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First record of the natural infection of *Chondrus tournefortianus* (Mollusca: Pulmonata) by Dicrocoeliidae (Digenea) larval stages in Kastamonu, Turkey

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Abstract. The prevalence of larval stages of Dicrocoeliidae in the land snail, *Chondrus tournefortianus* (Férussac, 1821), found in Kastamonu, Turkey, was investigated and some of its morphological and histological features were determined. The snail samples were collected in spring and autumn. *C. tournefortianus* has been reported for the first time as being an intermediate host in the life cycle of Dicrocoeliid (Trematoda: Digenea) species. The prevalence of the infection in Kastamonu, Turkey was 2.27%, with the highest value occurring in October.

Key words: *Chondrus tournefortinus*, Dicrocoeliidae, larval stage, Kastamonu, Turkey.

Dicrocoeliid species are parasites of the liver, gall bladder, pancreas and intestine of amphibians, reptiles, birds and mammals (Olsen 1974, Otranto & Traversa 2002, Gürelli & Göçmen 2007). Their biological cycle requires two intermediate hosts (a land snail and an arthropod) (Krull & Mapes 1952, Alunda & Rojo-Vazquez 1984, Otranto & Traversa 2002, Ducháček & Lamka 2003, Murvanidze et al. 2010, Gürelli et al. 2014). The land snails that are the first intermediate host of this parasite in Turkey are *Helicopsis derbentina*, *H. protea*, *Cernuella virgata*, *Trochoidea pyramidata*, *Cochicella acuta*, *Monacha carthusiana*, *Helicella candicans*, *Helix aspersa* and *H. lucorum* (Kalkan 1971, Gürelli & Göçmen 2007, Gürelli et al. 2014).

Chondrus tournefortianus (Férussac, 1821) is a land snail species spreading from North-Western Anatolia to Varna-Bulgaria. It shows regional endemism (Kebapçı 2007, Örstan 2013, Irikow & Mollov 2014). To date, no investigation has been reported about larval trematodes of this land snail. The aim of this study is to determine the prevalence of larval stages of Dicrocoeliidae in the land snail, *Chondrus tournefortinus* (Férussac, 1821) and investigate their (sporocysts and cercariae) histological and morphological features.

Chondrus tournefortinus (Férussac, 1821) were collected following rainfall in areas surrounding the Kastamonu province in March, April, May (spring) and September, October, November (autumn) in 2014. These areas were near farms where goats, sheep, cattle, horses and donkeys were present and crops were raised. A total of 88 land snails were collected and dissected while alive and their hepatopancreas were removed. These were placed on clean glass slides with a drop of 0.6% NaCl solution. Using a mounted needle, fluid was drawn out of the hepatopancreas and spread as a thin film on a slide for investigation of live larval stages of parasites. Samples were also fixed in 10% formaldehyde-alcohol and 70% alcohol and the larval stages (sporocysts and cercariae) of Dicrocoeliidae were observed under a light microscope. The fixed parasites were covered with an adhesive mixture and stained with Borax-Carmine. Photographs of the larval stages of this parasite were taken with Zeiss Imaging System.

Dicrocoeliid second generation sporocysts and cercariae were observed in the hepatopancreas of two specimens (2.27%) of the total 88 specimens examined (Table 1). The highest prevalence (4%) of sporocysts and cercariae by date was observed in October 2014 (Table 2).

Second Generation Sporocysts. The last stage of second generation sporocysts develop cercariae and become large elongated sacs. The tail, oral sucker and ventral sucker of cercariae were visible. Each sporocyst has 10-35 cercariae and the cercariae are released from a birth pore (Fig. 1a-b-c).

Cercaria. Cercariae have two suckers, oral and ventral, with an anterior stylet in the oral sucker. The bifurcated intestine is located behind the oral sucker and lasted near the ventral sucker. The excretory bladder is located behind the ventral sucker. Cercariae have long simple tails; however, shorter tails sometimes occur. In some of cercariae, the long tail is directed anteriorly. They are called Xiphidiocercariae (Fig. 1d-e-f).

This study reports for the first time the occurence of *C. tournefortianus* as an intermediate host

Table 1. Prevalence of *C. tournefortianus* infected with Dicrocoeliidae larval stages according to the number of dissected land snails.

