The family Agaricaceae: phylogenies and two new white-spored genera

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Abstract: A well resolved phylogeny of the Agaricaceae based on partial rpb2 sequences is presented from a wide geographic and systematic sampling of the family and compared to phylogenies based on nLSU and tef1 sequences. A smaller dataset of the family focusing on the Agaricus clade of nrITS sequences and a combined dataset were used to determine the position of several white-spored taxa from northern Thailand. Two new genera are described from Thailand. Coniolepiota accommodates Lepiota spongodes, a gray-lilac-purple floccose white-spored species with a wide distribution in tropical Asia; Eriocybe has a white wooly felt-like covering of pileus and stipe, white spores and is described with one new species E. chionea, so far known only from northern Thailand. These new genera are closely related to three genera with colored spores (viz. Agaricus, Heinemannomyces and Clarkeiinda) and not to other white-spored taxa.

Key words: biodiversity, gene sequences, molecular phylogeny, northern Thailand

INTRODUCTION

The circumscription of family Agaricaceae Chevall. has changed considerably since the application of molecular data to mycological classification (Moncalvo et al. 2000, 2002; Matheny et al. 2007b), and this is one of the few families that has seen a substantial influx of species (especially gasteroid and secotioid taxa from the Lycoperdaceae Chevall., Tulostomataceae E. Fisch. and Podaxaceae Corda). Morphological evidence for the close relationship between agaricoid and gasteroid taxa also has been provided; for example the structure of the rkhizomorphs of the agaricoid Agaricaceae and that of the former Lycoperdaceae was shown to be very similar (Agerer 2002). The family as a whole has been the focus of several molecular-phylogenetic studies (Johnson 1999, Johnson and Vilgalys 1998, Vellinga 2004), whereas some genera and sections of genera also have been studied extensively, for example Lepiota (Pers.) Gray s. str. by Vellinga (2003), Agaricus section Xanthodermatæ Singer by Kerrigan et al. (2006) and the gasteroid genera Lycoperdon Pers.: Pers. and Boletus Pers. by Larsson and Jeppson (2008). The geographic coverage of these studies was limited, and North America and Europe were the main focus (e.g. only northern European taxa were included in Larsson and Jeppson 2008) or collections from these two regions form the vast majority (e.g. Vellinga 2003, 2004). The attine ant cultivar studies of course treat South and Central American taxa (e.g. Chapela et al. 1994, Vo et al. 2009).

Phylogenetic or combined morphological/molecular studies have used nuclear ribosomal RNA genes (rDNA), which occur in a tandem repeat, such as the nuclear large subunit ribosomal RNA gene (nLSU) (e.g. Johnson and Vilgalys 1998, Larsson and Jeppson 2008) and internal transcribed spacers 1 and 2 flanking the 5.8S region (nrITS) or combinations of the two (e.g. Vellinga 2003, 2004). One study used atp6 sequence data to infer the phylogeny of genus Agaricus (Robison et al. 2001), but only 10 species were included; atp6 encodes subunit 6 of the F0F1 ATP synthase of mitochondria. Johnson (1999) included mitochondrial rDNA small subunit (mtSSU) sequences to a dataset composed of nLSU and nrITS sequences of a reasonably wide sampling (but still limited in numbers) of Agaricaceae. This gene contributed little to the resolution of the relationships within the family but can be used with more success at higher order phylogenies, especially in combination with other gene regions (Lutzoni et al. 2004).

For species-level studies the intergenic spacer region 1 (nrIGS) (Vellinga 2001), in the same tandem repeat nLSU and nrITS are a part of, or the single copy gene tef1, which encodes for the translation elongation factor 1-α gene, have been used (Vellinga et al. 2010, Vellinga 2010).

Here for the first time data from the rpb2 gene are used to establish a phylogeny of the Agaricaceae. The
The *rpb2* gene, a singly copy gene, encodes the second largest subunit of RNA polymerase II and has been used to infer fungal phylogenies at different systematic levels (Matheny 2005, Frøslev et al. 2005, Matheny et al. 2007a) on its own or in combination with *rpb1* (Matheny 2005) or other genes (Matheny et al. 2007b). All major clades of the Agaricaceae are included in this analysis, with representatives from Asia, Europe and North America.

In addition data from the *rpb2* gene, *tef1* sequences were produced and analyzed, again from a wide sampling of Agaricaceae. This gene also is used widely to infer fungal phylogenies; because of the presence of various highly variable introns its contribution to higher level phylogenies is smaller than that of the *rpb2* gene (Matheny et al. 2007b), but at the genus level it has been used successfully and it showed better resolution of the phylogeny than the nrITS region in the ascomycete genus *Beauveria* Vuill. (Rehner and Buckley 2005).

Morphological studies of the *Agaricaceae* from tropical regions often are based on collections made in the 19th and early 20th centuries (e.g. Pegler 1972, 1975, 1985); modern studies are rare, but a series of articles on white-spored species from southern India is a welcome exception (Kumar and Manimohan 2004, 2009a, b, c), and combined morphological-molecular-phylogenetic studies are rare; exceptions are the articles by Ge et al. (2008) and Liang et al. (2010).

