Contribution to the knowledge of mycobiota of Central European dry grasslands: *Phaeoclavulina clavarioides* and *Phaeoclavulina roellinii (Gomphales)*

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The paper reports on the occurrence of *Phaeoclavulina clavarioides* and *P. roellinii* in dry grasslands of rock steppes in the Czech Republic. Occurrence in this habitat is characteristic of both species, formerly considered members of the genus *Ramaria*, and they are apparently the only known representatives within the *Gomphales* with this ecology in Central Europe. The authors present macro- and microscopic descriptions and provide rDNA barcode sequence data for both species based on material collected at localities in Bohemia.

Key words: Ramaria, rock steppes, description, ecology, Bohemia.

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Kříž M., Jindřich O., Kolařík M. (2019): Příspěvek k poznání mykobioty středo-evropských suchých trávníků: *Phaeoclavulina clavarioides* a *Phaeoclavulina roellinii* (Gomphales). – Czech Mycol. 71(2): 137–150.

Článek informuje o výskytu kuřátek kyjankovitých – *Phaeoclavulina clavarioides* a kuřátek stepních – *P. roellinii* v suchých trávnících skalních stepí v České republice. Pro oba druhy, dříve řazené do rodu *Ramaria*, je výskyt v tomto habitatu typický a jedná se zatím zřejmě o jediné známé zástupce řádu *Gomphales* s touto ekologií v rámci střední Evropy. Autoři předkládají makroskopický a mikroskopický popis a uveřejňují sekvence rDNA obou druhů na základě materiálu sbíraného na lokalitách v Čechách.

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INTRODUCTION

The mycobiota of dry grasslands of rock steppes in Central Europe is unique and different from other habitats due to its extrazonal xerothermic character. The first aggregate list of fungal species of this habitat indicated as Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*) including 30 species of various degrees of fidelity to this habitat within the area of the Czech Republic was published by Hofmeister & Hošek (2016). Continued monitoring of localities comprising this habitat has provided further interesting finds. In recent years, two members of the order *Gomphales* were found and identified, viz. *Phaeoclavulina clavarioides* and *P. roellinii*. Both are new to the mycobiota of the Czech Republic. The aim of this paper is to summarise information on all the collections known to us from the Czech Republic to date, supplemented with authentic descriptions, colour photographs and rDNA barcode sequences of both species.

MATERIAL AND METHODS

Macroscopic characters were studied on fresh fruitbodies collected in northern and central Bohemia by the first author. Microscopic mounts were made from dried material in ammoniacal Congo Red, 5% KOH solution, L4 solution and cotton blue in lactophenol and studied under Olympus CX21 and CX41 light microscopes with an oil-immersion lens at a magnification of $1000\times$. At least 30 randomly selected spores from each collection were measured (extremely large spores were omitted). Spore sizes are presented in the form of the main data range, complemented with minimal and maximal values in parentheses. Q_{av} is the average value of spore length and width ratios. Colour photographs in situ of all collections were taken.

Herbarium specimens have been deposited in the Mycological Department, National Museum, Prague (PRM) and in the personal herbarium of Oldřich Jindřich. Data on geological conditions were taken from maps and descriptions at www.geologicke-mapy.cz (Bokr on-line).

DNA from dried specimens was isolated as described by Holec & Kolařík (2017). ITS-LSU rDNA was amplified using primers ITS1F and LR6. The same primers, together with ITS4 and NL1, were used for sequencing (see Holec & Kolařík 2017 for details). Sequences obtained in this study have been entered into the EMBL database.

RESULTS

Phaeoclavulina clavarioides (Schild) Giachini, Mycotaxon 115: 190, 2011

Figs. 1–5

≡ Ramaria clavarioides Schild, Z. Mykol. 64(1): 60, 1998

Macroscopic characters. Fruitbodies 20–30 mm high, in upper part about 15 mm wide, but often forming small clumps with a total width of 20–30 mm. The larger part of the height of the fruitbody is formed by an unbranched stipe up to 20 mm high and 2–5 mm wide. The top of the fruitbody is firstly clavate (resembling some *Clavaria* species), afterwards flattened-palmate with a few short hornlike branches protruding radially from its circumference – initially almost horizontally (the entire fruitbody then often resembling rather a *Cantharellus* than a *Ramaria*) and later curling upwards when extended, longitudinally wrinkled when old. The branching occupies only the upper half of height also in mature fruitbodies. When observed from above, the fruitbodies have a more or less depressed centre. The tips of the branches are more or less blunt and sparse. The colour of the fruitbody is yellow-ochre to ochre with concolorous tips, brown-ochre when old, white in stipe base. Mycelium white. Smell absent, taste bitter. Macrochemical reactions: context turning saffron orange with 5% KOH (including the white stipe base) – dried specimen tested.

