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The First Record of A Gregarine Pathogen from Altica hampei (Allard, 1867) (*Coleoptera: Chrysomelidae*)

Hilal YILDIRIM[1], Onur TOSUN[2], Çağrı BEKİRCAN[3]
[1]Giresun Üniversitesi, Espiye Meslek Yüksekokulu, Bitkisel ve Hayvansal Üretim Bölümü, Giresun, [2]Karadeniz Teknik Üniversitesi, Maçka Meslek Yüksekokulu, Veterinerlik, Laborant ve Veteriner Sağlık Ana Bilim Dalı, Trabzon, [3]Karadeniz Teknik Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, Trabzon
[1]https://orcid.org/0000-0002-6072-5543, [2]https://orcid.org/0000-0002-6763-5671, [3]https://orcid.org/0000-0002-5968-7359
✉ : hilalbakiesk@hotmail.com

ABSTRACT
A new gregarine pathogen from *Altica hampei* (Allard, 1867) has been reported (Coleoptera: Chrysomelidae) for the first time in Turkey. *A. hampei* causes considerable damage on *Cirsium* arvense (L.) Scop. (Asterales: Asteraceae). Therefore, detection of pathogens in which may be a biological control agent of *A. hampei* was aimed. Overall, 134 *A. hampei* adults were collected from Ordu province of Turkey from April to June of 2016. During the study, gut content of adults was examined thoroughly using a light microscope. Total infection rate was found as 1.5%. Different life stages of the gregarine pathogen, gamont, associative form, trophozoite, cyst and precyst were observed in the midgut of adults. Observed pathogen was a cephaline gregarine. The morphological features, characteristic measurements and ratios of the observed gregarine were not validation for the definition of species level. Up to the present, since there are no reports about presence of gregarine pathogen from *A. hampei*, the observed pathogen from *A. hampei* was significant as the first gregarine pathogen report for Turkey and for the world literature. Besides, observed gregarine pathogen in present study is a contribution to the pathogen biodiversity of Turkey.

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Altica hampei (Allard, 1867) (*Coleoptera: Chrysomelidae*)'den bir Gregarine Patojeninin İlk Kaydı

ÖZET
Bu çalışmada *Altica hampei* (Allard, 1867) (Coleoptera: Chrysomelidae)'den Türkiye'de ilk kez bir gregarine patojeni kaydedildi. Bu böceğin *Cirsium arvense* (L.) Scop. (Asterales: Asteraceae) bitkisi üzerinde önemli derecede zarara sebep olması nedeniyle biyolojik kontrol ajanı olabilecek patojenlerinin belirlenmesi amaçlanmıştır. Türkiye'de, 134 *Altica hampei* ergini 2016 yılında Nisan -Haziran ayları arasında Ordu ilinden toplanmıştır. Çalışma süresince *A. hampei* erginlerinin bağırsak kısımları ışık mikroskobu ile incelenmiştir. Toplam enfeksiyon oran ı %1,5 olarak bulunmuştur. Ergin böceklerin orta bağırsağında gregarine patojenine ait gamont, birleşme formu, trofozoit, kist ve prekist gibi farklı hayat safhaları gözlenmiştir. Görülen patojen bir cephaline gregarindir. Bu çalışmada gözlenen gregarinin morfolojik özellikleri, karakteristik ölçümleri ve oranları tür seviyesinde bir tanımlama yapmak için yeterli değildir. Fakat bugüne kadar *A. hampei*'den bir gregarine patojeni varlığına dair bir kayıt yoktur, bu nedenle *A. hampei* erginlerinde gözlenen gregarine patojeni Türkiye ve dünya literaturü için ilk kayıt olması sebebiyle önemlidir. Ayrıca bu çalışmada gözlenen gregarine patojeni, Türkiye'nin patojen biyoçeşitliliğine bir katkıdır.