Northern Thailand has been the epicenter of recent systematic mycological studies in southeastern Asia with the emphasis on gilled mushrooms (e.g. Le et al. 2007, Liu et al. 2009, Wannathes et al. 2009, Zhao et al. 2008), with only the small brown-spored genus *Mycopssalliotae* in the Agaricaceae treated so far (Zhao et al. in press). We focused on collecting white-spored taxa in the *Agaricaceae* in Chiang Mai Province. Several striking taxa with lilac-gray, lilac or white flocculose to woolly coverings of pileus and stipe, with white smooth spores and no clamp connections are the focus of the research presented here; their morphology is described, and the phylogenetic position of these taxa determined.

**MATERIALS AND METHODS**

**Morphology.**—Standard methods for describing basidiocarps were applied with the terminology of Vellinga and Noordeloos (2001). Color annotations in the macroscopic descriptions are from Kornerup and Wanscher (1974) and from Munseli soil color charts (1975). Microscopic observations were made on dried material. The notation [60,4,3] indicates that measurements were made on 60 spores in four samples in three collections. At least 15 spores were measured per collection. The lamellar characters and spore shape and size were observed in Congo red in 10% ammonia followed by 10% ammonia only, and the pileus covering was observed in 10% ammonia. These abbreviations are used: L for number of lamellae, l for number of lamellulae in between two lamellae, aw for average length, aw for average width, Q for quotient of length and width, and avQ for average quotient. The abbreviations *L*. is used for *Leptiola*, *La*. for *Leucoagaricus* and *Leucocybe* for *Leucoagaricus*. Herbarium abbreviations are according to Thiers (2010). Latin descriptions of new taxa were deposited in MycoBank.

**DNA extraction, primers, PCR and sequencing.**—Sequences were obtained from different sets of species and collections. A wide range of species from the Agaricaceae was sampled for the 5′ end of the nuclear large subunit ribosomal RNA gene (nLSU), between domain 6 and 7 of *rpb2*, and part of *tef1* (Supplementary Table I) with the emphasis on collections from Thailand; a subset was chosen for nrITS sequence analyses based on the results of the other analyses (Supplementary Table I).

DNA was extracted from dried material with a QIAGEN DNeasy® blood and tissue kit (QIAGEN, Valencia, California). These primer sets were used: LR0R, LR5, LR7 and LR16 for the 5′ part of the 25S LSU gene (www.biology.duke.edu/fungi/mycolab/primers.htm); brpb2-6F and brpb2-7.1R for part of *rpb2* (Matheny 2005); ef1-983F and ef1-1567R for part of the *tef1* gene according to the protocol developed by Rehner and Buckley (2005); and ITS-1F and ITS-4 for nrITS1, 5.8S and nrITS2 (Gardes and Bruns 1993). PCR reactions were performed with an MJ PTC-100™ thermo cycler (Applied Biosystems, Foster City, California) or an Eppendorf Mastercycler® gradient (Eppendorf, North America Inc., Westbury, New York).

PCR products were cleaned with 0.5 μL ExoSAP IT (USB Corp., Cleveland, Ohio) per reaction and cycled at 37 °C for 45 min, followed by 80 °C for 15 min. Sequencing was performed with big dye chemistry with the same primers as for PCR, and an ABI PRISM 3100 genetic analyzer (both from Applied Biosystems, Foster City, California). Sequences were edited and contigs assembled with Sequencer 4.2.2 (Gene Codes Corp., Ann Arbor, Michigan). All newly produced sequences were deposited in GenBank, under accession numbers HM488741–HM488956, and individual accession numbers are provided (Supplementary Table I).

**Phylogenetic analyses and visualization of the results.**—The sequences were aligned with the program MAFFT 6 (Katoh et al. 2002, Katoh and Toh 2008) with default settings. Minimal manual aligning was necessary, and no parts were excluded from the analyses. All alignments were based on the nucleotide sequences. Each database was analyzed separately by a maximum likelihood (ML) method with RAxML 7.2.3 (Stamatakis et al. 2008). All free-model parameters were estimated by RAxML with a general time-reversible (GTR) substitution matrix and a proportion of invariable sites estimate. One hundred rapid ML bootstraps were performed for each dataset calling a GTRCATI nucleotide model of substitution followed by a search for the tree with the highest ML. A combined dataset with representatives of the family (Supplementary Table I) as a
whole was analyzed thusly: RAxML, Bayesian Metropolis-coupled Markov chain Monte Carlo (Bi) conducted with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The general structure (i.e., the substitution model was defined with nst = 6 rates = gamma ngammacat = 4. Markov chains were run 10^6 generations with the sampling frequency set every 100th generation; a burn-in of 25% was used; and a bootstrap analysis under parsimony conditions in PAUP 4.0d (Swofford 2002).

