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Phylogeny of yeasts and related filamentous fungi within *Pucciniomycotina* determined from multigene sequence analyses

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Abstract: In addition to rusts, the subphylum *Pucciniomycotina* (*Basidiomycota*) includes a large number of unicellular or dimorphic fungi which are usually studied as yeasts. Ribosomal DNA sequence analyses have shown that the current taxonomic system of the pucciniomycetous yeasts which is based on phenotypic criteria is not concordant with the molecular phylogeny and many genera are polyphyletic. Here we inferred the molecular phylogeny of 184 pucciniomycetous yeast species and related filamentous fungi using maximum likelihood, maximum parsimony and Bayesian inference analyses based on the sequences of seven genes, including the small subunit ribosomal DNA (rDNA), the large subunit rDNA D1/D2 domains, the internal transcribed spacer regions (ITS 1 and 2) of rDNA including the 5.8S rDNA gene; the nuclear protein-coding genes of the two subunits of DNA polymerase II (*RPB1* and *RPB2*) and the translation elongation factor 1-α (*TEF1*); and the mitochondrial gene cytochrome *b* (*CYTB*). A total of 33 monophyletic clades and 18 single species lineages were recognised among the pucciniomycetous yeasts employed, which belonged to four major lineages corresponding to *Agaricostilbomycetes*, *Cystobasidiomycetes*, *Microbotryomycetes* and *Mixiomycetes*. These lineages remained independent from the classes *Atractiellomycetes*, *Classiculomycetes*, *Pucciniomycetes* and *Tritirachiomycetes* formed by filamentous taxa in *Pucciniomycotina*. An updated taxonomic system of pucciniomycetous yeasts implementing the ‘One fungus = One name’ principle will be proposed based on the phylogenetic framework presented here.

Key words: *Fungi*, *Basidiomycota*, *Pucciniomycotina*, Yeasts, Multigene phylogeny.

INTRODUCTION

Basidiomycetous yeasts are unicellular or dimorphic fungi that belong to the three lineages of the *Basidiomycota*, namely *Pucciniomycotina*, *Ustilaginomycotina* and *Agaricomycotina* (also previously known as *Urediniomycetes*, *Ustilaginomycetes* and *Hymenomycetes*, respectively) (Boekhout 1991, Bauer *et al.* 2006, Hibbett *et al.* 2007, Boekhout *et al.* 2011). At present, yeasts in the *Pucciniomycotina* comprise 28 genera, including 19 teleomorphic and 9 anamorphic ones (Bauer *et al.* 2009, Boekhout *et al.* 2011, Turchetti *et al.* 2011, Toome *et al.* 2013, de García *et al.* 2015). Our understanding of the phylogenetic relationships of these basidiomycetous yeasts and their systematics largely improved due to sequence analysis of parts of the ribosomal DNA (rDNA) (Fell *et al.* 2000a, Scorzetti *et al.* 2002), but the full taxonomic consequences of these studies have not yet been made. For instance, teleomorphic and anamorphic genera are still treated separately, and many anamorphic genera, such as *Rhodotorula* and *Sporobolomyces*, are polyphyletic (Fell *et al.* 2000a, Scorzetti *et al.* 2002, Boekhout *et al.* 2011, Hamamoto *et al.* 2011, Sampaio 2011a). Species of these two genera occur in three classes in the *Pucciniomycotina*, and some *Rhodotorula* species occur even in another subphylum *Ustilaginomycotina* (Boekhout *et al.* 2011, Sampaio 2011a).

Earlier results using sequence analysis of the small subunit (SSU or 18S) rDNA indicated that the yeast members within *Pucciniomycotina* could be divided into four groups, designated as the ***Agaricostilbum*/*Bensingtonia***, ***Erythrobasidium***, ***Sporidiales*** and **subbrunneus** clusters (Hamamoto & Nakase 2000, Nakase 2000). Sequence analyses of the large submit (LSU or 26S) rDNA D1/D2 domains and the internal transcribed spacer (ITS) region showed similar results and four lineages named ***Agaricostilbum***, ***Erythrobasidium***, ***Microbotryum*** and ***Sporidiobolus*** (Fell *et al.* 2000b, Scorzetti *et al.* 2002). In the 5th edition of *The Yeasts, a Taxonomic Study*, all known *Pucciniomycotina* yeast species were classified into four classes, namely *Agaricostilbomycetes* (the *Agaricostilbum* lineage), *Cystobasidiomycetes* (the ***Erythrobasidium*** lineage), *Microbotryomycetes* (the ***Sporidiobolus*** and ***Microbotryum*** lineages) and *Mixiomycetes* (Boekhout *et al.* 2011). The above listed studies provided a detailed grouping of species in many clades of these four classes, but the molecularly defined clades frequently lacked concordance or statistic support and many species remained unassigned (Boekhout *et al.* 2011). With the advent of the ‘One Fungus = One Name’ concept (Hawksworth 2011, Taylor 2011, McNeill *et al.* 2012) the anamorphic taxa have to be combined with the teleomorphic ones into a single taxonomy. Thus the boundaries of the clades and genera have to be reassessed by analyzing a robust molecular data set. For many of the yeast members of *Pucciniomycotina* SSU rDNA sequences were not yet available and also some LSU rDNA D1/D2 and ITS data were missing. In addition, protein coding gene sequences have rarely been used in molecular phylogeny studies of basidiomycetous yeasts. The multigene analysis of the fungal kingdom as presented by the Assembling the Fungal Tree of Life (AFTOL) consortium

(James *et al.* 2006) and its derived taxonomy (Hibbett *et al.* 2007) showed the potential of this kind of analysis to improve our understanding of fungal evolutionary relationships and taxonomy.

In the present work, we employed the six genes that were used in the AFTOL project (James *et al.* 2006) and an additional mitochondrial gene, cytochrome *b* (*CYTB*) that was used in phylogenetic analyses of some basidiomycetous yeast genera (Biswas *et al.* 2001, 2005, Yokoyama 2005, Wang & Bai 2008) to resolve the tree of life of the pucciniomycetous yeasts. The aim of this work is to recognise monophyletic clades and to improve the phylogeny and taxonomy of this group of eukaryotic microorganisms. In addition, by using available data, mainly generated from the AFTOL project (http://www.aftol.org/data.php), we also inferred the evolutionary relationships between the unicellular yeast taxa and the main groups of filamentous fungi in the *Pucciniomycotina*.

MATERIALS AND METHODS

Yeast and filamentous taxa employed

One hundred and ninety nine strains belonging to 184 yeast species within *Pucciniomycotina* were studied (Table 1). They were mostly type and authentic strains from CBS Fungal Biodiversity Centre (CBS-KNAW), Utrecht, The Netherlands, the China General Microbiological Culture Collection Center (CGMCC), Institute of Microbiology, Chinese Academy of Sciences, Beijing, China, and the Japan Collection of Microorganisms (JCM), RIKEN BioResource Center, Saitama, Japan. The type strains of all pucciniomycetous yeast species included in the latest edition of *The Yeasts, a Taxonomic Study* (Kurtzman *et al.* 2011) were employed. In addition, fifteen pucciniomycetous yeast species that were published after the publication of that treatment were used in this study. Fifteen representative filamentous taxa from the *Pucciniomycotina* were employed as references and two taxa from *Ustilaginomycotina* were used as an outgroup (Table 1). The alignments and trees were deposited in TreeBASE (No. 18076).

Sequencing and molecular phylogenetic analyses

A set of seven genes or loci were included in this study, including three rDNA regions, namely SSU, LSU D1/D2 domains and ITS (including 5.8S rDNA); three nuclear protein coding genes, namely the largest subunit of RNA polymerase II (*RPB1*), the second largest subunit of RNA polymerase II (*RPB2*), and translation elongation factor 1-α (*TEF1*); and the mitochondrial gene cytochrome *b* (*CYTB*). Sequencing of the ITS region and LSU D1/D2 domains were performed using methods described previously (Fell *et al.* 2000b, Wang & Bai 2004). SSU rDNA sequences were determined according to Wang *et al.* (2003). Sequences of *CYTB* were obtained as described by Wang & Bai (2008). PCR and sequencing primers for *RPB1*, *RPB2* and *TEF1* are listed in Table 2. PCR amplification and sequencing of the three nuclear protein-coding genes were performed using methods described previously (Wang *et al.* 2014). GenBank accession numbers for all the sequences determined in this study are listed in Table 1.

Sequences were aligned with the MAFFT program (Standley 2013) using the L-INS-I algorithm. The alignment datasets were analysed with Modeltest version 3.04 (Posada & Crandall 1998) using the Akaike information criterion (AIC) to find the most appropriate model of DNA substitution. A general time-reversible model of DNA substitution additionally assuming a percentage of invariable sites and Γ-distributed substitution rates at the remaining sites (GTR + I + G) was selected for Maximum likelihood (ML) and Bayesian inference (BI) analyses. ML analysis was conducted using RAxML-HPC 7.2.8 (Stamatakis 2006) with a rapid bootstrap analysis using a random starting tree and 1 000 bootstrap replicates searching for the best maximum-likelihood tree, and with GTRGAMMAI as the model of evolution. BI analysis was conducted using MrBayes 3.1.2 (Ronquist *et al.* 2012) with the GTR + I + G model and 5 000 000 to 10 000 000 generations, two independent runs and four chains. The other parameters were set as default. The analysis was stopped when the standard deviation of split frequencies between the trees generated in the independent runs was below 0.01. Twenty five percent of these trees were discarded, the remaining were used to compute a 50 % majority rule consensus tree to obtain estimates for posterior probabilities. Maximum parsimony (MP) analysis was performed using PAUP* 4.0b10 (Swofford 2002) with a heuristic search with 1 000 random additions and TBR. Bootstrap analysis was performed from 1 000 replicates using 10 random additions and TBR for each replicate. The gaps in the alignment were treated as missing data. MulTrees and Steepest descent options were not in effect. A bootstrap percentage (BP) of $\geq$70 % or a Bayesian posterior probability (PP) of $\geq$0.9 was considered as significantly supported in all constructed trees in this study.

RESULTS AND DISCUSSION

Sequence data obtained

From the sequences of the yeast strains employed here, 98.4 % (188/191) *TEF1*, 98.9 % (174/176) *RPB1*, 97.9 % (186/190) *RPB2*, 87.1 % (162/186) *CYTB*, 51.8 % (102/197) SSU, 9.1 % (18/198) LSU D1/D2 and 8.1 % (16/198) ITS sequences were newly determined in this study and the remaining sequences were retrieved from GenBank (Table 1). PCR amplification and sequencing of rDNA regions were successful for all the species studied. The success ratios of PCR amplification and sequencing of the *RPB1*, *RPB2*, *TEF1* and *CYTB* genes were 88 %, 91 %, 95 % and 93 %, respectively. The single gene sequences of the SSU rDNA, LSU rDNA D1/D2 domains, ITS + 5.8S rDNA, *TEF1*, *RPB1*, *RPB2* and *CYTB* were aligned using the MAFFT algorithm (Standley 2013), resulting in alignments of 1 773, 646, 1 252, 1 023, 796, 1 270 and 387 nucleotide lengths, respectively. Different data sets consisting of the three rDNA regions, the four protein coding genes, and the combined seven genes, respectively, were constructed. When available, the corresponding sequences from representative filamentous taxa in *Pucciniomycotina* were also incorporated in the data sets. In addition, a data set of SSU and LSU rDNA D1/D2 sequences from the yeast strains employed in this study and those from the representative filamentous taxa compared in Bauer *et al.* (2006), Schell *et al.* (2011) and Toome *et al.* (2013) was constructed, because of the scarcity of available ITS and protein gene

Table 1. List of pucciniomycetous yeasts and selected reference filamentous taxa employed. The sequences with GenBank numbers in bold are determined in this study.

Lineage/Clade	Species	Strain number	ITS	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
Agaricostilbomycetes									
Agaricostilbales									
Kondoaceae									
Kondoa	*Bensingtonia changbaiensis*	AS 2.2310^{T}	AY233339	AY233339	AY233339	**KJ708024**	**KJ708147**	**KJ707751**	**KJ707585**
	B. miscanthi	JCM 5733^{T}	AF444516	AF189891	D38236	**KJ708023**	**KJ708149**	**KJ707753**	**KJ707719**
	B. phyllada	JCM 7476^{T}	AF444514	AF189894	D38237	**KJ708022**	**KJ708152**	**KJ707756**	**KJ707727**
	B. sorbi	AS 2.2303^{T}	AY233343	AY233343	AY233343	**KJ708029**	**KJ708156**	**KJ707897**	**KJ707584**
	B. subrosea	JCM 5735^{T}	AF444565	AF189895	D38238	**KJ708027**	**KJ708157**	**KJ707895**	**KJ707640**
	B. thailandica	JCM 10651^{T}	AB040114	EF384207	AB040114	**KJ708026**	**KJ708159**	**KJ707898**	**KJ707661**
	B. yuccicola	JCM 6251^{T}	AF444518	AF189897	D38367	**KJ708025**	**KJ708161**	/	/
	Kondoa aeria	CBS 8352^{T}	AF444562	AF189901	**KJ708417**	**KJ708020**	**KJ708172**	**KJ707905**	/
	K. malvinella	AS 2.1946^{T}	AF444498	AF189903	D13776	**KJ708021**	**KJ708173**	**KJ707896**	**KJ707568**
Bensingtonia	*Bensingtonia ciliata*	AS 2.1945^{T}	AF444563	AF189887	D38233	**KF706509**	**KF706536**	**KF706486**	**KJ707567**
	B. naganoensis	JCM 5978^{T}	AF444558	AF189893	D38366	**KJ707960**	**KJ708151**	**KJ707755**	**KJ707722**
	B. pseudonaganoensis	AS 2.2601^{T}	DQ224375	DQ224374	**KJ708416**	**KJ707959**	**KJ708153**	**KJ707956**	**KJ707590**
Agaricostilbaceae									
ingoldii	*B. ingoldii*	JCM 7445^{T}	AF444519	AF189888	D38234	**KJ707961**	**KJ708148**	**KJ707752**	**KJ707726**
	B. musae	JCM 8801^{T}	AF444569	AF189892	D43946	**KJ707963**	**KJ708150**	**KJ707754**	**KJ707743**
Agaricostilbum	*Agaricostilbum hyphaenes*	CBS 7811	AF444553	AF177406	AY665775	**KJ707965**	**KJ708145**	**KJ707749**	**KJ707645**
	A. pulcherrimum	FO 29365 (ATCC MYA-4629)	AJ406402	GU291289	FJ641896	/	FJ623647	/	/
	Sterigmatomyces elviae	JCM 1822^{T}	AF444551	AF177415	**KJ708432**	**KJ707964**	**KJ708345**	/	**KJ707699**
	S. elviae	JCM 1602	AB038053	**KP216512**	**KP216516**	**KJ708077**	**KJ708208**	**KJ707852**	AB040614
	S. halophilus	AS 2.1935^{T}	AF444556	AF177416	D64119	**KJ707962**	/	**KJ707890**	**KJ707566**
Chionosphaeraceae									
Chionosphaera	*Chionosphaera apobasidialis*	CBS 7430	AF444599	AF177407	U77662	/	**KJ708163**	**KJ707883**	**KJ707641**
	C. cuniculicola	CBS 10063	**KJ778640**	**KJ708465**	**KJ708368**	**KJ707985**	**KJ708164**	**KJ707886**	**KJ707593**
	C. cuniculicola	CBS 10065	**KJ778641**	**KJ708466**	**KJ708369**	**KJ707984**	**KJ708165**	**KJ707887**	**KJ707594**
Kurtzmanomyces	*Kurtzmanomyces insolitus*	JCM 10409^{T}	AF444594	AF177408	**KJ708424**	**KJ707986**	**KJ708175**	**KJ707893**	**KJ707685**
	K. nectairei	AS 2.1950^{T}	AF444494	AF177409	D64122	**KJ707980**	**KJ708176**	**KJ707884**	**KJ707571**
	K. tardus	JCM 10490^{T}	AF444566	AF177410	**KJ708425**	**KJ707992**	**KJ708177**	**KJ707885**	**KJ707686**
sasicola	*Sporobolomyces sasicola*	AS 2.1933^{T}	AF444548	AF177412	AB021688	**KJ707990**	**KJ708335**	**KJ707900**	**KJ707565**
	S. taupoensis	JCM 8770^{T}	AF444592	AF177413	D66886	/	**KJ708339**	**KJ707901**	**KJ707741**

