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Detection of Neogregarine and Eugregarine (Apicomplexa) Infections from *Chrysolina herbacea* (Duftschmid 1825) (Coleoptera: Chrysomelidae) in Turkey

Türkiye'de *Chrysolina herbacea* (Duftschmid 1825) (Coleoptera: Chrysomelidae)'da Neogregarine ve Eugregarine (Apicomplexa) Enfeksiyonu Tespiti

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Abstract

Chrysolina herbacea (Duftschmid 1825) (Coleoptera: Chrysomelidae) also known as the mint leaf beetle, is a phytophagous, shiny green-gold colored beetle. These beetles are associated with plants belonging to the family Lamiaceae, especially the genus *Mentha*. They cause significant damage to the plants by consuming their leaves. In 2012-2013, a total of 264 individuals (54 larvae and 210 adults) were collected in the vicinity of Trabzon, Turkey and examined for infection with gregarine parasites. Prevalence rates of neogregarine and eugregarine infections were 4.5 ± 1.3 % and 40.2 ± 3.0 %, respectively. A co-infection with parasites of both groups was found in 2.3 ± 0.9 % beetles, but only in 2012. This is the first record of a neogregarine infection in this host. The eugregarine species is most likely *Gregarina munieri* (Schneider 1875), as inferred from morphological and life cycle characteristics.

Keywords: Chrysomelidae, *Chrysolina herbacea,* neogregarine, eugregarine, leaf beetle

Öz

Chrysolina herbacea (Duftschmid 1825) (Coleoptera: Chrysomelidae), parlak altınımsı yeşil renkli fitofag bir böcektir. Bu böcek türü, başta *Mentha* cinsi olmak üzere Lamiaceae familyasına ait bitkiler üzerinde etki yaparlar. Bahsi geçen bitkilerin yapraklarını tüketmeleri sonucu bu bitkiler üzerinde ciddi zararlar meydana getirirler. 2012-2013 yılları boyunca bu çalışmada gregarine enfeksiyonunu belirlemek için toplam 264 (54 larva ve 210 ergin) böcek disekte edilmiştir. Neogregarine ve eugregarine enfeksiyonu yoğunluğu sırası ile % 4,5 ± 1,3 ve % 40,2 ± 3,0 olarak tespit edilmiştir. 2012 yılında, böceklerin % 2,3 ± 0,9'un da bu iki patojen türünün oluşturduğu süper enfeksiyon tespit edilmiştir. Bu çalışma bu böcek türünde neogregarine enfeksiyonunun ilk kaydıdır. Bu böcekte tespit edilen eugregarine türü gerek morfolojik gerekse yaşam döngüsü özelliklerine bakıldığında *Gregarina munieri* (Schneider 1875)'dir.

Anahtar Kelimeler: Chrysomelidae, *Chrysolina herbacea,* neogregarine, eugregarine, yaprak böceği

1. Introduction

Chrysomelidae is one of the largest families of Coleoptera (Gavrilović and Ćurčić 2013). This family includes economically important leaf beetles, which pose a serious problem for agriculture all over the world (Aslan et al. 1999; 2007). Although this family is intensively studied, some species bionomics are still not well known, and this is true for the mint leaf beetle, *Chrysolina herbacea* (Duftschmid 1825). *C. herbacea* (Duftschmid 1825) belongs to the subgenus *Synerga* Weise 1900 within the complex genus *Chrysolina* Motschulski 1860 (Coleoptera: Chrysomelidae: Chrysomelinae). It is a shiny green-gold color beetle, associated with plants of the family Lamiaceae, especially the genus *Mentha*, which are used as spices and for medicinal purposes. *Mentha* plants are cultivated in mass productions all over the world (Verma 2006). The beetle causes significant damage to the plants by consuming their leaves (Bozsik 2006). Extensive use of synthetic insecticides from organophosphate compounds serves as routine control measure against this pest (PMSP 2002). This control strategy is however associated with undesired effects, including intoxication of environment, pest resistance development and affection of

non-target organisms (Vega and Kaya 2012). So there is growing interest to use environmental-friendly control screening for pathogens and parasites infecting the pest populations is of great concern. In the case of *C. herbacea,* there is only one such study by Lipa and Simchuk (1979). Similarly, in 2011, a cephaline gregarine and a mermithid were found for the first time in *Chrysolina fastuosa* (Scopoli 1763) by Yaman et al. (2011). In the present study we determine an infection with one neogregarine and one cephaline (septate) gregarine in *C. herbacea*.

