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Report and first molecular data of *Alternaria infectoria, Lecanicillium fungicola, Mycogone rosea, Sarocladium strictum* and *Sepedonum chrysospermum,* growing on wild mushrooms in Bulgaria

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Abstract

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Fungicolous fungi are the main group causing diseases on cultivated and wild mushrooms. This study aims to report six taxa growing on decaying mushrooms collected in Bulgaria, and to isolate and characterize five of them with molecular data: *Alternaria infectoria* on *Hortiboletus bubalinus*, *Hypomyces chrysospermus* on *Boletus edulis*, *Lecanicillium fungicola* on *Lactarius deliciosus*, *Mycogone rosea* on *Amanita caesarea*, and *Sarocladium strictum* on *Xerocomus subtomentosus*. Three of the mushroom hosts are known to be economically important, traditionally gathered by locals in Bulgaria. *Amanita caesarea* was red-listed in Bulgaria as Vulnerable (VU), and as Least Concern (LC) in the IUCN Red list of threatened species 2019. Additionally, *Cladobotryum verticillatum* (sexual morph *Hypomyces armeniacus*) is here reported and described from old and decaying basidiomata of *Lactifluus volemus* and *Russula* sp. in Forebalkan, and *Lactarius aurantiacus* in Vitosha Mt. Six collections were made in coniferous or deciduous habitats, and another was found under *Tilia cordata* trees. *Hypomyces chrysospermus* (asexual morph *Sepedonium chrysospermum*) is reported for the first time on fruit-bodies of *Boletus edulis* and *Xerocomus subtomentosus* from Forebalkan, Vitosha and Rhodopi Mts.

Keywords: Alternaria; fungicolous fungi; *Hypocreales*; ITS; *Sarocladium*

Introduction

Fungicolous ascomycetes are still not sufficiently studied in Bulgaria. The first reports of these fungi were connected to quite specific studies, i.e. *Dialonectria episphaeria* on *Quaternaria persoonii* by Klika (1926); *Hypomyces viridis* on *Lactarius deliciosus* by Barzakov (1933); *Hypomyces chrysospermus* on decaying bolete, and *Tolypocladium ophioglossoides* by Kuthan and Kotlaba (1981); *Trichoderma pulvinatum* on old *Fomitopsis pinicola* by Dörfelt and Müsch (1987). More recently, *Cosmospora coccinea* was found growing on old basidiomata of *Inonotus nodulosus* by Mihál et al. (2014), and *Hypomyces aurantius* was reported growing on *Lenzites warnieri* at the Balkan Range by Stoykov and Gyosheva (2017).

Another six fungicolous taxa with minute ascomata (*Cosmospora magnusiana* s.l., *Dialonectria episphaeria, Flamocladiella anomiae, Hypoxylon howeanum, Nectria* *decora* and *Tubeufia cerea*), growing predominantly on old effete stromata of *Diatrype sigma, Diatrypella quercina, Diatrypella* sp., *Quaternaria quaternata* and *Massaria anomia* were reported recently by Stoykov et al. (2018), Lechat et al. (2019), Stoykov (2020, 2021, 2023), Stoykov and Alvarado (2023). However, only two of these identifications were supported by molecular data.

The aim of the present work is to report the first presence of *Alternaria infectoria*, *Lecanicillium fungicola*, *Mycogone rosea*, *Sarocladium strictum,* and *Sepedonium chrysospermum* isolated from old, decaying basidiomata of *Hortiboletus bubalinus*, *Lactarius deliciosu*s, *Amanita caesarea, Xerocomus subtomentosusm,* and *Boletus edulis*, collected in different plant communities in Bulgaria. It also aims to report the first collections of *Cladobotryum verticillatum* (sexual morph *Hypomyces armeniacus*) on old or decaying basidiomata of *Lactarius aurantiacus, Lactifluus volemus* and *Russula* sp. *Alternaria infectoria*, a species found only on stems and leaves of graminicolous grasses, and petioles of broadleaved trees, was isolated from a mushroom cap of *H. bubalinus* under *Tilia cordata* in a city park.

