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Basidiolichen *Multiclavula corynoides* new to the Czech Republic and a remarkable occurrence of *Multiclavula mucida* on soil

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This paper reports the first occurrence of basidiolichen *Multiclavula corynoides* (*Agaricomycetes*, *Clavulinaceae*) in the Czech Republic, collected in the Šumava Mountains near Železná Ruda. Additionally, we discuss in detail a remarkable find of *M. mucida* growing atypically on soil in the Šumava foothills. In Europe, three species of *Multiclavula* are known: *M. corynoides*, *M. mucida*, and *M. vernalis*. *Multiclavula mucida* is traditionally considered a lignicolous species, typically found on decaying wood in old-growth forests, while *M. corynoides* is known to inhabit bare soil, often in association with bryophytes. *Multiclavula vernalis*, a boreo-montane terricolous species, has not yet been recorded in the Czech Republic. The identity of both specimens was confirmed by the nrITS DNA barcode. The discovery of *M. mucida* on soil is particularly surprising, as substrate type – wood versus soil – often serves as a crucial criterion for distinguishing the species and *M. corynoides* in widely used identification keys. The paper further discusses the macroscopic and microscopic characteristics of European *Multiclavula* species, highlighting small inconsistencies in diagnostic features, such as basidiocarp colour and spore dimensions, in various sources. Finally, we provide an identification key to these species.

Key words: DNA barcoding, identification key, Šumava Mountains.

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Malíček J., Janošík L., Mikulášková E., Palice Z. (2025): Bazidiolišejník *Multiclavula corynoides* poprvé zjištěn v České republice a pozoruhodný výskyt *Multiclavula mucida* na půdě. – Czech Mycol. 77(1): 27–36.

Tento článek informuje o prvním zaznamenaném výskytu bazidiolišejníku *Multiclavula corynoides* (*Agaricomycetes*, *Clavulinaceae*) v České republice; druh byl sbírán na Šumavě v blízkosti Železné Rudy. Dále se podrobně zabýváme pozoruhodným nálezem druhu *M. mucida* rostoucího atypicky na

půdě v šumavském podhůří. V Evropě jsou známi tři zástupci rodu *Multiclavula*: *M. corynoides*, *M. mucida* a *M. vernalis*. Tužnatka slizká (*M. mucida*) je tradičně považována za lignikolní druh, který se obvykle vyskytuje na tlejícím dřevě ve starých lesních porostech, zatímco *M. corynoides* je známa jako druh porůstající obnaženou půdu, často v asociaci s mechorosty. *Multiclavula vernalis*, boreo-montánní terikolní druh, nebyl v České republice dosud zaznamenán. Určení obou exemplářů bylo potvrzeno pomocí nrITS DNA. Překvapivý je zejména nález *M. mucida* na půdě, neboť typ substrátu (dřevo versus půda) často slouží jako klíčový znak pro odlišení tohoto druhu od *M. corynoides*. Příspěvek se dále zabývá makroskopickými a mikroskopickými charakteristikami evropských zástupců rodu *Multiclavula* a upozorňuje na drobné nesrovnalosti v diagnostických znacích (barva plodnic, rozměry spor) v různých zdrojích. Na závěr uvádíme určovací klíč k těmto druhům.

Introduction

Three *Multiclavula* species are known in Europe: *M. corynoides*, *M. mucida*, and *M. vernalis*. They are characterised by a gelatinous to granular, more or less green thallus from which the fruitbodies, basidiocarps, grow. These are simple to slightly branched, up to 2.5 cm high, and white, yellowish, pinkish to orange in colour. The fruitbodies are annual and form seasonally during summer and autumn (Stenroos et al. 2016). The photobiont genus *Elliptochloris* (Sanders & Masumoto 2021) is found only in the thallus. An interesting feature is the presence of asexual reproduction by means of so-called bulbils, which are ± spherical formations containing a mycobiont and a photobiont, which are formed on the thallus surface (Poelt & Obermayer 1990). At first glance, representatives of this genus resemble non-lichenised fungi of the genera *Clavulina* (Rico & Barrasa 2011), *Clavulinopsis*, and *Clavaria* (Petersen 1967). It is therefore not surprising that their occurrence is often recorded by mycologists.

