



Occurrence of *Rhabdochona fortunatowi* Dinnik, 1933 (Nematoda: Rhabdochonidae) in Two Endemic Cyprinid Species (*Capoeta* spp.) in Turkey

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Abstract: Two endemic freshwater cyprinid fishes, *Capoeta caelestis* Schöter, Özuluğ & Freyhof, 2009 and *C. angorae* (Hankó, 1925), have been studied for the presence of parasitic nematodes in Firmız Stream, K. Maraş, Göksu River, Antalya, in the Anatolian Region of Turkey. Only a species of the genus *Rhabdochona* was found in intestines and identified as *R. fortunatowi* Dinnik, 1933. Totally, 104 specimens of *R. fortunatowi* infected 19 out of 99 individuals of *C. caelestis* (prevalence 19.1 %, mean intensity 5.4). In *C. angorae*, 40 specimens of *R. fortunatowi* infected 15 out of 178 fish individuals (prevalence 8.4 %, mean abundance 2.6). Prevalence and intensity of *R. fortunatowi* infection were calculated across the seasons as well as in age groups and sexes of hosts. The present study provides the first record of *R. fortunatowi* in Turkey and the first host records of this parasite in both *C. caelestis* and *C. angorae*, thus expanding the known geographical and host range of this nematode species.

Key Words: parasitic nematode, *R. fortunatowi*, freshwater fish, *Capoeta* spp., new host record, Anatolian Region of Turkey

Introduction

The rhabdochonid nematodes are intestinal parasites of freshwater fishes, comprising 95 species worldwide (MORAVEC et al. 2012). The genus *Rhabdochona* Railliet, 1916 (Nematoda: Rhabdochonidae) is common in many cyprinid species and less frequent in bagrids and silurids (MORAVEC 2010). Only one species of this genus, *Rhabdochona denudata* (Dujardin, 1845), has been reported in Turkey (TOPCU 1993, KIR 1998, AYDOĞDU et al. 2001, 2011, AYDOĞDU & SELVER 2006, ASLAN 2009, OZTURK 2014, KOYUN et al. 2015). In addition, unidentified species of

Rhabdochona have been reported from several fish species (SAYGI & BARDAKÇI 1990, OGUZ & ÖZTÜRK 1993, YILDIRIM & ÜNVER 2006).

The fish genus *Capoeta* Valenciennes, 1842 is represented by 15 endemic species in Turkey. *Capoeta angorae* (Hankó, 1925) occurs across southern part of Anatolia and *C. caelestis* Schöter, Özuluğ & Freyhof, 2009 inhabits the Göksu River drainage system, which is also in southern Anatolia (AYDOĞDU et al. 2015a).

Two previous ichthyoparasitological studies have been carried out on *C. angorae* in Turkey. This species was surveyed for monogenean (AYDOĞDU et

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al. 2015) and digenean (AYDOĞDU et al. 2018) infections. *C. caelestis* in Turkey has only been studied for digeneans (AYDOĞDU et al. 2018). There were no previous studies of parasitic nematodes of either *C. angorae* or *C. caelestis*. Therefore, the present article is the first survey presenting nematological data on these two host species at the global scale.

The purpose of the present article is to provide data on the nematode infections in two species of *Capoeta* (*C. angorae* or *C. caelestis*) from localities in the Anatolian Region in Turkey and to describe their dependence on the season, host sex and host age.

Materials and Methods

Fish specimens were collected from Fırnız Stream, K. Maraş, Göksu River, Antalya, Anatolia, in 2012 using a small hand net. Each sample consisted of 19–37 individuals of *C. caelestis* and 30–79 individuals of *C. angorae*, randomly collected once in every three months. A total of 277 fish individuals, 99 of *C. caelestis* and 178 of *C. angorae*, were examined (Tables 1 and 2). The specimens were transported alive to the laboratory. Fish were kept in aquaria, sacrificed and examined for helminths approximately 4 h after capture. The age calculated by analysis of fish scales using the methods of LAGLER (1996) and BEGENAL (1978) as well as the sex of each fish were recorded (Table 3). The samples of