Material and Methods

The examined specimens were found on infected basidiomata of *Amanita caesarea*, *Boletus edulis*, *Hortiboletus bubalinus*, *Lactarius deliciosus*, *L. aurantiacus*, *Lactifluus volemus*, *Rusula* sp. and *Xerocomus subtomentosus*. They were collected between June and September of 2017 and 2018, in coniferous and deciduous forests in Forebalkan, Sofia region (city park), Vitosha region and Rhodopi Mts. Color photographs were taken *ex situ* with Canon PS A460, A95 and A1400 HD on Amplival Carl Zeiss and Boeco BM-120, and BM-180/T/SP LM. Potato dextrose agar (PDA) and water agar (WA) were used for the isolation and storage of fungal material. Nine monoconidial fungal isolates were produced for this study. Morphological observations were made on PDA after 14 days of cultivation. Conidial and spore morphological features were observed on semipermanent microscope slides under LM in water, and documented *ex situ* from living or dried materials from aleurioconidia, obtained from cultures on PDA. Spore and conidial size is usually presented in the form: (min. value-) mean value±1st dev (-max. value), n, where n – denotes the number of measurements, stdev – standart deviation. For the morphological determination of the taxa the studies of Brady and Gibson (1976), Simmons (1986), Rogerson and Samuels (1994), Sahr et al. (1999), Põldmaa (1999), Arnold and Yurchenko (2007), Gibson (2017), Berendsen et al. (2010) and Wu et al. (2021) were followed. Air-dried specimens of the infected basidiomata are preserved at the Mycological Collection, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia (SOMF).

Fungal biomass for molecular characterization was produced on PDA nutrient media for 10 days at 22°C. Total DNA was extracted from cultured isolates employing a modified protocol based on Murray and Thompson (1980), and amplified following Mullis and Faloona (1987) employing 35 cycles with an annealing temperature of 54ºC. The primers ITS1F and ITS4 (White et al., 1990; Gardes and Bruns, 1993) were used to amplify the ITS rDNA region. The resulting products were sequenced and compared with homologous sequences in public databases.

Results and Discussion

Alternaria infectoria E.G. Simmons, Mycotaxon 25, p. 298 (1986) Figures 1–2, 11–13, 24–25.

Sexual morph *Lewia infectoria* (Fuckel) M.E. Barr & E.G. Simmons, in Simmons, Mycotaxon 25, p. 296 (1986).

Syn.: *Pleospora infectoria* Fuckel, Jahrb. Nassau. Vereins Naturk. 23–24, p. 132 (1870).

Mycelium formed on PDA, fluffy, initially nearly white and floccose (Fig. 16) in front side, blackened on the reverse, with typical granular appearance of the colonies (Fig. 13), later greenish, dark green from below, especially around the center (Figures 11–12). Conidia forming white powdery mass on the cap surface of the host, growing from metulae-like conidiophores. Conidial cells hyaline, irregularly globose or more or less ellipsoid, 3–5 × 2.5–4 µm [4.1±0.7 × 3.5±0.6 on average], n = 10, obtained by scrapping portions of white mycelium with a preparatory needle (Fig. 1).

Specimens examined: Sofia Region, Sofia city, N 42.714461°, E23.250103°, alt. ca. 560 m, 25 July 2017, on old basidioma from *Horiboletus bubalinus* (Oolbekk. & Duin) L. Albert & Dima, under *Tilia cordata* Mill. tree, leg. D. Stoykov, SOMF 31623 (GenBank PP429847, PP429848); idem., 25 June 2017, leg. D. Stoykov, SOMF 31624.

Two ITS rDNA sequences obtained during the present work were deposited in GenBank (PP429847, PP429848). MegaBlast from the ITS of the duplicated culture isolate (Figs. 11–13): PP429847 showed 99.83% identity with homologous sequences of *Alternaria infectoria* (i.e. GU584953, OU989243, GQ376103, JX454532, OL691144, and 100% identity to *A. infectoria* MK513822; PP429848 is 100% identical to MW446952 (*A. infectoria*) and showed 99.83% identity with *A. infectoria* MT548676, OU989243, GQ376103, JX454532, and others.

