


Predators and parasitoids of the harlequin ladybird, *Harmonia axyridis*, in its native range and invaded areas

Piotr Ceryngier  · Oldřich Nedvěd · Audrey A. Grez · Eric W. Riddick · Helen E. Roy · Gilles San Martin · Tove Steenberg · Petr Veselý · Tania Zaviezo · Álvaro Zúñiga-Reinoso · Danny Haelewaters

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Abstract The harlequin ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae) has rapidly spread in several continents over the past 30 years and is considered an invasive alien species. The success of *H. axyridis* as an invader is often attributed to weak control by natural enemies. In this paper, we provide an overview of current knowledge on predators and parasitoids of *H. axyridis*. The common feature of predators and parasitoids is that they directly kill exploited organisms. Currently available data show

that *H. axyridis*, displaying a variety of chemical, mechanical, and microbiological anti-predator defenses, is usually avoided by predators. However, some birds and invertebrates can eat this ladybird without harmful consequences. The primary defenses of *H. axyridis* against parasitoids include immune response and physiological and nutritional unsuitability for parasitoid development. These defenses are probably relatively efficient against most ladybird parasitoids, but not against flies of the genus

P. Ceryngier (✉)
Faculty of Biology and Environmental Sciences, Cardinal Stefan Wyszyński University, Wóycickiego 1/3, 01-938 Warsaw, Poland
e-mail: p.ceryngier@uksw.edu.pl

O. Nedvěd · P. Veselý
Faculty of Science, University of South Bohemia, Branišovská 1760, 370 05 České Budějovice, Czech Republic

O. Nedvěd
Institute of Entomology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic

A. A. Grez
Facultad Ciencias Veterinarias y Pecuarias, Universidad de Chile, Casilla 2 Correo 15, La Granja, Santiago, Chile

E. W. Riddick
USDA-ARS, Jamie-Whitten Delta States Research Center, 59 Lee Road, Stoneville, MS 38776, USA

H. E. Roy
Centre for Ecology and Hydrology, Benson Lane, Oxfordshire OX10 8BB, UK

G. San Martin
Plant Protection and Ecotoxicology Unit, Life Sciences Department, Walloon Agricultural Research Centre, Rue de Liroux 2, 5030 Gembloux, Belgium

T. Steenberg
Department of Agroecology, Aarhus University, Forsøgsvej 1, 4200 Slagelse, Denmark

T. Zaviezo
Facultad Agronomía e Ing. Forestal, Pontificia Universidad Católica de Chile, Casilla 306 – 22, Santiago, Chile

Á. Zúñiga-Reinoso
Laboratorio de Genética y Evolución, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, 7800003 Ñuñoa, Santiago, Chile

Phalacrotophora. The latter are idiobiont parasitoids and hence can evade the host's immune response. Indeed, rates of parasitism of *H. axyridis* by *Phalacrotophora* in the Palaearctic region (both in the native range in Asia and in Europe) are relatively high. While strong evidence for enemy release on the invasive populations of *H. axyridis* is lacking, several cases of parasitoid acquisition have been recorded in Europe, North America, and South America. We conclude that enemy release cannot be excluded as a possible mechanism contributing to the spread and increase of *H. axyridis* in the early stages of invasion, but adaptation of parasitoids may lead to novel associations which might offset previous effects of enemy release. However, further work is required to elucidate the population-level effects of such interactions.

Keywords biocontrol · Coccinellidae · Enemy acquisition · Enemy release · Natural enemies

Introduction

The harlequin ladybird *Harmonia axyridis* (Pallas) is a species of Asiatic origin that became invasive in the late 1980s in North America and subsequently in other continents. Until now *H. axyridis* has colonized extensive areas in North and South America, Europe, and to a lesser extent Africa (Brown et al. 2011; Roy et al. 2016; Camacho-Cervantes et al. 2017). It has started to spread in some parts of western Asia (Orlova-Bienkowskaja 2014; Ukrainsky and Orlova-Bienkowskaja 2014; Bukejs and Telnov 2015; Görür et al. 2015; Roy et al. 2016) and New Zealand (<https://www.mpi.govt.nz/document-vault/12261>). In its invaded areas, *H. axyridis* can quickly become very common, posing a threat to native insect communities, especially to other ladybirds (Roy et al. 2012, 2016; Grez et al. 2016). It can also cause economic losses in horticulture and winemaking, as well as become a nuisance and health problem to some people (Koch and Galvan 2008; Roy et al. 2016). Nevertheless, *H.*

axyridis is a highly effective predator of aphids in several cropping systems, especially soybean, pecan, and apple (Riddick 2017). There are emerging anecdotal reports of the success of this species in controlling pest insects in fruit crops in the UK.

The success of *H. axyridis* as an invader is often attributed to weak control of its populations by natural enemies, with the enemy release hypothesis (ERH) usually being quoted to explain this low level of biological control (Roy et al. 2008, 2011a, b; Berkvens et al. 2010; Firlej et al. 2012; Comont et al. 2014; Haelewaters et al. 2017). ERH states that an alien species invading a new geographic range will be less affected by natural enemies compared to native species, leading to population build-up of the alien species in its invasive range. It remains disputable, however, whether or not the enemy release is responsible for fast expansion of *H. axyridis* with frequently observed population outbreaks. Some evidence exists that the invasive *H. axyridis* may be less affected by enemies than native ladybirds (Shapiro-Ilan and Cottrell 2005; Koyama and Majerus 2008; Roy et al. 2008; Comont et al. 2014). However, it is often uncertain whether this is the effect of enemy release or of generally low susceptibility of *H. axyridis* to most enemies.

This review focuses on predators and parasitoids of *H. axyridis*. These two categories of natural enemies differ in many respects but are similar in that both usually directly kill exploited organisms rather than decrease their fecundity or other fitness parameters. The group of enemies of *H. axyridis*, in which sublethal fitness effects prevail over direct killing, i.e. multicellular true parasites, has recently been reviewed by Haelewaters et al. (2017). Here, we (1) compile the available data on the predators and parasitoids of *H. axyridis* in its native range and invaded areas, (2) analyze the importance of these enemies in limiting populations of *H. axyridis*, (3) discuss enemy release and enemy acquisition by invading *H. axyridis*, and (4) suggest future research directions concerning this topic. In addition, we conducted a meta-analysis of data from 21 studies about *Dinocampus coccinellae* and *Phalacrotophora* spp. in the literature and unpublished data. Based on the available data, we only analyzed rates of parasitism (and emergence in the case of *D. coccinellae*) between the native area of *H. axyridis* and its invaded regions, between different ladybird species, and over

D. Haelewaters
Department of Organismic and Evolutionary Biology,
Harvard University, 22 Divinity Avenue, Cambridge,
MA 02138, USA

time. The data used, R code, and report of the results from the meta-analysis are available for download from the figshare online repository (San Martin 2017).

Predators of *Harmonia axyridis*

Due to their toxic properties and low palatability, ladybirds in general are rarely attacked by predators. For many species, including *H. axyridis*, aposematic colouration and intense smell of pyrazines provide additional protection from predation (Daloze et al. 1995; Pasteels 2007; Ceryngier et al. 2012; Petterson 2012). Nevertheless, some animals can eat ladybirds, and these are usually generalist predators. The predators can be either intraguild or extraguild but the distinction, however, is not always obvious, because the guilds of many ladybirds and other predators may change depending on the season and food availability. *Harmonia axyridis*, for example, may switch among various kinds of prey or plant food (pollen, nectar, fruit) (Koch 2003; Lucas 2012). Many aphids and other hemipterans (Adelgidae, Psyllidae, Aleyrodidae), and even pollen were found to be essential food for this species (Berkvens et al. 2008; Hodek and Honěk 2009; Hodek and Evans 2012). Despite this broad polyphagy, *H. axyridis* can be regarded as principally aphidophagous (Koch 2003), and therefore we restrict the usage of the term “intraguild predators of *H. axyridis*” to other primarily aphidophagous species that can also prey on *H. axyridis*.

While determining the prevalence rates of pathogens, parasites and parasitoids in populations of their hosts is relatively simple, the rates of predation are difficult to measure (Naranjo and Hagler 2001; Michaud and Harwood 2012). Most frequently only qualitative assessment of the diet of predators has been determined, either through direct observation of predation events or identification of prey remnants in predator’s alimentary tract or faeces (Harwood and Obrycki 2005; Michalski et al. 2011). An attempt to quantify the effect of invertebrate predators on *H. axyridis* and the two-spot ladybird, *Adalia bipunctata* (L.), was made in northern Italy by Burgio et al. (2008). Using outdoor cage experiments, they found that the rate of predation on *H. axyridis* eggs did not exceed 2%, while up to 40% of the eggs of *A. bipunctata* were consumed or destroyed. Individual larval instars, however, were preyed on at similar rates in both species. The cage method (sensu Burgio et al.