These outgroup taxa were used: for nLSU Limacella glioderma and Pseudobasidiospora pyrfera as in Vellinga (2004); for rpb2 species from outside the Agaricaceae (viz. Strobilomyces strobilaceus, Amanita muscaria and A. brunnescens, Hebeloma olympianum and Tubaria vinicolor); for tef1 also taxa outside the Agaricaceae: Amanita brunnescens and Schizophyllum commune, for nrITS taxa from the Leucoagaricus/Leucocoprinus clade within the Agaricaceae (viz. Leucoagaricus barsi and L. Leucothites). ML trees were viewed with Figtree 1.3.1 (Rambaut 2009). The four alignments and ML trees are deposited in TreeBase under accession number 10621.

RESULTS

The phylogeny of the Agaricaceae and the position of the Thai taxa Coniolepiota gen. nov. and of Eriocybe gen. nov., which are described below, were determined by analyses of three datasets containing respectively 88 nLSU sequences (1001 positions) representing 82 species (with Limacella glioderma and Pseudobasidiospora pyrfera as outgroup), 94 rpb2 sequences (701 positions), representing approximately 73 species (with two Amanita species, Strobilomyces strobilaceus, Tubaria vinicolor and Hebeloma olympianum as outgroup) and third 107 tef1 sequences (approximately 84 taxa, 669 positions; Schizophyllum commune and Amanita brunnescens were used as outgroup) (Supplementary Table I). Most genera are well represented in these matrices. The datasets were not combined to show the differences in phylogenetic resolution and to show the position of the new genera based on these three different gene regions separately. A third reason for not combining the datasets is that for many species the sequence data are derived from different collections.

The phylogenetic hypothesis based on maximum likelihood analysis of the nLSU sequences (Supplementary Fig. 1) shows low support for the various clades in the Agaricaceae, with the Agaricus clade (Agaricus including sectoiotid species such as Agaricus aridicola [syn. Gyrophragmium dunaliil], Longula texensis, Barcheria willisiiana and Agaricus inapertus [syn. Endophyllum depressum]) as one of the few exceptions. Coniolepiota and Eriocybe are close to Agaricus and group together with species with colored spores (Clarkeinda trachodes, Heinennannomyces splendidissimus, Hymenagaricus taxa) and white-spored Thai species that resemble L. fuscovinacea, a European taxon without clamp connections, with a floccose-fibrillose vinaceous gray pileus covering. Based on this analysis only a few genera are monophyletic; Leptota for instance is split into two parts. Leucoagaricus and Leucocoprinus are represented by a relatively low number of taxa and together or separately are not monophyletic. However, although not supported by statistical evidence, Macrolepiota species make up a lineage, which includes Mycenastrum corium and so do the gasteroid species in Lycoperdon, Boivista and Calvatia.

The rpb2 dataset contained 87 new sequences, covering the family as a whole as well as possible, with representatives of the agaricoid, lepiotoid and various gasteroid lineages of the family. To evaluate the variability of this gene fraction within a species multiple collections of some species were sequenced. The results of the analysis of the rpb2 dataset (Fig. 1) show a much better resolved phylogeny of the Agaricaceae with significant bootstrap support for many clades and traditionally recognized genera (e.g. 76% for the Leptota s.l. clade, 100% for Chlorophyllum, 80% for a combined Leucoagaricus and Leucocoprinus and 89% for Macrolepiota). Coniolepiota and Eriocybe fall again in the Agaricus s.l. clade (with bootstrap support of 84%), which also contains Clarkeinda trachodes and Heinennannomyces splendidissimus. Interspecific variation of this region of rpb2 is small.

The tef1 dataset used six GenBank sequences from studies by other authors and more than 100 sequences generated by the first author from a wide sampling of the family, and yet it is more limited in representatives of the Agaricus s.l. clade because it does not contain Clarkeinda, Heinennannomyces, Micropsalliota or Hymenagaricus sequences; no tef1 sequence data are available for Eriocybe. Multiple collections per species were sequenced to assess the intraspecific variability of this gene. Some morphologically defined genera are well recovered (Supplementary Fig. 2), (e.g. Agaricus with 100% bootstrap support, Chlorophyllum with 96%), but as in the nLSU tree bootstrap support in general is low. Furthermore the Leucoagaricus/Leucocoprinus clade is divided into three parts that also includes Coprinus comatus, the genus Macrolepiota is monophyletic but contains Podaxis pistillaris and Tulostoma and Battarea are placed within the Leptota clade. However again Coniolepiota is placed close to Agaricus and not with white-spored taxa in one of the three Leucoagaricus and Leucocoprinus clades or Leptota. Two of the three Leucoagaricus/Leucocoprinus clades correspond with morphologically defined sections of genus Leucoagaricus (i.e. clade 1 with sect. Rubrotincta and clade 3 with sect. Piloselli). The latter includes the type species of
FIG. 1. rpl2 phylogeny of the Agaricaceae inferred by maximum likelihood (ML) analysis. Numbers at internodes refer to confidence estimates based on 100 rapid ML bootstraps (only those > 70 are given). Coniolepiota and Eriocybe are highlighted, and main clades are indicated. Amanita brunnescens and A. muscaria, Hebeloma olympianum, Strobilomyces strobilaceus and Tubaria vinicolor are outgroup taxa.

