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Transmission of *Microsporidium* sp. between different generations of *Crepidopdera aurata* (Coleoptera: Chrysomelidae)

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Abstract: Microsporidia are mostly very small, intracellular, spore-forming pathogens classified as Protista and reported in every major group of animals. Insects are one of the most widely distributed hosts for microsporidia. Many entomopathogenic microsporidia cause chronic infections in insects and their effects appear as reduced fertility and pupal weight, reduced fecundity and shortened longevity. *Crepidopdera aurata* (Coleoptera; Chrysomelidae) is one of the most destructive pests in poplar nurseries. The host-parasite relation in microsporidium infections is of great importance to understand the possibilities to use microsporidia in the host's biological control. In the present study, the transmission of the microsporidian pathogen *Microsporidium* sp. was studied between different generations (in spring and summer) of *C. aurata* in 2015, 2016, 2018, and 2019. The results showed that there is considerable difference in the infection rates of the offspring and parental beetles. The results confirm that the infection rates in the offspring beetles (new generation in the same year) are higher than that in parental beetles (first generation in the same year) in the 2 populations for all investigated years. On the other hand, statistical analysis showed that *Microsporidium* sp. does not favour either of the sexes in *C. aurata* populations. Possible transmission of the microsporidium between different generations is also discussed according to the literature.

Key words: Transmission, *microsporidium,* generation, *Crepidodera aurata*

1. Introduction

Microsporidia are mostly very small, intracellular parasites reported from every major group of animals. Their host spectrum ranges from unicellulars to metazoans. Insects are one of the most widely distributed hosts (Hausmann et al., 2003). Many entomopathogenic microsporidia cause chronic infections in insects and their effects appear as reduced fertility and pupal weight, reduced fecundity and shortened longevity. Therefore, such infections caused by microsporidia in insects are mostly desirable for biological control without exceptions in some beneficial insects such as honeybees, silkworms, predators and parasitoids (Becnel and Andreadis, 1999; Yaman et al., 2016, 2019a, 2019b).

The host-parasite relation in microsporidian infections is of great importance to understand the possibilities to use microsporidia in host's biological control. Transmission of the microsporidian pathogens is the beginning of the host-parasite relations (Sprague et al., 1992). The principal modes of microsporidial transmission seem to be twofold; horizontal (oral), and vertical (transovarial). Horizontal transmission is through direct oral ingestion of infectious spores found in food or liquid within the host insect's contaminated environment. Vertical transmission is transmission of parental to the new generation and can be transovum (with eggs contamination) and transovarial (through infected gonads, egg yolk, or embryo). Vertical transmission is transovarial via the contaminated eggs including microsporidium pathogens (Becnel and Andreadis, 1999).

Crepidopdera aurata (Coleoptera; Chrysomelidae), the willow flea beetle, is one of the most destructive pests in poplar nurseries. Studies on any natural enemies of this pest, suppressing its populations naturally, are of great interest for biological control. However, there are very limited studies on the entomopathogenic organisms of *C. aurata* for biological control (Yaman and Ertürk, 2016; Yaman et al., 2015, 2019a). Recently, Yaman et al. (2015) found 2 types of microsporidian pathogens infecting this pest for the first time and later the pathogens were identified as *Microsporidium* sp. (Yaman et al., 2019a). Data on the transmission and effect of these microsporidia in the *C. aurata* generations is not known. Knowledge of the effect of transmission is essential for full understanding of the potential of the pathogens in biological control of the pest insect. In the present study, the transmission of

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the microsporidian pathogen *Microsporidium* sp. was investigated in different generations of *C. aurata* and the infection level in male and female beetles was compared from 2015 to 2019 years for the first time.

2. Materials and methods

2.1. Insect samples

Crepidodera aurata has one generation per year. Beetles overwinter as imago and lay eggs in spring. Larvae develop during the summer and in the end of August, and the new generation appears (Urban, 2011). Therefore, adult beetles were collected from 2 different populations in Samsun locality (Turkey), where the infections were found both in springs and summers by Yaman et al. (2015). There is no chemical pesticide application and the population densities are similar. During the 4 years-2015, 2016, 2018, and 2019, 758 beetles in springs and 351 beetles in summers were collected from 2 populations, Havalanı (N 41°24'61"- E 36°54'66")

and Irmaksırtı (N 41°25′06″- E 36°58′07″) locations in Samsun, Turkey. Two hundred seventy-nine of the obtained beetles were identified as either male or female to compare the infection levels between both sexes.

