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The invasive harlequin ladybug *Harmonia axyridis* (Pallas 1773) (Coleoptera, Coccinellidae) in the Pârâul Pețea natural reserve, northwestern Romania

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Abstract. The Asian invasive ladybug *Harmonia axyridis* (Pallas 1773) was first observed and collected in the Pârâul Pețea natural reserve near Oradea, NW Romania, in May-June 2011, with several color forms. The presence of both imagos and larvae indicates that the ladybugs were reproducing within the reserve.

Introduction

The Asian harlequin ladybug *Harmonia axyridis* (Pallas 1773) (Coleoptera, Coccinellidae, Coccinellinae, Coccinellini) is native to temperate and subtropical Asia (China, Japan, Korea, Mongolia and Siberia), although its entire range has not been clearly recorded. It occupies many habitats, and in both its native and introduced ranges has been recorded in meadows, heathlands, riparian zones, reedbeds and crop systems. In recent times, there have been 155 attempts to control aphids and 613 to control coccids worldwide through the introduction of coccinellids, mainly unsuccessful regarding aphids, while *H. axyridis*, one of the introduced ladybugs, has become an invasive species in Europe, having far-reaching, unacceptable impact on biodiversity (Brown et al. 2008, Dixon 2000).

H. axyridis has been released as a biological control agent in North America in 1916 and in Europe in 1927 (in Georgia, then in western Europe in 1982 in France with a population from China, probably northeast, which provided also the individuals for the release in Greece in 1994), due to its polyphagous nature (it preys mainly upon various tree-dwelling homopterans) (Koch et al. 2006, Poutsma & al. 2008). While it feeds on aphids, coccids, adelgids, psyllids, it also attacks the eggs of many other insects, including coccinellids and lepidopterans (Roy & Migeon 2010). Although efficient in aphids control, its extremely polyphagous nature suggests also a negative impact on non-target species, mainly through its intra-guild predation potential (probably due to its larger size throughout its life cycle), which could disrupt the natural aphidophagous ladybug guilds in Europe (a guild is a community of specialist and generalist predator, parasitoid and pathogen species that share the same prey/host resource). Since the arrival of *H. axyridis* in the USA, it has replaced *Coccinella 7-punctata* as top aphidophagous predator, and there are already data indicating the decline of European coccinellids as well, with the only exception of the coniferous tree specialist *Anatis ocellata* (L. 1758), which might even act as a threat for *H. axyridis* (Pell et al. 2008, Ware & Majerus 2008), a fact which could be at least partly useful for the biological control of the invasive ladybug. Still, sibling and non-sibling larval cannibalism may act to some extent as a limiting factor for the populations of *H. axyridis*, but also as a survival strategy (Joseph et al. 1999, Osawa 1989, 1992).

H. axyridis is large, aggressive, polyphagous, with a tendency for intraguild predation, rapid dispersal with more than 50 km per year (144.5 km per year westwards in Great Britain), higher developmental rate, continuous breeding, with evidence of bi-voltinism in Great Britain and Belgium, and a more plastic phenotype, thus it has a reproductive advantage over native ladybugs and it may cause a reduction in biodiversity and declines in native predators and parasitoids of aphids and coccids (Adriaens et al. 2008, Brown et al. 2008, Labrie et al. 2006, Majerus 2006, Roy & Migeon 2010). Furthermore, it feeds on plant materials, including pollen and fruits, when insect prey is scarce, thus becoming a pest for wine and fruit, and its overwintering aggregates in urban areas are a nuisance to humans (Berkvens & al. 2008, van Lenteren et al. 2008).

