Arthropod-Plant Interactions
https://doi.org/10.1007/s11829-024-10040-1

ORIGINAL PAPER

Parasitism of ladybirds by *Hesperomyces* in the Canary Islands

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Received: 22 November 2023 / Accepted: 12 January 2024

Abstract
Between 2016 and 2022, the ladybird (Coleoptera: Coccinellidae) fauna was surveyed on seven main islands of the Canary Islands archipelago. Adult specimens were collected and screened for thalli of Laboulbeniales (Ascomycota: Laboulbeniomycetes). Altogether, 9,807 ladybird specimens belonging to 39 species were screened, of which 103, representing 10 species, were infected by Laboulbeniales of the genus *Hesperomyces*. Morphological and molecular examination allowed to distinguish at least seven host-specific *Hesperomyces* species belonging to three groups: *He. virescens* species complex (associated with *Adalia decempunctata, Harmonia axyridis, Hyperaspis vinciguerrae, Novius cruentatus, Parexochomus nigripennis*, and *P. quadriplagiatus*), *He. coccinelloides* species complex (*Scymnus nubilus, Sc. subvillosus durantae*, and *Stethorus tenerifensis*), and a species (on *Nephus flavopictus*) that cannot be assigned to either of the above species complexes or the currently described species in the genus. The most widespread and abundant species was *Hesperomyces* ex *St. tenerifensis*; it was recorded on all seven islands with prevalence rates ranging between 0.04 and 0.19.

Keywords Coccinellidae · Ectoparasites · Host–Parasite Association · Laboulbeniales · Ladybirds · Molecular phylogeny

Introduction

All members of the ascomycetous order Laboulbeniales (Fungi: Pezizomycotina) are obligate ectoparasites of arthropods, with beetles (Coleoptera) as their most frequent hosts. Laboulbeniales never show hyphal growth, but form minute thalli of often bizarre shapes on their host's cuticle (Blackwell et al. 2020; Haelewaters et al. 2021). Although these parasites have long attracted the attention of naturalists, their ecology, taxonomy, and evolution have not been thoroughly studied until recently. The currently observed intensification of research on Laboulbeniales is partly related to the fact that one of the members of this order was recently identified as a common parasite of the harlequin ladybird, *Harmonia axyridis* (Ceryngier and Twardowska 2013; Haelewaters et al. 2016, 2017), a highly invasive species that causes serious concern due to its negative impacts on the biodiversity in its invasive range, human health, and food production (Roy et al. 2016). This Laboulbeniales parasite associated with *Ha. axyridis* has for a long time been referred to *Hesperomyces virescens*, until it was recognized as one of several species in the *He. virescens* species complex (Haelewaters et al. 2018). It was only recently formally described as *He. harmoniae* for its unique association with *Ha. axyridis* (Haelewaters et al. 2022b).

Members of the genus *Hesperomyces* are most frequently recorded on ladybird hosts (Coleoptera: Coccinellidae). Only two of the 12 species currently included in the genus were reported parasitizing beetles from other families: *He. biphylli* on Biphyllidae (Sugiyama and Majewski 1985) and *He. catopii* on Mycetophagidae (Thaxter 1931). These two species, however, should be re-examined as to whether they actually belong to *Hesperomyces*. Of the ladybird-associated species of *Hesperomyces*, ten have been formally described (Thaxter 1931; Majewski and Sugiyama 1985; Goldmann et al. 2013; Das

Handling Editor: Salmah Yaakop.

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Published online: 12 February 2024

et al. 2018; Haelewaters and De Kesel 2020; Crous et al. 2021; Haelewaters et al. 2022b) while several others have been recognized as distinct species, but not yet described (Haelewaters et al. 2018; Van Caenegem et al. 2023b). Studies supported by molecular phylogenetic data have shown that species of *Hesperomyces* are highly host specific: individual species are usually associated with a single host genus or species (Haelewaters et al. 2018).

Our recent survey of Coccinellidae in the Canary Islands (Romanowski et al. 2019, 2020a, b, c, 2023) has revealed the presence of *Hesperomyces* on individuals belonging to ten ladybird species in eight genera. Based on this material, one new species of *Hesperomyces*, *He. parexochomi* associated with ladybirds of the genus *Parexochomus*, was formally described in Crous et al. (2021), and the identity of another species associated with *Hyperaspis vinciguerrae*, was discussed by Van Caenegem et al. (2023b). Most of the species, however, still await detailed taxonomic treatment. In this paper, we summarize the data on parasitism of the Canarian ladybirds by *Hesperomyces*.

Materials and methods

Study area and collection of *Coccinellidae* specimens

The Canary Islands are a volcanic archipelago located near the north-western coast of Africa. The archipelago consists of seven major islands and many islets. The impressive biodiversity of the Canary Islands, both in terms of the variety of ecosystem types and species richness, has been shaped by a combination of factors, including subtropical geographical location, high age of the older islands (up to 20.5 Myr), significant altitude variation (from sea level to 3718 m a.s.l.), influence of the north-eastern trade winds, and the intermediate degree of isolation (distance of individual islands from the African mainland ranges between 95 and 416 km). A considerable proportion of Canarian biodiversity consists of endemic taxa (Fernández-Palacios and Whittaker 2008).

Specimens for this study were collected at the main islands of the archipelago (Fuerteventura, Lanzarote, Gran Canaria, Tenerife, La Gomera, La Palma, El Hierro) between 2016 and 2022. They were collected by hand, or using a sweep net or 1 m × 1 m beating tray. Adult specimens were screened under a stereomicroscope for the presence of Laboulbeniales thalli (at 50× magnification). The thalli observed on infected specimens were subjected to further examination (slide mounting, molecular work).

Morphological and molecular study of Laboulbeniales

Thalli were removed from the host individual at the point of attachment, placed and arranged in a 1:1 mixture of glycerin and Hoyer's medium, and slide-mounted using a double-coverslip technique (Liu et al. 2020; Van Caenegem et al. 2023b). Studied slides are stored at the Farlow Herbarium at Harvard University (FH), Herbarium Universitatis Gandavensis (GENT), and the Kriebel Herbarium at Purdue University (PUL). The slide collection of thallus-forming Laboulbeniomycetes at GENT is in the process of being accessioned, with each slide receiving a unique GENTFL label (F for Fungi, L for Laboulbeniomycetes). Voucher host specimens are deposited at the collection of Taxon Expeditions, Leiden, The Netherlands (TXEX) and the Purdue Entomological Research Collection, West Lafayette, Indiana (PERC).

DNA was extracted from 1 to 8 thalli per each host individual, using the REPLI-g Single Cell Kit (Qiagen, Stanford, California) following the manufacturer's instructions. PCR amplifications were done of the following regions: small subunit (SSU) and large subunit (LSU) of the nuclear ribosomal RNA gene, *MCM7* that encodes a licensing factor required for DNA replication initiation and cell proliferation, and the gene encoding the translation elongation factor 1α (*TEF1*). Primers combinations used were NSL1/NSL2 for SSU (Haelewaters et al. 2015), LR0R/LR5, NL1/ NL4, and LIC24R/LR3 for LSU (Vilgalys and Hester 1990; Hopple 1994; Kurtzman and Robnett 1997; Miadlikowska and Lutzoni 2000), MCM7-709for/MCM7-1384rev for *MCM7* (Schmitt et al. 2009), and EF1-1018 F/EF1-1620R and Al33_alternative_f/EF1-1620R for *TEF1* (Stielow et al. 2015). Amplifications were performed in 25-µl reactions consisting of 13.3 µl of REDExtract Taq polymerase (Sigma-Aldrich, St. Louis, Missouri), 2.5 µl of each 10 µM primer, 5.45 µl of ddH_2O, and 1 µl of DNA extract. PCR conditions were as in Van Caenegem et al. (2023a, b): For SSU: initial denaturation at 94 °C for 5 min; 39 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 45 s, and extension at 72 °C for 90 s; and final extension at 72 °C for 10 min. For LSU: initial denaturation at 94 °C for 5 min; 34 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 45 s, and extension at 72 °C for 1 min; and final extension at 72 °C for 7 min. For *MCM7*, an initial denaturation at 94 °C for 5 min; 10 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C (− 1 °C/cycle) for 50 s, and extension at 72 °C for 1 min; 24 cycles of denaturation at 94 °C for 45 s, annealing at 47 °C for 50 s, and extension at 72 °C for 1 min; and final extension at 72 °C for 5 min. For *TEF1*: initial denaturation at 94 °C for 5 min; 10 cycles of denaturation at 94 °C for 50 s, annealing at 54 C (− 1 C/cycle) for 50 s, and extension at 72 °C for 50 s; followed by 40 cycles of denaturation at 94 °C for 50 s, annealing at 48 °C for 50 s, and extension at 72 °C for 50 s; and final extension at 72 °C for 7 min.