Multiclavula corynoides, described from New York State, USA, was established as the type species of the genus. It is characterised by yellowish, straw-coloured to pinkish fruitbodies (Petersen 1967). However, in dry seasons and in herbarium material they may change colour to orange (Stenroos et al. 2016). Towards the apex they tend to be subspathulate or laterally compressed. The basidia produce 4–6 spores (Petersen 1967). The lichen grows on bare (sandy) soil, often in association with bryophytes, or peat, e.g. in moist road cuts, gravel pits, and peat bogs (Høiland 1987, Brodo et al. 2001, Stenroos et al. 2016). Its distribution range covers the Holarctic realm, where it is most commonly found in the boreal zone (Nimis et al. 2018). Based on the GBIF database, most of its European records come from Fennoscandia and the Alps (GBIF on-line a). The species has not yet been reported from the Czech Republic.

Multiclavula mucida is a lichen with white to cream-coloured fruitbodies which are sometimes ochre to brown at the tips. The basidia produce four to most commonly six spores (Wirth et al. 2013, Nimis et al. 2018). The species grows on soft decaying wood usually of rather thick logs, but it can also occur on

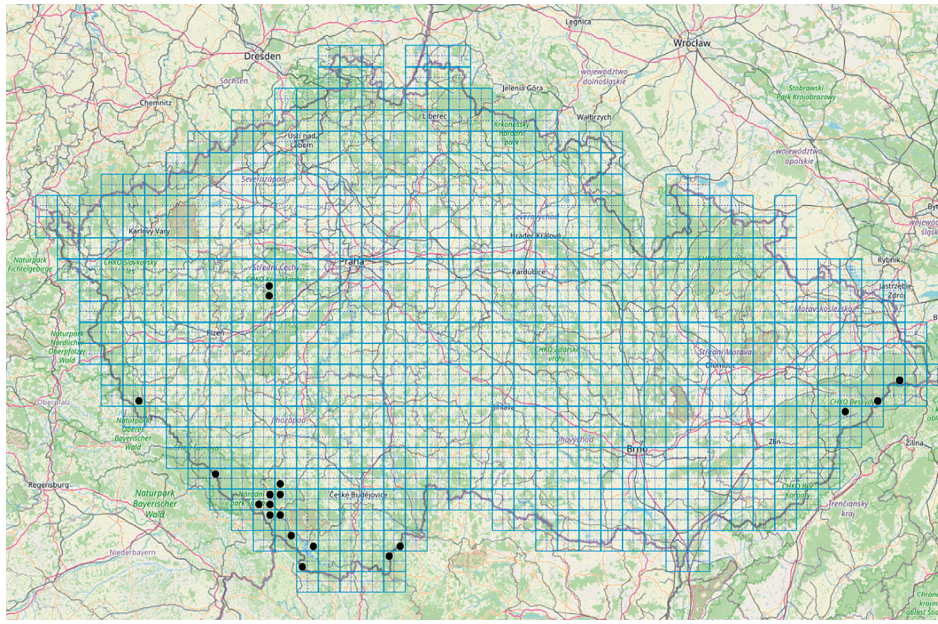

Fig. 1. Distribution of *Multiclavula mucida* in the Czech Republic, based on the DaLiBor database (6 May 2025).

stumps. It prefers old-growth forest stands (Stenroos et al. 2016), and in the Czech Republic, it is mainly associated with beech forests (Malíček et al. on-line), less often with spruce forests (personal observations). Nevertheless, *M. mucida* can also occur in humid managed forests with coarse deadwood debris, such as large stumps (Malíček et al. 2010). It is a widespread sub-cosmopolitan species extending to South America and Australia (Petersen 1967). In the Northern Hemisphere, *M. mucida* mostly inhabits temperate and boreal forests. In the Czech Republic, it is most common in foothill and mountain areas. It has so far been recorded in the Šumava Mts, Křivoklát region, Český les Mts, Novohradské hory Mts, and Beskydy Mts (Malíček et al. on-line; Fig. 1).

Multiclavula vernalis usually forms unbranched, at first cylindrical, soon becoming club-shaped or even spoon-shaped basidiocarps, which are creamy, yellowish to red-orange in colour. The basidia mostly bear four spores (Petersen 1967, Høiland 1987, Watling 2009, Stenroos et al. 2016). It grows on bare, acidic, sandy to humus-rich, sometimes peaty soil in humid areas (Wirth et al. 2013, Stenroos et al. 2016, Nimis et al. 2018). The European records of *M. vernalis* mainly originate from Fennoscandia, the Netherlands, Great Britain, and the Alps (GBIF on-line b). In Central Europe, it is a very rare high-mountain species which has not yet been found in the Czech Republic.

