

The Harlequin ladybird continues to invade southeastern Europe

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Abstract The ladybird *Harmonia axyridis* (Pallas) is considered one of the most serious invasive species around the globe. It has spread all over North America and Western Europe, while data from southeastern Europe, especially in the Mediterranean region, are scarce. In this study we present the first confirmed data of the spread of *H. axyridis* throughout Croatia. Specimens were sampled and identified during the period 2008–2010. The species was recorded at 18 localities in all three colour forms in various habitat types. Light trapping was found to be a satisfactory method for collecting *H. axyridis*. Since there is no evidence to suggest the deliberate introduction of *H. axyridis* in Croatia, it can be assumed that it has spread southwards from Central and Eastern Europe, and that it will probably continue

to spread. Further investigations should focus on monitoring and detailed mapping of *H. axyridis* in Croatia and neighbouring countries, especially in the Mediterranean region, to determine whether stable populations are present.

Keywords Harlequin ladybird · Invasion · Mediterranean region · Southeastern Europe

Introduction

Harmonia axyridis (Pallas), known as the harlequin ladybird or the multicoloured Asian ladybird, is considered one of the most serious invasive species on a global scale (Koch 2003; Brown et al. 2008; Roy and Wajnberg 2008). It has been intentionally spread from its native regions of China, Japan, Korea, Mongolia, Manchuria, southern Siberia and the Ryukyu Islands (see Brown et al. 2008; Soares et al. 2008), primarily as a biological control agent for the control of aphids on fruit trees. *H. axyridis* has a highly varied diet, feeding on numerous aphid species, other insects (Koch 2003), and even other species of ladybirds (Roy et al. 2006). It is a voracious, generalist predator that dominates in aggressive intra-guild interactions (Alyokhin and Sewell 2004; Majerus et al. 2006; Labrie et al. 2006). Therefore *H. axyridis*, as an invasive alien species, poses a threat to the biodiversity of invaded areas.

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The initial introduction of the harlequin ladybird in Europe occurred in 1990 (Brown et al. 2007), and it rapidly spread all over Europe soon thereafter (Brown et al. 2008). *H. axyridis* has been confirmed in a variety of habitats, from agricultural ones to more natural ones of conservation value (Adriaens et al. 2007). Its area of colonization in Europe, where it continues to spread, mostly matches the area predicted by microclimate-based simulations and its biological characteristics, though its boundaries towards the east and south have yet to be verified (Poutsma et al. 2008).

In addition to lack of knowledge about *H. axyridis* in southeastern Europe, data about its family (Coccinellidae) in Croatia are very scarce. The existing literature addresses only the interactions between specific coccinellid species and aphids (Gotlin Čuljak et al. 2006; Masten Milek 2007), and variations in native ladybird populations (Lorković 1928).

This paper is the first report of the spread of *H. axyridis* in Croatia; it describes the sites, colour forms of collected specimens and characteristics of their habitats. Together with previous work, these results suggest that *H. axyridis* has spread from the neighbouring countries of Slovenia and Hungary (see also Jelovčan 2006; Brown et al. 2008; Roy et al. 2009).

Materials and methods

Study site

Croatia is situated in southeastern Europe, at the intersection of several European geographical units: Pannonian-peripannonian, Dinarides and Mediterranean. It is divided into five geographical units with varying climatic and geographical characteristics: Central, Eastern, Mountain, Northern Croatian Coast and Southern Croatian Coast (Borovac 2002).

Data collection

During 2008–2010, specimens of *H. axyridis* were randomly sampled at 18 localities in all five geographical units in Croatia (Table 1) and identified. Samples were collected by sweep nets, visual search and light trapping in natural habitats, and by hand in buildings and houses. Specimens were preserved in 80% ethyl alcohol and identified in the laboratory.

