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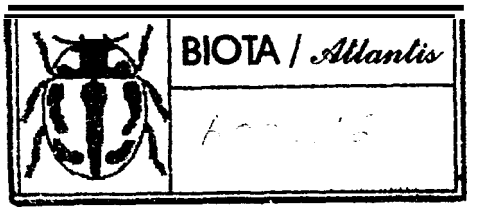

Gregarines (Eugregarinorida: Apicomplexa) in Natural Populations of *Dociostaurus maroccanus, Calliptamus italicus* and other Orthoptera

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Summary. *Gregarina acridiorum* Léger is redescribed based on material from *Calliptamus italicus* and *Dociostaurus maroccanus* collected in Spain and in *Locusta migratoria* in Uzbekistan. This gregarine has a wide range of hosts and is broadly distributed in Palearctic region. *Gregarina garnhami* Canning is considered to be a junior synonym of *G. acridiorum.* Intensity of parasitization in some host insects was very high which indicates a detrimental effect on gut functions. Gregarine infections have been also recorded in the natural populations of the acridids *Ailopus* sp., *Anacridium aegyptium,* and *Oedaleus decorus* and of the tettigoniid *Decticus albifrons* collected on Iberian Peninsula and Canary Islands.

Key words: *Ailopus* sp., *Anacridium aegyptium, Calliptamus italicus, Decticus albifrons, Dociostaurus maroccanus. Gregarina,* gregarines. insect parasites, *Locusta migratoria, Oedaleus decorus,* Orthoptera.

Abbreviations: LD - length of deutomerite, LP - length of protomentc, TL - total length of gamont or sporont, WD - width of deiitornerite. WP - width of protornerite.

INTRODUCTION

During the collaborative research programme on biological control of locusts by means of entomopathogens a survey for parasites and pathogens in populations of locusts and grasshoppers species is being conducted in Spain since 1989 (Hemandez-Crespo 1993). Special attention in this research is given to the Moroccan locust, *Dociostaurus maroccanus* (Thnb.) whose outbreaks have been numerous in the Iberian Peninsula until beginning of this century (Cañizo 1939, Vazqiiez Lesmes and Santiago-Alvarez 1994). Resulrs of studies on entomopathogenic fungi (Santiago-Alvarez 1991), mermithid parasites (Hernandez-Crespo and Santiago-Alvarez 1991) and entomoposvirus infection (Lipa et al. 1994) from various orthopterans have been recently published and they present an interest for microbial control of these pests (Bidochka and Khachatourians 1991).

During 1992 survey a gregarine infection was recorded among adults of *D. maroccanus.* Surprisingly. this locust was not among locusts and grasshopers listed by Uvarov (1928), Canning (1956) and Geus (1969) as hosts for gregarines (Gregarinomorpha). However, an infection caused by *Gregarina* sp. in *D. maroccanus* was reponed by Nurzhanov (1989), but without tasonomic identification of the species. When it become obvious to LIS that we recorded a new gregarine infection in *D. maroccanus* we

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undertook a specific research on this pathogen. In this paper we report on its morphological features and its occurrence among field host populations of *D. maroccanus* and *Calliptanus italicus* (L.) in Spain and also in a laboratory culture of *Locusta migratoria* (L.) from Uzbekistan. We also include here observations on gregarines recorded in five other orthopterans found during our studies.

MATERIALS AND METHODS

Specimens of different developmental instars of *D. maroccanus* and *C. italicus* were coliected regularly (7-15 day intervals) by means of a sweeping net in the permanent breeding area in La Serena region (Badajoz province, Spain) from March 24 to July 8, 1992. Specimens of these two species as well as of other Orthoptera were also coliected in the Valencia city, in Santa Marta (Albacete) and on islands El Hierro and Tenerife (Canary Isiands Archipelago).

Specimens of *L. migratoria* were taken from the laboratory culture kept at the All-Russian Institute of Plant Protection at Sankt Petersburg but the stock of that locust originated from Uzbekistan.

Using the stereoscopic microscope nymphs and adult insects were dissected, in a Petri dish filled with paraffin and physiological solution (0.6-0.8%NaCl) or with water, by cut open along the dorsal side of the body in order to have the access to visual examination of their **fat** body, intestine and other tissues. Recorded gregarines were transferred with the gut contents and a small volume of physiological solution on a microscopic slide and then measured and photographed. Permanent slides of gamonts and sporonts fixed in methyl alcohol for 2 min were stained with 0.25% Giemsa water solution.

