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 infer 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 Mixymycetes. These lineages remained independent from the classes Atractiellomycetes, Classicumycetes, Pucciniomycetes and Tritrichomycetes 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.

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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 Uredinomycetes, 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 Garcia 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 Agaricostilbomycetidae, Erythrobasidiomycetidae, Sporidiobolomycetidae and subbrunneus clusters (Hamamoto & Nakase 2000, Nakase 2000). Sequence analyses of the large subunit (LSU or 26S) rDNA D1/D2 domains and the internal transcribed spacer regions (ITS 1 and 2) 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 Mixymycetes. These lineages remained independent from the classes Atractiellomycetes, Classicumycetes, Pucciniomycetes and Tritrichomycetes 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.

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(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 invariant 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 GTR+GAMMAI 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 ≥70 % or a Bayesian posterior probability (PP) of ≥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
| Lineage/Clade          | Species                      | Strain number | ITS     | D1D2  | SSU    | RPB1   | RPB2   | TEF1    | CYTB    |
|-----------------------|------------------------------|---------------|---------|-------|--------|--------|--------|---------|---------|
| **Agaricostilbomycetes** |                              |               |         |       |        |        |        |         |         |
| **Agaricostilbales**   |                              |               |         |       |        |        |        |         |         |
| **Kondoaceae**         |                              |               |         |       |        |        |        |         |         |
| Kondo                      | B. miscanthi                  | JCM 5733T     | AF444516| AF189891| D38236 | KJ708023 | KJ708149 | KJ707753 | KJ707719 |
|                          | B. phylia                      | JCM 7476T     | AF444514| AF189894| D38237 | KJ708022 | KJ708152 | KJ707756 | KJ707727 |
|                          | B. sorbi                       | AS 2.2303T    | AF23343 | AF23343 | D38234 | KJ708029 | KJ708156 | KJ707897 | KJ707584 |
|                          | B. subrosea                    | JCM 5735T     | AF444565| AF189895| D38238 | KJ708027 | KJ708157 | KJ707895 | KJ707640 |
|                          | B. thailandica                 | JCM 10651T    | AB040114| EF384207| KJ708026 | KJ708159 | KJ707898 | KJ707661 |
|                          | B. yuccicola                   | JCM 6251T     | AF444518| AF189897| D38367 | KJ708025 | KJ708161 | /        | /        |
|                        | Kondo aera                     | CBS 8352T     | AF444562| AF189901| KJ708417 | KJ708020 | KJ708172 | KJ707905 | /        |
|                        | K. malvinella                  | AS 2.1946T    | AF444498| AF189903| D13776  | KJ708021 | KJ708173 | KJ707896 | KJ707586 |
| **Bensingtonia**        |                              |               |         |       |        |        |        |         |         |
|                          | B. ciliata                     | AS 2.1945T    | AF444563| AF189887| D38233 | KF706509 | KF706536 | KF706486 | KJ707567 |
|                          | B. naganoensis                 | JCM 5797T     | AF444558| AF189893| D38366 | KJ707960 | KJ708151 | KJ707755 | KJ707722 |
|                          | B. pseudonaganoensis           | AS 2.2601T    | DG224375| KJ707959 | KJ708026 | KJ708153 | KJ707956 | KJ707590 |
| **Agaricostilbaceae**   |                              |               |         |       |        |        |        |         |         |
|                          | B. ingoldii                    | JCM 7445T     | AF444519| AF189888| D38234 | KJ707961 | KJ708148 | KJ707752 | KJ707726 |
|                          | B. musae                       | JCM 8801T     | AF444569| AF189892| D34946 | KJ707963 | KJ708150 | KJ707754 | KJ707743 |
| **Agaricostilbum**      |                              |               |         |       |        |        |        |         |         |
|                          | Agaricostilbum hyphaenes       | CBS 7811      | AF444553| AF177406| AY657575| KJ707965 | KJ708145 | KJ707749 | KJ707645 |
|                          | A. pulcherinum                 | FO 29365      | AJ026402| GU91289 | FJ641966| KJ708424 | KJ708345 | /        | /        |
|                          | Sterigmatomyces elvisae        | JCM 1822T     | AF444551| AF177415| KJ708432 | KJ707964 | KJ708345 | /        | /        |
