

***Clitellocephalus ophoni* (Tuzet et Ormières, 1956)
(Apicomplexa: Eugregarinida: Gregarinidae) recorded
in Poland on strawberry seed beetle *Harpalus (Ophonus) rufipes*
(De Geer, 1774) (Coleoptera: Carabidae)***

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(Received on 16 April 2009; Accepted on 22 December 2009)

Abstract: The eugregarine *Clitellocephalus ophoni* (TUZET et ORMIÈRES, 1956), originally recorded and described on the strawberry seed beetle *Harpalus (Ophonus) rufipes* (De Geer, 1774) collected in France, is now reported from the same host insect in Poland. It is a new protozoan species in Polish fauna. Out of 280 adult beetles collected in Poland, 21 were parasitized by gregarines, but only 3 by *C. ophoni*. A morphometric and morphological analysis of *C. ophoni* trophozoites and gamonts found in the digestive tract of host specimens in France and Poland indicated a significant similarity. The prevalence of *C. ophoni* is low (about 1%), but the community of gregarines with prevalence of 8% could be an important factor of environmental resistance.

Keywords: *Clitellocephalus ophoni*, Apicomplexa, Eugregarinida, strawberry seed beetle, *Harpalus rufipes*, Coleoptera, Carabidae, Poland, biocontrol agent, prevalence

INTRODUCTION

Gregarines, which are parasites of invertebrates, are relatively poorly known protozoans. Only 0.31% of beetle species in the world have been checked for their occurrence (Clifton 2002). Thus further research on the occurrence of gregarines in Arthropoda will increase the knowledge of biodiversity. Little is known about which gregarine species occur in invertebrates in Poland and if they can regulate the size of populations of their host insects (biocontrol agent), including pests (Lipa 1967a, Kazubski 2003).

Clitellocephalus ophoni (Tuzet et Ormières, 1956), for the first time recorded in Poland, is known as a parasite of *Harpalus rufipes* (De Geer, 1774). The para-

* Research supported by grant no 2PO6R06630 of the Polish Ministry of Science and Higher Education

site's only locality known so far has been Sète in France (CLOPTON & NOLTE 2002, GEUS 1969, TUZET & ORMIÈRES 1956). The species was first classified in the genus *Gregarina*. After a review conducted by CLOPTON and NOLTE (2002), the species was then assigned to the genus *Clitellocephalus*. In spite of the local occurrence of this parasite, its host species is of economic importance even on the European scale, as it infests seed crops and strawberries (KRYZHANOWSKII 1983, BRIGGS 1965, DAVID'YAN & KABAK 2009). However, some authors highlight the importance of *Harpalus rufipes* as a vital natural factor in fighting weeds in crop fields (ZHANG et al. 1997; MARTINKOVÁ et al.2006).

MATERIAL AND METHODS

Adults of *Harpalus rufipes* were regularly collected during summer in 2006 and 2007, using pitfall traps (diameter 21 cm, height 17 cm) or collected manually from rye ears at 2 sites in mid-western Poland: (1) near Poznań city on the NATURA 2000 site "Biedrusko" PLH(SCI)300001 (UTM: XU31); and (2) on experimental fields of the Institute of Plant Protection near Winna Góra, Środa Wielkopolska county (UTM: XT68).

After bringing to the laboratory, adult beetles were dissected in physiological salt solution, and their digestive tract and other tissues were checked for the presence of gregarines and other protozoan infections. In total, 280 adults of *H. rufipes* were examined for gregarine presence, out of which 21 adults were found to be parasitized by various numbers of eugregarine developmental stages (Table 1). Only in 3 hosts some trophozoites and gamonts of *Clitellocephalus ophoni* were found.