Sequence alignment and phylogenetic analysis

Most sequences used in the phylogenetic analysis had been generated in previous studies and were downloaded from NCBI Genbank (http://www.ncbi.nlm.nih.gov/). We did not use SSU sequences in the phylogenetic analysis, because this region is too conserved to distinguish species. *TEF1* was also excluded from the analysis because we only generated four *TEF1* sequences representing two species. We used *Hesperomyces* ex *Azya orbigera*, *He. coccinelloides*, and *He. coleomegillae* as outgroup (Table 1; Haelewaters et al. 2022b). We aligned LSU and *MCM7* sequences by locus with the G-INS-I strategy and ITS with the E-INS-I strategy using the online version 7 of MAFFT (Katoh et al. 2019; Kuraku et al. 2013). Sequences were manually trimmed using BioEdit Sequence Alignment Editor version 7.2.6 (Hall 1999) and combined in SequenceMatrix 1.9 (Vaidya et al. 2011) to construct one concatenated dataset (ITS–LSU–*MCM7*). The final dataset included five partitions: the ITS1 and ITS2 spacer regions, the 5.8 S gene, LSU, and *MCM7*. Models for nucleotide substitution were selected for each partition with ModelFinder (Kalyaanamoorthy et al. 2017) according to the corrected Akaike Information Criterion (AICc). A maximum likelihood (ML) reconstruction was inferred using IQ-TREE (Nguyen et al. 2015) under partitioned models (Chernomor et al. 2016). Ultrafast bootstrapping (UFBoot2) was performed with 1000 replicates (Hoang et al. 2017).

Results

Altogether, 9,807 specimens representing 39 species of Coccinellidae were screened for the presence of Laboulbeniales ectoparasites. A total of 103 specimens (1.1% screened) belonging to 10 species (25.6%) were found to be hosts of *Hesperomyces* (Table 2). These are: *Adalia decempunctata*, *Harmonia axyridis* (tribe Coccinellini), *Hyperaspis vinciguerrae* (Hyperaspidini), *Nephus flavopictus*, *Scymnus nubilus*, *Sc. subvillosus durantae* (Scymnini), *Novius cruentatus* (Noviini), *Parexochomus nigripennis* and *P. quadriplagiatus* (Chilocorini), and *Stethorus tenerifensis* (Stethorini) (Fig. 1). The highest number of host species (six out of 22 total species) were recorded on Fuerteventura, the island closest to the mainland. On the remaining islands, between one and two host species were recorded. *Stethorus tenerifensis* was recorded as a host of *Hesperomyces* on all the Canarian islands, whereas other host species were found to be parasitized only on single islands (Fig. 2).

Species identifications of the recorded parasites

At least seven species of *Hesperomyces* can be found on ladybirds in the Canary Islands. Infected ladybirds of *A. decempunctata*, *Ha. axyridis*, *Hy. vinciguerrae*, *No. cruentatus*, *P. nigripennis*, and *P. quadriplagiatus* bore thalli of species belonging to the *He. virescens* species complex (Haelewaters et al. 2018; Van Caenegem et al. 2023b). The species associated with *Ha. axyridis* and *Parexochomus* spp. were recently described as *He. harmoniae* (Haelewaters et al. 2022b) and *He. parexochomi* (Crous et al. 2021), respectively, but those associated with *A. decempunctata*, *Hy. vinciguerrae*, and *No. cruentatus* are awaiting formal description. Thalli observed on *Scymnus nubilus*, *Sc. subvillosus durantae*, and *St. tenerifensis* were identified as *He. coccinelloides* sensu lato. Finally, *Ne. flavopictus* was found to host an undescribed member of the genus *Hesperomyces*. We have not been able to generate sequence data for this species; without molecular phylogenetic data, it cannot be assigned to either of the above species complexes.

Parasite prevalence rates

Parasite prevalence rates (across different islands) were highest for *P. quadriplagiatus* (0.18, $N=79$), followed by *P. nigripennis* (0.15, $N=124$), *Ha. axyridis* (013, $N=8$), *A. decempunctata* (0.11, $N=27$), *St. tenerifensis* (0.09, $N=609$), *Hy. vinciguerrae* (0.08, $N=12$), *No. cruentatus* (0.05, $N=77$) (Table 2). Prevalence rates for *Sc. subvillosus durantae* ($N=318$), *Ne. flavopictus* ($N=252$), and *Sc. nubilus* ($N=318$) were negligible (<0.01). In Table 3, we present detailed data on parasitism of ladybirds by *Hesperomyces* spp. on different islands. The parasite prevalence rates were usually low (<0.1), except for *A. decempunctata* from Gran Canaria (0.25, $N=12$), *Ha. axyridis* from Tenerife (0.13, $N=8$), *Parexochomus* spp. from Fuerteventura (0.18, $N=77$ for *P. quadriplagiatus* and 0.31, $N=59$ for *P. nigripennis*), and *St. tenerifensis* from Fuertaventura (0.11, $N=170$), La Gomera (0.19, $N=42$), and Tenerife (0.13, $N=89$).

Phylogenetic analysis

The concatenated ITS–LSU–*MCM7* dataset consisted of 2338 characters for 52 isolates. Selected models for each partition in our concatenated dataset were TVM + F + R2 (ITS1, 402 bp, -lnL = 3999.847), TNe (5.8 S, 143 bp, -lnL = 529.517), GTR + F + G4 (ITS2, 274 bp, -lnL = 3060.395), TIM + F + I (LSU, 894 bp, -lnL = 4485.782), and TIM3e + G4 (*MCM7*, 625 bp, -lnL = 2842.492). Sequences of *Hesperomyces* from five different host species collected on the Canary Islands were included. They represented four phylogenetic species

Table 1 Details of all isolates of fungi used in this study, with species name, country of collection, host species, and Genbank accession numbers.

Species	Isolate	Country	Host	SSU	ITS	LSU	MCM7	TEF1
He. aff. *coleomegillae*	D. Haelew. 1287b	Panama	*Coleomegilla maculata*		OL335932	MG745334		
He. aff. *coleomegillae*	D. Haelew. 1291c	Panama	*Coleomegilla maculata*		OL335933	MG745335		
He. coccinelloides	D. Haelew. 1428a	Spain (Canary Islands)	*Stethorus tenerifensis*		OL335930	OL335930	OP947141	OR800935
He. coccinelloides	D. Haelew. 1428b	Spain (Canary Islands)	*Stethorus tenerifensis*		OL335931	OL335915	OP947140	
He. halyziae	D. Haelew. 955b	The Netherlands	*Halyzia sedecimguttata*		MG757813			
He. halyziae	D. Haelew. 4209a	The Netherlands	*Halyzia sedecimguttata*		OP933656	OP933659		
He. harmoniae	D. Haelew. 1174a	The Netherlands	*Harmonia axyridis*		MG757815	MG745345		
He. harmoniae	D. Haelew. 1268b	Japan	*Harmonia axyridis*		MG757829	MG745357	OP037811	
He. harmoniae	D. Haelew. 1551b	Czech Republic	*Harmonia axyridis*		OL335935			
He. harmoniae	D. Haelew. 648c	South Africa	*Harmonia axyridis*		KU574864	KU574865		
He. parexochomi	D. Haelew. 1462a	Spain (Canary Islands)	*Parexochomus nigripennis*		MZ994855			
He. parexochomi	D. Haelew. 1463a	Spain (Canary Islands)	*Parexochomus nigripennis*		MZ994856			
He. parexochomi	D. Haelew. 1463b	Spain (Canary Islands)	*Parexochomus nigripennis*		MZ994857			
He. parexochomi	D. Haelew. 1690a	Spain (Canary Islands)	*Parexochomus nigripennis*					OR800937
He. parexochomi	D. Haelew. 1690b	Spain (Canary Islands)	*Parexochomus nigripennis*	MZ994882	MZ994861	MZ994872		
He. parexochomi	D. Haelew. 1690c	Spain (Canary Islands)	*Parexochomus nigripennis*	MZ994883	MZ994862	MZ994873		
He. parexochomi	D. Haelew. 1690d	Spain (Canary Islands)	*Parexochomus nigripennis*	MZ994884	MZ994863	MZ994874	OP947154	
He. parexochomi	D. Haelew. 1691a	Spain (Canary Islands)	*Parexochomus nigripennis*					OR800936
He. parexochomi	D. Haelew. 1691b	Spain (Canary Islands)	*Parexochomus nigripennis*					OR800938
He. parexochomi	D. Haelew. 1691c	Spain (Canary Islands)	*Parexochomus nigripennis*	MZ994885	MZ994864	MZ994875	OP947156	
He. parexochomi	D. Haelew. 1691d	Spain (Canary Islands)	*Parexochomus nigripennis*	MZ994886	MZ994865	MZ994876		
He. parexochomi	D. Haelew. 1693a	Spain (Canary Islands)	*Parexochomus nigripennis*	MZ994887	MZ994866	MZ994877		
He. parexochomi	D. Haelew. 1693b	Spain (Canary Islands)	*Parexochomus nigripennis*	MZ994888	MZ994867	MZ994878		
He. parexochomi	D. Haelew. 1465a	Spain (Canary Islands)	*Parexochomus quadriplagiatus*	MZ994881	MZ994860	MZ994871	OP947156	
He. parexochomi	D. Haelew. 1465b	Spain (Canary Islands)	*Parexochomus quadriplagiatus*	MZ994889	MZ994868	MZ994879	OP947155	
He. parexochomi	D. Haelew. 1584a	Spain (Canary Islands)	*Parexochomus quadriplagiatus*	MZ994880	MZ994858	MZ994869		
He. parexochomi	D. Haelew. 1584b	Spain (Canary Islands)	*Parexochomus quadriplagiatus*		MZ994859	MZ994870		