Comments. The ITS rDNA sequences obtained (PP429847, PP429848) showed a 99.83% identity with homologous sequences of *Alternaria infectoria* (i.e. GU584953, JX454532, GQ376103, and others). *Hortiboletus bubalinus* is an ectomycorrhizal fungus often found in urban parks and public gardens, usually under the trees of *Betula* and *Tilia* (Assyov and Stoykov, 2011). *Alternaria infectoria* was previously found during a PhD thesis work devoted to studying the leaf spots on *Triticum durum* (Nedyalkova, 2018). Its sexual morph, *Lewia infectoria* was described on *Hordeum* and *Triticum*. Most probably conidia of *A. infectoria* present in the wet soil, dead petioles of *Tilia cordata* or leaves of gramineous grasses presented in the park area infected bolete caps and pores. *Alternaria infectoria,* as hemibiotrophic organism is known to be the fungal agent responsible for drying of leaves in several grasses like *Agrostis*, *Arrhenatherum*, *Avena*, *Bromus*, *Dactylis*, *Festuca*, *Lolium*, *Poa, Salicornia, Triticum* (Dugan and Peever, 2002; Ondřej, 1996; Lawrence et al., 2014). The anamorph phase was originally described in 1986 on several gramineous plants by Simmons (1986). According to Simmons (1989) the most typical feature of *A. infectoria* and related species complex is the development of a *Lewia-t*ype sexual morph. However, Lawrence et al. (2014) stated that some species from this group lack a known sexual morph. Another useful diagnostic colony morphological character is the production of large clusters of conidia on nutrient poor PDA media, developing a distinctive granular surface appearance of the colonies (Simmons, 1989), a fact observed also in our cultured isolates (see Fig. 13).

Cladobotryum verticillatum (Link) S. Hughes, Can. J. Bot., 36, p. 750 (1958), Figures 3–5, 14, 26–30.

Acremonium verticillatum Link, Mag. Ges. Freunde, 3(1–2), p. 15 (1809).

=*Cladobotyum agaricina* (Link) Nees, Syst. Pilze, p. 56 (1816); *Verticillium agaricinum* (Link) Corda, Icon. Fung., 2, p. 15 (1838).

Sexual morph*: Hypomyces armeniacus* Tul. & C. Tul., Ann. Sci. Nat. Bot., sér. 4, 13, p. 12 (1860).

Mycelium white, cottony, with mould-like growth when fresh, covering hymenium surfaces of the host or its entire fruitbody. Phialides ca. (28–) 37–70 µm long, (3.7–) 4–5 (–6) µm wide; hyphae about 5–5.5 (–7) µm in diameter, up to 3 µm at the tips (on *Russula*). Conidia on *Russula* sp. 1-celled, ellipsoid or globose, hyaline (10–) 12.2±1.2 (–13.5) µm in diameter, n = 10, ellipsoid cells 14.5–16 × 10 µm. Aleuriospores produced on PDA: (16.5–) 20.1±2.1 (–22) µm in diameter, globose, hyaline, thick-walled. Conidia on *Lactifluus volemus* 1-celled, obovoid, ellipsoid, rarely globose, subglobose, pyriform, hyaline, (8.7–) 16.8±3.9 (–25.2) × (5.7–) 9.1±1.5 (–12) µm, n = 50. Conidia on *Lactarius aurantiacus* 1-celled, obpyriform, hyaline, (11–) 16.5±2.1 (–22) × (7–) 9.3±1.1 (–12.5) µm, n = 45.

Specimens examined: Forebalkan, Lovech District, Troyan Municipality, Golyama Zhelyazna village, Mikrenska Usoyna forest, N 43.001611°, E 24.495206°, alt. ca. 450 m, 25 June 2018, leg. D. Stoykov, on decaying fruit-body of *Lactifluus volemus* (Fr.) Kuntze (=*Lactarius volemus*), on soil in mixed oak-hornbeam forest, SOMF 29972; idem., Bardene locality, N 42.99755°, E 24.485039°, alt. ca. 412 m, 2 June 2018, leg. D. Stoykov, on dry, decaying fruit-body of *Russula* sp., on soil under oak-hornbeam trees, SOMF 31626; Vitosha Region, Vitosha Mt, Bistrishko Branishte Reserve, N 42.574706°, E 23.303081°, alt. ca. 1740 m, 3 August 2018, leg. D. Stoykov, on decaying fruit-bodies of *Lactarius aurantiacus* (Pers.) Gray, on soil in spruce forest, SOMF 31625, SOMF 31631.

Additional materials examined: A collection of *Cladobotyum mycophyllum* (Oudem.) W. Gams & Hooz. (Sofia, Lidl market, SOMF 31630) was examined under LM. It formed larger conidia than in *Cladobotryum verticillatum*, i.e. (18–) 23.5±3.6 (–32) × (8–) 9.2±1.4 (-13) µm, n = 20.

