

A SOUTHERN HEMISPHERE CONTRIBUTION TO THE PHYLOGENETIC STUDY OF THE AGARICS

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Abstract

Sequences for the 5' end of the nuclear large ribosomal subunit are compared for 18 species of *Gymnopilus*. Fourteen of these are for Australian collections, the remainder from the Northern Hemisphere. The sequences have been analysed in conjunction with 60 species of brown-spored genera from both hemispheres to place them in context. Results confirm the independent position of *Gymnopilus* in the overall phylogeny of agaric species.

Key words: Nuclear large ribosomal subunit RNA, *Gymnopilus*, *Cortinarius*, Agaricales, molecular phylogeny.

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Introduction

The process of revising macrofungal genera in Australia where so many new species await description, inevitably raises questions about the monophyly of the group under consideration and its relatedness to other genera. The monophyly of twenty species of the agaric genus *Gymnopilus* P. Karsten has been established in a cladistic analysis of sequences from the Internal Transcribed Spacer (ITS) region of ribosomal DNA using maximum parsimony (MP) and maximum likelihood (ML) analyses (Rees *et al.* 2002). In order to explore the diversity of the genus, species from both the Northern and Southern Hemispheres were included, resulting in eight groups of species, and two remaining taxa with unresolved affiliations. These groups bore no resemblance to the traditional morphological infrageneric classification of *Gymnopilus* based on the type of velar remains and spore size (Guzmán-Dávalos 1995, Hesler 1969, Romagnesi 1942, Singer 1951).

Before the use of molecular data, an ongoing debate existed about the inclusion of *Gymnopilus* in the agaric family Cortinariaceae (Pouzar) R. Heim, on the basis of similar spore wall structure and ornamentation (Bas 1988, Hesler 1969, Moser 1983, Singer 1951) or in the Strophariaceae Singer & Smith, on the basis of its saprotrophic mode of nutrition and the presence of styrylpyrone pigments produced by other members of that family (Høiland 1990, Kühner 1980). Høiland also proposed the idea that both *Gymnopilus* and *Cortinarius* Fr. may have arisen from a common, saprotrophic, *Gymnopilus*-like ancestor, with one line maintaining the saprotrophic lifestyle ultimately giving rise to the Strophariaceae and other dark-spored genera, while the other gave rise to a mycorrhizal line similar to *Dermocybe* (Fr.) Sacc. in the Cortinariaceae. Petersen (1968) has commented on the similar, cyanophilic reaction in the ornamented spore walls of both *Gymnopilus* and *Gomphus* S.F. Gray species, raising the possibility that brown-spored agarics may have arisen from a gomphoid-like ancestor. Moser (1985) advocated caution in the use of chemical data for determining relationships between genera, especially when styrylpyrones are found in seemingly unrelated genera such as *Inonotus* P. Karsten and *Phaeolus* Pat. The description of *Cortinarius* species which produce styrylpyrone pigments (Moser 1985, Watling *et al.* 1992) and of *Gymnopilus* species mycorrhizal on orchid *Cattleya* Linley species, tended to support the retention of *Gymnopilus* within the Cortinariaceae, but no conclusive decision could be arrived at owing to lack of further information.

Our initial attempts to arrive at a phylogeny for representative genera from the Cortinariaceae and Strophariaceae in the sense of Singer (1986) using ITS sequence data, resulted in problems with alignment of sequences and

largely unresolved consensus trees, with little light shed on the nature of the relationships. The publication of a phylogeny of 154 agaric taxa (Moncalvo *et al.* 2000) representing most families within the Agaricales using the 5' end of the nuclear large ribosomal subunit gene (nLSU) of ribosomal DNA with *Ganoderma lucidum* (Fr.) P. Karsten and *Ganoderma australe* (Pers.) Pat. as outgroups, heralded a new understanding of relationships in the order. The two families Cortinariaceae and Strophariaceae were found not to be monophyletic. No *Gymnopilus* species were included, but in a larger more recent analysis of 877 taxa (roughly one tenth of the described agaric species) using *Auricularia polytricha* (Mont.) Sacc. as outgroup, four species of *Gymnopilus* were to be found within a 'gymnopiloid' clade, together with a single *Hebelomina* Maire and *Galerina* Earle species. *Galerina*, which also emerges as non-monophyletic in the analysis, was regarded by Singer (1986) as the genus closest to *Gymnopilus* within the Cortinariaceae, but could be differentiated from it by a suite of features including the absence of styrylpyrone pigments. Since then, species of *Gymnopilus* have been described with undetectable levels of styrylpyrones and well-developed supra-hilar discs, and the boundary between the two genera in the Southern Hemisphere has become less well-defined (Rees *et al.* 1999, 2002). *Hebeloma* Kummer and *Crepidotus* Kummer appeared to be the most closely related large genera.

