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Hidden diversity: noteworthy cortinarioid fungi (Cortinarius s. l.) from relict deciduous forests of Strandzha Mountain, Bulgaria

***Boris Assyov*[1*], *Tzenka Radoukova*[2]**

[1]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Str., 1113 Sofia, BULGARIA

[2]University of Plovdiv, Faculty of Biology, Department of Botany and Biological Education, BULGARIA

*Corresponding author: contact@boletales.com

Abstract. The paper communicates interesting findings of cortinarioid fungi from relict deciduous forests in Strandzha Mt. (Bulgaria). Five species were revealed based on the above-ground collecting approach coupled with DNA-barcoding, targeting the nrITS region. These are *Calonarius albertii*, *Ca. violaceipes*, *Cortinarius pseudocollinitus*, *Phlegmacium perpallens*, and *P. scaurocaninum*. They are recorded for the first time from Bulgaria and represent their first molecularly supported findings in Southeastern Europe. The collections are illustrated and commented on, and brief morphological data are provided.

Key words: Balkan mycota, Bulgarian fungi, Cortinariaceae, DNA-barcoding, ITS.

Introduction

Cortinarioid fungi, i. e., the taxa formerly united in the genus *Cortinarius* s. l. are known for their vast richness, estimated to encompass over 2000 described species worldwide (Brandrud et al., 2018; Kibby & Tortelli, 2021). While the evaluation of the real diversity in Europe is still ongoing, Szabó et al. (2023) mentioned about 550 species registered in Norway alone, and Kuyper et al. (2024) treated over 280 species in Flora Agaricina Neerlandica, and undescribed species are frequently discovered (see e. g., Bidaud et al., 2021; Bellanger et al., 2022; Kuyper et al., 2024; Forejt & Vašutová, 2025). Due to this striking richness and their morphological variability, the identification of cortinarioid taxa is notoriously difficult (Calledda et al., 2021), particularly when a morphological approach is employed alone. The use of molecular techniques in studies of cortinarioid fungi became widespread in the last two decades, facilitating the resolution of the taxonomy of the group (see e. g. Liimatainen et al., 2014, and references therein).

The diversity of cortinarioid fungi in Bulgaria is far from exhaustively studied with some 114 species recorded up to date (Denchev & Assyov, 2010; Dima et al., 2014; Liimatainen et al., 2014; Bellanger et al., 2022; Assyov & Slavova, 2023), albeit the country has already proven to be attractive ground for cortinariological research providing type materials for the description of several new species, including *Cortinarius habros* Bojantchev et al., *C. uraceomajalis* Dima et al., *Cortinarius uraceonemoralis* Niskanen et al., and *Phlegmacium balteatibulbosum* (Kytöv et al.) Niskanen & Liimat., to name but a few (Dima et al., 2014; Liimatainen et al., 2014; Bellanger et al., 2022). The use of the DNA-barcoding approach also allowed the recovery of some previously unknown cortinarioid taxa for the Bulgarian mycobiota (Liimatainen et al., 2014; Assyov & Slavova, 2023). Despite this, profound molecularly backed knowledge on *Cortinarius* and the related genera in Bulgaria is apparently yet to develop.

Strandzha (or in Turkish "Istranca" or "Yıldız") is a moderately high mountain massif situated in South-eastern Bulgaria and North-western Turkey (Thrace). It is a known hotspot of biodiversity, harbouring representative examples of the Tertia-

ry relict flora in Europe (Turrill, 1929; Milne & Abbott, 2002; Asenov, 2006). An important refugium, Strandzha Mt. shelters relict oak forests (*Quercus* spp.) and also a considerable part of the very few Oriental beech (*Fagus orientalis* Lipsky) forests in Europe (Ważny et al., 2014). Both types of forests are characterised by the presence of numerous relict evergreen woody species, such as *Calluna vulgaris* (L.) Hull, *Daphne pontica* L., *Erica arborea* L., *Ilex colchica* Pojark., *Prunus laurocerasus* L., *Rhododendron ponticum* L., *Vaccinium arctostaphylos* L., as well as herbaceous euxinian plants (see e. g., Gussev, 2015; Gussev & Tzonev, 2015).

