

## Host diversity and seasonality of *Hyalospora hemerobii* (Apicomplexa: Eugregarinorida: Hirmocystidae) infections in lacewings

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**Abstract:** Lacewings are known as biological control agents, substantially reducing populations of aphids and spider-mites. The study focuses on gregarines parasitizing their brown lacewing hosts. The occurrence of the gregarine species *Hyalospora hemerobii* Geus, 1969 in brown lacewings (Hemerobiidae) in NE Slovenia was investigated. Seven brown lacewing species were surveyed and in five of them (*Hemerobius humulinus*, *H. micans*, *H. marginatus*, *Micromus variegatus* and *Symphorobius pygmaeus*) gregarines were present. Trophozoites, solitary gamonts, associations, syzygies and gametocysts were recognized. In this paper syzygy of *H. hemerobii* is illustrated for the first time. In some trophozoites the epimerite was present, associations were biassociative and caudofrontal. The occurrence of the parasite in *Hemerobius micans* was studied in 2011 and 2013. In 2011, gregarines occurred from June to September, and in 2013 from June to October. An infection rate within the range of 10–86.7% was detected. The average number of solitary gregarines per infested host increased progressively, from June to September/October. This is the first investigation of seasonal patterns in the occurrence of any known gregarine species parasitizing Neuropterida.

**Key words:** gregarines; brown lacewings; Hemerobiidae; phenology

### Introduction

Lacewings (Neuroptera) are economically important as beneficial predators of plant sucking insect pests and brown lacewings (Hemerobiidae) are one of three most significant neuropteran families involved in biological pest control (Szentkirályi 1992; Stelzl & Devetak 1999; McEwen et al. 2001). Although sporadic studies on ecology of brown lacewings have been conducted (e.g., Czechowska 1985; Monserrat & Marín 2001; Szentkirályi 2001a, b; Trouvé et al. 2002; Lara & Perioto 2003), present knowledge on the biology of the family is still insufficient. Due to the great importance of hemerobiids as pest control agents, there is need for further research to better understand the biology of brown lacewings. As parasites can shape the community structure of host organisms it is important to gain knowledge on the parasites of lacewings.

Gregarine apicomplexans infect insects and other invertebrates. In insects the majority of eugregarines are reported, nonetheless, our knowledge of their occurrence is poor. Gregarines have been reported from less than one percent of named insect species (Clopton 2002).

In Neuropterida only a few families have so far been recorded as hosts of gregarines. The first record of gregarines in lacewings (Neuroptera) dates back to 1969 when *Hyalospora hemerobii* Geus, 1969 was de-

scribed from the brown lacewing species *Hemerobius pini* Stephens, 1836 (Hemerobiidae) (Geus 1969). A few years later Achtelig (1974, 1975) described *Gregarina raphidiae* Achtelig, 1974 from snakeflies (*Raphidia*; Raphidiidae). Later, *Actinocephalus acanthaclisis* Marques & Ormières, 1978 was described from larval antlion *Synclisis baetica* (Rambur, 1842) (Myrmeleontidae) (Marques & Ormières 1978) and recently unidentified gregarines were reported from adult antlions (Devetak & Klokočovnik 2011). Gregarines recorded from lacewings seem to be non-pathogenic for their hosts, according to aforementioned literature.

In certain insect groups gregarine parasites present significant seasonal variation. Seasonally determined occurrence of gregarines is well documented in Blattaria, Odonata, Coeloptera, Siphonaptera and Diptera (Ghose & Haldar 1989; Zervos 1989; Vezzari & Wisnivesky 2006; Avelar & Linardi 2008; Albicocco & Vezzari 2009; Locklin & Vodopich 2010; Forbes et al. 2012; Alarcón et al. 2013; Bunker et al. 2013).

In two endemic New Zealand cockroaches, *Parallepsidion pachycercum* Johns, 1966 and *Celatoblatta peninsularis* Johns, 1966, incidence, intensity and gametocyst production of two unidentified gregarine species were only in certain parts of the season (Zervos 1989). A number of dragonfly species (Odonata) are well known hosts for gregarines with a clear seasonal pattern of prevalence (e.g., Locklin & Vodopich