Some clades in the enlarged agaric phylogeny reflect similarities in ecological, biochemical and nutritional habits rather than morphological similarities (Moncalvo *et al.* 2002), and several problematic taxa enjoy new and in some cases rather surprising affiliations. Although bootstrap support is not strong for some of the clades, *Phaeocollybia* R. Heim species are not too far removed from other mycorrhizal formerly Cortinariaceous genera. However, *Descolea gunnii* (Berk. : Masee) E. Horak with a hymeniform pileipellis occurs in the same clade as *Bolbitius* Fr., *Pholiotina* Fayod and *Conocybe* Fayod species as predicted by Singer on the basis of morphological similarities. *Phaeomarasmium erinaceus* (Pers. : Scherff.) Romagnesi is in close proximity to *Tubaria* (W.G. Smith) Gillet species which are well removed from other cortinariaceous former allies. *Melanotus* Pat. and *Kuehneromyces* Singer & A.H. Smith species are to be found near the base of the euagaric clade clearly separated from *Pholiota* Kummer but close to *Psilocybe* Kummer species.

As so few of the Australian species of the major agaric genera have been revised, most comprehensive phylogenetic analyses do not include data for Australian species, although many genera such as the former genus *Rozites* P. Karsten (now transferred to *Cortinarius* on molecular evidence), are thought to have co-evolved with *Nothofagus* Blume in the Southern Hemisphere (Bougher *et al.* 1994). It seems worthwhile therefore to offer the following sequence data for Australian species of brown-spored genera in the form of an analysis, placing them in context with exotic species of the same genera. Any anomalies, if they exist, will add to a more complete understanding of relationships within the agarics especially with regard to species and genera that have been difficult to place up to the present.

The analysis includes samples from most, but by no means all, of the agaric genera formerly included in the families Cortinariaceae and Strophariaceae by Singer (1986). Australian and some New Zealand taxa have been used where material or previously published sequences were available, supplemented by GenBank sequences already available for related genera and species from northern Europe and North America. A small number of genera thought to be derived from *Gymnopilus*-like ancestors have also been included, as well as a number of species and genera such as *Cortinarius abnormis* Watling & T.W. May, *Phaeomarasmium* Scherffel and *Descolea* Singer whose affiliations were considered intermediate or controversial.

Materials and methods

A total of 79 sequences were analysed. These included 21 collections of 18 species of *Gymnopilus* that were compared with two species of each brown-spored agaric genus as detailed in Table 1. Where possible each pair consisted of one Australian collection and one Northern Hemisphere collection, but not necessarily the same species. DNA was extracted from fresh or dried collections, and the most complete sequences were used for the analysis, although duplicates exist for some of the Australian *Gymnopilus* species. As it was not possible to determine the collection site for many of the Northern Hemisphere species, only voucher numbers are provided, and the work in which the species were published. Authorities for the taxa are included at the end of this paper in the Appendix.

Table 1. List of species included.

