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Galerina Earle: A polyphyletic genus in the consortium of dark-spored agarics

Gro Gulden[1]
Øyvind Stensrud

Natural History Museum, University of Oslo, P.O. Box 1172 Blindern, N-0318 Oslo, Norway

Kamran Shalchian-Tabrizi
Håvard Kauserud

Department of Biology, University of Oslo, P.O. Box 1045 Blindern, N-0316 Oslo, Norway

Abstract: The basidiomycete genus *Galerina* Earle accommodates more than 300 small brown-spored agarics worldwide, predominantly described from the Northern hemisphere. The delimitation of species and infrageneric units hitherto has been based on morphological and, to some extent, ecological characters. In this study we have analyzed nuclear ribosomal LSU and ITS sequences to reveal infrageneric phylogeny and the phylogenetic placement of *Galerina* among the dark-spored agarics. Sequences from 36 northern hemisphere *Galerina* species and 19 other dark-spored taxa were analyzed, some of them obtained from EMBL/GenBank. Our results, received from Bayesian and distance methods, strongly suggest that *Galerina* is a polyphyletic genus. The LSU analysis shows that *Galerina* is composed of three or four separate monophyletic main groups. In addition, a few species cluster together with other dark-spored agarics. The same groups are recognized in the ITS tree and they correspond roughly to previously recognized subgenera or sections in *Galerina.* With high support our LSU analysis suggests that *Gymnopilus* is a monophyletic genus and that *Gymnopilus* and one of the *Galerina* lineages (''mycenopsis'') are sister groups. The analyses further indicate that the *Galerina* lineages, as well as the genus *Gymnopilus,* could be referred to a strongly emendated family Strophariaceae, which corresponds largely to the family as circumscribed by Kühner (1980). Our results affirm that morphological characters often are highly homoplastic in the agarics. At the present stage formal taxonomic consequences or nomenclatural changes are not proposed.

Key words: Basidiomycota, Agaricales, brown-spored agarics, Cortinariaceae, *Galerina, Gymnopilus,* Strophariaceae

INTRODUCTION

The genus *Galerina* (Basidiomycota, Agaricales) has more than 300 species (Horak 1994), predominantly described from the Northern hemisphere. Most species have basidiocarps associated with living bryophytes, probably as saprophytes on dead parts, whereas others are confined to woody material or other plant debris. The delimitation of species and infrageneric units hitherto has been based on morphological and, to some extent, ecological characters. The basidiocarps of *Galerina* are small and mycenoid, although sometimes they are slightly fleshier and occasionally the stipe is provided with a membranous annulus. Important gross-morphological characters separating the species include ecology, fruit body size, veil conditions, surface features of the pileus, insertment of the lamellae, color and lepto-organic characters (flavor and odor). In the majority of the species, the spores are ornamented and have a ''plage'' (a delimited smooth area on the adaxial side above the apiculus), but in some species they are ornamented also over the plage area or smooth. The spores in species provided with a plage are exceptional by having an inner wall layer, endospore, which inflates after successive exposure to diluted base and acid solutions, and furthermore this endospore is strongly dextrinoid and cyanophilic (the ''ammoniaco-acetic treatment,'' Kühner 1972, 1980). A rich assortment of cystidia of different shapes and locations constitutes, in combination with spore characters, a fundament for recognition of the various *Galerina* species. In most cases microscopic examination is needed for species recognition and high quality optics often are required, and even then identifications may remain doubtful.

Current circumscription of the genus *Galerina* largely follows that of Kühner (1935), who excluded groups now placed in *Conocybe* s. lat. and included *G. marginata* and related annulate species that previously were referred to *Pholiota* (TABLE I). This circumscription of *Galerina,* followed by a later segregation of the *G. stagnina* group, has been generally accepted. However, Watling and Gregory (1993) in-

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[1] Corresponding author. E-mail: gro.gulden@nhm.uio.no

TABLE I. Overview of the historical infrageneric classification of *Galerina*

Kühner 1935	Kühner 1972	Smith and Singer 1964	Bon 1992	Watling and Gregory 1993	Gulden and Hallgrimsson 2000
				S.g. Kuehneromyces	
Sect. Tubariopsis	**S.g. Tubariopsis**	**S.g. Tubariopsis**	**S.g. Tubariopsis**	**S.g. Tubariopsis**	**S.g. Tubariopsis**
	Sect. Tubariopsis	Sect. Tubariopsis	Sect. Tubariopsis	Sect. Tubariopsis	Sect. Tubariopsis
	Sect. Hemitubariopsis		Sect. Hemitubariopsis	Sect. Hemitubariopsis	Sect. Hemitubariopsis
					Sect. Tibiicystis
Sect. Eugalerina	**S.g. Galerina**	**S.g. Galerina**	**S.g. Galerina**	**S.g. Galerina**	**S.g. Galerina**
	Sect. Galerina	Sect. Galerina	Sect. Galerina	Sect. Galerina	Sect. Galerina
	s.sect. Galerina	Sect. Naucoriopsis	Sect. Naucoriopsis	Sect. Naucoriopsis	
	s.sect. Triscopae		Sect. Pseudotubaria		Sect. Mycenopsis
	s.sect. Mycenopsidae	Sect. Mycenopsis		Sect. Mycenopsis	
			S.g. Mycenopsis	s.sect. Mycenopsidae	
	Sect. Tibiicystidiae	Sect. Calyptrospora	Sect. Mycenopsis	s.sect. Tibiicystidiae	
		Sect. Porospora	Sect. Calyptrospora	Sect. Calyptorspora	
		Sect. Inoderma	Sect. Tibiicystidiae		
		Sect. Physocystis		Sect. Physocystis	
		Sect. Inocyboides			
		Sect. Pseudotubaria	**S.g. Inocybula**	Sect. Inocyboides	
				Sect. Pseudotubaria	
Sect. Naucoriopsis	**S.g. Naucoriopsis**				**S.g. Naucoriopsis**
	Sect. Naucoriopsis				
	Sect. Styliferae				

cluded also the genus *Kuehneromyces* in *Galerina*. The delimitation of infrageneric units in *Galerina* and their taxonomic level vary between authors of current classifications (Bon 1992, Gulden and Hallgrímsson 2000, Smith and Singer 1964, Watling and Gregory 1993; TABLE I), but two of the units recognized already by Kühner (1935), *Tubariopsis* and *Naucoriopsis,* largely remain as in the original concept, while his third unit, *Eugalerina,* has been divided in various ways. Several species complexes of *Galerina* are in need of critical revision (e.g. the *G. marginata* [cf. Gulden et al 2001] and the *G. vittiformis* complexes), and numerous species delimitations remain controversial.

Fostered by our interest in the genus *Galerina,* this study originally was designed to examine its infrageneric taxonomy and to inspect unresolved species complexes in the genus by applying phylogenetic analyses of the nrDNA ITS region. It later was expanded with nrDNA LSU sequences from *Galerina* and other brown- and black-spored genera to pursue a supposedly polyphyletic origin (Moncalvo et al 2002) and to find the phylogenetic placement of its various groups. The genus *Gymnopilus* is of particular interest because some of its species share salient spore features with *Galerina* (e.g. plage and inflating endospore) (Kühner 1980). Furthermore many *Gymnopilus* species have cystidia similar to those of *Galerina* and the limit between the two genera, especially in southern hemisphere species, has been difficult to draw (Rees et al 1999).

