From effective biocontrol agent to successful invader: the Harlequin ladybird (*Harmonia axyridis*) an example of good ideas that could go wrong

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The use of biocontrol agents to eradicate pests is an alternative to pesticides and a tool to manage invasive alien species. Nevertheless, biocontrol agents' use is also a way of introduction of invaders worldwide. The Harlequin ladybird (*Harmonia axyridis*) is a native Asian biocontrol agent that has become a successful invader. We reviewed articles containing "*Harmonia axyridis*" to gather information on its presence and surveyed entomologists researching Coccinellidae around the world to investigate further insights about the current distribution, vectors of introduction, habitat use and threats this species pose. The Harlequin ladybird has established at least in 59 countries outside its native range. Nineteen scientists considered it a potential threat to native Coccinellidae. Published studies and scientists suggest *Adalia bipunctata*, native to Europe, is under the highest risk of population declines. Strict policies should be incorporated to prevent its arrival to non-invaded areas and to prevent further expansion range. Managing invasive species is key to prevent biodiversity loss and promote ecosystem services.

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From biocontrol agent to successful invader

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Abstract

The use of biocontrol agents to eradicate pests is an alternative to pesticides and a tool to manage invasive alien species. Nevertheless, biocontrol agents' use is also a way of introduction of invaders worldwide. The Harlequin ladybird (*Harmonia axyridis*) is a native Asian biocontrol agent that has become a successful invader. We reviewed articles containing "*Harmonia axyridis*" to gather information on its presence and surveyed entomologists researching Coccinellidae around the world to investigate further insights about the current distribution, vectors of introduction, habitat use and threats this species pose. The Harlequin ladybird has established at least in 59 countries outside its native range. Nineteen scientists considered it a potential threat to native Coccinellidae. Published studies and scientists suggest *Adalia bipunctata*, native to Europe, is under the highest risk of population declines. Strict policies should be incorporated to prevent its arrival to non-invaded areas and to prevent further expansion range. Managing invasive species is key to prevent biodiversity loss and promote ecosystem services.

Keywords

Coccinellidae, invasive threat, range expansion, biodiversity threats, perception, awareness

Introduction

Biocontrol is a management strategy that uses species that act as natural enemies to supress populations of other species that are considered pests and are causing a negative impact in crop

fields, human health or pose a threat to biodiversity (Greathead and Waage, 1983). Biocontrol agents have commonly been used in the last decades to control pests, diseases and weeds as an alternative to chemical pesticides (De Clercq et al., 2011). It is considered a more environmental-friendly way to deal with pests, nevertheless, if it is not used in a meticulous way, it has as much potential to be an efficient strategy to control pests as to be a route by which potentially damaging alien species are introduced and spread worldwide (Babendreier, 2007).

A famous example of biocontrol that went wrong is that of the Indian mongoose (*Herpestes auropuncatus*) which was intentionally introduced into over 60 islands mainly to control rats and is now a great contributor to the decline of native birds, mammals and herpetofauna in all these islands (Hays and Conant, 2007, Barun et al., 2011). Alike, the guppy (*Poecilia reticulata*) was introduced worldwide to control malaria by preying on mosquitoes' eggs and has now established in over 60 countries across the globe (Deacon et al., 2011). In México, it is known for threatening native topminnow species (Magurran, 2009, Valero et al., 2008). Nevertheless, if selected appropriately, biocontrol agents can provide a useful service without negative side effects. Pearson and Callaway (2005) suggest selecting biocontrol agents that are as specific and efficacious as possible to prevent indirect non-target effects. They point out that agents that are effective at reducing their target species will reduce their own populations through density-dependency.