2. Materials and Methods

In this study, 210 adults and 54 larvae of *C. herbacea* were collected in the vicinity of Trabzon, Turkey, between May and October 2012-2013. The adults and larvae were transported to the laboratory in plastic containers and dissected as soon as possible. The beetles were dissected in Ringer's solution and wet smear of insect tissues were examined under a light microscope at magnifications of 40× to 1000× for detection of protozoan infections (Yaman et al. 2012). The slides with neogregarine oocysts were air-dried and fixed with methanol for 3 min, rinsed with distilled water and stained for 10 hours in 5 % Giemsa stain solution (Carlo Erba, Code No. E45361301). Then the slides were rinsed, air-measures, including natural enemies of pest organisms (Tomalak 2003; Sezen et al. 2004). Consequently, the dried and re-examined under the light microscope (Undeen and Vávra 1997). Leica DM1000 microscope combined with Leica ICC50 digital camera and LAS EZ 1.0 Soft Imaging System was used for measurements and digital processing of images. In addition, some beetles were maintained in Petri dishes (9 cm in diam.) with wet filter paper for about 48 h to allow the collection of the feces. The obtained feces of the beetles were examined for the spore stages of gregarine according to Clopton et al. (1992). For gregarine pathogens, measurements (µm) of structures were performed in the following order: length of deutomerite (LD), length of protomerite (LP), total length (TL), width of deutomerite (WD), width of protomerite (WP), 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) (Yaman et al. 2011).

3. Results

During this study a total of 264 individuals, including 54 larvae and 210 adults, were dissected. While 12 examined beetles were infected with a neogregarine, 106 individuals were infected with a gregarine pathogen, corresponding to the overall prevalence rates of 4.5 ± 1.3 % and 40.2 ± 3.0 %, respectively (Table 1).

Table 1. Prevalence rates of infections with two pathogens in *Chrysolina herbacea* (Duftschmid 1825)

Total number of examined beetles			Number of infected insects (n) and prevalence rate (%±SE) of infection with					
			Neogregarine Infection		Gregarine Infection		Co-Infection (Neogregarine+Gregarine)	
			n	%	n	%	n	%
2012	Adult	96	9	9.4 ± 3.0	46	47.9 ± 5.1	6	6.3 ± 2.5
	Larvae	21	0	0	9	42.9 ± 10.8	0	0
2013	Adult	114	3	2.6 ± 1.5	48	42.1 ± 4.6	0	0
	Larvae	33	0	0	3	9.1 ± 5.0	0	0
	Total	**264**	**12**	**4.5 ± 1.3**	**106**	**40.2 ± 30**	**6**	**2.3 ± 0.9**

Although the infection with neogregarines was localized predominately in Malpighian tubules, it was also observed in midgut epithelium and hemocoel (Fig. 1). Mature oocysts of the neogregarine were lemon-shaped, with polar plugs and easily visible residual bodies (Fig. 2a-c). During non-synchronous development of oocysts, some irregular shaped stages and immature oocysts were observed possessing an ovoid shape and a large nucleus (Fig. 2b). Unfixed mature oocysts measured 9.34 ± 0.93 (6.67 - 12.56; number of examined cells n = 100) µm in length and 5.43 ± 0.73 (4.06 - 8.11; n = 100) µm in width. After fixation and staining, the oocysts measured 5.76 ± 0.39 (4.97 -6.38; n = 30) µm in length and 3.55 ± 0.29 (3.08 – 4.28; n = 30) µm in width (Fig. 2d). The infection was found only in adult beetles and its prevalence rate was higher in 2012 (9.4 ± 3.0 %) than in 2013 (2.6 ± 1.5 %), the difference being statistically significant at $p<0.05$.

