

Phylogenetic analyses of *Coprinopsis* sections *Lanatuli* and *Atramentarii* identify multiple species within morphologically defined taxa

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Abstract: Sections *Lanatuli* and *Atramentarii* of the genus *Coprinopsis* contain some of the best known and most important agaric species, including *C. cinerea* and *C. lagopus*, yet a critical, phylogeny-based assessment of the species limits has not been carried out. Taxa have been characterized chiefly on the basis of morphological characters, which however show little discriminatory power and/or considerable overlap between several species pairs. We used ITS and LSU sequence data of 29 described taxa in *Coprinopsis* sections *Lanatuli* and *Atramentarii* to infer species limits and the correspondence between morphological characters and species lineages, as well as to examine the phylogenetic affinities of sections *Lanatuli* and *Atramentarii*. Our analyses recovered three large clades, implying a paraphyly for section *Lanatuli*. Based on morphology and clade structure, we estimate ca. 38 species in the two sections, including several potentially new taxa, three of which are described herein. *Coprinopsis pachyderma*, *C. lagopus* var. *vacillans*, *C. acuminata*, *C. spelaiophila*, *Coprinus citrinovelatus* and *Cop. brunneistrangulatus* were found to be synonymous with other, earlier described species. Congruent with previous mating studies, our analyses recovered multiple, morpholog-

ically indistinguishable lineages within *C. lagopus*, which included *C. lagopus* var. *vacillans*, an ephemeral, developmental variant. Morphological traits supporting the inferred clade structure are discussed. Three new taxa (*C. fusispora*, *C. babosiae*, *C. villosa*), and one new combination (*C. mitraespora*) are proposed.

Key words: *Coprinopsis cinerea*, *C. lagopus*, *Coprinus* sensu lato, phylogeny, taxonomy

INTRODUCTION

Taxonomic research on species of *Coprinus* s.l. has played a pivotal role in forming our views on species concepts, ontogeny, as well as other aspects of the biology of fungi (Lange 1952; Kemp 1974, 1975, 1980, 1985a, b; Kües 2000). Having been subjected to tests of the biological species concept, section *Lanatuli* became the primary source of information about the mating system of Agaricomycetes and processes of speciation and reproductive isolation. As a forerunner of recent phylogenetic evidence for cryptic speciation and reproductive isolation without discernable morphological divergence in mushrooms (Kausserud et al. 2006, 2007; Matute et al. 2006, Le Gac et al. 2007, Sato et al. 2007, Crespo and Lumbsch 2011), mating tests recovered significant conflict between morphologically defined species and incompatibility groups, or biological species (Kemp 1975, Flynn 1990, Vilgalys 1991, Rowe 2011, Matute et al. 2006, Le Gac et al. 2007, Crespo and Lumbsch 2011). Recent phylogenetic studies have suggested the widespread occurrence of cryptic species in fungi (Bidochka et al. 2005, Alamouti et al. 2011, Chen et al. 2011), which in part could explain the discrepancy observed between the number of morphologically defined species and estimates of species diversity based on next generation sequencing technology (Hibbett et al. 2009, Nagy et al. 2011).

The taxonomy of section *Lanatuli* has been determined predominantly by morphological and ecological characters (Orton and Watling 1979, Uljé and Noordeloos 1999, Uljé et al. 2000, Uljé 2005). Macroscopic characters are largely uninformative for species identification, with the exception of the size of basidiomes, which can be used to delimit certain species. Of the micromorphological features, spore shape and size have the greatest diversity in section *Lanatuli*. Additionally, the number of sterigmata and

characteristics of the veil have been used to define species. Based on these traits, approx. three species of *Atramentarii* and 19 of *Lanatuli* (including one variety) were recognized in a recent monographic treatment of European species (Uljé 2005). A number of additional species have been described from the tropics, which fit either section *Lanatuli* or *Atramentarii*, including *C. brunneofibrillosus* (Dennis 1961), *C. macropus*, *C. africanus* and *C. fibrillosus* (Berkeley and Broome 1871, Pegler 1966). *Coprinus neolagopus* was described from Japan, while van de Bogart (1975, 1979) published several provisionally named species from North America. *Coprinopsis nevillei* and *C. annulopora* were described from dead herbaceous stems, from France and Germany respectively (Enderle 2004, Garcia and Vellinga 2010).

In addition to serving as a model for studies of a biological species concept, various taxa of section *Lanatuli* are model organisms in developmental biology, fungal cell biology or enzyme production (Kües 2000) and have been repeatedly isolated from human fungal mycoses (as the anamorph, *Hormoglyphiella aspergillata*, e.g. Lagrou et al. 2005). A complete genome sequence was published for *C. cinerea* (Stajich et al. 2010). In light of the popularity of *Lanatuli* species as experimental model organisms and the cumulative knowledge of their biology, a modern, phylogeny-based revision of the species in this group is highly desirable.

In this study we set out to test the monophyly of sections *Lanatuli* and *Atramentarii* and examine the species relationships within them. We infer trees from a combined alignment of sequences of two loci (ITS and LSU) and compare the phylogenetic patterns to established morphology-based classification to help delimit species in these groups.

MATERIALS AND METHODS

Taxon sampling.—The following strategy was used to design taxon sampling for this study: First, we attempted to collect specimens for all described species within sections *Atramentarii* and *Lanatuli* (FIG. 1), which were supplemented with materials selected by morphological examinations of specimens preserved in two of the largest collections of coprinoid fungi, L and SZMC (Szeged Microbiological Collections, each totaling > 1500 and > 2000 specimens respectively). Specimens with unusual characteristics and/or morphologically deviating characters were selected for inclusion in the phylogenetic analyses with the goals of recovering all potentially undescribed lineages and improving our understanding of the morphological plasticity of known species. We attempted to sample at least three specimens per species when possible. Basidiomes of *C. lagopus*, *C. fissolanatus*, *C. cinerea* and *C. radiata*, preserved from mating studies (Kemp 1975), also were involved in the

phylogenetic analyses. Furthermore, the LSU sequence from the recently published genome of *C. cinerea* (Stajich et al. 2010) has been extracted and included in the alignments, because most developmental studies utilize the sequenced strain Okoyama 7_130. An ITS sequence of *C. lagopus* (HM126487) was obtained from GenBank; additional GenBank sequences from this group were disregarded because of the unavailability of voucher specimens and the possibility of misidentifications. In total, 121 specimens were collected and analyzed. *Coprinus cortinatus* was used as outgroup. The abbreviations *C.* and *Cop.* are used for *Coprinopsis* and *Coprinus* respectively throughout the manuscript.

