Two new psathyrelloid species of *Coprinopsis* (Agaricales, Psathyrellaceae) from China

Gu Rao¹, Dan Dai¹, Hui-Nan Zhao¹, Yi Liang¹, Yu Li¹, Bo Zhang¹

¹ Engineering Research Center of Edible and Medicinal Fungi, Ministry of Education, Jilin Agricultural University, Changchun, Jilin 130118, Changchun, China

Corresponding authors: Yu Li (yuli996@126.com), Bo Zhang (zhangbofungi@126.com)

Academic editor: María P. Martín | Received 10 July 2021 | Accepted 20 August 2021 | Published 8 September 2021

Citation: Rao G, Dai D, Zhao H-N, Liang Y, Li Y, Zhang B (2021) Two new psathyrelloid species of *Coprinopsis* (Agaricales, Psathyrellaceae) from China. MycoKeys 83: 85–103. https://doi.org/10.3897/mycokeys.83.71405

Abstract
In this study, *Coprinopsis jilinensis* and *Coprinopsis pusilla* were introduced, based on their morphological characteristics, the internal transcribed spacer (ITS) and large subunit ribosomal (LSU) region sequences of nrDNA. These new psathyrelloid species were found in Jilin Province, China. *Coprinopsis jilinensis* has brown pileus, utriform pleurocystidia, brown, smooth, dextrinoid basidiospores and tiny pore. It mainly grows on humus. *Coprinopsis pusilla* has small basidiomata, greyish-white pileus, thick and distinct veil at edges, subcolourless and verrucose basidiospores. It is poreless and it grows on the decaying wood of broad-leaved trees. Both of them belong to the *C*. sect. *Melanthinae*. A supplementary description of *C*. sect. *Melanthinae* was given in combination with the newly-discovered taxa and an identification key to the fourteen psathyrelloid species of *Coprinopsis* is provided. *Coprinopsis* sect. *Canocipes* and *C*. sect. *Quartoconatae* were evaluated and the phylogenetic position of the psathyrelloid species of *Coprinopsis* was discussed. *Psathyrella subagraria*, as a confusing species, was also discussed in this study.

Keywords
Asia, molecular systematics, morphology, new taxa, taxonomy

Introduction

Coprinoid mushrooms are fascinating fungal taxa with the characteristic of deliquescent lamellae. *Coprinus* sensu lato is not monophyletic (Johnson and Vilgalys 1998; Hopple and Vilgalys 1999; Park et al. 1999a, 1999b; Moncalvo et al. 2002). Based on molecular studies, Redhead et al. (2001) subdivided *Coprinus* s. l. into four genera,
Coprinus Pers. (Agaricaceae), Corinopsis P. Karst. (Psathyrellaceae), Coprinellus P. Karst. (Psathyrellaceae) and Parasola Redhead, Vilgalys & Hopple (Psathyrellaceae). Combined with anatomical characteristics, 100 species were divided from Coprinus s. l. into Coprinopsis, which were then widely accepted. The subdivision of the Coprinus s. l. was full of controversies at the time. Noordeloos et al. (2005) argued that there were many unresolved problems in the phylogeny and it was too early to subdivide it into four genera.

Schafer (2010) used the classification system of Redhead et al. (2001) to divide Coprinopsis into five sections, Atramentarii, Lanatuli, Alachuani, Narcotici and Nivei, corresponding to Coprinus sect. Atramentarii (Fries 1836), C. sect. Lanatuli (Fries 1836), C. C. subsect. Alachuani (Singer 1949), C. C. subsect. Narcotici (Uljé and Noordeloos 1993) and C. C. subsect. Nivei (Citerin 1992), but the traditional section C. sect. Picacei (Kauffman 1918) was not covered. Wächter and Melzer (2020) subdivided the genus Coprinopsis into 20 sections according to the subclades of the phylogenetic tree (Cinereae, Filamentiferae, Melanthiniae, Alopeciae, Xenobiae, Phlyctidosporae, Kriegsteinerorum, Erythrocephalae, Geesteranorum, Mitraesporeae, Radiatae, Subniveae and Canocipes).

Coprinopsis is a worldwide fungal taxon that includes some well-known species, such as C. atramentaria, characterised by hyphal pileus cuticle, abundant powdery to floccose veil covering the whole pileus, coprophilous, growing in a terrestrial or lignicolous habitat (Redhead et al. 2001). According to Kirk et al. (2008), 200 species of Coprinopsis were known so far the world over. At the time of submission, there are 228 records of Coprinopsis in Index Fungorum (www.indexfungorum.org), including synonyms, varieties, forms and names. There are few studies on Coprinopsis in China. According to the report of Huang (2019), there were only 16 species of Coprinopsis in China, amongst which eight species were newly recorded in China and reported in 2019. Based on traditional morphology, sequence data and phylogenetic analyses, two new species of Coprinopsis were found in Jilin Province, China. They both belong to the C. sect. Melanthiniae and will be reported as follows.

