for *A. dan-*

Table 1. Prevalance (Pr), mean intensity (MI), mean abundance (MA) and intensity range (IR) values of digenean parasite species of fishes in the Sarıkum Lagoon Lake (n: examined fish number, ND: not detected, SE: standart error)

		Ascocotyle coleostoma	***Pygidiopsis genata***	***Cryptocotyle concava***	***Posthodiplostomum* sp.**
Aphanius danfordii (n: 210)	Pr MI±SE MA±SE IR	99.5% 157.96±6.97 157.21±6.95 10-641	80.9% 12.30±0.61 5.67±0.72 1-81	ND	89.5% 31.06±2.37 12.61±3.45 1-201
Proterorhinus marmoratus (n:50)	Pr MI±SE MA±SE IR	100% 618.82±61.95 618.82±61.95 13-1811	18.0% 10.89±5.35 1.96±1.09 1-39	96.0% 26.46±6.01 26.20±5.79 1-168	ND
Neogobius melanostomus (n: 46)	Prv MI±SE MA±SE IR	73.9% 32.15±9.65 23.76±7.41 1-279	15.2% 4.71±0.68 0.72±0.27 1-6	47.8% 11.68±2.90 5.80±1.64 1-56	ND
Platichthys flesus (n: 296)	Pr MI±SE MA±SE IR	75.4% 50.15±5.20 40.15±4.29 1-698	78.4% 12.18±0.94 4.74±0.60 1-131	ND	ND
Chelon auratus (n: 219)	Pr MI±SE MA±SE IR	49.3% 19.10±1.66 9.42±1.04 1-92	ND	ND	ND
Gasterosteus aculeatus (n: 85)	Pr MI±SE MA±SE IR	8.2% 2.43±0.81 0.20±0.09 1-6	ND	ND	ND

fordii. However, *Ascocotyle coleostoma* was found in all fish species examined.

Family Heterophyidae Leiper, 1909

***Ascocotyle coleostoma* (Looss, 1896)**

Site of infection: occasionally heart bulb and gills, rarely intestine or stomach wall (based on material ex *Proterorhinus marmoratus*, 10 encysted / 10 excysted fixed specimens).

Description (Fig. 2): The metacercariae of *A. coleostoma* are clearly visible in the bulbus arteriosus of fish (Fig. 2A). The shape and size of metacercariae were differing from each organs where they were found. Those from gills and the bulbus arteriosus of heart were oval, with maximum size 75 µm x 180 µm; cysts from other organs were spherical, with diameter up to 300 µm (Fig. 2). Metacercariae were oval (Fig. 2B) or spherical (Fig. 2C), in thin-walled cyst. Body of excysted metacercaria tear-shaped, with maximum size of 670 µm (Fig. 2D), with fully spinose tegument. Oral sucker subterminal; preoral lobe prominent, dorsal, with conical shape (Fig. 2E); posterior appendage long, curved to convoluted, reaching to pharynx. Oral sucker with 32 circumoral spines forming two complete rows, each of 16 spines (Fig. 2D-E); spines of posterior circlet smaller than those of anterior one. Prepharynx long. Pharynx muscular and oval. Oesophagus short. Intestinal caeca short and very wide. Ventral sucker round, postequatorial, filled with large lipoid droplets. Testes symmetrical, situated near to posterior extremity of body. Genital sac antero-sinistral to ventral sucker. Gonotyl inconspicuous. Ovary oval, dextral, pretesticular. Excretory vesicle Y-shaped (Fig. 2D).

***Pygidiopsis genata* Looss, 1907 (Fig. 3)**

Site of infection: intestine wall and stomach wall (based on material ex *Proterorhinus marmoratus*, 5 encysted / 5 excysted fixed specimens).

Description (Fig. 3): Metacercariae of *P. genata* were identified in *A. danfordii*, *N. melanostomus*, *P. marmoratus* and *P. flesus*. Cysts spherical, with thin wall; maximum diameter of cysts 300–350 µm (Fig. 3A). Body of excysted metacercaria pyriform, 576–580 µm long, 252–275 µm wide (Fig. 3B) at level of ovary. Tegument covered with scale-like spines, extending to testicular level. Preoral lobe absent. Oral sucker terminal and without oral spines. Ventral sucker at midbody. Prepharynx long. Pharynx muscular and spherical, situated at midway between oral sucker and intestinal bifurcation. Oesophagus longer than prepharynx. Caeca blind, extending to testicular level and surrounding ventral sucker. Ventral sucker spherical, situated at middle part of hindbody. Testes symmetrical, close to posterior end of body (Fig. 3C). Seminal vesicle long,