Notes. The asexual morph *Cladobotryum verticillatum* is more common in natural habitats, than the sexual stage, and was generally described with white mycelia, hyphae with fertile verticillate branches, and more or less oval hyaline conidia. The fruit-bodies of *Russula* sp. from Forebalkan, and *Lactarius aurantiacus* in Vitosha Mt, infected with *C. verticillatum,* decayed, became dry and covered with *mould*-like white mycelia. During the present study we succeeded to culture isolated spores on PDA and obtained a stage with thick-walled aleuriospores. The colour, size and shapes of the fertile hyphae, hyaline conidia and aleuoriospores observed conformed well with the data published by Rogerson and Samuels (1994), Arnold and Yurchenko (2007), and Gibson (2017) about *C. verticillatum* and its spore morphs. This species very rarely produces teleomorph with pimpled surface (Gibson, 2017). Arnold and Yurchenko (2007) commented that it differs from other *Cladobotryum* species because of its aseptate conidia with right outlines, except in cases of (0)1-septate conidia. Rogerson and Samuels (1994) considered *C. verticillatum* as the anamorphic stage of *Hypomyces armeniacus*. Later, Arnold and Yurchenko (2007) linked *Cladobotryum verticillatum* conidial sporulation to *Hypomyces ochraceus.* Gams et al. (2004) recorded it mainly as anamorph, and mentioned the ability of *C. verticillatum* to fully destroy the infected mushroom hosts. According to Gibson (2017) the mycelium of *H. armeniacus* shows typical mould-like growth on *Lactarius* and *Russula*, or occasionally on boletes. It covers the spore-bearing surface or the entire fruit-bodies, causing them to decay. The species was characterized by these authors as having up to three types of propagules: 1) conidia 11–24 × 7–12 µm, of various shapes; 2) aleuriospores, thick-walled cells about 20 µm in diameter; 3) ascospores (in the sexual morph), 32–40 × 5–8 µm, 2-celled, verrucose and apiculated. The host species, *Lactarius aurantiacus*, commonly known in Europe as Orange Milkcap, was reported recently by Gyosheva et al. (2017) from Bistrishko Branishte Reserve. Another host, *Lactifluus volemus* is also known as an edible mushroom, gathered by locals in some regions of Bulgaria.

Hypomyces chrysospermus (Bull.) Tul. & C. Tul., Ann. Sci. Nat. Bot., sér. 4, 13, p. 16 (1860).

Asexual morph: *Sepedonium chrysospermum* (Bull.) Fr., Syst. Mycol., 3, p. 438 (1832) Figures 21–23, 35.

Apiocrea chrysosperma (Tul. & C. Tul.) H. Syd., Ann. Mycol., 18, p. 187 (1921).

Colonies covered by dark orange thick conidial masses, almost reaching the whole edge of petri diameter; mycelium in yellow colour only in thin area near the Petri dish edge. Secondary, orange yellowish, powdery asexual morph covering the whole surface of the infected basidiomata. Phialoconidia ellipsoid, hyaline. Aleurioconidia 10.5–23.5 µm [(10.5–) 17.3±2.6 (–22.5) µm in diameter, Q ratio (0.82–) 0.99±0.1 (–1.29), n_1 = 50; (17–) 19.3±2.1 (–23.5) µm in diameter, n_2 = 15; (11.5–) 15.0±2.4 (–19) µm in diameter, Q ratio 1–1.1, n_3 = 10; globose to subglobose, verrucose, thick-walled, golden yellow to lemon yellow in water.

Specimens examined: Forebalkan, Lovech District, Troyan Municipality, Golyama Zhelyazna village, Gradjuvitsa locality, N 42.981056°, E 24.480833°, alt. ca. 405 m, 3 June 2018, leg. D. Stoykov, on decaying fruit-body of *Xerocomus subtomentosus* (L.) Quél., SOMF 31613 (n_3); Vitosha Region, Vitosha Mt, Bistrishko Branshte Reserve, alt. ca. 1680 m, 12 August 2017, leg. D. Stoykov, on decayed fruit-body of *Boletus edulis,* in spruce forest (n_1), SOMF 31628; Rhodopi Mts, Smolyan District, Pamporovo Resort, alt. ca. 1620 m, 9 September 2019, leg. D. Stoykov, on decaying basidioma of *Boletus edulis,* in spruce forest (n_2), SOMF 31622.

The ITS rDNA sequence obtained from SOMF 31622 (PQ436953) is 98.33% similar to *Sepedonium chrysospermum* (KT946851) and *Hypomyces chrysospermus* (ON416974), and others. The obtained sequence PQ436953 showed only 93% coverage and 98.2% similarity to AF054846 (*Sepedonium chrysospermum* var. *paxilli*).