Materials and methods
Collecting and morphological studies

The fresh basidiomata were collected from the Red Leaves Valley in Hanchongling (approximate 43°02′1.67″N, 127°59′36.55″E), Dunhua City, Yanbian Korean Autonomous Prefecture, Jilin Province, China. After dried at 45 °C for 1–2 days, they were stored in the Herbarium of Mycology of Jilin Agricultural University (HMJAU). Photos of fresh basidiomata were taken in the field. The macromorphology was observed from fresh basidiomata and the observation of microstructure was based on dry specimens under a light microscope (LEICA DM1000). The mainly used reagents are 5% potassium hydroxide (KOH) solution, 1% Congo Red and Melzer’s Reagent.
The morphological description referred to Largent et al. (1977 and 1978). The surface of the basidiospores was observed and photographed under a scanning electron microscope (SEM) (Hitachi SU8000) at 2.0 kV, with a working distance of 8 mm. The following symbols were used in the description: [n/m/p] indicates that ‘n’ randomly selected basidiospores from ‘m’ basidiomata of ‘p’ collections were measured, ‘avl’ means the average length of basidiospores, except the extreme values, ‘avw’ means the average width of the basidiospores, except the extreme values, ‘Q’ represents the quotient of the length and width of a single basidiospores in side view, ‘Q_m’ refers to the average Q value of all basidiospores ± standard deviation. Dimensions for basidiospores are given as (a) b–c (d). The range of b–c contains a minimum of 90% of the measured values. Extreme values (i.e. a and b) are given in parentheses.

**Research methods of molecular systematics**

The total DNA of the specimens was extracted by the new plant genomic DNA extraction kit from Jiangsu Kangwei Century Biotechnology Company Limited. The amplification primers of LSU nrDNA (LSU) were LROR and LR5 (Vilgalys and Hester 1990), the ITS nrDNA (ITS) regions were ITS1 and ITS4 (White et al. 1990; Gardes and Bruns 1993). The amplification reactions were carried out in a 25 μl system and the total amount of the reactions was as follows: ddH2O 13.5 μl, 10×Taq Buffer 5 μl, 10 mM dNTPs 1 μl, 10 mM upstream primer 1 μl, 10 mM downstream primer 1 μl, DNA sample 2 μl, 2 U/μl Taq Polymerase 1.5 μl. The cycle parameters were as follows: 4 min at 94 °C for 1 cycle; 40 s at 94 °C, 40 s at 54 °C, 1 min at 72 °C for 35 cycles; 10 min at 72 °C for 1 cycle; storage at 4 °C. The PCR product was subjected to 0.5% agarose gel electrophoresis to test strips. The sequencing work was entrusted to Shenggong Bioengineering (Shanghai) Company Limited and the sequencing results were clipped with Seqman 7.1.0 (Swindell and Plasterer 1997) and then submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/). The newly-obtained sequences are shown in Table 1. The sequences of relevant taxa were downloaded from GenBank and from the related articles (Larsson and Örstadius 2008; Nagy et al. 2010; Nagy 2012; Örstadius et al. 2015; Crous 2017; Melzer et al. 2017).

The ‘auto’ strategy and normal alignment mode of MAFFT (Katoh et al. 2005) were used for Sequence alignment and Gblocks (Castresana et al. 2000; Talavera and Castresana 2007) was used to obtain the conservative segments of sequences with the following parameters: the minimum number of sequences for a conserved/flank

| Taxon       | Voucher    | ITS nrDNA | LSU nrDNA |
|-------------|------------|-----------|-----------|
| *Coprinopsis pusilla*  | HMJAU 58779 | MZ398012  | MZ398067  |
| *C. pusilla*     | HMJAU 58780 | MZ398013  | MZ398068  |
| *C. pusilla*     | HMJAU 58781 | MZ398014  | MZ398069  |
| *C. jilinensis*  | HMJAU 58782 | MZ398015  | MZ398070  |
| *C. jilinensis*  | HMJAU 58783 | MZ398016  | MZ398071  |
position (12/12), the maximum number of contiguous non-conserved positions (8), minimum length of a block (10) and allowed gap positions (with half). ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fit models using the Bayesian Information Criterion (BIC). The Maximum Likelihood (ML) analyses were performed in IQTree 1.6.8 (Nguyen et al. 2015) and the Bayesian Inference phylogenies were performed in MrBayes 3.2.6 (Ronquist et al. 2012) (2 parallel runs, 2000000 generations), in which the initial 25% of sampled data were discarded as burn-in. The above software was integrated into PhyloSuite 1.2.2 (Zhang et al. 2020). The neighbour-joining (NJ) tree was carried out in Mega X (Kumar et al. 2018). The ML and NJ trees were evaluated by bootstrap analysis with 1000 replicates and the best models are shown in Table 2.

### Results

#### BLASTn results

In the BLASTn alignment, based on ITS sequences, *Coprinopsis pusilla* and *C. melanthina* KC992961 (Örstadius et al. 2015) had the highest sequence identities (94.92%–95.26%), with 34–36 base differences. *Corpinopsis jilinensis* and *C. uliginicola* MG712323 (Zhang 2019) had the highest sequence identity (99.27%), with five base differences. The sequence identities between *C. jilinensis* and *C. uliginicola* KC992960 Type (Örstadius et al. 2015) were 93.98%–94.35%, with 39–42 base differences. In BLASTn alignment, based on LSU sequences, *C. pusilla* and *C. melanthina* KC992961 (Örstadius et al. 2015) had the highest sequence identities (99.14%–99.25%), with 7–8 base differences. *Corpinopsis jilinensis* and *C. uliginicola* KC992960 Type (Örstadius et al. 2015) had the highest sequence identity (99.13%), with eight base differences.

