

First record of *Coprinopsis strossmayeri* (*Psathyrellaceae*) in Ukraine: morphological and cultural features

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The article presents data on the first record of the rare wood-rotting species of the *Coprinopsis strossmayeri* aggregate in Ukraine. A full description of its macro- and micromorphological features as well as an original drawing are provided. Morphological characters and data on mycelial growth on different agar media are reported. The growth optimum was observed on compost agar medium. Mycelial colonies of *C. strossmayeri* are white, cottony, very dense with fluffy aerial mycelium growing in concentric zones. Colonies have a characteristic yellow pigmentation and stain the agar yellowish. Microscopic features of vegetative mycelia are described. In the mycelium of *C. strossmayeri*, spherical structures inside storage hyphae, clamp connections, anastomoses, chlamydo-spores, and crystals on hyphae were observed.

Key words: Basidiomycetes, *Agaricales*, SEM, mycelium, morphological characteristics, growth rate.

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Prydiuk M.P., Lomberg M.L. (2021): První nález *Coprinopsis strossmayeri* (*Psathyrellaceae*) na Ukrajině: morfologické znaky a vlastnosti v kultuře. – Czech Mycol. 73(1): 45–58.

Článek přináší údaje o prvním nálezů vzácného dřevožijného hnojníku z okruhu *Coprinopsis strossmayeri* na Ukrajině. Je uveden kompletní popis makro- a mikromorfologických znaků spolu s jejich kresbou. Jsou též zaznamenány údaje o růstu mycelia na různých agarových médiích a morfologické znaky houby v kultuře. Jako nejlepší pro růst se ukázal kompostový agar. Myceliální kolonie *C. strossmayeri* jsou bílé, vatovité, velmi husté s pýřitým vzdušným myceliem, tvořící soustředné zóny. Jsou charakteristické žlutou pigmentací a barví též agar do žluta. Popsány jsou i mikroskopické znaky vegetativního mycelia; v něm byly pozorovány kulovité struktury uvnitř zásobních hyf, přezky, anastomózy, chlamydo-spory a krystaly na povrchu hyf.

INTRODUCTION

Coprinoid fungi of the family *Psathyrellaceae* have a wide distribution in Ukraine. At the end of 2014 a total of ninety-eight species of the family were registered in the country (Prydiuk 2015). Recently a very interesting representative of the *Psathyrellaceae* was found on wooden substrate in Kyiv (Ukraine) in May 2019 (Fig. 1). Later, in September and October 2020, its fruitbodies reappeared at the site, at the roots of a decayed stump, about half a metre from the previous location. Following examination, this specimen was identified as *Coprinopsis strossmayeri* (Schulzer) Redhead, Vilgalys et Moncalvo, a rather rare fungus in Europe. This find represents the first record of the species in Ukraine. It was successfully isolated from fresh basidiocarps into pure culture and is currently deposited at the IBK Mushroom Culture Collection (Bisko et al. on-line).

It should be mentioned that the species has antifungal, mitogenic/regenerative, proteolytic/caseinolytic/fibrinolytic properties. Besides, *C. strossmayeri* extracts revealed antimicrobial activity against Gram-positive and Gram-negative bacteria inhabiting human and animal bodies (*Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, etc.) (Badalyan et al. 2006, 2008). From the *C. strossmayeri* culture filtrate, two dimeric sesquiterpenes, bovistol B and D, and one monomeric sesquiterpene, strossmayerin, were isolated. These sesquiterpenes have potentially a wide range of applications including pharmaceutical and agricultural ones (Banks et al. 2020). Although the edibility of *C. strossmayeri* is unclear, its bioactive compounds mentioned above make this species promising as a medicinal mushroom (Gargano et Ferraro 2020).

Taking into account the rarity of the species and its potential importance as a producer of bioactive substances, we present a full description of the specimen including both macro- and microscopic details and cultural features.

MATERIAL AND METHODS

This paper is based on a study of both dried basidiocarps (deposited in the Herbarium of the M.G. Kholodny Institute of Botany (KW-M), National Academy of Sciences of Ukraine, Kyiv, Ukraine) and pure culture, isolated from the same specimen.

