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Notes on corticioid fungi of the Czech Republic. I. *Phlebia acanthocystis* and *Phlebia bispora* (*Meruliaceae*)

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Zíbarová L. (2017): Notes on corticioid fungi of the Czech Republic. I. *Phlebia acanthocystis* and *Phlebia bispora* (*Meruliaceae*). – Czech Mycol. 69(1): 65–76.

Two rare species of *Phlebia* s.l. (*Meruliaceae*) with hydnoid hymenophore are described and illustrated. Macro- and microscopic characters of *Phlebia acanthocystis* and *P. bispora* are described and supplemented with photographs of in situ fruitbodies and line drawings. Distribution and ecology in Europe are discussed for both species. They are compared to similar taxa found in Europe.

Key words: *Corticiaceae*, *Mycoacia*, *Mycoaciella*, distribution, rare species.

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V článku jsou popsány a ilustrovány dva vzácné druhy rodu *Phlebia* s. l. (*Meruliaceae*) s ostnitým hymenoforem. K oběma druhům je poskytnut makro- a mikroskopický popis doplněný o fotografie plodnic in situ a kresby mikroznaků. Je diskutováno jejich rozšíření a ekologie v Evropě a jsou srovnány s podobnými druhy v Evropě zaznamenanými.

INTRODUCTION

Corticioid fungi (*Corticiaceae* s.l.) are a diverse and heterogeneous group of macrofungi sharing gross morphology of resupinate or effused-reflexed fruitbodies with smooth, tuberculate, phlebioid, odontoid, hydnoid, merulioid or poroid hymenophores. Molecular phylogenetic studies show that these simple fruitbodies have evolved independently many times during the evolution of basidiomycetes (Binder et al. 2005, Larsson 2007). Therefore, the family *Corticiaceae* s.l. in its traditional sense is polyphyletic. Nevertheless, it is convenient and practical to study these taxa together without regard to their systematic position, since the collection and preparation procedures are the same. Most corticioid fungi are lignicolous saprotrophs, but mycorrhizal species are also present (Tedersoo et al. 2010).

Corticioid fungi are often overlooked by field mycologists, possibly because they have inconspicuous and simple fruitbodies and are difficult to identify. Information on the distribution of corticioid fungi is rather scanty, which is also the case in the Czech Republic. For example, species such as *Epithele typhae*, *Vuilleminia cystidiata* and *Steccherinum bourdotii* were formerly considered rare in the Czech Republic, but have been shown to be more common when focusing on them in targeted studies (Pouzar & Kotlaba 2015, 2017, Zíbarová & Kříž 2016a, 2016b).

Two rare species of the genus *Phlebia* Fr. with aculei are the subject of this article. Such species are included in *Mycoacia* Donk for taxa with monomitic or in *Mycoaciella* J. Erikss. & Ryvarden for taxa with di- and trimitic hyphal systems (Eriksson et al. 1978). Molecular phylogenetic analyses have shown that most species of *Phlebia* s.l. are clustered around the type species *Phlebia radiata* Fr. (core phlebioid clade) but other *Phlebia* species are placed in distant clades (Binder et al. 2005). In addition, *Phlebia* species with different hymenophore configuration are found together in cladograms (Chen & Cui 2014, Moreno et al. 2011). As the phylogeny of the *Polyporales* and *Meruliaceae* is still in flux, I choose to regard *Mycoacia* and *Mycoaciella* synonyms of *Phlebia* for the time being.

This article is a contribution to the knowledge and occurrence of some rare and lesser known corticioid species in the Czech Republic, which may help compiling an updated checklist of corticioid fungi of this country.

MATERIAL AND METHODS

The macroscopic descriptions are based on fresh material supplemented by colour photographs. Reactions with KOH were observed after applying a drop of 5% aqueous solution of KOH on fresh hymenium. Microscopic characters were observed in Melzer's solution, those on dried herbarium specimens using Carl Zeiss Jena Amplival. Heated cotton blue in lactophenol was used to observe cyanophilic reactions and 5% aqueous solution of KOH to describe the colour of spores and other microscopic characters. Measurements and microscopic drawings are based on Melzer's solution (Křísa & Prášil 1989) preparations under oil immersion at 1000× magnification. Spore measurements (excluding ornamentation) were obtained from 30 spores freely floating in medium or deposited on cystidia using an optical micrometer. Clearly abnormal or immature spores were not included in the measurements.