Long time under access restrictions as a border area in the past, the Bulgarian part of Strandzha Mt. has not been exhaustively studied from a mycological point of view, as this could be inferred from relevant publications (Denchev & Petrova, 2005; Denchev & Assyov, 2010; Lacheva & Radoukova, 2025). Moreover, the cortinarioid fungi are even less studied in the area, with merely six taxa on record (Denchev & Petrova, 2005; Denchev & Assyov, 2010; Lacheva & Radoukova, 2025). The first author had the opportunity to visit several areas of the Bulgarian part of the mountain during two field trips, which provided some interesting specimens of cortinarioid fungi. They are presented and discussed in this work.

Materials and Methods

Fungi were photographed and documented in the field, and wrapped individually in aluminum foil for transport. The alkali reaction was tested upon return indoors within a few hours with KOH 10%. The materials were further air-dried in a food dehydrator at 40°C for 24 h. Voucher specimens are deposited in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF). UV365 and UV395 were tested on dried samples. The geographical coordinates cited throughout the text refer to the WGS84 system and are approximated as far as possible from anchor points taken on route.

The microscopic study was performed under an AmScope T360B light microscope equipped with an AmScope MU900 digital camera. The microscopic observations were held on preparations from dried material, mounted in KOH 5%. Basidiospores were obtained from deposits on the velar remnants (Kibby & Tortelli, 2021) and measured with ornamentation included. From each collection, 30 random, but visibly normally developed spores were assessed, while abortive or malformed spores were excluded. All measurements were read on pre-calibrated microphotographs with the aid of Piximètre v. 5.10 (© A. Henriot & J.-L. Cheype). The brief descriptions below are based on the studied Bulgarian collections.

Sequences of the ITS region were obtained from dried material in ALVALAB (Spain) in accordance with the laboratory's standard protocol, communicated by the laboratory and previously presented in Assyov (2022). The newly generated sequences are deposited in GenBank. Their accession numbers are listed in Table 1. They were compared to existing sequences in GenBank by the BLASTn algorithm (Altschul et al., 1990). For the purposes of comparison, sequences from type specimens were selected preferentially. In cases where type sequences were unavailable or contained ambivalent nucleotide reads, comparison was also made using other reference sequences, released by authoritative sources. The respective works are cited throughout the text as appropriate.

The generic placement of the reported findings follows the system of Liimatainen et al. (2020), although there is an ongoing discussion in the mycological community about whether *Cortinarius* s. l. should be split at present (Gallone et al., 2024). Apart from the customary generic abbreviations ("*C.*" for "*Cortinarius*" and "*P.*" for "*Phlegmacium*"), "*Ca.*" is used throughout the text to denote "*Calonarius*".

Results and Discussion

Sequences comparison and specimens' identity

Sequences of the nrITS region were obtained from five collections. Their accession numbers and identity as accepted in this paper are shown in Table 1, along with their similarity to selected reference GenBank sequences. Five species were identified, namely *Calonarius albertii*, *Ca. violaceipes*, *Cortinarius pseudocollinitus*, *Phlegmacium perpallens*, and *P. scaurocaninum*. All of these appear to be less-known and rarely featured in the mycological literature, and are first recorded in Bulgaria (Denchev & Assyov, 2010) and the Balkan Peninsula (Zervakis et al., 1998; Ivančević, 2002; Mešić & Tkalčec, 2002; Assyov, 2018; Karadelev et al., 2018; Ramshaj et al., 2021, 2022).

Table 1. Comparison of the newly generated sequences with selected public reference sequences available on GenBank.

Sequence [GenBank ID]	Species identity	Reference sequence [GenBankID]	Similarity [%]
PZ055886	*Calonarius albertii*	NR_177453*	100
PZ055887	*Calonarius violaceipes*	NR_182784*	99.68
PZ055888	*Cortinarius pseudocollinitus*	PX221425	99.11
PZ055889	*Phlegmacium perpallens*	KJ421122*	99.88
PZ055890	*Phlegmacium scaurocaninus*	KF732422*	98.08

Asterisk (*) after accession numbers in the third column denotes sequences from respective holotypes.