2008) can provide some estimation of the overall predation but it cannot indicate with certainty which predators are involved. The effectiveness and prey preference of individual predators are often assessed in laboratory experiments, and this also applies to predation on ladybirds, including *H. axyridis* (Hough-Goldstein et al. 1996; Yasuda and Kimura 2001; De Clercq et al. 2003; Santi and Maini 2006; Sloggett 2010a; Průchová et al. 2014; Veselý et al. 2016).

Extraguild predators

Extraguild predation on Coccinellidae has been studied relatively often in birds (Ceryngier et al. 2012). However, bird predation specifically on *H. axyridis* has been rarely reported. In the Far East of Russia, within its native range, *H. axyridis* was reported to be eaten by eight bird species (Nechayev and Kuznetsov 1973). In sites of its mass overwintering aggregations in the Far East it was eaten frequently by the grey-headed woodpecker, *Picus canus* J. F. Gmelin (Pici-formes: Picidae) and the Eurasian nuthatch, *Sitta europaea* L. (Passeriformes: Sittidae) (Kuznetsov 1997). In the invasive range (Poland), *H. axyridis* was found to be preyed upon by house martins, *Delichon urbica* (L.) (Passeriformes: Hirundinidae), but much less frequently compared to native ladybirds (Electronic Appendix to Orłowski and Karg 2013). A single case of predation of *H. axyridis* by the black redstart, *Phoenicurus ochruros* (S. G. Gmelin) (Passeriformes: Muscicapidae) was reported by Romanowski and Ceryngier (2016).

Experimental studies showed that some birds, like the great tit, *Parus major* L. (Passeriformes: Paridae), strongly avoid attacking *H. axyridis*, both the red-and-black *succinea* form (Průchová et al. 2014) and the melanic *spectabilis* form (Veselý and Nedvěd, unpublished data). However, there were some attacks observed towards ladybirds modified by colour painting: 10% of *H. axyridis* were attacked when they were coloured brown with black spots and 25% were attacked when coloured brown without spots (Průchová et al. 2014). In contrast to great tits, tree sparrows, *Passer montanus* (L.) (Passeriformes: Passeridae), commonly attack *H. axyridis* as shown by Veselý et al. (2016). Naïve as well as experienced, adult sparrows attacked the *succinea* form as often as the brown-painted form. Furthermore, while great tits never killed or consumed any ladybird of several

species (Dolenská et al. 2009), including *H. axyridis* (Průchová et al. 2014), tree sparrows consumed *H. axyridis* (usually in a series of five individuals) showing no sign of discomfort or nausea (Veselý et al. 2016). Tree sparrows are obviously adapted to ingesting ladybirds and coping, physiologically, with their alkaloids and pyrazines.

Published data on predation of *H. axyridis* by vertebrates other than birds are lacking. There is an unpublished record of rodent predation on overwintering *H. axyridis*. In the vicinity of České Budějovice (Czech Republic), R. Vitek (pers. comm.) found elytra and wings of several tens of *H. axyridis* adults in an empty bee hive. As the hive was observed to be visited by the wood mouse (*Apodemus sylvaticus* (L.)), it is assumed that the beetles were preyed upon by this species.

Available data on invertebrates preying upon *H. axyridis*, albeit few and scattered, point at spiders and heteropteran bugs as the main predators in this category (Table 1). Both web-building (Araneidae, Pholcidae, Theridiidae) and actively hunting spiders (Oxyopidae, Philodromidae, Thomisidae) have been recorded consuming *H. axyridis*. However, some data indicate that *H. axyridis* is rather avoided by certain spiders, even if they prey on other ladybirds. Laboratory studies by Yasuda and Kimura (2001) demonstrated that *Ebrechtella tricuspoidata* (F.) (Thomisidae) hunts larvae of *Coccinella septempunctata* L. and *Propylea japonica* (Thunberg) but not those of *H. axyridis*. In contrast, *Araneus diadematus* Clerck (Araneidae) readily consumed both *C. septempunctata* and *H. axyridis* and chemical defences of these ladybirds had little deterrent or toxic effects on the spider (Sloggett 2010a, b). Another example of a spider that does not avoid *H. axyridis* as a prey is *Steatoda nobilis* (Thorell) (Theridiidae). This species, native to the Canary Islands and Madeira and invasive in Europe and North America, is since 2015 established in southern Chile (Taucare-Ríos et al. 2016) and has been found as a relatively frequent predator of *H. axyridis* soon after establishment. While numbers of *H. axyridis* in Concepción (Chile) rapidly increased in 2015, ladybirds were found trapped in webs of *S. nobilis* and the spiders were observed to prey on *H. axyridis*. Of 19 spider webs recorded by Á. Zúñiga-Reinoso et al. (unpublished data), eleven contained 21 corpses of *H. axyridis* (1–3 corpses per web). Other ladybird species, such as the native *Eriopis*

eschsoltzii Mulsant and the introduced *Adalia bipunctata* were also found in the webs, however, in much lower frequencies and always a single specimen per web. In 2016, *S. nobilis* individuals preying on *H. axyridis* were observed in Temuco, about 220 km SE of Concepción (F. Téllez, pers. comm.), in Pucón, about 290 km SE of Concepción (A. Grez, unpublished data, Fig. 1), and in Valdivia, about 335 km S of Concepción (O. Cerna, pers. comm.). These observations represent an interesting case of one invasive alien species providing some degree of limitation of the other invasive alien species.

Laboratory experiments with predatory Heteroptera again showed that some species accept and some reject *H. axyridis* as a food source. De Clercq et al. (2003) found that the older instars and adults of the pentatomid *Podisus maculiventris* (Say) readily preyed on eggs and larvae of *H. axyridis* but rarely attacked adult beetles. Larvae of *H. axyridis* were less suitable and less preferred food for nymphs of the bug than the caterpillars of the noctuid *Spodoptera littoralis* (Boisduval) and the pyralid *Galleria mellonella* (L.) moths, but much more suitable than the aphid *Myzus persicae* (Sulzer). *Podisus maculiventris* often successfully hunted *H. axyridis*, while the opposite outcomes of this interaction were not observed. Santi and Maini (2006) discovered that adults of the anthocorid *Orius laevigatus* (Fieber) rejected eggs of *H. axyridis* as well as of *Adalia bipunctata*. In the field in central Chile, the assassin bug *Zelus renardii* Kolenati (Reduviidae) preyed on *H. axyridis*. Interestingly, this is another case where one invasive alien species is found preying on another invasive alien species, both of which being associated with anthropogenic habitats (Faúndez 2015; Grez et al. 2016).

Intraguild predators

While much has been written about *H. axyridis* as an efficient intraguild predator (e.g. Yasuda and Ohnuma 1999; Yasuda et al. 2004; Pell et al. 2008; Ware and Majerus 2008; Raak-van den Berg et al. 2012), little data is available on *H. axyridis* as an intraguild prey. Indeed, in experiments testing the outcomes of intraguild interactions involving *H. axyridis*, it usually appears superior to other aphidophagous predators (Yasuda and Ohnuma 1999; Yasuda et al. 2004; Ware and Majerus 2008; Raak-van den Berg et al. 2012;

Table 1 Predatory invertebrates recorded to prey upon *Harmonia axyridis* in the field

| Predator | Region | <i>H. axyridis</i> stage preyed | References |
|------------------------------|-----------------|---------------------------------|---|
| Spiders | | | |
| Araneidae | | | |
| <i>Araneus diadematus</i> | The Netherlands | Adult | Sloggett (2010a) |
| <i>Araneus diadematus</i> | Britain | Adult | Roy et al. (2011c) |
| <i>Araneus diadematus</i> | Belgium | Adult | https://observations.be/waarneming/view/78196535 |
| <i>Araneus</i> sp. | Belgium | Adult | https://observations.be/waarneming/view/86650304 |
| Undetermined Araneidae | Belgium | Adult | https://observations.be/waarneming/view/109424277 , https://observations.be/waarneming/view/120356673 |
| Oxyopidae | | | |
| <i>Peucetia viridans</i> | Florida, USA | Adult | http://volusianaturalist.com/category/insects/ |
| Philodromidae | | | |
| <i>Thanatus</i> sp. | Quebec, Canada | Adult | Lucas (2012) |
| Pholcidae | | | |
| <i>Pholcus phalangioides</i> | Belgium | Adult | San Martin (unpublished data) |
| Theridiidae | | | |
| <i>Enoplognatha</i> sp. | Belgium | Prepupa | https://observations.be/waarneming/view/43870778 |
| <i>Steatoda nobilis</i> | Chile | Adult | Zúñiga-Reinoso et al. (unpublished data), Grez (unpublished data) |
| Thomisidae | | | |
| <i>Xysticus</i> sp. | Belgium | Adult | https://observations.be/waarneming/view/69819373 , https://observations.be/waarneming/view/84758433 , https://observations.be/waarneming/view/119360474 |
| <i>Xysticus</i> sp. | New York, USA | Larva | http://www.projectnoah.org/spotting/11321090 |
| Unspecified spiders | Japan | Larva | Hironori and Katsuhiko (1997) |
| Bugs (Heteroptera) | | | |
| Anthocoridae | | | |
| <i>Orius insidiosus</i> | Indiana, USA | Not specified | Harwood et al. (2009) |

