Coprinopsis rugosomagnispora: a distinct new coprinoid species from Poland (Central Europe)

Błażej Gierczyk¹ · Pamela Rodriguez-Flakus² · Marcin Pietras^{3,4} · Mirosław Gryc⁵ · Waldemar Czerniawski⁶ · Marcin Piątek⁷

Received: 3 November 2016 / Accepted: 31 March 2017 / Published online: 28 April 2017
© The Author(s) 2017. This article is an open access publication

Abstract A new coprinoid fungus, *Coprinopsis rugosomagnispora*, is described from Poland (Central Europe). Its macromorphological characters are similar to species belonging to the subsection *Nivei* of *Coprinus* s.l. However, *C. rugosomagnispora* has unique micromorphological characters: very large, ornamented spores, voluminous basidia and cystidia, and smooth veil elements. The large spores and pattern of spore ornamentation (densely pitted) make this species unique within all coprinoid species described so far. The structure (arrangement and shape) of veil elements in *C. rugosomagnispora* is intermediate between members of the subsections *Nivei* and *Lanatuli* of *Coprinus* s.l. Molecular phylogenetic analyses, based on single-locus (ITS) maximum likelihood and Bayesian

evolutionary trees recovered *C. rugosomagnispora* within a lineage containing species having morphological characters of the subsection *Lanatuli* (though within the so-called *Atramentarii* clade) that contradicts its morphological similarity to members of the subsection *Nivei*.

Keywords Agaricales · *Coprinopsis* · Coprinoid fungi · Molecular phylogeny · Spore ornamentation · Taxonomy

Introduction

The coprinoid fungi are a highly diverse and polyphyletic group of the order Agaricales, which species show a unique set of characters: presence of dark spores, deliquescent basidiocarps that often undergo autolysis, and pseudoparaphyses in the hymenium (Uljé 2005). These species were previously classified in one genus, *Coprinus* Pers., but molecular studies revealed that the genus is split into four main lineages assigned to four genera: *Coprinus* s. s., *Coprinellus* P.Karst., *Coprinopsis* P.Karst., and *Parasola* Redhead, Vilgalys & Hopple (Hopple and Vilgalys 1999; Moncalvo et al. 2002; Padamsee et al. 2008; Larsson and Örstadius 2008; Nagy et al. 2009, 2010a, b, 2011, 2012, 2013a). The coprinoid fungi with a veil composed of smooth, (sub)globose elements, and lacking pileocystidia belonged to subsection *Nivei* of *Coprinus* s.l. (Citérin 1992; Uljé 2005), now included in the genus *Coprinopsis* (Hopple and Vilgalys 1999; Moncalvo et al. 2002). In Europe, about 20 species from this subsection were reported so far (Uljé 2005; Vesterholt 2012). It cannot be ruled out that at least some of them are probably collective species, e.g., *Coprinopsis cortinata* (J.E.Lange) Gminder (Uljé 2005). This view may be supported by the molecular analyses of Keirle et al. (2004), which revealed significant genetic

Handling editor: Miroslav Kolařík.

✉ Błażej Gierczyk
hanuman@amu.edu.pl

¹ Faculty of Chemistry, Adam Mickiewicz University in Poznań, Umultowska 89b, 61-614 Poznań, Poland

² Laboratory of Molecular Analyses, W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, 31-512 Kraków, Poland

³ Department of Plant Taxonomy and Nature Conservation, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland

⁴ Laboratory of Symbiotic Associations, Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland

⁵ Elektryczna 1, 16-030 Ogrodniczki, Poland

⁶ Tyniecka 28, 32-050 Skawina, Poland

⁷ Department of Mycology, W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, 31-512 Kraków, Poland

diversity among the collections of *C. cordispora* (T.Gibbs) Gminder originating from Europe and Hawaii.

In this work, a distinct species of *Coprinopsis* is described on the basis of morphological and molecular analyses that differ from all other known species of the subsection *Nivei* of *Coprinus* s.l. (Citérin 1992; Uljé 2005) as well as from remaining recognized *Coprinopsis* species. This new species was collected several times in two different regions of Poland, indicating that unknown coprinoid fungi can still be found, even in relatively well-studied regions such as Europe.

Materials and methods

Specimen sampling, morphological analyses, and nomenclature of subsections

The study is based on dried herbarium specimens. The holotype is deposited in the fungal collection of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, Poland (KRAM F), while isotype and remaining specimens are in the personal collection of the first author (BG). The material was studied by light microscope (LM) and scanning electron microscope (SEM). For LM, samples were rehydrated in aqueous ammonia solution (10%) and stained with Congo red (0.5% in aqueous ammonia). Observations, measurements, and micrographs were made using a Bresser Science TRM 301 light microscope. Drawings of microcharacters were made from micrographs taken with a Bresser MicroCam 5.0 digital camera. All measurements were taken directly through the light microscope under an oil immersion objective (100×). The spore dimensions were established from measurements of 50 randomly selected, well-formed spores (the deformed or atrophied spores were excluded from analysis) per collection. The 95% population limits for the mean were calculated, and the lower and upper values are given. For basidia and cystidia, the extreme size values were presented. For these structures, dimensions were obtained after measuring 25 elements. For SEM, fragments of dried fungal hymenium were glued onto carbon tabs and fixed to an aluminum stub with double-sided transparent tape. The stubs were sputter-coated with carbon using a Cressington sputter coater and viewed under a Hitachi S-4700 scanning electron microscope, with a working distance of ca. 12 mm. SEM micrographs were taken in the Laboratory of Field Emission Scanning Electron Microscopy and Microanalysis at the Institute of Geological Sciences of Jagiellonian University (Kraków). Color notation used in the description is according to Kornerup and Wanscher (1984). In the following text, the names of subsections (*Alachuanii*, *Lanatuli*, *Narcotici*, *Nivei*) refer to

