

BARCODING AND MORPHOLOGICAL  
CHARACTERIZATION OF *Mycena cupressina*  
(AGARICALES, FUNGI), A LITTLE-KNOWN  
AND CONTROVERSIALLY TREATED MEMBER  
OF SECTION *SUPINAE*

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Received on January 17, 2022

Presented by D. Ivanov, Corresponding Member of BAS, on June 21, 2022

**Abstract**

The author presents recent collections of *Mycena cupressina*, a corticolous species on *Cupressus sempervirens*, described from the Mediterranean area and sometimes treated as conspecific with *M. juniperina*. The first ITS nrDNA sequence of the species from Balkan material was obtained. Its analysis demonstrated the affinity of the sequenced collection to section *Supinae*, although the results could not be unambiguously interpreted and suggest that in this section the ITS marker may be less informative, similarly to some other groupings of the genus. Description of *M. cupressina* is provided, based on Balkan collections. The studied *Cupressus*-affiliated specimens share smooth stipitipellis hyphae, deemed to be diagnostic character of the species, suggesting that for the time being the two are better retained as separate taxa. The first records for Bulgaria of four other species of the section, namely *M. juniperina*, *M. meliigena*, *M. pseudocorticola* and *M. supina*, are also included.

**Key words:** Balkan mycota, biogeography, *Cupressaceae*-associated fungi, *Mycenaceae*, taxonomy

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This work was supported by the National Science Programme “Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters”, approved by the Resolution of the Council of Ministers No. 577/17.08.2018 and supported by the Ministry of Education and Science (MES) of Bulgaria (Agreement No. D01-230/06.12.2018).

DOI:10.7546/CRABS.2022.11.06

**Introduction.** *Mycena* s. l. is one of the large groupings of agarics, comprising well over 200 species in Europe, with previously undescribed ones being continuously introduced in the last decades [1–4]. Its section *Supinae* Konrad & Maubl. unites several European small-sized, bark-dwelling species [2,4]. *Mycena cupressina* Antonín & Maas Geest. is a corticolous species of this section, described nearly a quarter of a century ago, based on materials from Italy [5], collected on bark of *Cupressus sempervirens* L. and *Arbutus* L. It remains rarely featured in the mycological literature up to date and is sometimes considered to be conspecific with another bark-inhabiting species, bound to *Cupressaceae* Bartlett, namely *M. juniperina* Aronsen [1,4,6]. In the last few years, the author was able to acquire several collections matching the original description of *M. cupressina*, which allowed morphological and molecular assessment of this controversially treated entity. The results are summarized herein.

**Materials and methods.** The materials of different members of *Mycena* section *Supinae* used in this work were collected by the author in Bulgaria and were supplemented with some extralimital specimens. They were documented in the field and are preserved in air-dried state in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF).

The morphological study was held with the aid of an AmScope T360B light microscope. Spore preparations were observed and measured in 1% ammonia solution of Congo red. The remaining microscopic structures were assessed from dry material after reviving in KOH 5% (~ 2 min), staining with Congo red in ammonia (~ 2 min), and final mounting in KOH 5%. Additional observations were held in Melzer's reagent without pretreatment, in order to assess the possible iodine reaction of different tissues. The spore dimensions (length, width and spore quotient Q) in the description are presented by the ranges of minimum, maximum and average values. For the remaining microscopic structures the minimum and maximum values are noted.

DNA extraction, amplification and sequencing were performed by ALVALAB (Oviedo, Spain) in accordance with the following protocol communicated by the laboratory. Total DNA was extracted from dry specimens employing a modified protocol based on MURRAY and THOMPSON [7]. PCR reactions [8] included 35 cycles with an annealing temperature of 54 °C. The primers ITS1F and ITS4 [9,10] were used to amplify the ITS rDNA region. PCR products were checked in a 1% agarose gel, and the amplicons were sequenced with one or both PCR primers. Sequences were corrected to remove reading errors in chromatograms.

The phylogenetic analysis included the sequence of *M. cupressina* obtained in this study, as well as publicly available on GenBank sequences of European taxa of section *Supinae* [11]. The dataset was assembled in BioEdit Sequence Alignment Editor v. 7.2.5 [12] and the alignment was trimmed as appropriate at the ends of ITS1-5.8S-ITS2 region. The phylogenetic analysis was run through the platform Phylogeny.fr in "One-click mode" [13], including analysis in PhyML 3.1/3.0 with

HKY85 substitution model, using the Shimodaira-Hasegawa-like aLRT-test for calculation of branch support [14]. Technical formatting of the resulting tree was applied in InkScape v. 1.0.2-2.