Morphological examinations.—Notes on macromorphology were made from freshly collected specimens. Dried herbarium specimens were revived in 5% KOH for light microscopy. Measurements were made with a calibrated ocular micrometer. Spores were measured at 1000×, whereas other details were measured at 400× magnification. At least 20 measurements were made on each cell type on each specimen. The numbers after the word “Basidiospores” refers to the number of spores measured and the number of basidiomes and collections they are originating from respectively. Terminology of shapes follows Vellinga (1988).

Laboratory protocols.—Genomic DNA was extracted from 1–10 mg dried basidiome, ground to a fine powder with micropestles, with the DNeasy Plant Mini Kit (QIAGEN) following the manufacturer’s instructions. We targeted the ITS1-5.8S-ITS2 locus and an approximately 1.5 kb portion of the large ribosomal subunit RNA gene (nrLSU) of the nuclear ribosomal gene cluster. PCR amplification and sequencing followed standard protocols and was performed with the primer combinations ITS1-ITS4 and LROR-LR7 (White et al. 1990). Sequencing was performed commercially by LGC Genomics (Berlin). Individual readings were assembled to contigs with the Pregap and Gap4 programs of the Staden package (Staden et al. 2000).

Alignments and phylogenetic analyses.—LSU sequences were aligned by Clustal W (Thompson et al. 2002), while the large number of indels in the ITS region necessitated a more sophisticated alignment strategy. First, we ran PRANK (Löytynoja et al. 2008), a probabilistic algorithm with the ability to distinguish insertions and deletions with default parameters and two replicates to build an initial alignment. This alignment, plus the LSU alignment was used to compute a maximum likelihood tree (see below), which was used as a guide tree in the second round of PRANK alignment, with the +F option specified. This resulted in many fewer incorrectly aligned residues (based on visual inspection) and more plausible placement of indels, as compared to Clustal W and MAFFT. Non-overlapping start and end positions of the alignments were trimmed. Unambiguously aligned indels were recoded as binary characters with the simple indel coding method, which treats multiple contiguous gapped sites as one evolutionary event, as implemented in FastGap 1.0 (Borchsenius 2007).

The combinability of ITS and LSU alignments was assessed by a comparison of single-gene ML bootstrap trees

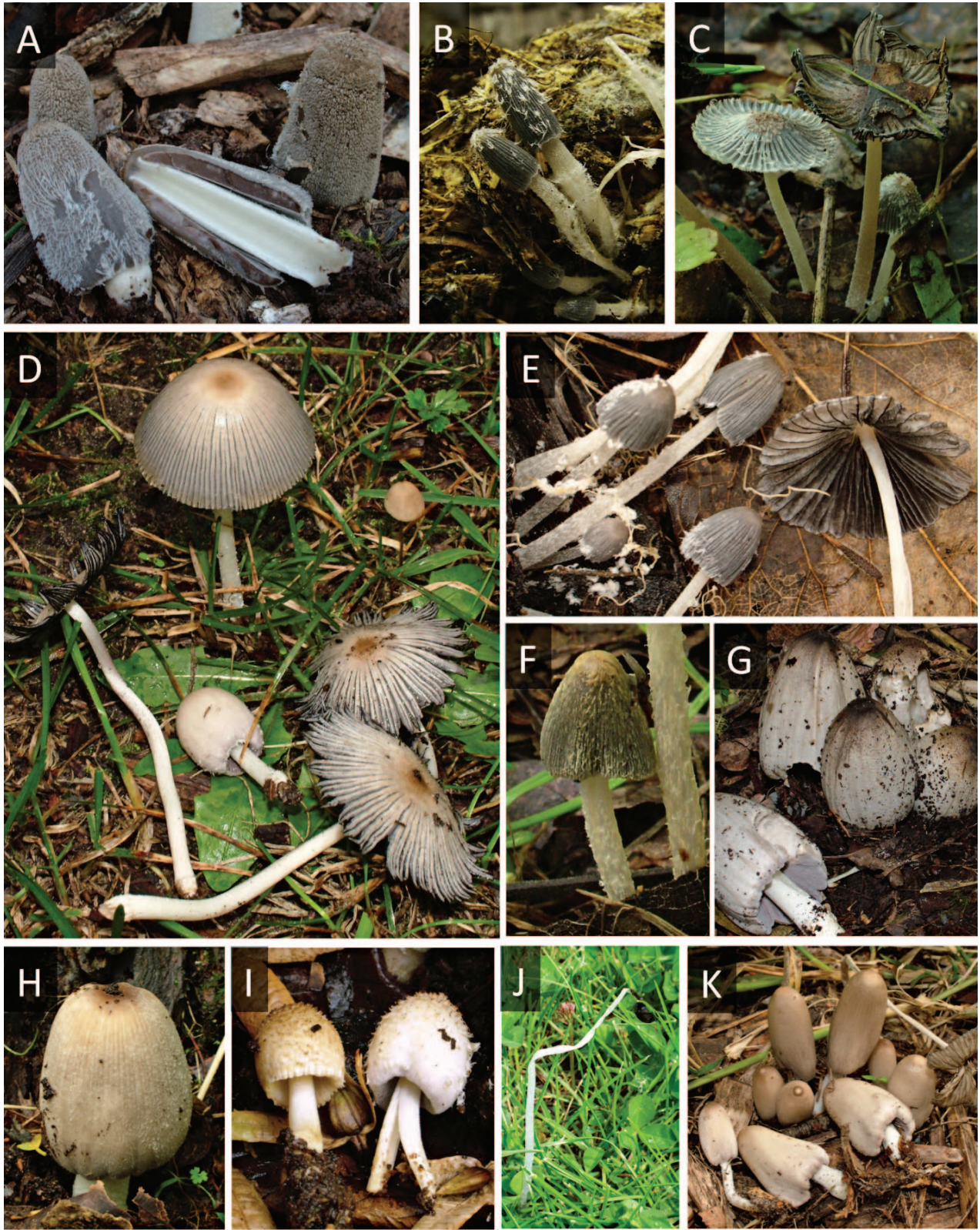


FIG. 1. Representatives of sections *Lanatuli* (A, B, C, E, J) and *Atramentarii* (D, F, G, H, K). A. *Coprinopsis lagopus*. B. *C. radiate*. C, E. *C. babosiae*. D. *C. fusispora*. F. *C. ochraceolanata*. G. *C. atramentaria*. H. *C. insignis*. I. *C. mitraespora*. J. *C. lagopus* var. *vacillans*. K. *C. krieglsteineri*.

inferred by RaxML (Stamatakis 2006) with 100 bootstrap replicates (see below). Mutually exclusive, strongly supported ($\geq 70\%$) clades were considered significant conflicts. Phylogenetic trees were inferred by maximum likelihood and Bayesian MCMC techniques in RaxML 7.0.2 (Stamatakis 2006), MrBayes 3.1.2 (Altekar et al. 2004) and BayesPhylogenies 1.0 (Pagel et al. 2004). Substitution models were selected for both analyses by the AIC_c criterion in jModeltest (Posada 2008), with invariant sites excluded (+I option), which accounts for the same phenomenon as the gamma-distributed rate heterogeneity, and parameter identifiability problems have been reported when both were estimated simultaneously (Rannala 2002, Stamatakis 2006).