(*continued on next page*)

Table 1. (Continued)

Lineage/Clade	Species	Strain number	ITS	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
	S. xanthus	AS 2.1957^{T}	AF444547	AF177414	D64118	**KJ707993**	**KJ708343**	**KJ707902**	**KJ707573**
lactophilus	*S. lactophilus*	JCM 7595^{T}	AF444545	AF177411	AB021675	/	**KJ708312**	**KJ707889**	**KJ707642**
	S. lophatheri	CBS 11272^{T}	AB126046	AB124561	AB126046	**KJ707988**	**KJ708315**	**KJ707880**	**KJ707608**
	Cystobasidiopsis nirenbergiae	BBA 65452^{T}	GQ180106	FJ536254	/	/	/	/	/
Single-species lineage	*Mycogloea nipponica*	CBS 11308	**KJ778629**	**KJ708456**	**KJ708370**	**KJ707982**	**KJ708194**	**KJ707882**	**KJ707609**
***Incertae sedis* in Agaricostilbales**									
ruber	*Sporobolomyces clavatus*	AS 2.2318^{T}	AY364839	AY364839	**KJ708406**	**KJ707979**	**KJ708295**	**KJ707894**	**KJ707586**
	S. diospyri	JCM 12157^{T}	AB126047	AB124560	AB126047	**KJ707989**	**KJ708298**	**KJ707904**	**KJ707696**
	S. dracophylli	AS 2.1959^{T}	AF444583	AF189982	D66882	**KJ707987**	**KJ708299**	**KJ707879**	**KJ707575**
	S. pyrrosiae	JCM 12159^{T}	AB126045	AB124562	AB126045	**KJ707981**	**KJ708330**	**KJ707903**	**KJ707697**
	S. ruber	AS 2.1958^{T}	AF444550	AF189992	AB021686	**KJ707983**	**KJ708333**	**KJ707899**	**KJ707574**
Single-species lineage	*Bensingtonia sakaguchii*	JCM 10047^{T}	AF444626	AF363646	AB001746	**KJ707958**	**KJ708155**	**KJ707891**	**KJ707671**
Spiculogloeales									
subbrunneus	*Sporobolomyces coprosmicola*	JCM 8767^{T}	AF444576	AF189981	D66879	/	**KJ708171**	**KJ707908**	**KJ707740**
	S. dimmenae	JCM 8762^{T}	AB038046	AB644404	D66881	**KJ707991**	**KJ708297**	**KJ707907**	**KJ707739**
	S. linderae	JCM 8856^{T}	AF444582	AF189989	D66885	/	/	**KJ707906**	**KJ707744**
	S. novozealandicus	JCM 8756^{T}	AB038048	**KJ708467**	**KJ708443**	**KJ708073**	**KJ708319**	**KJ707851**	**KJ707738**
	S. subbrunneus	JCM 5278^{T}	AF444549	AF189997	AB021691	/	/	**KJ707909**	**KJ707710**
Mycogloea	*Mycogloea* sp.	TUBFO40962	/	AY512868	DQ198791	/	/	/	/
Spiculogloea	*Spiculogloea* sp.	TUB RB1040	/	AY512885	/	/	/	/	/
Cystobasidiomycetes									
Cystobasidiales									
minuta	*Cystobasidium fimetarium*	DB1489	/	AY512843	AY124479	/	/	LM644071	/
	Rhodotorula benthica	JCM 10901^{T}	AB026001	AB026001	AB126647	**KJ708081**	**KJ708214**	**KJ707842**	**KJ707691**
	R. calyptogenae	JCM 10899^{T}	AB025996	AB025996	AB126648	**KJ708075**	**KJ708218**	**KJ707840**	**KJ707690**
	R. laryngis	JCM 10953^{T}	AB078500	AB078500	AB126649	**KJ708055**	**KJ708240**	**KJ707824**	**KJ707619**
	R. lysiniphila	JCM 5951^{T}	AB078501	AB078501	AB126650	**KJ708074**	**KJ708243**	**KJ707845**	**KJ707721**
	R. minuta	AS 2.1516^{T}	AF190011	AF189945	D45367	**KJ708059**	**KJ708246**	**KJ707825**	**KJ707562**
	R. pallida	JCM 3780^{T}	AB078492	AF189962	AB126651	**KJ708056**	**KJ708253**	**KJ707826**	**KJ707621**
	R. pinicola	AS 2.2193^{T}	AF444292	AF444293	AB126652	**KJ708057**	**KJ708257**	**KJ707827**	**KJ707579**
	R. slooffiae	JCM 10954^{T}	AF444627	AF444722	AB126653	**KJ708058**	**KJ708266**	**KJ707828**	**KJ707629**
Single-species lineage	*Occultifur externus*	JCM 10725^{T}	AF444567	AF189910	AB055193	**KJ708060**	**KJ708199**	**KJ707829**	**KJ707689**

Table 1. (Continued)

Lineage/Clade	Species	Strain number	ITS	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
Erythrobasidiales									
Erythrobasidium	*Erythrobasidium hasegawianum*	AS 2.1923^T	AF444522	AF189899	D12803	**KF706506**	**KF706534**	**KJ707776**	**KJ707563**
	Sporobolomyces elongatus	AS 2.1949^T	AF444561	AF189983	AB021669	**KJ708012**	**KJ708300**	**KJ707782**	**KJ707570**
	S. yunnanensis	AS 2.2090^T	AB030353	AB127358	AF229176	**KJ708015**	**KJ708344**	**KJ707779**	**KJ707576**
Bannoa	*Bannoa* sp.	MP 3490	DQ631900	DQ631898	DQ631899	/	DQ631901	DQ631902	/
	B. hahajimensis	JCM 10336^T	AB035897	AB082571	AB035897	**KJ708014**	**KJ708146**	**KJ707750**	**KJ707682**
	Sporobolomyces bischofiae	JCM 10338^T	AB035721	AB082572	AB035721	**KJ708018**	**KJ708292**	**KJ707777**	**KJ707684**
	S. ogasawarensis	JCM 10326^T	AB035713	AB082570	AB035713	**KJ708017**	**KJ708323**	**KJ707781**	**KJ707681**
	S. syzygii	JCM 10337^T	AB035720	AB082573	AB035720	**KJ708011**	**KJ708338**	**KJ707778**	**KJ707683**
Single-species clade	*Cyrenella elegans*	CBS 274.82	**KJ778626**	**KJ708454**	**KJ708360**	**KJ708080**	**KJ708168**	**KJ707830**	**KJ707620**
	Rhodotorula lactosa	CBS 5826^T	AF444540	AF189936	D45366	**KJ708016**	**KJ708239**	/	AB040633
Naohideales									
Naohidea	*Naohidea sebacea*	CBS 8477^T	DQ911616	DQ831020	**KP216515**	**KF706508**	**KF706535**	**KF706487**	**KJ707654**
	N. sebacea	CBS122592	/	/	/	**KJ708019**	**KJ708198**	**KJ707783**	**KJ707612**
***Incertae sedis* in Cystobasidiomycetes**									
aurantiaca	*Rhodotorula armeniaca*	JCM 8977^T	AF444523	AF189920	AB126644	**KP216521**	**KJ708211**	**KJ707762**	AB040615
	R. aurantiaca	JCM 3771^T	AF444538	AF189921	**KJ708436**	**KJ707970**	**KJ708212**	**KJ707757**	AB040616
	Sporobolomyces kluyveri-nielii	JCM 6356^T	AF444544	AF189988	AB021674	**KJ707977**	**KJ708310**	**KJ707760**	/
	S. phyllomatis	JCM 7549^T	AF444515	AF189991	AB021685	**KJ707976**	**KJ708328**	**KJ707761**	**KJ707728**
	S. salicinus	JCM 2959^T	AF444511	AF189995	AB021687	/	/	**KJ707758**	**KJ707703**
marina	*Rhodotorula marina*	JCM 3776^T	AF444504	AF189944	AB126645	**KJ707973**	**KJ708244**	**KJ707795**	AB040635
	Sporobolomyces coprosmae	JCM 8772^T	AF444577	AF189980	D66880	**KJ707966**	**KJ708296**	**KJ707798**	**KJ707742**
	S. foliicola	AS 2.2527^T	AF444521	AF189984	AB021671	**KJ707969**	**KJ708302**	**KJ707797**	**KJ707589**
	S. gracilis	JCM 2963^T	AF444578	AF189985	**KJ708433**	**KJ707968**	**KJ708304**	**KJ707799**	**KJ707705**
	S. oryzicola	JCM 5299^T	AF444546	AF189990	AB021677	**KJ707974**	**KJ708324**	**KJ707955**	**KJ707712**
	S. symmetricus	AS 2.2299^T	AY364836	AY364836	**KJ708350**	**KJ707975**	**KJ708337**	**KJ707800**	**KJ707582**
	S. vermiculatus	JCM 10224^T	AB030335	AF460176	AB030322	**KJ707967**	**KJ708342**	**KJ707801**	**KJ707675**
Sakaguchia	*Rhodotorula cladiensis*	CBS 10878^T	FJ008055	FJ008049	**KJ708354**	/	**KJ708219**	**KJ707847**	**KJ707603**
	R. lamellibrachii	CBS 9598^T	AB025999	AB025999	AB126646	**KJ708098**	**KJ708314**	**KJ707876**	**KJ707667**
	R. meli	CBS 10797^T	FJ807683	**KJ708452**	**KJ708355**	**KJ708085**	**KJ708245**	**KJ707855**	**KJ707602**
	R. oryzae	AS 2.2363^T	AY335160	AY335161	**KJ708352**	**KJ708100**	**KJ708250**	**KJ707853**	**KJ707587**
	R. oryzae	AS 2.3289	**KP216523**	**KJ708451**	**KJ708353**	**KJ708103**	**KJ708251**	**KJ707848**	**KJ707592**

(*continued on next page*)

Table 1. (Continued)

Lineage/Clade	Species	Strain number	ITS	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
	Rhodotorula sp.	JCM 8162	**KJ778625**	**KJ708453**	**KJ708356**	**KJ708079**	**KJ708268**	**KJ707858**	**KJ707732**
	Sakaguchia dacryoidea	JCM 3795^{T}	AF444597	AF189972	**D13459**	**KJ708102**	**KJ708348**	**KP216514**	**KJ707709**
	S. dacryoidea	CBS 7999	AF444571	AF444723	**KJ708351**	**KJ708099**	**KJ708346**	**KJ707878**	**KJ707647**
magnisporus	*Rhodotorula bloemfonteinensis*	CBS 8598^{T}	EU075189	EU075187	**KJ708359**	**KJ708082**	**KJ708215**	/	**KJ707657**
	R. orientis	CBS 8594^{T}	HM559719	HM559718	**KJ708358**	**KJ708078**	**KJ708249**	**KJ707843**	**KJ707656**
	R. pini	CBS 10735^{T}	EU075190	EU075188	**KJ708357**	**KJ708084**	**KJ708258**	**KJ707832**	**KJ707601**
	Sporobolomyces magnisporus	JCM 11898^{T}	AB112078	AB111954	**KJ708428**	**KJ708013**	**KJ708317**	**KJ707780**	**KJ707695**
Microbotryomycetes									
Sporidiobolales									
Rhodosporidium	*Rhodosporidium babjevae*	JCM 9279^{T}	AF444542	AF070420	AB073270	/	/	**KJ707874**	**KJ707746**
	R. diobovatum	JCM 3787^{T}	AF444502	AF070421	AB073271	**KJ708091**	**KJ708277**	**KJ707865**	**KJ707708**
	R. kratochvilovae	JCM 8171^{T}	AF444520	AF071436	AB073273	**KJ708095**	**KJ708205**	**KJ707863**	**KJ707733**
	R. paludigenum	JCM 10292^{T}	AF444492	AF070424	**KJ708422**	**KJ708094**	**KJ708206**	**KJ707870**	**KJ707676**
	R. sphaerocarpum	JCM 8202^{T}	AF444499	AF070425	AB073275	**KJ708086**	**KJ708207**	**KJ707867**	**KJ707734**
	R. toruloides	CBS 349	AF444489	AF070426	X60180	**KJ708090**	**KJ708278**	/	**KJ707623**
	R. toruloides	AS 2.1389	**KJ778637**	**KP216510**	**KJ708403**	**KJ708072**	**KJ708265**	**KJ707846**	**KJ707561**
	Rhodotorula araucariae	JCM 3770^{T}	AF444510	AF070427	**KJ708435**	**KJ708096**	**KJ708209**	**KJ707862**	AB041048
	R. dairenensis	CBS 4406^{T}	AF444501	AY033552	**KJ708411**	/	**KJ708276**	**KJ707866**	**KJ707625**
	R. evergladiensis	CBS 10880^{T}	FJ008054	FJ008048	**KJ708398**	/	**KJ708228**	**KJ707834**	/
	R. glutinis	JCM 8208^{T}	AF444539	AF070429	X69853	/	/	**KJ707869**	AB040626
	R. graminis	JCM 3775^{T}	AF444505	AF070431	X83827	**KJ708093**	**KJ708234**	**KJ707868**	AB040628
	R. mucilaginosa	JCM 8115^{T}	AF444541	AF070432	AB021668	/	**KJ708247**	**KJ707861**	**KJ707731**
	R. pacifica	CBS 10070^{T}	AB026006	AB026006	**KJ708397**	**KJ708087**	**KJ708252**	**KJ707860**	**KJ707595**
	R. taiwanensis	CBS 11729^{T}	GU646862	GU646863	**KJ708409**	**KJ708066**	**KJ708271**	**KJ707838**	**KJ707611**
	Sporobolomyces alborubescens	JCM 5352^{T}	AB030342	AF207886	**KJ708440**	**KJ708089**	**KJ708289**	**KJ707864**	**KJ707714**
Mixed *Rhodosporidium*/*Sporidiobolus*	*Rhodosporidium azoricum*	JCM 11251^{T}	AB073229	AF321977	AB073269	**KJ708053**	**KJ708202**	**KJ707813**	**KJ707693**
	R. fluviale	JCM 10311^{T}	AY015432	AF189915	AB073272	**KJ708046**	**KJ708204**	**KJ707816**	**KJ707679**
	R. lusitaniae	JCM 8547^{T}	AY015430	AF070423	AB073274	**KJ708047**	/	**KJ707812**	**KJ707737**
	Rhodotorula colostri	CBS 348^{T}	JN246563	AY372177	**KJ708399**	**KJ708051**	**KJ708220**	**KJ707818**	**KJ707622**
	Sporidiobolus microsporus	JCM 6882^{T}	AF444535	AF070436	**KJ708441**	**KJ708054**	**KJ708284**	**KJ707817**	**KJ707724**
	S. ruineniae	JCM 1839^{T}	AF444491	AF070434	AB021693	**KJ708052**	**KJ708286**	**KJ707820**	**KJ707700**
	Sporobolomyces nylandii	JCM 10213^{T}	AB030323	AF387123	AB030319	**KJ708050**	**KJ708321**	**KJ707822**	**KJ707674**
	S. odoratus	JCM 11641^{T}	**KJ778638**	AF387125	**KJ708427**	**KJ708045**	**KJ708322**	**KJ707819**	**KJ707694**

Table 1. (Continued)