#### Phylogenetic analyses

After Gblocks clipping, the ITS data matrix included 31 sequences of 588 nucleotide sites from 16 taxa and the data matrix included 20 sequences of 921 nucleotide sites from 14 taxa (gaps included). In the ITS and LSU phylogenetic trees (Figs 1, 2), *Coprinopsis pusilla* and *C. jilinensis* both belong to *C*. sect. Melanthinae. *Coprinopsis pusilla* and *C. melanthina* formed a sister clade, *C. jilinensis* and *C. uliginicola* formed a sister clade, both of which were strongly supported. In the phylogenetic trees, based on

### Table 2. The best models, based on ModelFinder and MegaX.

|             | BI     | ML     | NJ     |
|-------------|--------|--------|--------|
| ITS nrDNA   | SYM+G4 | TVMc+G4| T92+G5 |
| LSU nrDNA   | K2P+1  | TIM2+F+I| K2+G5  |

---

Note: The table shows the best models selected for ITS and LSU nrDNA sequences using ModelFinder and MegaX. Each model is identified by the code used in the software, indicating the type of model (e.g., SYM+G4 for symmetric model with gamma distribution).
Two new Psathyrellid species *Coprinopsis*

89

ITS sequences (Fig. 1), *C. submicrospora* and *C. marcescibilis/C. pseudomarcescibilis/C. musae/C. udicola* formed a sister clade in the ML and BI trees (MLBP/BIPP: 34/0.5), but *C. submicrospora* and *C. canoceps/C. pannucioides* formed a sister clade in the NJ tree (NJBP: 39). In the phylogenetic trees, based on LSU nrDNA sequences (Fig. 2), *C. marcescibilis* and *C. pseudomarcescibilis* formed a sister clade with strong support in the NJ and BI trees (NJBP/BIPP: 99/0.8). However, in the ML tree, *C. marcescibilis* formed a sister clade with all taxa, except the outgroups (MLBP: 100) and *C. pseudomarcescibilis* formed a sister clade with all taxa, except the outgroups and *C. marcesci-
bilis (MLBP: 30). In the analyses, based on different methods, the sister branch relationships of C. pannucioides, C. submicrospora, C. uliginicola and C. canoceps were different. In the BI tree, C. pannucioides and C. submicrosporal C. uliginicola/C. canoceps formed a sister clade with strong support (BIPP: 1), C. canoceps and C. submicrosporal C. uliginicola formed a sister clade (BIPP: 0.56), C. submicrospora and C. uliginicola formed a sister clade (BIPP: 0.62). In the ML tree, C. submicrospora and C. uliginicola/C. canoceps formed a sister clade with strong support (MLBP: 98), C. uliginicola formed a sister clade with C. pannucioides/C. canoceps (MLBP: 30) and C. pannucioides and C. canoceps formed a sister clade (MLBP: 50). In the NJ tree, C. pannucioides and C. submicrosporal C. uliginicola/C. canoceps formed a sister clade with strong support (NJBP: 99), C. submicrospora formed a sister clade with C. uliginicola/C. canoceps (NJBP: 38) and C. uliginicola and C. canoceps formed a sister clade (NJBP: 41).
Two new Psathyrelloid species Coprinopsis

Coprinopsis jilinensis G. Rao, H.N. Zhao, B. Zhang & Y. Li, sp. nov.
MycoBank No: 840297
Figures 3, 4, 7C, D

**Taxonomy**

**Coprinopsis jilinensis** G. Rao, H.N. Zhao, B. Zhang & Y. Li, sp. nov.

**Figures 3, 4, 7C, D**

**Typification.** CHINA. Red Leaves Valley in Hanchongling, Dunhua City, Yanbian Korean Autonomous Prefecture, Jilin Province, 22 August 2019, G. Rao & H.N Zhao (HMJAU 58782 Holotype!).

**Sequences ex holotype.** MZ398015 (ITS nrDNA), MZ398070 (LSU nrDNA).

**Etymology.** The epithet “jilinensis” refers to this species that was first discovered in Jilin Province, China.

**Description.** Basidiomata small to medium-sized. Pileus 33–52 mm broad, conical to convex, dark brown or clay brown, densely covered with white hairs, not sticky when dry or wet, not hygrophanous, veil remnants flocculent at edges. Lamellae close or crowded, grey-white to fleshy brown, brownish-black after drying, sinuate or adnexed, not the same length and width, edges slightly toothed, concolorous, not deliquescent. Stipe 80–95 × 5–9 mm, white to milky white, cylindrical, down slightly rough, fibrous, a little fragile, hollow, the base with white mycelium, dense or sparse, close to the stipe surface covered with brownish-yellow pubescent, no ring. Spore print without record.