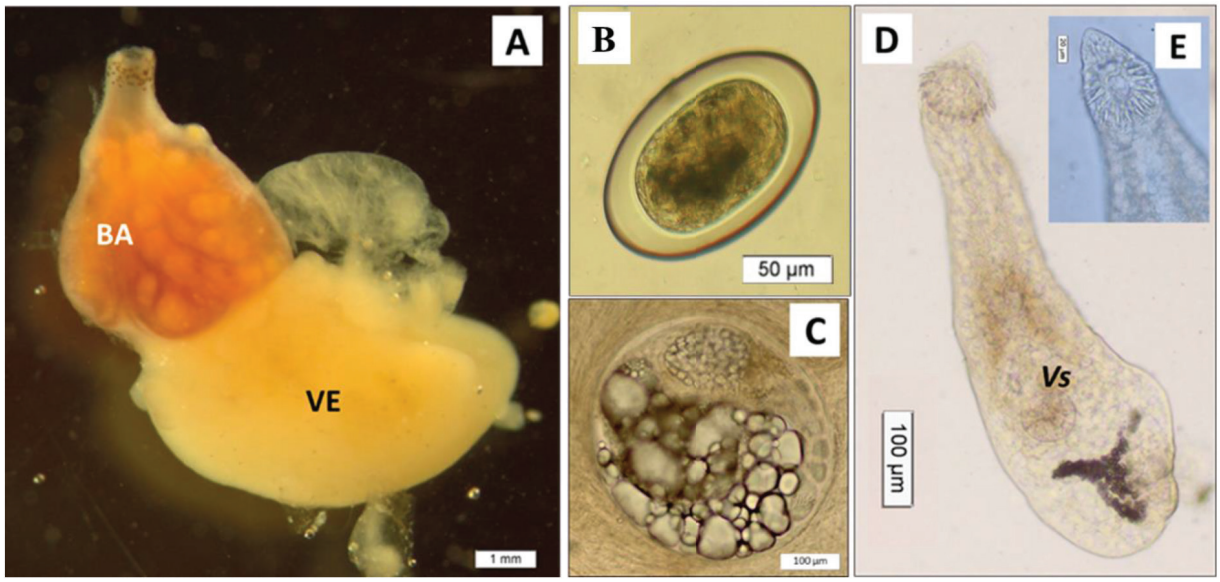

Fig. 2. Metacercariae of *Ascocotyle coleostoma* (Looss, 1896). A. Infected heart of *A. danfordii* with bulbing metacercariae within bulbus arteriosus (BA). B. Encysted metacercariae isolated from heart and gills, C. Encysted metacercaria isolated from intestine and stomach. D. Excysted metacercaria. E. Anterior ends with two rows-spined oral sucker. (VE: ventricle, Vs: ventral sucker)

