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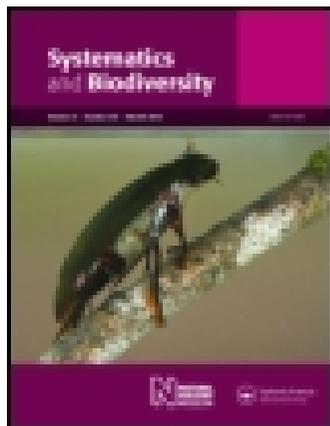
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### Crassisporium and Romagnesiella: two new genera of dark-spored Agaricales

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## Research Article

# *Crassisporium* and *Romagnesiella*: two new genera of dark-spored Agaricales

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A systematic study of a rare and enigmatic European species, *Galerina clavus* Romagn., is presented. Phylogenetic analyses show it to be most closely related to *Pachylepyrium carbonicola* (A.H. Sm.) Singer and *P. funariophilum* (M.M. Moser) Singer (Strophariaceae). Investigation of additional species of *Pachylepyrium* suggests this genus is polyphyletic as the type species, *P. fulvidula* (Singer) Singer, is nested in the Tubariaceae Vizzini based on multigene phylogenetic analyses. *Pachylepyrium nubicola* Singer is allied with *Pholiota* (Fr.) P. Kumm. based on high ITS similarity, and *P. carbonicola* and *P. funariophilum*, together with *G. clavus*, form a clade among a consortium of Strophariaceae Singer & A.H. Sm. and Hymenogastraceae Vittad. As a result, we propose *Romagnesiella* gen. nov. to accommodate *G. clavus*, for which a taxonomic description is given and lectotype and epitype are designated. The genus *Crassisporium* gen. nov. is proposed to encompass *Pachylepyrium funariophilum* (of which *P. carbonicola* is considered a younger taxonomic synonym), *P. chilense* M.M. Moser, and *P. squarulosum* Singer. *Crassisporium* is distinguished from *Romagnesiella* by its thick-walled basidiospores and occurrence in burnt habitats. The identities of the morphologically similar *Tubaria umbonata* S. Lundell, *T. embolus* (Fr.) Sacc. and *T. minima* J.E. Lange are also discussed.

**Key words:** Agaricoid clade, carbonicolous fungi, Hymenogastraceae, *Pachylepyrium*, Strophariaceae, taxonomy, taxon sampling, types

## Introduction

Considerable progress has been made to assess phylogenetic relationships in the Agaricales (Binder, Larsson, Matheny, & Hibbett, 2010; Garnica, Weiss, Walther, & Oberwinkler, 2007; Matheny, et al., 2006; Moncalvo et al., 2002), the largest order of mushroom-forming fungi with some 13 500 described species (Kirk, Cannon, Minter, & Stalpers, 2008). However, continued assessment of evolutionary relationships within the order is necessary. For instance, taxa from the tropics and southern hemisphere are in need of better integration into more inclusive molecular systematic treatments (Matheny et al., 2009; Rees, Midgley, Marchant, Perkins, & Orlovich, 2013), and some species are known only from type collections, of insufficient age for adequate gene

sampling, missing or unavailable (Ammirati, Parker, & Matheny, 2007; Baroni & Matheny, 2011).

The genus *Galerina* Earle (Agaricales), typified by *G. vittiformis* (Fr.) Singer, traditionally encompasses saprotrophic dark-spored agarics often with small and slender fruit bodies with a bell-shaped pileus (mycenoid or collybioid in habit), straight pileal margin, attached lamellae, presence of veil, and an ochre to rusty brown spore deposit. Spores of *Galerina* are typically yellow to dark tawny in KOH (potassium hydroxide), amygdaliform to elliptic, often verruculose or rugulose, and lack a well-defined germ pore. The spores of many species of *Galerina* are also characterized by a smooth region above the apiculus on the adaxial side of the spore (this is known as a plage) (Bon, 1992; Gulden, 2012; Watling & Gregory, 1993). The first (and only) detailed phylogenetic assessment of *Galerina* strongly suggests the genus is

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**Fig. 1–2.** Fruit bodies of *Galerina clavus* in situ (PAM06090110). Photo by P.-A. Moreau. Scale bar = 10 mm. (**Fig. 2**) Fruit bodies of *Pachylepyrium carbonicola* in situ (PBM2293, WTU). Scale bar = 10 mm. Photo by P.B. Matheny.

polyphyletic (Gulden, Stensrud, Shalchian-Tabrizi, & Kausserud, 2005).

*Galerina clavus* Romagn. (Fig. 1) is a small inconspicuous species published in 1944 by Romagnesi (1942) from Europe that displays a combination of anomalous characters for the genus: namely, its naucorioid habit (small size, pileus with a decurved margin), smooth spores without a plage, and absence of a veil. The combination of these traits cast doubts on an alliance with *Galerina* (de Haan & Walley, 2009; Moreau, 2009). Smith & Singer (1964) treated *G. clavus* in their world monograph of *Galerina* but placed it, together with the South American species *G. fuegiana* Singer, in an isolated section *Pseudotubaria* A.H. Sm. & Singer. This classification has been used by Bon (1992), Horak (2005), Moser (1978, 1983) and Singer (1986). Molecular systematic studies of *Galerina* and other dark-spored agarics (Aime, Vilgalys, & Miller, 2005; Garnica, Weiss, Walther, & Oberwinkler, 2007; Gulden, Stensrud, Shalchian-Tabrizi, & Kausserud, 2005; Matheny *et al.*, 2006, 2007a; Moncalvo *et al.*, 2002; Petersen, Knudsen, & Seberg, 2010; Walther, Garnica, & Weiß 2005) have not included *G. clavus*, and thus its systematic position remains ambiguous.

Based on preliminary phylogenetic analysis of nuclear ribosomal RNA (rRNA) gene sequences, samples of *G. clavus* clustered together with sequences of the North American species *Pachylepyrium carbonicola* (A.H. Sm.) Singer (Fig. 2). The genus *Pachylepyrium* Singer (Agaricales, Strophariaceae; type: *P. fulvidula* (Singer) Singer), however, contains seven accepted species that differ from *G. clavus* by their thick-walled spores typically with a germ pore and presence of a veil. Furthermore, most species are carbonicolous (Claridge, Trappe, & Hansen, 2009; McMullan-Fisher *et al.*, 2011), fruiting among burnt debris or on burnt ground in co-occurrence with bryophytes (*viz.* *Funaria* Hedwig) or on wood (lignicolous) (Moser, 2000; Singer, 1986). Thus, a substantial taxonomic emendation would be required to place *G. clavus* within *Pachylepyrium*.

To confirm phylogenetic and taxonomic relationships to other species of *Pachylepyrium* (*viz.* the type species of the genus, *P. fulvidula*), we produced molecular data from four of seven type collections of species accepted in this genus. We also studied a fifth type collection morphologically and several collections of European naucorioid fungi showing similar characters to *G. clavus*, including *Tubaria umbonata* S. Lundell, *T. minima* J.E. Lange and *T. embolus* (Fr.) Sacc.

Collections of *Pachylepyrium* are not common (Moser, 2000), probably owing to their specific habitat (mostly burnt areas), which is in decline in regions of Europe (Veerkamp 1998). While dense taxon sampling from broad geographic areas is a laudable goal, our focus is to produce a contemporary taxonomic revision based on available type materials. To accomplish this and a more thorough systematic comparison with *G. clavus*, we carried out a multi-gene phylogenetic analysis with an emphasis on the Agaricoid clade (Matheny *et al.*, 2006) to investigate the relationship of *G. clavus* to *Pachylepyrium*.

