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**Population of the invasive harlequin ladybird
Harmonia axyridis (Coleoptera: Coccinellidae)
from Pelješac Peninsula, southern Croatia**

Key words: *Harmonia axyridis*, invasion, Croatia, *Hesperomyces virescens*

SUMMARY

Samples of the invasive alien ladybird, *Harmonia axyridis*, were collected between 27 July and 8 August 2013 at four localities on the Pelješac Peninsula in southern Croatia. Altogether, 209 individuals were collected, of which 114 (54.5%) were males and 95 (45.5%) females. Three colour forms were present in the samples: the non-melanic form *succinea* and the melanic forms *spectabilis* and *conspicua*. The form *succinea* clearly predominated (90.5% of collected individuals) followed by *spectabilis* (8.1%) and *conspicua* (1.4%). The ectoparasitic fungus *Hesperomyces virescens*, not previously reported from Croatia, was found on the elytron of one *H. axyridis* individual. The localities in Pelješac Peninsula mentioned in this paper are the southernmost of all Croatian localities so far reported to be colonized by *H. axyridis*.

Introduction

The harlequin ladybird, *Harmonia axyridis* (Pallas), is a well known invasive species of Asiatic origin that threatens biodiversity in invaded areas, causes some economic losses in horticulture and winemaking and is a nuisance and health problem to some people (Kovach 2004, Koch & Galvan 2008, Roy et al. 2012).

Being a voracious predator of aphids and other soft-bodied arthropods that are often important agricultural pests, *H. axyridis* was commonly used in the 20th century as a biological control agent. Despite numerous introductions of this species in many parts of the world, starting with the introduction in 1916 in California (USA), for a long time *H. axyridis* could not establish anywhere outside its native range (Brown et al. 2008). In 1988, however, an established population of *H. axyridis* was detected in southeastern Louisiana, USA (Chapin & Brou 1991). From this initial finding a rapid spread of the beetle has been observed, at the beginning in North America and later also in other continents, including Europe, South America and Africa (Brown et al. 2011).

In Europe, the first feral populations of the harlequin ladybird were recorded in 1999 at two German localities: Hamburg and Frankfurt-Niederrad (Tolasch 2002, Brown et al. 2008). Until 2011 the species had established in at least 26 European countries (Brown et al. 2011). Currently, *H. axyridis* commonly occurs in the western and central parts of Europe and has also been reported from some areas in the east (Latvia, Ukraine, Belgorod region of Russia, North Caucasus) (Barševskis 2009, Verizhnikova 2011, Orlova-Bienkowskaja 2013, Belyakova & Reznik 2013, Ukrainsky 2013, Ukrainsky & Orlova-Bienkowskaja 2014). However, its spread to the north and south of the continent seems to be slower, probably due to climatic constraints. Available literature indicates that the southernmost areas of continental Europe, i.e. the Iberian, Apennine and Balkan Peninsulas, have so far only slightly been invaded by *H. axyridis*. In Spain, two harlequin ladybird individuals were found in 2007 in Loiu (Biscay Province, latitude 43°18'N) (Goldarazena & Calvo 2007), and in 2010-2012 established populations were recorded in several localities in Catalonia (northwestern Spain, approximate latitudes 41-42°N) (Carbonell & Sesma 2013). The progress in colonization of the Apennine Peninsula by *H. axyridis* is poorly known. Data on the distribution of *H. axyridis* in northern Italy, updated for 2008, were published by Burgio et al. (2008) and Uliana (2009). The southernmost of the localities in the Apennine Peninsula reported in these papers were in the Emilia-Romagna region (about 44°N). A few more recent reports indicate only a slight southward range shift: single *H. axyridis* individual was found in autumn 2010 in Monteguidi, Tuscany region (43°18'N) (<http://www.entomologiitaliani.net/public/forum/phpBB3/viewtopic.php?t=14012>), and the other one in winter 2011 in Fano, Marche region (43°50'N) (http://www.lavalledelmetauro.org/standard.php?lingua=it&id_sezione=8&id_sottosezione=31&id_sottosottosezione=10&record=11775).

More information is available on the presence of *H. axyridis* in the Balkan countries: Slovenia (Kus Veenvliet & Veenvliet 2009, Laznik et al. 2012), Croatia (Ivezić et al. 2011, Mičetić Stanković et al. 2011),

Serbia (Thalji & Stojanović 2008, Markó & Pozsgai 2009, Mihajlović & Stanivuković 2009, Tanasković et al. 2010), Romania (Ruicănescu & Alexandru 2009, Markó & Pozsgai 2009, Skolka & Preda 2010, Stan 2011), Bulgaria (Tomov et al. 2009, 2010, Harizanova et al. 2012) and Bosnia and Herzegovina (Kulijer 2010). The first records of *H. axyridis* in the Balkan Peninsula date back to 2008 and concern Maribor in northern Slovenia (46°33'N) (Kus Veenvliet & Veenvliet 2009), Pazin and Vela Traba in northwestern Croatia (45°15'N) (Mičetić Stanković et al. 2011), the Fruška Gora National Park in northern Serbia (about 45°N) (Thalji & Stojanović 2008), the natural reserve “Calcarele din Dealul Măgura” in western Romania (46°2'N) (Ruicănescu & Alexandru 2009) and Sofia in western Bulgaria (42°40'N) (Tomov et al. 2009). The southernmost Balkan localities so far reported to be colonized by *H. axyridis* are Smolyan and Ardino in southern Bulgaria (41°35'N) (Tomov et al. 2010).

