



Tracking an ectoparasitic fungus of *Harmonia axyridis* in the USA using literature records and citizen science data

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Abstract: *Hesperomyces harmoniae* nom. prov. (Ascomycota: Laboulbeniales) is a common fungal ectoparasite of *Harmonia axyridis* (Coleoptera: Coccinellidae), a globally invasive pest species. We launched an initiative to create a dataset encompassing all available records of the *H. axyridis* – *H. harmoniae* nom. prov. association in the USA. Reports have been gathered from the literature and from online citizen science platforms such as Bugguide and iNaturalist. A total of 163 curated records were downloaded from Bugguide and iNaturalist. Using these records, we built a distribution map available at <http://beetlehangars.org>. All occurrences are shown, each with location information, collection date, collector(s), and source – Bugguide, iNaturalist or literature. In time, the map will become searchable by date, as to be able to determine where in the USA the ladybirds–parasite association originated and track its distributional expansion over the years.

Key words: citizen science, distribution, *Harmonia axyridis*, *Hesperomyces*, Laboulbeniales

Introduction

Hesperomyces virescens is a fungal ectoparasite (Ascomycota: Laboulbeniales) that infects adult ladybirds (Coleoptera: Coccinellidae). We recently discovered that *H. virescens* is a complex consisting of multiple species, each specialized to a single host species or host species within a single genus (Haelewaters et al., 2018). One of these, *H. harmoniae* nom. prov., is only known from *Harmonia axyridis* from many countries around the world (Haelewaters et al., 2019). We do not know the place this fungus originated. Is it native to North America (Roy et al., 2011)? Was it imported into North America with *H. axyridis* upon introduction of the latter? Or did a host shift occur from a North American native ladybird to *H. axyridis*, after which the fungus became more successful on the invader?

From studies in the Netherlands and the USA, we know there is a significant gap between establishment of *H. axyridis* in the wild and the first observations of the fungus on this ladybird (Haelewaters et al., 2017). Supposedly, the ladybird acquired the fungus after a certain time lag, providing some support for the hypothesis that *H. harmoniae* nom. prov. is a North American native fungus. Here, we report preliminary results of our initiative to create a dataset encompassing all available reports of the *H. axyridis*–*H. harmoniae* nom. prov. association in North America.

Material and methods

We extracted information from Bugguide.net and the citizen-science initiative iNaturalist by searching for the following queries: “*Hesperomyces virescens*”, “green beetle hanger”, and “Laboulbeniales fungus”. We downloaded data from 2002 (Garcés and Williams, 2004) up to 2018. All records were subject to manual quality control; photographs of records were checked for correct host species identification and whether *Hesperomyces* fruiting bodies were indeed present. Duplicate records were filtered out based on date, locality, collector, and photographs. Bugguide records were not associated with geographic coordinates (contrary to records downloaded from iNaturalist), hence we used coordinates of the city or on county level from where records were made. All data were combined into a single database, which served as basis for a distribution map of the *H. axyridis* – *H. harmoniae* nom. prov. association in the USA.

The data from online sources were complemented by reports from the literature (Garcés and Williams, 2004; Riddick and Schaefer, 2005; Harwood et al., 2006 a; 2006 b; Riddick, 2006; 2010; Nalepa and Weir, 2007). We constructed a map showing all gathered occurrences of *H. harmoniae* nom. prov. associated with *H. axyridis* ladybirds, each with location information, collection date, name(s) of collector(s), and source (Bugguide / iNaturalist / literature). The distribution map is available at <http://beetlehangars.org>.

Results and discussion

We report a total of curated (= post-quality control) 163 records from the USA between 2002 and 2018. July-August 2002 marks the first published report of *H. harmoniae* nom. prov. on *H. axyridis*, from Ohio (Garcés and Williams, 2004). The number of records downloaded from Bugguide was 59, whereas the number of records from iNaturalist was 104 (Table 1). Early data are patchy; between 2002 and 2010 not enough records are available to make meaningful statements about targeted distributional spread of this association. It is likely that the association was present but that it has not been spotted or reported because not too many people – including researchers – are familiar with this laboulbenian fungus. Second, citizen science initiatives are only recently gaining more traction with the wide public, slowly accumulating publicly available species records.