Maximum likelihood trees were inferred in 1000 replicates with default settings and four partitions, corresponding to the ITS1, 5.8S, ITS2 and nrLSU fragments. Branch support was estimated by 1000 thorough bootstrap replicates in RaxML. The bootstrap trees were summarized as a consensus tree by the SumTrees script of the Dendropy package (Sukumaran and Holder 2010). Clades receiving a bootstrap value $\geq 70\%$ were considered significantly supported.

Partitioned Bayesian MCMC analyses were performed in two Bayesian packages, MrBayes 3.1.2 and BayesPhylogenies 1.0. These differ in their branch length priors, the structure of the evolutionary models implemented and in the MCMC proposal mechanism. BayesPhylogenies uses mixture models, which consist of n GTR+G models (with n different sets of parameters). At each step of the MCMC analysis, one of the n GTR+G matrices is fit to an alignment column (by reversible-jump MCMC), which when run long enough assigns the best-fit GTR+G matrix to each site of the alignment and offers a way to handle a priori unknown heterogeneities in the evolutionary processes generating the sequences. In setting up the mixture model for BayesPhylogenies, we specified three GTR+G matrices for each partition during the analyses of the two-gene dataset, which causes the program to fit three model matrices to each site in the alignment and finding the one that fits better, as opposed to the traditional way of using one GTR+G matrix only.

Both analyses were run 10 000 000 generations, with four incrementally heated chains and two replicates (eight chains in total) in the case of MrBayes and one chain in three replicates in BayesPhylogenies. The sampling frequency was set to every 1000th generation. Topological convergence was assessed by the CUMULATIVE and COMPARE functions of AWTY (Wilgenbusch et al. 2004). The burn-in value was set to include the widest possible sample after topological convergence. The abbreviations BPP_m, BPP_b, and MLBS stand for Bayesian posterior probability inferred by MrBayes, that by BayesPhylogenies and maximum likelihood bootstrap respectively.

RESULTS

Taxon sampling.—The phylogenetic analyses included 121 specimens of 29 described species (SUPPLEMENTARY TABLE I). Type materials of *C. candidolanata*, *C.*

calospora, *C. bicornis*, *C. geesterani*, *C. krieglsteineri*, *C. pachyderma*, *Cop. brunneistrangulatus*, *C. scobicola* and *Cop. citrinovelatus* were included. Both ITS and LSU genes were targeted in all specimens, however some ITS amplicons were mixed populations of either divergent copies of the same species or contaminating ascomycetes for which sequencing failed. An attempt was made to amplify the two loci but without successful DNA extraction or PCR amplification from the holotype materials of *Cop. brunneofibrillosus*, *C. ochraceolanata*, *C. spelaiophila*, *Cop. africanus*, *Cop. macropus* and *Cop. fibrillosus*. Altogether 173 new sequences were generated for this study (SUPPLEMENTARY TABLE I).

Sequencing of the type material of *Cop. brunneofibrillosus* failed, but two other specimens of this species were sequenced successfully. One of these grouped with the outgroup, which was supported by closest BLAST hits (97% similarity) in section *Alachuani* of *Coprinopsis*, such as *C. episcopalis* and *C. friesii*. The other specimen was nested in *C. cinerea*, which is supported by spore shape and size, with the only notable difference being the dark veil.

Alignments, congruence tests and phylogenetic analyses.—Separate alignments for the ITS (904 sites) and LSU (1329 sites) loci were assembled and analyzed both separately and combined. Unambiguously aligned indels of the ITS region were coded as a separate partition of binary characters, resulting in 184 characters. Single-gene ML analyses did not recover significant conflict between the ITS and LSU loci, so in all subsequent analyses we combined the two genes and the indel-coded characters in a supermatrix, resulting in 2417 characters in total.

We assessed branch support under two Bayesian models and maximum likelihood bootstrapping. Bayesian MCMC analyses converged quickly to the stationary distributions as judged from the convergence of likelihood values and tree split posterior probabilities. We established the burn-in value as 7 000 000 generations, pooled the remaining 3 000 trees from the two independent runs and computed a 50% Majority rule consensus tree in MrBayes or SumTrees (FIG. 2). The influence of recoded indel data on the results was examined by running analyses with and without the indel matrix. Consensus tree topologies inferred under the two methods differed only in the resolution of nodes around *C. ochraceolanata* and *C. sp. 5*, with these being resolved when gaps were excluded but collapsed to polytomy when included. Further, the exclusion of indel characters affected posterior probabilities to a minor extent (data not shown).

The phylogenetic analyses recovered three major clades (FIG. 2): one comprising *C. annulopora*, *C.*