Basidiospores [60, 2, 2] (8)8.5–10(10.2) × 4.5–5.9 (6) μm, avl = 9.1 μm, avw = 5.2 μm, Q = (1.62) 1.63–1.96 (2.02), Q_m = 1.77 ± 0.09, oval to long oval, brown, brownish-yellow or dark brown in 5% KOH solution, smooth, thick wall, dextrinoid, apical with small pores, 1–2 μm. Basidia 17–30 (39) × 8–10 (13) μm, clavate, 4-sterigmate up to 3–4 μm long, 2–3 sterigmate occasional. Pleurocystidia (30) 33–59 (60) × (11) 12–21 (23) μm, utriform and lageniform, sparse, smooth, hyaline. Cheilocystidia 27–56 × (10) 11–20 (22) μm, utriform and lageniform, smooth, hyaline, crowded in hymenium. Lamellar edge fertile. Pileipellis a cutis, up to 100 μm thick, hyphae (35) 42–111 (148) × (6) 7–34 (35) μm, ovoid, subcylindrical, with brownish-yellow to dark brown pigment, thick wall, encrusting pigment on the outer hyphae. Veil hyphae (5) 6–30 (33) μm wide, present dark encrusting pigment, thick wall, colourless to light yellow, cylindrical and subcylindrical. Stipitipellis a cutis, hyphae (5) 6–22 (32) μm diam., ovoid and subcylindrical, pale brown, with encrusting pigment, thick wall. Clamp connections present in all tissues.

**Habitat and distribution.** On humus of broad-leaved forest or coniferous and broad-leaved mixed forests in autumn.

**Additional specimens examined.** CHINA. Red Leaves Valley in Hanchongling, Dunhua City, Yanbian Korean Autonomous Prefecture, Jilin Province, 14 September 2019, Gu Rao (HMJAU 58783).

**Notes.** Coprinopsis jilinensis is characterised by its small to medium-sized basidiomata, brown pileus with white hairs, smooth and dextrinoid basidiospores with small
pores, pleurocystidia and cheilocystidia present. *C. jilinensis* forms a strongly-supported independent clade in ITS and LSU phylogeny trees (Figs 1, 2).

Morphologically and phylogenetic similar to *Coprinopsis jilinensis*, *C. uliginicola* is characterised by long basidiospores of 10–12(–15) μm, pleurocystidia absent and caulocystidia present, pileipellis no encrusting pigment (Smith 1972). Other similar species, *C. cineraria* is characterised by grey, hygrophanous and striate pileus, little short...
Two new Psathyrelloid species *Coprinopsis*

*C. melanthina* is characterised by little long and subcolourless basidiospores (avl × avw = 10.5 × 5.8 μm), pleurocystidia absent (Kits van Waveren 1985); *C. pusilla* has small basidiomata, grey pileus, subcolourless and verrucose basidiospores (this study) and *Psathyrella subagraria* has hygrophanous pileus, thick flesh and caulocystidia present (Smith 1972), both of which could be clearly distinguished from *C. jilinensis* in terms of morphology.

**Coprinopsis pusilla** G. Rao, B. Zhang & Y. Li, sp. nov.

MycoBank No: 840298

Figures 5, 6, 7A, B

**Typification.** CHINA. Red Leaves Valley in Hanchongling, Dunhua City, Yanbian Korean Autonomous Prefecture, Jilin Province, 21 August 2019, Gu Rao (HMJAU 58781 Holotype!).

**Sequences ex holotype.** MZ398014 (ITS nrDNA), MZ398069 (LSU nrDNA).

**Etymology.** The epithet “pusilla” refers to this species having small basidiomata.
**Description.** Basidiomata very small to small. Pileus 21–29 mm broad, bell-shaped to hemispherical when young, then convex, flat to slightly reflexed at edges, with inconspicuous bulge at the middle, grey or greyish-white when dry, no record when wet, densely covered with flocculent hairs, sometimes central with blackish-grey squamous tapering to the edges, not slime, sometimes the edges crack, hygrophanous no record, veil remnants dense at edges, triangular, subtriangular or massive, not easily disappearing. Lamellae close or crowded, subwhite, greyish-white or coffee brown,

**Figure 5.** Basidiomata and microscopic features of *Coprinopsis pusilla* A collection HMJAU 58780 B collection HMJAU 58781 C collection HMJAU 58779 D basidiospores E basidia and basidioles F cheilocystidia G pileipellis. Scale bars: 5 mm (A–C); 10 μm (D–G).
Two new Psathyrelloid species *Coprinopsis*

flesh blond after drying, sinuate or adnexed, sometimes with vertical teeth, edges slightly toothed, concolorous, not deliquescent. Stipe 35–57 × 3–7 mm, cylindrical, subcylindrical, subequal or a little rough towards the base, white, cream white, hollow, a little fragile, not easy to detach from the cap, densely covered with white and flocculent hairs, brown, brownish-grey to brownish-yellow near the base, veil present at the stalk and cap joints, easily disappearing, no ring, the base with white mycelium. Spore print without record.