The specimens were dried in a portable dryer within 48 hours after collection. Voucher herbarium specimens are deposited in the herbarium of the Museum of

Eastern Bohemia (HR) and personal herbarium of the author (herb. L.Z.). Major Czech herbaria (BRNM, CB, HR, PRC, PRM) were searched for herbarium material of both species during the second half of 2016. For herbarium acronyms, see Thiers (on-line). Herbarium labels in Czech were translated into English by the author. Data on the distribution in the Czech Republic based on herbarium records and literature is supplemented with codes of phytogeographical districts (Skalický 1988). Plant names follow Kubát (2002).

Abbreviations used: det. – identified by, leg. – collected by, M – Mesophyticum (Skalický 1988), n_{spec} – number of specimens from which the average value was calculated, Q – measured length/width ratio of spores, Q_{avg} – average Q values in individual specimens, rev. – revised by, T – Thermophyticum (Skalický 1988).

RESULTS AND DISCUSSION

Phlebia acanthocystis Nakasone & Gilbertson, Folia Cryptog. Estonica 33: 85, 1998 Figs. 1, 2

Macroscopic characters. Fresh basidiomata resupinate, effused, tightly adnate, ceraceous, hydnoid; subiculum and aculei base dirty ochraceous to livid, tips of aculei whitish, aculei up to 3 mm long, cylindrical to slightly tapering towards apex, margin sharply delimited, odour none, KOH reaction brown-red to vinaceous. Exsiccata tough coriaceous, ochraceous, colour unchanged.

Microscopic characters. Hyphal system in subiculum and subhymenium monomitic. Hyphae in subiculum tightly packed, agglutinated, individual hyphae difficult to discern, arranged more or less parallel to the substrate, only sparsely branched, smooth or partly incrusted with fine crystals, thin- to slightly thick-walled, 2–3 µm in diameter, septa with clamps. Hyphae in the core of aculei tightly packed, agglutinated, individual hyphae difficult to discern, only sparsely branched, smooth, thin-walled, 2–3 µm in diameter, septa with clamps. Hyphae in subhymenium tightly packed, agglutinated, individual hyphae difficult to discern, arranged more or less perpendicular to the surface, branched at sharp angles, smooth, thin-walled, 2.5–3.5 µm in diameter, septa with clamps, segments often short-celled.

Cystidia of hymenial origin, rare to abundant, narrowly fusiform, thin-walled, often with coralloid apex, 32–42 × 2.8–4 µm, projecting up to 18 µm. Terminal elements in the tips of the aculei cylindrical, 3–3.5 µm in diameter. Basidia narrowly clavate, clamped, tetrasterigmatic, 17.5–23 × 3–4.5 µm. Basidiospores ellipsoid, thin-walled, smooth, hyaline, with 1 or more guttules, inamyloid, indextrinoid,

non-cyanophilic, 3.5–5 × 2–2.5 µm (avg. 4.0–4.3 × 2.2–2.3 µm, n_{spec} = 2), Q = 1.5–2.3 (Q_{avg} = 1.8–2.0).

Taxonomic notes. Phylogenetically, *P. acanthocystis* is included in the core *Phlebia* clade, but distant from the generic type *Phlebia radiata* (Moreno et al. 2011, Kuuskeri et al. 2015). Of Central European species, *P. acanthocystis*, *P. nothofagi* (G. Cunn.) Nakasone, and young specimens of *P. fuscoatra* (Fr.) Nakasone have dull, pale-coloured fruitbodies compared to the usually brightly coloured *P. uda* (Fr.) Nakasone and *P. aurea* (Fr.) Nakasone. In addition, *P. nothofagi* has a distinctive odour, and older fruitbodies and exsiccatae of *P. fuscoatra* often blacken in contrast to *P. acanthocystis*. In micromorphology, the presence of leptocystidia with frequent coralloid apices is unique among hydnoid *Phlebia* species. However, these cystidia can be scarce, in which case the small ellipsoid spores may be used to identify *P. acanthocystis*.