Table 1 (continued)

Species	Isolate	Country	Host	SSU	ITS	LSU	MCM7	TEF1
He. virescens s.l.	D. Haelew. 1193 g	Denmark	*Adalia bipunctata*		MG757817	MG745346	OP947147	
He. virescens s.l.	D. Haelew. 1199 h	Sweden	*Adalia bipunctata*		MG757818	MG745347		
He. virescens s.l.	D. Haelew. 1231a	Italy	*Adalia bipunctata*		MG757821	MG745350	OP947146	
He. virescens s.l.	D. Haelew. 1248b	Italy	*Adalia decempunctata*		MG757823	MG745353		
He. virescens s.l.	D. Haelew. 1249a	Italy	*Adalia decempunctata*		MG757824			
He. virescens s.l.	D. Haelew. 655c	South Africa	*Cheilomenes propinqua*		MG757804	KU574867		
He. virescens s.l.	D. Haelew. 659b	South Africa	*Cheilomenes propinqua*		MG757805	MG745342		
He. virescens s.l.	D. Haelew. 1259a	South Africa	*Cheilomenes propinqua*		MG757828			
He. virescens s.l.	D. Haelew. 924a	Panama	*Cycloneda sanguinea*		MG757808			
He. virescens s.l.	D. Haelew. 1374a	Panama	*Cycloneda sanguinea*		MG757831			
He. virescens s.l.	D. Haelew. 3187a	Czech Republic	*Hippodamia tredecimpunctata*		OL335937	OL335923		
He. virescens s.l.	D. Haelew. 1809c	Chile	*Hippodamia variegata*			OL335922		
He. virescens s.l.	D. Haelew. 3939b	Spain (Canary Islands)	*Hyperaspis vinciguerrae*	OP933653		OP933649	OP947144	
He. virescens s.l.	D. Haelew. 3939c	Spain (Canary Islands)	*Hyperaspis vinciguerrae*	OP933654	OP933657	OP933650	OP947145	
He. virescens s.l.	D. Haelew. 3941b	Spain (Canary Islands)	*Novius cruentatus*	OR807744	OR807754	OR807749		
He. virescens s.l.	D. Haelew. 3941c	Spain (Canary Islands)	*Novius cruentatus*	OR807745	OR807755	OR807750		
He. virescens s.l.	D. Haelew. 3942c	Spain (Canary Islands)	*Novius cruentatus*	OR807746	OR807756	OR807751		
He. virescens s.l.	D. Haelew. 3942d	Spain (Canary Islands)	*Novius cruentatus*	OR807747	OR807757	OR807752		
He. virescens s.l.	D. Haelew. 4211a	Spain (Canary Islands)	*Novius cruentatus*	OR807748	OR807758	OR807753	OR800934	
He. virescens s.l.	D. Haelew. 1200 h	USA	*Olla v-nigrum*		MG757819	MG745348	OP947150	
He. virescens s.l.	D. Haelew. 3202a	Mexico	*Olla v-nigrum*		OL335938	OL335925		
He. virescens s.l.	JP352b	USA	*Olla v-nigrum*		MG757798	MG745337		
He. virescens s.l.	D. Haelew. 1250b	USA	*Psyllobora vigintimaculata*		MG757825	MG745354		
He. virescens s.l.	D. Haelew. 1250c	USA	*Psyllobora vigintimaculata*		MG757826	MG745355	OP947151	
He. virescens s.l.	D. Haelew. 1251b	USA	*Psyllobora vigintimaculata*		MG757827	MG745356	OP947152	
He. virescens s.l.	D. Haelew. 1444a	USA	*Chilocorus stigma*		MT373697	OL335916		
He. virescens s.l.	D. Haelew. 1444b	USA	*Chilocorus stigma*		MT373698	OL335917		
Hesperomyces sp.	D. Haelew. 928 g	Panama	*Azya orbigera*		MG745343	MG745343		

Table 2 Ladybird species collected on the Canary Islands and their infection by *Hesperomyces* spp. Presented ratios are the number of adults parasitized by *Hesperomyces* to the total number of adults collected

Ladybird species	L	F	GC	T	G	P	H	Total
Adalia bipunctata (Linnaeus, 1758)						0/17		
***Adalia decempunctata* (Linnaeus, 1758)**		0/11	**3/12**	0/4				**3/27**
Cheilomenes propinqua (Mulsant, 1850)			0/29	0/3				
Chilocorus canariensis Crotch, 1874				0/16		0/4	0/55	
Clitostethus arcuatus (Rossi, 1794)		0/25		0/37				
Coccinella miranda Wollaston, 1864			0/65	0/11	0/75	0/167	0/98	
Coccinella septempunctata algerica Kovář, 1977	0/18	0/56	0/54		0/4	0/4	0/66	
Cryptolaemus montrouzieri Mulsant, 1853	0/99	0/249	0/49	0/35	0/38	0/112	0/2	
Delphastus catalinae (Horn, 1895)	0/19	0/110	0/1	0/69	0/119	0/94		
Diomus sp.						0/1		
***Harmonia axyridis* (Pallas, 1773)**				**1/8**				**1/8**
Harmonia quadripunctata (Pontoppidan, 1763)						0/4		
Hippodamia variegata (Goeze, 1777)	0/2	0/133	0/83	0/26	0/2	0/26	0/3	
***Hyperaspis vinciguerrae* Capra, 1929**		**1/12**		0/2				**1/12**
Myrrha octodecimguttata (Linnaeus, 1758)			0/3	0/3		0/15	0/3	
Nephaspis bicolor Gordon, 1982				0/7		0/10		
***Nephus flavopictus* (Wollaston, 1854)**	0/5	0/42	0/14	0/45		0/50	**1/96**	**1/252**
Nephus incisus (Har. Lindberg, 1950)	0/30	0/161	0/10	0/44	0/71	0/2	0/14	
Nephus reunioni (Fürsch, 1974)				0/11		0/80		
Novius canariensis Korschefsky, 1931							0/6	
Novius cardinalis (Mulsant, 1850)	0/20	0/55	0/27	0/34	0/17	0/24	0/25	
***Novius cruentatus* (Mulsant, 1850)**			0/4	0/24		**4/49**		**4/77**
Oenopia doublieri (Mulsant, 1846)	0/1	0/21	0/4		0/1			
Olla v-nigrum (Mulsant, 1866)	0/2		0/10	0/2	0/38			
Parexochomus bellus (Wollaston, 1864)			0/9					
***Parexochomus nigripennis* (Erichson, 1843)**	0/27	**18/59**	0/3	0/27	0/8			**18/124**
***Parexochomus quadriplagiatus* (Wollaston, 1864)**	0/2	**14/77**						**14/79**
Pharoscymnus decemplagiatus (Wollaston, 1857)	0/12	0/38	0/42	0/121	0/7	0/124	0/45	
Pharoscymnus flexibilis (Mulsant, 1853)	0/39	0/122						
Rhyzobius litura (Fabricius, 1787)	0/2		0/1		0/1	0/1	0/1	
Rhyzobius lophanthae (Blaisdell, 1892)	0/118	0/157	0/147	0/70	0/38	0/82	0/28	
Scymnus canariensis Wollaston, 1864	0/330	0/683	0/390	0/515	0/80	0/1147	0/901	
Scymnus cercyonides Wollaston, 1864			0/1	0/9		0/2	0/3	
Scymnus medanensis Eizaguirre, 2007	0/1	0/5	0/1	0/4				
***Scymnus nubilus* Mulsant, 1850**	0/45	**1/155**	0/37	0/22	0/6	0/48	0/5	**1/318**
***Scymnus subvillosus durantae* Wollaston, 1854**	0/78	**3/174**	0/66					**3/318**
***Stethorus tenerifensis* Fürsch, 1987**	**2/31**	**18/170**	**5/130**	**12/89**	**8/42**	**1/15**	**11/132**	**57/609**
Stethorus wollastoni Kapur, 1948					0/7	0/1		
Tetrabrachys deserticola (Wollaston, 1864)		0/3						

Different islands are indicated with their first letter(s)

F Fuerteventura, *G* La Gomera, *GC* Gran Canaria, *H* El Hierro, *L* Lanzarote, *P* La Palma, *T* Tenerife

Host species of *Hesperomyces* spp. and non-zero ratio values are bolded

(Fig. 3). *Hesperomyces* ex *Novius cruentatus* (from La Palma) and *He. parexochomi* (from Fuerteventura) were retrieved as sister species with maximum support.

