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Occurrence of the Pathogens and Parasites of *Phyllotreta undulata* (Coleoptera: Chrysomelidae) in Turkey

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Abstract: The pathogens and parasites of *Pyllotreta undulata* Kutschera (Coleoptera: Chrysomelidae) collected from 5 localities in the Black Sea region of Turkey were investigated. Four different types of pathogens and parasites were recorded in adults of *P. undulata*: gregarine, microsporidium, and 2 nematodes, namely 1 *Howardula* species and 1 *Hexamermis* species. Gregarine and *Howardula* were the most common pathogen and parasite in *P. undulata* populations. There is the first record of a *Hexamermis* (Nematoda: Mermithidae) parasite from *P. undulata* in Turkey.

Key Words: Pathogen, parasite, *Hexamermis*, microsporidium, *Phyllotreta undulata*

Türkiye'de *Phyllotreta undulata* (Coleoptera: Chrysomelidae)'nın Patojen ve Parazitlerinin Varlığı

Özet: Bu çalışmada Türkiye'nin Karadeniz Bölgesi'ndeki beş ilden toplanan *Phyllotreta undulata* Kutschera (Coleoptera: Chrysomelidae)'nın patojen ve parazitleri araştırılmıştır. Gregarin, microsporidium ve *Howardula* ile *Hexamermis* cinslerine ait iki tip nematod olmak üzere 4 farklı patojen ve parazit türü gözlenmiştir. *Gregarina* ve *Howardula* cinslerine ait türler, *P. undulata* popülasyonlarında en çok bulunan patojen ve parazitler olarak tespit edilmiştir. *Hexamermis* cinsine ait parazit, *P. undulata* popülasyonlarında ilk kez tespit edilmiştir.

Anahtar Sözcükler: Patojen, parazit, *Hexamermis*, microsporidium, *Phyllotreta undulata*

Introduction

Phyllotreta undulata Kutschera (Coleoptera: Chrysomelidae) is a common and well-known pest of the genus *Brassica* throughout Europe, including Turkey (Aslan et al., 1999). Since flea beetles of the genus *Phyllotreta* cause damage to Cruciferae resulting in financial losses, natural enemies of *P. undulata* are of great interest nowadays. In detailed studies conducted in continental Europe, Sommer (1981) recorded several insect parasitoids as well as protists and nematodes from 8 *Phyllotreta* spp. Lipa and Ekbom (2003) reported microsporidian and eugregarine infections and nematode parasitisation of *P. undulata* in Sweden. Some studies on the parasites of *P. undulata* adults collected from some localities in Turkey in different periods were partially published by Yaman (2002a, 2002b) and Yaman and Radek (2005). In the present study we report on the occurrence of the parasites and pathogens of *P. undulata* from 5 localities in Turkey during 2007, adding new parasites.

Materials and Methods

Adult *P. undulata* specimens were collected from 5 localities (Samsun, Trabzon, Rize, Gümüşhane, and Hopa) in the central and eastern Black Sea regions of Turkey

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from April to September 2007. The samples were collected in summer and autumn from each locality with 2 exceptions. The first exception was Gümüşhane, where we could not obtain samples in spring or summer due to the lack of sampling sites as a result of harsh environmental conditions. The other exception was Trabzon, where we collected samples in early spring and summer from this locality because even the slightest change in climate causes large differences in the life cycle of these beetles (Table 1). Furthermore, we collected more samples from Samsun throughout the whole year because of its special location and because we knew from previous studies on *P. undulata* in Turkey that Samsun has the most diverse pathogen and parasite spectrum (Yaman, 2002a, 2002b; Yaman and Radek, 2005). Hopa, a district in Artvin province, has very harsh weather conditions and therefore only 2 samples could be examined from this locality. Collected *P. undulata* adults were put into sterilised glass bottles to prevent possible contamination and brought to the laboratory and dissected as soon as possible. Each beetle was dissected with Ringer's solution and examined under a light microscope at the magnifications of 40× to 1000× (Toguebaye et al., 1988; Undeen and Vavra, 1997). Detected pathogens and parasites were measured and photographed using an Olympus BX51 microscope with a DP-25 digital camera and DP2-BSW Soft Imaging System.

Table 1. Infections and parasitisations found in *Phyllotreta undulata* populations in various localities.

