

## New and noteworthy records of operculate discomycetes of the *Pyronemataceae* (*Pezizales*) from Ukraine

VERONIKA V. DZHAGAN<sup>1\*</sup>, YULIA V. SHCHERBAKOVA<sup>2</sup>, YULIA I. LYTVYENKO<sup>3</sup>

<sup>1</sup>Educational and Scientific Centre “Institute of Biology and Medicine”, Taras Shevchenko National University of Kyiv, 64/13 Volodymyrska Str., Kyiv, UA-01601, Ukraine; veronika.dzhagan@gmail.com

<sup>2</sup>The State scientific research forensic center of the Ministry of Internal Affairs of Ukraine, 10 Bohomoltsa Str., Kyiv, UA-01024, Ukraine; yulia.shcherbakova@ukr.net

<sup>3</sup>A.S. Makarenko Sumy State Pedagogical University, 87 Romenska Str., Sumy, UA-40002, Ukraine; lytvynenko2014@gmail.com  
\*corresponding author

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The article reports new data on the occurrence of three species of apothecial ascomycetes of the *Pyronemataceae* family collected in the Carpathian Biosphere Reserve. *Aleurina subvirescens* and *Smardaea purpurea* were found in Ukraine for the first time, *Ramsbottomia asperior* was previously found by us also at other localities in Ukraine, including the Carpathian Biosphere Reserve, but without any details and illustrations. For each species a description of the Ukrainian specimens, collection data, macro- and micrographs are provided here. In addition to morphological characters, ecological characteristics and data on the general distribution of these species are briefly discussed.

**Key words:** *Ascomycota*, *Pezizomycetes*, *Aleurina subvirescens*, *Ramsbottomia asperior*, *Smardaea purpurea*, biodiversity, Carpathian Biosphere Reserve.

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Dzhagan V.V., Shcherbakova Yu.V., Lytvynenko Yu.I. (2021): Nové a zajímavé nálezy operkulárních diskomycetů čeledi *Pyronemataceae* (*Pezizales*) z Ukrajiny. – Czech Mycol. 73(2): 137–150.

Článek přináší nové údaje o výskytu tří druhů apotheciálních askomycetů čeledi *Pyronemataceae* sbíraných v Karpatské biosférické rezervaci. *Aleurina subvirescens* a *Smardaea purpurea* představují první nálezy z území Ukrajiny, zatímco *Ramsbottomia asperior* už byla autory nalezena na jiných ukrajinských lokalitách, včetně Karpatské biosférické rezervace, ale k nálezům nebyly zveřejněny žádné detaily ani ilustrace. Ke všem druhům jsou uvedeny popisy ukrajinských položek a nálezová data, doplněná makro- a mikrofotografiemi. Kromě morfologických znaků jsou stručně diskutovány ekologické zvláštnosti a shrnutý údaje o celkovém rozšíření uvedených druhů.

## INTRODUCTION

*Pyronemataceae* is the largest and most heterogeneous family of the cup-fungi of the *Pezizales* order, comprising apothecial ascomycetes of mainly epigeous origin, some being semihypogeous to hypogeous (truffle-like fungi). Members of the family are diverse in ascoma shape, with discoid to pulvinate or cupulate, mostly sessile apothecia. The disc is predominantly coloured from whitish to yellow, orange, red or pink due to carotenoid pigments in the paraphyses, although in some species other colours occur too. The external surface is glabrous or with hyaline to dark brown hairs or thick-walled setae. The ectal excipulum is usually composed of a *textura globulosa* to *angularis*, and the medullary excipulum is made up of a *textura intricata*. Asci are operculate and iodine negative (inamyloid), cylindrical, with or sometimes without croziers. Ascospores are hyaline and vary in shape and ornamentation, in size, number of nuclei and lipid bodies; they can be globose to ellipsoid, sometimes fusoid, ovoid, also cylindrical, smooth or warty to spiny or reticulate, usually sheathless, but sheath occurring in some species such as *Lotinia verna* (Van Vooren et Vega 2018a), rarely with conical appendages, with very low to high lipid content. Anamorphs are known in many species and are hyphomycetous and blastic (Hansen et al. 2013, Jaklitsch et al. 2016, Ekanayaka et al. 2018, Döbbeler et al. 2021).

