Acta Mycologica

DOI: 10.5586/am.1117

Publication history
Received: 2018-02-25
Accepted: 2018-11-21
Published: 2019-04-18

Handling editor
Malgorzata Ruszkiewicz-Michalska, Institute for Agricultural and Forest Environment, Polish Academy of Sciences, Poland

Authors' contributions
HH: collected and examined the material, drafted the manuscript; JD and JR: examined the material, prepared the figure; contributed to manuscript preparation; AS: wrote the manuscript; EM: wrote the manuscript and identified ladybird species; CA: examined and identified the material, wrote the manuscript

Funding
The authors acknowledge CAPES, CNPq, and FAPERJ for funds supporting this research.

Competing interests
No competing interests have been declared.

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Citation
Inácio CA, de Paulo HH, Almeida JD, Rembinski J, Menezes ELA, Silva AC. A new report on *Hesperomyces coleomegillae* (Ascomycota, Laboulbeniales) parasitism of *Coleomegilla maculata* (Coleoptera, Coccinellidae) in Brazil. Acta Mycol. 2019;54(1):1117. https://doi.org/10.5586/am.1117

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ORIGINAL RESEARCH PAPER

A new report on *Hesperomyces coleomegillae* (Ascomycota, Laboulbeniales) parasitism of *Coleomegilla maculata* (Coleoptera, Coccinellidae) in Brazil

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Abstract
For the first time, the genus *Hesperomyces* has been reported to infect *Coleomegilla maculata* in laboratory mass rearing in Brazil. Thalli were found growing on several parts of this ladybird species, including the head, elytra, legs, and abdomen. Infested adults died after 60 days.

Keywords
host–parasitic interaction; Laboulbeniomycetes; ectoparasitic fungi; 12-spotted ladybird; biotrophic parasites; tropical fungi

Introduction

The order Laboulbeniales (Fungi: Ascomycota) comprises about 2,200 species in 141 genera [1] and was previously included in Basidiomycota and/or Zygomycota. Recent studies based on molecular data confirmed that Laboulbeniales are members of Ascomycota [2]. They are biotrophic ectoparasites of arthropods, and beetles (Insecta: Coleoptera) and flies (Insecta: Diptera) are their most common hosts [3–5]. These fungi are specialized and develop by forming a multicellular thallus in the tegument of living arthropods [6–8]. Seven species of Laboulbeniales parasitize ladybirds (Coleoptera, Coccinellidae) [6]: six species of the genus *Hesperomyces* (*H. chilomenes* Thaxt., *H. coccinelloides* Thaxt., *H. coleomegillae* W. Rossi & A. Weir, *H. papuanus* T. Majewski & K. Sugiy., *H. palustris* W. Rossi & A. Weir, and *H. virescens* Thaxt.) and one *Laboulbenia* species (*L. coccinellidicola* Haelew.) [6,9]. An eighth species, *H. hyperaspidis* Thaxt., was brought into synonymy with *H. virescens* based on morphological characteristics [10], although this decision might have been premature [6].

Coleomegilla maculata DeGeer (Coleoptera: Coccinellidae), the 12-spotted ladybird, is widely distributed in natural and managed ecosystems in North, Central, and South American regions, including Brazil [11–14]. It is primarily an aphidophagous insect, but its diet may include other insects (e.g., scales, psyllids, eggs, and neonate larvae of Lepidoptera and Coleoptera). Thus, this ladybird is considered an important agent for crop pest control [15–17]. *Coleomegilla maculata* adults are known to be hosts for *H. coleomegillae* and *H. palustris* in Ecuador, Costa Rica, and Cuba. The first observation of *C. maculata* acting as a host for *Hesperomyces* (*H. palustris*) occurred in 1951 in Cuba

[9]. *Coleomegilla maculata* adults were found with *H. palustris* and *H. coleomegillae* thalli in Costa Rica and Ecuador [18].

The Brazilian Laboulbeniales fungi have not yet been thoroughly investigated. There is a record of *Laboulbenia ecitonis* Blum from the *Eciton*, or army ant, genus (Hymenoptera: Formicidae, Ecitoninae) in Curitiba, State of Paraná [7,19]. Data in previous literature indicates that nearly 100 species of laboulbenialean fungi have been recorded in Brazil, the majority of which have been found on beetles [20,21]. In addition, Rossi and Bergonzo [21] documented the occurrence of 13 Laboulbeniales species in Brazil – all but one associated with beetles. The exception was *H. coccinelloides*, found on a ladybird, *Diomus seminulus* (Mulsant), in the State of Ceará.

This work aims to report *Hesperomyces* infecting *C. maculata* adults in mass-rearing laboratory conditions in Seropédica, Rio de Janeiro, Brazil.

