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# Distribution of microsporidial infection in the predator beetle, *Calosoma sycophanta* (Coleoptera:Carabidae)-rearing laboratories in Turkey

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**Abstract:** The present study aimed to determine the rate of microsporidiosis in larvae and adult beetles in *Calosoma sycophanta* L. (Coleoptera: Carabidae) mass-rearing laboratories. Eighty larvae and 7420 adults of *C. sycophanta* were dissected from the mass-rearing laboratories and investigated for the presence of microsporidiosis. Microsporidiosis was determined in all localities. Four hundred and forty-one adults of the dissected insects were found to be infected and the total infection rate was 5.94%. A total of 80 *C. sycophanta* larvae from Balıkesir and Isparta examined in 2016 showed no microsporidiosis. The infection ratio was varied among populations and years. The total infection rate per year was 2% in 2015, 3.85% in 2016, 5.7% in 2017, and 12.3% in 2018. It was observed that microsporidiosis increases year after year. In addition, infection rates reached considerable levels, such as 26.3% in İzmir, 31.1% in Antalya, 30.2% in Balıkesir, and 35.2% in Mersin localities. During the study, microsporidiosis in male and female beetles was also investigated. Twenty-four (2.49%) of 963 male and 41 (2.99%) of 1370 female beetles were found to be infected. These results confirm that the microsporidian pathogen has a high dispersal potential through the *C. sycophanta* mass-rearing laboratories and can be an undesirable suppressing factor in adult beetles.

**Key words:** Microsporidia, *Calosoma sycophanta,* mass-rearing, biological control

## 1. Introduction

*Calosoma sycophanta* L. (Coleoptera: Carabidae) is an effective predatory beetle that has been used efficiently in biological control of lepidopteran pests for more than a century (Ferrero, 1985; Weseloh et al., 1995; Schafer et al., 1999; Kanat and Özbolat, 2006). In Turkey, this beetle has been mass-reared in insectaries in large quantities for biological control of *Thaumetopoea pityocampa* (Den. & Schiff.) by investing a lot of effort at a great cost (Kanat and Özbolat, 2006). Higher biological control efficiency can be obtained with healthy beetles (Yaman et al., 2010; Bjørson and Oi, 2014).

However, pathogenic viruses, bacteria, fungi, protists, and nematodes can cause chronic diseases in predator insects that are reared in laboratories for biological control. Pathogenic organisms cause reduction of their foraging proficiency and fecundity (Yaman et al., 2010, 2012; Bjørnson and Oi, 2014). On the other hand, it is known that microsporidia from protist pathogens prolong larval and pupal development and reduce fecundity and survival of adult predator beetles (Bjørnson and Keddie, 1999; Bjørnson and Oi, 2014). For this reason, any infection by pathogenic organisms in *C. sycophanta* populations is undesirable. However, beneficial insects collected from fields or reared in insectaries for biological control of pests can be naturally infected by microsporidia (Yaman et al., 2010, 2016; Bjørnson and Oi, 2014). Recently, Yaman et al. (2016) recorded a microsporidian pathogen in *C. sycophanta* adults in mass-rearing laboratories in Turkey. That pathogen is the sole microsporidian recorded from *C. sycophanta*. There is no knowledge about its prevalence and distribution.

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In the present study, we studied the distribution of the microsporidian pathogen in terms of health conditions in the mass-rearing laboratories in Turkey over four years.

## 2. Materials and methods

### 2.1. Insect samples

During a period of four years (from 2015 to 2018), 7420 adults and 80 larvae of *C. sycophanta* were randomly sampled from sixteen *C. sycophanta* mass-rearing laboratories from ten provinces in five different regions of Turkey (Table 1). In order to compare the infection rate in both sexes, a total of 2333 *C. sycophanta* adults were distinguished as either male (963) or female (1370).

### 2.2. Microscopic examination

Collected adults and larvae of *C. sycopha* were brought to the laboratory as soon as possible. After macroscopic examination, they were dissected in Ringer's solution and wet smears were prepared. Host fat body, Malpighian tubules, gut epithelium, and hemolymph were examined for the presence of spores of the microsporidium pathogen under a light microscope at 400–1000× magnification. When infection was found, the slides were air-dried and fixed with methanol, then stained with freshly prepared 5% solution of Giemsa stain and re-examined under the microscope. Giemsa stain was used to differentiate the microsporidian pathogen from other artifacts and resistant stages of other organisms, such as fungi (Yaman and Radek, 2003).