Basidiospores [90, 4, 3] 8–12 × 5–6.5 (6.8) μm, avl = 9.8 μm, avw = 5.8 μm, Q = 1.45–2.2 (2.24), $Q_m = 1.70 \pm 0.18$ μm, oval, elliptic to long elliptic, subcolourless in 5% KOH and aqueous solution, surfaces verrucose, thin wall, no pores, not amyloid. Basidia (18) 19–32 (33) × 9–11 (12) μm, clavate, 4-sterigmate up to 3–4 μm long, 2-sterigmate occasional, without pseudoparaphyses. Pleurocystidia absent. Cheilocystidia (25) 27–53 (55) × (11) 13–21 μm, variable-shaped, subcylindrical, utriform, lageniform, reverse gourd-shaped and subcapitate, sphaeropedunculate elements present on gill edges, smooth, hyaline, thin wall to thick wall. Pileipellis a cutis, terminal hyphae (30) 31–84 (98) × (7) 8–17 (18) μm, with light brown pigment, mostly thick wall in the outer hyphae, present dark encrusting pigment, terminal hyphae present small cylindrical protrusions, about 3 × 3 μm. Veil hyphae (26) 27–100 (113) × (9)

Figure 6. Scatter plot of basidiospores size in *Coprinopsis pusilla*. 
10–19 (20) μm, without encrusting pigment, thick wall, colourless to yellowish, cylindrical, subcylindrical, clavate or irregular. Stipitipellis a cutis, hyphae (21) 22–87 (88) × (9) 10–19 (20) μm, encrusting pigment not observed, colourless to light yellow, cylindrical, subcylindrical, clavate or irregular, terminal hyphae present small cylindrical protrusions. Clamp connections present in all tissues.

**Habitat and distribution.** On the dead and rotten wood of broad-leaved forest or coniferous and broad-leaved mixed forests in autumn.

**Additional specimens examined.** China. Red Leaves Valley in Hanchongling, Dunhua City, Yanbian Korean Autonomous Prefecture, Jilin Province, 6 August 2019, G. Rao (HMJAU 58779, HMJAU 58780).

**Notes.** *Coprinopsis pusilla* has a variable macromorphology, but stable micromorphology, which is characterised by small basidiomata, greyish-white pileus, thick and distinct veil remnants at edges, subcolourless and verrucose basidiospores, no pore, the habitat on the decaying wood of broad-leaved trees. *C. pusilla* forms a strongly-supported independent clade in both ITS and LSU phylogeny trees (Figs 1, 2).

Morphologically and phylogenetically similar to *Coprinopsis pusilla*, *C. melanthina* is characterised by larger brown pileus, fibrous veil at edges, longer basidiospores (avl = 10.5 μm) (Kits van Waveren 1985). *C. uliginicola* is characterised by large basidiomata, brown-black basidiospores, pore present (Smith 1972). *C. cineraria* is characterised by fibrous veil at the edges easily disappearing, smaller basidiospores (6.5–8.5 × 4–5 μm), pileipellis an epithelium (Takahashi 2000).
Discussion

Here we report two new psathyrelloid species of *Coprinopsis* from northern China. There is no unified definition of “psathyrelloid”. Previously, “psathyrelloid” was mainly regarded as a species with the morphology of *Psathyrella*, including *Psathyrella* itself (Redhead et al. 2001). Örstadius et al. (2015) understood that “psathyrelloid” means that certain species of fungi with the morphology of *Psathyrella*, but belong to other genera in the phylogenetic analysis, so the species of *Psathyrella* were not included. These fungi were mainly distributed in *Coprinopsis* (Örstadius et al. 2015), *Typhrasa* (Örstadius et al. 2015; Wang et al. 2021), *Parasola* (Ganga and Manimohan 2019), *Homophron* (Örstadius et al. 2015), *Gystagaricus* (Örstadius et al. 2015), *Kauffmania* (Örstadius et al. 2015), *Lacrymaria* (Yan 2018) and so on. According to Redhead et al. (2001), coprinoid mushrooms were distributed in four genera: *Coprinus*, *Parasola*, *Coprinopsis* and *Coprinellus*. In Agaricaceae, Coprinoid mushrooms should also include *Montagnea*, *Podaxis* and *Xerocoprinus* (Moncalvo et al. 2002; Keirle et al. 2004). As there was no uniform standard, some fungi, such as *Parasola conopilea* (Ganga and Manimohan 2019), were intermediate between psathyrelloid and coprinoid species. With the introduction of molecular systematics, the species of coprinoid and psathyrelloid intersected with each other. Örstadius et al. (2015) studied the morphology of many psathyrelloid species of Psathyrellaceae and believed that the lack of the typical pattern of pseudoparaphyses was their main morphological characteristic. Since psathyrelloid species belong to different genera, their distributions were not indicated phylogenetically in their study. Worldwide, there were, currently, at least fourteen psathyrelloid species of *Coprinopsis* (Picón 2010; Portugalete 2011; Örstadius et al. 2015; Crous 2017; Larsson and Örstadius 2017; Melzer et al. 2017), which belong to C. sect. *Lanatulae* in Schafe’s grouping system (Schafe 2010) and to three sections in Wächter’s grouping system, *Melanthinae* (including *C. cineraria*, *C. melanthina*, *C. uliginicola*, *C. jilinensis* and *C. pusilla*), *Canocipes* (including *C. aesontiensis*, *C. canoceps*, *C. lotiniae*, *C. pannucioideus*, *C. submicrospora* and *C. udicola*) and *Quartoconatae* (including *C. marcescilis*, *C. musae* and *C. pseudomarcescilis*). Currently, the known sequences of these species in these three sections are all the psathyrelloid species of *Coprinopsis*.

