Distribution and occurrence of microsporidian pathogens of the willow flea beetle, *Crepidodera aurata* (Coleoptera: Chrysomelidae) in North Turkey

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In this study, microsporidian pathogens in *Crepidodera aurata* populations were investigated. Totally 1,728 *C. aurata* adults were examined for microsporidian pathogens and 78 of them were found to be infected. Two species of microsporidia; *Microsporidium* sp.1 and *Microsporidium* sp.2 were observed in the *C. aurata* populations from ten localities in North Turkey. They show considerable difference from each other in the spore morphology and dimension, infection rate and host locality. The spores of *Microsporidium* sp.1 were oval in shape and measured from 3.66 to 5.66 µm in length and from 1.35 to 2.22 µm in width (n=50). The spores of *Microsporidium* sp.2 were slightly curled and measured from 2.44 to 3.55 µm in length and from 1.25 to 1.55 µm in width (n=50). These microsporidia were recorded from *C. aurata* for the first time. Here we present occurrence and distribution of two microsporidia in *C. aurata* populations as natural potentially suppressing factors.

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1. Introduction

The family Chrysomelidae, one of the largest families of the order Coleoptera, includes phytophagous insects. A member of this family, the willow flea beetle *Crepidodera aurata* Marsham is a widely distributed species (Aslan *et al.* 2004, Urban 2011). This species naturally occurs in Europe, northern Africa, Caucasus, Anatolia (Turkey), Iran, Kazakhstan, Siberia, Mongolia, China and Korea (Aslan *et al.* 1999, Aslan & Warchalowski 2005, Baselga & Novoa 2007, Bukejs 2009, Urban 2011). It is an important pest species of poplars and willows in different countries (Aslan *et al.* 1999, Mikhailov & Hayashi 2002, Czerniakowski 2005, Urban 2011). Urban (2011) mentioned that this species is the most important species of the genus in forestry. Alternative to pesticides, biological control strategies or suppressing factors have not been yet searched to find possible effective methods to struggle this pest.

The members of the family Chrysomelidae are frequently infected by pathogenic protists.

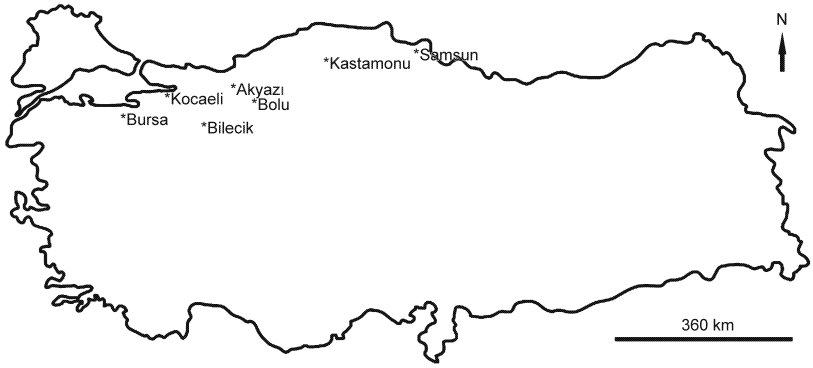

Fig. 1. Localities in where *Crepidodera aurata* adults were collected in Turkey.

Pathogenic and parasitic organisms infecting this family have aroused interest as potentially suppressing factors of chrysomelid populations (Toguebaye *et al.* 1988, Poinar 1988, Theodorides 1988, Hokkanen & Lipa 1995, Yaman 2002a,b, 2004, 2007, 2008, Yaman & Radek 2003, Martin *et al.* 2004, Ertürk *et al.* 2008, Yaman *et al.* 2008a, 2011a,b). Several new pathogen species from different groups have been isolated and characterized from these insects recently (Yaman & Radek 2003, Yaman *et al.* 2008b, 2010). However, there is no record on the entomopathogenic protists infecting *C. aurata*.

In the present study we aimed at searching for the first time microsporidian pathogens and documenting their occurrence and distribution in the populations of *C. aurata* as natural potentially suppressing factors of this important pest.

2. Materials and methods

2.1. Insect samples

Totally 1,728 *C. aurata* adults were collected from a wide geographical area (especially most poplar breeding areas from a piece of land of 779,000 km^2) with a high sampling ratio (10 localities and 26 samplings) in Turkey from April to September during 2013 and 2014 (Fig. 1).

2.2. Microscopic examination

Each of *C. aurata* adults was placed individually into a small drop of water on a microscopic slide and dissected in Ringer's solution by using dissection pins. Wet smears were examined under a microscope for identification of microsporidian pathogens. When an infection was observed, the slides were air-dried and fixed with methanol for 3 min. They were then washed with distilled water, stained for approximately ten hours in freshly prepared 5% solution of Giemsa stain, washed in running tap water and air-dried (Toguebaye *et al.* 1988). Giemsa-stained preparations were then carefully re-examined under a microscope. Detected fresh and stained spores were measured and photographed using an Olympus BX51 microscope with a DP-25 digital camera and a DP2-BSW Soft Imaging System.