Table 1 continued

| Predator | Region | <i>H. axyridis</i> stage preyed | References |
|----------------------------------|---------------------|---------------------------------|---|
| Pentatomidae | | | |
| <i>Arma custos</i> | Belgium | Adult | http://www.snavelinsecten.heuvelstraat37.be/page417/page1/Insecten/page99/page99.html |
| <i>Arma custos</i> | France | Adult | http://www.floredefrance.com/query.php?T=1&famille=Pentatomidae |
| <i>Arma custos</i> | Germany | Adult | http://www.wikiwand.com/de/Waldw%C3%A4chter |
| <i>Podisus maculiventris</i> | Europe ^a | Larva | De Clercq et al. (2003) |
| <i>Podisus maculiventris</i> | Delaware, USA | Larva | Hough-Goldstein et al. (1996) |
| <i>Zicrona caerulea</i> | Hungary | Adult | Bozsik (2016) |
| <i>Zicrona caerulea</i> | Maine, USA | Larva | http://bugguide.net/node/view/77611/bgimage |
| Reduviidae | | | |
| <i>Ariilus cristatus</i> | Missouri, USA | Adult | http://donnabrunet.com/images/stock/index.php?album=Predators%20and%20Parasitoids.alb&image=20040809_MO_7025.jpg |
| <i>Zelus renardii</i> | Chile | Adult | Faúndez (2015) |
| Lacewings | | | |
| <i>Chrysoperla carnea</i> | Britain | Larva | Fremlin (2007) |
| <i>Chrysoperla carnea</i> | Poland | Larva | http://swiatmaszkolorowy.blox.pl/tagi_b/39657/Harmonia-axyridis.html |
| Ladybirds | | | |
| <i>Coccinella septempunctata</i> | Japan | Egg | Hironori and Katsuhiko (1997) |
| <i>Coccinella septempunctata</i> | Belgium | Not specified | Hautier et al. (2013) |

^aBoth *P. maculiventris* and *H. axyridis* released as biocontrol agents in sweet pepper crops



Fig. 1 *Steatoda nobilis* preying upon *Harmonia axyridis* (Pucón, Chile, 27th September 2016, photographed and reported via www.chinita-arlequin.uchile.cl by Miguel Ángel Mellado)

Katsanis et al. 2013). A few exceptions include larvae of the ladybird *Anatis ocellata* (L.) (Ware and Majerus 2008; Katsanis et al. 2013) and of the lacewings *Chrysoperla carnea* (Stephens) (Nedvěd et al. 2010, 2013) and *C. rufilabris* (Burmeister) (Michaud and Grant 2003). Larvae of *A. ocellata* are covered with sclerotized spines and, additionally, are larger than larvae of *H. axyridis* of the same instar. These features seem to result in unidirectional IGP for encounters between *H. axyridis* and *A. ocellata* in which the latter dominates. The importance of spiny larval projections for anti-predator defence and probability to win in intraguild confrontation was further demonstrated by Ware and Majerus (2008) and Katsanis et al. (2013). They found that spiny larvae of other ladybird species, such as *Calvia quatuordeciguttata* (L.) or *Harmonia quadripunctata* (Pontopidan), although often preyed upon by larvae of *H. axyridis*, relatively frequently played a role of intraguild predators of the latter.

In laboratory experiments it has been shown that lacewing larvae can efficiently kill even large larvae of *H. axyridis*, although sclerotized spines are missing in the former. Instead, these lacewing larvae are equipped with very long and sharp jaws that deter predators from attacking them and, on the other hand, enable them to effectively attack predators (Michaud and Grant 2003; Nedvěd et al. 2010, 2013; Wells et al. 2017). Larvae of *C. carnea* have also been observed preying on the larvae of *H. axyridis* in the field (Fremlin 2007, Table 1).

Parasitoids of *Harmonia axyridis*

Most parasitoids recorded from *H. axyridis* are restricted in their host use to species of Coccinellidae. Known exceptions include polyphagous tachinid flies of the genera *Medina* and *Strongygaster*. A complete list of parasitoids of *H. axyridis* in its native range and invaded areas is given in Table 2.

Phalacrotophora spp. (Diptera: Phoridae)

The polyphyletic genus *Phalacrotophora* has a worldwide distribution and comprises over 50 described species (Disney 2012), of which nine have been recorded as gregarious parasitoids of the pupae of ladybirds in the tribe Coccinellini, and less often Chilocorini (Ceryngier et al. 2012). Five species parasitize *H. axyridis*. These are *P. berlinensis* Schmitz, *P. beuki* Disney, *P. delageae* Disney, *P. fasciata* (Fallén), and *P. philaxyridis* Disney (Table 2). For one of them, *P. philaxyridis*, *H. axyridis* is the only known host so far (Disney 1997). However, as this fly is only known from the few Japanese specimens of the original species description, it is difficult to say how widespread or host-specific it is. The other species of *Phalacrotophora* known to occur within the native range of *H. axyridis* are *P. fasciata*, *P. decimaculata* Liu, and *P. quadrimaculata* Schmitz (Ceryngier et al. 2012). *Phalacrotophora fasciata* was discovered in *H. axyridis* in Western Siberia and Far East of Russia (Filatova 1974; Kuznetsov 1975, 1987, 1997). *Phalacrotophora decimaculata* was found in Shaanxi province (central China) in an unidentified ladybird, and *P. quadrimaculata* was found in Guangdong province (SE China) without any host association (Liu 2001). The only known host of the latter species was recorded in New Caledonia. This is the imported (American) *Olla v-nigrum* (Mulsant) (Disney and Chazeau 1990). Several reports from continental Asia and Japan (Maeta 1969a; Osawa 1992; Park et al. 1996) mention parasitism of *H. axyridis* by undetermined species of *Phalacrotophora*.

Phalacrotophora have also been found in ladybirds within the areas invaded by *H. axyridis*, in Europe and South America (Ceryngier et al. 2012). In Europe, the members of four species are frequent parasitoids of ladybirds, including *H. axyridis* (Table 2), while in South America, *P. nedae* (Malloch) is known to

Table 2 Records of parasitoids of *Harmonia axyridis*

| | Native range | North America | South America | Europe | Africa |
|---|-----------------------|---------------|---------------|---|--------|
| Diptera: Phoridae | | | | | |
| <i>Phalacrotophora berolinensis</i> | | | | 3, 7, 34, 35, 41 | |
| <i>Phalacrotophora beuki</i> | | | | 7 | |
| <i>Phalacrotophora delageae</i> | | | | 2, 7 | |
| <i>Phalacrotophora fasciata</i> | 8, 19, 20, 21 | | | 3, 7, 10, 26, 34, 35, 41 | |
| <i>Phalacrotophora philaxyridis</i> | 5 | | | | |
| <i>Phalacrotophora</i> sp. | 24, 30, 31 | | | 38 | |
| Diptera: Tachinidae | | | | | |
| <i>Medina separata</i> | | | | 36 | |
| <i>Medina</i> sp. ^a | 19, 20, 31 | | | | |
| <i>Strongygaster brasiliensis</i> | | | 39 | | |
| <i>Strongygaster triangulifera</i> | | 15, 28, 29 | | | |
| Hymenoptera: Braconidae | | | | | |
| <i>Dinocampus coccinellae</i> | 8, 16, 17, 23, 25, 31 | 9, 14, 15, 22 | 36, 39 | 1, 4, 6, 10, 12, 13, 18, 32, 37, 38, 41 | 27, 35 |
| Hymenoptera: Eulophidae, Tetrastichinae | | | | | |
| <i>Aprostocetus neglectus</i> | | 33 | | | |
| <i>Oomyzus scaposus</i> | 8, 20, 21 | 33 | | 3, 41 | |
| undet. Tetrastichinae ^b | 19 | | | 34 | |
| Hymenoptera: Encyrtidae | | | | | |
| <i>Homalotylus</i> sp. ^c | 21, 40 | | | 11, 36 | |
| <i>Homalotylus terminalis</i> | | 33 | | | |
| Hymenoptera: Pteromalidae | | | | | |
| <i>Pachyneuron altiscuta</i> ^d | | 33 | | | |
| <i>Pachyneuron</i> sp. ^e | 20 | | | | |