According to the results of BLASTn analyses, ITS showed higher interspecific variability, so the ITS sequence was more advantageous in reflecting the interspecific relationship of *Coprinopsis* than the LSU sequence. The sequence identity between *C. jilinensis* from Jilin Province, China and *C. uliginicola* MG712323 from Hubei Province, China, was 99.27%. Based on the molecular sequence alone, *C. uliginicola* MG712323 and *C. jilinensis* could be the same species and subsequent re-examination of this specimen is recommended.

In this study, some sequences of *Coprinopsis* were selected, which belong to C. sect. *Melanthinae*, C. sect. *Canocipes* and C. sect. *Quartoconatae* in the grouping system proposed by Wächter and Melzer (2020). In the phylogenetic analyses (Figs 1, 2), whether in the phylogenetic trees, based on ITS or LSU, the tree shape of C. sect. *Melanthinae* obtained was consistent and the branches were stable. However, C. sect. *Canocipes* and C. sect. *Quartoconatae* were somewhat different from the grouping described by Wächter and Melzer (2020), based on different sequences and analyses. In the phylogenetic trees, based
on ITS, *C. udicola* and *C. submicrospora*, which originally belonged to *C.* sect. *canocipes*, formed a branch with three species belonging to *C.* sect. *Quartoconatae* in ML and BI trees. However, in the NJ tree, *C. udicola* formed a sister clade with three species belonging to *C.* sect. *Quartoconatae*. In the phylogenetic trees, based on LSU, *C.* sect. *Canocipes* and *C.* sect. *Quartoconatae* were well separated in the NJ tree, while in ML and BI trees, *C. musae* belonging to *C.* sect. *Quartoconatae* formed a sister clade with four species of *C.* sect. *Canocipes*. The branching relationships of phylogenetic trees, based on different molecular sequences and analyses may vary. Over-subdivision of sections would cause intersection. Subdivision of *C.* sect. *Canocipes* and *C.* sect. *Quartoconatae* may need to be reconsidered.

*Coprinopsis* sect. *Melanthinae* has a relatively clear systematic differentiation (Wächter and Melzer 2020), which is consistent with the results of this study. Currently, there are only six species in this section, including the *C. lignicola* nom. prov. (GenBank no.: MG966286 and MF163181). Combined with the two newly-discovered species, the expression of this section is modified as follows:

**Coprinopsis sect. Melanthinae Wächter & A. Melzer**

**Description.** Basidiomata very small to large, on humus or lignicolous. Pileus not radially sulcate, lamellae not deliquescent. Veil strongly developed, consisting of chains of subcylindrical, sometimes encrusted cells. Basidiospores medium to large-sized, ellipsoid to ovoid in side view, strikingly pale or brown, thin or thick-walled, germ pore absent or very indistinct, surfaces verrucose or smooth. Pseudoparaphyses absent. Basidia 4-sterigmate, 2–3-sterigmate occasional, always clavate, never polymorphic. Marginal cells of the lamellar edge predominantly utriform. Pleurocystidia present or absent. Cheilocystidia and clamps present.

*Coprinopsis pusilla* has a variety of macroscopic morphology, including the shape and colour of the pileus, the shape of the veil and the thickness and length of the stipe and so on, which makes it difficult to determine whether it is the same species during the collection process, when the size of the basidiomata and ecological habits seem to be stable. The microscopic morphology of *C. pusilla* is relatively stable, including the size, shape, colour and decoration of basidiospores, the type of pileipellis and the presence or absence of cystidia. Interestingly, Coprinoid fungi are thought to be a taxon of dark-coloured basidiospores (Noordeloos et al. 2005), but *C. pusilla*, including *C. melanthina*, which is closely related to *C. pusilla*, have subcolourless basidiospores. Collection HMJAU 58779 and collection HMJAU 58780 were collected at approximately the same time in the same forest, which were not far apart from each other. However, due to the significant difference in macroscopic morphology, they were made into two collections. The macroscopic morphology of Collection HMJAU 58781, collected later, was also significantly different from the two specimens collected previously. Through the observation of the microscopic morphology of these collections and the phylogenetic analysis, combined with ITS and LSU molecular sequences, the results showed that the three collections were the same species.