To further investigate the positions and relationships of the two new genera a smaller sampling with the emphasis on species of the *Agaricus* clade, *Chlorophyllum, Clarkeinda* and *Heinemannomyces*, was used for the nrITS region (Supplementary Table I). The nrITS dataset has a wider sampling of *Agaricus* species than the nrLSU, rpl2 and tef1 datasets and includes *A. trisulphuratus*, represented by two samples under that name that actually might be derived from two different species, the type species of *Agaricus* subg. *Lanagaricus*. This dataset contains 46 sequences (approximately 38 taxa) and has 857 positions. Some of the relevant results (Fig. 2) were that *Clarkeinda trachodes* is close to *Agaricus* (without significant bootstrap support); *Agaricus* including *A. trisulphuratus* again is well supported (98%) and the sister clade to *Agaricus* contains *Heinemannomyces, Hymenagaricus* species (*Agaricus* species with a hymeniform pileus covering; Heinemann 1981) and *Eriocybe* and *Coniolepiota*, plus again the two *Lepiota fuscovinacea*-like species from northern Thailand. This expanded *Agaricus* clade gets 100% bootstrap support and *Chlorophyllum* forms the sister group of the *Agaricus* s.l. clade again with 100% bootstrap support. It should be pointed out that in the nrLSU, rpl2 and nrITS trees *Micropsalliota* species do not group with *Agaricus* but are close to *La. meleagris* and *La. americanus* and allies.

To further evaluate the position of the new taxa and whether recognition at genus level of the separate Thai species is warranted a database combining the four gene regions was compiled and analyzed in which 40 collections are represented (Supplementary Table I, Fig. 5). The choice of taxa is focused just as in the nrITS dataset on *Agaricus, Chlorophyllum* and allies with relatively low numbers of representatives of the other clades and genera. Genus *Agaricus* and the wider *Agaricus* clade are well supported in all three analyses (ML, MP, MrBayes), a combination of *Coniolepiota, Eriocybe* and one of the taxa that resemble *L. fuscovinacea* (coll. ecv3556) gets high support in the Bayesian analysis only. All these taxa in the *Agaricus* s.l. clade are positioned on long branches.

Another outcome of the present analyses is the position of *Macrolepiota* in relation to that of *Chlorophyllum*. Just as in earlier studies (Vellinga et al. 2003, Vellinga 2004), in which only nrLSU and nrITS data were analyzed, the two together do not form a monophyletic clade and are not sister clades. The separation of the two in different genera seems to be completely justified by the results presented here based on non-ribosomal sequence data.

**DISCUSSION**

*Phylogeny of the Agaricaceae.*—The *rpl2* dataset with its 86 newly generated sequences significantly contributes to resolving the phylogeny of the Agaricaceae because in contrast to the nrLSU and tef1 phylogenies clades are well resolved and get relatively high bootstrap support. Matheny (2005) and Matheny et al. (2007a) showed the power of this gene region at different taxonomic levels, from genus (*Inocybe*) to phylum (Basidiomycota). Here despite a limited sampling the genera within family Agaricaceae are well supported, but taxon sampling has to be improved to allow for finer analyses of the *Leucoagaricus/Leucocoprinus* clade. In the present sampling the emphasis is on *Leucoagaricus* sect. *Pilovelli* from California and on sect. *Rubrotincti*, but tropical species, such as the attine ant cultivars and the *Leucocoprinus* species with spores without a germ pore, are severely underrepresented.

In the phylogenies based on a wide sampling of the family (Figs. 1, 3; Supplementary Figs. 1 and 2; Vellinga 2004) *Agaricus, Chlorophyllum, Macrolepiota*, and *Lepiota* (including *Cystolepiota*) clades are always recognized. The *Leucoagaricus/Leucocoprinus* clade is only monophyletic in the *rpl2* phylogeny (Fig. 1) presented here with high bootstrap support; in the other phylogenies it is split according to morphology.

The position of *Micropsalliota* is interesting because it forms a monophyletic group with species reckoned to *Leucoagaricus* or *Leucocoprinus* with reddening reactions when exposed to air and spores with a germ pore (i.e. the group of species around *La. americanus*). *Micropsalliota* was considered close to *Agaricus*, based on spore color and general basidiocarp morphology (Pegler and Rayner 1969, Heinemann 1977, Singer 1986). Two morphological facts support this position: The spores of the *La. americanus* group species often are colored, not white; and *Allopsalliota*, sister of *Micropsalliota*, shows the same kind of chemical reactions of the basidiocarps as the *La. americanus* group.