H. axyridis has spread rapidly from the countries where it was introduced as pest control to many others across Europe, particularly since 2002, so that it may become one of the most widely distributed coccinellids on the continent (Brown & al. 2008). It was first released in Europe in Georgia in 1927, then followed Ukraine (1964), Belarus (1968), France (1982), Portugal (1984), Greece (1994), Spain (1995), the Netherlands and Switzerland (1996), Germany and Belgium (1997),

and the Czech Republic (2003), as well as Italy (1990s) and Denmark (2000s). Its first European occurrence in the wild was reported in 1991 from France, then it was found in Greece (1998), Germany (1999), Belgium (2001), the Netherlands (2002), Spain (2003), Switzerland, Luxembourg and England (2004), Italy, the Czech Republic, Austria, Denmark, Wales, Poland, and Norway (2006), Liechtenstein, Sweden, Northern Ireland, and Scotland (2007), Serbia, Slovakia and Hungary (2008), Bulgaria and Romania (2009), and Bosnia and Herzegovina (2010) (Brown & al. 2008, Fekete & Merkl. 2010, Kulijer 2010, Merkl 2008, Poutsma & al. 2008, Przewoźny et al. 2007, Schneider & Loomans 2006).

It is now distributed in Europe in Albania, Austria, Belgium, Belarus, Bulgaria, the Czech Republic, Denmark, Germany, France (including Corsica), Great Britain, Greece (including Crete), Hungary, Israel, Italy (including Sicily), Lithuania, Luxembourg, the Netherlands, Norway, Portugal, Romania, Russia, Spain (including the Canaries), Slovakia, Sweden, Switzerland, and Ukraine. It was reported from various natural and urban or cultivated habitats, such as woodlands, parks, gardens, cultivated areas, and buildings, confirming the trend for alien coccinellids to occur in urban and cultivated habitats in Europe (Roy & Migeon 2010).

The aim of the present paper was to report the first occurrence of this invasive species in the Pârâul Pețea natural reserve, which is part of the ROSCI 0098 Natura 2000 site in northwestern Romania.

Material and methods

The Pârâul Pețea reserve is located in Băile 1 Mai spa, 9 km southeast from Oradea, Bihor County, in northwestern Romania, and was declared in 1932 around the hypothermal lake formed by some extensions of the brook (total length 1.5 km). The reserve has roughly two zones: first (A), a pond with thermal, underwater springs, having a depth of 0,1 – 3 m and an average temperature between 35 °C by the springs and 25 °C near the shore (Paina 1978). The second zone (B) is an elongated pond formed by another diverticulum of the rivulet.

Aquatic vegetation is abundant, consisting of species of *Potamogeton*, *Typha*, *Phragmites*, *Lemna*, *Butomus*, *Alisma*, *Spirodela*, *Cabomba*, *Elodea*, and the local endemic morph *Nymphaea lotus* L. var. *thermalis* (DC.) Tuzson 1908. Shore and terrestrial vegetation is also abundant, including species of *Carex*, *Equisetum*, *Cyperus*, *Alopecurus*, *Calamagrostis*, *Arrhenaterum*, *Anthoxanthum*, *Brachypodium*, *Bromus*, *Briza*, *Dactylis*, *Festuca*, *Holcus*, *Poa*, *Setaria*, *Juncus*, *Typha*, *Ranunculus*, *Corydalis*, *Chenopodium*, *Cerastium*, *Myosoton*, *Gypsophila*,

Saponaria, *Lychnis*, *Fallopia*, *Polygonum*, *Rumex*, *Erodium*, *Geranium*, *Lythrum*, *Epilobium*, *Vicia*, *Astragalus*, *Ononis*, *Euphorbia*, *Viola*, *Oxalis*, *Potentilla*, *Alyssum*, *Brassica*, *Camelina*, *Rorippa*, *Sisymbrium*, *Althaea*, *Lysimachia*, *Vinca*, *Gallium*, *Cuscuta*, *Lamium*, *Stachys*, *Glechoma*, *Mentha*, *Lycopus*, *Salvia*, *Veronica*, *Plantago*, *Verbascum*, *Angelica*, *Chaerophyllum*, *Pastinacum*, *Leucanthemum*, *Inula*, *Arctium*, *Crepis*, *Lactuca*, *Tragopogon*, *Sonchus*, *Knautia*, *Urtica*, *Sambucus*, *Robinia*, *Rosa*, *Alnus*, *Salix*, and *Tilia* (Danciu & Golban 2010). The fauna of the reserve includes characteristic elements such as the Cyprinid *Scardinius racovitzai* Müller 1958, endemic for the lake, the relict snail *Melanopsis parreyssii* Philippi 1847, and many other protected invertebrates and vertebrates. Odonata and Gerromorphans are abundant in many areas of the lake, the latter including the rare *Mesovelia thermalis* Horváth 1915.