Table 1 Geographical coordinates of localities in Croatia where *H. axyridis* was observed during the study period 2008–2010

Locality	Geographical coordinates	
Pazin	N 45°14'20.45"	E 13°55'31.62"
Vela Traba	N 45°14'48.66"	E 13°51'52.00"
Sovsko jezero	N 45°17'24.29"	E 18°01'29.11"
Zmajevac	N 45°47'55.60"	E 18°48'36.64"
Vukovar	N 45°20'30.85"	E 19°00'29.36"
Bizek quarry	N 45°49'41.01"	E 15°51'41.01"
Vugrovec	N 45°53'32.76"	E 16°06'00.42"
Pazin	N 45°14'20.45"	E 13°55'31.62"
Pazin	N 45°14'20.45"	E 13°55'31.62"
Plitvice lakes	N 44°21'59.87"	E 15°25'58.86"
Sinj	N 43°41'51.39"	E 16°38'26.05"
Pazin	N 45°14'20.45"	E 13°55'31.62"
Voloder	N 45°33'58.55"	E 16°40'15.74"
Ogulin	N 45°15'34.21"	E 15°14'18.83"
Klimen	N 46°05'22.86"	E 16°09'38.50"
Kaštel Žegarski	N 44°09'17.36"	E 15°51'31.00"
Opatovac	N 45°19'45.32"	E 17°26'07.10"
Zg Bot. Garden	N 45°47'14.35"	E 16°03'55.42"
NP Lonjsko polje	N 45°24'49.37"	E 16°37'50.39"
Papuk Mountain	N 45°31'32.46"	E 17°36'35.15"
Maksimir Park Zg	N 45°50'03.15"	E 16°01'13.38"

Zg Bot Garden-Zagreb Botanical Garden, NP Nature Park, Zg Zagreb

Each species record includes the recorder and determiner name. The morphological form of the identified species, life stage of recorded specimens and finding sites are given in Table 2.

Identification of collected material

Ladybirds were compared with specimens of the Petar Novak collection at the Croatian Natural History Museum, and later identified according to Chapin and Brou (1991), Koch (2003), Benisch (2010) and Jones and Boggs (2010). Three main colour forms were determined following Brown et al. (2007) and Brown et al. (2008).

Results

H. axyridis was recorded at 18 different localities throughout Croatia in the period 2008–2010 (Fig. 1).

Table 2 Localities, finding site characteristics, date of record, morphological forms and life stages of *H. axyridis* in Croatia

Locality	Finding site	Date of record	Forms	Life stage
Pazin	Household, UV lamp	18/07/2008	<i>Su, Sp, C</i>	A
Vela Traba	Household, UV lamp, meadow	14/08/2008	<i>Su, Sp, C</i>	A
Sovsko jezero	Grassland	28/03/2009	<i>Su</i>	A
Zmajevac	Grassland	11/07/2009	<i>Su</i>	A
Vukovar	Brushwood on road verge	14/07/2009	<i>Su</i>	A
Bizek quarry	Stones in quarry	15/07/2009	<i>C</i>	A
Vugrovec	Grassland and bushes	12/08/2009	<i>Su</i>	A
Pazin	Household, UV lamp	12/08/2009	<i>Su, Sp, C</i>	A
Pazin	Household, UV lamp	22/08/2009	<i>Su, Sp, C</i>	A
Plitvice lakes	Bushes on lake shore	17/11/2009	<i>Su</i>	A
Sinj	Household	27/11/2009	<i>Su, Sp, C</i>	A
Pazin	Household, UV lamp	12/12/2009	<i>Su, Sp, C</i>	A
Voloder	Household	10/04/2010	<i>Su, Sp, C</i>	A
Ogulin	Brushwood on road verge	18/04/2010	<i>Su</i>	A
Klimen	Household	28/04/2010	<i>Su, Sp</i>	A
Kaštel Žegarski	House (on external wall)	03/05/2010	<i>Su</i>	A
Opatovac	Flowers on road verge	14/05/2010	<i>Su</i>	A
Zg Bot. Garden	Trees	24/05/2010	<i>Su, Sp</i>	A
NP Lonjsko polje	Grasslands, trees, bushes	25/05/2010	<i>Su, Sp, C</i>	A;L
Papuk Mountain	Grasslands, trees, bushes	25/05/2010	<i>Su</i>	A
Maksimir Park Zg	Trees	28/05/2010	<i>Su</i>	A

A adult, L larvae, Zg Bot. Garden Zagreb Botanical Garden, NP Nature Park, Zg Zagreb, *Su succinea*, *Sp spectabilis*, *C conspiciua*

The first record of *H. axyridis* was in July 2008 in Istria (Pazin and Vela Traba), during field work to sample moth fauna using UV light-traps. Systematic light trapping in this locality had been conducted at these sites since 2006, with no previous record of *H. axyridis*. During the study period, 2009 was the year in which the seasonal appearance of *H. axyridis* was earliest (28 March) and latest (27 November).