Strain and medium. A dikaryotic culture of *C. strossmayeri* was isolated by means of the tissue plug method using wort agar (WA) medium and preserved at the IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany NAS of Ukraine under number IBK 2625 (Bisko et al. on-line).

Morphological and cultural studies of the *C. strossmayeri* culture were carried out in Petri dishes on three nutrient agar media: malt extract agar (MEA; 2% sugar, Oxoid, Great Britain), beer wort agar (WA; liquid beer wort, diluted with distilled water to a density of 4° on the Balling scale) and compost agar (CA). The latter is obtained by taking 75 g dried phase II compost, non-inoculated, adding 1 litre of boiling water, leaving untouched for 12 hours, then heating the mixture (compost and water) to boiling point and filtering it through triple layered cheese cloth, then supplementing the compost broth with distilled water to a volume of up to 1 litre. In the case of CA and WA media preparation we gently mixed 1 litre of liquid and 20 g agar. All media were adjusted to pH 6.0 with a 10% KOH and 1N HCl solution and sterilised by autoclaving at 121 °C for 30 minutes.

Growth rate and morphology study. Nutrient media were poured into sterile plastic Petri dishes (diameter 90 mm, 20 ml per dish), and cooled down in a laminar flow box. The inoculum was initially prepared by cultivating *C. strossmayeri* IBK 2625 mycelium for 10 days on WA in darkness at 25 °C. From the actively growing part of the colony, agar plugs (5 mm diameter) were cut out with a cork borer, placed into Petri dishes with fresh medium and plates were further incubated at 25 °C until the medium was covered by mycelia. The radial growth speed was calculated according to the method described by Lomberg et Solomko (2012). Average diameters of colonies were determined on days 7 and 10 and respective growth rates (mm/day) were calculated. Micro- and macro-morphological features of the vegetative mycelium were determined according to the standard methods proposed by Stalpers (1978). The type of colony, its colour and density, edge and character of the outer line, the colour of the reversum and the presence or absence of concentric circles were recorded.

Microscopic investigations. Microscopic structures were observed in dried material. Microscopic sections of lamellae were made at about half of the pileus radius and examined in 3% KOH. The spores were studied in water.

Spore sizes are based on at least 20 spore measurements per basidiocarp. For basidia and cystidia, the means of the smallest and the largest elements per basidiocarp are based on 10 measurements in each case.

In the descriptions the following abbreviations are used: av. B = average breadth of the spores in frontal view (according to Cléménçon 2004); av. L = average length of the spores; L = number of lamellae reaching stipe; l = number of short lamellae (not reaching stipe) between two long ones; Q = length divided by width; av. Q = average Q.

Microstructures of vegetative mycelium were investigated using an MBI-15 light microscope (LOMO, St. Petersburg, Russia; maximum magnification 1900×). Preparations for scanning electron microscopy (SEM) were prepared as described by Buchalo et al. (2009, 2011). Micrographs were prepared using a JSM-35C

scanning electron microscope (JEOL, Akishima, Japan; maximum magnification 180,000 \times).

Statistical analysis. All data were processed with statistical methods of analysis; values of standard deviations (SD), coefficients of variation, and confidence intervals were calculated using standard statistical packages, Microsoft Office Excel and StatSoft Statistica 6.0. Experimental data were expressed as mean \pm SD from quintuple measurements. The Student's *t*-test was applied to express the significance; values at $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Coprinopsis strossmayeri (Schulzer) Redhead, Vilgalys et Moncalvo in Redhead, Vilgalys, Moncalvo, Johnson et Hopple, *Taxon* 50: 231, 2001 Figs. 1, 2

- = *Coprinus strossmayeri* Schulzer, *Verh. zool.-bot. Ges. Wien* 28: 430, 1879
- = *Coprinus rhizophorus* Kawam., *Icons Jap. Fungi* 5: 559, 1954 (invalid) – *Coprinus rhizophorus* Kawam. ex Hongo et K. Yokoy., *Trans. mycol. Soc. Japan* 17: 140, 1976 – *Coprinopsis rhizophora* (Kawam. ex Hongo et K. Yokoy.) D.J. Schaf. et B. Douglas, *Field Mycol.* 21(1): 6–8, 2020
- = *Coprinus populicola* Mornand, *Docums Mycol.* 28 (nos. 109–110): 70, 1998 – *Coprinus strossmayeri* var. *populicola* (Mornand) Bon, *Docums Mycol.* 31 (no. 124): 21, 2002