New records for the Bulgarian mycobiota

Calonarius albertii (Dima, Frøslev & T.S. Jeppesen) Niskanen & Liimat. (Fig. 1)

Specimen examined. Bulgaria: Burgas Province, Malko Tarnovo municipality, Strandzha Mt., Uzunbudzhak Reserve, approx. 41.977300, 27.778892, elev. ca 250 m, in a mixed forest of *Quercus* spp. and *Fagus orientalis*, 17.10.2014, B. Assyov (SOMF 30984).

Morphological data. Pileus up to 5 cm across, hemispherical and tending to convex, with slightly protruding margin, white to off-white, with age, with yellowish-to-yellowish ochraceous areas but consistently retaining whitish colouration towards the pileal margin, glabrous, more or less dry, somewhat silky fibrillose. Lamellae free, in shades of ochre; margin slightly uneven. Stipe equal to slightly longer than pileal diameter, subcylindrical to clavate, with well-defined marginate basal bulb, white to off-white, in some basidiomata dingy in lower parts, fibrillose and with adhering abundant remnants of cortina. Context whitish; odour not remarkable; taste not documented. Macrochemical reactions: KOH pinkish red on pileipellis and bulbipellis. UV365 not distinctive; UV395 lavender on stipe surface. Basidiospores amygdaliform, coarsely ornamented, (9.2) 9.6–11 (11.1) × (5.7) 5.9–6.6 (6.9) µm; Q = (1.4) 1.5–1.7 (1.8); on average 10.3 × 6.3 µm; average Q = 1.6.

Notes

Calonarius albertii is a relatively recently introduced and less known species of the genus, described on materials from Hungary, Denmark, France, and Spain (Frøslev et al., 2006), and more recently found in Germany and Norway (Brandrud & Schmidt-Stohn, 2011; Brandrud et al., 2018). The latter authors believe it to be widespread in Europe, while its true distribution is apparently not well-known yet. It seems to be primarily related to different species of *Quercus*, although some collections with Fagus are also known (Frøslev et al., 2006; Brandrud & Schmidt-Stohn, 2011). Findings with *Tilia* have been reported recently (Brandrud et al., 2018), which deserve attention as the only record with a non-fagaceous host. The above Bulgarian collection extends the range of the species in a southeastern direction considerably. The habitat in Bulgaria is consistent with the majority of the previously documented findings, i. e., forests of Fagaceae (*Quercus*, *Fagus*) on lime-rich soils.

The Bulgarian collection of *Ca. albertii* seems to adhere reasonably well to the descriptions presented in Frøslev et al. (2006) and Brandrud et al. (2018). The considerable morphological similarity and the subtle distinction of *Ca. albertii* from the similar *Ca. catharinae* (Consiglio) Niskanen & Liimat. were discussed by the same authors. In our collection, the whitish stipe, entirely devoid of violet colours, is notable, while *Ca. catharinae* was said by Brandrud et al. (2018) to sometimes possess lilac tinges.

The sequence of the Bulgarian specimen in pairwise comparison with the one from the holotype of *C. albertii* NR_177453 (Frøslev et al., 2006) is completely identical (alignment length 645 bp). The comparison with the sequence DQ323961 from the holotype of the pale-coloured look-alike *C. catharinae* Consiglio showed them to be substantially different, with a similarity of 92.99% at 599 bp aligned.

Fig. 1. *Calonarius albertii* – basidiomata *in situ*.

Calonarius violaceipes (Bidaud & Consiglio) Niskanen & Liimat. (Fig. 2)

Specimen examined. Bulgaria: Burgas Province, Tsarevo municipality, Strandzha Mt., NW of Kosti village, at the road to Bulgari village and Tsarevo town, approx. 42.089278, 27.752083, elev. ca 295 m, in a mixed oak forest, 13.10.2014, B. Assyov (SOMF 30985).

Morphological data. Pileus 7 cm across, flattened convex, with slightly protruding margin, dull violet to pale violet, in places discoloured yellowish to yellowish ochraceous, at the margin with scattered flocci of cortina, glabrous, somewhat glutinose and shiny. Lamellae free, violaceous; margin slightly uneven. Stipe equal to slightly longer than pileal diameter, subcylindrical to clavate, with a well-defined marginate basal bulb, pale violet, in the lower part of the basal bulb, offwhite, fibrillose, and with notable remnants of cortina. Context whitish in the pileus and lower stipe, violaceous in uppermost parts of the stipe and below the stipe cortex, and somewhat yellowish in the bulb; odour not remarkable; taste not documented. Macrochemical reactions: KOH reddish brown on pileipellis and dull pinkish on bulbipellis. UV365 not distinctive; UV395 lavender on stipe surface. Basidiospores amygdaliform or occasionally citriform, coarsely ornamented, (9.7) 10–10.8 (11) × (5.4) 5.7–6.8 (7.1) µm; Q = (1.5) 1.55–1.8 (1.9); on average 10.4 × 6.3 µm; average Q = 1.7.