The analysis of Moncalvo et al (2002) indicates that the dark-spored agaric families Bolbitiaceae, Cortinariaceae, Coprinaceae, Crepidotaceae and Strophariaceae are polyphyletic as circumscribed in the widely accepted sense of Singer (1986). Singer's delimitation of these families has been strongly challenged also in morphology based taxonomy, especially by Kühner (1980) who, for example, referred *Galerina* and *Gymnopilus* to Strophariaceae while they, according to Singer, belong in Cortinariaceae. In the present study we have included mainly taxa from the dark-spored consortium Cortinariaceae-Strophariaceae and not from the taxonomically more remote families *Agaricaceae* and *Coprinaceae* (''*Psathyrellaceae*'') to investigate the family relationships of *Galerina*.

This study is a first contribution toward obtaining a new phylogeny of *Galerina*. Our data also made it possible to investigate family relationships among allied dark-spored agarics.

MATERIAL AND METHODS

Material.—A total of 95 specimens, representing 36 *Galerina* species and 20 specimens of seven genera of the dark-spored families Bolbitiaceae, Cortinariaceae, Strophariaceae and Tubariaceae were obtained from different herbaria (TABLE II). The origin of collections, the authors of taxa and GenBank accession numbers are presented (TABLE II). Our intention was to include, when possible, at least two samples from each species of *Galerina*. Species of the *G. marginata* complex were sampled more extensively to obtain a second dataset to compare with results published by Gulden et al (2001). The *G. atkinsoniana/G. vittiformis* complex also was sampled extensively. To verify identification and to evaluate selected characters, collections used in the molecular analyses were examined microscopically according to methods described in Gulden and Hallgrímsson (2000).

In addition to the sequences generated in this study, three ITS sequences of *Galerina pseudomycenopsis* (AJ300156-AJ300158), one LSU sequence of *G. semilanceata* (AY038309), one LSU sequence of *G. marginata* (AY219587) and two LSU sequences of *G. paludosa* (AF261528, AF261653) were retrieved from EMBL/GenBank and included in the analyses. Forty-six LSU sequences from these dark-spored genera also were retrieved from EMBL/GenBank and included in the LSU analysis (for accession numbers see FIG. 1): *Copelandia* (1), *Cortinarius* (4), *Descolea* (1), *Flammula* (1), *Flammulaster* (1), *Gymnopilus* (5), *Hebeloma* (3), *Hemipholiota* (3), *Hypholoma* (3), *Inocybe* (2), *Kuehneromyces* (2), *Melanotus* (1), *Mythicomyces* (1), *Naucoria* (1), *Pachylepyrium* (1), *Panaeolus* (1), *Phaeocollybia* (3), *Phaeogalera* (1), *Phaeomarasmius* (2), *Phaeonematoloma* (1), *Pholiota* (5), *Rozites* (1), *Stagnicola* (1) and *Tubaria* (1).

Molecular methods.—DNA extraction was performed with the 2% CTAB miniprep described by Murray and Thompson (1980) with minor modifications: DNA was resuspended in 100 μL dsH_2O at the final step of extraction, and DNA templates were diluted 50-fold before PCR amplification. PCR amplification was accomplished with the primers LROR/LR5 and ITS4/ITS5 (White et al 1990) for the nuclear partial LSU region and ITS. PCR was performed in 30 μL reactions containing 17.5 μL 50 × diluted template DNA and 12.5 μL reaction mix (final concentrations: 4 × 250 mM dNTPs, 0.625 mM of each primer, 2 mM MgCl2 and 1 unit DyNAzyme™ II DNA polymerase [Finnzymes Oy, Espoo, Finland]) on a Biometra PCR machine. The nrDNA LSU and ITS amplification program was initiated by a 4 min denaturation step at 94 C, followed by 37 cycles of 30 s at 94 C, 35 s at 54 C and 40 s at 72 C. The program was terminated with a 10 min elongation step at 72 C before storage at 4 C. Automated sequencing was performed on a MegaBACE™ 500 DNA Analysis System (Amersham Biosciences, Ohio) with the DYEnamic™ ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences, Buckinghamshire, England), according to the manufacturers recommendations. PCR products and cycle sequencing products were purified respectively with the ExoSAP-IT and AutoSeq96TM Dye Terminator Clean-up Kits according to the manufacturer's recommendations (Amersham Biosciences, Ohio). Both strands of the ITS and LSU amplicons were sequenced with the PCR primers.

TABLE II. Species analyzed, origin of the collections, and their GenBank accession numbers for the ITS and LSU sequences. The abbreviations of herbaria are according to Index Herbariorum and the nomenclature follows Nordic Macromycetes (Hansen and Knudsen 1992)

Species	Herbar. id.	Origin	GenBank Accession nos. ITS	LSU
Agrocybe arvalis (Fr.) Singer	O 152495	Norway		AJ871571
A. pediades (Fr.) Fayod	O 152544	Norway		AJ871493
A. praecox (Pers. : Fr.) Fayod	O 59617	Norway		AJ871494
A. putaminum (Maire) Singer	O 59981	Norway		AJ871495
Bolbitius reticulatus (Pers. : Fr.) Ricken	O 82057	Norway		AJ871561
Conocybe aporos Kits van Wav.	O 153351	Norway		AJ871563
C. intrusa (Peck) Singer	O 60165	Norway		AJ871559
C. lactea (J.E. Lange) Métrod	O 153415	Norway		AJ871560
C. vexans P.D. Orton	O 50693	Norway		AJ871562
Flammulaster carpophilus (Fr.) Earle var. *carpophilus*	O 83151	Norway		AJ871499
F. limulatus (Fr.) Watling var. *limulatus*	O 59850	Norway		AJ871498
Galerina allospora A.H. Sm. & Singer	O 73460	Scotland	AJ585452	
G. allospora—Paratypus	MICH 45915	USA: Michigan	AJ585511	
G. arctica (Singer) Nezdoym.	O 50535	Svalbard	AJ585441	
G. arctica	O 73465	Svalbard		AJ871557
G. arctica	O 73198	Greenland	AJ585442	AJ871556
G. atkinsoniana A.H. Sm. var. *atk.* f. *atkinsoniana*	O 73448	Germany	AJ871572	AJ871534
G. atkinsoniana var. *atk.* f. *atkinsoniana*	O 73459	Scotland	AJ585479	AJ871536
G. atkinsoniana var. *atk.* f. *quadrispora Gulden*	O 73188	Greenland	AJ585480	AJ871533
G. atkinsoniana var. *atk.* f. *quadrispora*	O 73217	Greenland	AJ585482	AJ871543
G. atkinsoniana var. *atk.* f. *quadrispora*	O 72933	Norway	AJ585478	AJ871537
G. atkinsoniana var. *atk.* f. *quadrispora*	CTB85.295	Greenland	AJ585481	
G. badipes (Pers.) Gulden (Holotypus *G. acris* Gulden)	O 72603	Norway	AJ585494	
G. badipes	O 73213	Greenland	AJ585495	AJ871517
G. calyptrata P.D. Orton	O 73449	Germany	AJ585465	AJ871503
G. calyptrata	O 154221	Norway		AJ871502
G. calyptrata	O 73454	France	AJ585466	AJ871501
G. cephalotricha Kühner	O 154146	Norway	AJ585462	AJ871513
G. chionophila Senn-Irlet	O 73463	Switzerland	AJ585506	
G. clavata (Velen.) Kühner	O 72166	Denmark	AJ585436	AJ871555
G. clavata	O 50544	Svalbard	AJ585437	AJ871554
G. fallax A.H. Sm. & Singer	O 154451	Norway	AJ585450	
G. fallax	O 154355	Norway	AJ585451	
G. cf. *fallax*	O 73450	Germany	AJ585449	AJ871508
G. fibrillosa A.H. Sm.—Holotypus	MICH 40850	USA: Washington	AJ58473	
G. harrisonii (Dennis) Bas & Vellinga	O 50711	Norway	AJ585463	AJ871506
G. harrisonii	O 73216	Greenland		AJ871507
G. hybrida Kühner	O 73458	France	AJ585444	
G. hybrida	O 73452	Germany	AJ585445	
G. hypnorum (Schrank : Fr.) Kühner	O 154362	Norway	AJ585468	
G. hypnorum	MICH 46292	USA: Michigan	AJ585469	
G. hypnorum	MICH 46302	USA: Michigan	AJ585470	
G. hypnorum forma	O 73206	Greenland	AJ585467	AJ871535
G. jaapii A.H. Sm. & Singer	O 50658	Norway	AJ585504	AJ871520
G. jaapii	O 154387	Finland	AJ585505	
G. laevis (Pers.) Singer	O 154389	Norway	AJ585438	
G. laevis	O 70903	Norway	AJ585439	
G. laevis	O 71160	Norway	AJ585440	AJ871558