Species name	Collection No	Sequence published	GenBank No
<i>Agrocybe parasitica</i>	BRQ 02/24	This work	AY219580
<i>Agrocybe praecox</i>	SAR 85/149	Moncalvo <i>et al.</i> (2000)	AF042644
<i>Bolbitius vitellinus</i>	SAR s.n.	Hopple & Vilgalys (1999)	AF041543
<i>Bolbitius vitellinus</i>	SAR 84/100	Rehner <i>et al.</i> (1944)	BVU11913
<i>Bolbitius demangei</i>	JMCR 137	Moncalvo <i>et al.</i> (2002)	AF261520
<i>Conocybe rickenii</i>	J 183	Hopple & Vilgalys (1999)	AF041546
<i>Coprinellus disseminatus</i>	C 221	Hopple & Vilgalys (1999)	AF041525
<i>Coprinellus micaceus</i>	C12	Hopple & Vilgalys (1999)	AF041513
<i>Cortinarius abnormis</i>	BRSA 02/03	This work	AY219581
<i>Cortinarius alboroseus</i>	NZ 8672	Peintner <i>et al.</i> (2002)	AY033127
<i>Cortinarius caperatus</i>	G 96/3	Moncalvo <i>et al.</i> (2002)	AF261497
<i>Cortinarius elaiochrous</i>	NZ 8701	Peintner <i>et al.</i> (2002)	AY033126
<i>Cortinarius iodes</i>	JM 96/23	Moncalvo <i>et al.</i> (2000)	AF042613
<i>Cortinarius limoneus</i>	IB 19740328	Peintner <i>et al.</i> (2002)	AY033126
<i>Cortinarius metallicus</i>	BRV 99/14	This work	AY291582
<i>Cortinarius sp.</i>	BRV 99/15	This work	AY219583
<i>Cortinarius traganus</i>	DAOM 212213	Moncalvo <i>et al.</i> (2002)	AF261500
<i>Cortinarius violaceus</i>	DAOM 216796	Moncalvo <i>et al.</i> (2002)	AF261499
<i>Crepidotus applanatus</i> var. <i>applanatus</i>	MCA 170	Aime (thesis)	AF205694
<i>Crepidotus applanatus</i> var. <i>globigera</i>	MCA 188.8	Aime (thesis)	AF205700
<i>Crepidotus sp.</i>	BRTAS 98/62	This work	AY219584
<i>Crepidotus sp.</i>	BRSA 99/15	This work	AY219585
<i>Dermocybe austroveneta</i>	1...647	Chambers <i>et al.</i> (2000)	AF112147
<i>Dermocybe marylandensis</i>	JM 96/24	Moncalvo <i>et al.</i> (2000)	AF042615
<i>Dermocybe splendida</i>	BRWA 99/62	This work	AY219586
<i>Descolea gunnii</i>	Duke 3001	Moncalvo <i>et al.</i> (2000)	AF261523
<i>Galerina marginata</i> 1	NSW 99/11	This work	AY219587
<i>Galerina marginata</i> 2	RM 3225	Moncalvo <i>et al.</i> (2002)	AF195590
<i>Galerina paludosa</i>	CBS 388.88	Moncalvo <i>et al.</i> (2000)	AF261653
<i>Galerina subcerina</i>	UNSW 99/31(a)	This work	AY219588
<i>Galerina tibiiformis</i>	UNSW 00/09	This work	AY219589
<i>Gomphus novae-zelandiae</i>	ZT 68-557	Moncalvo <i>et al.</i> (2002)	AF261547
<i>Gymnopilus aeruginosus</i>	CBS 296.36	Moncalvo <i>et al.</i> (2002)	AF261650
<i>Gymnopilus allantopus</i>	BRWA 99/7(b)	This work	AY219590
<i>Gymnopilus anomalus</i>	BRWA 99/5	This work	AY219591
<i>Gymnopilus austropicreus</i>	BRV 99/4	This work	AY219592
<i>Gymnopilus crociphyllus</i>	UNSW 98/34	This work	AY219593
<i>Gymnopilus dilepis</i>	LEECH 982	This work	AY219594
<i>Gymnopilus eucalyptorum</i>	BRV 99/10	This work	AY219595
<i>Gymnopilus ferruginosus</i>	BRV 99/5	This work	AY219596
<i>Gymnopilus junonius</i> 1	SJ84074	Moncalvo <i>et al.</i> (2000)	AF195591
<i>Gymnopilus junonius</i> 2	BRV 99/1	This work	AY219597
<i>Gymnopilus junonius</i> 3	BRSA 02/08	This work	AY219598
<i>Gymnopilus luteofolius</i>	AMM 12367	This work	AY219599
<i>Gymnopilus marginatus</i>	BRQ 02/13	This work	AY219600
<i>Gymnopilus megasporus</i>	BRSA 02/02	This work	AY219601
<i>Gymnopilus moabus</i>	UNSW 99/38	This work	AY219602
<i>Gymnopilus penetrans</i>	CBS 168.79	Moncalvo <i>et al.</i> (2002)	AF261652
<i>Gymnopilus picreus</i>	AS 97/103	This work	AY219603
<i>Gymnopilus purpuratus</i>	ZT 02/01	This work	AY219604
<i>Gymnopilus sp. A</i>	UNSW 02/02	This work	AY219605
<i>Gymnopilus sp. B</i>	KS 803/95	This work	AY210606
<i>Gymnopilus spectabilis</i>	CBS 489.90	Moncalvo <i>et al.</i> (2002)	AF261651
<i>Hebeloma crustuliniforme</i>	SAR 87/408	Rehner <i>et al.</i> (1994)	HCU 11918