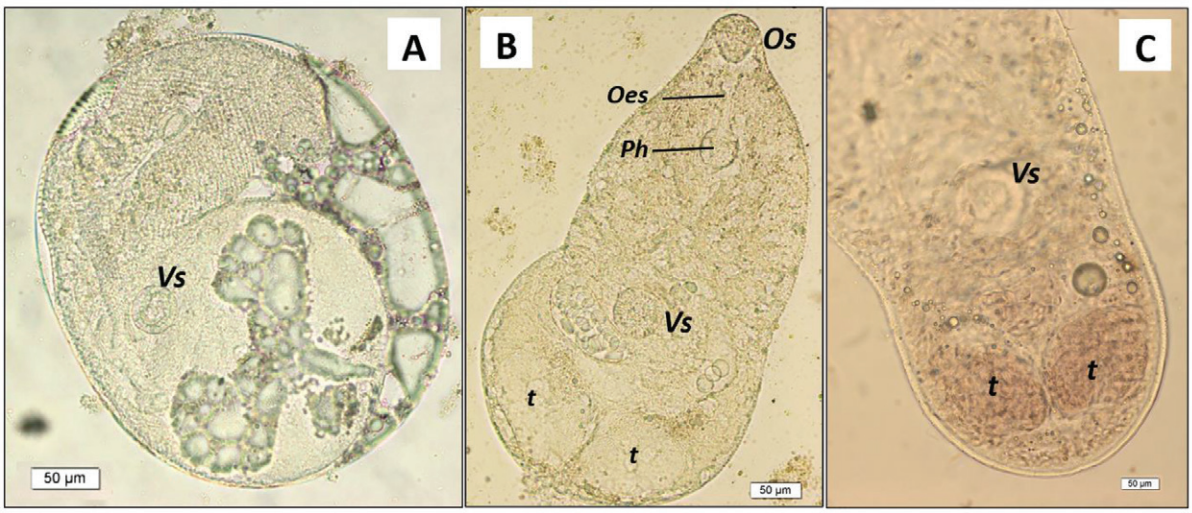

Fig. 3. Metacercariae of *Pygidiopsis genata* Looss, 1907. A. Encysted metacercaria. B. Excysted metacercaria. C. Posterior end of excysted metacercaria (Vs: ventral sucker, Os: oral sucker, Oes: oesophagus, t: testes)

sigmoid, postero-sinistral to ventral sucker. Genital sac antero-sinistral to ventral sucker, with slit-like aperture. Ovary dextral, slightly irregular in shape, anterior to right testis. Excretory bladder Y-shaped, with short and wide arms, or X-shaped with feebly developed posterior lobes (Fig. 3A).

***Cryptocotyle concava* (Creplin, 1825)**

Site of infection: fins and body surface (based on material ex *Proterorhinus marmoratus*, 7 encysted fixed specimens).

Description (Fig. 4): Metacercariae were in dermal cysts and easy to be recognised because of their black colour – clearly visible on the body surface and fins (Fig. 4A-B). Cysts spherical, with diameter 130–150 µm (Fig. 4C). Body of excysted metacercariae small and oval. Oral sucker subterminal. Prepharynx very short. Pharhynx elliptical. Oesophagus short. Ventral sucker small. Ovary spherical. Testes two, close to posterior extremity of body. Excretory bladder V-shaped (Fig. 4D).

Family Diplostomidae Poirier, 1886

***Posthodiplostomum* sp.**

Site of infection: body cavity, eyes, liver and intestine (based on material ex *Aphanius danfordi*, 10 encysted / 10 excysted fixed specimens).

Description (Fig. 5): Cysts oval or elongate-ov-

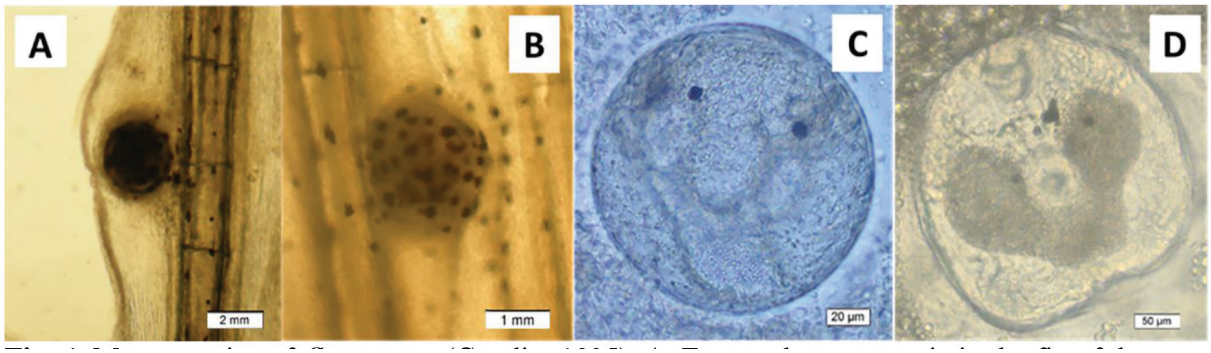

Fig. 4. Metacercariae of *C. concava* (Creplin, 1825). A. Encysted metacercaria in the fin of the round goby. B.Black pigmentation around encysted metacercaria. C. Encysted metacercaria. D. Excysted metacercaria.