3. Results

During the study, two possibly new microsporidian species were observed in the *C. aurata* populations. These microsporidia showed considerable difference in the spore morphology. The first Microsporidium (Isolate 1) was observed in Vezirköprü, Samsun provience. The spore of this microsporidium was oval in shape and measured from 3.66 to 5.66 µm in length and from 1.35 to 2.22 µm in width (n=50). The second microsporidian species (Isolate 2) was observed in the rest of the investigated localities. The spore of this microsporidium was slightly curled and measured from 2.44 to 3.55 µm in length and from 1.25 to 1.55 µm in width (n=50).

We observed microsporidial infections in seven of the ten investigated localities in the both years (Table 1). Isolate 1 showed infection from 1.7 to 5% and isolate 2 from 0.8 to 25%. Total infection was 5.4% in 2013 and 4.5% in 2014. The

Table 1. Occurrence of microsporidian pathogens in populations of *Crepidodera aurata* (Coleoptera: Chrysomelidae) in Turkey.

Locality	No. of examined beetles	Sampling date	No. of infected beetles	Infection rate (%)
Samsun				
1-Vezirköprü	118	25.4.2014	2	1.7
	40	05.9.2014	2	5
2-Irmaksırtı	30	03.6.2013	2	6.6
	100	23.4.2014	9	9
	84	01.6.2014	8	9.5
	44	28.6.2014	11	25
	14	28.6.2014	0	0
	20	20.9.2014	0	0
3-Kızılot	46	27.3.2013	3	6.5
	87	01.6.2014	5	5.7
	118	24.6.2014	6	5
	39	27.6.2014	2	5.1
	14	28.6.2014	0	0
	12	06.9.2014	0	0
	2	20.9.2014	0	0
4-Havaalanı	66	24.4.2014	6	9.1
	109	01.6.2014	4	3.6
	89	28.6.2014	6	6.7
Kastamonu	16	14.4.2013	0	0
	113	26.4.2014	1	0.8
	201	28.6.2014	4	2
Kocaeli	71	02.5.2014	0	0
Akyazı	62	03.5.2014	0	0
Bilecik	65	19.5.2014	0	0
Bursa	65	17.5.2014	2	3.1
Bolu	103	16.5.2014	5	4.8
Total	1,728		78	4.51

infection rates for both pathogens were variable between the localities (Table 1) and reached 25% in some localities. We did not observe any infection in three (Kocaeli, Akyazı and Bilecik) of the investigated localities. More details on the occurrence and distribution of the microsporidian pathogens are given in Table 1.

4. Discussion

In this study, two microsporidian pathogens, Isolate 1 and Isolate 2, are presented from *C. aurata* populations for the first time. To date there is no microsporidian report from *C. aurata*. Microsporidia have been known relatively host specific and the host affinity can be accepted as a valid taxonomic character for microsporidia taxonomy (Sprague *et al.* 1992). It seems that at least one *Microsporidium* species infects *C. aurata*. On the other hand, spore morphology and size, host locality and infection rates are also important characteristics to discriminate microsporidia infecting insects (Larsson 1999, Yaman & Radek 2003, Yaman *et al.* 2008b). Two microsporidian pathogens found in the *C. aurata* populations show considerable differences from each other in the spore shape and size (Table 2) as well as in infection capability (1.7 to 5% Isolate 1 and 0.8 to 25% for Isolate 2) and locality (Isolate 1 in Vezirköprü and Isolate 2 in the rest of the investigated localities) of the host population. Similarly, two new microsporidia were identified from the populations of *Chaetocnema tibialis* (Illiger) (Coleop-

Table 2. Microsporidian species first found in Chrysomelidae (Coleoptera).