Lineage/Clade	Species	Strain number	ITS	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
	S. poonsookiae	JCM 10207^{T}	AB030327	AF387124	AB030320	**KJ708048**	**KJ708329**	**KJ707821**	**KJ707672**
Sporidiobolus	*Sporidiobolus johnsonii*	AS 2.1927^{T}	AY015431	AF070435	L22261	**KJ708105**	/	**KJ707914**	**KJ707564**
	S. longiusculus	CBS 9655^{T}	JN246566	KJ708464	**KJ708400**	**KJ708109**	**KJ708282**	**KJ707929**	**KJ707668**
	S. metaroseus	CBS 7683^{T}	EU003482	EU003461	**KJ708415**	**KJ708068**	**KJ708283**	**KJ707841**	**KJ707644**
	S. pararoseus	JCM 5350^{T}	AF417115	AF070437	AB021694	**KJ708115**	**KJ708279**	**KJ707924**	**KJ707713**
	S. salmonicolor	JCM 1841^{T}	AY015434	AF070439	AB021697	**KJ708114**	**KJ708287**	**KJ707923**	**KJ707701**
	Sporobolomyces bannaensis	AS 2.2285^{T}	AY274824	AY274823	**KJ708405**	**KJ708120**	**KJ708290**	**KJ707934**	**KJ707581**
	S. beijingensis	AS 2.2365^{T}	AY364837	AY364837	**KJ708407**	**KJ708116**	**KJ708291**	**KJ707919**	**KJ707588**
	S. blumeae	JCM 10212^{T}	AB030331	AY213010	AB030321	/	**KJ708293**	**KJ707926**	**KJ707673**
	S. carnicolor	JCM 3766^{T}	AY069991	AY070008	**KJ708434**	**KJ708117**	**KJ708294**	**KJ707912**	**KJ707707**
	S. holsaticus	CBS 1522	AF444509	AF189975	AB021672	**KJ708106**	/	**KJ707916**	**KJ707614**
	S. japonicus	AS 2.2192^{T}	AY069992	AY158640	/	**KJ708123**	**KJ708307**	**KJ707932**	**KJ707578**
	S. jilinensis	AS 2.2301^{T}	AY364838	AY364838	**KJ708450**	**KJ708111**	**KJ708308**	**KJ707913**	**KJ707583**
	S. koalae	CBS 10914^{T}	EU276008	EU276011	**KP216519**	**KJ708063**	**KJ708311**	**KJ707850**	**KJ707604**
	S. marcillae	JCM 6883^{T}	AY015437	AF070440	**KJ708442**	**KJ708112**	**KJ708318**	**KJ707933**	**KJ707725**
	S. patagonicus	CBS 9658	AY552329	AY158656	**KP216518**	**KJ708108**	**KJ708326**	**KJ707930**	**KJ707669**
	S. patagonicus	CBS 9657^{T}	AY552328	AY158655	**KJ708421**	**KJ708110**	**KJ708325**	**KJ707928**	**KP216520**
	S. phaffii	AS 2.2137^{T}	AY069995	AY070011	**KJ708404**	**KJ708113**	**KJ708327**	**KJ707918**	**KJ707577**
	S. roseus	AS 2.1948^{T}	AY015438	AF070441	X60181	**KJ708119**	**KJ708331**	**KJ707917**	**KJ707569**
	S. ruberrimus	CBS 7550^{T}	AY015439	AF070442	**KJ708402**	**KJ708121**	**KJ708332**	**KJ707915**	**KJ707643**
	S. salmoneus	AS 2.2195^{T}	AY070005	AY070017	**KJ708401**	**KJ708107**	**KJ708334**	**KJ707920**	**KJ707580**
Kriegeriales									
Kriegeriaceae									
Kriegeria	*Kriegeria eriophori*	CBS 8387^{T}	AF444602	NR_119455	DQ419918	**KJ708144**	**KJ708174**	**KJ707936**	**KJ707649**
glacialis	*Rhodotorula glacialis*	CBS 10436^{T}	EF151249	EF151258	**KJ708381**	**KJ708067**	**KJ708233**	**KJ707831**	**KJ707597**
	R. psychrophenolica	CBS 10438^{T}	EF151246	EF151255	**KJ708382**	**KJ708071**	**KJ708259**	**KJ707859**	**KJ707598**
	R. psychrophila	CBS 10440^{T}	EF151243	EF151252	**KJ708383**	/	**KJ708260**	**KJ707833**	**KJ707599**
Single-species lineage	*Meredithblackwellia eburnea*	CBS12589	JX508799	JX508798	JX508797	/	/	/	/
	Rhodotorula rosulata	CBS 10977^{T}	EU872492	EU872490	**KJ708384**	**KJ708083**	**KJ708263**	**KJ707854**	**KJ707607**
Camptobasidiaceae									
Glaciozyma	*Glaciozyma antarctica*	JCM 9057^{T}	AF444529	AF189906	DQ785788	**KJ708131**	**KJ708182**	/	**KJ707745**

(*continued on next page*)

Table 1. (Continued)

Lineage/Clade	Species	Strain number	ITS	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
Leucosporidiales									
Leucosporidium	*Leucosporidium creatinivorum*	JCM 10699	KJ778627	KJ708455	**KJ708385**	**KJ708064**	**KJ708221**	**KJ707857**	**KJ707687**
	L. creatinivorum	CBS 8620^T	AF444629	AF189925	**KJ708418**	**KJ708036**	**KJ708178**	**KJ707789**	**KJ707658**
	L. fellii	JCM 9887^T	AF444508	AF189907	**KJ708449**	**KJ708030**	**KJ708184**	**KJ707784**	**KJ707748**
	L. fragarium	JCM 3930	AF444530	AF070428	**KJ708437**	**KJ708034**	**KJ708231**	**KJ707790**	AB040623
	L. fragarium	CBS 6254^T	AF444530	AF070428	**KJ708413**	**KJ708031**	**KJ708179**	**KJ707791**	AB040623
	L. golubevii	CBS 9651^T	AY212987	AY212999	**KJ708386**	**KJ708037**	**KJ708185**	**KJ707787**	/
	L. intermedium	JCM 5291^T	AF444630	AF189889	D38235	**KJ708132**	**KJ708188**	**KJ707785**	**KJ707711**
	L. muscorum	CBS 6921^T	AF444527	AF070433	**KJ708414**	**KJ708038**	**KJ708180**	**KJ707793**	AB040638
	L. scottii	JCM 9052^T	AF444495	AF070419	X53499	**KJ708033**	**KJ708186**	**KJ707788**	AB040658
	L. yakuticum	JCM 10701	AY212989	AF189971	**KJ708426**	**KJ708032**	**KJ708274**	**KJ707794**	**KJ707688**
	L. yakuticum	CBS 8621^T	AY212989	AY213001	**KJ708419**	/	**KJ708181**	/	**KJ707659**
Microbotryales									
Microbotryum	*Microbotryum reticulatum*	CBS 101451	**KJ778630**	**KJ708457**	**KJ708389**	**KJ708040**	**KJ708189**	**KJ707806**	**KJ707596**
	M. scabiosae	CBS 677.93	**KJ708459**	**KJ708459**	**KJ708390**	/	**KJ708195**	**KJ707808**	**KJ707633**
	M. scabiosae	CBS 176.24	**KJ708458**	**KJ708458**	**KJ708391**	**KJ708039**	**KJ708190**	**KJ707810**	**KJ707615**
	M. scorzonerae	CBS 685.93	**KJ708461**	**KJ708461**	**KJ708392**	/	**KJ708191**	**KJ707804**	**KJ707635**
	M. scorzonerae	CBS 364.33	**KJ708460**	**KJ708460**	**KJ708393**	**KJ708043**	**KJ708196**	**KJ707805**	**KJ707624**
	M. violaceum	CBS 143.21	**KJ708462**	**KJ708462**	**KJ708388**	**KJ708042**	**KJ708192**	**KJ707811**	**KJ707613**
	Sphacelotheca hydropiperis	CBS 179.24	**KJ708463**	**KJ708463**	**KJ708394**	**KJ708041**	**KJ708281**	**KJ707807**	**KJ707616**
	S. koordersiana	JAG 55	DQ832221	DQ832219	DQ832220	DQ832223	DQ832222	DQ832224	/
Single-species lineage	*Rhodotorula hordea*	JCM 3932^T	AF444524	AF189933	**AY657013**	/	**KJ708235**	**KJ707802**	/
Heterogastridiales									
Heterogastridium	*Heterogastridium pycnidioideum*	CBS 591.93	GU291276	GU291290	**KJ708412**	**KJ708009**	**KJ708170**	**KJ707770**	**KJ707630**
***Incertae sedis* in Microbotryomycetes**									
buffonii	*Rhodotorula bogoriensis*	JCM 1692^T	AF444536	AF189923	**KJ708363**	**KJ708130**	**KJ708216**	**KJ707949**	AB040619
	R. buffonii	JCM 3929^T	AF444526	AF189924	**KJ708362**	**KJ708127**	**KJ708217**	**KJ707946**	AB040620
	R. pustula	JCM 3934^T	AF444531	AF189964	**KJ708361**	**KJ708128**	**KJ708261**	**KJ707937**	AB040642
tsugae	*R. cresolica*	JCM 10955^T	AF444570	AF189926	**KJ708365**	**KJ708135**	**KJ708222**	**KJ707942**	/
	R. pilati	JCM 9036^T	AF444598	AF189963	**KJ708364**	**KJ708137**	**KJ708256**	**KJ707947**	**AB040641**
	Sporobolomyces tsugae	JCM 2960^T	AF444580	AF189998	AB021692	/	**KJ708340**	**KJ707945**	**KJ707628**
yarrowii	*Rhodotorula silvestris*	CBS 11420^T	GQ121045	GQ121044	**KJ708366**	**KJ708069**	**KJ708264**	**KJ707849**	**KJ707610**
	R. straminea	CBS 10976^T	EU872491	EU872489	**KJ708367**	**KJ708065**	**KJ708269**	**KJ707844**	**KJ707606**

Table 1. (Continued)

Lineage/Clade	Species	Strain number	ITS	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
	R. yarrowii	JCM 8232^{T}	AF444628	AF189971	AB032658	/	**KJ708275**	**KJ707938**	**KJ707735**
griseoflavus	*Sporobolomyces fushanensis*	JCM 12422^{T}	**KP216522**	AB176591	AB176530	**KJ708142**	**KJ708303**	**KJ707944**	**KJ707698**
	S. griseoflavus	JCM 5653^{T}	AF444557	AF189986	D66884	**KJ708143**	**KJ708305**	**KJ707950**	**KJ707717**
yamatoana	*Bensingtonia yamatoana*	AS 2.1956^{T}	AF444634	AF189896	D38239	**KJ708141**	**KJ708160**	**KJ707948**	**KJ707572**
	Rhodotorula arctica	CBS 9278	AB478857	AB478858	**KJ708371**	**KJ708070**	**KJ708210**	**KJ707856**	**KJ707666**
singularis	*R. lignophila*	CBS 7109^{T}	AF444513	AF189943	**KJ708372**	**KJ708139**	**KJ708241**	**KJ707953**	**KJ707637**
	Sporobolomyces singularis	JCM 5356^{T}	AF444600	AF189996	AB021690	**KJ708140**	**KJ708336**	**KJ707957**	**KJ707716**
Colacogloea	*Colacogloea peniophorae*	CBS 684.93	DQ202270	AY629313	DQ234565	DQ234569	DQ234550	DQ234566	/
	Rhodotorula cycloclastica	CBS 8448^{T}	AF444732	AF444631	**KJ708376**	**KJ707997**	**KJ708224**	**KJ707775**	**KJ707652**
	R. diffluens	JCM 1695^{T}	AF444533	AF075485	**KJ708380**	**KJ708125**	**KJ708226**	**KJ707939**	AB040621
	R. eucalyptica	CBS 8499^{T}	EU075185	EU075183	**KJ708377**	**KJ708061**	**KJ708227**	**KJ707839**	**KJ707655**
	R. foliorum	JCM 1696^{T}	AF444633	AF317804	**KJ708378**	**KJ708126**	**KJ708230**	**KJ707941**	AB040622
	R. philyla	JCM 3933^{T}	AF444506	AF075471	**KJ708438**	**KJ707995**	**KJ708254**	**KJ707772**	**KJ707631**
	R. retinophila	CBS 8446^{T}	AF444624	AF444730	**KJ708373**	**KJ707994**	**KJ708262**	**KJ707771**	**KJ707651**
	R. terpenoidalis	CBS 8445^{T}	AF444623	AF444729	**KJ708374**	**KJ707999**	**KJ708272**	**KJ707774**	**KJ707650**
	Sporobolomyces falcatus	JCM 6838^{T}	AF444543	AF075490	AB021670	**KJ708124**	**KJ708301**	**KJ707943**	**KJ707723**
vanillica	*Rhodotorula ingeniosa*	JCM 9031^{T}	AF444534	AF189934	**KJ708445**	**KJ708004**	**KJ708237**	**KJ707803**	AB040631
	R. vanillica	JCM 9741^{T}	AF444575	AF189970	**KJ708448**	**KJ708005**	**KJ708273**	**KJ707809**	**KJ707747**
sonckii	*R. auriculariae*	JCM 1597^{T}	AF444507	AF189922	**KJ708429**	**KJ708134**	**KJ708213**	**KJ707935**	AB040617
	R. sonckii	JCM 3935^{T}	AF444601	AF189969	**KJ708439**	**KJ708118**	**KJ708267**	**KJ707911**	AB040643
Curvibasidium	*Curvibasidium cygneicollum*	JCM 10310^{T}	AF444490	AF189928	**KJ708423**	**KJ708001**	**KJ708169**	**KJ707768**	**KJ707678**
	C. cygneicollum	JCM 9029^{T}	AB038090	**KP216511**	**KJ708444**	**KJ708062**	**KJ708232**	**KJ707836**	AB040625
	C. pallidicorallinum	CBS 9091^{T}	AF444641	AF444736	**KJ708420**	**KJ708000**	**KJ708167**	**KJ707767**	**KJ707665**
	Rhodotorula nothofagi	JCM 9034	AF444537	AF189950	**KJ708447**	**KJ708002**	**KJ708248**	**KJ707765**	AB040639
Reniforma	*Reniforma strues*	CBS 8263^{T}	AF444573	AF189912	**KP216517**	**KJ708122**	**KJ708200**	**KJ707927**	**KJ707648**
Single-species lineage	*Pseudoleucosporidium fasciculatum*	CBS 8786^{T}	KJ778628	AY212993	**KJ708387**	**KJ707998**	**KJ708183**	**KJ707769**	/
	Rhodotorula crocea	CBS 2029^{T}	FM957565	AY372179	**KJ708410**	**KJ708007**	**KJ708223**	**KP216513**	**KJ707618**
	R. ferulica	JCM 8231^{T}	AF444528	AF363653	**KJ708379**	**KJ708129**	**KJ708229**	**KJ707940**	/
	R. hylophila	JCM 1805^{T}	AF444622	AF363645	**KJ708431**	**KJ708008**	**KJ708236**	**KJ707764**	AB040630
	R. javanica	JCM 9032^{T}	AF444532	AF189935	**KJ708446**	**KJ708006**	**KJ708238**	**KJ707766**	AB040632
	Sporobolomyces inositophilus	JCM 5654^{T}	AF444559	AF189987	AB021673	**KJ708136**	**KJ708306**	**KJ707951**	**KJ707718**

(*continued on next page*)

Table 1. (Continued)

Lineage/Clade	Species	Strain number	ITS	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
Mixiomycetes									
Mixia	*Mixia osmundae*	CBS 9802	DQ831010	DQ831009	D14163	**KJ708076**	**KJ708193**	**KJ707837**	**KJ707670**
Tritirachiomycetes									
	Tritirachium oryzae	CBS 164.67	GQ329853	KF258732	JF779647	/	JF779648	JF779645	/
	Tritirachium sp.	CBS 473.93	JF779664	JF779649	JF779650	/	JF779646	JF779651	/
	Tritirachium sp.	CBS 265.96	JF779668	JF779652	JF779653	/	JF779654	/	/
Pucciniomycetes									
	Chrysomyxa arctostaphyli	CFB22246	DQ200930	AY700192	AY657009	/	DQ408138	DQ435789	/
	Endocronartium harknessii	CFB22250	DQ206982	AY700193	AY665785	/	DQ234551	DQ234567	/
	Helicobasidium mompa	CBS 278.51	AY292429	AY254179	U77064	/	/	EF100614	/
	Insolibasidium deformans	TDB183-1	/	AF522169	AY123292	/	/	/	/
	Platygloea disciformis	IFO32431	DQ234556	AY629314	DQ234563	/	DQ234554	DQ056288	/
	Puccinia graminis tritici	CRL75-36-700-3/ECS	AF468044	AF522177	AY125409	XM_003334476	XM_003321826	XM_003333024	/
	Septobasidium canescens	DUKE:DAH(323)	DQ241446	DQ241479	DQ241410	/	/	/	/
Atractiellomycetes									
	Helicogloea lagerheimii	FO 36341	AY512849	AY124476	/	/	/	/	/
	H. variabilis	KW 1540	L20282	U78043	/	/	/	/	/
	Platygloea vestita	DB 1280	AY512872	AY124480	/	/	/	/	/
Classiculomycetes									
	Classicula fluitans	ATCC 64713	AY512838	AY124478	/	/	/	/	/
	Jaculispora submersa	CCM 8127	AY512853	AY124477	/	/	/	/	/
Ustilaginomycotina									
	Rhodotorula phylloplana	JCM 9035^T	AB038131	AF190004	AJ496258	**KP322906**	**KP323063**	**KP323116**	AB041051
	Ustilago maydis	CBS 504.76/IFM 49220	AF453938	AY854090	X62396	XM401478	AY485636	AY885160	AB040663

Table 2. PCR and sequence primers used.