|                          | S. elvis                       | JCM 1602      | AB038053| KP216512| KP216516| KJ708077 | KJ708208 | KJ707852 | AB040614 |
|                          | S. halophilus                  | AS 2.1935T    | AF444556| AF177416| D64119  | KJ707962 | /        | /        | KJ707890 |
|                          | S. kasturi                     | CS 5730       | AF444559| AF177407| U77662  | /        | /        | KJ708163 | KJ707883 |
|                          | C. cuniculata                  | CBS 10086     | KJ708465| KJ708368 | KJ707985 | KJ708164 | KJ707886 | KJ707593 | KJ707594 |
|                          | C. cuniculata                  | CBS 10065     | KJ708466| KJ708369 | KJ707984 | KJ708165 | KJ707887 | KJ707594 | KJ707594 |
| **Kurtzmanomyces**      |                              |               |         |       |        |        |        |         |         |
|                          | Kurtzmanomyces insolitus       | JCM 10490T    | AF444594| AF177408| KJ708424 | KJ707986 | KJ708175 | KJ707893 | KJ707885 |
|                          | K. nectairei                   | AS 2.1950T    | AF444494| AF177409| D64122  | KJ707980 | KJ708176 | KJ707884 | KJ707571 |
|                          | K. tardus                      | JCM 10490T    | AF444566| AF177410| KJ708425 | KJ707992 | KJ708177 | KJ707885 | KJ707686 |
|                          | S. sasicola                    | AS 2.1933T    | AF444548| AF177412| AB201688| KJ708335 | KJ707900 | KJ707565 | KJ707565 |
|                          | S. taupoensis                  | JCM 8770T     | AF444592| AF177413| D68886  | /        | /        | KJ708339 | KJ707901 |

(continued on next page)
## Table 1. (Continued)

| Lineage/Clade | Species                  | Strain number | ITS         | D1D2       | SSU         | RPB1        | RPB2        | TEF1        | CYTB        |
|---------------|--------------------------|---------------|-------------|------------|-------------|-------------|-------------|-------------|-------------|
|               | S. xanthus               | AS 2.1957<sup>T</sup> | AF444547    | AF177414   | D64118      | KJ707993    | KJ708343    | KJ707902    | KJ707573    |
| lactophilus   | S. lactophilus           | JCM 7595<sup>T</sup> | AF444545    | AF177411   | AB021675    | /           | KJ708312    | KJ707889    | KJ707642    |
|               | S. iophatheri            | CBS 11272<sup>T</sup> | AB126046    | AB124561   | AB126046    | KJ707988    | KJ706315    | KJ707880    | KJ707608    |
|               | Cystobasidiopsis nirenbergiae | BBA 65452<sup>T</sup> | GG180106    | FJ536254   | /           | /           | /           | /           | /           |
|               | Single-species lineage   | Mycogloea nipponica | CBS 11308   | KJ778629   | KJ708456    | KJ708370    | KJ707802    | KJ708194    | KJ707882    | KJ707609    |
| Incertae sedis in Agaricostilbales | Sporobolomyces clavatus | AS 2.2318<sup>T</sup> | AY364839    | AY364839   | KJ708406    | KJ707917    | KJ708295    | KJ707894    | KJ707586    |
|               | S. diospyri              | JCM 12157<sup>T</sup> | AB126047    | AB124560   | AB126047    | KJ707989    | KJ708296    | KJ707904    | KJ707696    |
|               | S. dracophylli           | AS 2.1959<sup>T</sup> | AF444583    | AF189982   | D66882      | KJ707987    | KJ708299    | KJ707879    | KJ707575    |
|               | S. pyrosiae               | JCM 12159<sup>T</sup> | AB126045    | AB124562   | AB126045    | KJ707981    | KJ708330    | KJ707903    | KJ707697    |
|               | S. ruber                 | AS 2.1958<sup>T</sup> | AF444550    | AF189992   | AB021686    | KJ707983    | KJ708333    | KJ707899    | KJ707574    |
| Single-species lineage | Bensingtonia sakaguchii | JCM 10047<sup>T</sup> | AF444626    | AF363646   | AB001746    | KJ707958    | KJ708155    | KJ707891    | KJ707671    |
| Spiculogloeales | Sporobolomyces coprosmicola | JCM 8767<sup>T</sup> | AF444576    | AF189981   | D66879      | /           | KJ708171    | KJ707908    | KJ707740    |
|               | S. dimmenae              | JCM 8762<sup>T</sup> | AB038046    | AB644404   | D66881      | KJ707991    | KJ708297    | KJ707907    | KJ707739    |
|               | S. linderae              | JCM 8856<sup>T</sup> | AF444582    | AF189989   | D66885      | /           | KJ708296    | KJ707906    | KJ707744    |
|               | S. novozealandicus       | JCM 8756<sup>T</sup> | AB038048    | KJ708467   | KJ708443    | KJ708073    | KJ708319    | KJ707851    | KJ707738    |
|               | S. subbrunneus           | JCM 5278<sup>T</sup> | AF444549    | AF189997   | AB21691     | /           | KJ707906    | 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<sup>T</sup> | AB026001    | AB026001   | AB126647    | KJ708081    | KJ708214    | KJ707842    | KJ707691    |
