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The first record and occurrence of microsporidian pathogen, *Chytridiopsis* cf. *typographi* (Microspora) in *Pityokteines curvidens* (Coleoptera, Curculionidae) populations in Türkiye

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Abstract: *Pityokteines curvidens* is one of the most damaging bark beetles to coniferous trees, especially fir and it may cause significant tree death in Türkiye. Control methods are insufficient to control the pest due to its biology under tree bark. Entomopathogens can play a suppressive role on natural populations of pest insects. In the present study, the occurrence of *Chytridiopsis typographi*, the microsporidian pathogen of *P. curvidens* is presented for the Bolu region during four years. A total of 1.366 *P. curvidens* adult samples were dissected and searched for the microsporidian pathogen during the study. The first infection was found in *P. curvidens* adult samples collected in 2009 with a 1.6% infection rate. After 2009, the infection was also observed in the years, 2020 and 2021 as 0.8 and 3.6%, respectively. The infection ranged from 0.8 to 3.6%. The results of our study show that *C. typographi* has established a relatively stable infection in the *P. curvidens* populations over the last decade. This is the first study to show the occurrence of an entomopathogenic microsporridium in *P. curvidens* populations in Türkiye.
Keywords: *Chytridiopsis typographi*, *Pityokteines curvidens*, Bolu, Türkiye

Türkiye'de *Pityokteines curvidens* (Coleoptera, Curculionidae) popülasyonlarında microsporidian patojen *Chytridiopsis* cf. *typographi* (Microspora)'nın varlığı ve ilk kaydı

Öz: *Pityokteines curvidens*, başta köknar olmak üzere iğne yapraklı ağaçlara en çok zarar veren kabuk böceklerinden biridir ve Türkiye'de önemli ağaç ölümlerine neden olabilmektedir. Zararlının ağaç kabuğu altındaki biyolojisi nedeniyle mücadele yöntemleri yetersiz kalmaktadır. Entomopatojenler zararlı böceklerin doğal popülasyonları üzerinde baskılayıcı bir rol oynayabilir. Bu çalışmada, *P. curvidens*'in mikrosporidian patojeni *Chytridiopsis typographi*'nin Bolu bölgesinde dört yıl boyunca varlığı izlenmiştir. Çalışma sırasında toplam 1.366 adet *P. curvidens* ergin örneği disekte edilerek microsporidian patojeni arandı. İlk enfeksiyon, 2009 yılında toplanan *P. curvidens* ergin örneklerinde % 1,6 enfeksiyon oranıyla bulundu. 2009'dan sonra enfeksiyon oranı 2020 ve 2021 yıllarında da sırasıyla % 0,8 ve % 3,6 olarak görüldü. Enfeksiyon % 0,8 ila % 3,6 arasında gözlendi. Çalışma sonuçları, *C. typographi*'nin son on yılda *P. curvidens* popülasyonlarında nispeten stabil bir enfeksiyon oluşturduğunu göstermektedir. Bu çalışma, Türkiye'deki *P. curvidens* popülasyonlarında entomopatojenik bir mikrosporidian'ın varlığını gösteren ilk çalışmadır.
Anahtar kelimeler: *Chytridiopsis typographi*, *Pityokteines curvidens*, Bolu, Türkiye

1. Introduction

Bark beetles (Coleoptera: Curculionidae, Scolytinae) are distributed worldwide. Several bark beetle species known as important forest pests cause damage by attacking living coniferous and broad-leaved tree species trees when outbreaks occur. Natural enemies are important in limiting species distributions and pest status. Bark beetles have a large group of natural enemies (Ünal and Yüksel, 2005; Sarıkaya and Avcı, 2009; Wegensteiner et al., 2015). The occurrence and action of natural enemies of bark beetles are reviewed by Wegensteiner et al. (2015) recently. Among them, entomopathogens contribute to the natural regulation of insect populations. Their roles in pest populations stimulate scientists to study their occurrence in bark beetle populations. They play an important suppressive role in natural populations of bark beetles. Entomopathogens in bark beetles include viruses (especially entomopoxviruses), bacteria, fungi, microsporidia, protozoa, and nematodes.

Among the entomopathogenic organisms, Microsporidia generally has a diverse host range. However, some microsporidia can infect a rather restricted host range. Entomopathogenic microsporidia can have toxic effects on their hosts by reducing longevity and fertility (Becnel and Andreadis, 2014; Seatamanoch et al., 2022). *Chytridiopsis typographi* Weiser, 1954, the microsporidian pathogen of bark beetles, has been recorded from several bark beetle hosts (Wegensteiner et al., 2015). *C. typographi* was recorded from *Ips acuminatus* in Türkiye for the first time (Yaman et al., 2016). After that, there has been no any record from bark beetles.

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Pityokteines curvidens is one of the most damaging bark beetles to coniferous trees, especially fir and it may cause significant tree death in Türkiye (Sarıkaya and Avcı, 2011; Yüksel and Öztük, 2022; Sivrikaya et al., 2023).It causes serious economic losses in forest areas. For example, approximately 38 thousand cubic meters of wood raw material were damaged due to *P. curvidens* in Bartın Forestry Enterprise between 2008 and 2012 (Güngör and Daşdemir, 2014). *P. curvidens* was reported as one of the fir bark beetles that caused effective damage in the fir forests of Bolu and Aladağ Forestry between 2002-2003 (Serin et al., 2005). There is not any study on the natural enemies of *P. curvidens* in the Bolu region. In the present study, the occurrence of *Chytridiopsis typographi*, the microsporidian pathogen of *P. curvidens* is presented for the Bolu region during four years. This is the first study to show the occurrence of an entomopathogenic microsporridium in *P. curvidens* populations in Türkiye.