Fig. 1. *Clitellocephalus ophoni* (TUZET ET ORMIÈRES, 1956) found in the intestine of *Harpalus rufipes* (De Geer, 1774)

Table 1. Records of adult *Harpalus rufipes* (De Geer, 1774) collected and parasitized by *Clitellocephalus ophoni* (TUZET ET ORMIÈRES, 1956) and other gregarines in 2006–2007 on sites near Winna Góra (XT68) and in the NATURA 2000 Site “Biedrusko” (XU31)

Site (UTM code)	Date of collection (month/year)	Number of <i>H. rufipes</i> adults examined			Number of <i>C. ophoni</i> specimens	Observed developmental stages of <i>C. ophoni</i>
		total	parasitized by			
			<i>C. ophoni</i>	other gregarines		
XT31	05/2006	1	0	0	-	-
XT31	07/2006	2	0	1	-	-
XT31	08/2006	1	0	0	-	-
XT31	09/2006	2	0	1	-	-
XT31	06/2007	5	0	0	-	-
XT31	06/2007	4	0	1	-	-
XT31	08/2007	3	0	0	-	-
XT31	09/2007	1	1	0	418	associations, trophozoites
XT68	05/2006	7	0	0	-	-
XT68	06/2006	23	1	1	40	trophozoites
XT68	07/2006	108	1	8	300	associations, trophozoites, cephalonts
XT68	08/2006	21	0	2	-	-
XT68	09/2006	11	0	0	-	-
XT68	04/2007	1	0	0	-	-
XT68	06/2007	4	0	0	-	-
XT68	07/2007	7	0	2	-	-
XT68	08/2007	43	0	0	-	-
XT68	09/2007	34	0	1	-	-
XT68	10/2007	2	0	1	-	-
Total:		280	3 (1.07%)	18 (6.43%)	758	

For morphometric analysis, the sizes of 16 gamonts in syzygies (i.e. associations of 2 gregarines for the purpose of asexual exchange of genetic material) and of 20 solitary trophozoites and gamonts were measured. Additionally, the length of protomerites (LP) and deutomerites (LD) was measured as well as the width of protomerites (WP) and deutomerites (WD).

Table 2. Comparison of sizes of gamonts and trophozoites of *Clitellocephalus ophoni* (TUZET et ORMIÈRES, 1956) recorded in adult *Harpalus rufipes* (De Geer, 1774) in Poland and in France

Measurements	Minimum-maximum (mean±standard deviation)				
	Poland			France (CLOPTON 2002)	
	trophozoites	associations		association	
		primites	satellites	primites	satellites
Protomerite length (LP)	18.70-61.00 (41.53±11.90)	25.50-70.00 (48.30±12.22)	17.40-41.00 (31.65±7.34)	14.0-36.2 (23.6±6.5)	9.3-26.9 (18.8±5.2)
Protomerite width (WP)	14.80-35.00 (23.71±6.17)	17.10-35.00 (27.76±6.71)	14.00-35.00 (23.80±6.08)	18.8-47.5 (28.6±7.1)	19.9-52.4 (30.5±7.4)
Protomerite width to length ratio (WP/LP)	1.00-2.29 (1.76±0.31)	1.49-2.15 (1.75±0.25)	1.00-1.88 (1.36±0.30)	0.6-1.2 (0.8±0.2)	0.3-1.2 (0.6±0.2)
Deutomerite length (LD)	99.60-296.00 (191.10±51.25)	104.50-296.00 (197.36±65.79)	101.60-226.00 (163.99±39.29)	117.2-329.2 (224.1±68.9)	123.2-322.6 (215.7±63.2)
Deutomerite width (WD)	14.60-35.00 (22.79±5.69)	17.40-139.00 (27.70±6.59)	15.10-35.00 (24.86±5.49)	24.5-51.5 (34.4±6.8)	28.1-70.6 (38.8±9.0)
Deutomerite width to length ratio (WD/LD)	5.04-12.82 (8.54±1.91)	6.01-8.46 (7.00±0.91)	5.69-8.04 (6.60±0.68)	4.0-10.1 (6.6±1.8)	3.6-8.4 (5.7±1.6)
Body length (TL)	118.30-357.00 (232.63±61.08)	130.00-348.00 (245.66±75.75)	119.00-261.00 (195.64±43.88)	118.7-391.0 (273.2±86.1)	142.8-355.9 (243.8±69.0)
Protomerite to body length ratio (LP/TL)	4.15-7.69 (5.73±0.92)	4.21-6.69 (5.08±0.81)	4.1-7.46 (6.28±0.97)	8.0-15.3 (11.5±1.7)	7.9-19.3 (13.3±2.9)
Protomerite to deutomerite width ratio (WP/WD)	0.65-1.53 (1.05±0.20)	0.93-1.11 (1.00±0.05)	0.85-1.00 (0.95±0.06)	1.0-1.4 (1.2±0.1)	1.1-1.7 (1.3±0.1)
Total associacion lenght (TLA)		249.00-609.00 (441.30±118.29)		289.9-732.0 (511.9±145.3)	