The phylogenies presented here include agaricoid, secotioid and gasteroid taxa; this gives a realistic picture of the family. The family as a whole is well supported, even in the nrLSU based tree; this provides further evidence for the placement of families *Lycoperdaceae*, *Tulostomataceae* and *Podaxaceae* in synonymy with the *Agaricaceae*.

*Position of the new taxa.*—Phylogenies based on any of the four different gene regions are congruent in their
Fig. 2. Phylogeny of *Agaricus* and satellite genera in the Agaricaceae based on nrITS sequences, inferred by maximum likelihood (ML) analysis. Numbers at internodes refer to confidence estimates based on 100 rapid ML bootstraps (only those > 70 are indicated). *Coniolepiota* and *Eriocybe* are highlighted, and genus *Agaricus* and the *Agaricus* clade indicated. *Leucoagaricus barssii* and *La. leucothites* are outgroup taxa.
positioning of *Coniolepiota* and *Eriocybe* close to the *Agaricus* s. str. clade. This result came as a surprise because it was expected that the two white-spored, clampless species, *Coniolepiota spongodes* and *Eriocybe chionea*, would be situated within the *Leucoagaricus*/ *Leucocephrinus* clade or perhaps within the *Cystolepiota*/ *Lepiota* clade, although the vast majority of species in this clade have clamp connections. Clamp
connections are absent in the brown-spored Agaricus and related genera (Hymenagaricus, Heinemannomyces, Micropsalliota), but most species in these genera lack thick velar coverings with species in Agaricus subg. Lanagaricus as the exception.

The position of Coniolepiota and Eriocybe in the Agaricus s.l. clade is not just based on the analysis of one gene region but is apparent in all four phylogenetic trees (Figs 1, 2; SUPPLEMENTARY Figs 1, 2) separately and in the phylogenetic tree based on the combined data (Fig. 3).

After careful evaluation of all datasets and phylogenetic hypotheses, recognition of the two genera seems warranted because there is no support for monophyly of the two together and furthermore the branch lengths are long. No other taxa known yet share the characters of these taxa. All genera in this part of the family, for instance Heinemannomyces and Hymenagaricus, seem to be small, unique in characters and on long branches in the phylogenetic trees.

Eriocybe and Coniolepiota differ from each other in particular in the composition of the pileus and stipe covering. Furthermore in Eriocybe the spores display an immediate and strong dextrinoid reaction in Melzer’s reagent whereas in Coniolepiota the reaction is weak at the utmost. Eriocybe chionea has a strong and particular odor, whereas Coniolepiota taxa have a fungoid odor or smell like the rubber component in the odor of L. cristata, which is common in Lepiota and Leucoagaricus species. Both have relatively small ellipsoid smooth spores, a dense covering of pileus and stipe, which is most likely of velar origin, inconspicuous cylindrical to clavate cheilocystidia, no pleurocystidia, a similar hymenophoral trama made up of inflated hyphae, and they lack clamp connections.

Two other Thai taxa macroscopically resembling L. fuscovinacea (and H. splendidissima, except for spore color) are closely related to C. spongodes and E. chionea based on nrLSU and nrITS data. (Figs 2, 3; SUPPLEMENTARY FIG. 1). Further in-depth study might show that these positions are well supported and that separate genera for these taxa also may be warranted. But there is no support for including either or both with Eriocybe and Coniolepiota in one taxon (Fig. 3).

Spore color traditionally has been the first character to classify gilled mushroom species with a strict separation of light-spored genera and dark (brown to black) spored genera (e.g. Fries 1821). However a close relationship between the white-spored genus Lepiota and brown-spored Agaricus has long been postulated. Singer (1951, 1962, 1975, 1986) in all editions of “The Agaricales in modern taxonomy” united Lepiota and Agaricus in family Agaricaceae, but other authors (e.g. Bon 1981, 1993; Jülich 1982) kept Lepiota in a separate family. Molecular evidence has shown that within the Agaricales dark-spored taxa form a monophyletic clade (Matheny et al. 2007b) but some light-spored taxa (e.g. genus Laccaria) share ancestors with this clade. Vellinga (2004) showed clearly that the monophyletic Agaricaceae harbor species with a wide range of colored spores, with Chlorophyllum as an exemplary genus with white- and green-spored taxa. Here again this finding is repeated with spore color not a determining factor for phylogenetic placement. The Agaricaceae is clearly the family with the widest (and wildest) spore colors: from white to black, via pale lilac, bright greenish yellow, brown, green, dark blue and red.

The positions of Heinemannomyces splendidissima, Clarkeinda trachodes and the new taxa in relation to Agaricus and Hymenoagaricus are not completely resolved. In part this is caused by the different composition of the datasets for the phylogenetic analyses, in part by inherent differences in the various genes and DNA regions.

