Taxonomic studies in the Microbotryomycetidae: *Leucosporidium golubevii* sp. nov., *Leucosporidiella* gen. nov. and the new orders Leucosporidiales and Sporidiobolales

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The subclass Microbotryomycetidae (Basidiomycota, Urediniomycetes) comprises a remarkably diverse assemblage of fungi. This group includes phytoparasites, mycoparasites and probably also saprobes that show a wide range of ecological preferences. In order to study the phylogenetic relationships within the Microbotryomycetidae, and to develop a more natural classification system, mitosporic and meiosporic taxa were investigated using an integrated approach. Sequence data of 26S rDNA D1/D2 domains were analyzed using several procedures, including the Bayesian Markov chain Monte Carlo method of phylogenetic inference. Ultrastructural markers such as the type of septal pore and presence / absence of colacosomes were investigated and micromorphological and nutritional properties were compared. In this study the current concept of the genus *Leucosporidium* and its apparent polyphyletic nature were addressed, as well as the relationships of this genus with the Microbotryales and *Mastigobasidium*. The classification of the anamorphic species closely related to *Leucosporidium*, and the concepts of the order Sporidiales and family Sporidiobolaceae were also reviewed.

Taxonomic novelties: Leucosporidiales Sampaio, Weiss & Bauer; Leucosporidiaceae Sampaio, Weiss & Bauer; Sporidiobolales Sampaio, Weiss & Bauer; Sporidiobolaceae Moore emend. Sampaio, Weiss & Bauer; *Leucosporidiella* Sampaio; *Leucosporidiella creatinivora* (Golubev) Sampaio; *Leucosporidiella fragaria* (J.A. Barnett & Buhagiar) Sampaio; *Leucosporidiella muscorum* (di Menna) Sampaio; *Leucosporidiella yakutica* (Golubev) Sampaio; *Leucosporidium golubevii* Gadanho, Sampaio & Bauer

The subclass Microbotryomycetidae Swann (SWANN, FRIEDERS & MCLAUGHLIN 1999) includes a notably diverse assemblage of fungi that exhibit distinct life strategies. Some taxa like *Microbotryum* Lév. are phytoparasitic whereas other, such as *Colacogloea* Oberwinkler & Bandoni, are mycoparasitic (BAUER & OBERWINKLER 1991). A third group includes organisms usually regarded as saprobes, like *Leucosporidium* Fell, Statzell, Hunter & Phaff and *Rhodotorula* Harrison. However, since the life cycles of the species in the third group have not been investigated under natural conditions, parasitism cannot be completely ruled out. In fact, mycoparasitism has been observed in *Leucosporidium*, *Rhodosporidium* Banno and *Sporidiobolus* Nyland (BAUER, OBERWINKLER & VÁNKY 1997). The ecological preferences of the Microbotryomycetidae are also disparate. *Sporidiobolus* is normally found on the phylloplane, and some species of *Leucosporidium* and *Rhodosporidium* are associated with aquatic environments. In the present report, a new *Leucosporidium* species isolated from a fresh water environment, *L. golubevii* sp. nov., is described and compared with the other species in the genus. Some aspects of the classification of the Microbotryomycetidae above the species level are also addressed, namely (i) the current concept of the genus *Leucosporidium* and its apparent polyphyletic nature, (ii) the genus *Mastigobasidium* Golubev and its relationship with *Leucosporidium*, (iii) the classification of the anamorphic species closely related to *Leucosporidium*, and (iv) the order Sporidiales and family Sporidiobolaceae.

Material and methods

Yeast isolation

A water sample from river Olo, a mountain stream in the Alvão Natural Park (Northeast of Portugal), was collected in February 2000. Three 300 ml portions were immediately run through sterile 0.45 μm pore size and 47 mm diameter membrane filters. The filters were placed on MYP agar (malt extract 0.7 % w/v, yeast extract 0.05 % w/v, soytone 0.25 % w/v and agar 1.5 % w/v) plates supplemented with chloramphenicol 500 p.p.m. and incubated at 18 °C. The yeast colonies that formed on the membrane filters were purified in the

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usual way, using the same medium without antibiotic, and maintained in liquid nitrogen.