References: 1—Berkvens et al. (2010), 2—Ceryngier et al. (2012), 3—Comont et al. (2014), 4—Dindo et al. (2016), 5—Disney (1997), 6—Drumont (2011), 7—Durska and Ceryngier (2014), 8—Filatova (1974), 9—Firlej et al. (2005), 10—Francati (2015), 11—Glavendekić et al. (2010), 12—Hall et al. (2009), 13—Herz and Kleespies (2012), 14—Hoogendoorn and Heimpel (2002), 15—Katsoyannos and Aliniaze (1998), 16—Kawauchi (1984), 17—Koide (1961), 18—Koyama and Majerus (2008), 19—Kuznetsov (1975), 20—Kuznetsov (1987), 21—Kuznetsov (1997), 22—LaMana and Miller (1996), 23—Liu (1950), 24—Maeta (1969a), 25—Maeta (1969b), 26—Michie et al. (2009), 27—Minnaar et al. (2014), 28—Nalepa and Kidd (2002), 29—Nalepa et al. (1996), 30—Osawa (1992), 31—Park et al. (1996), 32—Raak-van den Berg et al. (2014), 33—Riddick et al. (2009), 34—Rondoni et al. (2013), 35—Roy et al. (2011b), 36—Roy et al. (2016), 37—Steenberg and Harding (2009), 38—Steenberg and Harding (2010), 39—Togni et al. (2015), 40—Trjapitzin (2011), 41—Ware et al. (2010)

^aReported as *Degeeria* (= *Medina*) *luctuosa* (probably *M. separata*)

^bProbably *Oomyzus scaposus*

^cReported as *H. flaminus* (probably *H. hemipterinus*)

^dProbably hyperparasitoid

parasitize several species of Coccinellini but has not been reported to attack *H. axyridis*.

As typical of idiobiont parasitoids, *Phalacrotophora* species are relatively polyphagous. A broad

host range has been reported for European species, *P. fasciata* and *P. berolinensis*, and to a lesser extent for *P. delageae*, and for a South American species, *P. nedae*. Possibly, *P. nedae* can also parasitize *H.*

axyridis, as it has been reported from other ladybirds in the same tribe (Coccinellini: *Cycloneda*, *Neda*, *Neocalvia*) (Ceryngier et al. 2012).

According to Filatova (1974) and Kuznetsov (1987), rates of parasitism of *H. axyridis* by *P. fasciata* in Asia did not differ considerably from the average rate for other ladybird hosts. In Europe, however, *H. axyridis* tends to be less affected by the species of *Phalacrotophora* than native ladybirds (Steenberg and Harding 2010; Ware et al. 2010; Comont et al. 2014). Furthermore, Ware et al. (2010) indicate that in the early phase of colonization of a new area *H. axyridis* might be either not parasitized or parasitized less compared to several years after arrival (Table 3).

To test the significance of these trends, we analyzed the data from Table 3 using binomial Generalized Linear Model (GLM) and Generalized Linear Mixed Model (GLMM). For comparisons between *H. axyridis* and other species, the study was used as random effect (see San Martin (2017) for the R code and detailed analysis output). However, as sample sizes were not mentioned in most data from the *H. axyridis* native range (Filatova 1974; Kuznetsov 1987), we imputed the missing sample sizes to conservative values of $N = 50$. The difference in the level of parasitism of *H. axyridis* and other species is well expressed in the invaded regions but not in the native range (highly significant Species \times Region interaction in a binomial GLMM, fixed effect = -2.8286 , $z = -3.802$, $p = 0.0001$). In the native range of *H. axyridis*, the average parasitism rate of *H. axyridis* (GLMM estimate = 16.2%) was very similar to that of the other species combined (15.19%), while in Europe the parasitism rate of *H. axyridis* was much lower (0.76%) than the rate for other species (10.76%) (Fig. 2a). There was a significantly lower parasitism rate of *H. axyridis* in Europe than in the native range (quasibinomial GLM, $F_{1, 15} = 19.68$, $p = 0.0005$, Fig. 2b). A weak trend toward an increase of the parasitism rate with time in the European *H. axyridis* was also observed (slope in a quasibinomial GLM = 0.2998, $t = 2.308$, $p = 0.038$) (Fig. 2c–d).

Tachinidae (Diptera)

Several tachinid flies attack coccinellids, mostly the juvenile stages of phytophagous species in the tribe Epilachnini (Ceryngier et al. 2012). However, two genera, *Medina* and *Strongygaster*, parasitize

Coccinellini adults, including *H. axyridis* (Ceryngier and Hodek 1996; Ceryngier et al. 2012; Togni et al. 2015). All reports of *Medina* parasitizing ladybirds are from the Palaearctic region, both within and outside of the native range of *H. axyridis* (Ceryngier and Hodek 1996). In the Chuncheon area in Korea, Park et al. (1996) recorded parasitism rates of *H. axyridis* by *Medina* sp. between 0.7 and 21.1%. In the Primorye region of the Russian Far East, Kuznetsov (1975) noted between 3.7 and 4.3% parasitism of *H. axyridis*. Outside of the native range, *H. axyridis* has been parasitized by *M. separata* (Meigen) in Britain (Roy et al. 2016). Moreover, tachinid larvae (probably of the genus *Medina*) were found in dissected adults of *H. axyridis* collected from overwintering sites in the vicinity of Warsaw (Poland) during February–April 2015. The recorded prevalence was low (0.48%, $n = 835$) and most of the tachinid larvae (3 of 4) were found dead and melanized (P. Ceryngier, unpublished data). In the Danish monitoring of natural enemies of ladybirds, tachinid larvae have not yet been recorded from *H. axyridis* ($n = 2299$), while they were found in very low prevalences in *C. septempunctata* (0.21%, $n = 483$) and *A. bipunctata* (0.31%, $n = 635$) (T. Steenberg, unpublished data).

Members of the genus *Strongygaster* are parasitoids of ladybirds in the Western Hemisphere. The North American *S. triangulifera* (Loew) is a polyphagous parasitoid of insects from several orders (Coleoptera, Lepidoptera, and Heteroptera) (Arnaud 1978; Golec et al. 2013). Nalepa et al. (1996), Katsoyannos and Aliniaze (1998) and Nalepa and Kidd (2002) found *S. triangulifera* attacking *H. axyridis* in the United States (North Carolina, Virginia, and Oregon). Parasitism rates ascertained by dissections of hosts fluctuated among years and localities from 0 to 31%. Rearing *H. axyridis* from a sample collected in Oregon yielded in emergence of the flies from 4.8% of the beetles, while the parasitism rate in the dissected subsample was 15.4% (Katsoyannos and Aliniaze 1998). Recently, another species of *Strongygaster*, *S. brasiliensis* (Townsend) was discovered parasitizing *H. axyridis* in Brazil (Togni et al. 2015).

Dinocampus coccinellae (Schrank) (Hymenoptera: Braconidae)

Dinocampus coccinellae is a species of nearly cosmopolitan distribution. It is a solitary endoparasitoid

Table 3 Rates of parasitism (and sample sizes if mentioned) by *Phalacrotophora* spp. of *Harmonia axyridis* (highlighted in bold) and other ladybird species at various localities within *H. axyridis* native and invasive ranges