<i>Hebeloma</i> sp.	BRBG 99/8	This work	AY219607
<i>Hebeloma westraliense</i>	BRWA 99/39	This work	AY219608
<i>Hypholoma aurantiacum</i> 1	H17(HB5)	Moncalvo <i>et al.</i> (2002)	AF261633
<i>Hypholoma aurantiacum</i> 2	BRBG 99/8	This work	AY219609
<i>Hypholoma sublateritium</i>	JM 96/20	Moncalvo <i>et al.</i> (2000)	AF042569
<i>Inocybe geophylla</i> var. <i>lilacea</i>	JM 96/25	Moncalvo <i>et al.</i> (2000)	AF042616
<i>Inocybe</i> sp.	BRTAS 98/27	This work	AY222740
<i>Kuehneromyces mutabilis</i>	GEL 4369	Langer (2001)	AJ406551
<i>Kuehneromyces</i> sp.	UNSW 02/39	This work	AY219610
<i>Melanotus horizontalis</i>	V208 CBS 101982	Moncalvo <i>et al.</i> (2002)	AF261609
<i>Melanotus phillipsii</i>	V077 CBS 101833	Moncalvo <i>et al.</i> (2002)	AF261608
<i>Melanotus subcuneiformis</i>	RV PR 64	Moncalvo <i>et al.</i> (2002)	AF261511
<i>Melanotus</i> sp.	UNSW 02/60	This work	AY219611
<i>Phaeocollybia jennyae</i>	DAOM 221500	Moncalvo <i>et al.</i> (2002)	AF261506
<i>Phaeocollybia ratticauda</i>	BRV 99/11	This work	AY219612
<i>Phaeomarasmius erinaceus</i>	SVH4 ECV 934	Moncalvo <i>et al.</i> (2002)	AF 261594
<i>Phaeomarasmius</i> sp.	GATES 0006	This work	AY219613
<i>Pholiota squarrosoides</i>	JJ7	Moncalvo <i>et al.</i> (2000)	AF042568
<i>Pholiota squarrosa</i>	Strain Phs 1	Jarosch & Besl (2001)	AF352047
<i>Pholiota</i> sp.	BVR 99/12	This work	AY219614
<i>Psilocybe stuntzii</i>	VT 1263 216	Moncalvo <i>et al.</i> (2002)	AF042567
<i>Psilocybe silvatica</i>	RV 5/7/1989	Moncalvo <i>et al.</i> (2000)	AF041544
<i>Stropharia rugosoannulata</i>	D 258	Hopple (1999)	AF041 544
<i>Tubaria furfuracea</i>	MCA 391	Moncalvo <i>et al.</i> (2002)	AF205710
<i>Tubaria rufofulva</i>	UNSW 98/19	This work	AF219615

Abbreviations for Australian voucher collections: All BR collections are currently housed at UNSW, GATES and KS are private collections held by Genevieve Gates (Tasmania) or Katrina Syme (now housed at PERTH). Information regarding overseas collections are detailed in Moncalvo *et al.* 2000, 2002 with the exception of ZT, AMM in WASH, and private collection LEECH, material of which are also held at UNSW.