During a meeting of young mycologists in 2024 near Černá v Pošumaví, Šumava foothills, L. Janošík and A. Jirsa discovered a population of *Multiclavula* growing on soil by a forest path. Based on its ecology, the species was presumed to be *M. corynoides*, which had not previously been reported from the Czech Republic. Almost two decades earlier, E. Loskotová (now E. Mikulášková) had brought a terricolous *Multiclavula* specimen from the Šumava Mts to the attention of the senior author (ZP) for further study. It was tentatively identified as *M. corynoides* but not studied in detail and never published. Both specimens have recently been sequenced, the results of which are presented below.

MATERIAL AND METHODS

Herbarium specimens are stored in the herbaria of the Faculty of Science of Charles University in Prague (PRC) and the Institute of Botany of the CAS in Průhonice (PRA). Living spores from the freshly collected material were measured in tap water at 1000× magnification. Values are given in the format (min)1st decil–median–9th decil(max). DNA was isolated from the fruitbodies using the ISOLATE II RNA Plant Kit (Bioline) according to the manufacturer's protocol. ITS and mitochondrial SSU barcodes were amplified according to Malíček et al. (2020). For the recent collection, primers ITS1F (Gardes & Bruns 1993) × ITS4 (White et al. 1990) were used. However, this combination was not successful for amplification of the older collection, where the combination of primers ITS1-F_KYO2 × ITS4_KYO2 (Toju et al. 2012) worked. Attempts to amplify mtSSU using the primers mrSSU1 × mrSSU3R (Zoller et al. 1999) failed. The sequences obtained (Tab. 1) were compared with previously published sequences from the GenBank database using the standard nucleotide BLASTN algorithm (Altschul et al. 1990). The field records were uploaded to the DaLiBor database (Man et al. 2022).

Tab. 1. Sequenced specimens and their NCBI accession numbers

Species	Voucher	nrITS
Multiclavula corynoides	PRA 22521	PV706826
Multiclavula mucida	PRA-Malíček 17053	PV706827

RESULTS

Although the spore dimensions of the recently collected *Multiclavula* specimen from bare clay soil – measuring (6.3)6.8–7.6–8.6(9.7) × (2.5)3.2–3.6–3.9(4.8) µm (N = 40) – were slightly larger than those previously reported in the literature (Tab. 2) and more closely resembled *M. corynoides* as described by Rico & Barrasa (2011) and Voitk (2017), ITS revealed the specimen to belong to *M. mucida*. The NCBI Blast search showed a sequence similarity of 98.3–99.8% with 12 sequences from the GenBank database.

Fig. 2. *Multiclavula corynoides*, Pamferova huť near Železná Ruda, Šumava Mts, 1 July 2006. Photo E. Mikulášková.

By contrast, the herbarium specimen from 2006 was identified as *M. corynoides*, based on its ecology, characteristic orange tinge and partly flattened basidiocarps. Its identification was confirmed by ITS, showing 99.7% and 98.6% similarity, respectively, with sequences from the USA (ON408375) and Iceland (U66440) in the GenBank database.

Specimens examined

Multiclavula corynoides (Fig. 2)

Czech Republic. Western Bohemia, Šumava Mts, Železná Ruda, Pamferova huť, sandpit 0.7 km NE–NNE of the settlement, alt. ca. 900 m, 49°09'14" N, 13°15'48" E, on bare sandy soil (associated with *Pogonatum aloides*, *Pohlia nutans*), 1 Jul 2006, leg. E. Loskotová (PRA 22521) [only a few basidiocarps present].

Multiclavula mucida (Fig. 3)

Czech Republic. Southern Bohemia, Černá v Pošumaví, road margin in mixed forest (*Fagus sylvatica*, *Picea abies*), on bare clay soil among bryophytes (*Scapania irrigua*, *Atrichum undulatum*, *Pohlia* sp.), alt. 751 m, 48°43'57.0" N, 14°07'23.6" E, 18 Oct 2024, leg. L. Janošík & A. Jirsa (PRC, dupl. in PRA-Malíček 17053).