Details of specimens examined

Hesperomyces coccinelloides (Thaxt.) Thaxt. sensu lato

CANARY ISLANDS. LAS PALMAS PROVINCE:

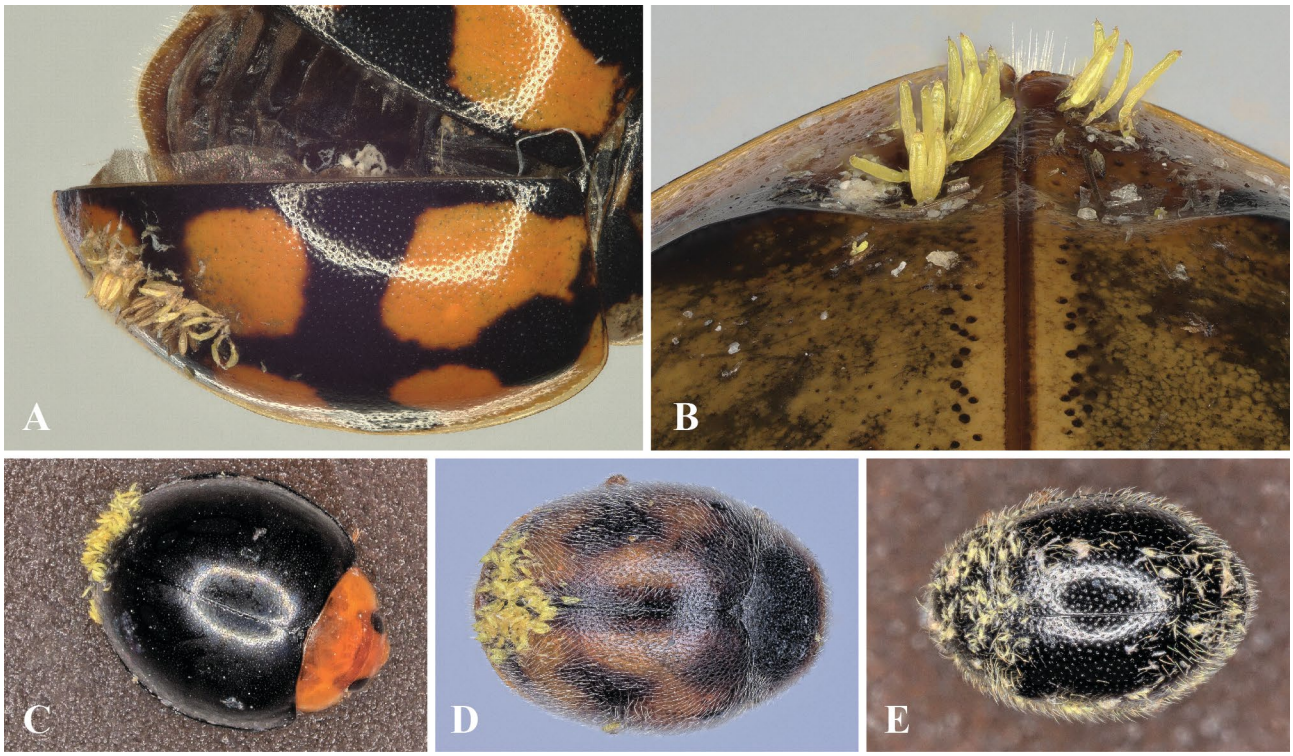

Fig. 1 In-situ photos of thalli of *Hesperomyces* on the integument of Canarian ladybirds. **A** *Adalia decempunctata*. **B** *Harmonia axyridis*. **C** *Parexochomus nigripennis*. **D** *Scymnus subvillosus durantae*. **E** *Stethorus tenerifensis*

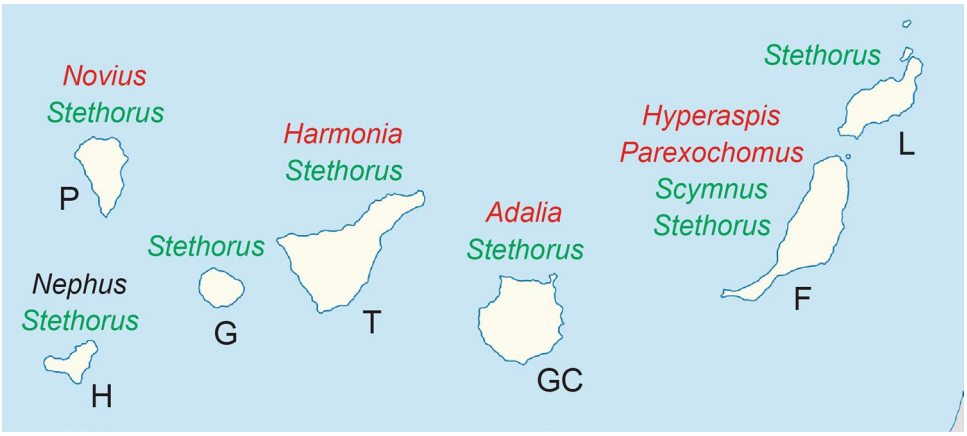

Fig. 2 Map of the Canary Islands, with indication of infected hosts by island. Host shown in red: parasitized by *Hesperomyces* sp. in the *He. virescens* species complex. Host shown in green: parasitized by *Hesperomyces* sp. in the *He. coccinelloides* species complex. Host shown in black: parasitized by *Hesperomyces* sp. Different islands are indicated with their first letter(s): *F* Fuerteventura, *G* La Gomera, *GC* Gran Canaria, *H* El Hierro, *L* Lanzarote; *P* La Palma; *T* Tenerife. Edited from an SVG map of the Canary Islands by NordNordWest (Wikimedia Commons, CC BY-SA 3.0)

Fuerteventura, Pájara, Costa Calma, 11 Feb 2017, on *Scymnus subvillosus durantae* Wollaston, 1854, *Jerzy Romanowski D. Haelew. 3863*, slide GENT:GENTFL00723 (6 adult thalli from elytral tips); Pájara, desert near Costa Calma, 28.1656111 N 14.2242778 W, 12 Feb 2017, on *Stethorus tenerifensis* Fürsch, 1987, *Jerzy Romanowski D. Haelew. 1428*, GenBank (isolate D. Haelew. 1428a, 2 adult thalli from left elytron): SSU = OL335930, ITS = OL335930, LSU = OL335930, *MCM7* = OP947141, *TEF1* = OR800935, GenBank (isolate D. Haelew. 1428b, 1 adult thallus from left elytron): ITS = OL335931, LSU = OL335915, *MCM7* = OP947140.

Table 3 Details on the parasitism of Coccinellidae by *Hesperomyces* in the Canary Islands

Species	Island	Sampling period	N screened	N parasitized	Parasite prevalence rate
Adalia decempunctata	GC	31.03–02.04.2019	12	3	**0.25**
Harmonia axyridis	T	23.02.2022	8	1	**0.13**
Hyperaspis vinciguerrae	F	30.09.2016	6	0	0
		09.02.2017	1	0	0
		30.03.2017	2	0	0
		05.08.2017	1	0	0
		27–29.11.2021	2	1	0.50
		Total	12	1	**0.08**
Nephus flavopictus	H	07–12.04.2019	96	1	**0.01**
Novius cruentatus	P	18–19.06.2021	49	4	**0.08**
Parexochomus nigripennis	F	10–13.02.2017	22	7	0.32
		23–30.03.2017	15	4	0.27
		12.08.2017	3	0	0
		20.10.2018	19	7	0.37
		Total	59	18	**0.31**
Parexochomus quadriplagiatus	F	10–13.02.2017	4	1	0.25
		22–30.03.2017	47	5	0.11
		04–12.08.2017	24	8	0.33
		20.10.2018	2	0	0
		Total	77	14	**0.18**
Scymnus nubilus	F	30.09.2016	23	0	0
		12–13.02.2017	16	0	0
		22–30.03.2017	34	1	0.03
		03–12.08.2017	82	0	0
		Total	155	1	**0.01**
Scymnus subvillosus durantae	F	30.09.2016	1	0	0
		08–13.02.2017	56	1	0.02
		21–30.03.2017	73	2	0.03
		03–12.08.2017	44	0	0
		Total	174	3	**0.02**
Stethorus tenerifensis	L	06–13.02.2018	31	2	**0.06**
	F	04.12.2016	1	0	0
		20.01–13.02.2017	34	5	0.15
		21–30.03.2017	82	13	0.16
		04–09.08.2017	53	0	0
		Total	170	18	**0.11**
	GC	31.03–05.04.2019	130	5	**0.04**
	T	08–11.09.2019	49	4	0.08
		22.06.2021	24	4	0.17
		23.02.2022	16	4	0.25
		Total	89	12	**0.13**
	G	24.02–01.03.2022	42	8	**0.19**
	P	17–20.06.2021	15	1	**0.07**
	H	07–12.04.2019	132	11	**0.08**

Different islands are indicated with their first letter(s)

F Fuerteventura, *G* La Gomera, *GC* Gran Canaria, *H* El Hierro, *L* Lanzarote, *P* La Palma, *T* Tenerife

