First records of lichenicolous species from the Bulgarian freshwater habitats

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Abstract. *Placopyrenium cinereoatratum* and *Stigmidium rivulorum*, lichenicolous lichen and fungus, respectively, are reported for the first time from Bulgaria. Both species are rare or otherwise overlooked worldwide. Descriptions, comments, and illustrations based on Bulgarian specimens are provided.

Key words: Bulgaria, freshwater fungi, *Mycosphaerellaceae*, Pirin Mts, *Verrucariaceae*

Introduction

Lichenicolous fungi form complex and diverse interactions with lichens (e.g. commensalistic, parasitic, saprotrophic). Some lichen-forming fungi are initially lichenicolous and form facultative associations with lichen hosts (Lawrey & Diederich 2003). Over 1800 species of lichenicolous fungi are currently known (Lawrey & Diederich 2016), whereas only 26 are known to inhabit freshwater lichens (Thüs & al. 2014). Sixteen obligate lichenicolous fungi have been published for Bulgaria (Mayrhofer & al. 2005; Otte 2005; Vondrák 2006; Ivanov 2010), and none of them is reported from freshwater habitats, nor is known to grow on freshwater lichens.

In this article, two lichenicolous species growing on freshwater lichens are reported for the first time from Bulgaria. Both species are also new for the Balkan Peninsula.

Material and methods

Seven specimens of freshwater lichens, with lichenicolous species from the Pirin Mts, Bulgaria were revised. The studied specimens are deposited in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research, Sofia (SOMF) and in the Collection of Natural History Museum, Slovak National Museum, Bratislava (BRA). The length, width and length/width (l/w) ratio of the ascospores are given as: (min–){$\bar{x}$–SD}–$\bar{x}$–{$\bar{x}$+SD}(–max), where min and max are the extreme values, $\bar{x}$ the arithmetic mean, and SD the corresponding standard deviation. With n is denoted the number of measured ascospores. The measurements of the ascospores were made in 10% KOH. For colour reactions and better observation of the microstructures, Lugol's solution (I) and Cotton Blue dissolved in water were used. The observations and measurements were made under Windaus Labortechnik D-38678 dissecting microscope equipped with a Canon PowerShot A630 digital camera and Boeco BM-180/T/SP microscope with mounted digital camera Boeco B-CAM10. All measurements of microstructures and observations are based on Bulgarian specimens. The photograph from the field was made with a Canon PowerShot SX710 HS.

Results and discussions

Placopyrenium cinereoatratum (Degel.) Orange (Plate I, Figs 1, 2)

Thallus 230–540 μm thick, initially thin, growing on *Staurothele fissa*, crustose to subsquamulose, palegrey to midbrown or yellowishbrown, cracked or form-