| Region | Years | Parasitoid | Host | % Parasitism (N) | References | |
|--------------------------|-----------|---------------------------------|----------------------------------|-----------------------------------|------------------------------|-------------------|
| Native range | | | | | | |
| Altai region, Russia | 1961 | <i>Phalacrotophora fasciata</i> | <i>Anatis ocellata</i> | 44.1 | Filatova (1974) | |
| | | | <i>Adalia bipunctata</i> | 25.0 | | |
| | | | <i>Harmonia axyridis</i> | 24.4 | | |
| | | | <i>Harmonia quadripunctata</i> | 9.6 | | |
| | 1968 | | | <i>Anatis ocellata</i> | 45.1 | |
| | | | | <i>Exochomus quadripustulatus</i> | 29.3 | |
| | | | | <i>Harmonia axyridis</i> | 25.8 | |
| | | | | <i>Harmonia quadripunctata</i> | 14.6 | |
| | | | | <i>Harmonia quadripunctata</i> | 8.5 | |
| | | | | <i>Hippodamia variegata</i> | 6.4 | |
| | | | | <i>Adalia bipunctata</i> | 5.9 | |
| | | | | <i>Coccinella septempunctata</i> | | |
| | | | | | | |
| Russian Far East | 1983 | <i>Phalacrotophora fasciata</i> | <i>Coccinella septempunctata</i> | 17.6 | Kuznetsov (1987) | |
| | | | <i>Harmonia axyridis</i> | 10.3 | | |
| | | | <i>Chilocorus inornatus</i> | 8.0 | | |
| Kyoto, Japan | 1987–1988 | <i>Phalacrotophora</i> sp. | <i>Harmonia axyridis</i> | 10.3 (146) | Osawa 1992 | |
| | 1990 | | <i>Harmonia axyridis</i> | 17.7 (249) | | |
| Chuncheon area, Korea | 1995 | <i>Phalacrotophora</i> sp. | <i>Harmonia axyridis</i> | 0.4–6.7 | Park et al. 1996 | |
| Invaded areas | | | | | | |
| London and Cambridge, UK | 2004 | <i>Phalacrotophora</i> spp. | <i>Coccinella septempunctata</i> | 2.2 (227) | Ware et al. 2010 | |
| | | | <i>Harmonia axyridis</i> | 0 (278) | | |
| | 2005 | | <i>Harmonia axyridis</i> | 0 (762) | | |
| | 2006 | | <i>Harmonia axyridis</i> | 0 (450) | | |
| | 2007 | | <i>Harmonia axyridis</i> | 0.4 (500) | | |
| | 2008 | | | <i>Coccinella septempunctata</i> | | 8.4 (119) |
| | | | | <i>Harmonia axyridis</i> | | 1.6 (1120) |
| | | | | | | |
| | 2009 | | <i>Coccinella septempunctata</i> | 10.5 (692) | | |
| | | | <i>Harmonia axyridis</i> | 0.4 (2574) | | |
| Copenhagen, Denmark | 2009 | <i>Phalacrotophora</i> sp. | <i>Coccinella septempunctata</i> | 55.4 (233) | Steenberg and Harding (2010) | |
| | | | <i>Adalia bipunctata</i> | 11.4 (404) | | |
| | | | <i>Harmonia axyridis</i> | 2.7 (4508) | | |
| | | | | | | |
| England | 2008–2011 | <i>Phalacrotophora</i> spp. | <i>Coccinella septempunctata</i> | 18.0 (727) | Comont et al. (2014) | |
| | | | <i>Harmonia axyridis</i> | 1.7 (3868) | | |
| Warsaw region, Poland | 2008–2012 | <i>Phalacrotophora</i> spp. | <i>Harmonia axyridis</i> | 5.3 (1054) | Durska and Ceryngier (2014) | |

Table 3 continued

| Region | Years | Parasitoid | Host | % Parasitism (N) | References |
|---------------------|-------|---------------------------------|--------------------------|--------------------------|-----------------|
| Bologna area, Italy | 2013 | <i>Phalacrotophora fasciata</i> | <i>Harmonia axyridis</i> | > 5.1 (98) ^a | Francati (2015) |
| | 2014 | | <i>Harmonia axyridis</i> | > 0.8 (125) ^a | |

Parasitism rates for species other than *H. axyridis* are only given in cases those species were sampled at the same period and region as *H. axyridis*

^asamples containing larvae and pupae (larvae are not parasitized by *Phalacrotophora*)

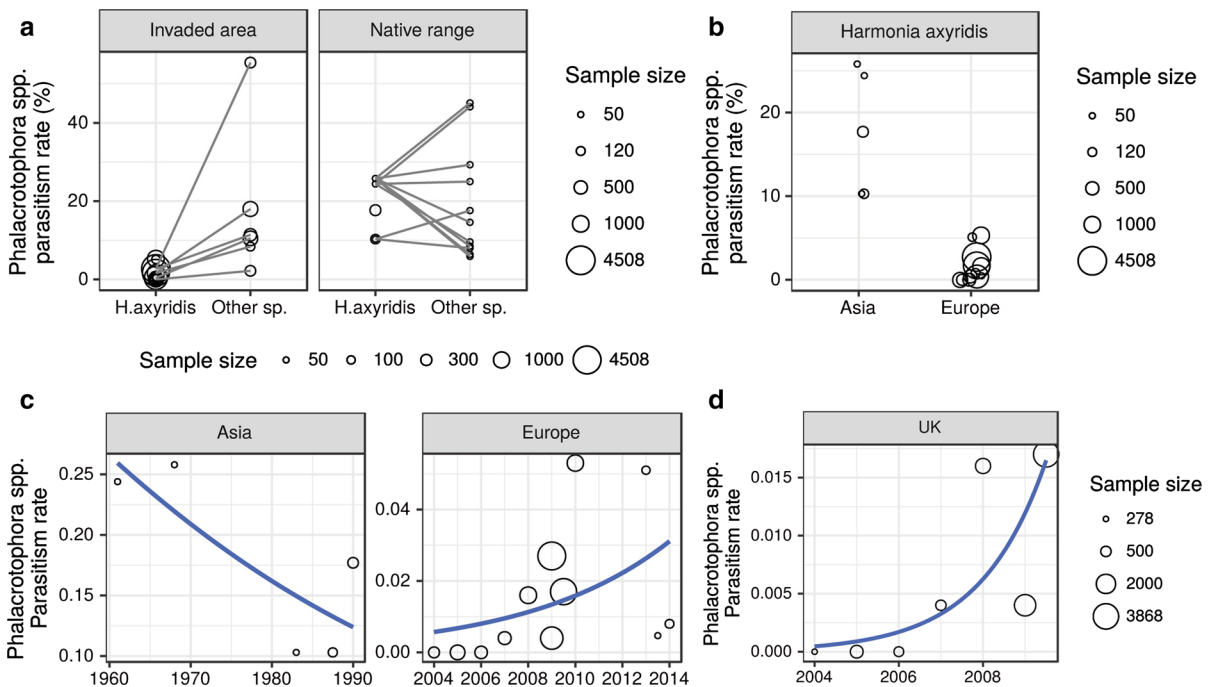


Fig. 2 Visualisation of the meta-analysis for parasitism by *Phalacrotophora* spp. **a** Parasitism rate of *H. axyridis* by *Phalacrotophora* spp. compared to all other ladybird species confounded. Lines connect observations from the same study and the same year. The level of parasitism of *H. axyridis* is significantly lower in the invaded areas, while in the native range it is similar to other host species. **b** Parasitism rate of *H. axyridis* in Europe compared to the native range There is a significantly lower rate in Europe. **c** Parasitism of *H. axyridis*

over time in the native range and in Europe. While the slope is negative in the native range (Asia), it was estimated with high imprecision; hence, the slope is not significantly different from 0. In Europe, there is a positive trend, which is significantly different from 0. **d** The UK is the only country with several years of data for parasitism of *H. axyridis* by *Phalacrotophora* (Ware et al. 2010; Comont et al. 2014). However, despite the strongly positive slope, it is not significantly different from 0. See San Martin (2017) for details

of many ladybird beetles, mostly in the tribe Coccinellini (54 host species reported) and much less frequently in Chilicorini (5 host species reported) (Ceryngier et al. 2012; Minnaar et al. 2014; Grez and Zaviezo unpubl. in Table 4).

Harmonia axyridis is a host of *D. coccinellae* (Fig. 3), with reports both within its native range and in all invaded continents (Table 2), but parasitism and emergence rates are usually low in comparison with other host species (Maeta 1969b; Hoogendoorn and

Table 4 Rates of parasitism of *Harmonia axyridis* and other ladybirds by *Dinocampus coccinellae* and rates of emergence of *D. coccinellae* from the hosts at various localities in the native and invasive ranges of *H. axyridis* (sample sizes in brackets)