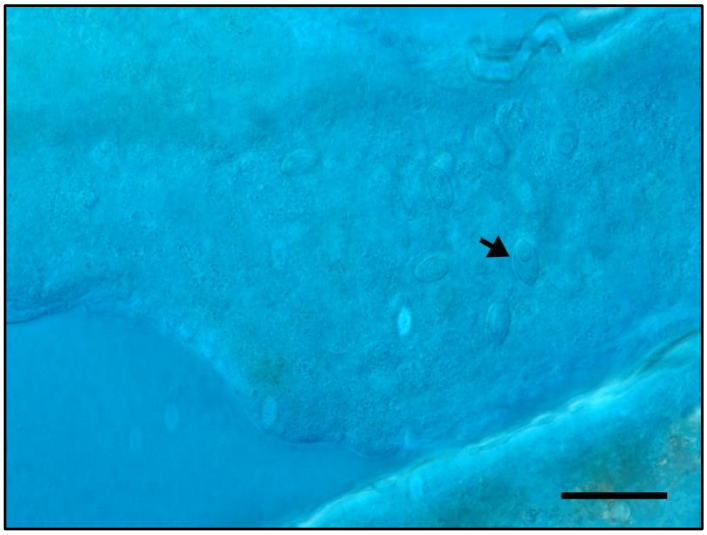

Figure 1. Malpighian tubules filled with neogregarine oocysts, bar = 25 µm.