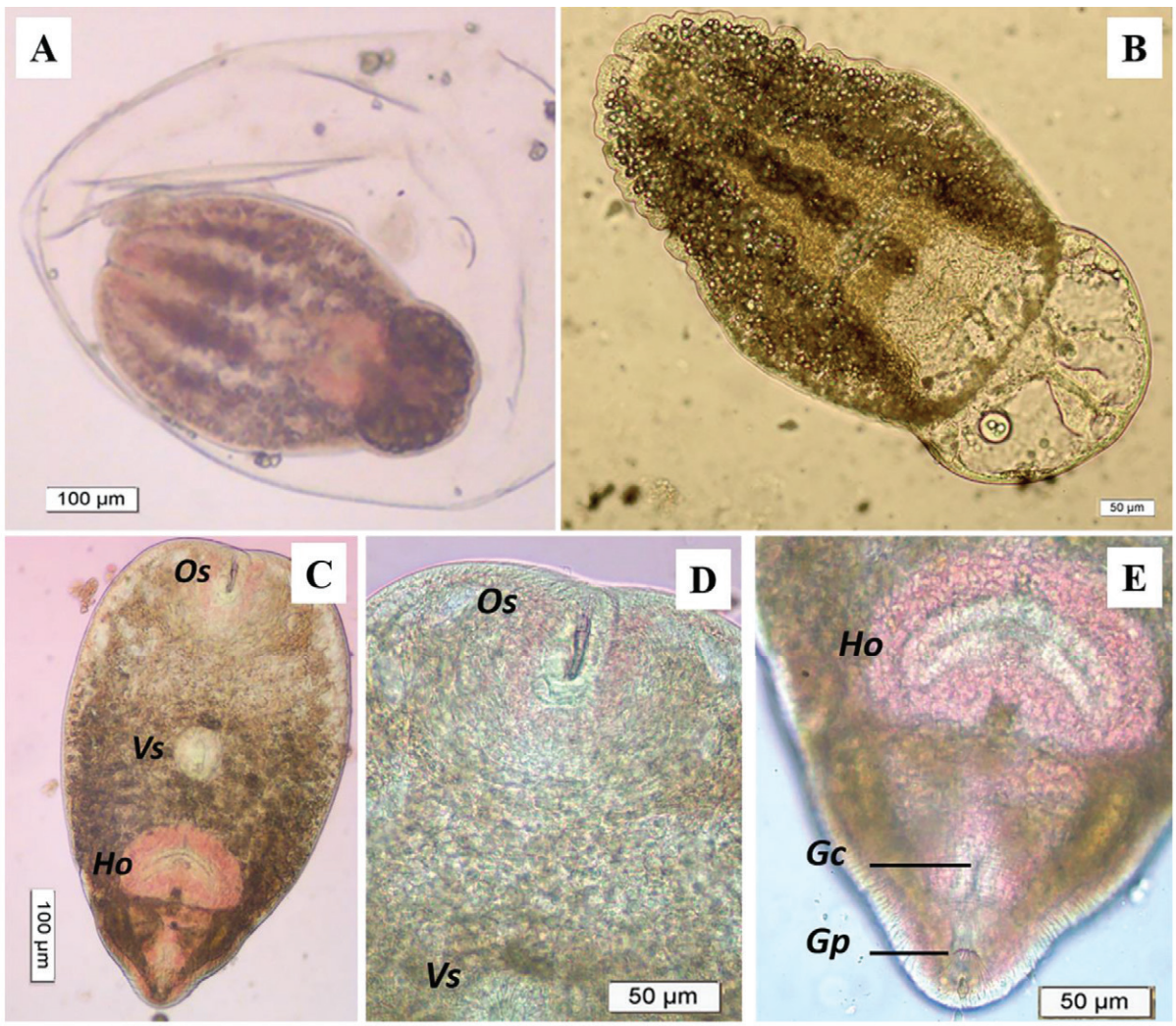

Fig. 5. Metacercariae of *Posthodiplostomum* sp. A. Encysted metacercaria with Semichon's acetocarmine stained. **B.** Live excysted *Posthodiplostomum* sp. from the vitreous humour of the eye of *A. danfordii*. C. Excysted metacercaria with Semichon's acetocarmine stained. D. Anterior end. E. Posterior end (Os: oral sucker, Vs: ventral sucker, Ho: holdfast organ, Gc: genital coni, Gp*:* genital pore)

al, with transparent, thin walls (Fig. 5A), 916–1100 (1020) µm long and 480–610 (560) µm wide. Body bipartite, consisting of forebody and hindbody (Fig. 5B). Total body size 571–604 (597) µm long and 300–313 (305) µm wide. Forebody 486–501 (490) µm long and 290–313 (302) µm wide (Fig. 5C). Pseudosuckers absent. Oral and ventral suckers feebly developed. Oral sucker subterminal, elongate-oval,

Table 2. The prevalence (%) and mean intensity ± stardard error values of digenean parasites in examined fishes with respect to seasons (n: examined fish number, ND: not detected, Meansfollowed by the same superscript letter are not significantly different)