Sampled Localities	Sampling Dates	Number of Examined Beetles	Infections / Parasitisations found in *P. undulata* populations							
			Gregarina sp.	%	Microsporidium	%	*H. phyllotreta*	%	Mermithidae	%
Trabzon	10.05.2007	19	10	52.63	0	0.00	4	21.05	0	0.00
	20.05.2007	47	34	72.34	0	0.00	6	12.77	0	0.00
	26.05.2007	55	31	56.36	0	0.00	4	7.27	0	0.00
	11.06.2007	68	32	47.06	0	0.00	7	10.29	0	0.00
	24.06.2007	68	35	51.47	0	0.00	24	35.29	0	0.00
	28.06.2007	8	2	25.00	0	0.00	0	0.00	0	0.00
	01.07.2007	42	18	42.86	0	0.00	18	42.86	0	0.00
Sum/Average		307	162	52.77	0	0.00	63	20.52	0	0.00
Samsun	08.04.2007	51	33	64.71	8	15.69	5	9.80	0	0.00
	15.05.2007	94	40	42.55	25	26.60	17	18.09	0	0.00
	24.06.2007	140	112	80.00	16	11.43	27	19.29	0	0.00
	20.07.2007	71	50	70.42	10	14.08	16	22.54	0	0.00
	20.08.2007	75	38	50.67	18	24.00	50	66.67	0	0.00
	03.09.2007	79	66	83.54	12	15.19	17	21.52	0	0.00
	15.10.2007	39	3	7.69	10	25.64	7	17.95	0	0.00
Sum/Average		549	342	62.30	99	18.03	139	25.32	0	0.00
Hopa	11.06.2007	146	61	41.78	0	0.00	52	35.62	0	0.00
	11.09.2007	66	47	71.21	0	0.00	36	54.55	4	6.06
Sum/Average		212	108	50.94	0	0.00	88	41.51	4	1.89
Rize	11.06.2007	136	47	34.56	0	0.00	65	47.79	0	0.00
	11.09.2007	84	32	38.10	0	0.00	23	27.38	0	0.00
Sum/Average		220	79	35.91	0	0.00	88	40.00	0	0.00
Gümüşhane	10.09.2007	102	18	17.65	0	0.00	11	10.78	0	0.00
Grand Total		1390	709	51.01	99	7.12	389	27.99	4	0.29

Results and Discussion

In this extensive study, 4 different pathogens and parasites of *P. undulata* (gregarine, microsporidium, and 2 nematodes) were determined. The total occurrence rate of all pathogens and parasites of *P. undulata* is very high (86.9%). Gregarine infection is 51.01%. The microsporidian pathogen, with a 7.12% infection rate, is not identified to species level. Two different nematodes were observed. The common nematode parasite is described in the genus *Howardula* with a 27.99% occurrence rate, and a new nematode parasite was identified as a member of Mermithidae. The locality records and rates of these pathogens and parasites are presented in Table 1 and the mixed infections and parasitisations are given in Table 2. Detailed information on the pathogens and parasites is given below.

Microsporidian infection

A microsporidian infection was found in the adults of *P. undulata* collected from only one locality, Samsun, during the study. The pathogen was determined in Malpighian tubules by microscopic examination. Spores in groups were only observed in this tissue (Figure 1). Measurements of fresh spores of the microsporidian pathogen were 3.24 ± 0.47 µm (n = 100) in length and 1.97 ± 0.25 µm (n = 100) in width, and the spores are oval and uninucleate. The first and unique microsporidium pathogen of *P. undulata* was *Nosema phyllotretae* Weiser (1961). *N. phyllotretae* has been commonly observed in *P. undulata* populations from different geographical regions. Martini (1955) first reported microsporidian infection from *P. nemorum* in Malpighian tubules, nerve ganglions, and gonads in England but did not describe this

Table 2. Mixed infections and parasitisations found in *P. undulata* populations.

