

# Natural Infection of *Helix aspersa* (Mollusca: Pulmonata) by Dicrocoeliidae (Digenea) Larval Stages in Izmir, Turkey

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**SUMMARY:** In this study, the prevalence of larval stages of Dicrocoeliidae in the garden snail *Helix aspersa* Müller, 1774 commonly found in the vicinity of Izmir, Turkey was investigated and some of its histological and morphological features were determined. The molluscs were collected during the spring of 2005. As the result of the investigation, a land snail species, *H. aspersa*, was found to be an intermediate host in the life cycle of Dicrocoeliidae and it had a prevalence of 0.97 % in the study area. In the present study, *H. aspersa* has been reported for the first time as an intermediate host of Dicrocoeliid species. The prevalence of infection was highest in March.

**Key Words:** *Helix aspersa*, larval stage, Dicrocoeliidae, intermediate host, Mollusca

## ***Helix aspersa* (Mollusca: Pulmonata)'nın Dicrocoeliidae Larval Safhalarıyla İzmir (Türkiye)'de Doğal Enfeksiyonu**

**ÖZET:** Bu çalışmada İzmir civarında dağılışı gösteren bahçe salyangozu *Helix aspersa* Müller, 1774 (Mollusca: Pulmonata)'daki Dicrocoeliidae larval safhalarının yaygınlığı araştırılarak, morfolojik ve histolojik özellikleri tespit edilmiştir. Yumuşakçalar 2005 yılının ilkbahar aylarında toplanmıştır. Araştırma sonucuna göre *H. aspersa*'nın çalışma alanında %0.97'lik yaygınlıkla Dicrocoeliidae yaşam döngüsüne ara konaklık yapan bir kara salyangozu türü olduğu tespit edilmiştir. Bu çalışmayla ilk kez *H. aspersa*'nın Dicrocoeliid türlerine ara konaklık yaptığı rapor edilmiştir. Enfeksiyon yaygınlığı Mart ayında en yüksek seviyededir.

**Anahtar Sözcükler:** *Helix aspersa*, larval safha, Dicrocoeliidae, ara konak, Mollusca

## INTRODUCTION

Dicrocoeliid species are parasites of the liver, gall bladder, pancreas and intestine of amphibians, reptiles, birds and mammals. The first intermediate host of Dicrocoeliidae is land snails (6). This family belongs to the superfamily Plagiorchioidea, suborder Plagiorchiata, order Plagiorchiida and superorder Epitheliocystida (4). To apply effective prophylactic measures to some indirect cycle of parasitosis linked to the grazing system, it is necessary to know previously the species of molluscs that intervene in their biological cycles as intermediate hosts, as well as their biology and ethology (3).

The prevalence of larval stages of Dicrocoeliidae in a garden snail *Helix aspersa* Müller, 1774 is the focus of this study. Its histological and morphological features were determined.

## MATERIAL AND METHODS

*Helix aspersa* Müller, 1774 was collected following rainfall in areas surrounding Izmir in March, April and May in 2005. These areas were near the farmers where sheep, goats, cattle, horses, donkeys live and agriculture makes.

A total of 205 snails (*H. aspersa*) were collected. They were dissected while alive and their livers were removed. These were placed on clean glass slide with a drop of %0.6 NaCl solution. The livers were incised with a mounted needle and a thin film of the liver fluid was drawn out on a slide for examination of live parasites. The larval stages of Dicrocoeliidae were observed under a light microscope, fixed in vapour of 2% osmic acid and 10% formaldehyde-alcohol. The fixed parasites were covered with adhesive mixture and stained with Borax-Carmine, Haematoxylin-Eosine and Ferric-Haematoxylin. Larval stages were measured with a calibrated ocular micrometer and photographs taken with an Olympus CX31-Altra 20 Soft Imaging System. Abbreviations about measurements are listed in Table 1.

SPSS (Vers. 10.0) was used to statistically analyse the observations on various morphological characteristics

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**Table 1.** Abbreviations about measurements.