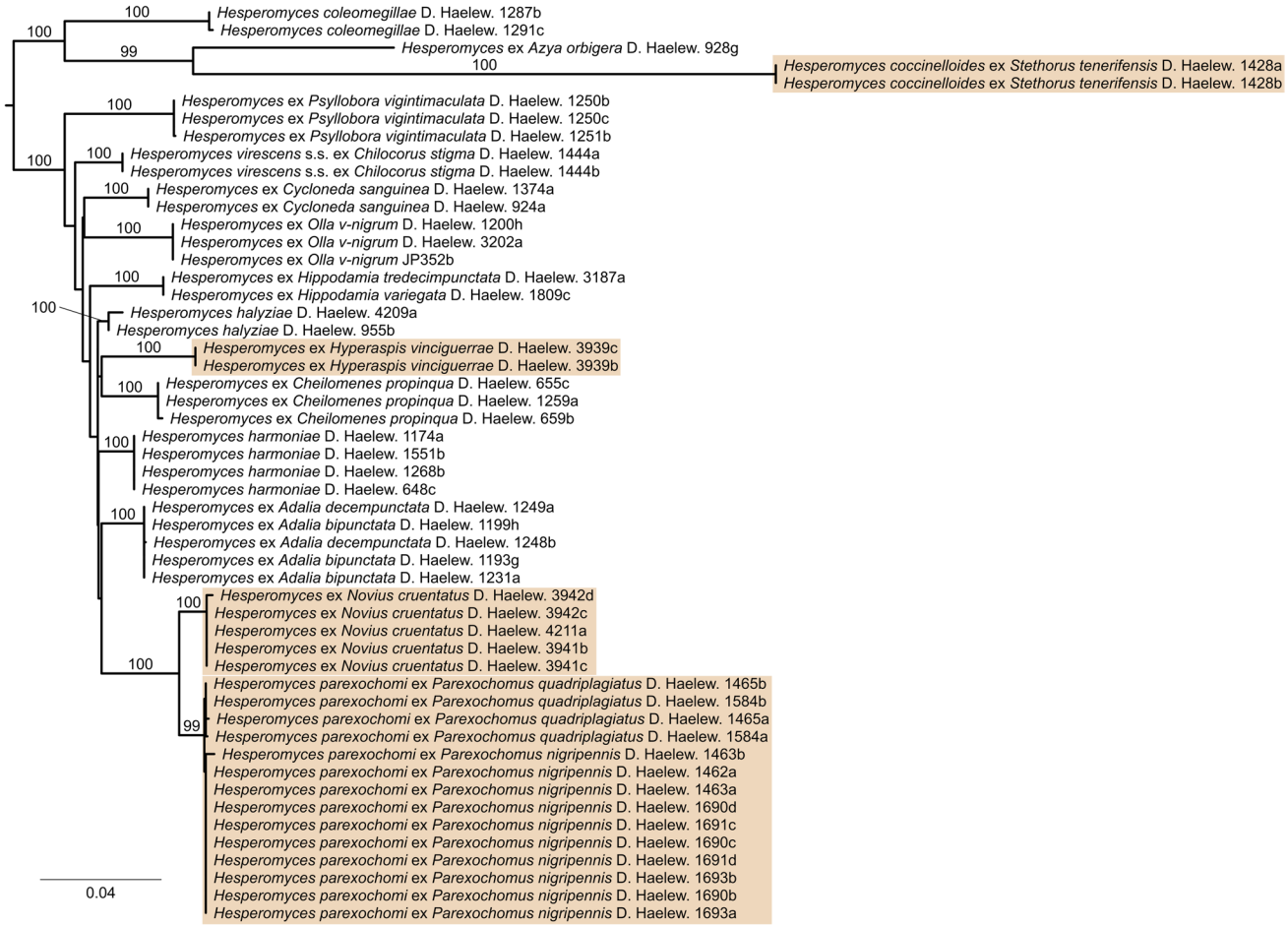

Fig. 3 Phylogeny of *Hesperomyces* species, reconstructed from an ITS–LSU–*MCM7* dataset. *Hesperomyces* ex *Azya orbigera*, *He. coccinelloides*, and *He. coleomegillae* were selected as outgroup taxa. UFBoot2 values ≥ 95 are indicated above the branch leading to each node, isolates originating from the Canary Islands are highlighted

CANADA. QUÉBEC PROVINCE: Saint-Nicolas, Chutes-de-la-Chaudière (now: city of Lévis), raspberry field, 12 Jul 1993, on *St. pusillus* [as *St. punctillum*], *Michèle Roy D. Haelew. 356*, slides FH:D. Haelew. 356a (1 juvenile thallus and 1 adult thallus from left elytral tip) and FH:D. Haelew. 356b (5 thalli from right elytron). PORTUGAL. AUTONOMOUS REGION OF THE AZORES: São Miguel Island, Nordeste Nursery Recreational Forest Reserve, 19 Apr 2019, on *Scymnus nubilus* Mulsant, 1850, *Isabel Borges D. Haelew. 1810*, GenBank (isolate D. Haelew. 1810a, 5 adult thalli from elytral tips): LSU = PP087140.

Hesperomyces harmoniae Haelew. & De Kesel

CANARY ISLANDS. SANTA CRUZ DE TENERIFE PROVINCE: Tenerife, Santa Cruz, 22 Feb 2022, on *Harmonia axyridis* (Pallas, 1773), *Piotr Ceryngier D. Haelew. 5009*, slide GENT:GENTFL01280 (8 adult thalli from right elytron).

Hesperomyces parexochomi Mironova & Haelew.

CANARY ISLANDS. LAS PALMAS PROVINCE: Fuerteventura, Antigua, Caleta de Fuste, 28.3865 N 13.8641389 W, 13 Feb 2017, on *Parexochomus nigripennis* (Erichson, 1843), *Jerzy Romanowski D. Haelew. 1377*, slides GENT:GENTFL00034 (paratype, 1 juvenile and 7 adult thalli from left metatrochanter) and GENT:GENTFL00035 (paratype, 9 thalli from sternites); ibid., on *P. nigripennis*, *D. Haelew. 1462*, GenBank (isolate D. Haelew. 1462a, 1 adult thallus from left epipleuron): ITS = MZ994855; ibid., on *P. nigripennis*, *D. Haelew. 1463*, GenBank (isolate D. Haelew. 1463a, 2 adult thalli from metacoxae and sternites): ITS = MZ994856, GenBank (isolate D. Haelew. 1463b, 1 adult thallus from metacoxae and sternites): ITS = MZ994857; ibid., on *P. nigripennis*, *D. Haelew. 1464* (PERC), slide PUL F29114; Fuerteventura, Lajares, 28.6789167 N 13.9370833 W, 20 Oct 2018, on *P. nigripennis*, *Jerzy Romanowski D. Haelew. 1690* (PERC), slide

GENT:GENTFL00071 (1 adult thallus from right elytron), GenBank (isolate D. Haelew. 1690a, 1 adult thallus from left elytral tip): *TEF1* = OR800937, GenBank (isolate D. Haelew. 1690b, 1 adult thallus thallus from left elytral tip): SSU = MZ994882, ITS = MZ994861, LSU = MZ994872, GenBank (isolate D. Haelew. 1690c, 1 adult thallus from left elytral tip): SSU = MZ994883, ITS = MZ994862, LSU = MZ994873, GenBank (isolate D. Haelew. 1690d, 2 adult thalli from left elytral tip): SSU = MZ994884, ITS = MZ994863, LSU = MZ994874, *MCM7* = OP947154; ibid., on *P. nigripennis*, *D. Haelew. 1691* (PERC), slide GENT:GENTFL00072 (paratype, 2 adult thalli from elytral tips); GenBank (isolate D. Haelew. 1691a, 2 adult thalli from left elytral tip): *MCM7* = PP097388, *TEF1* = OR800936; GenBank (isolate D. Haelew. 1691b, 2 adult thalli from left elytral tip): *TEF1* = OR800938; GenBank (isolate D. Haelew. 1691c, 2 adult thalli from left elytral tip): SSU = MZ994885, ITS = MZ994864, LSU = MZ994875; GenBank (isolate D. Haelew. 1691d, 3 adult thalli from left elytral tip): SSU = MZ994886, ITS = MZ994865, LSU = MZ994876; ibid., on *P. nigripennis*, *D. Haelew. 1694*, GENT:GENTFL00075 (paratype, 2 adult thalli from left antenna); ibid., on male *P. nigripennis*, *Jerzy Romanowski D. Haelew. 1693* (PERC), slides GENT:GENTFL00073 (paratype, 1 juvenile and 11 adult thalli from left elytral tip) and GENT:GENTFL00074 (paratype, 14 adult thalli from right elytral tip); GenBank (isolate D. Haelew. 1693a, 1 adult thallus from left elytral tip): SSU = MZ994887, ITS = MZ994866, LSU = MZ994877; GenBank (isolate D. Haelew. 1693b, 2 adult thalli from left elytral tip): SSU = MZ994888, ITS = MZ994867, LSU = MZ994878; Fuerteventura, Antigua, Caleta de Fuste, 30 Mar 2017, on *P. nigripennis*, *Jerzy Romanowsky D. Haelew. 3862*, slide GENT:GENTFL00576 (3 adult thalli from elytral tips); ibid., on *P. nigripennis*, *D. Haelew. 3864*, slide GENT:GENTFL00724 (1 juvenile thallus and 1 adult thallus from left metatrochanter); Fuerteventura, Pájara, desert near Costa Calma, 28.1656111 N 14.2242778 W, 27 Mar 2017, on *Parexochomus quadriplagiatus* (Wollaston, 1864), *Jerzy Romanowski D. Haelew. 1465* (PERC), slide GENT:GENTFL00050 (holotype, 1 subadult and 5 adult thalli from sternites); GenBank (isolate D. Haelew. 1465a, 1 adult thallus from left elytron): SSU = MZ994881, ITS = MZ994860, LSU = MZ994871, *MCM7* = OP947156; GenBank (isolate D. Haelew 1465b, 3 adult thalli from sternites): SSU = MZ994889, ITS = MZ994868, LSU = MZ994879, *MCM7* = OP947155; ibid., *D. Haelew. 1466* (PERC), slide GENT:GENTFL00051 (paratype, 1 subadult thallus and 1 adult thallus from right epipleuron and sternite); ibid., 22 Mar 2017, on *P. quadriplagiatus*, *D. Haelew. 1584*, GenBank (isolate D. Haelew. 1584a, 2 adult thalli from right elytron): SSU = MZ994880, ITS = MZ994858, LSU = MZ994869; GenBank (isolate D. Haelew. 1584b, 4 adult thalli from right elytron): ITS = MZ994859, LSU = MZ994870.