Fig. 1 Phylogenetic relationship among sampled *Coprinopsis* species, including the newly described *Coprinopsis rugosomagnispora*, based on Bayesian inference analyses of ITS sequences. The support values (ML—BP $\geq 60\%$, Bayesian analysis—PP ≥ 0.7) are placed above branches. Thickened branches represent clades well supported at least in one of the analyses (ML—BP $\geq 70\%$, Bayesian analysis—PP ≥ 0.9). The branch lengths denoted by double slash (//) were shortened (2×, 7×, 2×, 4×, respectively, starting from the top to the bottom of the tree) to improve visualization. The morphological subsections of *Coprinus* s.l. are demarcated with colored squares, and three *Agrocybe* species were included as outgroup. The species assigned to the so-called Atramentarii clade by Nagy et al. (2013a) are demarcated within lineage containing species showing characters of the morphological subsection *Lanatuli*

morphological subsections of *Coprinus* s.l. as delineated by Uljé (2005).

DNA extraction, PCR amplification, and sequencing

Fungal DNA was extracted from a small part of basidiocarp representing the holotype of *Coprinopsis rugosomagnispora*. DNA was extracted using the GeneMATRIX Plant and Fungi DNA Purification Kit (EURx, Poland), following the manufacturer's protocols. The final PCR reaction volume was 10 µl composed of 4 µl DNA template, 0.5 µl of each primer ITS5 and ITS4 (White et al. 1990), and 5 µl Type-it Microsatellite PCR Kit (Qiagen, Germany). PCR amplification was performed using the following thermocycling conditions: an initial denaturing step at 95 °C for 15 min, followed by 35 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and a final extension of 10 min at 72 °C. PCR product was visualized by running 5 µl DNA amplicon on 1.5% agarose gel for 30 min. The PCR product was sequenced at the Laboratory of Molecular Biology of Adam Mickiewicz University in Poznań. The obtained sequences fragments were checked, assembled, and manually edited using Geneious Pro, version 8.0. (Biomatters Ltd). The ITS sequence obtained in this study was deposited in the NCBI's GenBank database (<http://www.ncbi.nlm.nih.gov/genbank>) with accession number KX276859.

Phylogenetic analyses

The ITS sequence from the holotype of *Coprinopsis rugosomagnispora* generated in this study was checked by a Blast search (Altschul et al. 1997) against the ITS sequences deposited in GenBank to reveal closely related species. Subsequently, the sequence was analyzed within a dataset covering all *Coprinopsis* species available in GenBank, reduced to one sequence per species (with the exception of *Coprinopsis lagopus* (Fr.) Redhead, Vilgalys & Moncalvo s.l.), and including sequences generated by

experts of *Coprinopsis* taxonomy (Keirle et al. 2004; Navarro-González et al. 2006; Garcia and Vellinga 2010; Nagy et al. 2010a, 2011, 2013a, b; Geml et al. 2012; Osmundson et al. 2013; Ruiz et al. 2013; Örstadius et al. 2015; Elwess et al. 2016; and some unpublished sequences available in GenBank). A total of 85 sequences were aligned using MUSCLE algorithm and default settings (Edgar 2004) to obtain preliminary hypothesis on the phylogenetic placement of *Coprinopsis rugosomagnispora* (data not shown). For the final phylogeny, we reduced the dataset to 40 sequences representing lineage containing a new species and most closely related *Coprinopsis* species (from the subsection *Lanatulii*) and some more distantly related species (from other subsections, among others from the subsection *Nivei*). The three sequences of *Agrocybe* Fayod were chosen as outgroup following Nagy et al. (2013b). GenBank accession numbers are given in Fig. 1 and in Table 1. The alignment was deposited in TreeBASE (S20797).

Phylogenetic analyses were carried out using maximum likelihood (ML) implemented in RaxmlGUI version 0.9 beta 2 (Silvestro and Michalak 2012) and Bayesian inference in MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2003). The nucleotide substitution models were separately selected for each part of the ITS region (ITS1, 5.8S, ITS2) inferred with PartitionFinder version 1.0.1 (Lanfear et al. 2012) using the Bayesian Information Criterion (BIC) as optimality model criterion in a greedy search. In the likelihood analyses, a GTRGAMMAI substitution model and 1000 bootstrap resamples in separate partitions were performed, and for MCMC analyses, three independent parallel runs were started each with four incrementally heated (temperature = 0.15) chains. This Markov chain was allowed to run for 100 M generations sampling every 1000th tree and a 50% as the burn-in factor. Resulting phylogenetic trees were visualized in TreeView software (Page 1996).

