Phylogeny of tremellomycetous yeasts and related dimorphic and filamentous basidiomycetes reconstructed from multiple gene sequence analyses

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Abstract: The Tremellomycetes (Basidiomycota) contains a large number of unicellular and dimorphic fungi with stable free-living unicellular states in their life cycles. These fungi have been conventionally classified as basidiomycetous yeasts based on physiological and biochemical characteristics. Many currently recognised genera of these yeasts are mainly defined based on phenotypical characters and are highly polyphyletic. Here we reconstructed the phylogeny of the majority of described anamorphic and teleomorphic tremellomycetous yeasts using Bayesian inference, maximum likelihood, and neighbour-joining analyses based on the sequences of seven genes, including three rRNA genes, namely the small subunit of the ribosomal DNA (rDNA), D1/D2 domains of the large subunit rDNA, and the internal transcribed spacer regions (ITS 1 and 2) of rDNA including 5.8S rDNA; and four protein-coding genes, namely the two subunits of the RNA polymerase II (*RPB1* and *RPB2*), the translation elongation factor 1-α (*TEF1*) and the mitochondrial gene cytochrome *b* (*CYTB*). With the consideration of morphological, physiological and chemotaxonomic characters and the congruence of phylogenies inferred from analyses using different algorithms based on different data sets consisting of the combined seven genes, the three rRNA genes, and the individual protein-coding genes, five major lineages corresponding to the orders Cystofilobasidiales, Filobasidiales, Holtermanniales, Tremellales, and Trichosporonales were resolved. A total of 45 strongly supported monophyletic clades with multiple species and 23 single species clades were recognised. This phylogenetic framework will be the basis for the proposal of an updated taxonomic system of tremellomycetous yeasts that will be compatible with the current taxonomic system of filamentous basidiomycetes accommodating the 'one fungus, one name' principle.

Key words: Fungi, Basidiomycota, Tremellomycetes, Yeasts, Multigene phylogeny.

INTRODUCTION

Unicellular basidiomycetes and dimorphic basidiomycetes with a stable free-living unicellular state during their life cycles are recognised as basidiomycetous yeasts (Boekhout *et al.* 2011). They occur in all three subphyla of the Basidiomycota, namely Agaricomycotina, Pucciniomycotina and Ustilaginomycotina, which are presently recognised on the basis of molecular phylogenetic analyses (Fell *et al.* 2000, Scorzetti *et al.* 2002, James *et al.* 2006, Hibbett *et al.* 2007, Wuczkowski *et al.* 2011). Yeast taxa in the Agaricomycotina occur only in the basal Tremellomycetes lineage (Hibbett 2006, Boekhout *et al.* 2011, Weiß *et al.* 2014). Phenotypic and molecular analyses revealed a close affiliation of basidiomycetous yeasts with various groups of filamentous basidiomycetes (Millanes *et al.* 2011). However, yeasts and filamentous fungi have conventionally been studied by different scientific communities and classified using different criteria, resulting in the developments of hitherto independent taxonomic systems of the two groups of fungi. Recent molecular phylogenetic studies have shown the incompatibility between the taxonomic system of basidiomycetous yeasts and that of filamentous basidiomycetes. Furthermore many currently recognised genera of tremellomycetous yeasts, which are mainly defined based on phenotypic characters,

are strikingly polyphyletic. For example, species of the genus *Cryptococcus* are located in all currently recognised orders of Tremellomycetes and occur intermingled with species of other genera, such as *Bullera* (Fell *et al.* 2000, Boekhout *et al.* 2011, Fonseca *et al.* 2011, Millanes *et al.* 2011, Weiß *et al.* 2014). The polyphyletic nature of the traditionally defined teleomorphic genus *Tremella*, which is usually dimorphic, is also remarkable. Several monophyletic clades have been recognised among *Tremella* species which occur interspersed with other teleomorphic and anamorphic genera (Boekhout *et al.* 2011, Millanes *et al.* 2011, Weiß *et al.* 2014). These problems existing in the current taxonomic systems of both yeasts and filamentous fungi in the Tremellomycetes remain to be resolved.

The high-level classification of Basidiomycota has been updated with results from the Assembling the Fungal Tree of Life (AFTOL) project that used a multigene sequence analysis approach (Lutzoni *et al.* 2004, James *et al.* 2006, Hibbett *et al.* 2007). However, only a limited number of basidiomycetous yeast taxa were included in that project, making it impossible to propose a corresponding revision of the taxonomic system of basidiomycetous yeasts. Consequently, the artificial classification system of these organisms largely remained in the latest edition of 'The Yeasts, a Taxonomic Study' [hereafter referred to as 'The Yeasts'] (Kurtzman *et al.*

2011), due to the lack of reliable multigene phylogenetic studies of these yeasts.

The requirement for revising the taxonomic system of tremellomycetous yeasts has been emphasised by recent progress in biodiversity studies of yeasts and by the change of fungal nomenclature adopting the 'one fungus = one name' concept (Hawksworth 2011). Molecular taxonomic studies have resulted in the availability of a comprehensive sequence database of the large subunit of the ribosomal RNA gene (LSU rDNA) D1/D2 domains and the ITS (including 5.8S) regions of rDNA for almost all known basidiomycetous yeast species (Fell *et al.* 2000, Scorzetti *et al.* 2002, Wang & Bai 2008, Schoch *et al.* 2012). The rDNA sequence databases have become a molecular platform for rapid identification of yeasts, resulting in continued discovery of new taxa in recent years (Boekhout 2005, Wang & Bai 2008, Wuczkowski *et al.* 2011). The addition of these new species has contributed to the increase of the polyphyletic nature of many basidiomycetous yeast genera. For example, the distribution of *Bullera* species has expanded from Tremellales and Filobasidiales (Boekhout & Nakase 1998) to Trichosporonales (Nakase *et al.* 2002, Fungsin *et al.* 2006). With each new species being taxonomically misplaced, the chaos of the taxonomic system increases. Therefore, an updated taxonomic system is imperative for the correct placement of the vast amount of hidden yeast diversity.

Similar to filamentous fungi, many yeast species have separate teleomorphic and anamorphic names. As regulated by the new International Code of Nomenclature for algae, fungi and plants (Melbourne Code) (McNeill *et al.* 2012), after January 1 2013, only one name is legitimate regardless of whether or not a sexual state exists. An updated taxonomic system, especially the redefinition of genera based on a robust multigene phylogeny, will be required for the name choices and to minimise the possibility of name changes in the future. The purpose of this study is to confidently resolve the phylogenetic relationships among tremellomycetous yeasts and dimorphic fungi based on multiple gene sequence analyses, resulting in a framework that allows us to update the taxonomic system of yeasts and related taxa in the Tremellomycetes.

MATERIALS AND METHODS

Taxon sampling

A total of 294 tremellomycetous yeast strains were included in this study, which covered the type strains of 286 currently recognised species and varieties, the type strains of six synonyms, and two additional strains with mating types opposite to those of the type strains (Table 1). From the 240 tremellomycetous yeast species and varieties included in the latest edition of 'The Yeasts' (Kurtzman *et al.* 2011), 234 were included in this study. In addition, 52 tremellomycetous yeast species which were published too late for inclusion in the book were also employed in this study. The taxa sampled covered 16 teleomorphic and 19 anamorphic genera. The type strains of two pucciniomycetous and one ustilaginomycetous yeast species were employed as outgroup (Table 1).

DNA extraction, PCR, and sequencing

Genomic DNA was extracted from yeast cells actively growing on YPD medium using the method described in Bolano *et al.* (2001) with minor modifications. The UltraClean® Microbial DNA Isolation Kit (MO BIO, CA) was used when high quality DNA templates were required for PCR amplification of some protein genes. A set of six genes was selected and sequenced based on previous studies of the Assembling the Fungal Tree of Life (AFTOL-1) project (James *et al.* 2006, Hibbett *et al.* 2007). These genes included three rRNA genes, namely the small subunit (SSU or 18S) of the ribosomal DNA (rDNA), D1/D2 domains of the large subunit (LSU or 26S) rDNA, and the internal transcribed spacer regions (ITS 1 and 2) of the rDNA, including the 5.8S

Table 1. List of tremellomycetous yeasts and dimorphic taxa employed. The sequences with GenBank numbers in bold are determined in this study.

Lineage/clade	Species	Strain	ITS (5.8S)	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
Cystofilobasidiales									
Cystofilobasidium	*Cystofilobasidium bisporidii**	CBS 6346[T]	**KF036597**	EU085532	AB072225	**KF036419**	**KF036832**	**KF037103**	**KF423238**
	C. capitatum*	CBS 6358[T]	AF139627	AF075465	D12801	**KF036420**	**KF036833**	**KF037104**	/
	C. ferigula*	CBS 7202[T]	**KF036598**	CBS database	AB032628	/	/	**KF037105**	**KF423239**
	C. infirmominiatum*	CBS 323[T]	AF444400	AF075505	AB072226	/	**KF036834**	**KF037106**	**KF423240**
	C. lacus-mascardii*	CBS 10642[T]	EF613495	AY158642	**KF036665**	**KF036421**	**KF036835**	**KF037107**	**KF423241**
	C. macerans*	CBS 10757[T]	EU082231	EU082225	**KF036666**	**KF036423**	**KF036837**	**KF037109**	/
	C. macerans*	CBS 2206	AF444329	AF189848	AB032642	**KF036422**	**KF036836**	**KF037108**	**KF423242**
Guehomyces	*Guehomyces pullulans*	CBS 2532[T]	AF444417	EF551318	AB001766	**KF036478**	**KF036892**	**KF037155**	AF175778
	Tausonia pamirica	CBS 8428[T]	**KF036600**	EF118825	**KF036692**	/	/	/	/
huempii	*Cryptococcus huempii*	CBS 8186[T]	AF444322	AF189844	AB032636	**KF036377**	**KF036790**	**KF037062**	**KF423200**
	Mrakia curviuscula*	CBS 9136[T]	**KF036599**	EF118826	**KF036684**	**KF036510**	**KF036924**	**KF037185**	**KF423313**
Itersonilia	*Itersonilia perplexans*	CBS 363.85[T]	AB072233	AJ235274	AB072228	/	**KF036900**	/	**KF423296**
	Udeniomyces pannonicus	CBS 9123[T]	AB072229	AB077382	AB072227	**KF036579**	**KF036996**	**KF037251**	/
Mrakia	*Mrakia blollopis**	CBS 8921[T]	AY038826	AY038814	**KF036683**	**KF036509**	**KF036923**	**KF037184**	/
	M. frigida*	CBS 5270[T]	AF144483	AF075463	D12802	**KF036511**	**KF036925**	**KF037186**	/

Table 1. (Continued).

Lineage/clade	Species	Strain	ITS (5.8S)	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
	*M. nivalis**	CBS 5266^T	AF144484	AF189849	DQ831017	**KF036513**	**KF036927**	**KF037188**	/
	*M. gelida**	CBS 5272^T	AF144485	AF189831	**KF036685**	**KF036512**	**KF036926**	/	**KF423314**
	*M. stokesii**	CBS 5917^T	AF144486	AF189830	**KF036687**	**KF036515**	**KF036929**	**KF037190**	/
	*M. psychrophila**	CBS 10828^T	EU224267	EU224266	/	/	/	/	/
	*M. robertii**	CBS 8912^T	AY038829	AY038811	**KF036686**	**KF036514**	**KF036928**	**KF037189**	**KF423315**
	Mrakiella aquatic	CBS 5443^T	AF410469	AF075470	AB032621	**KF036516**	**KF036930**	**KF037191**	**KF423316**
	M. cryoconiti	CBS 10834^T	AJ866976	GQ911524	/	/	/	**KF037192**	/
	M. niccombsii	CBS 8917^T	AY029346	AY029345	**KF036688**	**KF036517**	**KF036931**	**KF037193**	**KF423318**
Udeniomyces	*Udeniomyces megalosporus*	CBS 7236^T	AF444408	AF075510	D31657	**KF036578**	**KF036995**	/	/
	U. puniceus	CBS 5689^T	AF444435	AF075519	D31658	**KF036580**	DQ836008	/	/
	U. pyricola	CBS 6754^T	AF444402	AF075507	D31659	**KF036581**	**KF036997**	**KF037252**	/
Phaffia	*Phaffia rhodozyma*	CBS 5905^T	AF139629	AF189871	**KF036689**	/	**KF036933**	**KF037195**	**KF423320**
	*Xanthophyllomyces dendrorhous**	CBS 7918^T	AF139628	AF075496	D31656	**KF036582**	**KF036998**	**KF037253**	**KF423356**
Filobasidiales									
aerius	*Cryptococcus aerius*	CBS 155^T	AF145324	AF075486	AB032614	**KF036336**	**KF036748**	**KF037019**	**KF423160**
	C. fuscescens	CBS 7189^T	AF145319	AF075472	AB032631	**KF036372**	**KF036784**	**KF037056**	**KF423195**
	C. keelungensis	CBS 10876^T	EF621565	EF621562	**KF036637**	/	**KF036792**	**KF037064**	**KF423202**
	C. phenolicus	CBS 8682^T	AF444351	AF181523	**KF036647**	**KF036394**	**KF036808**	**KF037079**	**KF423217**
	C. terreus	CBS 1895^T	AF444319	AF075479	AB032647	**KF036409**	**KF036823**	**KF037094**	**KF423231**
	C. elinovii	CBS 7051^T	AF145318	AF137604	**KF036631**	**KF036365**	**KF036777**	**KF037050**	**KF423189**
	C. terricola	CBS 4517^T	AF444350	AF181520	**KF036659**	**KF036410**	**KF036824**	**KF037095**	/
albidus	*C. adeliensis*	CBS 8351^T	AF145328	AF137603	**KF036610**	**KF036335**	**KF036747**	**KF037018**	**KF423159**
	C. albidosimilis	CBS 7711^T	AF145325	AF137601	**KF036612**	**KF036338**	**KF036750**	**KF037021**	**KF423162**
	C. albidus var. *Albidus*	CBS 142^T	AF145321	AF075474	AB032616	/	**KF036751**	**KF037022**	**KF423163**
	C. albidus var. *Kuetzingii*	CBS 922	AF444313	AF181504	**KF036613**	**KF036339**	**KF036752**	**KF037023**	**KF423164**
	C. albidus var. *Kuetzingii*	CBS 1926^T	AF145327	AF137602	AB032639	**KF036340**	**KF036753**	**KF037024**	**KF423165**
	C. albidus var. *Ovalis*	CBS 5810^T	AF145329	AF137605	**KF036614**	/	**KF036754**	**KF037025**	**KF423166**
	C. antarcticus var. *antarcticus*	CBS 7687^T	AF145326	AF075488	AB032620	**KF036345**	/	**KF037030**	**KF423169**
	C. antarcticus var. *circumpolaris*	CBS 7689^T	**KF036586**	CBS database	**KF036618**	**KF036346**	**KF036759**	**KF037031**	**KF423170**
	C. bhutanensis	CBS 6294^T	AF145317	AF137599	AB032623	**KF036352**	**KF036765**	**KF037037**	**KF423176**
	C. cerealis	CBS 10505^T	FJ473371	FJ473376	**KF036624**	**KF036356**	/	**KF037041**	**KF423180**
	C. diffluens	CBS 160^T	AF145330	AF075502	**KF036630**	**KF036363**	**KF036775**	**KF037048**	**KF423187**
	C. friedmannii	CBS 7160^T	AF145322	AF075478	AB032630	**KF036371**	**KF036783**	**KF037055**	**KF423194**
	C. liquefaciens	CBS 968^T	AF444345	AF181515	**KF036638**	**KF036381**	**KF036794**	**KF037066**	**KF423203**
	C. randhawai	CBS 10160^T	AJ876528	AJ876599	**KF036650**	/	/	/	/
	C. saitoi	CBS 1975^T	AF444372	AF181540	**KF036651**	**KF036400**	**KF036814**	**KF037085**	**KF423222**
	C. uzbekistanensis	CBS 8683^T	AF444339	AF181508	**KF036660**	**KF036412**	**KF036826**	**KF037096**	**KF423232**
	C. vishniacii	CBS 7110^T	AF145320	AF075473	AB032650	**KF036414**	/	**KF037098**	**KF423234**
cylindricus	*Bullera taiwanensis*	CBS 9813^T	/	AB079065	AB072234	/	/	/	/
	Cryptococcus cylindricus	CBS 8680^T	AF444360	AF181534	**KF036628**	**KF036360**	**KF036772**	**KF037045**	**KF423184**
	C. silvicola	CBS 10099^T	AY898956	AY898955	**KF036653**	**KF036402**	**KF036816**	**KF037087**	**KF423224**
Filobasidium	*C. chernovii*	CBS 8679^T	AF444354	AF181530	**KF036625**	**KF036357**	**KF036769**	**KF037042**	**KF423181**
	C. magnus	CBS 140^T	AF190008	AF181851	AB032643	**KF036383**	**KF036796**	**KF037068**	**KF423205**
	C. oeirensis	CBS 8681^T	AF444349	AF181519	**KF036644**	/	**KF036804**	**KF037076**	**KF423213**
	C. stepposus	CBS 10265^T	DQ222455	DQ222456	**KF036655**	**KF036405**	**KF036819**	**KF037090**	**KF423227**
	C. wieringae	CBS 1937^T	AF444373	AF181541	**KF036663**	**KF036416**	**KF036829**	**KF037100**	**KF423236**
	*Filobasidium elegans**	CBS 7640^{EXT}	AF190006	AF181548	**KF036678**	**KF036474**	**KF036888**	/	**KF423285**
	*F. floriforme**	CBS 6241^{EXT}	AF190007	AF075498	D13460	**KF036475**	**KF036889**	/	/
	*F. globisporum**	CBS 7642^{EXT}	AF444336	AF075495	AB075546	**KF036476**	**KF036890**	**KF037153**	**KF423286**

Table 1. (Continued).