Hesperomyces sp. ex *Adalia*

CANARY ISLANDS. LAS PALMAS PROVINCE: Gran Canaria, Municipal Town of Santa Lucía, 27.911111 N 15.540000 W, 680 m a.s.l., 31 Mar 2019, on *Adalia decempunctata* (Linnaeus, 1758), *Jerzy Romanowski D. Haelew. 4422* (TXEX.COL.1497), slide GENT:GENTFL00800 (7 adult thalli from left elytral tip).

Hesperomyces sp. ex *Hyperaspis*

CANARY ISLANDS. LAS PALMAS PROVINCE: Fuerteventura, Jandia, Ventura Shopping Center, 28.053 N 14.324 W, 27 Nov 2021, on *Hyperaspis vinciguerrae* Capra, 1929, *Jerzy Romanowski D. Haelew. 3939* (PERC), slides GENT:GENTFL00727 (9 adult thalli from left elytron) and GENT:GENTFL00728 (20 adult thalli from left elytron), GenBank (isolate D. Haelew. 3939b, 6 adult thalli from left elytron): SSU = OP933653, LSU = OP933649, *MCM7* = OP947144), GenBank (isolate D. Haelew. 3939c, 5 adult thalli from left elytron): SSU = OP933654, ITS = OP933657, LSU = OP933650, *MCM7* = OP947145.

Hesperomyces sp. ex *Novius*

CANARY ISLANDS. SANTA CRUZ DE TENERIFE PROVINCE: La Palma, Los Alamos, vicinity of 28.709838 N 17.766472 W, 18 Jun 2021, on *Novius cruentatus* (Mulsant, 1846), *Piotr Ceryngier, Jerzy Romanowski D. Haelew. 3941*, slide GENT:GENTFL00731 (1 juvenile thallus and 1 adult thallus from left elytral tip), GenBank (isolate D. Haelew 3941b, 3 juvenile thalli from right elytral tip): SSU = OR807744, ITS = OR807754, LSU = OR807749, GenBank (isolate D. Haelew 3941c, 2 juvenile thalli from right elytral tip): SSU = OR807745, ITS = OR807755, LSU = OR807750; ibid., on *No. cruentatus*, *D. Haelew. 3942*, slides GENT:GENTFL00732 (4 juvenile and 3 damaged thalli from left elytral tip) and GENT:GENTFL00733 (5 juvenile and 3 damaged thalli from right elytral tip), GenBank (isolate D. Haelew. 3942c, 4 juvenile and 2 subadult thalli from right elytral tip): SSU = OR807746, ITS = OR807756, LSU = OR807751, GenBank (isolate D. Haelew. 3942d, 2 juvenile and 6 subadult thalli from left elytral tip): SSU = OR807747, ITS = OR807757, LSU = OR807752; ibid., on *No. cruentatus*, *D. Haelew. 4211*, GenBank (isolate D. Haelew. 4211a (5 adult thalli from elytra): SSU = OR807748, ITS = OR807758, LSU = OR807753, *MCM7* = OR800934.

Discussion

Our study demonstrated that at least seven species of specialized ladybird parasites of the genus *Hesperomyces* occur in the Canary Islands. These seven *Hesperomyces* species were found to parasitize hosts belonging to various ladybird tribes: Chilocorini (*P. nigripennis*, *P. quadriplagiatus*), Coccinellini (*A. decempunctata, Ha. axyridis*), Hyperaspidini (*Hy. vinciguerrae*), Noviini (*No. cruentatus*), Scymnini (*Ne. flavopictus*, *Sc. nubilus*, *Sc. subvillosus durantae*), and Stethorini (*St. tenerifensis*). The recorded host species have various distribution patterns. Whereas some are endemic to the Canary Islands and neighboring areas of Madeira and the Azores (*Ne. flavopictus*, *St. tenerifensis*) or northwestern Africa (*P. quadriplagiatus*), other species are more widely distributed (*A. decempunctata*, *Hy. vinciguerrae*, *No. cruentatus*, *P. nigripennis*, *Sc. nubilus*, *Sc. subvillosus durantae*), and *Ha. axyridis* is an alien invasive species of nearly worldwide distribution (Kovář 2007; Roy et al. 2016; Soares et al. 2021).

Interestingly, probably the most abundant Canarian ladybird, *Scymnus canariensis*, with more than 4000 collected specimens that were all screened, is thus far uninfected. This could be explained by the idea that species of *Hesperomyces* maintain tight interactions with their hosts, possibly involving multiple adaptations and counteradaptations and leading to reproductive isolation (One-Host-One-Parasite; Haelewaters et al. 2022a). This would mean that the *Hesperomyces* species on *Sc. nubilus* and *Sc. subvillosus durantae* have coevolved with their hosts and thus are unable to infect an atypical host such as *Sc. canariensis*.

Many of the ladybird samples collected in this study were very small and those were usually uninfected. However, in some small samples (*A. decempunctata* from Gran Canaria, *Ha. axyridis* from Tenerife, *Hy. vinciguerrae* from Fuerteventura, *St. tenerifensis* from Lanzarote, La Gomera and La Palma) *Hesperomyces*-infected specimens were found. Prevalence rates for such samples should, of course, be treated with caution, but on the other hand, the detection of the parasite in a small sample may suggest the commonness of the relationship. The association of *He. harmoniae* with *Ha. axyridis* has become very common in several parts of the invasive range of the ladybird (eastern North America, Europe, South Africa) (Haelewaters et al. 2016, 2017), and furthermore, while invading new areas, the ladybird appears to contribute to the further spread of the parasite (Ceryngier and Romanowski 2017). The present finding seems to confirm this. In the Canary Islands, *Ha. axyridis* probably established very recently: Although recorded on Tenerife in 2003 and 2004 (Machado 2006; Eizaguirre 2007), it presumably did not establish then as its next record was only made in 2022 (Ceryngier et al. 2023). Interestingly, in the sample of only eight *Ha. axyridis* adults collected on 23 February 2022, two different natural enemies were recorded, the parasitic wasp *Dinocampus coccinellae* reported by Ceryngier et al. (2023) and *He. harmoniae* reported here.

Somewhat similar to the relationship of *Ha. axyridis* with *He. harmoniae* on Tenerife is that of *A. decempunctata* with *Hesperomyces* ex *Adalia* on Gran Canaria. This ladybird is presumably also a recent, still not abundant, arrival there (Romanowski et al. 2020b). Despite its paucity, it relatively often is found parasitized by *Hesperomyces*. The parasite, as yet an undescribed *Hesperomyces* species, is known to parasitize both *A. bipunctata* and *A. decempunctata* (Haelewaters et al. 2018). It has been commonly reported in Europe, mostly on *A. bipunctata* and less often on *A. decempunctata* in Austria, Denmark, England, France, Germany, Italy, the Netherlands, Poland, and Sweden (Webberley et al. 2006; Ceryngier and Twardowska 2013).

The most common and widespread species of *Hesperomyces* in the Canary Islands is that associated with a minute Macaronesian ladybird, *St. tenerifensis*. An analogous relationship with its continental relative, *St. pusillus* (syn. *St. punctillum*), was reported from Belgium (De Kesel 2011) and Poland (Ceryngier 2013). *Stethorus pusillus* was introduced in North America as a biological control agent against tetranychid mites in peach orchards halfway the 20th century, and unintentional introductions have resulted in its establishment in certain parts of Canada and the USA (Gordon 1985; Ragkou et al. 2004). The finding of *Hesperomyces* on this species in Canada reported by Haelewaters and De Kesel (2017) may be explained by an unintended co-introduction along with *St. pusillus* or a host shift event from another North American host species.

Based on morphology, the fungi associated with *Sc. nubilus*, *Sc. subvillosus durantae*, and *St. tenerifensis* are *He. coccinelloides* (Thaxter 1931). This species, originally described as *Stigmatomyces coccinelloides* (Thaxter 1917), has been reported from small-sized ladybird hosts in the Diomini, Scymnini, and Stethorini tribes in North America (Canada, Grenada, Jamaica, Panama, Trinidad and Tobago, USA), South America (Brazil, Ecuador), Europe (Belgium, Poland, Spain), Asia (Malaysian Borneo, Philippines), and Oceania (Fiji). While we were unable to generate sequences of *Hesperomyces* ex *Sc. nubilus* from the Canary Islands, we did generate an LSU sequence of *Hesperomyces* ex *Sc. nubilus* collected on São Miguel Island, Azores. This sequence shares a mere 87.96% identity with the LSU sequence of *Hesperomyces* ex *St. tenerifensis*. While these are limited data, they point to *He. coccinelloides* being a species complex, as previously also shown for *He. virescens* (Haelewaters et al. 2018, 2022b). In other words, *Hesperomyces* ex *Sc. nubilus* and *Hesperomyces* ex *St. tenerifensis* are two

different species in the species complex. Whether the segregation of species in the complex is at the host genus level or species level and whether a geographical component is involved as in the *He. virescens* species complex (Van Caenegem et al. 2023b), is currently unknown and requires an integrative taxonomy approach.