## Materials and methods

### Morphological analysis

Collections of fruit bodies were studied from the personal herbarium of M. Contu and material preserved at IB, K, LIP, MICH, MPU, PC and TENN. Herbarium designations follow Thiers (continuously updated). Colour designations in the format '(5E7)' refer to plate, column and row of Kornerup & Wanscher (1967). Microscopic observations were made in 5% KOH, Melzer's reagent and water mounts. Spores were measured on a Moticam 1000 video camera connected to a Nacet Andromede 0181 compound microscope or on a Nikon Eclipse 80i using NIS Elements (D) imaging software. First and ninth deciles (D1, 9) and average values (*italicized and in bold*) are presented according to Fannechère (2005, 2009).

## Type collections examined for molecular and taxonomic analyses

To evaluate the relationship between *Galerina clavus* and *Pachylepyrium*, we performed molecular and/or morphological annotations of five *Pachylepyrium* species, including type collections and the type species of the genus (*P. fulvidula*). The type of *G. clavus* is missing. *Pachylepyrium* types studied by us include: *P. carbonicola* AHS44640 (holotype of *Kuehneromyces carbonicola* A.H. Sm., MICH); *P. funariophilum* IB 1949/0008 (holotype of *Pholiotina funariophila* M.M. Moser); *P. nubicola* Singer K (M 181790) (holotype); *P. fulvidula* T1495 (isotype of *Phaeomarasmius fulvidulus*, MICH); and *P. chilense* M.M. Moser M3269 (paratype, MICH) (see also Appendix 1, online supplemental material, which is available from the article's Taylor & Francis Online page at <http://dx.doi.org/10.1080/14772000.2014.967823>). We obtained a loan of part of the holotype (IB) of *P. chilense*, but the material was inadequate for destructive sampling. Material of representative *G. clavus* sequenced included PAM06090110 (LIP) and Contu 15122007 (pers. herb.). To assess the taxonomic relationship of *G. clavus* to *Tubaria umbonata* and *T. embolus*, we examined the isotype of *T. umbonata* (ex Fungi exsiccati Suecici 2041 (PC)) and accessions labelled as '*Galerina embolus*' (Fr.) P.D. Orton (Bon 741120, Bon 70624) at LIP. Other taxa selected for phylogenetic analyses are listed in Appendix 1 (see supplemental material online).

## DNA extraction, PCR and Sanger sequencing

Procedures for DNA extraction, PCR and Sanger sequencing follow those outlined in Matheny et al. (2007b); Matheny, Austin, Birkebak, & Wolfenbarger (2010) except where mentioned below. For collections older than 30 years, we used an E.Z.N.A. High Performance (HP) Fungal DNA kit (Omega Bio-Tek, Norcross, Georgia, USA). We sampled the ITS region, including the 5.8S gene, using primers ITS1F and ITS4 (Gardes & Bruns 1993; White, Bruns, Lee, & Taylor, 1990); the 5' end of the nuclear 25S large subunit ribosomal RNA gene region (nLSU) using primers LR0R and LR7 or LR5 (Vilgalys & Hester, 1990); almost the entire nuclear 18S small subunit ribosomal RNA gene (nSSU) between primers PNS1 and NS8 (O'Donnell, Cigelnik, & Benny, 1998; White, Bruns, Lee, & Taylor, 1990); and the most variable region of rpb2 between conserved domains 6 and 7 using primers b6F and b7.1R (Matheny, 2005). All new sequences have been deposited in GenBank (shown in bold in Appendix 1, see supplemental material online).

## DNA alignments and phylogenetic analyses

We manually aligned 151 of our 153 new nLSU, nSSU, 5.8S and rpb2 sequences from 42 taxa, including type collections of *Pachylepyrium funariophilum* and *P. fulvidula* (type species of *Pachylepyrium*) with alignments

produced by Matheny et al. (2006) in MacClade 4.08 (Maddison & Maddison, 2005). We were only able to obtain ITS sequences from type collections of *Pachylepyrium nubicola* and *Pachylepyrium carbonicola*; thus, these were not added to our alignment due to insufficient variation across the 5.8S locus. Integration of ITS1 and ITS2 spacer sequences was not possible due to their high substitution rates. However, we did add sequences from two conspecific collections of *P. carbonicola* determined as such by A.H. Smith (sequences of which did not differ from the holotype). The datasets were pruned to exemplars of the Tricholomatoid clade and all members of the Agaricoid clade following Matheny et al. (2006). To these we added rRNA and/or rpb2 sequences of *Leucoagaricus barssii* (Zeller) Vellinga (Matheny et al., 2007b) and *Pseudoclitocybe cyathiformis* (Bull.: Fr.) Singer from Binder, Larsson, Matheny, and Hibbett (2010) and rRNA and/or rpb2 sequences of *Squamanita paradoxa* (A.H. Sm. & Singer) Bas, *Mycocalia denudata* (Fr. & Nordholm) J.T. Palmer and *Nidula niveotomentosa* (Henn.) Lloyd from Matheny & Griffith (2010). LSU and 5.8S sequences (AF261513, EF051055, EF051060) of '*Pachylepyrium funariophilum*' from Moncalvo et al. (2002) and '*Tubaria minima*' of Matheny et al. (2007a) were also added.

Alignments were concatenated in MacClade in a non-interleaved format with the final supermatrix composed of 170 taxa. Sixty-five taxa (38%) lacked rpb2 sequences, 21 (12%) lacked 5.8S sequences and 27 (16%) lacked SSU sequences. Several studies (Wiens, 2006; Wiens & Moen, 2008; Wiens & Tu, 2012) demonstrate that incorporation of incompletely sampled taxa in supermatrices improves phylogenetic accuracy, if the overall number of characters is sufficiently large, thus supporting a supermatrix approach. The concatenated alignment and tree files have been submitted to TreeBASE (S15353).

We converted the concatenated supermatrix from nexus format to a relaxed phylip format in Seaview version 4.2.4 (Gouy, Guindon, & Gascuel, 2010) after inspection for strongly supported conflict (>70%) between rRNA and rpb2 gene trees following Matheny (2005) using Maximum Likelihood (ML) bootstrapping as indicated below. The resulting concatenated rRNA and rpb2 phylip file contained 4508 total sites: 1451 sites from LSU, 1782 sites from SSU, 158 sites from the 5.8S gene and 1117 sites from the rpb2 gene region between conserved domains 5 and 7 after trimming staggered ends. A partition text file was created to model the rRNA gene regions (positions 1–3391) separately from first, second and third codon positions of rpb2 (positions 3392–4508) to allow separate GTRGAMMA models for each partition following model selection in Matheny et al. (2006) and recommendations made in the RAXML user manual (Stamatakis, 2006). Thus, four unique partitions were established with one for the rRNA gene regions and three separate partitions for each rpb2 codon position.

RAxML version 7.2.8 was used to generate 1000 rapid bootstraps and a final ML tree with all free model parameters estimated by the program. The same partitions were invoked using the parallel version of MrBayes 3.1.2 (Altekar, Dwarkadas, Huelsenbeck, & Ronquist, 2004; Ronquist & Huelsenbeck, 2003) for a Bayesian inference of the phylogeny with each partition modelled according to GTR+I+G following Matheny *et al.* (2006). This analysis entailed two independent runs for 50 million generations sampling trees and other parameters every 5000 generations on the Newton High Performance Computing cluster at the University of Tennessee. The average standard deviation of split frequencies was used as a metric to determine an appropriate burn-in. Trees were viewed in FigTree version 1.4.0 (Rambaut, 2009). *Pseudoclitocybe cyathiformis* was used for rooting purposes based on Binder *et al.* (2010). ML bootstrap proportions are referred to as MLBP and Bayesian posterior probabilities as BPP.

