LUCA ESZTER BALOG^a - OLEKSANDR HOLOVACHOV^b - JÚLIA KATALIN TÖRÖK^a

PREVALENCE AND DISTRIBUTION OF DIFFERENT NATURAL ENEMIES IN THE HARLEQUIN LADYBIRD *HARMONIA AXYRIDIS* (COLEOPTERA COCCINELLIDAE)

a - Dept. Systematic Zoology and Ecology, Eötvös Loránd University, Pázmány Péter sétány 1C, Budapest 1117, Hungary

b - Dept. Zoology, Swedish Museum of Natural History, Frescativägen 40, 114 18 Stockholm, Sweden

Corresponding Author: Luca Eszter Balog, e-mail: balogluca@caesar.elte.hu

Balog L.E., Holovachov O., Török J.K. - Prevalence and distribution of different natural enemies in the harlequin ladybird *Harmonia axyridis* (Coleoptera Coccinellidae).

The harlequin ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae) is one of the most common invasive alien species in the world, which has a global impact on biodiversity and agriculture, and causes economic loss and harm to humans. This insect can be infected by different natural enemies, but their interactions remain understudied. In the present study, 581 adult *Ha. axyridis* specimens were collected from 21 localities in Hungary with the help of citizen scientists and examined for natural enemies. The parasitic nematode *Parasitylenchus bifurcatus* (Nematoda: Allantonematidae) is reported in Hungary on the harlequin ladybird for the first time. The ladybirds were also infected by the ectoparasitic fungus *Hesperomyces virescens* (Ascomycota: Laboulbeniales) and parasitoid *Dinocampus coccinellae* (Hymenoptera: Braconidae). The nematode parasite was present in all localities where *Hesperomyces*-infected ladybirds appeared. The prevalence of recorded natural enemies varied by locality: for *P. bifurcatus* it ranged from 1% to 8%; for *He. virescens* from 2% to 36%; and for the parasitoid it was 4%. Besides, a statistically significant positive correlation was shown between the nematode and the fungus co-infection using Spearman's correlation. A study on the multiparasitism of harlequin ladybirds was not yet performed in Hungary. The study area is located between two distant areas in Europe where co-infections had already been observed, indicating that this is probably not a sporadic phenomenon, but that parasitism of the harlequin ladybird by multiple natural enemies might be prevalent throughout the area.

KEY WORDS: *Harmonia axyridis*; *Parasitylenchus bifurcatus*; *Hesperomyces virescens*; *Dinocampus coccinellae*; biological invasion; co-infection

INTRODUCTION

Today the harlequin ladybird *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) is the most common invasive ladybird species in the world. It is native to East Asia and was introduced in the USA starting in 1916, and in Western Europe during the early 1980s for biological control. *Harmonia axyridis* has been used against agricultural pests, such as *Aphis glycine* in soybean fields (KOCH, 2003; GARDINER and LANDIS, 2007), *A. spiraecola* in apple orchards (BROWN and MILLER, 1998), *Macrosiphum rosae* rose aphids (FERRAN *et al.*, 1996), pecan aphids (RICE *et al.*, 1998) and several citrus pests (KOCH, 2003). *Harmonia axyridis* is a highly effective predator of aphids and it has quickly spread globally during the last two decades (BROWN *et al.*, 2011; ROY *et al.*, 2016; CAMACHO-CERVANTES *et al.*, 2017; HILLER and HAELEWATERS, 2019), outcompeting local species (KATSANIS *et al.*, 2013). The harlequin ladybird is now considered a pest that can damage fruits and contaminate wine (KOCH and GALVAN, 2008; KOVACH, 2002).

When a new alien species is introduced into a new geographical area, this poses three scenarios with regard to the natural enemies it carries: (i) its natural enemies are co-introduced along with their host (co-invasion), (ii)

they follow the host into the new range after a given lag time (host pursuit), or (iii) while prevalent in the native range, they remain absent in the new area, and new natural enemies move from locally native hosts to the invasive species (host shifts) (PFLIEGLER *et al.*, 2018). Host shifts may also occur vice versa, from the invasive species to locally native species.

Not all introduced species cause biological invasion. However, *Ha. axyridis* spread very quickly to all parts of the world, and therefore is a suitable model organism to determine how certain species can be successful invaders (ROY and WAINBERG, 2008). The biological invasions of *Ha. axyridis* have significant impacts on biodiversity, agriculture, and the fruit industry, and they have caused economic loss and harm to humans (WAGNER and VAN DRIESCHE, 2010). The invasive success of *Ha. axyridis* can be explained partly by the predations on non-target species as well as by the intraguild predation competing for the same prey, consuming its competitors (PELL *et al.*, 2008; ROY *et al.*, 2016). Furthermore, *Ha. axyridis* has a superior immune system, which provides stronger resistance against parasites compared to native ladybird species. In particular, it can carry microsporidian pathogens, which are not tolerated by native ladybird species (VILCINSKAS *et al.*, 2015; VOGEL *et al.*, 2017). During winter, *Ha. axyridis* shows aggregation

behaviour and accumulates in man-made structures (NALEPA and KENNEDY, 2004), where it can rarely bite humans or cause allergic reactions in humans (KOVACH, 2002; GOETZ, 2007).

The citizen science method refers to the participation of non-professional volunteers in scientific research, most commonly in collecting and analysing data. This approach has been used in several parasitological surveys that involve the public in tackling issues that can directly affect them on the community level. Examples are investigations of the prevalence of pathogens of ticks (LAAKSONEN *et al.*, 2017; LERNOUT *et al.*, 2019; NIETO *et al.*, 2018) and monitoring the Asian tiger mosquito *Aedes albopictus* (BARTUMEUS *et al.*, 2018; PALMER *et al.*, 2017). More than 200 bioinvasion studies have utilized a citizen science approach (CRALL *et al.*, 2010). Several of these studies have focused on the presence of *Ha. axyridis* (COMONT and ASHBROOK, 2017; HILLER and HAELEWATERS, 2019), its regional distributional aspects (WERENKRAUT *et al.*, 2020), and tracking of its ectoparasitic fungus (HAELEWATERS *et al.*, 2019). Thus far, however, no citizen science-based study has examined the presence of the nematode parasites and co-infection of *Ha. axyridis* by different parasites.