## 3. Results

Microsporidiosis was observed in all mass-rearing laboratories (Figure 1). Four hundred and forty-one of the examined *C. sycophanta* adults (7420) were infected by the microsporidian pathogen. Total infection rate was 5.94%. Furthermore, the infection rate was considerably higher in İzmir (26.3%), Antalya (31.1%), Balıkesir (30.2%), and Mersin province (35.2%).

Reported microsporidiosis rates of the examined beetles were noticeably different between localities and years. In 2015, 955 beetles were examined, 19 of them were infected by the microsporidial pathogen and the infection rate was 2%. In 2016, among 2206 beetles that were examined, 85 were infected and the infection rate was 3.85% (Figure 2). Moreover, in 2017, 2842 beetles were examined, 162 of them were infected, and the infection rate was 5.7%; on the other hand, in 2018, 1417 beetles were examined, 175 of them were infected, and the infection rate was 5.94% (Figure 2).

However, the infection rate obtained in 2018 was higher than those obtained in 2015, 2016, and 2017. Thus, a regular increase in the infection rate was observed year by year. Noticeable differences were also observed among samples collected from laboratories in the same province. Furthermore, the infection rate of the samples from the same laboratory in different years was significantly dissimilar (Table 2). In Balıkesir province, the microsporidiosis rates were 6.97%, 4.77%, and 23.5% in 2015, 2016, and 2017, respectively. Despite the infection rate decrease in 2016, it

**Table 1.** Sampled localities and dates.

| | | |
|---|---|---|
| Marmara region | Bursa | 10.04.2015; 03.05.2015; 26.03.2016; 17.04.2016; 07.04.2017; 28.04.2017; 26.03.2018; 09.04.2018 |
| | Balıkesir, Burhaniye | 11.04.2015; 26.03.2016; 18.04.2016; 28.03.2017; 28.04.2017; 09.04.2018 |
| Aegean Region | Manisa, Merkez | 11.04.2015; 03.05.2015; 27.03.2016; 18.04.2016; 28.03.2017; 23.04.2017; 09.04.2018 |
| | Demirci | 11.04.2015; 18.04.2016 |
| | Akhisar | 11.04.2015; 18.04.2016; 28.03.2017 |
| | Gördes | 11.04.2015; 03.05.2015; 18.04.2016 |
| | İzmir, Bergama | 11.04.2015; 17.04.2016; 03.05.2015; 27.03.2016; 17.04.2016; 28.03.2017; 23.04.2017; 26.03.2018 |
| | Selçuk | 03.05.2015; 09.04.2017; 28.04.2017; 26.03.2018; 09.04.2018 |
| | Urla | 02.05.2015; 09.04.2017 |
| Mediterranean Region | Isparta | 25.03.2016; 18.04.2016; 18.03.2017; 01.05.2017; 26.03.2018 |
| | Antalya | 20.03.2015; 25.03.2016; 19.04.2016; 18.03.2017; 26.03.2018 |
| | Adana, Sarıçam | 21.03.2015; 08.04.2015; 14.04.2016; 06.04.2017 |
| | Karaisalı | 14.04.2016; 06.04.2017 |
| | Mersin | 20.03.2015; 08.04.2015; 06.04.2017 |
| Southeastern Anatolia Region | Gaziantep | 25.03.2015; 07.04.2017 |
| Black Sea Region | Çorum, Kargı | 01.05.2017 |


