

***Gregarina typographi* Fuchs, a Gregarine Pathogen of the Six-Toothed Pine Bark Beetle, *Ips sexdentatus* (Boerner) (Coleoptera: Curculionidae, Scolytinae) in Turkey**

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Abstract: A gregarine pathogen of *Ips sexdentatus* is reported in Turkey for the first time and described as *Gregarina typographi*. A mixed nematode parasitization and gregarine infection is reported. Gregarines were observed in the populations of *I. sexdentatus* in Şalpaazarı (Sisdağı), Trabzon. During the study, several life stages of the gregarine pathogen were observed, measured, and photographed. Measurements of gamonts and gametocysts of *G. typographi* are given and compared with other gregarine isolates from Turkey.

Key Words: Gregarine pathogen, *Ips sexdentatus*, *Gregarina typographi*

Türkiye’de *Ips sexdentatus* (Boerner) (Coleoptera: Curculionidae, Scolytinae)’un Bir Gregarin Patojeni *Gregarina typographi* Fuchs

Özet: Bu çalışmada *Ips sexdentatus*’un bir gregarin patojeni Türkiye’den ilk kez kaydedildi ve *Gregarina typographi* Fuchs. olarak tanımlandı. Zaman zaman aynı konakta gregarin ile bir nematod parazitinin birlikte neden olduğu çift enfeksiyon tespit edildi. Patojenik gregarinler Şalpaazarı (Sisdağı) (Trabzon)’ndaki ladin ormanlarında zararlı olan *Ips sexdentatus* popülasyonunda tespit edildi. Çalışma süresince, parazitin farklı safhaları gözlemlendi, bu safhaların ölçümleri yapılarak fotoğrafları çekildi. *G. typographi*’e ait gamont ve gametokist safhalarının ölçümleri verilmekte ve Türkiye’den izole edilen diğer böcek orijinli gregarin patojenleri ile bir karşılaştırılması yapılmaktadır.

Anahtar Sözcükler: Gregarin patojeni, *Ips sexdentatus*, *Gregarina typographi*

Introduction

The six-toothed pine bark beetle, *Ips sexdentatus* (Boerner) causes serious economic losses in spruce (especially *Picea orientalis*) forests in Turkey (Yüksel, 1998; Yüksel et al., 2000). Previously, strenuous effort has been expended to control this pest. Pheromone traps, and mechanical and chemical control strategies have been used for a long time, resulting in huge financial cost, and this pest still causes serious economic losses in Oriental spruce forests in Turkey. Moreover, these methods have a detrimental effect on the predators and parasites of this bark beetle, and on the ecosystem. In contrast, natural enemies of insects have certain advantages over chemicals as control agents. Consequently, any natural enemy of *I.*

sexdentatus is of great interest. Despite several studies on the parasites and pathogens of *Ips* spp. from different parts of the world (Fuchs, 1915; Theodorides, 1960; Lipa, 1967; Purrini, 1978; Wegensteiner and Weiser, 1995, 2004; Wegensteiner et al., 1996; Weiser et al., 1998; Weiser et al., 2006), there is no record of the pathogens of *I. sexdentatus* in Turkey. In this paper a gregarine of *I. sexdentatus* from Turkey is reported for the first time.

Materials and Methods

Adult *I. sexdentatus* specimens were collected from spruce (*Picea orientalis*) forests using pheromone traps in

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Şalpazarı, Trabzon (Turkey), in 2005 and 2006. Each beetle was dissected in a physiological solution (0.8% NaCl) and its intestine was examined microscopically at magnifications of 40×-400×. Several life stages of the detected pathogen were measured and photographed. Measurements were taken using a micrometer. Photomicrographs of live gregarines in physiological solution were taken using an Olympus BH-2 microscope with a camera attached.

The following abbreviations, according to Lipa (1967), Geus (1969), and Clopton (2004), are used to identify the parasite in this paper: 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; and WP:TL: ratio of the width of protomerite to total length. The systematic of the parasite was made by comparing the same or systematically close species given by Lipa (1967) and Geus (1969).