The knowledge about the natural enemies of *Ha. axyridis* is limited, but many of its predators (bugs, lacewings, stink bugs, ladybirds, and spiders), parasites (nematodes, fungi, mites), parasitoids (wasps and flies) and pathogens (bacteria, protozoa, fungi) and viruses are known in its native and invaded area (CERYNGIER *et al.*, 2012, 2018; RIDDICK *et al.*, 2009; HAELEWATERS *et al.*, 2017; RIDDICK *et al.*, 2009). Parasites are multicellular organisms living in the host without causing death, in contrast to parasitoids which kill the host organism (HAELEWATERS *et al.*, 2017). The European populations of harlequin ladybirds are mainly infected by the ectoparasitic fungus *Hesperomyces virescens* (Ascomycota: Laboulbeniales), the nematode *Parasitylenchus bifurcatus* (Nematoda: Allantonematidae), and the mite *Coccipolipus hippodamiae* (Acari: Podapolipidae) (RAAK-VAN DEN BERG *et al.*, 2014). *Harmonia axyridis* is also reported as a host for different parasitoid fly larvae, such as Phoridae or Tachinidae, as well as for parasitoid wasps, most notably *Dinocampus coccinellae* (Hymenoptera: Braconidae) (CERYNGIER *et al.*, 2018).

Research of the natural enemies of *Ha. axyridis* is essential to generate baseline data to develop strategies for controlling its invasive populations. The interactions with natural enemies and their impact on the host could be fundamental for an understanding of the ecology of co-infections, which offers a possibility for future development of biological control. Simultaneous application of *He. virescens* and an entomopathogenic fungus, *Beauveria bassiana* or *Metarhizium brunneum*, causes increased mortality, offering promising biological control tools against *Ha. axyridis* (HAELEWATERS *et al.*, 2020). Thus far, however, interactions among different parasites of *Ha. axyridis* are not well understood. Previous studies have described co-infections of 1) *He. virescens* – *C. hippodamiae* (RIDDICK, 2010), 2) *He.*

virescens – *P. bifurcatus* (HAELEWATERS *et al.*, 2017; ORLOVA-BIENKOWSKAJA *et al.*, 2018), 3) all three parasites (*He. virescens* – *P. bifurcatus* – *C. hippodamiae*) and a parasitoid wasp (*D. coccinellae*) (RAAK-VAN DEN BERG *et al.*, 2014), 4) *He. virescens* and an entomopathogenic fungus, either *B. bassiana* or *M. brunneum* (HAELEWATERS *et al.*, 2020).

The first occurrence of *Ha. axyridis* in Hungary was detected in 2008 (MERKL, 2014). Since then, it spread throughout the country. In Hungary, thus far, only *He. virescens* has been observed as a natural enemy of *Ha. axyridis* (PFLIEGLER, 2014; HAELEWATERS *et al.*, 2017). Here, we screened Hungarian populations of *Ha. axyridis* for the presence of multiple natural enemies. Based on our observations and previous work, we also aim to determine whether any of the reported natural enemies may have potential biological control properties against *Ha. axyridis*.

MATERIALS AND METHODS

COLLECTION OF INSECTS AND NEMATODE ISOLATION

Adult *Ha. axyridis* were collected at 21 localities in Hungary between 1 October, 2019 and 20 January, 2020 (Fig. I, Table 1). The samples were collected through a citizen science campaign using a poster distributed on Facebook. In response to the call for participation, people sent in ladybirds from different parts of the country. Each collection locality was treated as a separate ladybird population for further analysis, except for the four collection sites close to each other in the Bakony Mountains, which were treated as one. The number of collected insects varied from site to site, from one to eighty. The hand-picked specimens were transported alive to the laboratory. All ladybirds were stored in a refrigerator at a temperature of about +4 °C until dissection. A total of 581 specimens were dissected for this study. From four different places, only one *Ha. axyridis* specimen was collected and sent. These were dissected but were not included in all statistical analyses. Before dissection, the outer surface of each ladybird was checked in search of ectoparasites. Next, the body cavity was opened and examined for endoparasites. While dissecting specimens under the stereo microscope, we calculated the intensity of nematode and braconid infection, the number of individuals inside one host (MARGOLIS *et al.*, 1982). This method allows for detecting the natural enemies at various collection sites and finding different types of them in one host specimen.

MORPHOLOGICAL IDENTIFICATION OF THE NEMATODE, FUNGUS, AND BRACONID WASP

The identification of the natural enemies was based on morphological features. For light microscopic examination, the isolated nematodes and fungi were fixed in formalin-alcohol-acetic acid (FAA; 20 parts 90% ethanol, six parts 40% formalin, 1 part glacial acetic acid, and 40 parts water). After fixation, the samples were dehydrated in a solution of 9 parts glycerol and 1 part

ethanol, followed by a glycerol coating. Morphological studies were carried out with a Zeiss Axioscope using transmitted light and DIC illumination. We identified *He. virescens* based on the description by De Kesel (DE KESEL, 2011), while for the identification of *P. bifurcatus* we used the description of Poinar and Steenberg (POINAR and STEENBERG, 2012).

The identification of the parasitoid wasp was based on the morphology of the adult. Braconid larvae were found during the dissection of the ladybirds. One imago has emerged from a stored dissected *Ha. axyridis* body after the investigation and was identified by Dr. Gergely Várkonyi (Finnish Environment Institute, Finland).

Photographs for the identification were taken using a DCM130 (1.3 M pixels) camera, and measurements were made with the aid of the ScopePhoto 3.1 software (ScopeTek). Photographs for the figure were taken using a ColorView II camera equipped with analySIS® 5.0 image-analytical software (Olympus Soft Imaging Solutions, Münster, Germany).

A map illustrating sampling sites was created using QGIS Desktop 3.4.14 program with GRASS 7.8.1 software (<https://qgis.org/en/site/>), with an open source map (ESRI World Topo <https://qms.nextgis.com/geoservices/519/>).

STATISTICAL ANALYSIS

The relationship between the parasitic nematode and the parasitic fungi infection was assessed using Spearman correlation based on the data of all 581 examined ladybird individuals. Differences in parasitic infection among the localities were examined with Kruskal–Wallis H test. In the Spearman correlation and the H test analyses, only the presence of parasites was taken into account without considering the intensity of infection. Data for 577 individuals were used for the H test statistical analysis, which did not include localities with one collected individual.