2010; Forbes et al. 2012; Bunker et al. 2013). Forbes et al. (2012) found that both prevalence and intensity of gregarine parasitism in the damselfly *Nehalennia irene* (Hagen, 1861) were seasonally unimodal. One of the reasons for this pattern could be that, like density of hosts which peaks mid-season, the density of infective stages of directly transmitted parasites like gregarines also peaked mid-season (Forbes et al. 2012). For gregarine infection of two Coleoptera species, *Lophocateres pusillus* (Klug, 1832) and *Tribolium castaneum* (Herbst, 1797) a particular range of temperature and humidity is required and the combination of both factors influences seasonal prevalence of gregarines (Ghose & Hal-dar 1989). Significant seasonal differences in the occurrence of both gamonts and gametocysts of *Steinina ctenocephali* (Ross, 1909) in cat fleas (Siphonaptera) were revealed in Taiwan and Brazil (Avelar & Linardi 2008; Alarcón et al. 2013). Fleas were more likely to be infected with the gregarines during March – July period with high parasite prevalence, and the reason for that are high temperatures (Alarcón et al. 2013). In *Ascogregarina culicis* (Ross, 1895), parasitizing in adults and immature stages of *Aedes aegypti* (L., 1762) (Diptera), parasite prevalence and host abundance showed clear seasonal pattern (Vezzari & Wisnivesky 2006; Albicócco & Vezzari 2009). Parasite trophozoites were only found from January to May with a clear seasonal pattern of prevalence (Albicócco & Vezzari 2009).

In this study I address two questions concerning the *Hyalospora*-brown lacewings interactions in the field: (i) Does *H. hemerobii* occur in different host species; (ii) Are there any seasonal differences in gregarine occurrence. Data of seasonal phenology and the occurrence of the gregarines in brown lacewings are provided and discussed.

## Material and methods

Seasonal occurrence of gregarines in a brown lacewing *Hemerobius micans* Olivier, 1792 was studied in Piramida near Maribor (Slovenia), at the altitudes from 330 to 370 m a.s.l., with geographic position 46°34.3' N, 15°39.0' E, in a deciduous forest of *Carpinus betulus* L. and *Fagus sylvatica* L. *Hemerobius micans* was chosen for the seasonality part of this study as it is the most abundant species in the country. That collection site was inspected for the presence of adult insects once per month, in a period from April to November 2011 and in a period from April to November 2013. In the winter period, from December to March, adults of *H. micans* do not occur. Brown lacewings were collected using an insect net. In 2011 ten individuals and in 2013 thirty individuals were observed each month, total 320 individuals, and the following variables were recorded: percentage of infected hosts, number of solitary gregarines per host, number of associations per host.

Different species of brown lacewings were collected in localities in NE Slovenia, in a period from 2011 to 2013, to inspect them for the presence of gregarines. The brown lacewings were collected in the following sampling sites: Kamnica, Koblarjev zaliv; in vegetation along the river bank; 46°33'57.9" N, 15°37'03.9" E; Loče pri Poljčanah; in a deciduous forest; 46°29'03.7" N, 15°47'30.6" E;

Maribor; in a garden; 46°33'58.4" N, 15°39'15.3" E; Piramida, Maribor; in a deciduous forest; 46°34'17.5" N, 15°38'57.9" E; Savinjske Alpe: Matkov kot; on maple trees; 46°23'27.1" N, 14°35'55.1" E; Vurberk; in a deciduous forest; 46°29'03.7" N, 15°47'30.6" E.

Brown lacewings were eviscerated and their alimentary canals dissected in insect Ringer's solution (Laughton et al. 2011: 128 mM NaCl, 18 mM CaCl<sub>2</sub>, 1.3 mM KCl, 2.3 mM NaHCO<sub>3</sub>, 1 L dH<sub>2</sub>O, pH 7.2). Their intestine was examined microscopically at 100, 200 and 400-times magnifications. Gregarines were measured and photographed using a Nikon E 800 Microscope with a mounted digital camera Nikon DN100, and Eclipse Net version 1.16.3 software.

Gregarine ontogenic terminology follows Clopton (2002). Statistical analysis was performed using GraphPad Prism 4 (GraphPad Software Inc., San Diego, CA, USA). Normality was tested with Kolmogorov-Smirnov test. Mann Whitney *U*-test was used to test the null hypothesis that populations of gregarines occurring in two host species are of the same size. A threshold *P* value was 0.05.

## Results

### *Diversity of hosts*

Seven brown lacewing species were inspected for the presence of gregarines, and in five of them *Hyalospora* cf. *hemerobii* was present (Figs 1–6). The following species were observed:

### *Drepanopteryx phalaenoides* (L., 1758)

Locality and prevalence: Maribor, 18.VI.2012, 0/2 individuals infected.

