Phylogeny and species delimitation in the genus *Coprinellus* with special emphasis on the haired species

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**Abstract:** We inferred the phylogenetic structure and species limits within *Coprinellus* by using a newly generated multigene alignment of LSU, ITS and β-tubulin sequences. We sampled 154 specimens of 71 species out of ca. 80 known taxa in *Coprinellus* and inferred phylogenetic relationships by Bayesian MCMC and ML bootstrapping. Morphological and phylogenetic analyses revealed 17 new species, five of which are described herein. *Coprinellus* was split into three large clades, one comprising species with an initially continuous sheath of veil (Domestici/Micacei clade) and two consisting of primarily setulose (haired) species (eurysporoid and Core Setulosi clades). The separation of veiled species and the clade structure therein corresponds well to the morphology-based sectional classification, although the inclusion of certain setulose taxa (e.g. *C. disseminatus*, *C. verrucispermus*, *C. curtus* etc.) in these clades necessitates a new morphological definition for the sections. Morphological traits are discussed for all clades of *Coprinellus*. Species limits in the eurysporoid and Core Setulosi clades are scrutinized in detail. Future directions of species-rank research and an artificial key to the recovered setulose species of *Coprinellus* is presented.

**Key words:** *Coprinus* sensu lato, *Domestici, Micacei*, new species, pilocystidia, *Setulosi*

**INTRODUCTION**

Molecular phylogenies have significantly rearranged our views on the taxonomy of coprinoid mushrooms in the past few years (Hopple and Vilgalys 1994, 1999; Walther et al. 2005; Larsson and Örstedius 2008; Vasutova et al. 2008; Padamsee et al. 2008; Nagy et al. 2010, 2011). The splitting of species of the collective genus *Coprinus* into *Coprinopsis*, *Parasola*, *Coprinellus* and some residual species not placed in any of the former genera (Redhead et al. 2001, Redhead 2001, Simpson and Grgrunovic 2001) has received much attention and general acceptance among taxonomists. Yet the infrageneric relationships and the affinities of the residual species have remained largely untested on a molecular phylogenetic basis. Despite great morphological diversity, *Coprinopsis* and *Coprinellus* has not been investigated or split further while the paraphyly of *Psathyrella* has received more attention (Larsson and Örstedius 2008, Vasutova et al. 2008, Padamsee et al. 2008). Molecular studies revealed a noteworthy infrageneric phylogenetic structure both in *Coprinellus* and *Coprinopsis* (Nagy et al. 2010, 2011), whereas *Parasola* appeared to be uniform in accordance with its low morphological variation (Nagy et al. 2009). This was particularly striking in *Coprinellus*, where multigene phylogenies called the monophyly of the genus into question (Nagy et al. 2011). Subsequently the monophyly of the two large clades (*Coprinellus* p.p.1 and 2.) has been established and the conflicting results attributed to MCMC convergence or taxon sampling issues (Nagy pers comm). The previously inferred infrageneric structure is particularly noteworthy within *Coprinellus*. Studies outlined two large clades of *Coprinellus* (Walther et al. 2005; Vasutova et al. 2008; Nagy et al. 2010, 2011), one in a significant overlap with subsection *Setulosi*, the other with subsections *Domestici* and *Micacei* of a morphology-based subdivision of *Coprinus* s.l. (Partick 1977, Uljé 2005, Orton and Watling 1979). Despite the overlap these clades are in conflict with the sectional subdivisions proposed by Uljé (1991), Singer (1986), Kühner and Romagnesi (1953) and Orton and Watling (1979), which merits further study.

At the time of splitting *Coprinus* s.l. into *Coprinopsis*, *Coprinellus* and *Parasola*, in the absence of sequence information, several species with ambiguous affinities have not been placed in any of the newly erected genera (Redhead et al. 2001). These include several taxa of the cortinati group (sensu Uljé 2005), that is *C. cortinatus*, *C. bellulus* etc., certain members of section *Narcotici*, some lesser known taxa and *C. patouillardii* (= *C. cordisporus*). Subsequent to the rearrangement of *Coprinus* s.l. into segregate genera (Redhead et al. 2001), several species, including those described by Uljé and Keizer (2003), Uljé and Noordeloos (2003), Uljé and Verkeben (2002), Nagy (2006), have been recombined in *Coprinellus* based
on phylogenetic and/or morphological evidence (Simpson and Grgerinovic 2001). Of these taxa Doveri et al. (2011) recombined C. allovellus, C. canistri, C. callinus var. limicola, C. minutisporus and C. pseudoamphithallus while Házi et al. (2010) and Roux and Garcia (2006) placed C. doverii, C. parvatus, C. pusillul tus, C. pallidissimus and C. bipellis in Coprinellus respectively. However a classification in the new system of coprinoid fungi has not yet been proposed for several well known or recently described species.

Setulose species of Coprinellus have been subject to a commonly recognized, narrow morphological species concept that has been uniform among authors (Lange 1952, Lange and Smith 1953, Uljé and Bas 1991, Doveri et al. 2011, Nagy 2006, Házi et al. 2010). This is based on (conjectured) low morphological plasticity within species and was partially supported by the results of mating experiments (Kemp 1975, Lange 1952). Indeed most species have low intraspecific variability and are easily identified on the basis of micromorphology. However a comprehensive examination of herbarium materials revealed a large number of deviating specimens with considerable variation, primarily in spore shape, size and cheilocystidia. This variation would qualify the respective specimens as new species based on the currently accepted narrow morphological species concept. Molecular phylogenetic approaches permit a critical test of this morphological species concept by comparing the phylogenetic placement and branch lengths of the sequenced specimens to that of their morphological traits, which we undertake in this paper.

In view of the above-mentioned points in this study we assemble a new multigene dataset of Coprinellus and use the resulting phylogeny to analyze (i) the phylogenetic structure within Coprinellus, (ii) the large-scale polyphyly of species with hairs (pileo- and caulocystidia), that is those of subsection Setulosi, (iii) species limits within setulose species and (iv) whether a narrow species concept is applicable across setulose taxa as suggested previously.

**Materials and Methods**

**Taxon sampling.**—An attempt was made to sequence all described species of Coprinellus. Herbarium specimens were collected to achieve a uniform coverage across all taxa by selecting 2–6 specimens per species. Material was obtained from the herbaria WU, M, WTU, L, K, MICH as well as our own collection of coprinoid species (SZMC, Szeged Microbiological Collections). Specimens originating from two monographic treatments of coprinoid fungi of C.B. Uljé (Uljé and Bas 1991, Uljé and Keizer 2003, Uljé and Noordeloos 2003, Uljé and Verkeben 2002) and F. van de Bogart (1975) also were revised. Type collections were included whenever the condition of the material allowed destructive sampling. Previous work indicated a close relationship between Ps. candolleana and Coprinellus (e.g. Hopple and Vilgalys 1999, Padanmee et al. 2008, Nagy et al. 2011), so we used this group as outgroup in our analyses.

**DNA extraction and sequencing.**—Genomic DNA was extracted with the DNEasy Plant Mini Kit (QIAGEN), following the manufacturer’s protocol with minor adjustments to the fruiting bodies of certain species. Primer sets and PCR amplification of the ITS, LSU and β-tubulin genes were performed as described by Nagy et al. (2011). We amplified the ITS and β-tubulin fragments for all specimens, while the LSU region was targeted only for one or few representative collection(s) per species. In some cases only poor quality DNA extracts could be produced because of the diminutive fruiting bodies. In these cases PCR amplification of all loci employed the JumpStart Taq DNA polymerase (Sigma-Aldrich). Amplicons were sequenced commercially (by LGC Genomics Ltd, Berlin, Germany) from both directions and readings were assembled into contigs with the Pregap4 and Gap programs of the Staden package (Staden et al. 2000). All sequences were deposited in GenBank (Supplementary Table I.)