On May 25, 2011, two *H. axyridis* individuals were observed while monitoring the fauna in the reserve: an imago (f. *succinea*) on *Rosa canina* flowers, close to the end of zone B, which took flight and could not be retrieved, and a larva on a shrub by the lake shore, in zone A. Then, on June 22, 2011, two imagos (f. *succinea* and f. *conspicua*) were collected manually on shrubs near the end limit of zone B. No other individuals were found, not even by beating the shrubs over a collecting panel. Another f. *succinea* imago was found on October 12, 2011, entering the museum building in Oradea in search for a shelter for overwintering.

Results

The observed imagos were shortened oval in shape, moderately convex, with straw yellow pronotum with characteristic M-shaped black marks (except for the f. *conspicua* individual, which had the black marks extended and fused in a roughly trapezoidal form), black ventral side, and brownish legs (except for f. *conspicua*, which had black legs). The elytra coloration was as follows: reddish with black spots (observed f. *succinea*), yellow with 18 black spots (collected f. *succinea*), and black with two reddish spots with a black dot each (f. *conspicua*) (Fig. 1).

The larva (third instar) was elongated, somewhat flattened, blackish, with elongated tubercles and spines, with a prominent, typical, bright yellow-orange patch extending over the dorso-lateral lobes of abdominal segments 1-5 on each side (Adriaens & al. 2003, Chapin & Brou 1991, Gordon & Vanderberg 1991, Koch 2003, Michie & al. 2010, Przewoźny & al. 2007, Ruicănescu & Alexandru 2009). The collected specimens are deposited in the entomological collection of Țării Crișurilor Museum, Oradea.



Figure 1. Specimens of *Harmonia axyridis* (f. *succinea* and *conspicua*) collected in Pârâul Pețea reserve. Scalebar = 2 mm. (Photo Dr. Márton Venczel).

Discussion

Only one previous record of the presence of *H. axyridis* in Bihor county is available, mentioning two collectings from Oradea (10 April 2009, „one imago”) and Gurani (2 Aug. 2009, „more individuals”), with no other details and erroneously considered as the first records from Romania (Markó & Pozsgai 2009). The real first record in the wild in Romania was in the summer of 2008 (four individuals in the „Calcarele din Dealul Măgura”, a mountaineous area in Băița, Hunedoara county, central Romania), and afterwards the species was observed at the same spot in May 2009 and around Baia Mare (Maramureș county, northwestern Romania) in June 2009 (Ruicănescu & Alexandru 2009). In the fall of 2010, *H. axyridis* was already present, with many individuals, in southeast and central Romania: Dobrudja (Constanța, Măcin Mountains National Park, Celic Dere), Jiu Gorge (at Meri), and Olteț Gorge; then a huge population (thousands of individuals) was observed in October 2011 near Arad, west Romania (Skolka & Preda 2010, M. Skolka, pers. comm.). There is no information that *H. axyridis* was ever released for biological control purposes in Romania. As it invaded Hungary from western

and possibly northern directions and within one year (2009) it became one of the most common coccinellids (with f. *succinea* predominating, 91.3 %) (Markó & Pozsgai 2009), it could be more likely that the individuals in the Pârâul Pețea reserve originated from Hungary or the Baia Mare region, since no major natural barrier would prevent their spread along those directions.

It may not be possible to fully explain the mechanisms of spread of *H. axyridis* in Europe, because of the multiple introduction sites in at least twelve European countries over a period of approximately forty years, and time lags may occur between arrival, establishment and impact. The need for adaptation before increase in number and spread may explain the lack of a time lag between establishment and expansion in countries where *H. axyridis* was not introduced; individuals arriving in these countries from parts of the introduced range had already been through the adaptation phase. So after a period of adaptation of one or several populations in France, Netherlands, Belgium and/or Germany, a rapid spread of *H. axyridis* occurred in Europe (Brown et al. 2008).