Fig. 2A

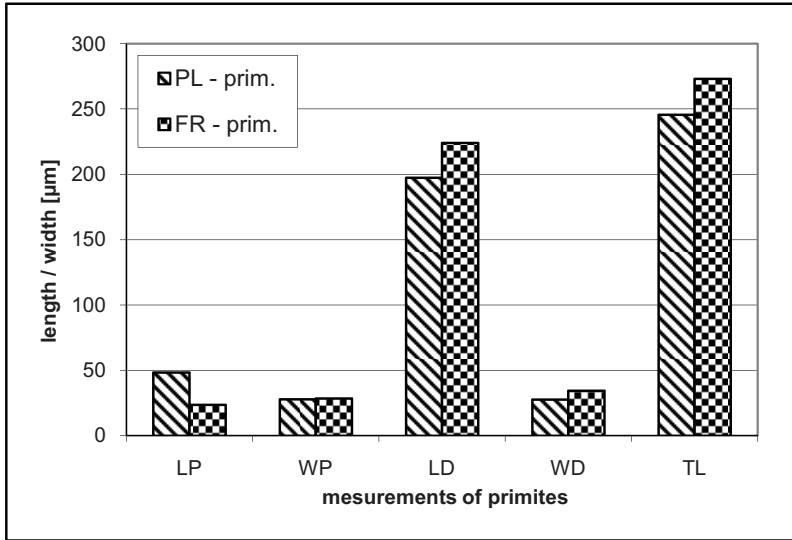


Fig. 2B

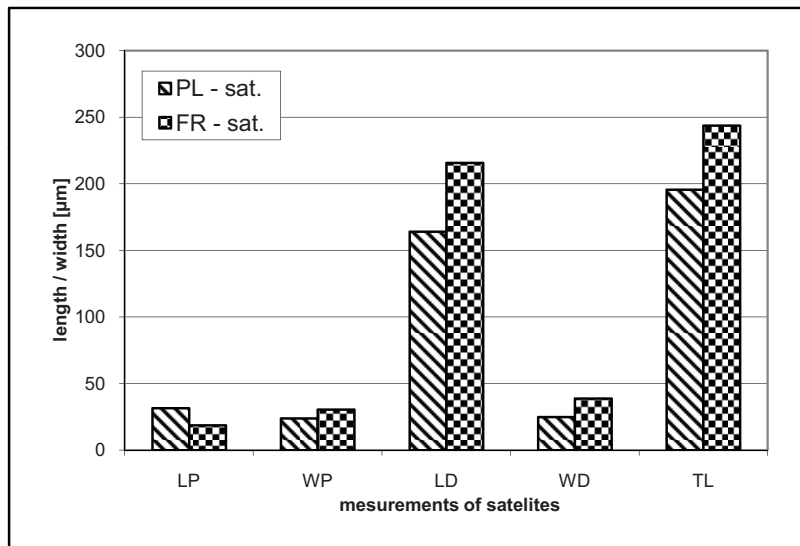


Fig. 2. Comparison of body length and width of primites (A) and satellites (B) of *Clitellocephalus ophoni* (TUZET et ORMIÈRES, 1956) from Poland (PL) and France (FR)

