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Short Communication
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A NEW TRITROPHIC ASSOCIATION: *Syspastospora parasitica* ON *Beauveria pseudobassiana* INFECTING *Corythucha arcuata*

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SUMMARY

While studying a sap-feeding insect oak lace bug (*Corythucha arcuata* Say 1832, Heteroptera: Tingidae) and its association with entomopathogenic fungi, a new tritrophic association was discovered: *C. arcuata* infected with entomopathogen *Beauveria pseudobassiana* Rehner & Humber, which in turn is hyperparasitised by mycoparasitic fungus *Syspastospora parasitica* (Tulasne) Cannon & Hawksworth. This hyperparasitic fungus has been reported on various entomopathogenic fungi including *B. pseudobassiana*, but never in tritrophic association with *C. arcuata*. Although fungal hyperparasitism is widespread in nature, it is still poorly studied. In a context of biological control, the interest for hyperparasitic fungi is growing, since entomopathogens have an important role in the regulation of many insect pest populations.

KEY WORDS: biological control, Croatia, entomopathogens, hyperparasitism, mycoparasite, natural infection, oak lace bug

INTRODUCTION

Beauveria pseudobassiana Rehner & Humber (Hypocreales: Cordycipitaceae) is a natural antagonist of oak lace bug (*Corythucha arcuata* Say 1832, Heteroptera: Tingidae), a sap-feeding insect native to North America that is causing foliar damage of oak trees (*Quercus* spp.), first observed in Croatia in 2013 (Hrašovec et al. 2013). Previous studies have shown that this entomopathogenic fungus is consistently present in *C. arcuata* populations and that it is the most common entomopathogen on this species (Kovač et al. 2020, 2021; Kovač 2021). While studying *C. arcuata* and its natural infections with entomopathogenic fungi on different locations in Croatia, we subsequently observed dark brown ascomata emerging from the insects infected with *B. pseudobassiana*. We inferred hyperparasitism, a phenomenon in which one fungus (the mycoparasite) parasitizes another fungus (the host that is also a parasite). A hyperparasitic interaction consists of at least three trophic levels: a primary host, which is parasitized by the primary parasite, which serves as secondary host to the secondary parasite or hyperparasite (Bermúdez-Cova 2023). The aim of this study was to report new tritrophic association with the hyperparasitic fungus on *C. arcuata* infested with *B. pseudobassiana* in natural populations in Croatia.

MATERIALS AND METHODS

During the previous research of *C. arcuata*, carried out in 2019, 2021 and 2022 in different localities in Croatia, many overwintering adults infected with entomopathogen *B. pseudobassiana* were sampled (Kovač 2021, Kovač et al. 2021). Methodology used for sampling and processing of the specimens is described in Kovač et al. (2021). Most of the collected samples were kept in a refrigerator at 4°C and used for the present research. Information on the locations and time of collection can be found in Table 1. Altogether 271 individuals infected with *B. pseudobassiana* were gathered and inspected for the presence of hyperparasitic fungus *S. parasitica*. Adults were first checked under a dissecting microscope (Olympus, model SZX7, Tokyo, Japan) at magnification 40X to visually discern the fungal structures, and the fruiting bodies (perithecia) with emerged spores were

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cut with a sterile needle and microscopically analysed with a phase-contrast microscope (Olympus, model BX53, Tokyo, Japan). The fungus was identified based on the morphology of the fruiting bodies, as well as the size and shape of the ascospores, same as described in Georgieva et al. (2020). The perithecium had globose bases, yellow-brown in colour, with necks of 80–95 μm × 40-42 μm in size. Ascospores were cylindrical, one-celled, 7.5-8 μm long, and 2.5–3 μm wide. The fungus was also isolated on the Potato Dextrose Agar (PDA) medium by taking the whole perithecia with a sterile needle and gently tapping the apices of perithecium necks with spores on a medium. The cultures were incubated for 2–3 weeks at ± 25°C, and purified when needed. After the development of mycelium they were microscopically examined.

RESULTS

After examination of 271 *C. arcuata* individuals infected with entomopathogenic *B. pseudobassiana* 34 of them exhibited mycoparasitic growth on *B. pseudobassiana* (Figure 1a–f). The mycoparasite was identified as *Syspastospora parasitica* (Tulasne) Cannon & Hawksworth (Ascomycota: Hypocreales). The incidence of hyperparasitism varied depending on the locality and collection period (Table 1).

