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Ancyrophora gracilis LEGER, 1892 and *Actinocephalus permagnus* Wellmer, 1910 (Eugregarinorida: Apicomplexa) in natural populations of ground beetles (Coleoptera, Carabidae) – hosts preferences, intensity and seasonal dynamic

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The study compares the parameters of parasitizing ground beetles (Coleoptera, Carabidae) by two gregarine species (Eugregarinorida: Apicomplexa): *Ancyrophora gracilis* LEGER, 1892 and *Actinocephalus permagnus* WELLMER. An analysis of parasite intensity in relation to body size and habitat, seasonal dynamic was conducted. The studies aimed to recognize the host specificity, intensity and dynamic of host parasitizing by eugregarines in natural conditions. The relationships between the intensity of parasitism and host characteristics as well as seasonal variation of *Ancyrophora gracilis* and *Actinocephalus permagnus* were analyzed using generalized linear models with Poisson distribution and log link-function. The density of *Ancyrophora gracilis* and *Actinocephalus permagnus* is highly species and host body size dependent. *Actinocephalus permagnus* is more likely densely appearing in the woodland meanwhile *Ancyrophora gracilis* have no significant division between woodland and meadow. Both species have significantly reduced density in the arable fields. The seasonal variation of both parasites were related to seasonal fluctuations of their hosts. We assumed that intensity of both parasites is highly host density dependent and reflects habitat and seasonal preferences of the hosts.

Key words: eugregarines, ground beetles, *Carabidae*, *Ancyrophora gracilis*, *Actinocephalus permagnus*, intensity, seasonal dynamic

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INTRODUCTION

Gregarines are among the most common protozan parasites of insects, occurring mainly in digestive tract. Although common and quite often abundant, their role in the control of host specimens is not clearly defined. On the other hand, a high mortality rate of host population is certainly caused by schizogregarines, which damage the tissue of mid-intestine (Lipa 1967, Geus 1969). Some authors claim that eugregarines significantly influences on the health of host population by prolonging the development of pre-imaginal stages and even cause high mortality in their populations (Johny et al. 2000, Kubilay & Gökçe 2005, Zuk 1987b). However, most researchers do not confirm a clear and direct impact of eugregarines on their hosts (Hecker et al. 2002, Govendasamy & Ganeshan 1999, Zuk 1987a). As the parasites of the epithelium, eugregarines may open infection possibilities for other pathogenic factors, thus indirectly adding to a fall in the population abundance.

Regardless of the state of knowledge on the role of eugregarines in density control of their hosts, various kinds of prevalence are reported for their hosts and seasonal dynamic of occurrence (Vezzani & Viskievski 2006, Locklin & Vodopich 2010). The studies aimed to recognize the host specificity, intensity and dynamic of host parasitizing by eugregarines in natural conditions. Such studies are time-consuming, because the possibility of occurrence of internal parasites will be influenced by the complexity of the habitat. There is however known the strict relations between the habitat quality the density of their hosts - Carabidae. Indication of significant role of eugregarines in controlling host population may become also a valuable source of practical information in terms of ecology as well as integrated pest management (Holland & Luff 2000).

MATERIAL AND METHODS

The field survey was provided in the years 2006-2007 (from April to October) on permanent study sites in western Poland. Almost 1100 specimens from family Carabidae were checked for appearance of *Ancyrophora gracilis* Leg. and *Actinocephalus permagnus* Well. A list of ground beetles collected in specific habitat type and abundance is shown in Table 1.

The collected live beetles were transported to a laboratory, where they were dissected and their digestive tracts were prepared. The cephalonts, trophosoits and gamonts were observed and counted under the light microscope. Each digestive content sample was morphologically measured and documented as digital images to classify into the proper species. In total 286 infestations by *A. gracilis* and 57 by *A. permagnus* were found.

The hosts of parasites were divided into two body size classes: medium size (15-23 mm) and broad size (26-36 mm). There was no records of infestation of small sized beetles (below 15 mm).