Figure 1. Trophozoite of *Gregarina typographi*. E: epimerite; P: protomerite; D: deutomerite; S: septum between the protomerite and the deutomerite (bar: 40 µm).

Results and Discussion

Gregarines were observed in the population of *Ips sexdentatus* collected from Şalpazarı (Sisdağı), Trabzon. Of the 31 beetles examined, 5 were infected by the pathogen. The pathogen was found in the anterior part of the intestine lumen of the bark beetle. Morphological features of the determined pathogen showed that it was a cephaline gregarine. The individual gregarine is divided into 2 parts, the protomerite and deutomerite. The gregarine pathogen showed a simultaneous incidence with nematodes in 3 of the 5 beetles.

During the study, several life stages of the gregarine pathogen, such as trophozoite with epimerite (Figures 1 and 2), gamont (Figure 3), and cyst (Figure 4) were observed in the intestinal lumen of the beetles. Epimerites measured up to 12.5 µm and were papilla-type in shape, and were divided by a septum from the rest of the protomerite (Figure 2). Gamonts were oval to elongate (Figure 3). Measurements (in µm) of gregarine gamonts are given in Table 1. Gametocysts were spherical and 90-100 µm in diameter (Figure 4).

In the literature there are several reports on the gregarine pathogens of bark beetles (Fuchs, 1915; Theodorides, 1960; Lipa, 1967; Purrini, 1978; Wegensteiner et al., 1996). The pathogen presented here is the first gregarine record from bark beetles in Turkey. Until now, 2 insect-originating gregarines, *Gregarina phyllostretae* (Yaman, 2002) and *Gregarina* sp. (Yaman,

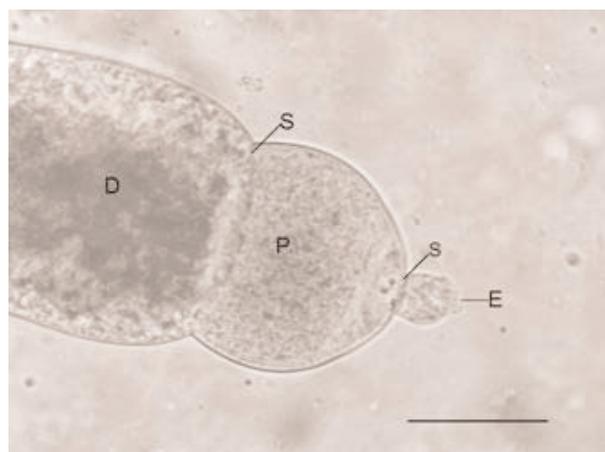


Figure 2. Papilla-type epimerite of *Gregarina typographi*. P: protomerite; D: deutomerite; S: septum E: epimerite divided by a septum from the rest of the protomerite (bar: 20 µm).



Figure 3. Young solitary gamont of *Gregarina typographi*. P: protomerite; D: deutomerite; S: septum between the protomerite and the deutomerite (bar: 50 μm).

2004) from the genus *Gregarina* have been reported. Both reported gregarines were found in chrysolmelides from Turkey. The gregarine presented in the present study differs from *Gregarina phyllotretae* recorded from *Phyllotreta atra* and *P. undulata* (Yaman, 2002), and *Gregarina* sp. recorded from *Chaetocnema tibialis* (Yaman, 2004) (Table 2). On the other hand, it shows

similarities to *Gregarina typographi* Fuchs, recorded by Fuchs (1915) from *Ips typographus* for the first, time in terms of its characteristics identified with the genus *Gregarina*, LP:TL and WP:WD (Table 2). However, it apparently differs from *G. typographi* by its larger size, though its larger size does not justify the creation of a new species. For example, while Geus (1969) reported that solitary gamonts measured from 480 \times 179 to 747 \times 238 μm and Theodorides and Jolivet (1959) observed gamonts 170-380 μm in length for *G. munieri*, Lipa and Simchuk (1979) observed they were smaller than those reported by both authors. In another example, Yaman (2002) found *G. phyllotretae* from *P. atra* and *P. undulata* in Turkey to be larger than *G. phyllotretae* recorded by Hoshide (1953) from *Phyllotreta vittata* in Japan. On the other hand, Turkey is a country of 779,000 km^2 , is located between Europe and Asia, serves as a bridge between 3 continents, and is surrounded on 3 sides by seas with substantially different characteristics. Each region of Turkey constitutes a different climatic zone and thus accommodates different groups of plants and animals (The Ministry of Environment, 1992). Therefore, the reported gregarine is considered to be *G. typographi*, described by Fuchs (1915) as a pathogen of