## Results

### *Pachylepyrium* is polyphyletic

152 ITS, 168 nLSU, 151 sSSU and 105 rpb2 sequences, 153 of which are new, were analysed for this study. The average standard deviation of split frequencies reached less than 0.01 by the 27 795 000th generation in the Bayesian inference analysis of the four-gene region supermatrix. We sampled trees every 5000 steps (producing a total of 10 001 trees for each of the two 50 million generation runs); thus, we conservatively burned the first 6001 trees, including the initial starting tree. Posterior probabilities were calculated from a total sample of 8000 trees (4000 from each run). Comparison of the rRNA-only ML phylogeny with that of the rpb2 ML phylogeny (data not shown) revealed no significantly supported conflicts.

The genus *Pachylepyrium* is polyphyletic (Fig. 3). The type species, *P. fulvidula*, clusters with strong support (80% MLBP, 0.99 BPP) among a grade of lineages that includes *Phaeomarasmius* Scherff., *Flammulaster* Earle and *Phaeomyces* E. Horak in the Tubariaceae. *Pachylepyrium carbonicola* and *P. funariophilum* cluster with strong support with *Galerina clavus* (90% MLBP, 1.0 BPP) forming a weakly supported sister group to exemplars of the Strophariaceae and Hymenogastraceae. ITS sequences of type collections of *Pachylepyrium carbonicola* and *P. funariophilum* differ only at two positions excluding polymorphic sites. A blast analysis of the ITS sequence of the holotype of *Pachylepyrium nubicola* strongly suggests this species belongs to the genus *Pholiota* (96% similar to *Ph. terrestris* HQ604756, 95% similar to *Ph. gummosa* JF908581 and 95% similar to numerous other ITS sequences of *Pholiota*).

Two collections (TENN053270, TENN05174) labelled *Pachylepyrium funariophilum* are incorrectly identified and not identical to each other. TENN053270, from Washington state, likely represents a species of *Psilocybe*

as first suggested by Walther, Garnica, and Weiß (2005), whereas TENN051714, collected in North Carolina, is an unidentified species of *Deconica*. Morphological examination of both collections affirms these results.

The monophyly of the Agaricoid clade of Matheny *et al.* (2006) is for the first time highly supported (77% MLBP, 1.0 BPP). Monophyly of the Agaricoid clade was recovered with significant BPP in Garnica *et al.* (2007) and Matheny *et al.* (2006), but with poor maximum parsimony bootstrap support. The Hydnangiaceae is indicated as the sister group to the rest of the Agaricoid clade but with high BPP only (0.99). A grouping of the Cortinariaceae, Bolbitiaceae, Tubariaceae, Inocybaceae, Crepidotaceae, Strophariaceae, Hymenogastraceae, and *Agrocybe erebia* receives poor bootstrap support but high BPP (0.99). Taken together, eight of 12 families in the Agaricoid clade (Inocybaceae, Tubariaceae, Bolbitiaceae, Cortinariaceae, Agaricaceae, Psathyrellaceae, Nidulariaceae, Hydnangiaceae) receive strong statistical support in our analyses (MLBP >70% and BPP >0.95). The Hymenogastraceae and Crepidotaceae are supported with only high BPPs (0.99). The Squamanitaceae is recovered as monophyletic, but with poor support. Similar to Matheny *et al.* (2006) the Hymenogastraceae and Strophariaceae are recovered as sister groups with a high posterior probability. Unlike Matheny *et al.* (2006) samples of *Gymnopilus*, which previously were placed in an isolated position with the Agaricoid clade, now cluster with samples of *Galerina* in the Hymenogastraceae but without strong support.

The lineage containing *Pachylepyrium funariophilum* and *P. carbonicola* is proposed as a new genus based on molecular, morphological and ecological distinctions between it and the lineage containing *Galerina clavus*, and due to the placement of the type of *Pachylepyrium* in the Tubariaceae. A separate genus is proposed to accommodate *G. clavus* due to differences in morphology and ecology with respect to *Pachylepyrium funariophilum* and *P. carbonicola*. All three taxa, however, are united by their basidiospores that darken to various shades of reddish brown in KOH. The spores of *Pachylepyrium fulvidula* are brownish yellow to yellowish brown in water mounts and darken to brown (not reddish brown) in KOH.

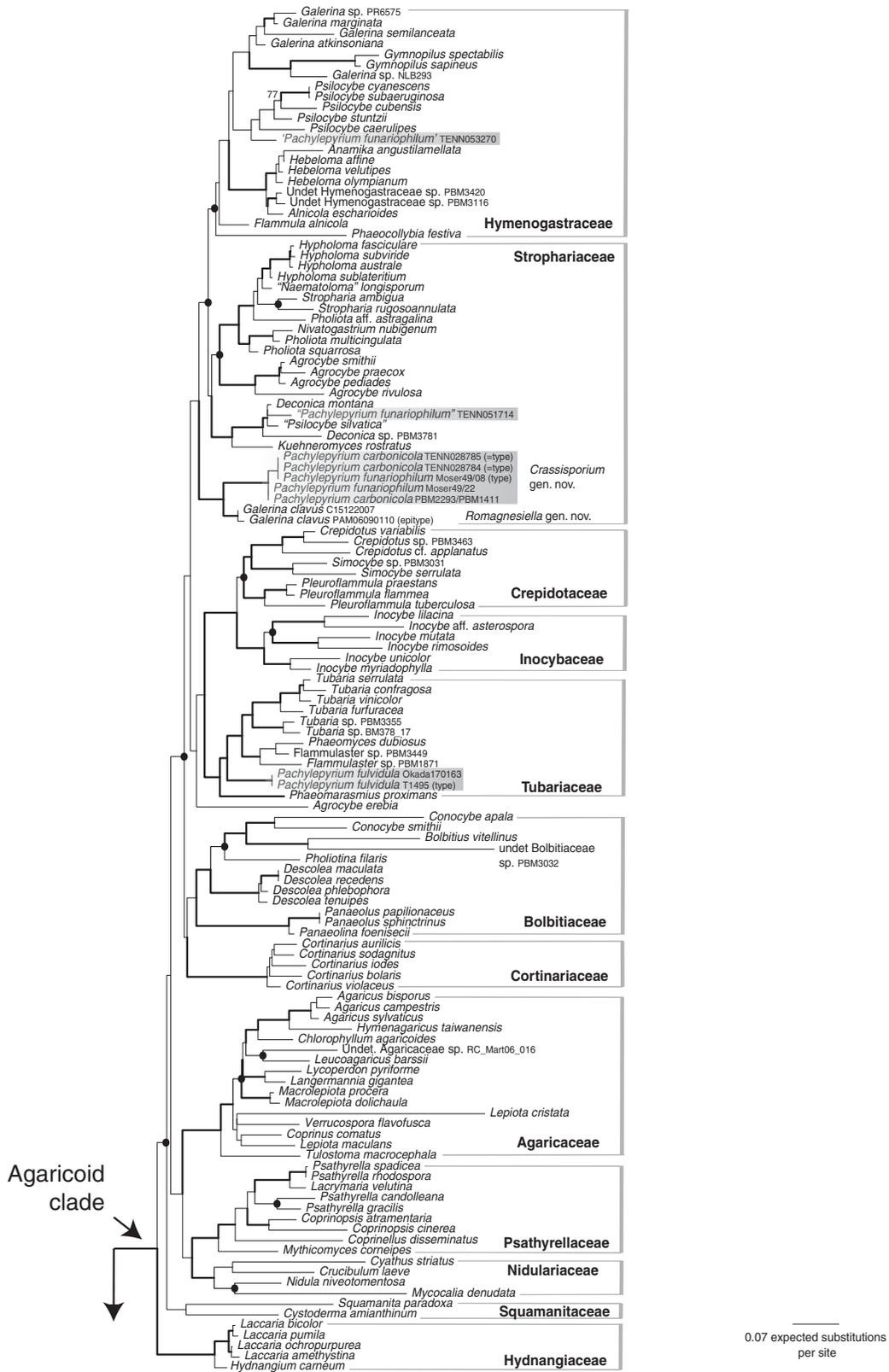
## Taxonomy

*Crassisporium* Matheny, P.-A. Moreau & Vizzini  
gen. nov.