Results

Morphological analyses

The morphological characteristics of new species, *Coprinopsis rugosomagnispora*, are included in the species description and depicted in section Taxonomic treatment.

Phylogenetic analyses

The current phylogenetic studies included 43 sequences: 40 sequences of *Coprinopsis* species and three outgroup sequences of *Agrocybe* species (following Nagy et al. 2013b), with 514 unambiguously aligned nucleotide

positions. The consensus tree topology and branch supports were assessed under maximum likelihood (ML) and Bayesian inference analyses. Although the consensus tree topology under ML and Bayesian inference yielded a similar pattern, the Bayesian phylogenetic tree is presented in this study, in which several major *Coprinopsis* clades are clearly delimited, and the ML bootstrap values and posterior probabilities are placed above moderately or well-supported branches (Fig. 1).

The molecular analyses revealed *Coprinopsis rugosomagnispora* as sister species to *C. insignis* (Peck) Redhead, Vilgalys & Moncalvo and *C. fusispora* L.Nagy, Vágvölgyi & T.Papp, members of the Atramentarii clade (Nagy et al. 2013a), which altogether formed a lineage showing strong support in the Bayesian analysis (PP = 0.99) and moderate support in ML analysis (BP = 64%). The clade is sister and basal to the large assemblage of 18 *Coprinopsis* species belonging to the subsection *Lanatulii* of *Coprinus* s.l. (Lange 1915; Uljé 2005). *Coprinopsis goudensis* (Uljé) Redhead, Vilgalys & Moncalvo, a member of the subsection *Alachuanii* of *Coprinus* s.l., is nested inside this assemblage and therefore probably should be reassigned to subsection *Lanatulii*. The remaining sampled *Coprinopsis* species (sister to the lineage containing members of the subsection *Lanatulii*) were split into distinct groups: (1) *Coprinopsis filamentifer* (Kühner) Redhead, Vilgalys & Moncalvo (subsection *Alachuanii*) that forms a long branch by its own, (2) a highly supported paraphyletic clade containing species with morphologies similar to members of the genus *Psathyrella* (Fr.) Quél. (we informally call this lineage ‘*Psathyrella*-like’), and (3) a not supported lineage including species from the subsection *Nivei* (Fig. 1).

Discussion

Many of the recently described coprinoid fungi differ only slightly from the earlier recognized species or are even indistinguishable without molecular data (Raut et al. 2011; Nagy et al. 2013a). By contrast, *Coprinopsis rugosomagnispora* described here has a unique and distinct combination of characters (Figs. 2, 3). Its macromorphological characters, such as small dimensions of basidiocarps and granular veil forming conical warts, are similar to that of *Coprinopsis cordispora* and morphologically similar species in the subsection *Nivei*. Likewise, the smooth veil elements suggest affinity of *C. rugosomagnispora* to members of this subsection. On the other hand, the structure (arrangement and shape) of veil elements in *C. rugosomagnispora* is intermediate between that of members in the subsections *Nivei* and *Lanatulii*. Specifically, veil elements are arranged in chains as in the subsection *Lanatulii* (in the subsection *Nivei*, veil elements are composed of

Table 1 List of species used in the molecular analyses, with countries of origin, collector, voucher information, GenBank accession numbers (ITS), and references