2.2. Microscopy and statistical analysis

Each insect was dissected in insect Ringer's solution and wet smears were prepared and examined for presence of microsporidium spores under a bright-field microscope at a magnification of 40×–1000×. The water-mounted preparation was dried at room temperature, fixed in methanol for 3 min, and stained with 5% Giemsa solution for 12 h. Stained preparations were then carefully examined. Microscopic observations were carried out according to Yaman et al. (2015). The results were statistically analysed using R.3.4.3.

3. Results

The present study is the first report on the transmission of a microsporidian pathogen from one generation to the next in *C. aurata* populations. Microsporidian infection was firstly observed by Yaman et al. (2015) in *C. aurata* populations in Turkey. In the present study, totally 1109 of *C. aurata* adults were examined for microsporidian infection. The transmission of the microsporidian pathogen was studied by including 2 populations in which the microsporidian infection was found in both spring and summer. Some populations (Vezirköprü and Çarşamba) in which the infection was not observed in both spring and summer or the samples collected only in spring or summer were not included into the study. During the 4 years, 1109 beetles were dissected and 56 of them were found to be infected by the microsporidian pathogen (Table 1). Total infection for 4 years in both localities was 5.04%. Thirty-five of totally 758 parental beetles collected in spring were infected by the microsporidian pathogen (Table 1). Total infection average was 4.61% for the parental beetles. On the other hand, 21 of totally 351 offspring beetles collected in summers during the 4 years were infected by the microsporidian pathogen. Total infection average was 5.98% for the offspring beetles.

Microsporidian infections in the offspring beetles collected in summers were found to be higher than those in the parental beetles collected in springs during the 4 years (Figure 1).

As a part of this study, the infection levels in male and female *C. aurata* beetles were also documented. It was established that 23 of the 279 examined males and 4 of the 91 female beetles were infected by the microsporidian pathogen. The infection levels were 8.24% in the male and 4.39% in the female beetles (Table 2, Figure 2). No statistical difference between both sexes was found.

Table 1. Microsporidian infection in parental and offspring individuals of *Crepidodera aurata* populations.

Sampling period	Localities								
	Havaalanı			Irmaksırtı			Total (Havaalanı + Irmaksırtı)		
	Examined beetles	Infected beetles	Infection rate (%)	Examined beetles	Infected beetles	Infection rate (%)	Examined beetles	Infected beetles	Infection rate (%)
2015 Spring /Summer	34/73	0/1	0/**1.4**	131/49	2/2	1.5/**4.08**	165/122	2/3	1.21/**2.46**
2016 Spring /Summer	126/44	3/4	2.38/**9.09**	244/56	24/7	9.83/**12.5**	370/100	27/11	7.29/**11.0**
2018 Spring /Summer	55/41	2/2	3.63/**4.87**	78/38	3/2	3.85/**5.26**	133/79	5/4	3.76/**5.06**
2019 Spring /Summer	20/22	0/1	0/**4.56**	70/28	1/2	1.42/**3.57**	90/50	1/3	1.11/**6.0**

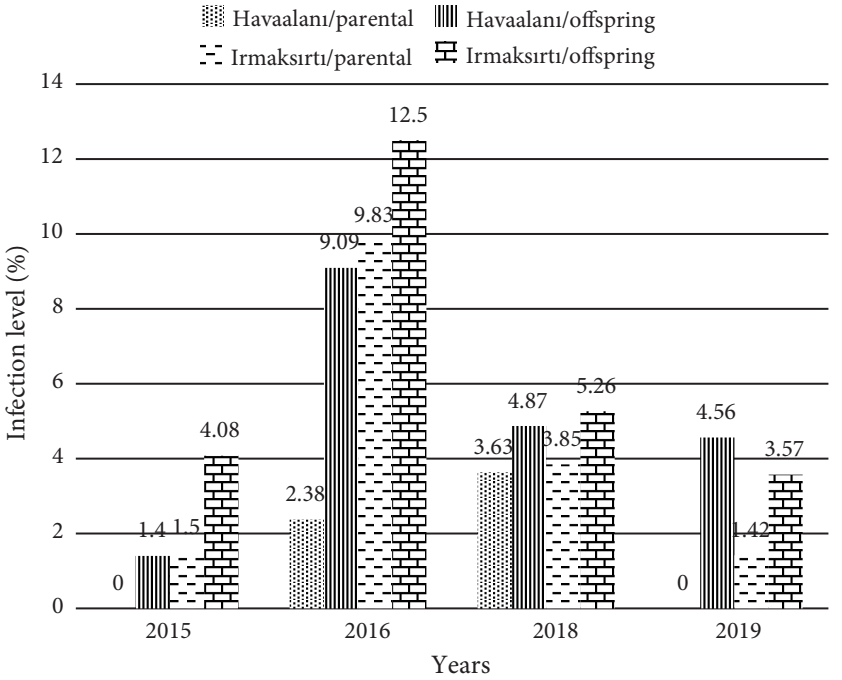

Figure 1. Microsporidian infection in different generations of *Crepidodera aurata* in 2 populations.