MYCOBANK No: MB 807853.

TYPE SPECIES: *Pholiotina funariophila* M.M. Moser, 1954.

ETYMOLOGY: crassus, Latin, means thick, and sporium, Latin, spore; in reference to the thick-walled basidiospores (gender: neuter).



**Fig. 3.** Phylogeny of the Agaricoid clade based on a Maximum Likelihood and Bayesian Inference analysis of a supermatrix of four nuclear gene regions (5.8S rRNA, LSU-rRNA, SSU-rRNA and rpb2 conserved domains 5–7). Thickened branches indicate ML bootstrap support >70% and Bayesian posterior probability >0.95. Nodes that receive Bayesian posterior probabilities >0.95 but with <70% ML bootstrap are indicated by small black-filled circles. Clade nomenclature follows Matheny et al. (2006). Grey shaded taxon labels indicate placement of species of *Pachylepyrium* or collections mislabelled *Pachylepyrium*.

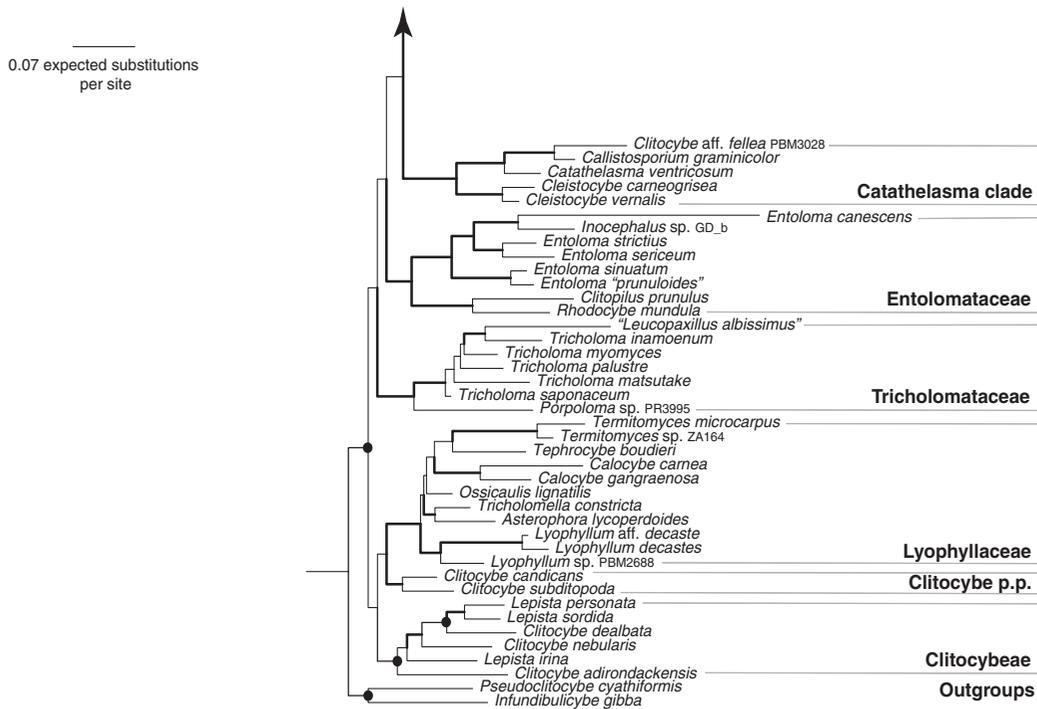


Fig. 3. (Continued)

DIAGNOSIS: Basidiomata naucorioid, pileus hygrophalous, veil present. Lamellae attached (adnate). Basidiospores smooth, ovate or subangular, wider in face view than in profile, thick-walled ( $>0.5 \mu\text{m}$  thick) and with a broad or conspicuous germ pore (often  $>0.5 \mu\text{m}$  wide), brownish yellow to golden yellow in water mounts, becoming rusty brown to reddish brown or rich reddish cinnamon in KOH. Pleurocystidia and chrysocystidia absent, cheilocystidia present. Pileipellis a cutis, not gelatinized. Hymenophoral trama regular to subregular, subhymenium not gelatinized. Clamp connections present. Carbonicolous. Typus: *Pholiotina funariophila* M.M. Moser, in Kühner & Romagnesi, Bull. Soc. nat. Oyonnax 8: 43, 1954.

*Crassisporium chilense* (M.M. Moser) Matheny, P.-A. Moreau & Vizzini, comb. nov.

MYCOBANK no: MB 807854.

BASIONYM: *Pachylepyrium chilense* M.M. Moser, Hoppea 61: 268, 2000, holotype seen. Chile.

### Remarks

The carbonicolous habit and the smooth thick-walled basidiospores (*c.*  $1.0 \mu\text{m}$  thick) with a distinct broad germ pore and rust brown colouration in KOH support transfer to *Crassisporium*. The species has been recorded from high-elevation (550 m) Nothofagus forests in Chile and differs from *P. funariophilum* by the somewhat smaller spores

( $7.0\text{--}8.5 \times 4.5\text{--}6.0 \mu\text{m}$  versus  $7.5\text{--}10.0 \times 5.5\text{--}7.0 \mu\text{m}$ ) and a more weakly developed and ochraceous veil, which is more strongly developed and white in *P. funariophilum* (Moser, 2000). Unfortunately, material sent to us from the holotype collection was not sufficient for examination and DNA extraction. However, upon examination of materials labelled *Pachylepyrium funariophilum* located at MICH, we found the spores of one collection (M3269, Valdivia, Chile, including one small fruit body) to measure  $7.0\text{--}8.5 \times 4.5\text{--}6.0 \mu\text{m}$ . Based on the taxonomic key below, this material keys out to *Crassisporium chilense* due to the smaller spores and occurrence in Chile. Moser (2000) mistakenly cites two different collections (M3208 in the Latin diagnosis and M3269 in the German description) as the isotype of *Pachylepyrium chilense*. We suspect the designation of M3269 as isotype is an error as the referenced collection date (3 Mar. 1963) in Moser's publication does not match that for M3269 (31 Mar. 1963). Under this scenario M3269 is the paratype. Unfortunately, the collection date on the holotype packet (IB 1963/0210) is 30 Mar. 1963 (unlike the protologue), but the locality is exactly that of M3269. Permission to extract DNA from the one small fruit body of M3269 (paratype) was not granted owing to the inadequate condition of this material.

*Crassisporium funariophilum* (M.M. Moser) Matheny, P.-A. Moreau & Vizzini, comb. nov.

(Fig. 2, as *Pachylepyrium carbonicola*).

MYCOBANK No: MB 807855.

BASIONYM: *Pholiotina funariophila* M.M. Moser, in Kühner & Romagnesi, Bull. Soc. nat. Oyonnax 8: 43, 1954, holotype seen. Austria.

≡ *Pachylepyrium funariophilum* (M.M. Moser) Singer, in Singer & Moser, Mycopath. Mycol. Appl. 26(2-3): 171, 1965.

= *Kuehneromyces carbonicola* A.H. Sm., Beihefte zur Sydowia 1: 53, 1957. Holotype seen. Idaho.

≡ *Pachylepyrium carbonicola* (A.H. Sm.) Singer, Sydowia 11: 321, 1958 [1957].

≡ *Pholiota subangularis* A.H. Sm. & Hesler, The North American Species of Pholiota: 44, 1968.