			Mixed Infections / Parasitisations found in *P. undulata* populations													
Sampled localities	Sampling dates	Number of examined beetles	Gregarine + Microsporidium	%	Gregarine + Nematode	%	Gregarine + Microsporidium + Nematode	%	Gregarine + Parasitoid	%	Gregarine + Nematode + Acarid	%	Nematode + Microsporidium	%	Nematode + Parasitoid	%
	10.05.2007	19	4	21.05	10	52.63	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	20.05.2007	47	0	0.00	3	6.38	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	26.05.2007	55	0	0.00	1	1.82	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Trabzon	11.06.2007	68	0	0.00	3	4.41	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	24.06.2007	68	0	0.00	11	16.18	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	28.06.2007	8	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	01.07.2007	42	0	0.00	6	14.29	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Sum/Average		307	4	1.30	34	11.07	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	08.04.2007	51	3	5.88	3	5.88	1	1.96	0	0.00	0	0.00	0	0.00	0	0.00
	15.05.2007	94	10	10.64	7	7.45	0	0.00	0	0.00	0	0.00	2	2.13	0	0.00
	24.06.2007	140	13	9.29	17	12.14	3	2.14	0	0.00	0	0.00	0	0.00	0	0.00
Samsun	20.07.2007	71	6	8.45	7	9.86	3	4.23	0	0.00	0	0.00	0	0.00	0	0.00
	20.08.2007	75	2	2.67	22	29.33	4	5.33	0	0.00	0	0.00	2	2.67	0	0.00
	03.09.2007	79	8	10.13	14	17.72	2	2.53	0	0.00	0	0.00	0	0.00	0	0.00
	15.10.2007	39	1	2.56	1	2.56	0	0.00	0	0.00	0	0.00	1	2.56	0	0.00
Sum/Average		549	43	7.83	71	12.93	13	2.37	0	0.00	0	0.00	5	0.91	0	0.00
Hopa	11.06.2007	146	0	0.00	26	17.81	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	11.09.2007	66	0	0.00	28	42.42	0	0.00	1	1.52	1	1.52	0	0.00	1	1.52
Sum/Average		212	0	0.00	54	25.47	0	0.00	1	0.47	1	0.47	0	0.00	1	0.47
Rize	11.06.2007	136	0	0.00	23	16.91	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	11.09.2007	84	0	0.00	6	7.14	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Sum/Average		220	0	0.00	29	13.18	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Gümüşhane	10.09.2007	102	0	0.00	2	1.96	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Grand Total		1390	47	3.38	190	13.67	13	0.94	1	0.07	0	0.00	5	0.36	1	0.07

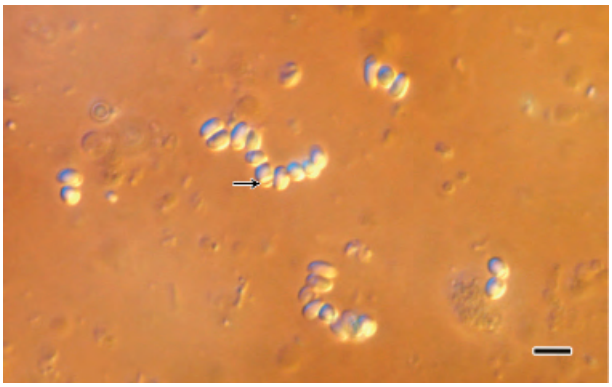

Figure 1. Microsporidian pathogen spores in fresh smear of *P. undulata* under phase contrast microscope (the arrows indicate the posterior vacuole of the pathogen), (bar: 5 µm).

microsporidian. Weiser (1961) named this microsporidian *Nosema phyllotretae* and reported *N. phyllotretae* from *P. undulata* collected from Czechoslovakia. Sommer (1981) studied natural enemies of *Phyllotreta* spp. in Austria, Hungary, Germany, and Poland, and found only one specimen of *P. undulata* infected with *Nosema* sp. Later Lipa and Ekbom (2003) reported *N. phyllotretae* from *P. undulata* in Sweden. Unfortunately all observations on this pathogen were by light microscope. More recently, Yaman et al. (2005a, 2005b) studied the ultrastructure of this microsporidium from different hosts in detail. Furthermore, Yaman and Radek (2005) showed that *Nosema phyllotretae* is not the sole microsporidium pathogen of *P. undulata*, determining a monokaryotic microsporidium from Turkey. They observed this new microsporidium only in Malpighian tubules with group of spores and measured fresh spores were oval and 2.0 to 4.75 µm in length and 1.90 to 2.40 µm in width; the spores were uninucleate. They ultrastructurally confirmed that this pathogen is monokaryotic. Measurements, tissue specificity, infection rate, and location of the microsporidium in our study show several similarities with the new microsporidium reported by Yaman and Radek (2005) from *P. undulata* populations in Samsun (Turkey). We found the infection in the same locality (Samsun) and in the same tissue (only Malpighian tubules) of the adults of the host, whereas *N. phyllotretae* infects Malpighian tubules, fat body, gonads, and nerve ganglions. Additionally, we measured 3.24 ± 1.97 µm for spore size of the pathogen, different from *Nosema phyllotretae* recorded by Weiser (1961), Lipa and Ekbom (2003), and Yaman et al. (2005a, 2005b). Similarly Sommer (1981) recorded *Nosema* infection in *P. undulata* and did not mention the species name, because the spore measurements did not match the size of *N. phyllotretae* given by Weiser (1961).