The relationships between the intensity of parasitism and host characteristics as well as

Table 1. List of species and the number of adult Carabidae (Coleoptera) studied under a microscope for eugregarine presence (Eugregarinorida, Apicomplexa)

Host	Type of habitat	Number of specimens studied
Carabus coriaceus L.	forest	23
Carabus hortensis L.	forest	57
Carabus nemoralis Muell.	forest	174
Carabus nemoralis Muell.	meadow	114
Carabus granulatus L.	forest	110
Pterostichus niger (Schall.)	forest	114
Carabus convexus F.	forest	30
Pterostichus melanarius (Ill.)	forest	98
Pterostichus melanarius (Ill.)	meadow	187
Pterostichus melanarius (Ill.)	field	136

seasonal variation of *Ancyrophora gracilis* and *Actinocephalus permagnus* were analyzed using generalized linear models with Poisson distribution and log link-function (Wald test or Chi-square-statistic, $p<0.05$).

Characteristics of hosts and parasites

Ancyrophora gracilis and *Actinocephalus permagnus* (Photo 1) are among the most frequently observed gregarine species feeding on ground beetles of the genera *Carabus* L. and *Pterostichus* Bon. (Wellmer 1911, Geus 1969, Foerster 1939a, 1939b, Lipa 1967, Levine 1988a, 1988b). The parasitism spectra of both gregarine species overlap only partly. This is probably related to the size of host's body and size of parasites. *Actinocephalus permagnus* is one of larger gregarines with body length reaching up to 2 mm, while *A. gracilis* is a smaller protozoan (0.5-1.0 mm on average). In consequence, assuming the presence of competition mechanisms among them, it can be expected that the host, will be to various extent infested by both gregarine species. So far *A. gracilis* has been recorded in 17 Carabidae species, while *A. permagnus* only in 8 species (Table 2) (Foerster 1939a, Geus 1969, Levine 1988b, Lipa 1967). Among the available group of hosts 6 species were parasitized by both eugregarines, 11 species were parasitized solely by *A. gracilis* and only 2 species solely by *A. permagnus* - including one of bigger European species - *Carabus coriaceus* L.

The hosts of the parasites are predators feeding usually in epigeal zone. They are common in their habitat, living often in high density, which

Table 2. A list of ground beetle species in which *Ancyrophora gracilis* Leg. and *Actinocephalus permagnus* Well were recorded, based on Levines (1988a) records and own observations.

L. p.	Host	Mean body length [mm]	*Ancyrophora gracilis* Leg.	*Actinocephalus permagnus* Well.
1	*Abax parallelepipedus* PILL.	18.6	recorded	*no records*
2	*Carabus arvensis* Herbst	14	recorded	*no records*
3	*Carabus auratus* L.	23.5	recorded	recorded
4	*Carabus auronitens* F.	26	recorded	*no records*
5	*Carabus cancellatus* Ill.	24.5	recorded	recorded
6	*Carabus problematicus* Herbst	25	*no records*	recorded
7	*Carabus convexus* F.	17	recorded	*no records*
8	*Carabus coriaceus* L.	36	*no records*	recorded
9	*Carabus glabratus* Payk.	28	recorded	*no records*
10	*Carabus granulatus* L.	19	recorded a	recorded a
11	*Carabus hortensis* L.	26	recorded	recorded a
12	*Carabus intricatus* L.	30	recorded	*no records*
13	*Carabus nemoralis* Muell.	23	recorded	recorded a
14	*Carabus nitens* L.	15.5	recorded	*no records*
15	*Carabus ullrichi* Germ.	27	recorded	*no records*
16	*Carabus violaceus* L.	28.5	recorded	recorded
17	*Pristonychus algierinus* Gory	16	recorded	*no records*
18	*Pterostichus melanarius* (Ill.)	15.5	recorded	*no records*
19	*Pterostichus niger* (Schall.)	18.5	recorded	*no records*