	Spring	Summer	Autumn	Winter	P value
***Ascocotyle coleostoma* (Looss, 1896)**					
A. danfordii	98.9% 134.81±9.76[a] (n:90)	100% 182.93±11.62[b] (n:90)	100% 151.73±14.29[b] (n:30)	ND	0.0046
P. marmoratus	ND	ND	ND	100% 618.82±61.95 (n:50)	
N. melanostomus	50% 8.50±1.50 (n:4)	70.8% 14.41±3.60 (n:24)	83.3% 55.40±20.29 (n:18)	ND	0.1037
P. flesus	51.5% 20.86±2.70[a] (n:53)	63.6% 12.74±1.85[a] (n:106)	100% 72.22±9.22[b] (n:96)	100% 75.27±15.54[b] (n:41)	<0.001
C. auratus	12.9% 4.75±2.53[a] (n:62)	80% 21.00±2.30[b] (n:75)	84.2% 21.90±2.92[b] (n:38)	18.2% 8.00±4.26[a] (n:44)	0.0016
G. aculeatus	13.2% 2.00±0.77 (n:38)	25% 3.50±2.50 (n:8)	ND	0 0.00±0.00 (n:39)	0.4548
***Pygidiopsis genata* Looss, 1907**					
A. danfordii	70.0% 10.48±0.67	78.3% 11.42±0.99	88.1% 13.23±2.50	ND	0.0709
P. marmoratus	ND	ND	ND	18.0 10.89±5.35	*
N. melanostomus	0 0.00±0.00	8.3% 5.00±1.00	27.8% 4.60±0.93	ND	2.2892
P. flesus	15.1% 10.13±6.88[ab]	69.8% 3.61±0.43[a]	88.5% 9.00±0.95[b]	75.6% 6.10±1.62[b]	<0.001
Cryptocotyle concava (Creplin, 1825)					
P. marmoratus	ND	ND	ND	96.0% 26.46±6.01	*
N.melanostomus	0 0.00±0.00	41.7% 3.9±1.07	72.2% 17.77±4.48	ND	0.123
***Posthodiplostomum* sp.**					
A. danfordii	92.2% 22.54±3.65[a]	85.6% 38.18±6.65[b]	93.3% 37.11±3.81[b]	ND	<0.05

43–58 (49) µm long and 27–33 (31) µm wide. Ventral sucker oval, 50–55 (51) µm long and 40–44 (42) µm wide, at mid-length of forebody and close to holdfast organ (Fig. 5D); distance from ventral sucker to anterior extremity of body 222–304 (253) µm; distance from ventral sucker to holdfast organ 101–110 (109) µm. Holdfast organ cashew-shaped (Fig. 5D), 83 x 61 µm. Genital cone surrounded by prepuce (Fig. 5E).

Seasonal occurrence

In general, high parasite burden was observed in the autumn and low parasite burdens was detected during the spring. Digenean parasites were detected in all fish species throughout the year but there was a seasonal trend in prevalence and mean intensity of *A. coleostoma*. Statistically significant differences were obtained in seasonal occurrences of *A. coleostoma* detected in all fish species (P <0.01) (Table 2). The highest mean intensity of *A. coleostoma* was detected in *P. marmoratus* caught only in the winter (100%, 618.82 ± 61.95). In contrast, *P. marmoratus*, *N. melanostomus* and *A. danfordii* were not caught only in the winter. In *N. melanostomus*, the prevalence and mean intensity values were high dur-