|               | R. calyptogena           | JCM 10899<sup>T</sup> | AB025996    | AB025996   | AB126648    | KJ708075    | KJ708218    | KJ707840    | KJ707690    |
|               | R. laryngis              | JCM 10953<sup>T</sup> | AB078500    | AB078500   | AB126649    | KJ708055    | KJ708240    | KJ707824    | KJ707619    |
|               | R. lysiniphila           | JCM 5951<sup>T</sup> | AB078501    | AB078501   | AB126650    | KJ708074    | KJ708243    | KJ707845    | KJ707721    |
|               | R. minuta                | AS 2.1516<sup>T</sup> | AF190011    | AF189945   | D45367      | KJ708059    | KJ708246    | KJ707825    | KJ707562    |
|               | R. pallida               | JCM 3780<sup>T</sup> | AB078492    | AF189962   | AB126651    | KJ708056    | KJ708253    | KJ707826    | KJ707621    |
|               | R. pinicola              | AS 2.2193<sup>T</sup> | AF444292    | AF444293   | AB126652    | KJ708057    | KJ708257    | KJ707827    | KJ707579    |
|               | R. stooffiae             | JCM 10954<sup>T</sup> | AF444627    | AF444722   | AB126653    | KJ708058    | KJ708266    | KJ707828    | KJ70629    |
| Single-species lineage | Occultifur externus | JCM 10725<sup>T</sup> | AF444567    | AF189910   | AB055193    | KJ708060    | KJ708199    | KJ707829    | KJ707689    |
| Lineage/Clade | Species                        | Strain number | ITS         | D1D2       | SSU         | RPB1         | RPB2         | TEF1         | CYTB         |
|--------------|--------------------------------|---------------|-------------|------------|-------------|---------------|---------------|---------------|--------------|
| **Erythrobasidiales** |                                |               |             |            |             |               |               |               |              |
| Erythrobasidium | Erythrobasidium hasegawianum  | AS 2.1923T    | AF444522    | AF189899   | D12803      | KF706506     | KF706534     | KJ707776     | KJ707563     |
|              | Sporobolomyces elongatus       | AS 2.1949T    | AF444561    | AF189983   | AB021669    | KJ708012     | KJ708300     | KJ707782     | KJ707570     |
|              | S. yunnanensis                 | AS 2.2090T    | AB030353    | AB127358   | AF229176    | KJ708015     | KJ708344     | KJ707779     | KJ707576     |
| Bannoa       | Bannoa sp.                     | MP 3490       | DQ631900    | DQ631898   | DQ631899    | /             | DQ631901     | DQ631902     |              |
|              | B. hahajimensis                | JCM 10336T    | AB035897    | AB082571   | AB035897    | KJ708014     | KJ708146     | KJ707750     | KJ707682     |
|              | Sporobolomyces bischofiae      | JCM 10338T    | AB035721    | AB082572   | AB035721    | KJ708018     | KJ708292     | KJ707777     | KJ707684     |
|              | S. ogasawarensis               | JCM 10326T    | AB035713    | AB082570   | AB035713    | KJ708017     | KJ708323     | KJ707781     | KJ707681     |
|              | S. syzygií                     | JCM 10337T    | AB035720    | AB082573   | AB035720    | KJ708011     | KJ708338     | KJ707778     | KJ707683     |
| Single-species clade |                                 |               |             |            |             |               |               |               |              |
|              | Cyrenella elegans              | CBS 274.82    | KJ778626    | KJ708454   | KJ708360    | KJ707980     | KJ708168     | KJ707830     | KJ707620     |
|              | Rhodotorula lactosa            | CBS 5826T     | AF444540    | AF189936   | D45366      | KJ708016     | KJ708239     | /             | AB040633     |
| **Naohideales** |                                |               |             |            |             |               |               |               |              |
| Naohidea     | Naohidea sebacea               | CBS 8477T     | DQ911616    | DQ831020   | KP216515    | KF706508     | KF706535     | KF706487     | KJ707654     |
|              | N. sebacea                     | CBS122592     | /           | /           | /           | KJ708019     | KJ708198     | KJ707783     | KJ707612     |
| **Incertae sedis in Cystobasidiomycetes** |                             |               |             |            |             |               |               |               |              |
|              |                                  |               |             |            |             |               |               |               |              |
|              | R. aurantiaca                  | JCM 8977T     | AF444523    | AF189920   | AB126644    | KP216521     | KJ708211     | KJ707762     | AB040615     |
|              | R. aurantiaca                  | JCM 3771T     | AF444538    | AF189921   | KJ708436    | KJ707970     | KJ708212     | KJ707757     | AB040616     |