| Region | Years | Host | % Parasitism (N) | % Emergence (N) | References |
|---------------------|-----------|---|-------------------------|-------------------------|--|
| Native range | | | | | |
| Kurume, Japan | 1960 | <i>Coccinella septempunctata brucki</i> | 15.8 (265) ^a | – | Maeta (1969b) |
| | | <i>Harmonia axyridis</i> | 7.5 (129) ^a | – | |
| Ina, Japan | 1961 | <i>Harmonia axyridis</i> | 1.2 (164) | – | |
| Invaded areas | | | | | |
| Oregon, USA | 1993–1994 | <i>Harmonia axyridis</i> | – | < 1 (> 2000) | LaMana and Miller (1996) |
| Oregon, USA | 1997 | <i>Harmonia axyridis</i> | 1 (101) | 0 (42) | Katsoyannos and Aliniaze (1998) |
| Minnesota, USA | 1999 | <i>Harmonia axyridis</i> | 23.8 (63) | – | Hoogendoorn and Heimpel (2002) |
| | | <i>Coleomegilla maculata</i> | 18.2 (435) | – | |
| | | <i>Coleomegilla maculata</i> | 14.5 (110) | 10.5 (57) | |
| Quebec, Canada | 2000 | <i>Harmonia axyridis</i> | 8.9 (282) | 1.2 (84) | Firlej et al. (2005) |
| | | <i>Coleomegilla maculata</i> | 32.1 (521) | 5.9 (472) | |
| London, UK | 2007 | <i>Coccinella septempunctata</i> | 10.0 (110) | 11.9 (219) | Koyama and Majerus (2008) |
| | | <i>Harmonia axyridis</i> | 7.6 (237) | 0.2 (474) | |
| UK | 2008 | <i>Harmonia axyridis</i> | – | 0.8 (1120) ^a | Hall et al. 2009 |
| | | <i>Coccinella septempunctata</i> | – | 0 (119) ^a | |
| UK | 2011–2012 | <i>Harmonia axyridis</i> | – | 4–5 ^a | R. F. Comont (pers. comm.) in Raak-van den Berg et al. 2014 (supplementary material) |
| Germany | 2008–2009 | <i>Harmonia axyridis</i> | 1 (1085) | 0 (1085) | Herz and Kleespies (2012) |
| The Netherlands | 2004–2008 | <i>Harmonia axyridis</i> | 0 (1049) | – | Raak-van den Berg et al. (2014) |
| | 2009 | <i>Harmonia axyridis</i> | 0.2 (633) | 0 (18,952) | |
| | 2010 | <i>Harmonia axyridis</i> | 0.1 (1652) | < 0.1 (1453) | |
| Copenhagen, Denmark | 2010 | <i>Coccinella septempunctata</i> | 28.6 (14) | – | Steenberg, unpubl. |
| | | <i>Harmonia axyridis</i> | 27.4 (339) | – | |

Table 4 continued

| Region | Years | Host | % Parasitism (N) | % Emergence (N) | References |
|----------------------------------|-------|----------------------------------|------------------------------|-------------------------------|---------------------------|
| | 2014 | <i>Coccinella septempunctata</i> | 4.4 (45) | – | |
| | | <i>Harmonia axyridis</i> | 3.5 (172) | – | |
| Bologna area, Italy | 2010 | <i>Coccinella septempunctata</i> | – | 6.2 (113) | Dindo et al. (2016) |
| | | <i>Harmonia axyridis</i> | – | 3.9 (1348) | |
| | | <i>Hippodamia variegata</i> | – | 2.4 (40) | |
| | | <i>Adalia bipunctata</i> | – | 2.3 (43) | |
| Bologna area, Italy | 2013 | <i>Harmonia axyridis</i> | – | 4.1 (98)^a | Francati (2015) |
| | 2014 | <i>Harmonia axyridis</i> | – | 19.2 (125)^a | |
| West Cape Province, South Africa | 2013 | <i>Adalia flavomaculata</i> | – | 11 (37) | Minnaar et al. (2014) |
| | | <i>Hippodamia variegata</i> | – | 9 (78) | |
| | | <i>Cheilomenes lunata</i> | – | 4 (68) | |
| | | <i>Exochomus flavipes</i> | – | 3 (35) | |
| | | <i>Harmonia axyridis</i> | – | 1 (276) | |
| Santiago, Chile | 2016 | <i>Eriopis chilensis</i> | 29.2 (24) ^b | – | Grež and Zaviezo. unpubl. |
| | | <i>Hippodamia variegata</i> | 46.4 (28) ^b | – | |
| | | <i>Harmonia axyridis</i> | 10.8 (37)^b | – | |
| | | <i>Eriopis chilensis</i> | – | 16.6 (253) ^c | |
| | | <i>Hippodamia variegata</i> | – | 14.6 (280) ^c | |
| | | <i>Harmonia axyridis</i> | – | 4.7 (43)^c | |
| České Budějovice, Czech Republic | 2015 | <i>Harmonia axyridis</i> | – | 0.4 (280) | Nedvěd, unpubl. |
| | 2016 | <i>Harmonia axyridis</i> | – | 26.1 (46) | |

Parasitism rate determined by dissection of field collected ladybirds, emergence rate—by rearing field collected ladybirds. Data for species other than *H. axyridis* are only given in cases those species were sampled at the same period and region as *H. axyridis*, data for *H. axyridis* are highlighted in bold

^aFrom hosts collected as larvae or pupae

^bAdults collected in alfalfa in autumn (March–May)

^cAdults collected in alfalfa in spring (August–December)

Heimpel 2002; Firlej et al. 2005, 2007, 2010; Koyama and Majerus 2008; Ceryngier et al. 2012; Minnaar et al. 2014; Table 4). Koyama and Majerus (2008) demonstrated that *D. coccinellae* of Japanese origin developed in British *H. axyridis* more successfully (about 17% successful parasitism rate) compared to wasps of British origin (no successful parasitism). On the other hand, about 25% of the Japanese as well as British wasps successfully developed in *H. axyridis* from Japan, suggesting that non-invasive ecotypes of *H. axyridis* may be more susceptible to various strains or geographic races of *D. coccinellae* than the invasive ecotype. However, some recent sequential data suggest that *D. coccinellae* may become better adapted to



Fig. 3 *Harmonia axyridis* with a cocoon of its parasitoid *Dinocampus coccinellae* (photographed by Jean-Yves Baugnée)

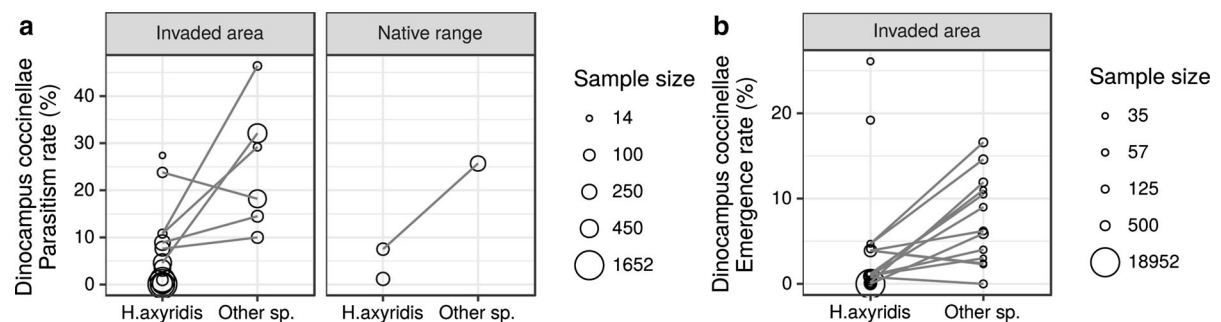


Fig. 4 Visualisation of the meta-analysis for parasitism by and emergence of *Dinocampus coccinellae*. **a** Parasitism rate of *H. axyridis* by *D. coccinellae* relative to all other ladybird species confounded. Lines connect observations from the same study and the same year. The level of parasitism of *H. axyridis* is significantly lower than of other host species. When considering the invaded areas only, the lower parasitism of *H. axyridis* is

the invasive populations of *H. axyridis* (see data of Francati (2015) and Nedvĕd, unpubl. in Table 4).

Dindo et al. (2016) found no significant differences in the rates of emergence of *D. coccinellae* from *H. axyridis* and three native ladybird species (*C. septempunctata*, *Adalia bipunctata* and *Hippodamia variegata* (Goeze)) in Italy. However, the samples of the two species with low suitability for *D. coccinellae* (*A. bipunctata* and *H. variegata*) were very small. Our GLMM analysis of the data from Table 4 reveals a significantly lower parasitism rate (fixed effect = 1.1099, $z = 2.439$, $p = 0.015$) of *H. axyridis* in its invaded areas compared to the other species pooled (Fig. 4a). The same is true for the emergence rate (fixed effect = 1.8531, $z = 3.334$, $p = 0.0009$) (Fig. 4b). The data from the native range are too scarce to be used. Furthermore, we did not observe any temporal trend in the parasitism and emergence rates in this dataset.