Species	Country	Collector	Voucher	GenBank acc. no. (ITS)	References
<i>Agrocybe praecox</i>	USA	N/A	MSC 378486	AY194531	Hallen et al. (2003)
<i>Agrocybe praecox</i>	N/A	N/A	AFTOL-ID 728 (WTU)	AY818348	Yang et al. (2005)
<i>Agrocybe semiorbicularis</i>	USA	N/A	MSC 378490	AY194536	Hallen et al. (2003)
<i>Coprinosia ammophilae</i>	N/A	R. Watling	Watling 24982	HQ847008	Nagy et al. (2013b)
<i>Coprinosia babosiae</i>	Hungary	L. Nagy	SZMC-NL-2641	JX118684	Nagy et al. (2013a)
<i>Coprinosia bellula</i>	Hungary	L. Nagy	SZMC-NL-2341	FM163176	Nagy et al. (2011)
<i>Coprinosia bicornis</i>	The Netherlands	C.B. Uljé	Uljé 1216 HOLOTYPE	JX118690	Nagy et al. (2013a)
<i>Coprinosia brunneistragulata</i>	North America	F. van de Bogart	FVDB 3821 HOLOTYPE	JX118724	Nagy et al. (2013a)
<i>Coprinosia candidolanata</i>	Italy	F. Doveri	? HOLOTYPE	JX118682	Nagy et al. (2013a)
<i>Coprinosia caniceps</i>	Sweden	L. Örstadius	LÖ 148-95	KC992964	Örstadius et al. (2015)
<i>Coprinosia coniotheca</i>	N/A	L. Nagy	SZMC-NL-3414	FN396122	Nagy et al. (2011, 2013b)
<i>Coprinosia cortinata</i>	N/A	L. Nagy	SZMC-NL-1621	FN396121	Nagy et al. (2011, 2013b)
<i>Coprinosia cothurnata</i>	USA (Hawai'i)	D.E. Hemmes	SFSU DEH 548	AY461833	Keirle et al. (2004)
<i>Coprinosia filamentifer</i>	N/A	L. Nagy	SZMC-NL-0380	HQ847035	Nagy et al. (2013b)
<i>Coprinosia fusispora</i>	Hungary	L. Nagy	SZMC-NL-1227 HOLOTYPE	JX118740	Nagy et al. (2013a)
<i>Coprinosia goudensis</i>	N/A	L. Nagy	SZMC-NL-4139	FN396128	Nagy et al. (2011)
<i>Coprinosia insignis</i>	Hungary	L. Nagy	SZMC-NL-1510	JX118738	Nagy et al. (2013a)
<i>Coprinosia jonesii</i>	Sweden	L. Nagy	SZMC-NL-0154	JX118726	Nagy et al. (2013a)
<i>Coprinosia lagopides</i>	N/A	N/A	N/A	KM373258	Elwess et al. (2016)
<i>Coprinosia lagopus</i>	Hungary	L. Nagy	SZMC-NL-0995	HQ847020	Nagy et al. (2013a)
<i>Coprinosia lagopus</i>	Hungary	L. Nagy	SZMC-NL-2493	HQ872014	Nagy et al. (2013a)
<i>Coprinosia lagopus</i>	Hungary	L. Nagy	SZMC-NL-1558	JX118711	Nagy et al. (2013a)
<i>Coprinosia lagopus</i>	Sweden	L. Nagy	SZMC-NL-3658	JX118717	Nagy et al. (2013a)
<i>Coprinosia macrocephala</i>	N/A	L. Nagy	SZMC-NL-1376	FN396126	Nagy et al. (2011)
<i>Coprinosia marcescibilis</i>	Hungary	L. Nagy	SZMC-NL-2140	FM878020	Nagy et al. (2010a)
<i>Coprinosia musae</i>	Denmark	J. Vesterholt	JV 06-179 HOLOTYPE	KC992965	Örstadius et al. (2015)
<i>Coprinosia neolagopus</i>	N/A	N/A	NBRC 100013	AB097564	Suzuki et al. (unpublished data)
<i>Coprinosia nevillei</i>	France	G. Garcia	GG 08090401 (LIP) HOLOTYPE	HM126488	Garcia & Vellinga (2010)
<i>Coprinosia nivea</i>	Italy	E. Bizio	MCVE 4585	JF907848	Osmundson et al. (2013)
<i>Coprinosia pachyderma</i>	North America	F. van de Bogart	FVDB 3237 HOLOTYPE	JX118731	Nagy et al. (2013a)
<i>Coprinosia pannucioides</i>	N/A	L. Nagy	SZMC-NL-3528	FN396143	Nagy et al. (2011)
<i>Coprinosia poliommallus</i>	Hungary	L. Nagy	SZMC-NL-2336	FM163182	Nagy et al. (2009)
<i>Coprinosia pseudonivea</i>	Hungary	L. Nagy	SZMC-NL-2340	FM163181	Nagy et al. (2009)
<i>Coprinosia pseudoradiata</i>	Norway	L. Nagy	SZMC-NL-0956	JX118687	Nagy et al. (2013a)
<i>Coprinosia radiata</i>	Hungary	L. Nagy	SZMC-NL-1244	HQ847024	Nagy et al. (2013a)
<i>Coprinosia rugosomagnispora</i>	Poland	M. Gryc	KRAM F-58717 HOLOTYPE	KX276859	this study
<i>Coprinosia scobicola</i>	United Kingdom	P.D. Orton	Orton 964 HOLOTYPE	HQ847021	Nagy et al. (2013a)

Table 1 continued

Species	Country	Collector	Voucher	GenBank acc. no. (ITS)	References
<i>Coprinopsis submicrospora</i>	N/A	L. Nagy	SZMC-NL-0635	HQ847053	Nagy et al. (2013b)
<i>Coprinopsis tectispora</i>	North America	F. van de Bogart	FVDB 6016 HOLOTYPE	JX118665	Nagy et al. (2013a)
<i>Coprinopsis udicola</i>	Germany	A. Melzer	AM 1240 HOLOTYPE	KC992967	Örstadius et al. (2015)
<i>Coprinopsis utrifer</i>	N/A	L. Nagy	SZMC-NL-0591	FN396140	Nagy et al. (2011, 2013b)
<i>Coprinopsis villosa</i>	Germany	L. Nagy	SZMC-NL-1758	HQ847031	Nagy et al. (2013a)
<i>Coprinus fissolanatus</i> nom. prov.	The Netherlands	C.B. Uljé	Daams 71-121	JX118733	Nagy et al. (2013a)

N/A not available

Fig. 2 Basidiocarps of *Coprinopsis rugosomagnispora* from the Puszcza Knyszynska forest, NE Poland: **a, b** general view; **c** lamellae; **d, e** young basidiocarps; **f** old specimen

