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Occurrence of *Gregarina typographi* (Apicomplexa, Gregarinidae) and *Metschnikowia typographi* (Ascomycota, Metschnikowiaceae) in *Ips sexdentatus* (Coleoptera: Curculionidae, Scolytinae) Populations in Kastamonu (Turkey)

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Abstract: In this study, *Ips sexdentatus* (Coleoptera: Curculionidae, Scolytinae) populations from Kastamonu (Turkey) were investigated for the occurrence of entomopathogens. Two species were encountered in the duration of this study and these were *Gregarina typographi* (Apicomplexa, Gregarinidae) and *Metschnikowia typographi* (Ascomycota, Metschnikowiaceae). Within the *I. sexdentatus* samples used in this study total infection rate of the pathogens *G. typographi* and *M. typographi* was calculated as 28.9%. Measurements of the *Metschnikowia* species identified from *I. sexdentatus* were as follows: mean length 16.86±2.2 μm and mean width 2.8±0.3 μm (n = 125). The ascospores of this pathogen were characteristically needle-shaped and therefore, easily distinguishable under the light microscope. The occurrence rate for the ascomycete pathogen (*M. typographi*) was 3.1%. The gregarine pathogen measured 108.1-203.5 μm and was identified as *Gregarina typographi*. The occurrence rate for the Gregarine pathogen was 25.8%. The first report for Turkey (Kastamonu) of an ascomycete fungus, *Meschnikowia typographi* from the pine bark beetle *Ips sexdentatus* (Boerner) is given in this study.

Key words: *Ips sexdentatus*, *Gregarina typographi*, *Metschnikowia typographi*, occurrence, Kastamonu, Turkey

INTRODUCTION

The pine bark beetle *Ips sexdentatus* (Boerner) causes serious economic losses in spruce and pine forests in Europe and Turkey (Yuksel, 1998; Yuksel *et al*., 1999; Wegensteiner *et al*., 2005). Pheromone traps, mechanical and chemical control strategies have been used to control this pest for a long time in Turkey (Yuksel, 1998). Despite all the attempts, this pest still causes serious economic losses in oriental spruce and pine forests in various parts of Turkey including Kastamonu. Moreover, chemicals have a detrimental effect on the predators and parasites of these bark beetles and on the ecosystem. In an attempt to destroy the bark beetles, its predators and parasites are killed by chemicals conveniently enabling *Ips* sp. to thrive. On the other hand, natural enemies of *Ips* sp. are very interesting and certainly have advantages over chemicals as control agents. Although, there are several studies on the parasites and pathogens of *Ips* sp. from different parts of the world (Fuchs, 1915; Wegensteiner and Weiser, 1995; Wegensteiner *et al*., 1996; Weiser *et al*., 1998; Weiser *et al*., 2003; Wegensteiner and Weiser, 2004; Wegensteiner *et al*., 2005; Weiser *et al*., 2006), there are only two studies on *I. sexdentatus* in Turkey (Yaman, 2007; Yaman *et al*., 2008). Therefore, there is very little information on parasite and pathogen spectra of *Ips* sp. occurring in various parts of Turkey. The present study is significant because it reports on the occurrence of *G. typographi* and *M. typographi* in *I. sexdentatus* from Kastamonu region (Turkey) for the first time.

MATERIALS AND METHODS

In 2007, adults of *I. sexdentatus* were collected from pine forests from three localities in the Kastamonu district of Turkey, i.e., Saraycik, Ahlatcik and Subasi. During the collection, the specimens were randomly collected, without discriminating between male and female or mature adult and callow adult. Each beetle was dissected in a physiological solution (0.8% NaCl) and wet smears were examined under a light microscope at a magnification of 40-1000x. Several life stages of the detected pathogens were measured and photographed. Positive smears were fixed with methanol and stained with Giemsa's dye according to Wegensteiner *et al*. (1996). Pathogens and

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parasites found throughout the period of observation were measured and recorded. Infection was also confirmed under TEM microscope (Yaman and Radek, 2005).