The Harlequin ladybird (*Harmonia axyridis*, Pallas 1773) is native to Asia, from the Kazakhstan in the West to the Pacific Coast in the East, including Kyrgyzstan, and from southern Siberia in the north to southern China in the south (Orlova-Bienkowskaja et al., 2015). However, it has

69 established populations in many countries outside its native range due to its introduction as
70 biocontrol and subsequent range expansion (Brown et al., 2011b, Roy et al., 2016). The
71 Harlequin ladybird is a generalist species widely used as biocontrol mainly due to its
72 voraciousness (Pervez and Omkar, 2006). Harlequin ladybirds have been used as biocontrol
73 agent of aphids and coccids since the 1910's (Gordon, 1985) and are considered a successful
74 invasive species due to its polyphagy, wide host range and ability to survive under scarcity of
75 preys to feed on (Pervez and Omkar, 2006). When preys are scarce they recur to prey on other
76 ladybird larvae or cannibalism to survive (Katsanis et al., 2013). As an invasive species, the
77 Harlequin ladybird is considered to be noxious in crop fields as well as to humans (Orlova-
78 Bienkowskaja, 2014, Koch et al., 2006a, Brown et al., 2011b). When they cannot locate preys,
79 they could feed on fruit from the crop fields, in vineyards it was reported that they can taint the
80 wine flavour as individuals get into the wine making process (Koch, 2003). In urban areas it is
81 considered a household pest when it shelters during winter inside houses and buildings (Koch et
82 al., 2006a). In addition, its bites to humans are compared to those of mosquitoes; however, these
83 are not very common (Ramsey and Losey, 2012).
84
85 Studies suggest that the Harlequin ladybird may be contributing to displace native Coccinellidae
86 in the areas where it has established (Roy et al., 2012, Yasuda and Ohnuma, 1999, Snyder et al.,
87 2004, Brown et al., 2011a, Bahlai et al., 2015, Honek et al., 2016). As other invasive species, the
88 Harlequin ladybird has the potential to homogenize ladybird diversity were it establishes and
89 with this they might be threatening ecosystem services that can be provided only by an
90 assemblage of species (Cardinale et al., 2003). In this study we aim to explore the Harlequin
91 ladybird invasion worldwide and use it as a case study to exemplify and raise awareness on the

92 importance of appropriate agent selection. Our objectives are to document the non-native range
93 of the Harlequin ladybird and the origins of the introductions that have led to their current
94 distribution. We reviewed published records and surveyed entomologists working with
95 Coccinellidae worldwide to produce a current distribution map and gather insights from the
96 academic community working on the subject.
97
98 Methods
99
100 *Published records review*
101 In order to assess the places were the Harlequin ladybird has been seen or collected, building on
102 Brown et al. (2011b), we used the search engine Web of Science ™ to find published articles
103 from January 2010 to June 2015 that contained the word "*Harmonia axyridis*". We reviewed all
104 the articles found (over 600) to see how many contained information on the presence of the
105 Harlequin ladybird in a particular place or region. From all the articles reporting Harlequin
106 ladybird presence we built a database (attached as supplementary material) that contains the site
107 where this species was seen or collected, the year of sight (when absent year of publication of the
108 article was included with an asterisk), the geographical coordinates of the site, and name and
109 affiliation of the first author of the article.
110
111 *Survey*
112 To complement our literature review, we designed a survey that allowed us to document
113 entomologists' non-published records of presence of the Harlequin ladybird and their perceptions
114 on its invasion routes and threats to local species. We acknowledge records and perceptions from

115 our survey might not be as strict as published records, nevertheless this information could be
116 useful for future policy design, conservation plans and environmental education programs
117 (Martin et al., 2012). In order to mitigate the effects of psychosocial and motivational bias in our
118 survey, we followed Sutherland and Burgman (2015) recommendations to design our survey and
119 select the specialists we were to contact. All our queries were open questions so we could
120 document as many details as possible. It was essential for us to give the respondents the
121 opportunity to detail their views in their own words. In this sense, this section of our study can be
122 considered within a qualitative approach (Maxwell, 2013). Our survey was designed to address
123 the following questions: 1) Are you aware of harlequin ladybirds within your surroundings or
124 where you carry out your research? 2) If yes, are they throughout the region or in localised parts
125 only? 3) Do you know anything about the origin of their introduction? 4) Are you aware of any
126 negative effects the Harlequin ladybird might be causing? 5) Are you aware of any native species
127 gone extinct due to the presence of the Harlequin ladybird? Aside from these questions we gave
128 respondents the option to add any other information they felt could be relevant to us.
129
130 The survey was intended for Coccinellidae specialists. To obtain a list of researchers studying
131 Coccinellidae, using the Web of Science ™ engine, we searched for all published articles from
132 December 2000 to April 2015 that contained the word “coccinellidae”. We collected the name,
133 affiliation and e-mail of corresponding authors of all the articles we could. With this we ensured
134 to contact scientist that are familiar with ladybirds’ species. In total we were able to gather the
135 contact information of 473 scientists from 65 different countries. We sent an email to all of them
136 including the survey and our goals. Emailing the survey was the fastest way to contact scientist
137 while allowing them to dedicate as much time as they needed to respond our survey and attach

any further information they felt was relevant. We organised all the responses we received to construct summary figures and tables that include all opinions expressed.