Locus	Primers (5′–3′)
RPB1	RPB1-Af: GAR TGY CCD GGD CAY TTY GG RPB1-Cr: CCN GCD ATN TCR TTR TCC ATR TA
RPB2	fRPB2-5F: GAY GAY MGW GAT CAY TTY GG fRPB2-7cR: CCC ATR GCT TGY TTR CCC AT bRPB2-6F: TGG GGY ATG GTN TGY CCY GC gRPB2-6R: GCA GGR CAR ACC AWM CCC CA
TEF1	EF1-983F: GCY CCY GGH CAY CGT GAY TTY AT EF1-2218R: AT GAC ACC RAC RGC RAC RGT YTG EF1-1577F: CAR GAY GN TAC AAG ATY GGT GG EF1-1567R: AC HGT RCC RAT ACC ACC RAT CTT
CYTB	E1M4: TGR GGW GCW ACW GTT ATT ACT A E2mr3: GGW ATA GCA CGT ARA AYW GCR TA
18S rDNA	P1 F: ATC TGG TTG ATC CTG CCA GT 570 F: CGC GGT AAT TCC AGC TCC A 934 F: CTG CGA AAG CAT TTG CCA AGG 1272 F: ATG GCC GTT CTT AGT TGG T U1 R: TGG AAT TAC CGC GGC TGC TGG CAC C U2 R: CCG TCA ATT CCT TTA AGT TTC AGC C U3 R: GAC GGG CGG TGT GTA CAA AGG GCA G
ITS + 5.8S rDNA	ITS1: GTC GTA ACA AGG TTT CCG TAG GTG ITS4: TCC TCC GCT TAT TGA TAT GC
D1D2 of 26S rDNA	NL1: GCA TATC AAT AAG CGG AGG AAA AG NL4: GG TCC GTG TTT CAA GAC GG

sequences of filamentous taxa in the *Pucciniomycotina*. Each of the data sets was subjected to ML, MP and BI analyses. The trees obtained were visually compared to inspect the phylogenetic concordance among the taxa analysed, based on which monophyletic lineages and clades were recognised and defined (Table 3). As expected, among the trees drawn from different data sets analysed, the seven genes-based trees exhibited the highest resolution with strongest support values (Table 3). The backbones of the trees shown here were obtained from ML analysis. The seven genes-based ML tree was used as the primary basis for lineage and clade recognition and definition, and as the starting point for the subsequent comparison and discussion.

Major lineages

The higher-level phylogenetic classification of the *Pucciniomycotina* proposed in Aime *et al.* (2006) and Bauer *et al.* (2006) mainly based on SSU and LSU rDNA sequence analyses was adopted in Hibbett *et al.* (2007) and Boekhout *et al.* (2011). They distinguished eight classes, namely *Agaricostilbomycetes*, *Atractiellomycetes*, *Classiculomycetes*, *Cystobasidiomycetes*, *Cryptomycocolacomycetes*, *Microbotryomycetes*, *Mixiomycetes* and *Pucciniomycetes*. Schell *et al.* (2011) proposed a new class *Tritirachiomycetes* in this subphylum based on multiple gene analyses and septal pore ultrastructure to accommodate the anamorphic genus *Tritirachium* that was once classified in the *Pezizomycotina* (*Ascomycota*). This affiliation was recently confirmed by Manohar *et al.* (2014) and Aime *et al.* (2014).

In agreement with Boekhout *et al.* (2011) our phylogenetic analyses based on the seven-gene dataset showed that the majority of the yeast species employed belonged to four major lineages corresponding to *Agaricostilbomycetes*, *Cystobasidiomycetes*, *Microbotryomycetes* and *Mixiomycetes* (Fig. 1). The phylogenetic analyses of the three rDNA genes and four protein coding genes (Figs 2, 3) showed a similar result to that obtained from the analysis of the seven-gene dataset. However, the position of the *Spiculogloeales* varied. In the seven genes-based tree this order showed a close relationship to the *Mixiomycetes* with 94–99 % BP and 1.0 PP support values

Table 3. Statistical supports to the monophyletic clades with multiple strains resolved in pucciniomycetous yeasts based on different data sets using different algorithms.

Lineage/Clade	Seven genes	rDNA	Protein genes	SSU + D1D2
	BP^1/BP^2/PP	BP^1/BP^2/PP	BP^1/BP^2/PP	BP^1/BP^2/PP
Agaricostilbomycetes	nm/nm/nm	86/59/1.0	nm/nm/nm	88/89/1.0
Agaricostilbales	88/100/1.0	100/100/100	100/100/1.0	99/100/1.0
Kondoa	100/100/1.0	100/100/1.0	100/100/1.0	99/100/1.0
Bensingtonia	100/100/1.0	100/100/1.0	98/88/1.0	99/99/1.0
ingoldii	100/100/1.0	100/99/1.0	99/86/1.0	98/90/1.0
Agaricostilbum	100/100/1.0	99/100/1.0	100/100/1.0	71/66/ns
Chionosphaera	100/100/1.0	100/100/1.0	100/96/1.0	53/98/1.0
Kurtzmanomyces	100/98/1.0	59/ns/1.0	99/95/1.0	63/97/1.0
sasicola	100/100/1.0	100/100/1.0	100/100/1.0	99/100/1.0
lactophilus	88/100/1.0	100/100/1.0	100/100/1.0	99/100/1.0
ruber	100/100/1.0	100/100/1.0	100/100/1.0	99/99/1.0
Spiculogloeales	100/100/1.0	100/100/1.0	100/100/1.0	99/100/1.0
subbrunneus	100/100/1.0	100/100/1.0	100/100/1.0	100/100/1.0
Cystobasidiomycetes	100/98/1.0	100/100/1.0	99/100/1.0	99/100/1.0
Cystobasidiales	100/100/1.0	100/100/1.0	100/100/1.0	99/100/1.0
minuta (*Cystobasidium*)	98/98/1.0	71/83/1.0	95/91/1.0	75/79/1.0
Erythrobasidiales	100/nm/1.0	nm/nm/1.0	95/nm/1.0	nm/nm/nm

(*continued on next page*)

Table 3. (Continued).

Lineage/Clade	**Seven genes**	**rDNA**	**Protein genes**	**SSU + D1D2**
	BP^1/BP^2/PP	**BP^1/BP^2/PP**	**BP^1/BP^2/PP**	**BP^1/BP^2/PP**
Erythrobasidium	100/nm/1.0	97/86/1.0	nm/nm/nm	93/91/1.0
Bannoa	100/100/1.0	100/100/1.0	100/100/1.0	99/100/1.0
Incertae sedis				
aurantiaca	100/100/1.0	100/100/1.0	100/100/1.0	99/100/1.0
marina	100/99/1.0	100/100/1.0	100/78/1.0	99/93/1.0
Sakaguchia	100/100/1.0	81/56/1.0	99/100/1.0	84/85/1.0
magnisporus	100/97/1.0	97/78/1.0	91/76/1.0	98/96/1.0
Microbotryomycetes	100/100/1.0	100/100/1.0	99/59/1.0	99/100/1.0
Sporidiobolales	88/100/1.0	99/100/1.0	82/100/1.0	91/93/1.0
Rhodosporidium	100/100/1.0	100/100/1.0	88/nm/ns	94/97/1.0
Mixed *Rhodosporidium*/*Sporidiobolus*	88/89/1.0	100/100/1.0	82/nm/1.0	98/98/1.0
Sporidiobolus	100/100/1.0	100/100/1.0	74/nm/1.0	98/100/1.0
Kriegeriales	ns/nm/nm	nm/nm/nm	nm/nm/nm	nm/nm/nm
glacialis	99/100/1.0	86/92/1.0	100/93/ns	52/ns/1.0
Leucosporidiales	95/99/1.0	98/97/1.0	91/96/1.0	74/70/1.0
Leucosporidium	95/99/1.0	98/97/1.0	91/96/1.0	74/70/1.0
Microbotryales	81/nm/1.0	100/100/1.0	nm/nm/nm	66/74/ns
Microbotryum	100/100/1.0	100/100/1.0	100/100/1.0	ns/75/1.0
Incertae sedis				
buffonii	100/100/1.0	100/100/1.0	100/100/1.0	99/100/1.0
tsugae	82/93/nm	nm/nm/nm	93/94/1.0	nm/nm/nm
yarrowii	100/100/1.0	100/100/1.0	100/100/1.0	99/99/1.0
griseoflavus	100/100/1.0	100/100/1.0	100/100/1.0	99/99/1.0
yamatoana	100/100/1.0	100/100/1.0	100/100/1.0	93/98/1.0
singularis	100/100/1.0	100/100/1.0	100/100/1.0	99/100/1.0
Colacogloea	99/89/1.0	67/86/ns	72/65/1.0	nm/nm/nm
vanillica	100/100/1.0	100/100/1.0	100/100/1.0	99/100/1.0
sonckii	100/100/1.0	100/100/1.0	100/100/1.0	99/100/1.0
Curvibasidium	100/100/1.0	100/100/1.0	100/100/1.0	99/100/1.0

Note. BP^1 and BP^2, bootstrap values from the maximum likelihood and maximum parsimony analyses, respectively; PP, Bayesian posterior probability; nm: not monophyletic; ns, not supported.

(Fig. 4). The *Mixiomycetes* contains only one species *Mixia osmundae*, which is a fern parasite occurring on *Osmunda* ferns (Nishida *et al.* 1995, 2011). The close affinity of the *Spiculogloeales* with *Mixia osmundae* was also revealed and strongly supported in the trees drawn from the four protein-coding genes (Fig. 3). However, in the trees constructed from the three rDNA regions, the *Spiculogloeales* formed a lineage basal to *Agaricostilbomycetes* with 59–91 % BP and 1.0 PP support values, while *Mixia osmundae* was located as a branch basal to the *Microbotryomycetes* lineage with 1.0 Bayesian PP support (Fig. 2).

The phylogenetic relationships between the yeast species and the filamentous fungal lineages recognised within *Pucciniomycotina* so far (Aime *et al.* 2006, 2014, Bauer *et al.* 2006, Boekhout *et al.* 2011, Schell *et al.* 2011) are shown in the tree constructed from the SSU and LSU rDNA D1/D2 domains sequences (Fig. 5). The yeast lineages mentioned above and the filamentous lineages, *Atractiellomycetes*, *Classiculomycetes*, *Pucciniomycetes* and *Tritirachiomycetes*, were separated as independent lineages. *Microbotryomycetes* exhibited a close relationship to the filamentous fungal lineage *Classiculomycetes* with moderate BP (56–79 %) and strong PP (1.0) support, being in agreement with Aime *et al.* (2006, 2014) and Bauer *et al.* (2006). However, the phylogenetic relationships among the remaining lineages were not confidently resolved. The *Spiculogloeales* was located as a deep lineage basal to the *Agaricostilbomycetes* with 88–89 % BP and 1.0 PP support (Fig. 5), being similar to the result shown in the tree based on the three rDNA regions (Fig. 2). This result suggests that the *Spiculogloeales* may represent a distinct class, supporting Bauer *et al.* (2006) and Aime *et al.* (2014) who indicated that the *Agaricostilbomycetes* might not be monophyletic and need to be separated into two classes because of the weakly supported monophyly of the class obtained from SSU rDNA sequence analysis.

Agaricostilbomycetes

The two orders *Agaricostilbales* and *Spiculogloeales* in this class (Aime *et al.* 2006, 2014, Bauer *et al.* 2006, Hibbett *et al.* 2007, Boekhout *et al.* 2011) were resolved with strong statistical support values in all the trees drawn from different data sets using different algorithms (Table 3, Figs 2–5). However, as shown above, the *Spiculogloeales* formed a sister lineage to *Mixiomycetes*, rather than to the *Agaricostilbales* in the trees drawn from the seven genes and the four protein-coding genes (Figs 3, 4). The order *Spiculogloeales* was proposed by Bauer *et al.* (2006) for a well-supported clade formed by two unidentified teleomorphic species, *Spiculogloea sp.* RB 1040 and *Mycogloea sp.* FO 40962, resulted from phylogenetic analyses of the joint SSU/LSU data set. *Sporobolomyces* (*pro parte*) was included in this order due to the fact that *Sporobolomyces coprosmicola* showed a close relationship with *Spiculogloea sp.* RB 1040 in the tree from the LSU rDNA sequences (Bauer *et al.* 2006). In the *Spiculogloeales* lineage recognised from the seven-gene dataset obtained in this study, five anamorphic species of the genus *Sporobolomyces*, namely *S. linderae*, *S. coprosmicola*, *S. subbrunneus*, *S. dimmenae* and *S. novazealandicus*, formed the **subbrunneus** clade which was resolved and strongly supported in all the trees constructed in this study (Figs 2–5). The SSU and LSU rDNA D1/D2 tree showed that this clade was closely related with *Spiculogloea sp.* RB 1040and *Mycogloea sp.* FO 40962 formed a branch basal to *Spiculogloea sp.* RB 1040 and the **subbrunneus** clade with strong BP and PP support (Fig. 5). The species of *Mycogloea* shared some phenotypic characters with those of *Spiculogloea*, including the presence of dimorphism, mycoparasitism and presence of tremelloid haustorial cells subtended by clamp connections (Bandoni 1998). However, previous molecular analyses (Aime *et al.* 2006, 2014, Bauer *et al.* 2006) and this study (Fig. 5) indicated that *Mycogloea* does not appear monophyletic. The genus *Spiculogloea* contains four described species with *S. occulta* as the type (Roberts 1996, 1997, Hauerslev 1999, Trichiès 2006). However, molecular data are not available from any of them at present. Additional molecular analyses on a better taxonomic sampling including the type species are needed to resolve the phylogenetic placements of *Mycogloea* and *Spiculogloea* species.

In the *Agaricostilbales* lineage, nine well-supported clades with yeasts species occurred, namely ***Agaricostilbum***, ***Bensingtonia***, ***Chionosphaera***, ***Kondoa***, ***Kurtzmanomyces***, **ingoldii**, **lactophilus**, **ruber** and **sasicola**. In addition, *Bensingtonia sakaguchii* and a filamentous species, *Mycogloea nipponica* that has a yeast stage, were each recognised to represent a clade (Table 3, Figs 2–4).

The ***Agaricostilbum*** clade contained two teleomorphic *Agaricostilbum* species and two anamorphic *Sterigmatomyces* species. The type species of both genera were included in this clade. *Agaricostilbum* species form synnemata-like basidiomata and have a stable yeast state with buds usually produced on short denticles (Wright 1970, Wright *et al.* 1981, Bandoni & Boekhout 2011). The *Sterigmatomyces* species produce conidia on stalks and appear to lack a filamentous stage (Fell 1966, 2011a). Species of *Agaricostilbum* and *Sterigmatomyces* occurred together in trees drawn from the LSU rDNA D1/D2 domains (Fell *et al.* 2000b), ITS (Scorzetti *et al.* 2002) and from all data sets generated in this study (Figs 2–5), suggesting that they represent a robust single clade.

The two *Bensingtonia* species, *B. musae* and *B. ingoldii*, which were assigned to the ***Agaricostilbum*** clade in Scorzetti *et al.* (2002) and to the *Agaricostilbaceae* in Bauer *et al.* (2006) and Boekhout *et al.* (2011), formed the **ingoldii** clade distinct from, but closely related to the ***Agaricostilbum*** clade with strong support values in all the trees obtained in this study (Figs 2–5). These two *Bensingtonia* species form ballistoconidia but do not form conidiogenous stalks (Nakase *et al.* 1989, 2011, Takashima *et al.* 1995), thus being different from the *Agaricostilbum* and *Sterigmatomyces* species. Therefore, the two *Bensingtonia* species are assigned in a separate clade in this study.