Lineage/clade	Species	Strain	ITS (5.8S)	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
	*F. uniguttulatum**	CBS 1730^T	AF444302	AF075468	AB032664	KF036477	KF036891	KF037154	KF423287
gastricus	*Cryptococcus aciditolerans*	CBS 10872^T	**KF036583**	AY731790	**KF036609**	/	KF036746	KF037017	KF423158
	C. agrionensis	CBS 10799^T	**KF036584**	EU627786	**KF036611**	KF036337	KF036749	KF037020	KF423161
	C. gastricus	CBS 2288^T	AF145323	AF137600	AB032633	KF036373	KF036785	KF037057	AB040652
	C. gilvescens	CBS 7525^T	AF444380	AF181547	AB032634	KF036374	KF036786	KF037058	KF423196
	C. ibericus	CBS 10871^T	**KF036592**	AY731791	**KF036636**	KF036379	KF036791	KF037063	KF423201
	C. metallitolerans	CBS 10873^T	**KF036594**	AY731789	**KF036639**	KF036385	KF036798	KF037070	KF423207
single-species clade	*C. arrabidensis*	CBS 8678^T	AF444362	AF181535	**KF036621**	KF036349	KF036762	KF037034	KF423173
	*Filobasidium capsuligenum**	CBS 1906^T	AF444381	AF363642	AB075544	**KF036473**	KF036887	KF037152	AB040654
Holtermanniales									
Holtermanniella	*Holtermanniella festucosa*	CBS 10162^T	AY462120	AY462119	**KF036633**	KF036367	KF036779	KF037052	KF423191
	H. mycelialis	CBS 7712^T	AF408417	AJ311450	**KF036641**	KF036388	KF036801	KF037073	KF423210
	H. nyarrowii	CBS 8804^T	AY006481	AY006480	**KF036643**	KF036390	KF036803	KF037075	KF423212
	H. wattica	CBS 9496^T	FJ473373	AY138478	**KF036662**	KF036415	KF036828	KF037099	KF423235
	H. takashimae	CBS 11174^T	FM246501	FM242574	**KF036679**	KF036486	/	KF037163	KF423295
Holtermannia	*Holtermannia corniformis**	CBS 6979^R	AF410472	AF189843	AF053718	**KF036485**	/	/	**KF423294**
Tremellales									
amylolyticus	*Cryptococcus amylolyticus*	CBS 10048^T	**KF036585**	AY562134	**KF036616**	KF036343	KF036757	KF037028	/
	C. armeniacus	CBS 10050^T	**KF036587**	AY562140	**KF036620**	KF036348	KF036761	KF037033	KF423172
	C. bromeliarum	CBS 10424^T	EU386359	DQ784566	**KF036623**	KF036353	KF036766	/	KF423177
	C. tibetensis	CBS 10456^T	EF363146	EF363143	EF363152	KF036411	KF036825	/	/
aurantia	*Tremella aurantia**	CBS 6965^R	AF444315	AF189842	**KF036693**	KF036522	KF036937	KF037199	KF423323
	*T. encephala**	CBS 8207^R	AF042402	AF042220	**KF036697**	KF036526	KF036942	KF037204	KF423327
	*T. encephala**	CBS 6968	AF410474	AF189867	**KF036698**	KF036525	KF036941	KF037203	/
	*T. indecorata**	CBS 6976^R	AF042432	AF042250	**KF036704**	KF036532	KF036948	KF037209	KF423333
aureus	*Cryptococcus aureus*	CBS 318^T	AB035045	AB035041	AB085795	KF036350	KF036763	KF037035	KF423174
	C. flavescens	CBS 942^T	**KF036590**	AB035042	AB085796	KF036368	KF036780	KF037053	KF423192
	C. terrestris	CBS 10810^T	EU200782	EF370393	**KF036658**	KF036408	KF036822	KF037093	KF423230
Auriculibuller	*Auriculibuller fuscus**	CBS 9648	AF444669	AF444763	**KF036604**	KF036314	KF036727	KF036999	KF423141
	Bullera japonica	CBS 2013^T	AF444666	AF444760	/	/	/	/	/
	Cryptococcus taeanensis	CBS 9742^T	AY686645	AY422719	**KF036656**	KF036406	KF036820	KF037091	KF423228
Bandoniozyma	*Bandoniozyma glucofermentans*	CBS 10381^T	JN381033	AY520334	**KF036605**	KF036315	KF036728	KF037000	/
	B. noutii	CBS 8364^T	AF444391	AF444700	**KF036606**	KF036316	KF036729	KF037001	KF423142
	B. complexa	CBS 11570^T	GU321089	GU321090	**KF036607**	KF036317	KF036730	KF037002	KF423143
	B. tunnelae	CBS 6123^T	AF444333	AF444687	**KF036608**	KF036318	KF036731	KF037003	/
Bulleribasidium	*Bulleribasidium oberjochense**	CBS 9110^T	GU327541	AF416646	GU327541	KF036333	KF036744	/	KF423157
	Mingxiaea begonia	CBS 10762^T	AB118874	AB119462	AB118874	KF036500	KF036914	KF037177	KF423304
	M. foliicola	CBS 11407^T	GQ438834	GQ438834	GQ438834	KF036501	KF036915	KF037178	KF423305
	M. hainanensis	CBS 11409^T	GQ438828	GQ438828	GQ438828	KF036502	KF036916	KF037179	KF423306
	M. panici	CBS 9932^T	AY188386	AY188387	AY188386	KF036503	KF036917	KF037180	KF423307
	M. pseudovariabilis	CBS 9609^T	AF453288	AF544247	AF453290	KF036504	KF036918	KF037181	KF423308
	M. sanyaensis	CBS 11408^T	GQ438831	GQ438831	GQ438831	KF036505	KF036919	KF037182	KF423309
	M. setariae	CBS 10763^T	AB118875	AB119463	AB118875	KF036506	KF036920	KF037183	KF423310
	M. siamensis	CBS 9933^T	AY188389	AY188388	AY188389	/	/	/	/
	M. variabilis	CBS 7347^T	AF314965	AF189855	D31654	KF036507	KF036921	/	KF423311
	M. wuzhishanensis	CBS 11411^T	GQ438830	GQ438830	GQ438830	KF036508	KF036922	/	KF423312
Bulleromyces	*Bullera unica*	CBS 8290^T	AF444441	AF075524	D78330	KF036332	/	KF037015	KF423155
	*Bulleromyces albus**	CBS 501^T	AF444368	AF075500	X60179	KF036334	KF036745	KF037016	KF423156
Cryptococcus	*Cryptococcus amylolentus**	CBS 6039^T	AF444306	AF105391	AB032619	KF036342	KF036756	KF037027	KF423168
	*Tsuchiyaea wingfieldii**	CBS 7118^T	AF444327	AF177404	D64121	KF036577	KF036994	KF037250	AB040662

Table 1. (Continued).

Lineage/clade	Species	Strain	ITS (5.8S)	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
	Cryptococcus neoformans*	CBS 132[T]	AF444326	AF075484	HQ596559	KF036472	KF036886	KF037151	AB040655
	C. gattii*	CBS 6289[T]	AF444444	AF075526	KF036677	KF036470	KF036884	KF037149	KF423283
	Filobasidiella depauperata*	CBS 7841[T]	FJ534881	FJ534911	AJ568017	KF036471	KF036885	KF037150	KF423284
Derxomyces	Derxomyces anomala	CBS 9607[T]	AF453289	EF682504	AF453291	KF036424	KF036838	KF037110	KF423243
	D. boekhoutii	CBS 10824[T]	EU517057	EU517057	EU517057	KF036425	KF036839	KF037111	KF423244
	D. boninensis	CBS 9141[T]	AB022933	AY487568	AB022928	KF036426	KF036840	KF037112	KF423245
	D. cylindrical	CBS 9744[T]	AY487563	AY487563	EU517071	KF036427	KF036841	KF037113	KF423246
	D. hainanensis	CBS 10820[T]	EU517056	EU517056	EU517056	KF036428	KF036842	KF037114	KF423247
	D. hubeiensis	CBS 9747[T]	AY487567	AY487566	EU517069	KF036429	KF036843	KF037115	KF423248
	D. huiaensis	CBS 8287[T]	AB022931	AB118870	D78331	KF036430	KF036844	KF037116	KF423249
	D. komagatae	CBS 10153[T]	AF314977	AF544249	AF314995	KF036431	KF036845	KF037117	KF423250
	D. linzhiensis	CBS 10827[T]	EU517058	EU517058	EU517058	KF036432	KF036846	KF037118	KF423251
	D. mrakii	CBS 8288[T]	AB022932	AB118871	D78325	KF036433	KF036847	KF037119	KF423252
	D. nakasei	CBS 9746[T]	AY487565	AY487564	EU517070	KF036434	KF036848	KF037120	KF423253
	D. pseudocylindrica	CBS 10826[T]	EU517059	EU517059	EU517059	KF036435	KF036849	KF037121	KF423254
	D. pseudohuiaensis	CBS 7364[T]	AF314970	AF544250	AF314994	KF036436	KF036850	/	KF423255
	D. pseudoschimicola	CBS 7354[T]	AF314979	AF416647	AF314997	KF036437	KF036851	KF037122	KF423256
	D. qinlingensis	CBS 10818[T]	EU517060	EU517060	EU517060	KF036438	KF036852	KF037123	KF423257
	D. schimicola	CBS 9144[T]	AB022936	AY487570	AB022930	KF036439	KF036853	KF037124	KF423258
	D. simaoensis	CBS 10822[T]	EU517062	EU517062	EU517062	KF036440	KF036854	KF037125	KF423259
	D. waltii	CBS 9143[T]	AB022935	AY487569	AB022929	KF036441	KF036855	KF037126	KF423260
	D. wuzhishanensis	CBS 10825[T]	EU517063	EU517063	EU517063	KF036442	KF036856	KF037127	KF423261
	D. yunnanensis	CBS 10821[T]	EU517064	EU517064	EU517064	KF036443	KF036857	KF037128	KF423262
dimennae	Bullera globispora	CBS 6981[T]	AF444407	AF075509	D31650	KF036323	KF036736	KF037007	KF423148
	Cryptococcus carnescens	CBS 973[T]	KF036588	AB035054	AB085798	KF036354	KF036767	KF037039	KF423178
	C. dimennae	CBS 5770[T]	AF410473	AF075489	AB032627	KF036364	KF036776	KF037049	KF423188
	C. heimaeyensis	CBS 8933[T]	KF036591	DQ000317	KF036635	KF036376	KF036788	KF037060	KF423198
	C. peneaus	CBS 2409[T]	AB035047	AB035051	AB085799	KF036392	KF036806	KF037077	KF423215
	C. tephrensis	CBS 8935[T]	DQ000318	DQ000318	KF036657	KF036407	KF036821	KF037092	KF423229
	C. victoriae	CBS 8685[T]	AF444469	AF363647	KF036661	KF036413	KF036827	KF037097	KF423233
Dioszegia	Dioszegia Antarctica	CBS 10920[T]	DQ402529	FJ640575	KF036667	KF036444	KF036858	KF037129	KF423263
	D. athyri	CBS 10119[T]	EU070926	EU070931	KF036668	KF036445	KF036859	KF037130	KF423264
	D. aurantiaca	CBS 6980[T]	AB049613	AB104689	AB049615	KF036446	KF036860	KF037131	KF423265
	D. buhagiarii	CBS 10054[T]	AY885687	AY562151	EU517065	KF036447	KF036861	KF037132	KF423266
	D. butyracea	CBS 10122[T]	EU070924	EU070929	KF036669	KF036448	KF036862	KF037133	KF423267
	D. catarinonii	CBS 10051[T]	AY562154	AY562142	KF036670	KF036449	KF036863	/	KF423268
	D. changbaiensis	CBS 9608[T]	AY242817	AY242819	AY242817	KF036450	KF036864	KF037134	KF423269
	D. crocea	CBS 6714[T]	AB049612	AF075508	D31648	KF036451	KF036865	KF037135	AB040649
	D. cryoxerica	CBS 10919[T]	FJ640565	FJ640562	KF036671	KF036452	KF036866	KF037136	KF423270
	D. fristingensis	CBS 10052[T]	AY562158	AY562146	EU517066	KF036453	KF036867	/	KF423271
	D. hungarica	CBS 4214[T]	AB049614	AF075503	AB032638	KF036454	KF036868	KF037138	KF423272
	D. statzelliae	CBS 8925[T]	AY029342	AY029341	/	/	/	/	/
	D. takashimae	CBS 10053[T]	AY562160	AY562149	KF036672	KF036455	KF036869	/	KF423273
	D. xingshanensis	CBS 10120[T]	EU070923	EU070928	KF036673	KF036456	KF036870	KF037139	KF423274
	D. zsoltii	CBS 9127[T]	AF385445	AF544245	AF385443	KF036457	KF036871	KF037140	KF423275
Fellomyces	Fellomyces borneensis	CBS 8282[T]	AJ608642	AF189877	AB032659	KF036458	KF036872	KF037141	KF423276
	F. horovitziae	CBS 7515[T]	AF444404	AF189856	AB001033	KF036461	KF036875	KF037143	/
	F. penicillatus	CBS 5492[T]	AF444337	AF177405	AB001034	KF036464	KF036878	KF037144	/
	F. polyborus	CBS 6072[T]	AF444411	AF189859	KF036676	KF036465	KF036879	KF037145	/

Table 1. (Continued).