The prevalence of infection was analysed separately and only for localities that contained more than 15 examined insect specimens, that is why, the Pápa-Tapolcafé, the János Mountain, the Börzsöny Mountains,

Mountains) samples were not analysed. Besides, four localities in the Bakony Mountains (Güsz-pihenő, Kinizsi Pál Lookout Tower, Némethánya, and Miklós Pál Mountain) were geographically close to each other in the same habitat, thus, the collected ladybirds were considered as members of the same population. Prevalence for these four localities was the average of the separately calculated prevalences. Statistical analysis was performed by SPSS (IBM SPSS Statistics, Ver. 25) under the terms of the campus-licence at the Eötvös Loránd University's software base.

RESULTS

In 43 *Ha. axyridis* adults, three natural enemies, *He. virescens*, *P. bifurcatus*, and parasitoid larvae, one of them identified as *D. coccinellae*, were detected.

Hesperomyces virescens thalli were observed in different positions of the host integument, including mouthparts, pronotum, elytra, sternites, and legs (Fig. I). Fungal infections were found in 33 adult ladybirds from 11 localities. Next, juveniles, first-generation females, vermiform females, and subsequent generation females of *P. bifurcatus* were found in the hemocoel of 8 ladybird individuals from 5 different localities (Fig. III, Table 1.). Three male specimens were also found, but they were damaged during preparation and could not be identified with confidence – they were excluded from statistical analysis. The first, the subsequent, and the vermiform generations of females were usually present simultaneously within the same host. The intensity of the first-generation females was very low, with only 1-2 nematodes inside the two host individuals where they were found. The intensity of subsequent females varied from 5-30 per host individual, whereas the intensity of vermiform nematodes was higher, approximately from 15 to about 200 per ladybird host.

In six of 21 localities, *Ha. axyridis* specimens were infected only by *He. virescens*; in three localities, they were parasitized by both *He. virescens* and *P. bifurcatus*. In three localities, only one parasitoid larva was found inside the host individuals. One of the parasitoid larvae escaped from the ladybird, completed its development and the adult was identified based on morphology. At the population level, four populations exhibited fungal infection, whereas two presented fungus – nematode co-infection (regarding the three localities where co-infection took place, two belonged to the same Bakony Mountains population). In addition, ladybirds in one population were infected just by the parasitoid, while in another population, parasitic fungus and braconid co-infection occurred. Only one population (Budakeszi) was found where all three species of natural enemies were detected.

In total, out of 581 dissected beetles, 530 beetles were not infected, 11 individuals were infected with nematodes, four individuals hosted parasitoids, 36 individuals were infected with fungus, and only four individuals were co-infected by *He. virescens* and *P. bifurcatus* (Fig. III, Table 1.).

Fig. I – *Hesperomyces virescens* on *Harmonia axyridis* the Velence Mountains, and the Güsz-pihenő (Bakony

Fig. II – *Parasitylenchus bifurcatus*. Vermiform (infective) female (1); subsequent generation female (2); tail tip of the subsequent generation female (3); subsequent generation female. Developing eggs and larvae can be seen in the body cavity (4). Scalebars: 1,4 = 100 μ m, 2 = 200 μ m, 3 = 20 μ m,

The prevalences were different between the parasites and between the infected localities. *Parasitylenchus bifurcatus* infection showed prevalence between 1.3-8%, which is lower than the prevalence of the parasitic fungus. In *He. virescens* it varied between 2.2-36.4%.

Prevalence calculation was possible in only one braconid-infected population (Budakeszi), representing a prevalence of 4%. The prevalence of co-infected individuals was the lowest, comprising 1.6% (Table 1).

Table 1 - Examined *Harmonia axyridis* individuals, *Parasitylenchus bifurcatus*, and *Hesperomyces virescens* infection and prevalence per localities. Bakony Mountains' prevalence calculated from Bakony, Günszi-pihenő, Kinizsi Pál Lookout Tower, Németbánya and Miklós Pál Mountain localities' data (lines on a grey background)

Map code	Locality	Date	N (dissected <i>Ha. axyridis</i> individuals)	<i>P. bifurcatus</i>	<i>He. virescens</i>	<i>P. bifurcatus</i> and <i>He. virescens</i> co-infection	Parasitoid larvae
1	Budaörs	30.09.2019	1				
2	Budapest XI. Dayka Gábor Street 4.	09.10.2019	54				
3	Budapest XI. Hengermalom Street 3.	14.11.2019	1				
4	North East of Budapest (Town Göd)	03.10.2019	1				
5	South Hungary (Village Magyarszék)	13.10.2019	40				
6	Pápa – Tapolcafü	12.10.2019	15				1
7	Velence Mountain (Village Sukoró)	13.10.2019	15				
8	Szombathely	18.10.2019	75				
9	Sződ – Debegio Hill	01.10.2019	1				
10	Visegrád Mountains	01.10.2019	12				
11	Bakony, Günszi-pihenő	20.10.2019	11		1		
12	Budapest II.	20.01.2020	22		8		
	Prevalence				36.36%		
13	North Hungary (Village Borsodgeszt)	07.10.2019	30		2		
	Prevalence				6.67%		
14	János Mountain	14.10.2019	7		1		
15	Velence Mountain (Village Nadap)	08.10.2019	15		1		1
	Prevalence						
16	Central Hungary (Village Tóalmás)	10.10.2019	45		1		
	Prevalence				2.22%		
17	Bakony, Kinizsi Pál Lookout Tower	21.10.2019	60	2	6	2	
	Prevalence			3.33%	10.00%	3.33%	
18	Bakony, Németbánya	22.10.2019	40	3	3	1	
	Prevalence			7.50%	7.5%	2.50%	
19	Bakony, Miklós Pál Mountain, Dr. Majer Antal Lookout Tower	23.10.2019	80	1	2		
	Prevalence			1.25%	2.50%		
20	Börzsöny Mountains	03.10.2019	6	1	2	1	
	Prevalence						
21	Budakeszi	12.10.2019	50	4	9		2
	Prevalence			8.00%	18.00%		
	Bakony population (No. 11, 17, 18, 19 sites)		191	6	12	3	
	Prevalence			3.14%	6.28%	1.57%	
Mean prevalence of the infected population (above 15 examined individuals)				5.57%	13.9%		

Table 2 - Application of citizen science approaches in previous *Harmonia axyridis* research