The localities recorded in this study vary greatly in climatic characteristics (winter and summer temperatures, precipitation). The westernmost locality is Vela Traba (Istria), the easternmost Vukovar (Slavonia, Eastern Croatia), the northernmost locality is a village near Konjščina (Central Croatia) and the southernmost locality is Kaštel Žegarski, situated in the Mediterranean part of Croatia (Dalmatia) (Fig. 1).

Overwintering in houses was recorded at four localities (Table 2), though only one locality, Voloder, had an overwintering colony that was estimated at more than 500 individuals.

All three morph types (forms) of *H. axyridis* were recorded (Table 2). The most common was the

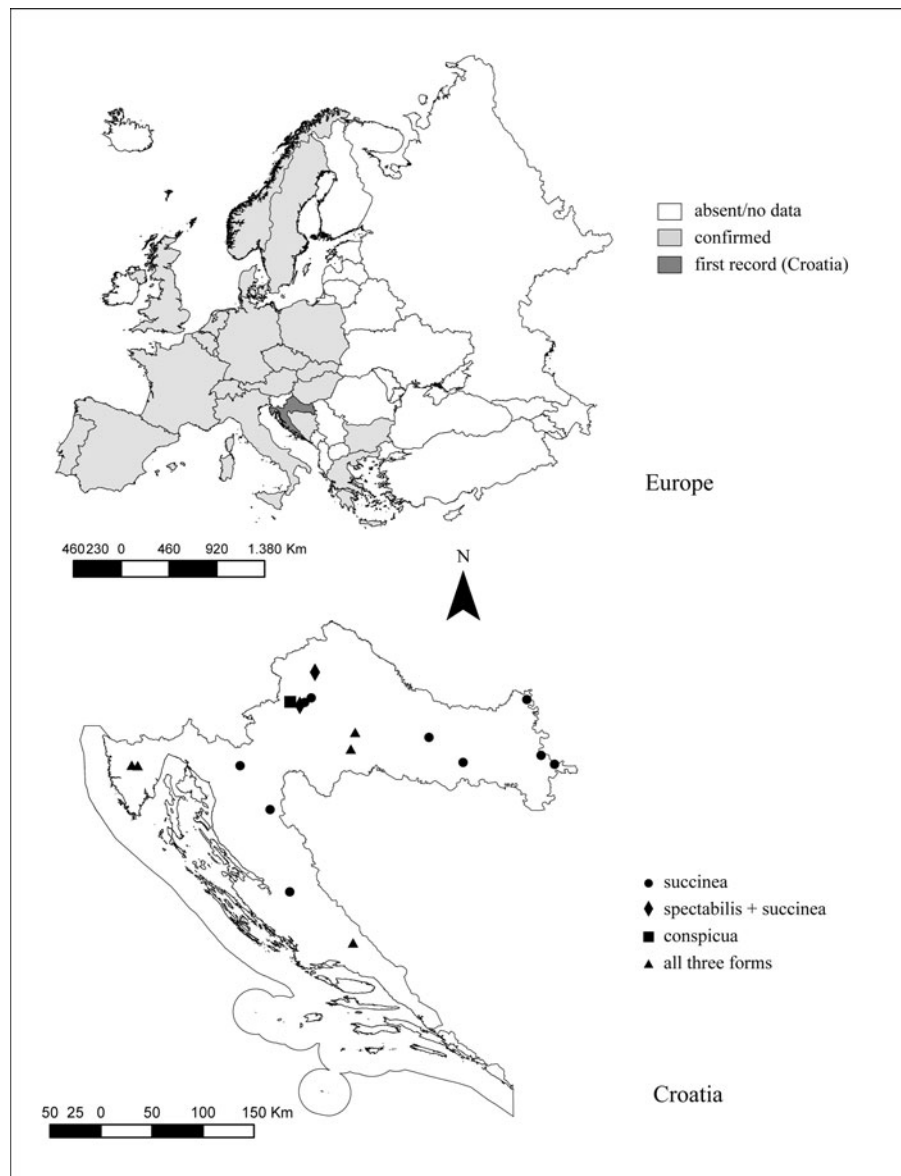
succinea form, recorded at 17 localities, while the forms *conspiciua* and *spectabilis* were recorded at six and seven localities, respectively. The co-occurrence of all three forms was recorded at five localities. Solitary populations of the *spectabilis* form were never observed, while one solitary population was recorded for the *conspiciua* form. Solitary populations of *succinea* were recorded at 10 localities.