|              |                                  | JCM 6356T     | AF444544    | AF189988   | AB021674    | KJ707977     | KJ708310     | KJ707760     |              |
|              |                                  | JCM 7549T     | AF444515    | AF189991   | AB021685    | KJ708328     | KJ707761     | KJ707728     |              |
|              |                                  | JCM 2959T     | AF444511    | AF189995   | AB021687    | /             | /             | KJ707758     | KJ707703     |
|              |                                  | JCM 3776T     | AF444504    | AF189944   | AB126645    | KJ707973     | KJ708244     | KJ707995     | AB040635     |
|              |                                  | JCM 8772T     | AF444577    | AF189980   | D66880      | KJ707966     | KJ708296     | KJ707798     | KJ707742     |
|              |                                  | JCM 2252T     | AF444521    | AF189984   | AB021671    | KJ707969     | KJ708302     | KJ707977     | KJ707589     |
|              | S. foliosa                     | JCM 2963T     | AF444576    | AF189985   | KJ708433    | KJ707968     | KJ708304     | KJ707799     | KJ707705     |
|              | S. gracilis                    | JCM 5299T     | AF444546    | AF189990   | AB021677    | KJ708324     | KJ707955     | KJ707712     |              |
|              | S. oryzicola                   | JCM 5299T     | AF444546    | AF189990   | AB021677    | KJ708324     | KJ707955     | KJ707712     |              |
|              | S. symmetricus                 | AS 2.2299T    | AY364836    | AY364836   | KJ708350    | KJ708337     | KJ707800     | KJ707582     |              |
|              | S. vermiculatus                | JCM 10247T    | AB030335    | AF460176   | AB030322    | KJ707967     | KJ708342     | KJ707801     | KJ707675     |
| Sakaguchia   | Rhodotorula cladiensis         | CBS 10878T    | FJ008055    | FJ008049   | KJ708354     | /             | KJ708219     | KJ707647     | KJ707603     |
|              | R. lamelibrachii               | CBS 9598T     | AB025999    | AB025999   | AB126646    | KJ708098     | KJ708314     | KJ707876     | KJ707667     |
|              | R. meli                        | CBS 10797T    | FJ807883    | KJ708452    | KJ708355    | KJ708085     | KJ708245     | KJ707555     | KJ707602     |
|              | R. oryzae                      | AS 2.2363T    | AY335160    | AY335161   | KJ708352    | KJ708100     | KJ708250     | KJ707853     | KJ707587     |
|              | R. oryzae                      | AS 2.3289     | KP216523    | KJ708451    | KJ708353    | KJ708103     | KJ708251     | KJ707848     | KJ707592     |
| 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 3795T                         | AF444597              | AF189972  | D13459     | KJ708102   | KJ708348   | KJ707709   |            |
| **Rhodotorula magnisporus** | CBS 7999                          | AF444571              | AF444723  | KJ708351   | KJ708099   | KJ708346   | KJ707878   | KJ707647   |
| **Rhodotorula bicembroneinensis** | CBS 8598T                         | EU075189              | EU075187  | KJ708359   | KJ708082   | KJ708215   | /          | KJ707657   |
| **S. dacryoidea** | JCM 3789T                         | AF155971              | AF444723  | KJ708351   | KJ708099   | KJ708346   | KJ707878   | KJ707647   |
| **S. magnisporus** | JCM 11898T                        | AB112078              | AB111954  | KJ708428   | KJ708013   | KJ708317   | KJ707780   | KJ707695   |
| **Microbotryomycetes** | **Sporidiobolales**               | ****                  | **        | **         | **         | **         | **         | **         | **         |
| **Rhodosporidium** | JCM 9279T                         | AF444542              | AF070420  | AB073920   | /          | /          | KJ707874   | KJ707746   |
| **R. diobovatum** | JCM 3787T                         | AF444502              | AF070421  | AB073921   | KJ708091   | KJ708277   | KJ707865   | KJ707708   |
| **R. kratochvilaevae** | JCM 8171T                         | AF444520              | AF071436  | AB073923   | KJ708095   | KJ708205   | KJ707863   | KJ707733   |
| **R. paladgenum** | JCM 10292T                        | AF444492              | AF071434  | AB073924   | KJ708094   | KJ708206   | KJ707870   | KJ707676   |
| **R. sphaerocarpum** | JCM 8120T                         | AF444499              | AF071435  | AB073925   | KJ708086   | KJ708207   | KJ707867   | KJ707734   |
| **R. toruloides** | CBS 349                           | AF444498              | AF071436  | AB073926   | KJ708090   | KJ708278   | /          | KJ707623   |
| **R. toruloides** | AS 2.1389                         | KJ77637               | KJ708092   | KJ708265   | KJ707866   | KJ707866   | KJ707625   |            |