The family is ecologically highly diverse. Members of *Pyronemataceae* are mainly saprobic on all types of soil or dead plant material, but also develop on dung and burnt substrates. Some form ectomycorrhizal associations with vascular plants, some have been detected as symbionts of orchids (Hansen et al. 2013). Several *Pyronemataceae* are known to be bryoparasitic and are associated with mosses and liverworts (species in the genera *Filicupula*, *Lamprospora*, *Neottiella*, *Octospora* and *Octosporopsis*) (Egertová et al. 2015, Egertová et al. 2018). Only *Pyropyxis rubra* has been recognised as a facultative pathogen of vascular plants (Hansen et al. 2013, Filippova et al. 2016). The family is mainly distributed from temperate to arctic-alpine regions, with a few taxa known from the tropics (Perry et al. 2007).

The circumscription of *Pyronemataceae* is recorded to vary from 34 genera and 358 species (Jaklitsch et al. 2016) through 58 genera and 677 species (Ekanayaka et al. 2017) to 78 genera and ca. 660 species (Kirk et al. 2008). According to the most recent data, the family includes 62 genera encompassing approximately 575 species (Wijayawardene et al. 2020).

Molecular phylogenetic studies (Perry et al. 2007, Hansen et al. 2013, Ekanayaka et al. 2018) have shown that the *Pyronemataceae* is a highly polyphyletic group of the *Pezizales* and have led to some genera being included into or excluded from the family. The group is being studied intensively, and many new taxa have also been described in recent years (Pfister et al. 2013, Stielow et al. 2013, Van

Vooren et al. 2015, Lindemann et Bohning 2016, Van Vooren et Vega 2018b, Van Vooren et al. 2021).

In Ukraine, the *Pyronemataceae* family is represented by 98 species (Smitskaya 1980, Minter et Dudka 1996, Andrianova et al. 2006). In the Ukrainian Carpathians, 50 species of this family are known today (Dudka et al. 2019) since this territory has attracted the attention of mycologists for a long time. During field excursions to the Carpathian Biosphere Reserve in 2014–2016, the second author found ascomata of some interesting *Pyronemataceae* fungi: *Aleurina subvirescens* (Velen.) W.Y. Zhuang et Korf, *Ramsbottomia asperior* (Nyl.) Benkert et T. Schumach., and *Smardaea purpurea* Dissing. The records of *A. subvirescens* and *S. purpurea* are the first for Ukraine; while *R. asperior* was preliminarily reported by us in lists of fungal species from other localities in Ukraine (Shcherbakova et Dzhagan 2015, Dudka et al. 2019), however without a description or illustration.

Here we provide the main diagnostic features of these fungi, updated information about their general distribution, the exact location, and original illustrations of the recently collected Ukrainian specimens.

#### MATERIAL AND METHODS

Specimens of the species listed below were collected in 2014–2016 during a field survey to the Carpathian Biosphere Reserve (Transcarpathian Region, Ukraine). Macrophotographs of the fungi were made with a digital camera, macroscopical descriptions of the collected specimens are based on fresh ascomata. After that, they were dried, and microscopically examined during the following weeks. Samples were rehydrated in tap water (H<sub>2</sub>O) and/or KOH (5%). The following mounts were used to observe microscopic characters: Congo Red solution (CR), Melzer's reagent (MLZ), Lugol's solution (IKI) and Lactophenol Cotton Blue solution (LPCB). Micromorphological characteristics were examined with a XY-B2T light microscope (Ulab, China) and a Primo Star light microscope (Carl Zeiss, Germany) at a magnification of up to 1000×. Spore statistics included length and width of a minimum of 20 spores from each collection. The quotient (Q) between spore length and width was calculated. The range of spore size was determined following the algorithm by Kušan (2015), i.e. arithmetic mean ± standard deviation, with extreme values in parentheses. Microphotographs were taken with a ScienceLab DCM 520 digital camera using the Tsview 7 modular software (Fuzhou Tucsen Imaging Technology Co., China).

Analysis of the general distribution of the species is based on data from many bibliographic sources (Dissing 1985, Zhuang et Korf 1986, Yao et Spooner 1995, Benkert 2005, Medardi 2006, Van Vooren et Mourgues 2009, Moyne et al. 2010)

and on critically revised open internet resources as well as the Global Biodiversity Information Facility (GBIF, <https://www.gbif.org/>). Specimens are deposited in the Herbarium of Taras Shevchenko National University, Kyiv (KWHU).

## RESULTS AND DISCUSSION

With the present study, *Aleurina subvirescens* and *Smardaea purpurea* were added to the Ukrainian *Pyronemataceae* as new records. Since diagnoses of these species have been provided in many resources, we restrict our report to a brief description of morphological features only based on the recent Ukrainian collections.