Maps

We used Google Earth© software to create the databases containing the different places mentioned both in the literature review and the survey to create the maps. The files generated were then transferred to ArcGIS ® for processing, layer overlapping and layout design. Both maps show in darker colour the native distribution reported by Brown et al. (2011b) and Koch et al. (2006a). Reports found in the literature review and through the survey are all referred to administrative units, ranging from locality to country, thus some countries are entirely coloured. In these cases, Harlequin ladybirds do not necessarily occur throughout the country. Figure 1 shows in lighter colour the invasion reported by Brown *et al* (2011). Black dots represent all the reports found in the literature revised. Figure 2 displays two clouds of points of presence and absence. In the survey scientists reported Harlequin ladybird's presence at a local, province or state and region scale; in order to keep valuable information provided by the respondents, different symbols representing different geographical scale were used. Since we are using a global scale, invasion in bigger countries might be appreciated as bigger than invasion in smaller countries. However, impacts in human livelihoods are of local attention no matter the country size.

Results

Published records review

160 The Harlequin ladybird was reported present or collected in published articles in 58 countries
161 outside its native range (7 in Africa, 12 in America, 2 in Asia, 36 in Europe, and 1 in Oceania;
162 including reports from Brown et al. (2011b)).
163
164
165 *Survey*
166 From the 473 surveys we sent, we received back 74 filled (15.6%) from 35 different countries (1
167 in Africa, 10 in America, 5 in Asia, 17 in Europe, 2 in Oceania). Additionally we were able to
168 identify the presence of the ladybird in eight more locations through reports from colleagues that
169 included a picture. The Harlequin ladybird was reported present in 28 countries, but not in
170 Australia, Cuba and the Philippines (Figure 2). However, certain areas of Brazil, Chile, Czech
171 Republic, India, Iran, New Zealand, Portugal, Turkey and the USA were mentioned to be free of
172 the Harlequin Ladybird (Figure 2). Harlequin ladybirds were reported to inhabit urban areas,
173 crop fields, greenhouses, pasture fields, meadows, forests and natural reserves (Figure 3). From
174 the 74 surveyed scientists, 19 (25.7%) mentioned the Harlequin ladybird might be posing a threat
175 to native Coccinelidae and other arthropods diversity, *Adalia bipunctata* was mentioned by 5
176 experts as the most likely to present population declines after the Harlequin ladybird invasion
177 (Figure 4). As for the vectors of introduction, range expansion and biocontrol were the most
178 mentioned (Figure 5). Some scientists reported this ladybird arrived to their countries
179 accidentally by human related activities (transportation and trade of goods). The Harlequin
180 ladybird is considered a nuisance in China and the USA and in Brazil Czech Republic, USA and
181 Venezuela was reported to compete for resources with native species. In Switzerland is
182 considered to be the most abundant ladybird (Table 1).
183

184 Discussion

185

186 We found the Harlequin ladybird is reported present in 59 countries outside its native range, 11
187 more countries (Ecuador, Egypt, India, Lithuania, Moldova, New Zealand, Pakistan, Swaziland,
188 Tanzania, Turkey and Venezuela) since Brown et al. (2011b) review. Presence of the Harlequin
189 ladybird in Pakistan was reported from our survey only, to the best of our knowledge there are
190 not published records from this country yet. We found expansion range and biocontrol are the
191 most recognised vectors of introduction. Nevertheless, certain countries where the Harlequin
192 ladybird is banned as a biocontrol agent have established populations due to a "natural"
193 expansion range of the ladybird as well as accidental introductions by humans. From our survey
194 and our literature review, we found there is concern about the Harlequin ladybird being a threat
195 to native arthropod diversity.