## Remarks

*Crassisporium funariophilum* is geographically widespread occurring in Europe, northern Africa and western North America (where it has been referred to as *Pachylepyrium carbonicola* and *Pholiota subangularis*) and may be expected elsewhere. Singer & Moser (1965) and Singer (1969) also report it from Argentina, but this material has not been revised in light of description of *P. chilense* (see above). Moser (2000) describes collections of *P. carbonicola* with a white fugacious veil and similar ecology to *P. funariophilum*, but with somewhat larger spores ( $8.2\text{--}12.1 \times 6.5\text{--}8.3 \mu\text{m}$ ) than for *P. funariophilum* ( $7.6\text{--}10.0 \times 5.3\text{--}7.1 \mu\text{m}$ ). However, pairwise comparison of ITS sequences from the type collections of *C. funariophilum* and *P. carbonicola* differ at only two nucleotide positions (excluding three polymorphic sites among the five sequences considered) strongly suggesting the two species are conspecific. As such, *P. funariophilum* has nomenclatural priority.

*Crassisporium squarulosum* (Singer) Matheny, P.-A. Moreau & Vizzini, comb. nov.

MYCOBANK no: MB 807856.

BASIONYM: *Pachylepyrium squarulosum* Singer, Beih. Nova Hedwigia 29: 281, 1969, holotype not seen. Chile.

## Remarks

We have not studied material of *C. squarulosum*, but the thick spore wall with a truncate germ pore and intense 'ferruginous' colouration in KOH described by Singer (1969) are consistent with placement in *Crassisporium* rather than with the type of *Pachylepyrium* in the Tubariaceae or with *Romagnesiella*. The species is associated with burnt debris and occurs at high elevations (1000 m) in Chile. The type (M 6550) is reportedly at SGO. The species differs most readily from *C. chilense* by the

flocculose-squarrose pileus surface and longer spores ( $12.0\text{--}14.0 \times 6.5\text{--}8.0 \mu\text{m}$ ).

*Pholiota nubicola* (Singer) Matheny & P.-A. Moreau, comb. nov.

MYCOBANK No: MB 807857.

BASIONYM: *Pachylepyrium nubicola* Singer in Dennis, Kew Bull. 15(1): 139, 1961, holotype seen. Venezuela.

## Remarks

The ITS sequence produced from the holotype strongly suggests that *Pachylepyrium nubicola* is a species of *Pholiota*. Consistent with this placement are the caespitose and lignicolous habit, paler (yellowish) pigmented basidiospores with a thinner wall than in *Crassisporium*, strongly gelatinized pileipellis composed of coarsely incrustated yellowish hyphae, and gelatinized subhymenial trama. Add to this the slightly phaseoliform spores with a distinct germ pore ( $0.8\text{--}1.0 \mu\text{m}$  wide) and the squamulose stipe covering, it is not surprising *P. nubicola* would be closely related to *Ph. gummosa* (Lasch: Fr.) Singer as described by Holec (2001).

In contrast to the protologue, our examination of the type revealed a gelatinized pileipellis and cylindrical to subphaseoliform non-dextrinoid basidiospores, these with a distinct germ pore. The spores measure  $7.5\text{--}8.8 \times 4.5\text{--}4.8 \mu\text{m}$ , which is consistent with the protologue. The basidia measure  $17\text{--}28 \times 7\text{--}8 \mu\text{m}$  with yellowish contents when mature. The lamellar edge was observed to be sterile and yellow but without reviving elements. The presence or absence of chrysocystidia could not be confirmed, but given the high sequence similarity to ITS sequences labelled *Ph. gummosa* and *Ph. terrestris*, we predict chrysocystidia will be found in this species.

## A taxonomic key to species of *Crassisporium*

- 1(a) Pileus surface flocculose-squarrose, spores  $12\text{--}14 \times 6.5\text{--}8 \mu\text{m}$ . . . . . *C. squarulosum* Singer  
 1(b) Pileus surface glabrous or with marginal fibrils, spores  $7\text{--}11.5 \times 5.5\text{--}7 \mu\text{m}$ . . . . . 2  
 2(a) Spores mostly  $8\text{--}11.5 \times 5.5\text{--}7 \mu\text{m}$ , in north temperate forests of Europe, North Africa and western North America (also reported from southern South America, but this is likely *C. chilense*); veil well developed, white. . . . . *C. funariophilum* (M.M. Moser) Singer  
 2(b) Spores mostly  $7\text{--}9 \times 4.5\text{--}6 \mu\text{m}$ , in *Nothofagus* forests in southern South America; veil weakly developed, ochraceous. . . . . *C. chilense* M.M. Moser

*Romagnesiella* Contu, P.-A. Moreau, Vizzini & A. de Haan, gen. nov.

MYCOBANK No.: MB 519559.

TYPE SPECIES: *Galerina clavus* Romagn., 1944 [1942].

ETYMOLOGY: named in honour of Henri Romagnesi, French mycologist (1912–1997) (gender: feminine).

DIAGNOSIS: Basidiomata naucorioid, lamellae distant, adnate to subdecurrent; pileus dry, not hygrophanus; stipe smooth, without a partial veil. Basidiospores smooth, more or less ovate, not subangular, yellow in water mounts, reddish ochre in KOH, not dextrinoid, germ pore absent; necrobasidia numerous; cheilocystidia present, edges of lamellae smooth and (sub)sterile, pleurocystidia present but dispersed and infrequent, pileipellis filamentous, hymenophoral trama regular, clamp connections frequent. On unburnt soil or sand among mosses and grasses.

Typus: *Galerina clavus* Romagn., Bull. Trimest. Soc. Mycol. France 58(4): 149 (1944 [1942]).

*Romagnesiella clavus* (Romagn.) Contu, P.-A. Moreau, Vizzini & A. de Haan, comb. nov.

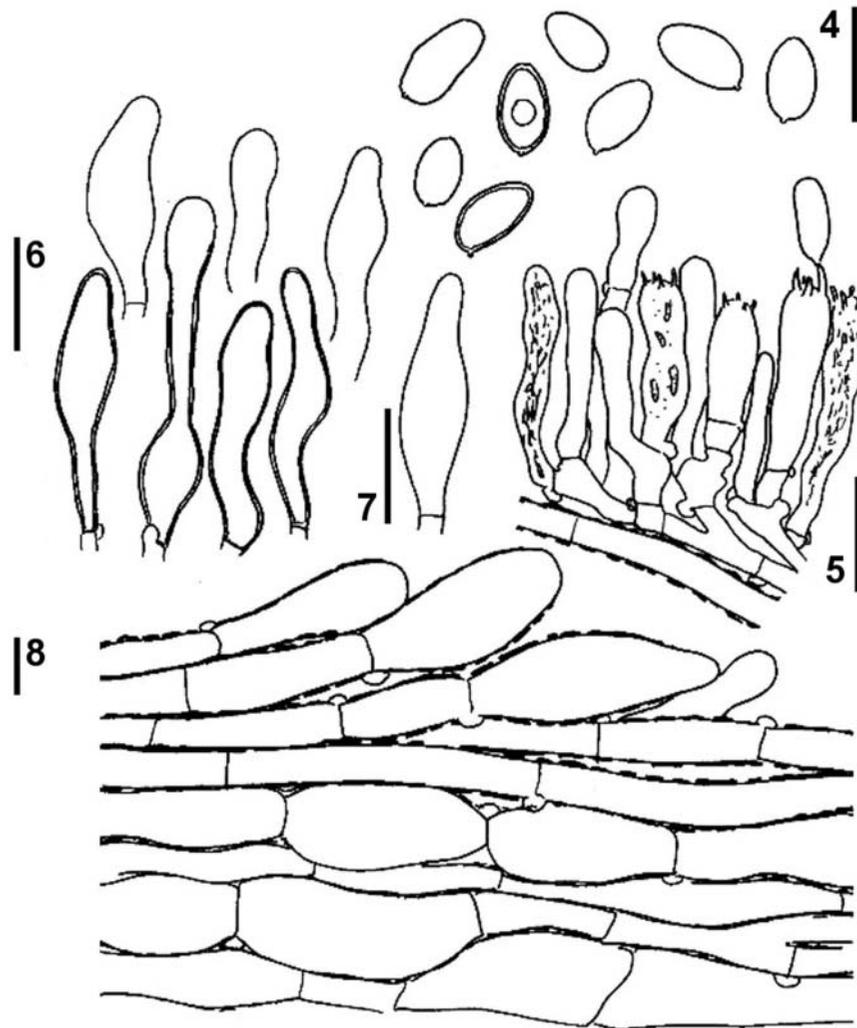
(Figs 1, 4–8).