The ***Kondoa*** clade accommodated two *Kondoa* species including the type species of this genus, *K. malvinella*, and seven anamorphic species of the genus *Bensingtonia* (Table 1, Fig. 4). The ***Bensingtonia*** clade contained *B. ciliata*, the type species of the genus, and two other species *B. naganoensis* and *B. pseudonaganoensis*. Each of the ***Kondoa*** and the ***Bensingtonia*** clades received strong support values in all the trees obtained from different data sets (Table 3, Figs 2–5). The ***Bensingtonia*** clade was assigned to the *Agaricostilbaceae* in Bauer *et al.* (2006) and Boekhout *et al.* (2011) based on LSU rDNA sequence analyses. However, Wang *et al.* (2012) indicated that this clade was closely related to the ***Kondoa*** clade. The close relationship of this clade with the ***Kondoa*** clade was strongly supported in the trees constructed from different data sets in this study (Figs 3–5), suggesting that the ***Bensingtonia*** clade should be assigned to the *Kondoaceae*.

From the species included in the *Chionosphaeraceae* in Bauer *et al.* (2006, 2009) and Boekhout *et al.* (2011), five distinct clades and two single species lineages were distinguished (Table 3, Figs 2–5). The three anamorphic *Kurtzmanomyces* species including the type species of this genus formed a distinct clade closely related to the teleomorphic species *Mycogloea nipponica* that forms auricularioid basidia (Bandoni 1998). Though the latter has a *Kurtzmanomyces*-like state, the connection between *Kurtzmanomyces* and *M. nipponica* needs to be addressed further as discussed in Sampaio (2011b). The original description of *M. nipponica* based on a Japanese collection did not include a living culture (Bandoni 1998). The culture from which molecular data were obtained was isolated from a collection made in Taiwan (Kirschner *et al.* 2003). It is not clear whether the *Kurtzmanomyces* species have a sexual *Mycogloea*-like stage and if the remaining five *Mycogloea* species (Bandoni 1998) have a *Kurtzmanomyces*-like yeast stage. The present and previous (Aime *et al.* 2006, 2014, Bauer *et al.* 2009) studies indicate that the genus *Mycogloea* is polyphyletic and species of this genus occur in the *Agaricostibales* and *Spiculogloeales*. Thus, at present, we consider it better to treat *M. nipponica* as representing a clade separated from the ***Kurtzmanomyces*** clade. The two teleomorphic *Chionosphaera* species including the generic type *Ch. apobasidialis* formed an independent clade with a close affinity to the ***Kurtzmanomyces*** clade and *M. nipponica* (Fig. 4). The genus *Chionosphaera* is characterised by holobasidia that are different from the gasteroid basidia of *Mycogloea nipponica* (Bandoni 1998, Kwon-Chung 2011).

The ten *Sporobolomyces* species in the family *Chionosphaeraceae* employed in this study were separated into three different clades, namely the **sasicola** clade with three species, the **lactophilus** clade with two species, and the **ruber** clade with five species (Table 1, Fig. 4). The **lactophilus** and **sasicola** clades showed a close relationship in all the trees obtained (Figs 2–5). The **sasicola** clade recognised in Scorzetti *et al.* (2002) based on LSU rDNA D1/D2 sequence analysis included *Sporobolomyces lactophilus*, however, the inclusion of this species in the **sasicola** clade was not supported in the ITS tree (Scorzetti *et al.* 2002). The close relationship of the three species in the **sacicola** clade and the two species in the **lactophilus** clade was

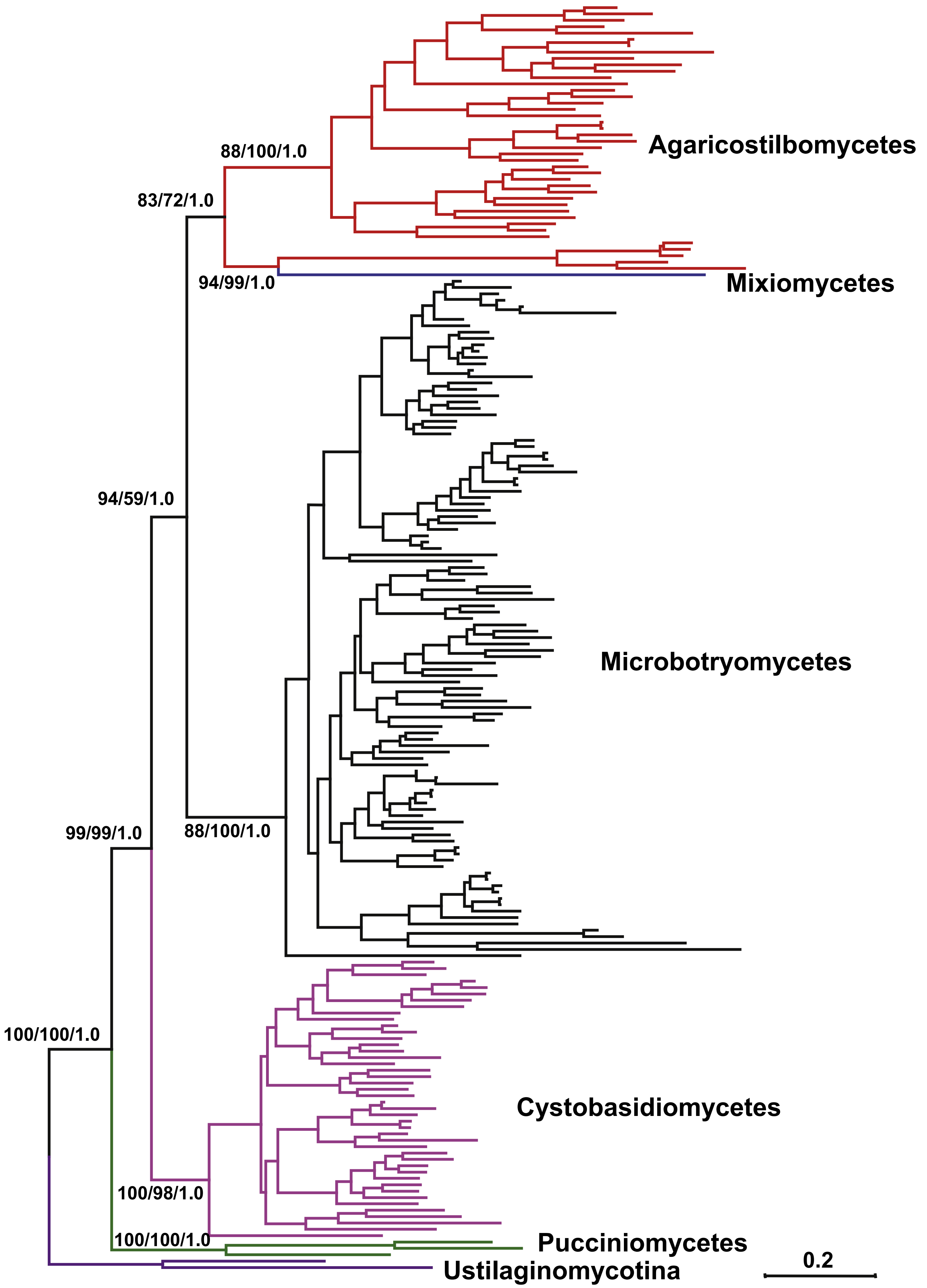

Fig. 1. Phylogeny of yeast species in the *Pucciniomycotina* inferred from the combined sequences of the SSU rDNA, LSU rDNA D1/D2 domains, ITS regions (including 5.8S rDNA), *RPB1*, *RPB2*, *TEF1* and *CYTB*. The tree backbone was constructed using maximum likelihood analysis. Bootstrap percentages of maximum likelihood and maximum parsimony analyses over 50 % from 1 000 bootstrap replicates and posterior probabilities of Bayesian inference above 0.9 are shown respectively from left to right on the deep and major branches resolved. Bar = 0.2 substitutions per nucleotide position.

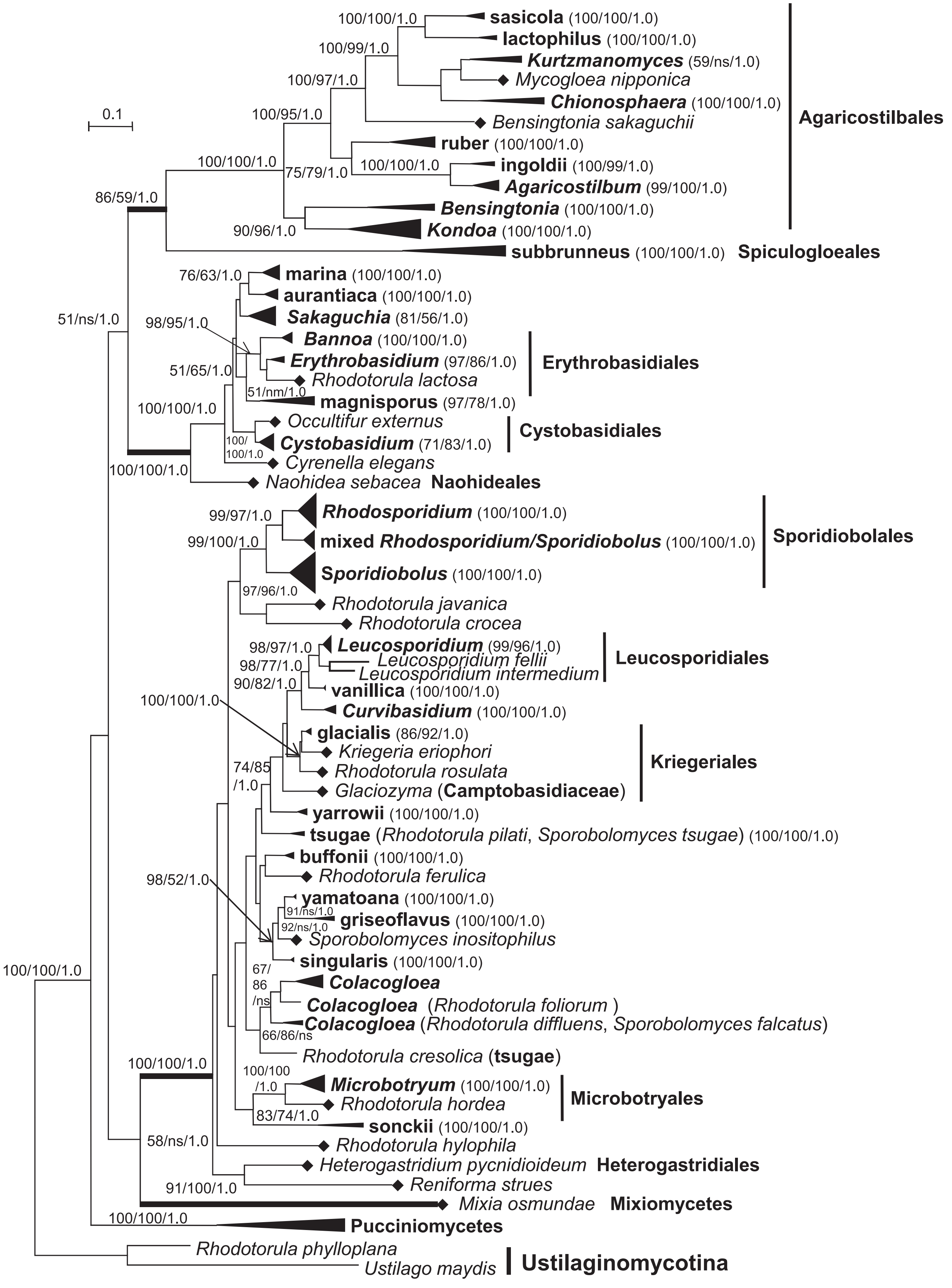

Fig. 2. Phylogeny of yeast species in the *Pucciniomycotina* inferred from the combined sequences of the SSU rDNA, LSU rDNA D1/D2 domains, and ITS regions (including 5.8S rDNA). The tree backbone was constructed using maximum likelihood analysis. Bootstrap percentages (BP) of maximum likelihood and maximum parsimony analyses over 50 % from 1 000 bootstrap replicates and posterior probabilities (PP) of Bayesian inference above 0.9 are shown respectively from left to right on the deep and major branches and in the brackets following the clades resolved. The branches ending with filled diamonds represent single-species clades. Bar = 0.1 substitutions per nucleotide position. Note: ns, not supported (BP < 50 % or PP < 0.9); nm, not monophyletic.

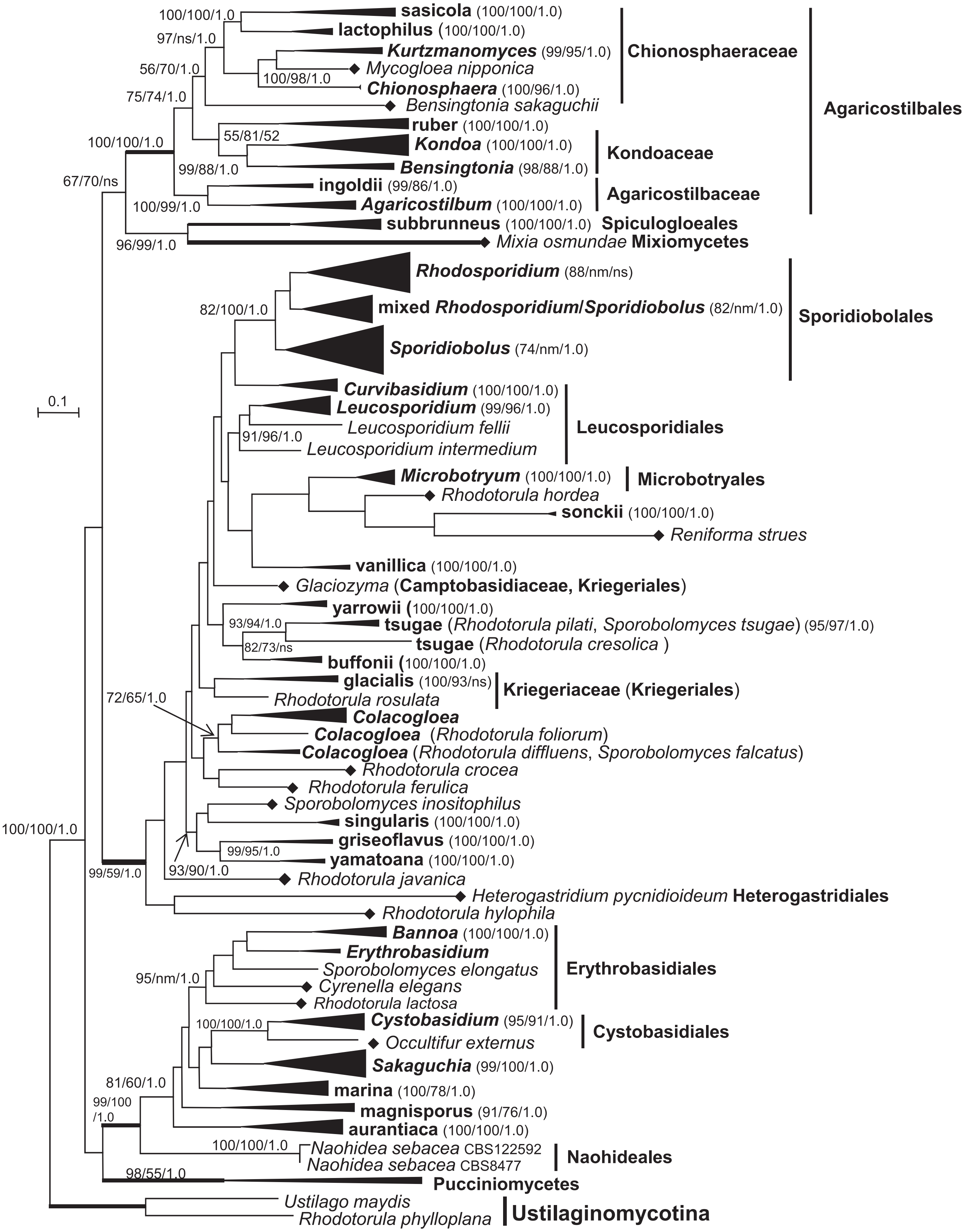

Fig. 3. Phylogeny of yeast species in the *Pucciniomycotina* inferred from the combined sequences of *RPB1*, *RPB2*, *TEF1* and *CYTB*. The tree backbone was constructed using maximum likelihood analysis. Bootstrap percentages (BP) of maximum likelihood and maximum parsimony analyses over 50 % from 1 000 bootstrap replicates and posterior probabilities (PP) of Bayesian inference above 0.9 are shown respectively from left to right on the deep and major branches and in the brackets following the clades resolved. The branches ending with filled diamonds represent single-species clades. Bar = 0.1 substitutions per nucleotide position. Note: ns, not supported (BP < 50 % or PP < 0.9); nm, not monophyletic.