Fig. 3 Microcharacters of *Coprinosia rugosomagnispora* (holotype: KRAM F-58717): **a** spores; **b, c** ornamentation of immature spore surface; **d** basidia; **e** cheilocystidia; **f, g** veil from pileus surface; **h** veil from stipe surface. Scale bars 10 μ m

spherical elements emerging from thin hyphae), but particular cells of veil elements are (sub)globose in shape, as in the subsection *Nivei* (in the subsection *Lanatuli*, the veil elements are cylindrical to fusiform). Remarkably, the molecular phylogenetic analyses indicate a relationship of *C. rugosomagnispora* with the species possessing morphological traits typical for the subsection *Lanatuli*. None of the described species of the subsection *Nivei* have ornamented spores. In the subsection *Lanatuli*, ornamented spores are characteristic only for *C. insignis* and *C. calospora* (Bas & Uljé Redhead, Vilgalys & Moncalvo. Indeed, the molecular phylogenetic analyses indicated that

C. rugosomagnispora and *C. insignis* are closely related (Fig. 1). In contrast, *C. calospora* is related to *C. cinerea* (Schaeff.) Redhead, Vilgalys & Moncalvo as shown by Nagy et al. (2013a). The well-developed episporium in *Coprinosia rugosomagnispora* is not typical for species assigned to the subsections *Lanatuli* and *Nivei* but is characteristic for *Coprinosia* species from the subsection *Narcotici* of *Coprinus* s.l. (Uljé 2005); however, ornamented spores are rare in that subsection (Uljé 2005). It is noteworthy that *Coprinosia rugosomagnispora* is phylogenetically related to *C. fusispora* and *C. insignis* that were assigned to the so-called Atramentarii clade (Nagy et al.

Fig. 4 SEM micrographs of the spores of *Coprinopsis rugosomagnispora* (holotype: KRAM F-58717): **a, d** general view; **b** ornamentation pattern; **c** germ pore close-up. Scale bars: 10 μ m

2013a), despite of their morphological similarity to members of the subsection *Lanatuli*. Since the sectional and subsectional taxonomy within *Coprinopsis* is still not clarified, we tentatively retain with assignment of these species to the morphological subsection *Lanatuli* (Fig. 1).

The spore ornamentation pattern of *Coprinopsis rugosomagnispora* is unique among coprinoid fungi. Some of the recently described or redescribed species have warts (*C. echinospora* (Buller) Redhead, Vilgalys & Moncalvo and related taxa, *C. insignis*) or nodules (*C. calospora*) (Uljé 2005; Raut et al. 2011; Fukiharu et al. 2011, 2013, 2014). Spores of *Coprinellus* species may have warts (*Coprinellus silvaticus* (Peck) Gminder) or low crest and rows composed from minute warts (*Coprinellus verrucispermus* (Joss. & Enderle) Redhead, Vilgalys & Moncalvo) (Uljé 2005; Nagy et al. 2012), while spores of *Coprinopsis rugosomagnispora* are densely pitted. This pattern is poorly visible on mature spores, due to their dark color, but it is easy to observe on immature spores when they are just red-brown in color. The ornamentation is also hardly visible on SEM micrographs, as the episporium is smooth and persistent, so only some darken areas or rarely hollows are present on the spore surface (Fig. 4). The spore size of *C. rugosomagnispora* is larger than in all known coprinoid taxa. Only *Coprinus xerophilus* Bogart and *Coprinus sterquilinus* (Fr.) Fr. have spores, which may be longer than 20 μ m, but both these taxa differ by having a smooth spore surface and different veil structure (Uljé 2005). This makes the species newly described here easy to recognize, even if the collection is incomplete, namely autolysed and decayed.

Taxonomic treatment

Coprinopsis rugosomagnispora Gierczyk, Pietras, Piątek, Gryc, Czerniawski & Rodriguez-Flakus, **sp. nov.**—TYPE: Poland, Podlaskie Province: Puszcza Knyszyńska forest, S from Supraśl village, on soil in mixed forest, 24 Aug 2013, M. Gryc s.n. (holotype: KRAM F-58717!; isotype: BGF/BF/MG/130824/0005!; GenBank: KX276859). [MycoBank no. MB 820057].

Etymology: The Latin *rugoso* refers to the ornamentation of the spores, *magni* refers to their large dimensions, and *spora* refers to spore.