Morphological features. Pileus at first 7–40 \times 5–25 mm, ovoid, ellipsoid to paraboloid, then obtusely conical, campanulate to convex-paraboloid, sometimes with small umbo, finally with recurved margin, up to 60 mm in diameter, young completely covered by white veil, later splitting up into small felty patches and flocks, the latter at first white or cream, later yellowish or dirty yellow in cap centre; pileus pale grey to ochraceous grey below veil, later dirty grey, towards margins paler, in centre darker, ochre-brown. Lamellae free, very crowded, $L > 50$, $l = 3–7$, at first white then grey, grey-brown to blackish-brown, finally black. Stipe 10–120 \times 4–8 mm, hollow, cylindrical, slightly tapering upwards, with clavate base, smooth to slightly fibrous, with scattered velar remnants, whitish, later often yellowish; at base with well developed orange-brown to brown rhizomorphs up to 100 mm in length and up to 2–3 mm thick. Flesh in pileus and stipe whitish, with mild taste and fungoid smell. Rhizomorphs with strong unpleasant smell resembling mould. Spore print black.

Spores 8.5–10.0(11.0) \times 5.0–6.0 \times 4.5–5.0 μm , $Q = 1.58–1.91$, av. $L = 9.6 \pm 0.66$ μm , av. $B = 5.5 \pm 0.35$ μm , av. $Q = 1.74 \pm 0.08$; slightly lentiform, in frontal view ovoid to ellipsoid, sometimes slightly mitriform, with rounded basis and apex, in lateral view ellipsoid, dark red-brown, germ pore central, up to 1.5 μm wide. Basidia 17–24 \times 8–8.5 μm , 4-spored, surrounded by 3–5 pseudoparaphyses. Cheilocystidia laterally compressed, 120–180 \times 33–41 \times 12–16 μm , utriform, subfusiform, sometimes nearly sublageniform. Pleurocystidia 134–254 \times 22–46 \times



Fig. 1. *Coprinopsis strossmayeri* (KW-M 71194) – source of strain IBK 2625. Photo M.L. Lomberg.

12–18 μm , similar to cheilocystidia but more elongated. Veil consisting of branched, weakly diverticulate, thin-walled elements $25\text{--}50 \times (2.5)5\text{--}8 \mu\text{m}$ in size. Clamp-connections present.

Habitat and distribution. In very large clusters (up to several dozens of basidiocarps) on rotten wood of broadleaved trees in forests and parks. Known from Europe (Austria, Belgium, Croatia, Denmark, France, Germany, Great Britain, Italy, Netherlands, Norway, Russia, Slovakia, Spain, Switzerland, and Ukraine), Africa (Morocco), Asia (Armenia, India, Japan, South Korea) and North America (Canada, USA) (Enderle et Bender 1990, Immerzeel 1997, Cacialli et al.



Fig. 2. *Coprinopsis strossmayeri* (KW-M 71194): **a** – basidiocarps; **b** – basidia; **c** – cheilocystidia; **d** – pleurocystidia; **e** – veil elements; **f** – spores. Scale bars = 1 cm for basidiocarps, 10 μ m for microstructures. Del. M.P. Prydiuk.

1999, Fraiture et Vanholen 2000, Lanconelli 2000, Vizzini 2001, Červenka 2003, Uljé 2005, Badalyan et al. 2011a, Vesterholt 2012, El Akil et al. 2014, Champion 2018, Venanzoni et al. 2019, Anonymus 2019, Douglas et al. 2020, Gargano et Ferraro 2020). *Coprinopsis strossmayeri* is very rare everywhere, and details of its distribution in Ukraine are still unknown.

Specimen examined

Ukraine. Kyiv, Tereshchenkivska St., 50°26'42.7" N, 30°30'56.2" E, on rotten stump of *Aesculus hippocastanum*, 17 May 2019, leg. M.L. Lomberg, det. M.P. Prydiuk (KW-M 71194).