MYCOBANK No.: MB 519560.

BASIONYM: *Galerina clavus* Romagn., Bull. Trimest. Soc. Mycol. France 58(4): 149, 1944 [1942], lectotype designated here (Fig. 14, p. 145, Romagnesi (1944) [1942], MBT177567); epitype designated here (P.-A. Moreau 06090110, LIP, MBT177568). Switzerland.

≡ *Naucoria clavus* (Romagn.) Kühner & Romagn., Fl. Anal. Champ. Sup.: 239 (1953, comb. inval., Art. 33.4).

MISAPPLICATIONS: *Tubaria minima* J.E. Lange sensu Moreau in Matheny *et al.* (2007a: 571); *Galerina embolus*



**Figs. 4–8.** Anatomical features of *Romagnesiella clavus* (PAM06090110, epitype). (**Fig. 4**) Spores. (**Fig. 5**) Basidia and subhymenium. (**Fig. 6**) Cheilocystidia. (**Fig. 7**) Pleurocystidium. (**Fig. 8**) Pileipellis. Scale bars = 10  $\mu$ m.

(Fr.) Sacc. sensu Orton (1960: 239), sensu de Haan & Walley (2009: 64).

**BIBLIOGRAPHY:** Romagnesi (1942: 144, protologue); Kühner & Romagnesi (1953: 239; description); Smith & Singer (1964: 336); de Haan & Walley (2009: 64, 66; description, picture); North African collections: Hausknecht & Zuccherelli (1993: 47), Moreau (2009: 199).

## Description

Pileus 5–9 (12) mm diam, hemispheric-umbonate then ± depressed around umbo, margin early inrolled becoming shortly crenulate when old, even, not striate, densely furfuraceous-micaceous, grey-brown with somewhat purplish tones when fresh, paler at margin, quickly fading from margin to uniformly fleshy-ochre, without any trace of veil. Lamellae adnexed-ventricose at first, becoming shortly uncinata in age, distant with 14–16 L reaching stipe, interspersed by 1–2 series of lamellulae, dull rusty ochre even when young; edges smooth but (sub)sterile, pale yellow. Stipe 15–25 × 1 mm, flexuose, slightly attenuate at base and inflated at apex, pruinose-floccose just below lamellae, fibrillose below then glabrous against a uniform dirty brown ground colour, slightly purplish when young; no perceptible trace of veil (primordia not observed). Context dark brown when fresh, pale ochre when dry. Odour and taste fungoid, not remarkable.

Basidiospores (5.6) 6.2–6.7–7.3 (8.5) × (3.6) 3.9–4.2–4.4 (5.0) μm, Q = 1.51–1.62–1.73 (n = 48), ovate to obovate but longer spores more fusiform, smooth, germ pore absent; bright yellow in water, amber yellow in Melzer's, warm reddish ochre in KOH, wall thickened up to 0.3 (0.5) μm; content with a large central droplet, often elongate. Basidia four-spored, 28–36 × 7–9 μm, broadly clavate, with long sterigmata, content often microguttulate; necrobasidia abundant, with reddish-brown content. Cheilocystidia 22–45 × 5.5–7 μm, cylindrical-flexuose with slightly thickened yellowish wall, mixed with fascicles of terminal hyphae issued from trama with pear-shaped to subglobose terminal cells, 9–14 μm wide, lamella edge fertile to locally substerile. Pleurocystidia 38–42 × 7.5–13 μm, cylindrical to subutriform, not very distinct but not rare. Hymenophoral trama regular, with strongly encrusted hyphae, 3–5 μm wide. Pileipellis a superficial layer of short cells, these lobate, digitate, puzzle-like, fusiform or pyriform, 12–16 μm wide, more or less erected to nearly hymeniform towards margin, pale in KOH, smooth, issued from hyphae of subpellis; subpellis filamentous, coarsely encrusted, thick-walled, deep yellow to reddish brown in KOH, continuous with pileus context. Stipitipellis a cutis with sparse to fasciculate (at apex) caulocystidia measuring 16–25 × 5–12 μm, cylindrical to clavate-pyriform, very rare below apex; superficial hyphae slender, 2–3.5 μm wide. Clamp connections frequent.

**Habitat and distribution:** Often on calcareous, mineral-rich, sandy or alluvial substrates in pioneer or disturbed habitats including fixed coastal dunes and banks of trails or paths among mosses and grasses. Less frequent in secondarized dunes, scattered and never abundant. Europe (Belgium, France, Switzerland) and reported from Italy, the Netherlands, and North Africa. Fruiting Sept.–Nov.

**Material studied:** BELGIUM. Antwerpen: Antwerpen-Linkeroever, Het Rot, 4 Sep 2004; 10 specimens, among grass and mosses (*Tortula ruralis* and *Ceratodon purpureus*) on sandy, calcareous soil, herb. A. de Haan n° 04101; Antwerpen-Linkeroever, Blokkersdijk, 9 Sep 2004, 2 specimens, among mosses on sandy, calcareous soil, herb. A. de Haan n° 04113. Namur: Oignies-en-Thiérache, l'Estache, 23 Sep 1999, 1 specimen on wet calcareous soil, herb. A. de Haan n° 99100. Oost-Vlaanderen: Zwiinaarde, Rijvisschepark, 15 Oct 1989, about ten carpophores, in bare spot in mossy lawn, on sandy slightly loamy soil, leg. P. Van der Veken, herb. A. de Haan n° 89017; same location, leg. P. Van der Veken, 4 specimens, 29 Oct 2003, herb. A. de Haan n° 03088. West-Vlaanderen: De Panne, Calmeijnbos, 3 Nov 1997, 4 specimens, on humus-rich, calcareous soil, herb. A. de Haan n° 97088; Oostduinkerke, Doornpanne, 1 Nov 2001, 2 specimens, among moss and lichens, on calcareous dune sand, herb. A. de Haan n° 01080 (as '*Galerina embolus*'). FRANCE. Pas-De-Calais: Equihen-Plage, dunes d'Ecault, 7 November 2004, five specimens in Phleo-Tortuletum with *Calamagrostis epigeos*, calcareous fixed dune, leg. A. Brabant & P.-A. Moreau, 7 Nov 2004, herb. P.-A. Moreau n° 04110710 (LIP); same location, along a sandy path amongst *Calamagrostis epigeos*, fixed calcareous dunes with *Hippophae rhamnoides*, leg. C. Hanoire & P.-A. Moreau, 31 Oct 2008, herb. P.-A. Moreau n° 08103102 (LIP). Seine: Paris, bois de Vincennes, 1 Oct 1932, herb. R. Kühner (G, as '*Tubaria oligophylla*', ined.). ITALY: Sardinia, prov. Olbia, Golfo Aranci, Golfo di Marinella, in troops on sandy soil in a coastal grassland, leg. M. Contu, 15 Dec 2007, herb. M. Contu (C15122007, TENN 063957). SWITZERLAND. Gräubunden: Rothenbrunnen, edge of path, riparian *Alnus incana* forest, on black alluvial humus, 1 Sep 2006, leg. B. Senn-Irlet & P.-A. Moreau, herb. P.-A. Moreau n° 06090110 (epitype LIP), TENN 063587, TENN 063976.

## Remarks

Our interpretation of *Galerina clavus* is based on the detailed protologue of Romagnesi (1944 [1942]), which matches collections from Belgium and Switzerland. Unfortunately, no original material of *Galerina clavus* exists. The herbarium packet corresponding to one of the two collections cited by Romagnesi (1942: 145) Yverres, bois de Cerçay, 18 Jun 1942, kept in herb. H. Romagnesi

(PC) was empty. The other cannot be located. Because a figure that depicts *G. clavus* exists in the protologue, this must serve as the lectotype. Original drawings of *G. clavus* made by Romagnesi also exist at PC. Thus, we designate PAM06090110 (LIP) as an epitype.