Description: Basidiocarps small (Fig. 2). Pileus 5–10 $\times$ 4–8 mm, first subglobose to broadly ellipsoid, expanding to convex campanulate, up to 15 mm broad; surface radially striate to plicate, covered by mealy to granulose veil forming squarrose, conical to nipple-shaped warts, veil whitish (1B2) to ocher-brown (6D6). Lamellae free, crowded ($L = 40$ –60), first whitish, then gray, finally black, deliquescent and undergoing autolysis with age. Lamellar edge strongly pruinose (Fig. 2c), white. Stipe 10–30 $\times$ 1–1.5 mm, white, covered by white fibrils, rather fragile, hollow. Stipe base equal, not rooting. Flesh very thin, white, odorless. Basidiospores (19.5–)21.0–21.5 (–24.0) $\times$ (12.0–)13.0–13.5 (–15.0) $\times$ (12.0–)12.5–13.0 (–15.0) μ m (av. 21.3 $\times$ 13.1 $\times$ 12.9 μ m), $Q = 1.56$ –1.64 (av. 1.60), black in mass, very dark red-brown (10G8) to almost black under light microscope, limoniform with prominent apiculus (apiculus length 1.3–2.0 μ m) and with central, papillate germ pore 2.0–2.5 μ m broad, not

Fig. 5 Drawings of microcharacters of *Coprinopsis rugosomagnispora* (holotype: KRAM F-58717): **a** spores; **b** basidia; **c** veil from the stipe surface; **d** cheilocystidia; **e** veil from pileus surface. Scale bars 10 μ m

flattened (Figs. 3a, 4, 5a). Ornamentation of spore surface prominent, up to 0.5 μ m high, covered with smooth, tight, persistent episporium. Ornament composed of hollows that make rugulose or pitted appearance of the spore wall (pitted spores; Figs. 3b, c, 5a). Basidia 4-spored, dimorphic, long type $52\text{--}56 \times 16\text{--}17 \mu\text{m}$, short type $34\text{--}38 \times 15\text{--}16 \mu\text{m}$ (measured without sterigmata; Figs. 3d, 5b). Sterigmata $5.0\text{--}5.5 \mu\text{m}$ long. Cheilocystidia $50\text{--}100 \times 32\text{--}60 \mu\text{m}$, subglobose, broadly ellipsoid to broadly clavate (Figs. 3e, 5d). Pleuro-, caulo- and pileocystidia absent. Veil on the pileus composed of (sub)globose to broadly ellipsoid, smooth, thin-walled, colorless elements, $24\text{--}60 \times 24\text{--}45 \mu\text{m}$, and with scattered

cylindrical hyphae up to $25 \mu\text{m}$ broad (Figs. 3f, g, 5e). Stipe covering made up of cylindrical hyphae, $35\text{--}85 \times 10\text{--}30 \mu\text{m}$, forming chains, not or moderately constricted at septa. On upper part of the stipe veil consists also of broadly ellipsoid elements up to $35 \mu\text{m}$ broad. Terminal elements of the stipe veil conically tapering with obtuse, $5\text{--}8 \mu\text{m}$ broad apex (Figs. 3h, 5c). Clamp-connections present.

Diagnosis: Species characterized by a veil composed of smooth, globose elements in chains, 4-spored, large basidia and rugulose (pitted), limoniform spores up to $24 \mu\text{m}$ long, with a tight episporium.

Fig. 6 Known localities of *Coprinopsis rugosomagnispora* in Poland (•)

Phenology: Specimens found between July and September.

Distribution area: Poland—Podlaskie Province and Małopolska Province. *Coprinopsis rugosomagnispora*-known localities in Poland are over 450 km apart (Fig. 6); therefore, it is not a local species and should occur in other parts of Poland as well as in other European countries. Further studies are needed to determine the distribution of *Coprinopsis rugosomagnispora*.

Habitats: *Coprinopsis rugosomagnispora* grows in forests. It has been found in both coniferous forest and mixed forest dominated by deciduous trees. At all three known stands, it was found on wet, muddy soils.

Additional specimens examined: Poland, Podlaskie Province: Puszcza Knyszyńska forest, 2 km S from Supraśl village, near Krasny Las forest ranger's lodge, on muddy soil on dirt road side in mixed forest (*Picea abies* (L.) H.Karst., *Alnus glutinosa* (L.) Gaertn.), 7 Jul 2013, *M. Gryc s.n.* (BGF/BF/MG/130707/0002!), 24 Aug 2013, *M. Gryc s.n.* (BGF/BF/MG/130824/0005!), 1 Sep 2013, *M. Gryc s.n.* (BGF/BF/MG/130901/0002!); Poland, Podlaskie Province: Puszcza Knyszyńska forest, 6 km NE from Supraśl village, near on muddy soil on dirt roadside in mixed forest, Sep 2013, *M. Gryc s.n.* (BGF/BF/MG/130901/0002!); Poland, Małopolska Province: Pogórze Wielickie foothills, Jasienica near Sułkowice village, on clayed soil mixed with sandstone rubble in silver fir (*Abies alba* Mill.) forest, Aug 2010, *W. Czerniawski s.n.* (BGF/BF/WCz/100801/0002!).