| **Rhodotorula araucariae** | JCM 3770T                         | AF444510              | AF070424  | AB073927   | KJ708096   | KJ708209   | KJ707862   | AB041048   |
| **R. dairenensis** | CBS 4046T                         | AF444501              | AF070425  | AB073928   | KJ708097   | KJ708210   | KJ707866   | KJ707625   |            |
| **R. evergladiensis** | CBS 10880T                        | FJ008054              | FJ008084  | KJ708398   | /          | KJ708228   | KJ707834   |            |
| **R. glutinis** | JCM 8208T                         | AF444539              | AF070426  | X89653     | /          | /          | KJ707869   | AB040626   |
| **R. graminis** | JCM 3775T                         | AF444505              | AF070431  | X89687     | KJ708093   | KJ708234   | KJ707868   | AB040628   |
| **R. mucilaginosa** | JCM 8115T                         | AF444541              | AF070432  | AB21668    | /          | /          | KJ707861   | KJ707731   |
| **Sporobolomyces magnisporus** | JCM 11898T                        | AB030342              | AF207886  | KJ708404   | KJ708089   | KJ708209   | KJ707864   | KJ707714   |
| **Sporidiobolus microsporus** | JCM 6882T                         | AF444535              | AF070436  | KJ708441   | KJ708054   | KJ708284   | KJ707817   | KJ707724   |
| **Sporidiobolus microsporus** | JCM 6882T                         | AF444535              | AF070436  | KJ708441   | KJ708054   | KJ708284   | KJ707817   | KJ707724   |
| **Microbotryomycetes** | **Sporidiobolales**               | ****                  | **        | **         | **         | **         | **         | **         | **         |
| **R. fluviatile** | JCM 10311T                        | AF189915              | AF070437  | AB073922   | KJ708046   | KJ708204   | KJ707816   | KJ707679   |
| **R. lusitaniae** | JCM 8547T                         | AF189915              | AF070437  | AB073924   | KJ708047   | /          | KJ707812   | KJ707737   |
| **Rhodotorula colostris** | CBS 349                           | JN246563              | JN246563  | KJ708399   | KJ708051   | KJ708220   | KJ707818   | KJ707622   |
| **S. ruinesiae** | JCM 1839T                         | AF444591              | AF070434  | AB021693   | KJ708052   | KJ708286   | KJ707820   | KJ707700   |
| **S. odoratus** | JCM 1164T                         | KJ778628              | KJ708045  | KJ708322   | KJ707819   | KJ707694   |            |            |
### Table 1. (Continued)

| Lineage/Clade       | Species      | Strain number | ITS    | D1D2   | SSU   | RPB1  | RPB2  | TEF1 | CYTB   |
|---------------------|--------------|---------------|--------|--------|-------|-------|-------|------|--------|
| **Kriegeriales**    |              |               |        |        |       |       |       |      |        |
| **Kriegeriaceae**   |              |               |        |        |       |       |       |      |        |
| Kriegeria           | K. eriophori | CBS 8387T     | AF444602 | NR_119455 | DQ419918 | KJ708144 | KJ708174 | KJ707936 | KJ707649 |
| glacialis           | R. glacialis  | CBS 10436T    | EF151249 | EF151258 | KJ708381 | KJ708067 | KJ708233 | KJ707831 | KJ707597 |
| R. psychrophenoica  | CBS 10438T   | EF151246 | EF151255 | KJ708382 | KJ708071 | KJ708259 | KJ707859 | KJ707598 |        |
| R. psychrolithica   | CBS 10440T   | EF151243 | EF151252 | KJ708383 | / | KJ708260 | KJ707833 | KJ707599 |        |
| **Single-species**  |              |               |        |        |       |       |       |      |        |
| **Camptobasidiaceae** |              |               |        |        |       |       |       |      |        |
| Glaciozyma          | G. antarctica | JCM 9057T  | 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 creatinivorum | JCM 10699     | KJ778627    | KJ708455    | KJ708385     | KJ708064    | KJ708221    | KJ707857    | KJ707687    |
|               | L. creatinivorum               | CBS 8620T     | AF444629    | AF189925    | KJ708418     | KJ708036    | KJ708178    | KJ707789    | KJ707658    |
|               | L. fellii                     | JCM 9887T     | AF444508    | AF189907    | KJ708449     | KJ708030    | KJ708184    | KJ707784    | KJ707748    |
|               | L. fragarium                  | JCM 9330      | AF444530    | AF070428    | KJ708437     | KJ708034    | KJ708231    | KJ707790    | AB040623    |
|               | L. fragarium                  | CBS 6254T     | AF444530    | AF070428    | KJ708413     | KJ708031    | KJ708179    | KJ707791    | AB040623    |
|               | L. golubevi                   | CBS 9651T     | AY212987    | AY212999    | KJ708386     | KJ708037    | KJ708185    | KJ707787    | /           |
|               | L. intermedium                | JCM 5291T     | AF444630    | AF189889    | D38235       | KJ708132    | KJ708188    | KJ707785    | KJ707711    |