Morphological characterization and studies of sexual compatibility

For microscopy, cultures were grown on MYP agar at room temperature (20–23 °C) and studied with an Olympus BX50 microscope, using phase contrast optics. For determination of sexual compatibility, pairs of 2–4 days old cultures were crossed on SG agar (soytone 0.2 % w/v, glucose 0.2 % w/v and agar 1.5 % w/v), incubated at room temperature and regularly examined for the production of mycelium and teliospores.

Physiological characterization

Physiological and biochemical characterization was carried out according to the techniques described by YARROW (1998). Additional assimilation tests were performed using aldaric acids and aromatic compounds, as described by FONSECA (1992) and SAMPAIO (1999), respectively. The numerical analysis of physiological traits was done using the results of 68 tests, the Simple Matching coefficient, the UPGMA clustering method (SNEATH & SOKAL 1973), and the software NTSYSpc, version 2.02h (ROHLF 1998).

Ultrastructure

For transmission electron microscopy samples were fixed overnight in 2 % glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2. Following six transfers in 0.1 M sodium cacodylate buffer, the material was post-fixed in 1 % osmium tetroxide in the same buffer for 2 h in the dark, washed in distilled water, and stained in 1 % aqueous uranyl acetate for 1 h in the dark. After five washes in distilled water, samples were dehydrated in acetone, using 10 min changes at 25 % (v/v), 50 %, 70 % and 95 % and three times in 100 % acetone. The material was embedded in SPURR'S (1969) plastic. Serial sections (65–75 nm) were cut with a Reichert-Jung Ultracut E (Leica, Nußloch), equipped with a diamond knife. Sections were mounted on Formvar-coated single slot copper grids, stained with lead citrate (REYNOLDS 1963) at room temperature for 3-5 min, and washed again with water. The thin sections were examined at 80 kV with a Zeiss EM 109 transmission electron microscope.

Sequence analyses

The species studied are listed in Table 1. For rDNA sequence analysis, total DNA was extracted using the protocol of SAMPAIO et al. (2001) and amplified using primers ITS5 (5' GGA AGT AAA AGT CGT AAC AAG G) and LR6 (5' CGC CAG TTC TGC TTA CC). Cycle sequencing of the 600-650 base pair region at the 5' end of the 26S rDNA D1/D2 domains employed forward primer NL1 (5' GCA TAT CAA TAA GCG GAG GAA AAG) and reverse primer NL4 (5'GGT CCG TGT TTC AAG ACG G). Sequences were obtained with an Amersham Pharmacia ALF Express II automated sequencer using standard protocols. Alignments were made with MegAlign (DNAStar) and visually corrected using Se-Al (RAMBAUT 1996). For the phylogenetic analyses, 58 nucleotide sites from the final alignment were excluded due to ambiguous aligning possibilities. To estimate phylogenetic relationships we used several approaches. We applied a Bayesian Markov chain Monte Carlo method of phylogenetic inference (MCMC; LARGET & SIMON 1999) as implemented in the computer program MrBayes (HUELSENBECK & RONQUIST 2001). This method allows estimation of the *a posteriori* probability that groups of taxa are monophyletic given the DNA alignment (i.e., the probability that corresponding bipartitions of the species set are present in the true unrooted tree including the given species). This method has been applied to efficiently reconstruct phylogenetic relationships, e.g., by MURPHY et al. (2001) for mammalian phylogeny, and by MAIER et al. (2003) and GARNICA, WEIß & OBERWINKLER (in press) for different fungal groups. Four incrementally heated simultaneous Monte Carlo Markov chains were run over 2000000 generations using the general time reversible model of DNA substitution with gamma distributed substitution rates (GTR+G; see SWOFFORD et al. 1996), random starting trees, and default starting parameters of the DNA substitution model. Trees were sampled every 100 generations resulting in an overall sampling of 20000 trees. From those trees that were sampled after the process had reached a stationary stage, a 50 % majority rule consensus tree was computed to obtain estimates for the *a posteriori* probabilities. This Bayesian approach to phylogenetic analysis was repeated several times on a Macintosh G4 computer, always using random starting trees and default starting values for the model parameters to test the reproducibility of the results. The second approach was the neighbor-joining method (SAITOU & NEI 1987) in the BIONJ modification of GASCUEL (1997) as implemented in PAUP*, version 4b10 (SWOFFORD 2001), using genetic distances derived from the TrN+I+G model of DNA substitution allowing one class of substitution rates for transversions, two classes of substitution rates for transitions, unequal nucleotide frequencies and assuming a portion of invariable nucleotide sites with gamma distributed mean substitution rates of the remaining sites (see SWOFFORD et al. (1996) for a survey of these parameters). This model of DNA substitution was suggested by a series of hierarchical likelihood ratio tests as implemented in Modeltest, version 3.06 (POSADA & CRANDALL 1998). BIONJ analysis was validated using 1000 rounds of bootstrap analysis (FELSENSTEIN 1985) with PAUP*. Using the same software, we additionally applied heuristic maximum parsimony analysis (MP; 100 rounds of heuristic search with TBR branch swapping, starting from trees obtained by random addition of sequences, multrees option on, deepest descent option off, in each round saving no more than 10 trees with less than 1002 steps) and heuristic maximum likelihood analysis (ML; heuristic search using TBR branch swapping with the BIONJ tree as starting tree; multrees option on, deepest descent option off).