### *Hemerobius humulinus* L., 1758

Localities and prevalence: Kamnica, Koblarjev zaliv, 8.IX.2011, 1/1 individual infected; Piramida, Maribor, 11.IX.2011, 1/1 individual infected; Piramida, Maribor, 16.IX.2011, 8/9 individuals infected.

Trophozoites (Fig. 3), solitary gamonts, associations, syzygies (Fig. 6) and gametocysts were noted.

### *Hemerobius micans* Olivier, 1792

Localities and prevalence: Loče pri Poljčanah, 18.IX.2011, 1/1 individual infected; Piramida, Maribor, 11.IX.2011, 6/8 individuals infected; Piramida, Maribor, 15.IX.2011, 0/1 individual infected; Vurberk, 18. IX.2011, 1/2 individuals infected.

Trophozoites (Fig. 4), solitary gamonts (Fig. 5), associations (Fig. 5) and gametocysts were noted.

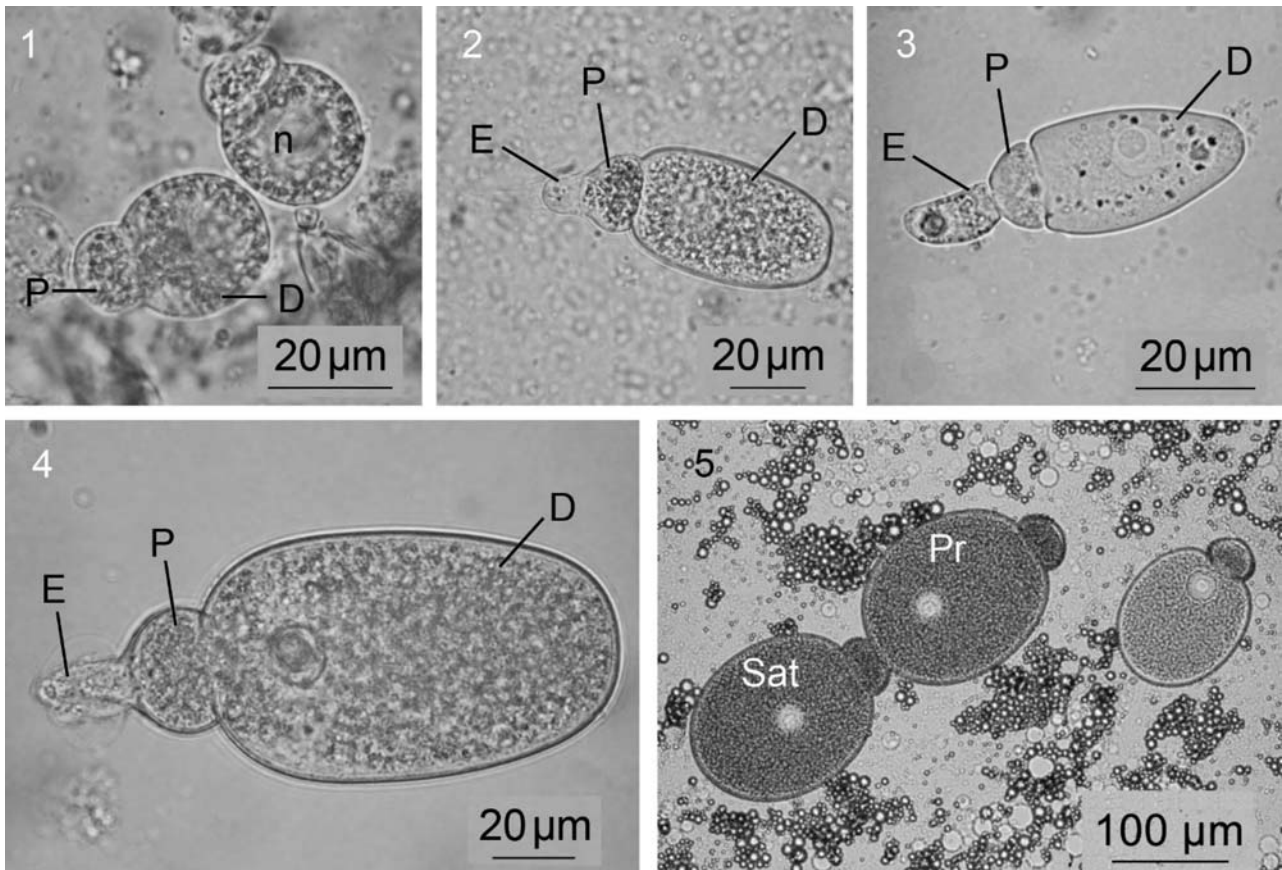
### *Hemerobius marginatus* Stephens, 1836

Localities and prevalence: Maribor, Piramida, 29.VI.2012, 0/2 individuals infected; Savinjske Alpe: Matkov kot, 12.VIII.2013, 4/9 individuals infected.