Recorded a - based on authors'observations

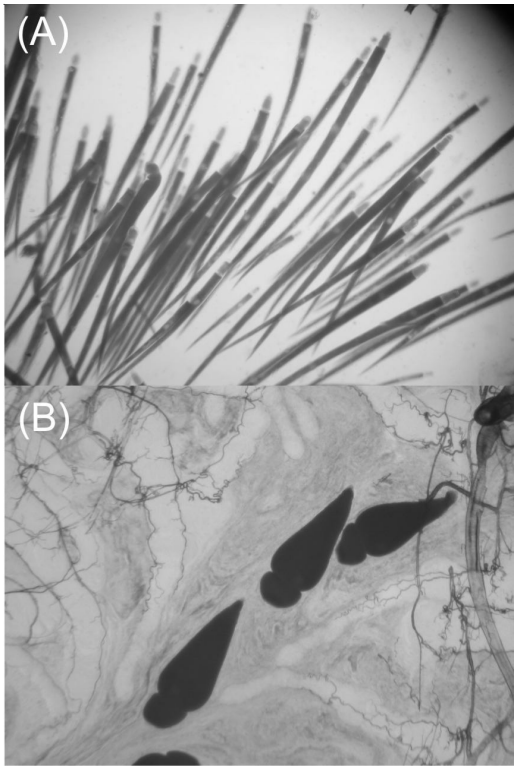

Photo 1. Trophozoits of *Actinocephalus permagnus* (A) and *Ancyrophora gracilis* (B) in the alimentary duct of ground beetles

favours infestation by gregarines (Lindroth 1945, Foerster 1939b, Stachurska 1969). The availability of the particular host species is also season-dependent. Two basic breeding types, spring and summer are usually specified in relation to the period of adult hosts occurrence (Larson 1939). Among both breeding groups there are potential hosts of *A. gracilis* and *A. permagnus.*

RESULTS

During the survey, *Ancyrophora gracilis* was recorded in seven Carabidae species of the genus *Carabus* and *Pterostichus,* meanwhile *Actinocephalus permagnus* was the parasite of the 5 species from genus *Carabus*. Four species of ground beetles were hosts of both eugregarine species, *A. gracilis* was specific for *Carabus convexus, Pterostichus niger* and *P. melanarius*, however *A. permagnus* was recorded solely in the digestive system of *Carabus coriaceus* (Table 2).

The density of *Ancyrophora gracilis* and *Actinocephalus permagnus* is highly species and host body size dependent (Table 3). There is however different pattern of parasite density between size classes of hosts. *Actinocephalus permagnus* prefers bigger species, meanwhile *Ancyrophora gracilis* is more likely preferring medium sized beetles (Fig. 1).

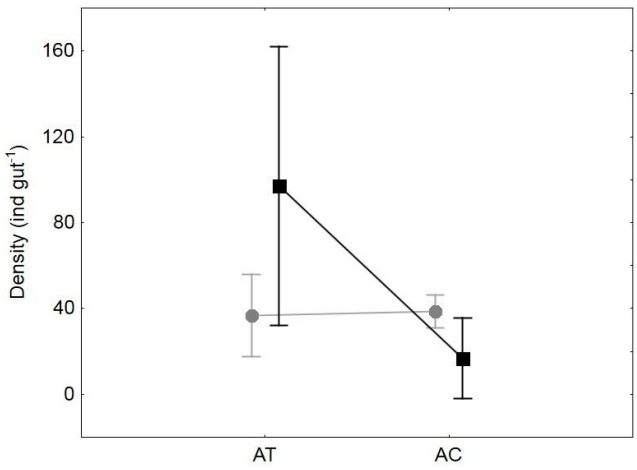

Fig. 1. Mean density of *Actinocephalus permagnus (AT)* and *Ancyrophora gracilis* (AC) in relation to host size classes. circle – medium sized, squares – broad sized hosts

There are also different parasite habitat preferences (Fig. 2). *Actinocephalus permagnus* is more likely densely appearing in the woodland meanwhile *Ancyrophora gracilis* have no significant division between woodland and meadow. It's however remarkable elimination of both eugregarine in the field (Table 4).