The forms occupied different habitat types. While all three forms co-occurred only in anthropogenic habitats (houses and buildings), the *succinea* form was observed in more natural habitats, such as bushes, grasslands and trees.

Discussion

H. axyridis has spread throughout Europe in a very short time and it continues to spread eastward and northward. The most recent reports of the establishment of *H. axyridis* have occurred in Hungary, Slovakia and Bulgaria (Roy et al. 2009). It is likely

Fig. 1 Presence of *H. axyridis* in Europe (upper panel) and Croatia (lower panel). The upper panel is based on Adriaens et al. (2008), Brown et al. (2008), Poutsma et al. (2008), Roy et al. (2009). The lower panel is based on records of different morphological types in Croatia (present study)



present, though still undetected, in more eastern European countries such as Slovenia (Brown et al. 2008). The first record of *H. axyridis* in Croatia was in the western part, Istria, approximately 20 km from the border with Slovenia, indicating the establishment and spread of species *H. axyridis* from that country.

Habitat preference varied greatly among *H. axyridis* specimens in Croatia. They were recorded in various habitat types, including anthropogenic ones (houses) and more natural ones (grasslands, bushes); these results are similar to those of several other investigators (Roy et al. 2006; Adriaens et al. 2007;

Brown et al. 2008). In addition, *H. axyridis* was recorded in all five geographical regions of Croatia (Borovac 2002), which differ significantly in climate and altitude. It was present in dry grasslands to swamps and marshlands, from gardens to road verges. In all cases, the species occurred in the continental part of Croatia, not on the Adriatic coast or islands, which have a climate more similar to that of Greece or southern France. The southernmost locality was Kaštel Žegarski, located in the southern part of continental Croatia, in the region of Dalmatia. There the climate reflects the combination of

Mediterranean influence and highlands with diverse topography and altitudes. Since *H. axyridis* has a limited distribution in the Mediterranean region (Kontodimas et al. 2008; Soares et al. 2008), further investigations should include detailed mapping of *H. axyridis* in Croatia, with an emphasis on the southernmost populations. Detailed monitoring of *H. axyridis* and recording of the number of generations is also required.

Brown et al. (2008) proposed that the *succinea* form is predominant in European populations, and the present study confirms this. The melanic forms (*spectabilis* and *conspicua*) were recorded at seven localities, in much higher numbers than in studies in other European countries.

Light trapping proved to be a satisfactory method for *H. axyridis* collection, especially during July and August; immediately after placing males and females on a lamp sheet, they began to copulate. These results support a previous suggestion that light trapping can be used as a control mechanism in the management of *H. axyridis* (Kenis et al. 2008). Management of this species is an important issue for a wine-growing country such as Croatia, since it is known as a pest of fruit production (Koch 2003) and it can have a serious impact on wine composition and aging (Pickering et al. 2005; Pickering et al. 2006). In the present study, an overwintering *H. axyridis* colony with more than 500 individuals was recorded in a household near a vineyard in a well-known wine production area of Croatia (Voloder vineyards). To date, no cases of wine tainting by *H. axyridis* have been reported in Croatia; however, this can be expected to become an issue in the near future.

Taking the first confirmed report of *H. axyridis* in 2008 as a point of reference, we conclude that the species has had a very short expansion period in Croatia. Since there is no evidence to suggest deliberate introduction of *H. axyridis* in Croatia, it can be assumed that its distribution is the result of its spread southward, from Central and Eastern Europe to the northern- and westernmost sites in Croatia, where its presence was first recorded. From there, it appears to have spread east and south within Croatia. Unfortunately, we predict that it will continue to spread southward and eastward, which may explain a very recent report of *H. axyridis* establishment in Bosnia and Herzegovina (Kulijer 2010).

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