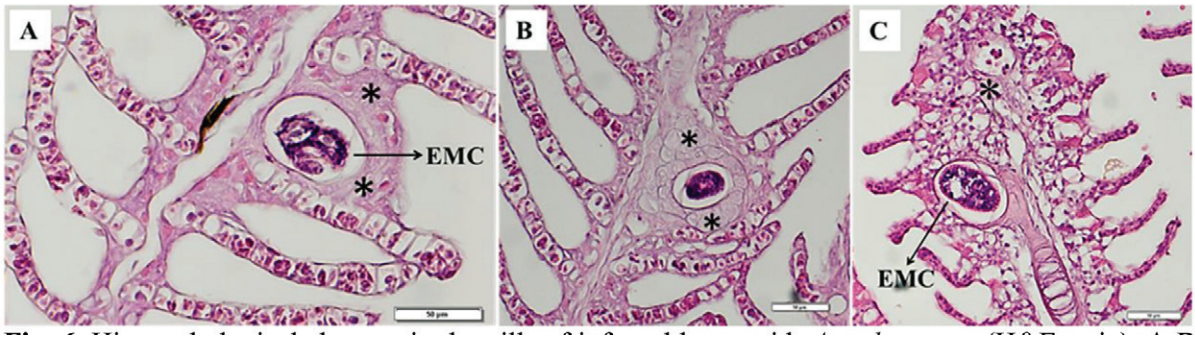

Fig. 6. Histopathological changes in the gills of infected hosts with *A. coleostoma* (H&E stain). A-B. Cartilage proliferation induced by a *A. coleostoma* metacercaria (*), in *P. flesus* and *A. danfordii* respectivelly. C. Hyperplasia, hypertrophy and fusion of the gill filament and lamellae around the cyst (*) in *P. marmoratus.*

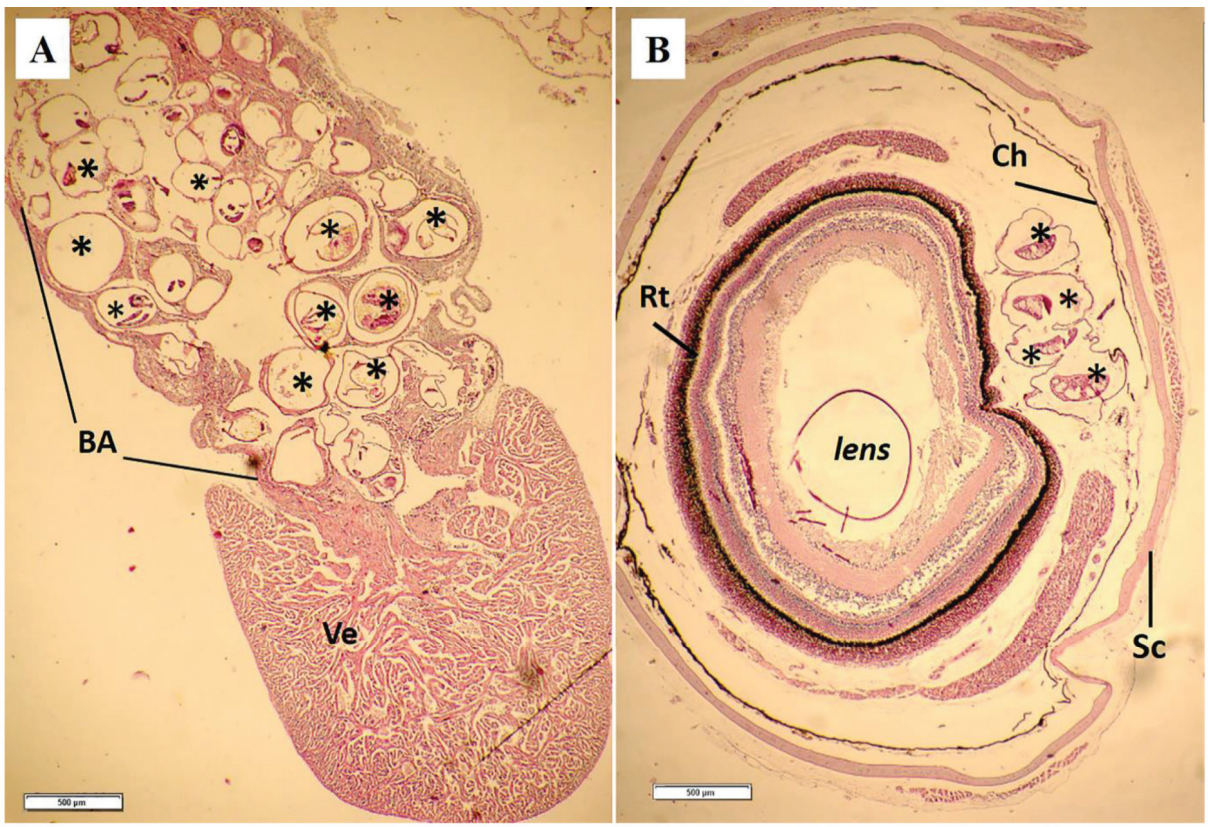

Fig. 7. Longitudial sections of infected heart and eyeball of *A. danfordii* (H&E stain). A. Histological section of heart infected with *A. coleostoma* metacercariae (*), B. Histological section of eyeball infected with *Posthodiplostomum* sp. metacercariae (*), (BA: bulbus arteriosus,Ve: ventriculus, Sc: sclera, Ch: choroid, Rt: retina).