Ecology. Little can be said of the ecology of the species in the Czech Republic since it is only known from two localities. Regarding the substrate, it was reported from wood of numerous angiosperms of several families in Hawaii (Gilbertson et al. 2002). In Europe, it was reported from *Cotoneaster salicifolius* and *C. delsianus* (Duhem 2008), *Quercus ilex* (Saitta et al. 2014), *Prunus avium* (Martini 2016), *Fagus sylvatica* and *Tilia* sp. (this study). In my experience, branches of *Fagus* and *Tilia* frequently host also other, more common species of hydnoid *Phlebia* species, such as *P. aurea*, *P. fuscoatra*, and *P. uda*.

The localities in the Czech Republic are different in character. The locality of Opičák at the periphery of the city of Liberec is a clay pit abandoned for decades with a wet microclimate, supporting a small fragment of herb-rich beech forest. In contrast, locality Na Voskopě in the Bohemian Karst is a mosaic of dry and warm thermophilous oak and oak-hornbeam forests on limestone. *Phlebia acanthocystis* appears to thrive in diverse habitats, being reported from Hawaii and mangrove forests (Maekawa et al. 2003).

Distribution. In the Europe *P. acanthocystis* was found in France (Duhem 2008), Switzerland (Martini 2016), and Italy (Saitta et al. 2014, Saitta & Losi 2016). Outside of Europe, it was reported from Hawaii (Nakasone & Gilbertson 1998), Japan (Maekawa et al. 2003), Argentina (Gorjón et al. 2012), Chile (Gorjón & Hallenberg 2012), and the island of Réunion (Duhem 2008). This is the first report from the Czech Republic and is also the northernmost record of the species. No additional specimens of *P. acanthocystis* have been found in any of the searched Czech herbaria.

Distribution in the Czech Republic. T: 8; M: 48b.

Fig. 1. Fruitbody of *Phlebia acanthocystis* (HR 103557). Scale bar = approx. 1 cm. Photo L. Zíbarová.