Trophozoites with epimerite were noted.

### *Micromus variegatus* (F., 1793)

Localities and prevalence: Piramida, Maribor, 18.IX.2011, 0/1 individual infected; Piramida, Maribor, 28.IX.2011, 1/1 individual infected.



Figs 1–5. *Hyalospora hemerobii* in different brown lacewing species. 1: Young trophozoites in *Sympherobius pygmaeus*; 2–4: Trophozoites with epimerite in *Micromus variegatus* (2), *Hemerobius humulinus* (3) and in *Hemerobius micans* (4); 5: An association and a gamont in *Hemerobius micans*. Abbreviations: D – deutomerite; E – epimerite; n – nucleus; P – protomerite; Pr – primate; Sat – satellite.



Fig. 6. *Hyalospora hemerobii* in syzygy isolated from *Hemerobius humulinus*.

Trophozoites with epimerite were noted (Fig. 2).

*Micromus angulatus* (Stephens, 1836)  
 Locality and prevalence: Piramida, Maribor, 3.X.2011, 0/1 individual infected.

*Sympherobius pygmaeus* (Rambur, 1842)  
 Locality and prevalence: Kamnica, Koblarjev zaliv, 4.X.2011, 1/1 individual infected.

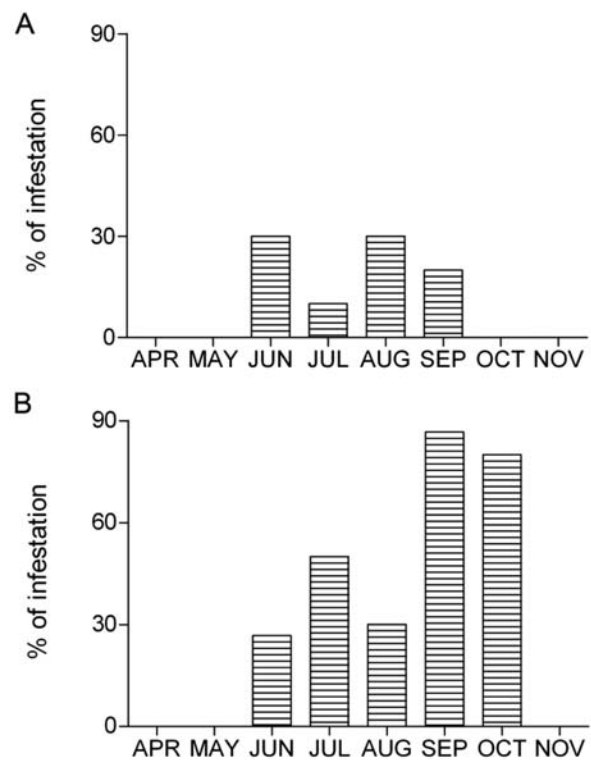


Fig. 7. Seasonal percentage of infestation of *Hyalospora hemerobii* in *Hemerobius micans*: A: 2011 ( $n = 10$  host individuals/month); B: 2013 ( $n = 30$  host individuals/month).

Table 1. Measurements (in  $\mu\text{m}$ ) of solitary gregarines without epimerite in three host species. Data from *Hemerobius pini* were obtained from Geus (1969).

| Host species                             | Statistical measure | TL    | PL   | DL    | PWE  | PWM  | DWE   | DWM   |
|--|---------------------|-------|------|-------|------|------|-------|-------|
| <i>Hemerobius micans</i>                 | Mean                | 103.3 | 20.2 | 83.1  | 29.4 | 30.9 | 57.7  | 60.6  |
|  | SD                  | 22.2  | 3.3  | 20.7  | 4    | 4.2  | 13.3  | 15.6  |
|  | Min                 | 61.7  | 14.8 | 44.6  | 22   | 21.7 | 41.7  | 41.7  |
|  | Max                 | 151.9 | 26.7 | 128.5 | 36.3 | 38.3 | 85.5  | 92.4  |
| <i>Hemerobius humulinus</i>              | Mean                | 116.9 | 25.5 | 91.4  | 37.2 | 37.1 | 76.4  | 79.2  |
|  | SD                  | 42.8  | 2.5  | 40.9  | 10.7 | 6.9  | 32.8  | 30.4  |
|  | Min                 | 86.5  | 22.2 | 62    | 22.2 | 31.6 | 32.4  | 53.1  |
|  | Max                 | 268.4 | 32   | 236.4 | 66.8 | 61.3 | 183.5 | 183.5 |
| <i>Hemerobius pini</i> (after Geus 1969) | Mean                | 115.4 | 20.1 | 95.2  | 27.6 | –    | 57.5  | –     |
|  | SD                  | 15.9  | 3.6  | 12.6  | 1.7  | –    | 9.1   | –     |
|  | Min                 | 95    | 16   | 79    | 26   | –    | 50    | –     |
|  | Max                 | 138   | 26   | 112   | 31   | –    | 78    | –     |