TABLE II. Continued

Species	Herbar. id.	Origin	GenBank Accession nos. ITS	LSU
G. lubrica A.H. Sm.	O 154034	Norway	AJ585471	AJ871525
G. lubrica	O 73455	France	AJ585472	
G. luteolosperma A.H. Sm. & Singer	O 154076	Norway	AJ585453	AJ871509
G. marginata (Batsch) Kühner	O 71328	Norway	AJ585498	
G. marginata	O 72101	Norway		AJ871526[7]
G. marginata	O 72135	Norway		AJ871529
G. marginata (Holotypus *G. veneata* A.H. Sm.)	O 300011[1]	USA: Oregon	AJ585497	
G. marginata	O 72434	USA: Oregon	AJ585499	
G. marginata	O 72429	USA: Oregon	AJ585502	AJ871527
G. marginata	O 72501[2]	USA: Michigan		AJ871522
G. marginata	O 72427	USA: Oregon	AJ585500	AJ871530
G. marginata	O 72507[3]	USA: Michigan	AJ585496	AJ871521
G. minima (Peck) A.H. Sm. & Singer	O 73467	Greenland	AJ585489	AJ871540
G. minima	O 73468[5]	Greenland	AJ585483	AJ871514
G. (cf.) *mniophila* (Lasch) Kühner	O 154072	Norway	AJ585456	AJ871538
G. (cf.) *mniophila*	O 50679	Norway	AJ585457	AJ871516
G. mniophila	O 50545	Svalbard	AJ585458	
G. mniophila	O 60574	Norway	AJ585459	AJ871515
G. mniophila	O 73175	Greenland	AJ585460	AJ871512
G. mniophila	MICH 29880	USA: Idaho	AJ585461	
G. nana (Petri) Kühner	O 153723	Norway	AJ585490	AJ871518
G. paludosa (Fr.) Kühner	O 153974	Norway	AJ585446	
G. paludosa	O 153987	Norway	AJ585448	AJ871500
G. paludosa	O 73462	Estonia	AJ585447	
G. pruinatipes A.H. Sm.	O 73438	France	AJ585510	AJ871531
G. pruinatipes—Holotypus	MICH 29836	USA: Washington	AJ585509	
G. pseudobadipes Joss. ex A.H. Sm. & Singer	O 154252	Norway	AJ585474	AJ871548
G. pseudobadipes	O 154186	Norway		AJ871549
G. pseudobadipes	O 154254	Norway		AJ871547
G. pseudocamerina Singer	O 73471	Germany	AJ585507	
G. pseudocamerina	O 73481	Germany	AJ585508	AJ871519
G. pseudocerina A.H. Sm. & Singer	O 153998	Norway	AJ585431	
G. pseudocerina	O 50547	Svalbard	AJ585432	AJ871552
G. pseudocerina	O 154004	Norway	AJ585433	AJ871553
G. pseudomycenopsis Pilát	O 73464	USA: Alaska	AJ585503	AJ871523
G. pseudomycenopsis	O 50526	Svalbard	AJ585501	AJ871524
G. pumila (Pers. : Fr.) M. Lange var. *pumila*	O 73067	Greenland	AJ585476	AJ871545
G. pumila var. *pumila*	O 73440	Germany	AJ585477	AJ871546
G. salicicola P.D. Orton	K 99448	England	AJ585493	
G. sphagnicola (G.F. Atk.) A.H. Sm. & Singer	O 73441	Estonia	AJ585464	AJ871505
G. sphagnicola	O 71238	Finland		AJ871504
G. sphagnorum (Pers. : Fr.) Kühner	O 70913	Norway	AJ585454	AJ871511
G. sphagnorum	O 154094	Norway	AJ585455	AJ871510
G. stordalii A.H. Sm.	O 154179	Norway	AJ585434	AJ871551
G. stordalii	O 154169	Norway	AJ585435	
G. stordalii	O 73436	Norway		AJ871550
G. stylifera (G.F. Atk.) A.H. Sm. & Singer	O 73457	France	AJ585475	
G. tibiicystis (G.F. Atk.) Kühner	O 72930	Norway	AJ585443	
G. triscopa (Fr.) Kühner	O 73453	France	AJ585491	
G. triscopa	O 73451	Germany	AJ585492	
G. triscopa	O 154505	Norway		AJ871532
G. vittiformis (Fr.) Singer var. *vitt.* f. *tetraspora* A.H. Sm. & Singer	O 154565	Norway	AJ585487	

TABLE II. Continued

Species	Herbar. id.	Origin	GenBank Accession nos. ITS	LSU
G. vittiformis var. *vitt.* f. *tetraspora*	O 73466[4]	Greenland		AJ585488
G. vittiformis var. *vitt.* f. *tetraspora*	O 73427	USA: Washington		AJ871542
G. vittiformis var. *vitt.* f. *tetraspora*	O 73470	Greenland		AJ871539
G. vittiformis var. *vitt.* f. *tetraspora*	O 154480	Norway		AJ585486
G. vittiformis var. *vitt.* f. *vittiformis*	O 7312	Greenland	AJ585484	AJ871544
G. vittiformis var. *vitt.* f. *vittiformis*	O 73469[6]	Greenland	AJ585485	AJ871541
Gymnopilus decipiens (W.G. Sm.) P.D. Orton	O 82172	Norway		AJ871564
G. junonius (Fr. : Fr.) P.D. Orton	O 171053	Norway	AJ871568	
G. oidinii (Fr.) Kühner & Romagn.	O 82167	Norway	AJ871566	
G. penetrans (Fr.) Murrill	O 95835	Norway	AJ871565	
G. sapineus (Fr.) Maire	O 154821	Norway	AJ871567	
Naucoria amarescens Quél.	O 155460	Norway	AJ871497	
N. escharioides (Fr.) P. Kumm.	O 155488	Norway	AJ871496	
Phaeogalera stagnina (Fr.) Pegler & T.W.K. Young	O 73215	Greenland	AJ871570	
Phaeogalera stagnina	O 73191	Greenland	AJ871569	

[1] = MICH 10698.
[2] = MICH 27694.
[3] = MICH 27691.
[4] = C 3681.
[5] = C 1539.
[6] = C 6345.
[7] Genbank accession no. AJ871528 is also derived from this collection.

Alignments and phylogenetic analyses.—Sequence chromatograms were inspected visually and sequences manually aligned with the program BioEdit Sequence Alignment Editor version 5.0.9 (Hall 1999). Regions that could not be aligned reliably were excluded from the analyses. Due to alignment problems only *Galerina* sequences were included in the ITS alignment, although this represented a polyphyletic dataset (see below).