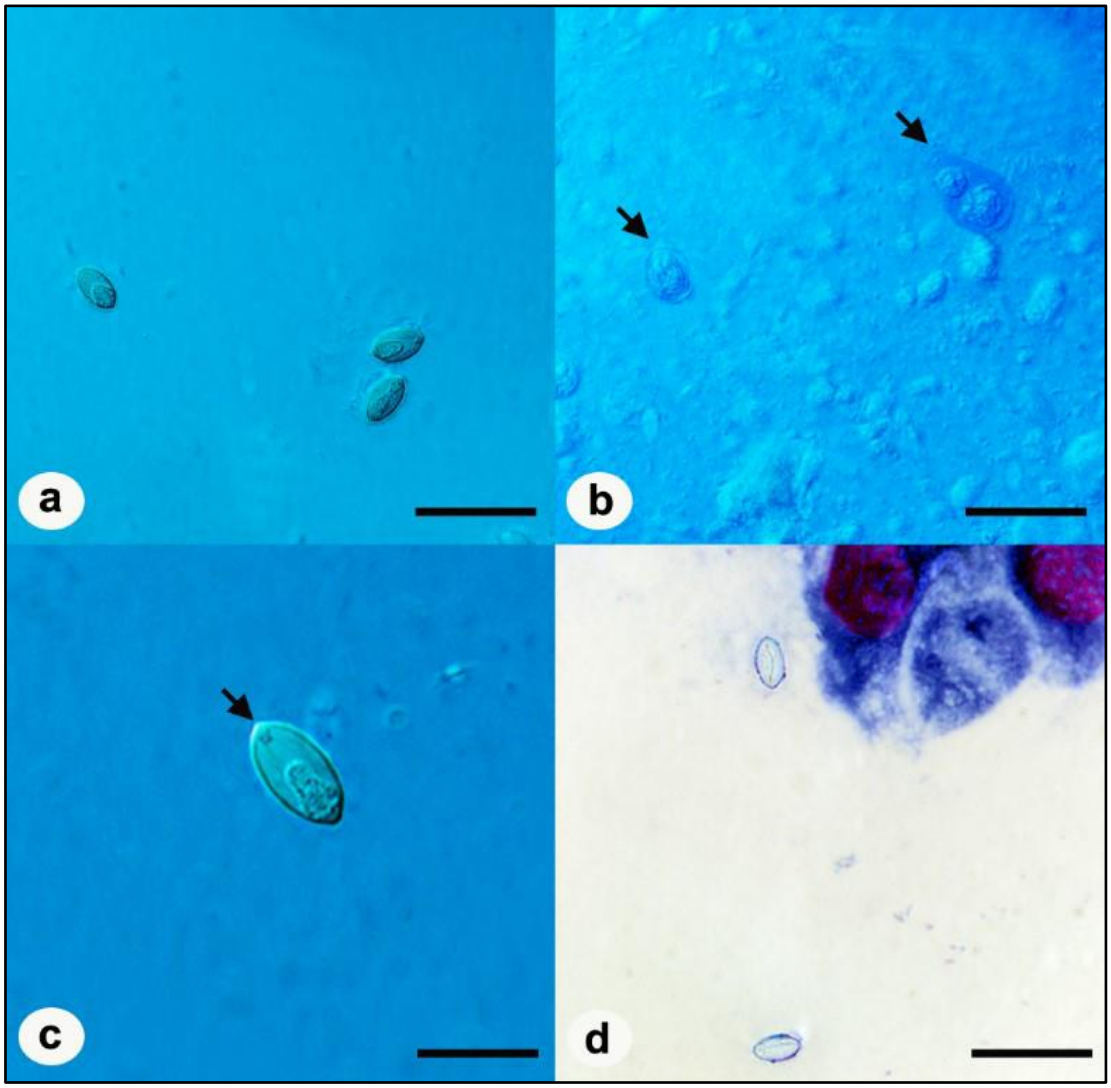

Figure 2. Light micrographs of different neogregarine stages infecting *C. herbacea*. a – c: Mature oocysts of determined neogregarine pathogen, note that residual bodies and polar plugs are easily seen and marked by arrow, bar = 30 – 10 µm. b: Non-synchronous development of oocysts in irregular shaped stage and immature oocysts were seen which were ovoid shape and with a large nucleus, were marked arrow, bar = 20 µm. d: Stained mature oocysts with Giemsa, bar = 15 µm.

Gregarine infections were determined in the midgut of the host. The observed gregarine was composed of one protomerite and one deutomerite, separated by septum. During examination, the following life cycle stages were detected: trophozoite, gamont, associative form (syzygy), associative form prior to cyst, cyst and spore stages (Fig. 3).

The trophozoites attracted attention with quite large epimerite (Fig. 3a). The gamonts were ellipsoidal to ovoidal in shape (Fig. 3b). Gamonts in associations possessed ovoidal or elongate shape. The nuclei were well seen in both gamonts and associative forms (Fig. 3b - d). Additionally, while cysts of the observed gregarine possessed round shape, spores were in barrel shape (Fig. 3e - f).

Based on the observed life cycle stages and their morphological characteristics, we were able to identify the eugregarine as a cephaline (septate) gregarine. The morphological measurements are summarized in Table 2. The infection was found both in adults and larvae and its prevalence rates usually exceeded 40 % while in larvae sampled in 2013 it reached but 9.1 ± 5.0 %, being significantly lower than in other samples ($p<0.01$).

In 2012, it is determined that six adult *C. herbacea* beetles were co-infected by neogregarine and gregarine pathogens, resulting in overall prevalence rate of 2.3 ± 0.9 % (Table 2). It was noted that co-infected beetles moved slower as compared to those non-infected or bearing a single infection.

Table 2. Measurements of different life cycle stages (trophozoite, gamont, syzygy, cyst and spore stage) of the gregarine pathogen (in µm) (minimal and maximal, TL; total length, LE; length of epimerite, LP; length of protomerite, LD; length of deutomerite, WE; width of epimerite, 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).