Material and methods

A colony matrix of *C. maculata* was reared in a climate-controlled laboratory (25°C ±1°C, 60% ±10% RH, and 12-hour photoperiod) at the Integrate Pest Management Center (CIMP) of the Department of Entomology and Plant Pathology (DEnF) of the Universidade Federal Rural do Rio de Janeiro (UFRRJ) on the Seropédica campus in RJ, Brazil.

This colony originated from adults collected from the organic farm Fazendinha Agroecológica Km 07, also known as Integrated System of Agroecological Production (SIPA) in the municipality of Seropédica, RJ (22°45′24″ S, 43°40′29″ W) in 2010 [22]. Adults and larvae were reared continuously and fed ad libitum on living larvae of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). The offspring of adults collected in this farm were introduced annually in the colony matrix to maintain the vigor of the colony. The field adults were separated from those of the colony matrix and held in plastic containers.

Adults were stored in disposable 1-liter transparent plastic containers sealed with organza to enable gas exchange. Due to the difficulty in visually determining the sex [23], six adults were kept per container; however, when mating had not been observed in a 24-hour period, random exchanges among containers of some individuals were performed to ensure the presence of at least one viable pair per container. Filtered water was provided using cotton wool placed in plastic bottle caps. The larvae of *C. maculata* were individually kept in 20-mL glass vials closed with hydrophilic cotton from the second instar until adulthood.

From September to December 2015 and January to May 2016, *C. maculata* adults (6 months to 1-year old) with yellowish structures in some parts of their integument were observed in the colony matrix at CIMP. Each individual was observed under a dissecting microscope in the Laboratory of Mycology (DEnF, UFRRJ). Examining these adults revealed that the visible structures were thalli of Laboulbeniales fungi. Their position on the host integument was recorded (Tab. 1).

Tab. 1 Number of mature thalli of *H. colleomegillae* on different body parts of several host individuals.

Body part	Number of thalli*	% of thalli
Head	25	8.9
Leg	44	16.0
Thorax	46	30.1
Abdomen	61	22.0
Elytra	64	23.0
Total	281	100.0

* Taken from 50 naturally infected individuals.

To identify the parasite, the thalli were gently removed from the host's cuticle using a needle as well as dissecting and optical microscope techniques. The thalli were mounted on permanent slides stained with cotton-blue/lactoglycerol or floxin/KOH glycerol. Measurements and pictures were taken using an Olympus BX41 optical microscope with a digital camera and micrometer. The following morphological characteristics were determined: total length from foot to perithecial tip, length from foot to tip of uppermost antheridium, length and width of perithecium, and length and width of ascospores. Both the Philco-Hitachi TM 1000 electron microscope, located at the Health and Sciences Biological Institute – ICBS/UFRRJ (Seropédica campus), and Evo LS 10 (Carl Zeiss), located at EMBRAPA Agrobiology (Seropédica, RJ), were

utilized for some measurements, and pictures were acquired. Identification of the fungal parasite followed de Kesel [24] and Goldmann et al. [18]. Sampled material and voucher slides were deposited at the Phytopathological Herbarium Verlande Duarte Silveira (DEnF/UFRRJ), UFRJ.

Results

The fungus was identified in the genus *Hesperomyces*, described by Thaxter in 1891 (Ascomycota, Laboulbeniomycetes, Laboulbeniales) [25].

Hesperomyces coleomegillae W. Rossi & A. Weir (Fig. 1)

Material examined. Brazil, Rio de Janeiro, Seropédica: main campus of UFRRJ, inside a building of CIMP, coordinates 22°46′10″ S, 43°41′38″ W, on elytra of the ladybird *Coleomegilla maculata* DeGeer, November 14, 2015, H. H. Paulo No. 3 (UFRRJ – 12.305).

The fungus formed ascomata on several parts of *C. maculata* adults, including the prothorax, mesothorax, and head (antennae and mouthparts, such as palpi); however, formation primarily occurred on the elytra (dorsal part) (Fig. 1A–G). The fore, middle, and hind legs and abdomen also exhibited infection (Tab. 1). Ascomata showed the following characters: length from foot to top of perithecium: up to 770 µm; length from foot to tip of uppermost antheridium: 108–156 µm; perithecium: 92–563(–670) × 34–80(–96) µm, showing apical outgrowths (26–36 µm) (Fig. 1J–L); ascospores: 68–96 × 5–6 µm, hyaline, one-septate, spindle-shaped, with thick a mucilaginous layer (Fig. 1L) (Tab. 2).

Discussion

This paper reports the first record of living *C. maculata* adults hosting *Hesperomyces* in laboratory mass-rearing in Brazil, originating from specimens collected in the field. Eggs and larvae were never infected, which was expected as Laboulbeniales fungi only infect living adults [4,6]. This fungus was identified as *H. coleomegillae* based on morphological characters described in previous literature [18] (also see Tab. 2).