Phylogenetic trees were constructed from the ITS (85 sequences and 579 characters) and LSU (130 sequences and 733 characters) alignments. Phylogenetic analyses were performed with MrBayes v. 3 (Ronquist and Huelsenbeck 2003) applying a general time reversible (GTR) substitution model, gamma (G) and proportion of invariable site parameters (I) to accommodate variable rates across sites. Other prior settings were set to default values. The Markov chain Monte Carlo (MCMC) chains lasted 2 000 000 generations, and trees were saved each 100 generation, in all counting 20 000 trees. The MCMC analysis implemented four chains (three heated and one cold) starting from random tree topologies. Burn-in was set to 300 000 generations based on the stationarity of the MCMC chains, leaving 17 000 trees for calculation of the consensus tree and posterior probability values. To test the convergence of the MCMC chains, the Bayesian inference was done twice and from different random, starting trees. Comparison of the runs showed almost identical tree topologies, mean likelihood scores and the posterior probability values, and therefore we considered it likely that the MCMC had lasted long enough to converge. Phylogenetic analysis with distance methods were done in PAUP* v. 4.0b10 (Swofford 2000) by inferring trees with maximum likelihood (ML-distance) and LogDet distances. The ML-distance parameters were estimated from a K2P tree generated with NJ, and included these parameters: GTR, G+I and nucleotide frequencies. In the LogDet analysis the I parameter was used by excluding invariable sites in proportion to nucleotide frequency estimated from constant sites only.

In preliminary LSU analyses, where we tested different potential outgroup taxa distantly related to the ingroup, *Inocybe* clustered basically and was chosen as outgroup in the LSU analysis. The *Galerina* group ''tubariopsis,'' appearing as clearly defined and monophyletic in the LSU analysis, was chosen as outgroup in the ITS analysis.

RESULTS

To infer the infrageneric phylogenetic relationships in *Galerina,* 85 ITS nrDNA sequences from 36 *Galerina* species were analyzed. In initial phylogenetic analyses, various brown-spored taxa were evaluated as potential outgroups (results not shown). In these analyses different brown-spored taxa interfered and clustered within the *Galerina* phylogeny, indicating a poly- or paraphyletic status of *Galerina.*

To evaluate the potential polyphyletic status of *Galerina* and the relationships to other dark-spored taxa, we established another dataset, with the first part of

the LSU nrDNA gene. Fifty-eight LSU sequences from 28 *Galerina* species were generated together with 20 sequences from 19 other dark-spored species. In addition 51 sequences representing 50 species were retrieved from GenBank from various dark-spored taxa and included in the alignment. A Bayesian analysis of the LSU alignment was performed (as stated above). GenBank sequences from *Inocybe agardhii* and *Inocybe curvipes* (AY380366 and AY239022) were used as outgroup sequences in the analyses of LSU. The resulting consensus phylogram is shown (FIG. 1). Although low support was obtained for basal branches in the tree, the topology indicates that *Galerina* is a highly polyphyletic genus, clustering into four more or less independent lineages referred to provisional names as ''mycenopsis,'' ''naucoriopsis,'' ''galerina'' and ''tubariopsis'' (shown in different colors in FIG. 1). The ''galerina'' group obtained low support in the LSU analysis. These four groups largely reflect already recognized morphology-based subgenera or sections within *Galerina* (TABLE I). However some *Galerina* species were not included in these four main groups (cf. species in red in FIG. 1). Of note, the genus *Gymnopilus* was found to be monophyletic and *Gymnopilus* and ''mycenopsis'' occurred together as a highly supported group (FIG. 1). Phylogenetic inferences of the LSU data with distance methods by inferring trees with maximum likelihood (ML-distance) and LogDet-distance methods, resulted in highly congruent topologies (results not shown).

Although being a polyphyletic dataset, a Bayesian analysis of the *Galerina* ITS dataset also was performed (as described above) to infer phylogenetic relationships between *Galerina* taxa, independently of other dark-spored agarics. The ''tubariopsis'' group was used as outgroup in this analysis. Most *Galerina* species grouped into the same four groups as in the LSU tree (FIG. 2) and largely the same species had intermediate positions in the ITS tree (cf. taxa in red in FIG. 2). The ''mycenopsis'' group obtained low support in the ITS analysis. Of note, the 36 species included in the ITS alignment grouped into monophyletic groups except for *Galerina marginata* and *Galerina atkinsoniana* (cf. FIG. 2). Phylogenetic analysis of the ITS dataset with distance methods by inferring trees with maximum likelihood (ML-distance) and LogDet-distances gave largely the same topology (results not shown).

DISCUSSION

Galerina, a polyphyletic genus.—In this study, we have analyzed nuclear ribosomal LSU and ITS sequences to reveal phylogenetic relationships on family, generic as well as specific level and to further pursue indications of polyphyly in *Galerina*. Our results from the LSU analysis strongly suggest that *Galerina* is a polyphyletic genus (cf. FIG. 1), as earlier suggested by Moncalvo et al (2002). The LSU tree shows that *Galerina* has 3–4 separate main groups and some odd species interspersed between other dark-spored genera. Roughly the same groups are recognized in the ITS tree (FIG. 2). However the ITS data constitute otherwise a highly polyphyletic dataset by not including other brown-spored taxa. Bearing in mind its ''artificial'' status, the ITS dataset nevertheless can be used to investigate species relationships within the four main groups of *Galerina*. The resolved lineages correspond more or less to previously recognized subgenera or sections in *Galerina,* and we accordingly have named them ''mycenopsis,'' ''naucoriopsis,'' ''galerina'' and ''tubariopsis.'' These groups are discussed separately below as are the isolated species. The LSU tree also indicates that the genera *Phaeogalera* (represented by *P. stagnina*) and *Kuehneromyces* (represented by *K. mutabilis* [Schaeff.] Singer & A.H. Sm. and *Pholiota lignicola* [Peck] Jacobsson [= *K. lignicola* {Peck} Redhead]) are correctly kept separate from *Galerina* as currently done (TABLE I). These two genera occur at the basis of a /psilocybe clade in the analysis of Moncalvo et al (2002).

Family concepts and affinities of the different Galerina lineages.—The surrounding agaric consortium of our LSU analysis was chosen, through initial phylogenetic analyses, from the ochre- to black-spored range of agarics generally referred to the families Bolbitiaceae, Coprinaceae, Cortinariaceae, Crepidotaceae and Strophariaceae in the sense of Singer (1986). The sample corresponds to the old groups Dermini (with ochre to rusty brown spores), Pratelli (with brown to purplish-black spores) and a small part of the black-spored Coprinarii of Fries (1836–1838). These black-spored agarics of the Coprinarii (the *Panaeolus/Copelandia* group [= subfamily Panaeoloidae of Coprinaceae]) differ from the other members of Coprinaceae by having spore pigments that do not discolor in H_2SO_4 (Kühner 1980). In the widely accepted family scheme of Singer (1986), *Galerina* belongs in the family Cortinariacae. *Galerina* belongs in Strophariaceae, according to Kühner (1980), who drastically changed Singer's circumscription of the dark-spored families in the Dermini-Pratelli-Coprinarii range. Strophariaceae in the sense of Kühner includes only saprophytic species with a spore color range from brown to black, and it further assembles all species containing styryl-pyroles and psilocybin/psilocin, as well as all species with the special kind of cystidia termed chrysocystidia. Cortinariaceae sensu