Microscopic characters. Basidiospores (5.2)6.0–8.5(10.0) \times 3.2–4.4 µm, Q = 1.50–2.27, Q_{av} = 1.90, drop-shaped, ellipsoid to subcylindrical with suprahilar depression (in some collections markedly concave on adaxial side and then suballantoid), but mostly enlarged towards apex, in side view sometimes almost narrowly pyriform (important feature), with distinct hilar appendix, ornamentation cyanophilic, echinate, with fine but distinct spines of often variable height, up to 0.5–0.7 µm. Basidia ca. 40–60 \times 6–8 µm, narrowly clavate, 4-spored, with basal clamp, sterigmata up to 6 µm long. Hyphal system monomitic. Rhizomorphs with clamped septa covered with polymorphous crystals of variable size but mostly small, up to 5–7 µm in diameter, hyphae ampullately swollen at septa (up to 6–7 µm broad). Globular cystidia on rhizomorphs present, yellowish, with finger-like protuberances, total width mostly 8–11 µm.

Material examined

Czech Republic. Bohemia. Vlastislav (North Bohemia, České středohoří Mts.), Plešivec hill, rock steppe, 20 Nov. 2016, leg. & det. M. Kříž, rev. O. Jindřich (PRM 945442; herb. Jindřich 43/2016). – Libochovany (North Bohemia, České středohoří Mts.), Kalvárie Nature Reserve, Kalvárie hill, rock steppe, 25 Dec. 2014, leg. & det. M. Kříž, rev. O. Jindřich (PRM 945440, EMBL accession no. LR723646, LR723645; herb. Jindřich 145/2014). – Žalhostice (North Bohemia, České středohoří Mts.), Radobýl Nature Monument, rock steppe, 18 Jan. 2014, leg. L. Tmej & P. Brůžek, det. J. Christan, rev. O. Jindřich (herb. Jindřich 1/2014); ibid., 20 Oct. 2015, leg. L. Zíbarová, det. O. Jindřich (herb. Jindřich 14/2015); ibid., 27 Oct. 2016, leg. & det. M. Kříž, rev. O. Jindřich (PRM 945441, EMBL accession no.



Fig. 1. Phaeoclavulina clavarioides, Plešivec hill, Vlastislav, České středohoří Mts., Czech Republic, 20 Nov. 2016 (PRM 945442). Photo M. Kříž.



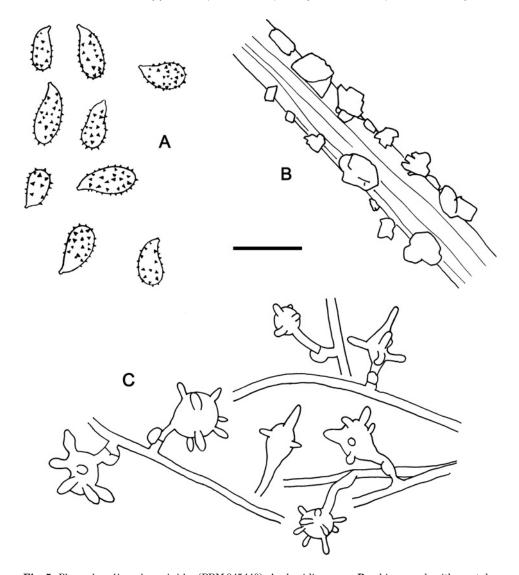
Fig. 2. Phaeoclavulina clavarioides, Kalvárie Nature Reserve, Libochovany, České středohoří Mts., Czech Republic, 25 Dec. 2014 (PRM 945440). Photo M. Kříž.



 $\textbf{Fig. 3.} \ Phaeoclavulina\ clavarioides, Zlončická rokle ravine, Zlončice, Czech Republic, 26 \ Dec.\ 2016 \ (PRM\ 945443). \ Photo\ M.\ Kříž.$



 $\textbf{Fig. 4.} \ Phaeoclavulina\ clavarioides, Zlončická rokle ravine, Zlončice, Czech Republic, 26 \ Dec.\ 2016\ (PRM\ 945443). \ Photo\ M.\ Kříž.$