Tab. 1. List of taxa and respective accession numbers of D1/D2 26S rDNA sequences used in the molecular phylogenetic analyses. Identical sequences are indicated with an * and were not included in the phylogenetic trees (Figs. 12 and 13).

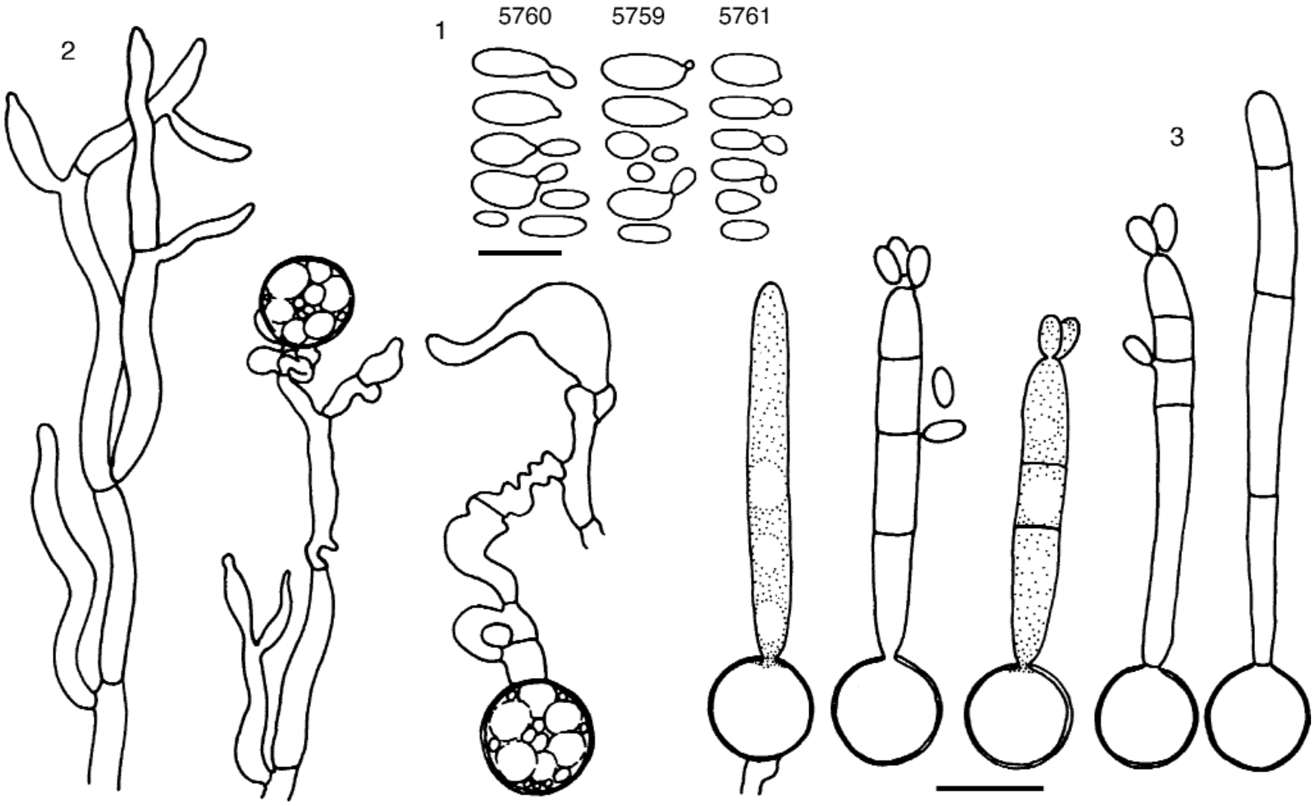
Taxon	Strain		GenBank
Leucosporidiales			
Leucosporidium scottii	CBS 5930^{T}	= PYCC 4405	AY213000
Leucosporidium scottii	CBS 5932	= PYCC 4425	AF189908
Leucosporidium fellii	CBS 7287^{T}	= PYCC 4403	AF199907
Leucosporidium golubevii	PYCC 5759^{T}	= A358	AY212997
*Leucosporidium golubevii**	PYCC 5760	= A366	AY212998
*Leucosporidium golubevii**	PYCC 5761	= A371	AY212999
*Leucosporidium golubevii**	KCTC 17091		AF459708
Mastigobasidium intermedium	CBS 7226^{T}	= PYCC 5340	AF189889
*Mastigobasidium intermedium**	CBS 7281	= PYCC 5458	AF189890
Leucosporidiella creatinivora	CBS 8620^{T}		AF189925
*Leucosporidiella creatinivora**	KCTC 17084		AF459701
Leucosporidiella fragaria	CBS 6254^{T}	= PYCC 4494	AF070428
*Leucosporidiella fragaria**	PYCC 5272		AY212996
*Leucosporidiella fragaria**	CBS 6253		AY212994
*Leucosporidiella fragaria**	CBS 6256		AY212995