RESULTS

Morphology of gregarines in *Dociostaurus maroccanus* and *Calliptamus italicus*

Ganionts

The gamonts (trophozoites) are solitary with the pale appearance as compared with sporonts (Figs. 1, 2). The knoblike epimerite is transparent. and being from 7 to 9 µm long and 9 to 10.6 µm wide. After the epimerite is detached the scar on the top of protoniente reinains well seen for some time but later becomes healed. The protomerite of gamont is oval or slightly conical and separated from the ovoidal deutomerite by very distinct constriction. Deutomerite is ovoidal with well seen ectocyte. Its endocyte is much less granular than of sporonts and thanks to that the gamonts easily change the shape of their bodies while moving (Fig. 2).

Sporonts

The sporonts are biassociative as only two sporonts made syzygies; associations of three sporonts or more were never observed. Small and large sporonts enter into the syzygial phase biit their future size and shape greatly depends on the fact whether they are primites or satellites. In general, primites are shorter and ovoidal while satellites are longer and cylindrical or ellipsoidal (Figs. 3. 4). The largest observed syzygies were up 1006 µm long (Table 1).

Primites. Protomerite semicircular, wider than long with well seen ectocyte about 5-7 µm thick. The endocyte of protomerite is coarsely granular and not translucent. Deutomerites of the majority of primites are ovoidal and relatively wide. with the maximum width 467 µm. Their ectocyte is thinner than in protomerite. The endocyte is coarsely granular and less translucent than of protonierite. The end of primite's deutomerite is oval or slightly flattened.

Due to densely granular endocyte the nucleus is weakly seen or not seen at all. Only using the highly diluted solution of TAF, which clears the endocytic granules, it is possible to denionstrate the location of niiclei. The nuclei of the primite have the diameter from 46 to 56 µm and contains several small size karyosomes (Fig. 8).

Satellites. Protomerites of the satellites are much shorter and wider than protomerites of primite and have smaller ratios WP:LP (Table 1). In most cases the ectocyte of the satellite's protornerite is very thick (up to 11.4µm) and forms a peculiar circular ridge (Fig. 5).

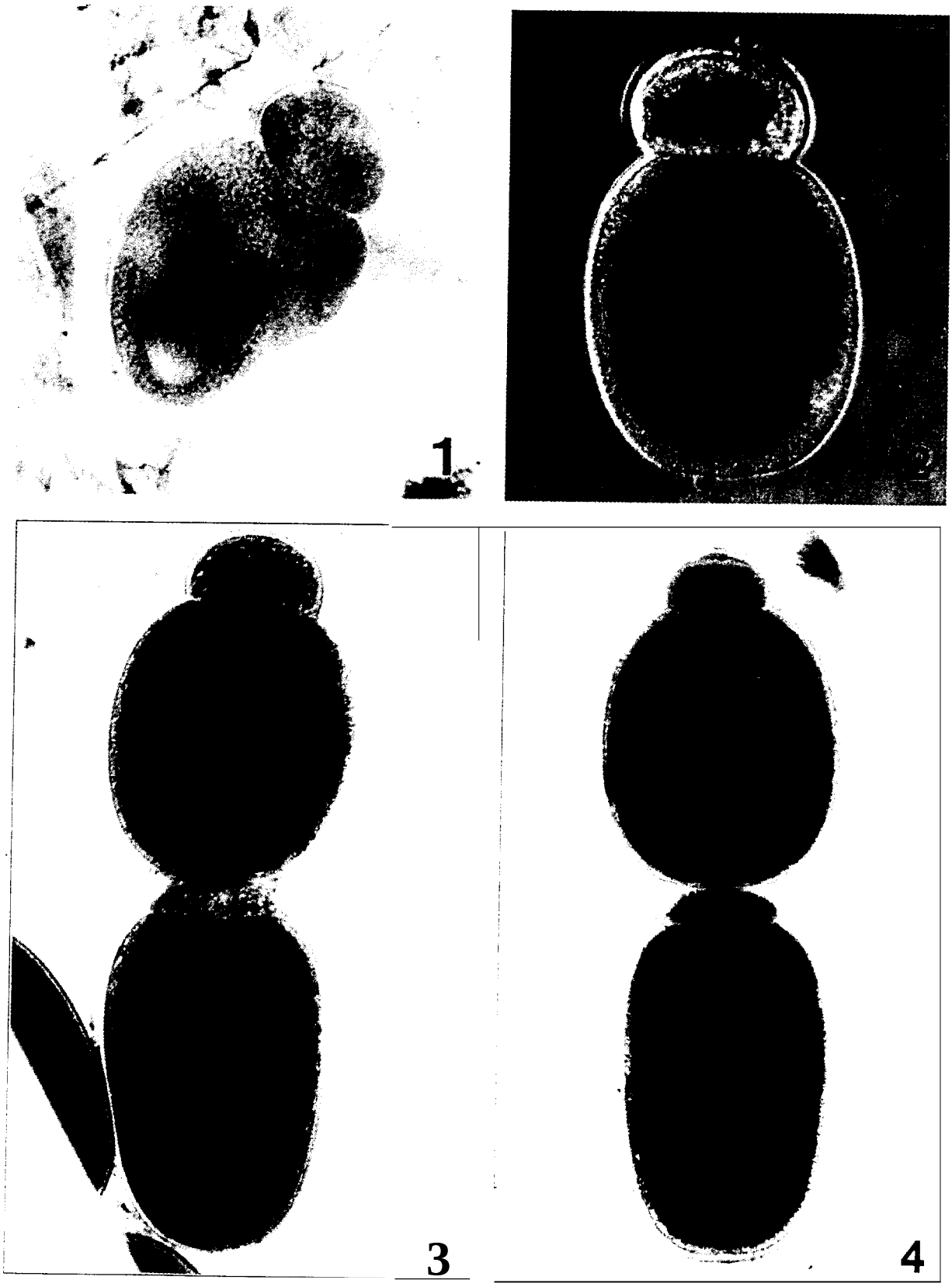
The apical part of the protonierite, surrounded by this circular ridge, is elevated up to the ridge edges or slightly less. This protomerite structure allows the satellite to attach firmly to the posterior end of the primite deutomerite at the caudo-frontal association. Attachment is so firni that syzygies are not broken when gut is dissected and the gregarines are released into the dissecting pan (Fig. 6). The deutomerite of the satellite is cylindrical or ellipsoidal and narrows toward the posterior end which is flattenrd. The ectocyte is well seen and the endocyte is coarsely graniilar. The nucleus is located in the centre of the satellite's deutomerite and has the diameter of 46 to 56 µm.