	Number of records	Time period	Number of records per one year	Source	References
Recording bumblebees as a group and <i>Ha. axyridis</i> as a flagship for ladybirds	32957	35 year (1980-2015)	942	iRecord, Harlequin Ladybird Survey website and others, such as e-mail	COMONT and ASHBROOK, 2017
Presence of <i>Ha. axyridis</i> in Central America	1096	12 year (2006-2018)	91	iNaturalist, Flickr, and insect collections at universities and museums	HILLER and HAELEWATERS, 2019
Distribution of <i>He. virescens</i> on <i>Ha. axyridis</i> in the USA	163 59 104	16 year (2002-2018)	10 4 7	BugGuide, iNaturalist	HAELEWATERS et al., 2019
Distribution of <i>Ha. axyridis</i> in Argentina	370 152 86 45 27 16 18	1 year (June 2018- July 2019)	370 152 86 45 27 16 18	iNaturalist, WhatsApp, e-mail, Facebook, Google Forms, other	WERENKRAUT et al., 2020
Prevalence and distribution of natural enemies of <i>Ha. axyridis</i> in Hungary	581	4 mounts (October 2019 - January 2020)		Facebook poster	Present study

	Year, season	Number of the collection localities	<i>Harmonia axyridis</i> (dissected)	Prevalence of natural enemies				Co-infection prevalence	Reference
				<i>D. coccinellae</i>	<i>C. hippodamiae</i>	<i>P. bifurcatus</i>	<i>He. virescens</i>		
USA	2007–2008 (winter)	5	361♂ 346♀	1.8 – 17.4%		3.6 – 43.5%		0 – 33.3% 2.7 – 19.15%	RIDDICK, 2010
Russia, Caucasus	2018 (spring)	6	61♂ 152♀			1.6% 6%	61% 44%	4% 4.6%	ORLOVA-BIENKOWSKAJA et al., 2018
Czech Republic	2014 (autumn)		49			47%	24%	14%	HAELEWATERS et al., 2017
Hungary	2014 (spring)		40			5%			PFLIEGLER, 2014
	2019	21	581	4%		3.14 – 8%	2.22 – 36.36%	1.57 – 3.33% (<i>P. bif.</i> + <i>He. vir.</i>)	Present study
Netherlands	2003–2011	20	3490	0.004 – 1.3%	0.38 – 2.6%	5.4 – 65.4%	0 – 20%	1.5% (<i>P. bif.</i> + <i>He. vir.</i>) 0.14% (<i>C. hip.</i> + <i>He. vir.</i>)	RAAK-VAN DEN BERG et al., 2014

Table 3 - *Parasitylenchus bifurcatus*, *Dinocampus coccinellae*, *Coccipolipus hippodamiae* and *Hesperomyces virescens* co-infection and prevalence in *Harmonia axyridis* in previous and present studies

Fig. III – *Hesperomyces virescens* parasitic fungus and *Parasitylenchus bifurcatus* parasitic nematode infections of *Harmonia axyridis* in Hungary. Filled square: non infected, triangle: *Hesperomyces virescens*, star: *Parasitylenchus bifurcatus* and *Hesperomyces virescens* co-infected, numbers in squares: braconid infected localities

The Kruskal-Wallis test confirmed the infection differences between the locations. The distribution of nematode infected samples and the fungi infected samples are not the same across the studied localities (nematode $p = 0.033^*$; fungi $p < 0.001^{***}$).

The co-infection of *He. virescens* and *P. bifurcatus* showed a significant positive correlation (Spearman's correlation coefficient $= 0.176$ $p < 0.001^{***}$).

DISCUSSION

CITIZEN SCIENCE CAMPAIGN

The citizen science method allowed us to obtain specimens from a broad range of habitats across the country, such as mountain forests and urban areas (Tables 1–3). However, due to the nature of this approach, sampling was not systematic which limited our analysis. On several occasions, only few specimens were sent from certain areas, making their inclusion into statistical evaluation impossible. In our case, physical examination and dissection of the animals were possible and essential for revealing the natural enemies, in contrast to previous studies, which used the same

approach (Table 2) but evaluated only photographs and completed questionnaires.

PARASITYLENCHUS BIFURCATUS

This study reports for the first time the occurrence of parasitic nematode species *P. bifurcatus* in *Ha. axyridis* in Hungary. This species was originally described by Poinar and Steenberg (POINAR and STEENBERG, 2012) based on specimens collected in *Ha. axyridis* from Denmark in 2010. The species was subsequently reported in Netherlands, Slovenia, Czech Republic, Poland, Germany, and Russia (HAELEWATERS *et al.*, 2017; Table 3). The *P. bifurcatus* parasitises only in *Ha. axyridis* and the real origin of this nematode species in Europe is as yet unknown. It could either come from Asia with the ladybird individuals introduced for biocontrol measures or, more likely, acquired from endemic European ladybird beetles (POINAR and STEENBERG, 2012; RAAK-VAN DEN BERG *et al.*, 2014). In addition, the nucleotide sequences of the Caucasian *P. bifurcatus* are identical to the ones from the European populations of this nematode (ORLOVA-BIENKOWSKAJA *et al.*, 2018). Broad geographic distribution of *P. bifurcatus* suggests that this parasitic nematode species

could be present in many localities in Europe where the harlequin ladybird beetle has spread, and it was only a matter of time before it was found in Hungary. In our investigation, the entomoparasitic nematodes were found in five out of twenty-one studied localities, in mountain forest habitats (Fig. III).

First-generation, subsequent generation, and vermiform females, juveniles, and males of *P. bifurcatus* are systematically found together inside one adult host individual, with the highest intensity of vermiform females (Fig. II). ORLOVA-BIENKOWSKAJA *et al.* (2018) detected between 5 and 32 subsequent generation females and approximately 200 vermiform females per host in the *Ha. axyridis*. Contrary to other species in the genus *Parasitylenchus*, only the fertilized vermiform females leave the host in the life cycle of *P. bifurcatus* and *P. coccinellinae*. For this reason, therefore multiple development forms such as first-generation and subsequent generation females, vermiform females, as well as males and juveniles appear inside the same host individual. Nutrient supply alone determines the number of individuals and generations (IPERTI and VAN WAEREBEKE, 1968; POINAR and STEENBERG, 2012).