*Ramsbottomia asperior* was first reported by us in swamps in Drevlanskiy Nature Reserve (Zhytomyr Region, Polissya) (Shcherbakova et Dzhagan 2015). Later we found it in the two protected areas of the Ukrainian Carpathians – Synevyr National Nature Park and Carpathian Biosphere Reserve (Dudka et al. 2019). In the mentioned works only the fact of finding *R. asperior* was stated, lacking a description and illustration. Below we provide information about our records from the territory of the Carpathian Biosphere Reserve.

***Aleurina subvirescens*** (Velen.) W.Y. Zhuang et Korf, Mycotaxon 26: 377, 1986  
Figs. 1–2

- ≡ *Humaria subvirescens* Velen., České Houby 4–5: 855, 1922 (basionym)
- ≡ *Jafneadelphus subvirescens* (Velen.) Svrček, Česká Mykol. 31(2): 69, 1977

**Macroscopic and microscopic features.** Apothecia discoid, about 2–2.5 mm diam., sessile, hymenium ochraceous brown, flat; receptacle surface light brown, scabrous. Ectal excipulum made up of a *textura angularis*, 170–200 µm thick, with polyhedral isodiametric and ovoid cells, brown or light brown, 30–70 µm in diam. Anchor hyphae present, pale brown, 4.5–5.5 µm wide. Medullary excipulum made up of a *textura intricata*, up to 120 µm thick, hyphae pale brown, 4.0–6.0 µm wide. Asci 195–265 × 14.5–16.5 µm, operculate, cylindrical, 8-spored, inamyloid. Ascospores (18.5)19.3–21.2(22.3) × (11.1)12.2–13.8(14.7) µm, Q = 1.60–1.76, uniseriate, ellipsoidal, hyaline, in rehydrated state with one large, sometimes two or more small lipid bodies. Perispore ornamented with small round to ellipsoid warts, 0.4–0.6(1.0) µm in diam., which sometimes fuse. Paraphyses 3.8–4.2 µm wide, straight, septate, unbranched, apical cells up to 6 µm wide.

### Specimen examined

Ukraine. Transcarpathian Region, Rakhiv District, Svydovetskyi Mountain Range of the Carpathian Biosphere Reserve, vicinity of Dragobrat, 48°14'48.9" N, 24°14'59.5" E, alt. 1250 m, mixed