Notes

Calonarius violaceipes is a relatively recently introduced and less known species of the genus, described on materials from France (Bidaud et al., 2001), and several of its molecularly confirmed findings originate from the same country (Bellanger et al., 2018), but published barcoded European collections are also known from Italy, Germany, and Spain (Garnica et al., 2009; Mahiques et al., 2018). Here we provide the first evidence for its occurrence in the Balkans, but this is not unexpected, as it has already been shown that the range of *Ca. violaceipes* stretches as far as Lebanon (Sleiman et al., 2021). Primarily related to oak-dominated habitats, to which the Bulgarian finding fits well, being found in thermophilous oak-dominated stands on calcareous soils, the species is also known from forests of *Fagus* (Garnica et al., 2009).

There has been a long-standing controversy over the relationships between *Ca. violaceipes* and *Ca. parasuaveolens* (Bon & Trescol) Niskanen & Liimat., which is now settled. For a detailed discussion on this matter, the reader is referred to the works of Bellanger (2015), Clericuzio et al. (2017), and Bellanger et al. (2018). The sequence of the Bulgarian collection in pairwise comparison with the sequence NR_182784 from the holotype of *C. violaceipes* (Frøslev et al., 2007) resulted in high similarity (99.68%) at 627 bp alignment, with a difference in two substitutions. At the same time, the

comparison with the one from the holotype of *C. parasuaveolens* (KY290688; Bellanger et al., 2018), albeit short, produced a similarity rate of merely 94.04% with 8 gaps and 10 substitutions (alignment length 302 bp). The above studied collection is thus referred to as *Ca. violaceipes*.

The Bulgarian collection of *Ca. violaceipes* consists of a single fruitbody, which, albeit with obvious violet tinges, shows a notable tendency to discolouration to yellowish ochraceous. The colouration of pilei in the protologue of *C. violaceipes* has been said to have fueled the difficulties in the application of the names *C. violaceipes* and *C. parasuaveolens* Bon & Trescol until both types were successfully sequenced (Bellanger, 2015; Clericuzio et al., 2017; Bellanger et al., 2018).

Fig. 2. *Cortinarius violaceipes* – basidioma *in situ*.

Cortinarius pseudocollinitus Rob. Henry & Ramm (Fig. 3)

Specimen examined. Bulgaria: Burgas Province, Malko Tarnovo municipality, Strandzha Mt., Sredoka Reserve, approx. 42.029153, 27.563678, elev. ca 290 m, in a mixed oak and *Carpinus* forest, 16.10.2014, B. Assyov (SOMF 30986).

Morphological data. Pileus up to 8 cm across, hemispherical to convex, viscid when wetted, somewhat shiny in dry weather, beige to ochraceous or orange ochraceous, in places spotted brownish or discolouring to off-white, finely innately fibrillose, with age subtly striate along the margin. Lamellae adnate to notched, in shades of beige or fawn; margin slightly uneven, paler than the lamellar surfaces. Stipe equal to slightly longer than pileal diameter, subcylindrical, smooth or sometimes squamulose, white to off-white, in some basidiomata pale violaceous tinted at the apex, with scarce remnants of cortina. Context whitish; odour and taste not documented. Macrochemical reactions: KOH nil on pileipellis and stipitipellis. UV365 not distinctive; UV395 lavender on stipe surface. Basidiospores oblong to amygdaliform, coarsely ornamented, (12.3) 12.4–15.2 (15.6) × (7.8) 8.1–9.1 (9.6) µm; Q = (1.4) 1.44–1.7 (1.8); on average 13.7 × 8.7 µm; average Q = 1.6.