 $\label{eq:Fig. 5. Phaeoclavulina clavarioides} \ (PRM\ 945440). \ {\bf A}-{\bf basidiospores}, \ {\bf B}-{\bf rhizomorph}\ {\bf with\ crystals}, \ {\bf C}-{\bf globular\ cystidia\ with\ finger-like\ protuberances\ on\ rhizomorphs}.\ Scale\ {\bf bar}=10\ \mu{\rm m}.\ {\bf Del.\ M}.\ {\bf K\'r\'iz}.$

LR723647; herb. Jindřich 44/2016). – Zlončice (Central Bohemia, Pražská plošina plateau), Zlončická rokle ravine, rock steppe, 26 Dec. 2016, leg. & det. M. Kříž, rev. O. Jindřich (PRM 945443; herb. Jindřich 41/2016). – Praha-Sedlec (Central Bohemia, Pražská plošina plateau), Podbabské skály rocks, rock steppe, 27 Nov. 2016, leg. & det. M. Kříž, rev. O. Jindřich (PRM 945444; herb. Jindřich 42/2016).

Phaeoclavulina roellinii (Schild) Giachini, Mycotaxon 115: 194, 2011

Figs. 6–10

≡ Ramaria roellinii Schild, Schweiz. Z. Pilzk. 56: 97, 1978

Macroscopic characters. Fruitbodies 20–35 mm high, 10–25 mm wide, conspicuously branched, starting already in the lower part (or the middle), branches oriented upward, crowded and longitudinally wrinkled, more distinctly in robust fruitbodies. Ends of branches with abundant minute acute points. Shape of the bifurcation in the form of a narrow letter U or V. Stipe 1–4 mm wide. Colour of the fruitbody ochraceous to watery ochre-brown, tips of branches concolorous or somewhat paler, ultimate base white. Mycelium white. Upper part of the fruitbodies seemingly somewhat dishevelled. Context whitish, unchanging when cut. Smell absent or faintly earthy, taste insignificant. Macrochemical reactions: context without reaction on 5% KOH, but hymenium distinctly darkening to vividly brown or chestnut brown – dried specimens tested.

Microscopic characters. Basidiospores (5.0)5.7–7.8(8.5) \times 3.0–4.3 µm, Q = 1.45–2.33, Q_{av} = 1.88, narrowly drop-shaped to cylindrical-ellipsoid with suprahilar depression, with distinct hilar appendix, ornamentation cyanophilic, delicately warty/echinate, with fine spines of 0.2–0.5 µm in height. Basidia 38–70 \times 5–7(8) µm, narrowly clavate, 4-spored, with basal clamp, sterigmata up to 6(–7) µm long. Hyphal system monomitic. Rhizomorphs with clamped septa and abundant stellate crystals (asterocystidia) of mostly 6–13 µm in diameter, hyphae markedly ampullately swollen at septa (5–12 µm broad).

Material examined

Czech Republic. Bohemia. Máslovice (Central Bohemia, Pražská plošina plateau), Máslovická stráň Nature Reserve, rock steppe, among mosses, 26 Dec. 2016, leg. & det. M. Kříž, rev. O. Jindřich (PRM 945446, EMBL accession no. LR723648; herb. Jindřich 45/2016). – Roblín (Central Bohemia, Pražská plošina plateau), village of Kuchařík, site named "Na skalkách", rock steppe, among mosses and grass, 22 Nov. 2016, leg. M. Kříž & P. Mikuš, det. M. Kříž, rev. O. Jindřich (PRM 945445, EMBL accession no. LR723649; herb. Jindřich 46/2016). – Praha-Radotín (Central Bohemia, Pražská plošina plateau), Cikánka I National Nature Monument, rock steppe, among mosses, grass and stones, 19 Nov. 2017, leg. J. Schneider, det. O. Jindřich (herb. Jindřich 23/2017).



Fig. 6. *Phaeoclavulina roellinii*, nearby village of Kuchařík, Czech Republic, 22 Nov. 2016 (PRM 945445). Photo M. Kříž.



 ${\bf Fig.~7.}\ Phaeoclavulina\ roellinii,\ Máslovická stráň\ Nature\ Reserve,\ Máslovice,\ Czech\ Republic,\ 26\ Dec.\ 2016\ (PRM\ 945446).\ Photo\ M.\ Kříž.$



Fig. 8. Phaeoclavulina roellinii, Máslovická stráň Nature Reserve, Máslovice, Czech Republic, 26 Dec. 2016 (PRM 945446). Photo M. Kříž.