C. caelestis were clustered in four and those of *C. angorae* were in eight age classes (Table 4). During dissection in saline, the gastrointestinal tracts were opened and their content was removed and examined using stereomicroscope. Specimens of *Rhabdochona fortunatowi* found in each dissected fish were counted, isolated from the intestines, washed in physiological saline, fixed in hot 4% formalin and preserved in 70% ethanol. Subsequently, to confirm taxonomic identification, they were cleared in mixture of glycerine and water in different proportions. Scanning electron microscopy (SEM) microphotographs (prostomal teeth and deirids) and light microscope photographs (spicules, eggs and papillae) showing the most important taxonomic characters of *R. fortunatowi* were obtained for the purposes of the identification. For SEM, individuals were first dried using Critical Point Dryer and then coated with Gold-Palladium 40-50 nm thickness with the help of BAL–TEC SC D005 coating device; they were examined at 20-30 kV in Carl Zeiss/Evo 40 stereoscam modes SEM. Nematode species were identified according to identification keys by YAMAGUTI (1985) and MORAVEC (1994). Prevalence and mean intensity of infection were calculated as defined by BUSH et al. (1997).

The distribution of the data was examined by the Shapiro–Wilk Test. The Mann–Whitney U Test was used for comparison between two groups that were not normally distributed. The Kruskal–Wallis Test was used in comparisons between three or more groups that were not normally distributed. Descriptive statistics of the data were presented as the median followed by the range (min-max). Spearman Correlation Analysis was applied in the study of the relationship between numerical variables. All statistical analyses were performed using IBM SPSS Statistics ver. 21.0 (IBM Co., Armonk, NY, USA); the level of significance was set at $\alpha=0.05$.

Table 1. Prevalence and mean intensity of *R. fortunatowi* in two *Capoeta* spp. from Turkey.

Infection parameters	<i>C. caelestis</i>	<i>C. angorae</i>
Examined Fish Number	99	178
Infected Fish Number	19	15
Prevalence (%)	19.1	8.4
Mean intensity	5.4	2.6
Intensity range (min–max)	1-12	1-20

Table 2. Prevalence and mean intensity of *R. fortunatowi* in two *Capoeta* spp. from Turkey across seasons in 2012.

Host species	Infection parameters	Winter	Spring	Summer	Autumn
<i>Capoeta caelestis</i> (N = 99)	Examined Fish Number	23	37	19	20
	Infected Fish Number	8	4	3	4
	Prevalence (%)	34.8	10.8	15.8	20.0
	Mean intensity	7.1	4.0	4.0	4.8
	Total parasite no	57	16	12	19
<i>Capoeta angorae</i> (N = 178)	Examined Fish Number	79	30	33	36
	Infected Fish Number	9	6	0	0
	Prevalence (%)	11.4	20.0	0	0
	Mean intensity	1.1	2.0	0	0
	Total parasite no	28	12	0	0

Table 3. Prevalence and intensity values of *R. fortunatowi* in two *Capoeta* spp. from Turkey, according to host sex

Fish sex groups	Infection parameters	<i>C. caelestis</i>	<i>C. angorae</i>
Female	Examined Fish Number	35	73
	Infected Fish Number	9	4
	Prevalence (%)	25.1	5.4
	Mean intensity	5.1	1
	Total parasite no	46	4
Male	Examined Fish Number	64	105
	Infected Fish Number	10	11
	Prevalence (%)	15.6	10.4
	Mean intensity	5.8	3.2
	Total parasite no	58	36

Table 4. Prevalence and intensity of *R. fortunatowi* in two *Capoeta* spp. from Turkey according to the host age

Host	Infection parameters	0	I	II	III	IV	V	VII	VIII
<i>C. angorae</i> (N = 178)	Examined Fish Number	10	7	60	53	25	20	1	2
	Infected Fish Number	0	0	6	6	2	1	0	0
	Prevalence (%)	0	0	10.0	11.3	8.0	5.0	0	0
	Mean intensity	0	0	5.0	1.0	1.5	1	0	0
	Total parasite no	0	0	30	6	3	1	0	0
Host	Infection parameters	0	I	II	III				
<i>C. caelestis</i> (N = 99)	Examined Fish Number	12	33	53	1				
	Infected Fish Number	2	4	13	0				
	Prevalence (%)	16.6	13.7	24.5	0				
	Mean intensity	3.0	4.2	6.2	0				
	Total parasite no	6	17	81	0				