ing autumn (83.3%, 55.40 ± 20.29) and low during spring (20.0%, 8.50 ± 1.50). The prevalence and mean intensity values of *A. coleostoma* from *A. danfordii* were very high in summer and autumn (100%, 182.93 ± 11.62 and 151.73 ± 14.29, respectively). *P. flesus* and *C. auratus* were caught in all seasons. In *P. flesus,* the prevalence and mean intensity of *A. coleostoma* were high in autumn and winter (100%, 72.22 ± 9.22 and 100%, 75.27 ± 15.54, respectively) and low in spring and summer (51.5%, 20.86 ± 2.70 and 63.6%, 12.74 ± 1.85, respectively). Differences in the seasonal mean intensity of *A. coleostoma* in the six fish species were statistically significant (P <0.01) (Table 2). In general, the intensity and prevalance of *A. coleostoma* varied seasonally in six fish species.

Metacercariae of *P. genata* were found in four out of the 6 fish species examined in this study (Table 1). The highest prevalence and mean intensity were detected in *A. danfordii* and *P. flesus* (80.9%, 12.30 ± 0.61 and 78.4%, 12.8 ± 0.94, respectively) (Table 1). There was a distinct seasonal trend in prevalence

and mean intensity (Table 2). Seasonally, the prevalence in the four fishes was high in autumn and low in spring (Table 2).

Metacercariae of *C. concava* were only found in *P. marmoratus* and *N. melanostomus.* Both prevalence and mean intensity were higher in *P. marmoratus* than in *N. melanostomus* (Table 1). The prevalence and mean intensity values in the two gobiid hosts were seasonally different. In *N. melanostomus*, prevalence and mean intensity were high during autumn (72.2%, 17.77 ± 4.48).

In *A. danfordii,* the prevalence and mean intensity were high in autumn (92.5%, 37.48 ± 3.81) (Table 2). Differences in the seasonal mean intensity of *Posthodiplostomum* sp. were statistically significant ($P < 0.01$) (Table 2).

Histopathological effects

The heart and gill tissues of fishes infected with *A. coleostoma* were examined. In the histopathological sections of gills, some pathological abnormalities were observed. In sections of the gill tissue, *A. coleostoma* was detected to cause histopathological changes such as cartilage proliferation (Fig. 6A-B), hyperplasia, hypertrophy and fusion (Fig. 6C) around the cyst in the tissue. In heart tissue sections, the metacercariae were intensively detected in the lumen and bulbo-ventricular regions of the bulbus arteriosus of the heart. Moreover, bulbus arteriosus region has been determined to be completely full with encysted metacercariae (Fig. 7A).

Eyeball sections of fishes infected with *Posthodiplostomum* sp. were examined. Histopathological changes in eyes are presented in Fig.7B. The metacercariae were observed between the choroid and pigmented retina (within the aqueous humor) and no metacercaria were detected in other locations in the eye.

Discussion

In the present study, four digenean parasite species were identified from six fish species (Table 1) and their seasonal occurrence and histopathological effects were determined (Fig. 6–7, Table 2). The present study provides new data on four digenean parasites infection in six fish species from Sarıkum Lagoon Lake in Sinop, Turkey. The most common parasite found during this study was *A. coleostoma* detected in all fish species examined (Table 1).