Number of dissected *C. tournefortianus*	Number of infected *C. tournefortianus*	Prevalence of Infection (%)
88	2	2.27

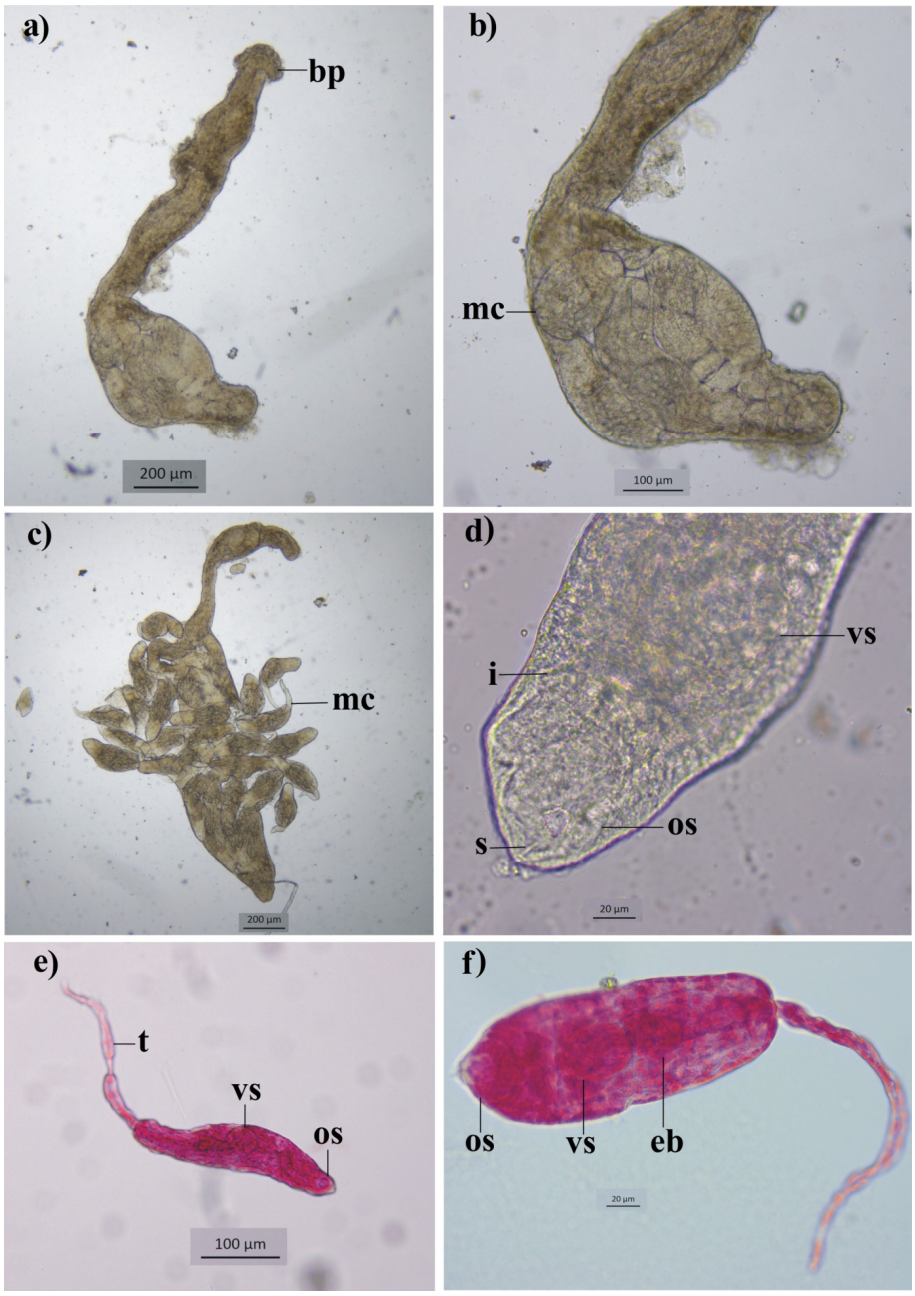

Figure 1. Different stages of larval trematodes. **a-b-c.** Last stage of second generation sporocysts, **d.** cercaria, **e-f.** cercariae stained with Borax-Carmine. Bp: birth pore, eb: excretory bladder, mc: mature cercaria, i: intestine, os: oral sucker, s: stylet, t: tail, vs: ventral sucker.