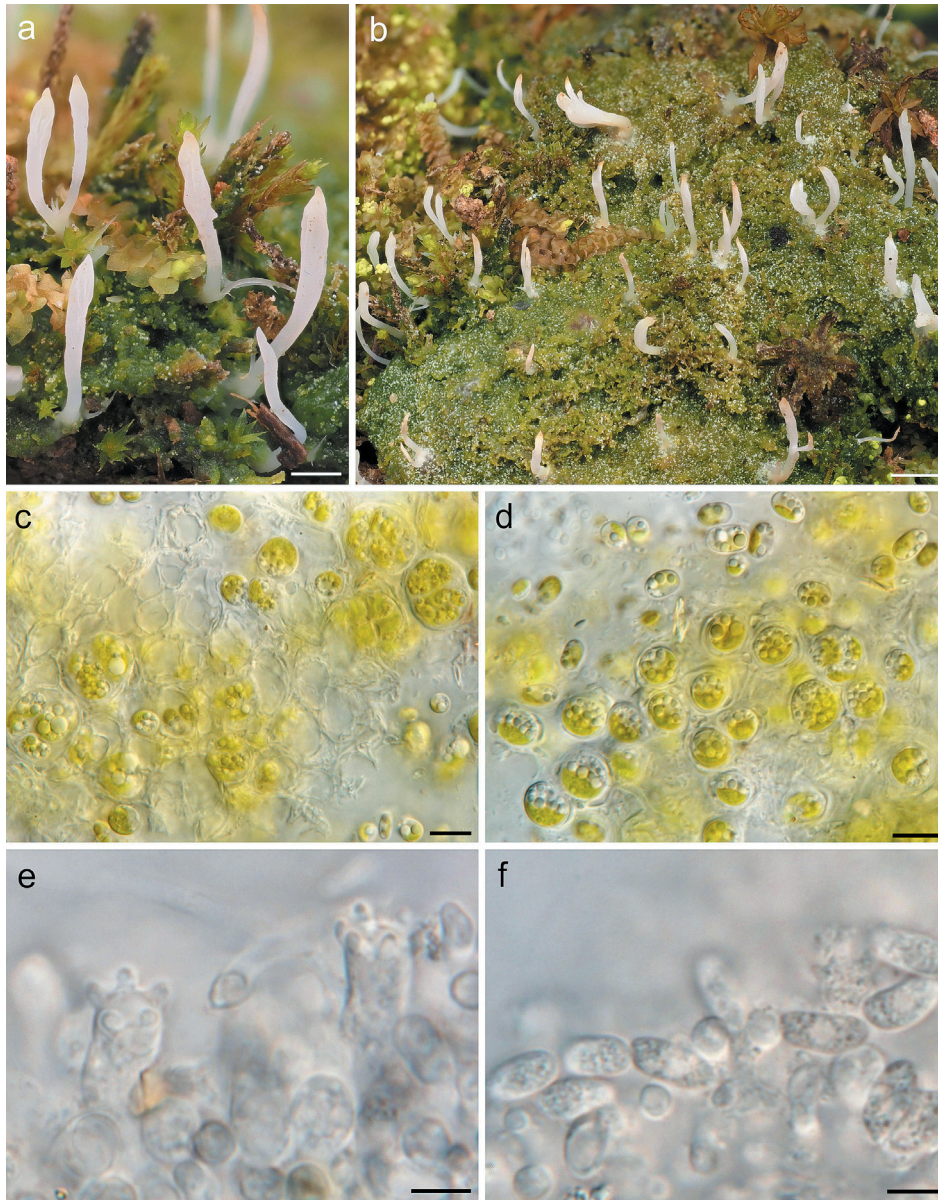

Fig. 3. *Multiclavula mucida* collected on soil near Černá v Pošumaví (PRC). **a, b** – habitus in situ; **c, d** – photobiont cells with abundant vesicles of *Elliptochloris* cf. *subsphaerica* from the thallus, including autospores (oval cells in the upper part of d); **e** – basidia with 5 and 6 sterigmata; **f** – spores. Scale bars: a = 2 mm; b = 5 mm; c, d = 10 µm; e, f = 5 µm. Photo L. Janošík.

Discussion

The possible occurrence of *M. mucida* on soil seriously complicates identifications of terricolous *Multiclavula* species, since *M. corynoides* and *M. mucida* are often distinguished by substrate in practice (e.g. Høiland 1987, Brodo et al. 2001, Rico & Barrasa 2011, Nimis 2025). On the other hand, the occurrence of *M. mucida* on other substrates than wood is not a novelty in lichenology – two terricolous records of this species were reported by Stofer et al. (2019) from Switzerland, one from the Polish Tatra Mountains (Wilk et al. 2025), and there is even an old report on loamy forest soil near Letovice in Moravia, Czech Republic (Niessl 1865, as *Clavaria mucida*), which however needs revision. The species may also grow on turf or peaty soil in peatbogs (Z. Palice, unpublished observation). Besides, a lignicolous population was also found at the same locality near Černá v Pošumaví, which looked suspicious from the beginning.

