A “clean” alien species? Parasites of the invasive ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae)

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**Abstract.** The multicoloured Asian ladybird *Harmonia axyridis* is an invasive insect that can negatively influence biodiversity and human economy in invaded areas. According to the enemy release hypothesis, invasive alien species are often little affected by parasites and other enemies. We studied the prevalence of common parasites of insects infesting and infecting *H. axyridis* in NW Poland. A large sample of 2351 individuals was collected and divided into two groups: 1180 beetles were dissected and examined for the presence of eugregarines, nematodes and Laboulbeniales fungi, and 751 were checked for phoretic mites. Our results show that *H. axyridis* is indeed parasitized infrequently. The prevalence of eugregarines and nematodes was very low (1.5% and 0.4%, respectively). No specimens of Laboulbeniales or phoretic mites were found. Our study indicates that in NW Poland *H. axyridis* is rarely infested or infected by parasites. This paper reports for the first time the infection of *H. axyridis* by the eugregarine *Gregarina barbarara*.

**INTRODUCTION**

One of the reasons for rapid and successful invasions by non native species might be their escape from natural enemies – “enemy release hypothesis” (Torchin et al., 2003; Colautti et al., 2004; Heger & Jeschke, 2014). Many studies have shown that invaders are less parasitized and more prone to attack from predators in invaded areas than native species, and this is recorded for many species of plants (Keane & Crawley, 2002; Mitchell & Power, 2003) and animals (Torchin et al., 2002; Carroll et al., 2005). Apart from the studies supporting the enemy release hypothesis, there are also many studies questioning it (Heger & Jeschke, 2014). There is some evidence, however, that the enemy release hypothesis might partly explain one of the most rapid and widespread insect invasions of recent years, namely that of the multicoloured Asian ladybird *Harmonia axyridis* (Roy et al., 2011; Haelewaters et al., 2017).

The main goal of this study was to search for common parasites of ladybirds infesting *H. axyridis* in an invaded area (NW Poland): eugregarines, fungi and mites. These organisms are often found to infect/infest insects, so they may decrease insect survival and play an important role in the worldwide spread of *H. axyridis*. However, in this study we did not investigate parasitoids, such as *Dinocampus coccinellae* and *Phalacrotophora* spp., which may also contribute to the suppression of the invader (Ware et al., 2010).

**Eugregarines**

Eugregarines are protozoans that commonly parasitize insects (Clopton et al., 2002). Gregarine infections may decrease insect fitness and percentage survival (Logan et al., 2012; Bollatti & Ceballos, 2014), and massive infestation of the gut may be even lethal for hosts (Lipa, 1967). A laboratory study of the Blattodea has shown that infestation by gregarines predisposes the hosts to parasitism by other, more dangerous microorganisms (Lopes & Alves, 2005). Gregarines vary in specificity, some infest hosts from closely related taxonomic groups but most have a broad range of hosts (Geus, 1969). In the guts of Coccinellidae, 12 species of gregarines, with host spectra ranging between 1 and 9 coccinellid species, are recorded (Ceryngier et al., 2012). Species with a broad spectrum of hosts include *Gregarina barbarara* (7 hosts) and *G. katherina* (9 hosts). Both of these species of gregarine occur in the USA and the central part of Europe (Ceryngier et al., 2012). However, there is a lack of published data on gregarines infecting *H. axyridis* (Roy et al., 2011).
Nematodes

The Coccinellidae are mostly infested by nematodes of the families Allantonematidae and Mermithidae (Ceryngier et al., 2012). Nematodes penetrate into the body cavity of coccinellids through tracheae and soft parts of the cuticle. Transmission may be facilitated when coccinellids aggregate during winter (Ceryngier & Hodek, 1996). Consequences of infestation vary from general weakness to death. Parasitylenchus coccinellinae retards maturation of the ovaries and decreases fat supplies (Ceryngier & Hodek, 1996). Howardula sp. causes a reduction in the body size of its hosts, larvae of Adalia bipunctata (El-Hariri, 1966). Infestation by merismid nematodes is fatal for ladybirds, as the nematodes destroy organs of the insects, resulting in death 17 days after infestation (Rhamhalinghan, 1987b, a). Laboratory studies have shown lethal effects of Steinernema carpocapsae on H. axyridis (Lemire et al., 1996), but there are no reports of infections by this nematode in the field. European populations of H. axyridis are infested by Parasitylenchus bifurcatus (Poinar & Steenberg, 2012; Haelewaters et al., 2017).