HESPEROMYCES VIRESCENS

The biotrophic fungus, *He. virescens* (DE KESEL, 2011; HAELEWATERS and DE KESEL, 2017) was found with or without the parasitic nematode infection in 11 localities in Hungary (Fig. I, Fig. III). In contrast to the nematode, the fungus was found in Hungary earlier by Pfliegler in 2014 (PFLIEGLER, 2014). *Hesperomyces virescens* is widespread in Europe, with reports in Austria, Belgium, Czech Republic, Croatia, Germany, France, Hungary, the Netherlands, Poland, Slovakia, and the UK (HAELEWATERS *et al.*, 2017), while the spread of the parasitic nematode is known to a much lesser extent. The origin of the *He. virescens* is unknown yet. HAELEWATERS *et al.* (2014) reported this species on specimens of *Ha. axyridis* collected in Asia in the 1930s. The *Ha. axyridis*' parasite *He. virescens* may have been co-introduced along with their host (co-invasion). But on the other hand, *He. virescens* is a species complex that contains different ladybird host-specific genetic lineages, but not geographically delineated (HAELEWATERS *et al.*, 2018). This raises the possibility that some populations identified as "*He. virescens*" can originate by the transfer of a lineage from another native coccinellid species to *Ha. axyridis*.

DINOCAMPUS COCCINELLAE, PARASITOID WASPS

Parasitoid larvae were found in four host individuals. Previous studies have shown that only one braconid species, the cosmopolitan *D. coccinellae* parasitizes *Ha. axyridis*. This parasitoid species has a wide host range of more than 50 different coccinellid host-species and was already present in *Ha. axyridis* in all invaded continents (CERYNGIER *et al.*, 2018). In Europe, *D. coccinellae* was detected for the first time in Belgium in 2005 (BERKVENs *et al.*, 2010). Since then it has

emerged in several other countries (CERYNGIER *et al.*, 2018), but was not reported in Hungary up until now. Though *D. coccinellae* reduces the hosts' reproductive capacities and the aphid consumption rates, they cannot detain its population growth (BERKVENs *et al.*, 2010). One ladybird specimen recently collected from the Budakeszi site produced an adult braconid identified as *D. coccinellae*. This adult specimen proves that the widespread parasitoid is also present in Hungary. Other discovered parasitoid larvae are similar morphologically to *D. coccinellae* and were discovered in the same locality – they are likely to belong to the same species, but are treated as "parasitoids" in this paper.

PREVALENCE OF NATURAL ENEMIES OF *HARMONIA* *AXYRIDIS*

The prevalence of both *P. bifurcatus* and *He. virescens* varied within a wide range, similar to previously published results (Table 3): nematode prevalence ranged from 1.25% to 8%; fungus prevalence from 2.5% to 36.36% (Table 1, Table 3). The 4% prevalence we obtained for the parasitoid wasp larvae in one examined population falls within the published prevalence range of the parasitoid *D. coccinellae* [between 0.1% to 27.4% in (CERYNGIER *et al.*, 2018)]. Note that this study was based on ladybirds that were collected non-systematically; the entire Hungarian population of *Ha. axyridis* was not studied. Gender-specific or seasonal differences were not investigated, either. Previous work has revealed that infection rates of both parasites differ among years, between host genders, and depending on abiotic (local environmental conditions) and biotic (ladybird behavior) factors. In winter, more *Ha. axyridis* males are infected by *He. virescens*, because of the mating behavior of the beetles (RAAK-VAN DEN BERG *et al.*, 2014). The parasite transmission of both the nematode and fungus is affected by the overwinter aggregation behaviour of the ladybirds. *Harmonia axyridis* produces β -caryophyllene aggregation pheromone, which triggers aggregation behaviour before dormancy (VERHEGGEN *et al.*, 2007). The route of the nematode transmission is unknown yet. Proposed hypotheses suggest that the process can happen through the trachea or soft parts of the cuticle during sexual contact or during contact while in aggregation (HARDING *et al.*, 2011; POINAR and STEENBERG, 2012).

CO-INFECTIONS BY TWO NATURAL ENEMIES OF THE *HARMONIA* *AXYRIDIS*

The knowledge concerning natural enemies of *Ha. axyridis* is continuously improving but reveals that rare infections have only limited potential for controlling population densities of their host (CERYNGIER *et al.*, 2018; HAELEWATERS *et al.*, 2017; RIDDICK, 2010; ROY and WAJNBERG, 2008). The multiple infections by the parasites, parasitoids, pathogens, and their impact on the natural host populations are poorly studied and hard

to interpret. However, HAELEWATERS *et al.* (2020) have recently published a laboratory assay testing the impact of co-infection of *He. virescens* and an entomopathogenic fungus (*Beauveria bassiana* or *Metarhizium brunneum*) on host mortality. The native ladybird species showed increased mortality when co-infected with both *He. virescens* and an entomopathogenic fungus. On the other hand, compared to *Hesperomyces*-only infected specimens, *Ha. axyridis* mortality did not increase significantly when co-infected with an entomopathogenic fungus (HAELEWATERS *et al.*, 2020).

Co-infection of *Ha. axyridis* by *P. bifurcatus* and *He. virescens* was for the first time recorded in the Netherlands (RAAK-VAN DEN BERG *et al.*, 2014). The nematode-fungus co-infection was present only in one examined locality in Tilburg. The authors wrote that this co-infection might result in lower survival. This co-infection was also detected in the Caucasus, but correlation analysis could not be performed due to the low number of specimens collected (ORLOVA-BIENKOWSKAJA *et al.*, 2018).

In every case, the beetles from nematode-infected populations were also infected by the parasitic fungus; we did not find any populations where beetles were infected by the parasitic nematodes only. This is consistent with the results found in the Caucasus, where no nematode-infected population were found without the *He. virescens* among the examined localities (ORLOVA-BIENKOWSKAJA *et al.*, 2018). In contrast, the 2008 and 2009 winter populations of ladybirds from the Netherlands were only nematode-infected (RAAK-VAN DEN BERG *et al.*, 2014). Furthermore, in a Hungarian population affected by all three natural enemies simultaneously, individual ladybird specimens were often infected with one of them only, either the nematode or the fungus or the braconid larvae. Whereas, co-infection of the same ladybird specimen has not been recorded. In the Caucasus, the *P. bifurcatus* infection also appeared in host specimens with and without the *He. virescens* co-infection (ORLOVA-BIENKOWSKAJA *et al.*, 2018). The present study provides new support for the co-infection in a geographic location in between the two preceding co-infection records (Netherlands and Caucasus).