Notes

The sequence from the Bulgarian specimen matches closely two GenBank sequences labelled as *C. pseudocollinitus* (Kuyper et al., 2024), both from the Netherlands and related to *Quercus* or *Fagus* (no coniferous host trees recorded). The similarity to PX221425 is 99.11% (672 bp alignment length), differing in 6 base pairs. The second sequence, PX221135, in comparison with our sequence shows 98.81% identity (672 bp alignment length), differing by 8 base pairs (7 substitutions and one ambivalently read nucleotide). It seems feasible that the Bulgarian collection is identical to the two mentioned finds from the Netherlands. It also appears more or less consistent with the pro-

tologue of *C. pseudocollinitus* (Bidaud et al., 2000), a species described on material collected in France, on siliceous terrain under *Picea*, although at relatively low elevation. The spores of the Bulgarian specimen are consistent with the measurements in Bidaud et al. (2000) and larger than the size given in Kuyper et al. (2024). The Bulgarian collection was found in a thermophilous oak and hornbeam forest on calcareous soil. As there is no publicly available sequence from the holotype of the name to conclusively corroborate the identity, and considering the similarity with the mentioned sequences labelled as *C. pseudocollinitus*, we apply this name to our collection with due caution.

Fig. 3. *Cortinarius pseudocollinitus* – basidiomata *in situ*.

Phlegmacium perpallens (Chevassut & Rob. Henry) Niskanen & Liimat. (Fig. 4)

Specimen examined. Bulgaria: Burgas Province, Malko Tarnovo municipality, Strandzha Mt., Sredoka Reserve, approx. 42.029153, 27.563678, elev. ca 290 m, in a mixed oak, *Fraxinus* and *Carpinus* forest, 16.10.2014, B. Assyov (SOMF 30987).

Morphological data. Pileus up to 8 cm across, convex to flattened, with slightly protruding margin, pale violaceous, in places discolouring to yellowish ochraceous or cream, dry, with numerous, faint, darker violet innate fibrils. Lamellae free, ochraceous with some pinkish or dull violet tinges; margin irregularly finely dentate, concolorrous. Stipe shorter or equal to pileal diameter, subcylindrical to subclavate, with well-defined marginate basal bulb and hint of volva-like structure, very pale violet throughout and tending towards off-white at the bulb, fibrillose and with adhering more or less copious remnants of cortina. Context violaceous in the upper parts of the stipe, elsewhere off-white to pale ochraceous in the base, whitish in pileus; odour faint and not distinctive, taste not documented. Macrochemical reactions: KOH not distinctive, brownish on pileipellis, on stipe nil. UV365 not distinctive; UV395 lavender on stipe surface. Basidiospores oblong, rarely amygdaliform, coarsely ornamented, (7.8) 8.1–9.2 (9.7) × (5) 5.4–5.9 (6.2) µm; Q = (1.4) 1.5–1.7 (1.9); on average 8.7 × 5.6 µm; average Q = 1.6.

Notes

Phlegmacium perpallens is an appallingly little-known species, the knowledge on which is based on its holotype collection from France and one more documented sample from the same country (Chevassut & Henry, 1978; Bidaud et al., 1993; both as *Cortinarius perpallens* Chevassut & Rob. Henry). An additional report from Italy (Clericuzio et al., 2022), backed with nrITS sequence, provides only locality data. The difference between the habitats of the known findings is noteworthy. The holotype was collected under *Picea* and *Fagus* (Chevassut & Henry, 1978), while the finding of Bidaud et al. (1993) appeared in a habi-

tat with *Abies* and *Picea*. Strikingly, the Italian collection came from thermophilous oak forests. The soil types of the three encounters were not specified. The collection reported here comes from mixed, thermophilous broadleaf forests on limestone, a habitat that seems at least partly compatible with the Italian find.

The Bulgarian collection, by its morphology, fits reasonably well the existing descriptions in the literature (Chevassut & Henry, 1978; Bidaud et al., 1993). Viscous pilei were not observed in our specimen, but this is probably due to the sun-exposed situation where the species was found. Nonetheless, the original description also notes that viscous pilei become dry later. The Bulgarian collection features copious cortina, unlike the French findings (Chevassut & Henry, 1978; Bidaud et al., 1993). The basidiospores of the studied collection generally fit the data in the above works, although mostly on the lower part of the ranges.