Table 2. Microsporidian infection in male and female beetles of *Crepidodera aurata* in 2 populations.

Sampling plots	♂♂			♀♀		
	Dissected beetles	Infected beetles	%	Dissected beetles	Infected beetles	%
Irmaksırtı	175	20	11.4	69	4	5.8
Havaalanı	104	3	2.9	22	0	0
Total	279	23	8.2	91	4	4.4

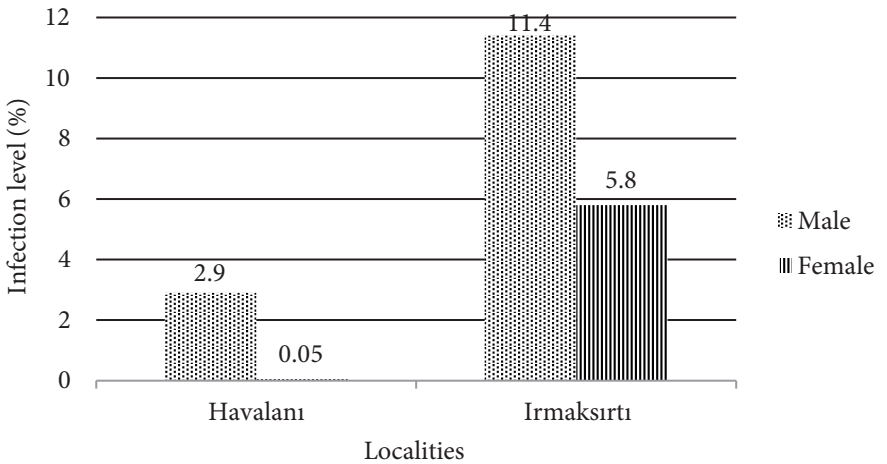

Figure 2. Microsporidian infection in male and female beetles of *Crepidodera aurata* in 2 populations.

4. Discussion

Distribution and occurrence of microsporidian pathogens of *C. aurata* in northern Turkey were studied in detail by Yaman et al. (2015). They recorded different infection rates from different populations of *C. aurata* in Turkey. However, transmission of the microsporidian pathogen from one generation to the next generation has not been documented. Here the potential of the transmission of the microsporidian pathogen in different generations of *C. aurata* and the infection levels in male and female beetles from 2015 to 2019 years was documented for the first time.

As seen in Table 1 and Figure 1, the infection rates in the offspring beetles (new generation in the same year) are higher than those in parental beetles (overwintered in the same year) in the 2 populations for all investigated years. Statistical analysis supported the difference in the year 2015, however, higher insect numbers need to be analysed for the 2016, 2018, and 2019. Similarly Yaman et al. (2015) recorded that the infection levels in the beetles collected in summers were higher those in the beetles collected in springs in the same year in most populations. Results confirm that microsporidium infections increased in all populations of the offsprings during the 4 years (Figure 1). Chemical pesticide application, host population density and climatic conditions are the main factors affecting microsporidian infections in insect populations (Lipa and Hokkanen, 1992; Yaman, 2007, 2008).

Use of chemical pesticides affects infection rate of a microsporidian pathogen in the host's populations. As Rosicky (1951) demonstrated, infected insects are more sensitive to chemical pesticides than healthy ones. In a study, Lipa and Hokkanen (1992) suggested that pesticide usage at or close to sampling sites may play a role in microsporidian infections, because most samples from which *Nosema* were detected originated from areas where few pesticides are used. Yaman (2007) did not observe *Nosema meligethi* infection in the populations of *Meligethes aeneus* in the main *Brassica oleracea* growing areas where farmers commonly use chemical pesticides to control pest insects, while he found microsporidium-infected populations from uncultivated areas in which any chemical pesticide has not been applied. Similarly, Yaman (2008) speculated that microsporidian infection in *Chaetocnema tibialis* populations in Trabzon is lower than that of in Samsun because the farmers cultivating vegetables in Trabzon use chemical pesticides to control pest insects. Furthermore Yaman (2007, 2008) suggested that pesticide usage can be a reason for the low infection level in host populations. However, chemical pesticide usage cannot be the reason of the differences on the infection rates between the both offspring and parental beetles addressed in this study, because the investigated *C. aurata* populations were selected from nonchemical application areas.