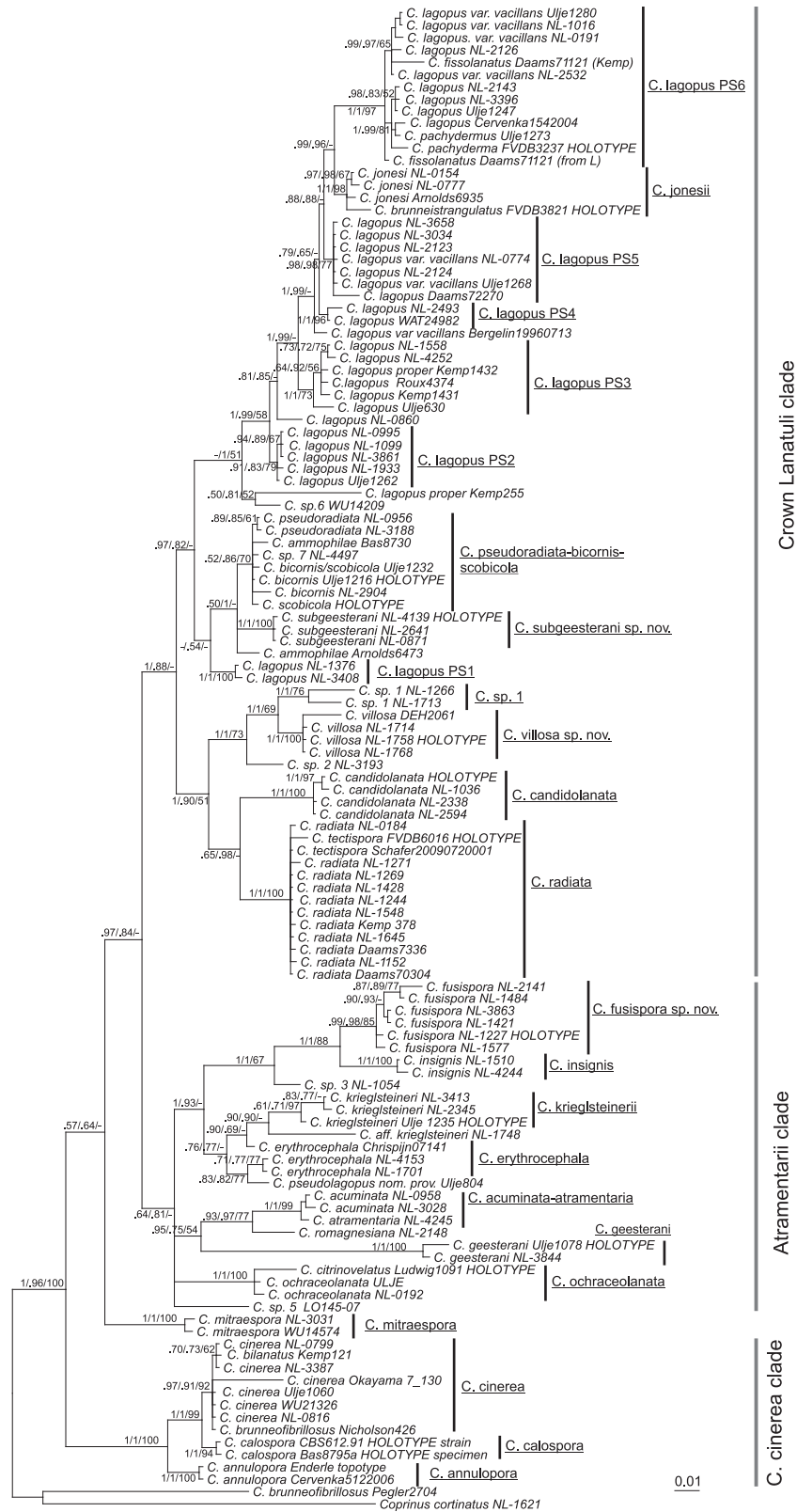


FIG. 2. Fifty percent majority rule consensus phylogram inferred from the ITS + LSU dataset with Bayesian MCMC (BayesPhylogenies). Numbers above branches represent Bayesian posterior probabilities from BayesPhylogenies MrBayes and ML bootstrap values respectively. Only support values greater than 0.5 or 50% are shown. Bar indicates 0.01 expected substitutions per site.

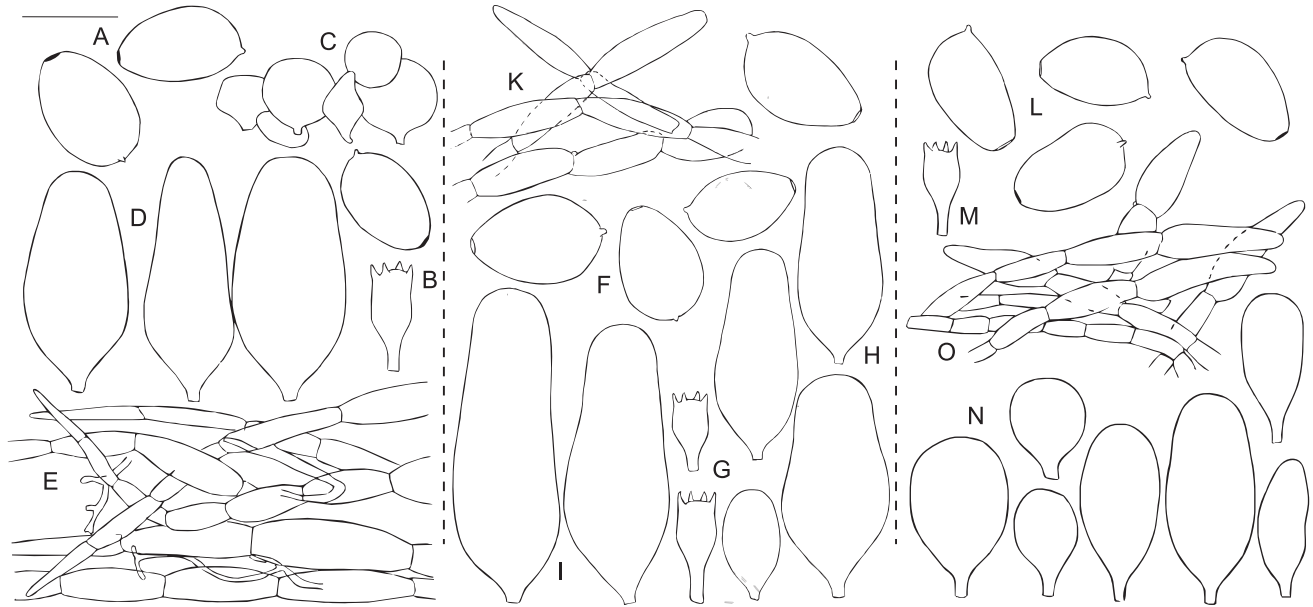


FIG. 3. Micromorphological features of the newly described species. A–E. *Coprinopsis villosa*. A. Basidiospores. B. Basidia. C. Cheilocystidia. D. Pleurocystidia. E. Veil structure. F–K. *Coprinopsis fusispora*. F. Basidiospores. G. Basidia. H. Cheilocystidia. I. Pleurocystidia. K. Veil structure. L–O. *Coprinopsis babosiae*. L. Basidiospores. M. Basidia. N. Cheilocystidia. O. Veil structure. Bar (near A) means 10 μm for spores, 24 μm for all other details (basidia, cystidia, veil).

calospora and *C. cinerea* (Cinerea clade, BPP_m: 1.00, BPP_b: 1.00, MLBS: 100%), the second (Atramentarii clade, BPP_m: 0.81, BPP_b: 0.64, MLBS:–) comprising *C. geesterani*, *C. atramentaria* (incl. var. *squamosus*), *C. ochraceolanata*, *C. pseudolagopus*, *C. erythrocephala*, *C. krieglsteineri*, *C. insignis*, *C. sp. 3*, *C. sp. 5* and a new species described here as *C. fusispora*. The third major clade, referred hereafter to as the crown Lanatuli clade (BPP_m: 0.88, BPP_b: 1.00, MLBS:–) contains *C. radiata* (including *C. tectispora*), *C. candidolanata*, *C. scobicola*, *C. bicornis*, *C. pseudo-radiata*, *C. ammophilae*, *C. macrocephala*, *C. jonesii*, *C. babosiae*, *C. villosa*, *C. sp. 1,2,6,7* as well as the *C. lagopus* complex. In our study (Nagy et al. 2011), both section *Atramentarii* (including *C. insignis*, *C. krieglsteineri* and *C. fusispora* /as *C. cinerea*/) and section *Lanatuli* appeared monophyletic in a phylogeny of the Psathyrellaceae, based on four genes. However, support was weak for the basal nodes, as it was in the case of a wider sampling of 70 species of *Coprinopsis* (Nagy et al. 2012). Statistical support for the earliest divergences is likewise weak on the tree inferred for this study (FIG. 2). However, most likely tree topologies suggest that both sections are paraphyletic.