Explanations: TL – total length; PL – length of protomerite; DL – length of deutomerite; PWE – width of protomerite at equatorial axis; PWM – maximum width of protomerite; DWE – width of deutomerite at equatorial axis; DWM – maximum width of deutomerite. Number of measurements: *H. micans*:  $n = 15$ ; *H. humulinus*:  $n = 15$ ; *H. pini*:  $n = 8$ .

Table 2. Testing the statistical data of solitary gregarines in three host species with Mann Whitney  $U$ -test.

|       | TL m         | TL h         | TL p        |
|-------|--------------|--------------|-------------|
| TL m  | –            | $U = 85.00$  | $U = 37.00$ |
| TL h  | $P = 0.2628$ | –            | $U = 44.00$ |
| TL p  | $P = 0.1465$ | $P = 0.3171$ | –           |
|       | PL m         | PL h         | PL p        |
| PL m  | –            | $U = 25.00$  | $U = 60.00$ |
| PL h  | $P = 0.0003$ | –            | $U = 12.50$ |
| PL p  | $P = 0.9742$ | $P = 0.0024$ | –           |
|       | DL m         | DL h         | DL p        |
| DL m  | –            | $U = 98.00$  | $U = 32.00$ |
| DL h  | $P = 0.5615$ | –            | $U = 29.50$ |
| DL p  | $P = 0.0759$ | $P = 0.0528$ | –           |
|       | PW m         | PWM h        | PWM p       |
| PWE m | –            | $U = 39.50$  | $U = 43.00$ |
| PWE h | $P = 0.0026$ | –            | $U = 8.500$ |
| PWE p | $P = 0.2867$ | $P = 0.0010$ | –           |
|       | DWE m        | DWE h        | DWE p       |
| DWE m | –            | $U = 65.00$  | $U = 55.50$ |
| DWE h | $P = 0.0513$ | –            | $U = 23.00$ |
| DWE p | $P = 0.7962$ | $P = 0.0185$ | –           |

Explanations: TL – total length; PL – length of protomerite; DL – length of deutomerite; PWE – width of protomerite at equatorial axis; DWE – width of deutomerite at equatorial axis; m – *H. micans*, h – *H. humulinus*, p – *H. pini*;  $P$  – level of significance.

Numerous young trophozoites (Fig. 1) were noted.

Gregarines were found in *Hemerobius humulinus*, *H. micans*, *H. marginatus*, *Micromus variegatus* and *Symphorobius pygmaeus*. In some trophozoites the epimerite was noted (Figs 2–4). Associations were bi-associative, caudofrontal (Fig. 5) and were preceded by syzygy (Fig. 6).

#### Measurements of gregarines

In two hosts, namely *Hemerobius micans* and *H. humulinus*, gregarines occurred in high numbers. Gregarines obtained from the two host species were measured and the results compared with the measurements of the parasite in nominant species – *H. pini* (Geus, 1969).

Measurements of solitary gregarines are given in Table 1. As Mann Whitney  $U$ -test revealed, the solitary gregarines occurring in three hemerobiid species significantly do not differ according to their total length and length of deutomerite (Table 2). There are no significant differences between the solitary gregarines occurring in *H. pini* and *H. micans* when length of protomerite, length of deutomerite, width of protomerite at equatorial axis and width of deutomerite at equatorial axis are compared (Table 2). Gregarines in *H. humulinus* have significantly longer protomerite than in *H. micans* and *H. pini* ( $P < 0.05$ ).