0.1
Inocybe agardhii AY380366
Inocybe curvipes AY239022
Conocybe intrusa O60165
Conocybe lactea O153415
Bolbitius reticulatus O82057
Stagnicola perplexa AF261509
Descolea gunnii AF261523
Conocybe vexans O50693
Conocybe aporos O153351
Cortinarius bolaris AY293173
Cortinarius badiovinaceus AF388786
Cortinarius bulliardii AF388782
Rozites caperatus AF261497
Cortinarus anomalus AF388769
Hemipholiota populnea AF195593
Hemipholiota destruens AF261647
Hemipholiota lucifera AF261646
Galerina paludosa O153987
Galerina paludosa AF261528
Galerina paludosa AF261653
Gymnopilus decipiens O82172
Gymnopilus penetrans O95835
Gymnopilus odini O82167
Gymnopilus aeruginosus AF261650
Gymnopilus crociphyllus AY219593
Gymnopilus sapineus O154821
Gymnopilus penetrans AF261652
Gymnopilus sapineus AY380362
Gymnopilus junonius O171053
Gymnopilus junonius AY219598
Galerina calyptrata O73454
Galerina calyptrata O154221
Galerina calyptrata O73449
Galerina sphagnicola O71238
Galerina sphagnicola O73441
Galerina harrisonii O50711
Galerina harrissonii O73216
Galerina cf. fallax O73450
Galerina luteolosperma O154076
Galerina sphagnorum O154094
Galerina sphagnorum O70913
Galerina mniophila O73175
Galerina cephalotricha O154146
Galerina cf. mniophila O154072
Galerina mniophila O60574
Galerina cf. mniophila O50679
'mycenopsis'
Strophariaceae
sensu Kühner (1980)
Galerina badipes O73213
Phaeogalera stagnina O73191
Phaeogalera stagnina O73215
Phaeogalera stagnina AF261512
Agrocybe arvalis O152495
Agrocybe pediades O152544
Agrocybe praecox O59617
Agrocybe putaminum O59981
Naucoria escharioides AF261517
Naucoria escharioides O155488
Pholiota lignicola AF195594
Mythicomyces corneipes AF261381
Hebeloma westraliense AY219608
Hebeloma fastibile AY033139
Hebeloma longicaudum AF261515
Galerina nana O153723
Galerina pseudocamerina O73481
Galerina jaapii O50658
Galerina marginata AY219587
Galerina marginata O72507
Galerina marginata O72501
Galerina pseudomycenopsis O73464
Galerina pseudomycenopsis O50526
Galerina marginata O72434
Galerina marginata O72101
Galerina marginata O72429
Galerina marginata O72101
Galerina marginata O72135
Galerina marginata O72427
'naucoriopsis'
Galerina pruinatipes O73438
Galerina triscopa O154505
Flammula alnicola AF195588
Galerina nana AF261524
Phaeocollybia jennyae AF261506
Phaeocollybia festiva AY509119
Phaeocollybia redheadii AF261503
Galerina atkinsoniana O73188
Galerina atkinsoniana O73448
Galerina hypnorum forma O73206
Galerina atkinsoniana O73459
Galerina atkinsoniana O72933
Galerina minima O73467
Galerina vittiformis O73470
Galerina cf. minima O73468
Galerina vittiformis O73469
Galerina vittiformis O73427
Galerina atkinsoniana O73217
Galerina vittiformis O73122
'galerina'
Galerina pumila O73067
Naucoria amarescens O155460
Pachylepyrium funariophilum AF261513
Flammulaster linulatus O59850
Phaeomarasmius proximans AY380410
Tubaria hiemalis AF205689
Flammulaster carpophilus O83151
Phaeomarasmius erinaceus AF261492
Galerina pumila O73440
Flammulaster rhombospora AF261493
Melanotus phillipsii AF261608
Kuehneromyces sp. AY219610
Kuehneromyces mutabilis AJ406551
Pholiota conissans AF195606
Pholiota lundbergii AF195607
Pholiota highlandensis AF261644
Pholiota lenta AF261643
Hypholoma aurantiacum AY219609
Phaeonematoloma myosotis AF195599
Hypholoma capnoides AF195595
Hypholoma sublateritium AF042569
Galerina pseudobadipes O154254
Galerina pseudobadipes O154252
Galerina pseudobadipes O154186
Copelandia cyanescens AF261526
Panaeolus sphinctrinus AB104646
Galerina stordalii O73436
Galerina stordalii O154179
Galerina pseudocerina O50547
Galerina pseudocerina O154004
Galerina semilanceata AY038309
Galerina clavata O50544
Galerina clavata O72166
Galerina arctica O73198
Galerina arctica O73465
Galerina laevis O71160
'tubariopsis'

Kühner contains only ectomycorrhizal genera and only those with ochre to rusty brown spores.

The LSU tree (FIG. 1) includes a large unsupported clade that embraces all the *Galerina* species of our analysis. This clade corresponds largely to the family Strophariaceae sensu Kühner (1980) (indicated by arrow in FIG. 1), except for the fact that only a fragment of the family Bolbitiaceae occurs here (the genus *Agrocybe*), while Kühner included the whole family Bolbitiaceae in his Strophariaceae (the other genera of Bolbitiaceae, *Bolbitius, Conocybe* and *Descolea* occur in the upper part of the tree). In addition the two ectomycorrhizal genera *Hebeloma* and *Phaeocollybia* are irregular in the essentially saprophytic Strophariaceae of Kühner. None of our *Galerina* lineages appear close to genera of the Cortinariaceae, neither do the isolated *Galerina* species, and we conclude that *Galerina* species belong in the family Strophariaceae, not in the Cortinariaceae.

Gymnopilus and ''mycenopsis'' constitute one monophyletic group.—With high support our LSU analysis suggests that *Gymnopilus* is a monophyletic genus, as previously suggested by Rees et al (2002) and Guzmán-Dávalos et al (2003) inferred from ITS analyses, and that *Gymnopilus* and our ''mycenopsis'' constitute a highly supported group. A close relationship between *Galerina* and *Gymnopilus* as suggested by the LSU topology is highly interesting because relationships and delimitation of these genera have been much disputed (Kühner 1980, Singer 1986, Rees et al 1999, Rees et al 2002). ''Presence or the absence of styrylpyrones bis-noryangonin and hispidin provides the only clear-cut means of differentiation between the two genera,'' according to Rees et al (1999). Rees et al (2002) however state that some species of *Gymnopilus* has been found lately without these pigments. A close relationship between the two genera is not evident from comparison of fruit body aspects of northern hemisphere *Galerina* and *Gymnopilus* species. The relatively fleshy, often caespitose and lignicolous, bitter-tasting *Gymnopilus* species are quite different from the typically small and bryophilous *Galerina* species. *Galerina pruinatipes,* also described as *Gymnopilus laricicola* J. Favre, may serve as an odd example of fruit body similarity. However, in the southern hemisphere, a group of small, mostly eccentrically stipitate species of *Galerina* are difficult to distinguish from small-statured *Gymnopilus* species in the field and even after microscopic examination (Rees et al 1999). Typical of these *Gymnopilus* species apart from their small fruit bodies are more or less tibiiform cystidia and dextrinoid spores with a more or less distinct plage. Interestingly, our ''mycenopsis'' group that forms the sister group of *Gymnopilus* does not include the *Galerina* species with tibiiform cystidia.

More than anything, spore features seem to unite *Galerina* and *Gymnopilus*. Typical of both genera are ochre to rusty brown, amygdaloid-ellipsoid and ornamented spores often provided with a plage. An inflating, dextrinoid/cyanophilic endospore appears to be unique to species of these genera (Kühner 1972, 1980). This kind of endospore is found in the ''mycenopsis'' group (in fact in all the groups except that of ''tubariopsis'') and it has been demonstrated in these *Gymnopilus* species: *G. junonius, G. penetrans, G. liquiritiae, G. bellulus, G. flavus* and *G. fulgens* (Kühner 1980).

''mycenopsis''.—Most species included in the ''mycenopsis'' lineage in our ITS and LSU analyses have a typical ''*Galerina*'' appearance; that is a mycenoid fruit body, presence of a more or less fugacious veil, and yellow to dark red-brown color. They are bryophilous, and some are strictly sphagnophilous. *Galerina paludosa* is a parasite on *Sphagnum* (Redhead 1981). In current classifications the species of this group are referred to subgenus/section *Mycenopsis* (or subsection *Mycenopsidae*) and to section *Calyptrospora* (TABLE I). They are characterized microscopically by faintly ornamented to practically smooth spores that in some species are more or less calyptrate (i.e. with an outer wall layer that tends to separate and form smaller or larger blisters, particularly in the area around the apiculus) and by cystidia that are lageniform to ventricose-capitate and restricted to the lamellae edge (cheilocystidia).