DNA Extraction

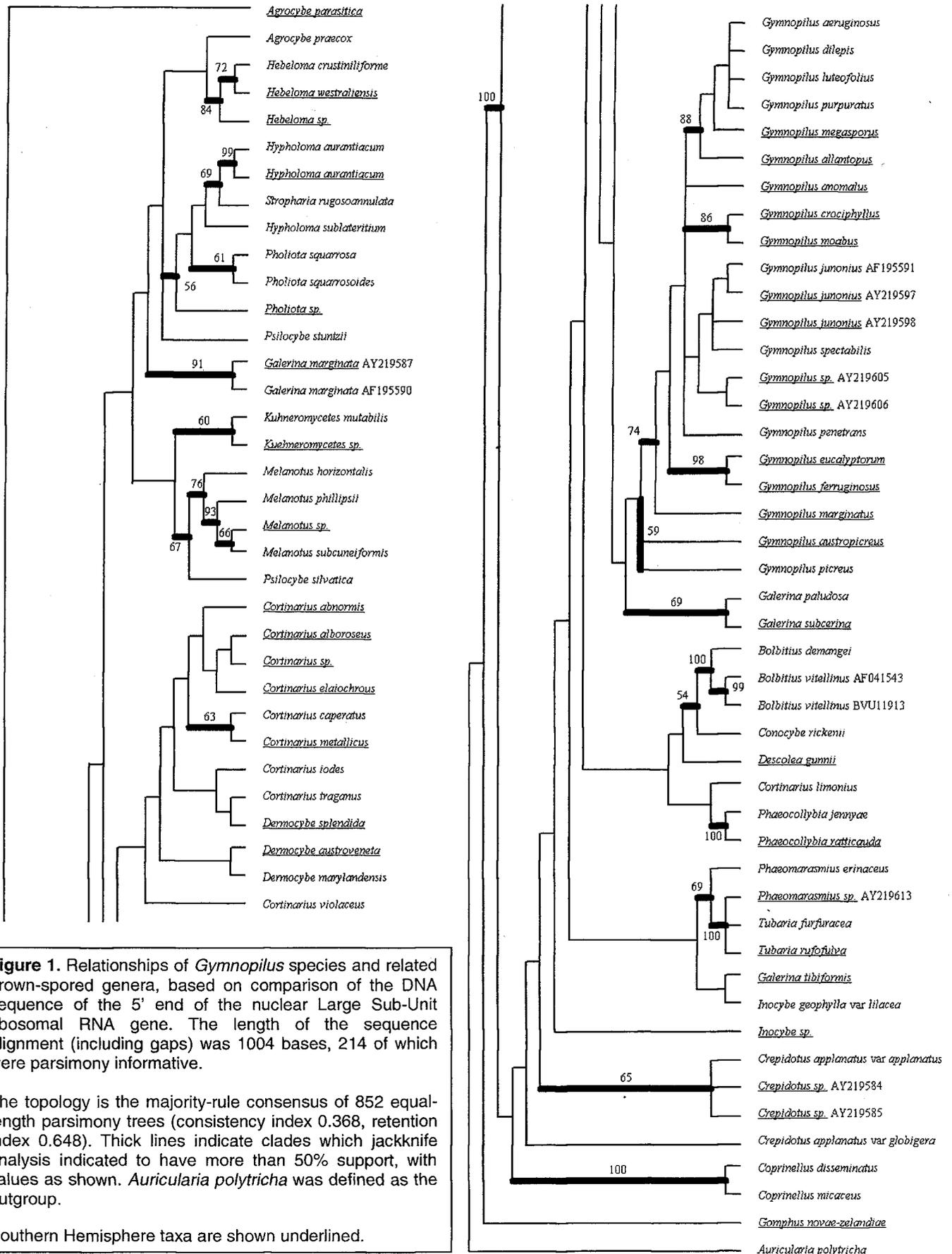
DNA was extracted in CTAB buffer using the following procedure. Tissue (approximately 10–50 mg) was ground in microfuge tubes in extraction buffer (2% CTAB, 0.1 M Tris-HCl (pH 8.0), 1.4 M NaCl, 0.02M EDTA, 1% PEG 8000). Samples were then placed in a waterbath at 65°C for 30 min. mixing occasionally. Samples were spun for 10 min. at 12K G and then extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1), mixed and spun again for 10 min. The aqueous phase was then extracted with an equal volume of chloroform:isoamyl alcohol (24:1), spun, and the aqueous phase removed to a fresh microfuge tube. An equal volume of 100% isopropanol was added, the tube inverted several times and placed at room temperature for 30 min. The sample was centrifuged for 30 min. at 12K G and decanted. The DNA pellet was washed in 70% ethanol, air-dried and 50 µl of TE buffer was added. 1 µl of DNase-free RNase A was added and the sample incubated at 37°C for 15 min. The sample was then frozen until use.

Amplification and sequencing of the 5' end of Large Sub-Unit nuclear Ribosomal RNA gene

The primers LR7 (TACTACCACCAAGATCT) (detailed in Moncalvo *et al.* (2000) and the Vilgalys web site at (<http://www.botany.duke.edu/fungi/mycolab/printers.htm>)), and ITS5 (GGAAGTAAAAGTCGTAACAAGG) (White *et al.* 1990), were used to amplify a roughly 17kbp fragment of DNA including the 5' end of the LSU, as well as the entire internal transcribed spacer region.