Comments. *Hypomyces chrysospermus* is common on edible boletes in Bulgaria, but so far it was reported only twice in this country, from the territory of Ropotamo Reserve, Southern Black Sea Coast – as *Sepedonium chrysospermum* (Kuthan and Kotlaba, 1981) and in Vitosha region, Vitosha Mt on *Boletus* sp. – as *Hypomyces chrysospermus* (Gyosheva and Stoykov, 2017). It was noted as a potentially harmful agent on fruit-bodies of *Boletus parasiticus* Bull. in the Red Data Book of the Republik of Bulgaria (Assyov and Denchev, 2015). Sahr et al. (1999) reported sepedonin and derivates as isolated pigments from *Hypomyces chrysospermus*.

Lecanicillium fungicola (Preuss) Zare & W. Gams, Mycol. Res., 112(7), p. 818 (2008) Figures 6–7, 15–16, 31.

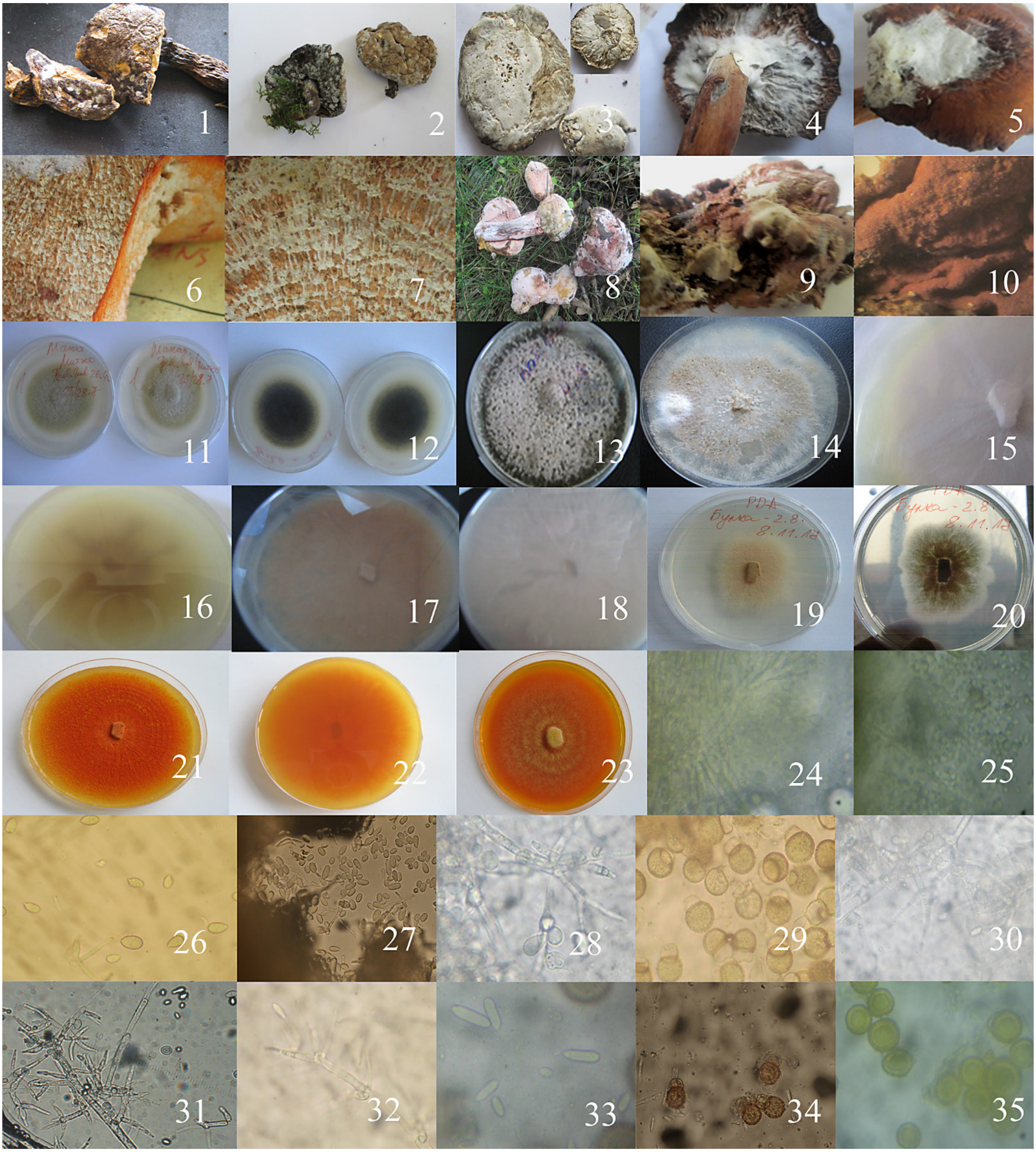
Acrostalagmus fungicola Preuss, Linnaea, 24, p. 96 (1851); *Verticillium fungicola* (Preuss) Hassebr., Phytopath. Z., 9, p. 514 (1936); *Verticillium malthousei* Ware, Ann. Bot., 47, p. 781 (1933).