Lineage/clade	Species	Strain	ITS (5.8S)	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
Fibulobasidium	Fibulobasidium inconspicuum*	CBS 8237^R	AF444318	AF363641	D64123	KF036468	KF036882	KF037147	KF423281
	F. murrhardtense*	CBS 9109^T	GU327540	AF416648	GU327540	KF036469	KF036883	KF037148	KF423282
flavus	Cryptococcus flavus	CBS 331^T	AF444338	AF075497	AB032629	KF036369	KF036781	/	KF423193
	C. paraflavus	CBS 10100^T	AY395800	AY395799	KF036645	KF036391	KF036805	/	KF423214
	C. podzolicus	CBS 6819^T	AF444321	AF075481	AB032645	KF036396	KF036810	KF037081	/
foliacea	C. fagi	CBS 9964^T	DQ054534	DQ054535	KF036632	KF036366	KF036778	KF037051	KF423190
	C. skinneri	CBS 5029^T	AF444305	AF189835	AB032646	KF036403	KF036817	KF037088	KF423225
	Tremella foliacea*	CBS 6969^R	AF444431	AF189868	KF036700	KF036528	KF036944	KF037206	KF423329
	T. neofoliacea*	CBS 8475^R	AF042415	AF042236	KF036706	/	/	/	/
hannae	Bullera hannae	CBS 8286^T	AF444486	AF363661	D78327	KF036324	KF036737	KF037008	/
	B. penniseticola	CBS 8623^T	AF444471	AF363649	AB005452	KF036329	KF036741	KF037012	KF423152
Hannaella	Hannaella coprosmaensis	CBS 8284^T	AF444485	AF363660	D78326	KF036479	KF036893	KF037156	KF423288
	H. kunmingensis	CBS 8960^T	AF325171	AB109558	AF325169	KF036480	KF036894	/	KF423289
	H. luteola	CBS 943^T	AF444323	AF075482	AB032641	KF036481	KF036895	KF037158	KF423290
	H. oryzae	CBS 7194^T	AF444413	AF075511	D31652	KF036482	KF036896	KF037159	KF423291
	H. sinensis	CBS 7238^T	AF444468	AF189884	D78328	KF036483	KF036897	KF037160	KF423292
	H. surugaensis	CBS 9426^T	AB100440	AB100440	AB100440	KF036484	KF036898	KF037161	KF423293
Kockovaella	Fellomyces chinensis	CBS 8278^T	AF444460	AF189878	AB032660	KF036459	KF036873	KF037142	KF423277
	F. distylii	CBS 8545^T	AF444475	AF363652	AB001036	/	/	/	/
	F. fuzhouensis	CBS 8243^T	AF444484	AF363659	KF036674	KF036460	KF036874	/	KF423278
	F. lichenicola	CBS 8315^T	AF444462	AF363643	AB032661	KF036462	KF036876	/	KF423279
	F. mexicanus	CBS 8279^T	AJ608667	AJ627906	KF036675	KF036463	KF036877	/	/
	F. ogasawarensis	CBS 8544^T	AF444474	AF363651	AB001035	/	/	/	KF423280
	F. sichuanensis	CBS 8318^T	AF444461	AF189879	AB032662	KF036466	KF036880	/	/
	F. thailandicus	CBS 8308^T	AJ608647	AF363644	AB044804	KF036467	KF036881	/	/
	Kockovaella barringtoniae	CBS 9811^T	AB052631	AB292854	AB052631	KF036487	KF036901	KF037165	KF423297
	K. calophylli	CBS 8962^T	AB042227	AB292852	AB042222	KF036488	KF036902	KF037166	/
	K. cucphuongensis	CBS 8959^T	AB042225	AB292853	AB042220	KF036489	KF036903	KF037167	/
	K. imperatae	CBS 7554^T	AB054091	AF189862	KF036680	KF036490	KF036904	KF037168	KF423298
	K. litseae	CBS 8964^T	AB042223	AB292850	AB042218	KF036491	KF036905	KF037169	KF423299
	K. machilophila	CBS 8607^T	AB054092	AF363654	AB005479	KF036492	KF036906	KF037170	/
	K. phaffii	CBS 8608^T	AB054093	AF363655	AB005480	KF036493	KF036907	/	KF423300
	K. sacchari	CBS 8624^T	AB054094	AF363650	AB005453	KF036494	KF036908	KF037171	/
	K. schimae	CBS 8610^T	AB042228	AF363656	AB005482	KF036495	KF036909	KF037172	/
	K. thailandica	CBS 7552^T	AB054095	AF075516	D64133	KF036496	KF036910	KF037173	KF423301
	K. vietnamensis	CBS 8963^T	AB042226	AB292851	AB042221	KF036497	KF036911	KF037174	/
Kwoniella	Bullera dendrophila	CBS 6074^T	AF444443	AF189870	D31649	KF036320	KF036733	KF037005	KF423145
	Cryptococcus bestiolae	CBS 10118^T	FJ534873	FJ534903	KF036622	KF036351	KF036764	KF037036	KF423175
	C. dejecticola	CBS 10117^T	AY917103	AY917102	KF036629	KF036362	KF036774	KF037047	KF423186
	C. heveanensis*	CBS 569^T	AF444301	AF075467	AB032635	FJ534921	KF036789	KF037061	KF423199
	C. pinus	CBS 10737^T	EF672246	EF672245	KF036648	KF036395	KF036809	KF037080	KF423218
	C. shivajii	CBS 11374^T	FM212571	FM212446	KF036652	KF036401	KF036815	KF037086	KF423223
	Kwoniella mangroviensis*	CBS 8507^T	AF444646	AF444742	KF036681	KF036498	KF036912	KF037175	KF423302
laurentii	Cryptococcus laurentii	CBS 139^T	AF410468	AF075469	AB032640	KF036380	KF036793	KF037065	AB040653
	C. rajasthanensis	CBS 10406^T	AM262325	AM262324	KF036649	KF036398	KF036812	KF037083	KF423220
melastomae	Bullera formosana	CBS 10306^T	AB118873	AB119465	AB118873	KF036321	KF036734	/	KF423146
	B. melastomae	CBS 10305^T	AB118872	AB119464	AB118872	KF036327	/	/	KF423150
moriformis	Tremella moriformis*	CBS 7810^R	AF444331	AF075493	U00977	KF036534	KF036950	KF037211	KF423335
	T. nivalis*	CBS 8487^R	AF042414	AF042232	KF036707	/	/	/	/
Papiliotrema	Cryptococcus nemorosus	CBS 9606^T	AF472628	AF472625	KF036642	KF036389	KF036802	KF037074	KF423211

Table 1. (Continued).

Lineage/clade	Species	Strain	ITS (5.8S)	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
	C. perniciosus	CBS 9605[T]	AF472627	AF472624	**KF036646**	**KF036393**	**KF036807**	**KF037078**	**KF423216**
	*Papiliotrema bandonii**	CBS 9107[T]	GU327539	AF416642	GU327539	**KF036518**	**KF036932**	**KF037194**	**KF423319**
pseudoalba	*Bullera pseudoalba*	CBS 7227[T]	AF444399	AF075504	D31660	**KF036330**	**KF036742**	**KF037013**	**KF423153**
	Cryptococcus cellulolyticus	CBS 8294[T]	AF444442	AF075525	AB032624	**KF036355**	**KF036768**	**KF037040**	**KF423179**
	C. anemochoreius	CBS 10258[T]	DQ830986	DQ384929	**KF036617**	**KF036344**	**KF036758**	**KF037029**	/
Tremella	*Tremella brasiliensis**	CBS 6966[R]	AF444429	AF189864	**KF036694**	/		**KF036938**	**KF037200**	**KF423324**
	*T. cinnabarina**	CBS 8234[R]	AF444430	AF189866	**KF036695**	**KF036523**	**KF036939**	**KF037201**	**KF423325**
	*T. coalescens**	CBS 6967[R]	**KF036601**	AF189865	**KF036696**	**KF036524**	**KF036940**	**KF037202**	**KF423326**
	*T. flava**	CBS 8471[R]	AF042403	AF042221	**KF036699**	**KF036527**	**KF036943**	**KF037205**	**KF423328**
	*T. fuciformis**	CBS 6970[R]	AF444316	AF075476	**KF036701**	**KF036529**	/		**KF037207**	**KF423330**
	*T. globispora**	CBS 6972[R]	AF444432	AF189869	**KF036703**	**KF036531**	**KF036947**	**KF037208**	**KF423332**
	*T. mesenterica**	CBS 6973[R]	AF444433	AF075518	**KF036705**	**KF036533**	**KF036949**	**KF037210**	**KF423334**
	*T. resupinata**	CBS 8488[R]	AF042421	AF042239	**KF036708**	**KF036535**	**KF036951**	**KF037212**	**KF423336**
	*T. taiwanensis**	CBS 8479[R]	AF042412	AF042230	**KF036709**	**KF036536**	**KF036952**	**KF037213**	**KF423337**
	*T. tropica**	CBS 8483[R]	AF042433	AF042251	**KF036710**	**KF036537**	**KF036953**	**KF037214**	/
single species clade	*Bullera arundinariae*	CBS 9931[T]	AF547662	AF547661	AF547660	**KF036319**	**KF036732**	**KF037004**	**KF423144**
	B. miyagiana	CBS 7526[T]	AF444409	AF189858	D31651	**KF036328**	**KF036740**	**KF037011**	**KF423151**
	B. sakaeratica	CBS 9934[T]	AY217651	AY211546	AY211544	**KF036331**	**KF036743**	**KF037014**	**KF423154**
	Cryptococcus allantoinivorans	CBS 9604[T]	AY315664	AY315662	**KF036615**	**KF036341**	**KF036755**	**KF037026**	**KF423167**
	C. cistialbidi	CBS 10049[T]	**KF036589**	AY562135	**KF036626**	**KF036358**	**KF036770**	**KF037043**	**KF423182**
	C. cuniculi	CBS 10309[T]	CBS database	DQ333885	**KF036627**	/	/	/	/
	C. mujuensis	CBS 10308[T]	**KF036595**	DQ333884	**KF036640**	**KF036386**	**KF036799**	**KF037071**	**KF423208**
	C. spencermartinsiae	CBS 10760[T]	EU249514	DQ513279	**KF036654**	**KF036404**	**KF036818**	**KF037089**	**KF423226**
	*Cuniculitrema polymorpha**	CBS 9644[T]	**KF036596**	AY032662	**KF036664**	**KF036418**	**KF036831**	**KF037102**	/
	*Sirobasidium intermedium**	CBS 7805	AF444330	AF075492	**KF036690**	**KF036519**	**KF036934**	**KF037196**	/
	*S. magnum**	CBS 6803	AF444314	AF075475	**KF036691**	**KF036520**	**KF036935**	**KF037197**	**KF423321**
	*Tremella giraffa**	CBS 8489[R]	AF042453	AF042271	**KF036702**	**KF036530**	**KF036946**	/	**KF423331**
	*Trimorphomyces papilionaceus**	CBS 443.92	AF444483	AF075491	**KF036726**	**KF036576**	**KF036993**	**KF037249**	**KF423355**
Trichosporonales									
cutaneum	*Trichosporon cutaneum*	CBS 2466[T]	AF444325	AF075483	**KF036712**	**KF036545**	**KF036961**	**KF037221**	AB175752
	T. debeurmannianum	CBS 1896[T]	AY143556	AY143554	**KF036713**	**KF036546**	**KF036962**	**KF037222**	**KF423340**
	T. dermatis	CBS 2043[T]	AY143557	AY143555	AB035585	**KF036548**	**KF036964**	**KF037224**	**KF423342**
	T. jirovecii	CBS 6864[T]	AF444437	AF105398	AB001758	/	**KF036974**	**KF037234**	AB175765
	T. moniliiforme	CBS 2467[T]	AF444415	AF105392	AB001761	**KF036562**	**KF036979**	**KF037238**	AB175772
	T. mucoides	CBS 7625[T]	AF444423	AF075515	AB001763	**KF036564**	**KF036981**	**KF037240**	AB040665
	T. smithiae	CBS 8370[T]	AF444397	AF444706	**KF036720**	**KF036570**	**KF036987**	**KF037244**	**KF423350**
	T. terricola	CBS 9546[T]	AB031517	AB086382	**KF036722**	**KF036572**	**KF036989**	/	**KF423352**
formosensis	*Bullera formosensis*	CBS 9812[T]	AY787859	AY787858	AB072235	**KF036322**	**KF036735**	**KF037006**	**KF423147**
	B. koratensis	CBS 10484[T]	AY919655	AY313006	AY863105	**KF036325**	**KF036738**	**KF037009**	**KF423149**
	B. lagerstroemiae	CBS 10483[T]	AY313033	AY313010	AY313033	**KF036326**	**KF036739**	**KF037010**	/
	Cryptococcus tepidarius	CBS 9427[T]	AB094045	AB094046	/	/	/	/	/
gracile/brassicae	*Trichosporon dulcitum*	CBS 8257[T]	AF444428	AF075517	AB001755	**KF036551**	**KF036967**	**KF037227**	AB175755
	T. gracile	CBS 8189[T]	AF444440	AF105399	AB001756	**KF036554**	**KF036970**	**KF037230**	AB175761
	T. laibachii	CBS 5790[T]	AF444421	AF075514	AB001760	**KF036559**	**KF036976**	**KF037235**	AB175769
	T. multisporum	CBS 2495[T]	AF414695	AF139984	AB001764	**KF036565**	**KF036982**	**KF037241**	AB175775
	T. loubieri	CBS 7065[T]	AF444438	AF075522	AB001759	**KF036561**	**KF036978**	**KF037237**	AB175771
	T. mycotoxinivorans	CBS 9756[T]	AJ601389	AJ601388	**KF036718**	**KF036566**	**KF036983**	**KF037242**	**KF423347**
	T. vadense	CBS 8901[T]	AY093425	AY093426	**KF036723**	**KF036573**	**KF036990**	**KF037246**	**KF423353**
	T. veenhuisii	CBS 7136[T]	AF414693	AF105400	**KF036724**	**KF036574**	**KF036991**	**KF037247**	AB175781

Table 1. (Continued).

Lineage/clade	Species	Strain	ITS (5.8S)	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
	T. brassicae	CBS 6382^T	AF444436	AF075521	AB001731	**KF036541**	**KF036957**	**KF037218**	AB175750
	T. domesticum	CBS 8280^T	AF444414	AF075512	AB001754	**KF036550**	**KF036966**	**KF037226**	AB175753
	T. montevideense	CBS 6721^T	AF444422	AF105397	AB001762	**KF036563**	**KF036980**	**KF037239**	AB175774
	T. scarabaeorum	CBS 5601^T	AF444446	AF444710	**KF036719**	**KF036569**	**KF036986**	**KF037243**	**KF423349**
haglerorum	*Cryptococcus arboriformis*	CBS 10441^T	AB260936	AB260936	**KF036619**	**KF036347**	**KF036760**	**KF037032**	**KF423171**
	C. haglerorum	CBS 8902^T	AY787857	AF407276	**KF036634**	**KF036375**	**KF036787**	**KF037059**	**KF423197**
porosum	*Trichosporon dehoogii*	CBS 8686^T	AF444476	AF444718	**KF036714**	**KF036547**	**KF036963**	**KF037223**	**KF423341**
	T. gamsii	CBS 8245^T	**KF036602**	AF444708	**KF036716**	**KF036553**	**KF036969**	**KF037229**	AB175780
	T. lignicola	CBS 219.34^T	AY370684	AY370685	DQ836003	**KF036560**	**KF036977**	**KF037236**	**KF423346**
	T. porosum	CBS 2040^T	AF414694	AF189833	AB051045	**KF036568**	**KF036985**	/	**KF423348**
	T. sporotrichoides	CBS 8246^T	AF444470	AF189885	**KF036721**	**KF036571**	**KF036988**	**KF037245**	**KF423351**
	T. wieringae	CBS 8903^T	AY315667	AY315666	**KF036725**	**KF036575**	**KF036992**	**KF037248**	**KF423354**
Trichosporon	*T. aquatile*	CBS 5973^T	AF410475	AF075520	AB001730	**KF036538**	**KF036954**	**KF037215**	AB040664
	T. asahii	CBS 2479^T	AY055381	AF105393	AB001726	**KF036539**	**KF036955**	**KF037216**	AB175744
	T. asteroids	CBS 2481^T	AF444416	AF075513	AB001729	**KF036540**	**KF036956**	**KF037217**	AB175746
	T. caseorum	CBS 9052^T	AJ319758	AJ319757	AJ319754	**KF036542**	**KF036958**	/	**KF423338**
	T. coremiiforme	CBS 2482^T	AF444434	AF139983	AB001727	**KF036544**	**KF036960**	**KF037220**	AB175751
	T. dohaense	CBS 10761^T	FJ228475	FJ228471	**KF036715**	**KF036549**	**KF036965**	**KF037225**	**KF423343**
	T. faecal	CBS 4828^T	AF444419	AF105395	AB001728	**KF036552**	**KF036968**	**KF037228**	AB175758
	T. inkin	CBS 5585^T	AF444420	AF105396	AB001757	**KF036555**	**KF036971**	**KF037231**	AB175764
	T. insectorum	CBS 10422^T	**KF036603**	AY520383	AY520254	**KF036556**	**KF036972**	/	**KF423344**
	T. japonicum	CBS 8641^T	AF444473	AF308657	AB001749	**KF036557**	**KF036973**	**KF037233**	**KF423345**
	T. lactis	CBS 9051^T	AJ319759	AJ319756	AJ319755	**KF036558**	**KF036975**	/	/
	T. ovoides	CBS 7556^T	AF444439	AF075523	AB001765	**KF036567**	**KF036984**	/	AB175776
Vanrija	*Vanrija albida*	CBS 2839^T	AB035578	AB126584	AB039285	**KF036399**	**KF036813**	**KF037084**	**KF423221**
	V. humicola	CBS 571^T	AF410470	AF189836	AB032637	**KF036378**	DQ645517	DQ645519	AB176693
	V. longa	CBS 5920^T	AB035577	AB126589	AB035586	**KF036382**	**KF036795**	**KF037067**	**KF423204**
	V. musci	CBS 8899^T	AB035579	AB126586	AB039378	**KF036387**	**KF036800**	**KF037072**	**KF423209**
	V. pseudolongus	CBS 8297^T	AB051048	AB126587	AB051047	**KF036397**	**KF036811**	**KF037082**	**KF423219**
single species clade	*Cryptococcus curvatus*	CBS 570^T	AF410467	AF189834	AB032626	**KF036359**	**KF036771**	**KF037044**	**KF423183**
	C. daszewskae	CBS 5123^T	AB035580	AB126588	AB035582	**KF036361**	**KF036773**	**KF037046**	**KF423185**
	C. fragicola	CBS 8898^T	AB035588	AB126585	AB035588	**KF036370**	**KF036782**	**KF037054**	/
	C. marinus	CBS 5235^T	**KF036593**	AF189846	AB032644	**KF036384**	**KF036797**	**KF037069**	**KF423206**
	Cryptotrichosporon anacardii	CBS 9551^T	AY549985	AY550002	DQ242636	**KF036417**	**KF036830**	**KF037101**	**KF423237**
	Trichosporon chiarellii	CBS 11177^T	GQ338074	EU030272	**KF036711**	**KF036543**	**KF036959**	**KF037219**	**KF423339**
	T. guehoae	CBS 8521^T	AF410476	AF105401	**KF036717**	/	/	/	AB175763
Pucciniomycotina									
	Leucosporidium scottii	CBS 5930^T	AF444495	AF070419	**KF036682**	**KF036499**	**KF036913**	**KF037176**	**KF423303**
	Sterigmatomyces halophilus	CBS 4609^T	AF444556	AF177416	D64119	**KF036521**	**KF036936**	**KF037198**	**KF423322**
Ustilaginomycotina									
	Ustilago maydis		AY854090	AF453938	X62396	XM754917	AY485636	AY885160	AB040663

The asterisks indicate teleomorphic taxa; CBS database: sequences are available from the CBS database at http://www.cbs.knaw.nl/Collections/Biolomics.aspx?Table=CBS strain database.

rDNA; and three nuclear protein-coding genes, namely the two subunits of RNA polymerase II (*RPB1* and *RPB2*) and translation elongation factor 1-α (*TEF1*). In addition, the mitochondrial gene cytochrome *b* (*CYTB*) was also included. The primers used for PCR amplification and sequencing of these genes are listed in Table 2. Because of the degenerate nature of the primers used for PCR amplification of the protein-coding genes, sometimes faint or multiple PCR bands were generated from PCR amplification or direct sequencing of amplicons failed. In these cases, amplicons were cloned using the pGEM®-T Easy Vector Systems (Promega Corporation, Madison) following the protocol of the kit. Positive colonies with an insert of expected size were chosen for sequencing.