The Cinerea clade.—The Cinerea clade includes *C. cinerea* and its close relative, *C. annulopora*, a specimen of *Cop. brunneofibrillosus* as well as *C. calospora*. *C. mitraespora* was inferred as a sister group of the Atramentarii + Crown Lanatuli clade, although its position is not strongly supported. This species is

better known under the names *C. spelaiophila* and *Coprinus extingtorius*, however a type study shows that the name *C. mitraespora* is the oldest for this species (see below).

Specimens initially identified as *C. cinerea* were placed in three species clades in this study, of which two are described here as new species (*C. villosa*, *C. fusispora*). A fourth putative species, *Coprinopsis* sp. 3, morphologically closely resembles *C. fusispora*; however, there are numerous sites and indels in the ITS and LSU alignments that discriminate it from that species.

Of the above-mentioned species, morphological features (including completely ellipsoid spores, often radicate stipes and white veil) of the third clade conform best to what is currently understood as *C. cinerea* and what most likely was described under the name *Agaricus cinereus* by Schaeffer in 1774. Therefore, we designated this clade as *C. cinerea*. Although the protolog of the species is obscure and could apply to many species of *Coprinopsis*, in accordance with the current usage of the name (including the strains for which a genome sequence is available), and the widely accepted synonymy with *Coprinus fimetarius* and *C. macrorrhizus*, we refer to this species as *C. cinerea*. The nrLSU sequence of the *Okayama 7* (#130) strain, for which a complete genome sequence have been published (Stajich et al. 2010) is nested in the clade of true *C. cinerea*.

A close relationship between *C. cinerea* and *C. annulopora* (Enderle 2004) is supported by the

phylogeny inferred in this study. However, specimens identified as *C. annulopora* were split into two clades, one in the *C. cinerea* clade (including original material obtained from the type locality) and the other in the crown *Lanatuli* clade (as *C. sp. 1*). Both lineages bear an annulus-like protrusion on the apex of spores and share habitat preferences and habit. Because no morphological differences were found to support the phylogenetic results, more material is needed before a formal description of *C. sp. 1*.

The Atramentarii clade.—The species nested in this clade are characterized by robust, stout basidiomes with scarce silvery veils. This is most characteristic in *C. krieglsteineri*, *C. erythrocephala*, *C. atramentaria*, *C. atramentaria* var. *squamosa* (= *C. bresadoliana*), *C. acuminata* and *C. insignis*, whereas other species have more well developed and/or hairier, radially splitting veils. The pale, grayish silvery basidiomes support the phylogeny and can be used in a morphological circumscription of the clade. Although previous classifications placed these species in section *Lanatuli*, it seems more appropriate to place them in section *Atramentarii*. Thirteen species are nested in this clade, of which four appear to be previously undescribed (*C. fusispora*, *C. aff. krieglsteineri*, *C. sp. 3*, *C. sp. 5*). *Coprinopsis* aff. *krieglsteineri* is close to *C. krieglsteineri* (sharing the typical grayish pileus and habit), but it has a non-radiculate stipe and lacks the long, utriform pleurocystidia with concavely attenuating neck, typical for *C. krieglsteineri*. *Coprinopsis* sp. 5 is reminiscent of *C. pseudoradiata*, but its spores are somewhat longer and the base and apex of the spores are not obtuse. The species designated as *C. sp. 3* could not be separated from *C. fusispora* morphologically, whereas the phylogeny provides strong support for its status as a separate species. Therefore, more specimens and research are needed to recover potential morphological traits supporting its separation. The type specimen of *Cop. citrinovelatus* is nested in *C. ochraceolanata*, which indicates that *Cop. citrinovelatus* is its younger synonym (also noted by Uljé 2005).

The crown Lanatuli clade.—The crown *Lanatuli* clade comprises species with *C. lagopus*-like appearance, rich, fibrillose to hairy veil and ellipsoid spores. The young basidiomes are whitish to pale gray and the veil is white in all but one of the species (*C. villosa*). The phylogeny supported most of the morphologically established species, with multiple lineages of *C. lagopus*.

The clade formed by specimens of *C. bicornis*, *C. pseudoradiata*, *C. scobicola*, *C. ammophilae*, *C. babosiae* and *Coprinopsis* sp. 7 appears to have the smallest mean genetic divergence among the species, which on the other hand are well separated on the basis of

morphology. Although the phylogenetic analyses do not support the recognition of these species as separate (except *C. babosiae*), on the basis of clear-cut morphological differences, we raise the possibility that more variable loci would provide unambiguous support for them.

The Radiata clade.—Specimens of *C. radiata* seem to be genetically highly uniform, as compared to other common taxa of section *Lanatuli*. We found that *C. radiata* includes *C. tectispora* and the tentatively named *C. pseudolagopus* (Kemp unpubl). Perplexingly, we found that the low genetic divergence is coupled with a considerable morphological plasticity in this species, observable in basidiome size and spore shape. Many collections with unusual, medially constricted, subcylindrical or subamygdaliform spores were encountered, but the phylogeny did not provide evidence for the autonomy of these variants. Also, because both sequenced collections of *C. tectispora* clustered in *C. radiata* the loosening of the perisprium, the main diagnostic character for *C. tectispora* (Uljé 2005), cannot be considered a taxonomically important character or a good predictor of phylogenetic relationships. *Coprinopsis* sp. 2 has affinity to the *C. radiata*-*C. villosa* clade. This species is characterized by a *C. radiata*-like habit, coprophilous habitat, but its spores are much more slender than those of *C. radiata*, *C. villosa*, *C. candidolanata* or *C. sp. 1*. Specimens initially labeled *C. macrocephala*, a widely known but dubious species, clustered together with *C. radiata*, which is in agreement with previous morphology based conclusions that specimens identified as *C. macrocephala* are actually *C. radiata* with large basidiomes (Uljé 2005).