Mycelium superficial on host, white, cottony near the cap edge, forming whitish patches on the surface of lamellae (Fig. 10–11). The cultured fungal isolate covers the entire surface of petri dishes in 10 days, looking white in front view and light yellow from the reverse side, mycelium superficial, slightly cottony (Figures 15–16). Hyphae examined near the edges of the cap about 3.5 µm in diameter. Conidiophores 24–39 × 3–3.4 µm [31±7.3 × 3.2±0.15, on average], n = 5, septate, hyaline, verticillate, main branches erect, septate, more or less rounded at the end. Conidia 6–7.7 × 4–4.8 µm [6.6±0.9 × 4.3±0.4), n = 5, hyaline, oblong ellipsoidal.

Material examined: Rhodopi Mts, Pamporovo Resort, alt. ca 1650 m, 9 September 2017, isolate obtained from white cottony mycelium from wet surface of old basidioma of *Lactarius deliciosus* (L.) Gray*,* in conifer forest. This voucher specimen, from which an isolate on PDA was obtained on 12 September 2017, was not preserved.

MegaBlast search of ITS rDNA sequence obtained from the cultured isolate (PQ436948) showed 99.82% identity with homologous sequences of *Lecanicillium fungicola* (i.e. OW983022, OW982935, OW983021), isolates from beech leaves and *Clitocybe infundibuliformis*, and is 99.47% identical to cultures *L. fungicola* (MH859667).

Notes. In Europe *L. fungicola* was recorded in Denmark, France, the Netherlands, Poland, Spain, and Ireland, while on the Balkan Peninsula it was reported from Serbia (Piasecka, 2011). The fungus is known to cause dry bubble disease in cultivated mushrooms *Agaricus bisporus* (Lange) Sing. and *A. bitorquis* (Quel.) Sacc. as well as others, i.e. *Coltricia, Clitocybe, Henningsomyces, Hypholoma, Laccaria, Marasmiellus, Pleurotus* and *Thelephora* (Berendsen et al., 2010). Indeed, the presence of white cottony mycelia can be observed in decaying material of *Lactarius deliciosus* preserved in domestic fridge after just a couple of days.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Figs. 1-2 *Hortiboletus bubalinus* infected with *A. infectoria*;
Fig. 3. *Cladobotryum verticillatum* on *Russula* sp.;
Figs. 4–5. *C. verticillatum,* mould-like growth on *Lactifluus volemus*;
Figs. 6–7. *Lecanicillium fungicola* on gills of *Lactarius deliciosus*;
Fig. 8. *Mysogone rosea* on *A. caesarea*, ex situ, SOMF 31627;
Figs. 9–10. Close view of *M. rosea* on the host surface, SOMF 31629;
Figs. 11–12. 30 days colonies of *Alternaria infectoria*, front and reverse;
Fig. 13. *Alternaria infectoria,* 7 days colony, front;
Fig. 14. *C. verticillatum*, colony with aleuriospores*,* front;
Fig. 15–16. *Lecanicillium fungicola,* white and light-yellow colonies, front and reverse;
Fig. 17-20. *M. rosea*, roseous and salmon colonies, front and reverse;
Fig. 21–23. *Sepedonium chrysospermum*, colonies in front, reverse and front, SOMF 31622;
Figs. 24–25. Conidiophores and conidia from cap of *H. bubalinus*;
Figs. 26–27. Conidia of *C. verticillatum* on *L. aurantiacus*;
Fig. 28. *Cladobotryum verticillatum,* conidiophores and conidia on *Russula* sp.;
Fig. 29. *C. verticillatum*, aleuriospores on *Russula* sp.;
Fig. 30. *C. verticillatum*, conidiophores and conidia on *L. volemus*;
Fig. 31. *Lecanicillium fungicola*, conidiophores and conidia on *L. deliciosus*;
Fig. 32. *Mycogone rosea*, verticillate conidiophore;
Fig. 33. *M. rosea*, hyaline phialoconidia on host;
Fig. 34. Two-celled aleuriospores of *M. rosea*;
Fig. 35. *Sepedonium chrysospermum*, aleuriospores, SOMF 31622