Gametocyst

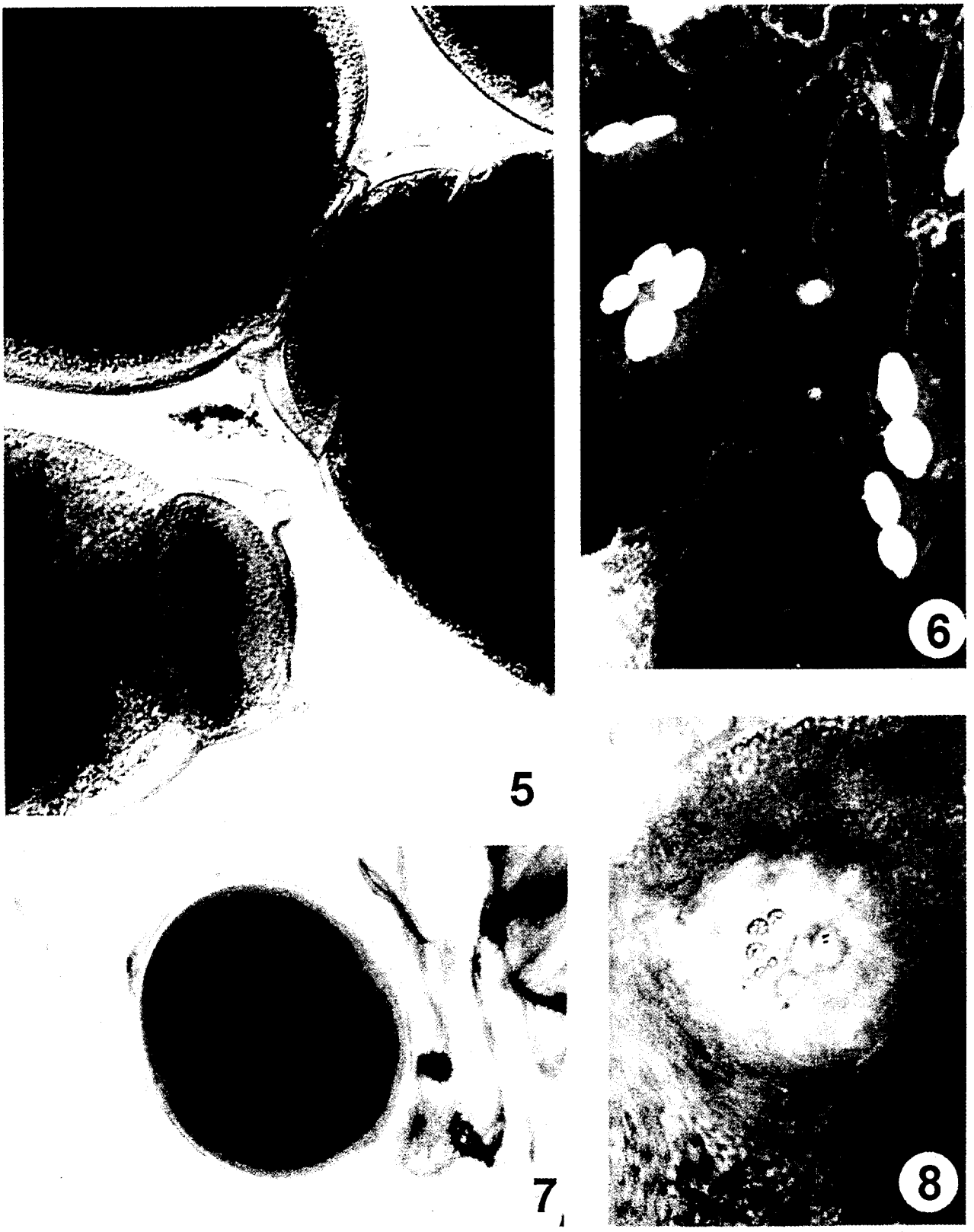
Gametocysts were rather rarely observed with the diameter from 473 x 422 to 650 x 590 µm. The young gametocyst has a more traiislucent body while gametocyst at the advanced maturation process is opaque (Fig. 7).