The occurrence of *A. gracilis* and *A. permagnus* in their hosts was characterized by different seasonal dynamic. Only intensity of infestation was species and season related (Wald's $W = 452.1$, $p < 0.001$), while the prevalence have no seasonal significance. Both species had different seasonal pattern of occurrence (Fig. 3). The mean density of *Actinocephalus permagnus* was higher in spring and summer and then decreased in autumn. The high variation in both "higher" seasons was also visible. The density of *Ancyrophora gracilis* however increased during the consecutive seasons and density fluctuation

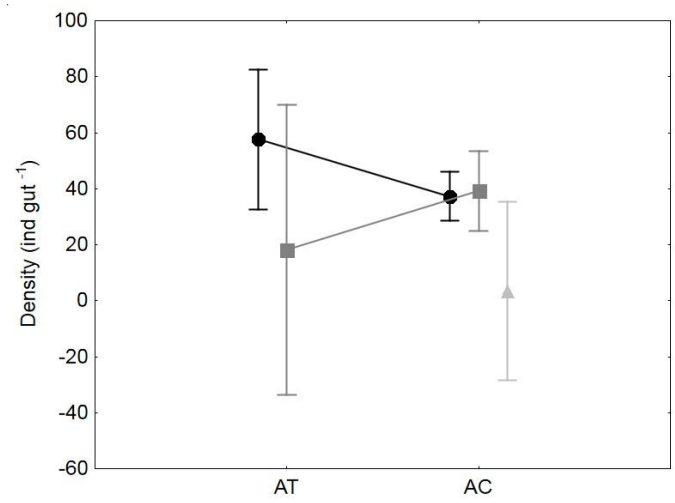

Fig. 2. Mean density of *Actinocephalus permagnus (AT)* and *Ancyrophora gracilis* (AC) in relation to habitat. Circle – woodland, square – meadow, triangle – arable land

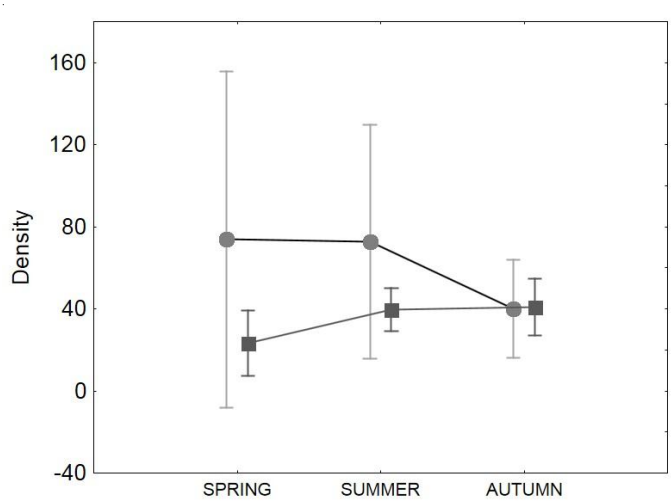

Fig.3. The mean density ± SE of *Actinocephalus permagnus* (circle) and *Ancyrophora gracilis* (square) in consecutive seasons

Table 3. Results of generalized linear model test of significance (Wald statistics) of the effect of species, host size a on density of parasitizing gregarines

	Wald's Stat.	p
Intercept	33731.30	0.00
Species	453.48	0.00
Size	3.18	0.07
Species*Size	505.21	0.00

Table 4. The effect of host species and habitat type on density of eugregarins derived from generalized linear model

	Log-function	Chi-square	p
Intercept	-13126.03		n.s.
Species	-12977.03	298.00	0.00
Habitat	-12924.93	104.20	0.00
Species*Habitat	-12852.01	145.82	0.00

was similar and much lower during whole host activity year.