Tetrastichinae (Hymenoptera: Eulophidae)

Of several species in this subfamily that are known as ladybird parasitoids, the most frequently reported is *Oomyzus scaposus* (Thomson). This widely distributed minute wasp is a larval-pupal endoparasitoid of many ladybird species, mostly in the tribes Coccinellini and Chilocorini (Ceryngier et al. 2012). It parasitizes *H. axyridis* in its native range in Western Siberia (Filatova 1974) and the Russian Far East (Kuznetsov 1987, 1997) as well as in the invasive

close to significant. **b** Emergence rate of *D. coccinellae* from *H. axyridis* relative to all other ladybird species confounded. No data are available for emergence rate from the native range. Again, the rate of emergence of *D. coccinellae* when using *H. axyridis* as a host is significantly lower compared to other host species. See San Martín (2017) for details

areas in North America (Riddick et al. 2009) and Britain (Ware et al. 2010; Comont et al. 2014). Riddick et al. (2009) reported that 44% of 48 *H. axyridis* pupae collected in North Carolina (USA) were parasitized by *O. scaposus*. In the same sample, also another species of Tetrastichinae, *Aprostocetus neglectus* (Domenichini), was recorded. Ware et al. (2010) found *O. scaposus* as an occasional parasitoid of *H. axyridis* in Britain; between 2004 and 2008 no parasitism by this species was recorded, while in 2009, two of 2574 *H. axyridis* pupae (0.08%) were parasitized. In Denmark *O. scaposus* occasionally parasitizes *C. septempunctata* (1.3%, $n = 397$) and *A. bipunctata* (0.5%, $n = 730$), but it has not been recorded in pupae of *H. axyridis* (11,394 pupae examined between 2007 and 2016) (Steenberg and Harding, unpublished data).

Homalotylus spp. (Hymenoptera: Encyrtidae)

Wasps of the genus *Homalotylus* are endoparasitoids of ladybird larvae and pupae. Approximately 30 of 67 described species parasitize Coccinellidae, and the remaining species are also thought to parasitize ladybirds as well (Ceryngier et al. 2012; Noyes 2017). The taxonomy of the *Homalotylus* species most frequently reported from the hosts in the tribe Coccinellini, including *H. axyridis*, has often been confused during the past 100 years. Usually it has erroneously been determined as *H. flaminus* (Dalman) (the species parasitizing ladybirds in the tribe Scymnini) and less frequently as *H. eytelweini* (Ratzeburg) (described from Germany as a parasitoid of *Anatis ocellata*). Noyes (2010) and Trjapitzin (2013), however, suggest that the proper name of this species probably is *H. hemipterinus* (De Stefani). It was reported parasitizing *H. axyridis* in the Russian Far East (Kuznetsov 1997; Trjapitzin 2011), in Croatia (Glavendekić et al. 2010), and in Britain (Roy et al. 2016). In North Carolina (USA), *H. axyridis* was attacked by yet another species, *H. terminalis* (Say) (Riddick et al. 2009).

Potential of predators and parasitoids to limit *H. axyridis* numbers

Harmonia axyridis is protected chemically against predation and more so than many other coccinellids (Sloggett et al. 2011). Many laboratory assays indicate

its higher toxicity and/or unpalatability compared to other members of aphidophagous guilds (ladybirds, lacewings) (Phoofolo and Obyrcki 1998; Cottrell 2004; Sato and Dixon 2004; Kajita et al. 2010; Nedvěd et al. 2010). Further protection of the larvae is provided by spiny projections on their cuticle. Apart from these chemical and mechanical defenses, *H. axyridis* is also equipped with a biological weapon against some predators—symbiotic microsporidia, which are lethal to potential intraguild predators, *C. septempunctata* and *A. bipunctata* (Vilcinskas et al. 2013, 2015; Vogel et al. 2017; but see Sloggett 2013; Solter et al. 2013; Gegner et al. 2015).

A broad repertoire of *H. axyridis* defenses combined with limited evidence of predation by vertebrate and invertebrate carnivores indicate that predators are unlikely to play a considerable role in reducing harlequin ladybird populations. Although some birds (*Delichon urbica*, *Passer montanus*) and invertebrates (*Araneus diadematus*, *Steatoda nobilis*, *Podisus maculiventris*, *Chrysoperla carnea*) do not avoid *H. axyridis* as a prey, their contribution to the control of the latter is probably not high.

The chemical and morphological defenses protecting *H. axyridis* against predators are probably less effective against parasitoids. Instead, the ladybird's immune responses to and physiological and nutritional unsuitability for the development of parasitoid eggs and larvae may represent the main defensive strategy. These questions were studied in detail by Firlej et al. (2007, 2012) using the *H. axyridis*—*D. coccinellae* system. They found that *H. axyridis* possesses an effective immune system that is able to destroy the parasitoid eggs through encapsulation by hemocytes (Firlej et al. 2012). If the egg is not destroyed and the larva hatches, its development may be impeded or delayed due to a reduced number and inappropriate growth pattern of teratocytes, cells originating from the serosa of the parasitoid egg that serve as food for the parasitoid larva (Firlej et al. 2007). It is also possible that the microsporidia discovered in *H. axyridis* (Vilcinskas et al. 2013) contribute to its low suitability for *D. coccinellae*. No empirical confirmation of this exists so far, but the finding of Sluss (1968) that the successful development of *D. coccinellae* was prevented in microsporidia-infected ladybirds (*Hippodamia convergens* Guérin) makes this hypothesis plausible. It should also be tested whether other pathogens and parasites of *H. axyridis*, especially the

nematode *Parasitylenchus bifurcatus* Poinar and Steenberg (Harding et al. 2011; Poinar and Steenberg 2012; Haelewaters et al. 2017), can interfere with *D. coccinellae* larval development.

Another hypothesis that needs testing assumes that *H. axyridis* may be more resistant than other ladybirds to the *D. coccinellae* paralysis virus. This virus, after transmission from the parasitoid larva to the host ladybird nervous system, replicates in the cerebral ganglia causing paralysis of the ladybird. This enables the emerging larva to spin a cocoon between the host's legs and consequently gain protection beneath the aposematically coloured and chemically defended host body (Dheilly et al. 2015). The virus-induced paralysis is temporary and ladybirds can sometimes recover and even resume their reproductive activity (Triltsch 1996; Maure et al. 2014). If the intensity of paralysis is weak and/or its duration short, the role of parasitoid bodyguard played by the ladybird may be incomplete. It seems probable that *H. axyridis* often provides incomplete bodyguard protection to its *D. coccinellae* parasitoid. Quick resumption of walking and feeding ability of *H. axyridis* following *D. coccinellae* emergence was observed several times and in one case the emerged parasitoid was consumed by the host ladybird (P. Ceryngier, unpubl.).

Despite its low suitability for development of *D. coccinellae*, *H. axyridis* is no less preferred for oviposition by this wasp than more suitable hosts (Hoogendoorn and Heimpel 2002; Koyama and Majerus 2008; Firlej et al. 2010). Furthermore, interacting with *D. coccinellae* females, *H. axyridis* adults display more behavioural defenses than do the adults of suitable hosts, such as *Coleomegilla maculata* (DeGeer), which increases the handling time of the parasitoid and hence further reduces its fitness (Firlej et al. 2010). Finally, although *H. axyridis* larvae are more suitable for development of *D. coccinellae* than adults (Firlej et al. 2007, 2010; Berkvens et al. 2010), the latter are more readily parasitized by *D. coccinellae* (Firlej et al. 2010). In conclusion, *H. axyridis* populations within the invaded range seem unlikely to be reduced to a significant extent by *D. coccinellae*. Instead, *H. axyridis* may represent an ecological trap or at least a sink for *D. coccinellae* eggs (Hoogendoorn and Heimpel 2002; Firlej et al. 2010).

Physiological interactions between *H. axyridis* and parasitoids other than *D. coccinellae* have not been investigated yet, but it can be expected that these

interactions might limit the developmental success of koinobiont parasitoids, such as *O. scapوسus* and *Homalotylus* spp. Both occasionally attack *H. axyridis* larvae and pupae at low prevalences (but see Riddick et al. 2009), which may be attributed to either low oviposition rates or low successful parasitism. Idiobiont parasitoids, like flies of the genus *Phalacrotophora*, quickly kill their hosts and then develop in host corpses. Therefore, physiological host-parasitoid interactions, if established at all, will be weak and short-lasting. Perhaps due to this, the reported rates of parasitism of *H. axyridis* by *Phalacrotophora* spp. (especially in the native range of *H. axyridis*) are higher than those attained by the koinobionts. On the other hand, the branched spiny projections on *H. axyridis* larvae may hamper oviposition of females of Tetrastichinae and *Homalotylus*. This is not the case with *Phalacrotophora* females which oviposit (during ladybird ecdysis to the pupal stage) into smooth and soft pupal skin (Ceryngier et al. 2012). Of *H. axyridis* parasitoids in the Palearctic region, *Phalacrotophora* flies might be the most important, although their role is still poorly known.

Enemy release hypothesis and enemy acquisition

Populations of a non-native species introduced in a new area may escape from its natural enemies as proposed by the enemy release hypothesis (ERH) but may also, often after a certain time lag, become prone to attacks from native predators, parasitoids, parasites, and pathogens. The latter process, when an association of a non-native species with a native enemy is established, is referred to as "enemy acquisition".