Habitar

Young gamonts are developing within midgut epithelium or in the gastric intestinal caeca (ampules)



Figs. 1-4. *Gregarina acridiorum* from *Calliptamus italicus* and *Dociostaurus maroccanus*. 1 - gamont from *C. italicus* (x 500), 2 - gamont from *D. maroccanus* (x 400). 3 - sporonts in syzygy from *C. italicus* (x 200). 4 - sporonts in syzygy from *D. maroccanus* (x 200)



Figs. 5-8. *Gregarina acridiorum* froni *Calliptamus italicus* and *Dociostaurus maroccanus*. 5 - ridge structure of the satellitess protomerite which secures the firni lock with primite and ridge on the top of the protonierite of sporonts froni *C. italicus* (x 500). 6 - a group of sporonts in syzygies from *C. italicus* released from the gut of *D. maroccanus* into the dissecting tluid (x 40). 7 - an early gametocyst from *C. italicus* (x 100). 8 - nucleus of sporont from *C. italicus* with karyosomes (x 800)

Table 1. Comparison of sizes of gregarines from *D. maroccanus* and *C. italicus* with data given for *Gregarina acridiorum* by Geus (1967)

		TL	LP	LD	WP	WD	LP:TL	WP:WD	WP:LP
from *Dociostaurus maroccanus*									
Gamonts (n=9)	Range	183-328	51-85	128-246	71-125	94-101	1:2.9-4.0	1:1.2-1.6	1:0.7-0.9
	Mean	243.4± 54.9	70.5±13.4	172.8± 43.6	89.6± 17.6	125.5 ±31	1:3.4±0.4	1:1.4±0.1	1:0.8±0.1
Sporonts (n=16)									
Primites	Range	250-518	62-102	218-416	68-176	132-380	1:3.1-6.2	1:1.4-2.3	1:0.5-1.0
	Mean	351.5±107.7	79.5±13.6	271.9±97.4	119.6±36.0	216.2±81.5	1:4.4±0.9	1:1.8±0.3	1:0.7±0.1
Satellites	Range	232-587	44-72	175-524	62-224	120-352	1:4.1-10.9	1:1.2-2.1	1:0.3-0.8
	Mean	376.6±122.4	55.3±7.2	321.2±121.8	130.6±44.3	200.4±77.1	1:6.9±2.3	1:1.5±0.2	1:0.5±0.2
from *Calliptamus italicus*									
Gamonts (n=10)	Range	150-360	44-80	108-294	64-128	94-185	1:3.2-5.5	1:1.4-1.6	1:0.4-0.9
	Mean	259.8±57.6	63.7±10.4	196.1±52.4	96.7±19.0	142.9±29	1:4.1±0.8	1:4.1±0.8	1:0.7±0.1
Sporonts (n=22)									
Primites	Range	284-570	62-114	210-456	106-210	180-467	1:3.8-5.9	1:1.2-2.5	1:0.4-0.9
	Mean	427.1±77.1	89.3±13.2	337.7±67.7	152.3±27.7	281.8±72.3	1:4.8±0.6	1:1.8±73	1:0.6±0.1
Satellites	Range	301-576	40-47	243-518	110-205	171-342	1:5.2-12.4	1:1.3-1.8	1:0.2-0.5
	Mean	451.0±87	53.8±9.0	397.1±87.5	153.9±27.4	245.±55.4	1:8.6±2.3	1:1±0.1	1:0.4±0.1
Gregarina acridiorum									
Gamonts (n=6)	Range	405-622	80-118	325-504	90-130	108-157	1:4.9-5.3	1:1-1.4	1:0.8-0.9
	Mean	492.2±77.5	96.5±15	395.7±64.3	108.3±15.0	130.5±15.7	1:5.1±0.1	1:1.2± 0.1	1:0.9±0.1
Sporonts (n=5)									
Primites	Range	52 1-802	103-159	418-643	116-163	129-203	1:5.0-5.7	1:1.1-1.3	1:0.9-1.0
	Mean	695.6±121.0	1< 16±23.6	564.0±98.6	141.4±20.2	169.4±33.3	1:5.3±0.3	1:1.±0.1	1:0.±0.0
Satellites	Range	103-789	98-147	405-642	122-175	137-208	1:5.1-5.9	1:1.1-1.2	1:0.8-0.9