Of the species of *Hesperomyces* recorded in this study, the most interesting is perhaps that associated with *Ne. flavopictus*. Because *Ne. flavopictus* belongs to the Scymnini tribe, like *Sc. nubilus* and *Sc. subvillosus durantae*, its parasite likely also belongs to *He. coccinelloides* sensu lato. Given our findings with the LSU sequences of *Sc. nubilus* and *St. tenerifensis*, it may be assumed that *Hesperomyces* ex *Ne. flavopictus* represents yet another species in the *He. coccinelloides* species complex. However, we were unable to generate sequence data for the fungus and thus cannot identify it to species level with confidence. We only collected a single *Ne. flavopictus* individual infected with this parasite, out of 96 collected specimens. The continuation of our study will hopefully result in more parasitized specimens of *Ne. flavopictus*.

Acknowledgements Thanks are due to Isabel Borges (University of the Azores) for sending infected ladybirds to the last author, and to Joseph Moisan-De Serres and colleagues to accommodate a study trip by D.H. to the Collection d'insectes du Québec in December 2014, with support from the Department of Organismic and Evolutionary Biology at Harvard University.

Funding Open access publishing supported by the National Technical Library in Prague. This study was supported in part by the Research Foundation–Flanders (Senior Postdoctoral Fellowship 1206024N to D.H.), Czech Science Foundation (Grant 21-06446S), Santander Universidades (2017 and 2019 Mobility Grants to J.R.), and En Arche (Grant 2020/01/15 to J.R.).

Declarations

Competing interest The authors have no competing interests to declare that are relevant to the content of this article

References

Blackwell M, Haelewaters D, Pfister DH (2020) Laboulbeniomycetes: evolution, natural history, and Thaxter's final word. Mycologia 112(6):1048–1059. https://doi.org/10.1080/00275514.2020.1718442

Ceryngier P (2013) *Stethorus Pusillis* (Coleoptera: Coccinellidae) as a host of the ectoparasitic fungus *Hesperomyces coccinelloides* (Ascomycota: Laboulbeniales: Laboulbeniaceae) in Poland. Pol J Entomol 82(1):13–18. https://doi.org/10.2478/v10200-012-0018-7

Ceryngier P, Twardowska K (2013) *Harmonia axyridis* (Coleoptera: Coccinellidae) as a host of the parasitic fungus *Hesperomyces virescens*: a case report and short review. Eur J Entomol 110:549–557. https://doi.org/10.14411/eje.2013.075

Ceryngier P, Romanowski J (2017) *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) and its parasite in south-western Bulgaria and northern Greece. BioInvasions Rec 6(4):307–310. https://doi.org/10.3391/bir.2017.6.4.02

Ceryngier P, Franz KW, Romanowski J (2023) Distribution, host range and host preferences of *Dinocampus coccinellae* (Hymenoptera: Braconidae): a worldwide database. Eur J Entomol 120:24–34. https://doi.org/10.14411/eje.2023.004

Chernomor O, von Haeseler A, Minh BQ (2016) Terrace aware data structure for phylogenomic inference from supermatrices. Syst Biol 65:997–1008. https://doi.org/10.1093/sysbio/syw037

Crous PW, Osieck ER, Jurjević Ž, Boers J, van Iperen AL, Starink-Willemse M, Dima B, Balashov S, Bulgakov TS, Johnston PR, Morozova OV, Pinruan U, Sommai S, Alvarado P, Decock CA, Lebel T, McMullan-Fisher S, Moreno G, Shivas RG, Zhao L, Abdollahzadeh J, Abrinbana M, Ageev DV, Akhmetova G, Alexandrova AV, Altés A, Amaral AGG, Angelini C, ArenasF Asselman P, Badali F, Baghela A, Bañares A, Barreto RW, Baseia IG, BellangerJM Berraf-Tebbal A, Biketova AY, Bukharova NV, Burgess TI, Cabero J, CâmaraMPS Cano-Lira JF, Ceryngier P, Chávez R, Cowan D, Lima AF, de Oliveira LR, DenmanS Dang QN, Santos Vieira WA, Dovana F, Duarte IG, Eichmeier A, Erhard A, Esteve-Raventós F, Fellin A, Ferisin G, Ferreira RJ, Ferrer A, Finy P, Gaya E, Geering ADW, Gil-Durán C, Glässnerová K, Glushakova AM, Gramaje D, Guard FE, Guarnizo AL, Haelewaters D, Halling RE, Hill R, Hirooka Y, Hubka V, IliushinVA Ivanova DD, Ivanushkina NE, Jangsantear P, Justo A, Kachalkin AV, Kato S, Khamsuntorn P, Kirtsideli IY, Knapp DG, Kochkina GA, Koukol O, Kovács GM, KruseJ Kumar TKA, Kušan I, Læssøe T, Larsson E, Lebel T, Lebeuf R, Levicán G, Loizides M, Lúcio PSM, Luangsa-ard JJ, Lukina EG, Magaña-Dueñas V, Maggs-KöllingG Malysheva EF, Malysheva VF, Martín B, Martín MP, Matočec N, McTaggart AR, Mehrabi-Koushki M, Mešić A, Miller AN, Mironova P, Moreau PA, Morte A, MüllerK Nagy LG, Nanu S, Navarro-Ródenas A, Nel W, Nguyen TH, Nóbrega TF, NoordeloosME Olariaga I, Overton BE, Ozerskaya SM, Palani P, Pancorbo F, Papp V, Pawłowska J, Pham TQ, Phosri C, Pichai S, Popov ES, Portugal A, Pošta A, Reschke K, Reul M, Ricci GM, Rodríguez A, Romanowski J, Ruchikachorn N, Saar I, Safi A, Sakolrak B, Salzmann F, Sandoval-Denis M, Sangwichein E, Sanhueza L, Sato T, Sastoque A, Senn-Irlet B, Shibata A, Siepe K, Somrithipol S, Spetik M, Stchigel AM, Stuskova K, Suwannasai N, Tan YP, Thangavel R, Tiago I, Tiwari S, Tkalčec Z, Tomashevskaya MA, Tonegawa C, Tran HX, Tran NT, Trovão J, TrubitsynVE Wyk J, Vila J, Visagie CM, Vizzini A, Volobuev SV, Vu DT, Wangsawat N, Yaguchi T, Ferreira BW, de Souza AP, Vieira BS, Groenewald JZ (2021) Fungal Planet description sheets: 1284–1382. Persoonia 47:178–374. https://doi.org/10.3767/persoonia.2021.47.0

Das K, Rossi W, Leonardi M, Ghosh A, Bera I, Hembrom ME, Bajpai R, Joseph S, Nayaka S, Upreti DK, Wang X, Hofstetter V, Buyck B (2018) Fungal Biodiversity Profiles 61–70. Cryptogam Mycol 39:381–418. https://doi.org/10.7872/crym/v39.iss4.2018.381

De Kesel A (2011) *Hesperomyces* (Laboulbeniales) and coccinellid hosts. Sterbeeckia 30:32–37

Eizaguirre S (2007) Revisión De Los coleópteros coccinéllidos de las islas canarias (Coleoptera: Coccinellidae). Bol Soc Entomol Aragon 41:101–118

Fernández-Palacios JM, Whittaker RJ (2008) The canaries: an important biogeographical meeting place. J Biogeogr 35:379–387. https://doi.org/10.1111/j.1365-2699.2008.01890.x

Goldmann L, Weir A, Rossi W (2013) Molecular analysis reveals two new dimorphic species of *Hesperomyces* (Ascomycota, Laboulbeniomycetes) parasitic on the ladybird *Coleomegilla maculata* (Coleoptera, Coccinellidae) Fungal Biol 117:807–813. https://doi.org/10.1016/j.funbio.2013.10.004. Coleoptera

Gordon RD (1985) The *Coccinellidae* (Coleoptera) of America north of Mexico. J New York Entomol Soc 93:1–912

Haelewaters D, De Kesel A (2017) De Schimmel *Hesperomyces virescens*, een natuurlijke vijand van lieveheersbeestjes. Entomol Ber 77(3):106–118

Haelewaters D, De Kesel A (2020) Checklist of thallus-forming Laboulbeniomycetes from Belgium and the Netherlands, including *Hesperomyces halyziae* and *Laboulbenia quarantenae* spp. nov. MycoKeys 71:23–86. https://doi.org/10.3897/mycokeys.71.53421

Haelewaters D, Gorczak M, Pfliegler WP, Tartally A, Tischer M, Wrzosek M (2015) Bringing Laboulbeniales into the 21st century: enhanced techniques for extraction and PCR amplification of DNA from minute ectoparasitic fungi. IMA Fungus 6:363–372. https://doi.org/10.5598/imafungus.2015.06.02.08

Haelewaters D, Minnaar IA, Clusella-Trullas S (2016) First finding of the parasitic fungus *Hesperomyces virescens* (Laboulbeniales) on native and invasive ladybirds (Coleoptera, Coccinellidae) in South Africa. Parasite 23:5. https://doi.org/10.1051/parasite/2016005