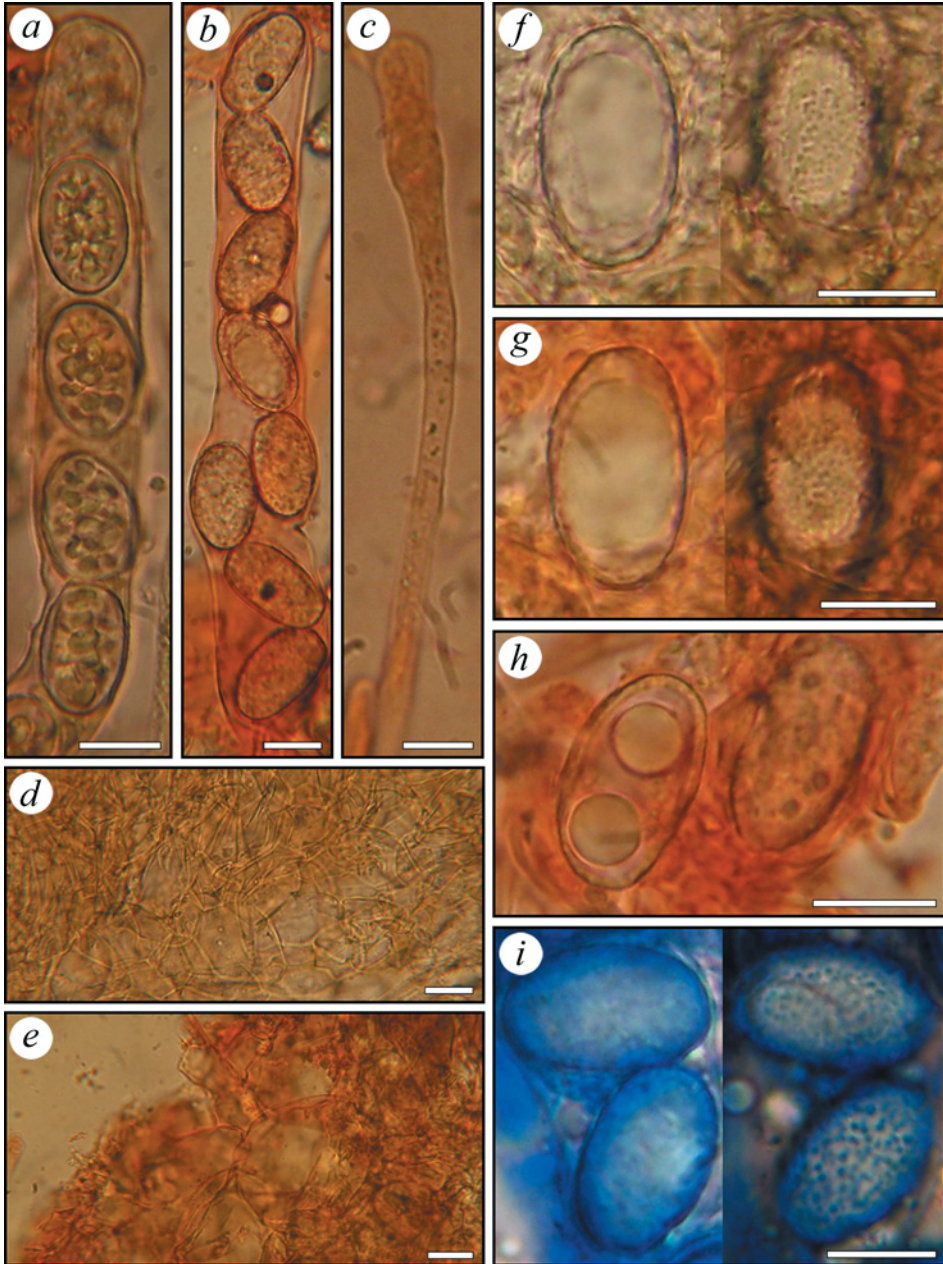


**Fig. 1.** *Aleurina subvirescens* (KWHU 162309/6). Fresh apothecium on substrate. Scale bar = 2 mm. Photo Yu. Shcherbakova.

forest dominated by *Picea abies*, near stream, on wet piece of decaying wood (immersed in soil), among protonemata of mosses, 23 September 2016, leg. Yu. Shcherbakova, det. V. Dzhagan et Yu. Shcherbakova (KWHU 162309/6).

**General distribution.** Europe: Czech Republic, Germany, Norway, Ukraine (Zhuang et Korf 1986, Anonymus 2015, GBIF on-line 1, this study).

**Notes.** According to various data, the genus *Aleurina* Masee comprises 10 (Wijayawardene et al. 2020) to 15 species (Jaklitsch et al. 2016). Three of them are known from Europe, i.e. *A. olivaceofusca* (Svrček et J. Moravec) W.Y. Zhuang et Korf, *A. subvirescens*, and *A. tenuiverrucosa* Dougoud et Roffler (Dougoud et Roffler 2006). *Aleurina subvirescens* differs from the other two species in the morphology of the perispore and the much larger size of the ascospores. According to available data (Zhuang et Korf 1986, GBIF on-line 1), the species is very rare, found only in the Czech Republic, Germany and Norway. In Ukraine, species of the genus *Aleurina* had not previously been registered and the current locality of *A. subvirescens* is the easternmost known one in its distribution range.



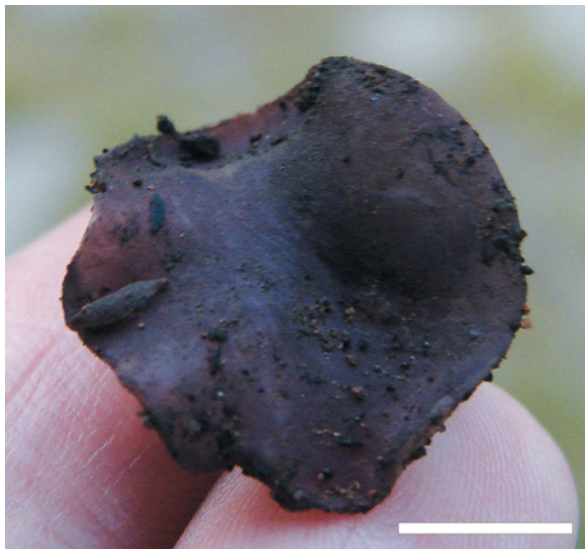
**Fig. 2.** *Aleurina subvirescens* (KWHU 162309/6): **a** – fragment of immature ascus (H<sub>2</sub>O); **b** – mature ascus (IKI); **c** – paraphyse (IKI); **d, e** – cells of ectal excipulum (d – H<sub>2</sub>O, e – CR); **f-i** – ascospores (f – H<sub>2</sub>O, g, h – CR, i – LPCB). Scale bars = 10 µm (a, b, d, f-i), 5 µm (c), 20 µm (e).