Table 1. Measurements of gamonts of *Gregarina typographi* in *Ips sexdentatus* (in μm).

	TL	LP	LD	WP	WD	LP:TL	WP:WD	WP:LP
Gamonts	100	37.5	62.5	35	42.5	2.67	1.21	1.07
	200	50	150.0	65	75	4.00	1.15	0.77
	200	62.5	137.5	62.5	67.5	3.20	1.08	1.00
	100	37.5	62.5	37.5	45	2.67	1.20	1.00
	175	55	120.0	55	62.5	3.18	1.14	1.00
	200	37.5	162.5	57.5	75	5.33	1.30	0.65
	275	57.5	217.5	78.4	100	4.78	1.28	0.73
	263	62.5	200.0	70	87.5	4.20	1.25	0.89
	80	25	55.0	42	50	3.20	1.19	0.60
	105	30	75.0	45	55	3.50	1.22	0.67
	100	30	70.0	55	65	3.33	1.18	0.55
	90	25	65.0	32	40	3.60	1.25	0.78
Min.	80	25	55	32	40	2.7	1.1	0.5
Max.	275	63	218	78	100	5.3	1.3	1.1
Mean	157.3	42.5	114.8	52.9	63.8	3.6	1.2	0.8
SD	69.9	14.3	58.0	14.7	18.5	0.8	0.1	0.2

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.

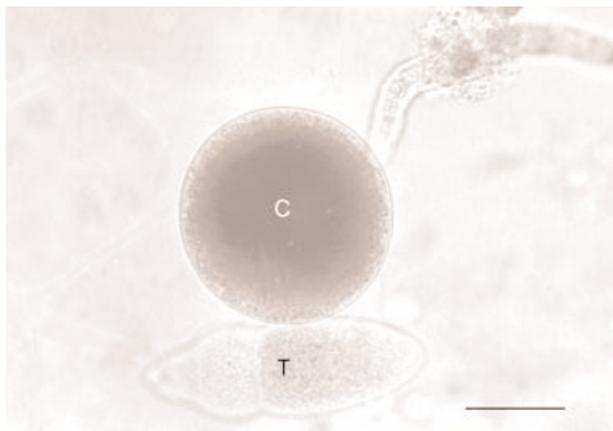


Figure 4. Gametocyst of *Gregarina typographi* (bar: 50 µm). C: Gametocyst; T: Trophozoite.



Figure 5. Young trophozoite and juvenile nematode in *Ips sexdentatus*. P: protomerite; D: deutomerite; E: epimerite; S: septum between protomerite and deutomerite; N: nematode (bar: 50 µm).

Table 2. The comparative characters of 3 gregarines observed in beetles (Coleoptera) from Turkey compared to *Gregarina typographi* Fuchs (µm).

Characters	<i>G. phyllotretae</i>	<i>Gregarina</i> sp.	<i>G. typographi</i>	<i>G. typographi</i> Fuchs
TL ^a	63-235	75-185	80-275	78-118
LP:TL ^a	1: 3-8	1: 3.3-9.8	1:2.7-5.3	1: 2.2-5
WP:WD ^a	1:1.1-1.7	1: 1.0-3.2	1:1.1-1.3	1: 1.1-2
Gametocyst ^a	hemi-ellipsoidal 117 × 98	-----	Spherical 90 to 100	-----
Host	<i>Phyllotreta undulata</i>	<i>Chaetocnema tibialis</i>	<i>Ips sexdentatus</i>	<i>Ips typographus</i>
Reference	Yaman, 2002	Yaman, 2004	Present study	Lipa, 1967

^a: minimal and maximal; 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 deutomerite.