196

197 The most cost-effective strategy to diminish biodiversity loss due to invasive species is to
198 prevent them arriving rather than mitigate their effects afterwards (Mack et al., 2000).
199 Identifying vectors of introduction of species outside its native range is key to avoid invasion and
200 identify management options (Puth and Post, 2005, Hulme et al., 2008). The most mentioned
201 vectors of introduction for the Harlequin ladybird according to our survey are biocontrol and
202 expansion range. The Harlequin ladybird had a rapid period of invasive expansion range
203 after the introduction in eastern North America, which acted as source of colonists for
204 Europe, South America and Africa (Lombaert et al., 2010). A reduced dependence on
205 photoperiod to trigger reproduction was thought to contribute to the development of this
206 invasive population (Reznik et al., 2015).

207

208 Dispersal is the least studied part of biocontrol, once the species has been released little or any
209 monitoring is carried out in the introduced region to find out what happened with the agents (De
210 Clercq et al., 2011). When assessing the appropriateness of using biocontrol agents many
211 factors must be evaluated, such as climate conditions that may affect the establishment
212 success of biocontrol organisms (Norris et al., 2002). Our results show the Harlequin
213 ladybird is present almost continuously from North to South in America and is widely
214 spread in Europe, limited data from Africa does not allow us to see if this species is present
215 along the latitudinal gradient from Europe to Africa but we can see it is present in Egypt,
216 Tanzania and South Africa. We believe the information gap in most of Africa (both in the
217 literature review and the survey) is due to the lack of research done for the species in the area.
218 Given that the Harlequin ladybird has been found in 59 countries outside its native range (as South
219 as Punta Arenas in Chile and as North as Oslo in Norway), we are inclined to think that it might
220 be able to survive in all type of habitats and tolerate a wide climate range (Roy et al., 2016). We
221 predict the Harlequin ladybird is present in many more countries than what we currently know;
222 including central Africa.

223

224 Some countries where the Harlequin ladybird has been found present it is prohibited to be used
225 as a biocontrol agent. Switzerland, for example, indicated Harlequin ladybird's use as biocontrol
226 agent there is not permitted; nevertheless it is now the most abundant Coccinellidae species in
227 that country (Table 1). Each country, and in some countries each state, is autonomous to
228 permit or not the use of certain biocontrol agents; lack of or deficient regulations can affect
229 neighbouring entities. In the U.S. A., for example, each state has its own system to permit or

not biocontrol agents (Messing and Wright, 2006), which can end up in species being imported to an area and then "naturally" spread where they were not permitted.

Potential risks of biological control can be divided in three categories: risks to human and animal health, production and ecological risks. While in its native range, as the responses to our survey from China and Japan entomologists suggest, the Harlequin ladybird is not considered a threat, rather it is perceived as a beneficial biocontrol agent; its establishment outside its native range can bring negative effects on the three risk categories (Koch et al., 2004, Koch et al., 2006b, Orlova-Bienkowskaja, 2014, Galvan et al., 2008). Despite the well studied nuisance that the Harlequin ladybird represents, in North America it was believed to be more efficient than other native Coccinellidae to control pests and therefore it was encouraged to be used as a biocontrol agent (Lucas et al., 2002).

Biocontrol does not necessarily have negative outcomes; in some cases (using the right species and with the right management) it can promote biodiversity conservation as well as being a greener alternative to chemical treatment for pests (Davies and Britton, 2015). Nevertheless, managers must always assess the effect altering the food web structure may have on ecosystems, as the invasive species population could be contained or eradicated but native species could get harmed. Recently it has been established that they carry spores of a parasitic microsporidia that, while not harming them, are lethal pathogens for other species (Vilcinskas et al., 2013) and they are less parasitized than native species (Comont et al., 2014), as a result there could be a displacement of native species.