**Results. Analysis of ITS sequences.** The pairwise comparison of the ITS1-5.8S-ITS2 region of the new sequence of *M. cupressina* and NR173926 from the holotype of *M. juniperina* showed 99.07% identity, the two sequences differing by two substitutions and one indel. The sequence JF908478 of *M. juniperina* on the other hand differed from the one from the holotype of the species by three substitutions and one indel. In the obtained phylogenetic tree (Fig. 1) the new sequence of *M. cupressina* clustered together with another deposited under the same name, the two publicly available of *M. juniperina*, and unexpectedly, with two sequences of *M. meliigena* (Berk. & Cooke) Sacc. The sequence from the holotype of *M. juniperina* appears to be somewhat divergent, but this difference is likely insignificant and possibly resulting from three ambiguously read nucleotides. Another sequence of *M. meliigena* appeared in a sister clade, comprising also one labelled as *M. pseudocorticola* Kühner and one of *M. supina* (Fr.) P. Kumm. Further on, the second publicly available sequence of *M. pseudocorticola* appeared as a sister branch to an accession of *M. polygramma* (Bull.) Gray, a species not related to the members of section *Supinae*. The tree, obtained with application of procedure to remove poorly aligned positions and divergent regions (not shown) demonstrated similar topology with regards to the two former clades.

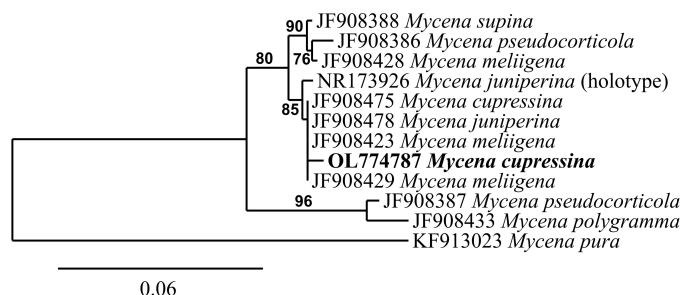


Fig. 1. PhyML phylogenetic tree of ITS sequences of *Mycena* section *Supinae*. Figures next to branches indicate branch support as percentage, obtained by SH-like aLRT test. The sequence generated in this work is shown in boldface

**Description of the species.** *Mycena cupressina* Antonín & Maas Geest., Persoonia, 16(4): 545 (1998), (Fig. 2, 3).

Pileus up to 10 mm across, hemispherical then convex, at the centre flattened or slightly depressed, translucent striate, at first finely whitish pruinose, then almost glabrous, usually darker in the centre and with small dark umbo and paler towards the margin, pale cinnamon, pale sienna, buff, pale buff, yellowish buff; pileal margin finely crenulated, sometimes almost white. Lamellae 9–12(–16),



Fig. 2. Basidiomata of *Mycena cupressina* on *Cupressus sempervirens*: a – on bark; b – on cones. Scale bar = 5 mm

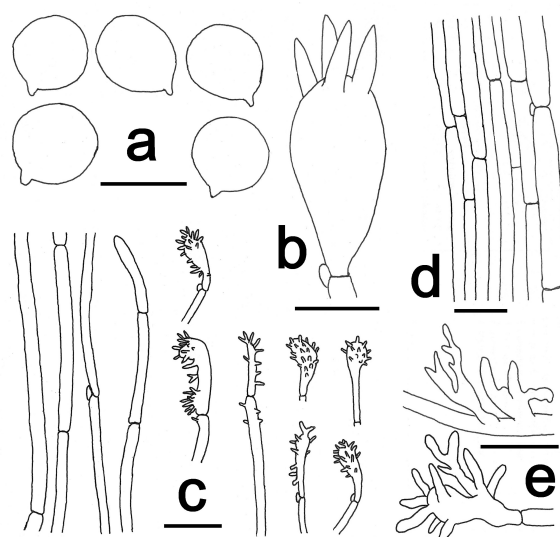


Fig. 3. Micromorphological features of *Mycena cupressina*: a – basidiospores; b – basidium; c – hyphae of the cortical layer of stipe and caulocystidia; d – hyphae of internal stipe strata; e – hyphae of pileipellis. Scale bars = a, b, e = 10  $\mu$ m; c, d = 20  $\mu$ m