DISCUSSION

Eugregarines as parasites of insects' digestive tracts, are generally considered to be slightly virulent or even avirulent towards their hosts. Only few studies (Zuk 1987b, Rhein et al. 2000, Kubilay & Gökçe 2005) showed that they have a negative influence on hosts life and population development. *Ancyrophora gracilis* and *Actinocephalus permagnus* are some of the most common species of eugregarines parasitizing mainly species from genus *Carabus* (Insecta, Coleoptera, Carabidae). The hosts play important role in terrestrial habitats. They are top predators in most terrestrial ecosystems and play important role in integrated pest management approach of agroecosystems (Holland & Luff 2000). They are also protected by law in many countries and in some regions are threatened with extinction (eg. Blab et al. 1985, Głowaciński & Nowacki 2004, Pawłowski et al. 2002). Understanding the mechanisms of infestation of ground beetles by parasites, seasonal dynamic of their occurrence, their prevalence allow to work the strategy of sufficient protection system.

As the host species of both eugregarine species partly overlap (Table 2), so the simultaneous infestation could be expected. However, *Ancyrophora gracilis* and *Actinocephalus permagnus* have not been found to occur together so far. Some mechanisms of exclusion allowing only one parasite species to infest the host could be developed. Although the hosts of both protozoa belong to relatively large ground beetles, a statistically significant differences in preferred size of carabids were revealed (Table 3). There is, however, strict relation between the size of host and parasite. *A. permagnus* which is much larger preferred much more broad size carabids (more than 20 mm long) while smaller *A. gracilis* was much more densely occurring in smaller species (Fig. 1). It's worth however to express, that the mean densities of both gregarine species in medium sized hosts are on the same level. The differences between host sizes may be explained by host habitat preferences. The mean density of *A. permagnus* is significantly higher in woodland than in meadows, although no specimens were recorded in the arable fields. *A. gracilis* density

in the forest and meadows is on the same level, however more significantly lower in arable fields. This picture reveal clearly habitat preferences of host species. Most large sized Carabus species are forest species, appearing in gradient of disturbance in smaller densities (Skalski et al. 2010, Skalski et al. 2011). On the other hand most medium sized species are more abundant in open areas. Positive effect of host density of insect and parasite intensity was very well documented (Vezzani & Wisnivesky 2006, Lindsey et al. 2009, Locklin & Vodopich 2010). *A. permagnus* density decreased along the gradient of habitat disturbance from natural forest to arable field. *A. gracilis* density however is on similar level in the forest and meadows and significantly decreases in arable field. Medium sized ground beetles have similar habitat preferences between open and forested areas. Their decrease is observed in the field (Kosewska et al. 2014).

The first data of the *A. permagnus* and *A. gracilis* prevalence and intensity are based on the material from single findings of gregarines in various parts of Germany. *A. permagnus* was reported from May to October (Foerster 1939b, Wellmer 1911). Most findings of this parasite was concentrated in autumn. These studies carried out by the authors on a high number of specimens systematically partly collected corroborate those observations. The prevalence of *A. permagnus* seemed to be higher in summer and autumn than in spring but statistical analysis didn't confirm this pattern. This can be explained by the occurrence of suitable hosts (large *Carabus* spp.) during whole season (even species belonging to autumn breeders occur in spring and summer in the next period) and earlier observations were mainly related species (*C. coriaceus*) occurring in large numbers in autumn.