not supported in the LSU rDNA D1/D2 tree constructed in Boekhout *et al.* (2011) either. Thus, we prefer to maintain the **lactophilus** and the **sasicola** clades as distinct clades. Bauer *et al.* (2009) described the teleomorphic genus *Cystobasidiopsis* with only one species, *C. nirenbergiae*, and showed that it clustered together with *S. lactophilus* based on neighbour-joining analysis of the LSU rDNA D1/D2 sequences. Our ML, MP and BI analyses of the LSU rDNA D1/D2 sequences

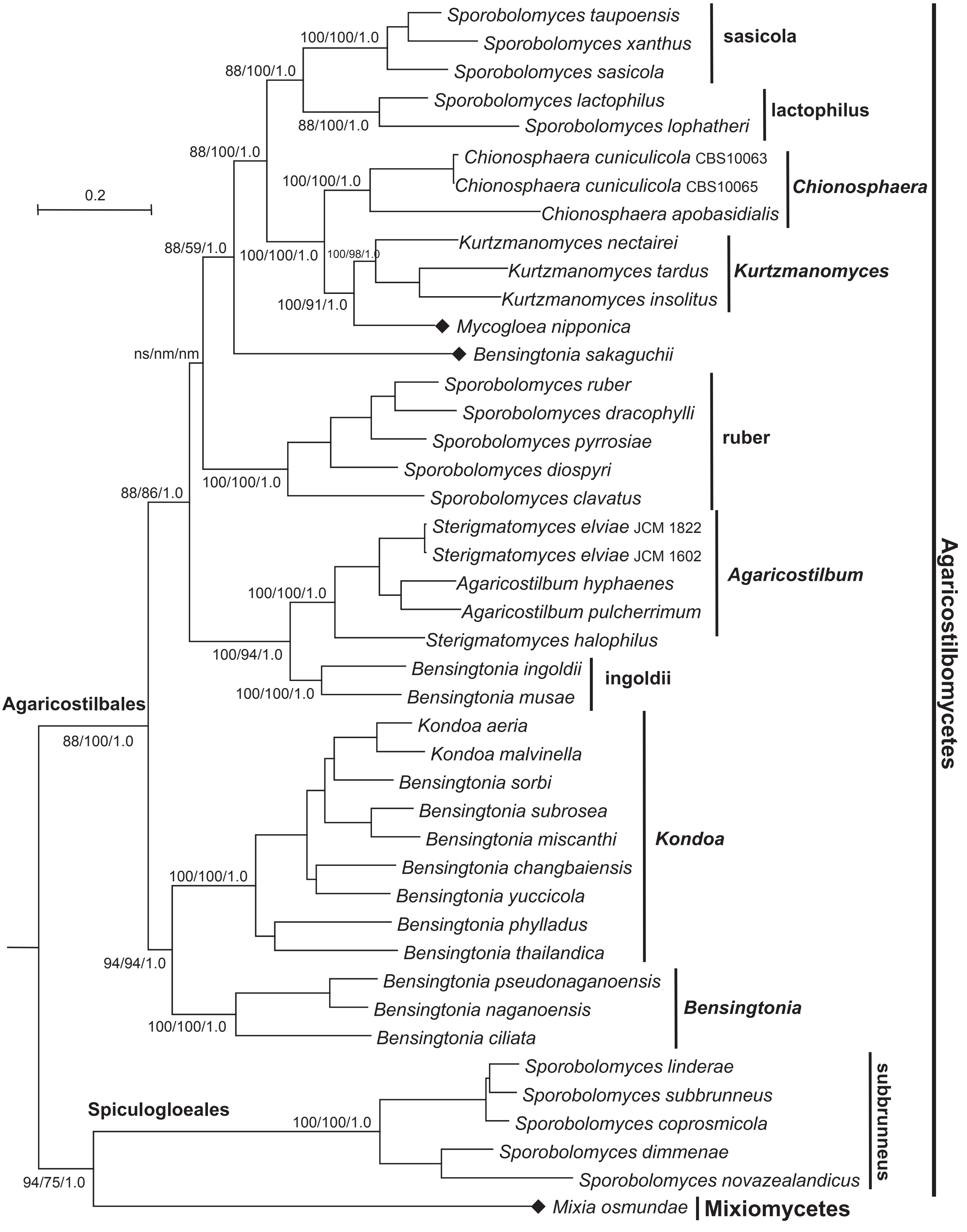

Fig. 4. Phylogeny of yeast species in the *Agaricostilbomycetes* and *Mixiomycetes* inferred from the combined sequences of the SSU rDNA, LSU rDNA D1/D2 domains, ITS regions (including 5.8S rDNA), *RPB1, RPB2*, *TEF1* and *CYTB*. The tree backbone was constructed using maximum likelihood analysis. Bootstrap percentages (BP) of maximum likelihood and maximum parsimony analyses over 50 % from 1 000 bootstrap replicates and posterior probabilities (PP) of Bayesian inference above 0.9 are shown respectively from left to right on the deep and major branches and clades resolved. The branches ending with filled diamonds represent single-species clades. Bar = 0.2 substitutions per nucleotide position. Note: ns, not supported (BP < 50 % or PP < 0.9); nm, not monophyletic.

also clustered *C. nirenbergiae* together with *S. lactophilus* and *S. lophatheri* with 71–98 % BP and 1.0 PP supports (data not shown). More sequence data are needed to confirm the relationship of *C. nirenbergiae* with the ***lactophilus*** clade. The close relationship of the **lactophilus** and the **sasicola** clades with the ***Chionosphaera*** and ***Kurtzmanomyces*** clades occurred in all trees obtained in this study, supporting that they belong to the *Chionosphaeraceae*.

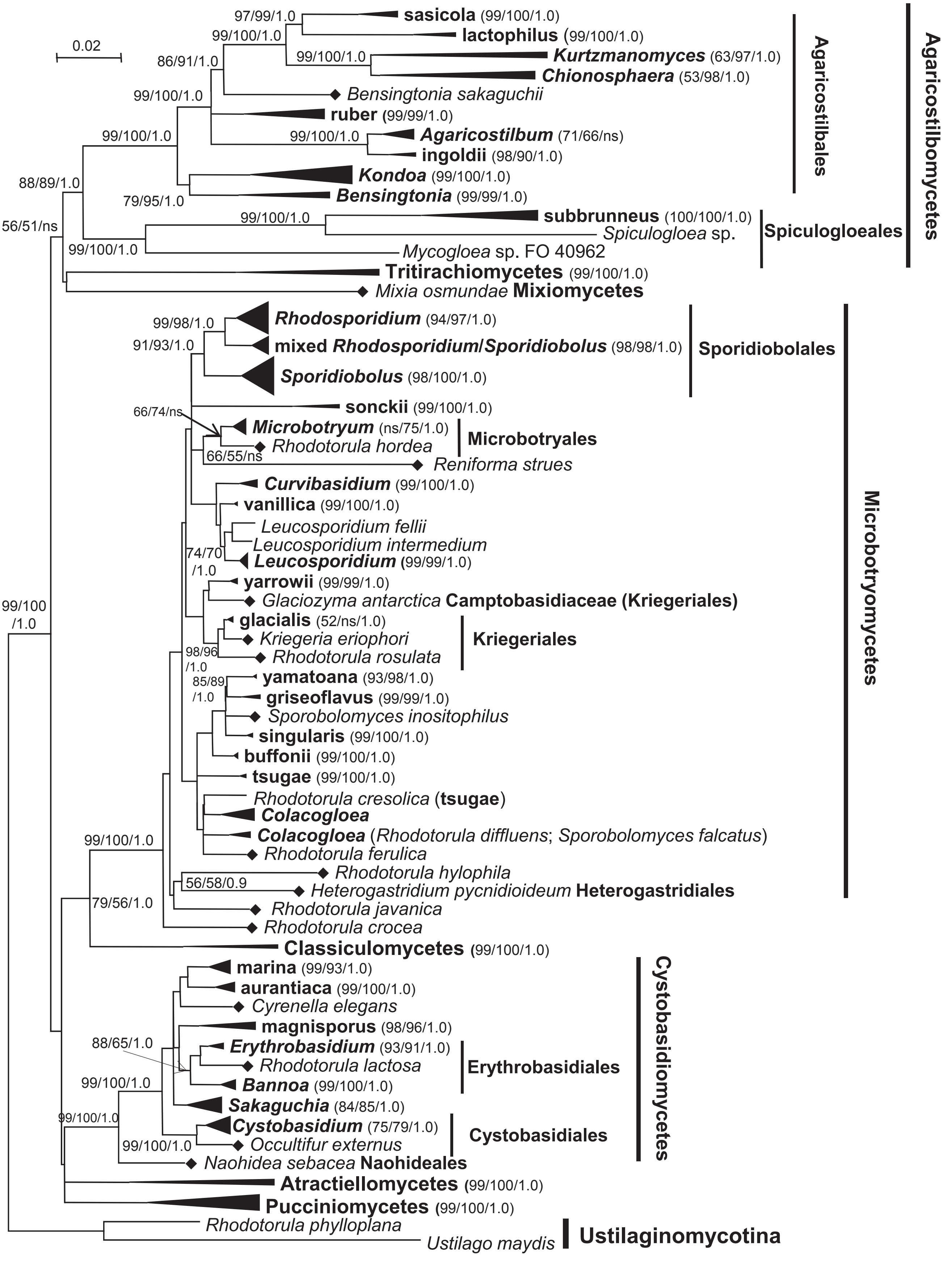

Fig. 5. Phylogeny of yeast taxa and filamentous fungi in the *Pucciniomycotina* inferred from the combined sequences of SSU rDNA and LSU rDNA D1/D2 domains. The tree backbone was constructed using maximum likelihood analysis. Bootstrap percentages (BP) of maximum likelihood and maximum parsimony analyses over 50 % from 1 000 bootstrap replicates and posterior probabilities (PP) of Bayesian inference above 0.9 are shown respectively from left to right on the deep and major branches and clades resolved. The branches ending with filled diamonds represent single-species clades. Bar = 0.02 substitutions per nucleotide position. Note: ns, not supported (BP < 50 % or PP < 0.9).

The **ruber** clade was assigned to the *Chionosphaeraceae* in Boekhout *et al.* (2011), but its affinity to the other clades of this family mentioned above was not supported in this study. In trees drawn from the rDNA regions and the four protein-coding genes, the **ruber** clade was located as a sister lineage to the *Agaricostibaceae* and the *Kondoaceae*, respectively (Figs 2, 3). In the seven genes-based tree, this clade was resolved as a sister lineage to the other families within *Agaricostilbomycetes* (Fig. 4), which suggests that the **ruber** clade represents a separate family in this class.

Bensingtonia sakaguchii was consistently located as a separate lineage basal to the family *Chionosphaeraceae* in different trees with strong BP and PP support values (Figs 2–5). Phenotypically, this species has Q9 as the major ubiquinone that differs from the other species in the *Chionosphaeraceae* that have Q10 (Boekhout *et al.* 2011).

Cystobasidiomycetes

This class mainly consists of taxa known from yeast stages only. Three orders, *Cystobasidiales*, *Erythrobasidiales* and *Naohideales*, were distinguished by Aime *et al.* (2006, 2014), Bauer *et al.* (2006) and Boekhout *et al.* (2011) based on LSU rDNA sequence analyses. However, the circumscription of the *Erythrobasidiales* in Aime *et al.* (2006) is different from that in the latter two studies. In addition to the three orders, we observed four more sister clades in the *Cystobasidiomycetes* in the tree from the seven genes (Fig. 6), which were also largely resolved and supported in the trees from the rDNA and the four protein gene datasets (Figs 2, 3).

The teleomorphic species *Naohidea sebacea* in the *Naohideales* formed a basal branch in the *Cystobasidiomycetes* in all the trees constructed in this study (Figs 2, 3, 5, 6), being in agreement with Boekhout *et al.* (2011) and Sampaio & Chen (2011). This species is mycoparasitic, forms cream-colored colonies, has ‘simple’ septal pores and reproduces by long and slender basidia without probasidia (Oberwinkler 1990, Sampaio & Chen 2011).

The *Cystobasidiales* proposed in Bauer *et al.* (2006) contains two teleomorphic genera, *Cystobasidium* and *Occultifur*, and some anamorphic *Rhodotorula* species based on SSU and LSU rDNA sequence analyses. Recently, Yurkov *et al.* (2015) confirmed the close relationship of nine described *Rhodotorula* species in the ***R. minuta*** clade with *Cystobasidium fimetarium*, the type species of the genus, based on ML analysis of SSU, ITS, LSU rDNA D1/D2 and *TEF1* sequences. They transferred the *Rhodotorula* species to the genus *Cystobasidium*. The monophyly of the ***Cystobasidium*** clade was shown in all the trees generated in this study with strong support values (Figs 2, 3, 5, 6). Though the separation of *Occultifur externus* from the other taxa in the *Cystobasidiales* was not resolved in Sampaio & Oberwinkler (2011) based on LSU rDNA D1/D2 sequence analysis, it was located as a distinct branch basal to the ***Cystobasidium*** clade in all the trees obtained in this study (Figs 2, 3, 5, 6), being in agreement with Nagahama *et al.* (2006), Boekhout *et al.* (2011) and Yurkov *et al.* (2015). *C. fimetarium* and *O. externus* share some morphological characters, including the presence of clamp connections and haustoria, a similar basidial morphology and mode of basidiospore germination. The former species differs, however, from the latter by the presence of probasidia (Sampaio *et al.* 1999, Scorzetti *et al.* 2002, Sampaio & Oberwinkler 2011). The phylogenetic and phenotypic comparisons suggest that *O. externus* represents a separate clade.

The yeast species with hydrogenated coenzyme Q10 system (Q-10H_2) formed two clades in the *Erythrobasidiales*, namely the ***Bannoa*** and ***Erythrobasidium*** clades, which was proposed by Bauer *et al.* (2006). The ***Bannoa*** clade included a teleomorphic species *Bannoa hahajimensis*, an undescribed *Bannoa* species MP 3490 (Scorzetti *et al.* 2002) and three *Sporobolomyces* species (Table 1, Fig. 6). The ***Erythrobasidium*** clade contained the monotypic teleomorphic genus *Erythrobasidium* and two *Sporobolomyces* species (Table 1, Fig. 6). The close phylogenetic relationship of the two clades was resolved in almost all the trees obtained, but their sexual life cycles are distinguishable. *Erythrobasidium hasegawianum* produces unicellular basidia without mating (Hamamoto 2011, Hamamoto *et al.* 1988), while *Bannoa hahajimensis* produces unicellular basidia on a clamp connection formed after mating (Hamamoto *et al.* 2002).

Two anamorphic species *Rhodotorula lactosa* and *Cyrenella elegans* were located as basal branches to the two clades in the *Erythrobasidiales* in the trees drawn from the seven genes and the four protein coding genes (Figs 3, 6). The affinity of *R. lactosa* with the *Erythrobasidiales* was also supported in the rDNA trees, which located *R. lactosa* as a sister branch to the ***Erythrobasidium*** clade (Fig. 2). This result is consistent with Boekhout *et al.* (2011) and Sampaio (2011a), though the major CoQ of *R. lactosa* is Q-9 (Yamada & Kondo 1973). The phylogenetic position of *Cy. elegans* remains uncertain. In contrast to the results obtained from the seven-gene and four protein coding gene sequence analyses, this species was located in a branch basal to the *Cystobasidiales* and *Erythrobasidiales* in the tree obtained from the three rDNA genes with strong support (Fig. 2), being in agreement with the result shown in Sampaio (2011c) based on LSU rDNA D1/D2 sequence analysis. *Cy. elegans* is an unusual species as it forms conidia with radiate appendages resembling those of aquatic hyphomycetes. It also forms clamp connections in the hyphae and teliospores, although germination of teliospores with basidia has not been observed (Gochenaur 1981, Sampaio 2011c). The phylogenetic and phenotypic comparisons suggest that *Cy. elegans* represents an independent lineage in *Cystobasidiomycetes*.