Acknowledgements We thank Anna Łatkiewicz (Kraków) for help with SEM micrographs. The work of Marcin Piątek and Pamela

Rodriguez-Flakus was partly supported by the statutory funds of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, Poland.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl Acids Res* 25:3389–3402. doi:[10.1093/nar/25.17.3389](https://doi.org/10.1093/nar/25.17.3389)
- Citérin M (1992) Clé analytique du genre *Coprinus* Pers. *Doc Mycol* 22:1–28
- Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinform* 5:113. doi:[10.1186/1471-2105-5-113](https://doi.org/10.1186/1471-2105-5-113)
- Elwess NL, Levere S, Latourelle SM (2016) DNA barcoding and phylogenetic characterization of mushrooms within the Adirondack Park. *Int J Res Developm Organ J Agric Res* 2:28–43
- Fukiharu T, Bougher N, Buchanan P, Suzuki A, Tanaka C, Sagara N (2011) *Coprinopsis austrophlyctidospora* sp. nov., an agaric ammonia fungus from Southern Hemisphere plantations and natural forests. *Mycoscience* 52:137–142. doi:[10.1007/s10267-010-0077-0](https://doi.org/10.1007/s10267-010-0077-0)
- Fukiharu T, Shimizu K, Li R, Raut JK, Yamakoshi S, Horie Y, Kinjo N (2013) *Coprinopsis novorugosobispora* sp. nov., an agaric ammonia fungus from Beijing, China. *Mycoscience* 54:226–230. doi:[10.1016/j.myc.2012.09.014](https://doi.org/10.1016/j.myc.2012.09.014)
- Fukiharu T, Shimizu K, Utsunomiya H, Raut JK, Goto R, Okamoto T, Kato M, Horigome R, Furuki T, Kinjo N (2014) *Coprinopsis asiaticophlyctidospora* sp. nov., an agaric ammonia fungus from Amami and Okinawa, southern Japan. *Mycoscience* 55:355–360. doi:[10.1016/j.myc.2013.12.002](https://doi.org/10.1016/j.myc.2013.12.002)
- Garcia G, Vellinga EC (2010) Une nouvelle espèce de coprin sur tiges de *Polygonatum multiflorum*: *Coprinopsis nevillei* sp. nov. *Bull Féd Assoc Mycol Médit* 37:37–58
- Geml J, Timling I, Robinson CH, Lennon N, Nusbaum HC, Brochmann C, Noorderloos ME, Taylo DL (2012) An arctic community of symbiotic fungi assembled by long-distance dispersers: phylogenetic diversity of ectomycorrhizal basidiomycetes in Svalbard based on soil and sporocarp DNA. *J Biogeogr* 39:74–88. doi:[10.1111/j.1365-2699.2011.02588.x](https://doi.org/10.1111/j.1365-2699.2011.02588.x)
- Hallen HE, Watling R, Adams GC (2003) Taxonomy and toxicity of *Conocybe lactea* and related species. *Mycol Res* 107:969–979. doi:[10.1017/S0953756203008190](https://doi.org/10.1017/S0953756203008190)
- Hopple JS Jr, Vilgalys R (1999) Phylogenetic relationships in the mushroom genus *Coprinus* and dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: divergent domains, outgroups, and monophyly. *Molec Phylogen Evol* 13:1–19. doi:[10.1006/mpev.1999.0634](https://doi.org/10.1006/mpev.1999.0634)