The species features some morphological and microscopic variation. The epitype (sequenced here), showed purplish-grey tones on the stipe as well as the pileus, but the protologue only mentions this colour on the stipe. Belgian collections described by de Haan & Walley (2009) describe a more convex pileus, broadly adnate instead of subdecurrent lamellae, a ‘weakly farinaceous’ taste, and habitat in dry mineral spots in urban grasslands. However, all collections show gradual variation in the pileus shape, colour and lamellae attachment.

Coastal collections are probably better considered as variants of *R. clavus*. Despite some variation in spore dimensions we could not find any support for specific or infraspecific distinctions. Detailed spore measurements illustrate continuity in these variation patterns but apparent differences (Appendix 2, see supplemental material online).

North African and Sardinian collections of *Galerina clavus*, as described by Hausknecht & Zuccherelli (1993) and Malençon (Moreau, 2009), differ from continental collections by somewhat larger basidiospores [(7.0) 7.6–8.2–9.0 (10) × (4.0) 4.9–5.2–5.5 (5.7) μm, Q = 1.48–1.59–1.70], slightly larger cystidia, and a filamentous pileipellis with a more or less continuous suprapellis of slender cylindrical hyphae with sparse slightly upraised terminal elements. It is possible that Mediterranean collections may represent distinct populations. One collection from Sardinia was sequenced (C15122007, leg. M. Contu), in which the ITS1 region reveals nine site differences with *R. clavus* PAM06090110, four of which, however, are polymorphic in C15122007. *Galerina clavus* has also been reported from the Netherlands (www.verspreidingsatlas.nl/046620).

*Romagnesiella clavus* is probably often confused with other naucorioid species frequent in the same environment, such as *Galerina graminea* (Velen.) Kühner, *Psilocybe pratensis* P.D. Orton, or *Tubaria* spp. The distant lamellae and absence of a veil on the stipe are good distinctive field characters. However, we discuss below three additional species with which *R. clavus* could be confused.

***Tubaria umbonata*** S. Lundell in Lundell & Nannfeldt (1953: 23).

(Figs 9–13).

MYCOBANK No. 307168.

ISOTYPE: SWEDEN. Upland: Uppsala, Slottsbaken, NW part below Gunillaklaken, 50 m from Stockholmsvägen, 6 August 1944, leg. S. Lundell, ex Fungi exsiccati Suecici 2041 (PC, about 20 well-preserved specimens).

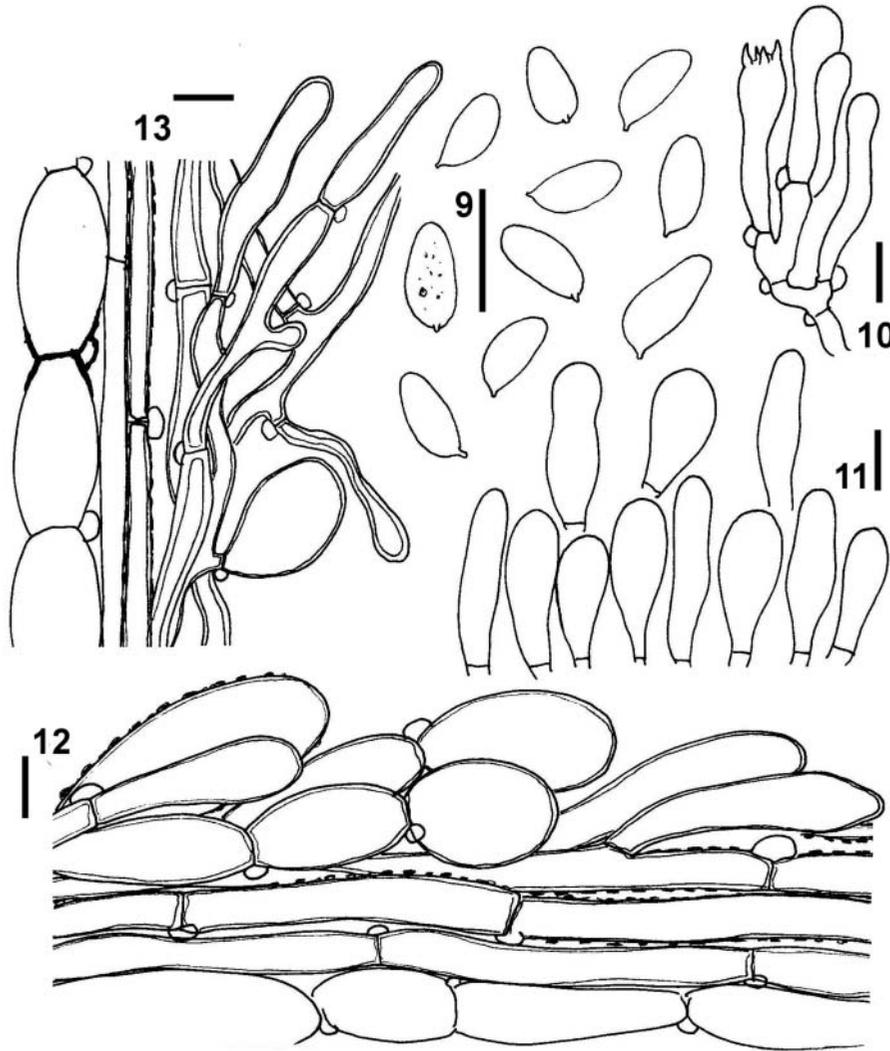
## Description

Exsiccata small to minute (2–6 mm), very slender, entirely dark brown, without visible veil, with arcuate and distant lamellae. Basidiospores (6.2) 6.5–7.2–8.0 (9.0) × (3.0) 3.2–3.7–4.2 μm, Q = 1.70–1.96–2.24 (n = 23; see also Appendix 2, see supplemental material online), pale yellow, slightly thick-walled (<0.5 μm thick), ochraceous yellow in KOH, not collapsing, narrowly ovo-ellipsoidal to ellipsoidal, with slightly guttulate content, not dextrinoid. Basidia 22–34 × 6.5–7.5 μm, 4-spored (occasionally 2-spored), clavate more or less capitate, often strangulate under apex before maturity, hyaline; subhymenium 12–15 μm thick, filamentous-ramose, with hyphae 2–2.5 μm wide. Cheilocystidia 16–30 × 6–11.5 μm, often clustered–ampullaceous, clavate, ellipsoidal, utriform, cylindrical, with a thin and smooth wall, intermixed with some fertile basidia; lamella edge almost sterile. Pleurocystidia if present, not studied. Hymenophoral trama regular, yellowish, made of slender hyphae 2–6 μm wide with thick encrusted wall. Pileipellis with a discontinuous suprapellis made of +/- erected wide ellipsoidal to cylindrical catenulate elements, 18–30 × 6–13 μm, pale, slightly thick-walled, not or only locally encrusted; subpellis made of slender hyphae 3.5–8 μm wide, distinctly encrusted by granular pigment remaining yellow in KOH. Stipitipellis with sparse traces of filamentous veil towards apex, composed of slender hyphae 3–5 μm wide; wall yellowish, up to 2 μm thick and encrusted, terminal cells cylindrical with some vesicular cells up to 20 μm wide; superficial hyphae slender, these 3–5 μm wide, with yellow walls up to 0.5 μm thick, intermixed with large cylindrical hyphae 9–16 μm wide, locally encrusted by gold-yellow pigment (KOH), especially at septa, and some sparse pale gleoplerous hyphae. Clamp connections present at septa.

