

Rapid Communication

Harmonia axyridis (Pallas, 1773) (Coleoptera: Coccinellidae) and its parasite in south-western Bulgaria and northern Greece

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Abstract

Samples of the invasive harlequin ladybird (*Harmonia axyridis*) were collected in June 2017 in four localities in western Bulgaria and three localities in northern Greece. Established (reproducing) populations of *H. axyridis* were found in each of the surveyed localities and were commonly infected with a fungal ectoparasite, *Hesperomyces virescens* (Ascomycota: Laboulbeniales). This parasite was recorded on 33% and 22% of the collected old-generation *H. axyridis* males and females, respectively. Our findings represent the first records of established *H. axyridis* populations for Greece and the first records of *H. virescens* for Bulgaria.

Key words: Balkan Peninsula, harlequin ladybird, *Hesperomyces virescens*, range expansion

Introduction

The harlequin ladybird, *Harmonia axyridis*, is native to the temperate climate areas in Asia; some records of its occurrence in the subtropics and tropics (southern China, northern Vietnam) need confirmation as they may refer to the sibling species, *H. yedoensis* (Takizawa) (Orlova-Bienkowskaja et al. 2015). Since the late 1980s, *H. axyridis* has spread and become invasive in North and South America, Europe and southern Africa, and started to re-colonize Asia (Roy et al. 2016). In 2016 established populations were found in the North Island of New Zealand (NatureWatch NZ 2017).

In Europe, the invasive populations of *H. axyridis* have been recorded since 1999 (Brown et al. 2008). While colonization of most of Europe proceeded rapidly, northern and southern peripheries of the continent still seem to be largely free of *H. axyridis*, probably because of climatic constraint (Roy et al. 2016). One of the European regions relatively slowly colonized by *H. axyridis* is the Balkan Peninsula.

First records of the harlequin ladybird in this region are from 2008, when it was found in Slovenia (Kus Veenvliet and Veenvliet 2009), Croatia (Mičetić Stanković et al. 2011), Serbia (Thalji and Stojanović 2008), Romania (Ruicănescu and Alexandru 2009) and Bulgaria (Tomov et al. 2009). In 2009 *H. axyridis* was recorded in Montenegro for the first time (Gligorović et al. 2016), in early 2010 (January and February) in Bosnia and Herzegovina (Kulijer 2010), in 2011 in the European part of Turkey (Aysal and Kivan 2014), in 2012 in Albania (Ibrahimi et al. 2016) and in 2015 in Macedonia (Kulijer 2016). Between 1994 and 2002, mass introductions of *H. axyridis* were made in southern Greece, but the released beetles failed to establish (Kontodimas et al. 2008). Until now, no more recent data on the occurrence of *H. axyridis* in Greece have been published.

The spread of *H. axyridis* after invasion is believed to contribute to the increase in numbers and subsequent expansion of some ladybird parasites. For example, since the early 2000s an ectoparasitic fungus *Hesperomyces virescens* Thaxter (Ascomycota: Laboulbeniales) has been recorded on *H. axyridis* in

many localities in North America, Europe, South America and southern Africa, with the prevalence often exceeding 50%. To a lesser extent, the invasive populations of *H. axyridis* are infected with the mite *Coccipolipus hippodamiae* (McDaniel & Morrill) (Acari: Podapolipidae) and the nematode *Parasitylenchus bifurcatus* Poinar & Steenberg (Nematoda: Allantonematidae) (Ceryngier and Twardowska 2013; Haelewaters et al. 2017). As yet, *C. hippodamiae* and *P. bifurcatus* have not been reported infecting *H. axyridis* in its native range. However, *H. virescens* was found as a rare parasite of *H. axyridis* in Asia. While examining Coccinellidae from museum collections, Haelewaters et al. (2014) revealed two infected specimens of *H. axyridis* that had been collected in the 1930s in Sichuan (China).

All parasites mentioned above infect only adult ladybirds. Although the mode of transmission of one of them, the endoparasitic *P. bifurcatus*, remains unknown, the ectoparasites, *H. virescens* and *C. hippodamiae*, are mainly sexually transmitted. Newly-emerged ladybird adults must mature before they can be infected, usually through sexual contact with an infected individual of the old generation (Ceryngier et al. 2012).

Our survey aimed at assessing the occurrence of *H. axyridis* and its ectoparasites in the south-western part of Bulgaria and northern part of Greece.

Material and methods

Between 14th and 19th of June 2017, we collected samples of *H. axyridis* in four localities in western Bulgaria and three localities in northern Greece (Table 1). We used standard methods of collecting ladybirds, such as shaking the insects down from various trees and shrubs on a 1 m × 1 m beating sheet, sweep-netting of herbaceous vegetation and directly picking the observed beetles.