In this paper, we report on the further spread of the harlequin ladybird in Croatia. The population found in 2013 in southern part of this country is characterized as to the sex ratio and frequency of colour forms. Since the invasive populations of *H. axyridis* have recently appeared to be susceptible to some ectoparasitic organisms, such as the fungus *Hesperomyces virescens* Thaxter (Ascomycota: Laboulbeniales, Laboulbeniaceae) or the mite *Coccipolipus hippodamiae* (McDaniel & Morrill) (Acari: Podapolipidae) (Ceryngier & Twardowska 2013), we also checked the ladybirds in our samples for the presence of ectoparasites.

Material and methods

We conducted field surveys for *H. axyridis* at Pelješac Peninsula (southern Dalmatia, Croatia) from 27 July till 8 August 2013. We recorded *H. axyridis* at four sites: (1) Divna (43°01'N, 17°12'E), (2) Duba (43°01'N, 17°10'E), (3) Kucište (42°59'N, 17°06'E) and (4) Perna camp (42°58'N, 17°09'E). Sites were located along northern (Divna, Duba)

and southern (Kucište, Perna camp) coasts of Pelješac Peninsula. At each site sweep nettings and visual observations were carried out. At Divna and Perna camp additional censuses for *H. axyridis* attracted by light were conducted near buildings after dusk and this method enabled collection of the largest sample (200 individuals at Divna). Collected beetles were sexed and their colour form was determined. Additionally, the body surface and underside of the elytra of each beetle were examined for the presence of ectoparasites.

Results and discussion

H. axyridis was found at each of the four localities surveyed, although at three of them very few individuals were collected (Table 1). The reason of those low numbers is not necessarily related to the rarity of the ladybird at the sites. As *H. axyridis* is mainly arboreal species (Brown et al. 2011), sweep netting on herbaceous vegetation may not be an effective method of collecting this species.

Table 1. Numbers of male and female *Harmonia axyridis* of individual colour forms collected at four localities in Pelješac Peninsula.

Locality	Colour form						Total
	succinea		spectabilis		conspicua		
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	
Divna	80	101	9	8	1	1	200
Duba		1					1
Kucište	1						1
Perna camp	4	2				1	7
Total	85	104	9	8	1	2	209

More males (54.5%) than females (45.5%) were found in our samples, but this proportion does not differ statistically from the 1:1 sex ratio (two-tailed z-test for proportions: $z = 1.286$, $P < 0.05$). Proportion of males, however, was often lower than 50% in other countries (Nedvědová et al. 2013).

Three colour forms, *succinea*, *spectabilis* and *conspicua*, were collected with the non-melanic *succinea* being a clear dominant (90.5%; Table 1). Of the two melanic forms, *spectabilis* was more frequent (8.1%) than *conspicua* (1.4%). The frequencies of colour forms did not differ from those found in other European countries (Belgium, England, Italy, Hungary, Bulgaria, Denmark, Czech Republic), where *succinea* constituted 71–98% of the populations followed by *spectabilis* (2–19%) and *conspicua* (0–7%) (Brown et al. 2008, Burgio et al. 2008, Bozsik 2009, Tomov et al. 2009, Steenberg & Harding 2010, Nedvědová et al. 2013). In some European populations of the harlequin ladybird the fourth colour form, *axyridis*, occurs at low frequencies (Steenberg & Harding 2010). This rare form, although present in the Balkan populations (Tomov et al. 2009, Izević et al. 2011, Horizonova et al. 2012), was not recorded in our survey.