[SGSLI]	Second Generation Sporocyst Length I
[SGSWI]	Second Generation Sporocyst Width I
[SGSLII]	Second Generation Sporocyst Length II
[SGSWII]	Second Generation Sporocyst Width II
[SGSLIII]	Second Generation Sporocyst Length III
[SGSWIII]	Second Generation Sporocyst Width III
[CBL]	Cercaria Body Length
[CBW]	Cercaria Body Width
[CTL]	Cercaria Tail Length
[CTW]	Cercaria Tail Width
[OSL]	Oral Sucker Length
[OSW]	Oral Sucker Width
[VSL]	Ventral Sucker Length
[VSW]	Ventral Sucker Width

## RESULTS

Dicrocoeliid second generation sporocysts and cercariae were observed in the livers of 0.97% of the total 205 specimens of examined (Table 2). The highest prevalence of sporocysts and cercariae by date was observed in March 2005 (1.81%) (Table 3).

**Table 2.** Prevalence of *H. aspersa* Müller, 1774 infected with Dicrocoeliidae larval stages according to the number of dissected snails.

Number of dissected <i>H. aspersa</i>	Number of infected <i>H. aspersa</i>	Prevalence of Infection (%)
205	2	0.97

**Table 3.** Prevalence of *H. aspersa* infected with Dicrocoeliidae larval stages according to the date of collection.

Number of dissected snails according to the date of collection	Number of infected snails	Infection prevalence (%)
55 (March 2005)	1	1.81
57 (April 2005)	-	0
93 (May 2005)	1	1.07

### Second Generation Sporocyst

First stage of second generation sporocysts has undifferentiated germinal masses and become small simple elongated sacs (Table 4, Figure 1A).

Second stage of second generation sporocysts has germinal masses containing an outline of cercariae or immature cercariae and become medium elongated sacs (Table 4, Figure 1B).

Final stage of second generation sporocysts has developed cercariae and become large elongated sacs. Each sporocysts have 8-37 cercariae and cercariae are released from a birth pore (Table 4, Figure 1C).

### Cercaria

Cercariae possess two suckers, oral and ventral suckers (Figure 1E, G). They have an anterior stylet in the oral sucker (Figure 1D, G). The bifurcated intestine is located behind the

oral sucker. The excretory bladder is situated behind the ventral sucker (Figure 1E). Mucous penetration glands are located on the dorsal side of the cercariae (Figure 1F). Cercariae have long simple tails (Figure 1E), and occasionally tails are shorter and directed anteriorly (Table 5). They are xiphidiocercariae.

**Table 4.** Measurements and biometric data belonging to the first, second and final stage of second generation sporocysts (n: Number of sample, SD: Standard deviation, SE: Standard error).

Measurements	First, second, final stage of second generation sporocysts (n=30)			
	Range	Mean	SD	SE
[SGSLI]	550-1420	930.33	202.95	37.06
[SGSWI]	50-167.5	95.5	31.33	5.72
[SGSLII]	937-337.5	1498.08	339.24	61.93
[SGSWII]	100-250	180.08	43.07	7.86
[SGSLIII]	1062.5-2912.5	2064.75	450.56	82.26
[SGSWIII]	150-260	217.16	28.42	5.18