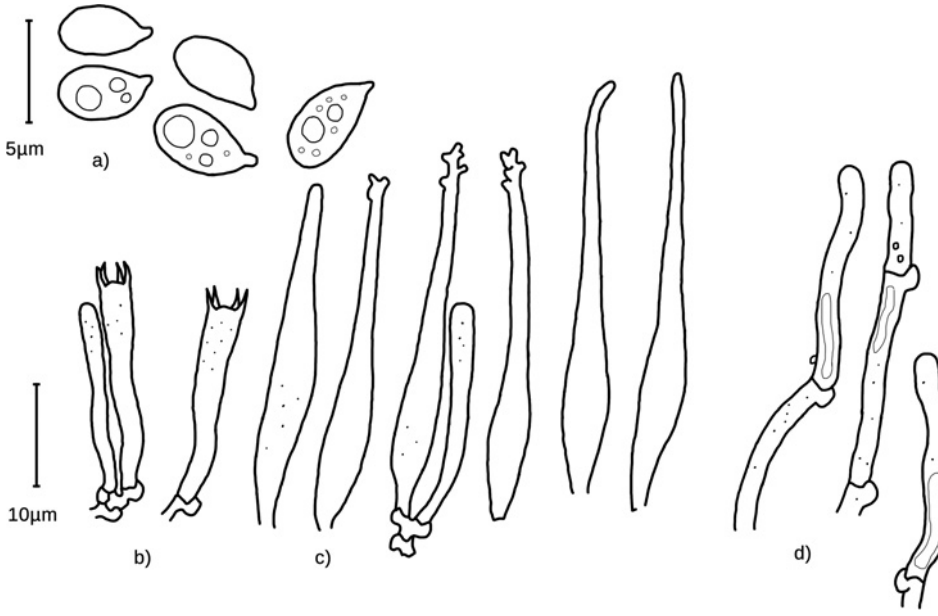
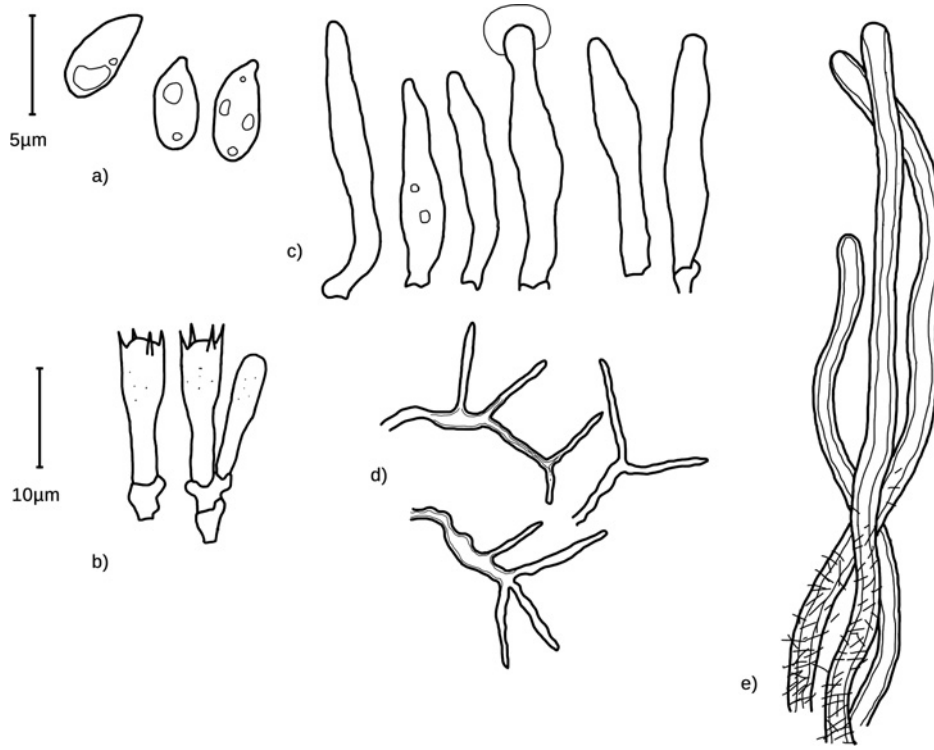


Fig. 2. Microscopic characters of *Phlebia acanthocystis* (HR 103557): **a** – spores, **b** – basidia, **c** – hymenial cystidia, **d** – terminal elements of aculei. Scale bar = 5 µm for spores, 10 µm for other elements. Del. L. Zíbarová.

Fig. 3. Fruitbody of *Phlebia bispora* (HR 103559). Scale bar = approx. 1 cm. Photo L. Zíbarová.



Specimens studied

Czech Republic. North Bohemia, Liberec (Liberec District), Opičák, former clay pit, 390 m a.s.l., small fragment of herb-rich beech forest, fallen branch of *Tilia* sp., 3 Jun 2014, leg. L. Zíbarová, J. Gaisler & I. Mráček, det. L. Zíbarová (herb. L.Z. 6370); ibid., 6 Oct 2014, leg. L. Zíbarová, J. Gaisler & I. Mráček, det. L. Zíbarová (HR 103557). – Central Bohemia, Bohemian Karst [Český kras] Protected Landscape Area, Suchomasty (Beroun District), Na Voskopě Nature Reserve, 455 m a.s.l., oak-hornbeam forest on limestone, fallen branch of *Fagus*, 5 Jun 2016, leg. L. Zíbarová & A. Lepšová, det. L. Zíbarová (herb. L.Z. 5718, HR 103560).

Phlebia bispora (Stalpers) Nakasone, Mycotaxon 81: 481, 2002 Figs. 3, 4

Synonyms:

≡ *Resinicium bisporum* Stalpers. – *Mycoaciella bispora* (Stalpers) J. Erikss. & Ryvarden. – *Mycoacia bispora* (Stalpers) Spirin & Zmitr.

= *Acia denticulata* (Pers.) Bourd. & Galz. sensu Cejp (1926) et Pilát (1930).

Macroscopic characters. Fresh basidiomata resupinate, effused, tightly adnate, ceraceous, hydnoid, subiculum and aculei ochraceous, subiculum up to 150 µm thick, aculei up to 5 mm long, cylindrical to slightly conical, margin sharply delimited, odour not noted, KOH reaction negative (brownish). Exsiccata toughly coriaceous, ochraceous, in parts darkening to dark brown.