|               | L. muscorum                   | CBS 6921T     | AF444527    | AF070433    | KJ708414     | KJ708038    | KJ708180    | KJ707793    | AB040638    |
|               | L. scotti                     | JCM 9052T     | AF444495    | AF070419    | X53499       | KJ708033    | KJ708186    | KJ707788    | AB040658    |
|               | L. yakuticum                  | JCM 10701     | AY212989    | AY189971    | KJ708426     | KJ708032    | KJ708274    | KJ707794    | KJ707688    |
| Microbotryales | Microbotryum reticulatum     | CBS 101451    | KJ778630    | KJ708457    | KJ708389     | KJ708040    | KJ708189    | KJ707806    | KJ707596    |
|               | M. scabiosae                  | CBS 677.93    | KJ708459    | KJ708459    | KJ708390     | /           | KJ708195    | KJ707808    | KJ70633    |
|               | M. scabiosae                  | CBS 176.24    | KJ708458    | KJ708458    | KJ708301     | KJ708039    | KJ708190    | KJ707810    | KJ70615    |
|               | M. scorzonerae                | CBS 685.93    | KJ708461    | KJ708461    | KJ708392     | /           | KJ708191    | KJ707804    | KJ70635    |
|               | M. scorzonerae                | CBS 364.33    | KJ708460    | KJ708460    | KJ708393     | KJ708043    | KJ708196    | KJ707805    | KJ70624    |
|               | M. violaceum                  | CBS 143.21    | KJ708462    | KJ708462    | KJ708388     | KJ708042    | KJ708192    | KJ707811    | KJ70613    |
|               | Sphacelotheca hydropiperis    | CBS 179.24    | KJ708463    | KJ708463    | KJ708394     | KJ708041    | KJ708281    | KJ707807    | KJ70616    |
|               | S. koordersiana               | JAG 55        | DQ832221    | DQ832219    | DQ832220     | DQ832222    | DQ832222    | /           | /           |
|               | Single-species lineage        | JCM 3932T     | AF444524    | AF189933    | AY657013     | /           | KJ708235    | KJ707802    | /           |
| Heterogastridiales | Heterogastridium           | CBS 591.93    | GU291276    | GU291290    | KJ708412     | KJ708009    | KJ708170    | KJ707770    | KJ70630    |
| Incertae sedis in Microbotryomycetes | H. hordeae           | JCM 1692T    | AF444536    | AF189923    | KJ708363     | KJ708130    | KJ708216    | KJ707949    | AB040619    |
|               | Rhodotorula bogoriensis       | JCM 3929T     | AF444526    | AF189924    | KJ708362     | KJ708127    | KJ708217    | KJ707946    | AB040620    |
|               | R. buffonii                   | JCM 3934T     | AF444531    | AF189964    | KJ708361     | KJ708128    | KJ708261    | KJ707937    | AB040642    |
|               | R. creolica                   | JCM 10955T    | AF444570    | AF189926    | KJ708365     | KJ708135    | KJ708222    | KJ707942    | /           |
|               | R. pilati                     | JCM 9036T     | AF444598    | AF189963    | KJ708364     | KJ708137    | KJ708265    | KJ707947    | AB040641    |
|               | Sporobolomyces tsugae         | JCM 2960T     | AF444580    | AF189998    | AB021692     | /           | KJ708340    | KJ707945    | KJ707628    |
|               | tsugae                        | JAG 55        | DQ836223    | DQ836222    | DQ836222     | DQ836222    | DQ836222    | /           | /           |
|               | yarrowii                      | CBS 11420T    | GO121045    | GO121044    | KJ708366     | KJ708069    | KJ708264    | KJ707849    | KJ707610    |
|               | R. straminea                  | CBS 10976T    | EU872491    | EU872489    | KJ708367     | KJ708065    | KJ708269    | KJ707844    | KJ707606    |
| Lineage/Clade | Species | Strain number | ITS | D1D2 | SSU | RPB1 | RPB2 | TEF1 | CYTB |
|--------------|---------|---------------|-----|------|-----|------|------|------|------|
|              | R. yarrowii | JCM 8232<sup>T</sup> | AF444628 | AF189971 | AB032658 | / | KJ708275 | KJ707838 | KJ707735 |
|              | griseoflavus | JCM 12422<sup>T</sup> | KP216522 | AB175591 | AB176530 | KJ708142 | KJ708303 | KJ707944 | KJ707698 |
|              | S. griseoflavus | JCM 5653<sup>T</sup> | AF444557 | AF189986 | D66884 | KJ708143 | KJ708305 | KJ707950 | KJ707717 |
|              | yamatoana | Benningtonia yamatoana | AS 2.1956<sup>T</sup> | AF444634 | AF189896 | D38239 | KJ708141 | KJ708160 | KJ707948 | KJ707572 |
|              | Rhodotorula arctica | CBS 9278 | AB478857 | AB478858 | / | KJ708371 | KJ708070 | KJ708210 | KJ707856 | KJ707666 |
|              | singularis | R. lignophila | CBS 7109<sup>T</sup> | AF444513 | AF189943 | / | KJ708372 | KJ7081139 | KJ708241 | KJ707953 | KJ707637 |
|              | Colacogloea | Colacogloea peniophorae | CBS 684.93 | DQ202270 | AY629313 | DQ234565 | DQ234569 | DQ234550 | DQ234566 | / |
|              | Rhodotorula cycloclastica | CBS 8448<sup>T</sup> | AF444732 | AF444631 | / | KJ708376 | KJ707997 | KJ708224 | KJ707775 | KJ707652 |