The initial BLAST search on the sequence from the Bulgarian finding returned a number of highly similar *Phlegmacium* sequences, of which the closest match appeared to be KJ421122, produced from the holotype of *P. perpallens* (Liimatainen et al., 2014). The pairwise comparison produced 99.88% similarity (833 bp alignment length) due to a single discordant position. Such a similarity should firmly place the studied specimen in *P. perpallens*.

Fig. 4. *Phlegmacium perpallens* – basidiomata *in situ*.

Phlegmacium scaurocaninum (Chevassut & Rob. Henry) Niskanen & Liimat. (Fig. 5)

Specimen examined. Bulgaria: Burgas Province, Tsarevo municipality, Strandzha Mt., NW of Kosti village, at the road to Bulgari village and Tsarevo town, 42.090694, 27.754361, elev. ca 290 m, in a mixed oak forest, 19.10.2024, B. Assyov (SOMF 30988).

Morphological data. Pileus up to 8 cm across, convex to flattened, with slightly protruding margin, ochraceous, gradually discolouring to yellowish ochraceous, tending to off-white at the margin; surface dry, with numerous, dark brown, innate fibrils. Lamellae free to adnexed, at first violaceous, with age pale ochraceous, finally ochraceous; margin irregularly uneven, concolorous. Stipe more or less equal to pileal diameter, subcylindrical to subclavate, with moderately developed basal bulb, at first pale violet throughout and tending towards off-white at the bulb, then gradually discolouring to off-white or yellowish, fibrillose and with adhering more or less copious remnants of cortina. Context violaceous throughout the stipe, more intense in the upper parts, pale pastel orange in the stipe base, whitish in the pileus; odour faint and not distinctive, taste not documented. Macrochemical reactions: KOH slowly dark reddish brown on pileipellis. UV365 not dis-

tinctive; UV395 lavender on stipe surface. Basidiospores oblong to amygdaliform, occasionally citriform, finely ornamented, (6.4) 6.6–7.5 (7.9) × (4.1) 4.3–5.1 (5.2) µm; Q = (1.3) 1.4–1.6 (1.8); on average 7.1 × 4.7 µm; average Q = 1.5.

Notes

Phlegmacium scaurocaninum was described from under *Quercus ilex* L. from France (Chevassut & Henry, 1982; as *Cortinarius scaurocaninus* Chevassut & Rob. Henry) and is a relatively less-known species of the genus, likely having a distribution somewhat centered on southern Europe (France, Italy, Spain), albeit records are also known outside the Mediterranean region, e. g., in Austria, Germany, and Hungary (Krisai-Greilhuber et al., 2017). It is reported here for the first time for the Bulgarian and the Balkan mycobiota, but recent records exist from the neighbouring Romania (Szabo et al., 2025).

The species shows a preference for thermophilous oak forests on lime-rich soils, albeit collections of *P. scaurocaninum* with *Fagus* are not unknown (see e. g. Liimatainen, 2014; Schmidt-Stohn et al., 2016; Maletti, 2020). The Bulgarian collection presented here was found in a pure forest of *F. orientalis* on calcareous soil.

The studied specimen produced an nrITS sequence that, on BLASTn-comparison, matches a number of *Phlegmacium* sequences. It aligns with GenBank KF732641 of *C. scaurocaninus* from Liimatainen et al. (2014) with 100% identity (702 bp length of alignment). The pairwise comparison with GenBank KF732422 (Liimatainen et al., 2014) from the holotype of *C. scaurocaninus* produced an alignment of 676 bp with 98.08% identity. The lower similarity appears to be mostly due to 10 ambivalently read nucleotides in the holotype sequence.

Fig. 5. *Phlegmacium scaurocaninus* – one of the basidiomata *ex situ.*

Conclusion

This study revealed the presence in Bulgaria of five interesting and little-known cortinarioid fungi, some of which are ostensibly rare and only documented throughout Europe by a couple of collections. While this output is a result of a purely opportunistic sampling approach, it demonstrates the great potential of barcoding of cortinarioid fungi in aid of the inventory of their diversity in this and other Balkan countries. On the other hand, the Balkans, even though a well-known biodiversity hotspot, remain scarcely covered in terms of the use of DNA sequencing in cortinariological research. Thus far, the region remains a considerable gap in knowledge on this fungal group in Europe, and without a doubt, more rigorous research on cortinarioid fungi is worth pursuing.

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