Photos: Yu. Shcherbakova.

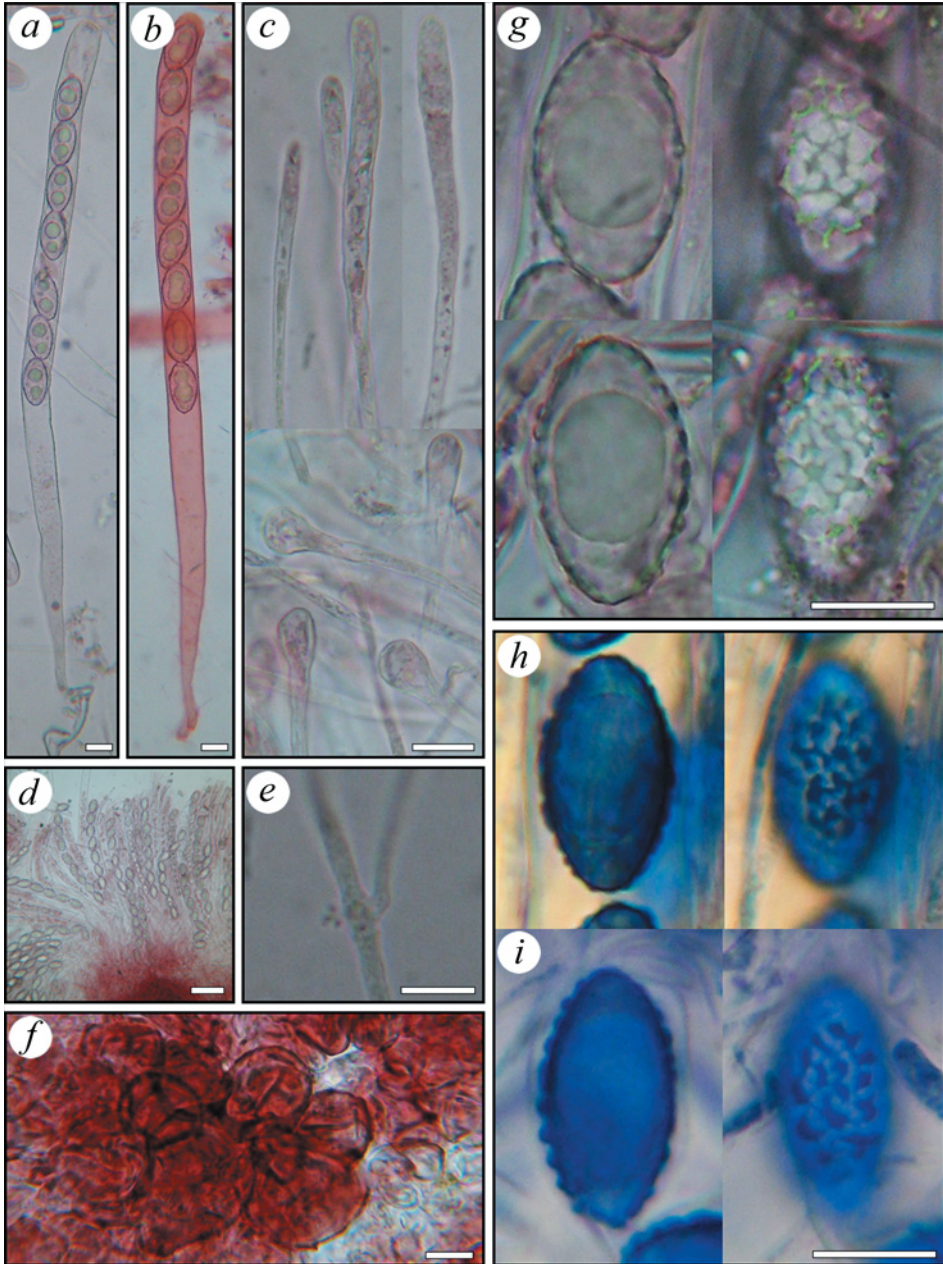
*Smardaea purpurea* Dissing, Sydowia 38: 35–40, 1985