Table 2. PCR and sequencing primers used in this study.

Primer name	Nucleotide sequence (5′–3′)	Reference
ITS and D1/D2		
V9	TGC GTT GAT TAC GTC CCT GC →	Boekhout et al. 2003
RLR3R	← GGT CCG TGT TTC AAG AC	Boekhout et al. 2003
ITS4	← TCC TCC GCT TAT TGA TAT GC	White et al. 1990
NL1	GCA TAT CAA TAA GCG GAG GAA AAG →	O'Donnell 1993
SSU		
NS1	GTA GTC ATA TGC TTG TCT →	White et al. 1990
NS24	← AAA CCT TGT TAC GAC TTT TA	Gargas & Taylor 1992
Oligo3	← GTA CAC ACC GCC CGT C	Hendriks et al. 1989
Oligo10	← TGG YRA ATG CTT TCG C	Hendriks et al. 1989
Oligo13	← ATA ACA GGT CTG TGA TGC CC	Hendriks et al. 1989
Oligo14	ATA ACA GGT CTG TGA TGC CC →	Hendriks et al. 1989
RPB1		
RPB1-Af	GAR TGY CCD GGD CAY TTY GG →	Stiller & Hall 1997
RPB 1-Cr	← CCN GCD ATN TCR TTR TCC ATR TA	Matheny et al. 2002
RPB2		
f RPB2-5F	GAY GAY MGW GAT CAY TTY GG →	Liu et al. 1999
RPB2-6F	TGG GGK WTG GTY TGY CCT GC →	Liu et al. 1999
RPB2-6R	← GCA GGR CAR ACC AWM CCC CA	Liu et al. 1999
RPB2-7R	← CCC ATW GCY TGC TTM CCC AT	Liu et al. 1999
bRPB2-7.1R	← CCC ATR GCY TGY TTM CCC ATD GC	Matheny 2005
TEF1		
EF1-983F	GCY CCY GGH CAY CGT GAY TTY AT →	Rehner & Buckley 2005
EF1-2218R	← ATG ACA CCR ACR GCR ACR GTY TG	Rehner & Buckley 2005
EF1-2212R	← CCR ACR GCR ACR GTY YGT CTC AT	Rehner & Buckley 2005
1577F	CAR GAY GTB TAC AAG ATY GGT GG →	Rehner & Buckley 2005
1567R	← ACH GTR CCR ATA CCA CCR ATC TT	Rehner & Buckley 2005
CYTB		
E1M4	TGR GGW GCW ACW GTT ATT ACT A →	Biswas et al. 2003
E2M4	← GGW ATA GMW SKT AAW AYA GCA TA	Biswas et al. 2003

Molecular phylogenetic analyses

Sequences were inspected and assembled using the SeqMan program in the Lasergene 7 software package (DNASTAR Inc., Madison) and were then aligned with Clustal X 1.83 (Thompson et al. 1997). Spliceosomal intron regions were inferred from the insertions with canonical splice sites (GT-AG, GC-AG, AT-AC) (Babenko et al. 2004) in the nucleotide sequence alignments between our data and reference cDNA sequences from Gen-Bank. Exon sequences of the protein-encoding genes RPB1, RPB2, TEF1 and CYTB were manually aligned using MEGA 5 (Tamura et al. 2011). Positions deemed ambiguous to align were excluded manually. Thereafter, multiple sequence alignments for ITS, D1/D2, SSU, RPB1, RPB2, TEF1, and CYTB were concatenated as a combined file.

Maximum likelihood (ML), neighbour-joining (NJ), and Bayesian analyses were conducted for separate and combined nucleotide data sets using RAxML v8.1.X (Stamatakis 2014), MEGA 5.0 (Tamura et al. 2011) and MrBayes 3.2.1 (Ronquist et al. 2012), respectively. ML analysis was implemented with the novel fast bootstrap algorithm with 100 replicates and a

subsequent search for the best maximum-likelihood tree in conjunction with the GTRGAMMAI model approximation (Stamatakis 2014). NJ analysis was performed on the evolutionary distance data calculated from Kimura's two-parameter model (Kimura 1980). Bootstrap analyses (Felsenstein 1985) were performed from 1 000 random re-samplings in both ML and NJ analyses. A bootstrap proportion (BP) support above 70 % obtained from the ML and NJ analyses was considered as significant (Hillis & Bull 1993).

Bayesian analysis was implemented using heterogeneous models to the data set with seven unlinked partitions, one for each gene. The best-fit evolution model of each gene fragment in the data set was determined using the Bayesian Information Criterion (BIC) in jModeltest (Posada 2008). The ITS, D1D2, and SSU rDNA gene sequences were fitted to TPM3uf+G, TIM3+G, and TIM2+T+G models, respectively. The protein-coding genes RPB1 and CYTB both used the GTR+I+G model; whereas RPB2 and TEF1 used the TPM3uf+I+G and TPM1uf+G models, respectively. Six to fifty million generations were run with four Markov chains (three heated and one cold), sampling every 500 generations. The average standard deviation of split frequencies,

Table 3. Nucleotide sequence data sets constructed for phylogenetic analyses.

Data set	No. of strains	No. of taxa	Length of alignment	Parsimony informative characters (%)
rDNA[1]	297	285	3 208	1 447 (45)
RPB1	271	262	758	615 (81)
RPB2	273	263	1 133	872 (77)
TEF1	249	238	909	498 (55)
CYTB	246	238	388	279 (71)
Seven-gene	281	269	6 298	3 623 (57)

[1] The rDNA data set includes 296 ITS, 297 LSU D1/D2, and 292 SSU rDNA sequences.

below 0.01, was examined to identify the convergence of the two independent runs. Clades with posterior probabilities (PP) above 0.95 were considered as significantly supported (Larget & Simon 1999).

RESULTS

Sequences generated and data sets constructed for phylogenetic analyses

A total of 1 147 new sequences were produced in this study, including 21 ITS, 123 SSU, 269 *RPB1*, 270 *RPB2*, 249 *TEF1*, and 215 *CYTB* sequences. In addition, a total of 777 previously published sequences of these genes from the type strains of tremellomycetous yeast taxa were retrieved from GenBank (Table 1). Different data sets consisting of the three rRNA genes (rDNA), the individual protein-coding genes, and the combined seven genes were constructed from the 1 924 sequences

employed in this study (Table 3). In addition, a data set of 5.8S and LSU rDNA D1/D2 domain sequences was constructed to include more *Tremella* species whose sequences were determined from herbarium specimens (Millanes *et al.* 2011).

These data sets were subjected to phylogenetic analyses using Bayesian, ML and NJ algorithms, respectively. The topologies of the trees obtained were compared visually to inspect the phylogenetic concordance among the taxa analysed, based on which monophyletic lineages and clades were recognised and defined (Table 4). As expected, among the trees drawn from different data sets analysed, the seven-gene trees exhibited the clearest resolution and strongest supports; and among the algorithms employed, the Bayesian analysis usually showed the most robust phylogeny (Table 4). Thus, the Bayesian tree constructed from the seven-gene data set was used as the primary basis for lineage and clade recognition and definition, and as the starting point for the subsequent comparison and discussion. The phylogenetic trees inferred from the rDNA data set containing all the taxa employed in this study were used as references to judge the phylogenetic positions of a minority of taxa which were absent in the seven-gene tree because of failure in sequencing of the protein coding genes.

Major lineages recognised among tremellomycetous yeasts

In the Bayesian tree constructed from the seven-gene data set, five lineages corresponding to the orders Tremellales, Trichosporonales, Filobasidiales and Cystofilobasidiales recognised by Boekhout *et al.* (2011) and the order Holtermanniales proposed by Wuczkowski *et al.* (2011) were resolved (Table 4, Fig. 1). The posterior probability for each of the Trichosporonales, Holtermanniales, Filobasidiales and Cystofilobasidiales lineages was 1.0. The support for the Tremellales was weak (PP = 0.51) when the basal foliacea clade of the lineage formed by *Cryptococcus fagi*, *C. skinneri*, *C. spencermartinsiae*, and *Tremella foliacea*

Table 4. Monophyletic clades resolved in tremellomycetous yeasts and dimorphic taxa based on different data sets using different algorithms.

Lineage/Clade	*RPB1* PP/BP[1]/BP[2]	*RPB2* PP/BP[1]/BP[2]	*TEF1* PP/BP[1]/BP[2]	*CYTB* PP/BP[1]/BP[2]	rDNA PP/BP[1]/BP[2]	Seven-gene PP/BP[1]/BP[2]
Cystofilobasidiales	nm/nm/nm	1.0/100/99	.90/64/70	nm/nm/nm	1.0/87/100	1.0/100/100
Cystofilobasidium	1.0/100/100	1.0/100/99	nm/nm/nm	nm/nm/nm	1.0/100/100	1.0/100/100
Guehomyces	S	S	S	S	1.0/100/nm	S
huempii	1.0/100/100	1.0/100/100	1.0/100/100	1.0/100/99	1.0/100/100	1.0/100/100
Itersonilia	S	1.0/100/100	S	S	1.0/100/100	1.0/100/100
Mrakia	1.0/90/83	1.0/96/89	nm/nm/ns	nm/nm/nm	1.0/100/100	1.0/100/100
Udeniomyces	1.0/100/87	1.0/100/99	S	–	1.0/100/100	1.0/100/100
Phaffia	S	1.0/100/100	1.0/100/100	1.0/99/99	1.0/100/100	1.0/100/100
Filobasidiales	1.0/100/98	1.0/100/100	nm/nm/ns	nm/nm/nm	1.0/83/98	1.0/100/100
aerius	1.0/100/100	1.0/100/100	ns/nm/nm	nm/nm/nm	nm/nm/nm	1.0/100/85
albidus	1.0/100/100	1.0/100/100	1.0/90/99	nm/nm/nm	1.0/94/99	1.0/100/100
cylindricus	1.0/100/100	1.0/100/100	1.0/100/100	ns/55/ns	1.0/100/100	1.0/100/100
Filobasidium	1.0/99/100	1.0/100/100	1.0/97/92	nm/nm/nm	1.0/100/100	1.0/100/100
gastricus	1.0/100/99	1.0/100/98	nm/nm/nm	nm/nm/nm	1.0/99/91	1.0/100/100
Holtermanniales	1.0/100/99	1.0/100/100	1.0/100/100	.99/66/ns	1.0/100/100	1.0/100/56
Holtermanniella	1.0/62/78	1.0/100/100	1.0/100/100	nm/nm/nm	1.0/99/100	1.0/100/80

Table 4. (Continued).

Lineage/Clade	RPB1 PP/BP¹/BP²	RPB2 PP/BP¹/BP²	TEF1 PP/BP¹/BP²	CYTB PP/BP¹/BP²	rDNA PP/BP¹/BP²	Seven-gene PP/BP¹/BP²
Tremellales/Trichosporonales	1.0/100/99	1.0/100/97	ns/ns/68	nm/nm/nm	.99/99/nm	1.0/100/55
Tremellales	nm/nm/nm	.95/72/nm	nm/nm/nm	nm/nm/nm	nm/nm/nm	ns/nm/nm
amylolyticus	1.0/83/84	1.0/100/100	1.0/97/88	1.0/99/99	1.0/100/100	1.0/100/100
aurantia	1.0/62//99	1.0/100/100	ns/ns/63	nm/nm/nm	nm/nm/nm	1.0/100/100
aureus	1.0/100/100	1.0/100/100	.99/85/98	nm/nm/nm	1.0/100/100	1.0/100/100
Auriculibuller	ns/59/nm	1.0/100/100	ns/ns/89	1.0/94/64	1.0/100/nm	1.0/100/100
Bandoniozyma	1.0/100/100	1.0/100/100	1.0/96/95	1.0/100/99	1.0/100/100	1.0/100/100
Bulleribasidium	.94/57/nm	1.0/67/56	nm/nm/nm	nm/nm/nm	1.0/100/100	1.0/100/100
Bulleromyces	1.0/100/100	S	1.0/94/98	1.0/99/99	1.0/100/100	1.0/100/100
Cryptococcus	1.0/98/80	1.0/100/99	nm/nm/nm	1.0/85/92	1.0/100/100	1.0/100/100
Derxomyces	.93/ns/nm	ns/ns/nm	nm/nm/nm	ns/ns/57	1.0/100/100	1.0/100/100
dimennae	1.0/96/93	1.0/99/100/	nm/nm/nm	nm/nm/nm	1.0/99/83	1.0/100/100
Dioszegia	1.0/96/89	1.0/93/99	nm/nm/nm	1.0/98/99	1.0/100/100	1.0/100/100
Fellomyces	nm/nm/nm	nm/nm/nm	1.0/69/63	S	1.0/87/98	1.0/64/nm
Fibulobasidium	1.0/100/100	1.0/100/100	1.0/99/100	1.0/100/99	1.0/100/100	1.0/100/100
flavus	nm/nm/nm	nm/nm/nm	S/S/S	1.0/96/99	.99/83/98	.97/72/82
foliacea	1.0/100/100	1.0/100/99	nm/nm/nm	nm/nm/nm	1.0/100/100	1.0/100/100
hannae	1.0/100/100	1.0/100/100	1.0/100/100	S	1.0/100/100	1.0/100/100
Hannaella	nm/nm/ns	1.0/99/95	nm/nm/nm	1.0/72/97	1.0/100/100	1.0/100/100
Kockovaella	1.0/62/74	nm/nm/nm	1.0/100/99	nm/nm/nm	1.0/100/100	1.0/96/96
Kwoniella	1.0/75/92	1.0/100/99	nm/nm/nm	nm/nm/nm	1.0/100/100	1.0/100/100
laurentii	1.0/100/99	1.0/100/69	.97/ns/nm	nm/nm/nm	1.0/100/92	1.0/100/100
melastomae	1.0/96/98	S	–	1.0/96/98	1.0/99/100	1.0/100/100
moriformis	S	S	S	S	1.0/100/95	S
Papiliotrema	1.0/100/100	1.0/100/100	1.0/95/97	1.0/99/99	1.0/100/nm	1.0/100/100
pseudoalba	.98/89/96	1.0/100/98	1.0/75/59	.97/100/99	.96/89/nm	1.0/100/100
Tremella	1.0/100/99	1.0/100/100	nm/nm/nm	nm/nm/nm	1.0/100/99	1.0/100/55
Trichosporonales	1.0/95/65	0.92/ns/nm	nm/nm/ns	nm/nm/nm	nm/nm/nm	1.0/100/100
gracile/brassicae	.90/61/nm	1.0/97/91	nm/nm/nm	ns/ns/nm	1.0/98/100	1.0/100/100
cutaneum	.96/ns/76	ns/ns/61	nm/60/86	nm/nm/nm	1.0/100/100	1.0/100/98
formosensis	1.0/100/99	1.0/100/95	1.0/92/66	1.0/100/99	1.0/100/100	1.0/100/100
Vanrija	1.0/100/100	1.0/100/100	1.0/100/100	1.0/98/99	1.0/97/77	1.0/100/100
haglerorum	nm/ns/nm	nm/ns/nm	nm/nm/ns		1.0/91/100	1.0/78/91
porosum	ns/80/100	1.0/100/100	1.0/51/86	nm/nm/nm	1.0/100/100	1.0/100/100
Trichosporon	1.0/100/99	1.0/100/100	1.0/100/99	nm/nm/nm	1.0/100/100	1.0/100/100