Measurements of gregarines in associations are presented in Table 3. Mann Whitney  $U$ -test revealed that the gamonts in associations in *H. micans* are similar

Table 3. Measurements (in  $\mu\text{m}$ ) of gamonts in association in three host species. Data from *H. pini* were obtained from Geus (1969).

| <i>Hemerobius micans</i>                 |         |        |       |        |       |        |        |
|--|---------|--------|-------|--------|-------|--------|--------|
| Statistical measure                      |         | TL     | PL    | DL     | PWM   | DWE    | DWM    |
| Pr                                       | Mean    | 141.40 | 26.67 | 114.8  | 44.16 | 111.50 | 111.70 |
|  | SD      | 33.46  | 5.89  | 30.52  | 9.05  | 35.84  | 35.92  |
|  | Minimum | 79.40  | 14.00 | 63.80  | 25.00 | 41.80  | 41.80  |
|  | Maximum | 185.70 | 32.30 | 157.00 | 55.20 | 158.0  | 158.00 |
| Sat                                      | Mean    | 132.30 | 18.37 | 114.00 | 48.13 | 110.50 | 112.00 |
|  | SD      | 35.38  | 3.95  | 31.89  | 12.29 | 38.57  | 38.90  |
|  | Minimum | 73.00  | 12.30 | 59.10  | 23.40 | 35.50  | 35.90  |
|  | Maximum | 183.50 | 26.30 | 157.20 | 69.00 | 152.20 | 156.50 |
| <i>Hemerobius humulinus</i>              |         |        |       |        |       |        |        |
| Statistical measure                      |         | TL     | PL    | DL     | PWM   | DWE    | DWM    |
| Pr                                       | Mean    | 87.79  | 16.77 | 71.02  | 29.21 | 56.53  | 56.95  |
|  | SD      | 9.42   | 2.61  | 9.48   | 3.90  | 13.56  | 13.54  |
|  | Minimum | 70.60  | 13.00 | 54.00  | 20.30 | 37.90  | 37.90  |
|  | Maximum | 99.50  | 22.90 | 84.00  | 35.00 | 79.00  | 79.00  |
| Sat                                      | Mean    | 91.39  | 13.63 | 77.75  | 28.89 | 47.71  | 48.57  |
|  | SD      | 8.10   | 1.53  | 7.58   | 4.70  | 13.84  | 13.13  |
|  | Minimum | 81.80  | 10.20 | 67.80  | 23.80 | 30.50  | 33.30  |
|  | Maximum | 105.40 | 16.20 | 89.70  | 36.70 | 72.00  | 72.00  |
| <i>Hemerobius pini</i> (after Geus 1969) |         |        |       |        |       |        |        |
| Statistical measure                      |         | TL     | PL    | DL     | PWM   | DWE    | DWM    |
| Pr                                       | Mean    | 139.20 | 24.50 | 114.80 | 37.17 | 75.17  | –      |
|  | SD      | 15.07  | 3.15  | 12.04  | 3.76  | 11.48  | –      |
|  | Minimum | 123.00 | 21.00 | 102.00 | 33.00 | 56.00  | –      |
|  | Maximum | 162.00 | 30.00 | 132.00 | 42.00 | 89.00  | –      |
| Sat                                      | Mean    | 130.00 | 17.83 | 112.20 | 39.17 | 67.67  | –      |
|  | SD      | 15.75  | 1.60  | 15.09  | 6.88  | 7.94   | –      |
|  | Minimum | 111.00 | 17.00 | 94.00  | 32.00 | 53.00  | –      |
|  | Maximum | 146.00 | 21.00 | 129.00 | 52.00 | 74.00  | –      |

Explanations: TL – total length; PL – length of protomerite; DL – length of deutomerite; PWM – maximum width of protomerite; DWE – width of deutomerite at equatorial axis; DWM – maximum width of deutomerite; Pr – primite; Sat – satellite. Number of measurements: *H. micans*:  $n = 15$ ; *H. humulinus*:  $n = 15$ ; *H. pini*:  $n = 6$ .

sized as in *H. pini* (Table 4), and the gregarines in *H. humulinus* are significantly smaller than in *H. micans*.

Measurements of two syzygies in *H. micans*: length of primite: min – max: 82–142  $\mu\text{m}$ ; length of satellite: min – max: 81.5–101  $\mu\text{m}$ . Measurements of five syzygies in *H. humulinus*: length of primite: min – max: 46.8–62.8  $\mu\text{m}$ , mean: 54.8  $\mu\text{m}$ ; length of satellite: min – max: 51.8–69.9  $\mu\text{m}$ , mean: 63.6  $\mu\text{m}$ .