Mycogone rosea Link, Obs. in Ordines, 1, p. 18 (1809), Mag. Gesell. Naturf. Freunde, 3(1–2), p. 18 (1809) Figures 8–10, 17–20, 32–34

Syn.: *Mycogone incarnata* Pers., Mycol. Eur., 1, p. 26 (1822); *Sepedonium roseum* Fr., Syst. Mycol., 3, p. 438 (1832).

Cultures on PDA initially with salmon colour, later stained with a faint roseous to pinkish colour. Spore mass on the surface of basidiomata dark roseous or pink with white patches. Conidiophores under LM of *Verticillium*-type, observed both in cultured isolates and from dried fungal material of infected host. Phialoconidia 7.5–11 × 4–5 µm, ellipsoid, hyaline, smooth, present in both studied fungal specimens. Aleurioconidia typically 2-celled; 3-celled formed on fresh material. Top cell 23–30 × 25–30 (–36) µm in diameter, [(23.5–) 27.8±2.4 (–34) × (26.3–) 30.1±2.8 (–36) µm, n_1=15, ratio (0.8–) 0.93±0.06 (–1.1); (26–) 29.5±2.3 (–34) × (28–) 32±2.4 (–36) µm, n_2 = 15, coloured, more or less globose, thick-walled, finely warted. Second lower cell 11.5–16.5 × 16.5–23.5 µm in diameter [(11.5–) 14.6±1.7 (–16.5) × (16.5) 21±2.3 (–23.5) µm, n = 12], hyaline, subglobose, with thickened wall. Third lower cell when formed (11–) 13.4±1.3 (–16) × (13.5–) 15±1.1 (–16.5) µm in diameter, n = 6, hyaline, subglobose.

Specimens examined: Forebalkan, Lovech District, Troyan Municipality, Golyama Zhelyazna village, Mikrenska Usoyna forest, N 43.001611°, E 24.4945°, alt. ca 445 m, 31 July 2017, old decaying basidioma of *Amanita caesarea* (Scop.) Pers., leg. D. Stoykov, SOMF 31629; idem., 8 August 2018, leg. D. Stoykov, SOMF 31627.

Comments. *Amanita caesarea* is an edible species protected in many European countries. It has been evaluated in Bulgaria as Vulnerable (VU) by Gyosheva et al. (2006), and in the IUCN Red list of threatened species for 2019 as Least Concern (LC) by Conçalves (2019). Our cultured fungus fits well with the morphological descriptions of *M. rosea* in Brady and Gibson (1976) and Du et al. (2021). After these authors, *M. rosea* could be distinguished from *Mycogone perniciosa* because of the much brighter colour of its colonies, and at the ultramicroscopic level by the ornamentation on the surface of its aleurioconidia. Unlike *Mycogone perniciosa* (Fries, 1832; Umar et al., 2000), *M. rosea* is not host-specific and was registered in Bulgaria growing on basidiomata of *Amanita caesarea* and *A. rubescens* (Pavel Nedelev, pers. comm., https://manatarka.org/mycogone-rosea/).

The sequences produced from three distinct cultures of SOMF 31629 (PQ436949-PQ436951) showed a 99.83% identity to homologous ITS rDNA sequences of *Mycogone rosea* (PP850323, OP681765, OW982351).

Indeed, 1) PQ436949 showed 99.65% identity with ITS rDNA sequences PP294823, PP850323 and 99.83% identity with PP695216, OP681765; 2) PQ436949 showed 99.83% identity with PP850323, OP681765, OW982351), 99.48-

99.66% identity with PP695216, PP294823, PP850323; and 3) PQ436951 showed 99.15-99.49% identity with OW982351, PP695216, OP681765.