It has already been reported that species in the genus *Hesperomyces* parasitize *C. maculata* adults; specifically, *H. coleomegillae* in Central America (Costa Rica) and *H. palustris* in Costa Rica, Cuba, and Ecuador [18,26].

Laboulbeniales fungi do not have a free-living stage, and the propagation of their sticky ascospores is triggered or promoted by the activity of the host (grooming, copulation, or other contact) [4,6,27]. A number of factors may have led the ladybird colony to become infected with *H. coleomegillae*. It is likely that a field-collected adult bearing mature thalli (having passed unnoticed by the collector) was enough to initiate propagation among the adults in the rearing containers. In addition, when mating had not been observed within 24 hours, there was random exchanges of individuals among the containers until the presence of at least one viable pair per container was observed. However, new adult collections should be carried out on the same farm to more accurately detect natural infection of *C. maculata* by *Hesperomyces* species.

Individual *Cycloneda sanguinea* (Linnaeus), *Eriopis connexa* (Germar), *Harmonia axyridis* (Pallas), and *Hippodamia convergens* Guerin-Meneville adults have also been reported to occur at SIPA [13,28,29], and *H. virescens* Thaxt. has been reported to infect adult lady beetles of the above species in other countries [6,25]. Therefore, new records of species in *Hesperomyces* on wild populations of these lady beetles in Brazil should be expected. However, based on studies by Cottrell and Riddick [30] and Riddick [31] the chances of transmission taking place from *H. virescens*-infected *H. axyridis* to *C. maculata* adults at SIPA are likely low. These authors demonstrated that intraspecies transmission of this fungus in *H. axyridis* was common, whereas interspecies transmission of *H. axyridis* to *Coccinella septempunctata* L. and *Olla v-nigrum* (Mulsant), as well