The C. lagopus complex.—Our phylogenetic analyses revealed a high diversity within the 38 sequenced specimens of *C. lagopus*, with at least six well supported lineages (FIG. 2, phylogenetic species PS 1–6), plus *C. jonesii* placed in between clades formed by *C. lagopus* specimens. Of interest, *C. jonesii* (previously *C. lagopides*) was found formerly to be compatible with species of the *C. lagopus* complex (Kemp 1975). Upon re-examination of specimens of the various clades (concentrating on spore size, shape, surface and size of veil cells, position and size of germ-pore, veil color and basidiome size), we found no morphological synapomorphies that would support the observed phylogenetic structure. This, in combination with low branch support values and the unstable branching order, suggests a recent split between these phylogenetic species. The existence of multiple “species” within *C. lagopus* is congruent with the observations made by Kemp (1975) using mating tests. In that work, the examined specimens

formed four incompatible groups, interpreted as biological species. Of these, we included specimens of two in our analyses (groups B and C, all material of A is missing, Kemp 341/1 [group D] failed in sequencing). In our analyses, however a representative specimen of *C. lagopus* group C (Kemp 378), clustered within *C. radiata*, that of *C. lagopus* group B (Kemp 1432 and Kemp 1431) in *C. lagopus* PS 3. *Cop. fissolanatus* (both original specimen and culture-grown basidiome, identified later as *C. lagopus* var. *vacillans*) was placed in *C. lagopus* PS 6.

Two specimens of *C. pachyderma* (including the type material, van de Bogart 1979), a species separated from *C. lagopus* on account of the thickened wall of veil cells, were nested in *C. lagopus* phylogenetic species 6. Examination of other specimens in this clade did not reveal a tendency for thickened wall of the veil cells.

TAXONOMY

Based on the availability of specimens, phylogenetic evidence and known diagnostic morphological features, we describe three new species, *C. fusispora*, *C. babosiae* and *C. villosa*, and propose one new combination.

Coprinopsis babosiae L. Nagy, Vágvölgyi and Papp, sp. nov.

Mycobank MB800684

Etymology: Named after the Hungarian mycologist Margit Babos (1931–2011), who made the first extensive mycological inventory of the area where the species is known to occur.

Diagnosis: Pileus 3–10 × 2–5 mm when closed, cylindrical to ellipsoid, later campanulate, finally applanate, up to 20–25 mm when fully expanded, whitish to pale ochraceous when young, becoming grayish to blackish on aging, plicate when expanded; surface longitudinally translucently striate, covered with a whitish, floccose to woolly, but compact veil, with a soggy appearance; veil soon disappearing. Lamellae up to 1.5 mm broad, ventricose, white when young, blackish and distant when mature, with whitish fimbriate edge. Stipe 30–50 × 0.5–2 mm, slender, hollow, fragile, covered with fine, floccose to woolly white veil remnants, whitish or pale grayish. Odor and flavor indistinct.

Basidiospores [40,1,1] 7.8–9.7 × 5.0–6.0 μm, on average 8.9 × 5.5 μm, Q = 1.44–1.64 (1.76), Q_{av} = 1.6 in frontal view ellipsoid, often with one side somewhat depressed or straight, giving a subphaseoliform outline, ellipsoid to subamygdaloid in lateral view, not lentiform in polar view, dark reddish brown in KOH, almost opaque, with small hilum and a

central, 1.4–1.7 μm wide germ pore. Basidia four-spored, bimorphic, clavate or spathulate with a median constriction, 23–28 × 7–8 μm. Pleurocystidia absent. Cheilocystidia abundant, 27–48 × 10–18 μm, variable shape, ranging from almost globose to subglobose, vesiculose, ellipsoid to broadly utriform, most frequently vesiculose with short pedicel. Veil made up of sausage-like hyphae constricted at the septa, with fusiform-cylindrical end cells (40–78 × 18–22 μm). Clamp connections present.

Holotype: HUNGARY. Alföld: Ásotthalom, mixed forest of *Populus alba*, *Robinia pseudoacacia* and *Quercus robur*, growing on rotten bark of *Populus*, 2 Nov 2008. L. Nagy (SZMC-NL-4139, deposited in the Hungarian National History Museum /Bp/).

Other specimens examined: HUNGARY. Alföld: Bócsai-forest, mixed forest of *Populus alba*, *Robinia pseudoacacia* and *Pinus sylvestris*, growing on rotten bark of *Populus*, 24 Oct 2009. L. Nagy (SZMC-NL-0871); Alföld: Méntelek-Belsőnyír: *Populus alba* forest, on rotten bark, 14 Nov 2009. L. Nagy (SZMC-NL-2641); Alföld: Nyárjas: continental dune *Quercus robur*-*Populus alba* forest, under *Crataegus*, among leaf-litter, 22 May 2010. L. Nagy (SZMC-NL-2630); Alföld: Ásotthalom: mixed forest of *Celtis*, *Populus alba* and *Robinia pseudoacacia* on decaying logs, 26 Oct 2009. L. Nagy (SZMC-NL-3070); *ibid.*, on decaying logs, 29 Jun 2009. L. Nagy (SZMC-NL-3858).

Distribution: Known only from Hungary, where it was found at humid places in mixed *Populus alba* forest between continental sand dunes. Although the tree species could not always be verified, *C. babosiae* seems to have an affinity to *Populus*.

Observations: There are only a handful of species with such small spores in section *Lanatulii*, namely *C. geesterani*, *C. pseudoradiata*, *C. candidolanata* and *C. jonesii*. Of these, *C. pseudoradiata* and *C. candidolanata* are strictly coprophilous and have pleurocystidia. *C. jonesii* has much larger basidiomes and differently shaped spores. *C. geesterani* shares both its habitat and spores size, however, it has pleurocystidia, the shape of the spores is different (rhomboid) and they usually are paler.

Coprinopsis fusispora L. Nagy, Vágvölgyi and Papp, sp. nov.

Mycobank MB 800685

Diagnosis: Pileus up to 15 × 20–30 mm when young, ellipsoid to subglobose, later expanding to convex to plano-convex, finally applanate with up-rolled margin, up to 45 mm broad; pale ocher, cream to beige when young, darker brownish in the center, surface smooth when young, becoming radially sulcate upon cap expansion, covered with whitish floccose to filamentous veil, soon vanishing and remaining only in the center as brownish fibrils and

at the margin. Lamellae free, crowded, strongly ventricose, up to 3(4) mm broad, white when young, then grayish, finally blackish, strongly deliquescent. Stipe 35–70 × 2–3 mm, slender, firm, fistulose, white all over; surface silky, smooth, at base with whitish veil remnants when young. Odor and flavor indistinct.

Basidiospores [40,1,1] 9.2–12.5 × 5.8–7.3 μm, on average 10.6 × 6.7 μm, Q = 1.52–1.71, Q_{av} = 1.58, in frontal view ovoid to ellipsoid with protruding apex, in lateral view broadly fusiform, subamygdaliform or submitriform, not lentiform, medium dark reddish brown in KOH, not opaque, mostly with one large oil inclusion and a small hilum; germ pore central, 1.4–1.7 μm wide. Basidia four-spored, clavate or spathulate with median constriction, 20–30 × 8–11 μm. Cheilocystidia abundant, 62–120 × 22–46 (60) μm, predominantly utriform but also ellipsoid to cylindrical, with obtuse apex. Pleurocystidia abundant, 55–110 × 25–38 μm, chiefly utriform, sometimes cylindrical or ellipsoid. Veil made up of chains of elongate to cylindrical or fusiform, 13–25 μm wide cells; end cells fusiform to cylindrical or ellipsoid. Clamp connections present.