Notes. *Mycogone perniciosa* and *M. rosea* are known to cause the wet bubble disease of cultivated and wild mushrooms (Brady and Gibson, 1976; Umar et al., 2000; Wu et al., 2021, etc.). *Mycogone perniciosa* has 2-celled thick-walled, brown pigmented resting-spores, and hyaline phialoconidia formed on *Verticillium*-type conidiophores (Umar et al., 2000). The *Verticillium* state is a feature not included in the original description of *Mycogone rosea*. Nicot (1962) considered *M. rosea* a parasite, infecting basidiomata from *Amanita gemmata*, *A. muscaria*, *A. phalloides, Boletus* sp., as well as *Clavaria formosa*, *Clavaria* sp. in Asia, *Hygrophorus limacinus*, *H. olivaceo-albus*, and other agarics. This species is similar to *Sepedonium chrysospermum* on boletes (Nicot, 1962) and produces abundant intensively coloured aleurioconidia when mature, tending to cover completely the host surface with pink-brown spore powder. It is associated by Nicot (1962) with some *Verticillium* forms, closely resembling *Verticillium agaricinum* (Link) Corda. This fact was observed from the morphological studies of both Bulgarian collections of *M. rosea* from Forebalkan.

Gams (1983) reported some *Clavaria fennica* and *Ramaria* sp. from Austria apparently infected by another closely related species of *Mycogone*, *M. calospora* (Karst.) Höhn. This species forms white fluffy colonies on 2% MEA, repeatedly verticillate conidiophores, phialoconidia 7–13 × 3–6 µm, pale ochraceous-salmon after 7-days of culture, 2-celled aleuroconidia 25–40(–52) µm in diameter, with 1 or several supportive lateral hyphal cells, and basal cells 8–24 × 7–14 µm.

Brady and Gibson (1976) reported *Mycogone rosea* on cultivated mushrooms, on *Amanita rubescens*, *A. caesarea*, *Inocybe* sp., and *Trichloloma terreum* regionally distributed in Germany, Hungary, Uganda, UK and New South Wales. Du et al. (2021) recently examined numerous isolates from mushroom farms in China. According to the online resources of the Global Biodiversity Information Facility (GBIF, https://www.gbif.org/species/5251292, a map), *M. rosea* has a worldwide distribution.

Sarocladium strictum (W. Gams) Summerb., in Schummerbell et al., Stud. Mycol., 68, p. 158 (2011).

Acremonium strictum W. Gams, Cephalosporium-artige Schimmelpilze, p. 42 (1971).

A single isolate was obtained from an infected basidioma of *Xerocomus subtomentosus* with two white and yellow *Sepedonium* fungal morphs, grown on PDA medium and the ITS rDNA region was sequenced (PQ436952).

The sample originated from Forebalkan, Lovech District, Troyan Municipality, Golyama Zhelyazna village, Gradjuvitsa locality, N 42.981056°, E 24.480833°, alt. ca. 405 m, 3 June 2018, on decaying fruit-body of *X. subtomentosus.* It was found under *Carpinus orientalis,* near the edge of a *Quercus cerris*-*Carpinus betulus* forest.

Comments. ITS rDNA sequence PQ436952 showed 99% identity with *Sarocladium strictum* MH864442 (culture CBS 127151).

This species has been often isolated from soil and plant debris. Summerbell et al. (2011) noticed that *S. strictum* and similar taxa can grow together on natural substrata and therefore could be isolated accidentally in nature.

Conclusions

First molecular data of five fungicolous species, *Alternaria infectoria*, *Hypomyces chrysospermus*, *Lecanicillium fungicola*, *Mycogone rosea*, and *Sarocladium strictum* from Bulgaria is here reported. ITS sequence data about *Mycogone rosea* are the first ones for South-East Europe. It was observed that *Cladobotryum verticillatum* can cause mould-like growth and decay of fruit-bodies from *Russula* sp., *Lactarius aurantiacus* and *Lactifluus volemus*. The fungicolous species studied in the present work, excepting *Alternaria infectoria* and *Sarocladium strictum*, are closely associated to their mushroom hosts. *Lecanicillium fungicola* has been reported and isolated for the first time from decaying lamellae of *Lactarius deliciosus* in Bulgaria. *Mycogone rosea*, a well-known fungicolous species occurring in most European countries and North America, was not registered before on *Amanita caesarea* in oak-hornbeam forests in Bulgaria. *Hypomyces chrysospermus* (asexual stage *Sepedonium chrysospermum*), probably one of the most widespread fungal parasites on edible boletes, is reported on basidiomata of *Boletus edulis* and *Xerocomus subtomentosus.*

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Conflict of interest

The authors declare no conflict of interests.

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