SUITABILITY OF *PARASITYLENCHUS BIFURCATUS* AND *HESPEROMYCES VIRESCENS* FOR BIOLOGICAL CONTROL OF *HARMONIA AXYRIDIS*

PARASITYLENCHUS BIFURCATUS

The present study did not investigate the influence exerted by *P. bifurcatus* on the fitness and mortality of the host. Based on previous studies, *P. bifurcatus* is not specifically entomopathogenic, unlike nematodes from the genera *Steinernema* and *Heterorhabditis* (KAYA and GAUGLER, 1993). Although *P. bifurcatus* is supposed to have a significant impact on the host, even be a prominent mortality factor (POINAR and

STEENBERG, 2012), the presence of co-infected specimens with *He. virescens* suggest that the nematode infection does not necessarily result in short term destruction of its host. The evolutionary strategy of *P. bifurcatus* maybe somewhere between necromenic and entomopathogenic (SUDHAUS, 2008). Due to its apparent host specificity, *P. bifurcatus* may have potential as a biocontrol agent.

HESPEROMYCES VIRESCENS

Hesperomyces virescens is a complex of multiple species, each of which has specific ladybird hosts (HAELEWATERS *et al.*, 2018). Horizontal transmission of *Hesperomyces* species among different ladybird species is thought to be rare (COTTRELL and RIDDICK, 2012; RIDDICK, 2010). *Hesperomyces virescens* harms *Ha. axyridis* population by decreasing the mating frequency/success since sensing ability and movements of infected females are hindered by the high number of heavy thalli on legs, mouthparts, head, and wings (NALEPA and WEIR, 2007). Besides, heavily infected coccinellid males have lower survival rates during winter (RIDDICK, 2010). Laboratory assays confirm that this parasitic fungus could weaken the host's condition and make it more susceptible to infection by other natural enemies. *Harmonia axyridis* co-infected with *He. virescens* and an entomopathogenic fungus (*Beauveria bassiana* or *Metarhizium brunneum*) do not suffer the same effects compared to a native coccinellid species (HAELEWATERS *et al.*, 2020). Nonetheless, currently *He. virescens* is not suitable for biological control because its negative impact occurs too late in the host life cycle to regulate population (HAELEWATERS *et al.*, 2017). On the other hand, its host specificity and negative impact on the host may allow its future use in biocontrol in combination with an entomopathogenic species.

CO-INFECTIONS

Agreeing with the conclusion of HAELEWATERS *et al.* (2020), we hypothesize that parasitic infection results in lower host resistance, allowing for a second natural enemy to infect (*sensu* HAELEWATERS *et al.* 2020). The results of the previous and present studies do not allow us to draw global conclusions about the parasites' spread, seasonal change, preferred habitats. The differences in the sampling methods, the number of dissected individuals, and the number of sampled localities are the main reasons (Table 3) for this. More robust prevalence data could be obtained through well-organized international collaborations.

The low numbers of co-infection in the previous studies are in line with our results (ORLOVA-BIENKOWSKAJA *et al.*, 2018; RAAK-VAN DEN BERG *et al.*, 2014). The co-infection might involve a shorter life span of the host and, consequently, the lower incidence in the collected samples. Competition can be assumed to occur between nematodes and braconid larvae, which requires additional investigation.

CONCLUSIONS

We evidenced naturally occurring infections of *Ha. axyridis* with *P. bifurcatus*, *D. coccinellae*, and *He. virescens* in the host's invaded range in Central Europe. In accordance with the conclusion of RAAK-VAN DEN BERG *et al.* (2014), we suggest that the low incidence numbers of co-infection by *P. bifurcatus* and *He. virescens* might reflect the lower survival rates of the co-infected host. Further experimental studies need to be done to check the life span of native, experimentally co-infected ladybirds to confirm the above assumption about the decline of the host's condition and draw precise conclusions about the *P. bifurcatus*, *He. virescens*, and *D. coccinellae* co-infection affecting *Ha. axyridis*. The research of the interaction between *P. bifurcatus*, *D. coccinellae*, and *He. virescens*, as well as their collective impact on the host, is imperative for the understanding of the ecology of co-infection and can offer a possibility for biological control development.

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REFERENCES

- BARTUMEUS F., OLTRA A., PALMER J.R.B., 2018 – *Citizen Science: A Gateway for Innovation in Disease-Carrying Mosquito Management?* - Trends in Parasitology, 34: 727-729.
- BROWN M.W., MILLER S.S., 1998 – *Coccinellidae (Coleoptera) in apple orchards of eastern West Virginia and the impact of invasion by Harmonia axyridis*. - Entomological News, 109: 143-151.
- BROWN P.M.J., THOMAS C.E., LOMBAERT E., JEFFRIES D.L., ESTOUP A., HANDLEY L.J.L., 2011 – *The global spread of Harmonia axyridis (Coleoptera: Coccinellidae): distribution, dispersal and routes of invasion*. - BioControl, 56: 623-641.
- CAMACHO-CERVANTES M., ORTEGA-ITURRIAGA A., DEL-VAL E., 2017 – *From effective biocontrol agent to successful invader: the harlequin ladybird (Harmonia axyridis) as an example of good ideas that could go wrong*. - PeerJ, 5: e3296.
- CERYNGIER P., NEDVĚD O., GREZ A.A., RIDDICK E.W., ROY H.E., SAN MARTIN G., STEENBERG T., VESELÝ P., ZAVIEZO T., ZÚÑIGA-REINOSO Á., HAELEWATERS D., 2018 – *Predators and parasitoids of the harlequin ladybird, Harmonia axyridis, in its native range and invaded areas*. - Biological Invasions, 20: 1009-1031.
- CERYNGIER P., ROY H.E., POLAND R.L., 2012 – *Natural Enemies of Ladybird Beetles*, In: Ecology and Behaviour of the Ladybird Beetles (Coccinellidae), Hodek I., van Emden H.F., Honek A., Ed., Blackwell Publishing Ltd, pp.375-443.
- COMONT R.F., ASHBROOK K., 2017 – *Evaluating promotional approaches for citizen science biological recording: bumblebees as a group versus Harmonia axyridis as a flagship for ladybirds*. - BioControl, 62: 309-318.
- COTTRELL T.E., RIDDICK E.W., 2012. – *Limited transmission of the ectoparasitic fungus Hesperomyces virescens between lady beetles*. - Psyche, 2021: 814378.
- CRALL A.W., NEWMAN G.J., JARNEVICH C.S., STOHLGREN T.J., WALLER D.M., GRAHAM M., 2010 – *Improving and integrating data on invasive species collected by citizen scientists*. - Biological Invasions, 12: 3419-3428.
- FERRAN A., NIKNAM H., KABIRI F., PICART J.L., DE HERCE C., BRUN J., IPERTI G., LAPCHIN L., 1996 – *The use of Harmonia axyridis larvae (Coleoptera: Coccinellidae) against Macrosiphum rosae (Hemiptera: Sternorrhyncha: Aphididae) on rose bushes*. - European Journal of Entomology, 93: 59-67.
- GARDINER M.M., LANDIS D.A., 2007 – *Impact of intraguild predation by adult Harmonia axyridis (Coleoptera: Coccinellidae) on Aphis glycines (Hemiptera: Aphididae) biological control in cage studies*. - Biological Control, 40: 386-395.
- GOETZ D.W., 2007 – *Harmonia axyridis ladybug hypersensitivity in clinical allergy practice*. - Allergy & Asthma Proceedings, 28: 50-57.
- HAELEWATERS D., DE KESEL A., PFISTER D.H., 2018. – *Integrative taxonomy reveals hidden species within*