Holotype: HUNGARY. Alföld: Belsőnyír, among grass on sandy soil in a continental, semidesert *Quercus* forest, 22 Aug 2005. L. Nagy & Zs. Gorliczai (SZMC-NL-1227, deposited in Bp.).

Other specimens examined: HUNGARY. Alföld: Tőserdő, *Fraxinus-Alnus* swamp forest, among leaf-litter, 14 Oct 2006. L. Nagy & Zs. Gorliczai (SZMC-NL-1553); Alföld: Bácsalmás-Bajmok, grazed alkaline meadow with *Cynodon* and *Festuca*, 25 Oct 2008. L. Nagy (SZMC-NL-0777); Alföld: Kecskemét, among grass on clay soil, 13 Jun 2004., L. Nagy & Zs. Gorliczai (SZMC-NL-1577); *ibid.*, among grass, 24 Sep 2005. L. Nagy & Zs. Gorliczai (SZMC-NL-1421); Alföld: Ásotthalom: mixed forest of *Populus alba*, *Robinia pseudoacacia* and *Quercus robur*, growing on wood-chips, 8 Aug 2006. L. Nagy (SZMC-NL-1484); Alföld: Bácsalmás: fertilized pasture, on soil, 1 Nov 2008. L. Nagy (SZMC-NL-3863).

Distribution: Known only from dry continental areas in Hungary with grazed calcareous fertile meadows or pastures, more rarely from leaf litter or woodchips in forests. Recorded both from clayey and sandy, dry and wet soils.

Observations: Morphologically this species is close to *C. cinerea*, however *C. cinerea* has ellipsoid spores with obtuse apex whereas *C. fusispora* has broadly fusiform or submitriform spores. Although according to a traditional, morphological species concept, this difference would not qualify to be separated, the two species are phylogenetically distinct.

Coprinopsis mitraespora (Bohus) L. Nagy, Vágvölgyi and Papp, comb. nov.

Mycobank MB800686

Basionym: *Coprinus mitraesporus* Bohus, Bot. Közl. 57:18. 1970.

= *Coprinus spelaiophilus* Bas & Uljé, Persoonia 17:179. 1999; *Coprinopsis spelaiophila* (Bas & Uljé) Redhead, Vilgalys & Moncalvo, Taxon 50:231. 2001.

Misapplied name: *Coprinus extincorius* sensu Romagn., Rev. Mycol. 6:112. 1941; sensu Kühner & Romagnesi, Flore Analytique Champ. Sup.: 387.1953; sensu Moser, Die Röhrlinge und Blätterpilze Ed. 5:256. 1983; sensu Orton & Watling, British Fung. Flora 2:39. 1979.

Specimen examined: HUNGARY, Budapest, Szilágyi Erzsébet fasor, “in cavitate trunci *Aesculi hippocastanum*”, 7 Oct 1967, G. Bohus (BP 43.893).

Observations on the type specimen: The holotype is a well preserved Herpell-exsiccatum, consisting of many basidiomes, representing all stages of development, stuck to two paper cards.

Pileus (conico-)elliptical when closed, up to 20 × 15 mm, later conico-campanulate, conico-convex when old, up to 45 mm diam, surface covered with flocculose-subsquamulose pale yellowish veil, pale beige-isabelline on dried specimens, light ochraceous, more grayish brownish when old. Lamellae narrowly adnate to free, 4 mm broad, slightly ventricose, whitish when young, black on aging. Stipe 3–6 × 25–50 mm, cylindrical to somewhat tapering upward, whitish, thick and firm.

Basidiospores [35,1,1] 8.9–11.7 × 6.8–8.2 × 5–7 μm, on average 10.8 × 7.4 × 5.8 μm, in face view broadly fusiform to rhomboid, rarely with elliptical outline, in lateral view broadly fusiform, sometimes amygdaloid, dark reddish brown in KOH; germ-pore central, 1.6–2 μm wide. Pleurocystidia ± abundant, ellipsoid-oblong, with some utriform ones, thin-walled, smooth, 67–138 × 30–50 μm. Cheilocystidia densely packed, mainly ellipsoid-subcylindrical, some oblong or subutriform present, 30–100 × 15–4 μm. Veil hyphoid, composed of elongate elements slightly constricted at septa, terminal cells cylindrical not or hardly inflated, 97–130 × 10–20 μm, unclamped; in exsiccata the veil is compact, difficult to dissolve in KOH 5%, Pileipellis a cutis of cylindrical, clamped hyphae; caulo- and pileocystidia absent.

Observations: The study of the types of *Coprinus mitraesporus* revealed that it is conspecific with species known currently as *C. spelaiophila*. However, since the epithet *mitraesporus* (Bohus 1970) is older than *C. spelaiophilus* (described in 1999 to suppress the misuse of *C. extincorius*, Uljé & Noordeloos 1999) it has nomenclatural priority over the latter (Art. 11.4).

Coprinopsis villosa L. Nagy, Desjardin, Vágvölgyi and Papp, sp. nov.

Mycobank MB 800683

Original diagnosis: Pileus 8–25 × 4–10 mm when closed, ellipsoid to cylindrical with acute apex, later

campanulate, then bell-shaped, applanate when mature, up to 35 mm diam; surface when young covered with a thick layer of floccose-fibrillose, chestnut-brown veil, translucently striate below veil, brownish to chestnut brown, but paler, almost whitish below the veil, grayish to blackish upon aging, strongly deliquescent. Lamellae crowded, free, ventricose, up to 3 mm broad, whitish when young, black when mature, with whitish, finely fimbriate edge. Stipe 30–90 × 2–5 mm, cylindrical or somewhat broadening toward base, fistulose, fragile, covered with whitish flocks all over, white. Context thin without characteristic odor or flavor.

Basidiospores [31,2,1] 9.8–12 × 6.0–7.8 μm, on average 11.1 × 7.1 μm, Q = 1.47–1.64. Q_{av} = 1.57, ellipsoid to ovoid in frontal view, ellipsoid to subamygdaloid or sometimes slightly phaseoliform in lateral view, regular, not lentiform in polar view, dark reddish brown+/-opaque, with small hilum and a 1.4–1.7 μm wide, central germ pore. Basidia four-spored, bimorphic, clavate or spatulate with a median constriction, 21–33 × 8–10 μm. Pleurocystidia sparse, 42–70 × 26–30 μm, utriform, ellipsoid or rarely utriform with obtuse apex. Cheilocystidia 10–21 μm diam, poorly developed, variable shape, mostly globose-subglobose, a few utriform, in size hardly larger than the pseudoparaphyses. Veil made up of two types of elements: (i) chains of elongate-ellipsoid inflated cells (40–88 μm diam), gradually attenuating toward the apex, terminating in inconspicuous, acute or flagellate fusiform cells; (ii) narrow, diverticulate or branched but not coralloid, 2–5 μm wide hyphae. Clamp connections not found.