253 Some of the mechanisms behind Harlequin ladybird replacement of native ladybirds are its high
254 fecundity and voracity. An entomologist from the USA reported intraguild predation to other
255 ladybirds by the Harlequin ladybird has been observed in the field (Table 1). Harlequin ladybirds
256 outcompete other ladybirds when foraging and prey on other ladybird eggs and larvae (Hentley
257 et al., 2016, Cottrell and Yeargan, 1998). These characteristics are fairly common among
258 successful invaders (Ehrenfeld, 2010) and sometimes among useful biocontrol agents too (De
259 Clercq et al., 2011, Simberloff and Stiling, 1996). Laboratory rearing of biocontrol species
260 promotes fast life history strategies, such as early reproduction and short life spam, which might
261 be contributing to the species becoming a successful invader (Tayeh et al., 2015).
262
263 Due to the ever-growing global trade and transport, unintended introductions of exotic species
264 are occurring more often (Mack et al., 2000). Interdisciplinary collaboration is needed for a
265 better management of invasive species mobility to analyse and potentially predict introduction
266 and spread of invasive species (Banks et al., 2015). For example, Australia has implemented
267 advanced detection, prevention and impact mitigation programmes that include species
268 distribution models and pre-border risk assessments before importing species (Pheloung et al.,
269 1999). Indeed, a scientist from Australia shared with us that the Harlequin ladybird has been seen
270 in the airport and prevented from entering Australia more than once. Identifying areas where
271 policies could benefit from synergies between climate predictions, land use change and invasive
272 species management can help prevent future invasion (Bellard et al., 2013).
273
274 Citizen science – engaging the public in a scientific project (Bonney et al., 2014) – has
275 invaluably contributed to the monitoring of invasive species while engaging the public in a

hands-on way with ecological problems and its potential solutions. Getting the general public involved in the gathering of scientific data is of great use to address spatial questions, such as the distribution of an invasive species (Kobori et al., 2016). Along with the distribution maps produced using published records in scientific articles, like ours, there are increasing efforts to engage the public in the monitoring of the Harlequin ladybird to be more accurate when describing its expansion. Indeed, in Chile (Chinita Arlequin), France (L'Observatoire de la Coccinelle asiatique en France), United Kingdom (The Harlequin Ladybird Survey) and the United States of America (The Lost Ladybug Project) there is a continuous monitoring of the Harlequin laybird dispersion with the help of citizens.

Biocontrol strategies' outcomes can be quite complicated to predict, especially when agents used are exotic to the area where they are to be used (Louda and Stiling, 2004). Biocontrol is a useful tool to manage pests, in agro-ecosystems as well as in natural areas. However, it should be treated carefully; to prevent large effects on ecosystems biocontrol species should be at least specialists and free of parasites. Assessments that consider the risk of introducing a biological control agent and the risk of not doing it must be carried out for every case; however we must acknowledge biocontrol agents will always present a certain environmental risk (de Lange and van Wilgen, 2010).

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302
303

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477 Table 1. Insights from experts on the Harlequin ladybird invasion in their countries that were
478 included in the survey responses. *Countries where the Harlequin ladybird is native.

Country	Insights
China*	Harlequin ladybirds are considered beneficial to control agricultural pests.
	Some people perceive them as a nuisance.
	Scientists are still working on developing effective ways of mass rearing them for biocontrol purposes.
Japan*	Considered a beneficial natural enemy.
	Other ladybirds avoid trees ad crops where the Harlequin ladybird is present
Australia	Harlequin ladybirds have been intercepted in the airport several times.
Brazil	Biocontrol companies are rearing them to sell them.
	Not seen yet in preserved areas.
	Not yet records of other species extinction after the Harlequin ladybird invasion.
	Harlequin ladybirds are competing with native aphidophagous species.
Chile	Some people are concerned about the negative effects of the Harlequin ladybird's invasion and are keen to eradicate them.
	Some people like them and are keen to promote their establishment in the country.
	The Harlequin ladybird has been seen in company of other native species (*Adalia sp., Cycloneda sp., Eriopis sp.*) and other introduced species (*Adalia bipunctata*, *Hippodamia sp.*).
	The most abundant species in Chile is the Harlequin ladybird.
Czech Republic	Harlequin ladybirds are competing with native species for food resources.
France	Harlequin ladybird has been sheltered inside overwintering aggregations of native species.
India	Unconfirmed reports of Harlequin ladybird presence in the North-Eastern Himalayan region of India.
Ireland	The Harlequin ladybird arrived to Ireland in celery hearts shipped to a supermarket.
	Establishment of this ladybird is believed to be delimited by climate conditions.
Netherlands	The Harlequin ladybird is being now attacked by natural enemies in the Netherlands.
South Africa	The Harlequin ladybird feeds on the senescent oak leaves' aphids.
Sweden	Entire half of Sweden could be suitable habitat for the Harlequin ladybird given current climate warming trends.
Switzerland	Harlequin ladybird has never been allowed for purposeful release in Switzerland.
	Harlequin ladybird is now the dominant ladybird in Switzerland.
USA	Harlequin ladybirds overwinter inside buildings, as winters in the wild can be too harsh for them.
	Perceived as a nuisance by people because of fall aggregations and overwintering site selection (inside buildings).
	Harlequin ladybirds coexists with many other ladybeetle species (native and other introduced) in the USA.
	Intraguild predation to other ladybirds has been observed in the field.
	Perceived as beneficial because they are voracious predators that control aphids.
	The Harlequin ladybird is outcompeting native species for food resources.
Venezuela	The Harlequin ladybird is believed to be competing with native species for