Note. PP, Bayesian posterior probability; BP¹ and BP², bootstrap values from the maximum likelihood and neighbour-joining analyses, respectively; nm: not monophyletic; ns, not supported (PP < 0.9 or BP < 50 %); S: single species clade. Data sets that produce both significant PP (≥0.95) and BP (≥70 %) values have dark grey shaded cells; and data sets that produce either a significant PP or BP support value have light grey shaded cells.
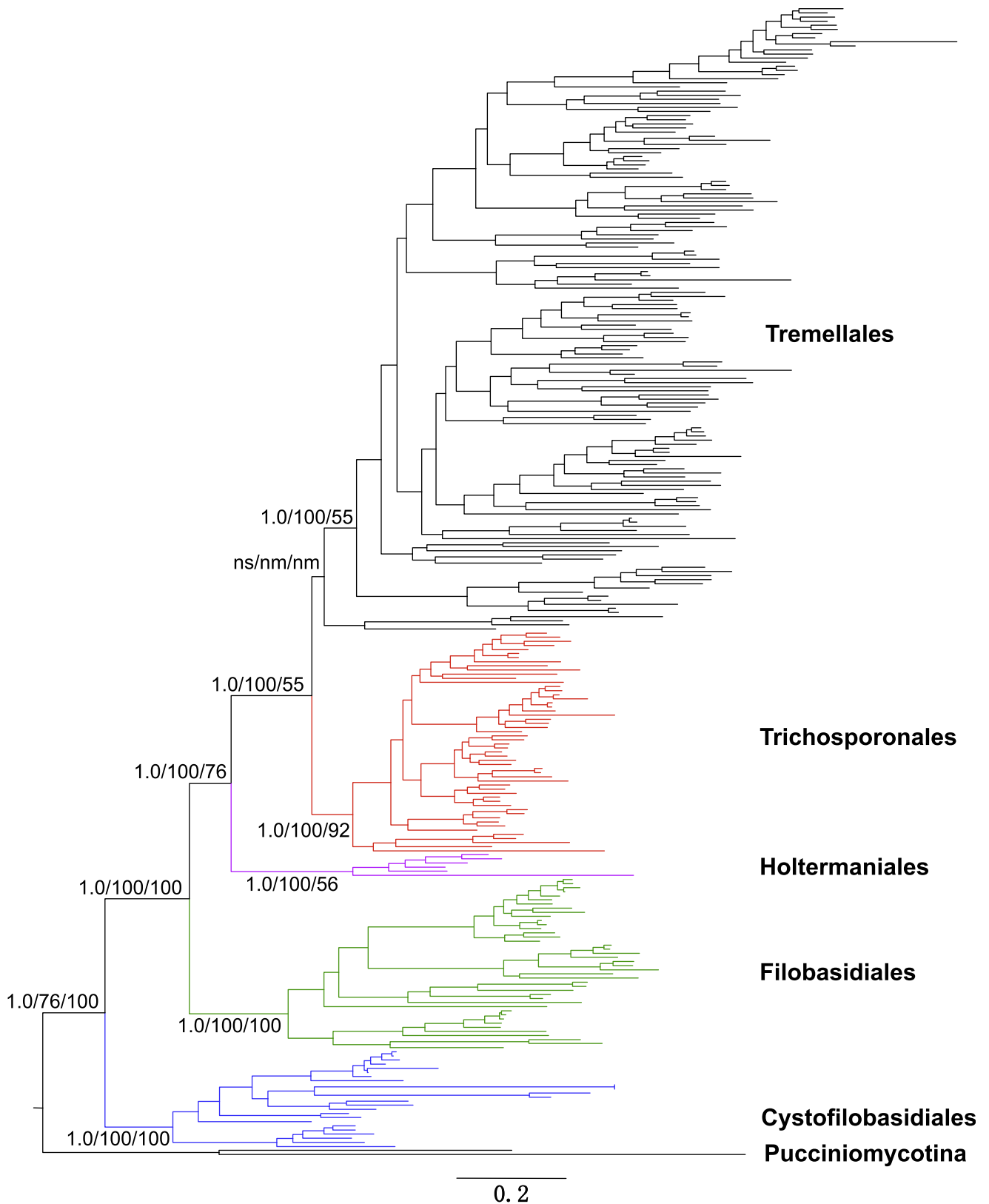
was included. However, when this clade was not included, the Tremellales taxa formed a well-supported lineage with a PP value of 1.0 (Fig. 1).

The five lineages were also clearly recognised in the ML and NJ trees constructed from the seven-gene data set (Table 4, Fig. 1), though the statistic support values varied. The Cystofilobasidiales, Filobasidiales, and Trichosporonales lineages received 100 % bootstrap supports, while the Holtermanniales received a moderate bootstrap support (56 %) in the NJ tree, but a strong support (100 % BP) in the ML tree. In the ML tree, the foliacea clade was located basal to the Trichosporonales lineage. In the NJ tree, the foliacea clade was located basal to the Trichosporonales and Tremellales lineages, but the bootstrap support for this topology was weak (Fig. 1).
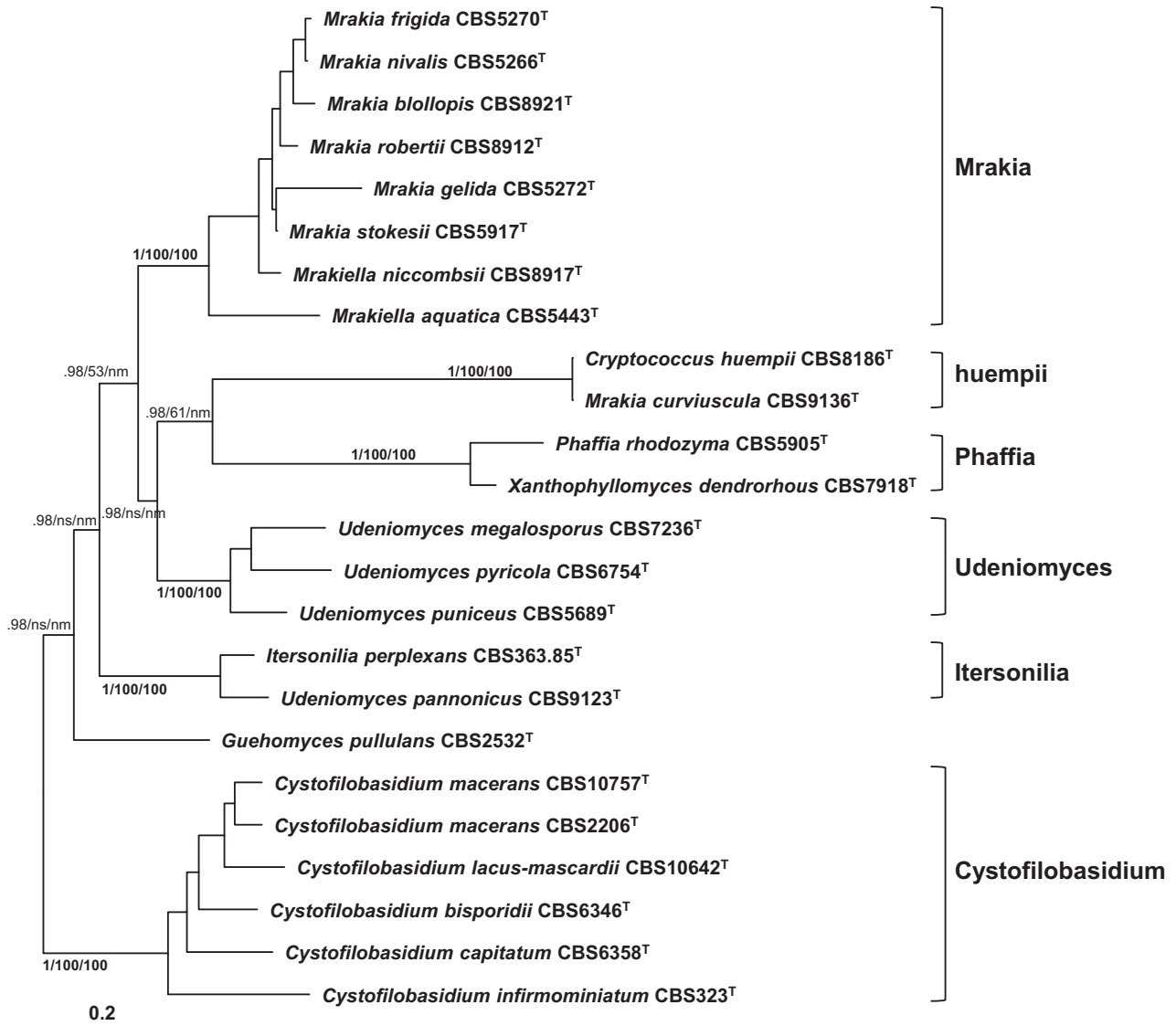
Cystofilobasidiales

The species of the Cystofilobasidiales clustered into seven well-supported clades in the Bayesian tree drawn from the seven-gene data set (Table 4, Fig. 2), being in agreement with Boekhout et al. (2011). Each of the clades was strongly supported with a posterior probability of 1.0. The **Mrakia** clade contained all the *Mrakia* and *Mrakiella* species, except *Mrakia curviuscula* that formed a separated clade together with

Fig. 1. An outline of the phylogeny of tremellomycetous yeasts and dimorphic taxa inferred from a seven-gene data set including sequences of three rDNA genes, *RPB1*, *RPB2, TEF1* and *CYTB*. The tree backbone is constructed using Bayesian analysis. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site. The Bayesian posterior probabilities (PP) and bootstrap percentages (BP) of maximum likelihood and neighbour-joining analyses from 1 000 replicates are shown respectively from left to right on the deep and major branches resolved. Note: ns, not supported (PP < 0.9 or BP < 50 %); nm, not monophyletic.

Cryptococcus huempii. Three of the four currently recognised *Udeniomyces* species formed the **Udeniomyces** clade, while the other species of the genus, *U. pannonicus*, formed a clade together with *Itersonilia perplexans*. All six recognised

Cystofilobasidium species clustered together in a single clade. *Phaffia rhodozyma* CBS 5905[T] and its proposed teleomorph, *Xanthophyllomyces dendrorhous* CBS 7918[T], formed a well separated clade, but the type strains of the two taxa differ clearly

Fig. 2. The phylogenetic relationships among species of the Cystofilobasidiales inferred from a seven-gene data set including sequences of three rDNA genes, *RPB1*, *RPB2*, *TEF1* and *CYTB*. The tree backbone is constructed using Bayesian analysis. The Bayesian posterior probabilities (PP) and bootstrap percentages (BP) of maximum likelihood and neighbour-joining analyses from 1 000 replicates are shown respectively from left to right on the deep and major branches and clades resolved. Note: nm, not monophyletic; ns, not supported (PP < 0.9 or BP < 50 %).

in protein gene sequences, suggesting that they may represent different species. Previous studies showed that *Guehomyces pullulans* CBS 2532[T] and *Tausonia pamirica* CBS 8428[T] clustered together (Boekhout *et al.* 2011, Fell & Guého-Kellermann 2011, Sampaio 2011b). Unfortunately, due to the unsuccessful amplification and sequencing of the protein genes of *T. pamirica* CBS 8428[T], this species was not included in the seven-gene data set. However, in the tree drawn from the rDNA data set, CBS 2532[T] and CBS 8428[T] formed a well-supported clade (Fig. 3). The seven clades were also all recognised and well-supported with bootstrap value of 100 % in the trees drawn from the ML and NJ analyses (Table 4).

Filobasidiales

Bayesian analysis on the seven-gene data set recognised five strongly supported clades within the Filobasidiales, namely **aerius**, **albidus**, **cylindricus**, *Filobasidium*, and **gastricus**, being largely in agreement with Boekhout *et al.* (2011) (Fig. 4). The **albidus** clade containing 17 *Cryptococcus* species and

varieties was clearly separated from the rest of the Filobasidiales. The *Filobasidium* clade contained four teleomorphic *Filobasidium* species including the type species of the genus, *F. floriforme*, and five *Cryptococcus* species. The affinity of *F. uniguttulatum* to this clade was not supported in Boekhout *et al.* (2011) and Weiß *et al.* (2014), but this study clearly showed that this species belongs to the *Filobasidium* clade with 1.0 posterior probability and 100 % bootstrap supports. This species was located in a basal branch of the *Filobasidium* clade together with *C. wieringae* (Fig. 4).