RESULTS AND DISCUSSION

During this study, two pathogens were observed in the population of *Ips sexdentatus* collected from Kastamonu. The pathogens observed are species of genera *Metschnikowia* and *Gregarina*. *M. typographi* belonging to the order Ascomycota is a spore forming scrounging yeast infecting the mid-gut lumen and surrounding cells. Asci of the fungus are also found free in the hemolymph in large numbers. *G. typographi* is a typical member of Apicomplexa, Gregarinidae, its gamonts are found in the mid-gut lumen and its trophozoites bind to the mid-gut epithelium.

The morphological features of the observed fungus include navicular asci having two needle-shaped ascospores attenuated at both ends and vegetative forms resembling typical round to tubular fungal cells. Measurements of asci are reported as mean length 17.6±2.4 μm and width 2.8±0.3 μm (11.5-24.1×1.4-4 μm) (n = 182) and mean sizes of ascospores are 16.88±1.4 μm in length and 1.54±0.3 μm (13.7-19.8×1.06×2.13) in width. This pathogenic fungus which forms two ascospores per ascus was observed with a light microscope (Fig. 1a). Of the 426 beetles examined, 13 were infected by the fungus *M. typographi*. Total pathogen infection rate of *I. sexdentatus* is 28.9%. Total rate of *Metschnikowia* infection is 2.81%. The rate of ascomycete infection from Saraycik is 3.5% and the infection rate from Subasi is 2.9%. We did not determine any *Metschnikowia* pathogen from Ahlatcik.

In this study, we also observed a gregarine pathogen from these beetles and observed several life stages of this pathogen, such as trophozoite with epimerite, gamont and cyst (Fig. 2) in anterior part of the intestinal lumen of the bark beetles. The measurements of gamonts of the observed gregarine parasite are 108-203 μm (Table 1). A 110 of the examined beetles were infected with *G. typographi*. Total gregarine infection rate is 25.8%. Gregarine infection rate is 33.3% at Ahlatcilik 31.7% at Saraycik and 16.6% at Subasi.

The pathogens observed in the present study were known species in *Ips* sp. And they were identified as *Metschnikowia typographi* and *Gregarina typographi* according to the study (Weiser *et al*., 2003; Wegensteiner *et al*., 2005; Yaman, 2007; Yaman and Radek, 2008).

Previously, Weiser *et al*. (2003) isolated and identified *M. typographi* from the bark beetles *Ips typographus* and *Ips amitinus* (Coleoptera: Curculionidae, Scolytinae). Wegensteiner *et al*. (2005) also found *M. typographi* from *I. sexdentatus* in Austria.

Morphological features obtained from the electron and light microscopical (Fig. 1a and b) observations of the determined fungus pathogen suggest that it is *Meschnikowia typographi*. Yaman and Radek (2008) described an ascomycete from *Dendroctonus micans* (Kugelann) from Turkey and identified it as

Table 1: The measurements of gregarine parasite from *Ips sexdentatus*

I. sexdentatus	TL	LP	LD	WP	WD	LP:TL	WP:WD
Mean (n: 34)	152.56±25.15	36.98±5.67	115.58±22.70	48.2±7.71	57.87±11.98	4.17±0.67	1.19±0.11
	203.5-108.1	48.6 - 26	163.6-77.5	65.5-34.1	80.4-34.4	6.05-2.99	1.42-0.97

TL: Total Length; LP: Length of Protomerite; LD: Length of Deutomerite; WP: Width of Protomerite; WD: Width of Deutomerite; LP:TL: Ratio of the length of protomerite to total length; WP:WD: Ratio of the width of protomerite to the width of deutomerite