Heinemannomyces splendidissima has a dark blue spore print and a floccose-squamose dark red-brown pileus covering composed of cylindrical hyphae (Fig. 5D). Watling (1999) suggested that Heinemannomyces is close to Cystoagaricus Singer, but the type species of the genus and the only one so far phylogenetically investigated, C. strobilomyces (Mur- rill) Singer, belongs to the Psathyrellaceae (Vellinga 2004, Padamsee et al. 2008). The data presented here clearly support the position of Heinemannomyces in the Agaricaceae, close to Agaricus s. str. Singer (1947) included Agaricus trisulphuratus Berk. in genus Cystoagaricus, but this too is not supported by the evidence from the phylogenetic analyses (Fig. 2), in which it is placed within genus Agaricus s. str.

Clarkeinda trachodes does not resemble Eriocybe, Coniolepiota or Heinemannomyces because it forms large basidiocarps resembling those of Chlorophyllum rachodes with a universal veil leaving white patches on the pileus and a volva at the base of the stipe, a brown patchy to squamose covering under the white velar patches, an ample but thin annulus, greenish yellow spores with a relatively wide germ pore and no clamp connections (ECV pers obs, Pegler 1985) (Fig. 5C).

Other tropical genera.—A number of tropical white-spored genera in the Agaricaceae has been described for which no sequence data are available. The morphology of each is briefly discussed.

Hiatulopsis Singer & Grinling was described for H. amara (Beeli) Singer & Grinling from Africa, which has large brown detersile scales on whitish background, a cutis as pileus covering, ornamented
spores, no cheilocystidia and no clamp connections (Singer and Grinling 1967). A second species, *H. aureoflava* Singer, described from Brazil (Singer 1989), has a gold to yellow subtly and densely flocculose pileus, no volva, no annulus, ornamented white spores, no cystidia, no clamp connections and a pileus covering composed of irregular hyphae. (Singer 1989).

*Smittiomyces mexicanus* (Murrill) Singer from North America and the Caribbean has white basidiocarps with crowded lamellae, a farinaceous odor, nests of spherical cells among hyphae of the pileus surface, small truncate and ornamented spores, and it is has clamp connections (Vellinga 1999). A position close to genus *Lepiota* seems plausible.

*Januaria amazonica* Singer described from Brazil forms small basidiocarps with a pileus covering with one or more layers of spherical cells, vesiculose cheilocystidia, finely spinulose-punctate spores with a germ pore and no clamp connections (Singer 1986). It is known only from the type collection.

*Rugosocephala* Heinem. with two species (*R. ochraceobadia* [Beeli] Heinem. from Africa and *R. pseudorubiginosa* [Cifuentes & Guzmán] Guzmán & Bandala from Mexico south into South America) with the aspect of *Leucogaricus* in sect. *Pilosellii* has distinctly ornamented, metachromatic spores, clamp connections and a hymeniform pileus covering (Heinemann 1973, Guzmán et al. 1989, Franco-Molano 1995). These four genera are based on one or two species from tropical Africa and America, and all have ornamented spores and a squamose to smooth pileus covering.

Further research of southeastern Asian taxa and tropical Agaricaceae in general is necessary to establish the position of *Micropsalliota* and *Hymenogaricus*. With ongoing research and exploration of tropical regions it is almost certain that more new lineages will be discovered.

**TAXONOMY**

**Coniolepiota** Vellinga, gen. nov.

MycoBank MB518493

Basidiocarpium velo crasso obtectum cellulis laxe dispositis breviter cylindricis saepe ramosis parietibus lileaceis, sporae albae laves sine poro germinali dextrinae, cheilocystidia adsunt, lamellarum trama cellulis inflatis compositis, fibulae desunt.

*Typus generi.—Agaricus spongodes* Berk. & Broome.

**Etymology.**—From the Greek κοφις, dust, and the genus name *Lepiota* because of the pulverulent-scaly aspect of pileus and stipe.

**Coniolepiota spongodes** (Berk. & Broome) Vellinga, comb. nov. Figs. 4A, B, 6

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Pileus 15–110 mm, campanulate to wide-conical, plano-convex to apllanate with slightly inflexed margin, when young covered in lilac, purple-gray (K. & W. 12E F4–5, 11E4, 11–12D3 to 13C3) powdery velar warts, later still covered in center, but in outer zone often gone and showing white to whitish background; covering thick at center, and the powdery warts a bit spread out in outer 1.5 cm, some overhanging margin; marginal zone smooth or slightly fissured, when young exceeding lamellae. Lamellae, L = c. 80, 1 = 1–3, free and remote from stipe with distinct gutter, crowded, not or slightly ventricose, 3–9 mm wide, in some specimens furcate to anastomosing, off white, pale cream to pale yellow, with even to eroded, concolorous edge. Stipe 40–100 × 4–11 mm, cylindrical or tapering upward and slightly wider (up to 8 mm) at base, off white at apex, in lower four-fifths of length with bands of powdery material as on pileus (e.g. 12D3), brown in basal part from handling, hollow, with white tomentose mycelial growth at base. Annulus thin membranous to cortina-like, often attached to pileus margin, white-flocculose above, flocculose and gray-lilac (13C3) on underside. Context thick in pileus, white and dull, in stipe satiny, whitish to brownish in lower one-third, rarely pinkish. Odor like that of *Lepiota cristata* but without the sweetness, or unremarkable and fungoid. Taste fungoid, mild. Spore print white.