**Alignments and phylogenetic analyses.**—LSU and β-tubulin sequences were aligned with Clustal W (Thompson et al. 2002), ITS was aligned by PRANK (Probabilistic alignment kit, Löytynoja et al. 2008). The latter is a model-based alignment approach that distinguishes insertions from deletions and reconstructs the multiple alignment with a two-step progressive algorithm that uses an accurate ML phylogeny for the assembly of the second multiple alignment. For the distinction between insertions and deletions we used the +F option, which produces alignments with more gaps but has been shown to be more accurate in finding positional homologies than all previously available alignment approaches (Löytynoja et al. 2008). Overly divergent intron regions of the β-tubulin gene and leading and trailing gaps of all alignments were trimmed before analysis. Indels in the ITS region were recoded by means of the simple indel-coding algorithm (Simmons and Ochoterena 2001) as implemented in FastGap 1.2.1 (Borchsenius 2007). The simple indel coding algorithm treats multiresidue indels as a single evolutionary event (Simmons and Ochoterena 2001) and has been shown to compete well with the performance of the complex indel-coding algorithm (Simmons et al. 2007). Combinability of the genes was assessed by inspecting ML bootstrap values obtained for single-gene alignments. Mutually exclusive, strongly supported (>70%) relationships were interpreted as significant conflict between the alignments. Models of nucleotide substitution were selected for each alignment with AICc in jModelTest (Posada 2008). During model selection we disregarded the invariant sites (I) option because this accounts for the same phenomenon as the gamma-distributed rate heterogeneity model and simulation studies revealed a risk of parameter nonidentifiability when the two are applied simultaneously (Rannala
Alignments and trees were deposited in TreeBASE (study 11515).

Maximum likelihood bootstrapping was performed in the MPI-enabled parallel version of RaxML 7.5.1 (Stamatakis 2006). The dataset was partitioned as above, but the indel matrix was omitted and the GTR + G model was invoked for each partition separately. The gamma distribution was separated into four rate categories. One thousand nonparametric bootstrap replicates were run. Each bootstrap replicate was started from a maximum parsimony tree built in RaxML.

We inferred phylogenies under maximum likelihood and two Bayesian methods. For Bayesian estimation we used MrBayes 3.1.2 (Altekar et al. 2004) and BayesPhylogenies 2.0 (Pagel and Meade 2007). MrBayes was launched with default priors and these settings: chain length 30 000 000 generations, sampling frequency 100, two replicates, with three heated and one cold chains per replicate; while in BayesPhylogenies no metropolis coupling was applied, the sampling frequency was set to 1000, replicates to three and chain length was 15 000 000. The concatenated alignment was partitioned into ITS1, 5.8S, ITS2, β-tubulin, LSU and indel matrices, and the parameters of the evolutionary model were estimated separately for each partition. In BayesPhylogenies we invoked a mixture model with three patterns for each alignment site in each partition (Pagel et al. 2004). The burn-in was established by inspecting the convergence of likelihood values in Tracer (Rambaut 2004) and tree-split posterior probabilities in AWTY (Wilgenbusch et al. 2004). Post burn-in trees were used to compute a 50% majority rule consensus tree in the SumTrees script of the Dendropy package (Sukumaran and Holder 2010). To examine the optimal phylogenetic placement of C. verrucispermus and C. deliquescentes we performed a maximum likelihood constraint analysis as described in Nagy et al. (2010).

Morphological studies.—Characters suitable for differentiation among species were collected from literature searches and microscopic examination of dried herbarium specimens. Microscopic examination was performed on a Zeiss Axiolab light microscope after mounting the dried materials in 10% NH₄OH and Congo red. Dimensions and shape of cauloc-, cheilo-, pleuro- and pilocystidia, veil remnants, basidia, basidiospores, the angle and presence of a germ pore were noted. Cheilo- and pleurocystidia were observed separately by cutting the gill edge from the rest of the gill to avoid blending of the two types of cystidia. Measurements of basidia and basidiospores included sterigmata and the hilum respectively. Q values refer to the length divided by the width of the spores in frontal view. Macromorphological characters were described from fresh collections or were taken from herbarium notes. Line drawings and measurements were carried out at 400× or 1000× magnification. Line drawings were converted to vector graphics format in CorelDraw (Corel Co., Canada) after optimizing filter and smoothing values while tracing bitmaps.

**Results**

Altogether 160 new sequences were produced for this study; accession numbers are provided (Supplementary Table I). The concatenated dataset contained 154 specimens of 71 species. We did not obtain specimens for *Coprinellus ditectus*, *C. minutisporus* and *C. allovellus*, while PCR amplification or sequencing failed for *Coprinellus singularis*, *C. pyrrhanthes*, *C. heptemerus* and *C. ephemerus*. The latter turned out to be much rarer than previously assumed, with most specimens labeled *C. ephemerus* actually representing *Coprinus congreatus*. Out of 154 specimens ITS, β-tubulin and LSU were available respectively for 153, 134 and 79. Sequencing of ITS fragments failed for *C. simulans*, so this species was represented only by the β-tubulin and LSU genes. Both the ITS and the β-tubulin sequences of *C. parvulus* had an excessive number of ambiguities and potentially misidentified bases, so we excluded this taxon from the analyses.

After the exclusion of nonoverlapping leading and trailing regions as well as introns, the alignments spanned 1022 characters for ITS, 369 for β-tubulin and 1296 for LSU regions, producing a concatenated alignment of 2689 nucleic acid sites. This was supplemented with 238 binary characters obtained by recoding the indels in the ITS alignment. Based on the AICc distances, the best fit model was found to be the GTR + G for all partitions except the 5.8S region, for which the JC + G was proposed. The indel partition was modeled by a two-state Markov model implemented for restriction sites in MrBayes. In both Bayesian and ML analyses the ITS region was partitioned further into ITS1 (1–450), 5.8S (451–631) and ITS2 (632–1022), following the results of Nagy et al. (2011). The BayesPhylogenies analysis was stopped after 15 000 000 generations because AWTY analyses indicated sufficient topological convergence. The burn-in was established respectively as 25 000 000 and 10 000 000 generations for the MrBayes and BayesPhylogenies runs. Thus posterior probabilities were calculated for 2 × 50 000 and 3 × 5000 trees from the output of the two programs. Unexpected placements of *C. verrucispermus* and *C. silvaticus* (basally in the Domestici clad) were examined by constraint analyses. When these taxa were constrained from the output of the two programs. Unexpected placements of *C. verrucispermus* and *C. silvaticus* (basally in the Domestici clad) were examined by constraint analyses. When these taxa were constrained
Micacei/Domestici (BPP: 0.50 MLBS: 56% MBPP: 0.86) clade (Fig. 1). The latter was split into two subclades, one containing species of the morphologically discerned section Domestici plus *C. pusillus* (= *C. heptemerus f. parvisporus*), *C. curta* and *Coprinellus* sp. 7 (SZMC-NL-0150), hereafter referred to as Domestici clade (BPP: 0.50 MLBS: − MBPP: 0.76) and the other corresponding to section Micacei (Micacei clade, BPP: 1.00 MLBS: 89% MBPP: 0.94), including the setulose taxa *C. disseminatus*, *C. aureocranatus*, *C. silvicola*, *C. verrucispermus* and *C. xylophilus*. The position of *Coprinellus* sp. 8 (SZMC-NL-2933) has proved difficult to resolve due to a highly divergent ITS sequence. Based on morphological features (rich, thick-walled, colored veil), it would fit well in section Domestici but is not conspecific with any of the described species therein. All analyses place it in a transitional position between the Micacei/Domestici and the setulose clades. Similarly *C. simulans* could not be assigned a certain position in any of the analyses.

**Taxonomy**

*Coprinellus cinereopallidus* L. Nagy, Házi, Papp & Vágvölgyi, sp. nov.  

Figs. 2, 3  

MycoBank MB561851  

Pileus 3–6 (7) × 3–4 mm in iuventue atque clausus, usque ad 10–20 mm latus si plane expansus, ellipsipodeus vel subglobosus, deinde convexus, apllanatus in maturitate, leviter deliquescens, pellucidally striatus usque ad centrum si usus, radialiter canaliculatus, plicatus in expansis speciminius, omnino tenuissimis micaceis pilis obsectus, sine velo, pallidissimae ochraceae vel pallide griseo-roseus, in maturitate paene albidus vel pallide griseolo-ochraceus. Lamellae libereae, confertae, usque ad 1–1.5 mm latae, in iuvente albidae, deinde griseolae, demum nigrungentes, acie minute fimbrata albida. Stipes 0.5–11 mm crassus, 30–80 mm longus, elatus, fistulosus, fragilis, cylindricus vel inconspicue inflatus basim versus, minutissimae omnia albis pilis pruinosus, albidus vel pallide ochraceus in maturitate. Caro inconspicua, fragilis, sine odorle vel sapore conspicuo.