## Remarks

*Tubaria umbonata* has not been revised nor documented since its publication in Fungi Exsiccati Suecici (Lundell & Nannfeldt 1953). We studied the isotype at PC (Fungi Exsiccati Suecici fasc. 41–42). Based on our morphological analysis, we conclude that *T. umbonata* represents a genuine species of *Tubaria* (W.G. Sm.) Gillet, but with rather narrow spores. DNA extraction of the PC material of *T. umbonata* yielded no PCR amplicons. Illustrations of anatomical features (Fig. 5) and their description (above) from the isotype are presented.

Two additional species could be confused with *R. clavus*. The name *Tubaria minima* J.E. Lange (Lange, 1940) was misapplied by Moreau (in Matheny *et al.*, 2007a) to collections of *R. clavus*. Although Bon (1992) maintains *T. minima* as an autonomous species, Romagnesi (1942) considers it to be a synonym of *T. minutalis*



**Figs. 9–13.** Anatomical features of *Tubaria umbonata* (Fungi Exsiccati Suecici fasc. 41–42, isotype). (**Fig. 9**) Spores. (**Fig. 10**) Basidia and subhymenium. (**Fig. 11**) Cheilocystidia. (**Fig. 12**) pileipellis. (**Fig. 13**) Stiptipellis. Scale bars = 10  $\mu\text{m}$ .

Romagn. (Romagnesi, 1937), a position followed by modern authors. This species (sensu Lange (1940), non Moreau) differs from *G. clavus* by its hygrophanous piles and smaller spores ( $5.2\text{--}6.0 \times 3.2\text{--}3.8 \mu\text{m}$ ), features that reinforce its conspecificity with *T. minutalis*.

*Tubaria embolus* (Fr.) Sacc. is rather frequently cited in the literature but has been interpreted several different ways. Lange (1938: 655, pl. 127B) illustrates as '*Tubaria embola*' a species with broadly adnexed lamellae and yellow tones especially in the context (conforming to Fries' protologue, 1836–1838: 206), which seems to represent *Agrocybe pusiola* (Fr.) R. Heim. Bon (1992) cites the species in the genus *Galerina*, but examination of his materials (LIP) showed that his concept was unclear: coll. 741120 is *Galerina uncialis* (Britzelm.) Kühner, and coll. 70624 (as '*Galerina cf. embolus*') is a species with pleurocystidia close to *Galerina*

*vittaeformis* (Fr.) Kühner. Orton (1960: 176) mentions five reports of *T. embolus* (as '*Galerina embolus*') from sand dunes, with comparable microscopical characters (but with notably long spores,  $9\text{--}11 \times 4.5\text{--}6 \mu\text{m}$ , compatible with our coastal collections of *R. clavus*), but pleurocystidia and necrobasidia are not mentioned. Moreover, yellow tones are described towards the pileus margin when dry, incompatible with any species known to us. In addition de Haan & Walley (2009) describe without illustrations a collection of *G. embolus* (reported here as *R. clavus*) and also found in fixed dunes in Belgium. Considering the ambiguities of the protologue (Fries, 1836–1838), and the diversity of interpretations proposed by various authors, we reject the name here. Additional morphological and molecular study is required to unravel the taxonomic relationships of these variously interpreted collections to Romagnesiella.

## Discussion

### Polyphyly of *Pachylepyrium* and recognition of *Crassisporium* and *Romagnesiella* as new genera

Our results strongly support the polyphyletic status of *Pachylepyrium*. The type species of the genus, *P. fulvidula*, lacks several of the features attributed to the residual species. Originally described in *Phaeomarasmius*, *P. fulvidula* fruits on non-burnt woody debris and lacks the broad germ pore observed in other species. Horak (1968) reports seldom seeing any germ pore at all in the type of *P. fulvidula*. Our examination of the isotype at MICH confirms this observation (a germ pore was not observed). Thus, it is not surprising to see phylogenetic placement of *P. fulvidula* apart from the residual *Pachylepyrium* species. *Pachylepyrium fulvidula* resides in the Tubariaceae (Fig. 3) where it is closely related to other species of *Flammulaster*, *Phaeomyces*, *Phaeomarasmius* and *Tubaria* (W.G. Sm.) Gillet, all of these also lacking a broad germ pore (Horak, 2005).

We place three residual species of *Pachylepyrium* in the new genus *Crassisporium* united by a combination of basidiospore features (thick-walled spores with a broad germ pore and rusty to reddish brown colouration in KOH), anatomical features (non-gelatinous cutis, absence of pleurocystidia and chrysocystidia, absence of a gelatinous subhymenial layer) and ecology (carbonicolous habit). However, before our phylogenetic analysis based on molecular data, we did not suspect that *Galerina clavus* would be related to *Crassisporium* more so than to any other group of Hymenogastraceae or Strophariaceae. In order to point out differences between typical carbonicolous species with thick-walled pored spores (*Crassisporium* species) and non-carbonicolous species with thinner-walled (<0.5  $\mu\text{m}$ ) spores such as *G. clavus*, we have proposed a new genus *Romagnesiella* to accommodate the latter. No extra-European or North African species are unequivocally attributable to *Romagnesiella* at present without the addition of detailed morphological and molecular study. *Galerina fuegiana* Singer from Patagonia (Smith & Singer, 1964) is a possible candidate.

The inclusion of sequences of *Crassisporium* and *Romagnesiella* in a multigene phylogenetic analysis of the Agaricoid clade shows these two taxa form a well-supported group (Fig. 3) sister to the Strophariaceae s.lat. consortium (Gulden *et al.*, 2005), including the families Hymenogastraceae and Strophariaceae s. str. of Matheny *et al.* (2006). Inclusion of *Crassisporium* and *Romagnesiella* in Strophariaceae s. str. would render the family paraphyletic in this analysis. Consideration of a more broadly conceived Hymenogastraceae, subsuming the Strophariaceae, could be made since the name Hymenogastraceae Vitt. 1831 pre-dates that of the Strophariaceae Singer & A.H. Sm. 1946. However, additional taxon and

gene sampling are needed to resolve the relationship between these two families.

An alternative scenario to consider is inclusion of the three species of *Crassisporium* into one genus with *R. clavus*, thereby describing only a single genus as new. Samples of each group form a clade with strong support, a synapomorphy of which are the basidiospores that deepen various shades of reddish brown in KOH. However, we favour separate genera for the two lineages for several reasons: (1) species of *Crassisporium* are carbonicolous, whereas those of *Romagnesiella* are non-carbonicolous; (2) the lamellae are adnate to subdecurrent in *Romagnesiella* but never subdecurrent in *Crassisporium*; (3) a veil is absent in *Romagnesiella* but present in *Crassisporium*; (4) the basidiospores of *Crassisporium* feature walls >0.5  $\mu\text{m}$  thick, a wide germ pore typically 1.0–1.5  $\mu\text{m}$  thick, and are subangular in face view. These features may be correlated with the fire ecology in that heat may be required to induce germination (Claridge, Trappe, & Hansen, 2009). Basidiospores of *Romagnesiella* have thinner walls (<0.5  $\mu\text{m}$  thick), no germ pore and are not subangular; (5) pleurocystidia are present in *Romagnesiella* but absent in *Crassisporium*; and (6) the relative branch length differences between the two lineages correspond to branch length differences between other genera of Strophariaceae and Hymenogastraceae. Therefore, we prefer to recognize the two lineages as separate genera.

The monophyly of the Agaricoid clade is recovered here for the first time with high bootstrap and significant Bayesian posterior probability. Most species in the Agaricoid clade tend to have pigmented and thick-walled spores, perhaps indicative of adaptations to novel environments (e.g. dung, burnt sites) (Garnica *et al.*, 2007). Many species of the Agaricoid clade also feature multiple nuclei per spore and an open pore type of hilum (Matheny *et al.*, 2006).

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## Supplemental data

Supplemental data for this article can be accessed here.

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