The rate of the microsporidian pathogen in the present study is 18.03% in Samsun and 7.12% in total; the highest rate of the microsporidian pathogen is 26.6%, in May in Samsun. The microsporidium pathogen occurred at different rates. Several factors can be the reason for the changes in the rates within the year. Population density of the insect, microsporidian transmission, and use of chemical pesticides in the field could be factors that affect the infection rate of a microsporidian parasite in host populations (Yaman, 2008). As Rosicky (1951) demonstrated, infected insects are more sensitive to chemical pesticides than healthy ones (Lipa and Hokkanen, 1992; Hokkanen and Lipa, 1995). One of these factors can be a reason for the low infection level in the *P. undulata* population. Lipa and Ekbom (2003) also found a microsporidian infection from *P. undulata* in Sweden and reported the infection rate to be 47.38%; the highest rate was 76.4%, in September. The pathogen rate reported by Lipa and Ekbom (2003) is higher than the rate reported in the present study. Moreover, the microsporidian in this study is only observed in Samsun throughout the Black Sea region of Turkey. Yaman and Radek (2005) also reported the new microsporidium from *P. undulata* populations in Samsun (Turkey). This shows that this microsporidian species is found only in this locality and the fact that it was not found in any other locality suggests that the microsporidian infection was not distributed outside Samsun.

Gregarine pathogen: *Gregarina phyllotretae* (Apicomplexa, Gregarinidae)

The gregarine pathogen, in contrast with the microsporidium pathogen, was observed in all localities during the study. Stages of the life cycle of the gregarine pathogen: trophozoite, gamont, associative form, and cyst were observed. The pathogen was found in the midgut epithelium. Each trophozoite was elongate with a spherical papilla epimerite, the anterior half of the protomerite was transparent and conoidal, deutomerite was hemicylindrical with a spherical nucleus and small, round karyosome, and cysts were observed in the midgut lumen (Figure 2). The present gregarine pathogen shows similarities with *Gregarina phyllotretae* reported by Yaman (2002a) from *P. undulata* and *Phyllotreta atra* in

measurements and morphological features; hence we identified it as *G. phyllotratae*. The total rate of the gregarine pathogen is 51.01%. The localities with the highest and lowest infection rates were Samsun and Gümüşhane, respectively. The highest rate of gregarine pathogen is 83.54%, in September in Samsun, while the lowest rate of gregarine pathogen is 7.69%, in October in Samsun. Yaman (2002a) reported the gregarine infection rate to be 57.48% and the highest rate was 90.3%, in August in Turkey. The results show that the gregarine infection in *P. undulata* populations was relatively stable between 2000 and 2007. However, Lipa and Ekbom (2003) reported the infection rate to be 36.1% and the highest rate was 70.5% in September 2002 in Sweden. It is seen that the gregarine pathogen rate in Turkey is higher than that in Sweden.