The **gastricus** clade contained six *Cryptococcus* species, including three species isolated from acid rock drainage (ARD) from a pyrite mine in Portugal. The three *Cryptococcus* species were recognised as the ARD ecoclade (Gadanho & Sampaio 2009). This ecoclade was supported by Bayesian and ML analyses based on the seven-gene data set, but not by NJ analysis. Therefore, we included this ecoclade in the **gastricus** clade.
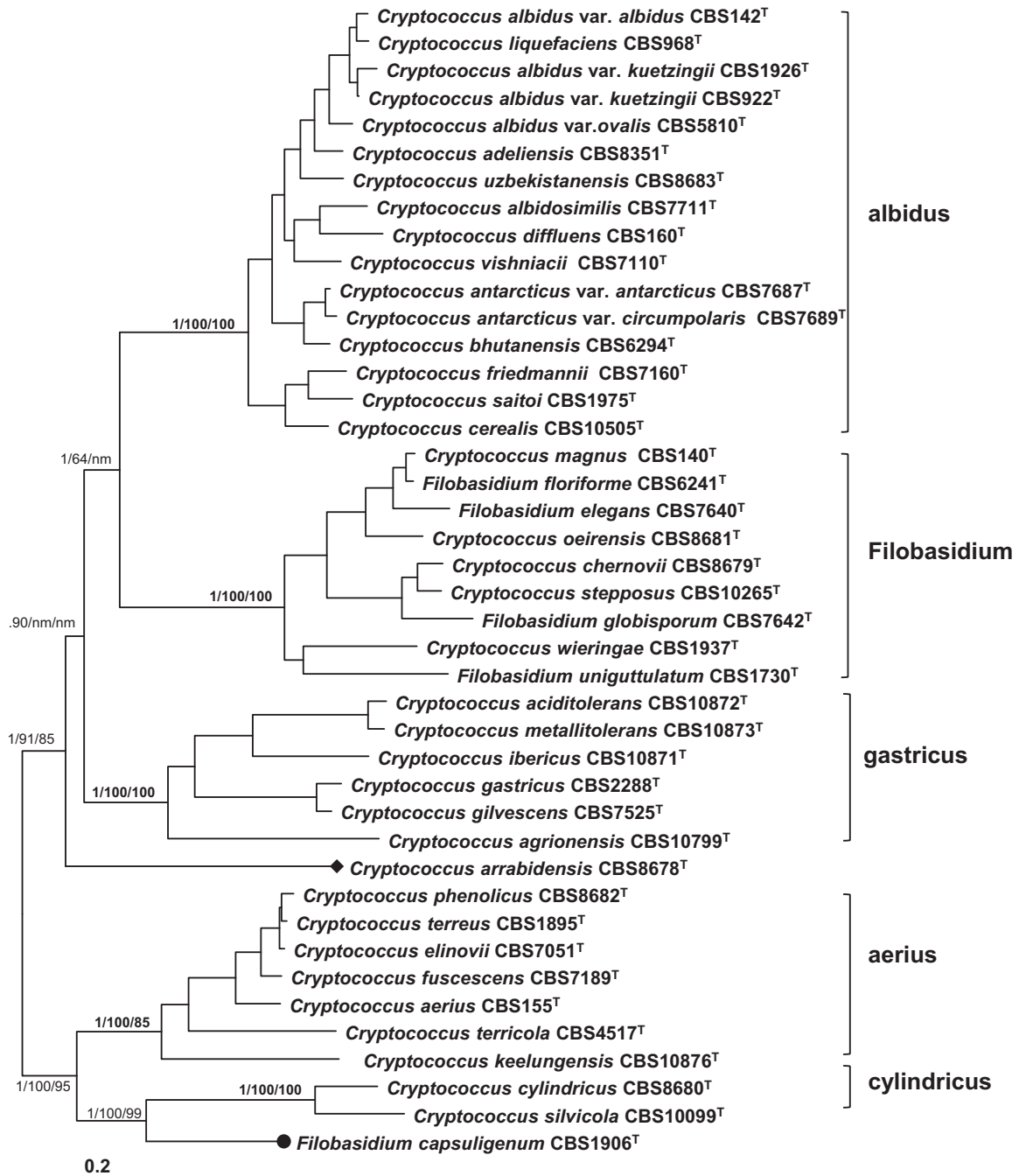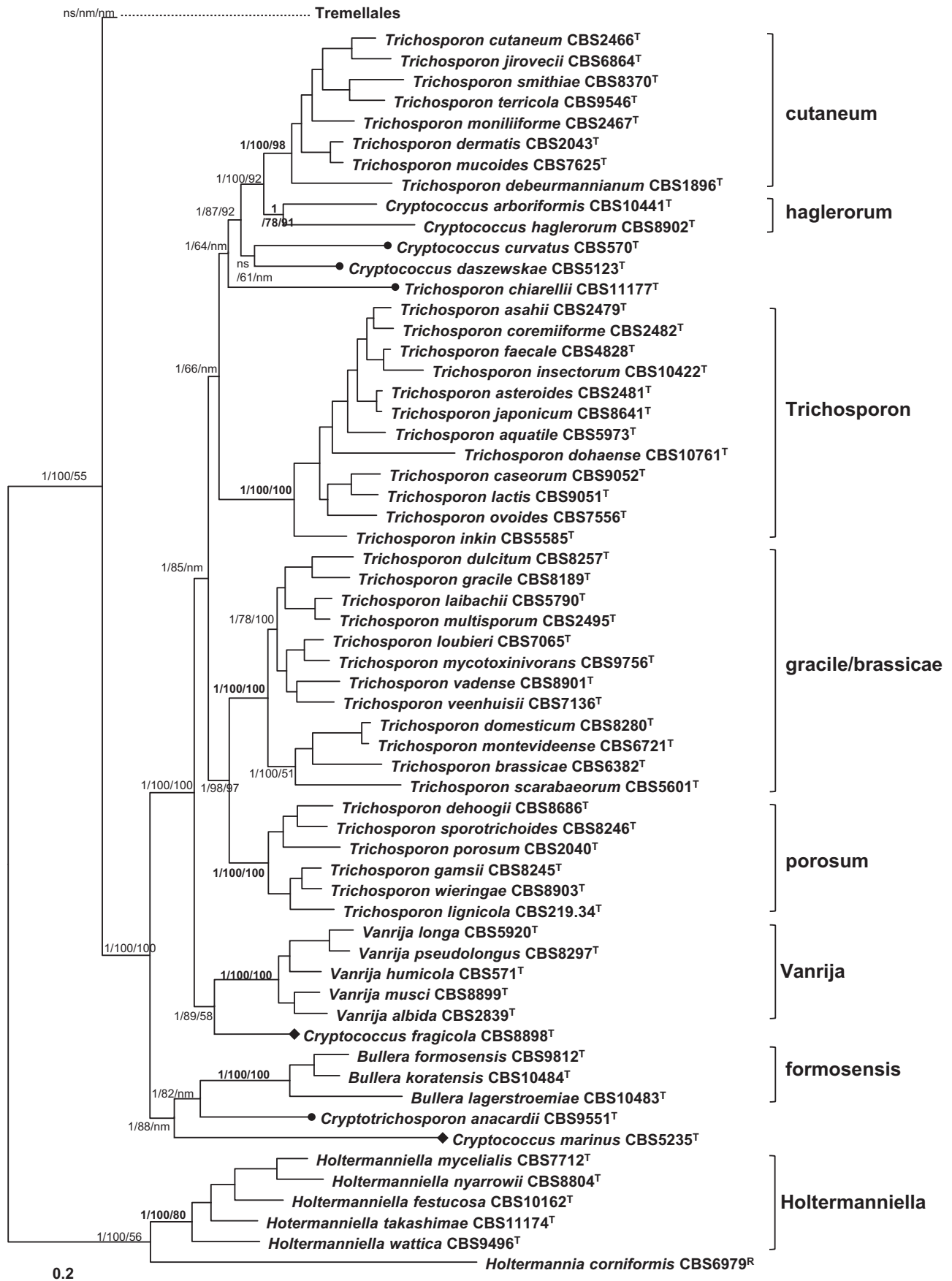
The **aerius** and **cylindricus** clades contained seven and two *Cryptococcus* species in the seven gene tree, respectively (Fig. 4). Analyses based on the rDNA data set showed that *Bullera taiwanensis*, whose protein gene sequences were not

Fig. 3. Phylogeny of tremellomycetous yeasts and dimorphic taxa based on the rDNA data set containing ITS, D1/D2, and SSU rDNA sequences. The tree backbone is constructed using Bayesian analysis. The Bayesian posterior probabilities (PP) and bootstrap percentages (BP) of maximum likelihood and neighbour-joining analyses from 1 000 replicates are shown respectively from left to right on the deep and major branches and in brackets following the clades resolved. Notes: nm, not monophyletic; ns, not supported (PP < 0.9 or BP < 50 %).

successfully determined, also clustered in the **cylindricus** clade with strong statistical supports (PP = 1.0) (Table 4, Fig. 3). The two clades together with *Filobasidium capsuligenum*, which represent a separate monotypic clade, formed a well-supported lineage (PP = 1.0; BP > 95 %). *Cryptococcus arrabidensis* was not included in any of the clades recognised in the Filobasidiales

and remained as a separate branch in the trees constructed using different algorithms (Fig. 4).

Fig. 4. The phylogenetic relationships among species of the Filobasidiales inferred from a seven-gene data set including sequences of three rDNA genes, *RPB1*, *RPB2*, *TEF1* and *CYTB* sequences. The tree backbone is constructed using Bayesian analysis. The Bayesian posterior probabilities (PP) and bootstrap percentages (BP) of maximum likelihood and neighbour-joining analyses from 1 000 replicates are shown respectively from left to right on the deep and major branches and clades resolved. The branches ending with a filled cycle and a diamond represent single-species clades with a stable and unstable position, respectively. Note: nm, not monophyletic.

Holtermaniales

Five anamorphic *Holtermanniella* species proposed by Wuczkowski *et al.* (2011) and the teleomorphic species *Holtermannia corniformis* were included in this small lineage (Fig. 5). This lineage was well separated from other groups of tremellomycetous yeasts and strongly supported in the seven-gene Bayesian and ML trees, though it was weakly supported in the NJ tree. *Holtermannia corniformis* was located as a basal branch in this lineage and its affinity with the *Holtermanniella* species was weakly supported by NJ analysis (Fig. 5), implying that this teleomorphic species may represent a distinct clade.

Trichosporonales

Seven well-supported clades with multiple species and seven single species clades were recognised in this order (Table 4, Fig. 5). The *Trichosporon* species were separated into four clades, namely **cutaneum**, **gracile**, **porosum**, and *Trichosporon* (also referred to as ovoides), supporting the classification of Middelhoven *et al.* (2004). The **brassicae** clade recognised in Sugita *et al.* (2004), Boekhout *et al.* (2011) and Sugita (2011) was also resolved in the Bayesian and ML trees based on the seven-gene data set, however, its separation from the **gracile** clade was only weakly supported in the seven-gene NJ tree. Furthermore, the distinction of the two clades was not

Fig. 5. The phylogenetic relationships among species of the Trichosporonales and Holtermaniales inferred from a seven-gene data set including sequences of three rDNA gene, *RPB1*, *RPB2*, *TEF1* and *CYTB* sequences. The tree backbone is constructed using Bayesian analysis. The Bayesian posterior probabilities (PP) and bootstrap percentages (BP) of maximum likelihood and neighbour-joining analyses from 1 000 replicates are shown respectively from left to right on the deep and major branches resolved. The branches ending with filled cycles and diamonds represent single-species clades with a stable and unstable position, respectively. Note: nm, not monophyletic; ns, not supported (PP < 0.9 or BP < 50 %).

supported in the Bayesian tree drawn from the rDNA data set. Therefore, we combined these two clades into a single clade. Among the *Trichosporon* species employed in this study, *T. chiarellii* could not be assigned to any clade. *Trichosporon guehoae*, whose protein gene sequences were not successfully amplified, was also located in a single branch in the tree drawn from the rDNA data set (Fig. 3).

In addition to the *Trichosporon* species, seven *Cryptococcus*, three *Bullera* and five *Vanrija* species and the monotypic genus *Cryptotrichosporon* (Okoli *et al.* 2007) were included in the Trichosporonales lineage (Table 1, Fig. 5). The genus *Vanrija* which was recently reinstalled by Weiß *et al.* (2014) for the five *Cryptococcus* species in the **humicola** clade recognised before (Boekhout *et al.* 2011, Fonseca *et al.* 2011) was confirmed to be a monophyletic group (Fig. 5). Two *Cryptococcus* species, *C. arboriformis* and *C. haglerorum*, formed the **haglerorum** clade which were resolved and well-supported in the seven-gene and the rDNA trees (Table 3, Figs 3 and 5). Other four *Cryptococcus* species, *C. curvatus*, *C. daszewskae*, *C. fragicola*, and *C. marinus*, occurred in single species branches. The three *Bullera* species formed a basal **formosensis** clade with strong statistical support (Table 4, Fig. 5). The thermotolerant species *Cryptococcus tepidarius* was located in this clade with a close relationship to *B. lagerstroemiae* based on rDNA sequence analysis (Fig. 3), being in agreement with Takashima *et al.* (2009). The protein gene sequences of *C. tepidarius* were not successfully determined. A close phylogenetic relationship of the **formosensis** clade with *Cryptococcus marinus* and *Cryptotrichosporon anacardii* was shown in the seven-gene Bayesian and ML trees, but the latter two species were located in separate clusters in the NJ tree (Fig. 5). In the trees drawn from the rDNA and single protein gene data sets, these two species did not cluster together, suggesting they represent different clades.

The affinity of *Cryptococcus marinus* within the Trichosporonales was strongly supported in the seven gene tree. It was located in a basal cluster of the order together with the formosensis clade and *Cryptotrichosporon anacardii* with strong support values from the Bayesian and ML analyses, but its phylogenetic position was not resolved by the NJ analysis (Fig. 5).

Tremellales

The majority of the taxa employed in this study belong to this lineage. Most of the clades recognised in Boekhout *et al.* (2011) were confirmed here with improved resolution and stronger support values. While most of the species can be assigned into clear clades, some remained undetermined and the boundaries of some clades need to be examined further.

Twenty five well-supported clades were recognised among the 160 strains included in the Bayesian tree drawn from the seven-gene data set (Tables 1 and 4, Fig. 6). Five recently proposed or redefined genera based on molecular phylogenetic analyses were confirmed as monophyletic groups, including *Bandoniozyma* (Valente *et al.* 2012), *Bulleribasidium/Mingxiaea* (Sampaio *et al.* 2002, Wang *et al.* 2011), *Derxomyces*, *Dioszegia*, and *Hannaella* (Takashima *et al.* 2001, Wang & Bai 2008). Each of these clades received a posterior probability value of 1.0 in the Bayesian tree and bootstrap values of 100 % in the ML and NJ trees drawn from the seven-gene data set, respectively (Table 4, Fig. 6). These clades were also clearly