The separate position of *G. paludosa,* as a sister group of *Gymnopilus* in the LSU tree, corresponds to the findings of Moncalvo et al (2002) where *G. paludosa* is included in the /gymnopiloid clade as a sis-

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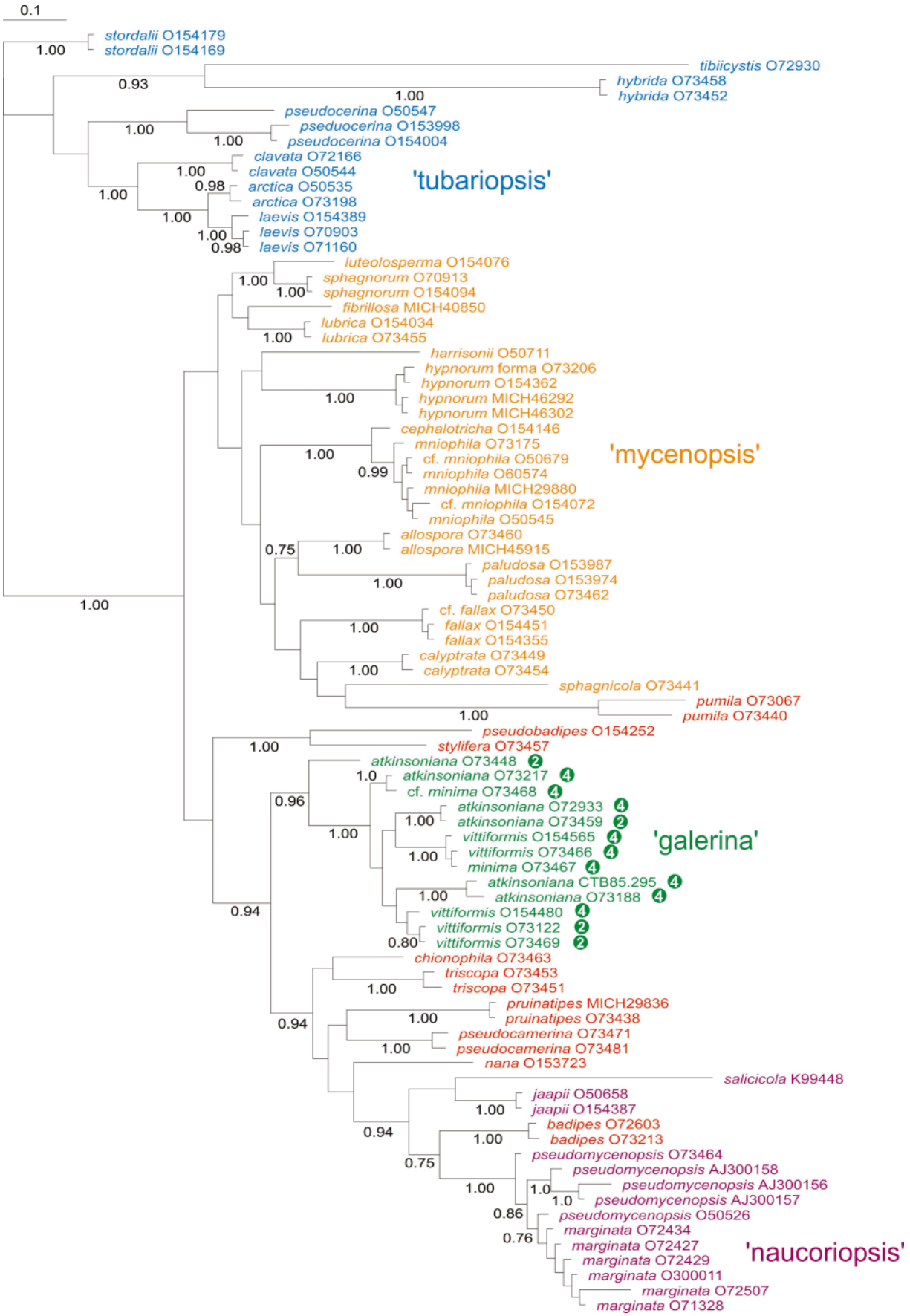
FIG. 1. Consensus phylogram of 20 000 trees resulting from Bayesian analysis of 130 LSU sequences. *Inocybe agardhii* and *I. curvipes* were used as outgroup. Bayesian posterior probabilities (BPP) are given below branches. Boldface indicates sequences generated in this study (Herbarium accession codes are shown behind sequences). GenBank accession numbers are shown behind sequences downloaded from GenBank. The four *Galerina* main lineages are shown in different colors and *Galerina* species not included among them are in red. Numbers within circles in the ''galerina'' group indicate 2- or 4-spored basidia. The main monophyletic grouping corresponds largely with Kühner's (1980) concept of Strophariaceae and is indicated by an arrow.


0.1
stordalii O154179
stordalii O154169
tibiicystis O72930
hybrida O73458
hybrida O73452
pseudocerina O50547
pseduocerina O153998
pseudocerina O154004
clavata O72166
clavata O50544
arctica O50535
arctica O73198
laevis O154389
laevis O70903
laevis O71160
'tubariopsis'
luteolosperma O154076
sphagnorum O70913
sphagnorum O154094
fibrillosa MICH40850
lubrica O154034
lubrica O73455
harrisonii O50711
hypnorum forma O73206
hypnorum O154362
hypnorum MICH46292
hypnorum MICH46302
cephalotricha O154146
mniophila O73175
cf. mniophila O50679
mniophila O60574
mniophila MICH29880
cf. mniophila O154072
mniophila O50545
'mycenopsis'
allospora O73460
allospora MICH45915
paludosa O153987
paludosa O153974
paludosa O73462
cf. fallax O73450
fallax O154451
fallax O154355
calyptrata O73449
calyptrata O73454
sphagnicola O73441
pumila O73067
pumila O73440
pseudobadipes O154252
stylifera O73457
atkinsoniana O73448
atkinsoniana O73217
cf. minima O73468
atkinsoniana O72933
atkinsoniana O73459
vittiformis O154565
vittiformis O73466
minima O73467
atkinsoniana CTB85.295
atkinsoniana O73188
vittiformis O154480
vittiformis O73122
vittiformis O73469
'galerina'
chionophila O73463
triscopa O73453
triscopa O73451
pruinatipes MICH29836
pruinatipes O73438
pseudocamerina O73471
pseudocamerina O73481
nana O153723
salicicola K99448
jaapii O50658
jaapii O154387
badipes O72603
badipes O73213
pseudomycenopsis O73464
pseudomycenopsis AJ300158
pseudomycenopsis AJ300156
pseudomycenopsis AJ300157
pseudomycenopsis O50526
marginata O72434
marginata O72427
marginata O72429
marginata O300011
marginata O72507
marginata O71328
'naucoriopsis'

ter group of a monophyletic gymnopilus clade. *Galerina pumila,* which currently is referred to section *Mycenopsis* or its equivalents and which also occurs in the ''mycenopsis'' clade in the ITS tree, is found in two separate positions outside the clade in the LSU tree. The species differs from the other species of the ''mycenopsis'' clade by having truly smooth spores and elongate, narrow cystidia. It has been interpreted as the *Agaricus mycenopsis* of Fries and is the type species of section *Mycenopsis* A.H. Sm. & Singer (subgenus *Mycenopsis* Bon 1992). When a splitting of the genus *Galerina* is implemented in forthcoming studies, the position of *G. pumila* becomes important for nomenclatural reasons.