Pileus 4–7 × 3–5 mm when closed, ellipsoidal to subcylindrical, expanding to convex-plano-convex then convex, 10–30 mm wide when fully expanded, surface translucently striate up to center when young, later radially sulcate-grooved, densely covered with short, hardly noticeable hairs, pale grayish brown to beige, darker in the center, grayish toward margin when moist, pale ochraceous brown to almost whitish when dry, weakly deliquescent; lamellae distant, free to narrowly adnate, verrucinose, up to 1–1.5 mm broad, white when young becoming grayish then blackish upon spore maturation, with whitish, fimbrariate edge; stipe 0.8–1.5 × 20–50 mm, fragile, fistulose, equal or slightly broadening downward, pure white, densely covered with glistening, short hairs; context insignificant, brittle without peculiar odor or flavor.

Basidiospores 9.2–13.3 × (5)5.5–7 μm, on average 12.1 × 6.5 μm, Q = 1.76–2.18, in frontal view ellipsoid to subcylindrical with obtuse base and often acute or subpapillate apex, in lateral view ellipsoid to subamlygdaloid, not lentiform, with small hilum and a (strongly) eccentric, ca. 1.8–2.1 μm wide germ pore; Basidia four-spored, clavate or elongate with a median constriction, biform, 21–33 × 8–10 μm; Cheilocystidia lageniformia with a tapering neck and capitata apex, sparse, 35–40 × 10–12 μm; pleurocystidia absent; pilocystidia sparse, lageniform with a tapering, rarely cylindrical neck and obtuse, rounded or subcapitate, often definitely capitata apex, 67–88 × 12–22 μm; caulocystidia seldom similar to pilocystidia but generally with a larger rounded base, lageniform with cylindrical or tapering neck and rounded apex 57–113 × 15–27.5 μm; cylindrical to diverticulately veiled elements present on pileus surface. Clamp connections present.


**Habitat and distribution.** Growing solitary on mossy, clay-like soil or leaf litter. Known from Hungary and the Netherlands.

**Remarks.** On account of the diverticulate veil elements on the pileus, this species belongs to the group around *C. hiascens*, which also is supported by the phylogeny. Within this group it is closest to *C. velatoprainatus* both morphologically and phylogenetically. The molecular phylogeny however clearly
FIG. 1. Bayesian 50% majority rule consensus phylogram for 154 specimens of Coprinellus with the Psathyrella candollea clade as outgroup. The partitioned BMCMC analysis was performed on the combined ITS + β-tubulin + nrLSU alignment and the tree and posterior probabilities were computed from 100 000 and 15 000 trees for the MrBayes MCMC and BayesPhylogenies mixture-model MCMC analyses respectively. Bootstrap values were estimated in 1000 replicates.
separates it from the three sequenced collections of C. velatopruinatus (BPP: 1.00 MLBS: 100% MBPP: 1.00). Although C. cinereopallidus resembles C. velatopruinatus in many respects, it differs in having distinctly capitate pileocystidia and lageniform cheilocystidia. The latter character is shared with C. heterothrix, in which the apices of the cheilocystidia are not capitate and both ends of the spores are obtuse. The grayish brown cap of C. cinereopallidus, which has no or faint ochraceous hues, also distinguishes it from C. velatopruinatus and C. heterothrix, which has a mainly ochraceous cinnamon-brown cap, similarly to most other species of the C. hiascens clade. Another species in this group is C. minutisporus, distinguished by its small, 7–8.5 μm long, basidiospores, a central germ pore and diminutive basidiocarps.

Coprinellus deminutus (Enderle) L. Nagy, Házi, Vágvölgyi and Papp, comb nov.

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**Coprinellus fuscocystidiatus** L. Nagy, Házi, Papp & Vágvölgyi, sp. nov.  
*Fig. 2, 4*

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Pileus 5–12 × 4–7 mm in juvanten, ellipsoideus vel plus minuscule cylindraceus, deinde convexus vel plano-convexus in maturitate, usque ad 25 mm latus, valde deliquescent; in juventute pelluciude striatus et radioriter canaliculatus usque ad centrum, deinde radiatim sulcatus, sine manifes- 
ings veli relicuis, minuitissime, vix manifeste, omnino prunusus; ochraceus vel pallide brunneorubebuleus si usus, griseobrunneus vel pallide ochraceus si sicus, centro obscurior. Lamelleae libereae, confertae, ventricosae, usque ad 2 mm latae, albae in juventute, deinde griseoleae vel nigrescentes, acie minite fimbrirata pallidii. Stipes 1– 
2 mm crassus, 40–90 mm longus, filiformis, cylindricus, fragilis, fistulosus, tenuibus hyalinis pilis dense obtectus

Cheilocystidia (i) globosa, vesiculosa, dense collecta, 15–25 
m diam, et (ii) satis numerosa, lageniformia, collo valde attenuato et apice obtuso, 20–43 × 10–15 
m. Pileocystidia moderate numerosa, lagoeniformia, collo attenuato et apice obtuso, basi pallide castanea sub lente, laevia, haud incrustata, 80–145 × 16–22 
m. Sclerocystidia absentia. Caulocystidia numerosa, lagoeniformia, basi ampla vel cylindricala, collo attenuato, hyalina, basi haud brunneea, 57–109 × 17–28 
m. Velum abest. Fibulae absunt.

Pileus 5–12 × 4–7 mm when young, ellipsoid to +/− cylindrical, expanding to convex/plano-convex when old, up to 25 mm across, strongly deliquescent; surface when young translucently striate and radially grooved up to center, later radially sulcate, without visible veil remnants and with fine, faintly pruinose; ochraceous pale reddish brown when moist, beige to pale ochraceous when dry, darker in the center. Lamelleae free, crowded, ventricose up to 2 mm broad, white when young, then grayish blackish, with finely frimbriate, paler edge. Stipe 1–2 × 40–90 mm, filiform, cylindricale, fragile, fistulosus, densely covered with fine hyaline hairs over its length, whitish, vitreous to pale ochraceous. Context without characteristic odor or flavor, thin, whitish.

Basidiospores 11.7–15 × 6.4–7.5 × 5.8–6.5 

**Remarks.** This species can be recognized by the striking brown base of pileocystidia in combination with both globose and lageniform cheilocystidia. No other species in *Coprinellus* is known to have colored pileocystidia. Both of the collections examined by us possess both types of cheilocystidia, so we included it as a diagnostic character, even though it is not always taxonomically reliable. Macroscopically this species is similar to *C. callinus*, although the pileus is somewhat darker and the neck of its pileocystidia is tapering. Phylogenetically its closest relative is *C. amphithallus*, a two-spored species with similar colors but lacking globose cheilocystidia.

**C. pallidus** L. Nagy, Házi, Papp & Vágvölgyi, sp. nov.  
*Fig. 5*

MycoBank **MB561849**

Pileus 3–6 (7) × 3–4 mm in juventute atque clausus, usque ad 10–20 mm latus si plane expansus, ellipsoideus vel subglobosus, deinde convexus, planulatus in maturitate, leviter deliquescent, pellicula striatus usque ad centrum si usus, radioriter canaliculatus, plicatus in expansis speciminius, omnino tenuissimis micaceis pilis obtestus, sine vello, pallidissime ochraceus vel pallide griseulus, in maturitate paene albidus vel pallide griseolo-ochraceus. Lamelleae libereae, confertae, ventricosae, usque ad 1–1.5 mm latae, in juventute albidae, deinde griseoleae, demum nigrescentes, acie minite fimbrirata albida. Stipes 0.5–1 mm crassus, 30–80 mm longus, albus, fistulosus, fragilis, cylindricus vel inconstipue infatus basim versus, minuitissime omnino albis pilis prunosus, albidus vel pallide ochraceus in maturitate. Caro inconspicua, fragilis, sine odore vel sapore conspicuo.
Fig. 5. *Coprinellus pallidus*. A. Spores. B. Basidia. C. Pileocystidia. D. Caulocystidia. Bars: A = 5 μm, B = 10 μm, C, D = 20 μm.
Sporae 8.9–14.2 × 5.8–7 μm, plerumque 12.6 × 6.6 μm, Q = 1.75–2.03, in fronte ellipsoideae apice fusiformi vel conico vel cylindrico atque basi obtusa, a laterae fusiformes vel cylindricae, haud lentiformes, mediocriter rubrobrunneae, poro germinativo valde excentrico, 2–2.3 × inflato, interdum capitato, 62–93 parva, elata, apice paene eadem latitudine, rotundato-globosa-clavata. Pileocystidia numerosissima, delicata, laticida absunt. Velum abest. Fibulae absunt.