Microscopic characters. Hyphal system in subiculum and subhymenium monomitic; dimitic in core of aculei; trimitic in basal area of aculei. Hyphae in subiculum arranged more or less parallel with substrate, tightly packed, H-connections frequent, hyaline, clamped, with numerous crystal rhomboid aggregates, 3–5 µm in diameter. Binding hyphae in base of aculei rare, originating from generative hyphae, branched up to 3 orders, thin- to slightly thick-walled, 1–3 µm in diameter. Skeletal hyphae in core of aculei originating from subiculum or base of aculei, arranged more or less parallel to aculei axis, unbranched, smooth or with fine rod-like crystals arranged perpendicular to hyphae, yellowish to brownish, variously thick-walled (walls up to 2 µm) but lumen always present, 4–5.5 µm in diameter, septa rare, clampless. Generative hyphae in core of aculei more randomly arranged, richly branched, smooth or with rhomboid crystal clusters, thin-walled, agglutinated, septa with clamps. Hyphae in subhymenium tightly packed, agglutinated, individual hyphae difficult to discern, arranged more or less perpendicular to surface, branched at sharp angles, H-connections frequent, smooth, thin-walled, 3–4 µm in diameter, septa with clamps, segments often short-celled.

◀ **Fig. 4.** Microscopic characters of *Phlebia bispora* (HR 103559): **a** – spores, **b** – basidia, **c** – hymenial cystidia, **d** – micro-binding hyphae, **e** – terminal parts of skeletal hyphae in tips of aculei. Scale bar = 5 µm for spores, 10 µm for other elements. Del. L. Zíbarová.

Cystidia of hymenial origin, rare to scattered, narrowly fusiform to cylindrical, thin-walled, apex obtuse, rarely with resinous cap, 24–42 × 3.5–5 µm, projecting up to 10 µm. Terminal elements in tips of aculei consisting of a mixture of generative and skeletal hyphae, not differentiated. Basidia narrowly clavate to clavate, clamped, with 4 sterigmata, 13.5–17 × 4–5 µm. Basidiospores ellipsoid to subcylindrical, thin-walled, smooth, hyaline, with 1 or more guttules, inamyloid, indextrinoid, non-cyanophilic, 4.3–5.5 × 2–3 µm (avg. 4.9 × 2.4 µm), Q = 1.7–2.3 (Q_{avg} = 2.0).

Taxonomic notes. The species has been placed in the genus *Mycoaciella* J. Erikss. & Ryvarden and differentiated from *Mycoacia* Donk by its dimitic hyphal system (Eriksson et al. 1978). However Nakasone (2002) synonymised *Mycoaciella* with *Phlebia*. Later Spirin & Zmitrovich (2004) transferred the species to *Mycoacia*. *Mycoaciella* is in the phlebioid clade (Moreno et al. 2011), but the taxonomy of *Phlebia* s.l. is still in flux, so I prefer keeping the species in *Phlebia*.

A hyphal system described as trimitic for micro-binding hyphae was reported in the subiculum of *Phlebia bispora* by Nakasone (2002). This is a unique feature among *Mycoaciella* species, but also inconspicuous and maybe therefore they were not mentioned in the descriptions by Bernicchia & Gorjón (2010), Eriksson et al. (1978), Große-Brauckmann (1983), and Karasiński et al. (2009). A confusing issue is that Bernicchia & Gorjón (2010) actually mentioned a trimitic hyphal system in their key. I observed micro-binding hyphae near the base of aculei but never in the subiculum between the aculei, which was consistently monomitic. The presence of bisporic basidia is variable, as they were not observed in my specimens nor by Nakasone (2002), although they were reported by others (Bernicchia & Gorjón 2010, Eriksson et al. 1978, Große-Brauckmann 1983).