- a common fungal parasite of ladybirds*. - Scientific Reports, 8: 1-16.
- HAELEWATERS D., DE KESEL A., 2017 – *De schimmel Hesperomyces virescens, een natuurlijke vijand van lieveheersbeestjes*. - Entomologische Berichten, 77: 106-118.
- HAELEWATERS D., HILLER T., PAN F.Y., PAN I.Y., 2019 – *Tracking an ectoparasitic fungus of Harmonia axyridis in the USA using literature records and citizen science data*. - Benefits and Risks of Exotic Biological Control Agents IOBC-WPRS Bulletin, 145: 17-22.
- HAELEWATERS D., ZHAO S.Y., CLUSELLA-TRULLAS S., COTTRELL T.E., DE KESEL A., FIEDLER L., HERZ A., HESKETH H., HUI C., KLEESPIES R.G., LOSEY J.E., MINNAAR I.A., MURRAY K.M., NEDVĚD O., PFLIEGLER W.P., RAAK-VAN DEN BERG C.L., RIDDICK E.W., SHAPIRO-ILAN D.I., SMYTH R.R., STEENBERG T., VAN WIELINK P.S., VIGLÁŠOVÁ S., ZHAO Z., CERYNGIER P., ROY H.E., 2017 – *Parasites of Harmonia axyridis: current research and perspectives*. - BioControl, 62: 355-371.
- HAELEWATERS D., HILLER T., KEMP E.A., VAN WIELINK P.S., SHAPIRO-ILAN D.I., AIME C.M., NEDVĚD O., PFISTER D.H., COTTRELL T.E., 2020 – *Mortality of native and invasive ladybirds co-infected by ectoparasitic and entomopathogenic fungi*. - PeerJ, 8: e10110.
- HILLER T., HAELEWATERS D., 2019 – *A case of silent invasion: Citizen science confirms the presence of Harmonia axyridis (Coleoptera, Coccinellidae) in Central America*. - PLoS ONE, 14: e0220082.
- KATSANIS A., BABENDREIER D., NENTWIG W., KENIS M., 2013 – *Intraguild predation between the invasive ladybird Harmonia axyridis and non-target European coccinellid species*. - BioControl, 58: 73-83.
- KAYA H.K., GAUGLER M., 1993 – *Entomopathogenic nematodes*. - Annual Review of Entomology, 38: 181-206.
- DE KESEL A., 2011 – *Hesperomyces (Laboulbeniales) and Coccinellid hosts*. - Sterbeekia, 30: 32-37.
- KOCH R.L., 2003 – *The multicolored Asian lady beetle, Harmonia axyridis: A review of its biology, uses in biological control, and non-target impacts*. - Journal of Insect Science, 3: 1-16.
- KOCH R.L., GALVAN T.L., 2008 – *Bad side of a good beetle: The North American experience with Harmonia axyridis*. - BioControl, 53: 23-35.
- KOVACH J., 2002 – *Impact of Multicolored Asian Lady Beetles as a Pest of Fruit and People*. - American Entomologist, 50: 159-161.
- LAAKSONEN M., SAJANTI E., SORMUNEN J.J., PENTTINEN R., HÄNNINEN J., RUOHOMÄKI K., SÄÄKSJÄRVI I., VESTERINEN E.J., VUORINEN I., HYTÖNEN J., KLEMOLA T., 2017 – *Crowdsourcing-based nationwide tick collection reveals the distribution of Ixodes ricinus and I. persulcatus and associated pathogens in Finland*. - Emerging Microbes and Infections, 6: 1-7.
- LERNOUT T., DE REGGE N., TERSAGO K., FONVILLE M., SUIN V., SPRONG H., 2019 – *Prevalence of pathogens in ticks collected from humans through citizen science in Belgium*. - Parasites and Vectors, 12: 1-11.
- MARGOLIS L., ESCH G.W., HOLMES J.C., KURIS A.M., SCHAD G.A., 1982 – *The Use of Ecological Terms in Parasitology (Report of an Ad Hoc Committee of the American Society of Parasitologists)*. - The Journal of Parasitology, 68: 131-133.
- MERKL O., 2014 – *A harlekinkatica (Harmonia axyridis Pallas) Magyarországon (Coleoptera: Coccinellidae). (First record of the harlequin ladybird (Harmonia axyridis Pallas) in Hungary (Coleoptera: Coccinellidae))*. - Növényvédelem, 44: 239-242.
- NALEPA C.A., KENNEDY G.G., 2004 – *Orientation of Multicolored Asian Lady Beetles to Buildings*. - American Entomologist, 50: 165-166.
- NALEPA C.A., WEIR A., 2007 – *Infection of Harmonia axyridis (Coleoptera: Coccinellidae) by Hesperomyces virescens (Ascomycetes: Laboulbeniales): Role of mating status and aggregation behavior*. - Journal of Invertebrate Pathology, 94: 196-203.
- NIETO N.C., PORTER W.T., WACHARA J.C., LOWREY T.J., MARTIN L., MOTYKA P.J., SALKELD D.J., 2018 – *Using citizen science to describe the prevalence and distribution of tick bite and exposure to tick-borne diseases in the United States*. - PLoS ONE, 13: e0199644.
- ORLOVA-BIENKOWSKAJA M.J., SPIRIDONOV S.E., BUTORINA N.N., BIENKOWSKI A.O., 2018 – *Coinvasion by the ladybird Harmonia axyridis (Coleoptera: Coccinellidae) and its parasites, Hesperomyces virescens (Ascomycota: Laboulbeniales) and Parasitylenchus bifurcatus (Nematoda: Tylenchida, Allantonematidae), in the Caucasus*. - PLoS ONE, 13: 1-18.
- PALMER J.R.B., OLTRA A., COLLANTES F., DELGADO J.A., LUCIENTES J., DELACOUR S., BENGIOA M., ERITJA R., BARTUMEUS F., 2017 – *Citizen science provides a reliable and scalable tool to track disease-carrying mosquitoes*. - Nature Communications, 8: 1-13.
- PELL J.K., BAVERSTOCK J., ROY H.E., WARE R.L., MAJERUS M.E.N., 2008 – *Intraguild predation involving Harmonia axyridis: a review of current knowledge and future perspectives*. - BioControl, 53: 147-168.
- PFLIEGLER W.P., 2014 – *First Hungarian record of the fungus Hesperomyces virescens (Ascomycota: Laboulbeniales), parasitic on the harlequin ladybird (Coccinellidae: Harmonia axyridis) A Hesperomyces virescens (Ascomycota: Laboulbeniales) gombafaj első magyarországi előfordulása*. - E-Acta Naturalia Pannonica, 7: 139-142.
- PFLIEGLER W.P., BÁTHORI F., WANG T.W., TARTALLY A., HAELEWATERS D., 2018 – *Hesperomyces ectopa-*