Pleurocystidia is present in many species of *Coprinopsis*, such as *C. cinerea*, *C. jonesii* and *C. pseudoradiata* (Redhead et al. 2001), but in psathyrelloid species of *Coprinopsis*, spe-
Two new Psathyrelloid species Coprinopsis

Species with pleurocystidia are rare. The pleurocystidia are sparsely distributed in the hymenium of *C. jilinensis*, but not present in *C. uliginicola*, which is closely related to *C. jilinensis*. In psathyrelloid species of *Coprinopsis*, only *C. pannucioides* (Larsson and Örstadius 2008), *C. udicola* (existing but rare) (Örstadius et al. 2015) and *C. jilinensis* have pleurocystidia.

*Psathyrella subagraria* is a confusing species, described by Smith (1972) as it is very similar to *P. uliginicola* morphologically, with the main difference being that this species has pleurocystidia, which are mainly growing on humus. Since Smith (1972) introduced *P. subagraria*, no further reports have been made. Örstadius et al. (2015) moved *P. uliginicola* to *Coprinopsis*, based on molecular studies, the taxonomic status of *P. subagraria* being questionable. There are some differences between *P. subagraria* and *C. jilinensis* in macroscopic morphology, but they are similar in microscopic morphology. Smith (1972) described *P. subagraria* as having two basidiospores sizes [8–10 × 4–5 (10–12 × 4.5–5.5) μm], one of which was very close to the size of *C. jilinensis* [(8) 8.5–10 (10.2) × 4.5–5.9 (6) μm], requiring re-examination of the type specimens of *P. subagraria* in future studies.

### Key to fourteen psathyrelloid species of *Coprinopsis*

1. Basidiospores no pore ................................................................. 2
   - Basidiospores with pore .................................................... 6
2. Basidiospores distinctly pigmented ...................................... *C. submicrospora*
   - Basidiospores hyaline to very pale brown ........................... 3
3. Pileus < 20 mm, glabrous; pseudoparaphyses often present ...... *C. musae*
   - Pileus > 20 mm, hairy; pseudoparaphyses absent or unrecorded... 4
4. Pileus hygrophanous, striate; basidiospores avl < 9 μm; pileipellis an epithe-lium ......................................................... *C. cineraria*
   - Pileus not or scarcely hygrophanous, not striate; basidiospores avl > 9 μm; pileipellis a cutis .......................................................... 5
5. Pileus 25–80 mm, brown, fibrous veil at edges; basidiospores avl = 10.5 μm. ........................................................... *C. melanthina*
   - Pileus 21–29 mm, grey or greyish-white, veil dense at edges; basidiospores avl = 9.8 μm .............................................................. *C. pusilla*
6. Basidiospores avl > 11.5 μm ......................................................... 7
   - Basidiospores avl < 11.5 μm .................................................. 10
7. Pileus mostly brown, almost not striate, flocci at cap margin ........ 8
   - Pileus mostly white or grey, striate, no flocci at cap margin .......... 9
8. Basidiospores 13.3–14.5 μm; irregular flocci on cap margin ............ *C. pseudomarcescibilis*
   - Basidiospores 11.6–12.8 μm; denticulate flocci on cap margin .......... *C. marcescibilis*
9. Pseudoparaphyses present, pleurocystidia absent; caulocystidia present ....... *C. lotinae*
   - Pseudoparaphyses absent; pleurocystidia rare, close to gill edge; caulocystidia absent ........................................... *C. udicola*
|   | Description                                                                                           | Acknowledgements                                                                                                                                                                                                 |
|---|-------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|10 | Pleurocystidia present; cheilocystidia no long neck                                                 | We are grateful to the editors and reviewers for improving the manuscript. This study is funded by Key Project on R&D of Ministry of Science and Technology (No. 2018YFE0107800), Jilin Province Science and Technology Development Plan Project (No. 20190201026JC), Modern Agroindustry Technology Research System (No. CARS20) and Scientific and Technological Tackling Plan for the Key Fields of Xinjiang Production and Construction Corps (No. 2021AB004). |
|  | – Pleurocystidia absent; cheilocystidia part with long neck                                         |                                                                                                                                                                                                                  |
|11 | Pileus yellowish-white, papillate-umbonate; caulocystidia present; tufty                             |                                                                                                                                                                                                                  |
|  | – Pileus brown, not papillate-umbonate; caulocystidia absent; solitary or scattered                  | C. pannucioioides                                                                                                                                                                                                |
|12 | Basidiomata medium-sized to large-sized (pileus > 50 mm); pileus pallid to greyish                   | C. jilinensis                                                                                                                                                                                                     |
|  | – Basidiomata very small to small (pileus < 50 mm); pileus not grey                                 | C. uliginicola                                                                                                                                                                                                    |
|13 | Stipe < 60 mm long; basidia 4-sterigmate; cheilocystidia not inflated fusiform                      | C. canoceps                                                                                                                                                                                                       |
|  | – Stipe > 60 mm long; basidia 1-, 2-, 4-sterigmate; cheilocystidia part inflated fusiform            | C. aesontiensis                                                                                                                                                                                                    |

