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Short note

First Record of Therrya fuckelii (Rhytismatales, Ascomycota) in Bulgaria, with Ecological Remarks

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Abstract. The short note presents the first record in Bulgaria of *Therrya fuckelii*, an ascomycetous fungus developing on hosts of the genus *Pinus* L. The Bulgarian collection was found on the relict and subendemic Bosnian Pine (*Pinus heldreichii*). Brief description and illustrations of the Bulgarian specimen are provided and some aspects of the ecology of the species are briefly discussed.

Key words: Bulgarian mycota, endophytic fungi, Leotiomycetes, forest pathogen, Rhytismataceae.

Introduction

In 2021 during studies of the fungal diversity in stands of *Pinus heldreichii* H. Christ. the authors collected a remarkable member of the family *Rhytismataceae* Chevall., preliminary identified in the field as a member of the genus *Therrya* Sacc. Further study of this collection revealed that it belongs to *T. fuckelii* (Rehm) Kujala, a fungal pathogen new for the Bulgarian mycota. This finding is presented herein.

Material and Methods

The examined specimens were photographed and documented in the field, as well as *ex-situ*. The microscopic study was held with an AmScope T360B light microscope with an AmScope MU900 digital camera. All microscopic observations were held in cotton blue in lactoglycerol (lactic acid : glycerol :

Ecologia Balkanica https://ecologia-balkanica.com water = 2 : 1 : 1 v/v) after heating. Melzer's reagent was used for testing the amyloid reaction of asci. Measurements of microscopic structures were obtained from pre-calibrated digital images with the aid of Piximetre 5.10 software. Air-dried voucher specimens are preserved in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF).

Results and Discussion

Therrya fuckelii (Rehm) Kujala, Comm. Inst. Forest. Fenn. 38(4): 48 (1950); *Coccophacidium fuckelii* (Rehm) Krieg., Fung. Saxon. Exsicc., Pilze Sachsen's 15: no. 735 (1892); *Coccophacidium pini* var. *fuckelii* Rehm in Rabenh. Krypt.-Fl., Edn 2, 1.3(lief. 29): 99 (1888)/[1896].

Macroscopic features. Ascomata in groups or scattered on dead, attached

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First Record of Therrya fuckelii (Rhytismatales, Ascomycota) in Bulgaria, with Ecological Remarks

branches and twigs, initially covered with thin bark layer, then erumpent from bark, rounded or irregular in top view, at first covered by black fungal layer, which later disrupts by radial splits into several coriaceous rays, curving backwards, producing star-like structure and revealing up to 4 mm broad, hazel, sepia to blackish brown coloured disk with somewhat waxy appearance.



Fig. 1. Ascomata of *Therrya fuckelii* on branch of *Pinus heldreichii*. Scale bar = 10 mm.

Microscopic features. Ascospores 103.5- $121.7 \times 4.2-5.5 \ \mu m$, straight or to a different degree curved, 7-10-septate and slightly constricted at septa, hyaline, smooth, at the apices tapering into thread-like tips. Asci cylindrical, 128–175 × 10–17 μ m, 4-spored with parallel spore arrangement, clavate, with flattened apex, thin-walled, inamyloid, visible predefined without opening structures. Paraphyses slightly longer than asci, filiform, 2-3.5 µm wide, with widened tips (up to 8 µm) at the apex, thin-walled, septate, hyaline, enveloped in mucous sheath.

Specimen examined. Bulgaria: Znepole Region, Konyavska Mt, below Viden Peak (Kyustendil Province), 42°20'47.6"N, 22°50'21.4"E, elev. ca 1455 m, on branches of *Pinus heldreichii* in an artificial plantation (Fig. 3), 30.09.2021, B. Assyov (SOMF 30423).

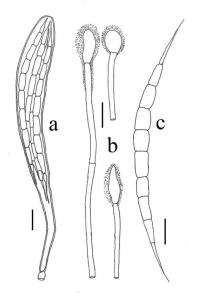


Fig. 2. Microscopic features of *Therrya fuckelii*: a – ascus with ascospores; b – paraphyses (mucous sheaths presented as dotted outline); c - ascospore. Scale bars = 10 μm.

The morphological features of the Bulgarian collection of *T. fuckelii* (Fig. 1-2) are consistent with the characters, accounted by Reid & Cain (1961) and Minter (1996). The only notable difference is that in our collection of T. fuckelii on P. heldreichii we have not observed the presumed characteristic bright red-brown colouration of the colonized twigs (also illustrated in Kwaśna & Lakomy, 2011) and this is a character that might be related to some peculiarities of the host tree species. The closest European species to T. fuckelii is T. pini (Alb. & Schwein.) Höhn., which has considerable similarity in gross morphology. The former species is however easily distinguished upon microscopic observations by the strictly four-spored asci and ascospores, which are 7- to 11-septate and with tips narrowing in thread-like appendages (Reid & Cain, 1961; Minter, 1996). Therrya pini has 8spored asci and very different, predominantly 3-septate (by exception 7-septate) ascospores, with abruptly tapered apices, not elongated in thread-like tips (Reid & Cain, 1961; Minter, 1996).



Fig. 3. The habitat of *Therrya fuckelii* on the slopes of Mount Vezhen in Konyavska Mts.

Therrya fuckelii seems to be а widespread species in Europe and has been previously reported to occur on several native species of two-needled pines, but most records are said to be in association with P. sylvestris L. (Minter, 1996). Previous findings of T. fuckelii with P. heldreichii (as P. leucodermis Antoine) are of unspecified origin (Minter, 1996) and may originate from Italy or Greece. Furthermore, the Bulgarian collection seems to be the second record of the species on the Balkan Peninsula after the mention from Greece (Minter, 1996).

Therrya fuckelii is the second rhytismataceous fungus to be reported on P. heldreichii in Bulgaria, the first being Zeus olympius, an uncommon species, as far as currently known strictly confined to this particular host (Minter, 1996; Stoykov et al., 2014). The two species share the presumed endophytic lifestyle, as well as localization of the ascomata, which are believed to appear after the death of host tissues (Minter, 1996). It was noted that T. fuckelii and another European species, T. pini, occur on dead fallen twigs (Minter, 1996). On the contrary, we observed ascomata of T. fuckelii only on branches and twigs still attached to the tree, including (albeit rarely) on not yet defoliated twigs. Fallen branches had merely remnants of ascomata, possibly developed in previous seasons and prior to shedding. Furthermore, branch such remnants were observed on still attached twigs. Ascomata or remnants of them were seen on nearly every tree in the plantation at Mount Viden. This seems to be consistent with the proposed role of the fungus in the trees self-pruning (Minter, 1996) and the high incidence of T. fuckelii in the studied area might be related to the high density of the pine plantation. Kwaśna & Lakomy (2011) studied the colonization of fungi of *P*. sylvestris following lightning damage. They found T. fuckelii to be the first colonizer and the noted co-occurrence of necrotic conductive tissues in the invaded branches. The colonization by *T. fuckelii* was followed several other fungi with known bv pathogenicity. Similarly to our observations, Solheim et al. (2013) noted two cases of occurrence of the fungus on living branches and proposed that the members of this genus would have endophytic growth phase. This habit, along with the observed in the Bulgarian locality ascomata on nondefoliated, living twigs, suggests that the species discussed here may be an opportunistic pathogen, capable of developing ascomata on host individuals weakened by damage or physiological stress.

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First Record of Therrya fuckelii (Rhytismatales, Ascomycota) in Bulgaria, with Ecological Remarks

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