I. typographus (L). Lipa (1967), Purrini (1978), and Wegensteiner et al. (1996) recorded this pathogen from the same host. Theodorides (1960) reported *G. typographi* from *I. sexdentatus*. *G. typographi* presented in this paper is the first gregarine pathogen from bark beetles in Turkey.

I. sexdentatus causes serious economic losses in spruce forests in Turkey and Europe. The gregarine pathogen presented in this paper can indirectly reduce the damage of this insect in Oriental spruce forests. In the case of eugregarines parasitizing in the cells of the midgut epithelium, the greatest pathological changes are observed in the period of intracellular and intercellular development of trophozoites. The damage to epithelium

by trophozoites is mechanical and physiological (Lipa, 1967). The pathogenic effect of eugregarines depends on mechanical damage to gut epithelium, physiological influence through excretion of metabolites, and through toxins in the process of pinocytosis, as well as intracellular and intercellular development of trophozoites in the gut epithelium, the transport of intestinal juice of the gut lumen with trophozoites and gamonts, and providing the port of entry for other microorganisms into the body cavity of the host (Lipa, 1967). Several studies support these ideas. For example, there are several entomopathogenic bacteria reported to control plant pest insects (Yaman, 2003). Bacterial septicemia was frequently observed among Insecta heavily infected with

gregarines (Lipa, 1967). It indicates that bacteria enter the body cavity of arthropods through openings made by sporozoites or trophozoites of gregarines in the gut epithelium. However, Tanada and Kaya (1993) reported that the damage to the midgut can be easily repaired (by cells developed in regeneration crypts) and the pathological effects of gregarines are generally weak; the very wide distribution all over Europe down to Turkey supports the estimation that *G. typographi* most probably is not a very virulent pathogen.

Conversely, during the present study a mixed infection of gregarine with nematode parasitization in 3 of 5 beetles infected by gregarine was observed (Figure 5). Nematodes were found in the gut lumen of the adult beetles. Nematode parasites and their effect on *I. sexdentatus* were studied previously (Lieutier, 1980, 1982, 1984). These 2 natural enemies may play an important role in the biological control of these insects by reducing the life span and reproductive potential of the host.