ERH can be considered at a biogeographical and community scale (Colautti et al. 2004). Biogeographical studies compare diversity and impact of natural enemies attacking a given species in its native and introduced ranges, while community studies compare diversity and impact of enemies of co-occurring native and non-native species. Below we briefly discuss these two approaches limiting our considerations to parasitoids. Too few data are available on *H. axyridis* predators and, on the other hand, it is not very likely that these principally generalist zoophages may be involved in the enemy release phenomenon.

At a biogeographical scale, no conclusive statements can be made as to whether the ERH is pertinent to *H. axyridis* invasion. The recorded richness of *H.*

axyridis parasitoids in the native range (at least 6 species of primary parasitoids and one hyperparasitoid) is higher than in most of invaded continents (5 species of primary parasitoids plus 1 hyperparasitoid in North America, 2 species in South America, 1 species in Africa), but lower than in Europe (8 species) (Table 2). These figures, however, do not necessarily reflect the actual differences among regions, but may be related to the unequal state of knowledge on this topic in different geographic regions. Indeed, more studies on ladybird parasitoids have been conducted in Europe than in the remaining continents invaded by *H. axyridis* and the native area in Asia as well.

The most common parasitoids of ladybirds of the tribe Coccinellini in the Palaearctic region are hymenopterans *D. coccinellae*, *O. scaposus*, and *H. hemipterinus*, and dipterans of the genus *Phalacrotophora*. (Ceryngier et al. 2012). All of them have been reported to parasitize *H. axyridis* in its native range. Yet, the hymenopterans mentioned above also occur within the invasive ranges of *H. axyridis*, not only in the Palaearctic (Europe) but also in South America, North America (maybe with the exception of *H. hemipterinus*), and Africa (Noyes 2010, 2017; Ceryngier et al. 2012). As to species of *Phalacrotophora* parasitic in Coccinellini, they have been reported from the invasive ranges of *H. axyridis* in Europe and South America. Thus, according to the present knowledge on the distribution of *H. axyridis* parasitoids, the enemy release in the literal meaning only concerns escape of a few invasive populations of the harlequin ladybird from parasitism by a few parasitoids, e.g. escape of North American and African populations from parasitism by *Phalacrotophora*. When considering the enemy release not as a simple presence/absence opposition, but in terms of differences in the parasitism rates reached in the native and invaded areas, some support for the ERH can be found: rates of parasitism of *H. axyridis* by *Phalacrotophora* spp. tend to be higher in Asia than in Europe (Table 3).

Stronger support for the ERH is provided by the community approach. Comparing parasitism of the invasive *H. axyridis* with that of the native ladybirds, lower prevalences are usually recorded in the former. This applies to parasitism by both *Phalacrotophora* spp. (Table 3) and *D. coccinellae* (Table 4). While discussing applicability of the enemy release, however, the community studies should not be considered

in isolation from the biogeography comparisons and research on the physiological, immunological or behavioural host-enemy interactions. It is well documented that *H. axyridis* belongs to hosts of low suitability for *D. coccinellae* not only in the areas outside of the ladybird native range, but also within this range (Maeta 1969b; Koyama and Majerus 2008; Ceryngier et al. 2012). Nonetheless, study by Koyama and Majerus (2008) suggests that *D. coccinellae* from the native area of *H. axyridis* may be better adapted to exploit it.

Although some evidence, both at the level of biogeography and community, indicates that the European populations of *H. axyridis* may have experienced partial release from parasitism by *Phalacrotophora* spp., the faunistic data point at enemy acquisition rather than enemy release. While two species of *Phalacrotophora* (*P. fasciata* and *P. philaxyridis*) are known to affect the native populations of *H. axyridis*, four species (*P. fasciata* and three other species) have been recorded in Europe. Two of these three species other than *P. fasciata* (*P. berolinensis* and *P. delageae*) are not known to occur outside of Europe, and the third one (*P. beuki*) is known from Europe and Israel (Ceryngier et al. 2012; Mostovski 2016). Interestingly, none of the native European ladybirds has been found to host so many *Phalacrotophora* parasitoids as the invasive *H. axyridis*. All four species of *Phalacrotophora* were found parasitizing *H. axyridis* in Warsaw and its surroundings (Poland) during the studies conducted between 2008 and 2012. From among 1054 collected pupae of *H. axyridis*, 56 (5.3%) were parasitized by phorid flies, and of them the most common was *P. delageae*, followed by *P. fasciata*, *P. berolinensis* and *P. beuki* (Durska and Ceryngier 2014). The latter species was previously reported only from *Anatis ocellata* (Durska et al. 2003). That *H. axyridis* may be parasitized by a wider array of *Phalacrotophora* parasitoids than the native European species is also confirmed by studies conducted in Britain. Ware et al. (2010) and Comont et al. (2014) found *H. axyridis* parasitized by *P. fasciata* and *P. berolinensis*, while *C. septempunctata* only by *P. fasciata*.

Further examples of parasitoid acquisition by the invasive *H. axyridis* include flies of the genus *Strongygaster* (*S. triangulifera* and *S. brasiliensis*) and the wasp *H. terminalis* (Table 2). As those species

are only known from the New World, *H. axyridis* almost certainly is their novel host.

Several parasitoids reported from *H. axyridis* are widely distributed in many parts of the world. This concerns *D. coccinellae*, *O. scaposus*, *H. hemipterinus* and to a lesser degree *P. fasciata*. Perhaps populations of these species from different regions have different requirements and host-use adaptations and hence should be regarded as distinct ecological entities. Studies by Koyama and Majerus (2008) showing differences between the Japanese and British *D. coccinellae* support this view. Such an approach, however, implies that *H. axyridis* invading a new area may escape from one geographic ecotype or race of a given parasitoid to acquire another one, often after a certain time lag.

To conclude this section, in the initial phase of *H. axyridis* invasion the enemy release has possibly contributed to the spread and increase of the invader's populations. Later, however, novel associations might start to establish mitigating previous effects of the enemy release. Acquisition of new enemies by *H. axyridis* in the invaded areas does not only apply to parasitoids. True parasites, such as the mite *Coccipolipus hippodamiae* (McDaniel and Morrill), the nematode *Parasitylenchus bifurcatus*, and the fungus *Hesperomyces virescens* Thaxter, are also believed to have been acquired by the populations of the harlequin ladybird in the invaded range (Ceryngier and Twardowska 2013; Haelewaters et al. 2016, 2017).

Conclusions and perspectives

There is little evidence of population limitation of *H. axyridis* by predators or parasitoids. Predatory vertebrates and invertebrates usually avoid preying on *H. axyridis* and those that accept it as a prey, being broad generalists, do this opportunistically. There may be more opportunities for such generalist predators to attack *H. axyridis* during population outbreaks. However, whether or not predators can provide any degree of control of *H. axyridis* during such events requires observational and experimental data.

Rates of parasitism of *H. axyridis* by the hymenopteran parasitoids (*D. coccinellae*, *Homalotylus* sp., Tetrastichinae) are generally low, both in the native and invaded areas of the ladybird. Possible reasons of this are (1) low rates of oviposition into *H. axyridis* by

the parasitoid females, (2) low nutritional suitability of *H. axyridis*, and/or (3) its effective immune response. Physiological and immunological interactions may also play a role in lowering the successful parasitism of *H. axyridis* by the tachinid flies of the genera *Medina* and *Strongygaster*.

The most promising control agents of *H. axyridis* among parasitoids seem to be phorid flies of the genus *Phalacrotophora* (many species involved, relatively high parasitism rates—see Table 3). They are idio-biont parasitoids and hence can evade the effects of *H. axyridis* immune system. There are many species of *Phalacrotophora* parasitizing various species of Coccinellini ladybirds. Field studies to be conducted in different regions would clarify the host spectra of individual *Phalacrotophora* species and show which of them parasitize *H. axyridis* and to what extent. The host range, geographic distribution, and abundance of *P. philaxyridis* is especially worth checking. Currently, this species is only known as a parasitoid of *H. axyridis* with very scarce data coming from only one locality in Japan (Disney 1997).

Although the present state of knowledge indicates that the majority of ladybird parasitoids are unable to exert any significant effect on *H. axyridis* populations, it cannot be excluded that some of the parasitoids native to the areas invaded by the harlequin ladybird will adapt to exploit it more efficiently. Moreover, bearing in mind great potential of the invasive populations of *H. axyridis* for long-distance translocations (Lombaert et al. 2010; Brown et al. 2011), it is also possible that some of the parasitoids may disperse with the hosts to other regions (co-colonization of new areas). This may especially be the case with imaginal parasitoids, such as *D. coccinellae*. For examining geographic variation of *H. axyridis* parasitoids and their possible host-driven spreading, large-scale genetic comparisons are required.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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