 $\textbf{Fig. 9.} \ Phaeoclavulina\ roellinii,\ Máslovická stráň\ Nature\ Reserve,\ Máslovice,\ Czech\ Republic,\ 26\ Dec.\ 2016\ (PRM\ 945446).\ Photo\ M.\ Kříž.$

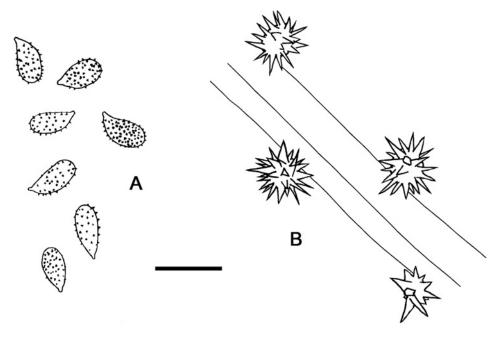


Fig. 10. Phaeoclavulina roellinii (PRM 945445, PRM 945446). $\bf A$ – basidiospores, $\bf B$ – rhizomorph with crystals (asterocystidia). Scale bar = 10 μ m. Del. M. Kříž.

DISCUSSION

Taxonomic position and similar taxa

The genus *Phaeoclavulina* Brinkmann is a molecularly well-supported clade in the order *Gomphales* (Giachini et al. 2010). Its ramarioid representatives were formerly included in the genus *Ramaria* Fr. ex Bonord., subgenera *Asteroramaria* Christan & C. Hahn, *Echinoramaria* Corner (e.g. in Jindřich 1999, Christan 2008) and partly in *Lentoramaria* Corner, depending on the classification of the respective authors. In addition, the genus *Phaeoclavulina* comprises some cantharelloid-gomphoid species (Giachini et al. 2010: 229), but none of them occur in Europe. The ramarioid species, such as *P. clavarioides* and *P. roellinii*, are morphologically distinct from *Phaeoclavulina* species described by Brinkmann, being bisporic with large spores and granular basidium content. In addition, the bootstrap support for the *Phaeoclavulina* clade was not ultimate (84/73), so this clade is not necessarily monophyletic and some of its lineages can be placed outside of it. But this study does not deal with the taxonomy of the

genus and since it is obvious that the former genus *Ramaria* is polyphyletic, we adopt the generic concept presented by Giachini et al. (2010).

Phaeoclavulina clavarioides is characteristic due to its small, poorly branched fruitbodies with bifurcations only in the upper part. When young it looks like a small Clavariadelphus with a tuberculate apical part. Concerning fungi from other genera, it may resemble primarily Clavulinopsis corniculata (Schaeff.) Corner, which also grows in open, grassy (but ± colder, mesophilic) habitats. The fruitbodies of C. corniculata are mostly larger and more conspicuously branched, branches are longer, thinner and of more fragile consistency, and the colour is more vividly yellow. Microscopically, it clearly differs in its smooth globose basidiospores. Ramariopsis luteoochracea (Cavara) R.H. Petersen has much thinner fruitbodies and grows in deciduous woods and unfertilised meadows. Phaeoclavulina quercus-ilicis (Schild) Giachini, occurring in Mediterranean maquis, is a somewhat questionable species. Although it has been described by the same author and in the same work as in the case of *P. clavarioides* (Schild 1998), we admit the possibility that both species are identical. Although Schild mentions broader spores [(2.8)3.2–5.4(5.6) µm] in the original description of P. quercusilicis, the investigation of the holotype performed by Christan (2008: 175) revealed a spore width of only 4.3 µm, which is in agreement with *P. clavarioides*. In spite of this, Christan maintained Schild's original but confusing measurement for keying out P. quercus-ilicis. Neither Giachini acceded to synonymisation and recombined both species into the genus Phaeoclavulina (Giachini & Castellano 2011). Franchi & Marchetti (2001) classified P. clavarioides in section Flaccidae and P. quercus-ilicis in section Grandisporae, subgenus Echinoramaria, of the genus Ramaria. ITS and LSU rDNA sequences of both studied specimens of Phaeoclavulina clavarioides (PRM 945440, PRM 945441) were identical. The ITS barcode sequence had 99.5% similarity with an unpublished, and thus doubtful, P. quercus-ilicis sequence with accession number AJ408382 (voucher MA-Fungi 47984, Spain), suggesting also that both species are identical. Out of other species, P. argentea (R.H. Petersen) Giachini (JQ408234, unpublished, 84% similarity) had the highest similarity. For LSU rDNA, the best hit belonged also to P. argentea (JQ408231, unpublished, 96% similarity).

