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MG and MT collected and examined the material; all authors contributed to manuscript preparation

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# **Competing interests**

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### SHORT COMMUNICATION

# First record of *Hesperomyces virescens* (Laboulbeniales, Ascomycota) on *Harmonia axyridis* (Coccinellidae, Coleoptera) in Poland

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## **Abstract**

*Hesperomyces virescens* Thaxt. is a fungal parasite of coccinellid beetles. One of its hosts is the invasive harlequin ladybird *Harmonia axyridis* (Pallas). We present the first records of this combination from Poland.

# **Keywords**

harlequin ladybird; invasive species; ectoparasitic fungi

# Introduction

Harmonia axyridis (Pallas) is a ladybird of Asiatic origin considered invasive in Europe, Africa, and both Americas [1]. One of its natural enemies is Hesperomyces virescens Thaxt., an obligatory biotrofic fungal ectoparasite of the order Laboulbeniales. This combination was described for the first time in Ohio, USA in 2002 [2] and soon afterwards recorded in many other localities in North America ([3] and references therein). Four years later it was recorded in Belgium [4] and subsequently in the Netherlands (2008), Germany (2008), the United Kingdom (2011), the Czech Republic (2013), Croatia (2013), and Hungary (2014) [3-7]. Recently (2013), H. virescens was also recorded on H. axyridis in South Africa [8]. Although H. virescens was reported on different coccinellid hosts from many countries it was never observed on Harmonia axyridis in its native range. Haelewaters et al. [9] presented historical record of the fungus on Harmonia axyridis collected in the 1930s in Sichuan Province, China and there is no more recent record of this combination from that region. Hesperomyces virescens infection is considered a rare example of acquiring novel parasite by invasive species as it is assumed that invasive Harmonia axyridis strain was originally free of infection [9,10]. Despite extensive studies of Laboulbeniales, the first report of Hesperomyces from Poland was only in 2013: H. coccinelloides on Stethorus pusillus [11]. Here we report the presence of a second species, Hesperomyces virescens, in Poland.

# Material and methods

Harmonia axyridis beetles were collected in vicinity of the University of Warsaw Botanic Garden, Warsaw, Poland (52°13′1″ N, 21°1′38″ E), on following days: 2014-10-30 – 2014-11-06, 2015-03-09 – 2015-03-10, and 2015-10-26 – 2015-11-17. Insects

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**Fig. 1** a Hesperomyces virescens Thaxt. thalli on pronotum of Harmonia axyridis (Pallas). **b** Hesperomyces virescens Thaxt. mature thallus.

were killed with ethyl acetate vapors or by freezing in  $-20^{\circ}$ C, then observed for the presence of Laboulbeniales fungi using a dissecting microscope (Nikon SMZ800). Several thalli were mounted in glycerin as described by Majewski [12], which are deposited at the Warsaw University Herbarium (WA) under numbers WA0000052035 to WA0000052040. Photographs were taken using a NIKON DX-1200 camera (Fig. 1). Some thalli were used for generating ribosomal DNA sequences, which have been already published in Haelewaters et al. [13]. All insects are in the authors' collection except for four specimens, which were sent to Harvard University.

# **Results**

Of the total 1443 ladybirds screened, 16 (1.11%) were infected by *Hesperomyces virescens*. Both autumn 2014 and spring 2015 collections were rather accidental with only a small number of beetles screened (Tab. 1). During autumn 2015 more systematic collection was performed aiming to gather all *H. axyridis* from one overwintering site, on the outer walls of the building. The number of ladybirds caught was 1340, of which 14 (1.03%) were infected. Thirteen of the infected ladybirds bore parasites on the posterior part of their elytra. Four *H. axyridis* bore about 20 mature thalli. In other specimens the thallus density was much lower, between one to ten mature thalli per insect.

# Discussion

Despite a long tradition of Laboulbeniales studies in Poland neither Janina and Wincenty Siemaszko nor Tomasz Majewski ever found any fungus of the genus

 Tab. 1
 Number of collected and infected Harmonia axyridis.

Date	N collected	N infected	% infected
October 2014	30	0	0.00%
November 2014	30	1	3.33%
March 2015	23	1	4.35%
2015-10-26	182	5	2.75%
2015-10-27	181	1	0.55%
2015-10-28	540	0	0.00%
2015-10-29	317	5	1.58%
2015-11-12	140	3	2.14%
Autumn 2015	1360	14	1.03%
Total	1443	16	1.11%

Hesperomyces [11]. In his book Kozłowski [14] showed a photo of Adalia bipunctata from Poland infected by Laboulbeniales ectoparasitic fungi, probably H. virescens. However the finding was never published formally. Since the specimen is not available for study, the proper identification of the fungus is impossible. It most probably belonged to H. virescens as this is the only known species of Hesperomyces found on A. bipunctata. However in Toruń, Kuyavian-Pomeranian Voivodship, more than 4000 A. bipunctata were collected and screened but no infection was detected [15]. In 2013 Ceryngier reported from Poland a related species, H. coccinelloides, on another coccinellid beetle, Stethorus pusillus [11].

Although our finding is neither the northernmost nor easternmost in Europe it moves the boundary of known distribution of H. virescens on Harmonia axyridis [3]. Comparing to infection rates recorded in Belgium (96.5%, n = 86) [4], Germany (79.1%, n = 134) [3], and South Africa (29.4%, n = 1794) [8], the infection rate in our studied population was very low

(1.1%). This may indicate that conditions for fungus development are suboptimal or that the fungus was only recently acquired by the host. However, it should be noted that infection rate of *H. virescens* is known to change greatly between localities and years or even seasons [3,4,8,9,16]. Further studies on *H. virescens* on *Harmonia axy-ridis* and other lady beetle species in Central and Eastern Europe may improve our understanding of this parasite-host combination.

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