Fungi

The importance of fungi that could keep the population of insects at a low level is well known and described in many papers (Nikiforov, 1970; Vorontsov, 1995; Tarasco et al., 1997). Many fungi are considered to be biological control agents of insects damaging agricultural, horticultural and forest crops (Inglis et al., 2001; Pell et al., 2001). Coccinellids in their natural environment are infested by many species of fungi e.g. endoparasitic Hesperomyces virescens (Haelewaters et al., 2014), and entomopathogenic Beauveria bassiana (Cottrell & Shapiro-Ilan, 2003), Metarhizium anisopliae (Ginsberg et al., 2002), Isaria farinosa, I. fumosorosea, and Lecanillicillum lecanii (Ceryngier & Hodek, 1996).

Majority of the knowledge about fungi in ladybirds is from studies on fungi as biopesticides in which coccinellids are non-target insects (Pingel & Lewis, 1995; Todorova et al., 1996; Roy & Pell, 2000; Pell & Vandenberk, 2002). H. axyridis is known as a host of Hesperomyces virescens in invaded areas in the Americas, Europe and South Africa (Haelewaters et al., 2017). Studies on B. bassiana have shown that H. axyridis is rather resistant to this pathogen (Cottrell & Shapiro-Ilan, 2003; Roy et al., 2008). Vilecinskias et al. (2013) suggest that this resistance may be related to a variety of antimicrobial peptides synthesized by H. axyridis.

Mites

Mites associated with ladybirds may be either parasitic or phoretic (Ceryngier et al., 2012). Phoresy is a specific nonparasitic relationship, wherein the mites (Acarina) use different taxonomic groups of animals only for transport (Gwiazdowicz, 2000). Nonetheless, phoresy is a strong stressor for the host insect (Gudowska et al., 2015). Moreover, mites could be vectors of a wide range of bacterial, fungal and protozoan pathogens or viruses (Poinar & Poinar, 1998). Thus determining the presence of these organisms complements the search for parasites and pathogens of H. axyridis. In the context of phoretic relationships, H. axyridis has never been investigated. Other coccinellids carry some phoretic species of mites (Hurst et al., 1997).

MATERIAL AND METHODS

Collection of material

The beetles used in this study were collected on a wind farm located west of Golanez in the Wielkopolska region in NW Poland (52°57´N, 17°14´E). The wind farm is in an intensive agricultural landscape dominated by oilseed rape and corn fields. Insects were collected in October and November 2015 on 16 wind turbines (for details of habitat see Dudek et al., 2015). The total of 2351 individuals of H. axyridis was divided randomly into 2 groups. The largest group of 1180 individuals was used for searching for eugregarines, nematodes and Laboulbeniales fungi, and the remaining 751 beetles were checked for mites.

Laboulbeniales, eugregarines and nematodes

The group of 1180 individuals of H. axyridis were sexed, checked under a stereomicroscope for the presence of Laboulbeniales, and then their guts were dissected and searched for parasites and pathogens. The digestive tract was dissected in a saline solution and checked under a microscope for the presence of gregarines and nematodes. The total number of gregarines and nematodes in the gut contents was recorded. Gregarines were identified to species on the basis of microscopic measurements, according to Geus (1969) and Lipa (1967). The nematodes were determined using the online key by Nguyen (2010). It is important to know whether the nematodes infecting coccinellids live inside their body cavities, thus searching the digestive tract may detect only individuals penetrating through the gut wall.