Although winter temperatures below 12 °C. are needed for induction of diapause, too cold winters may prevent *H. axyridis* from establishing (Poutsma & al. 2008, Soares & al. 2008), but selection of human houses as overwintering sites may constitute a cold-free space, which could explain its great invasive success in northern regions (Labrie & al. 2008). The larvae favor temperatures of 18-30 °C. and no development occurs in adults above 25 °C., while larvae and pupae are capable of developing at temperatures between 0 and 35 °C., although very slowly below 15 °C., with an optimum for development in adults from 5 to 25 °C. and for larvae and pupae from 5 to 15 °C. (Acar & al. 2004). Ovipositing females showed strong preference for *Urtica dioica* infested with the aphid *Microlophium carnosum* (Alhmedi & al. 2008), an interesting fact to be correlated with the interactions between *H. axyridis* and the native aphidophagous guilds in crops and other habitats.

Being a colonizing species which frequently encounters new habitats, climates, resources and selection pressures, *H. axyridis* displays great phenotypic plasticity on a genetic basis, among the tested traits being the ability to enter into quiescence during periods of low resource availability and the ability to forage for a variety of different food sources, including through cannibalism (Grill & al. 1997, Lombaert & al. 2008); yet more comparative studies between colonizing and established populations are needed to clarify the role of plasticity in colonization and range expansion.

Three color forms of adult *H. axyridis* have been found in Europe: the non-melanic f. *succinea* (predominating), and the melanic f. *spectabilis* and f. *conspicua*,

while the typical form *axyridis*, which predominates in central Asia, has not been recorded in Europe yet. The broad consistency in the color form frequency data from Europe suggests a genetic similarity of populations, possibly the result of spread from very limited points of origin in Europe (Brown & al. 2008), a fact which is to be verified through genetic analyses of *H. axyridis* samples from different countries and regions. Another melanic form (*nigra*, completely black) had lower voracity, biomass, longevity and reproductive capacity than the *aulica* form (mainly red) in laboratory conditions (Soares et al. 2001), a fact which may change under different conditions in various habitats. Therefore, the polymorphism in *H. axyridis* may have an adaptative valuability if different genotypes were selectively favoured in different habitats or at different times, resulting in an increased tolerance of a wide range of environmental conditions. Melanic forms would be in advantage in colder climates, being able to attain higher body temperatures when exposed to sunlight, and therefore greater fitness, as melanization is a phenotypical response of the highly plastic f. *succinea* to colder temperatures (Michie & al. 2010, Poutsma & al. 2008). Indeed, the melanic f. *conspicua* predominates in southwestern Siberia and northern Japan (Chapin & Brou 1991), but an increase in non-melanic f. *succinea* from spring to summer and in melanic forms from summer to autumn was observed, caused by non-random mating based not only on elytral color (Osawa & Nishida 1992).

Various techniques and trapping methods to prevent ladybugs from entering and aggregating in buildings or on grapes in autumn or to remove aggregates inside buildings have been proposed, as well as several natural enemies, but the latter showed very limited potential as biological control agents (Kenis & al. 2008), thus it is unlikely that any highly specific predator or parasitoid would be effective against *H. axyridis* in the field in the colonized regions.

Further spreading in Europe is likely, particularly northwards and eastwards (Brown et al. 2008). The data about the presence of *H. axyridis* in Romania indicate a fast spreading and numerous populations in southern, central and western regions, this ladybug being probably already distributed in the whole country. The low number of individuals in the area of Oradea and the Pârâul Pețea reserve (compared to the abundance in central, southeastern and western regions of Romania), the presence of different color forms and the lack of reports of overwintering groups in that northwestern region suggest that, while certainly reproducing, *H. axyridis* may still be in the process of establishing itself in the Pârâul Pețea reserve and the surrounding zone, but its impact on native species in the protected area needs investigation.

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