The section *Calyptrospora,* according to its authors, is based on a single character (Smith and Singer 1964) and its taxonomic value is questionable also because the spores in a single species vary with regard to degree of calyptration (i.e. percentage of spores affected and size of the blisters). The distinctly calyptrate *G. calyptrata* and *G. sphagnicola* appear together in both trees; *G. fallax,* which is variable with regard to calyptration, takes different positions within the clade in both trees, while *G. hypnorum,* present only in the ITS analysis, takes a position among the noncalyptrate species of the clade. This species has been variously interpreted and, according to Smith and Singer (1964), has noncalyptrate spores and cystidia with acute to obtuse necks. The analyzed material collected by Smith (MICH 46292, 46302), however, has many spores with small blisters and mostly ventricose-capitate cystidia and corresponds in these respects to the included Norwegian collection (O 154362). Apparently the taxonomic importance of calyptration should be tuned down and the section *Calyptrospora* abandoned. The collection identified as a form of *G. hypnorum* (O 73206) has aberrant, voluminous cystidia (Gulden in press). It occurs with the other *G. hypnorum* collections in the ITS tree, but has a strange position in the LSU tree together with species of the ''galerina'' lineage.

An annulate thickening is seen near the spore apex in *G. allospora* and has been considered unique for this species. However we found the same character in the holotype material of *G. fibrillosa* when this was studied microscopically. *Galerina fibrillosa* is a rare species that, according to its author, reminds one of a *Phaeomarasmius* and differs from the typical galerinas by being dark (Verona brown, snuff brown, dark, dull, vinaceous cinnamon, etc.) with a convex-umbonate and coarsely matted-fibrillose pileus (Smith and Singer 1964). It was made the type species of the small section *Inoderma* A.H. Sm. & Singer; its name refers to the fibrillose pileus. An inclusion here of *G. fibrillosa* in the 'mycenopsis' clade as indicated in the ITS tree does not seem unnatural in view of the spore apex thickening shared with *G. allospora,* but also because other species in the group has a more or less scurfy-subscaly pileus deviating from the normal smooth pattern in *Galerina. Galerina harrisonii,* for example, originally was described as *Phaeomarasmius harrisonii* and shares a more convex, less conic-campanulate pileus with *G. fibrillosa.*

''*naucoriopsis*''.—The core group of the ''naucoriopsis'' lineage includes the more fleshy *Galerina* species, in particular by having an incurved pileus margin and an annulus or annulate zone on the stipe. The ''naucoriopsis'' species vary considerably with regard to habitat-substrate preferences and often form fruit bodies on woody and herbal debris or grow in mossy or grassy sites, and some of the species seem little specific in this regard being able to switch from a lignicolous to a terricolous habit. In the ''naucoriopsis'' lineage the spores are more or less distinctly ornamented, and large ventricose-fusoid cystidia occurring on the edge as well as on the sides of the lamellae (pleurocystidia) are characteristic.

Our ''naucoriopsis'' lineage includes the toxic species *G. marginata,* which according to morphological and molecular studies, was considered identical with inter alia *G. venenata* and *G. autumnalis* (Gulden et al 2001). Material identified as *G. marginata, G. autumnalis* and *G. venenata* have been shown to contain amatoxins and have caused intoxications (Enjalbert et al 2004, Tyler and Smith 1963, Tyler et al 1963). *G. marginata* in the broad sense contains amanitins (α, β and γ) in different concentrations related to substrate conditions and sometimes even in higher amounts than in fruit bodies of *Amanita phalloides,* according to Enjalbert et al (2004). Also *G. badipes,* with an aberrant position in the LSU analysis (FIG. 1), contains amatoxin (γ-amanitin) (Besl et al 1984) and fits in our ''naucoriopsis'' lineage in this respect.

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FIG. 2. Consensus phylogram of 20 000 trees resulting from the Bayesian analysis of 85 ITS sequences. The ''tubariopsis'' group was used as outgroup. Bayesian posterior probabilities (BPP) are given below branches. Herbarium accession codes are shown behind sequences and GenBank accession numbers behind sequences downloaded from GenBank. The four *Galerina* main lineages are shown in different colors and *Galerina* species not included are in red. Numbers within circles in the ''galerina'' group indicate 2- or 4-spored basidia.

In fact all reported amanitin-containing *Galerina* species belong in section Naucoriopsis (Enjalbert et al 2004).

The phylogenetic relationship between the two central species of ''naucoriopsis,'' *G. marginata* and *G. pseudomycenopsis,* was studied by Gulden et al (2001) with ITS sequences. Ecologically and morphologically there are clear differences between these species (Horak and Miller 1992, Gulden and Vesterholt 1999). It was concluded, however, that the applied molecular methods could not distinguish the two species. The two species did not separate well in our present analyses. A status of *G. pseudomycenopsis* as a distinct species may be inferred from a limited mating experiment conducted at the lab of Ronald Petersen, Knoxville, Tennessee, in 2002, where single-spore isolates of *G. pseudomycenopsis* from Greenland were crossed with single-spore isolates of *G. autumnalis* from North America (Washington) and of *G. marginata* from Europe (Norway and Austria). All crosses of *G. pseudomycenopsis* with *G. marginata/G. autumnalis* were incompatible, while all crosses between *G. marginata* and *G. autumnalis* were compatible (Peterson personal communication). *Galerina pseudomycenopsis* has not been analyzed with respect to amanitin-content.

In both analyses the aberrant *G. jaapii* forms a sister group of the core group. The strictly bryophilous *G. jaapii* is a slender species of a more *Galerina*-like look than the other species of the group, but it generally has a well developed annulus. In contrast to the core species it has cystidia of the ''mycenopsis'' type, present only as cheilocystidia, and almost smooth spores with an apical pore borne on 2-spored basidia. The spore characters are shared with *G. badipes* that occurs close to *G. jaapii* in the ITS tree. *Galerina jaapii* generally has been considered a species of section *Mycenopsis,* while *G. badipes,* which has typical ''naucoriopsis'' cheilo- and pleurocystidia, in all classifications has been included in section/subgenus *Naucoriopsis.* A monophyletic ''naucoriopsis'' clade includes also *G. salicicola,* according to the ITS tree. *Galerina salicicola,* which gross-morphologically fits well in ''naucoriopsis,'' was placed in section *Physocystis* together with *G. pruinatipes* and a few more species (Smith and Singer 1964) on account of somewhat special (utriform-broadly lageniform) but rather polymorph cystidia (cheilo- and pleurocystidia). Orton (1960), who originally described the species, mentioned *G. badipes* and *G. nana* as probably the nearest relatives. A more thorough phylogenetic analysis with multiple genetic markers is necessary to draw further taxonomic conclusions in this important group of *Galerina* species.

''*galerina*''.—The ''galerina'' lineage includes bryophilous species with typical *Galerina* habit and yellow, amber to red brown color. They generally are referred to section *Galerina.* The species of this lineage have the same type of cystidia as the species of ''naucoriopsis,'' but these occur also as caulocystidia, covering more or less the entire stipe, and on the pileus (pileocystidia) in some taxa. The spores are ornamented distinctly and often borne on 2-spored basidia, as also frequently seen in the ''naucoriopsis'' lineage. The ''galerina'' and the ''naucoriopsis'' lineages appear closer related to each other than to any other of the *Galerina* lineages as inferred from the LSU analyses, but apart from the cystidia and the tendency to develop 2-spored forms they do not appear to have characters in common that also are not shared with species of the other *Galerina* groups. The ''galerina'' group includes the type species of the genus *Galerina* Earle, *Agaricus vittaeformis* Fr.