Holotype: GERMANY. Baden-Württemberg: Tübingen, on horse dung, 10 Oct 2006, L. Nagy (SZMC-NL-1758, deposited in Bp).

Other specimens examined: HUNGARY. Alföld: Bugac, on horse dung, 14 Dec 2005, L. Nagy (SZMC-NL-1714); SWEDEN: Öland, Ned Vasterstad, on horse dung, 19 Sep 2006, L. Nagy & T. Knutsson (SZMC-NL-1155); *ibid.* 20 Nov 2006., L. Nagy & T. Knutsson (SZMC-NL-1768); USA. HAWAII: Hawai'i, Agriculture Farm, fruited in moist chamber, 10 Feb 2001, D. Hemmes, D. Desjardin, M. Keirle (DEH 2061; SFSU).

Distribution: So far known only from horse dung but seems to have a broad distribution including continental semidesert to boreal habitats. Known from Germany, Sweden, Hungary and Hawaii (Keirle et al. 2005).

Observations: *Coprinopsis villosa* was first reported from Hawaii, from horse dung (Keirle et al. 2005). Both morphology and the phylogenetic analyses confirm the conspecificity of European specimens with those reported from Hawaii. *C. villosa* can be recognized by the brownish veil, coprophilous

habitat, broadly ellipsoid spores around 11 μm, and optionally by the presence of diverticulate hyphae in the veil. Based on morphology and previous identification keys, this species is closest to *C. cinerea*, especially because the diverticulate hyphae are often difficult to discover. However, *C. cinerea* lacks the diverticulate elements, has somewhat larger basidiomes with white veil and grows on wood-chips, straw (mixed with dung) and rarely directly on manure. The coprophilous habitat is shared with *C. candidolanata*, which has a considerably overlapping spore size, but smaller basidiomes bearing pure white veil flocks, and a preference for deer and sheep dung. Furthermore, the diverticulate veil elements are different; those of *C. candidolanata* bear numerous small, rounded excrescences on their surface (like certain *Mycena* species), while in *C. villosa* the hyphae are branched and their surface is almost smooth.

The basidiomes in the holotype collection are larger, as compared with other materials: pileus 2–5 × 4–13 mm when young and closed, up to 25 mm when fully expanded.

DISCUSSION

This study aimed to infer phylogenetic relationships among species of sections *Lanatuli* and *Atramentarii*. An attempt was made to sequence all *Coprinopsis* species described to date with a veil composed of chains of elongate, cylindrical cells without diverticulate or coralloid elements (Orton and Watling 1979, Singer 1986, Uljé and Noordeloos 1999, Uljé 2005). As a result, specimens for 45 published names have been collected, of which 41 were included in phylogenetic analyses. Our results (FIG. 2) indicate an incongruence of morphology-based sectional classification and molecular results. Section *Lanatuli* appears paraphyletic with regard to section *Atramentarii* and the latter appears paraphyletic with regard to a number of species (*C. kriegsteineri*, *C. geesterani*, *C. insignis*, *C. erythrocephala*) classified in section *Lanatuli* by Orton and Watling (1979), Uljé and Noordeloos (1999) and Uljé (2005). We consider it unlikely that outgroup choice may have influenced the assessment of monophyly in our case because we used a representative of the basal-most clade of *Coprinopsis* as outgroup (*C. cortinatus*, pseudonivea clade, see Nagy et al. 2011). We conclude that more genes may be needed to infer basal relationships robustly and to clarify the sectional taxonomy in *Coprinopsis* and whether species with an apparently uniform veil coverage evolved independently twice or form a monophyletic clade.

The morphological diversification between species lineages does not appear uniform across the tree. The

phylogenetic analyses recovered three new species, two of which (*C. villosa* and *C. fusispora*) could have been confused with *C. cinerea* due to overlapping spore size and habitat but differing in straightforward morphological characters. Similarly, multiple lineages corresponding to what is currently understood as *C. lagopus* have been recovered, but in this case we could not identify any morphological differences that would support the phylogenetic divergence. On the other hand, we observed a high morphological plasticity in spore shape and basidiome size in *C. radiata* coupled with negligible genetic divergence. This reveals the limitations of uniform species concepts and/or phylogenetic markers even within limited groups of fungi and prompts for the examination of species level relationships using more specimens and more variable loci in future studies. Although it is well known that the rate of molecular and phenotypic evolution can vary widely, even across closely related species, this is rarely accounted for in taxonomic studies. Whether more variable loci would provide evidence for spore shape variants of *C. radiata*, including *C. tectispora*, or whether and how phenotypic diversification could be traced in the *C. lagopus* complex remain to be explored.

Cryptic species have been reported in several fungal groups, including *Hebeloma*, *Coniophora*, anther smuts, lichen-forming fungi etc. (Aanen and Kuyper 2004, Kauserud et al. 2006, 2007; Le Gac et al. 2007, Crespo and Lumbsch 2011), although deciding whether the taxa are indeed cryptic depends strongly on the nature and accessibility of phenotypic differences sought for as well as the approach used to detect them. Many studies have reported morphologically identical species with different geographic and/or ecological amplitudes. Chorological data available for the studied specimens did not reveal any consistent patterns for phylogenetic species within *C. lagopus*. Therefore, following (Crespo and Lumbsch 2011), we denote PS1-PS6 as the *C. lagopus* complex and refrain from formally describing any of them because of the lack of supporting morphological evidence and strong support for all groups. The only species that can be separated unambiguously is *C. jonesii*, differing by significantly shorter (up to 10 µm), ovoid spores. We raise the possibility that sampling more specimens with better documented (ecological) data might reveal the patterns of phenotypic or ecological diversification of these fungi.

Coprinopsis lagopus var. *vacillans*—a polyphyletic phenotypic mutant?—We sequenced eight specimens assigned to *C. lagopus* var. *vacillans*, which differs from var. *lagopus* in having the ephemeral basidiomes and fragile stipes, that can barely hold the weight of

the pilei (Uljé 2005). Phylogenetic analyses did not support the monophyly of these specimens but placed these in two of the four phylogenetic species of *C. lagopus* (PS 5 and PS 6). This suggests on one hand that the taxonomic status of this variety cannot be maintained and on the other hand that switches from normal to vacillans-like ephemeral basidiomes can occur frequently in these mushrooms, even within one species. Similar high rates of switches between basidiome morphologies have been reported for several, chiefly ectomycorrhizal groups, such as *Suillus* (Bruns et al. 1989), and shed light on the tempo of basidiome evolution.

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