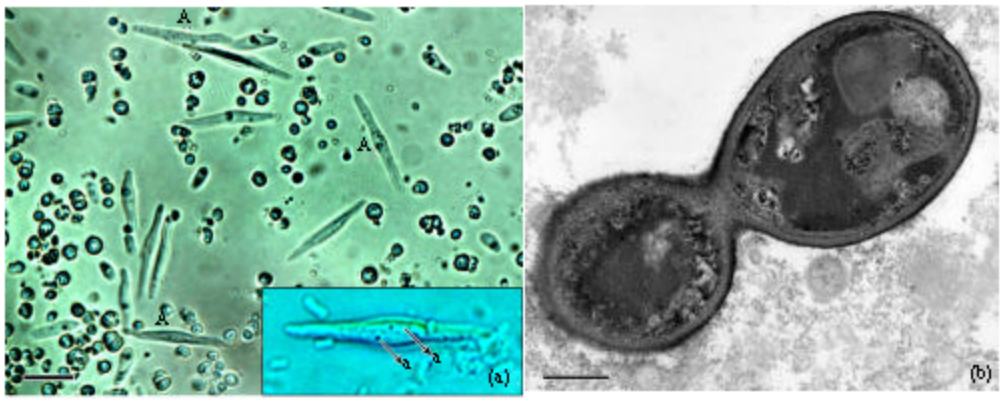

Fig. 1: a) Differential interference contrast micrographs of *M. typographi* from *I. sexdentatus*; asci (A) and ascospores (a). Bar: 10 μm, b) Thin section of *M. typographi* showing the early stage in the formation of a bud. Bar: 500 nm

Table 2: Comparison of measurements of *Gregarina typographi* from different studies

	G. typographi (reference)			
Characters	Lipa (1967)	Yaman (2007)	Takov *et al.* (2007)	Present study
TL*	78-118	80-275	55-237	108-203
LP:TL*	1:2.2-5	1:2.7-5.3	1:0.09-0.38	1:2.9-6.0
WP:WD*	1:1.1-2	1:1.1-1.3	1:0.22-1.8	1:0.9-1.4
Host	*I. typographus*	*I. sexdentatus*	*I. sexdentatus*	*I. sexdentatus*

*: Max.-Min.: TL: Total Length; LP:TL: Ratio of the Length Of protomerite to Total Length; WP:WD: Ratio of the width of protomerite to the width of deutomerit

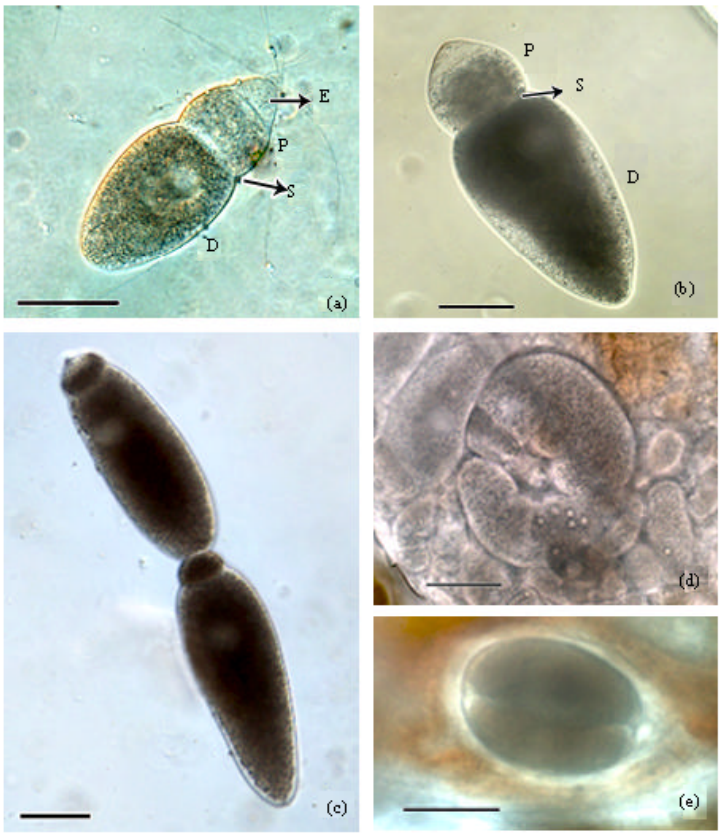

Fig. 2: Life stages of *Gregarina typographi*: A-trophozoite, B-gamont, C-associative form, D- two gamonts in the process of rotation previous to cyst formation E-cyst still in rotation with thin cyst wall. (E: Epimerite; P: Protomerit; D: Deutomerite; Pr: Primate; St: Satellite). Bar: 50 µm