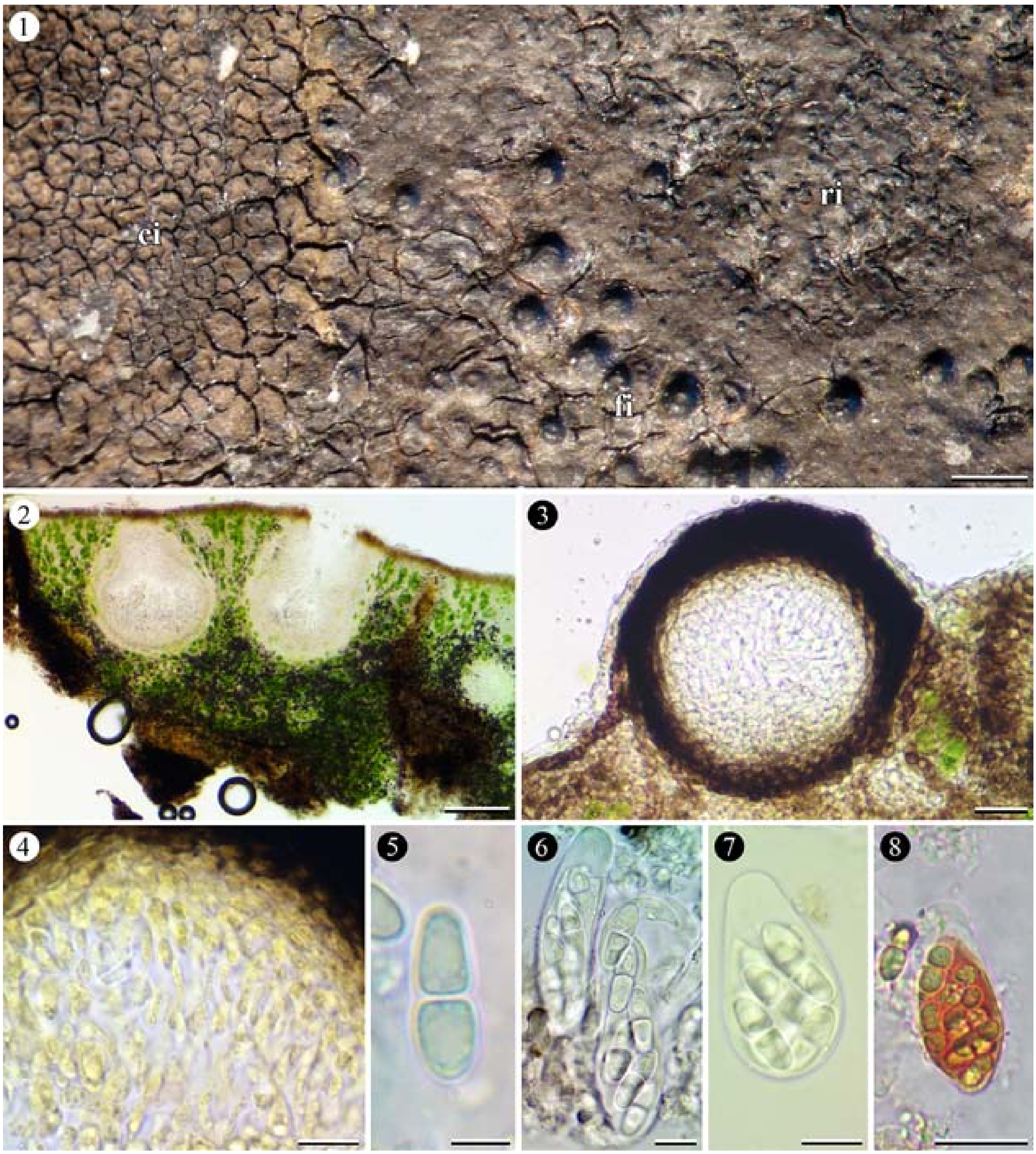

Plate I. General habit and microstructures: Fig. 1. *Placopyrenium cinereoatratum* and *Stigmidium rivulorum* co-inhabiting a thallus of *Staurothele fissa*: ci – *P. cinereoatratum,* fi – *S. fissa* with dark-brown thallus and large perithecia, ri – *S. rivulorum* with small dot-like perithecia (SOMF 29 728). Scale bar = 1 mm. **Fig. 2.** *P. cinereoatratum,* vertical section with perithecia (SOMF 29 746). Scale bar = 100 µm. **Figs 3–8.** *S. rivulorum.* **Fig. 3.** Well-developed perithecum and pale-brown vegetative hyphae (SOMF 29 728). Scale bar = 20 µm. **Fig. 4.** Pseudoparaphyses with elongated distal cells in I (SOMF 29 728). Scale bar = 10 µm. **Fig. 5.** Ascospore with a thin perispore in water solution of Cotton Blue (SOMF 29 726). Scale bar = 5 µm. **Figs 6, 7.** Asci of different shapes (BRA-CR 1 701). Scale bars = 10 µm. **Fig. 8.** Ascus in I (SOMF 29 728). Scale bar = 25 µm.

ing discrete angular areoles; upper surface plane. Prothallus not observed. Upper cortex brown, with epinecral layer. Photobiont cells arranged more or less in columns. Black basal layer well-developed or absent. **Perithecia** immersed in the thallus, several to many per areole. Exciple 140–200 µm in diameter, entirely hyaline or palebrown, near the apex usually brown. Involucrellum absent. Asci 8-spored. Ascospores (13.0–)15.4–**18.0**–20.6(–24.0) × (5.5–)5.9–**6.7**–7.5(–8.5) µm, l/w ratio (2.1–)2.3–**2.7**–3.1(–3.8), n = 25, ellipsoid, simple, hyaline, perispore not observed. **Conidiomata** not observed.

Specimens examined: Bulgaria, Pirin Mts, river Demyanitsa, on *Staurothele fissa* together with *Stigmidium rivulorum*, on siliceous rock in the splash water zone, 41.79079° N, 23.46477° E, alt. 1436 m, 21 Jul 2016, V.V. Shivarov (SOMF 29 728, 29 746).

Ecology and distribution. Initially growing on *Staurothele fissa*, on siliceous rocks in freshwater habitats; a rare species, known only from Europe (Austria, Finland, France, Great Britain, Norway, Sweden) (Hafellner & Türk 2001; Orange 2009; Roux & al. 2017).

Comments. *Placopyrenium cinereoatratum* was found growing on *Staurothele fissa* together with *Stigmidium rivulorum* (Fig. 1). According to Roux & al. (2017), in France the species is extremely rare, and is categorized as Critically Endangered (CR). It is probably a rare species, but also overlooked worldwide. The initial thallus of *P. cinereoatratum* could be easily missed due to external similarity with that of the host (Orange 2009).

Placopyrenium cinereoatratum is characterized by the host preference (the only member of *Placopyrenium* known to grow on *Staurothele fissa*), ascospore sizes 15–21 × 6–8 µm, and angular areoles with plane upper surface (for more information see Orange 2009).