#### Phenology

Individuals of *Hyalospora hemerobii* infecting *H. micans* occurred from June to September in 2011 and from June to October in 2013 (Fig. 7). The lowest infection rate in 2011 was noted in July (10%) and in 2013 it was in June (26.7%). The highest infection rate in 2011 was detected in June and August (30%) and in 2013 it was in September (86.7%). The average number of solitary gregarines per infested host varied in 2011 from 13.7 to 165 individuals/host (Fig. 8A) and in 2013 from 16 to 220 individuals/host (Fig. 8B). The number of associa-

tions found in all inspected hosts was 8 in 2011 and in 2013.

#### Discussion

The family Hirmocystidae Grassé, 1953 sensu Clopton (2002) comprises 16 genera and more than 70 species. Genus *Hyalospora* Schneider, 1875 contains 10 named species and these were noted from the following insect orders: Archaeognatha, Zygentoma, Psocoptera, Heteroptera, Neuroptera, and Coleoptera (Geus 1969; Larson et al. 1992; Hoshide & Sacho 1994; Devetak et al. 2013).

*Hyalospora hemerobii* was described for the first time in *Hemerobius pini* in Germany (Geus 1969). Since then, it has been reported only once, from *H. humulinus* in Slovenia (Devetak et al. 2013). In surveyed localities in Slovenia *H. pini* did not occur. Searching for gregarines in other potential hosts revealed that *H. hemerobii* seems to be a common species occurring in brown lacewings (Hemerobiidae). Even though the

Table 4. Testing the statistical data of gamonts in association with three host species with Mann Whitney *U*-test.

|          |                   |                   |                   |
|----------|-------------------|-------------------|-------------------|
| PrTL m   | PrTL m            | PrTL h            | PrTL p            |
| PrTL h   | –                 | <i>U</i> = 20.00  | <i>U</i> = 42.00  |
| PrTL p   | <i>P</i> = 0.0001 | –                 | <i>U</i> = 0.00   |
|          | <i>P</i> = 0.8457 | <i>P</i> = 0.0005 | –                 |
| PrPL m   | PrPL m            | PrPL h            | PrPL p            |
| PrPL h   | –                 | <i>U</i> = 25.00  | <i>U</i> = 27.50  |
| PrPL p   | <i>P</i> = 0.0003 | –                 | <i>U</i> = 1.00   |
|          | <i>P</i> = 0.1857 | <i>P</i> = 0.0007 | –                 |
| PrDL m   | PrDL m            | PrDL h            | PrDL p            |
| PrDL h   | –                 | <i>U</i> = 18.50  | <i>U</i> = 44.00  |
| PrDL p   | <i>P</i> = 0.0001 | –                 | <i>U</i> = 0.0000 |
|          | <i>P</i> = 0.9690 | <i>P</i> = 0.0005 | –                 |
| PrDWE m  | PrDWE m           | PrDWE h           | PrDWE p           |
| PrDWE h  | –                 | <i>U</i> = 29.00  | <i>U</i> = 18.00  |
| PrDWE p  | <i>P</i> = 0.0006 | –                 | <i>U</i> = 13.50  |
|          | <i>P</i> = 0.0392 | <i>P</i> = 0.0158 | –                 |
| SatTL m  | SatTL m           | SatTL h           | SatTL p           |
| SatTL h  | –                 | <i>U</i> = 34.00  | <i>U</i> = 38.00  |
| SatTL p  | <i>P</i> = 0.0012 | –                 | <i>U</i> = 0.0000 |
|          | <i>P</i> = 0.6129 | <i>P</i> = 0.0005 | –                 |
| SatPL m  | SatPL m           | SatPL h           | SatPL p           |
| SatPL h  | –                 | <i>U</i> = 25.50  | <i>U</i> = 40.00  |
| SatPL p  | <i>P</i> = 0.0003 | –                 | <i>U</i> = 0.0000 |
|          | <i>P</i> = 0.7259 | <i>P</i> = 0.0005 | –                 |
| SatDL m  | SatDL m           | SatDL h           | SatDL p           |
| SatDL h  | –                 | <i>U</i> = 34.50  | <i>U</i> = 40.00  |
| SatDL p  | <i>P</i> = 0.0013 | –                 | <i>U</i> = 0.0000 |
|          | <i>P</i> = 0.7259 | <i>P</i> = 0.0005 | –                 |
| SatDWE m | SatDWE m          | SatDWE h          | SatDWE p          |
| SatDWE h | –                 | <i>U</i> = 27.00  | <i>U</i> = 18.00  |
| SatDWE p | <i>P</i> = 0.0004 | –                 | <i>U</i> = 10.00  |
|          | <i>P</i> = 0.0392 | <i>P</i> = 0.0073 | –                 |