Figs. 3–4

Macroscopic and microscopic features. Apothecia 2.5–12 mm diam., discoid, sessile, with a broad base, purple or purplish brown (including the hymenial layer), margin distinct, broad, finely scaly on the outside. Ectal excipulum made up of spherical to widely cylindrical (barrel-shaped) cells, with purplish pigmented walls, becoming elongated to club-shaped towards the margin,  $15\text{--}30 \times 6\text{--}10 \mu\text{m}$ , tending to form subparallel rows as noted by Dissing (1985). The excipular ‘scales’ are due to clusters of globular to subglobular thick-walled cells,  $10\text{--}20 \mu\text{m}$  wide. Marginal cells sometimes elongated and resembling hyphoid hairs, strongly staining blue in LPCB. Medullary excipulum up to  $200 \mu\text{m}$  wide, consisting of tightly intertwined multicellular, branched hyphae  $3\text{--}5 \mu\text{m}$  broad, with purplish pigment in the walls. Hymenium up to  $300 \mu\text{m}$  thick. Asci  $290\text{--}330 \times 13.5\text{--}15.0 \mu\text{m}$ , operculate, cylindrical, inamyloid. Ascospores  $(20.5)22.0\text{--}24.2(25.0) \times (10.1)11.5\text{--}13.6(14.8) \mu\text{m}$ ,  $Q = 1.54\text{--}2.29$ , elongated-ellipsoidal, rarely ellipsoidal, often inequilateral with respect to the longitudinal axis, with two rounded prominent lipid bodies, sometimes with de Bary bubbles. Ornamentation of the perispore of mature ascospores consisting of irregularly pigmented (purple), strongly cyanophilic ridges and warts,  $1.5 \mu\text{m}$  high. Paraphyses  $2.5\text{--}3.5 \mu\text{m}$  wide, straight, septate, branching, with purple cell walls, apical cells slightly dilated at the apex, up to  $3.3\text{--}4.2 \mu\text{m}$ , or club-shaped, rarely up to  $6.0 \mu\text{m}$  wide.



**Fig. 3.** *Smardaea purpurea* (KWHU 153006/1). Fresh apothecium. Scale bar = 5 mm. Photo Yu. Shcherbakova.



**Fig. 4.** *Smardaea purpurea* (KWHU 153006/1): **a, b** – mature ascus with ascospores (a – H<sub>2</sub>O, b – CR); **c** – paraphyses (H<sub>2</sub>O); **d** – fragment of the hymenial layer (CR); **e** – fragment of paraphyses; **f** – cells of ectal excipulum (CR); **g–i** – ascospores (g – H<sub>2</sub>O, h, i – LPCB). Scale bars = 10 μm (a–c, e–i), 50 μm (d). Photos: V. Dzhagan et Yu. Shcherbakova.



**Specimen examined**

Ukraine. Transcarpathian Region, Rakhiv District, Svydovetskyi Mountain Range of the Carpathian Biosphere Reserve, village of Chorna Tysa, Chorna Tysa river valley, 48°18'23.9" N, 24°17'12.7" E, alt. 850 m, spruce-fir-beech forest, on wet soil, 30 June 2015, leg. Yu. Shcherbakova, det. V. Dzhagan et Yu. Shcherbakova (KWHU 153006/1).

**General distribution.** Asia: Kyrgyzstan; Europe: Austria, France, Germany, Slovakia, Switzerland, Ukraine (Dissing 1985, Benkert 2005, Moyne et al. 2010, Polhorský 2016, Anonymus 2018, GBIF on-line 3, this study).

**Notes.** Because of the spore ornamentation consisting of irregular ridges and warts, *Smaradaea purpurea* is easily distinguished from the very similar *S. amethystina* (W. Phillips) Svrček, having fusiform ascospores showing a regular ornamentation of prominent, rounded warts, and differing from another similar species, *S. proteae* W.Y. Zhuang et Korf, by narrower ascospores and larger warts.

Eleven species of the genus *Smaradaea* Svrček are known today (Index Fungorum database), eight of which have been documented from Europe: *S. amethystina*, *S. marchica* (Benkert et J. Moravec) Benkert, *S. ovalispora* (Grélet) Van Vooren, *S. planchonis* (Dunal ex Boud.) Korf et W.Y. Zhuang, *S. proteae*, *S. purpurea*, *S. reticulosperma* (Donadini, Rioussset et G. Rioussset) Benkert, and *S. verrucispora* (Donadini et Monier) Benkert (Donadini 1980, Benkert 2005, Van Vooren 2009). *Smaradaea purpurea* is recorded for the first time in Eastern Europe.