2. Materials and methods

A long-term and patient sampling study was designed to determine the presence of the microsporidian pathogen in *P. curvidens* populations. Adult beetles were collected from pheromone traps as much as possible with a random sampling in the area between Aladağlar and Abant in Bolu. For this, the first sampling was done in 2009 and after a waiting period of 10 years, the sampling process was repeated for three consecutive years, from 2020 to 2022. After each sampling, adult beetles were dissected in a drop of physiological water (0.8%) on a microscope slide, and wet smears were examined for the presence of the microsporidian infection under a light microscope at a magnification of 200–1000 × (Yaman and Radek, 2017). Midgut epithelium was the main target organ to examine for the presence of the pathogen. When infection was found, the slides were air-dried and fixed with methanol, then stained with a freshly prepared 5% solution of Giemsa stain. They were then washed in running tap water, air-dried, and re-examined under the microscope. Detected spores were measured and photographed using an Optika B-293PLi microscope with a digital camera and Optika Proview Digital Camera Software. The data of fresh oocysts are presented as mean values ± standard deviation.

Diagnosis of the microsporidium was carried out according Weiser (1969) and Wittner and Weiss (2000). Giemsa-stained slides are deposited at BAİBÜ-Insect Pathology and Biological Control Laboratory.

3. Results and discussion

After the dissection of the infected adults of *P. curvidens*, their guts were examined for pathogen infections. Thick-walled cyst-like sporophorous vesicles in the host's midgut epithelium are evidence for the presence of the microsporidium pathogen, *C. typographi* (Figure 1). The infection was visible due to unique thick-walled cysts. Thick-walled cysts were regularly spherical in shape (Figure 1).

A total of 1.366 *P. curvidens* adult samples were dissected and searched for the microsporidian pathogen, *C. typographi* in Bolu region during the study. The infection ranged from 0.8 to 3.6%. The first infection was found in *P. curvidens* adult samples collected in 2009 with a 1.6% infection rate. After 2009, the infection was also observed in the years, 2020 and 2021 as 0.8 and 3.6%, respectively. However, no infection was observed in 2022. It is known that climatic conditions are the main factors affecting microsporidian infections in insect populations (Yaman and Radek, 2020). Considering that the infection rate in previous years was low in the populations, it is thought that the infected individuals in that year could not fly to the pheromone traps because they could not withstand the hard winter conditions and died. Twenty of the examined beetles were infected by the pathogen (Table 1), and the total infection rate was found as 1.5%. The results of our study show that *C. typographi* has established a relatively stable infection in the *P. curvidens* populations over the last decade.

C. typographi generally causes infection at different rates in bark beetles. Haidler et al. (2003) searched the microsporidian infections in different bark beetle species and found that *C. typographi* occurs in 7.7% of *Hylurgops cunicularius*, 0.5% in *H. glabratus*, 11.4% in *Pityogenes pityographus*, 2% *P. chalcographus*, 16.6% in *Ips typographus*, 15.9% in *I. aminitus* in Austrian forest. Weiser (1969) found 2% infection in the Douglas Fir Beetle, *Dendroctonus pseudotsugae*. Purrini and Weiser (1982) found a considerable infection rate, of 20% in the population of the bark beetle, *Hylastes cunicularius.* Pernek et al. (2009) found 5.6% in *P. curvidens* in Croatia. Yaman et al. (2016) found 0.9% in *Ips acuminatus* in Turkey. According to those results, it can be stated that *C. typographi* is a more generalist species found in different bark species as mentioned before by Pernek et al. (2009). On the other hand, Wegensteiner and Weiser (1996) observed the frequency of *C. typographi* in an *Ips typographus* laboratory stocks during six generations and found that 3.2% *C. typographi*-infection in the beetles of the parental generation increased up to 52.5% in the F2-generation and 49.6% in the F3-generation, decreased to 27.9% in the F4-generation and 10.6% in the F5-generation and then increased again in the F6-generation.

On the other hand, although another microsporidian pathogen, *Nosema curvidentis* infects *P. curvidens* (Weiser, 1961) we observed only *C. typographi* infection in *P. curvidens* populations in Bolu region. Up to now, there is no microsporidian record from *P. curvidens* populations in Türkiye. In this study, *C. typographi* is recorded from *P. curvidens* populations in Türkiye for the first time.

4. Conclusions

C. typographi generally causes infection at different rates in bark beetles. However, there is no record from bark beetles in Türkiye. This is the first study to show the occurrence of an entomopathogenic microsporidium in *P. curvidens* populations in Türkiye. The results of our study show that *C. typographi* has established a relatively stable infection in the *P. curvidens* populations over the last decade.

Table 1. Infection of *Chytridiopsis* cf. *typographi* in *Pityokteines curvidens* populations in Bolu region.

Sampling year	Number of examined beetles	Number of infected beetles	Infection rate (%)
2009	320	5	1.6
2020	391	3	0.8
2021	330	12	3.6
2022	325	-	0
Total	1.366	20	1.5

Figure 1. Cyst of *Chytridiopsis* cf. *typographi* in *Pityokteines curvidens*

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