Pileus 3–6(7) × 3–4 mm when young and closed, up to 10–20 mm broad when fully expanded, ellipsoid-subglobose, then convex, flattened when mature, weakly deliquescent, transluently striate up to center when moist, radially grooved, plicate when expanded, the surface is covered with fine glimmering hairs, veil not seen, pale ochraceous to pale grayish, fading to almost whitish, pale grayish ochraceous when mature; lamellae free, crowded, ventricose, up to 1–1.5 mm broad, whitish when young, then grayish, finally blackish, with minutely fimbriate whitish edge; stip 0.5–1 × 30–80 mm, slender, fistulose, fragile, cylindrical or insignificantly broadening toward base, surface covered with fine whitish pruinose hairs, whitish to pale ochraceous to pale grayish, fading to almost whitish, pale grayish when mature. Context insignificant, brittle, with no noticeable odor or flavor.

Basidiospores 8.9–14.2 × 5.8–7 μm, on average 12.6 × 6.6 × 6.5 μm, Q = 1.75–2.03, in frontal view ellipsoid with fusiform, conical or cylindrical apex and obtuse base, in lateral view fusiform to cylindrical, slightly lentiform, medium red-brown, with 2–2.5 μm wide, strongly eccentric germ pore and small a hilum; basidia four-spored, bimorphic, clavate or with median constriction, 23–30 × 8–10 μm; pleurocystidia absent; cheilocystida not seen, probably globose-clavate; pileocystidia abundant, delicate, lageniform with cylindrical, or often sinuous, neck and a small slender base and almost equally broad rounded-enlarged, sometimes capitate apex, 62–93 × 9–18 μm, apex often filled with yellowish refractive material; interspersed with the before-mentioned type, a small number of robust, firmer cystidia (110 × 15 μm) mainly toward the center of the pileus; caulocystidia abundant, lageniform, with cylindrical, mostly sinuous neck and enlarged-clavate apex with delicate rounded tip, 55–88 × 17–29 μm; sclerocystidia absent; veil absent; clamp connections absent.


**Additional specimens examined.** HUNGARY, Szeged, Népliget, in mown lawn, 14 May 2008, L. Nagy, NeSl; Szeged Botanical Garden, among grass on clay-like soil, 9 May 2008, L. Nagy, NL-0625, SZMC.

**Habitat and distribution.** So far known from humid places with mossy, clay-like soil from three localities in Hungary.

**Remarks.** The pale colors and the slender, capitate pileocystidia with frequently sinuous neck are distinctive for this species. Capitate or subcapitate pileocystidia also are found in other species of *Coprinellus*, such as *C. curtus*, *C. plagioioporus*, *C. marculentus*, *C. heterothrix*, *C. velatopruinatus*, *C. subdisseminatus* and *C. cinereopallidus*. Of these *C. curtus* differs in having strongly flattened spores, more vivid colors and a rich coverage of velar spherocysts on the pileus; the fruiting bodies of *C. plagioioporus* and *C. marculentus* are dark brown and pileocystidia are broader. *C. heterothrix* possesses diverticulate veil elements on the pileus surface unlike *C. pallidus*, which can vanish quickly from the maturing fruiting bodies. In such cases the fusiform spores and the narrow, slender pileocystidia are indicative of *C. pallidus*.

**Coprinellus ramosocystidiatius** (Bender) L. Nagy, Házi, Vágvölgyi and Papp, comb nov.

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**Coprinellus sabulicola** L. Nagy, Házi, Papp & Vágvölgyi, sp. nov.

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Pileus primo ellipsoidesus vel etiam conicus, usque ad 3 × 2 mm in expanso tempore, deinde campanulatus cum obtuso centro, ad postremum explanatus vel proprium revolutus, usque ad 15 mm latus, etiam in iuvenili tempore usque ad centrum radialis sulcatus sed periluciditatem non striatus, in senectute fissuratus, non deliquescens. Cuticula opaca, primo levis atque pallide ochraceofusca griseis cum varietatibus, deinde etiam fuscous in cuticula opaca, deinde pallidor flos lactis-ochracea griseis cum varietatibus atque austeriori papilla. Lamellae remotae, ventricose, usque ad 1 mm latae, cum albo nigricantes, cum albidore margine. Stipes 20–45 × 0.5–0.9 mm, filiformis, haud bulbosus, fragilis, dense pruinosis, albovitreus, ad basim pallide ochraceus. Odor atque sapor nulli.

Sporae 8.9–14.2 × 5.8–7 μm, plerumque 12.6 × 6.6 μm, Q = 1.75–2.03, in fronte ellipsoideae apice fusiformi vel conico vel cylindrico atque basi obtusa, a laterae fusiformes vel cylindricae, haud lentiformes, mediocriter rubrobrunneae, poro germinativo valde excentrico, 2–2.3 × inflato, interdum capitato, 62–93 parva, elata, apice paene eadem latitudine, rotundato-globosa-clavata. Pileocystidia numerosissima, delicata, laticida absunt. Velum abest. Fibulae absunt.

Basidiospores 8.9–14.2 × 5.8–7 μm, on average 12.6 × 6.6 × 6.5 μm, Q = 1.75–2.03, in frontal view ellipsoid with fusiform, conical or cylindrical apex and obtuse base, in lateral view fusiform to cylindrical, slightly lentiform, medium red-brown, with 2–2.5 μm wide, strongly eccentric germ pore and small a hilum; basidia four-spored, bimorphic, clavate or with median constriction, 23–30 × 8–10 μm; pleurocystidia absent; cheilocystida not seen, probably globose-clavate; pileocystidia abundant, delicate, lageniform with cylindrical, or often sinuous, neck and a small slender base and almost equally broad rounded-enlarged, sometimes capitate apex, 62–93 × 9–18 μm, apex often filled with yellowish refractive material; interspersed with the before-mentioned type, a small number of robust, firmer cystidia (110 × 15 μm) mainly toward the center of the pileus; caulocystidia abundant, lageniform, with cylindrical, mostly sinuous neck and enlarged-clavate apex with delicate rounded tip, 55–88 × 17–29 μm; sclerocystidia absent; veil absent; clamp connections absent.

Pileus ellipsoid to conical when young, up to 3 × 2 mm when closed, later campanulate with more or less obtuse center, becoming applanate or, more typically concave when mature, not or weakly deliquescent. Margin strongly radially sulcate up to center even when young, becoming strongly fissured when old, in young stages not or slightly translucently striate. Surface mat, smooth when young, veil or setules not visible to the naked eye but somewhat pruinose when very young. In young stages pale ocher-brown with grayish hue to warm reddish brown (like typical young *Parasola misera*), becoming paler ochraceous-beige with grayish hue due to maturing spores, typically with a darker button in center when mature. Rarely the mature pileus is just pale gray to almost whitish (mostly in dry environment). Lamellae free, distant (almost like *P. misera*), ventricose up to 1 mm broad, white when young,
becoming grayish-blackish with age, but the gill edge remains white for a long time, strongly contrasting with the sides of the gills. Stipe 0.5–0.9 × 20–45 mm long, slender, fistulose, fragile, equal without basal bulb, surface densely pruinose from short caulocystidia, whitish, vitreous to pale ochraceous toward base. Context thin, insignificant, odor and flavor indistinct.

Basidiospores 15–21.8 × 10–13 µm, on average 17.3 × 10.9 µm, Q = 1.43–1.74, ellipsoid-broadly ellipsoid to almost ovoid, with strongly obtuse apex and large hilum, not or slightly lentiform, dark blackish reddish brown, opaque, germ pore large, strongly eccentric, hilum, not or slightly lentiform, dark blackish reddish to almost ovoid, with strongly obtuse apex and large 2005, L. Nagy

Brown, opaque, germ pore large, strongly eccentric, hilum, not or slightly lentiform, dark blackish reddish to almost ovoid, with strongly obtuse apex and large

2008, L. Nagy

clavate, not bimorphic, two-spored, ca. 32.5–11.3 µm. Basidia clavate, not bimorphic, two-spored, ca. 32.5–11.3 µm.