Basidiospores [99,5,5] in side view 4.4–6.4 × 2.7–3.7 μm, avl × aw = 4.8–5.1 × 3.0–3.2 μm, Q = 1.4–1.8, av Q = 1.55–1.6, ellipsoid to oblong with flattened abaxial side and rounded apex, ellipsoid to oblong in frontal view, thick-walled, with guttule, with congophilous contents, blue in cotton blue, with pink line around dark blue contents and non-colored wall in cresyl blue, slightly and slowly turning orange in Melzer’s reagent. Basidia 11–21 × 5.0–7.0 (–8.0) μm, four-spored. Lamella edge sterile, with a band of inconspicuous cheilocystidia. Cheilocystidia 13–25 × 6–13 μm, elavate to broadly clavate, some narrowly clavate, hyaline, not colored, sometimes difficult to find. Pleurocystidia not observed. Lamella trama made up of non-colored, inflated long cells, 5–20 μm diam. Pileus covering a powdery layer made up of cylindrical to irregularly branched hyphae, t-shaped.
or with more side branches, forming a three-dimensional structure; individual cylindrical cells 29–60 × 4–10 μm, with refractive connecting points, with lilac parietal, sometimes fine incrusting, pigment. Stipe covering as pileus covering. Clamp connections absent.

Habitat and distribution.—Solitary or in small groups, saprotrophic, on clayey soil, on banks and trails, in disturbed areas of native rainforest, or in lowland rainforest, fruiting during rainy season. Jun-Sep northern Thailand, Singapore, Malaysia, also known from Sri Lanka.


Comments.—Pegler (1972) studied the type collections of C. spongodes and L. euconiata and concluded, just like Petch (1917), that C. spongodes represents an older version of L. euconiata; he described the species.
as follows: “This is a large attractive species, readily recognized by the purplish to lilaceous pulverulent covering of the pileus and stipe.” This description perfectly fits the here described and illustrated species. Microscopically it is striking because of the three-dimensional structure of the pileus-covering elements, the absence of clamp connections, inconspicuous cheilocystidia (hard to find in some specimens) and relatively small, smooth spores.

Pegler (1972, 1986) noted that the spores of *L. spongodes* are dextrinoid. In the Thai material studied the spores are pale or not colored at all in Melzer’s reagent. *Hymenagaricus ardosiaecolor* (Heinem.) Heinem. has comparable elements in the pileus covering but has a brown floccose pileus, brown stipe, brown spores and brown contents in the cheilocystidia and pileus covering elements (Heinemann 1956).

**Coniolepiota aff. spongodes**

Figs. 4C, D, 7

Pileus 53 mm, more or less plano-convex with slightly uplifted margin; center covered in a thick layer of pink-brown-purplish (Munsell 5 YR 5/4) powder, around center with concentric rings and more toward margin patches of irregular heaps of powder (concolorous with center), which are easily rubbed off, on whitish background; background tomentose, not radially fibrillose. Lamellae crowded, free, but not remote from stipe, slightly ventricose up to 5 mm broad, yellowish white, with white irregularly dentate edge. Stipe 30 × 6 mm, more or less cylindrical, whitish, a bit pink, innately lengthwise fibrillose, with 0.5 cm above base a band, 3–5 mm wide, of pink-brown-purplish powdery material, as on pileus. Context white and thick in pileus. Odor fungoid-lepiotoid.

**Basidiocarpium velo albino lanato e hyphis parallelis composito, sporae alvae laeves sine poro germinali dextrinoideae, cheilocystidia adsunt, lamellarum trama hyphis inflatis; fibulis desunt.**

**Typus generis.—** *Eriocybe chionea* Vellinga

**Etymology.—** From the Greek ἔριον = wool and κυβή = head

**Eriocybe Vellinga, gen. nov.**

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Basidiocarpium velo albo lanato e hyphis parallelis composito, sporae alvae laeves sine poro germinali dextrinoideae, cheilocystidia adsunt, lamellarum trama hyphis inflatis; fibulis desunt.

**Typus generis.—** *Eriocybe chionea* Vellinga

**Habitat and distribution.—** Solitary, terrestrial, on open dirt road, in lowland dipterocarp forest, only found in northern Thailand. July.

**Collection examined.—** THAILAND, Chiang Mai Province, Amphur Mae Taeng District, Ban Pao Subdistrict, Sri Lanna National Park, near headquarters, solitary, terrestrial, on dirt road, 12 Jul 2007, E.C. Vellinga ecv3613 (MFLU).

**Comments.—** The differences with *C. spongodes* are the more pink-reddish tinges of the basidiocarp and the cylindrical cheilocystidia (clavate in *C. spongodes*). The color difference might have been caused by the much more open habitat in which this specimen grew. It also differs slightly in nrITS, tef1, and rpb2 sequences. It clearly belongs to the same clade, but because the variability of the morphological and molecular characters is not sufficiently known and only one collection is available it is not described as a new species.