	Mean	684.2±121.2	123.6±29.7	560.6±101.5	151.6±22.3	178.8±30.5	1:5.5±0.3	1:1.2± 0.1	1:0.8±0.1

but with the maturation they move to the gut lumen uhere they meet other gamonts and enter into the syzygies as sporonts. However, some gamonts evidently fail to move into the intestine lumen maturing within the caeca.

The intensity of parasitization in some adult locusts was extremely high and in some specimens the whole intestine was filled with hundreds of gamonts, sporonts in associations and gametocysts which are easily visible in the dissected insects through the midintestine wall (Fig. 9). Such great number of gregarines without doubt has the detrimental effect on food movement and its assimilation in the insect host gut.

It is generally thought that gregarines (Eugregarinorida) do not affect seriously the life processes of their hosts. However, Lipa (1967) and Brooks and Jackson (1990) demonstrated that some eugregarine species can greatly increase the host mortality due to serious damage of the gut epithelium and intestinal caeca. This effect interferes with the proper assimilation of the food by their hosts or make port of entry to the haemcoel for intestinal bacteria what results in the septicemia.

Parasitization level

The populations of *D. maroccanus* and *C. italicus* were regularly monitored in La Serena (Badajoz province) for the presence of pathogens beginning from March 24, 1992. Although over a thousand of nymphs were examined no gregarine infection was observed. The adult insects which appeared in May were also free from infection, but starting on Junr 19 eleven adults of *D. maroccanus* out of 110 and one *C. italicus* out of three examinecl were found infected. On July 8, one out of fourteen *D. maroccanus* adults and six out of sixteen *C. italicus* were found infected.

In addition, nine out of sixty one adults of *D. maroccanus* collected in La Laguneta (Tenerife Island) on July 15. 1992 and two out of eight collected in the same place on October 3, 1992 were found infected.

One infected *C. italicus* was found in Santa Marta (Albacete) out of three collected on July 4,1993.

Late occurrence of gregarine infection among popiilations of *D. maroccanus* and *C. italicus* registered by us is not surprising. Bush (1928) observed that grasshoppers in the spring were free from infection but later in the season practically every grashopper was infected.

Gregarine in *Locusta migratoria*

An eugregarine was also found in two adults out of six examinecl from laboratory rearing of *Locusta migratoria* in Sankt Petersburg but the insects were originally collected in Uzbekistan Republic. Morphology of gamonts, type of syzygies and size parameters (Table 1) indicate that it can be considered as identical with the eugregarine observed in *D. maroccanus* and *C. italicus*.