Distinguishing characters. This species can be rather easily identified due to its relatively large basidiocarps growing in clumps. Although its fruitbodies look somewhat like those of *Coprinopsis atramentaria* (Bull.: Fr.) Redhead, Vilgalys et Moncalvo or *C. romagnesiana* (Singer) Redhead, Vilgalys et Moncalvo, they clearly differ in several important features. The first of them is the presence of rather thick white veil on its pileus, soon breaking up into small felty flocks and scales, while *C. atramentaria* has almost smooth caps and *C. romagnesiana* differs by the shape, consistence and colour of its veil-scales (red-brown, not felty). A second noticeable distinguishing feature of *C. strossmayeri* is the development of rather thick orange-brown rhizomorphs at the stipe base, which moreover have a strong smell resembling mould. Microscopically, *C. strossmayeri* is characterised by diverticulate veil hyphae and ellipsoid-ovoid spores.

The spores of our specimen were longer [8.5–10.0(11.0) × 5.0–6.0 × 4.5–5.0 μm] than indicated by many authors: 6.5–8.5 × 4–5 μm (Červenka 2003), 7.0–9.0 × 4.5–6.0 μm (Uljé 2005, Vesterholt 2012), 9.1 × 5.8 μm (Gargano et Ferraro 2020). On the other hand, they are rather close to the spore measurements given by Douglas et al. (2020): 8.1–10.6 × 4.7–6.1 μm, albeit these authors did not notice any lentiform spores. Similarly, cystidia of the Ukrainian specimen of *C. strossmayeri* were much longer compared to published data. This concerns particularly the pleurocystidia reaching up to 254 μm in length, while they are 70–180 μm according to Uljé (2005) and 60–130 μm long according to Gargano et Ferraro (2020). However, Douglas et al. (2020) also observed relatively long pleurocystidia (125–220 μm) on their specimen.

It must be pointed out that phylogenetic analysis of several collections of *C. strossmayeri* from different countries has revealed a whole complex of closely related taxa (Douglas et al. 2020). Nevertheless, these authors presented a phylogenetic tree showing particular lineages, but their taxonomical rank still remains uncertain. On the whole, macro- and microscopic features of the fruitbodies collected in Ukraine allow us to identify them as *C. strossmayeri* agg. with confidence, but the exact taxonomical position of the specimen still needs further investigation (it has however some morphological similarity to the British collection). Therefore a wide species concept is retained in our study.

Micromorphological characteristics of vegetative mycelium. The vegetative mycelial microstructures of *C. strossmayeri* IBK 2625 were studied using light and scanning electron microscopes (Fig. 3). It was shown that the studied strain is characterised by the presence of such micromorphological features as clamps (Fig. 3c), anastomoses (Fig. 3a, d), chlamydo-spores (Fig. 3a, e), crystals of different shapes (Fig. 3a, d), which had already been reported for this species (Badalyan et al. 2011a, 2011b). Clamp connections are characteristic features of dikaryotic mycelia of many basidiomycetes. The presence and dislocation of clamp connections on hyphae are essential taxonomic characteristics for some species (Buchalo et al. 2009, 2011). Dikaryons of *Coprinopsis* species usually have oval to round clamp cells at the hyphal septae. On the contrary, dikaryons of *Coprinellus* species are mainly clampless, with occasional fused clamps or pseudoclamps (Badalyan et al. 2011b). The studied species, *Coprinopsis strossmayeri*, forms mostly single round, arch-shaped clamps, often seen at the hyphal septa of the dikaryotic strains, although septa without clamp cells have also been reported (Badalyan et al. 2011a). Intercalary chlamydo-spores may de-