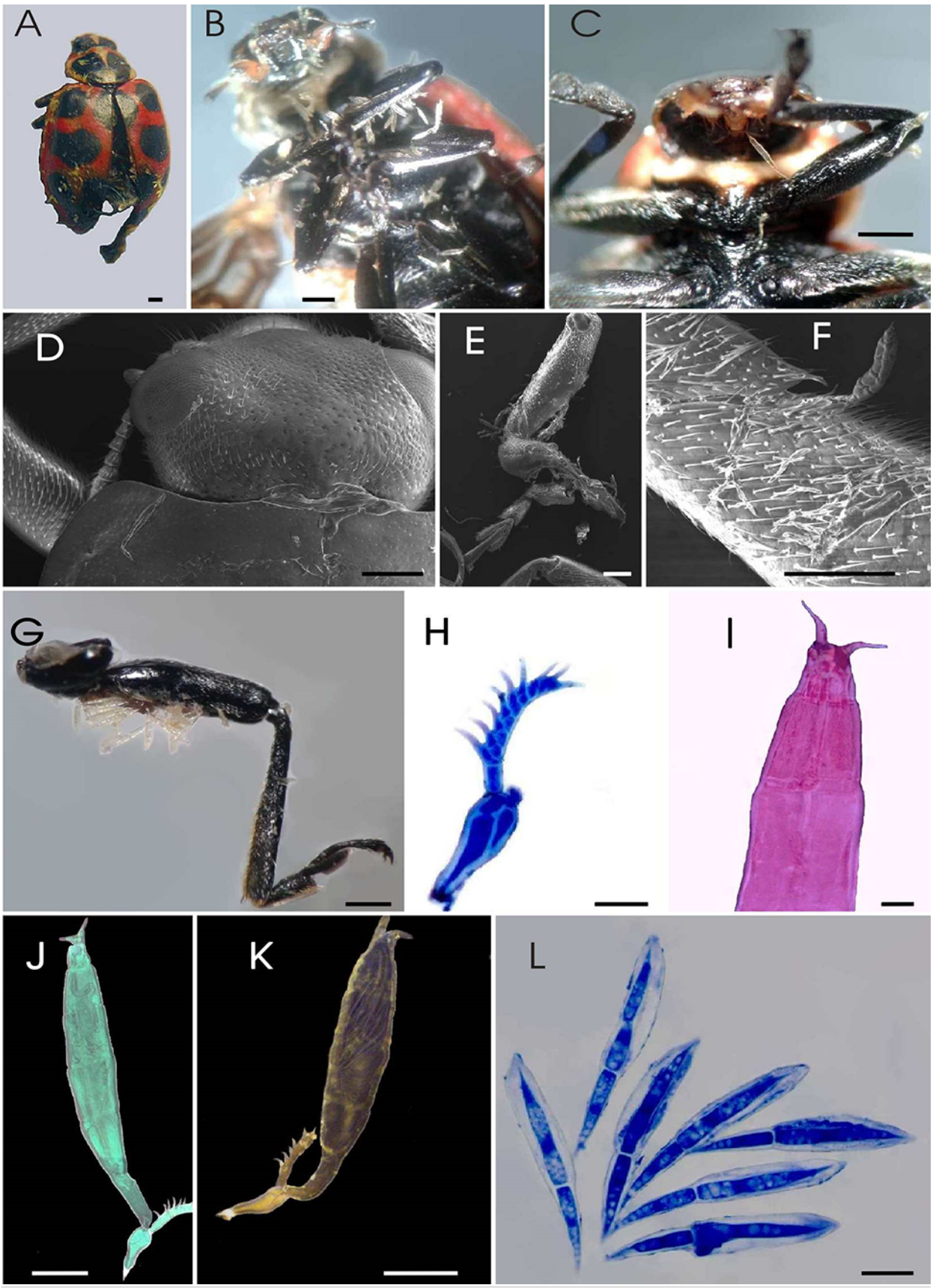

Fig. 1 (**A–G**) Body parts of *Coleomegilla maculata* infected by *Hesperomyces*. (**A**) Pronotum and dorsal part of the elytra of *C. maculata* with thalli (bar: 1 mm). (**B,C**) Legs and prosternum of *C. maculata* with ascomata (bar: 500 µm). (**D–F**) Pictures obtained from a scanning electron microscope (SEM). (**D**) Detail of the fungus on the head and pronotum of *C. maculata* (bar: 5 mm). (**E**) Infection on leg (bar: 600 µm). (**F**) Detail of infection of tibia (bar: 600 µm). (**G**) Detail of infection, mainly of femur (bar: 600 µm). (**H–L**) *Hesperomyces* structures. (**H**) Detail of young antheridium with appendages (bar: 15 µm). (**I**) Apical outgrowth of perithecia (bar: 100 µm). (**J,K**) Perithecia (bar: 100 µm). (**L**) Ascospores (bar: 20 µm).

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Tab. 2 Comparison of morphological characters (in µm) of *Hesperomyces* species associated with Coccinellidae (Coleoptera) reported from Central and South America.

Species	Country	Length from foot to perithecial tip	Length from foot to tip of uppermost antheridium	Length and width of perithecium	Perithecial outgrowth	Length and width of ascospores
H. coccinelloides[1]	Panama	120–240	-	24–30 × 85–110	35–40	-
H. coleomegillae[2]	Costa Rica, Ecuador	370–600	130–150	55–70 × 210–350	27–33	75–77
H. hyperaspidis[3]	Trinidad Tobago	180	-	25 × 110	20	-
H. palustris[2]	Costa Rica, Ecuador	(355)400–675	105–130	55–70 × 245–355	50–70	-
H. coleomegillae[4]	Brazil	Up to 770	108–156	34–80(–96) × 92–563(–670)	26–36	68–96 × 5–6

[1] Thaxter [38] (host: *Scymnus tardus* Mulsant); [2] Goldmann et al. [19] (host: *Coleomegilla maculata*); [3] Thaxter [38] (host: *Hyperaspis* sp.); [4] present study (host: *Coleomegilla maculata*).

as from *O. v-nigrum* to both *C. septempunctata* L. and *H. convergens* Guerin-Meneville, was notably uncommon. The same authors [30,31] did not observe infection of *C. maculata* adults after confinement with *H. axyridis* after placed in a vial and tumbled on a vial roller for 1 h.

In general, Laboulbeniales fungi cause little or no harm to their arthropod hosts and do not seem to kill them [32–35]. However, there are indications that species in the genus *Hesperomyces* deviate from this because of the penetrating haustorium in the exoskeleton of their hosts, and some negative effects on hosts have been reported [36,37]. Hosts with high numbers of thalli, such as those from *H. virescens* on *H. axyridis* with more than 100 thalli (sometimes >400 thalli), on the outer parts of their body (e.g., elytra, eyes, antennae, mouthparts, and/or legs) may no longer be able to fly, mate, or detect their prey [6,37]. In this study, it was observed that 100% of naturally colonized adults died after 60 days and the number of eggs laid by infected adults was affected compared to noninfected adults of the same age. It was also observed that *C. maculata* adults under 6 months of age were not infected by *H. coleomegillae*. This result suggests that older adults may be more susceptible to infection by this species. Additional research is needed to support this hypothesis.

Acknowledgments

The second author acknowledges CAPES for grants supporting his master's degree program. The authors are obliged to Karinne Moura de Freitas (EMBRAPA Agrobiologia) and Jose Antonio (UFRRJ /ICBS) for their technical assistance with electron microscope; C. A. Inácio is grateful to the Foundation for Aid Research in the State of Rio de Janeiro (FAPERJ) and National Council for Scientific and Technological Development (CNPq).

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