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INTRODUCTION

The subfamily Alticinae, the largest subfamily of Chrysomelidae, contains several significant agricultural pests (Aslan et al., 1999; 2007). Members of this subfamily cause substantial damage on the foliage of herbaceous plants, bushes and trees in commonly angiosperm families and some gymnosperms (Aslan et al., 1999). *Altica hampei* is one of the most important pests belonging to subfamily Alticinae. Adults and larvae of this species particularly feed on plant leaves and cause considerable damage on *Cirsium arvense* (Asterales: Asteraceae) in Black Sea Region of Turkey. *C. arvense* has wide distribution areas over east and northeast of Turkey (Tiley, 2010: Pala et al., 2018). Common name of the plant is Köygöçüren in Turkey. This plant is known as an invasive weed, however, it has also some benefits. In both cases, the pathogens of insect pest of the plants are important. If the plant isn't invasive, there is a need to control against to *A. hampei.* The use of pesticides to control the pests has negative effects on the ecosystem. Natural pathogens and parasites of pests are alternative biological control agent to the chemical insecticides. According to a common believe, entomopathogenic organisms may reduce insect population intensity (Tosun et al., 2008; Baki, 2016; Bekircan et al., 2016). If the plant is an invasive weed for us, the pest is gaining importance. In this situation, pathogens of the pest are undesirable factors because of reducing the pest populations or killing the pest (Yaman et al., 2009a). Gregarine pathogens were infected several kinds of insects in Chrysomelidae family (Théodoridés, 1988; Clopton et al., 1992; Yaman, 2004; Yaman et al., 2008; 2011). Especially, natural enemies of *A. hampei* are of great attention, because there is no record about pathogens or parasites of *A. hampei* in the literature so far.

In this study, gregarine pathogen was observed from *A. hampei* in Ordu province in Black Sea Region of Turkey. This study was the first report of gregarine pathogen from *A. hampei* from Turkey for world literature.

MATERIALS and METHODS

Samples of *A. hampei* were collected by forceps from nature in Ordu province of Turkey, in 2016. A total of 134 adults were collected during the April, May and June were transferred to laboratory with sterile cruet and dissected with Ringer's solution. Midgut of the insects was examined under light microscope at a magnification of a 100× to 1,000× for the presence of pathogen according to Tosun et al. (2008). Gregarine pathogens usually live in intestine of insects. In the study, intestine content of *A. hampei* were examined for several life stages (cyst, associative form, trophozoite, precyst and gamont) of the pathogen. Observed gregarines measured and photographed with Nicon Eclips E-400 and Nicon Eclips Ci microscope with digital camera DS-fi 2. The following gregarine pathogen structures were measured (µm): total length (TL), length of protomerite (LP), length of deutomerite (LD), length of epimerite (LE), width of protomerite (WP), width of deutomerite (WD), ratio of the width of protomerite to the width of deutomerite (WP:WD) and ratio of the length of protomerite to total length (LP:TL)according to Lipa (1967) and Clopton (2004). These measurements are significant to identify the gregarine pathogen. Number of infected samples by Gregarin were recorded both for male and female insects differently to compare the infection between both sexes. Infection rate was calculated using direct proportion, as calculated by Eq. (1):

$$\text{Infection rates} = \frac{\text{number of infected beetle}}{\text{number of examined beetle}} \times 100 \quad (1)$$

RESULTS

During the study, the several life stages of the gregarine pathogen, including cyst, gamont, precyst, associative form and trophozoite, were observed in the midgut epithelium of the host (Fig. 1). The gregarine parasite has two segments. First segment called protomerite was separated by a septum from the second segment called deutomerite. Observed ellipsoidal shape trophozoites have a spherical shape epimerite (Fig. 1a). The ellipsoidal to ovoidal shape gamonts were well seen, and the anterior quarter of the protomerite was transparent and globular or conoidal. The septum of gamont was clearly showed in Fig. 1b. Deutomerite was ellipsoidal to ovoidal with spherical nucleus with small karyosome (Fig. 1b). Gamonts are ovoidal or elongate. The associative form of gregarine was well seen in Fig. 1c and the cyst with ovodial shape was observed well in Fig. 1d. Only spore stage was not observed during the study (Fig.1e). Morphological shapes and measurements of life stages characteristic of the observed pathogen in this study showed that it was a gregarine pathogen. Structural and morphological measurements of gamonts of the gregarine was determined average as following: TL: 171 ± 40.2 µm; LD: 131.4 ± 27.9 µm; LP: 39.5 ± 14.5 µm; WD: 63.9 ± 18.5 µm; WP: 42.5 ± 8.1 µm; WP:WD: 1.5 ± 0.3 µm; LP:TL: 4.5 ± 0.6 µm dimension. The detail of the morphological measurements gamonts and primites-satellites in association form of gregarine are given in Tables 1 and 2. The cyst form was measured as 202.9 µm× 200 µm to 205.9 µm× 202.3 µm. Two of 134 adults of *A. hampei* were infected by the gregarine pathogen, and the total infection rate is 1.5%. The highest rate of gregarine infection was 6.2%, while the lowest infection rate was 3.9% (Table 3).