lamellulae 1–2 between lamellae, arcuate, adnate, whitish or with some yellowish tint. Stipe up to  $10 \times 0.3$  mm, central, slightly widened towards the apex and the base, arising from a small rounded mat of whitish hyphae, fragile, curved, concolorous with pileus or nearly so; surface whitish pulverulent, later becoming smooth or almost so. Context thin. Odour not distinctive. Basidiospores  $8.9\text{--}(10.5 \pm 0.7)\text{--}12.0 \times 6.9\text{--}(9.1 \pm 0.7)\text{--}10.5$   $\mu$ m,  $Q=1.0\text{--}(1.2 \pm 0.1)\text{--}1.3$  ( $n = 30$ ), subglobose to globose, apiculate, smooth, pluriguttulate, hyaline, amyloid in Melzer's reagent (pale greyish blue). Basidia 4-spored,  $25.0\text{--}33.0 \times 10.8\text{--}13.1$   $\mu$ m, clavate,

with clamps; sterigmata 6–8  $\mu\text{m}$  long. Cheilocystidia 16.5–29.2  $\times$  8.5–13.6  $\mu\text{m}$ , clavate, covered mostly in the upper half with moderately dense, simple papillae or cylindrical excrescences up to 4  $\mu\text{m}$  long; furcate or branched excrescences not seen. Pleurocystidia not encountered. Lamellar trama reddish brown in Melzer's reagent (dextrinoid). Pileipellis a cutis of 2–4  $\mu\text{m}$  wide, branched and sometimes anastomosing hyphae with numerous simple or furcate excrescences; walls 0.7–0.8  $\mu\text{m}$  thick. Hypodermium of somewhat inflated, septate hyphae, 8–20  $\mu\text{m}$  wide, cystidioid or ovoid free hyphal tips occasionally seen; walls 0.5–0.7  $\mu\text{m}$  thick; clamps not seen. Hyphae of the cortical layer of stipe 1.5–3.5  $\mu\text{m}$  wide, with walls 0.5–0.8  $\mu\text{m}$  thick and frequent clamps, usually not or scarcely branched, smooth or only by very rare exception (mostly close to the terminal element) with a few short excrescences; terminal elements of two types: 1) simple, cylindrical, smooth, with acute or obtuse apex; and 2) caulocystidia very variable by shape and size, with few, mostly simple excrescences. Hyphae of inner stipe stratum 6–10  $\mu\text{m}$  broad, dextrinoid; wall 0.4–0.5  $\mu\text{m}$ , smooth, septate, clamps rarely seen; hyphal tips where allocated, with rounded apex.

Specimens examined. *Mycena cupressina*: Bulgaria, ca 4 km SW of Mikrevo village, 41.605000°, 23.173250°, 430 m, on bark, 31.01.2019 (SOMF 30450); Greece, above Sidirokastro village, 41.232861°, 23.398444°, 140 m, on bark and cones, 12.11.2017 (SOMF 30449); ca 1 km NE of Sidirokastro village, 41.254778°, 23.405500°, 140 m, on bark, 05.03.2019 (SOMF 29982; GenBank OL774787); North Macedonia, S of Peshtani village, 40.999167°, 20.802778°, 725 m, on cones, 20.10.2016 (SOMF 30448); all on *C. sempervirens*; *M. juniperina*: Bulgaria, in the vicinity of Kamenitsa village, 41.649361°, 23.163333°, 195 m, on *J. deltoides* R.P. Adams, 31.01.2019 (SOMF 30439); *idem*, 03.11.2021 (SOMF 30447); in the vicinity of Kresna town, 41.731444°, 23.155083°, on *J. deltoides*, 27.11.2021 (SOMF 30446); Sofia city, Vrana Park, 42.639056°, 23.435917°, 560 m, on *J. sabina* L., 17.10.2020 (SOMF 30445); *M. meliigena*: Bulgaria, Logodazh village, in the vicinity of the quarry, 42.008111°, 22.923361°, 860 m, on *Quercus* sp., 21.02.2016 (SOMF 30444); in the vicinity of Pancharevo dam, 42.602778°, 23.411139°, 665 m, on *Quercus* sp., 14.03.2019 (SOMF 30443); Sofia city, West Park, 42.705639°, 23.256611°, 575 m, on *Salix alba* L., 16.11.2019 (SOMF 30442); *M. pseudocorticola*: Bulgaria, Sofia city, West Park, 42.703056°, 23.254889°, 615 m, on *Tilia* sp., 24.11.2019 (SOMF 30441); *M. supina*: Sofia city, West Park, 42.705639°, 23.256611°, 575 m, on *S. alba*, 16.11.2019 (SOMF 30440); all on bark.