resources.

481
482 Figure 1. Published records of the presence of the Harlequin ladybird in the world.

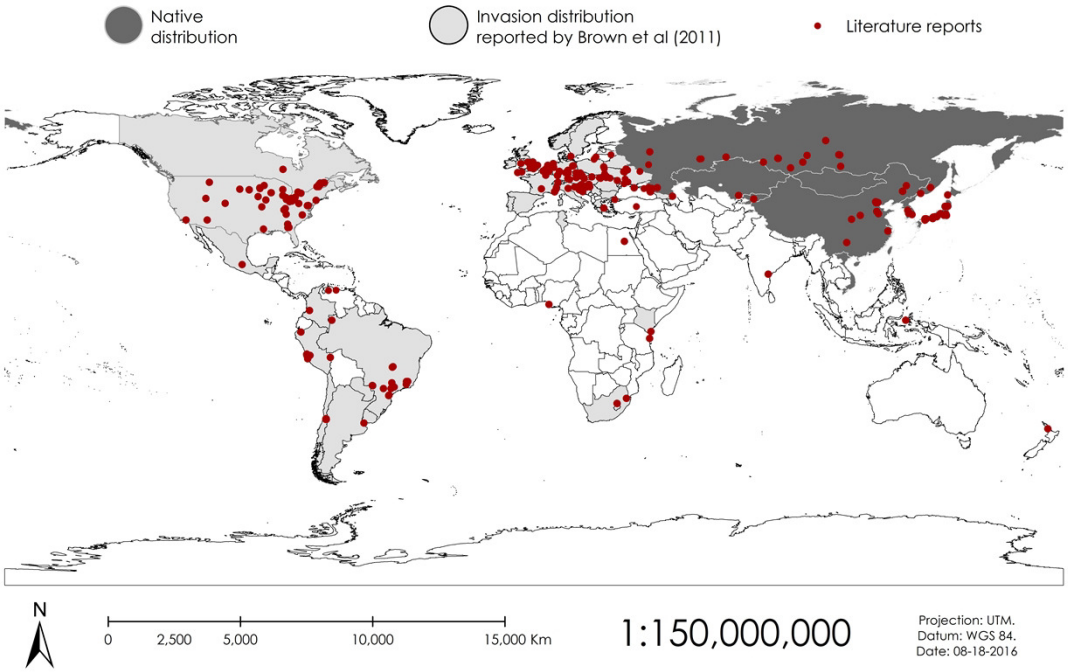

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Figure 2. World distribution of the Harlequin ladybird from survey records. Presence is represented by filled circles and absence is represented by empty squares.