Stigmidium rivulorum (Kernst.) Cl. Roux & Nav.-Ros. (Plate I, Figs 1, 3–8)

Vegetative hyphae palebrown, I–, developed in the host cortex. **Perithecia** (40–)55–80(–90) µm in diameter, black, globose to subglobose, two-thirds immersed to superficial on thalli of freshwater *Verrucariaceae*. Exciple paraplectenchymatous; upper half darkbrown and thick; lower half thin, palebrown or seldom hyaline, in some superficial states perithecia entirely brown. Hamathecium of periphyses, and pseudoparaphyses composed of a short basal cell and elongated distal cell (pseudoparaphyses 'type a' sensu Roux & Triebel 1994). Hymenial gel I–. Asci 35–48 × 14–20 µm, 8-spored, narrowly ovoid, clavate or seldom cylindrical; endotunica apically thickened; ascoplast (epiplasm) I+ red to red-brown. Ascospores (13.5–)14.2–**15.2**–16.2(–17.5) × (4.0–)4.8–**5.5**–6.2(–7.5) µm, l/w ratio (2.3–)2.5–**2.8**–3.1(–3.6), n = 33, 1-septate, hyaline, a thin perispore present or absent. **Conidiomata** not observed.

Specimens examined: Bulgaria, Pirin Mts, Belemetski Lakes, below Mt Kamenitsa, on *Dermatocarpon miniatum* var. *complicatum* (sub *D. luridum*), on periodically inundated siliceous rocks in river Mozgovitsa, alt. 2480 m, 7 Aug 1983, I. Pišút (BRA-CR 1 717, 80 116); Mt Kralev Dvor, below the saddle Kraledvorska Porta, on *D. miniatum* var. *complicatum* (sub *D. luridum*), on frequently moistened siliceous rock, alt. 2550 m, 7 Aug 1983, I. Pišút (BRA-CR 1 701); river Demyanitsa, on *Staurothele fissa*, on rock in the splash water zone, 41.79079° N, 23.46477° E, alt. 1436 m, 21 Jul 2016, V.V. Shivarov (SOMF 29 720, 29 726).

Ecology and distribution. The species grows on freshwater lichens of *Verrucariaceae* (*Dermatocarpon rivulorum*, *Sporodictyon cruentum*, *Staurothele clopimoides*, *S. fissa*, *Verrucaria aquatilis*, *V. hydrophila*, *V. pachyderma,* and *V. praetermissa*). It occurs in mountain rivers, from the forest belt up to the alpine belt. It is reported from Europe (Austria, Denmark: Faroe Islands, France, Germany, Great Britain, Italy, Luxembourg, Poland) and Asia (Russia: Siberia) (Alstrup & al. 1994; Molitor & Diederich 1997; Zhurbenko & Hafellner 1999; Hawksworth 2003; Thüs & Dornes 2003; Berger & Türk 2015; Łubek & Kukwa 2017; Roux & al. 2017). The recent record of *Stigmidium rivulorum* on *Agonimia cavernicola,* on a rock near the seashore to South Korea (Kondratyuk & al. 2015) is in contrast to the species overall ecological preferences of freshwater habitats.

Comments. The species can vary in ascospore sizes, if a great number is available for measurement. Only three ascospores sized above 16.2 × 6.2 µm were measured. They were found in different specimens on both hosts. Mention deserves the fact that the ascospores were measured in 10 % KOH, which can slightly increase their size, compared to water. The ascospores of Bulgarian specimens agree with the sizes provided in the protologue, 15–16 × 5–6 µm (Arnold 1893). Other authors report smaller ascospores 12.5–14.3 × 5.4–6.3 µm (Vouaux 1912), 11–15 × 5–6 µm (n = 5, in water) (Zhurbenko & Hafellner 1999). This variation is probably due to the absence of a sufficient number of mature spores for measuring. *Stigmidium rivulorum* is characterized by the host preference, freshwater *Verrucariaceae*, and spore sizes 14–16 × 5–6 µm. *Stigmidium stygnospilum* (Minks) R. Sant. also occurs on subaquatic members of *Dermatocarpon* (Santesson & al. 2004), but has larger ascospores, 17–20 × 4.5–5 µm (Hawksworth & al. 2010).

Three specimens of *Dermatocarpon miniatum* var. *complicatum* from alpine freshwater habitats were found, with a lichenicolous fungus belonging to *Stigmidium rivulorum*, but there is also a coexisting species of *Stigmidium* with similar morphology and I+ blue reaction of the exciple wall. *Stigmidium rivulorum* is already known to grow on *Dermatocarpon* in freshwater habitats (Berger & Türk 2015).

Molitor & Diederich (1997) reported that *Stigmidium rivulorum* does not infect *Hydropunctaria rheitrophila*, *Verrucaria elaeomelaena* and *V. margacea*. These species can survive long periods of complete submersion (see Shivarov & al. 2017), which might be unfavorable for the lichenicolous fungus. It was not found to inhabit the thalli of *Verrucaria,* which are in places with continuous periods of inundation even on the rock from where the specimens of *S. rivulorum* were collected (Fig. 9).

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Fig. 9. The rock in river Demyanitsa, predominantly covered with brown thalli of *Staurothele fissa*; an arrow shows the place where *Placopyrenium cinereoatratum* and *Stigmidium rivulorum* are growing.

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