Within the ''galerina'' group, the studied material was separated on three species as follows: Specimens with pileocystidia were identified as *G. atkinsoniana*; the *atkinsoniana*-collections had either predominantly 2-spored or 4-spored basidia and were referred to two distinct forms. Material without pileocystidia was identified either as *G. minima* or *G. vittiformis.* Specimens with a well developed veil, forming a whitish brim on the pileus or persisting as fibrils on the stipe were identified as *G. minima* (= *G. terrestris* V.L. Wells & Kempton [Gulden in press]). All collections of this species had 4-spored basidia. Typical features of *G. minima* are also a farinaceous flavor and occurrence in pioneer vegetation in cold climates. The material of *G. vittiformis* had no veil, the odor/flavor was not always documented, and the collections had either 2- or 4-spored forms corresponding to the situation in *G. atkinsoniana.* Neither of our analyses renders support or any clear clue to the differentiation of the taxa in this lineage, and apparently the 2- and 4- spored states are not stable conditions.

''*tubariopsis*''.—The ''tubariopsis'' lineage includes bryophilous species with a typical *Galerina* appearance but aberrant microscopic features. Clamp connections, otherwise present at all septa in all *Galerina* species, occur inconsistently in this group, and important spore characters vary as well. All species of the lineage have tibiiform cystidia that occur in most parts of the fruit bodies but never as pleurocystidia. The lineage corresponds to the morphologically defined subgenus *Tubariopsis,* but includes in addition a few species that generally are placed in subgenus *Galerina,* in section *Tibiicystidiae* or its equivalents. These are *G. pseudocerina,* included in the LSU anal-

ysis, and *G. tibiicystis* and *G. hybrida,* in the ITS analysis.

Core species of the lineage, *G. clavata, G. laevis, G. arctica* and *G. semilanceata,* are characterized by a total lack of clamp connections and furthermore by spores that differ from the *Galerina* spores in general by lacking the plage as well as the inflating/dextrinoid/cyanophilic endospore. These species currently are placed in section *Tubariopsis* of the subgenus, while *G. stordalii* (= *G. dimorphocystis* ss. Kühner 1972) belongs in a section of its own, *Hemitubariopsis,* characterized by sporadic clamp connections (found only at the base of basidia and at some hyphal septa). *Galerina stordalii* occurs well separated from the core species in both trees. The spores of *G. stordalii* lack a plage as is common in the subgenus but differ fundamentally from the typical pattern of the subgenus by having the inflating/dextrinoid/cyanophilic endospore and an apical pore. This latter feature led Smith and Singer (1964) to place the species in section *Porospora* (subgenus *Galerina*).

The last three species of the lineage (viz. *G. pseudocerina, G. tibiicystis* and *G. hybrida)* have clamp connections at all septa and dextrinoid spores with a more or less delimited plage just like species of the other subgenera of *Galerina*. Of these *G. pseudocerina* differs from all others of the lineage by having a somewhat more hemispheric and fleshy pileus and by its coarsely ornamented spores. *Galerina tibiicystis* and *G. hybrida,* which are similar species, share an exclusively sphagnophilous habit with *G. stordalii,* while some of the other species of the lineage occasionally may occur on *Sphagnum*.

Galerina species without clear affinities.—*Galerina nana, G. pruinatipes, G. pseudocamerina* and *G. triscopa* occur in various positions close to the ''naucoriopsis'' lineage in the LSU tree. In the ITS tree they also take positions close to ''naucoriopsis'' together with *G. chionophila* (only present in the ITS analysis). All these species have typical *Galerina* spores (i.e. amygdaliform, ornamented with plage, dextrinoid and cyanophilic). They are born on two-spored basidia in *G. nana, G. pseudocamerina* and *G. pruinatipes* just like in *G. jaapii* and *G. badipes* of the ''naucoriopsis'' lineage. All but the bryophilous or terricolous *G. chionophila* also share the lignicolous habit with the typical species of this lineage. In current classifications these four species are referred to widely different infrageneric taxa: *G. nana* is the type species of section *Inocyboides* Singer (= subgenus *Inocybula* [Singer] Bon) that is particular by presence of thick-walled cheilo- and pleurocystidia (reminding of those found in the genus *Inocybe*). Except for these cystidia, *G. nana* is similar to the *G. marginata* group. The species occurs linked to the /panaeoloideae clade in the analysis of Moncalvo et al (2002). *Galerina pruinatipes* was placed in section *Physocystis* by Smith and Singer (1964) on account of its large, broad-headed cystidia that are present as cheilo-, pleuro-, caulo- and pileocystidia, and in section *Galerina* by Bon (1992). The species also has been placed in the genus *Gymnopilus* (as *G. laricicola* J. Favre). *Galerina pseudocamerina* and *G. triscopa* are most often placed in section *Tibiicystidiae* on account of their cystidia. Our LSU tree indicates that these four species together with the two groups ''galerina'' and ''naucoriopsis'' may constitute a single monophyletic genus (*Galerina* Earle emnd.). However this unit will have to encompass a fairly broad variation in cystidial shape and topology. More data are necessary to draw conclusion on this topic.

Galerina pseudobadipes and *G. stylifera* are morphologically similar species that occur at a distance from the other *Galerina* species in both trees. Their spores differ clearly from the typical *Galerina* spore by being smooth, blunt-ellipsoid and only faintly dextrinoid. These species have tibiiform cystidia and form fruit bodies on woody substrate. They traditionally have been referred to section *Tibiicystidiae* or to a new section *Styliferae* of subgenus *Naucoriopsis* Kühner (1972). In the LSU phylogeny *G. pseudobadipes* occurs in the vicinity of the ''tubariopsis'' lineage where the other *Galerina* species with tibiiform cystidia are found, but separated from the others by a group of *Panaeolus/Copelandia* species. More species and genetic markers need to be included before a phylogenetically statistical supported position of *G. stylifera* and *G. pseudobadipes* can be reached.

Concluding remarks and further perspectives.—This study represents a first attempt to investigate the phylogeny of *Galerina* and its allied genera. Our investigation demonstrates clearly that *Galerina* is a polyphylethic genus, that *Gymnopilus* is monophyletic, and that a part of *Galerina* (the ''mycenopsis'' lineage) is closely related to *Gymnopilus*. It also affirms isolated positions for *Phaeogalera* and *Kuehneromyces* in relation to *Galerina*. On the family level it demonstrates that the family Strophariaceae in the sense of Kühner agrees fairly well with the molecular phylogenies and that all the different lineages of *Galerina* species fall within the frame of Strophariaceae sensu Kühner. A high correspondence between the resolved lineages and previously defined subgenera/sections indicates that traditional morpho-taxonomy in *Galerina* operates fairly well at lower taxonomic levels. However our analyses affirm previous findings by e.g. Larsson et al (2004) that many morphological characters (e.g. fruit body morphology, cystidial

shape) are highly homoplastic. On the specific level our analyses yield little additional information for solving taxonomically intricate complexes.

Judging from the present selection of *Galerina* taxa, where southern hemisphere species are not included, this study demonstrates that the old genus *Galerina* collapses and must be segregated in at least three different monophyletic genera. It further indicates that some morphologically based infrageneric units should be abandoned (e.g. the sections *Calyptrospora* A.H. Sm. & Singer, *Inoderma* A.H. Sm. & Singer and *Physocystis* A.H. Sm. & Singer). At the present stage, due to limited amount of data we are not ready to proclaim formal taxonomic consequences or nomenclatural changes. Several studies have shown that multiple unlinked loci should be analyzed before formal taxonomic conclusions are drawn (e.g. Rokas et al 2003). This also is indicated in our results by some incongruent topological patterns in the ITS and LSU trees (e.g. the placements of *G. badipes* and *G. pumila*). Our preliminary results will be pursued by more collaborated analyses of multiple loci and a broader taxonomic sample.

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