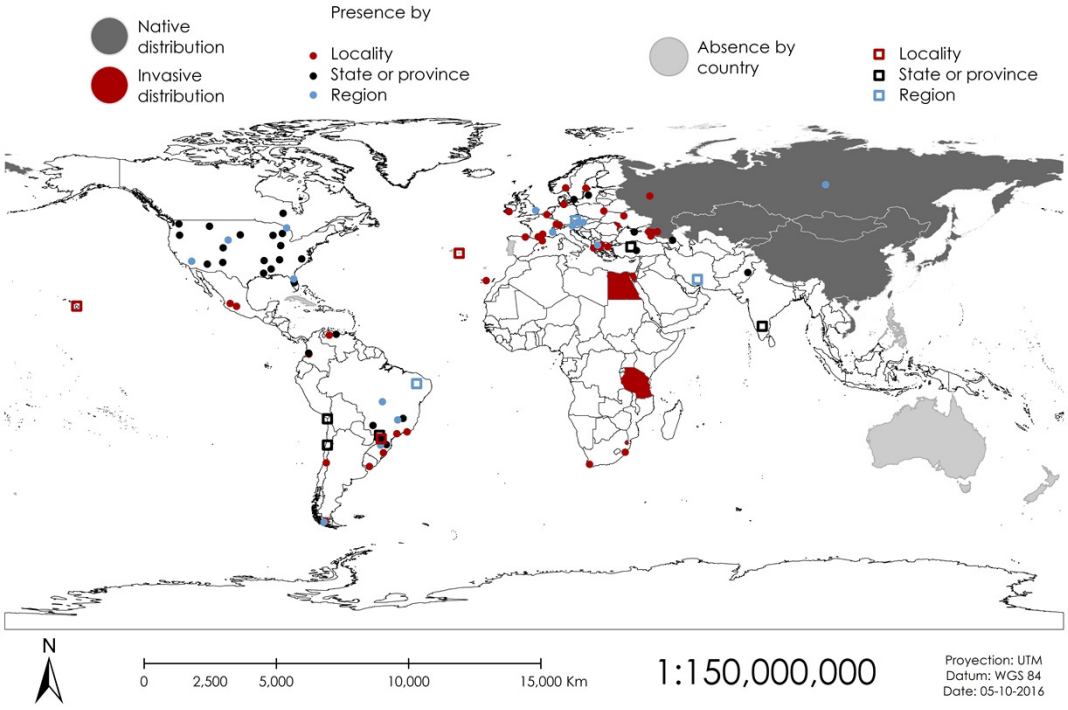
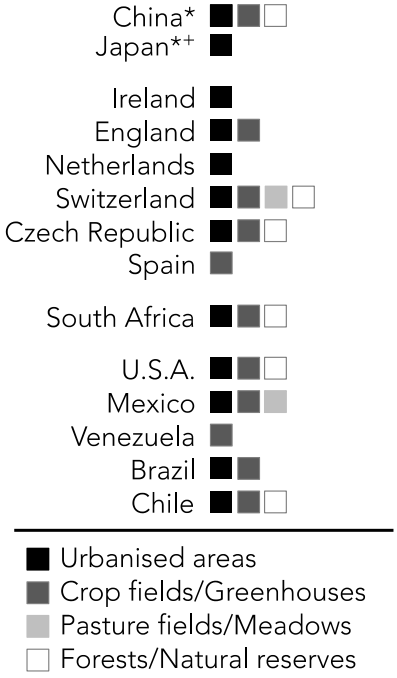


Figure 3. Habitat types´ entomologists report the Harlequin ladybird uses by countries.
*Countries where the Harlequin ladybird is native. + The Harlequin ladybird is known to be established in other than urbanised areas in Japan, though this was the only habitat mentioned by an expert in the survey.



497
498 Figure 4. Number of scientists that mentioned concern about the Harlequin ladybird being a
499 threat to native Coccinellidae and species they mentioned.

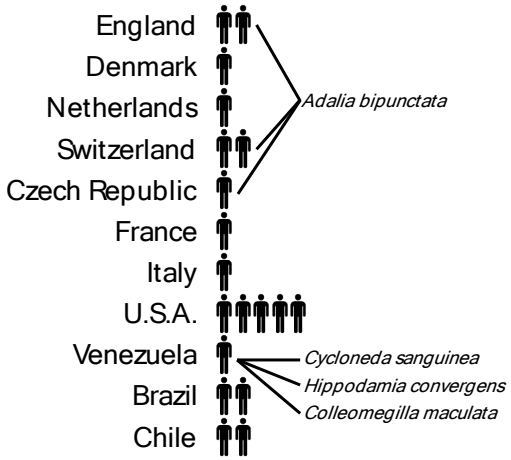


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Figure 5. Reported vectors of Harlequin ladybird introduction.