- Huelsenbeck JP, Ronquist F (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574. doi:[10.1093/bioinformatics/btg180](https://doi.org/10.1093/bioinformatics/btg180)
- Keirle MR, Hemmes DE, Desjardin DE (2004) Agaricales of the Hawaiian Islands. 8. Agaricaceae: *Coprinus* and *Podaxis*; Psathyrellaceae: *Coprinopsis*, *Coprinellus* and *Parasola*. *Fungal Diversity* 15:33–124
- Kornerup A, Wanscher JH (1984) *Methuen handbook of colour*, 3rd edn. Methuen London Ltd, Copenhagen
- Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molec Biol Evol* 29:1695–1701. doi:[10.1093/molbev/mss020](https://doi.org/10.1093/molbev/mss020)
- Lange JE (1915) Studies in the Agarics of Denmark. Part II. *Amanita*, *Lepiota*, *Coprinus*. *Dansk Bot Ark* 3:1–53
- Larsson E, Örstadius L (2008) Fourteen coprophilous species of *Psathyrella* identified in the Nordic countries using morphology and nuclear rDNA sequence data. *Mycol Res* 112:1165–1185. doi:[10.1016/j.mycres.2008.04.003](https://doi.org/10.1016/j.mycres.2008.04.003)
- Moncalvo JM, Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hofstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jacobsson S, Clémence H, Miller OK Jr (2002) One hundred and seventeen clades of euagarics. *Molec Phylogen Evol* 23:357–400. doi:[10.1016/S1055-7903\(02\)00027-1](https://doi.org/10.1016/S1055-7903(02)00027-1)
- Nagy GL, Kocsubé S, Papp T, Vágvolgyi C (2009) Phylogeny and character evolution of the coprinoid mushroom genus *Parasola* as inferred from LSU and ITS nrDNA sequence data. *Persoonia* 22:28–37. doi:[10.3767/003158509X422434](https://doi.org/10.3767/003158509X422434)
- Nagy LG, Urban A, Örstadius L, Papp T, Larsson E, Vágvolgyi C (2010a) The evolution of autodigestion in the mushroom family Psathyrellaceae (Agaricales) inferred from maximum likelihood and Bayesian methods. *Molec Phylogen Evol* 57:1037–1048. doi:[10.1016/j.ympev.2010.08.022](https://doi.org/10.1016/j.ympev.2010.08.022)
- Nagy LG, Vágvolgyi C, Papp T (2010b) Type studies and nomenclatural revisions in *Parasola* (Psathyrellaceae) and related taxa. *Mycotaxon* 112:103–141. doi:[10.5248/112.103](https://doi.org/10.5248/112.103)
- Nagy LG, Walther G, Házi J, Vágvolgyi C, Papp T (2011) Understanding the evolutionary processes of fungal fruiting bodies: correlated evolution and divergence times in the Psathyrellaceae. *Syst Biol* 60:303–317. doi:[10.1093/sysbio/syr005](https://doi.org/10.1093/sysbio/syr005)
- Nagy LG, Házi J, Vágvolgyi C, Papp T (2012) Phylogeny and species delimitation in the genus *Coprinellus* with special emphasis on the haired species. *Mycologia* 104:254–275. doi:[10.3852/11-149](https://doi.org/10.3852/11-149)
- Nagy LG, Desjardin DE, Vágvolgyi C, Kemp R, Papp T (2013a) Phylogenetic analyses of *Coprinopsis* sections *Lanatuli* and *Atramentarii* identify multiple species within morphologically defined taxa. *Mycologia* 105:112–124. doi:[10.3852/12-136](https://doi.org/10.3852/12-136)
- Nagy LG, Vágvolgyi C, Papp T (2013b) Morphological characterization of clades of the Psathyrellaceae (Agaricales) inferred from a multigene phylogeny. *Mycol Progr* 12:505–517. doi:[10.1007/s11557-012-0857-3](https://doi.org/10.1007/s11557-012-0857-3)
- Navarro-González M, Domingo-Martínez A, Navarro-González SS, Beutelmann P, Kües U (2006) Monstrosities under the inkcap mushrooms. In: Pisabarro AG, Ramírez L (eds) *Genetics and cellular biology of Basidiomycetes VI*. Universidad Pública de Navarra, Pamplona, pp 113–122
- Örstadius L, Ryberg M, Larsson E (2015) Molecular phylogenetics and taxonomy in Psathyrellaceae (Agaricales) with focus on psathyrelloid species: introduction of three new genera and 18 new species. *Mycol Progr* 14:25. doi:[10.1007/s11557-015-1047-x](https://doi.org/10.1007/s11557-015-1047-x)
- Osmundson TW, Robert VA, Schoch CL, Baker LJ, Smith A, Robich G, Mizzan L, Garbelotto MM (2013) Filling gaps in biodiversity knowledge for macrofungi: contributions and assessment of an herbarium collection DNA barcode sequencing project. *PLoS ONE* 8:e62419. doi:[10.1371/journal.pone.0062419](https://doi.org/10.1371/journal.pone.0062419)
- Padamsee M, Matheny PB, Dentinger BTM, McLaughlin DJ (2008) The mushroom family Psathyrellaceae: evidence for large-scale polyphyly of the genus *Psathyrella*. *Molec Phylogen Evol* 46:415–429. doi:[10.1016/j.ympev.2007.11.004](https://doi.org/10.1016/j.ympev.2007.11.004)
- Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applic Biosci* 12:357–358. doi:[10.1093/bioinformatics/12.4.357](https://doi.org/10.1093/bioinformatics/12.4.357)
- Raut JK, Suzuki A, Fukiharu T, Shimizu K, Kawamoto S, Tanaka C (2011) *Coprinopsis neophlyctidospora* sp. nov., a new ammonia fungus from boreal forests in Canada. *Mycotaxon* 115:227–238. doi:[10.5248/115.227](https://doi.org/10.5248/115.227)
- Ruiz A, Iglesias P, Rodríguez B, Muñoz G (2013) *Coprinopsis xenobia*, descripción y primeras localizaciones en España. Comparación filogenética con *Coprinopsis luteocephala*. *Bol Micol FAMCAL* 7:63–70
- Silvestro D, Michalak I (2012) RaxmlGUI: a graphical front-end for RAXML. *Organisms Diversity Evol* 12:335–337. doi:[10.1007/s13127-011-0056-0](https://doi.org/10.1007/s13127-011-0056-0)
- Ulje CB (2005) *Coprinus*. In: Noordeloos ME, Kuyper TW, Vellinga EC (eds) *Flora Agaricina Neerlandica*, vol 6, Critical monographs on families of agarics and boleti occurring in the Netherlands. Taylor & Francis, Boca Raton, pp 22–109
- Vesterholt J (2012) *Coprinopsis* P. Karst. In: Knudsen H, Vesterholt J (eds) *Funga Nordica*. Agaricoid, boletoid, clavarioid, cyphelloid and gasteroid genera. Nordsvamp, Copenhagen, pp 672–687
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*, vol. 4. Academic, New York, pp 315–322
- Yang ZL, Matheny PB, Ge ZW, Slot JC, Hibbett DS (2005) New Asian species of the genus *Anamika* (euagarics, hebelomatoid clade) based on morphology and ribosomal DNA sequences. *Mycol Res* 109:1259–1267. doi:[10.1017/S0953756205003758](https://doi.org/10.1017/S0953756205003758)