Furthermore, key characters of both species are not reported in a completely consistent manner across the literature. For example, the basidiocarp colour for European populations of *M. mucida* is reported to be creamy white (e.g. Nimis 2025), but Brodo et al. (2001) reported dark yellow to orange basidiocarps for American populations. Whereas the spore dimensions of *M. mucida* reported in the literature are 4.5–8 × 2–3.5 µm (Tab. 2), those of *M. corynoides* are more variable, from 5–8.5 × 2–3.5 µm (Petersen 1967, Høiland 1987, Brodo et al. 2001, Nimis 2025) up to 6–10.5 × (2)2.5–3.5(5) µm (Rico & Barrasa 2011, Voitk 2017). Our spore measurements slightly exceed the upper limit reported for *M. mucida* and fill the interval reported by Rico & Barrasa (2011) and Voitk (2017) for *M. corynoides*. The inconsistencies in spore dimensions may be caused by non-standardised measurements. These should be made on mature basidia with well-developed, flattened sterigmata bearing mature basidiospores, which are often impossible to detect in limited material (Voitk 2017). Measurements may be biased if they include a high proportion of immature basidiospores. The differences in measurements may also reflect the used mounting media and the condition of the studied material (fresh or rehydrated herbarium material), as these factors are known to strongly influence spore dimensions in other groups of fungi (Baral 1992).

The third species occurring in Europe, *M. vernalis*, is well distinguishable from the two previous taxa due to its unbranched, clavate, robust and pigmented (creamy to red-orange) fruitbodies with a ± sharply delimited, short cylindrical pale stalk (e.g. Voitk 2017, Masumoto & Degawa 2020). Its basidia are 2–4-spored compared to (4–)6-spored ones in *M. corynoides* and *M. mucida*. Moreover, the spores of *M. vernalis* are slightly larger, 8–12 × 2.5–3.5 µm (e.g. Petersen 1967, Voitk 2017). Unfortunately, the characters of basidia and spores are difficult to observe, as are the macroscopic characters on herbarium material. Therefore, we recommend confirmation by DNA for problematic material.

Tab. 2. Overview of spore dimensions (µm) in European *Multiclavula* species published in the literature.

Reference	*M. corynoides*	*M. mucida*	*M. vernalis*
Petersen 1967	5.6–8.2 × 2.1–3.5	4.5–7.7 × 1.8–3.2	8–12 × 2.5–3.5
Høiland 1987	(5)5.5–7.5(8) × (2)2.5–3	4.5–7.5 × 2–3	(6)6.5–9.5 × 2–3.5
Brodo et al. 2001	5–8 × 2–3.5	–	–
Watling 2009	–	–	8–10 × 2.5
Rico & Barrasa 2011	(6.5)7–10 × (2)2.5–3.5	(4.5)5–7.5(8) × 2–3	6–10(12) × 2.5–3.5
Wirth et al. 2013	–	5.5–6.5(8) × 2–3	5–10(12) × 1.5–3.5
Stenroos et al. 2016	6.5–8.5 × 2.5–4	4.5–7.5 × 2–3	8–12 × 2.5–3
Voitk 2017	5.8–10.1 × 2.8–5.2	5.8–8.7 × 1.9–2.9	6.7–12.4 × 2.5–3.4
Masumoto & Degawa 2020	–	–	(6.7)7.8–9.5(10.6) × (2.8)3.4–4(5)
Nimis 2025	5–8.5 × 2–3.5	(4.5)5.5–6.5(8) × 2–3.5	(5)8–10(12) × 1.5–3.5

Identification key to European *Multiclavula* species

1a Basidiocarps simple, robust, clavate, cream-coloured to red-orange, basidia with (2–)4 spores, spores (5)8–12 µm long; on bare or peaty soil ..... ***M. vernalis***

1b Basidiocarps simple to branched, cylindrical to clavate or compressed, white, yellowish or pinkish, basidia with 4–6 spores, spores 4.5–8.5(10.5) long 2

2a Basidiocarps white to cream-coloured, sometimes ochre to brownish at the tips, horn-like at the tips; on decaying wood in forests, rarely terricolous at humid sites ..... ***M. mucida***

2b Basidiocarps yellowish to pinkish, often compressed, tips blunt to fairly acute; on bare or peaty soil at open sites ..... ***M. corynoides***

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GBIF (on-line b): *Multiclavula vernalis* (Schwein.) R.H.Petersen. GBIF Backbone Taxonomy. – https://www.gbif.org/species/2555271 [accessed 29 May 2025]

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