Metschnikowia sp. and it measured 18.48±2.05 µm (14.7-22.3) in length and 2.1±0.4 µm in width. Weiser *et al.* (2003) measured asci of *M. typographi* as 13-17×1.5-2.5 µm from *I. typographus* and 17-22×2-2.5 µm from *I. amitinus*. Additionally *I. typographus* and *I. sexdentantus* are from the same beetle family (Coleoptera: Curculionidae) and they share the same terrestrial habitat therefore they also share pathogens. *I. sexdentatus* and *D. micans* are also pests that share the same habitat in Turkey. Although, *I. sexdentatus* and *D. micans* cannot be found in the same habitat elsewhere, in Turkey these bark beetles infest the same spruce stands together. Therefore, it would not be surprising to find the same pathogens in these bark beetles. The spores of *Metschnikowia* sp observed in *I. sexdentatus* in the present study and in *D. micans* by Yaman and Radek (2008) have the same physiological and morphological characters and ultrastructural studies prove that the *Metschnikowia* sp. determined from *I. sexdentatus* certainly is *Metschnikowia typograpi*.

According to these facts, *Metschnikowia* sp. has two different hosts which are *D. micans* and *I. sexdentatus* in Turkey. Besides, the occurrence and ultrastructure of *M. typographi* isolated from *I. sexdentatus* is reported for the first time from Turkey in the present study.

The rate of ascomycete infection from Saraycik with 3.5% is higher than the infection rate from Subasi with 2.9%. We did not determine any *Metschnikowia* pathogen from Ahlatcik. Wegensteiner *et al.* (2005) reported that the rates of *M. typographi* infection from *I. sexdentatus* varied between 0.9-51.4%. Weiser *et al.* (2003) reported that the rate of *M. typographi* infection was 1.8% from *I. sexdentatus* and 5.9% from *I. amitinus*.

On the other hand Yaman and Radek (2008) found *Metschnikowia* infection up to 50% in *Dendroctonus micans* populations at Black sea region of Turkey. Observed infection level in this study is not as high as that recorded from *I. sexdentatus* by Wegensteiner *et al.* (2005) and *D. micans* by Yaman and Radek (2008). It is similar with that recorded from *I. sexdentatus* and *I. amitinus* by Weiser *et al.* (2003).

There are several reports in the literature about the occurrence of *G. typographi* from two *Ips* species (*I. sexdentatus* and *I. typographus*) which are most economically important in Turkey. These studies include Purrini (1978) and Wegensteiner *et al.* (1996)'s report on *G. typographi* from *I. typographus* (L), Theodorides (1960)'s report on the same pathogen from *I. sexdentatus*, Takov *et al.* (2007) report from *I. sexdentatus* in Bulgaria, Kereselidze and Wegensteiner (2007)'s report from *I. typographus* in Georgia and Yaman (2007)'s report from *I. sexdentatus* in Turkey. *G. typographi* was also reported from Bulgaria and Georgia therefore, this shows that *G. typographi* is also present in adjacent countries indicating the distribution of this pathogen.

The measurements of gamonts of the observed gregarine parasite are similar with *G. typographi* reported by Yaman (2007) in Turkey measuring 80-275 μm. Morphological features and characters show that this gregarine pathogen is *Gregarina typographi*. The measurements of *G. typographi* are compared with the results of Lipa (1967), Takov *et al.* (2007) and Yaman (2007) in Table 2.

Rate of gregarine pathogen infection from Saraycik and Ahlatcik are higher than Subasi. Wegensteiner *et al.* (2005) reported that the rates of *G. typographi* infection from *I. sexdentatus* varied between 16.7-45.5%. Yaman (2007) found 16.1% gregarine infection in *I. sexdentatus* from Trabzon. Presented gregarine infection rate from Kastamonu is higher than the rate reported by Yaman (2007) but lower than the rate recorded by Wegensteiner *et al.* (2005).

CONCLUSION

Till now, one pathogen, *G. typographi* has been recorded from *I. sexdentatus* in Trabzon (Turkey). In this study, additional to this pathogen, *M. typographi* is reported in *I. sexdentatus* from Turkey for the first time confirming *G. typographi* in *I. sexdentatus* populations in Kastamonu.

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