**Eriocybe chionea Vellinga, sp. nov.**

Figs. 5A, B, 8

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Sporeae 4.0–5.2 μm longae 2.3–3.1 μm latae, odor valide foetido.

**Holotypus.—** Thailand, Chiang Mai Province, Mae Taeng District, Ban Pha Deng, Mushroom Research Centre, 19°17.123′N 98°44.009′E, 900 m, 12 Jul 2007, E.C. Vellinga ecv3616 (MFLU).

**Etymology.—** *Chioneus* is the Latin transliteration of the Greek χιόνεος, snow white.

**Pileus** 20–28 mm, plano-convex with deflexed margin to more or less planate set with cotton-wool-like broad fluffy warts, composed of hyphae, pure white, with margin exceeding lamellae. Lamellae, 1 = 0–1(–3), crowded to crowded free, not remote from stipe, rounded near stipe, ventricose, 2–3 mm wide, (pale) cream white, with white, even to subtly cystidiose edge. Stipe 18–22 × 4 mm, slightly narrower at apex, white, pale cream white and smooth above annular zone, below that with white fluff in bands or all over floccose, hollow. Annulus a widened
white cottony, slightly flaring zone on stipe. Context white in pileus and stipe, thick in pileus. Odor spicy, camphor-like, totally different from any other species (ecv3560); strongly unpleasant, like rancid coconut (ecv3616). Taste not tested.

Basidiospores [40,2,2] in side view 4.0–5.2 × 2.3–3.1 μm, avl × awv = 4.5–4.6 × 2.9 μm, Q = 1.45–1.85, avQ = 1.55–1.6, ellipsoid to oblong, in frontal view ellipsoid to oblong, slightly thick-walled, smooth, without germ pore, often with guttule, congophilous, immediately dextrinoid in Melzer’s reagent, in cresyl blue with non-colored wall, and a pink line around blue contents, with blue walls in cotton blue. Basidia 13–16 × 5.0–7.0 μm, four-spored, with scattered two-spored basidia. Lamella edge sterile, set with cystidia. Cheilocystidia 12–28 × 4.5–10 μm, cylindrical to narrowly clavate in one collection (ecv3616), and clavate, pedunculate fusiform, to narrowly clavate in the other (ecv3560). Pleurocystidia absent. Hymenophoral trama composed of cylindrical to inflated non-colored hyphae, 7–12 μm diam. Pileus covering a big felt-like mat composed of slightly inflated to cylindrical, non-pigmented hyphae with elements 45–100 × 9.0–17 μm on top, with a big layer of narrow, 2.0–4.0 μm wide cylindrical, non-pigmented hyphae. Stipe covering as pileus covering. Clamp connections absent.

Habitat and distribution.—Solitary, terrestrial, on road verge in mixed rain forest and on steep vertical red clay bank, 900–1300 m, in northern Thailand, fruiting during the rainy season (Jun–Jul). Found only twice so far.

Collections examined.—THAILAND, Chiang Mai Province, Chiang Mai district, Doi Suthep National Park, 26 Jun 2007, E.C. Vellinga ecv3560 (MFLU); Mae Taeng District, Ban Pha Deng, Mushroom Research Centre, 19°17.123’N, 98°44.009’E, 900 m, MRC grounds, 12 Jul 2007, E.C. Vellinga ecv3616 (HOLotype, MFLU).
Comments.—Eriocybe chionea is characterized by the small relatively short-stiped white felted-woolly basidiocarps with an unusual odor among the Agaricaceae; microscopically it is defined by the combination of spore size, narrowly clavate to clavate-fusiform cheilocystidia, the thick pileus covering composed of wide parallel hyphae and the absence of clamp connections.

**Fig. 6.** Coniolepia spongodes, microscopical characters: A, E. spores; B, F. basidia; C. cheilocystidia; D, G. pileipellis elements. (A–D from collection png012; E–G from collection ecv3816). Bar = 10 μm.

**Fig. 7.** Coniolepia aff. spongodes, microsporic characters: A. spores, B. basidia, C. cheilocystidia, D. pileipellis elements (from collection ecv3613). Bar is 10 μm.
Agaricus subg. Lanagaricus Heinem. comes close morphologically but has brown spores. Agaricus trisulphuratus Berk., the type species of this subgenus, has basidiocarps covered in pointed orange scales, which are composed of parallel cylindrical hyphae with incrusting pigment, a regular hymenophoral trama composed of cylindrical hyphae up to 7 µm diam and subutriform cheilocystidia (Vellinga pers obs).

Species of Agaricus subg. Conioagaricus Heinem. also resemble Eriocybe because they have a velvety, powdery or squamulose pileus covering composed of ellipsoid to inflated short cells, which often have incrusted cell walls or an ornamentation composed of warts or spines (Heinemann 1956), but the stipe lacks this covering and of course the spores are brown. This subgenus has, as does Agaricus subg. Lanagaricus, a mainly tropical distribution.

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