**Discussion.** *Mycena cupressina* shows certain degree of similarity to the remaining European members of the section *Supinae*. The most similar species is apparently *M. juniperina*, both by morphology and by the affinity to different representatives of *Cupressaceae*. This considerable similarity led some authors to discuss the possible synonymy between the two species [<sup>1,4,6</sup>]. In his monograph of the genus *Mycena* (Pers.) Roussel, however, ROBICH [<sup>2,3</sup>] treated them separately and used the pileal colour to key out *M. cupressina* and *M. juniperina*.

The present author is uncertain as to what extent this difference may be useful, for considerable variability is known in other European members of the section [4]. However, from the original description, to which the studied here specimens agree well, the most striking character separating the two species is the morphology of the stipe cortical hyphae. *Mycena cupressina* has smooth hyphae, while in *M. juniperina* they bear obvious excrescences. While some authors consider this a character that may be within the range of variability of *M. juniperina* [1,4,6], at least from the Balkan specimens it seems that it could be reliable separating feature. In addition, from the published descriptions and illustrations it seems that cheilocystidia in *M. juniperina* are with longer and seemingly often branched excrescences [1,4,6], while such are not seen in the examined here specimens of *M. cupressina* (albeit branched excrescences are not unknown in this species according to its original description [5]). It is noted, however, for the Turkish collection of *M. juniperina*, that cheilocystidia have shorter excrescences than the type and due to the smaller spores the authors also presume that the collection may not be fully mature [15]. On the other hand, some Italian collections of *M. cupressina* are described and illustrated as having numerous flexuose, simple or branched excrescences [2]. Certain variability of this feature is apparently not unknown in some other species of the section [4]. Further on, the already mentioned smooth hyphae of the cortical layer of the stipe also set apart *M. cupressina* from the remaining corticolous mycenae of this section in Europe, namely *M. conicoalba* M. Villarreal & Esteve-Rav., *M. meliigena*, *M. pseudocorticola*, and *M. supina* [1,2,4,16]. The morphological distinction was also supported by study of comparative specimens of the three latter species from Bulgaria.

In general section *Supinae* is scarcely represented with sequences in public databases. One of the studied here specimens of *M. cupressina* produced nrITS sequence. The phylogenetic analysis (Fig. 1) did not conclusively separate the European members of the section as for the currently accepted morphology-based concept [1,2,4]. As a matter of fact, the difficulties concerning the molecular identification in section *Supinae* were already discussed in some other sources [17]. Two possible explanations could be considered in order to tackle the outcomes of the phylogenetic analysis. It may be argued that some sequences of section *Supinae* may come from mixed specimens as the members of the section may indeed occur in close proximity. Should this be the case, at least two lineages may be recognized in the section, the one comprising sequences NR173926, JF908429, JF908423, JF908475, JF908478, and the sequence of our specimen of *M. cupressina*, and the other – consisting of sequences JF908388, JF908386 and JF908428. The second lineage may in fact host more than one phylogenetic entity. Mixed specimens could hardly explain the clustering of sequence JF908387 of *M. pseudocorticola* with the one of *M. polygramma*, for the two species bear little, if any, morphological similarity and are thus unlikely to be mistaken, while the two sequences demonstrate 98% similarity. In such case, relying on the outcomes of