- rasitic fungi (Ascomycota, Laboulbeniales) are globally distributed by their invasive cockroach hosts and through the pet trade industry.* - Mycologia, 110: 39-46.
- POINAR G.O., STEENBERG T., 2012 – *Parasitylenchus bifurcatus n. sp. (Tylenchida: Allantonematidae) parasitizing Harmonia axyridis (Coleoptera: Coccinellidae).* - Parasites and Vectors, 5: 1-8.
- RAAK-VAN DEN BERG C.L., VAN WIELINK P.S., DE JONG P.W., GORT G., HAELEWATERS D., HELDER J., VAN LENTEREN J.C., 2014 – *Invasive alien species under attack: natural enemies of Harmonia axyridis in the Netherlands.* - BioControl, 59: 229-240.
- RICE N.R., SMITH M.W., EIKENBARY R.D., ARNOLD D., TEDDERS W.L., WOOD B., LANDGRAF B.S., TAYLOR G.G., BARLOW G.E., 1998 – *Density for low-input pecan management Assessment of legume and nonlegume ground covers on Coleoptera: Coccinellidae density for low-input pecan management.* - American Journal of Alternative Agriculture, 13: 111-123.
- RIDDICK E.W., COTTRELL T.E., KIDD K.A. 2009 – *Natural enemies of the Coccinellidae: Parasites, pathogens, and parasitoids.* - Biological Control, 51: 306-312.
- RIDDICK E.W., 2010 – *Ectoparasitic mite and fungus on an invasive lady beetle: parasite coexistence and influence on host survival.* - Bulletin of Insectology, 63: 13-20.
- ROY H., WAJNBERG E., 2008 – *From biological control to invasion: the ladybird Harmonia axyridis as a model species.* - BioControl, 53: 1-4.
- ROY H.E., BROWN P.M.J., ADRIAENS T., BERKVEN N., BORGES I., CLUSELLA-TRULLAS S., COMONT R.F., DE CLERCQ P., ESCHEN R., ESTOUP A., EVANS E.W., FACON B., GARDINER M.M., GIL A., GREZ A.A., GUILLEMAUD T., HAELEWATERS D., HERZ A., HONEK A., HOWE A.G., HUI C., HUTCHISON W.D., KENIS M., KOCH R.L., KULFAN J., LAWSON HANDLEY L., LOMBAERT E., LOOMANS A., LOSEY J., LUKASHUK A.O., MAES D., MAGRO A., MURRAY K.M., SAN MARTIN G., MARTINKOVA Z., MINNAAR I., NEDVED O., ORLOVA-BIENKOWSKAJA M.J., RABITSCH W., RAVN H.P., RONDONI G., RORKE S.L., RYNDEVICH S.K., SAETHRE M-G., SLOGGETT J.J., SOARES A.O., STALS R., TINSLEY M.C., VANDEREYCKEN A., VAN WIELINK P., VIGLÁŠOVÁ S., ZACH P., ZAVIEZO T., ZHAO Z., 2016 – *The harlequin ladybird, Harmonia axyridis: global perspectives on invasion history and ecology.* - Biological Invasions, 18: 997-1044.
- ROY H.E., RHULE E., HARDING S., HANDLEY L.-J.L., POLAND R.L., RIDDICK E.W., STEENBERG T., 2011 – *Living with the enemy: parasites and pathogens of the ladybird Harmonia axyridis.* - BioControl, 56: 663-679.
- SUDHAUS W., 2008 – *Evolution of Insect Parasitism in Rhabditid and Diplogastrid Nematodes.* - Advances in arachnology and developmental biology, 12: 143-161.
- VERHEGGEN F.J., FAGEL Q., HEUSKIN S., LOGNAY G., FRANCIS F., HAUBRUGE E., 2007 – *Electrophysiological and Behavioral Responses of the Multicolored Asian Lady Beetle, Harmonia axyridis Pallas, to Sesquiterpene Semiochemicals.* - Journal of Chemical Ecology, 33: 2148-2155.
- VILCINSKAS A., SCHMIDTBERG H., ESTOUP A., TAYEH A., FACON B., VOGEL H., 2015 – *Evolutionary ecology of microsporidia associated with the invasive ladybird Harmonia axyridis.* - Insect Science, 22: 313-324.
- VOGEL H., SCHMIDTBERG H., VILCINSKAS A., 2017 – *Comparative transcriptomics in three ladybird species supports a role for immunity in invasion biology.* - Developmental and Comparative Immunology, 67: 452-456.
- WAGNER D.L., VAN DRIESCHE R.G., 2010 – *Threats Posed to Rare or Endangered Insects by Invasions of Nonnative Species.* - Annual Review of Entomology, 55: 547-568.
- WERENKRAUT V., BAUDINO F., ROY H.E., 2020 – *Citizen science reveals the distribution of the invasive harlequin ladybird (Harmonia axyridis Pallas) in Argentina.* - Biological Invasions, 22: 2915-29