The classification of the heterophyid trematodes is based mainly on features of adults such as number and arrangement of oral spines, morphology of the terminal genitalia, length of oral appendage, length of intestinal caeca, position of uterine loops and distribution of vitelline follicles. However, the identification of metacercariae from fish is much more difficult, being based mainly on the armament of the oral sucker (Scholz et al. 1997). *Ascocotyle coleostoma* resembles *A. felippei* Travassos, 1929 in the number and arrangement of oral spines. The geographical distribution of these two species possessing 32 circumoral spines is rather different. *Ascocotyle coleostoma* was described from Egypt and later reported from Iraq, Italy, Ukraine, Russia, Azerbaijan and Turkmenistan (Mhaisen & Abus-Eis 1992, Mhaisen & Khanees 1995, Al-Jawda & Asmar 2015, Bhair & Al-Rudainy 2018). *A. felippei* has so far been recorded only from the Nearctic and Neotropical regions and has never been recorded from Palaearctic (Scholz et al. 2001, Hicks & Steele 2003, Santos et al. 2007, Brock & Font 2009, Shoaibi Omrani et al. 2010).Quite recently, in 2017, *A. felippei* were reported in the Sarıkum Lagoon Lake, outside of its natural range (Özer & Öztürk 2017). *Ascocotyle coleostoma* has been found in European waters including in the Black Sea basin and has so far been reported from the Palaearctic (Gaevskaya et al. 1975). Therefore, *A. coleostoma* was incorrectly recognized as *A. felippei* from the same fish hosts (except *A. danfordii*) in Sarıkum lagoon Lake. Thus, in the present study *A. coleostoma* is reported in *A. danfordii* for the first time. Other than these, the presence of *Ascocotyle longa* Ransom, 1920 was reported in *L. aurata* and other mugilid fish species (Scholz 1999, Scholz et al. 2001, Özer & Kirca 2013), but it was not found in the present study. The presence of *A. coleostoma* in all fish species examined can be evaluated as an indicator that this heterophyid metacercariae has no host specificity. On the other hand, differences in the infection parameters of *A. coleostoma* in the six species of fish living in the same sampling area most likely reflect variations in host preference and/or susceptibility. Hence, the significantly higher prevalence of *A. coleostoma* in *P. marmoratus* and *A. danfordii* than in the other four fish species indicates that these fish species are more preferable (susceptible) than *G. aculeatus, N. melanostomus, P. flesus,* and *C. auratus* as intermediate hosts to this heterophyid metacercariae in Sarıkum Lagoon Lake, Turkey.

Two other heterophyid species, *Pygidiopsis genata* and *Cryptocotyle concava,* have previously been reported from various gobiids in the NW Black Sea (Kvach 2004,2005). Similarly, *P. genata* has been reported in *N. melanostomus* from the Black Sea coasts of Sinop (Özer 2007). This study provides infection parameters of *P. genata* and *C. con-*

cava from southern Black Sea in *P. marmoratus* for the first time.

Species of the genus *Posthodiplostomum* are distributed around the world (Niewiadomska 2002). In Europe, two species of this genus, *P. cuticola* (von Nordmann, 1832) Dubois, 1936 and *P. brevicaudatum* (von Nordmann, 1832) Dubois, 1936, have been reported from various fish species and localities (Sudarikov et al. 2002). The third species in Europe, *Posthodiplostomum centrarchi* Hoffman, 1958 was reported as non-native invader (Stoyanov et al. 2017). In the present study, *Posthodiplostomum* sp. has been identified at the generic level only, since adequate morphological data of our metacercariae were not available. Further morphological and molecular data of these metacercariae are needed in order to identify them at the species level.

In this study, although both prevalence and mean intensity values of digenean metacercarial cysts detected in each fish species showed significant differences between seasons, the general trend was observed to being high in autumn and low in winter (except for *P. marmoratus*). This may be explained by the fact that the two infection indicators of metacercarial cysts in fish are affected by the same factors. It has been documented that the prevalence and mean intensity of digenean metacercarial infection rise and fall seasonally, which can be explained by the temperature-dependent release of cercariae (Poulin 2006). Furthermore, it has been reported that the release of the cercariae from the first intermediate host and the successful contamination to the second intermediate host is highly dependant on environmental temperature (Elsheikha & Elhasly 2008).

There are few reports on pathogenicity of *A. felippei* metacercariae (Hicks & Steele 2003, Shoaibi-Omrani et al. 2010). Shoaibi-Omrani et al. (2010) observed a reaction that the production of fibrous capsule of collagen surrounding the cyst produced by associated host fibroblasts and in the affected gill filaments of *Xiphophorus maculatus* (Güther, 1866), there were cartilage proliferation, hyperplasia, hypertrophy and fusion, especially around metacercarial cysts. The histopathological findings from gills detected in the present study agree with those reported by the above mentioned authors. Although there are no serious pathological findings in the heart and eye tissues of infected fish, it should be considered that the blood flow pumped to the gills may be slowed due to the metacercarial density in the bulbus arteriosus. Similarly, visual sharpness for infected fish can be slightly hindered or lost due to metacercariae burden. As a result, it can be said that slow blood circulation and poor vision may adversely affect the defence and feeding of fishes.

In conclusion, this paper is the first report on digenean parasite species in six fish species from Sarıkum Lagoon Lake in Sinop, Turkey. The findings of this study contain valuable data to contribute to our current knowledge of the distribution of digenean parasites in the Black Sea region.

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