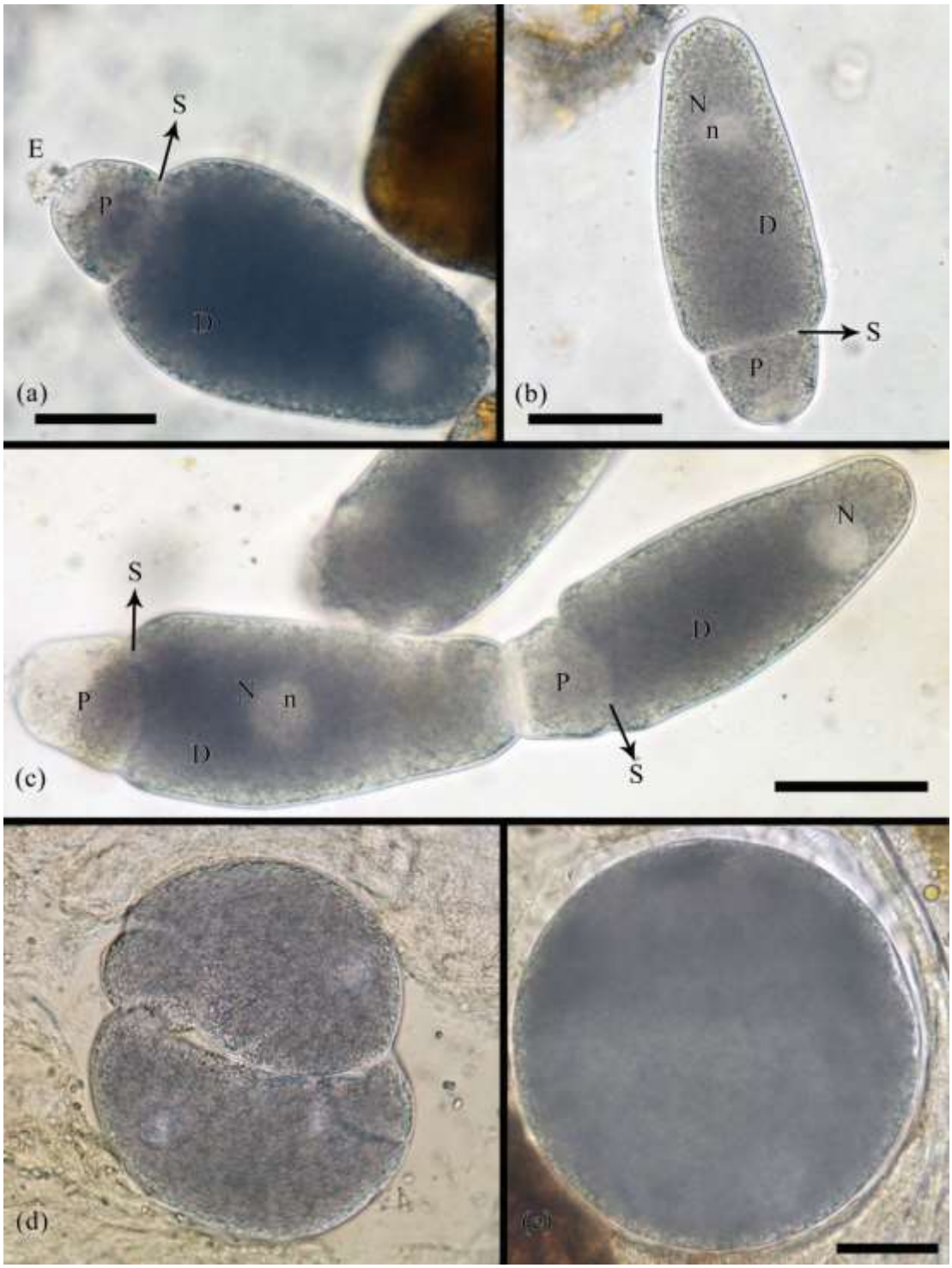

Fig. 1 Trophozoite (a), gamont (b), associative form (c, d) and precyst (e) of the gregarine parasite. E: epimerite, S: septum, D: deutomerite, P: protomerite, N: nucleus, n: karyosome (bar: 50µm).