Chellocystidia abundant, globose to vesiculose or broadly ellipsoid, typically with short but broad pedicel or no pedicel at all, and then the cystidium ends in a broad flat base, 17–32 × 12.5–27 µm. Pleurocystida absent. Pseudoparaphyses present. Piloseptidae of two types; (i) normal lageniform cystidia with tapering neck and capitate apex, these cystidia are small compared to other species of Setulosi, they have an inconspicuous neck and apex (somewhat similar to that of C. heptemerus), basidial part sometimes inconspicuous, making the cystidium just fusiform, sometimes a more pronounced basidial part is observable, incrusted with crystal not dissolving in NH₄OH in most cases, 22–63 × 8–19 µm; (ii) typically mucronate with an ellipsoid basidial part ending in a short neck with obtuse, noncapitate apex, basidial part sometimes incrusted, 30–33 × 12–14 µm. Veil not seen but several hyphae lying on the pileipellis are incrusted. Caulocystidia abundant, lageniform to fusiform with a tapering neck and generally large capitulum, incrustation scarce, 41–60 × 10–20 µm. Pileipellis cells unusually narrowly clavate but mostly somewhat angular. Hyphae underneath pileipellis layer rather narrow (2.5–4 µm wide), richly diverticulate; veil absent; clamp connections present.


Habitat and distribution. Grows in sand dunes always in the vicinity of smaller open, sandy areas, often found on naked sand but is always emerging from dead sedges, grasses or dung buried in sand (Euphorbia cyparissias, Artemisia absinthium, Stipa arenaria); the type material was growing on bark of Populus nigra exposed on bare sand. Rarely found on pure dung (deer, horse). So far known only from Hungary.

Remarks. This species bears some spectacular features compared with other Coprinellus species; among these are relatively large basidiospores, the lack of a pedicel on the cystidia, a habitat in dry, sandy sites and short, capitate pileocystidia with incrusted base. These characters readily distinguish it from any other member of Coprinellus. Macroscopically C. sabulicola has superficial resemblance to C. curtus or C. deminutus, both of which differ in having much smaller basidiospores, globose veil and four-spored basidia. Furthermore, unlike in other coprinoid species, its basidia are not bis- or trimorphic. Bis- and trimorphic basidia have been found to be linked to the presence of pseudoparaphyses, plicate pileus, increased size of cystidia and autodigestion across the entire Psathyrellaceae, which has been referred to as coprinoidization (Nagy et al. 2011). Being an exception to this rule, three of these traits can be found in C. sabulicola, that is plicate pileus, increased cell size and pseudoparaphyses, whereas the basidia are nonomorphic. Thus we hypothesize that C. sabulicola represents a transitional state between deliquescent and nondeliquescent species, reflecting the characteristics of ancestral members of the genus Coprinellus. In agreement with this assumption it has a basal, albeit somewhat uncertain phylogenetic position, either as a sister group to the eurysporoid clade, or between the Domestici + Micacei clades and the Core Setulosi + eurysporoid clade. Both alternatives received low support in the Bayesian and ML analyses. A third group to the eurysporoid clade, or between the uncertain phylogenetic position, either as a sister
**Coprinellus uljéi** L. Nagy, Házi, Papp & Vágvölgyi, sp. nov.  
Figs. 2, 7

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Pileus 4–10 × 3–7 mm in iuventute, ellipsoides vel cylindricus, unde convexus vel plano-convexus obtuso umbone centrali praeditus, demum planocystidiose vel margine revoluto, valde pellucido striatus unde ad centrum in iuventute, radialiter sulcatus in maturitate, tenuibus micaeis pilis obsectus, brunneo-ochraceus in iuventute, brunneo-ochraceus in maturitate, deliquescentes. Lamellae liberae, conformatae, ventricose, unde ad 1 mm lateae, albcae in iuventute, unde griseoleae, demum nigrescoentes, acie albidae fimbriatae. Stipes 30–90 longus, 0.8–1.5 mm crassus, elatus, fragilis, fistulosus, cylindricus vel leviter inflatus basim versus, unde prinuousus per cunctam longitudinalinem, albidas vel pallide ochraceas. Caro inconspicua, odore et sapore in conspicuo.

Spores 11.3–14 × 6.2–8 μm, plerumque 13.1 × 7.1 μm, Q = 1.75–1.98, ellipsoidae, latiores basim versus, haud lentiformes, poro germinativo valde excentrico, 1.7–1.9 μm lato, praeditae. Basidia tetrasporigera, biformia, clavata vel bimorphic, clavata vel lageniformia, collo attenuato, lato, atque Sclerocystidia sparsa vel sparsissima, moderate crassitunicata, lageniformia-fusiformia, ca. 62–125 μm. Pleurocystidia abundantissima, lageniformia, collo attenuato et apice obtuso, inflato vel clavato, sed haud manifeste capitato, 65–115 × 12–25 μm. Sclerocystidia sparsa vel sparsissima, moderate crassitunicata, lageniformia-fusiformia, circiter 62 × 10 μm. Basidiospores 11.3–14 × 6.2–8 μm, on average 13.1 × 7.1 μm, Q = 1.75–1.98, ellipsoid, somewhat broader toward base, not lentiform, with a strongly eccentric, 1.7–1.9 μm wide germ pore; Basidia four-spored, biformia, clavata vel with median constriction, 18–27 × 8–10 μm; pleurocystidia absent; cheilocystidia predominantly globose, vesiculoso, 20–30 μm diam, interspersed with several lageniform cystidia, 45–65 × 17–20 μm; Pileocystidia abundant, lageniform with a tapering, broad neck and obtuse, enlarged apex; veil absent; clamp connections not seen.

**HOLOTYPE.** SLOVAKIA, Muranska Planina National Park, Studna, on wood chips in Piceetum, 10 Oct 2008, L. Nagy, SZMC-NL-3985 (HOLOTYPE, BP).


**Habitat and distribution.** Growing on soil and wood chips in broadleaf and coniferous forests. Solitary or in large troops. So far known only from warm, dry habitats of Öland (Sweden) and Slovakia.

**Remarks.** The presence of lageniform cheilocystidia in addition to globose ones is somewhat uncertain for two out of the three examined collections. Morphologically this species is close to *C. sclerocystidiosus* with which it shares the obtuse-enlarged apex of the pileocystidia, eccentric germ-pore and spore size but differs in having globose and (optionally) lageniform cheilocystidia, whereas *C. sclerocystidiosus* has globose-ellipsoid cheilocystidia, more abundant sclerocystidia and the apices of its pileocystidia are never enlarged. In addition *C. sclerocystidiosus* is phylogenetically well separated from *C. uljéi*. Another similar species is *C. plagioporus*, which can have pileocystidia with enlarged apex and also possesses similar basidiospores, but has a different cap color (dark brown) and exclusively globose cheilocystidia and lacks sclerocystidia.

