First records of fungi pathogenic on spiders for the Republic of Serbia

Dragiša Savić, Gordana Grbić, Eleonora Bošković & Ambros Hänggi

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Abstract. During an investigation into parasitic fungi on arthropods in the mixed forests of Mt. Fruška Gora, Republic of Serbia, two pathogenic species were found: *Cordyceps thaxteri* Mains (anamorph: *Akanthomyces aranearum* (Petch) Mains) and *Torrubiella arachnophila* (J.R. Johnst.) Mains (anamorph: *Gibellula leiopus* (Vuill. ex Maubl.) Mains (Hypocreales, Cordycipitaceae). Both specimens were found in the anamorphic (asexual) stage. Previously, there have been no investigations of this group of fungi in this region, thus these are the first records of pathogenic fungi for both Mt. Fruška Gora and the entire territory of the Republic of Serbia. Collected specimens are deposited in the Herbarium of the National Park Fruška Gora, Republic of Serbia.

Keywords: *Akanthomyces,* Araneae, *Cordyceps, Gibellula*, pathogenic fungi*, Torrubiella*

Zusammenfassung. Erstnachweise von arachnophagen Pilzen für Serbien. Im Rahmen einer Untersuchung von Schlauchpilzen (Ascomyceten = Ascomycota), die auf Arthropoden parasitieren, wurden im Untersuchungsgebiet Fruška Gora (Vojvodina, Serbien) zwei spinnen-parasitierende Arten gefunden: *Cordyceps thaxteri* Mains (Anamorph: *Akanthomyces aranearum* (Petch) Mains) und *Torrubiella arachnophila* (J.R. Johnst.) Mains (Anamorph: *Gibellula leiopus* (Vuill. ex Maubl.) Mains (Hypocreales, Cordycipitaceae). Beide Exemplare wurden im anamorphen, ungeschlechtlichen Stadium gefunden. Bisher wurden in dieser Region keine Untersuchungen zu dieser Pilzgruppe gemacht. Die Funde sind somit Erstmeldungen für das Fruška Gora Gebirge und für Serbien. Die gesammelten Individuen sind im Herbar des Nationalparks Fruška Gora, Serbien, hinterlegt.

Fruška Gora is a small mountain (highest peak 539 m) located in the southern region of the Pannonian plain. In the upper parts of this mountain the dominant vegetation type is mixed deciduous forest (*Fagus*, *Tilia*, *Quercus*), while the lower parts are covered with meadows, steppe, arable land, orchards, vineyards and settlements (Janković & Mišić 1980). The spider fauna of Mt. Fruška Gora is very diverse and 267 species have been recorded so far (Grbić et al. 2015).

In their natural habitats spiders can be infected with numerous parasitic fungi, exhibiting anamorphic (asexual morph) and teleomorphic (sexual morph) stages. Fungi are named according their teleomorphs, but names of their anamorphic stages (formerly considered separate species) can also be found in the recent literature; even though they represent synonyms (Norvell 2011). The mode of infection and mortality are similar to mechanisms found in numerous entomopathogenic species (Evans & Samson 1987). After contact of the fungal spores with the body of an insect or spider, the spores germinate and grow through the exoskeleton into the haemocoel, gradually occupying the whole body of its host by decomposing soft tissues with digestive enzymes. After a few weeks, the spider dies and fungal structures grow out through the exoskeleton and form sporulating structures (conidiophores) on the surface of the body (Hughes et al. 2016). Arachnophagous fungi can be found in the same habitats as spiders, but they require much humidity and shade for their development (Hajek & Leger 1994).

The anamorphic genus *Akanthomyces* (Cordycipitaceae) consists of 13 species, all parasites of spiders and insects (Lepidoptera and Coleoptera) except one that lives as a saprobe on decaying plant material (Mains 1950, Samson & Evans 1974, White et al. 2003). Species of the genus *Akanthomyces* are characterized by producing white, cream or flesh-coloured cylindrical, attenuated synnemata (large, erect reproductive structures, bearing compact conidiophores) covered with a hymenium (tissue layer on the fungal fruiting body where cells develop reproductive structures) of phialides (flask-shaped projection from the top of the conidiophore).