Mites

Sample of ladybirds was stored in 75% ethanol before checking for the presence of mites. Both the body surface and the spaces under the elytra were examined under a stereomicroscope.

Statistical analyses

Statistical analyses of the prevalence of gregarines and nematodes, confidence limits, and infestation levels were determined using Quantitative Parasitology 3.0 software (Rózsa et al., 2000), and chi-square tests using SPSS ver. 21 software.

RESULTS

The large sample of overwintering H. axyridis ladybirds collected from the wind farm was strongly female-biased: 778 females and only 402 males (chi-square with Yates correction \( \chi^2 = 60.81, p < 0.0001 \)). Only gregarines and nematodes (and no Laboulbeniales and mites) were recorded.

Gregarines were found in 18 specimens (11 males and 7 females). Total prevalence was 1.5%, with a median number of 49 (90% confidence interval: 5–110) individuals per host, and dispersion index (variance/mean ratio) of 249.84. Infestation with gregarines was sex-biased, with a higher prevalence in males (2.7% with a median number of 60 individuals per host vs. 0.9% with a median number of 21, respectively; chi-square with Yates correction \( \chi^2 = 4.605, p = 0.031 \)). All the gregarines were identified as Gregarina barbarara Watson, 1915 (Fig. 1).
Nematodes were found in 5 specimens. Infestation was strongly sex-biased, as the parasites were present only in male ladybirds. Nematodes were found in 0.4%, with a median number of 10 individuals per host (90% confidence interval: 3–14), and a variance/mean ratio of 10.58. All the nematodes belonged to the genus *Parasitylenchus* Wachek, 1955 (Fig. 2).

**DISCUSSION**

The population of ladybirds studied was strongly female biased. This strongly skewed sex ratio might be related to the occurrence of male-killing bacteria, like *Wolbachia* spp. (Hurst et al., 1999b), *Rickettsia* spp. (Werren et al., 1994) and *Spiroplasma* spp. (Hurst et al., 1999a), however, their prevalence in the population studied was low (Dudek et al., 2017).

Although the gregarine *G. barbarara* was found only in 1.5% of the *H. axyridis* sampled, this finding is the first solid evidence of a gregarine infecting *H. axyridis* (Roy et al., 2011). The three-fold higher prevalence and infestation intensity in males than females are hard to explain. The low prevalence of gregarines and a scarcity of previous data on the infestation of the multicoloured Asian ladybird (only Kuznetsov, 1997, which mentions that *H. axyridis* is infected by *Gregarina* in the Far East of Russia) may indicate a recent adaptation of these pathogens to this non-native species or fluctuations in the prevalence level. Further studies are needed to explain this phenomenon.

The nematodes were identified as *Parasitylenchus* sp. Members of this genus are already known as parasites of *H. axyridis* in several European countries (Harding et al., 2011; Poinar & Steenberg, 2012; Haelewaters et al., 2017). The recorded proportion of infected ladybirds was low (0.4%). However, as only digestive tracts and not body cavities were examined, the actual nematode prevalence was certainly higher.

During this study, no infection by the Laboulbeniales fungi was recorded. This is surprising because studies on some American and European populations show a high prevalence of *H. virescens* in *H. axyridis* (Garcés & Williams, 2004; Naleta & Weir, 2007; Ceryngier & Twardowska, 2013; Haelewaters et al., 2017), and this prevalence tends to be highest in winter [55–60% (Garcés & Williams, 2004) and 62% (Naleta & Weir, 2007)].

Neither parasitic nor phoretic mites were recorded on the individuals of *H. axyridis* examined, so it can be concluded that the mites do not use, or rarely use this ladybird species as a host or a means of transport. Lack of reports of observations of phoretic mites on *H. axyridis* in the literature indicates that this coccinellid is avoided of them, like majority of the other species of ladybirds.

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