As our survey was conducted at the time of emergence of a new generation *H. axyridis* adults, we distinguished between freshly emerged and old adults based on the colour intensity and the hardness of their elytra. Not fully coloured adults were considered very recently emerged and, as such, not collected, although their numbers were noted. Adults with fully coloured elytra were collected and their age was assessed in the laboratory. Beetles with elytra yielding when pressed with a dissecting needle were classified as young (newly emerged), and those with not yielding (inflexible) elytra, as the old generation adults.

Old generation *H. axyridis* adults were screened for parasite infection under a stereomicroscope. Ladybird body surface was examined for fungal infection and the underside of the elytra for presence of mites.

Table 1. Details of the sampling localities of *Harmonia axyridis* in Bulgaria and Greece.

Country	Locality	Coordinates
Bulgaria	Sofia	42°42'N; 23°19'E
	Rila	42°07'N; 23°08'E
	Blagoevgrad	42°01'N; 23°05'E
	Melnik	41°31'N; 23°23'E
Greece	Promachonas	41°22'N; 23°21'E
	Charopo	41°15'N; 23°22'E
	Asprovalta	40°45'N; 23°43'E

Results

We detected *H. axyridis* adults and juveniles (larvae and pupae) in all the localities sampled. Three colour forms, *succinea*, *spectabilis*, and *conspicua*, were found, with the former being most common (Table 2).

No parasitic mites were found on *H. axyridis* adults. However, the laboulbenian fungus, *H. virescens*, commonly occurred on the old-generation beetles. It was recorded in the samples from each locality except Melnik, where old-generation adults were low in number at the time of sampling (Table 3). In all samples pooled, 33% of males and 22% of females were infected, but the difference was not significant (chi-square test: chi-square = 1.78, df = 1, P = 0.18).

Discussion

Although some of our samples were small, all of them contained juvenile stages of *H. axyridis* indicating that the ladybird has already established in the localities included in the survey. The presented data show that the current southern limit of *H. axyridis* distribution in the Balkan Peninsula has reached at least the northern coast of the Aegean Sea in Greece (Asprovalta). Further investigations in Greece are needed to determine extent of the spread of *H. axyridis* to the south of the peninsula. Published records on the occurrence of *H. axyridis* in the Eastern Mediterranean region do not only concern the Balkan Peninsula. The species was also reported from the Anatolian part of Turkey (Bukejs and Telnov 2015; Görür et al. 2015) and even the Douma District of Syria (latitude of about 33°N) (Abo Kaf et al. 2008). This might suggest that the invasive populations of *H. axyridis* have adapted to dry and hot Mediterranean summers.

The frequent occurrence of *H. virescens* on the Bulgarian and Greek *H. axyridis* confirms the previous observations that *H. axyridis* is especially susceptible to *H. virescens* and contributes to its geographical dispersal and increase in numbers (Ceryngier and Twardowska 2013; Haelewaters et al. 2017). Although *H. virescens* is a relatively polyphagous

Table 2. Numbers of *Harmonia axyridis* larvae, pupae and adults of different colour forms collected in individual sampling localities.

Locality	larvae	pupae	adults		
			<i>succinea</i>	<i>spectabilis</i>	<i>conspicua</i>
Sofia	10	–	17	1	1
Rila	24	6	26	1	1
Blagoevgrad	75	15	110	5	5
Melnik	22	9	4	–	–
Promachonas	–	1	4	1	–
Charopo	5	2	14	1	1
Asprovalta	28	–	7	1	1

Table 3. Occurrence of *Hesperomyces virescens* on female and male adults of the old-generation *Harmonia axyridis*. N – number of collected old-generation *H. axyridis* individuals, n_{paras.} – number of parasitized old-generation *H. axyridis* individuals.

Locality	Females		Males		Total	
	N	n _{paras.}	N	n _{paras.}	N	n _{paras.}
Sofia	8	1	4	2	12	3
Rila	18	5	6	3	24	8
Blagoevgrad	63	14	34	11	97	25
Melnik	3	0	–	–	3	0
Promachonas	1	1	2	0	3	1
Charopo	6	1	–	–	6	1
Asprovalta	2	1	2	1	4	2

parasite known to infect about 30 ladybird species (Ceryngier et al. 2012; Haelewaters et al. 2017), *H. axyridis* is nowadays its most common host, not only in Europe, but also in North and South America, and southern Africa (Ceryngier and Twardowska 2013; Haelewaters et al. 2016, 2017). In the Balkan Peninsula, the association *H. axyridis* – *H. virescens* was reported from Croatia (Ceryngier et al. 2013), and recently it was also observed in Montenegro (1st of May 2017, Bistrice: 42°18'N; 19°10'E) (S. Karjalainen, unpubl., see photograph in Karjalainen 2017). *Hesperomyces virescens* has not previously been reported from Bulgaria. In Greece, it was found in 2001 on two ladybird species other than *H. axyridis*, *Coccinula quatuordecimpustulata* (L.) and *Tytthaspis sedecimpunctata* (L.) (Castaldo et al. 2004).

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