Amplification conditions were as follows: each 50µL reaction contained 5µL of BioTaq 10X NH₄ buffer (BioLine Co.), 2.5µL of 50mM MgCl₂, 5µL of a mixture of four deoxynucleotide triphosphates, each at a concentration of 2.5mM, 1µL of each of the primers at a concentration of 20µM, 35µL sterile water, and 0.5µL of BioTaq (BioLine Co.) Taq polymerase, at a concentration of 5 units/µL.

A touchdown PCR program was used, with an initial denaturation of 95°C for five minutes. The initial denaturation temperature was 65°C, reducing to 45°C, with two cycles at each of the higher temperatures, followed by thirty repetitions of the final cycle. Each cycle had a denaturation step of 30 seconds at 95°C, and an extension step of 1 min. 30 sec. at 72°C.



PCR products were purified by a Wizard PCR Preps (Promega Co.), and sequenced using ABI cycle sequencing chemistry and electrophoresis equipment (Perkin-Elmer Co.), using the primers LS7 and ITS3 (GCATCGATGAAGAACGCAGC) (White *et al.* 1990), with additional internal primers in some cases, where necessary.

The results of sequencing the internal transcribed spacers will be analysed and reported in another publication.

Analysis of sequence data

Sequences were edited using the program 'Sequencher' (GeneSearch), and aligned 'by eye'. The alignment was cladistically analysed using the program PAUP* (Swofford 2001). The sequence from *Auricularia polytricha* was defined to be the outgroup. Trees were generated using Maximum Parsimony criteria, and support for clades was estimated by Jackknife analysis (100 replicates, with 37% character replacement, and the 'Jac resampling' option chosen).

New sequences generated in this study have been deposited in GenBank.

Results

A sequence alignment of total length (including inserted gaps) 1004 bases was generated (however, some of the sequences obtained from GenBank for comparison with the sequences generated in this study were shorter than this).

Parsimony analysis yielded 852 equal-length trees (length 1218, consistency index 0.368, retention index 0.648). Figure 1 is the majority-rule consensus of these trees. Jackknife analysis indicated very low support for most of the clades shown. Those with jackknife support of more than 50% are indicated by thick lines. Bootstrap analysis (results not shown) produced similar levels of support for these clades.

With *Auricularia polytricha* defined as the outgroup, *Gomphus novae-zealandiae* appears as sister to all the other taxa analysed, this clade having 100% jackknife support. Within this clade, there were two large clades of interest. One of these contained all the *Gymnopilus* species, and was clearly separate from another clade containing genera formerly included in the Cortinariaceae and Strophariaceae as defined by Singer (1986), both of which appear to be polyphyletic. The convention of using '/' followed by a lower case name for a clade name has been followed, to avoid confusion between clade names and conventional generic names.

The genus *Galerina* appears to be polyphyletic, with two species (*G. paludosa* and *G. subcerina*) appearing as sister to *Gymnopilus*. *Galerina marginata* is associated with genera formerly placed in the Strophariaceae rather than Cortinariaceae, while *Galerina tibiformis* is associated with an *Inocybe* species.

Within *Gymnopilus* itself, *G. picreus* and *G. austropicreus* appear the most isolated species, with others (apart from *G. marginatus*, *G. penetrans* and *G. anomalus*) forming closely related groups. These groups correspond roughly with groups derived from ITS data (Rees *et al.* 2002), but LSU data was not as informative as ITS sequences in distinguishing between species. *Gymnopilus aeruginosus*, which has green colours in the pileus surface and flesh when young, is to be found in a small clade with red-coloured species.

The /gymnopiloid clade is well isolated from the genus *Cortinarius* which contains former *Rozites* and *Cuphocybe* species as well as representatives of the traditional subgenera *Cortinarius*, *Dermocybe*, *Myxacium* and *Telamonia*. *Cortinarius abnormis* is clearly contained within *Cortinarius* in association with *C. alboroseus*, *C. elaiochrous* and an unnamed, former *Cuphocybe* species from Australia. With the small number of species used, other species apart from *Cortinarius violaceus* were not well-resolved, owing in part to the shortness of the sequence for some species, including *Cortinarius iodes*. Relationships in this large genus are more satisfactorily resolved using ITS sequence data.