**Artificial key to the setulose (hairy) species of *Coprinellus***

1. Pileus with a macroscopically visible, either continuous or abundant granulose veil coverage when young .......................................................... 2
2. Veil on pileus absent or observable only under the microscope in the form of discontinuous sphaerocysts or hyphae ........................................ 11
2a. Basidiospores ovoid with conical base, mitriiform in lateral view often amygdaliform ...... 3
3. Basidiospores ovoid with obtuse base or elliptoid ................................................. 6
3a. Basidiospores ornamented ........................................... 4
4. Basidiospores smooth ........................................... 5
4a. Basidia four-spored ......... C. deliquescentes (= *C. silvaticus*)
5. Basidiospores two-spored .......... C. verrucispermus
5a. Pileus up to 20 mm wide when expanded, fruiting bodies small, fragile .......... C. disseminatus
5b. Pileus 20–50 mm broad when expanded, fruiting bodies robust, habit like *C. impatiens* or *C. micaceus* ........................................... C. xylophilus
6 Pileocystidia capitate and veil cells with up to
5 μm thick, brown walls ................... 7
6 Pileocystidia not capitate or veil cell walls
< 2 μm ................... 8
7 Pileus 4–13 mm diam, veil patches pale ocher, on
dung ..................................  C. curtus
7 Pileus up to 25 mm diam, veil patches blackish, on
soil .................................... C. sp. 7 (SZMC-NL-0159)
8 Apex of pileocystidia obtuse to capitate .... 9
8 Apex of pileocystidia acute ............... 10
9 Pileus bright golden yellow-orange ........
............................................. C. aureogranulatus
9 Pileus dull ................................ C. pyrhanthes
10 Basidiospores 12–16 μm long ........... C. heptemerus
10 Basidiospores up to 7–11 μm long ....
............................................. C. psittulus (= C. heptemerus f. parvisporus)
11 Basidia two-spored ...................... 12
11 Basidia four-spored ..................... 19
12 Basidia monomorphic, basidiospores 15–
21 μm long ................................ C. sabulicola
12 Basidia bi-(tri)morphic, basidiospores smaller
......................................... 13
13 Germ pore central, pileocystidia with a tapering
neck and acute apex, habitat on sandy places 
............................................. Coprinellus sp. 2 (SZMC-NL-1356)
13 Not with this combination of characters .... 14
14 Cheilocystidia globose to ellipsoid ....... 15
14 Cheilocystidia lageniform ............... 17
15 Sclerocystidia present .................. C. sassi
15 Sclerocystidia lacking ................... 16
16 Velar spherocysts present on pileus 
............................................. Coprinellus sp. 4 (SZMC-NL-3843 and 2644)
16 Velar spherocysts lacking ............... C. bisporus and C. bisporiger
17 Sclerocystidia present, av. Q of basidiospores > 2
............................................. C. pseudoamphithallus
17 Sclerocystidia present, spore length/width ratio
smaller .................................. 18
18 Germ pore eccentric, av. Q of basidiospores
1.7–1.8 .................................... C. amphithallus
18 Germ pore central, av. Q of basidiospores 1.3–
1.5 ......................................... C. singularus
19 Diverticulate or hyphal veil elements present on
pileus ..................................... 20
19 Veil, if present composed of sphaerocysts ..... 25
20 Base of basidiospores obconical, germ pore
central, cheilocystidia lageniform .... C. hiascens
20 Base of basidiospores obtuse ........... 21
21 Basidiospores 7–8.5 μm long ............. C. minutisporus
21 Basidiospores longer .................... 22
22 Veil on pileus unbranched, hyphal (like
section Lainati) C. allocaus (doubtful spe-
cies, known only from one collection and one
fruiting body) ................................
22 Veil diverticulate ...................... 23
23 Cheilocystidia globose-ellipsoid .... C. velatopraunatus
23 Cheilocystidia lageniform .............. 24
24 Cheilocystidia with capitate apex ........
............................................. C. cinereopallidus
24 Cheilocystidia with equal or acute apex 
............................................. C. heterothrix
25 Basidiospores hexagonal, nodulose or mitriform 26
25 Basidiospores ellipsoid, ovoid, but not angular .... 29
26 Basidiospores mitriform, fruiting body robust,
pileus 30–70 mm when expanded ........
............................................. C. angulatus
26 Basidiospores hexagonal or nodulose .... 27
27 Basidiospores hexagonal ............... C. marcellus
27 Basidiospores nodulose ................ 28
28 Basidiospores 6–8 μm long, sclerocystidia
lacking .................................... C. doverri
28 Basidiospores 9–11 μm long, sclerocystidia
present .................................... C. mitrinodulisporus
29 Apex of pileocystidia obtuse or capitate, neck
cylindrical or tapering ..................... 30
29 Apex of pileocystidia +/- acute, neck tapering .... 37
30 Sclerocystidia present ................ C. pseudoamphithallus
30 Sclerocystidia absent ................. 31
31 Cheilocystidia globose-ellipsoid . C. sclerocystidius
31 Cheilocystidia globose-ellipsoid plus lageniform .... 32
32 Pileocystidia with enlarged to subcapitate
apex ..................................... C. uljoi
32 Pileocystidia with acute or equal apex ....
............................................. C. subimpatiens
33 Color dark brown, with or without purplish tints or
grayish .................................. 34
33 Color of young fruiting bodies ocher melleous,
yellow-brown or cinnamon brown ....... 36
34 Color of fruiting bodies pale gray, pileocysti-
dia slender, delicate, with capitate apex ..... 36
34 Fruiting bodies dark brown, pileocystidia
stout ..................................... 35
35 Color purplish, apex of pileocystidia equal, or
enlarged, not capitate ................... C. subparvus (C. fallax, a doubtful species
comes close, should differ in a reddish spore print)
35 Color without purplish hues, pileocystidia (sub)-
capitate .................................. C. plagioporus
36 Cheilocystidia lageniform, basidiospores sub-
cylindrical (Q = 1.8–1.9) ................. C. subdisseminatus
36 Cheilocystidia globose, basidiospores elliptical
(Q < 1.8) .................................... C. simulans
37 Cheilocystidia lageniform or globose plus lageni-
form ........................................ 38
37 Cheilocystidia globose-ellipsoid ........ 44
38 Cheilocystidia of two types, globose and
lageniform ................................ 39
38 Cheilocystidia lageniform only .......... 41
39 Base of pileocystidia brown but not incrusted
............................................. C. fuscoscydiiatus
39 Base of pileocystidia not brown ........ 40
40 Pileus bright orange-brown, sclerocystidia ab-
sent ................. C. diletus (based on the careful
redescription of Schaefer) (see also C. pelliculus, which
occasionally can have lageniform cheilocystidia too)
40 Pileus dull, sclerocystidia present ........ C. uljoi
41 Pileus dull, sclerocystidia present 
............................................. Coprinellus sp. 9 (SZMC-NL-1070)
Pileus up to 2–3 mm when expanded, habitat on dung ........................................ C. parvulus
Pileus larger, noncoprophilous taxa ........................................ 42
Basidiospores 11–13 μm long, sclerocystidia present ............... 46
Basidiospores > 10 μm, sclerocystidia absent ....................... 43
Sclerocystidia absent ....................................................... 59
Sclerocystidia present .................................................... C. callinus

41 Fruiting body nondeliquescent, germ pore central ................. C. impatiens
43 Fruiting body deliquescent, germ pore eccentric ................ C. heterosetulosus
44 Pleurocystidia present ............................................. 45
44 Pleurocystidia absent ............................................... 49
45 Noncoprophilous species ............................................ C. canistris
45 Coprophilous taxa .................................................... 46
46 Basidiospores 11–13 μm long; pileocystidia up to 70 μm long .... 47
46 Basidiospores larger (> 12 μm); pileocystidia longer ........... 48
47 Caulocystidia unbranched .......................................... C. breviseptulosus
47 Caulocystidia branched .............................................. C. pellucidus

53 Germ pore eccentric, on dung ...................................... C. heterosetulosus
53 Germ pore central, on wood chips ................................ C. pellucidus

54 Basidiospores ovoid to subglobose, Q = 1.1–1.3 .................. 55
55 Basidiospores ellipsoid to somewhat mitriform .................. 56
56 Cheese cystidia lageniform ........................................ C. eurysporus
56 Cheese cystidia globose ............................................ C. aff. eurysporus (SZMC-NL-1761, 3418, Arnolds 4845, Ulj 1191, Hoijer 95067)

60 Average spore length < 10 μm ................................... 57
60 Average spore length > 10 μm .................................... 59
61 Basidiospores submitriform, strongly lentiform 7–9 μm long, on dead grasses .................. C. disseminatus
62 Basidiospores ellipsoid, not lentiform, on dung ............... 58
63 Basidiospores 8–11 μm long, stipe often radicate in the substrate ................... C. radicellus
64 Basidiospores 6–9 μm long, stipe not radicate ................ C. pellucidus

59 Sclerocystidia absent ................................................ C. limicola + Coprinellus sp. 1 (SZMC-NL-0195 and 1751) (the two species seem morphologically identical but are phylogenetically separated)

58 Cheese cystidia absent ............................................. C. eurysporus

DISCUSSION

In this study we analyzed the phylogeny and diversity of the genus Coprinellus, one of the most species-rich groups in the Psathyrellaceae. The phylogeny inferred here, with an almost complete species-level dataset (71 out of ca. 80 taxa sampled), does not provide evidence for the monophyly of morphologically based sections of previous classifications (Uljé 2005, Orton and Watling 1979, Kühner and Romagnesi 1953, Patrick 1977, Schafer 2010, Keirle et al. 2005, Singer 1986) or for the monophyly of species with pileo- and caulocystidia. Below we attempt to synthesize morphological synapomorphies to explain the phylogenetic distribution of the species.