There are four other species of *Mycoaciella* distributed in the tropics (Hjortstam & Ryvarden 2009). *Phlebia badia* (Pat.) Nakasone and *Mycoaciella dusenii* Hjortstam & Ryvarden lack clamps, whereas *M. brunnea* (Jülich) Hjortstam & Spooner and *M. hinnulea* (Bres.) Hjortstam & Ryvarden have clamped generative hyphae. *Mycoaciella brunnea*, known from the type locality only, differs from *Phlebia bispora* by lacking cystidia and having subglobose spores and smaller basidia, whereas *M. hinnulea* has larger spores [5.5–7(8) × 3–4 µm; Nakasone 2002].

A related species is *Phlebia pyrenaica* Duhem described from Mediterranean France, which has micro-binding hyphae but lacks skeletals (Duhem 2009). Macromorphologically, the fruitbodies of *P. bispora* are similar to those of the more common *Phlebia aurea* with its negative reaction to a KOH solution (Henrici 2002), but the latter species is easily separable by the absence of skeletal hyphae and its small, allantoid spores.

E c o l o g y. *Phlebia bispora* has been reported from wood and bark of various angiosperms (Nakasone 2002), in Europe *Arbutus unedo*, *Carpinus*, *Quercus*, *Phyllirea angustifolia* (Bernicchia & Gorjón 2010), *Alnus incana* (Ryvarden et al. 2003), *Alnus glutinosa* (Pilát 1926), *Fagus* (Cejp 1930), *Populus tremula* (?) (Karasiński et al. 2009), *Populus* sp., *Salix* sp. (Kriegelsteiner 2000), *Salix alba* (Große-Brauckmann 1983, this study) and *Fraxinus* (L. Hagara in litt.) but was also reported from *Pinus* by Hagara (2014). In my specimens, the underlying wood was decorticated and moderately decayed. The locality of Libický luh is one of the last more or less preserved fragments of riparian forest along the Elbe river in Bohemia which possess a rich mycobiota of lignicolous species (Zíbarová 2014).

D i s t r i b u t i o n. *Phlebia bispora* is widely distributed in Europe, being reported from Belgium, Denmark, France, Germany, Greece, Ireland, Italy, Macedonia, Norway, Poland, Switzerland, the United Kingdom (Bernicchia & Gorjón 2010), Austria (Hagara 2014), the Netherlands (NDFF & NMV on-line) and Slovakia (Pilát 1926), but also from outside Europe (Nakasone 2002, Hjortstam & Ryvarden 2009). In Germany it is known from ten states (Ostrow & Dämmrich 2010), and there are recent records of the species from Austria (Styria; Österreichische Mykologische Gesellschaft on-line) and Slovakia (Podunajská pahorkatina and Slanské vrchy hills; Hagara in litt.).

The first record from former Czechoslovakia was by Pilát (1926) [Matliare (= Tatranské Matliare in nowadays Slovakia), 1200 m a.s.l., fragment of wood of *Alnus glutinosa*]. The species was reported from the Czech Republic (as *Acia denticulata*) for the first time by Cejp (1930), but I have not been able to locate his collection in PRC nor PRM, and it is probably lost. The only specimen of *Acia denticulata* from Cejp's herbarium in PRC (unnumbered specimen from Iowa, USA) is in fact rather typical *Hyphodontia arguta* (Fr.) J. Erikss. Nevertheless Cejp's description fits the current concept of *Phlebia bispora* well.

Since Cejp's report (Cejp 1930), no other records of the species from the Czech Republic had been found nor are there any specimens deposited in Czech herbaria under the name *Phlebia bispora* or its synonyms. While corticioid fungi are not often collected by field mycologists, *P. bispora* is a conspicuous species, which could attract even non-corticiologists, so it may be reasonably concluded that it is rather rare in the Czech Republic. Therefore inclusion of *P. bispora* into next version of the Red list of macromycetes of the Czech Republic would be appropriate. I suggest classifying it into the Data Deficient category to raise awareness of the species.

Distribution in the Czech Republic. T: 11e.

Specimen studied

Czech Republic. Central Bohemia, Libice nad Cidlinou (Nymburk District), Libický luh National Nature Reserve, 190 m a.s.l., riparian willow-poplar forest, fallen trunk of *Salix alba*, 6 Oct 2013, leg. L. Zíbarová, det. L. Zíbarová (HR 103559).

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