The seasonal dynamic of prevalence for *Ancyrophora gracilis* was described by Foerster (1939b) and Wellmer (1911) in which the most data on the occurrence of this protozoan were reported for April, May and June. As in the case of *A. permagnus* this pattern was not confirmed. Maybe it's because more of the hosts of *A. gracilis* occurs in highest number on spring and the beginning of summer and this same there are easy to handcatching. To this group of species belong common carabids like *Carabus nemoralis, C. granulatus C. convexus* and *Pterostichus niger* (Larsson 1939). At the other hand the species with spring breeders in which *A. gracilis* was found, are characterised by the fact that they reappear in a smaller number in autumn. Hence a possibility to use them as hosts occurs basically throughout the season from spring to autumn (similar to the observation of Stachurska (1969) in *Polydesmus complanatus* (L.) population). For this reason, the prevalence in the season should be similar.

While studies have shown significant seasonal differentiation in level of infestation of both protozoans, which also differed among themselves (Fig. 3). The mean density of *A. permagnus* was highest in spring and summer and decreased in autumn. The variation of this parameter was 'stronger' then in case of *A. gracilis*. Infestation of *A. gracilis* differ also - higher values were observed in summer and autumn. This results suggest that further casual observation of seasonal dynamic of both gregarines may be inexact. However confirmed earlier information, that level of infestation of *A. permagnus* is average higher then infestation of *A. gracilis*. Such situation was described by Wellmer (1911), who observed that *A. permagnus* can occur in such big numbers that it can block the intestine. In our survey in digestive tract of *C. coriaceus* was hundreds of individuals (about 400/ind) of this protozoan.

It seems to be interesting relationship between parasite intensity, host body size and habitat (Fig. 2). We found that especially broad size ground beetles but also medium size carabids living in semi natural meadow have relatively highest mean density than hosts living in arable land. In woodland intensity of medium size carabids was similar to these from meadow. This is probably related to the degree of human impact on habitat. Arable land belongs to unstable habitats,

which falls frequent changes in the season (as a result of cultivation). This kind of habitat is often defeated by the hosts of both gregarines, especially large *Carabus* and constant changes do not allow maintain and accumulate parasite reproductive material. A different situation occurs in the well preserved woods and other seminatural open habitats like uncultivated meadow. The hosts occur in such habitat in large number and the circumstance are stable. Similar results were obtained by Stachurska (1969) in population of *Polydesmus complanatus* (L.) inhabiting well preserved and managed hornbeam forests.

Due to an important role of *Carabidae* as a model insect group in ecological and monitoring studies the next step of consideration should be focused on practical role of eugregarines in controlling of the populations of those beetles in respect of their pre-imaginal stages.

REFERENCES

Blab J., Nowak E., Trautmann W., Sukopp H. 1985. Rote Liste der gefährdeten Tiere und Pflanzen in der Bundesrepublick Deutschland. Kilda-Verlag, 245 pp.

Foerster H. 1939a. Beobachtingen über das Auftreten von Gregarinen in Insekten. Zeitschrift für Parasitenkunde 10: 644-673.

Foerster H. 1939b. Gregarinen in Schlesischen Insekten. Zeitschrift für Parasitenkunde 10: 157-209.

Geus A. 1969. Sporentierchen, Sporozoa – Die Gregarinen der land- und süßwasserbewohnenden Arthropoden Mitteleuropas. Die Tierwelt Deutschlands 57: 1-608.

Głowaciński Z., Nowacki J. 2004. Polish Rred Data Book of Animals. Invertebrates. Instytut Ochrony Przyrody PAN – Akademia Rolnicza im. A. Cieszkowskiego. 448 pp.

Govendasamy M., Ganeshan S. 1999. Gregarine (Protozoa: Apicomplexa: Eugregarinida) infection in sugar cane white grubs (Coleoptera: Scarabaeidae) in Mauritius. Food and Agricultural Research Council, Réduit, Mauritius, p. 83-86.

Hecker K. R., Forbes M. R., Leonard N. J. 2002. Parasitism of damselflies (Enallagma boreale) by gregarines: sex biases and relation to adult survivorship. Canadian Journal of Zoology 80: 162-168.