C. herbacea	TL	LP	LD	WP	WD	LP:TL	WP:WD
Gamont (n=40)	463.88 ± 113.96 320.04-787.84	87.89 ± 22.82 62.85-149.78	375.64 ± 94.15 243.42-639.43	113.35 ± 25.72 80.14-176.61	193.72 ± 58.24 132.72-362.53	1:5.34 ± 0.82 1:3.80-7.31	1:1.71 ± 0.36 1:1.16-3.13
Syzygy							
Pirimite (n=14)	342.35 ± 81.22 253.85-508.69	64.14 ± 14.36 33.56-88.59	278.21 ± 71.06 204.53-432.73	101.89 ± 26.12 67.14-151.99	165.69 ± 57.63 96.96-291.34	1:5.44 ± 1.02 1:4.06-7.88	1:1.62 ± 0.32 1:1.06-2.22
Satallite (n=14)	329.13 ± 90.38 196.49-524.44	47.99 ± 15.00 25.04-83.92	281.13 ± 77.24 171.45-440.52	107.05 ± 28.67 58.84-153.44	167.71 ± 63.83 95.7-326.46	1:7.01 ± 1.02 1:5.69-9.37	1:1.54 ± 0.23 1:1.28-2.12
	TL	**LE**	**LP**	**LD**	**WE**	**WP**	**WD**
Trophozoite (n=3)	115.72 ± 53.47 74.55-176.16	10.98 ± 5.17 5.46-11.77	28.92 ± 18.77 12.84-49.56	74.09 ± 31.73 46.4-108.72	11.95 ± 5.14 6.81-17.11	39.29 ± 18.61 20.63-57.85	59.63 ± 36.28 25.86-97.99
Cyst (n=5)	322.11 ± 27.19 (298.78-367.13) × 306.04 ± 6.96 (299.96-311.64)			**Spore** (n=70)	8.31 ± 0.34 (7.35-9.46) × 4.66 ± 0.35 (3.93-5.79)		

4. Discussion

The present study is the first attempt to determine pathogens of *Chrysolina herbacea* after the report of Lipa and Simchuck (1979). Those authors detected for the first time *Gregarina munieri* (Schneider 1875) and *Gregarina crenata* (Bhatia and Setna 1924) infections in *C. herbacea.* Similarly, one cephaline (septate) gregarine was found in the present study. This situation was expected because cephaline gregarine infections are common in Chrysomelidae (Geus 1969; Clopton et al. 1992; Thomas et al. 1999; Yaman 2002; Tosun et al. 2008; Yaman et al. 2008; 2009; 2011). Until now, several cephaline gregarine species were reported from chrysomelid beetles: *Gregarina crenata* (Bhatia and Setna 1924), *Gregarina phyllotretae* (Hoshide 1953; Yaman 2002), *Gregarina ampullaria* (Hoshide and Hoshide 1969), *G. munieri* (Geus 1969; Lipa and Simchuk 1979), *Gregarina chaetocnemae* (Sarkar 1984), *Gregarina phaedoni, Gregarina hoplosomae, Gregarina juengeri* (Théodoridés et al. 1984) and *Gregarina coronata* (Clopton et al. 1992). As a result of the morphological examinations of the collected gregarine samples during present study, it was concluded that the samples identified as *G. munieri* (Table 3).

For the first time, we were able to describe a neogregarine infection in *C. herbacea*. Neogregarines (order Neogregarinorida) are important pathogens of different insect groups with significant pathogenic effects on their hosts (Valigurova and Koudela 2006). Neogregarines are differentiated from Eugregarinida due to the presence of the merogonial stage and higher virulence (Lacey 2012; Vega and Kaya 2012). The examined neogregarines prefer the Malpighian tubules as the site of infection, which might be a possible cause for tissue malfunction and disorders at organism level. Effect of co-infection on the beetle mobility indicates interactions between the pathogens of synergistic nature.