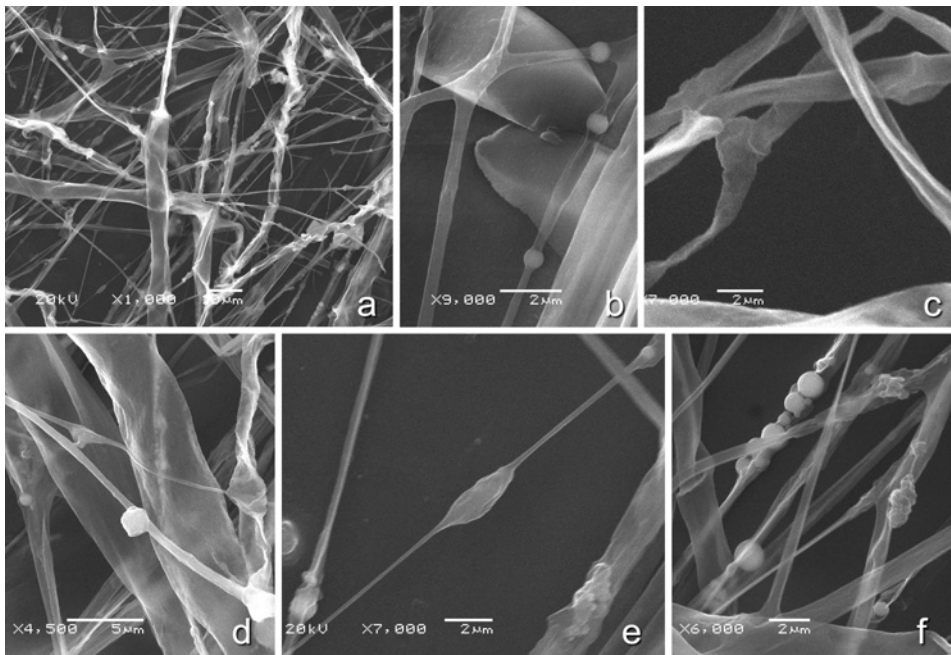


Fig. 3. Micromorphological features of *Coprinopsis strossmayeri* IBK 2625 on MEA medium: anastomoses, chlamydo-spores, crystals, storage hyphae (**a**); storage hyphae (**b**, **d**, **f**); clamp connection (**c**); anastomosis and crystals on hyphae (**d**); chlamydo-spore (**e**). Scale bars = 10 µm (**a**), 5 µm (**d**), 2 µm (**b**, **c**, **e**, **f**). Photo M.L. Lomberg.

velop on its hyphae (Fig. 3a). Our observations are on the whole consistent with literature data (Badalyan et al. 2011a, 2011b).

As for the round structures inside the hyphae seen in SEM photos (Fig. 3a, b, f), we suspect that they are accumulations of some nutrients, such as glycogen (although they may also be lipids, polyphosphates or some proteins). They are often accumulated inside the hyphae (so-called 'storage hyphae'). According to Cléménçon (2004), inclusion of glycogen can be found in such hyphae in laboratory cultures of *Coprinopsis cinerea* (Schaeff.) Redhead, Vilgalys et Moncalvo and *C. trispora* (Kemp et Watling) Redhead, Vilgalys et Moncalvo, which are representatives of the same genus. We suppose that these structures are 'storage hyphae' and are important features in the vegetative stage of development of *C. strossmayeri* which could be added to the species diagnosis. However, it is not clear which substance storage hyphae contain; whether it is glycogen or something else, it still needed to be investigated. Nevertheless, the study carried out using SEM has made it possible to establish some morphological mycelium characteristics which can be used to identify and verify *C. strossmayeri* strains in vegetative stage.

Growth and colony morphology. Literature data indicate that some cultural and morphological characteristics, as well as growth rate of vegetative mycelium, and the morphology of colonies on certain reference agar media may serve as essential taxonomic characteristics of the species (Stalpers 1978, Badalyan et al. 2011a, 2011b, Lomberg et Solomko 2012, Lomberg 2016). Our study was carried out on natural and complex nutrient media such as MEA, WA and CA. Previously, MEA was mostly used as a nutrient medium for *C. strossmayeri* cultivation (Badalyan et al. 2011a, 2011b). On the other hand, many saprotrophic species, for example *Coprinus comatus* (O.F. Müll.) Pers., *Lepista nuda* (Bull.) Cooke, some *Agaricus* species, etc. prefer CA for fast vegetative growth and storage in culture collections (Lomberg et Solomko 2012, Lomberg 2016, Mykhaylova et al. 2019).