Results

Of the 277 fishes of the two species examined, 31 were infected. Only one species of nematodes was recovered. Our detailed light and scanning electron microscopy studies on morphology of the rhabdochonid nematodes identified that the species was *Rhabdochona fortunatowi* Dinnik, 1933 (Fig. 1 and 2). The prevalence of infection was higher in *C. caelestis* than in *C. angorae* (19.1 % and 8.4 %, respectively, see Table 1).

For *C. caelestis*, the highest prevalence was in winter (34.8%) while the lowest rate was in spring (10.8%) (Table 2). For *C. angorae*, seasonal prevalence of *R. fortunatowi* was highest in spring (20%). The species was not detected in the summer and autumn samples (Table 2). The mean intensity of *R. fortunatowi* varied between 1.1 and 7.1, being highest in *C. caelestis* in winter samples (7.1) (Table 2). Comparing occurrence of *R. fortunatowi* in *Capoeta* spp. over seasons, statistically no significant difference was found in both host species across seasons ($p=0.208$ and $p=0.090$) (Table 5).

When prevalence and mean intensity of *R. fortunatowi* were evaluated according to sex groups of *Capoeta* spp., prevalence was higher in female and male individuals of *C. caelestis* than in *C. angorae* (Table 3). However, with respect to mean intensity, it was higher in males of *C. caelestis* and in *C. angorae* females (Table 3). There was no statistically significant difference in the numbers of *R. fortunatowi* between sexes in both *C. angorae* and *C. caelestis* ($p=0.220$ and $p=0.257$, respectively) (Table 5). The prevalence and mean intensity of *R. fortunatowi* according to age groups of hosts are presented in Table 4. In *C. angorae*, the prevalence was highest in three-year-old fishes, while mean intensity was higher in two-year-old fishes. There was no correlation between the age of *C. angorae* and the numbers of *R. fortunatowi* ($p=0.530$) (Table 6).

As for *C. caelestis*, the prevalence and mean intensity levels in *R. fortunatowi* were higher in two-year-old fishes (Table 4). However, there was no infection in three-year-old hosts. There was no significant correlation of the numbers of *R. fortunatowi* parasites between ages ($p=0.104$) (Table 6).

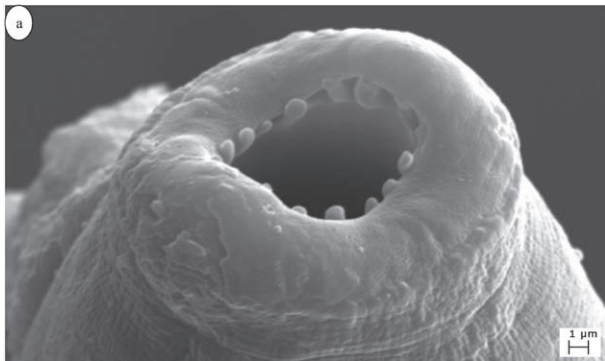


Fig. 1. *Rhabdochona fortunatowi* from *Capoeta caelestis* in Turkey, scanning electron micrographs, (a) mouth, apical view, (b) deirids.