the phylogenetic analysis of ITS sequences one may argue that both *M. cupressina* and *M. juniperina* may be conspecific with *M. meliigena* and that the latter name is applied to two distinct entities. The present author is reluctant to follow such a thread, for the five species of section *Supinae* involved in the analysis seem to have certain delimiting morphological characters and with exception of the different views on *M. cupressina*, are recognized as separate in recent treatments [2,4] and the specimens used to obtain the mentioned GenBank sequences are related to Robich's monographic study of the genus [2,3]. Another possible explanation for the observed discrepancy may be that infraspecific and interspecific variability in this group overlap, and nrITS may not be suitable marker for resolving the phylogenetic relationships in section *Supinae*. Considered that cases are already known in other groups of *Mycena* s. l., where ITS-inferences were shown to underestimate the phylogenetic diversity [18], as well as that from morphological point of view several taxa could be delimited within the section, this hypothesis is here given preference. Taking into account that specimens of *M. cupressina*, studied here, show certain morphological differences from *M. juniperina*, *M. meliigena* and *M. pseudocorticola*, it is suggested that the proposed synonymy of the two former is not uncritically accepted until further phylogenetic studies of this group become available, based on more extensive sampling and assessment of additional loci.

*Mycena cupressina* is recorded here for the first time for the mycobiota of the Balkan Peninsula, supported by specimens from Bulgaria and two neighbouring countries. It is found as corticolous or strobilicolous on bark and cones of *Cupressus sempervirens*. When developing on bark, it produces small, scattered basidiomata, usually in places where water precipitates and lichen mats are developed. The occurrence on cones seems to happen rarely, but in this particular case, basidiomata are notably larger, up to three times than average-sized fruit-bodies from bark. The species seems rare in its habitats, but it apparently spreads in artificial plantations as all here described findings come from such stands. In the sole known Bulgarian locality it was found on a single tree among over 30 surveyed. At Ohrid Lake it was located on a single cypress cone. In one of the Greek localities, in a large *Cupressus* plantation, basidiomata were found only on four trees out of about fifty screened. Again, a single cone with *M. cupressina* was discovered in the same place. In spite of extensive search for five consequent seasons in another spot with extensive plantings of *C. sempervirens* (Stratoni, Chalkidiki), the fungus was never spotted there. The continuous search for corticolous mycenae on *Juniperus excelsa* M. Bieb. in Bulgaria so far yielded no results, albeit the two species pertain to the same plant family and have certain similarity in the bark texture. However, *J. excelsa* has been reported as substrate plant of *M. juniperina* in North Macedonia and Turkey, with the published description of the Turkish collection closely corresponding to the ones of this species from the northern parts of Europe [15,19]. In the original description of the species

[<sup>5</sup>] occurrence of *M. cupressina* on bark of *Arbutus* was also noted. The present author had the opportunity to conduct numerous visits to several stands with abundant presence of both *A. unedo* L. and *A. andrachne* L. on the eastern coast of Chalkidiki Peninsula in Greece, but his attempts to obtain *Arbutus*-associated collections of any member of section *Supinae* so far produced no results. As of present, the degree of affinity to substrata of the different species of this section is unclear and some of the members are known to occur on a variety of trees [<sup>2,4</sup>], e. g. *M. meliigena*, which in our studies was collected on bark of *Quercus* spp., *Corylus avelana* L., *Acer campestre* L. and *Salix alba*. Others seem so far bound to a single tree species or different species of a single genus, like *M. conicoalba* by now only known from *Q. ilex* L. [<sup>16</sup>], and *M. juniperina*, which is found on different species of *Juniperus* L.

The examined specimens of *M. juniperina*, *M. meliigena*, *M. pseudocorticola* and *M. supina* in addition represent the first records of those species for the Bulgarian mycobiota [<sup>20</sup>]. Their morphological features compare well to recent authoritative descriptions [<sup>2,4</sup>]. Of those three the former seems to be somewhat uncommon species. In the other Balkan countries *M. juniperina* has been documented so far by findings in North Macedonia [<sup>19</sup>]. Of the two former, *M. meliigena* is likely common and widespread in Bulgaria. The present author examined several Bulgarian specimens of this species, of which for space-saving purposes only a few representative ones are mentioned here. *Mycena pseudocorticola* and *M. supina* will probably also appear to be more widespread, albeit a single specimen of each species was examined in this work. As a matter of fact, all species of the section could easily pass unrecorded due to their small size and occurrence throughout the colder months of the year.

**Acknowledgements.** Dr. Pablo Alvarado (ALVALAB, Spain) is thanked for obtaining the sequence of *M. cupressina* released in this study.

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