Anamorphic fungi from the genus *Gibellula* (Cordycipitaceae), with 16 species described so far, are typical pathogens of spiders (White et al. 2003). Most *Gibellula* species are tropical, while there is a small number of species present in the temperate region (Mains 1950). Detailed descriptions of *Gibellula* species can be found in the studies of Petch (1932), Samson and Evans (1977, 1992), Kobayashi & Shimizu (1982), Humber & Rombach (1987), Tzean et al. (1997a, 1998) and Kubátová (2004). Around half of the *Gibellula* species are connected with *Torrubiella* teleomorphs. *Gibellula* usually forms synnemata bearing aspergilloid or penicillate conidiophores with phialidic conidiogenous cells (Kubátová 2004).

The aim of this study was to describe two pathogenic fungi, *Cordyceps thaxteri* Mains and *Torrubiella arachnophila* (J.R. Johnst.) Mains, collected on Mt. Fruška Gora which represent the first records of these species for the Republic of Serbia. We also hope that this paper will draw arachnologist's attention to infected spider specimens, since there is a need for more comprehensive research into this group of fungi.

Material and methods

Sampling

In the period from 2013 to 2015 investigation into fungi of the phylum Ascomycota was conducted in the mixed deciduous forests of Mt. Fruška Gora. Two dead spiders infected with parasitic fungi were collected. The specimens were macro-photographed in situ. Both samples were deposited as dry specimens in the Herbarium of the Fruška Gora National Park, labelled FG266 and FG262 respectively.

Dragiša SAVIĆ, National Park Fruška Gora, Zmajevtrg 1, 21208 Sremska Kamenica, Republic of Serbia; E-mail: dragita@gmail.com
Gordana GRBIĆ, Educons University, Faculty of Environmental Protection, Vojvodeputnika 87, 21208 Sremska Kamenica, Republic of Serbia; E-mail: gordana_grbicns@yahoo.com
Eleonora BOŠKOVIĆ, University of Novi Sad, Faculty of Sciences, Department for Biology and Ecology, Trg Dositeja Obradovića 3, 21000 Novi Sad, Republic of Serbia; E-mail: eleonora.boskovic@dbe.uns.ac.rs
Ambros HÄNGGI, Naturhistorisches Museum Basel, Augustinergasse 2, 4001 Basel, Switzerland; E-mail: ambros.haenggi@bs.ch

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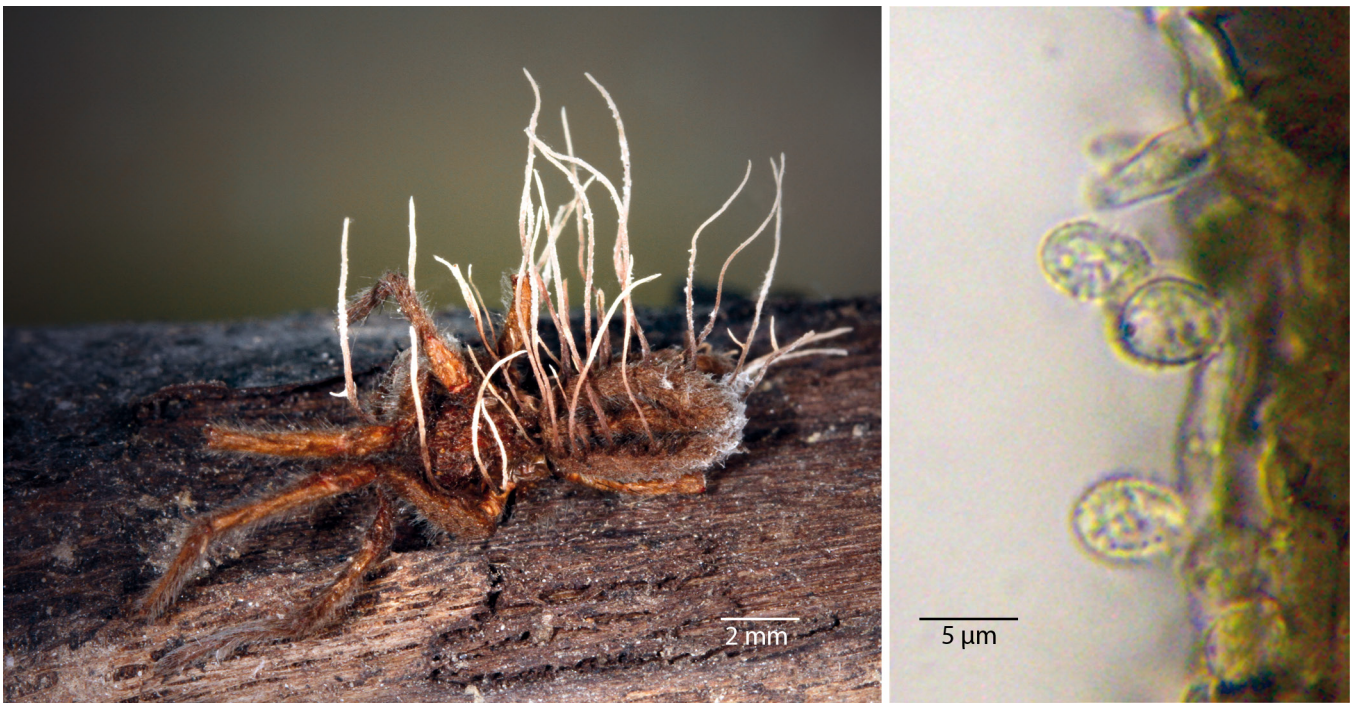