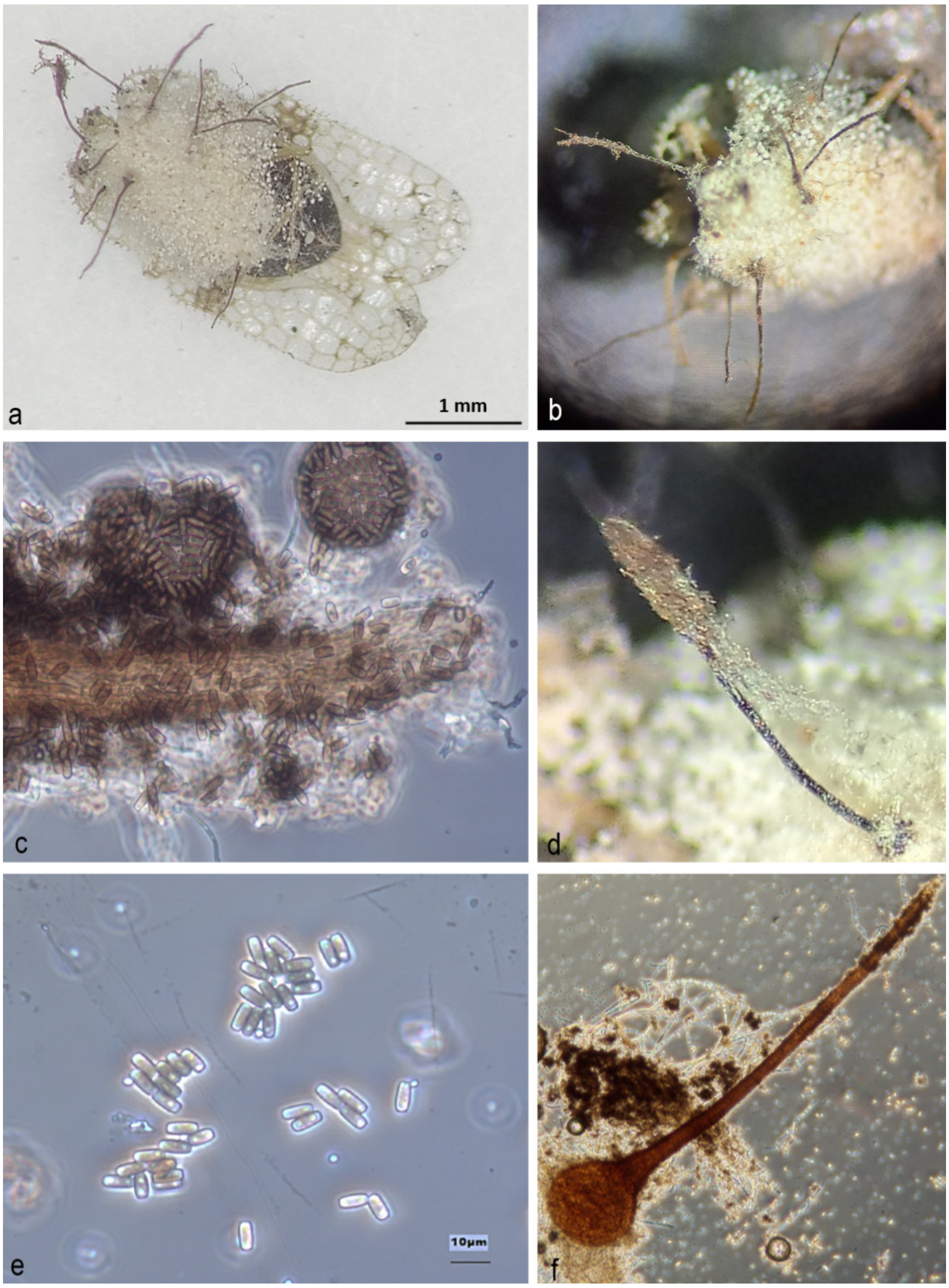

Figure 1 (a) *Corythucha arcuata* adult infested with *B. pseudobassiana* and hyperparasitised by *S. parasitica*; (b) mature *S. parasitica* perithecia growing on *B. pseudobassiana*; (c) ascospores moving to the tip of the perithecium; (d) *S. parasitica* perithecium up close; (e) mature ascospores; (f) dissected perithecium with released ascospores.