Phaeoclavulina roellinii is a species similar to P. flaccida (Fr.) Giachini with almost identical, on average only slightly longer spores ($Q_{av} = 2.0$; Christan 2008, as $Ramaria\ flaccida$). The latter differs especially by its growth in forests. $Phaeoclavulina\ roellinii$ is distinguished from P. clavarioides by its standard ramarioid (not clavarioid) appearance, faint (not bitter) taste, and, microscopically, stellate (not polymorphous) crystals on the rhizomorphs. $Phaeoclavulina\ murrillii$ (Coker) Franchi & M. Marchetti, a species not known from the Czech Republic yet, differs from both P. roellinii and P. clavarioides by its hirsute stipe base without rhizomorphs, somewhat divergent spore size, and in addition more

golden-yellow to orange branch tips. It is also a species growing outside of woods but not on rock steppes. According to Christan (2008, as *Ramaria murrillii*) it occurs on nutrient-poor heather meadows. The ITS sequences of both studied specimens of *Phaeoclavulina roellinii* (PRM 945446, PRM 945445) were identical and showed the highest similarity to sequences deposited as *P. flaccida* (Fr.) Giachini (AJ408390, unpublished, 94%) and *P. abietina* (Pers.) Giachini (JN649369, Sjökvist et al. 2012, 94%).

Ecology and distribution

Phaeoclavulina clavarioides was described by Schild (1998) based on a collection from a pine forest in Sardinia, Italy. The distribution of this species is unknown to us – except for Italy and now the Czech Republic information is missing. Also Phaeoclavulina roellinii was described by Schild (1978), namely from a dry place with mosses near Geneva, Switzerland. Krieglsteiner (2000) reports this species from neighbouring Germany and mentions three finds in Baden-Württemberg, but the fruitbodies on a photo from Ostalb represent P. murrillii (Christan, pers. comm.). Dämmrich et al. (2016) regard P. roellinii an extremely rare species in Germany. It is also reported from the Netherlands (https://waarneming.nl/species/597879/), Sweden (Knutsson 2014), Denmark and Italy (Petersen 1981). Mueller et al. (on-line) state unconfirmed records from some other countries.

The localities of both species in the Czech Republic are predominantly narrow-leaved dry grasslands without significant occurrence of orchids. This habitat is a component of the Semi-natural dry grasslands and scrubland facies on calcareous substrates habitat in terms of the Natura 2000 classification (Chytrý et al. 2010). In a list of fungal species of this habitat (Hofmeister & Hošek 2016), representing an aggregate of mycobiota of particular related habitats, in particular broad-leaved dry grasslands host a partly different range of species than narrowleaved dry grasslands. For example, Clavaria fragilis Holmsk., Clavulinopsis corniculata and Entoloma incanum (Fr.) Hesler are species more typical of broad-leaved dry grasslands and also mesic meadows or even grasslands in submountainous areas. Compared to that, the localities of Phaeoclavulina clavarioides and P. roellinii are xeric rock steppes, representing Central European extrazonal vegetation. The south- and southwest-facing slopes in the warmest and driest area in Bohemia are covered by thermophilous flora with typical steppe plant communities and host also very valuable insect fauna with many rare species, especially beetles and butterflies. These preserved relics of natural communities of Central European continental steppes have extraordinary importance from the perspective of nature conservation and can be regarded as a treeless analogue of old-growth forests. Other interesting macromycetes occurring together with one of the discussed species in the same habitat are e.g. *Clitocybe collina* (Velen.) Klán, *Mycena chlorantha* (Fr.) P. Kumm., *M. pseudopicta* (J.E. Lange) Kühner, and, characteristically, many rare gasteromycetes such as *Calvatia fragilis* (Vittad.) Morgan, *Geastrum floriforme* Vittad., *G. pouzarii* V.J. Staněk (see e.g. Kříž 2015b), and *Tulostoma pulchellum* Sacc. (see e.g. Kříž 2015a). In the case of Kalvárie Nature Reserve, the fruitbodies of *P. clavarioides* grew directly next to *Geastrum campestre* Morgan.

For the time being, we do not know any locality where both discussed species occur together. Concerning the geological bedrock, the localities of *P. clavarioides* are mainly of volcanic origin constituted by various types of basalt subsoil: olivinic nephelinite, analcimite and leucitite or alkalic olivinic basalt, basanite and limburgite, but some of them are of Palaeozoic to Proterozoic age: greenschist or greywacke, siltite and possibly also slate. The bedrock of the currently known localities of *P. roellinii* was developed in the Palaeozoic: limestone or basalt, andesite-basalt and tuffs.

We propose including both *Phaeoclavulina clavarioides* and *P. roellinii* into the next edition of the Red list of macromycetes of the Czech Republic.

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