Fig. 1: *Cordyceps thaxteri*: Body of dead spider (Lycosidae) with synnemata, right: Conidiophore and conidiogenous cells

Identification

The collected species were identified on the basis of macroscopic and microscopic morphological characteristics according to specific identification keys published in Samson & Evans (1992) and Hsieh et al. (1997). Microscopic features were observed in water using transmission light microscope and bright-field technique. Spiders were identified according to Loksa (1969), Blauwe (1973), Wang (2002) and Nentwig et al. (2015).

Results

A first sample of a dead spider infected with an anamorphic stage of *C. thaxteri* (Fig. 1) was found on 29 September 2013 on the forest floor of the oak/hornbeam forest (*Aculeato-Querco-Carpinetum*) in the locality Iriški venac (45°8'58.43"N, 19°49'49.67"E). Most of the spider's body was covered with yellowish/white mycelium from which numerous elongated synnemata were growing out. Synnemata were dark brown at the base and yellowish white on the upper part, covered with a thick layer of spherical to ellipsoidal phialides, measuring 6-10 x 4-6 µm. The conidia were hyaline, globular with constrictions at the top, 5-10 x 2.1- 2.4 µm (Fig. 1). A teleomorph was not found.

Macroscopic and microscopic characters of the specimen correspond to the description given by Hsieh et al. (1997). Due to severe damage, identification of the dead spider to species level was not possible, but it was determined that it belongs to the family Lycosidae.

The second sample of a dead spider infected with an anamorphic stage of *T. arachnophila* (Fig. 2) was found on 6 October 2015 under decaying leaves of beech forest (*Tilio-Fagetum submontanum)* in the locality Papratski do (45°8'7.18"N, 19°38'23.62"E). The spider's body was covered with white mycelium and numerous conical to cylindrical synnemata, approximately 1 mm long and 400 µm in diameter (Fig. 2). Conidiophores were short (42-75 x 6-8 µm), septate, at the tip expanding into vesicles 5-10 µm in diameter with metulae in the upper part. The metulae were wide and globular with numerous cylindrical phialides. The conidia were hyaline, ellipsoid to cylindrical, 4-5 x 1.6-1.9 µm (Fig. 2). A teleomorph was not found.

Macroscopic and microscopic characters of our specimen correspond to the characters of *Gibellula leiopus* described by Tzeanet al. (1997). The spider belongs to the species *Urocoras longispinus* (Kulczyński, 1897), Agelenidae.

Discussion

C. thaxteri has a global distribution, and was recorded e.g., in North America (Mains 1950a), Taiwan (Hsieh et al. 1997), the United Kingdom (NBN Gateway 2015), Ghana (Samson & Evans 1974), The Netherlands (Samson & Evans 1974), Japan (NBRC 2015), Thailand (Hywel-Jones 1996) and New Zealand (GBIF 2015), while this finding represents the first record for Serbia.

G. leiopus is distributed widely and has been recorded e.g., in North America (Mains 1950), Trinidad (Evans & Samson, 1987), the Czech Republic (Fassatiova 1960), Poland (Bałazy 1970), France (in 1975, CBS Fungi database 2015), Sweden (Lundquist 1998), Greece (Kubátová 2004), Austria (Tkaczuket al. 2011), Ghana (Samson & Evans 1973), Russia (Koval 1974) and Taiwan (Tzeanet al. 1997). The finding presented in this paper is the first record for Serbia.