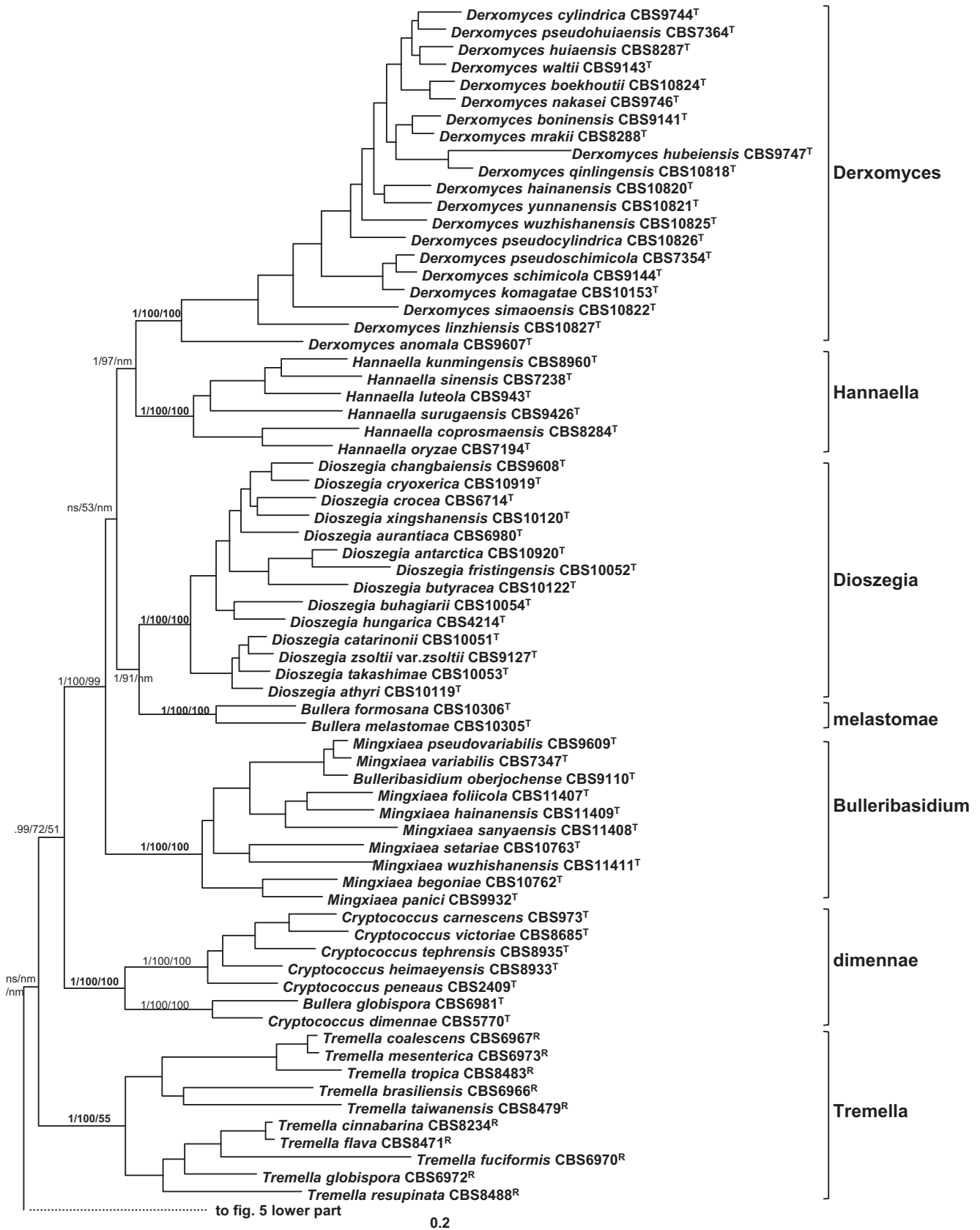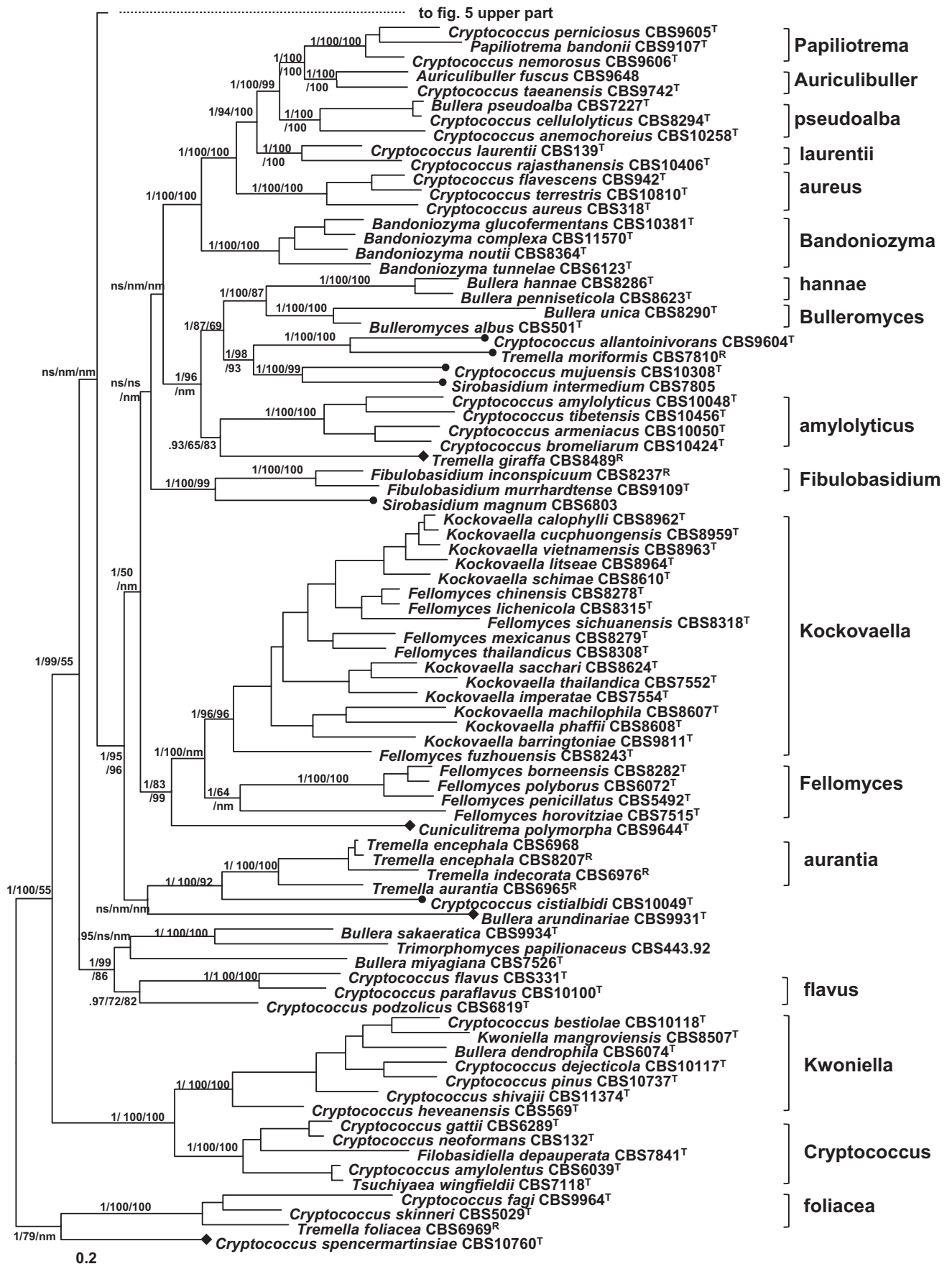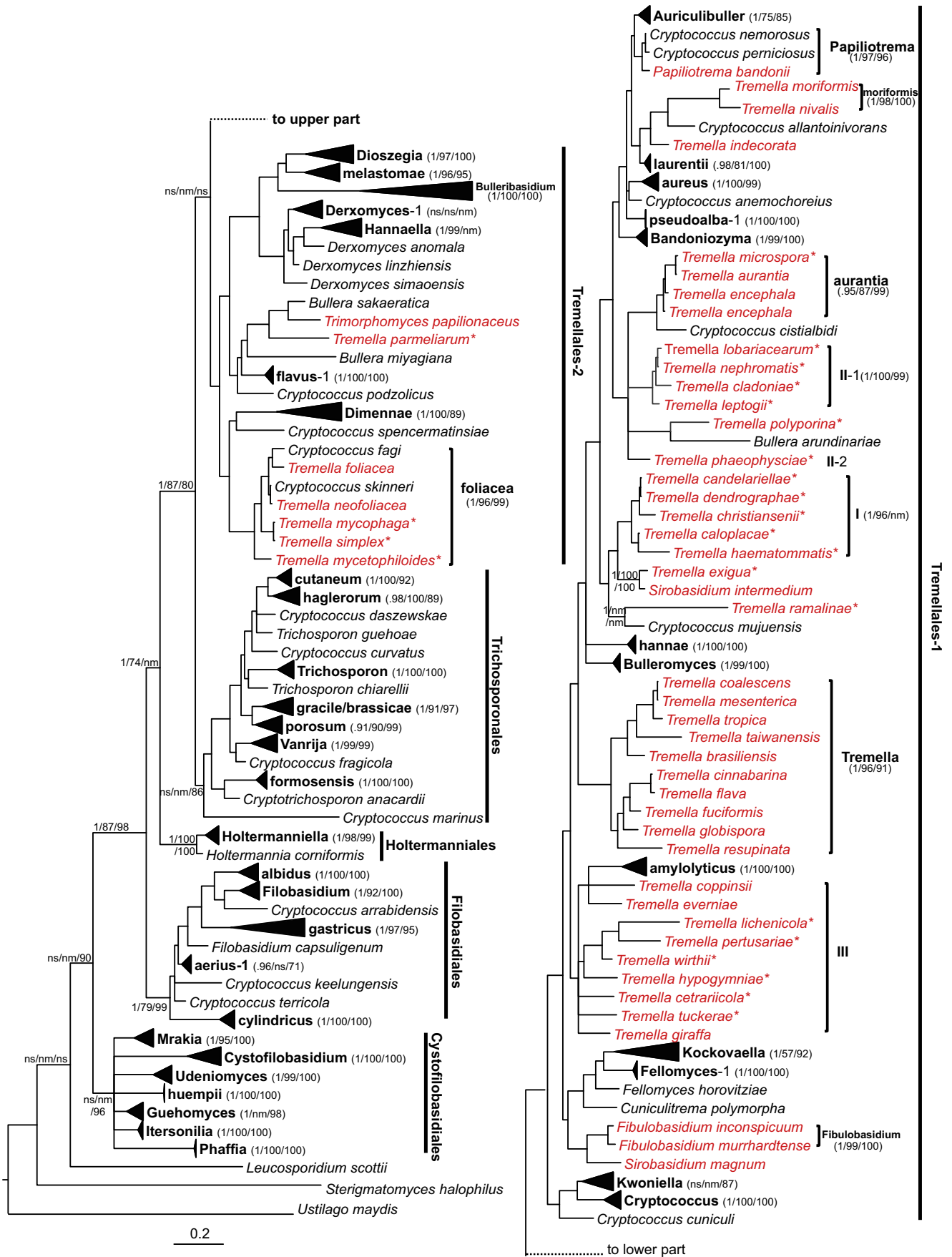resolved in the analyses using the rDNA and single protein gene data sets (Table 4).

In addition to the monotypic teleomorphic genus *Cuniculitrema*, the Cuniculitremaceae designated by Kirschner *et al.* (2001) contained *Fellomyces* and *Kockovaella* species. The species of the latter two anamorphic genera clustered into a well-supported cluster. However, two subclades represented by the type species of the two genera, *F. polyborus* and *K. thailandica*, respectively, could be recognised in the seven-gene Bayesian and ML trees (Fig. 6). The two subclades were also resolved in the NJ tree, with *F. horovitziae* being located as a basal branch to the two subclades. In the Bayesian and ML trees, this species was basal to the Fellomyces subclade with a PP and BP value of 1.0 and 64 %, respectively (Fig. 6).

The phylogenetic relationships among the species tentatively assigned to the *Bulleromyces/Papiliotrema/Auriculibuller* group by Boekhout *et al.* (2011) were resolved in this study (Fig. 6). The teleomorphic species *Bulleromyces albus* and three anamorphic *Bullera* species occurred in a distinct group with two clades being recognised, namely the **Bulleromyces** clade containing the anamorphic species *Bullera unica*, and the **hannae** clade formed by *B. hannae* and *B. penniseticola*. However, in the trees drawn from the rDNA data set, the close relationship of the two clades was not resolved (Fig. 3).

The monotypic teleomorphic genera *Papiliotrema* and *Auriculibuller* formed a well-supported group with one *Bullera* and 10 *Cryptococcus* species. This group showed a close relationship to the **Bandoniozyma** clade with strong support (Fig. 6). Five clades were recognised in this group (Table 4, Fig. 6). The **Papiliotrema** clade contained two other *Cryptococcus* species, namely *C. nemorosus* and *C. perniciosus*; *C. taeanensis* showed a close affinity to the **Auriculibuller** clade. The **pseudoalba** clade contained a *Bullera* species and two *Cryptococcus* species, *C. anemochoreius* and *C. cellulolyticus*. The **laurentii** and the **aureus** clades contained two and three *Cryptococcus* species, respectively.

Four recently described *Cryptococcus* species with orange coloured colonies (Inácio *et al.* 2005, Wang *et al.* 2007, Landell *et al.* 2009) clustered together in a well-supported **amylolyticus** clade. Two *Bullera* species described from Taiwan (Nakase *et al.* 2004), which were assigned to the **Dioszegia** clade in Boekhout *et al.* (2011), formed a distinct **melastomae** clade closely related with the **Dioszegia** clade. Other clearly supported clades consisting of species with only or mainly yeast forms were the **Cryptococcus**, **dimennae**, and **Kwoniella** clades. The **dimennae** clade, which was also resolved by Boekhout *et al.* (2011) but was referred to as the **victoriae** clade by Fonseca *et al.* (2011), consisted of six *Cryptococcus* species and one *Bullera* species (*B. globispora*). In addition to the teleomorphic species *Kwoniella mangroviensis*, five *Cryptococcus* and one *Bullera* species were included in the **Kwoniella** clade. The opportunistically pathogenic species in the *Cryptococcus neoformans* complex and their teleomorphs were included in the **Cryptococcus** clade together with *Filobasidiella depauperata*, *C. amylolentus* and *Tsuchiyaea wingfieldii*.

The *Tremella* species employed in the present study separated into different clades. Ten of them, including the type species of the genus, *T. mesenterica*, clustered in the **Tremella** clade. No species with mainly yeast forms in their life cycle were located in this clade. Three *Tremella* species formed the **aurantia** clade. *Tremella moriformis* was located in a group containing two *Cryptococcus* species (*C. allantoinivorans* and

Fig. 6. The phylogenetic relationships among species of the Tremellales inferred from a seven-gene data set including sequences of three rDNA genes, *RPB1*, *RPB2*, *TEF1* and *CYTB* sequences. The tree backbone is constructed using Bayesian analysis. The Bayesian posterior probabilities (PP) and bootstrap percentages (BP) of maximum likelihood and neighbour-joining analyses from 1 000 replicates are shown respectively from left to right on the deep and major branches resolved. The branches ending with filled cycles and diamonds represent single-species clades with a stable and unstable position, respectively. Note: nm, not monophyletic; ns, not supported (PP < 0.9 or BP < 50 %).

Fig. 6. (Continued).

C. mujuensis) and *Sirobasidium intermedium*, a teleomorphic species. This group, which was tentatively included in the *Bulleromyces/Papiliotrema/Auriculibuller* group in Boekhout *et al.* (2011), was also resolved as a separate group in the ML and NJ trees with 93–98 % bootstrap supports (Fig. 6). However, *C. mujuensis* and *S. intermedium* were separated from the other species of this group in the tree drawn from the rDNA data set (Figs 3 and 7). With the consideration that the four species in this

Fig. 7. Phylogeny of tremellomycetous yeasts and dimorphic taxa based on 5.8S and LSU D1/D2 rDNA sequences from strains employed in this study and 26 more *Tremella* species employed in Millanes *et al.* (2011). The tree backbone is constructed using Bayesian analysis. The Bayesian posterior probabilities (PP) and bootstrap percentages (BP) of maximum likelihood and neighbour-joining analyses from 1 000 replicates are shown respectively from left to right on the deep and major branches and in brackets following the clades resolved. The species names in red represent fruiting-body forming taxa and those with a star superscript indicate that the sequences are from herbarium specimens of lichen-inhabiting species. Note: nm, not monophyletic; ns, not supported (PP < 0.9 or BP < 50 %).

group exhibit quite different morphological characters from each other, they were regarded as representing four separate single species clades. *Tremella nivalis* and *T. moriformis* formed the **moriformis** clade with 1.0 PP and with over 95 % bootstrap support in the tree drawn from the rDNA sequence data set (Fig. 3). Another *Sirobasidium* species employed in this study, *S. magnum*, was located in a branch basal to the **Fibulobasidium** clade (Figs 3 and 6). *Tremella foliacea* and two *Cryptococcus* species (*C. fagi* and *C. skinneri*) clustered in the **foliacea** clade, which was located at the basal position of the Tremellales lineage in the seven-gene Bayesian tree (Fig. 5). The rDNA tree showed that *T. neofoliacea* was also located in this clade (Fig. 3).

Another group containing both yeast and filamentous taxa is the *Trimorphomyces* group. Two *Bullera* species and three *Cryptococcus* species were located in this group together with *Trimorphomyces papilionaceus*, a basidiocarp-forming species with a yeast state (Fig. 6). *T. papilionaceus* was regarded as representing a distinct clade because of its unique sexual reproductive structures (Bandoni & Boekhout 2011, Boekhout et al. 2011). The three *Cryptococcus* species, *C. flavus*, *C. paraflavus* and *C. podzolicus*, were assigned to the **flavus** clade since they clustered together in the seven-gene and rDNA Bayesian trees with 0.97–0.99 PP supports (Figs 3 and 6). The two *Bullera* species in this group, *B. sakaeratica* and *B. miyagiana*, was separated by *T. papilionaceus* in the seven-gene and the rDNA trees (Figs 3 and 6). Therefore, they were regarded as representing two different single species clades.

The following species in the Tremellales lineage, *Bullera arundinariae*, *Cryptococcus cistialbidi*, *Cryptococcus spencermatinsiae*, *Cuniculitrema polymorpha*, and *Tremella giraffe*, could not be assigned to any recognised clade or group, because of their unstable or unresolved phylogenetic positions, or their unique phenotypic characters. *Bullera arundinariae* and *C. cistialbidi* were located as basal branches to the **aurantia** clade formed by four *Tremella* species in the seven-gene Bayesian tree (Fig. 6). While the close relationship of *C. cistialbidi* to the **aurantia** clade was consistent in different trees, *B. arundinariae* was located in different positions in the seven-gene NJ tree and the trees resulting from the rDNA data set (Figs 3 and 6). *Cryptococcus spencermartinsiae* was located in a branch basal to the **foliacea** clade with strong statistical support in the Bayesian and ML trees drawn from the seven-gene data set, but the species was located at a different position in the seven-gene NJ tree and the trees drawn from the rDNA data set (Figs 3 and 6). The teleomorphic species *Cuniculitrema polymorpha* (anamorph: *Sterigmatosporidium polymorphum*) was located in a branch basal to the *Fellomyces/ Kockovaella* group. *Tremella giraffa* was located as a basal branch to the **amylolyticus** clade in the seven-gene tree with weak to moderate support values (Fig. 6), but its position was not resolved in the rDNA tree (Fig. 3).

In order to investigate further the relationships of yeasts with filamentous taxa in the Tremellomyetes, we retrieved the 5.8S and LSU rDNA sequences of 26 lichen-inhabiting *Tremella* species employed in Millanes et al. (2011) that were absent in the current data set. These sequences were determined from herbarium specimens (Millanes et al. 2011). The Bayesian tree obtained from the combined 5.8S and LSU D1/D2 rDNA sequence data set showed a largely identical topology with that obtained from the seven-gene data set and the five major lineages were also clearly resolved (Fig. 7). The majority of the additional 26 *Tremella* species were located in clades I, II and III

as defined by Millanes et al. (2011) which mainly contained lichen-inhibiting *Tremella* species; one in the **aurantia** clade containing *Tremella* taxa only; three in the **foliacea** clade containing both *Tremella* and *Cryptococcus* species; and one in the *Trimorphomyces* group (Fig. 7).

DISCUSSION

In this study, we inferred the phylogeny of basidiomycetous yeasts and related dimorphic and filamentous basidiomycetes in the Tremellomycetes based on analyses of seven gene sequences using different phylogenetic algorithms. The majority of the yeast taxa and dimorphic basidiomycetes that have free-living unicellular states in their life cycles in the Agaricomycotina were employed. Five major lineages corresponding to the five orders currently recognised in the Tremellomycetes (Boekhout et al. 2011, Millanes et al. 2011, Weiß et al. 2014) were resolved. A total of 45 strongly supported monophyletic clades with multiple species and 23 single species clades were recognised. This phylogenetic framework will be the basis for an improved modern taxonomy unifying both yeast-like and filamentous species in the Tremellomycetes as well as anamorphs and teleomorphs occurring in this class. The result is also helpful for a better understanding of the evolution of characters and different life styles by integrating the phylogeny with biochemical, morphological and reproductive characteristics of unicellular, dimorphic and filamentous basidiomycetes in the Tremellomycetes.