Table 1 Measurements (µm) of gamonts of gregarine pathogen

G Gamonts (n = 34)	TL	LP	LD	WP	WD	LP:TL	WP:WD
Mean	171.0 ± 40.2	39.5± 14.5	131.4± 27.9	42.5± 8.1	63.9± 18.5	4.5 ± 0.6	1.5± 0.3
Range	(72.1-332.1)	(19.8-113.0)	(52.2-218.9)	(22.1-66.7)	(29.5-120.7)	(2.9-5.6)	(0.6-2.5)

WP:WD—ratio of the width of protomerite to the width of deutomerite; LP:TL—ratio of the length of protomerite to total length; WD—width of deutomerite; WP—width of protomerite; LD—length of deutomerite; LP—length of protomerite; TL—total length.

Table 2 Measurements (μm) of associative forms of gregarine pathogen

Gregarine pathogen	TL	LP	LD	WP	WD	LP:TL	WP:WD
Primite (n = 7)							
Mean	167.1 ±48.3	36.5±10.3	130.5±40.6	42.5±13.3	72.3±24.9	4.5±0.8	1.7±0.2
Range	(40.5-227.5)	(8.8-47.5)	(31.2-180.0)	(8.1-55.0)	(13.2-107.5)	(2.9-6.3)	(1.4-2.0)
Satellite (n = 7)							
Mean	130.7±59.9	29.8±14.5	100.9±47.2	39.1±20.5	42.5±19.9	4.7±1.1	1.2±0.3
Range	(33.1-182.2)	(27.2-137.9)	(27.2-137.9)	(8.9-63.3)	(11.8-60.6)	(3.4-5.8)	(0.6-1.4)

WP:WD—ratio of the width of protomerite to the width of deutomerite; LP:TL—ratio of the length of protomerite to total length; WD—width of deutomerite; WP—width of protomerite; LD—length of deutomerite; LP—length of protomerite; TL—total length.

Table 3 Gregarine pathogenof the *A.hampei*

Sampled dates	Sexuality	Number of examined beetles	Infection found in *A. hampei*population	
			Gregarine	%
29.04.2016	♀	26	1	2.08
	♂	22	0	
20.05.2016	♀	18	0	
	♂	16	1	2.94
28.06.2016	♀	28	0	-
	♂	24	0	
Total		134	2	1.5

♀:female, ♂: male

DISCUSSION

In the present study, a gregarine pathogen was reported from *A. hampei* even the members of the family Chrysomelidae in literature for the first time. In the literature, there are several species of Gregarinidae reports from different Chrysomelid hosts, such as *G. chaetocnemae, G. munieri*, *G. phyllotretae*, *G. crenata*, *G. hoplosomae, G. juengeri*, *G. coronate* and *G. phaedoni* (Bhatia and Setna, 1924; Hoshide, 1953; Lipa and Simchuk, 1979; Sarkar, 1984; Théodoridés et al., 1984; Clopton et al., 1992). Additionally, there are several study about gregarine pathogen reported from Turkey, for example, some undefined species and one identified species as *G. phyllotretae* (Tosun et al., 2008;Yaman, 2002; 2004; Yaman et al., 2008; 2009b; 2011; Yaman and Baki, 2010) The observed gregarin pathogen from *A. hampei* in this study has same morphological features with others gregarine pathogens in literature with one exception for *G. munieri*, which has larger size than others gregarine pathogens (Table 4). Observed gregarine pathogens in this study have same morphological characters and size with gregarine species observed from *Psylloides cupreu*s (Yaman et al., 2008), *Gregarine* specie observed from *Chrysolina fastuosa* (Lipa, 1967), *G. phyllotretae* observed from *P. atra, P. undulata* (Yaman, 2002) and *Gregarine* species from *P. atra* (Tosun et al., 2008) from Turkey (Table 4). Additionally characteristic measurements and ratios, such as TL, LP:TL and WP:WD were similar with gregarine observed from *P. cupreus* (Yaman et al,. 2008), *G. phyllotretae* observed from *P. undulate* and *P. atra* (Yaman, 2002) and gregarine from *P. atra* (Tosun et al., 2008) (Table 4). The gregarine in this study morphological characteristic measurements and ratios were not adequate for the definition of species. For instance, G. phyllotreta, reported from P. atra and P.undulata in Turkey, has greater morphological measurements than Phllotreta vittata which was described by Hoshide (1953) in Japan (Yaman, 2002). Since the spores, diagnostic phase at gregarine systematics, could not been observed in this study, the observed gregarine pathogen was not identified at the species level.