Since the spider fauna is quite diverse in all the types of habitats on Mt. Fruška Gora (Grbić et al. 2015), it can be assumed that other species of arachnophagous fungi may also occur in this region.

There is very little information about the distribution of arachnophagous species of fungi, especially in temperate regions. This is partly because there is a much smaller number of species present in comparison with tropical regions, and fewer investigations have been conducted (Tzean et al. 1997, Kubátová 2004).

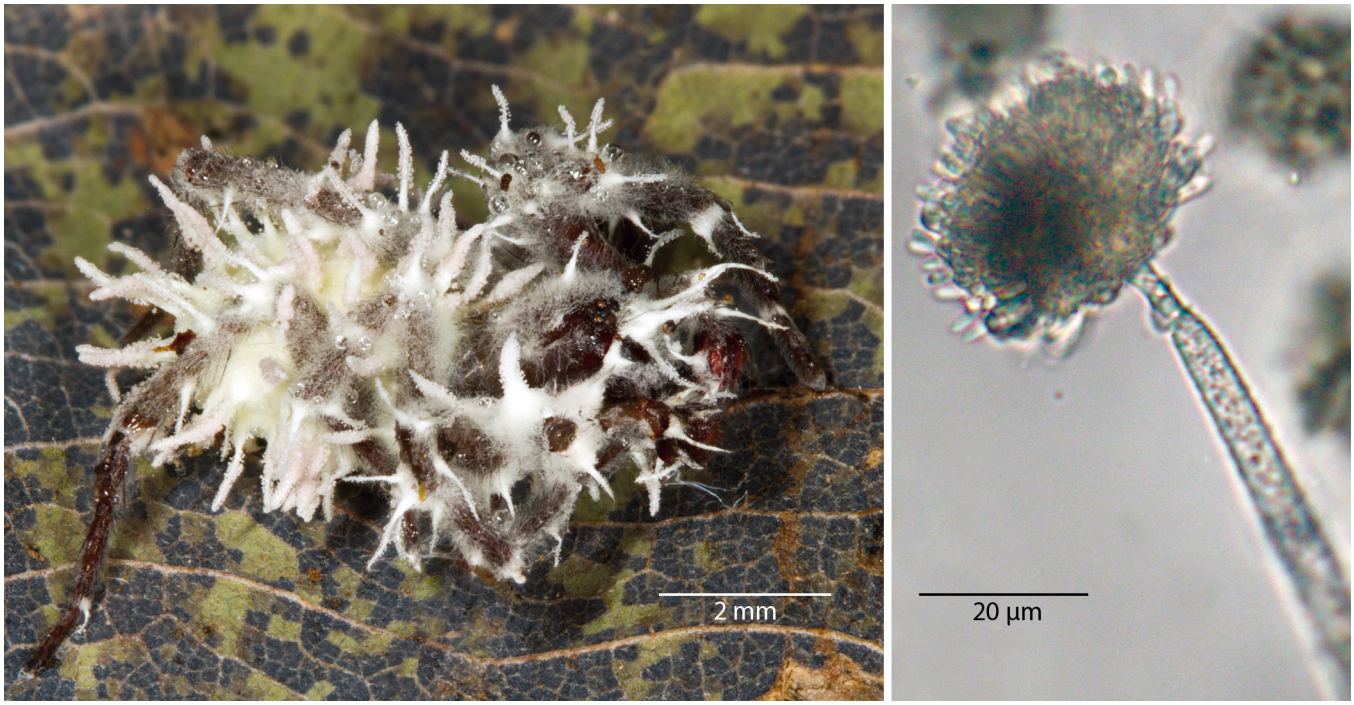

Fig. 2: *Torrubiella arachnophila*: Body of dead spider (*Urocoras longispinus*, Agelenidae) with synnemata, right: Conidiophore and conidiogenous cells

The effect of pathogenic fungi on spider populations is only poorly known (Evans 2013), so it is of great importance for arachnologists to be more involved in the investigations of arachnophagous fungi, since the data they collect during fieldwork can be very valuable.

Also, it is worth mentioning that antimicrobial activity of the species presented in this study was confirmed (Kuephadungphan et al. 2014) and their potential as producers of bioactive compounds should be investigated in the future.

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