The **marina** clade included *Rhodotorula marina* and five *Sporobolomyces* species (Table 1, Fig. 6). Interestingly, all *Sporobolomyces* species in this clade form nearly symmetrical ballistoconidia, differing from the other *Sporobolomyces* species that typically form asymmetrical ballistoconidia (Shivas & Rodrigues de Miranda 1983, Wang & Bai 2004). The **aurantiaca** clade contained two *Rhodotorula* and three *Sporobolomyces* species (Table 1, Fig. 6). The **marina** and **aurantiaca** clades were also recognised in Scorzetti *et al.* (2002), Nagahama *et al.* (2006) and Boekhout *et al.* (2011). A close relationship of these two clades was shown in the tree from the three rDNA genes (Fig. 2), but was not supported in the trees from the four protein-coding genes and the seven genes (Figs 1, 3). Species from these two clades were included in the *Erythrobasidiales* in Aime *et al.* (2006). This conclusion, however, was not supported in the present study. In the rDNA and the four protein-coding genes-based trees, the position of these two clades varied (Figs 2, 3). In the seven genes-based tree, the **marina** and **aurantiaca** clades were resolved as sister lineages to the *Erythrobasidiales* (Fig. 6).

The **magnisporus** clade consisted of *Sporobolomyces magnisporus* and three *Rhodotorula* species described recently

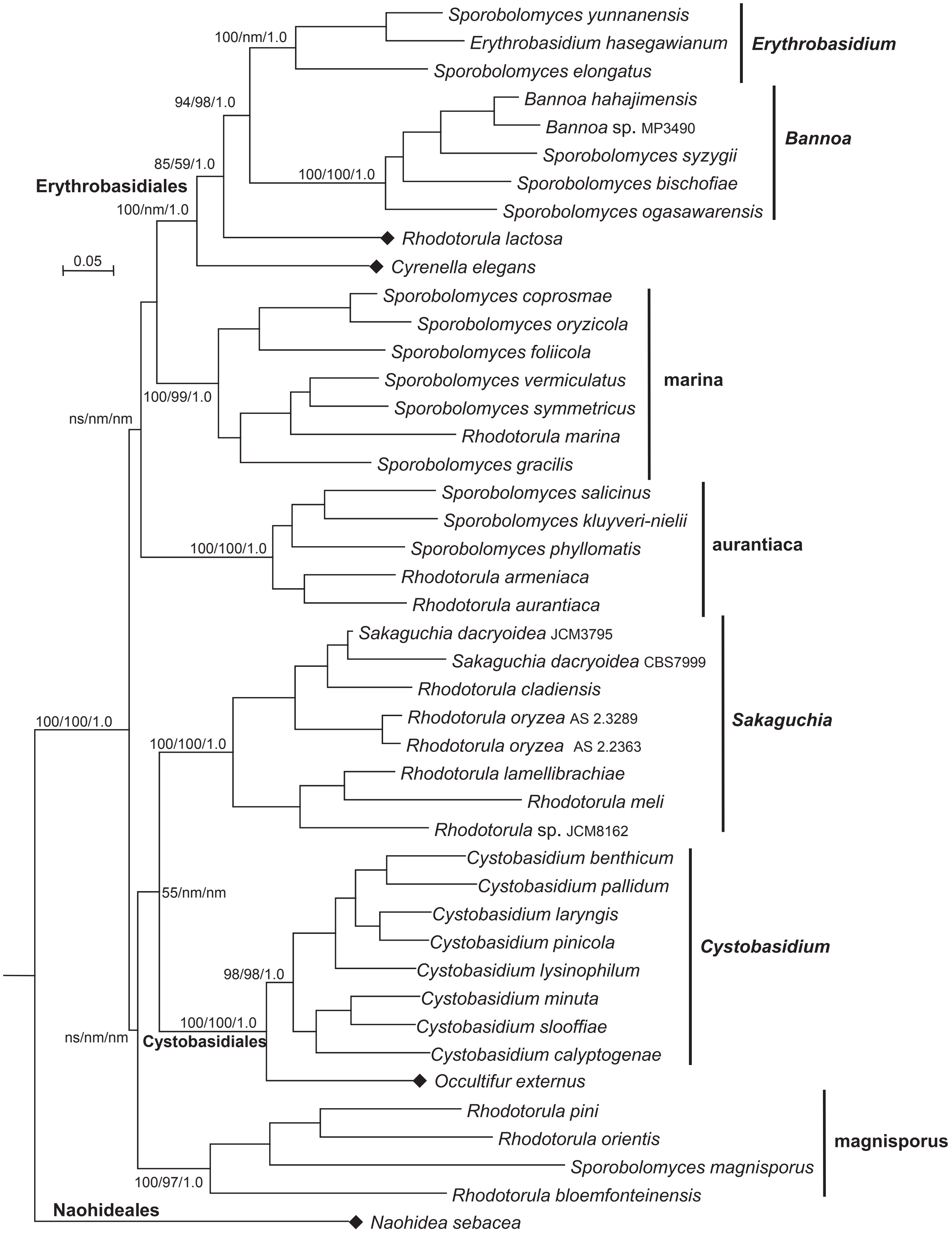

Fig. 6. Phylogeny of yeast species in the *Cystobasidiomycetes* inferred from the combined sequences of SSU rDNA, LSU rDNA D1/D2 domains, ITS regions (including 5.8S rDNA), *RPB1*, *RPB2*, *TEF1* and *CYTB*. The tree backbone was constructed using maximum likelihood analysis. Bootstrap percentages (BP) of maximum likelihood and maximum parsimony analyses over 50 % from 1 000 bootstrap replicates and posterior probabilities (PP) of Bayesian inference above 0.9 are shown respectively from left to right on the deep and major branches and clades resolved. The branches ending with filled diamonds represent single-species clades. Bar = 0.05 substitutions per nucleotide position. Note: ns, not supported (BP < 50 % or PP < 0.9); nm, not monophyletic.

by Pohl *et al.* (2011). *Sporobolomyces magnisporus* was assigned to the *Erythrobasidiales* in Boekhout *et al.* (2011). The close relationship of the **magnisporus** clade with the *Erythrobasidiales* was shown in Pohl *et al.* (2011) and in the rDNA genes-based tree in this study (Fig. 2). However, in the trees from the four protein coding genes and the seven genes, the relationships of the **magnisporus** clade with the other clades in *Cystobasidiomycetes* were not resolved (Figs 3, 6).

The ***Sakaguchia*** clade included the monotypic teleomorphic genus *Sakaguchia* and five anamorphic *Rhodotorula* species (Table 1, Fig. 6). This clade was consistently resolved and strongly supported in all the trees constructed in this (Table 3, Fig. 6) and previous studies (Nagahama *et al.* 2006, Boekhout *et al.* 2011). The genus *Sakaguchia* was treated as '*incertae sedis*' in Aime *et al.* (2006), Bauer *et al.* (2006) and Boekhout *et al.* (2011), but was assigned to the *Erythrobasidiales* in Fell (2011b). The close phylogenetic relationship of the ***Sakaguchia*** clade with the clades in *Cystobasidiomycetes* was not resolved in any of the trees generated in this study (Figs 2, 3, 5, 6). Furthermore, *Sakaguchia dacryoidea* produces teliospores (Yamada *et al.* 1994, Fell & Statzell-Tallman 1998), that are different from the sexual structures of *Bannoa* and *Erythrobasidium* species in the Erythrobasidiales. Our results suggest that the ***Sakaguchia*** clade together with the **marina**, **aurantiaca** and **magnisporus** clades represent lineages distinct from the currently recognised orders in the *Cystobasidiomycetes*.

Microbotryomycetes

More than half of the yeast species compared in this study belong to the class *Microbotryomycetes*. Within this class, six and nine clades were distinguished by Scorzetti *et al.* (2002) and Boekhout *et al.* (2011), respectively. Five orders, namely *Heterogastridiales*, *Kriegeriales*, *Leucosporidiales*, *Microbotryales* and *Sporidiobolales*, have been proposed in this class mainly based on SSU, LSU and ITS-5.8S rDNA sequence analyses (Sampaio *et al.* 2003, Aime *et al.* 2006, 2014, Bauer *et al.* 2006, Hamamoto *et al.* 2011, Toome *et al.* 2013). These orders were also recognised in this study. In addition to the clades that could be assigned to the five orders, we observe a considerable number of clades that did not belong to any of the orders.

The *Sporidiobolales* was resolved as a monophyletic group with strong BP and PP support values (Table 3, Fig. 7). Three clades, namely ***Rhodosporidium***, ***Sporidiobolus*** and mixed ***Rhodosporidium*/*Sporidiobolus*** clades (Fig. 7), are in agreement with Boekhout *et al.* (2011). The ***Rhodosporidium*** clade was composed of nine *Rhodotorula* and six *Rhodosporidium* species and *Sporobolomyces alborubescens*, including the type species of the former two genera (*Rhodotorula glutinis* and *Rhodosporidium toruloides*). The ***Sporidiobolus*** clade contained 15 *Sporobolomyces* and five *Sporidiobolus* species, including the type species of these two genera (*Sporobolomyces roseus* and *Sporidiobolus johnsonii*). The mixed ***Rhodosporidium*/*Sporidiobolus*** clade consisted of nine species from the four genera mentioned above (Table 1, Fig. 7). The three clades were well-supported in the trees drawn from the seven-gene and the rDNA datasets with 100 % BP and 1.0 PP supports (Figs 2, 7). In the tree derived from the four protein coding gene dataset, each of the three clades was also resolved as monophyletic group by ML and BI analyses with strong support values (Table 3), but was not resolved as a monophyletic group by MP analysis (Fig. 3).

The *Leucosporidiales* included two teliospore-forming yeast genera, namely *Leucosporidium* and *Mastigobasidium*, and the anamorphic genus *Leucosporidiella* (Table 2, Fig. 7). The latter was proposed by Sampaio *et al.* (2003) as the anamorphic counterpart of *Leucosporidium* to accommodate the *Rhodotorula* species that belong to the *Leucosporidiales*. In this study, the described *Mastigobasidium, Leucosporidium* and *Leucosporidiella* species except *Leucosporidium fasciculatum* were located in the monophyletic ***Leucosporidium*** clade, which was resolved in all the trees constructed from different data sets (Figs 2, 3, 5, 7). The assignment of *Leucosporidium fellii* and *Mastigobasidium intermedium* to the *Leucosporidiales* is uncertain in Sampaio *et al.* (2003) because of their clustering with the *Microbotryales* in the Bayesian Markov chain Monte Carlo (MCMC) analysis of LSU rDNA D1/D2 sequences. The affinity of *L. fellii* and *Ma. intermedium* with the ***Leucosporidium*** clade was also not supported in Boekhout *et al.* (2011). In the present study, the close relationship of these two species within the ***Leucosporidium*** clade was resolved and strongly supported in all the trees obtained (Figs 2, 3, 5, 7), being in agreement with Yurkov *et al.* (2012) and de García *et al.* (2015). Yurkov *et al.* (2012) described *Leucosporidium drummii*, that produces hyphae without clamp connections and intercalary teliospores. The teliospores germinate with either typical basidia for species of the genus *Leucosporidium* or produce, depending on the conditions, hyphae that originated from curved metabasidia similar to those of *Mastigobasidium intermedium* (Golubev 1999, Sampaio *et al.* 2003, Yurkov *et al.* 2012). Recently, Laich *et al.* (2014) described an anamorphic species as *Leucosporidium escuderoi f.a.* based on the new code for fungal nomenclature (McNeill *et al.* 2012). de García *et al.* (2015) transferred the species of the genera *Mastigobasidium* and *Leucosporidiella* into the genus *Leucosporidium* and proposed a new genus *Pseudoleucosporidium* to accommodate the species *Leucosporidium fasciculatum*. Another *Leucosporidium* species, *L. antarcticum*, was transferred to the genus *Glaciozyma* which was proposed for a group of psychrophilic yeasts from various cold environments, such as soil, seawater and sediment, in Antarctica and European glaciers (Turchetti *et al.* 2011). Recently, a new species *Glaciozyma litorale* was isolated from silt, alga and coastal sand in the White Sea intertidal zone, supporting the psychrophilic nature of this genus (Kachalkin, 2014). The genus *Glaciozyma* was assigned to the family *Camptobasidiaceae* in the *Kriegeriales* by Toome *et al.* (2013) based on LSU rDNA D1/D2 sequence analysis.

Six species from the order *Kriegeriales* proposed by Toome *et al.* (2013) were employed in this study, including *Glaciozyma antarctica* representing the family *Camptobasidiaceae*, and *Kriegeria eriophori* and four *Rhodotorula* species representing the family *Kriegeriaceae* (Table 1). These species were located together in a cluster in the seven-gene tree (Fig. 7). The affinity of *G. antarctica* with the species in the *Kriegeriaceae* was not supported by ML and MP analyses. In the rDNA and the four protein-coding genes-based trees, *G. antarctica* was not located in the same cluster with the *Kriegeriaceae* species (Figs 2, 3), suggesting that the order *Kriegeriales* defined by Toome *et al.* (2013) may not be monophyletic. Among the four *Rhodotorula* species in this order, *R. glacialis*, *R. psychrophenolica* and *R. psychrophila* (Margesin *et al.* 2007) formed a strongly supported clade labeled as **glacialis** in all the trees obtained (Table 3, Figs 2, 3, 5, 7). The close relationship between the monotypic teleomorphic genus *Kriegeria* and the **glacialis** clade was shown in different trees, but the statistic support values were

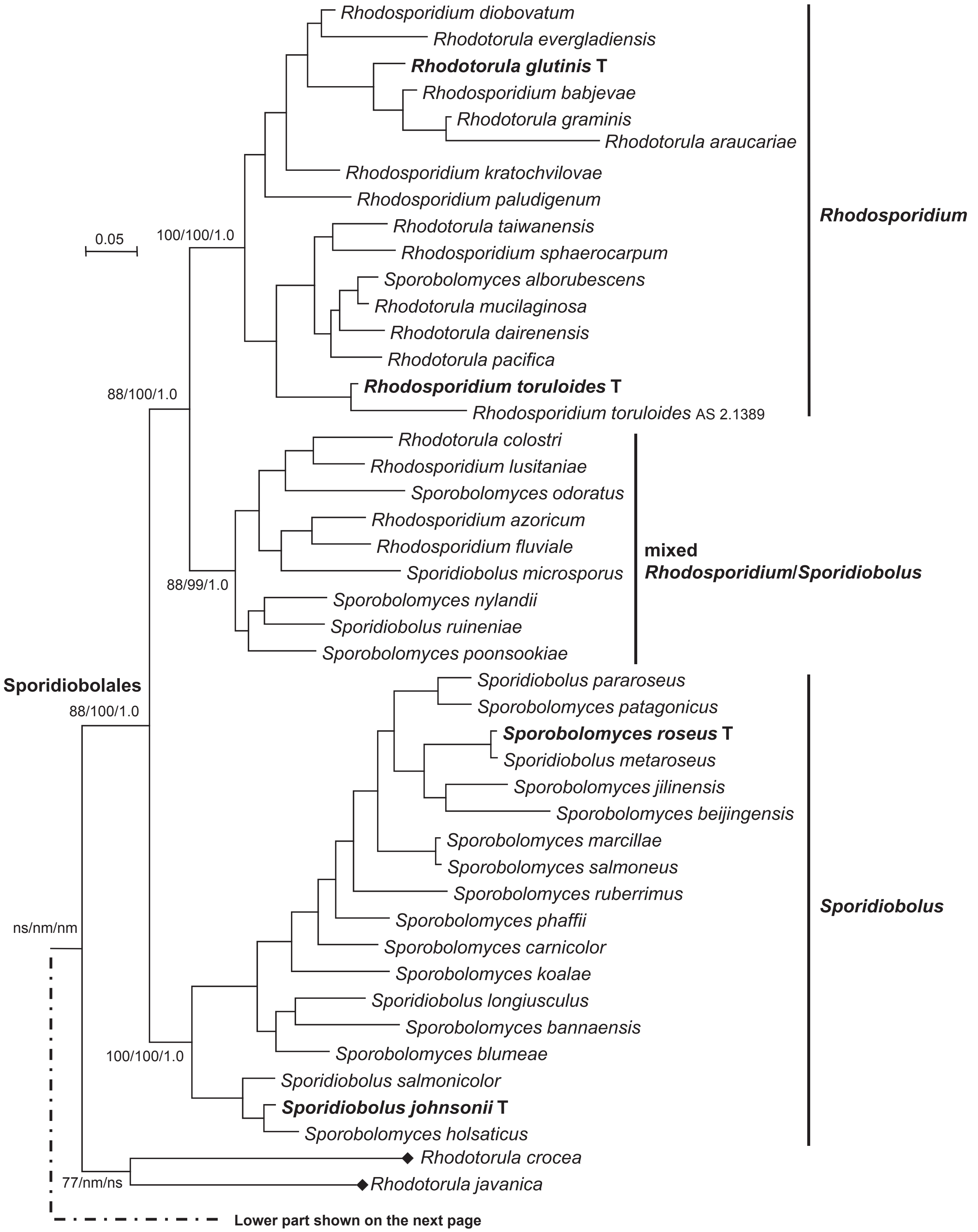

Fig. 7. Phylogeny of yeast species in the *Microbotryomycetes* inferred from the combined sequences of SSU rDNA, LSU rDNA D1/D2 domains, ITS regions (including 5.8S rDNA), *RPB1*, *RPB2*, *TEF1* and *CYTB*. The tree backbone was constructed using maximum likelihood analysis. Bootstrap percentages (BP) of maximum likelihood and maximum parsimony analyses over 50 % from 1 000 bootstrap replicates and posterior probabilities (PP) of Bayesian inference above 0.9 are shown respectively from left to right on the deep and major branches and clades resolved. The branches ending with filled diamonds represent single-species clades. Bar = 0.05 substitutions per nucleotide position. Note: ns, not supported (BP < 50 % or PP < 0.9); nm, not monophyletic.