The gregarine observed from Chrysomelidae family was listed by Théodoridés (1984). There was not a gregarine record from *A. hampei* in the list. Additionally, so far, there is no record about presence of gregarine pathogen in *Altica* genus. Total range of gregarine infection in *A. hampei* was not at a high level (1.5%). Infection rate in May was higher (2.94%) than that in April (2.08%). It is important to clarify of pathogen host relationships in which season the pathogen is more intense. In this study, the infections in males were observed higher than those in females. However, a lot of studies are needed to study sex differences of this gregarine pathogen. Gregarines have some potential as biological control agents for several insects (Tanada and Kaya 1993; Lange and Lord 2012; Rueckert and Devetak 2017). If the eugregarine is pathogenic, these forms enter the midgut ephitalia and grow (Tanada and Kaya 1993).

Table 4 The comparative characters of seven gregarine species.

Characters	*G. munieri*	*G. crenata*	*G. phyllotretae*	*G. phyllotretae*	*Gregarine* sp.	*Gregarine* sp.	*Gregarine* sp.
TL (µm)	303.0	220.0	102.0	150.0	462.8 ± 103.7	177.2 ± 44.4	171.0± 40.2
Sporadin	Ovoidal, ellipsoidal	Elongate	Elongate ovoidal to ellipsoidal	Elongate to ellipsoidal	Ellipsoidal to ovoidal	-	-
Protomerite	Globular or oval	Rhomboidal	Hemispherical	Conical to hemispherical	Globular or oval	Ellipsoidal to ovoidal	Globular or conoidal
Gametocyst	Ellipsoidal (303.0 × 239.0 to 442.0 × 311.0)	Ovoidal (220.0 ×188.0 to 232.0 ×183.0)	Ellipsoidal (120.0 × 80.0)	Ellipsoidal (117.0 × 98.0)	Ovodial (314.0 × 266.0 to 427.0 × 372.0)	-	Ovoidal (202.9×200.0 to 205.9×202.3)
LP:TL	1:4.8-1:6.8	1:4.3-1:6.2	1:4.0-1:8.1	1:3.0-1:8.0	1: 3.9-1:11.7	1:3.9-1:7.1	1: 2.9-1:5.6
WP:WD	1:1.1-1:2.0	1:1.1-1:1.8	1:1.0-1:1.5	1: 1.1-1:1.7	1:0.9-1:1.9	1:1.1-1:2.3	1:0.6-1:2.5
Host	*Chrysomela menthastri*	*Chrysomela menthastri*	*Phyllotreta vittata*	*Phyllotreta undulata*, *P. atra*	*Chrysolina fastuosa*	*Psylloides cupreus*	*A.hampei*
Reference	Lipa and Simchuk, 1979	Lipa and Simchuk, 1979	Hoshide, 1953	Yaman, 2002	Yaman et al., 2011	Yaman et al., 2008	In the present study

LP:TL—ratio of the length of protomerite to total length; WP:WD—ratio of the width of protomerite to the width of deutomerite; TL—total length.

The gregarine pathogens were found in only midgut of *A. hampei.* Determination of the gregarine species from insects is a first step and prerequisite for the probable using of these pathogens as biological control agents in the future (Rueckert and Devetak 2017). Observed the pathogen from *A. hampei* was the first report to literature. Therefore, it has been reported as a new gregarine from *A. hampei* from Turkey and world literature. It was also a contribution to the pathogen biodiversity of Turkey.

CONCLUSIONS

According to the results, gregarine pathogen from *A. hampei* has been reported for the first time in Turkey. Gregarine pathogen was detected from Ordu. Based on morphological features, the pathogen revealed different life stages, including ascyst, gamont, precyst, associative form and trophozoite. TL of the ellipsoidal to ovoidal shape gamonts of the gregarine parasite was 171 ± 40.2 µm. The pathogen was observed in mid gut epithelium of the host. Gregarine was also found in both male and female samples and there was no remarkable difference between sexes.

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