***Ramsbottomia asperior*** (Nyl.) Benkert et T. Schumach., *Agarica* 6(12): 35, 1985 Figs. 5–6

- ≡ *Peziza asperior* Nyl., Not. Sällsk. Fauna et Fl. Fenn. Förh. 10: 21, 1869 (basionym)
- ≡ *Leucoloma asperior* (Nyl.) Rehm, Ber. Naturh. Ver. Augsburg 26: 6, 1881
- ≡ *Sphaerospora asperior* (Nyl.) Sacc., Syll. Fung. 8: 188, 1889
- ≡ *Sphaerosporula asperior* (Nyl.) Kuntze, Rev. Gen. Plant. 3: 530, 1898
- ≡ *Ciliaria asperior* (Nyl.) Boud., Hist. Classif. Discom. Europe: 62, 1907
- ≡ *Scutellinia asperior* (Nyl.) Dennis, Kew Bull. 10(4): 571, 1956

**Macroscopic and microscopic features.** Apothecia up to 3 mm diam., saucer-shaped, lenticular or pillow-shaped, sessile. Hymenium smooth, apricot-orange to egg-yellow, flat to convex; receptaculum concolorous with the hymenium, glabrous or with short hyphoid cylindrical hairs arising from the excipulum towards the margin, indistinctly visible. Ectal excipulum made up of a *textura globulosa* to *globulosa-angularis*. Asci 258–295 × 16.6–18.9 µm, operculate, cylindrical, 8-spored, inamyloid, internal content of the apical part of the asci turning reddish brown after adding Lugol's solution. Ascospores (16.5)18.4–21.5(22.0) × (14.4)15.7–17.8(18.4) µm, Q = 1.10–1.29, uniseriate, hyaline, globose to broadly ellipsoidal, with one large lipid body in rehydrated state. Perispore ornamented with conical spines reaching 2.0–2.5 µm in length.



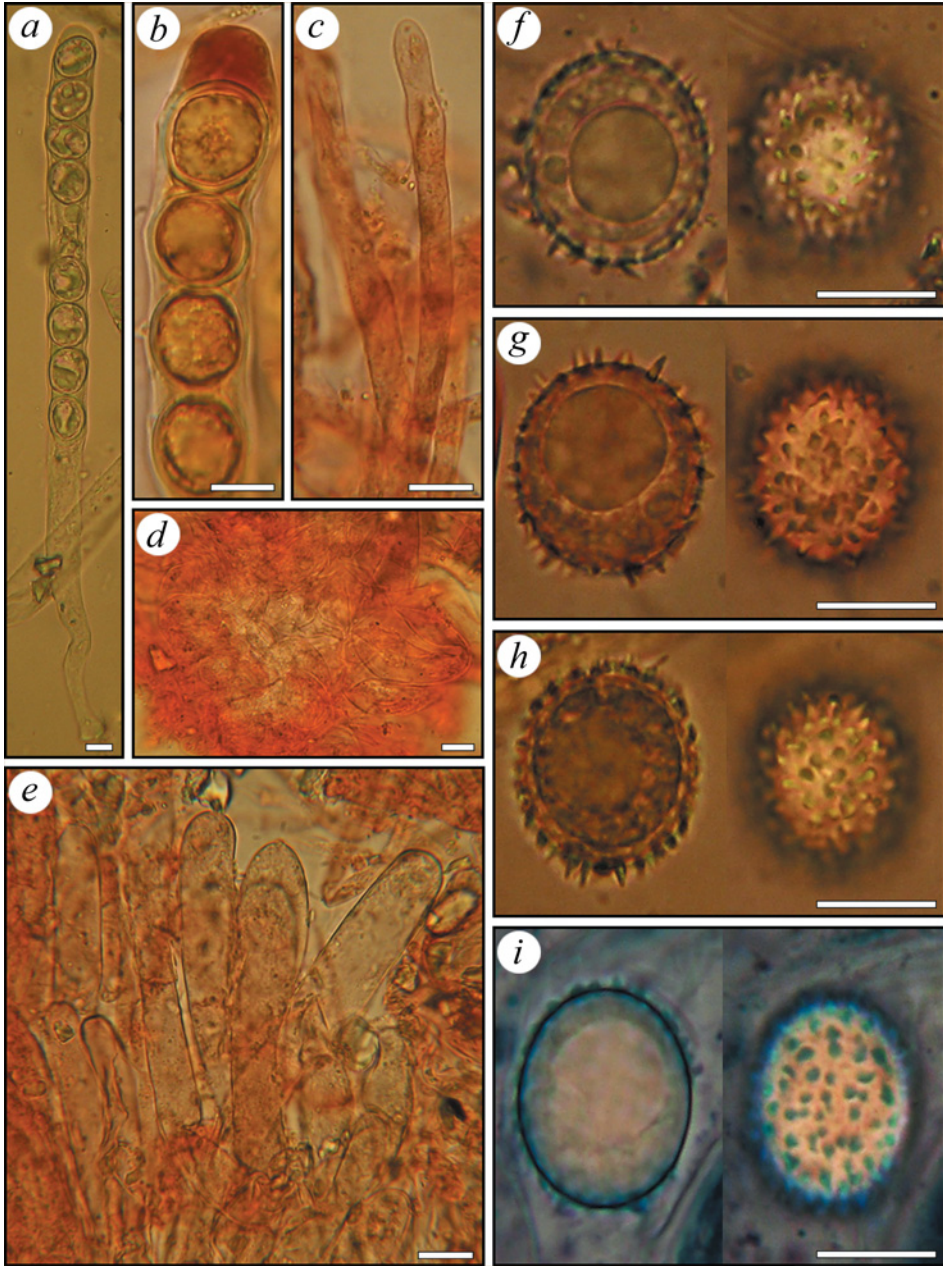
**Fig. 5.** *Ramsbottomia asperior* (KWHU 152308/2). Fresh apothecia on substrate. Scale bar = 2 mm. Photo Yu. Shcherbakova.