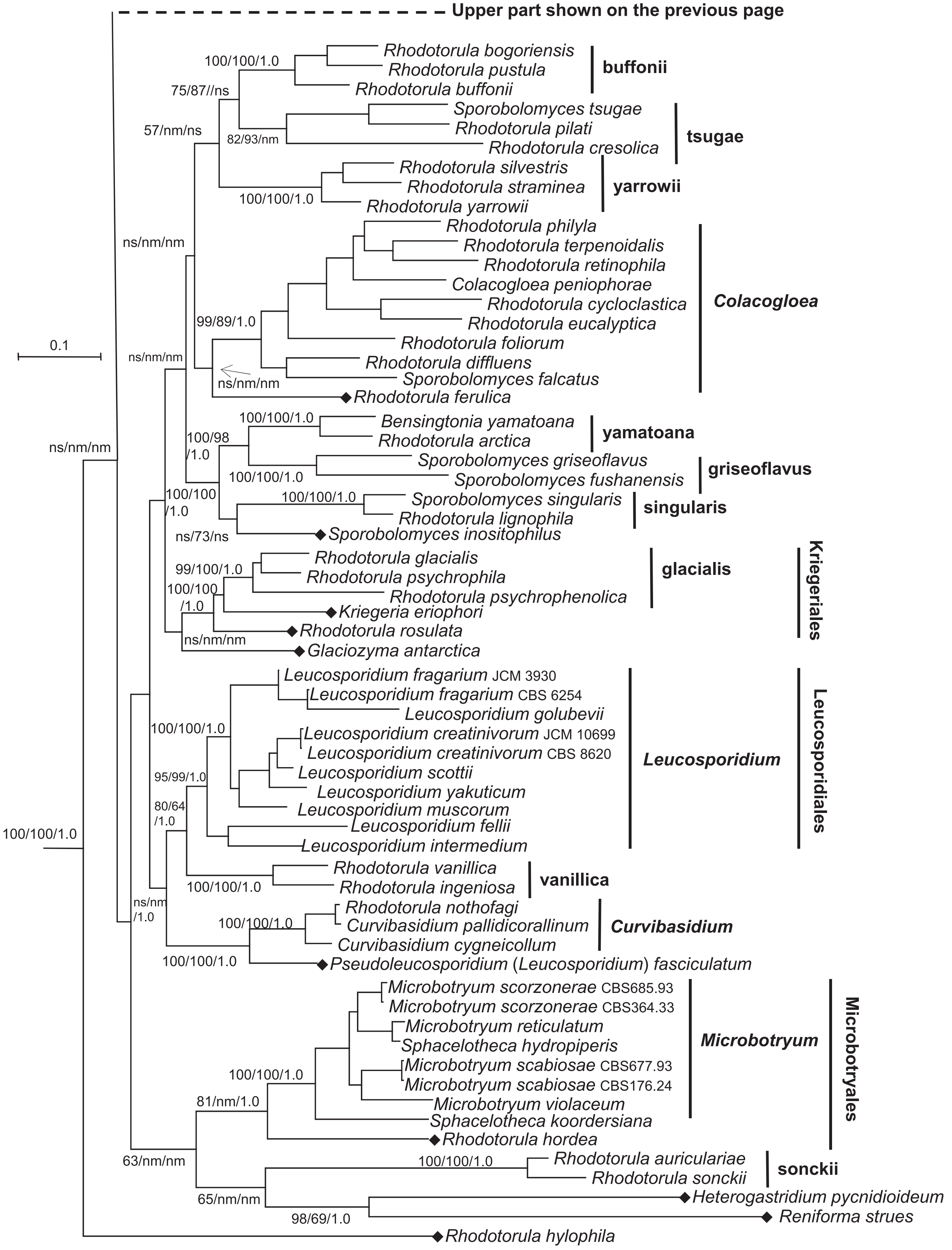

Fig. 7. (Continued).

low or lacking (Table 3), suggesting they represent separate clades. The species *Rhodotorula rosulata* formed a branch basal to the ***Kriegeria*** and the **glacialis** clades in the trees from the seven genes and the three rDNA genes with 100 % BP and 1.0 PP supports values (Figs 2, 7), suggesting that *R. rosulata* represents another clade in the *Kriegeriales*. Toome *et al.* (2013) showed that *R. rosulata* was closely related to *Meredithblackwellia eburnea* in their ML analysis of LSU, SSU and ITS sequences. These authors, however, did not transfer *R. rosulata* to *Meredithblackwellia* because of the lack of statistic support. The relationship between *R. rosulata* and *Me. eburnea* needs to be addressed further.

Within the *Microbotryales* as defined by Bauer *et al.* (1997) only one known anamorphic yeast species *Rhodotorula hordea* was included based on LSU rDNA D1/D2 sequence analysis (Boekhout *et al.* 2011, Sampaio 2011a). In agreement with Boekhout *et al.* (2011) this species was located as a basal branch of the order with strong support value in the trees from the seven genes (Fig. 7) and the rDNA genes (Fig. 2) in this study. However, in the tree from the four protein-coding genes, the affinity of the species with the *Microbotryales* was not resolved (Fig. 3). The closest relative of *R. hordea* is *Ustilentyloma fluitans*, a parasite of *Glyceria* (*Graminiae*) plants (Vánky 2002). In the LSU rDNA D1/D2 domains, *R. hordea* differs from *Ustilentyloma fluitans* by only one mismatch (Sampaio 2011a), suggesting that the former represents a yeast stage of *U. fluitans* or a closely related *Ustilentyloma* species. No yeast species is included in the *Heterogastridiales* which includes the genus *Heterogastridium*.

The species that could not be assigned to any recognised orders in *Microbotryomycetes* formed 10 clades and 7 monotypic lineages. In addition to the four *Rhodotorula* species which were included in the ***Colacogloea*** clade in Boekhout *et al.* (2011) and Sampaio (2011a), two *Rhodotorula* species and *Sporobolomyces falcatus* (Table 2, Fig. 7) were included in this clade together with the dimorphic mycoparasite *Colacogloea peniophorae*, which forms minute basidiocarps in nature (Sampaio *et al.* 2011). In the phylogenetic trees obtained from the seven genes, *Rhodotorula foliorum*, *Rhodotorula diffluens* and *Sporobolomyces falcatus* clustered in the ***Colacogloea*** clade (Fig. 7). Though the affinity of these three species with this clade was weak or not supported in the trees from the rDNA genes (Figs 2, 5), this was supported in the tree from the four protein-coding genes (Fig. 3).

The ***Curvibasidium*** clade contained two teleomorphic *Curvibasidium* species (Table 1). *Leucosporidium fasciculatum* was located basal to this clade with 100 % BP and 1.0 PP support values in the trees from the seven genes, the rDNA and the four protein-coding genes (Figs 2, 3, 7). The close relationship of *L. fasciculatum* with the ***Curvibasidium*** clade was also shown in previous studies (Sampaio *et al.* 2004, Boekhout *et al.* 2011, Sampaio, 2011e), however, in contrast to *Curvibasidium*, *L. fasciculatum* lacks clamp connections and forms septate basidia (phragmobasidia) (Sampaio 2011d). Therefore, *L. fasciculatum* has been placed in a new genus *Pseudoleucosporidium* by de García *et al.* (2015). The **vanillica** clade contained two *Rhodotorula* species as recognised by Sampaio *et al.* (2004) and Boekhout *et al.* (2011). The ***Curvibasidium*** and **vanillica** clades and *L. fasciculatum* were located basal to the *Leucosporidiales* in the trees from the seven-genes with moderate PP support values (Fig. 7) and in the tree from the rDNA genes with strong supports by all algorithms employed (Fig. 2). However, in the tree from the four protein-coding genes, the close relationships of these two clades with the *Leucosporidiales* were not resolved (Fig. 3). Phenotypically, the *Curvibasidium* species form non-septate basidia, which is a unique feature in the *Pucciniomycotina* (Sampaio *et al.* 2004).

Among the species tentatively assigned to the yamatoana/*Leucosporidium antarcticum* group in Boekhout *et al.* (2011), three (*Kriegeria eriophori*, *Camptobasidium hydrophilum* and *Leucosporidium antarcticum*) were assigned to the *Kriegeriales* by Toome *et al.* (2013). From the remaining species of this group, four clades and two single-species lineages were distinguished in this study (Fig. 7). The **buffonii** clade contained three *Rhodotorula* species, the **tsugae** clade included *Sporobolomyces tsugae* and two *Rhodotorula* species, and the **yarrowii** clade comprised three *Rhodotorula* species. These three clades clustered together in the ML and MP trees based on the seven genes with weak ML BP support (Fig. 7). The BI tree from the seven genes, and the trees from the rDNA and the four protein coding genes did not support the close relationship of these three clades (Figs 2, 3, 7). *Rhodotorula cresolica* was located in the **tsugae** clade in the tree from the four protein-coding genes with 93–94 % BP and 1.0 pp support values (Fig. 3). This phylogeny was also supported by the ML and MP analyses of the seven genes, though not supported in the BI tree from the seven genes and the trees from the rDNA dataset (Figs 2, 5, 7).

The **griseoflavus** clade containing two *Sporobolomyces* species, the **yamatoana** clade with *Bensingtonia yamatoana* and *Rhodotorula arctica*, and the **singularis** clade with *Sporobolomyces singularis* and *Rhodotorula lignophila*, clustered together with high BP and PP values in all the phylogenetic trees constructed (Figs 2, 3, 5, 7). Each of these clades received strong support values in the trees. *Sporobolomyces inositophilus* was located in the same cluster with these three clades with strong support values (Fig. 7), however, its relationship to each of the clades was not resolved by ML and BI, suggesting that this species may represent a separate clade. In addition, species of the **griseoflavus** and **yamatoana** clades were characterised by the presence of Q10 and Q9, respectively, supporting their separation as two clades.

Rhodotorula auriculariae located in the yamatoana/*Leucosporidium antarcticum* group in Boekhout *et al.* (2011) was shown to be closely related with *Rhodotorula sonckii*, which was located as a basal branch of the *Microbotryomycetes* in Boekhout *et al.* (2011). The **sonckii** clade formed by these two species clustered with the *Microbotryales* and *Heterogastridiales* in the tree from the seven genes (Fig. 7). The close relationship of this clade with the *Microbotryales* was also supported in the tree from the rDNA genes (Fig. 2), but not supported in the tree from the four protein-coding genes (Fig. 3). The relationship of the **sonckii** clade with the *Heterogastridiales* was not resolved in the analyses of the rDNA and the four protein-coding genes (Figs 2, 3). *Rhodotorula ferulica* was also placed in the yamatoana/*Leucosporidium antarcticum* group by Boekhout *et al.* (2011). This species was located basal to the ***Colacogloea*** clade in the ML tree from the seven genes but the BP support was lack. This relationship was, however, not resolved by the other algorithms used in this study (Fig. 7, Table 3).

The following *Rhodotorula* species, *R. crocea*, *R. hylophila*, and *R. javanica*, occupied isolated positions in the *Microbotryomycetes* with their closest relatives not being resolved. Their phylogenetic positions changed in different trees constructed from different data sets using different algorithms (Figs 2, 3, 5, 7). The species *Reniforma strues*, which was located at the deepest branch in the *Microbotryomycetes* in Boekhout *et al.* (2011), exhibited a relationship with *Heterogastridium pycnidioideum* (*Heterogastridiales*) in the trees from the seven and the rDNA genes with strong BP and PP support values (Figs 2, 7). However, the position of the former was uncertain in the tree from the four protein-coding genes (Fig. 3). *Reniforma strues* is a morphologically unique anamorphic yeast species, forming reniform cells and buds (Pore & Sorenson 1990, Pore & Fell 2011).

CONCLUSION

The molecular phylogeny of yeasts and related dimorphic and filamentous basidiomycetes in the *Pucciniomycotina* was inferred based on analyses of sequences of seven genes using different phylogenetic algorithms. The major phylogenetic groupings of pucciniomycetous yeasts observed in previous studies based on the LSU rDNA D1/D2 domains or ITS-5.8S sequences (Fell *et al.* 2000b, Scorzetti *et al.* 2002, Boekhout *et al.* 2011) were confirmed in the present study. In each of the major groups, more robust topologies with higher resolution were achieved in this study than obtained before. The yeast taxa employed were assigned into four major lineages, namely *Agaricostilbomycetes*, *Cystobasidiomycetes*, *Microbotryomycetes* and *Mixiomycetes*. These lineages are independent from *Atractiellomycetes*, *Classiculomycetes*, *Cryptomyco colacomycetes*, *Pucciniomycetes* and *Tritirachiomycetes* that are formed by filamentous taxa in the *Pucciniomycotina*.

The orders distinguished in previous studies except the *Kriegeriales* were all resolved as monophyletic groups in this study. The order *Spiculogloeales* was resolved as a sister lineage of *Mixiomycetes*, rather than of the order *Agaricostilbales* in the *Agaricostilbomycetes*. This suggests that the *Spiculogloeales* may represent a new class in *Pucciniomycotina*. In the *Cystobasidiomycetes*, four independent groups with sisterhood to the orders *Cystobasidiales*, *Erythrobasidiales*, and *Naohideales* were resolved, suggesting that additional orders remain to be discerned in this class. In addition to the five existing orders *Heterogastridiales*, *Kriegeriales*, *Leucosporidiales*, *Microbotryales*, and *Sporidiobolales* in the class *Microbotryomycetes*, several groups that seem to represent new orders were recognised. The boundaries of some of these new groups remain to be defined.

A total of 33 monophyletic clades and 18 single species lineages were recognised among the pucciniomycetous yeasts employed in this study (Tables 1, 3). As shown previously, the majority of the currently anamorphic genera are polyphyletic. For example, *Rhodotorula* and *Sporobolomyces* species occurred in 17 and 23 clades, respectively. These genera and related teleomorphic ones need to be redefined. A considerable number of new genera need to be proposed to accommodate the monophyletic clades that do not include any generic type species. The next step will be to propose an updated taxonomic system for yeasts and related taxa within *Pucciniomycotina* based on the phylogenetic framework presented here and to implement the 'One fungus = One Name' principle.

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