Haelewaters D, Zhao SY, Clusella-Trullas S, Cottrell TE, De Kesel A, Fiedler L, Herz A, Hesketh H, Hui C, Kleespies RG, Losey JE, Minnaar IA, Murray KM, Nedvěd O, Pfliegler WP, Raak-van den Berg CL, Riddick EW, Shapiro-Ilan DI, Smyth RR, Steenberg T, van Wielink PS, Viglášová S, Zhao Z, Ceryngier P, Roy HE (2017) Parasites of *Harmonia axyridis*: current research and perspectives. Biocontrol 62(3):355–371. https://doi.org/10.1007/s10526-016-9766-8

Haelewaters D, De Kesel A, Pfister DH (2018) Integrative taxonomy reveals hidden species within a common fungal parasite of ladybirds. Sci Rep 8(1):15966. https://doi.org/10.1038/s41598-018-34319-5

Haelewaters D, Blackwell M, Pfister DH (2021) Laboulbeniomycetes: intimate fungal associates of arthropods. Annu Rev Entomol 66:257–276. https://doi.org/10.1146/annurev-ento-013020-013553

Haelewaters D, Lubbers M, De Kesel A (2022a) The haustorium as a driving force for speciation in thallus-forming Laboulbeniomycetes. IMA Fungus 13:1. https://doi.org/10.1186/s43008-021-00087-7

Haelewaters D, Van Caenegem W, De Kesel A (2022b) *Hesperomyces harmoniae*, a new name for a common ectoparasitic fungus on the invasive alien ladybird *Harmonia axyridis*. Sydowia 75:53–74

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2017) UFBoot2: improving the Ultrafast bootstrap approximation. Mol Biol Evol 35:518–522. https://doi.org/10.1093/molbev/msx281

Hopple JS (1994) Phylogenetic investigations in the genus *Coprinus* based on morphological and molecular characters. PhD thesis. Duke University

Kalyaanamoorthy S, Minh B, Wong T, von Haeseler A, Jermiin L (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14:587–589. https://doi.org/10.1038/nmeth.4285

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinf 20:1160–1166. https://doi.org/10.1093/bib/bbx108

Kovář I (2007) Coccinellidae. In: Löbl I, Smetana A (eds) Catalogue of Palaearctic Coleoptera, vol 4. Apollo Books, Stentrup, pp 71–74

Kuraku S, Zmasek CM, Nishimura O, Katoh K (2013) aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. Nucleic Acids Res 41:22–28. https://doi.org/10.1093/nar/gkt389

Kurtzman CP, Robnett CJ (1997) Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5' end of the large-subunit (26S) ribosomal DNA gene. J Clin Microbiol 35:1216–1223. https://doi.org/10.1128/jcm.35.5.1216-1223.1997

Liu J, Haelewaters D, Pfliegler WP, Page RA, Dick CW, Aime MC (2020) A new species of G*loeandromyces* from Ecuador and Panama revealed by morphology and phylogenetic reconstruction, with a discussion of secondary barcodes in Laboulbeniomycetes taxonomy. Mycologia 112(6):1192–1202. https://doi.org/10.1080/00275514.2020.1781496

Machado A (2006) El sarantontón asiático *Harmonia axyridis* (Pallas, 1773) presente en canarias (Coleoptera: Coccinellidae). Vieraea 34:71–72

Majewski T, Sugiyama K (1985) The Laboulbeniomycetes from eastern Asia. IV. On ten species from Japan including four new species. Trans Mycol Soc Japan 26:295–313

Miadlikowska J, Lutzoni F (2000) Phylogenetic revision of the genus *Peltigera* (lichen-forming Ascomycota) based on morphological, chemical, and large subunit nuclear ribosomal DNA data. Int J Plant Sci 161:925–958. https://doi.org/10.1086/317568

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274. https://doi.org/10.1093/molbev/msu300

Ragkou VS, Athanassiou CG, Kavallieratos, Tomanović Ž (2004) Daily consumption and predation rate of different *Stethorus punctillum* instars feeding on *Tetranychus urticae*. Phytoparasitica 32(2):154–159. https://doi.org/10.1007/BF02979781

Romanowski J, Ceryngier P, Větrovec J, Szawaryn K (2019) The Coccinellidae (Coleoptera) from Fuerteventura. Canary Islands Zootaxa 4646(1):101–123. https://doi.org/10.11646/zootaxa.4646.1.6

Romanowski J, Ceryngier P, Szawaryn K (2020a) New data on the Coccinellidae (Coleoptera) from Lanzarote, Canary Islands. Coleopt Bull 74(1):188–194. https://doi.org/10.1649/0010-065X-74.1.188

Romanowski J, Ceryngier P, Větrovec J, Piotrowska M, Szawaryn K (2020b) Endemics versus newcomers: the ladybird beetle (Coleoptera: Coccinellidae) fauna of Gran Canaria. Insects 11(9):641. https://doi.org/10.3390/insects11090641

Romanowski J, Ceryngier P, Zmuda C, Větrovec J, Szawaryn K (2020c) The Coccinellidae (Coleoptera) from El Hierro, Canary Islands. Bonn Zool Bull 69(2):249–261

Romanowski J, Ceryngier P, Větrovec J, Szawaryn K (2023) The ladybird beetles (Coleoptera: Coccinellidae) of La Palma. Insects 14(7):655. https://doi.org/10.3390/insects14070655

Roy HE, Brown PMJ, Adriaens T, Berkvens N, Borges I, Clusella-Trullas S, Comont RF, De Clercq P, Eschen R, Estoup A, Evans EW, Facon B, Gardiner MM, Gil A, Grez AA, Guillemaud T, Haelewaters D, Herz A, Honek A, Howe AG, Hui C, Hutchison WD, Kenis M, Koch RL, Kulfan J, Lawson Handley L, Lombaert E, Loomans A, Losey J, Lukashuk AO, Maes D, Magro A,

Murray KM, San Martin G, Martinkova Z, Minnaar I, Nedved O, Orlova-Bienkowskaja MJ, Rabitsch W, Ravn HP, Rondoni G, Rorke SL, Ryndevich SK, Saethre M-G, Sloggett JJ, Soares AO, Stals R, Tinsley MC, Vandereycken A, van Wielink P, Viglášová S, Zach P, Zaviezo T, Zhao Z (2016) The harlequin ladybird, *Harmonia axyridis*: global perspectives on invasion history and ecology. Biol Invasions 18(4):997–1044. https://doi.org/10.1007/s10530-016-1077-6

Schmitt I, Crespo A, Divakar PK, Fankhauser JD, Herman-Sackett E, Kalb K, Nelsen MP, Nelson NA, Rivas-Plata E, Shimp AD, Widhelm T, Lumbsch HT (2009) New primers for promising single-copy genes in fungal phylogenetics and systematics. Persoonia 23:35–40. https://doi.org/10.3767/003158509X470602

Soares AO, Calado HR, Franco JC, Aguiar AF, Andrade MM, Zina V, Ameixa OMCC, Borges I, Magro A (2021) An annotated checklist of ladybeetle species (Coleoptera, Coccinellidae) of Portugal, including the Azores and Madeira Archipelagos. ZooKeys 1053:107–144. https://doi.org/10.3897/zookeys.1053.64268

Stielow B, Lévesque CA, Seifert K, Meyer W, Irinyi L, Smits D, Renfurm R, Verkley GJM, Groenewald M, Chaduli D, Lomascolo A, Stephane W, Lesage-Meessen L, Favel A, Al-Hatmi A, Damm U, Yilmaz N, Houbraken J, Lombard L, Robert V (2015) One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. Persoonia 35:242–263. https://doi.org/10.3767/003158515X689135

Sugiyama K, Majewski T (1985) Notes on the Laboulbeniomycetes of Bali Island, Indonesia II. Trans Mycol Soc Japan 26:125–144

Thaxter R (1917) New Laboulbeniales, chiefly dipterophilous American species. Proc Am Acad Arts Sci 52(10):649–721. https://doi.org/10.2307/20025703

Thaxter R (1931) Contribution towards a monograph of Laboulbeniaceae. Part V. Mem Am Acad Arts Sci 16:1–435. https://doi.org/10.2307/25058136

Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27:171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

Van Caenegem W, Blondelle A, Dumolein I, Santamaria B, Dick CW, Hiller T, Liu J, Quandt CA, Villarreal Saucedo RV, Verbeken A, Haelewaters D (2023a) Five new species of *Gloeandromyces* (Fungi, Laboulbeniales) from tropical American bat flies (Diptera, Streblidae), revealed by morphology and phylogenetic reconstruction. Mycologia 115(5):714–737. https://doi.org/10.1080/00275514.2023.2230114

Van Caenegem W, Ceryngier P, Romanowski J, Pfister DH, Haelewaters D (2023b) *Hesperomyces* (Fungi, Ascomycota) associated with *Hyperaspis* ladybirds (Coleoptera, Coccinellidae): rethinking host specificity. Front Fungal Biol 3:1040102. https://doi.org/10.3389/ffunb.2022.1040102

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Webberley KM, Tinsley MC, Sloggett JJ, Majerus MEN, Hurst GDD (2006) Spatial variation in the incidence of a sexually transmitted parasite of the ladybird beetle *Adalia bipunctata* (Coleoptera: Coccinellidae). Eur J Entomol 103(4):793–797. https://doi.org/10.14411/eje.2006.108