**Figure 1.** Prevalence of microsporidiosis in *Calosoma sycophanta* populations in Turkey.

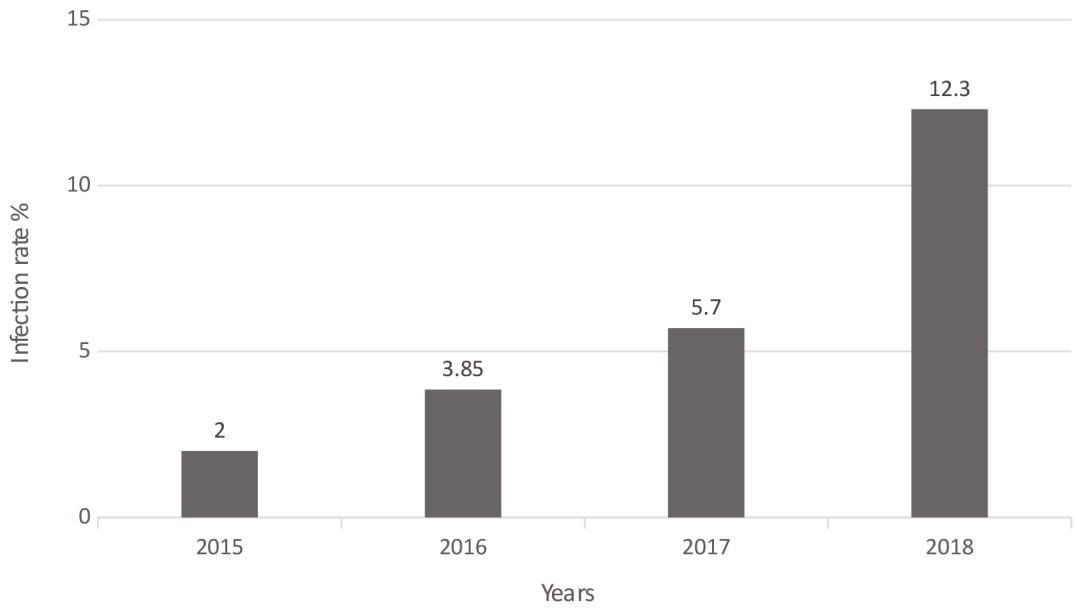


**Figure 2.** Microsporidiosis in *Calosoma sycophanta* between the years 2015 and 2018.

**Table 2.** Distribution of microsporidiosis in *C. sycophanta*-rearing laboratories.

| Rearing laboratory localities | | Collection date | Number of insects | | Percentage |
|---|---|---|---|---|---|
| | | | Dissected | Infected | % |
| Manisa | Merkez | 11.04.2015 | 46 | 0 | 0 |
| | | 03.05.2015 | 69 | 0 | 0 |
| | | 27.03.2016 | 154 | 3 | 1.9 |
| | | 18.04.2016 | 171 | 0 | 0 |
| | | 28.03.2017 | 68 | 8 | 11.7 |
| | | 23.04.2017 | 375 | 2 | 0.5 |
| | | 09.04.2018 | 62 | 0 | 0 |
| | Demirci | 11.04.2015 | 69 | 2 | 2.9 |
| | | 18.04.2016 | 15 | 0 | 0 |
| | Akhisar | 11.04.2015 | 62 | 0 | 0 |
| | | 18.04.2016 | 276 | 0 | 0 |
| | | 28.03.2017 | 62 | 0 | 0 |
| | Gördes | 11.04.2015 | 38 | 0 | 0 |
| | | 03.05.2015 | 87 | 0 | 0 |
| | | 18.04.2016 | 105 | 6 | 5.7 |
| İzmir | Bergama | 11.04.2015 | 78 | 0 | 0 |
| | | 03.05.2015 | 9 | 0 | 0 |
| | | 27.03.2016 | 179 | 1 | 0.5 |
| | | 17.04.2016 | 387 | 13 | 3.4 |
| | | 28.03.2017 | 120 | 4 | 3.3 |
| | | 23.04.2017 | 173 | 11 | 6.4 |
| | | 26.03.2018 | 298 | 8 | 2.68 |
| | Selçuk | 03.05.2015 | 92 | 4 | 4.3 |
| | | 09.04.2017 | 446 | 3 | 0.6 |
| | | 28.04.2017 | 283 | 1 | 0.3 |
| | | 26.03.2018 | 148 | 39 | 26.3 |
| | | 09.04.2018 | 262 | 13 | 4.96 |
| | Urla | 02.05.2015 | 90 | 0 | 0 |