Howardula phyllotretae Oldham (Tylenchida: Allantonematidae)

In this study the nematode parasite was observed in all localities in Turkey. The total rate of the nematode parasite is 27.99%. The female of the nematode parasite is cylindrical, elongated, and measured as 1491.44 µm in length and 152.2 µm in width; the juvenile forms measured 310.9 µm in length and 20.8 µm in width (Figure 3). The nematode parasite was identified as

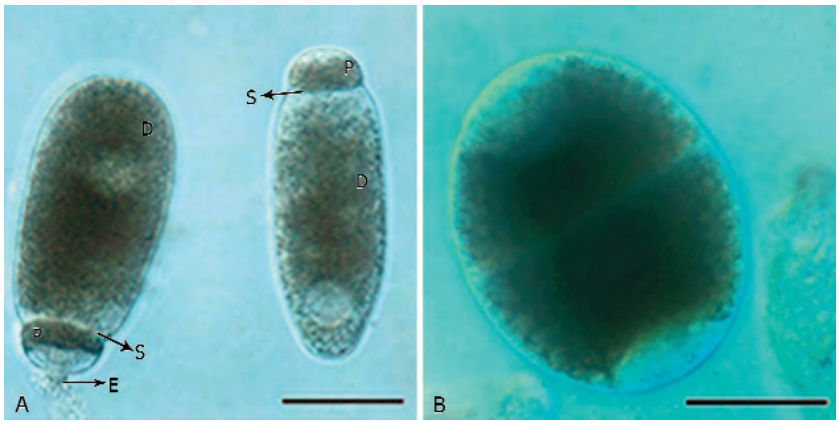

Figure 2. *Gregarina phyllotretae* from *P. undulata*. A: trophozoite (left) and gamont (right) (E: epimerite, S: septum, P: protomerite, D: deutomerite); B: gamont cyst. (bar: 50 µm).

Figure 3. *Howardula phyllotretae* from *P. undulata*. A: female adult form (bar: 100 µm); B: juvenile form; C: gregarine infection plus nematode parasitisation (bar: 50 µm).

Howardula phyllotretae according to Zakharenkova (1996) and Yaman (2002b). *Howardula phyllotretae* is a very common parasite of flea beetles (*Phyllotreta* spp.) in Europe (Oldham, 1933, 1935; Sommer, 1981; Zakharenkova, 1996). Sommer (1981) found that *P. undulata* is more frequently parasitised by *H. phyllotretae* than other *Phyllotreta* spp. Similar to the gregarine pathogen, the localities with the highest and the lowest nematode parasitisation rates were Samsun and Gümüşhane provinces, respectively. The highest rate of the nematode parasite is 66.67%, in August in Samsun, while the lowest rate of nematode parasite is 7.27%, in May in Trabzon. Yaman (2002b) reported the rate of *H. phyllotretae* to be 13.54% from *P. undulata* in Turkey and the highest was 19.6%, in April in 2002. The parasitisation rate in the present study is higher than that recorded by Yaman (2002b). The results show that the *Howardula* parasitisation in *P. undulata* populations was not stable between 2000 and 2007. Ekbom (1990, 1991) reported 11%-16% parasitisation of *P. undulata* by nematodes without specific identification. Lipa and Ekbom (2003) reported the rate of *H. phyllotretae* to be 44.51% in Sweden with the highest being 58.8%, in September. The *Howardula* parasitisation rate in Sweden also increased from 1990 to 2003. The parasitisation rate in Turkey is lower than the rate in Sweden. Sommer (1981) reported the rate of nematode to be 65% for *P. undulata* in different parts of central Europe. Compared to central Europe the *Howardula* parasitisation rate in Turkey is lower. The most important reason for this difference could be climatic conditions.