The results of the study of *C. strossmayeri* IBK 2625 growth on agar nutrient media of different composition show the highest growth rate being on CA – up to 6.23 ± 0.36 mm/day at 25 °C and pH 6.0 (Fig. 4). Mycelial colonies were white and cottony. We also observed the densest colonies with fluffy aerial mycelium growing in concentric zones exactly on this medium (Fig. 5a, b). Comparative data of growth rate on the studied media classifies this species into the middle-range group of fungi with an average radial mycelium growth rate of 4 to 8 mm/day. This group includes the majority of cultures in the IBK Collection: *Coprinus comatus*, *Agaricus* spp., *Flammulina velutipes* (Curtis) Singer, *Hericium erinaceus* (Bull.) Pers., *Hypsizygus marmoreus* (Peck) H.E. Bigelow, *Kuehneromyces mutabilis* (Schaeff.) Singer et A.H. Sm., *Lentinula edodes* (Berk.) Pegler, *Pleurotus eryngii*

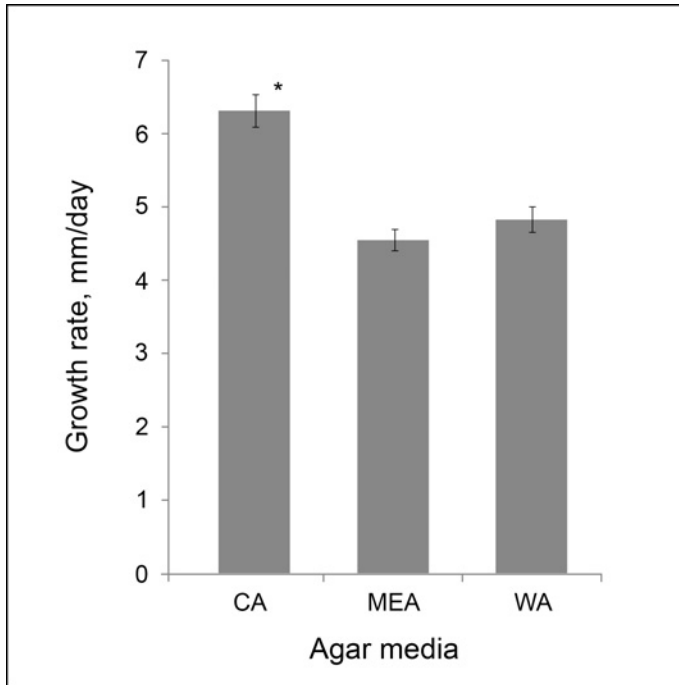


Fig. 4. Effect of medium on *Coprinopsis strossmayeri* IBK 2625 growth rate. Values are given as mean ± SD (n = 5).

* p < 0.05 in comparison with corresponding growth rate on MEA and WA media.

(DC.) Qué. etc. (Lomberg et Solomko 2012, Mykchaylova et al. 2019). As documented by Badalyan et al. (2011a), at 25 °C and pH 6.0, mycelia of the various *C. strossmayeri* strains grew fast on MEA (growth rates of 5.3 to 7.2 mm/day), colonies were characterised by prominent radial cords and little fluffy aerial mycelium. We observed a similar morphology of the strain on MEA (Fig. 5c) but the growth rate was somewhat lower – 4.33 ± 0.16 mm/day. This can be explained by strain variability. On WA, mycelia formed silky, smooth, not very dense colonies with radial-parallel mycelial strands (Fig. 5d). On all media, the mycelium was initially pearl white. On aging, the colonies on CA became dirty white and the concentric growth zones stained yellow, whereas WA and MEA colonies showed a pale yellow colourisation (Fig. 5). In all cases, the agar became light yellow pigmented over time.

Therefore, since the composition of agar media significantly affects the growth characteristics of the vegetative mycelium, the obtained data allow for determining the optimum conditions ensuring a maximum growth rate of *C. strossmayeri*.