Abbreviations: PrDL – length of deutomerite of the primate; PrDWE – width of deutomerite of the primate at equatorial axis; PrPL – length of protomerite of the primate; PrTL – total length of the primate; SatDL – length of deutomerite of the satellite; SatDWE – width of deutomerite of the satellite; SatPL – length of protomerite of the satellite; SatTL – total length of the satellite; m – *H. micans*, h – *H. humulinus*, p – *H. pini*.

general morphology under the light microscope looked similar in different hosts, the differences in size show that the identities of the species in different hemerobiid species should be confirmed by means of molecular methods. To elucidate potential presence of the gregarine species in other families of the order Neuroptera, further surveys should be conducted. There are still open questions, e.g., whether the gregarines have any impact on the host organisms and therefore, have an effect on the host population.

We have no explanation how do the hemerobiids get infected. Brown lacewings feed on soft bodied arthropods like aphids, therefore one possibility to ingest the parasite is that oocysts are ingested as brown lacewings feed on aphids carrying the oocysts on their bodies and the another possibility is drinking oocyst-contaminated water.

In original description provided by Geus (1969) trophozoites, solitary gamonts, associations and gametocysts are figured. In this paper syzygy is illustrated and measurements provided for the first time.

The present study represents the first investigation

of seasonal patterns in the occurrence of *H. hemerobii* parasitizing *H. micans*. The infection rate was substantially lower in 2011 than in 2013, when it reached maximal value with 86.7% in host insects collected in September. A possible explanation for low infection rate in 2011 could be the fact that spring and summer in that year were drier than during normal season, but this hypothesis needs to be verified.

One can only speculate on the reasons for the seasonal occurrence of the gregarines in hemerobiids. In damselflies density of gregarines is in a high positive correlation with density of hosts (Forbes et al. 2012). Locklin & Vodopich (2010) provide possible explanation for strong seasonality of gregarine parasitism in dragonflies. The possible reason for low parasite levels at the beginning of the season and high levels towards the end are oocyst viability and/or oocyst accessibility to hosts (Locklin & Vodopich 2010). Adult dragonflies were present from April to November, but adults occurring from September to November had highest probabilities of infection and carried more parasites. This mode of seasonal occurrence suggests that insect exposure to

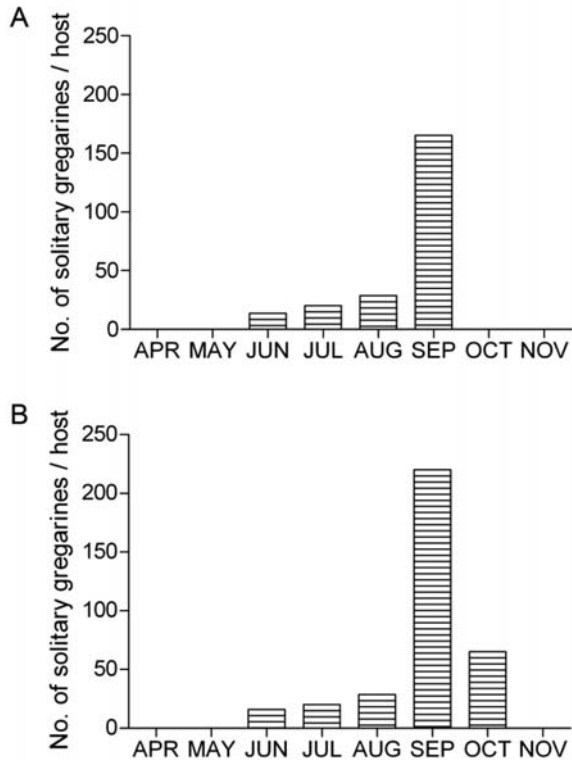


Fig. 8. Seasonal changes of the average number of solitary gregarines per infested host *Hemerobius micans*: A: 2011 ( $n = 10$  host individuals/month); B: 2013 ( $n = 30$  host individuals/month).

viable and accessible oocysts increased with the season (Locklin & Vodopich 2010). Brown lacewings occurring at the end of the season carried more gregarines, thus the exposure to accessible oocysts increased with the season, therefore the possible explanation for increasing parasite level towards the end of the season in *H. micans* is oocyst accessibility to hosts.

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