Fig. 3A

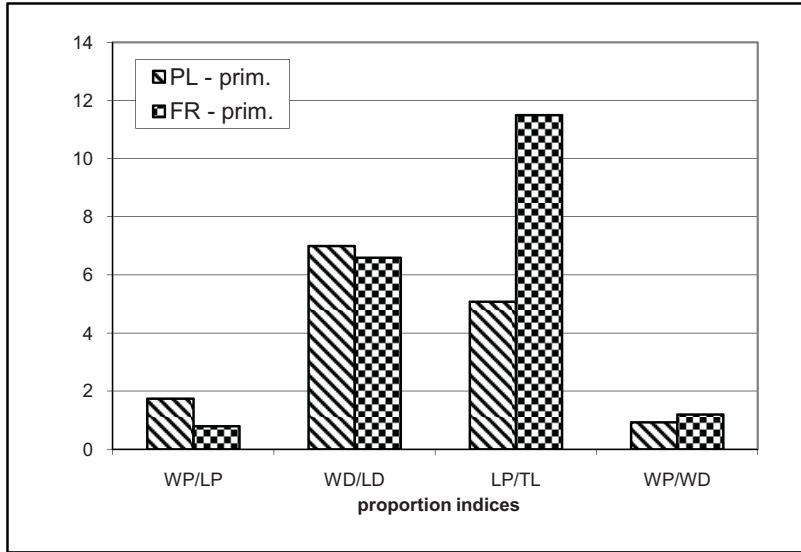


Fig. 3B

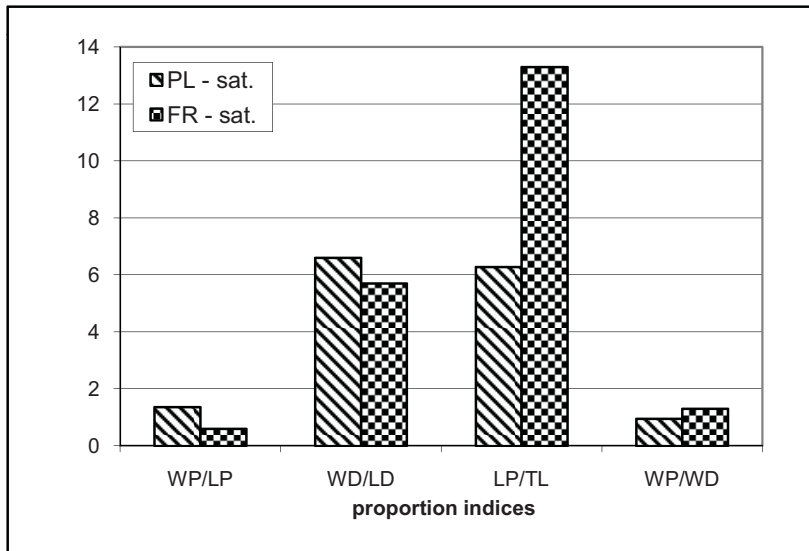


Fig. 3. Comparison of body proportion indices of primites (A) and satellites (B) of *Clitellocephalus ophoni* (TUZET et ORMIÈRES, 1956) from Poland (PL) and France (FR)

RESULTS AND DISCUSSION

This is the first record of *Clitellocephalus ophoni* in *Harpalus rufipes* from Poland. This species was not previously listed by LIPA (1967a, b; 1975). The sizes of primites and satellites from Poland are on average smaller than those described from France by TUZET & ORMIÈRES (1956). The maximal length of gamonts in Poland was 357 µm, compared to 391 µm in France (Table 2, Fig. 2). Also their proportions showed some differences between the populations from the 2 countries (Table 2, Fig. 3). The individuals from Poland are on average shorter and broader than those from France. Even so, a morphometric analysis of *C. ophoni* gamonts extracted from host specimens from France and Poland indicated significant similarity (Fig. 1).

As mentioned above, the host species of *C. ophoni* are among economically important pests of strawberries and seed crops (e.g. cereals). Thereby the role of gregarines as a biological agent is interesting. The prevalence of gregarine community in the examined material of 208 individuals of *H. rufipes* is 8 %, out of which the prevalence *C. ophoni* is only about 1% (Table 1). It means that gregarines might be an important biological agent among environmental resistance factors.

In terms of biodiversity of Polish protozoans, the modest list of 144 known gregarine species, has been expanded to include *Clitellocephalus ophoni* (KAZUBSKI 2003, SIENKIEWICZ & LIPA 2008).

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