Indeed, citizen science projects are increasingly important for the detection of easily recognizable species, such as the harlequin ladybird, *H. axyridis*. Examples are the Lost Ladybug Project (<http://www.lostladybug.org/>) and the UK Ladybird Survey (<http://www.ladybird-survey.org/>). To date, 1,000s and 1,000s of people have contributed observations of ladybirds through this UK initiative (Roy et al., 2015; Brown et al., 2018) aiding us to better understand post-invasion effects of *H. axyridis*. However, the next step – citizen scientists providing photographs of natural enemies of ladybirds – comes with

difficulties, as it becomes more than simply taking a photograph and uploading it to a website. There is a need to actively screen the observed specimen for fungal growth and take close-up photographs for good documentation. Much less citizen scientists will be willing to go that far. Those who will contribute, however, are expected to provide high-quality data.

Table 1: Number of records extracted from Bugguide & iNaturalist, by year and post-quality control.

Year	Bugguide	iNaturalist
2002		
2003		
2004	2	
2005	3	
2006	4	
2007	2	
2008	1	
2009	6	
2010	13	
2011	4	1
2012	3	
2013	6	4
2014	5	5
2015	2	7
2016	3	19
2017	3	22
2018	2	46

What we did in this study was making use of already available photographs from online sources to screen ladybirds for *Hesperomyces*. Fruiting bodies may not be observed if photographs are of poor quality or if fruiting bodies are only present ventrally whereas the photograph only shows the elytra (or *vice versa*). In other words, there are limitations to this approach, but we have been able to gather over 100 records of *H. hamoniae* nom. prov. from *H. axyridis*, accounting for some first state-wide records in the USA (Haelewaters et al., 2017). In 2018 alone, data downloaded from iNaturalist included new state records of *H. axyridis* – *H. hamoniae* nom. prov. for Indiana, Missouri, and Wisconsin.

As a next step, we aim to incorporate records submitted to the photo-sharing website [Flickr.com](https://www.flickr.com). When searching for “ladybug” + “fungus”, the number of observations is 757, but following quality control this number is likely to be much decreased. In addition, we will add data from personal collections in North America and from screening dried ladybirds in natural history collections. We further aim to transform this into citizen science project, through which users can submit sightings of *H. hamoniae* nom. prov. on *H. axyridis*. In the future, the map will be searchable by date, as to be able to determine where in North America the association originated and track its distributional expansion over the years.