Gregarines in other Orthoptera

During late summer of 1992 eugregarine infections were recorded in adults of the following orthopterans collected in the Iberian Peninsula (Albacete, Badajoz and Valencia) and in the Canary Islands (El Hierro and Tenerife):

Anacridium aegyptium L. (Orthoptera, Acrididae). One out of three collected at Valencia city on July **2,** 1992 was infected with *Gregarina* sp. but lack of sufficient number of measurements make impossible to idetify this gregarine to species.

Oedaleus decorus (Germ.)(Orthoptera, Acrididae). One out of five collected at La Laguneta (Tenerife Island) on July 15, 1992 was infected with *Gregarina* sp. forming syzygies (Table 2). Only three syzygies were observed, possibly by young sporonts, with the maximum size of 356 pm. Therefore, although this eugregarine was similar to the eugregarine observed in *D. maroccanus*, we cannot make a firm conclusion that they are identical.

Ailopus sp. (Orthoptera; Acrididae). In one out of four collected at La Laguneta (Tenerife) on October 3, 1992 only solitary gamonts (sporonts) with the maximum size of 257 µm were present (Fig. 10) (Table 2) having the characteristic of *Leidyana* genus.

Decticus albifrons (E) (Orthoptera, Tettigoniidae). In one collected at El Cascajo (El Hierro Island) three solitary gamonts were present having the maximum length up to 553 µm (Table **2).** Although it resembled the eugregarine observed in *D. maroccanus* we cannot make a firm conclusion that they are identical, due to lack of siifficient data.

DISCUSSION

Eugregarines are common parasites of Orthoptera and several species have been described or recorded on various continents (Watson 1916a. b, 1922: Geus 1969; Levinr 1988).However, for the taxonomic consideration we have taken into account only gregarine species recorded in orthopterans occurring in the Palearctic region from which originates our research material.

Table 2. Size of gregarines from *Ailopus* sp., *Decticus albifrons*, *Locusta migratoria* and *Oedaleus decorus*

		TL	LP	LD	WP	WD	LP:TL	WP:WD	WP:LP
Ailopus sp.									
Gamonts (n=6)	Range	143-257	29-60	114-200	51-81	68-123	1:3.8-4.9	1:1.3-1.6	1:0.6-0.9
	Mean	206.8±42.1	49.7±157.2	157.2±31.4	62.5±11.8	92.0±19.1	1:4.2±0.5	1:1.5±0.1	1:0.8±0.1
Decticus albifrons									
Gamonts (n=3)	Range	205-553	60-171	143-450	63-154	89-342	1:1.3-5.4	1:1.4-2.2	1:0.7-2.7
	Mean	228.7±194.6	111.3±56.6	250.0±173.3	93.3±52.5	177.0±143.0	1:3.4±2.0	1:1.7±0.4	1:1.4±1.1
Locusta migratoria									
Gamonts (n=5)	Range	79-468	22-82	57-383	35-126	46-246	1:3-5.7	1:1.3-2.0	1:0.6-0.9
	Mean	215.4±170.1	48.8±28.4	165.4±141.5	70.2±43.7	109.8±84.2	1:4.1±1.0	1:1.5±0.3	1:0.7±0.1
Sporonts (n=5)									
Primites	Range	201-410	55-82	146-338	137-526	115-219	1:3.7-5.3	1:1.3-1.9	1:0.5-0.8
	Mean	334.4±80.0	65.8±11.0	268.6±74.5	105.2±22.0	166.2±40.7	1:5.1±0.1	1:1.6±0.2	1:0.6±0.1
Satellites	Range	262-438	38-72	224-394	104-115	115-191	1:4.3-10.0	1:1.1-1.7	1:0.3-0.7
	Mean	348.2±70.9	48.4±13.5	299.8±74.3	107.2±4.9	146.6±28.3	1:7.6±2.2	1:1.4±0.2	1:0.5±0.1
Oedaleus decorus									
Gamonts (n=1)	Range	-	-	-	-	-	-	-	-
	Mean	118	52	136	84	116	1:2.3	1:1.4	1:0.6
Sporonts (n=3)									
Primites	Range	280-304	72-76	208-228	100-108	148-192	1:3.9-4.0	1:1.5-1.8	1:0.7-0.8
	Mean	293.3±12.2	74.7±2.3	218.7±11.1	102.±4.6	170.7±22.0	1:3.9±0.1	1:1.7±0.2	1:0.7±0.1
Satellites	Range	256-356	28-36	220-320	100-112	148-188	1:7.1-12.1	1:1.5-1.7	1:0.3-0.4
	Mean	317.3±53.7	33.3±4.6	284.0±55.6	105.3±6.1	165.3±20.5	1:9.7±2.5	1:1.6±01	1:0.3±0.1