Paraphyses abundant, cylindrical, straight, septate, unbranched, slightly clavate in the upper part, up to 6–7  $\mu\text{m}$  wide, with intracellular orange pigment. Hairs cylindrical-clavate, up to  $120 \times 15\text{--}16 \mu\text{m}$ , slightly brownish.

**Specimens examined**

Ukraine. Transcarpathian Region, Rakhiv District, Svydovetskyi Mountain Range of the Carpathian Biosphere Reserve, vicinity of Dragobrat, 48°14'52.5" N, 24°15'12.2" E, alt. 1200 m, thickets of *Scirpus sylvaticus*, on wet soil near stream, 7 July 2014; *ibid.*, spruce forest, 23 August 2015, on wet soil, both leg. et det. Yu. Shcherbakova (KWHU 152308/2).

General distribution. Africa: Morocco; Asia: Japan, Russia; Europe: Austria, Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Great Britain, Iceland, Italy, the Netherlands, Norway, Poland, Russia, Spain, Sweden, Switzerland, Ukraine; North America: USA (Yao et Spooner 1995, Medardi 2006, Van Vooren et Mourgues 2009, Shcherbakova et Dzhagan 2015, Anonymus 2017, Dudka et al. 2019, GBIF on-line 2, this study).



**Fig. 6.** *Ramsbottomia asperior* (KWHU 152308/2): **a** – mature ascus with eight ascospores ( $H_2O$ ); **b** – fragment of ascus (IKI); **c** – paraphyses (CR); **d** – ectal excipulum cells (CR); **e** – hyphoid hairs (CR); **f-i** – ascospores (f –  $H_2O$ , g – CR, h – IKI, i – LPCB). Scale bars = 10  $\mu m$ . Photos: Yu. Shcherbakova.

Notes. *Ramsbottomia asperior* and other known species of *Ramsbottomia* W.D. Buckley [*R. crechqueraultii* (P. Crouan et H. Crouan) Benkert et T. Schumach., *R. lamprosporoidea* W.D. Buckley, and *R. macrantha* (Boud.) Benkert et T. Schumach.] have the same habitus, but the three last-mentioned are distinguished from *R. asperior* by their completely spherical ascospores. Besides, *R. lamprosporoidea* and *R. macrantha* have larger ascospores ornamented with very long spines (3–6 µm) (Yao et Spooner 1995, Van Vooren et Mourgues 2009).

As a result of our investigations, today three localities of *R. asperior* are known in Ukraine. Based on this fact and on the wide general distribution of this species in the world, it can be assumed that this species is also common in Ukraine.

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