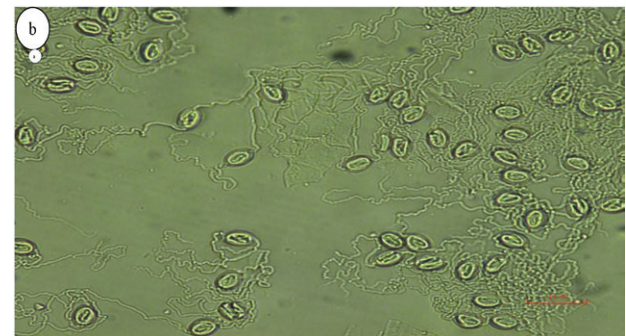
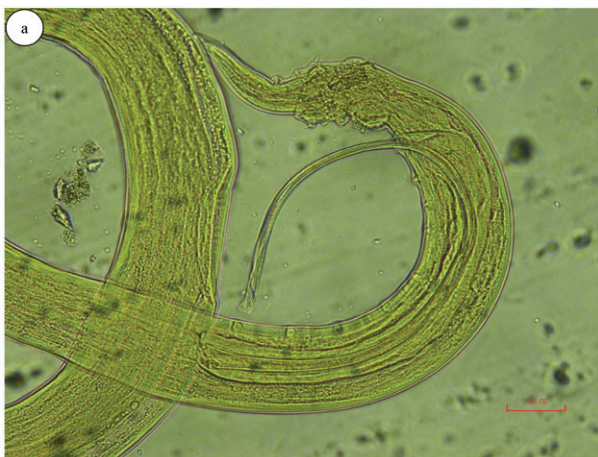
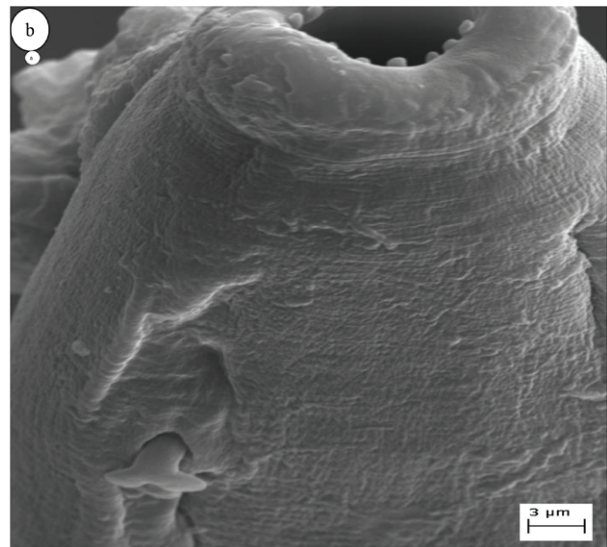


Fig. 2. *Rhabdochona fortunatowi* from *Capoeta caelestis* in Turkey: (a) posterior end of male, lateral view; (b) fully-developed eggs. (Scale bar = 100 μm).

Discussion

In this study, the occurrence of nematode parasites in two endemic species of *Capoeta* from the Anatolian Region of Turkey was investigated. Only one nematode species was identified, i.e. *Rhabdochona fortunatowi*. The specimens were identified on the basis of number and arrangement of prostomal teeth (Fig. 1a), size of deirids (conspicuously large) (Fig. 1b), structure of the distal tip of the left spicule (Fig. 2a), presence of long filaments on mature eggs (Fig. 2b), length of the left spicule and presence of lateral preanal papillae (Fig. 2a). On the basis of these characters, *R. fortunatowi* can easily be distinguished from the other congeneric species. Members of the genus *Rhabdochona* exhibit a rather high degree of host specificity. *R. fortunatowi* is a specific parasite of *Capoeta* spp. The nematode species *R. fortunatowi* is reported for the first time parasitising these two endemic species, *Capoeta caelestis* and *C. angorae*.

The present study raises the number of *rhabdochoniid* species recorded in Turkey to two and the

nematode *R. fortunatowi* is reported for the first time from Turkey. Two endemic species, *Capoeta angorae* and *C. caelestis*, are new hosts for *R. fortunatowi*. Therefore, the present study adds new data to the geographical distribution and the host range of this parasite species.

The results show that *C. caelestis* is more infected by this species (16.2%) than *C. angorae* (8.4%) (Table 1). The differences in the prevalence of *R. fortunatowi* in the two host species may be due to host resistance, different habitat use, behaviour of fish, environmental conditions and diet (ESCH & FERNANDEZ 1993, MARCOGLIESE 2002, KNUDSEN et al. 2004, MWITA & NKWENGULILA 2008, NUNN et al. 2008, MWITA & LAMTANE 2014).