| | | 09.04.2017 | 234 | 16 | 6.8 |
| Balıkesir | Burhaniye | 11.04.2015 | 172 | 12 | 7 |
| | | 26.03.2016 | 146 | 15 | 10.2 |
| | | 18.04.2016 | 168 | 0 | 0 |
| | | 28.03.2017 | 261 | 79 | 30.2 |
| | | 28.04.2017 | 83 | 2 | 2.4 |
| | | 09.04.2018 | 46 | 0 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| Bursa | Merkez | 10.04.2015 | 16 | 0 | 0 |
| | | 03.05.2015 | 63 | 0 | 0 |
| | | 26.03.2016 | 38 | 5 | 13.1 |
| | | 17.04.2016 | 100 | 11 | 11 |
| | | 07.04.2017 | 168 | 20 | 11.9 |
| | | 28.04.2017 | 119 | 0 | 0 |
| | | 26.03.2018 | 132 | 28 | 21.2 |
| | | 09.04.2018 | 12 | 1 | 8.3 |
| Adana | Sarıçam | 21.03.2015 | 13 | 0 | 0 |
| | | 08.04.2015 | 23 | 0 | 0 |
| | | 14.04.2016 | 48 | 0 | 0 |
| | | 14.04.2016 | 23 | 0 | 0 |
| | | 06.04.2017 | 58 | 0 | 0 |
| | Karaisalı | 14.04.2016 | 38 | 1 | 2.6 |
| | | 06.04.2017 | 120 | 0 | 0 |
| Mersin | Davultepe | 20.03.2015 | 6 | 0 | 0 |
| | | 08.04.2015 | 4 | 0 | 0 |
| | | 06.04.2017 | 14 | 5 | 35.7 |
| Antalya | Merkez | 20.03.2015 | 5 | 1 | 20 |
| | | 25.03.2016 | 59 | 10 | 16.9 |
| | | 19.04.2016 | 65 | 19 | 29.2 |
| | | 18.03.2017 | 37 | 6 | 16.2 |
| | | 26.03.2018 | 241 | 75 | 31.1 |
| Isparta | Merkez | 25.03.2016 | 142 | 1 | 0.7 |
| | | 18.04.2016 | 92 | 0 | 0 |
| | | 18.03.2017 | 7 | 1 | 14.2 |
| | | 01.05.2017 | 146 | 1 | 0.6 |
| | | 26.03.2018 | 216 | 11 | 5.09 |
| Gaziantep | Merkez | 25.03.2015 | 13 | 0 | 0 |
| | | 07.04.2017 | 30 | 1 | 3.3 |
| Çorum | Kargı | 01.05.2017 | 38 | 2 | 5.2 |
| Total | | | 7420 | 441 | 5.94 |