Higher level relationships within Coprinellus.—Phylogenetic analyses do not support the monophyly of Coprinellus species with pileo- and caulocystidia. These, formerly classified in subsection Setulosi of section Pseudocoprinus (Uljé and Bas 1991, Uljé 2005, Orton and Watling 1979, Kühner 1928), are distributed throughout Coprinellus and are found in all four larger clades of the genus. The relationship of certain setulose species to veiled ones accords with formerly published phylogenies; the affinities of C. disseminatus to veiled Coprinellus species, in particular to C. micaceus, has been noted (Vasutova et al. 2008; Larsson and Örstadius 2008; Walther et al. 2005; Nagy et al. 2011, 2010) and the position of C. curtis and C. heptemerus in the vicinity of C. domesticus and C. radians also has been inferred (Larsson and Örstadius 2008; Nagy et al. 2010, 2011), although satisfying morphological synapomorphies could not be defined in either case. In fact, although the general appearance of C. disseminatus and C. micaceus is different, the presence of a rich, granulose veil and the shape of the basidiospores are indicative of their relationship. In addition to C. disseminatus two other setulose species, C. auropgranulatus and C. pyrrhanthes, belong to this clade, although the latter conclusion is based only on morphological similarity in the shape of basidiospores and pileocystidia as well as veil characters. The mitriform spores further support the placement of C. silvaticus and C. verrucispermus in the Micacei clade, which has been judged plausible by the constraint analyses as well. Thus we propose that mitriform spores can be regarded as a synapomorphy for this clade and can be used in the taxonomic circumscription of section.
Coprinellus. Although the basidiospores of C. truncorum, C. aureogranulatus and two incompletely known taxa, C. pallidissimus and C. saccharinus, are not explicitly mitriform, they do have a tendency to produce (sub)mitriform spores, which is evident when spore populations of mature specimens are inspected.

How morphological traits support the inclusion of C. curtus and C. pusillus (and C. heptemerus) in the Domestici clade is more difficult to establish. These two species share a rich, granulose veil that forms an almost continuous sheath over the pileus on young fruiting bodies and strongly flattened basidiospores with an eccentric germ pore, the latter being unusual for the rest of the clade. The only character that shows an overlap between the setulose and veiled species of this clade is the tendency of terminal veil cells to have thick, often brownish walls with crystalline incrustation. To our best knowledge no other Coprinellus species possess thick-walled, colored and/or incrusted veil cells and thus an almost continuous brownish velar sheath on the young pilei, so these can be considered unique synapomorphic characters for this group. Our results further support the affinity of C. deminutus to the Domestici clade, which is well supported by morphological features. Another tiny species with morphological features of the Domestici clade is C. ramosocystidiatus, a rare or overlooked taxon with granular veil and ellipsoid-subglobose pileipellis cells, which bear finger-like projections. Because both C. deminutus and C. ramosocystidiatus fit well into the Domestici clade we propose new combinations for them in Coprinellus (see below).

Two clades consist entirely of setulose species. Of the four major clades of Coprinellus the Micacei and Domestici clades are easy to separate from the Core Setulosi and eurysporoid clades on the basis of a rich veil coverage on the pileus. The Core Setulosi plus the eurysporoid clades together compose about 90% of the species classified in subsection Setulosi (Uljé and Bas 2003, Uljé 2005, Schafer 2010, Keirle et al. 2005, Orton and Watling 1979). To our best knowledge subsection Setulosi has not been subdivided further, which reflects the morphological uniformity of this group. The key characters that have been used for guidance in identification keys are among others the number of sterigmata on the basidia, the shape of the pileocystidia (tapering/cylindrical), the shape of the basidiospores, the presence or absence and shape of pleuro- and cheilocystidia, however none of the characters show a correlation with the phylogenetic structure of these clades. Thus a morphology-based distinction between the Core Setulosi and the eurysporoid clades is not straightforward. The latter contains robust, soil-inhabiting species without veil, although these characters do not separate it unambiguously from the Core Setulosi clade. Further study is necessary to establish reliable characters that can be used in identification keys.

The core setulosi clade.—The backbone of the Core Setulosi clade has proved difficult to resolve, probably because of an episode of accelerated speciation in the evolution of this group (Nagy et al. unpubl). Consistent with this the most significant incongruence between the ML and the two Bayesian analyses involved the branching order at the backbone of the Core Setulosi clade. Although the majority of nodes could not be resolved, several strongly supported and/or morphologically homogeneous clades were recovered in the phylogenetic analyses. These, hereafter named C. bisporus, C. callinus, C. hiascens and C. pellucidus clades, can be unambiguously circumscribed morphologically, as presented below.

C. bisporus clade. Although this clade received low support values (BPP: 0.51, MLBS: –, MBPP: 0.53), it is worth mentioning because all taxa are characterized by two-spored basidia except for C. canistri, for which the inclusion of more materials might reveal a more plausible position in other parts of the tree. Four morphologically established species, C. canistri, C. sp. 11, C. bisporus as well as C. hisporiger.

C. callinus clade. On the basis of morphology four species can be discerned in this group. However only three of these could be inferred as monophyletic. Two collections (SZMC-NL-0195 and 1751) differ from C. callinus in having somewhat smaller basidiospores (perhaps only statistically), central or hardly eccentric germ pore and the lack of sclerocystidia. However SZMC-NL-3101 is completely different, growing on dung, up to 2–3 mm diam, with sclerocystidia and navicular spores. Coprinellus sp. 2 is characterized by two-spored basidia, Parasola-like appearance and 8–10 µm long ellipsoid spores, which are obtuse at both ends. Formal description of the mentioned taxa will require more specimens.

C. hiascens clade. C. hiascens is delimited here in a broad sense, including C. heterothrix, which implies a considerable morphological plasticity for C. hiascens. Traditional characters used to delimit these two species (shape of the spore base, angle of germ pore and the shape of the pileocystidia; Orton and Watling 1979, Uljé 2005) have considerable overlap between collections and no correlation with clade structure can be recognized. In addition to C. hiascens the phylogeny provides evidence for two species, C. velatopraimitatus and C. cinereopallidus, with diverticulate veil elements on the pileus. Although the phylogeny clearly separates the latter two taxa (BPP: 1.0, MLBS: 100%, MBPP: 1.0), morphologically they are difficult
to separate. Both species differ from *C. hiascens* in having globose-ellipsoid cheilocystidia, whereas the cheilocystidia of *C. hiascens* are lageniform.

*Coprinus* ‘crassitunicatus’, a species informally described by van de Bogart (1975) also is nested in this clade. For this species a scattered universal veil of globose, thick-walled elements has been reported. Despite this type of veil, the phylogeny suggests a close relationship to or conspecificity with *C. hiascens*.

*C. pellucidus* clade. *C. brevisetulosus*, *C. radicellus* and *C. pellucidus* form a well supported monophyletic group (BPP: 1.0, MLBS: 68%, MBPP: 0.97), characterized by 7–11 μm long ellipsoid basidiospores with a central germ pore, four-spored basidia and short, up to ca. 90 μm long pileocystidia, and a habitat on dung. The cheilocystidia are globose, except for *C. pellucidus*, in which lageniform cheilocystidia intermixed with globose ones have been encountered frequently. The identity of such collections has been questioned and the possibility of being separate taxa have been raised, however our results confirm that these collections belong to *C. pellucidus*.