Table 2. Prevalence of *C. tournefortianus* infected with Dicrocoeliidae larval stages according to the date of collection.

Number of dissected *C. tournefortianus* according to the date of collection	Number of infected *C. tournefortianus*	Prevalence of Infection (%)
10 (March 2014	-	0
20 (April 2014)	-	0
22 (May 2014)	1	4.54
10 (September 2014)	-	0
20 (October 2014)	1	5
6 (December 2014)	-	0

Table 3. Comparision of the prevalence in different land snail species infected with Dicrocoeliidae larval stages.

Land snail species	Prevalence (%)	Country	Reference
Helicella candicans	4.3	Turkey	Kalkan 1971
Helicopsis derbentina	4.0	Turkey	Kalkan 1971
Helicopsis krynickii	2.6	Turkey	Kalkan 1971
Trochoidea pyramiadata	0.2	Turkey	Kalkan 1971
Monacha carthusiana	2.8	Turkey	Kalkan 1971
Cernuella virgata	1.0	Turkey	Kalkan 1971
Helicopsis protea	0.8	Turkey	Kalkan 1971
Cochlicella acuta	0.4	Turkey	Kalkan 1971
Helicella itala	5.68	Turkey	Kalkan 1971
Helicella obvia	26.8	Germany	Schuster 1993
Helicella itala	2.98	Spain	Manga-González 1987, Manga-González et al. 2001
Helicella corderoi	1.06	Spain	Manga-González et al. 2001
Helix aspersa	0.97	Turkey	Gürelli & Göçmen 2007
Helix lucorum	27.6	Turkey	Gürelli et al. 2014
Chondrus tournefortianus	2.27	Turkey	Present study

in the life cycle of Dicrocoeliidae. It occurred with a 2.27% prevalence of infection in the study area. This land snail species is spreading from North-Western Anatolia to Varna-Bulgaria (Kebapçı 2007, Örstan 2013, Irikow & Mollov 2014). Due to its important distribution, the spread of infection of this parasite to second intermediate hosts in this area could be higher. The infection rate was the highest in October and the rate of infection spreading to second intermediate hosts (arthropods) was also higher in October.

The second generation sporocysts are divided into three groups according to their morphological features. The first stage of second generation sporocysts have undifferentiated germinal masses and they are like thin long sacs. The second stage of second generation sporocycts have germinal masses containing the outline of cercariae or immature cercariae and the last stage of second generation sporocysts have developed cercariae (González-Lanza et al. 1997, Gürelli 2006). In this study, only the last stage of second generation sporocycts were observed. Otranto & Traversa (2002) and Ducháček & Lamka (2003) have reported that in snails, the larval stages evolved in about 3-4 months from the miracidia, which pass into the snails with the eggs, to first and second generation sporocysts, which represent asexual reproduction. Therefore, *C. tournefortianus,* which was found infected in May may have been infected the previous year. After being infected, *C. tournefortianus* could go into winter hibernation and in spring, when they awaken, the larvae in their hepatopancreas continue to develop. Thus, *C. tournefortianus,* which was found in the snails in October may have been originated from being infected in the previous spring or summer.

C. tournefortianus was collected near farms and villages where herbivorous animals were grazing, so the larval stages could be *D. dendriticum*. If they are larval stages of *D. dendriticum*, the risk factor of infection of other animals is increasing.

The life history of the mollusc intermediate hosts is of great epidemiological interest, as regards both the ingestion periods of Dicrocoeliidae eggs, dependent on the mollusc's activity and the survival of the parasite in them. Species, age and nutritional state of the molluscs, infective dose, ambient temperature and relative humidity, amongst other aspects, all influence the development of larval stages of this digenean in the first intermediate hosts (Manga-González et al. 2001).

The reason for selecting spring and autumn for the investigation is because of the interest in the activity time of land snails. They are in hibernation during the winter and awake because of warmth and rain in spring. Activated land snails could get the miracidia of parasites, so the risk factor could be increased. In the summer land snails could be in an aestivation period and they might be activated again in autumn, because of wet weather.

When the prevalence of infection among different land snails in different countries was compared, a large variation was observed (Table 3).

In conclusion, *Chondrus tournefortianus* seems to play an important role as an intermediate host of Dicrocoeliid species.

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