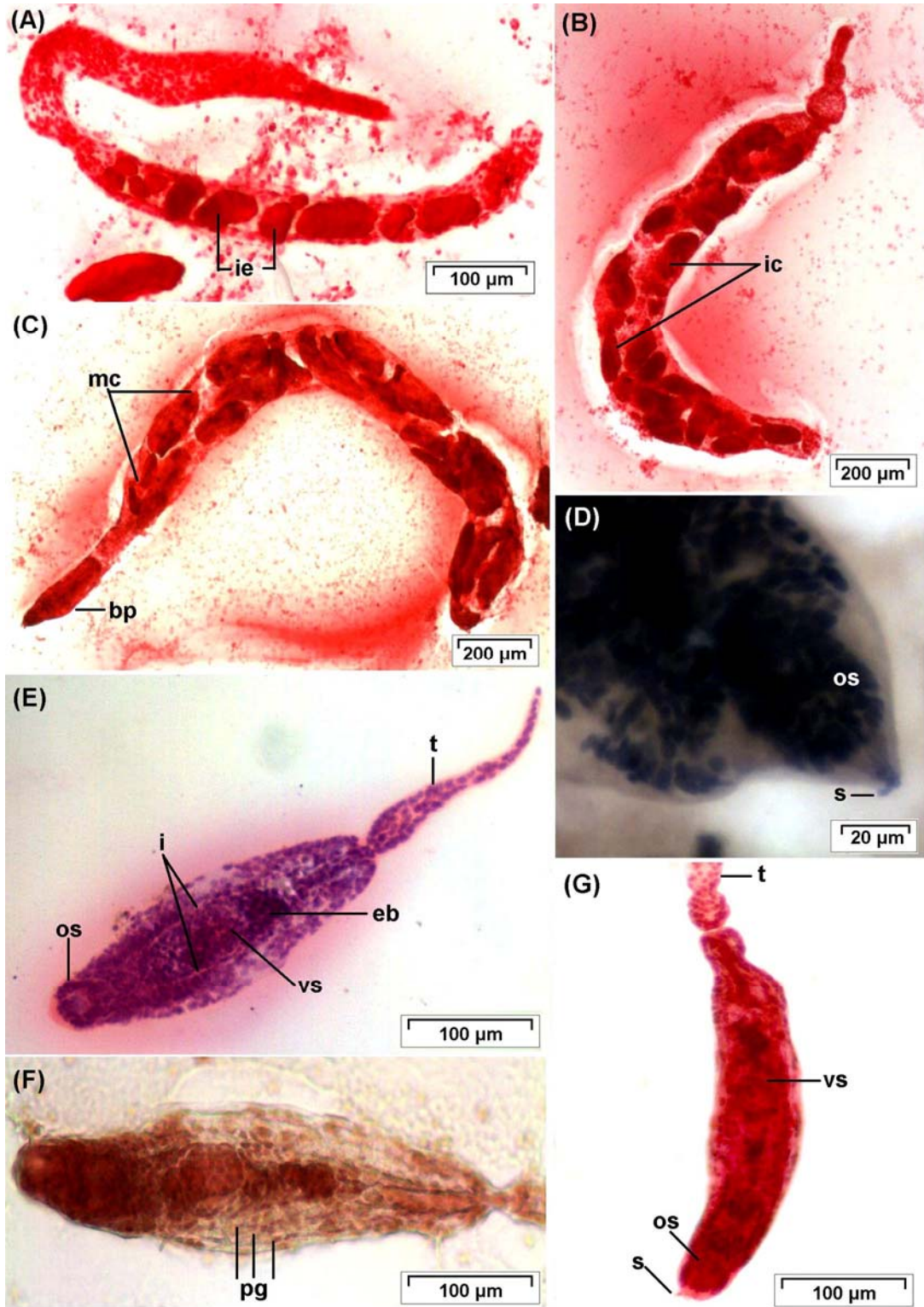
**Table 5.** Measurements and biometric data belonging to the cercariae.

Measurements	Cercaria (n=30)			
	Range	Mean	SD	SE
[CBL]	150-450	333.33	64.41	11.75
[CBW]	62.5-147.5	104.66	21.45	3.91
[CTL]	42.48-87.5	339.91	99.44	18.15
[CTW]	22.5-100	36.16	19.45	3.55
[OSL]	30-70	48.75	9.01	1.64
[OSW]	25-70	42.08	9.51	1.73
[VSL]	25-55	41.58	8.16	1.48
[VSW]	27.5-80	48.16	11.76	2.14

## DISCUSSION

In this study *H. aspersa* Müller, 1774 was determined as a species of land snail which is the intermediate host in the life cycle of Dicrocoeliidae, with a 0.97% prevalence of infection in the study area. This study reports for the first time *H. aspersa* as an intermediate host of Dicrocoeliidae. The importance of *H. aspersa* in Dicrocoeliosis epidemiology is great due to the wide distribution and abundance of the species not only in Turkey, but also throughout the world.