Species	Spore size and shape (µm)	Infected organ	Host
Nosema phyllotretae Weiser, 1961	4.2×2–3	Adipose body	*Phyllotreta atra* *Phyllotreta undulata*
Nosema gastroideae Hostounský & Weiser, 1973	3–4.8×2.5–3	Overall infestation	*Gastrophysa polygoni* and several experimental hosts
Nosema polygrammae Hostounský & Weiser, 1975	4.8×2.05	Gut	*Polygramma undecemlineata*
Nosema equestris Hostounský & Weiser, 1980	4–5×3	General infestation	*Gastrophysa viridula* *Leptinotarsa decemlineata*
Nosema couilloudi Toguebaye & Marchand, 1984	3.4–4×1–1.5	Gut	*Nisotra* sp.
Nosema birgii Toguebaye & Marchand, 1986	6.2×3.5	Eggs and general infestation, larvae and imago	*Mesoplatys cincta*
Nosema nisotrae Toguebaye & Marchand, 1989	5.8×3.1	General infestation	*Nisotra* sp.
Nosema galerucellae Toguebaye & Bouix, 1989	4.95×2.89	Gut principally, adipose body, muscles, tracheae, malpighian tubules	*Galerucella luteola*
Nosema chaetocnemae Yaman & Radek, 2003	3.52×2.09	Gut, tracheae, muscles, malpighian tubules	*Chaetocnema tibialis*
Nosema tokati Yaman *et al.*, 2008	3.82×1.3	Malpighian tubules	*Chaetocnema tibialis*
Nosema leptinotarsae Lipa, 1968	2–5×1.9–3.3	Haemolymph	*Leptinotarsa decemlineata*
Nosema leptinotarsae Yaman *et al.*, 2011	4.69×2.43	General infestation	*Leptinotarsa decemlineata*
Unikaryon bouixi Toguebaye & Marchand, 1983	ovoid 1.6–2.5×1.5–1.6	Gut and malpighian tubules	*Euryope rubra*
Unikaryon matteii Toguebaye & Marchand, 1984	ovoid 3.72×1.96	gut, malpighian tubules and muscles	*Nisotra sp.*
Unikaryon nisotrae Toguebaye & Marchand, 1986	ovoid 2.33×1.66	gut and adipose tissue	*Nisotra sjoestedti*
Unikaryon phyllotretae Yaman *et al.*, 2010	spherical to ovoid, 3.80×1.90	Malpighian tubules	*Phyllotreta undulata*
Microsporidium sp.1 (this study)	ovoid 3.66–5.66×1.35–2.22	Haemolymph	*Crepidodera aurata*
Microsporidium sp.2 (this study)	Slightly curled 2.44–3.55×1.25–1.55	Haemolymph	*Crepidodera aurata*

tera; Chrysomelidae) in different localities (Yaman & Radek 2003, Yaman *et al.* 2008b). Yaman *et al.* (2008b) observed different infection rates between *Nosema chaetocnemae* Yaman & Radek and *N. tokati* Yaman, Radek & Toguebaye, infecting *C. tibialis* populations in Turkey.

There are different microsporidian species recorded from the members of the family Chrysomelidae. The results show that the microsporidian pathogens presented here differ from microsporidia infecting the family Chrysomelidae in spore size, type of infected tissue, infection rate, host affinities and locality of the host population (Table 2). The Isolate 2 from *C. aurata* clearly differs in spore size from microsporidian species infecting chrysomelids (Table 2). To identify both isolates at the species level we need to complete some morphological and ultrastructural studies on the different life stages and molecular phylogeny on the microsporidian pathogens in-

fecting chrysomelids. For now, we consider our organisms, tentatively classified in the collective group *Microsporidium*, to be two distinct, undescribed species and call Isolate 1 as *Microsporidium* sp.1 and Isolate 2 as *Microsporidium* sp. 2. Further studies, however, are needed to finally resolve the taxonomic status of the new microsporidia from *C. aurata*. Here we are focused on the occurrence and distribution of *Microsporidium* sp.1 and *Microsporidium* sp.2 in the *C. aurata* populations from several localities in a wide geographical area in Turkey.

Microsporidial infections were observed in the seven of ten investigated localities in the both years (Table 1). Yaman (2007) observed *Nosema meligethi* infection in only two of the seventeen *Meligethes aeneus* (Fabricius) (Coleoptera: Nitidulidae) populations investigated in Turkey. In another study, Yaman (2008) found *Nosema* infection in *Chaetocnema tibialis* (Coleoptera: Chrysomelidae) adults from two of the ten localities. Further, Aydın *et al.* (2009) observed *Nosema phyllotretae* Weiser infection in *Phyllotreta atra* (Fabricius) (Coleoptera: Chrysomelidae) populations from only one of the five localities. The results confirm that the microsporidian pathogens in the present study have an extensive distribution in the populations of *C. aurata*. However, we did not observe any infection in *C. aurata* populations from the three localities (Kocaeli, Akyazı and Bilecik) which are close to each other geographically (Fig. 1).

Total infection rate for both microsporidia was 4.51%, varying among the localities from 0.8 to 25% (Table 1). Potentially high infection is promising for biological pest control. *Crepidodera aurata* has one generation per year. Beetles overwinter as imago and lay eggs in spring. Larvae develop during summer and in the end of August, and the new generation appears (Aslan *et al.* 1999, Urban 2011). Therefore, the beetles of *C. aurata* occur on poplars and willows from April to October and skeletonize leaves of these plants. During this developmental period, any disease factor affecting population density is of great important. The microsporidia presented here are the first pathogens recorded from *C. aurata*. This study confirms that the pathogens are found widely in the populations of *C. aurata* as natural potentially suppressing factor.

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