Holland J.M., Luff M.L. 2000. The effects of agricultural practices on Carabidae in temperate agroecosystems. Integrated Pest Management Reviews 5: 109-129.

Johny S., Muralirangan M. C., Sanjayan K. P. 2000. Parasitization potential of two cephaline gregarines, Leidyana subramanii Pushkala and Muralirangan and Retractocephalus dhawanii sp. n. on the tobacco grasshopper, Atractomorpha crenulata (Fab.). Journal of Orthoptera Research 9: 67-70.

Kosewska A., Skalski T., Nietupski M. 2014. Effect of conventional and non-inversion tillage systems on the abundance and some life history traits of carabid beetles (Coleoptera: Carabidae) in winter triticale fields. European Journal of Entomology 111: 669-676.

Kubilay Er M., Gökçe A. 2005. Effect of Diplocistis tipulae Sherlock (Eugregarinida: Apicomplexa), a coelomic gregarine pathogen of tipulids, on the larval size of Tipula paludosa Meigen (Tipulidae: Diptera). Journal of Invertebrate Pathology 89: 112-115.

Larsson S. G. 1939. Entwicklungstypen und Entwicklungszeiten der dänischen

Carabiden. Entomologische Meddelels 20: 270-560.

Levine D. N. 1988a. The Protozoan Phylum Apicomplexa. Vol I, CRC Press Inc., Boca Raton, 203 pp.

Levine D. N. 1988b. The Protozoan Phylum Apicomplexa. Vol II, CRC Press Inc., Boca Raton, 154 pp.

Lindroth C. H. 1945. Die fennoskandischen Carabidae – eine tiergeographische Studie. I spezialler Teil. Göteborg, 709 pp.

Lindsey E., Mehta M., Dhulipala V., Oberhauser K., Altizer S. 2009. Crowding and disease: effects of host density on response to infection in a butterfly–parasite interaction. Ecological Entomology 34: 551-561.

Lipa J.J. 1967. Studies on gregarines (Gregarinomorpha) of arthropods in Poland. Acta Protozoologica 5: 97-223.

Locklin J.L., Vodopich D.S. 2009. Bidirectional gender biases of gregarine parasitism in two coexisting dragonflies (Anisoptera: Libellulidae). Odonatologica 38: 133–140.

Pawłowski J., Kubisz D., Mazur M. 2002. Coleoptera - Chrząszcze. In: Głowaciński Z. (ed.), Czerwona Lista Zwierząt Ginących i Zagrożonych w Polsce. PAN i IOP Kraków, p. 88-110.

Skalski T., Gargasz K., Laskowski R. 2011. Does mixed diffuse pollution decrease ground beetle diversity? Baltic Journal of Coleopterology 11: 1-15.

Skalski T., Stone D., Kramarz P., Laskowski R. 2010. Ground beetle community responses to heavy metal contamination. Baltic Journal of Coleopterology 10: 1-12.

Stachurska T. 1969. Factors affecting the degree of infestation of population of Polydesmus complanatus (L.) by Eugregarinaria. Ekologia Polska Seria A. 17: 263-285.

Vezzani D., Wisnivesky C. 2006. Prevalence and seasonality of Ascogregarina culicis (Apicomplexa: Lecudinidae) in natural populations of Aedes aegypti (Diptera: Culicidae) from temperate Argentina. Journal of Invertebrate Pathology 96: 183–187.

Wellmer L. 1911. Sporozoen ostpreussischer Arthropoden. Schriften der Physikalisch-ekonomische Gesellschaft Königsberg 52: 103-164.

Zuk M. 1987a. Seasonal and individual variation in gregarine parasite level in the field cricets Gryllus veletis and G. pennsylvanicus. Ecological Entomology 12: 341-348.

Zuk M. 1987b. The effects of gregarine parasites on longevity, weight loss, fecundity and developmental time in the field crickets *Gryllus veletis* and *G. pennsylvanicus*. Ecological Entomology 12: 349-354.

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