Table 1 *C. arcuata* infected by *Beauveria pseudobassiana* and hyperparasitised by *Syspastospora parasitica*, and information on sampling periods and sites.

Collection site		Coordinates	Period of collection	The number of *C. arcuata* infected by *B. pseudobassiana* (n)	The number of *C. arcuata* infected by *B. pseudobassiana* and hyperparasitised by *S. parasitica* (n)	The share of the *C. arcuata* infected by *B. pseudobassiana* and hyperparasitised by *S. parasitica* (%)
Spačva	FO Otok MU Otočke šume	45°08′45.7″N 18°48′16.6″E	March 2019, 2021, 2022	99	26	19%
	FO Gunja MU Desićevo	44°59′41.1″N 18°48′33.6″E	March 2019, 2021, 2022	25	1	
	FO Vrbanja MU Vrbanjske šume	45°02′22.5″N 18°53′16.4″E	March 2019, 2021, 2022	15	1	
	FO Cerna MU Ceranski lugovi	45°10′41.3″N 18°43′54.9″E	December 2019	9	0	
Jaskanski lugovi	MU Jastrebarski lugovi, dep 25b	45°38'11.8"N 15°41'23.4"E	March 2021, 2022	10	0	5%
	MU Jastrebarski lugovi, dep 12a	45°37′25.0″N 15°41′45.5″E	March 2021, 2022	101	6	
	MU Jastrebarski lugovi, Crna Mlaka	45°36'35.0"N 15°42'38.3"E	March 2021, 2022	12	0	
				271	34	12.5%

*MU = Management unit, FO = Forest office, dep = department

DISCUSSION AND CONCLUSIONS

In this research a new tritrophic association is reported for the first time: a mycoparasitic fungus *Syspastospora parasitica* (syn. *Melanospora parasitica* (Tul.)) attacking *B. pseudobassiana* that had infected adult *C. arcuata*. This hyperparasitic fungus has been reported on various entomopathogenic fungi including *B. pseudobassiana* (Georgieva et al. 2020), but never in tritrophic association with *C. arcuata*. According to previous studies, *B. pseudobassiana* was established as a dominating species affecting *C. arcuata* in natural populations of Croatia (Kovač 2021, Kovač et al. 2021), so this new association is an interesting addition to that finding.

The fungus was first described as *Sphaeronema parasitica* Tul. on *Isaria crassa* by Tulasne (1857). So far, *Syspastospora parasitica* was recorded as a secondary parasite of the entomopathogenic fungi *B. pseudobassiana* and *B. varroae* on pine processionary moth *Thaumetopoea pityocampa* (Denis and Schiffermüller 1775) (Lepidoptera: Notodontidae) larvae and pupae (Georgieva et al. 2020), as well as on other *Beauveria* species such as *B. tenella* on *Melolontha* spp. (Coleoptera: Scarabaeidae) (Müller-Kögler 1961), and *B. bassiana* on ash weevil *Stereonychus fraxini* (Coleoptera: Curculionidae) (Markova 1991), Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Posada et al. 2004) *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), and on pupae of pine sawfly *Neodiprion sertifer* (Geoffroy) (Hymenoptera: Diprionidae) (Rosnev and Tsankov 1978). It has also been reported on other entomopathogenic fungi infecting insects such as *Paecilomyces tenuipes* (Peck) Samson attacking *Bombyx mori* L. (Lee and Nam 2000), *Spicaria farinosa* (= *Paecilomyces farinosus*) (Fr.) Vuill. infecting *Boarmia bistortata* Goeze (Lepidoptera: Geometridae), and *Spicaria fumosorosea* (= *P. fumosoroseus*) (Wize) Vassiljevsky infecting *T. pityocampa* (Müller-Kögler 1961).

Although fungal hyperparasitism is widespread in nature, it is still poorly studied, and not ecologically and evolutionarily well understood (Parratt and Laine 2016, Haelewaters et al. 2018, Bermúdez-Cova 2023). However, since entomopathogenic fungi are becoming an increasingly important factor in reducing the population of many insect pests, or at least in keeping their abundance at an acceptable level, in the context of biological control the interest in hyperparasitic fungi is growing.

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