Congruence of phylogenies inferred from analyses using different algorithms and data sets

Almost all currently recognised teleomorphic and anamorphic yeast species and dimorphic taxa in the Agaricomycotina were obtained from culture collections and revived for DNA isolation and PCR amplification in this study. Despite our best effort to obtain a complete sequence data set for all the genes and strains employed, the sequence of some genes, especially the nuclear protein-coding genes and the mitochondrial gene *CYTB*, could not be determined for a small percentage of strains because of failure in the PCR amplification or sequencing reactions. Specifically, 8.8 %, 8.1 %, 16.2 % and 17.2 % of the total 297 strains employed failed in the sequence determination of the *RPB1*, *RPB2*, *TEF1* and *CYTB* genes, respectively. This problem is known from all groups of fungi (Schoch et al. 2012). A previous study has shown that an inferred phylogeny is not sensitive to 25 % or even 50 % missing data for a sufficiently large alignments (*e.g.*, ~30 000 positions and 36 species) (Philippe et al. 2004). Though the length of the seven-gene alignment in this study is only about 6 300 positions, the amount of missing data is also much less. Thus, we assume that the relative minor amount of missing data in our study will not significantly influence the reliability of the resulting phylogeny.

The phylogenies of the taxa compared in this study were inferred from analyses using different data sets and algorithms. The topologies of the trees constructed using different algorithms performed on different data sets were largely congruent as examined visually, which make the delimitation of major lineages and clades more clear and confident. In addition to the Clustal X,

we also used the MAFFT program (Katoh & Standley 2013) to align the sequences and the alignments generated were subjected to ML analysis. The topologies of the trees obtained from the Clustal X and the MAFFT alignments were almost the same (data not shown). This further supports the notion that our inferred trees are reliable and not greatly influenced by the missing data as discussed above.

Bayesian analysis is usually believed to be more reliable compared to parsimony and neighbour-joining methods, especially for an extensive sampling with a high divergence occurring among the sequences (Alfaro *et al.* 2003, Holder & Lewis 2003, James *et al.* 2006). As expected, the Bayesian analysis of the seven-gene data set showed the most robust phylogeny among the analyses performed (Table 4). However, analyses aiming at comparing Bayesian and ML supports have revealed that PP and BP values show significant correlation, but the strength of this correlation is highly variable and sometimes very low. ML BP values are generally lower than PP values, and thus, ML BP might be less prone to strongly supporting a wrong phylogenetic hypothesis (Douady *et al.* 2003). Therefore, the boundaries of the lineages and clades recognised in this study were determined based not only on Bayesian analysis, but also on ML and NJ analyses, aiming to recognize reliable monophyletic groups.

Conflicts between phylogenies obtained from rDNA and protein-coding gene sequences have been observed in different studies on basidiomycetes (Matheny *et al.* 2002, Froslev *et al.* 2005, Matheny 2005, Matheny *et al.* 2006, 2007). However, in this study, the topologies of the trees and the clades resolved from the data sets of *RPB1* and *RPB2* were similar to those obtained from the rDNA data set (Table 4), except for the position of the Trichosporonales which was nested into the Tremellales in the *RPB1*-based phylogeny. Furthermore, *RPB1* and *RPB2* had an equivalent resolution power in the Cystofilobasidiales and Filobasidiales lineages. The Holtermanniales lineage was supported strongly (100 % BP) by the ML algorithm in the *RPB2*-based phylogeny but only received moderate support (62 % BP) in the *RPB1*-based phylogeny. The *RPB1* and *RPB2*-based phylogenies constructed from Bayesian analysis supported the same number of clades in the Tremellales, while the *RPB1*-based phylogeny constructed from ML or NJ analyses resolved one more clade if compared to the *RPB2*-based phylogeny. The *RPB1* and *RPB2*-based phylogenies drawn from Bayesian and ML analyses also resolved the same number of clades in the Trichosporonales. The *TEF1* and *CYTB* sequences showed less parsimony-informative characters for the inference of phylogenetic relationship in the tremellomycetous yeasts compared to the *RPB1* and *RPB2* sequences. The *TEF1* and *CYTB* data sets generated the lowest resolution across the Bayesian, ML and NJ trees, in which only 19 and 16 strongly supported clades were resolved with high BP and PP values, respectively (Table 4). The *TEF1* and *CYTB* data sets were unable to resolve higher level taxonomic relationships, such as the five orders in the Tremellomycetes, and they did not show strong support to some clades, such as the **Cystofilobasidium**, **Derxomyces**, **foliacea**, **Hannaella**, **Kwoniella**, and **Trichosporon** clades, which were strongly supported by the analyses based on the other data sets. Our results suggest that *RPB1* and *RPB2* are more useful to infer reliable phylogeny of tremellomycetous yeasts than the *TEF1* and *CYTB* genes. A previous study of basidiomycetes phylogeny also showed that the major clades at higher and lower taxonomic levels were more clearly resolved based on *RPB2* than on *TEF1* sequence data (Matheny *et al.* 2007).

More robust topologies and higher resolution were achieved in this study than those obtained in previous studies based on the LSU rDNA D1/D2 domains or ITS-5.8S sequences (Fell *et al.* 2000, Scorzetti *et al.* 2002, Boekhout *et al.* 2011). The consensus is that the major groups recognised in the previous studies were confirmed in the present study. Fell *et al.* (2000) studied 171 hymenomycetous yeast strains representing 116 species. They recognised four major lineages including the Cystofilobasidiales, Filobasidiales, Tremellales and Trichosporonales. However, the clades within each lineage were largely unresolved. In addition to the four major lineages, Scorzetti *et al.* (2002) recognised clades within each lineage. Most of the clades recognised in the Cystofilobasidiales, Filobasidiales and Trichosporonales were in agreement to those recognised in this study. However, the fine phylogenetic relationships among the taxa in the Tremellales remained largely unresolved in the previous studies. Boekhout *et al.* (2011) employed more strains and designated a fifth lineage containing the **Holtermanniella** clade and a teleomorphic species *Holtermannia corniformis* that was described as a separate order (Wuczkowski *et al.* 2011). Our study confirmed this fifth lineage as a separate order Holtermanniales with 1.0 PP and 100 % ML BP supports. The phylogenetic position of *Cryptococcus marinus* has been debated. It was considered to belong to the Tremellales according to a phylogenetic analysis of SSU rDNA sequences (Takashima & Nakase 1999). The phylogenetic position in the LSU rDNA D1/D2 tree suggested that this species may represent a separate order within the Tremellomycetes (Scorzetti *et al.* 2002, Fonseca *et al.* 2011, Weiß *et al.* 2014). However, the affinity of this species with the Trichosporonales lineage was strongly supported in this study (Fig. 5).

The major lineages and clades recognised in this study are similar to those recognised in Millanes *et al.* (2011) and Weiß *et al.* (2014), which sampled more teleomorphic and filamentous taxa in the Tremellomycetes. In their molecular phylogenetic study on the jelly fungi based on nuclear SSU, 5.8S and LSU rDNA sequences, Millanes *et al.* (2011) employed three more teleomorphic genera *Biatoropsis*, *Syzygospora* and *Tetragoniomyces*, but limited yeast taxa. In addition to the teleomorphic genera employed in Millanes *et al.* (2011), Weiß *et al.* (2014) listed seven other teleomorphic genera that were not employed in our study, including *Carcinomyces*, *Rhynchogastrema*, *Phyllogloea*, *Phragmoxenidium*, *Sigmogloea*, *Sirotrema*, and *Xenolachne* in the Tremellomycetes. However, the latter five genera were not included in their phylogenetic analysis based on LSU D1/D2 sequences, because no DNA data were available from these genera. In the trees presented in Millanes *et al.* (2011) and Weiß *et al.* (2014), the species of the teleomorphic and filamentous genera that were not included in this study were located in separated clades from those formed by yeast taxa.

Correlation between morphology, physiology and molecular phylogeny

Because of the morphological simplicity, it is not easy to find morphological characters that distinguish the five major lineages of tremellomycetous yeasts recognised by molecular phylogenetic analyses. Teleomorphic taxa belonging to the Tremellales usually form tremella-type basidia, *e.g.*, phragmobasidia with longitudinal primary septa; whereas those of the *Cystofilobasidiales* and *Filobasidiales* are usually characterised by forming holobasidia (Wells & Bandoni 2001, Boekhout *et al.*

2011). However, some species with holobasidia or transversely septate basidia, like *Auriculibuller fuscus* (Sampaio *et al.* 2004), *Papiliotrema bandonii* (Sampaio *et al.* 2002), *Tremella fuciformis*, *T. hypogymniae* (Millanes *et al.* 2011) and *Bulleribasidium oberjochense* (Sampaio *et al.* 2002) are also present in Tremellales. These observations show that different types of basidial septation can coexist in the same lineage. The sexual stage of the Trichosporonales species has not yet been observed. The majority of the species in this order are characterised by forming abundant true hyphae that disarticulate into arthroconidia. However, the filamentous species *Tetragoniomyces uliginosus* which was tentatively assigned to the Trichosporonales in Millanes *et al.* (2011) and Weiß *et al.* (2014) forms basidia in pustulate basidiocarps (Oberwinkler & Bandoni 1981).

The species in the genera *Fellomyces* and *Kockovaella* share a special morphological character of forming conidia on stalks (Nakase *et al.* 1991). These species were located together in a cluster with strong PP and ML BP supports (Fig. 6). The affinity of *F. horovitziae* to the *Fellomyces* clade was weakly supported in ML analysis and not supported in NJ analysis. We tentatively assign *F. horovitziae* to the **Fellomyces** clade with the consideration of minimising name changes in the subsequent taxonomic treatment.

The ability to form ballistoconidia has since long been shown to be an unreliable phylogenetic marker (Nakase *et al.* 1993). This observation is confirmed by the intermixture of species of the ballistoconidia-forming genera *Bullera* and *Kockovaella* with those of non ballistoconidia-forming genera *Cryptococcus* and *Fellomyces*. However, the morphology of ballistoconidia seems to be phylogenetically relevant. Ballistoconidia formed by species in the Cystofilobasidiales and Trichosporonales are usually bilaterally symmetrical, whereas those formed by species in the Tremellales and Filobasidiales are usually rotationally symmetrical (Boekhout *et al.* 2011).

Within the Tremellales, some clades may be distinguished by colony morphology. For example, the anamorphic genera *Derxomyces*, *Hannaella* and *Dioszegia* are closely related, but are distinguishable by forming whitish to yellowish colonies with a butyrous texture, whitish colonies with a highly mucoid texture, and orange-coloured colonies with a butyrous texture, respectively (Wang & Bai 2008). The two *Bullera* species in the **melastomae** clade were assigned to the **Dioszegia** clade by Boekhout *et al.* (2011). However, they are morphologically different by forming yellowish to brownish colonies compared to the orange-coloured colonies of *Dioszegia* species (Takashima *et al.* 2001, Wang & Bai 2008).

The physiological and biochemical differences among the major lineages are also quite elusive, though some trends have been observed (Sampaio & Fonseca 1995, Sampaio 2004). The majority of the Cystofilobasidiales and Filobasidiales species can utilise nitrate; whereas the Tremellales and Trichosporonales taxa are usually nitrate negative. The coenzyme Q (CoQ) system has been used as an important taxonomic criterion at the genus level in yeasts (Yamada & Kondo 1973). The major CoQ systems of the tremellomycetous yeasts are CoQ-8, CoQ-9 and CoQ-10 (Fell 2011, Fell & Guého-Kellermann 2011, Sampaio 2011a, b). The taxa with CoQ-8 are concentrated in the Cystofilobasidiales. The species within a strongly supported clade usually possess the same major CoQ type, which may be helpful to recognize and define homogenous clades. The species with the ability to ferment sugars, a rare trait among basidiomycetous yeasts, are concentrated in a few clades in the Cystofilobasidiales (**Mrakia** and **Phaffia**/**Xanthophyllomyces**) and Tremellales (**Bandoniozyma**).

One species in the Filobasidiales, *Filobasidium capsuligenum*, can also ferment glucose and maltose, while the other known *Filobasidium* species can not ferment glucose (Kwon-Chung 2011). *F. capsuligenum* was separated from the **Filobasidium** clade and located in a branch closely related with the **cylindricus** clade containing two *Cryptococcus* species with strong PP and BP supports (Fig. 4). Ultrastructurally, *F. capsuligenum* is also special by having cone-shaped vesicular parenthesomes (Moore & Kreger-van Rij 1972). Thus, we recognised this species as representing a distinct clade. Consequently, the **cylindricus** clade and the closely related **aerius** clade were recognised as separate clades.

Serological characteristics of *Trichosporon* species correspond to some extent with their phylogenetic clustering. Species in the **cutaneum**, **Trichosporon** and **brassicae** clades have serotypes I, II and III, respectively, while species in the gracile and porosum clades have serotype I-III, which is a serotype that reacts to both antisera I and III (Ikeda *et al.* 1996, Sugita & Nakase 1998, Sugita *et al.* 2004, Sugita 2011). However, the phylogenetic separation between the brassicae and gracile clades, which have different serotypes (III and I-III, respectively) was not supported in this study. The gracile and brassicae clades were recognised as separate clades based on D1/D2 rDNA sequence analyses and serological characteristics (Sugita *et al.* 2004, Boekhout *et al.* 2011, Sugita 2011). However, both clades lacked bootstrap supports in the NJ trees drawn from D1/D2 sequences (Boekhout *et al.* 2011, Sugita 2011). In this study, the monophyly of the gracile clades was not resolved and supported in the Bayesian tree drawn from the rDNA data set. Therefore, we combined the gracile and brassicae clades.

Life strategy evolution in Tremellomycetes

The multiple gene phylogeny of tremellomycetous yeasts is helpful for a better understanding on the evolution of different life styles and strategies. The tremellomycetous fungi present a high diversity of lifestyles, with many species being dimorphic, including both unicellular and filamentous growth forms (Bandoni 1995, Sampaio 2004, Boekhout *et al.* 2011). They are also nutritionally heterogeneous, comprising saprotrophs, animal parasites, and fungal-inhabiting (including lichen-inhabiting) species (Millanes *et al.* 2011, Weiß *et al.* 2014). A previous study on phylogeny and character evolution in tremellomycetous fungi based on three rDNA markers (nSSU, 5.8S and nLSU) showed that, in a broad sense, a specific life style or strategy is usually homoplastic; however, taxa with the same life strategy, for example, fungal- or lichen-inhabiting, usually form distinct clades (*e.g.*, clades I, II and III in Millanes *et al.* (2011)). The results of this study also show that taxa with different life styles (*e.g.*, dominated by unicellular and filamentous growth stages, respectively) usually form different clades, though clades with species having the same life styles may not be closely related.

This observation is also shown by the analysis based on an integrated 5.8S and LS D1/D2 sequence data set containing additional *Tremella* species as employed in Millanes *et al.* (2011). Though fruiting-body forming species were intermingled with yeast species throughout the Tremellales (Fig. 7), the former usually clustered into different groups from the latter. A few fruiting-body forming species, *e.g.*, *Papiliotrema bandonii*, *Tremella parmeliarum*, *T. polyporina*, *T. ramalinae*, *T. foliacea*, and *Trimorphomyces papilionaceus*, were located in the same

clusters together with some yeast taxa, but they usually formed distinct branches or clades. These results suggest that tremellomycetous fungi with the same life styles or nutritional strategies may be the result of convergent evolution as a result of early adaptation to different ecological niches or habitats.

Taxonomic consequences

As with many other groups of fungi, the taxonomic system of basidiomycetous yeasts needs to be updated to reflect the evolutionary relationships of the taxa concerned and to accommodate the requirements of the new nomenclatural code (McNeill *et al*. 2012). Based on the results of this study, we will propose an updated taxonomic system for tremellomycetous yeasts which will have the best approximation of the molecular phylogeny and that will be compatible with the current taxonomic system of filamentous basidiomycetes. A considerable number of genera need to be redefined to include only the species in the monophyletic clades that contain the type species of those genera, and, secondly, many new genera need to be proposed to accommodate monophyletic clades that do not include any generic type species. The names of many species will be changed due to the proposal of new genera and adaptation of the 'one fungus = one name' principle at this stage. We believe that this updated taxonomic system based on a reliable phylogeny and extensive phenotypical comparisons will be relatively stable and minimise the necessity of future name changes.

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