References

- Clopton, R.E. 2004. Standard nomenclature and metrics of plane shapes for use in gregarine taxonomy. *Comp. Parasitol.* 71: 130-140
- Fuchs, G. 1915. Die Naturgeschichte der Nematoden und einiger anderer Parasiten 1. des *Ips typographus* L. 2. des *Hylobius abietis* L. *Zool. Jb., Abt. Syst.* 38: 109-222.
- Geus, A. 1969. Sporentierchen Sporozoa, Die Gregarinida: Die Tierwelt Deutschlands. Teil 57, VEB Gustav Fischer, Jena. pp. 608
- Hoshide, H. 1953. Studies on the gregarines from Coleoptera in Japan II. *Bull. Fac. Educ. Yamaguchi Univ.* 2: 70-81.
- Lieutier, F. 1980. Le parasitisme d'*Ips sexdentatus* (Boern) (Coleoptera: Scolytidae) par les nématodes du genre *Parasitaphelenchus* Fuchs. Relations avec le parasitisme par *Contortylenchus diplogaster* (v. Lins.). *Rev. Nématol.* 3: 271-281.
- Lieutier, F. 1982. Effect of the endoparasitic nematodes on the oviposition of the bark beetle *Ips sexdentatus* Boerner (Insecta: Coleoptera). *Acta Oecol. Oecol. Appl.* 3: 191-204.
- Lieutier, F. 1984. Observations sur le parasitisme d'*Ips sexdentatus* (Insecta: Scolytidae) par le *Parasitorhabditis ipsophila* (Nematoda: Rhabditidae). *Ann. Parasitol. Hum. Comp.* 59: 507-520.
- Lipa, J.J. 1967. Studies on gregarines (*Gregarinomorpha*) of arthropods in Poland. *Acta Protozoologica.* V: 97-179.
- Lipa, J.J. and Simchuk, P. 1979. *Chrysomela menthastris* Suffr. as a host for *Gregarina munieri* (Schneider) and *Gregarina crenata* (Bhatia and Setna). *Bull. Acad. Pol. Sci., Ser. Sci. Biol.* 27: 105-108.
- Purrini, K. 1978. Protozoen als Krankheitserreger bei einigen Borkenkäferarten (Col., Scolytidae) im Königsee-Gebiet, Oberbayern. *Anz. Schädlingskunde, Pflanzenschutz, Umweltschutz.* 51: 171-175.
- Tanada, Y. and Kaya, H.K. 1993. Protozoan infection: Apicomplexa, Microspora. In: *Insect Pathology*, Academic Press, San Diego, pp 414-458.
- The Ministry of Environment, 1992. Turkey's importance in the world of living things. Bersay Publications, İstanbul.
- Theodorides, J. 1960. Parasites et phoretiques de coleopteres et de myriapodes de Richelieu (Indre-et-Loire). *Ann. de Parasitologie* XXXV: 488-503.
- Theodorides, J. and Jolivet, P. 1959. Eugregarines parasites de Coleopteres. Exploration du Parc National Albert, Congo Deuxieme Serie, Fasc. 8.
- Wegensteiner, R. and Weiser, J. 1995. A new entomopox virus in the bark beetle *Ips typographus* (Coleoptera: Scolytidae). *J. Invertebr. Pathol.* 65: 203-205.
- Wegensteiner, R., Weiser, J. and Führer, E. 1996. Observations on the occurrence of pathogens in the bark beetle *Ips typographus* L. (Coleoptera, Scolytidae). *J. Appl. Entomol.* 120: 199-204.
- Wegensteiner, R. and Weiser, J. 2004. Annual variation of pathogen occurrence and pathogen prevalence in *Ips typographus* (Coleoptera, Scolytidae) from the BOKU University Forest Demonstration Centre. *J. Pest Sci.* 78: 221-228.
- Weiser, J., Wegensteiner, R. and Zizka, Z. 1998. *Unikaryon montanum* sp.n. (Protista: Microspora), a new pathogen of the spruce bark beetle *Ips typographus* (Coleoptera: Scolytidae). *Folia Parasitol.* 45: 191-195.
- Weiser, J., Holusa, J. and Zizka, Z. 2006. *Larsoniella duplicati* n.sp. (Microsporidia, Unikaryonidae), a newly described pathogen infecting the double-spined spruce bark beetle, *Ips duplicatus* (Coleoptera, Scolytidae) in the Czech Republic. *J. Pest Sci* 79: 127-135.
- Yaman, M. 2002. *Gregarina phyllotretae* Hoshide 1953, a protozoan parasite of the flea beetles, *Phyllotreta undulata* and *P. atra* (Coleoptera: Chrysomelidae) in Turkey. *App. Entomol. Zool.* 37: 649-653.
- Yaman, M. 2003. Insect bacteria and hazelnut pests' biocontrol: The state of the art in Turkey. *Rivista di Biologia / Biology Forum.* 96: 137-144.
- Yaman, M. 2004. A newly recorded gregarine parasite of *Chaetocnema tibialis* (Coleoptera, Chrysomelidae) from Turkey. *Turk. J. Zool.* 28: 95-96.
- Yüksel, B. 1998. *Ips sexdentatus* (Boerner)'un biyolojisi ve mücadelesi, İ.Ü. Orman Fakültesi, Cumhuriyetimizin 75. Yılında Ormancılığımız Sempozyumu, Bildiriler Kitabı, İstanbul.
- Yüksel, B., Tozlu, G. and Şentürk, M. 2000. Sarıkamış sarıçam (*Pinus silvestris* L.) ormanlarında etkin zarar yapan kabuk böcekleri ve bunlara karşı alınabilecek önlemler, Doğu Anadolu Ormancılık Araştırma Enstitüsü, Teknik Bülten Yayın No: 3, Erzurum, 69 s.