|              | R. diffinens | JCM 1695<sup>T</sup> | AF444533 | AF075465 | / | KJ708380 | KJ708125 | KJ708226 | KJ707939 | AB040621 |
|              | R. eucalyptica | CBS 8499<sup>T</sup> | EU075185 | EU075183 | / | KJ708377 | KJ708061 | KJ708227 | KJ707839 | KJ707655 |
|              | R. folorum | JCM 1996<sup>T</sup> | AF444633 | AF317804 | / | KJ708378 | KJ708126 | KJ708230 | KJ707941 | AB040622 |
|              | R. philyra | JCM 3933<sup>T</sup> | AF444506 | AF075471 | / | KJ708438 | KJ708095 | KJ708254 | KJ707772 | KJ707631 |
|              | R. retinophila | CBS 8446<sup>T</sup> | AF444624 | AF444730 | / | KJ708373 | KJ708094 | KJ708262 | KJ707771 | KJ707651 |
|              | R. terpenoidalis | CBS 8445<sup>T</sup> | AF444623 | AF444729 | / | KJ708374 | KJ707999 | KJ708272 | KJ707774 | KJ707650 |
|              | Sporobolomyces falcatus | JCM 6838<sup>T</sup> | AF444534 | AF189934 | / | KJ708445 | KJ708004 | KJ708237 | KJ707803 | AB040631 |
|              | R. vanillica | JCM 9741<sup>T</sup> | AF444575 | AF189970 | / | KJ708448 | KJ708005 | KJ708273 | KJ707809 | KJ707747 |
|              | sonckii | R. auriculariae | JCM 1597<sup>T</sup> | AF444507 | AF188922 | / | KJ708429 | KJ708134 | KJ708213 | KJ707935 | AB040617 |
|              | R. sonckii | JCM 3935<sup>T</sup> | AF444601 | AF189969 | / | KJ708439 | KJ708118 | KJ708267 | KJ707911 | AB040643 |
|              | Curvibasidium | Curvibasidium cygneicollum | JCM 10310<sup>T</sup> | AF444900 | AF189928 | / | KJ708423 | KJ708001 | KJ708169 | KJ707768 | KJ707678 |
|              | C. cygneicollum | JCM 9029<sup>T</sup> | AB038090 | KP216511 | / | KJ708444 | KJ708062 | KJ708232 | KJ707836 | AB040625 |
|              | C. pallidicoralium | CBS 9029<sup>T</sup> | AF444641 | AF444736 | / | KJ708420 | KJ708000 | KJ708273 | KJ707809 | KJ707747 |
|              | Rhodotorula nothofagi | JCM 9034 | AF444537 | AF189950 | / | KJ708447 | KJ708002 | KJ708248 | KJ707765 | AB040639 |
|              | Reniforma | Reniforma strues | CBS 8263<sup>T</sup> | AF444573 | AF189912 | / | KJ708157 | KJ708122 | KJ708200 | KJ707927 | KJ707648 |
|              | Single-species lineage | Pseudoleucosporidium fasciculatum | CBS 8786<sup>T</sup> | KJ778628 | AY212993 | / | KJ708387 | KJ707998 | KJ708133 | KJ707769 | / |
|              | Rhodotorula crocea | CBS 2029<sup>T</sup> | FM957565 | AY372179 | / | KJ708410 | KJ708007 | KJ708223 | KJ708213 | KJ707618 |
|              | R. ferulica | JCM 8231<sup>T</sup> | AF444622 | AF363645 | / | KJ708431 | KJ708008 | KJ708236 | KJ707764 | AB040630 |
|              | R. hylophila | JCM 1805<sup>T</sup> | AF444622 | AF363645 | / | KJ708431 | KJ708008 | KJ708236 | KJ707764 | AB040630 |
|              | R. javanica | JCM 9032<sup>T</sup> | AF444532 | AF189935 | / | KJ708446 | KJ708006 | KJ708238 | KJ707766 | AB040632 |
|              | Sporobolomyces inositophilus | JCM 5654<sup>T</sup> | AF444559 | AF189987 | / | KJ708136 | KJ708306 | KJ707951 | KJ707718 | (continued on next page) |
| Lineage/Clade          | Species               | Strain number | ITS   | D1D2   | SSU   | RPB1       | RPB2       | TEF1       | CYTB          |
|------------------------|-----------------------|---------------|-------|--------|-------|------------|------------|------------|---------------|
| Mixiomyctes            | Mixia                 | 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 | TD8183-1   | /         | AF522169 | AY123292 | /           | /           | /           | /             |
|                        | Platygloea disciformis |IFO32431    | DQ234565 | AY629314 | DQ234563 | /           | DQ234554   | DQ056288   | /             |
|                        | Puccinia graminis tritic | CRL75-36-700-3/ECS | AF468044 | AF522177 | AY125409 | XM_00334476 | XM_003321826 | XM_00333024 | /             |
|                        | 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 | AJ486258 | KP322906   | KP323063   | KP323116   | AB041051      |
|                        | Ustilago maydis        |CBS 504.76/IFM 49220 | AF453938 | AY854090 | X62396 | XM401478 | AY485636 | AY885160 | AB040663      |
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 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_, _Classidulomycetes_, _Cystobasidiales_, _Cryptomycocolacomycetes_, _Microbotryomycetes_, _Mixiomyces_ 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_, _Cystobasidiales_, _Mixiomyces_ and _Pucciniomycetes_ (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 _Mixiomyces_ with 94–99 % BP and 1.0 PP support values.
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*, *Classicalimycetes*, *Pucciniomycetes* and *Tritirachiumycetes*, were separated as independent lineages. *Microbotryomycetes* exhibited a close relationship to the filamentous fungal lineage *Classicalimycetes* 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.