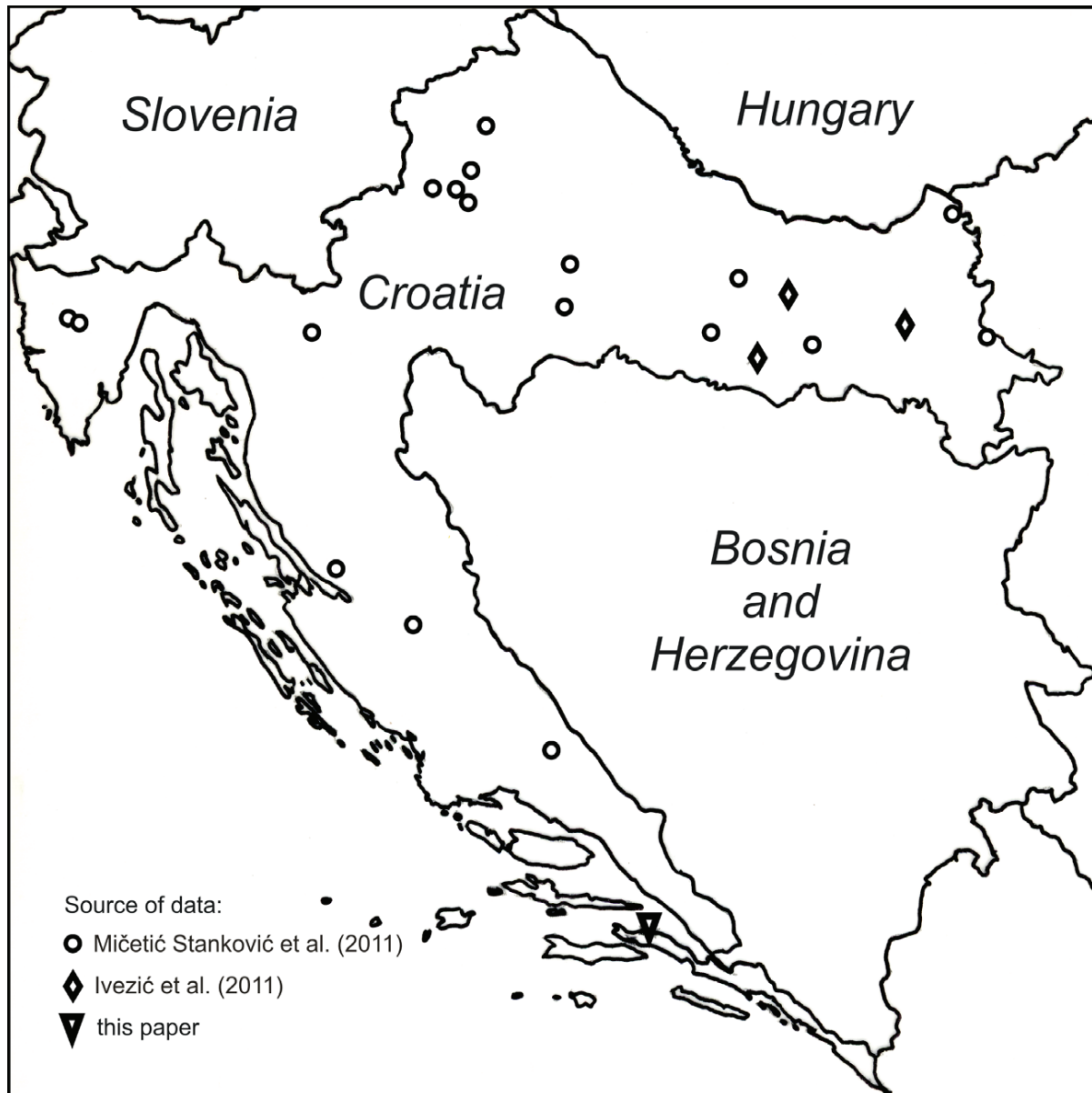
Examination of the beetles for the presence of ectoparasites revealed in the sample from Divna a female of the form *succinea*, which was infected with the fungus *Hesperomyces virescens*. On the posterior part of the left elytron of this female a group of *H. virescens* thalli was noticed. Some of those thalli were untypical in that they had no perithecial part developed (Fig. 1). According to our knowledge, *H. virescens* has not previously been reported from Croatia. The nearest known localities of this fungus are situated about 200 km SW of our study area, in Apulia region of Italy, and about 470 km SE of this area, in Epirus region of Greece, (Castaldo et al. 2004, Ceryngier & Twardowska 2013). Our finding of *H. virescens* on the harlequin ladybird confirms the hypothesis that the geographical expansion of the latter is associated with the expansion of some of its recently acquired parasites (Ceryngier & Twardowska 2013).

Fig. 1. *Hesperomyces virescens* thalli removed from the elytron of *Harmonia axyridis* from Divna. The arrow shows a group of thalli without perithecia.



The distribution of *H. axyridis* in Croatia, based on the records reported by Mičetić Stanković et al. (2011), Ivezić et al. (2011) and this paper, is shown in Fig. 2. Our records from Pelješac Peninsula are about 85-90 km SE of the town Sinj, the southernmost place in Croatia so far reported as a locality of *H. axyridis* (Mičetić Stanković et al. 2011).

Fig. 2. Distribution records of *Harmonia axyridis* in Croatia. Note that the four localities reported in this paper, as situated close to each other, are flagged by a single icon.



This indicates that the spread of the harlequin ladybird to the south of Croatia and the Balkan Peninsula is in progress. It is disputable, whether the invader is able to colonize the southern part of the region, including Albania, Macedonia, Greece and the European part of Turkey. Introductions of hundreds of thousands of *H. axyridis* individuals in central and southern Greece between 1994 and 1999 appeared a failure and no establishment of the species was achieved (Kontodimas et

al. 2008). On the other hand, according to the climatic model used by Poutsma et al. (2008), most of the Mediterranean Basin, including the Balkan Peninsula, is highly suitable for the establishment of *H. axyridis*. This high suitability, however, is not confirmed by the species distribution models developed by Bidinger et al. (2012). Predictions of these models suggest that the southern parts of the Balkan Peninsula may not be suitable to the harlequin ladybird. To check this, monitoring of the spread of *H. axyridis* in the Balkan countries should be continued.

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