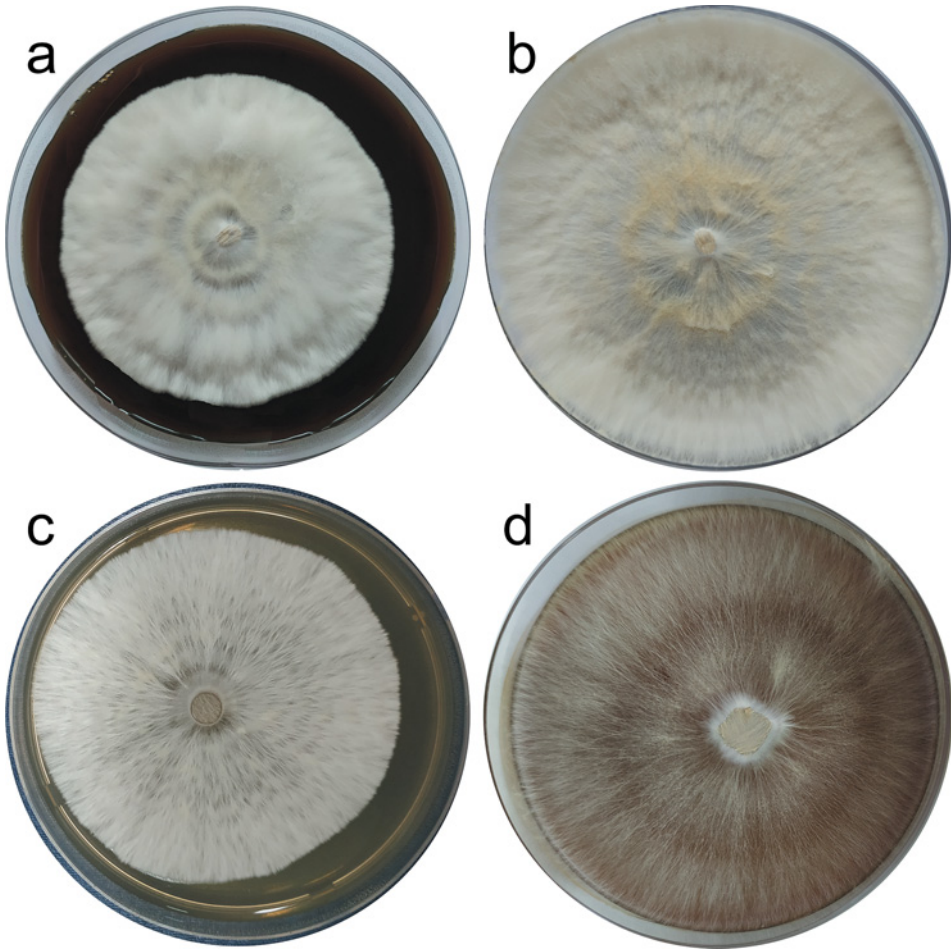


Fig. 5. Mycelial colonies of *Coprinopsis strossmayeri* IBK 2625 on CA (a, b), MEA (c), and WA (d) at 25 °C: **a** – on day 7; **b**, **c** – on day 10; **d** – on day 13 of cultivation. Photo M.L. Lomberg.

CONCLUSION

Our morphological study has showed a slightly larger variability in the spore-size of *C. strossmayeri* and a much higher variety of hymenial cystidia measurements than had been reported for this species. Similarly, large spores and long pleurocystidia have recently been reported from the United Kingdom, hence the Ukrainian specimen may belong to the same taxon within the species aggregate, but further investigation is needed.

Depending on media composition, morphological variations were observed in colonies, but over time the agar became light yellow pigmented in all cases. The densest white and cottony mycelial colonies with fluffy aerial mycelium growing in concentric zones were observed on compost agar medium, which also showed to have the highest growth rate. Regarding the obtained growth rate data this species belongs to the middle-range group of fungi. The studied strain is characterised by the presence of such micromorphological features as clamps, anastomoses, chlamydospores, and crystals of different shapes. We suppose that the spherical structures inside the hyphae are important features of the vegetative stage of development of *C. strossmayeri* and could be added to the species diagnosis.

The obtained results expand our knowledge of the biological characteristics of *C. strossmayeri* and are useful for the creation of reliable means of maintaining pure cultures under artificial conditions, required for the protection and preservation as well as the practical application of fungi.

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