**References**

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17(4): 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Citerin M (1992) Clé analytique du genre Coprinus. Documents Mycologiques 22(86): 1–28.
Two new Psathyrelloid species *Coprinopsis*
Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Dictionary of the Fungi, 10th edn. CABI Publishing, UK, 771 pp.
Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096
Largent DL (1986) How to identify mushrooms to genus I: macroscopic features. Mad River Press, Eureka, 166 pp.
Largent DL, Johnson D, Watling R (1977) How to identify mushrooms to genus : microscopic features. Mad River Press, Eureka, 148 pp.
Larsson E, Örstadius L (2008) Fourteen coprophilous species of Psathyrella identified in the Nordic countries using morphology and nuclear rDNA sequence data. Mycological Research 112(10): 1165–1185. https://doi.org/10.1016/j.mycres.2008.04.003
Melzer A, Ferisin G, Dovana F (2017) Coprinopsis aesontiensis, a new species found in Friuli Venezia Guilia, Italy. Micologia e Vegetazione Mediterrane 31(2): 123–132.
Moncalvo JM, Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hofstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jacobsson S, Clémençon H, Miller Jr OK (2002) One hundred and seventeen clades of euagarics. Molecular Phylogenetics and Evolution 23(3): 357–400. https://doi.org/10.1016/S1055-7903(02)00027-1
Nagy L (2012) An investigation of the phylogeny and evolutionary processes of deliquescent fruiting bodies in the mushroom family Psathyrellaceae (Agaricales). Szegedi Tudományegyetem, Szeged, 119 pp.
Nagy LG, Urban A, Örstadius L, Papp T, Larsson E, Vágvölgyi C (2010) The evolution of autodigestion in the mushroom family Psathyrellaceae (Agaricales) inferred from Maximum Likelihood and Bayesian methods. Molecular Phylogenetics and Evolution 57(3): 1037–1048. https://doi.org/10.1016/j.ympev.2010.08.022
Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300
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. Mycological Progress 14(5): 1–42. https://doi.org/10.1007/s11557-015-1047-x
Park DS, Go SJ, Kim YS, Seok SJ, Ryu JC, Sung JM (1999) Phylogenetic relationships of genera Coprinus and Psathyrella on the basis of ITS region sequences. The Korean Journal of Mycology 27(4): 274–279.
Park DS, Go SJ, Ryu JC (1999) Phylogenetic relationships of coprinoid taxa and an agaric-like gastroid taxon based on the sequences of internal transcribed spacer (ITS) regions. The Korean Journal of Mycology 27(6): 406–411.
Picón GRM (2010) Coprinopsis lotinae (Picón) Picón, comb. nov. (Psathyrellaceae). Zizak 7: 43–45.
Redhead SA, Vilgalys R, Moncalvo JM, Johnson J, Hopple Jr JS (2001) Coprinus Pers. and the disposition of Coprinus species sensu lato. Taxon 50(1): 203–241. https://doi.org/10.2307/1224525
Two new Psathyrelloid species *Coprinopsis*

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Schafer DJ (2010) Keys to sections of *Parasola*, *Coprinellus*, *Coprinopsis* and *Coprinus* in Britain. Field Mycology 11(2): 44–51. https://doi.org/10.1016/jfldmyc.2010.04.006

Singer R (1949) The Agaricales (mushrooms) in modern taxonomy. Chronica Botanica, New York, 832 pp.

Smith AH (1972) The North American species of *Psathyrella*. Memoirs of the New York Botanical Garden 24: 1–633.

Swindell SR, Plasterer TN (1997) Sequence data analysis guidebook. Humana Press, Totowa, 352 pp. https://doi.org/10.1385/0896033589

Takahashi H (2000) Two new species and one new variety of Agaricales from central Honshu, Japan. Mycoscience 41(1): 15–23. https://doi.org/10.1007/BF02464381

Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56(4): 564–577. https://doi.org/10.1080/10635150701472164

Uljé CB (2005) Flora Agaricina Neerlandica Volume 6, CRC Press, Boca Raton, 226 pp.

Uljé CB, Noordeloos ME (1993) Studies in *Coprinus* III—*Coprinus* section *Veliformes*. Subdivision and revision of subsection *Nivei* emend. Persoonia-Molecular Phylogeny and Evolution of Fungi 15(3): 257–301.

Van Waveren EK (1985) The Dutch, French and British species of *Psathyrella*. Persoonia-Supplement 2(1): 3–300.

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172(8): 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wächter D, Melzer A (2020) Proposal for a subdivision of the family Psathyrellaceae based on a taxon-rich phylogenetic analysis with iterative multigene guide tree. Mycological Progress 19(11): 1151–1265. https://doi.org/10.1007/s11557-020-01606-3

Wang SN, Hu YP, Chen JL, Qi LL, Zeng H, Ding H, Huo GH, Zhang LP, Chen FS, Yan JQ (2021) First record of the rare genus Typhrasa (Psathyrellaceae, Agaricales) from China with description of two new species. MycoKeys 79: 119–128. https://doi.org/10.3897/mycokeys.79.63700

Yan JQ (2018) Taxonomy and Molecular Phylogeny of *Psathyrella* and related genera in China. PhD Thesis, Jilin Agricultural University, Changchun, 193 pp.

Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096

Zhang X (2019) Diversity of macro-basidiomycetes in Xingshan County, Hubei province. Master Thesis, Nanjing Normal University, Nanjing, 98 pp.