Table 3. Characteristics of *Gregarina* species described from *Chrysolina herbacea* (Duftschmid 1825) (Coleoptera: Chrysomelidae)

	Gregarina munieri	*Gregarina crenata*	*Gregarina sp.*
Total length	303 µm	220 µm	463.88 ± 113.96 µm
Protomerite	Globular or oval	Rhomboidal	Globular or oval
Deutomerite	Ovoid or ellipsoidal	Elongate	Ellipsoidal
Cyst	Ellipsoidal 303-442 × 239-311	Ovoidal 220-232 × 183-188	Round 298.78-367.13 × 299.96-311.64
LP:TL	1: 4.8-6.8	1: 4-8.1	1:3.80-7.31
WP:WD	1: 1.1-2.0	1: 1-1.5	1: 1.16-3.13
Reference	Lipa and Simchuk 1979	Lipa and Simchuk 1979	In the present study

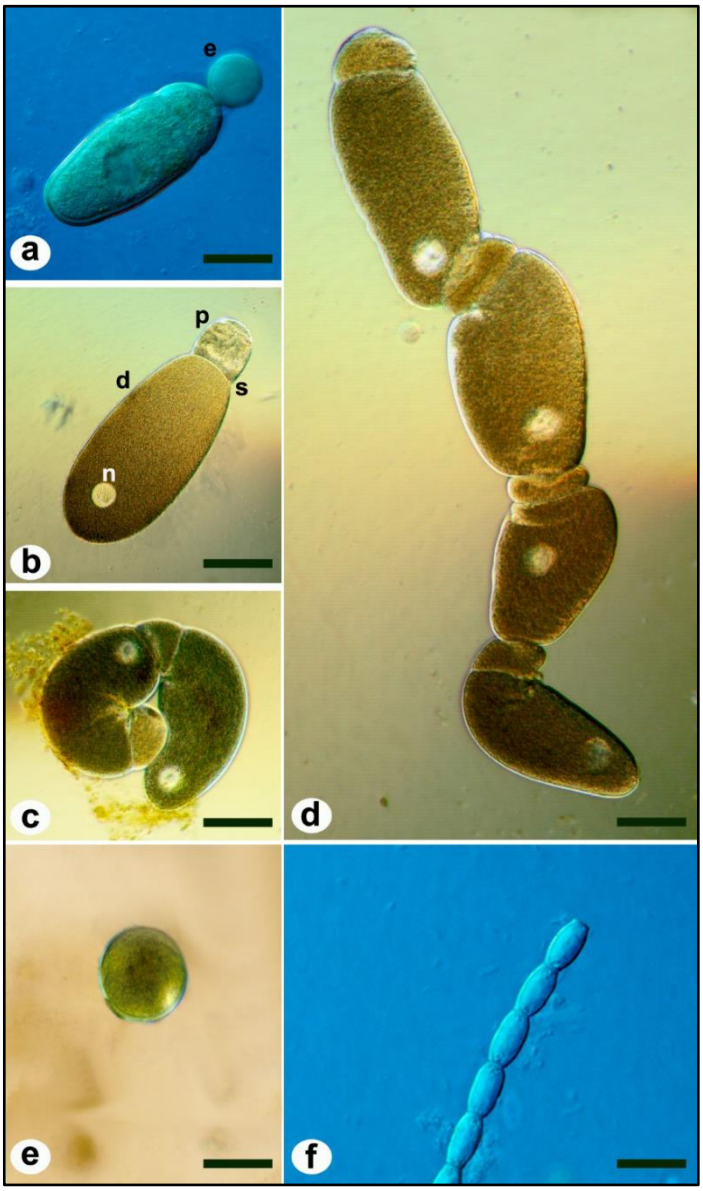

Figure 3. Different life cycle stages of cephaline gregarine determined in *C. herbacea*. a: trophozoite; epimerite (e), bar = 50 µm. b: gamont; protomerite (p), deutomerite (d), septum (s) and nucleus (n), bar = 140 µm. c: associative form prior to cyst, bar = 140 µm. d: associative form (syzygy), bar = 140 µm. e: cyst, bar = 300 µm. f: part of spore chain just after gametocyst dehiscence, bar = 15 µm.

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