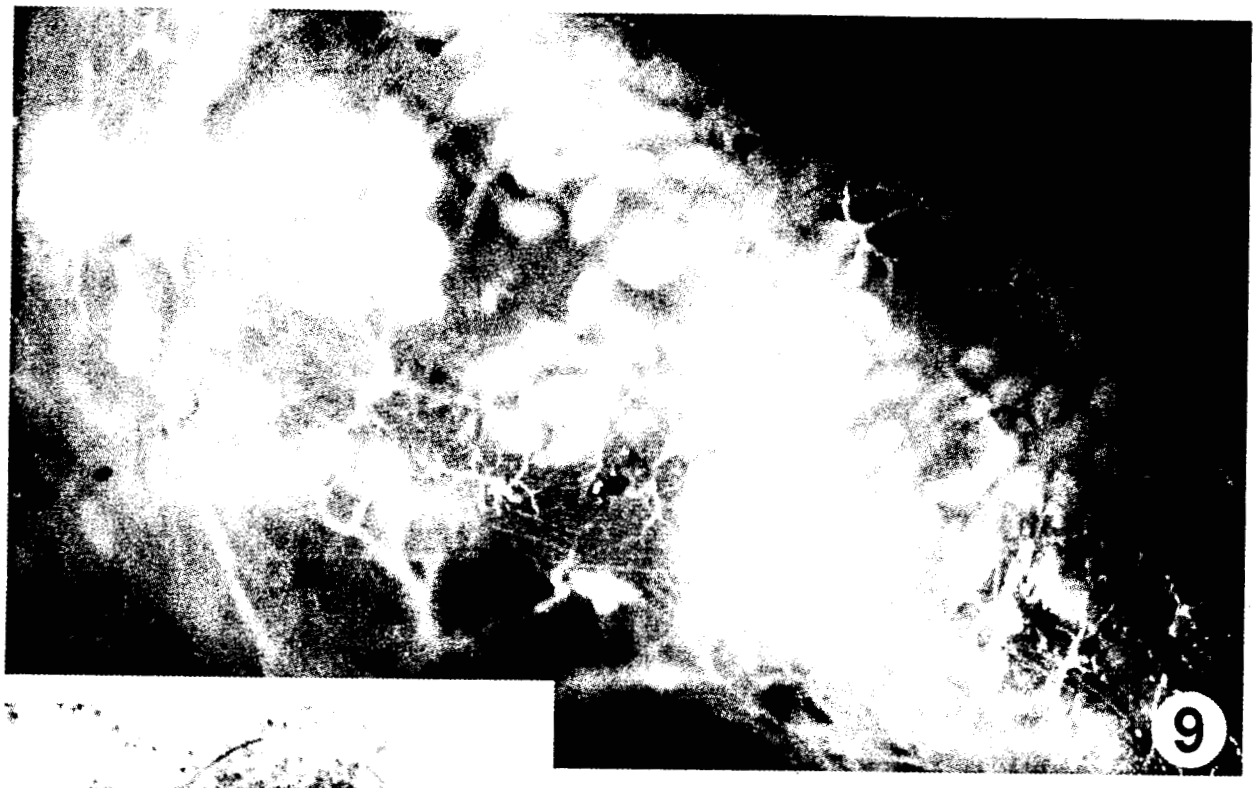


Fig. 9. Hundreds of syzygies of *Gregarina acridiorum* seen through the gut wall of *Calliptamus italicus* (x 40)

Figs. 10-11. *Leidyana* sp. from *Ailopus* sp. 10 - young gamont (x400). 11 - maturing gamont (x 400)

Table 3. Comparison of mean size of eugregannes from *D. maroccanus*, *C. italicus*, and *L. migratoria* with *Gregarina acridiorum* and *Gregnrinn garnhami*