In terms of seasonal variation of infection with *R. fortunatowi*, the highest levels of prevalence in *C. angorae* were recorded in spring, while prevalence was higher in winter in *C. caelestis*. KOYUN et al. (2015) reported that the prevalence of *R. denudata* was high in kura barbell in spring (23%) while AYDOGDU et al. (2001) recorded the highest prevalence in chub at

Table 5. Comparison of between various host characteristics and intensity of infection with *R. fortunatowi* in two *Capoeta* spp. from Turkey.

Host	Variable	Med (Min-Max)	p-value
<i>C. angorae</i> (n=178)	Host Sex [#]		
	Female	0 (0-1)	0.220
	Male	0 (0-20)	
	Season [#]		0.208
Winter 2012	0 (0-20)		
Spring 2012	0 (0-4)		
<i>C. caelestis</i> (n=99)	Host Sex [#]		
	Female	0 (0-12)	0.257
	Male	0 (0-11)	
	Season [#]		0.090
	Winter 2012	0 (0-12)	
	Spring 2012	0 (0-7)	
Summer 2012	0 (0-8)		
Autumn 2012	0 (0-9)		

#Values are represented as median (minimum – maximum). The values highlighted in bold are statistically ($p < 0.05$). No the seasonal comparison were made between *R. fortunatowi* and the summer – autumn periods in *Capoeta angorae* since *R. fortunatowi* was not encountered during these periods.

Table 6. Correlation of between ages and intensity of infection of *R. fortunatowi* in two *Capoeta* spp. from Turkey

	Number of <i>R. fortunatowi</i> parasites	
	r	p-value
<i>C. angorae</i> (n=178)	-0.047	0.530
<i>C. caelestis</i> (n=99)	0.164	0.104

the beginning of summer and at the end of winter (90 % and 87.5 %, respectively). ÖZTÜRK (2014) found high prevalence of infection by *R. denudata* in chub in summer (86.6%). The relationships between the level of endohelminth infection and sex of the fish have been studied by several researchers (ÖZTÜRK 2014, AYDOĞDU et al. 2014, 2015b-c, KOYUN et al. 2015, AÇIKEL & ÖZTÜRK 2016). ÖZTÜRK (2014) reported a difference in the relation between sex and infection of *R. denudata* in chub and the maximum number was found in male hosts. However, in the present study, the prevalence of *R. fortunatowi* in two *Capoeta* spp. was higher in females. The above-mentioned data suggest that this might be a result of food preferences of the host linked to the availability of infected ephemeropteran intermediate hosts. ROHDE (1993) found that if endoparasites infect sexes differently they often might have different feeding habits.

The higher prevalence of *R. denudata* in males in the present study supports this suggestion.

In our study, the prevalence of infection with respect to age is highest in three-year-old individuals of *C. angorae* and *C. caelestis*. The mean intensity of infection was higher in two-year-old hosts in both *Capoeta* spp. (Table 4). This agrees well with POJMANSKA & CHABROS (1993) who have found higher prevalence in common carps aged 0–2 years. Similarly, ÖZTÜRK (2014) found that *R. denudata* on chub heavily infected small-sized fish. AYDOĞDU et al. (2015c) found higher infections with *Saccocoeilium obesum* (Digenea) and *Neoechinorhynchus agilis* (Acanthocephala) in younger fish (age group II). According to ANDERSON (1974), POULIN (2007) and MARCOGLIESE & ESCH (1989), age and size may influence behaviour of the host in a way that its habitat or food preferences allow to benefit of the quantity of the potential intermediate hosts. The higher prevalence of *R. fortunatowi* in younger fish (age group II) in the present study supports these suggestions.

In conclusion, this is the first record of *R. fortunatowi* in Turkey expanding the number of identified rhabdochonid species in freshwaters of this country from one to two (except for unidentified species). *C. angorae* and *C. caelestis* are new hosts for *R. fortunatowi*. The present study reports infection parameters of the parasite linked to seasonal changes, host age and sex. There are 22 parasitic nematode species in fish in Turkey. Further extensive studies are needed in order to accurately record the diversity of parasitic nematodes in Turkey by studying more species of fish.

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