Other genera included by Singer (1986) in the Cortinariaceae are to be found scattered throughout the majority rule tree in positions well isolated from *Cortinarius* (Fig. 1). *Inocybe geophylla* var. *lilacea* shows an affinity with *Galerina tibiformis* albeit with weak support. The three *Hebeloma* species (which form a well-supported clade) are associated with *Agrocybe praecox*, separate from the *Conocybe* /*Bolbitius* clade. However, a clade

containing *Conocybe*, *Bolbitius*, *Descolea gunnii* and *Phaeocollybia* appears as a basal group distant from *Cortinarius* with which the latter two genera had previously been associated.

The anomalous placement of *Cortinarius limoneus* is undoubtedly owing to the sequence having a 300 base deletion, which deprives us of most of the phylogenetically informative material. The position of *Agrocybe parasitica* is also uncertain, as heteroplasmy prevented us from obtaining readable sequence from PCR product, for about half the length of the DNA region.

In this analysis, the boundary between traditional subfamilies Pholitoideae and Stropharioideae of Strophariaceae (*sensu* Singer 1986) has disappeared, but individual genera are to be found in company with other genera with weak support. *Kuehneromyces* and the dark-spored genera such as *Melanotus* form a separate clade with one, but not both species of *Psilocybe* species, which are widely separated.

Representatives of several little known or undescribed species from Australia such as *Cortinarius (Cuphocybe)*, *Kuehneromyces* and *Melanotus* species included in the analysis to see where they would emerge, are clearly related on the basis of LSU data to other similar exotic species. An Australian *Phaeomarasmium* species appears as sister to a group of *Tubaria* species despite the presence of a trichoderm in the pileus surface hyphae. This collection shows intermediate morphological characters worthy of further investigation.

Discussion

Many of the results from this analysis support findings of the giant phylogeny of the agarics recently published by Moncalvo *et al.* (2002), but others do not. It is offered as a setting in which to place new sequences for an expanded list of species of mainly Southern Hemisphere origin, and as a check on the identity of so many new Australian taxa whose affiliations have been hitherto uninvestigated. It also incorporates several taxa with transitional features such as *Cortinarius abnormis*, *Descolea gunnii*, *Tubaria*, *Phaeomarasmium* and *Agrocybe* species, which have been included in a variety of formerly conventional agaric families by a number of authors in the past. Support for clades in both analyses is often weak and the positions of taxa can often be reversed in such poorly supported clades.

Phylogenetic analysis of the 5' end of the LSU coding region of ribosomal DNA in this study, clearly demonstrates that the rust-brown spored genus *Gymnopilus* is monophyletic. This confirms findings of an earlier study based on ITS sequences (Rees *et al.* 2002). Regrettably LSU sequences were not available for all the species included in the previous study, including *Pyrrhoglossum pyrhum* (Berk. & M.A. Curtis) Singer now combined into *Gymnopilus* as *G. pyrhum* (Berk. & M.A. Curtis) B.J. Rees. Since then, sequences have been generated for two less frequently encountered species *Gymnopilus megasporus* and *G. marginatus* from Australia. While groups of species present within *Gymnopilus* bear some resemblance to those derived from ITS phylogeny, the LSU analysis is less informative as might be expected. There are also some differences, which reflect the incorporation of new species sequences into the data set. Northern Hemisphere *Gymnopilus junonius*/*G. spectabilis* species still form a cohesive group with Australian collections. The name *G. junonius* has priority (Orton 1960) for this species, but spore incompatibility studies may help resolve why this species is the only cosmopolitan species yet described from the genus from Australia.

In accordance with the predictions of Singer (1986), some of the *Galerina* species analysed are confirmed as most closely related to *Gymnopilus*, which according to Moncalvo *et al.* (2002) also contains *Hebelomina neerlandica* with which we as yet have no experience. In the present analysis, *Gymnopilus* can no longer be thought of as part of the Cortinariaceae or Strophariaceae which are both seen to be polyphyletic in this treatment and that of Moncalvo *et al.* (2002). *Gymnopilus* is a well-represented genus in Australia with over 40 species (Rees 1996), and seems to be more closely related to the /hebelomatoid and /tubarioid clades than to other former members of the Cortinariaceae or Strophariaceae.