	TL	LP	LD	WP	WD	LP:TL	WP:WD	WP:LP
Gamonts								
Dociostaurus maroccanus	243.4	70.5	172.8	89.6	125.5	3.4	1.4	0.8
Calliptamus italicus	259.8	63.7	196.1	96.7	142.9	4.1	1.5	0.7
Locusta migratoria	215.4	48.8	165.4	70.2	109.8	4.1	15	0.7
Gregarina acridiorum	492.2	96.5	395.7	108.3	130.5	5.1	1.2	0.9
Gregarina garnhami	n.a.	n.a.	n.a.	n.a.	n.a.	4.0	1.5	n.a.
Sporonts Primites								
Dociostaurus maroccanus	351.5	79.5	271.9	119.6	216.2	4.4	1.8	0.7
Calliptamus italicus	427.1	89.3	337.7	152.3	281.8	4.8	1.8	0.6
Locusta migratoria	334.4	65.8	268.6	105.2	166.2	5.1	1.6	0.6
Gregarina acridiorum	695.6	131.6	564.0	141.4	169.4	5.3	1.2	0.9
Gregarina garnhami	n.a.	n.a.	n.a.	n.a.	n.a.	4.0	1.4	0.6
Satellites								
Dociostaurus maroccanus	376.6	55.3	321.2	130.6	200.4	6.9	1.5	0.5
Calliptamus italicus	451.0	53.8	397.1	153.9	245.1	8.6	1.6	0.4
Locusta migratoria	348.2	48.4	299.8	107.2	146.6	7.6	1.4	0.5
Gregarina acridiorum	684.2	123.6	560.6	151.6	178.8	5.5	1.2	0.8
Gregarina garnhami	n.a.	n.a.	n.a.	n.a.	n.a.	5.5	1.4.	n.a.

n.a. - no data in original description

Type of epimerite, shape of gamonts and sporonts, type of syzygies, gametocysts and size features of the gregarines observed in *Callipatmus italiciis, Dociostaurus nzaroccaniis* and *Locusta migratoria* indicate that they represent the genus *Gregarina* Dufour.

Foerster (1938a, b), Semans (1943), Geus (1969) and Levine (1988) list a number of gregarines from Orthoptera from which only *Gregarina acridioriim* Léger (Léger 1893) and *Gregarina garnhami* Canning (Canning 1956) are taken into consideration of the taxonomic position of the recorded by us gregarines. It must be mentioned that none of these authors list *D. maroccanus* as host for any gregarine species, but *C. italicus* is mentioned bv them as host for *G. acridiorum* and *Locusta migratoria* as host for *G. garnhami*. Also Corbel (1964) reported *L. migratoria* as an experimental host for *G. garnhami*. However, a gregarine infection caused by *Gregarina* sp. in *D. maroccanus* was observed by Nurzhanov (1988).

In Table 3 we compare size parameters of gregarines recorded in *C. italicus, D. maroccanus* and *L. migratoria* with size parameters of *G. acridiorum* given by Geus (1969). These data indicate that all three acridids studied by us were infected by the same gregarine species which we identify as *G. acridioriim.*

Unfortunately, similar comparison with *G. garnhami* is not possible as at its original description Canning (1956) has not provided any data on size of protomerites and deutomerites of gamonts and gave only maximum size of sporonts, size of cysts, and ratio TL:LP, WP:WD, LP:WP. However, even such scarce data, together with illustrations demonstrating the ridge structures securing the firm caudo-frontal associations, indicate that *G. garnhami* is identical with our material of *G. ncridioriim.* This structure of the satellites protomerites was noticed and described by Canning (1956) but here we illustrate it by photographs. Such type of the attachement secures the metabolic communication between primite and satellite which is needed to coordinate movements while producing the gametocyst. This type of "lock" attachement, may indicate that vacuum suction may be involved as suggested by Canning (1956).

Our oppinion that *G. garnhami* is a synonym of *G. acridiorum* is based not only on morphology but also on size parameters. The maximum length of sporont of *G. garnhami* given by Canning is 554-643 µm and the diamater of cysts varied from 114 to 470 x 428 pm. In our material sporonts had up to 576 µm in length and cysts varied from 473 x 422 to 650 x 590 µm in diameter. It may be here mentioned that the maximum length of *G. acridioriim* given by Geus (1969) is 802 pm.

While describing *G. gnrnhami* Canning (1956) was not aware about existence in the literature of *G. acridioriinz* which she confirmed later (Canning 1992). It is also surprising that *G. garnhnmi* was not included by Geus (1969) in his monograph. These facts created to us some difficulties in interpreting the taxonomic position of the involved gregarines but we consider that our studies and this discussion provided grounds to conclude that *Gregarina garnhami* Canning (1956) is not a valid species but is a synonym of *Gregarina ncridioriim* Léger (1893).

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