Host population density is another reason affecting microsporidian infections in insect populations. Yaman (2008) found that *Nosema chaetocnemae* infection in *Chaetocnema tibialis* populations in Samsun is higher (25.20%) those (3.28%) in the populations in Trabzon. Although both infected provinces are on the same coast, Middle and East Black Sea Region of Turkey, the population density of *C. tibialis* in Samsun was very high when compared that of in Trabzon according to the field observation. Yaman (2008) suggested that population density can be a reason for the high infection level in host populations. Similarly, Malone and Wigney (1980) suggested that the high frequencies of infection at most sites may be a consequence of high population densities for the codling moth. However, population density cannot be the reason for the differences in the infection rates between both offspring and parental beetles analysed in this study because the investigated *C. aurata* populations were comparably similar.

These 2 factors, chemical pesticides and host population density cannot be the reason for the differences in the infection rates between both offspring and parental beetles. The results stimulate us to think that climatic conditions (winter seasons) can be one factor for the differences in the infection levels between both offspring and parental beetles. Infected parental beetles cannot survive the hard winter conditions and most of them killed without reaching the spring season. *C. aurata* has one generation per year. Beetles overwinter as imago under barks or debris. It is known that most microsporidia infecting insects induce sublethal effects on insect hosts, resulting in reduced fertility, shortened longevity and a loss of vigour (Brooks, 1988; Becnel and Andreadis, 1999). Because of the chronic nature of infections caused by many entomopathogenic microsporidia, effects on the host are commonly evaluated by measuring the impact on fitness. The individual or cumulative effects of lower survival, reduced longevity, and fertility can be used to pressure fitness.

The transmission of insect microsporidia is well explained by Becnel and Andreadis (1999). Based on this explanation, we think that the infection from the rest of infected parental beetles to the offspring beetles of *C. aurata* seems to be transferred in 2 ways schematized in Figure 3; horizontal (oral) and vertical (transovum via oral and transovarial via venereal and embryonal) as well as that in many insects. Horizontal transmission occurs by direct oral ingestion of infective spores in food or liquid in the host insect's environment. Environmental contamination by microsporidian pathogens is achieved when spores are released in faecal excrement or with the disintegration of infected tissues when an infected host dies (Becnel and Andreadis, 1999). These 2 ways are possible for *C. aurata*