**Eurysporoid clade.**—Species limits within this clade require more investigation. Several hitherto poorly defined species belong to this clade coupled with the lowest mean path length between specimens across the entire phylogeny, resulting in poor resolution of species limits and polyphyly of even well established species such as *C. angulatus* even when the clade is analyzed separately (results not shown). The high number of incongruent nodes inferred in the mixture-model and traditional Bayesian as well as the ML analyses also can be considered a symptom of the weak phylogenetic signal within this clade. Out of the species included *C. sclerocephalidiosus* and *C. subnompatis* were inferred as monophyletic with strong support (BPP/MLBS/MBPP 1.00/76/0.67 and 1.00/100/1.00 respectively). A weakly supported clade of specimens labeled *C. aff. eurousporus* could be discerned (BPP: 0.87 MLBS: − MBPP: 0.88), which on the other hand does not include the holotype specimen of *C. eurousporus*. However for the time being we refrain from splitting these specimens into two species because it would trigger a cascade of taxon splits, given the present phylogeny.

**Velar spherocysts as a phylogenetically informative character.**—The structure, color and existence of various veil structures have long been considered among the most important characters in coprinoid fungi for delimiting species or higher taxonomic groups. For instance taxa with globose veil elements have been classified in section Veliformes (Orton and Watling 1979, Ulić 2005, Keirle et al. 2005, Schafer 2010, Horak 2005), which proved polyphyletic (Hopple and Vilgalys 1999; Nagy et al. 2010, 2011; Keirle et al. 2005; Walther et al. 2005), included many species now placed in Coprinopsis (subsections Nivei and Narcotici p.p.). Of section Veliformes subsections Micacei and Domestici belong to Coprinellus and comprise most species in this genus with a rich granulose veil coverage on the pileus. However a number of representatives of the Core Setulosi clade (e.g. *C. doverii*, *C. mitrnodulisporus*, *C. plagioforpus*, *C. marculentus Coprinellus* sp. 4) possess globose veil elements, which renders this type of veil homoplastic. In other words the phylogenetic explanatory power of granulose veil is low not only in the Psathyrellaceae but also in *Coprinellus*. Although literature descriptions treat all granular-globose veil cells as one and the same character, their manifestation in the Core Setulosi and Micacei/Domestici clades represents two morphological types. Those found in the Core Setulosi clade are mostly smooth, hyaline, thin-walled and scarce, often difficult to notice under the microscope and never visible macroscopically or with hand lenses, whereas those in the Micacei/Domestici clades are more rich and macroscopically observable, usually incrusted or have thickened wall. Therefore we propose that these two types of veil can be used as phylogenetically informative characters for defining species groups when distinguished this way. On the other hand understanding the evolution of velar structures is an interesting future prospect.

**Shall we apply a narrow species concept in Coprinellus?**—The phylogeny inferred here provides evidence for the monophyly of most morphologically established species. During the years of research on *Coprinus* s.l. taxa a practice of using a narrow morphological species concept has become fixed among mycologists. For instance mating experiments confirmed the existence of both *C. congestatus* and *C. ephemerus*, two extremely closely related species differing only in the absence or presence of clamp connections, which are difficult to observe, especially when clamp connections are rare and cannot be found on each of the septa, and a hardly detectable difference in spore sizes (Lange 1952, Ulić 2005). Multi-specimen sampling in our dataset is suitable for testing this narrow species concept in a phylogenetic framework. We intentionally sampled many specimens that morphologically fall close to certain taxa in the Core Setulosi clade but showed one or more deviating characters (Table I). Of these specimens a smaller portion proved to be enigmatic lineages while those clustered in already described taxa contribute valuable information on the taxonomic value of morphological characters. The species affiliations (Table I) imply that the least reliable character is
the occurrence of lageniform cheilocystidia in species with predominantly globose-ellipsoid cystidia. Although the occurrence of both types of cystidia is known to be diagnostic for species such as *C. subimpatiens*, *C. dilectus*, *C. fuscocystidiatus* as well as *Coprinellus* sp. 9 (SZMC-NL-1070) (Ortega and Esteve-Raventos 2003, Uljé 2005, Uljé and Bas 1991, Schafer 2011, Lange and Smith 1953), our results suggest that they can occur occasionally in *C. dilectus*, *C. congreqatus* and *C. uljéi* as well. Similarly spore size and shape are usually considered taxonomically reliable characters with little amplitude; our phylogeny suggests that spore size and shape can be highly variable in *C. congreqatus* and the *C. hiascens* group respectively. Taken together molecular data do not support the taxonomic recognition of specimens that were easily distinguished morphologically, suggesting that the narrow morphological species concept proposed for setulose *Coprinellus* taxa is not universal, with at least the above-mentioned characters forming exceptions. Whether other characters show the same plasticity is an open question, however a phylogenetic approach for proving the autonomy of proposed new species is advisable, even in cases the candidate morphological difference seems constant across collections.

**Table I. Morphological amplitude of certain species of the Core Setulosi clade of Coprinellus**

<table>
<thead>
<tr>
<th>Voucher number*</th>
<th>Species affiliation</th>
<th>Morphological difference to typical specimens of that species</th>
</tr>
</thead>
<tbody>
<tr>
<td>SZMC-NL-0764</td>
<td><em>C. congreqatus</em></td>
<td>Spore up to 16 × 8.5 μm</td>
</tr>
<tr>
<td>SZMC-NL-1221</td>
<td><em>C. congreqatus</em></td>
<td>Spore up to 15 × 5 μm, Pileus consistently acute, like <em>C. sassi</em></td>
</tr>
<tr>
<td>SZMC-NL-1443</td>
<td><em>C. congreqatus</em></td>
<td>Lageniform cheilocystidia between globose-ellipsoid ones</td>
</tr>
<tr>
<td>SZMC-NL-1449</td>
<td><em>C. congreqatus</em></td>
<td>Lageniform cheilocystidia between globose-ellipsoid ones, spore up to 17 × 9.2 μm</td>
</tr>
<tr>
<td>SZMC-NL-3064</td>
<td><em>C. congreqatus</em></td>
<td>Base of pileocystidia with thickened walls, cheilocystidia globose (not ellipsoid) with some lageniform interspersed, pileus vivid orange-yellow</td>
</tr>
<tr>
<td>SZMC-NL-1128</td>
<td><em>C. congreqatus</em></td>
<td>Base of pileocystidia with slightly thickened walls, cheilocystidia globose (not ellipsoid) with some lageniform interspersed, pileus vivid orange-yellow</td>
</tr>
<tr>
<td>SZMC-NL-0636</td>
<td><em>C. congreqatus</em>?</td>
<td>Pileus up to 4–8 mm wide when fully expanded, cheilocystidia predominantly utriform, basidiospores 9.5–12(12.4) × 5.4–6.6 μm</td>
</tr>
<tr>
<td>SZMC-NL-0628</td>
<td><em>C. hiascens</em></td>
<td>Pileocystidia with cylindrical neck and +/- obtuse apex, basidiospores with a central germ pore and obtuse base</td>
</tr>
<tr>
<td>SZMC-NL-1329</td>
<td><em>C. hiascens</em></td>
<td>Pileocystidia with cylindrical neck and +/- obtuse apex, basidiospores with a central germ pore and obtuse base</td>
</tr>
<tr>
<td>SZMC-NL-1349</td>
<td><em>C. hiascens</em></td>
<td>Pileocystidia with cylindrical neck and +/- obtuse apex, basidiospores with a central germ pore and obtuse base</td>
</tr>
<tr>
<td>SZMC-NL-3856</td>
<td><em>C. bisporus</em></td>
<td>Cheilocystidia globose (not ellipsoid), basidiospores fusiform, with profoundly conical base</td>
</tr>
<tr>
<td>SZMC-NL-1233</td>
<td><em>C. heterosetulosus</em></td>
<td>Sclerocystidia absent, basidiospores not lentiform</td>
</tr>
<tr>
<td>SZMC-NL-2928</td>
<td><em>C. pellucidus</em></td>
<td>Scarce velar sphaerocysts present on pileus</td>
</tr>
<tr>
<td>SZMC-NL-1446</td>
<td><em>C. pellucidus</em></td>
<td>Basidiospores dark brown, with distinctly thickened wall, lageniform cheilocystidia present but scarce</td>
</tr>
</tbody>
</table>

*Deviating specimens qualifying to species or varieties under the narrow morphological species concept applied in subsection *Setulosi*, their species affiliation based on the phylogenetic analyses and the morphological differences as compared to typical appearance of the species they belong to. For the phylogenetic placement of these specimens, see Fig. 1.

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**Literature cited**


NAGY ET AL.: COPRINELLUS PHYLOGENY 273