The question of the evolutionary development of the brown-spored agarics is far from settled (Moncalvo *et al.* 2002), but any connection between *Gymnopilus* and present day *Gomphus* species (which share some spore wall and ornamentation features with *Gymnopilus*) is not immediately obvious.

It is confirmed that, despite the possession of styrylpyrone pigments, *Cortinarius abnormis* is definitely within *Cortinarius* on molecular grounds, within a clade containing former *Cuphocybe* and *Rozites* species, including an

undescribed *Cuphocybe* species from Australia. As a result, the ability to produce styrylpyrone pigments has arisen independently in at least two agaric genera, as well as in *Phaeolus* and *Inonotus*, regardless of preferred substrata. Although the position of *Descolea gunnii* is not totally resolved in this analysis, the species shows some affiliations with the *Bolbitoid* clade as Singer (1986) predicted on a morphological basis. A similar affinity was also apparent in the big data set of Moncalvo *et al.* (2002).

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APPENDIX

Authorities for included taxa: (follow Brummit & Powell 1992)

- Agrocybe parasitica* Stevenson
A. praecox (Pers. : Fr.) Fayod
Bolbitius vitellinus (Pers. : Fr.) Fr.
B. demangei (Quél.) Sacc. & D. Sacc.
Conocybe rickenii (Jul. Schaeff.) Kühner
Coprinellus disseminatus (Pers. : Fr.) Hopple & Vilgalys
C. micaceus (Bull. : Fr.) Hopple & Vilgalys
Cortinarius abnormis Watling & T.W. May
C. alboroseus (R. Heim) Peintner, E. Horak, M.M. Moser & Vilgalys
C. caperatus Fr.
C. elaiochrous E. Horak, M.M. Moser, Peintner & Vilgalys
C. iodes Berk. & M.A. Curtis
C. limoneus (Fr. : Fr.) Fr.
C. metallicus (Bougher, Fuhrer & E. Horak) Peintner, E. Horak, M.M. Moser & Vilgalys
C. traganus (Fr. : Fr.) Fr.
C. violaceus (L. : Fr.) S.F. Gray
Crepidotus applanatus (Pers. : Fr.) Staude var. *applanatus* Singer
C. applanatus (Pers. : Fr.) Staude var. *globiger* (Berk.) Sacc.
Dermocybe austroveneta (Cleland) M.M. Moser & E. Horak
D. marylandensis Ammirati
D. splendida E. Horak
Descolea gunnii (Berk. : Masee) E. Horak
Galerina marginata (Batsch) Kühner
G. paludosa (Fr.) Kühner
G. subcerina A.H. Smith & Singer
G. tibiiformis A.E. Wood
Gomphus novae-zelandiae Segedin
Gymnopilus aeruginosus (Peck.) Singer
G. allantopus (Berk.) Pegler
G. austropicreus B.J. Rees
G. crociphyllus (Cooke & Masee) Pegler
G. decipiens (W.G. Smith) P.D. Orton
G. eucalyptorum (Cleland) Singer
G. ferruginosus B.J. Rees
G. junonius (Fr.) P.D. Orton
G. luteofolius (Peck) Murrill
G. marginatus B.J. Rees
G. megasporus Grgurinovic
G. moabus Grgurinovic
G. odini (Fr.) Kühner & Romagnesi
G. penetrans (Fr. : Fr.) Murrill
G. picreus (Pers. : Fr.) P. Karsten
G. purpuratus (Cooke & Masee) Singer
G. spectabilis (Fr.) A.H. Smith
G. turficola M.M. Moser
Hebeloma crustuliniforme (Bull. : Fr.) Quélet
H. westraliense Bougher, Tommerup & Malajczuk
Inocybe geophylla (Sowerby) Quélet var. *lilacina* Gillet
Kuehneromyces mutabilis (Schaeff. : Fr.) Singer & A.H. Smith
Melanotus subcuneiformis (Murrill) Singer
M. phillipsi (Berk. & Broome) Singer
M. horizontalis (Bull.) P.D. Orton
Phaeocollybia jennyae (P. Karsten) R. Heim
P. ratticauda E. Horak
Phaeomarasmium erinaceus (Pers. : Scherff.) Romagnesi
Pholiota squarrosoides (Peck) Sacc.
P. squarrosa (O.F. Müll.) P. Kummer
Tubaria furfuracea (Pers. : Fr.) Gillet
T. rufofulva (Cleland) D.A. Reid & E. Horak

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