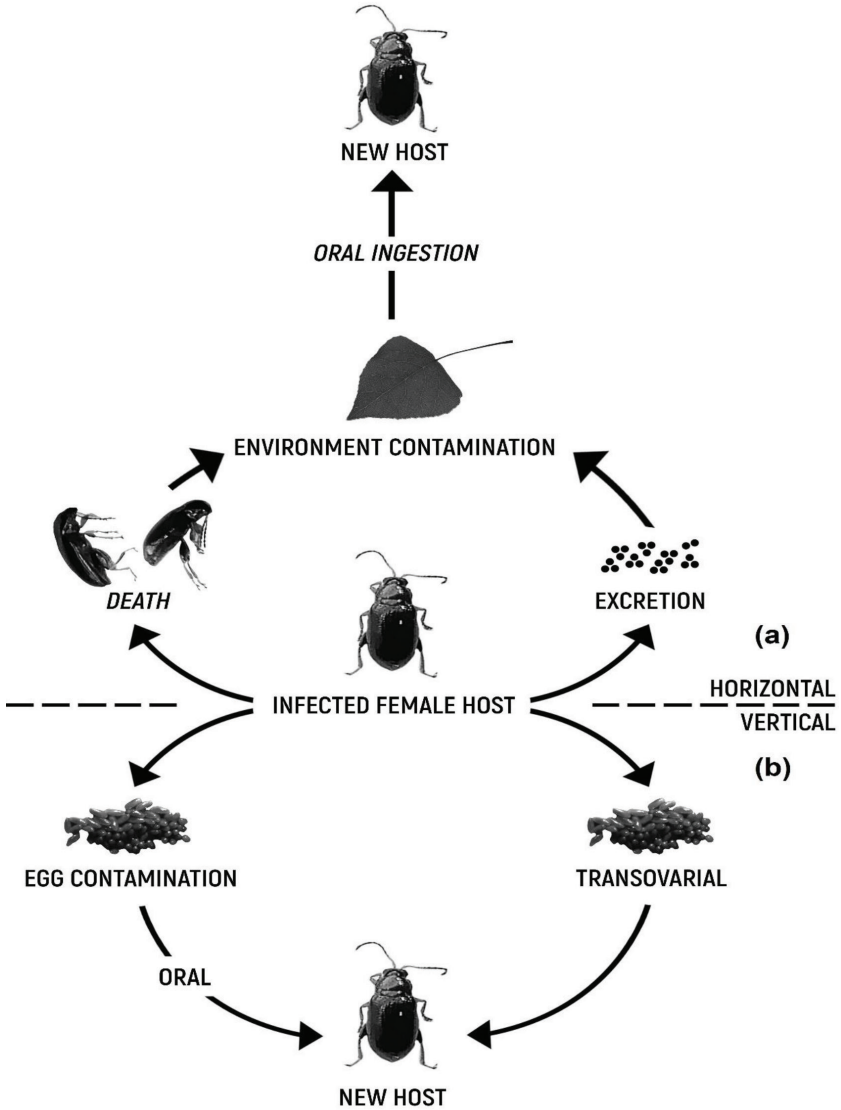

Figure 3. Transmission of *Microsporidium* sp. between different generations of *Crepidopdera aurata* (based on Becnel and Andreadis, 1999)

(Figure 3 (a)). Yaman et al. (2015, 2019a) found general infestation including haemolymph, Malpighian tubules, midgut, silk glands, and fat body in *C. aurata*. General infection observed in different tissue cells supports the contamination of poplar leaves with the microsporidian spores leaving from the host faeces or released after the host dies (Figure 3 (a)). As a result of the general infection, the accumulation of the pathogen spores in the infected cells causes the rupturing of all the infected tissue cells and the spread of the microsporidian spores from the dead host bodies to the host's environment (Agnew et al., 2003). Both *C. aurata* larvae and adults feed on the same young poplar leaves that their previous generation used to feed on. The transmission of the microsporidian pathogen occurs through direct oral ingestion of spores found on poplar leaves contaminated with spores in faeces or from the dead host body. As a result of this pathway, epithelial cells lining the host's gut are directly exposed to infection, and the new infection cycle commences after the spores are ingested by another host *C. aurata* larvae or adults (Figure 3 (a)).

The second transmission pathway for microsporidian pathogens is vertical transmission. In most host insects, vertical transmission occurs entirely through the female germline (Becnel and Andreadis, 1999). Vertical transmission is transovarial via the contaminated eggs including microsporidian pathogens. Microsporidium occurs within or on the surface of the egg. In that way, spores are transferred from parent to progeny directly (Becnel and Andreadis, 1999). Parental *C. aurata* beetles lay eggs

in spring. Larvae develop during the summer and in the end of August, the new generation appears (Urban, 2011). Yaman et al. (2019a) observed heavily infected midgut addition to gut, Malpighian tubules, haemolymph, silk glands, and adipose body in *C. aurata* adults, but they did not mention reproductive organs. Spores of microsporidia infecting the digestive tract are commonly disseminated in faeces (Weiser, 1961; Becnel and Andreadis, 1999). In the vertical transmission of the microsporidium in *C. aurata* the pathogen enters into the egg within the female host reproductive system or contaminates the external surface of the egg leaving from the anal opening of the infected digestive tract (Figure 3 (b)). This transmission results in the maintenance of the microsporidian pathogen from one generation to the next generation.

There are also other transmission ways for microsporidia in insects. In one of them, oral ingestion of microsporidian spores occurs through cannibalistic feeding. It seems that it is not possible for *C. aurata* populations since cannibalism does not occur between *C. aurata* larvae and adults.

On the other hand, the microsporidian infection levels in male and female beetles was also documented. The infections occur more frequently (8.24%) in male beetles than in female beetles (4.39%). However, there was no statistical difference between both sexes. Similar results were found by Yaman and Radek (2008) for *M. typographi* in *Dendroctonus micans* and Yaman et al. (2019b) for the microsporidian pathogen in *Calosoma sycophanta*. Statistical analysis showed that *Microsporidium* sp. dose not favour either of the sexes in *C. aurata* populations.

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