increased in 2017. In Mersin province, the infection rate was 35.7% in 2017. On the other hand, high infection rates were found in Antalya province during the four years: 20% in 2015, 23.3% in 2016, 16.2% in 2017, and 31.1% in 2018. A total of 80 *C. sycophanta* larvae from Balıkesir and Isparta were examined and showed no microsporidiosis in 2016.

During the study, the infection levels in male and female beetles were also compared. In total, 24 out of 963 male individuals and 41 out of 1370 female individuals were infected by the microsporidium pathogen. Infection levels were 2.49% in males and 2.99% in females (Table 3). There was no significant difference (Pearson $x^2$, $P > 0.05$) in infection levels between male and female beetles, which showed that the microsporidiosis does not favor either of the sexes.

**4. Discussion**

The first case of microsporidiosis in the predatory beetle *C. sycophanta* was reported by Yaman et al. (2016). In that study, a microsporidium from *C. sycophanta* was recorded for the first time and the pathogen was identified by using light and TEM microscopies; no data on distribution was reported. In the present study, the distribution of microsporidian pathogen in *C. sycophanta* populations from sixteen mass-rearing laboratories from ten provinces in five different regions of Turkey during four years (Table 1) is presented for the first time. There is a lack of data

**Table 3.** Infected males and females of *C. sycophanta*.

| Rearing laboratory localities | | Collection date | Male ♂♂ | | | Female ♀♀ | | |
|---|---|---|---|---|---|---|---|---|
| | | | D | I | % | D | I | % |
| Manisa | Merkez | 18.04.2016 | 54 | 0 | 0 | 84 | 0 | 0 |
| | | 23.04.2017 | 118 | 1 | 0.8 | 168 | 1 | 0.5 |
| | Demirci | 18.04.2016 | 4 | 0 | 0 | 6 | 0 | 0 |
| | Gördes | 18.04.2016 | 40 | 2 | 0.5 | 58 | 4 | 6.8 |
| | Akhisar | 18.04.2016 | 86 | 0 | 0 | 74 | 0 | 0 |
| Izmir | Bergama | 17.04.2016 | 106 | 4 | 3.77 | 122 | 5 | 4.09 |
| | | 23.04.2017 | 26 | 0 | 0 | 74 | 7 | 9.4 |
| | Selçuk | 09.04.2017 | 140 | 0 | 0 | 143 | 1 | 0.6 |
| | | 28.04.2017 | 67 | 1 | 1.4 | 110 | 0 | 0 |
| | Urla | 09.04.2017 | 70 | 9 | 12.8 | 128 | 6 | 4.6 |
| Bursa | Merkez | 17.04.2016 | 33 | 3 | 9.1 | 56 | 8 | 14.3 |
| | | 28.04.2017 | 42 | 0 | 0 | 52 | 0 | 0 |
| Balıkesir | Burhaniye | 18.04.2016 | 33 | 0 | 0 | 96 | 0 | 0 |
| | | 28.04.2017 | 20 | 1 | 5 | 43 | 0 | 0 |
| Adana | Sarıçam | 14.06.2017 | 14 | 0 | 0 | 9 | 0 | 0 |
| | | 06.04.2017 | 29 | 0 | 0 | 24 | 0 | 0 |
| | Karaisalı | 14.04.2016 | 3 | 0 | 0 | - | - | - |
| Antalya | Merkez | 19.04.2016 | 9 | 2 | 22.2 | 22 | 7 | 31.8 |
| Isparta | Merkez | 01.05.2017 | 51 | 0 | 0 | 81 | 1 | 1.23 |
| Çorum | Kargı | 01.05.2017 | 18 | 1 | 5.5 | 20 | 1 | 5 |
| Total | | | 963 | 24 | 2.49 | 1370 | 41 | 2.99 |

D: number of dissected beetles; I: number of infected beetles; %: percentage of infection

on the distribution of microsporidian in *C. sycophanta* populations. Some symptoms, such as reduced food consumption, prolonged larval and pupal development, deformed pupae and adults, reduced fecundity and longevity, can be observed in microsporidian-infected beneficial insects (Brooks and Cranford, 1972; Siegel et al., 1986; Zchori-Fein et al., 1992; Geden et al., 1995; Bjørson and Keddie, 1999; Idris et al., 2001; Steele and Bjørson, 2012). Due to the fact that host insects are more susceptible to microsporidia under stress, more noticeable effects are seen in mass rearing laboratories (Kluge and Caldwell, 1992). The effectiveness of predators depends on their health, besides many other factors such as biotic and abiotic factors and intrinsic factors (Yaman et al., 2012). We demonstrated that microsporidiosis varied in populations of *C. sycophanta* from different districts of Turkey in different years. Our results indicated that there were variations in microsporidiosis rates year by year, and that the infection ratio increased from 2015 to 2018. The total infection rates in 2015, 2016, and 2017 were clearly lower than that of 2018 (Figure 2). Noticeable differences were observed among the samples collected from laboratories in the same province and also from the same laboratory but in different years (Table 1; see, for example, three *C. sycophanta* mass-rearing laboratories in İzmir province, Bergama, Selçuk, and Urla). In 2017, the microsporidium infection rate was different in all three laboratories in İzmir; the infection rate was 5.11% in Bergama, 0.54% in Selçuk, and 6.8% in Urla. In total, no microsporidium infection was observed in 80 examined larvae of *C. sycophanta*.

In Balıkesir province, the microsporidium infection rate was 6.97% in 2015, 4.77% in 2016, and 23.5% in 2017. Despite infection rate decrease in 2016, it increased in 2017. In addition, in Mersin province, the infection rate was 35.7% in 2017. One of the most important results of this study is that high infection rates were found in the Antalya province during the four years: 20% in 2015, 23.3% in 2016, 16.2% in 2017, and 31.1% in 2018.

Other laboratories were provided with parent individuals of *C. sycophanta* from Antalya province. Therefore, parent individuals in Antalya province

have an important role due to the risk of spreading the pathogen infection. Infected female hosts transmit many microsporidian species to their offspring by transovarial transmission via eggs or embryos. Thus, the existence of microsporidian pathogens in host population may increase significantly (Dunn et al., 2001). In this study, the infection rates of the examined *C. sycophanta* male and female individuals showed a variation between 2016 and 2017. In total, microsporidium infections were observed in 24 of 963 male beetles and the infection rate was 2.49%. On the other hand, that microsporidium infections were observed in 41 of 1370 female beetles and the infection rate was 2.99%. According to the results, due to the fact that the number of microsporidian-infected females is higher than males, the host population is at risk in mass-rearing laboratories. Therefore, the infected individuals must be eliminated before outbreaks. In addition, we suggest that the adults of *C. sycophanta* selected as parents to produce a new generation in mass-rearing laboratories should be tested for microsporidiosis; adults for mating should be selected from noninfected populations.

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