A new mermithid (Nematoda: Mermithidae) parasite

In this study a mermithid parasite from *P. undulata* is reported from Turkey for the first time. The mermithid parasite was observed in a population of *P. undulata* collected in Gümüşhane province. In the literature several different nematodes belonging to the family Mermithidae were found in adults and larvae of *Phyllotreta* spp. Altogether 5 species could be reared in the study by Sommer (1981). They were identified as *Hexamermis albicans* Sieb, *Amphimermis elegans* Hagmeier, *Amphimermis* n.sp., *Psammomermis* n.sp., and a mermithid belonging to the *Hexamermis brevis* group. A mermithid parasite was recorded from *Euproctis chrysorrhoea (Lepidoptera, Lymantriidae*), which was collected from Gümüşhane province, Turkey, by Yaman et al. (2002). Mennan and Ertürk (2006) reported mermithid parasitisation in *Leptinotersa decemlineata* (Coleoptera: Chrysomelidae) in Turkey. They did not identify the mermithid but reported that measurements of examined juvenile forms were similar to those of the genus *Hexamermis*. Oldham (1933) reported a non-identified mermithid from *P. undulata*. Newton (1931) reported a mermithid parasite as *Mermis* sp. from *P. atra*. Observed juvenile forms of mermithids in this study measured 10.6-12.2 mm in length from anterior to posterior and 152-162 µm in width; the anterior end of the juvenile form was blunt and the posterior end had an appendage tail about 52 µm (Figure 4). Yaman et al. (2002) reported that the mermithids were 0.2-0.3 mm wide with a maximum length of 150 mm, and identified the mermithid as *Hexamermis albicans*. The mermithid reported by Yaman et al. (2002) had a tail at the posterior end and the anterior end was blunt; this characteristic feature is similar to that in the mermithid we observed. Mennan and Ertürk (2006) measured the mermithid as 0.2-0.3 mm wide with a maximum length of 150 mm; its anterior end was blunt and posterior end had a short finger-like tail about 40 µm. The lack of fully matured specimens does not allow us to make a complete taxonomic identification of the mermithid recovered from *P. undulata.* However, according to Sommer (1981), the *Hexamermis brevis* group nematodes can be distinguished by their body length being between 0.7 and 1.5 cm. Therefore, the mermithid found in this study is similar to the *Hexamermis brevis* group. The rate of mermithid parasitisation is 2.39% in Gümüşhane province. Yaman et al. (2002) reported that despite the rate of mermithid parasite being 87.5% in Gümüşhane, mermithid parasitisation was not observed in Trabzon. Moreover, in the present study mermithid parasitisation was not observed in any localities except Gümüşhane. Mennan and Ertürk (2006) reported the rate of mermithid parasitisation to be 0.1% in Turkey. This rate is similarly low to ours; the reason could be the fact that viability of eggs of mermithids depends on many factors in addition to rainfall that decrease the rate of parasitisation (Mennan and Ertürk, 2006).

The results show that at least 2 pathogens and 2 parasites may occur in *P. undulata* in Turkey. Two of them are very common in *P. undulata* populations as recorded from other parts of the world. However, the rest of the observed pathogens and parasites have been

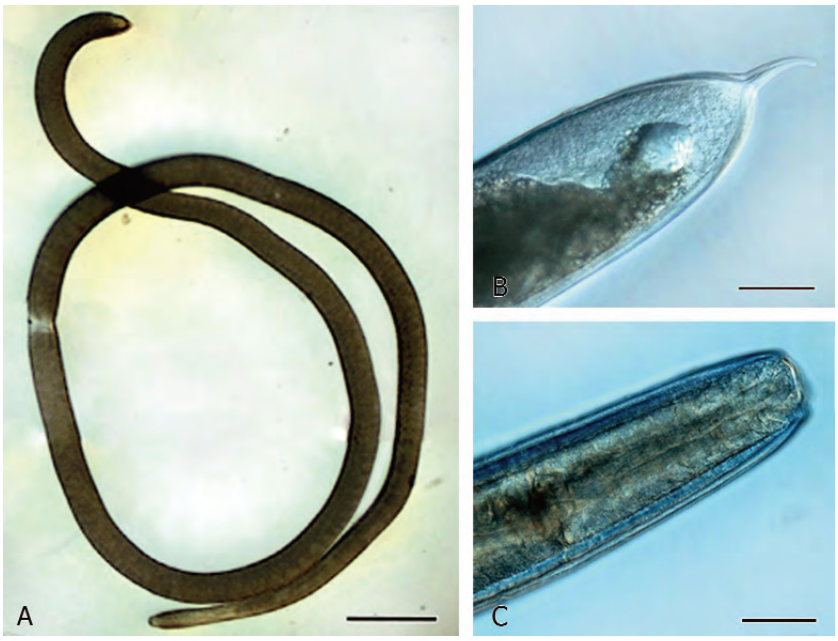

Figure 4. Mermithid parasite of *P. undulata*. A: full length juvenile mermithid (bar: 500 µm); B: posterior end of juvenile mermithid; C: anterior end of juvenile mermithid (bar: 50 µm).

only recorded from Turkey. This study also confirms that at least 2 microsporidium pathogens infect *P. undulata.* Therefore, further studies should be conducted to identify this new microsporidian pathogen and *Hexamermis* parasite to species level.

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