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References

- Brown, P. M. J., Roy, D. B., Harrower, C., Dean, H. J., Rorke, S. L. and Roy, H. E. 2018. Spread of a model invasive alien species, the harlequin ladybird *Harmonia axyridis* in Britain and Ireland. *Sci. Data* 5: 180239.
- Garcés, S. and Williams, R. 2004. First record of *Hesperomyces virescens* Thaxter (Laboulbeniales: ascomycetes) on *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). *J. Kansas Entomol. Soc.* 77: 156-158.
- Haelewaters, D. 2019. *Hesperomyces* “*harmoniae*” nom. prov. (Laboulbeniales), an ectoparasitic fungus specific to *Harmonia axyridis*. *IOBC-WPRS Bull.* 145: 53-55.
- Haelewaters, D., de Kesel, A. and Pfister, D. H. 2018. Integrative taxonomy reveals hidden species within a common fungal parasite of ladybirds. *Sci. Rep.* 8: 15966.
- Haelewaters, D., Zhao, S. Y., Clusella-Trullas, S., Cottrell, T. E., De Kesel, A., Fiedler, L., Herz, A., Hesketh, H., Hui, C., Kleespies, R. G., Losey, J. E., Minnaar, I. A., Murray, K. M., Nedvěd, O., Pfliegler, W. P., Raak-van den Berg, C. L., Riddick, E. W., Shapiro-Ilan, D. I., Smyth, R. R., Steenberg, T., van Wielink, P. S., Vigišová, S., Zhao, Z., Ceryngier, P. and Roy H. E. 2017. Parasites of *Harmonia axyridis*: current research and perspectives. *BioControl* 62: 355-371.
- Harwood, J. D., Ricci, C., Romani, R., Pitz, K. M., Weir, A. and Obrycki, J. J. 2006 a. Prevalence and association of the laboulbeniaceous fungus *Hesperomyces virescens* (Laboulbeniales: Laboulbeniaceae) on coccinellid hosts (Coleoptera: Coccinellidae) in Kentucky, USA. *Eur. J. Entomol.* 103: 799-804.
- Harwood, J. D., Ricci, C., Romani, R. and Obrycki, J. J. 2006 b. Historic prevalence of a laboulbeniaceous fungus infecting introduced coccinellids in the United States. *Antenna* 30: 74-79.
- Nalepa, C. A. and Weir, A. 2007. Infection of *Harmonia axyridis* (Coleoptera: Coccinellidae) by *Hesperomyces virescens* (Ascomycetes: Laboulbeniales): Role of mating status and aggregation behavior. *J. Invert. Pathol.* 94: 196-203.
- Riddick, E. W. 2006. Influence of host gender on infection rate, density and distribution of the parasitic fungus, *Hesperomyces virescens*, on the multicolored Asian lady beetle, *Harmonia axyridis*. *J. Insect Sci.* 6: 42, doi:10.1673/031.006.4201.
- Riddick, E. W. 2010. Ectoparasitic mite and fungus on an invasive lady beetle: parasite coexistence and influence on host survival. *Bull. Insectol.* 63: 13-20.
- Riddick, E. W. and Schaefer, P. W. 2005. Occurrence, density, and distribution of parasitic fungus *Hesperomyces virescens* (Laboulbeniales: Laboulbeniaceae) on multicolored Asian lady beetle (Coleoptera: Coccinellidae). *Ann. Entomol. Soc. Am.* 98: 615-624.
- Roy, H. E., Rhule, E., Harding, S., Lawson Handley, L.-J., Poland, R. L., Riddick, E. W. and Steenberg, T. 2011. Living with the enemy: parasites and pathogens of the ladybird *Harmonia axyridis*. *Biocontrol* 56: 663-679.
- Roy, H. E., Rorke, S. L., Beckmann, B., Booy, O., Botham, M. S., Brown, P. M. J., Harrower, C., Noble, D., Sewell, J. and Walker, K. 2015. The contribution of volunteer recorders to our understanding of biological invasions. *Biol. J. Linn. Soc.* 115: 678-689.

Roy, H. E., Brown, P. M. J., Adriaens, T., Berkvens, N., Borges, I., Clusella-Trullas, S., Comont, R. F., de Clercq, P., Eschen, R., Estoup, A., Evans, E. W., Facon, B., Gardiner, M. M., Gil, A., Grez, A., Guillemaud, T., Haelewaters, D., Herz, A., Honek, A., Howe, A. G., Hui, C., Hutchison, W. D., Kenis, M., Koch, R. L., Kulfan, J., Lawson Handley, L., Lombaert, E., Loomans, A., Losey, J., Lukashuk, A. O., Maes, D., Magro, A., Murray, K. M., San Martin, G., Martinkova, Z., Minnaar, I. A., Nedved, O., Orlova-Bienkowskaja, M. J., Osawa, N., Rabitsch, W., Ravn, H. P., Rondoni, G., Rorke, S. L., Ryndevich, S. K., Saethre, M.-G., Sloggett, J. J., Soares, A. O., Stals, R., Tinsley, M. C., Vandereycken, A., van Wielink, P., Vigišová, S., Zach, P., Zakharov, I. A., Zaviezo, T. and Zhao, Z. 2016. The harlequin ladybird, *Harmonia axyridis*: global perspectives on invasion history and ecology. *Biol. Invasions* 18: 997-1044.