*H. aspersa* was found infected in March 2005 contained second generation sporocysts with germinal masses containing outline of cercariae or immature cercariae, developed cercariae and free cercariae. *H. aspersa* was found infected in May 2005 contained second generation sporocysts with undifferentiated germinal masses, germinal masses containing an outline of cercariae or immature cercariae, developed cercariae and free cercariae. Otranto and Traversa (6) and Ducháček and Lamka (1) 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



**Figure 1.** Different stages of larval trematodes. **(A):** First stage of second generation sporocyst. **(B):** Second stage of second generation sporocyst. **(C):** Final stage of second generation sporocyst (Fixed in Formaldehyde-alcohol, stained with Borax-Carmine). **(D):** Cercaria (Fixed in Formaldehyde-alcohol, stained with Ferric-Haematoxylin). **(E):** Cercaria (Fixed in Formaldehyde-alcohol, stained with Borax- Carmine). **(F):** Cercaria (Fixed in Formaldehyde-alcohol, stained with Haematoxylin-Eosine). **(G):** Cercaria (Fixed in Osmium vapour, stained with Borax-Carmine). **ie:** immature embriyos, **ic:** immature cercariae, **mc:** mature cercariae, **bp:** birth pore, **os:** oral sucker, **s:** stylet, **vs:** ventral sucker, **i:** intestine, **eb:**excretory bladder, **t:** tail, **pg:** pentration glands.

generation sporocysts which represent asexual multiplication stages. Therefore, *H. aspersa* which was found, infected in March may have been infected the previous year, possibly even as early as the beginning of autumn. *H. aspersa* which was found infected in May, may have been infected at the beginning of March or the autumn of the previous year.

Especially, cercariae are liked of *Dicrocoelium dendriticum* (Rudolphi, 1819) cercariae from obtained other land snails by other researchers such as, Kalkan (2), Manga-González (3), Schuster (7), Manga-González *et al.* (4). In addition, due to molluscs obtained near the farmers, possibility was increased.

Sometimes different developmental stages of the parasites were found in a mollusc at the same time. This may indicate that either the mollusc was infected at a different time or that larval development of eggs ingested at the same time is diachronic (4).

When the percentage of infection by Dicrocoeliidae, possibly *D. dendriticum* observed in *H. aspersa* (0.97%) was compared with those given by other authors in different species of molluscs and countries (Table 6), a large degree of variability was observed.

**Table 6.** Comparison of the prevalence in different snail species infected with Dicrocoeliidae especially *Dicrocoelium dendriticum* and present study (PS).

Mollusc species	Prevalence (%)	Country	Reference
<i>Helicella candicans</i>	4.3	Turkey	2
<i>Helicopsis derbentina</i>	4.0	Turkey	2
<i>Helicopsis krynickii</i>	2.6	Turkey	2
<i>Trochoidea pyramidata</i>	0.2	Turkey	2
<i>Monacha carthusiana</i>	2.8	Turkey	2
<i>Cerņuella virgata</i>	1.0	Turkey	2
<i>Helicopsis protea</i>	0.8	Turkey	2
<i>Cochlicella acuta</i>	0.4	Turkey	2
<i>Helicella itala</i>	5.68	Spain	2
<i>Helicella obvia</i>	26.8	Germany	7
<i>Helicella itala</i>	2.98	Spain	4
<i>Helicella corderoi</i>	1.06	Spain	4
<i>Helix aspersa</i>	0.97	Turkey	PS

**Table 7.** Comparison of the measurements of second generation sporocysts obtained from different studies and present study. All measurements are in micrometers (PS: Present study).

Mollusc species	[SGSL]	[SGSW]	Country	Reference
<i>Helicella itala</i>	140-4005	50-231	Spain	3
<i>Helicella itala</i>	560-4160	150-501	Spain	4
<i>Helicella corderoi</i>	200-1540	20-170	Spain	4
<i>Helix aspersa</i>	550-2912.5	50-260	Turkey	PS

**Table 8.** Comparison of the measurements of cercariae obtained from Manga-González's (1987) and present study. All measurements are in micrometers (PS: Present study).

Mollusc species	[CBL]	[CBW]	Country	Reference
<i>Helicella itala</i>	495-1220	10-200	Spain	3
<i>Helix aspersa</i>	192.5-937.5	62.5-147.5	Turkey	PS

According to the measurements, mollusc species could possess an important effect on the measurement values of second generation sporocysts and cercariae.

Although 8-37 cercariae were observed in the second generation sporocysts by during this study, 10-40 cercariae were observed by Ducháček and Lamka (1).

In conclusion, *Helix aspersa* seems to be an important role as an intermediate host of Dicrocoeliid species.

When the measurements were compared between the second generation sporocysts and cercariae obtained in this study within *H. aspersa* with those given by other authors in different species of molluscs (Tables 7, 8), a large degree of variability was observed.

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