### Table 3. (Continued).

| Lineage/Clade                  | Seven genes  | rDNA          | Protein genes | SSU + D1D2       |
|-------------------------------|--------------|---------------|---------------|-----------------|
|                               | BP1/BP2/PP   | BP1/BP2/PP    | BP1/BP2/PP    | BP1/BP2/PP      |
| Erythrobasidium               | 100/100/1.0  | 97/86/1.0     | nm/nm/nm     | 93/91/1.0       |
| Banno                        | 100/100/1.0  | 100/100/1.0   | 100/100/1.0  | 99/100/1.0      |
| Incertae sedis                | 100/100/1.0  | 100/100/1.0   | 100/100/1.0  | 99/100/1.0      |
| auranicata                    | 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   | 99/99/1.0    | 99/100/1.0      |
| Sporidiobiales                | 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   | 88/100/1.0   | 94/97/1.0       |
| Mixed *Rhodosporidium*/Sporidiobolus | 88/89/1.0 | 100/100/1.0 | 82/100/1.0 | 98/98/1.0 |
| Sporidiobolus                 | 100/100/1.0  | 100/100/1.0   | 74/100/1.0   | 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                | 81/100/1.0   | 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                | 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      | 72/85/1.0    | 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. BP1 and BP2, bootstrap values from the maximum likelihood and maximum parsimony analyses, respectively; PP, Bayesian posterior probability; nm: not monophyletic; ns, not supported.
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 coprosimicola 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. coprosimicola, 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 1040 and 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, Trichies 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 phylodetic placements of Mycogloea and Spiculogloea species.

In the Agaricostilbales lineage, nine well-supported clades with yeasts species occurred, namely Agaricostilbum, Bensingtonia, Chionosphaera, Kondoia, 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 stipes 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 Kondoia clade accommodated two Kondoia 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 Kondoia 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 Kondoia clade. The close relationship of this clade with the Kondoia 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 Kondoia clade.

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 Sampiao (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 Agaricostilbales 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 sasicola 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 1000 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
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 Puccinioomycotina 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 1000 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 *Kondaceae*, 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 *Naohidea*, 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 *Naohidea* 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 *Cystobasidiomycetes* 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 fimetarum*, 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 *Cystobasidiomycetes* 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 *Cystobasidiomycetes* 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. fimetarum* 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, Scorza 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-10H2) 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 (Scorza 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 *Crenellina 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 *Cystobasidiomycetes* 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 telospores, although germination of telospores with basidia has not been observed (Gochanour 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 Scorza 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 study (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 dacyroidea produces teliospores (Yamada et al. 1994, Fell & Statzell-Tallman 1998), that are different from the sexual structures of Bannoia 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 comprised 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 Rhodesporidium, Sporidiobolus and mixed Rhodesporidium/Sporidiobolus clades (Fig. 7), are in agreement with Boekhout et al. (2011). The Rhodesporidium clade was composed of nine Rhodotorula and six Rhodesporidium species and Sporobolomyces alborubescens, including the type species of the former two genera (Rhodotorula glutinis and Rhodesporidium 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 Rhodesporidium/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 felli 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. felli and M. 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 Garcia 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 melabasidia 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 Garcia 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.
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 *Meredith-blackwellia 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 *Ustilytoma fluidans*, a parasite of *Glyceria* (Graminiae) plants (Vánky 2002). In the LSU rDNA D1/D2 domains, *R. hordea* differs from *Ustilytoma fluidans* by only one mismatch (Sampaio 2011a), suggesting that the former represents a yeast stage of *U. fluidans* or a closely related *Ustilytoma* species. No yeast species is included in the *Heterogastriales* which includes the genus *Heterogastrium*.

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 basidicarps in nature (Sampaio 2011). In the phylogenetic trees obtained from the seven genes, *Rhodotorula foliorum, Rhodotorula diffiusa* 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 *Pseudoleuco- sporidium* by de Garcia 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 *Puccinomyctolina* (Sampaio et al. 2004).

Among the species tentatively assigned to the *yamatoana/Leucosporidium antarcticum* group in Boekhout et al. (2011), three (*Kriegeria eriophorii, 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 *tsgae* clade included *Sporobolomyces tsgae* 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 *tsgae* 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/Leuco- sporidium 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 *Heterogastriales* 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 *Heterogastriales* 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 weak. 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 *Micro- botryomycetes* 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 *Heterogastriales pycnidio- deum* (*Heterogastriales*) 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 Puccinimycotina 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, Cystosporobolomyces, Microbotryomycetes, and Mixiomyces. These lineages are independent from Atractiellomycetes, Classicilumycetes, Cryptomyco colacomyces, Pucciniomycetes and Tritirachiomycetes that are formed by filamentous taxa in the Puccinimycotina.

The orders distinguished in previous studies except the Kriegeriales were all resolved as monophyletic groups in this study. The order Spiculogloeales were all resolved as monophyletic groups in this study. In addition to the orders distinguished in previous studies except the Kriegeriales, 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 Puccinimycotina based on the phylogenetic framework presented here and to implement the ‘One fungus = One Name’ principle.

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