Leucosporidiella muscorum	CBS 6921^{T}	= PYCC 4498	AF070433
*Leucosporidiella muscorum**	PYCC 4848		AY213008
Leucosporidiella yakutica	VKM Y-2837^{T}		AY213001
Microbotryales			
Aurantiosporium subnitens	MP 1173		AF009846
Fulvisporium restifaciens	HUV 17637		AF 009860
Liroa emodensis	MP 2520		AY212992
Microbotryum reticulatum	RB 2057		AY213003
Microbotryum violaceum	FO 31653	= PYCC 4279	AY213005
*Microbotryum violaceum**	FO 38227		AF009866
Microbotryum silenes-inflatae	GD 1291	= PYCC 4291	AY213004
Microbotryum violaceo-irregulare	GD 1357	= PYCC 4278	AY213006
Microbotryum cordae	GD 1675	= PYCC 4294	AY213002
Sphacelotheca polygoni-persicariae	GD 1655	= PYCC 4293	AF189974
Ustilentyloma fluitans	RB 900		AF009882
Rhodotorula hordea	CBS 6403^{T}	= PYCC 4527	AF189933
Sporidiobolales			
Rhodosporidium azoricum	PYCC 5062^{T}		AF321977
Rhodosporidium babjevae	CBS 7808^{T}	= PYCC 5168	AF070420
Rhodosporidium diobovatum	CBS 6085^{T}	= PYCC 4364	AF070421
Rhodosporidium fluviale	CBS 6568^{T}	= PYCC 4701	AF070422
Rhodosporidium kratochvilovae	CBS 7436^{T}	= PYCC 4583	AF071436
Rhodosporidium lusitaniae	CBS 7604^{T}	= PYCC 4641	AF070423
Rhodosporidium paludigenum	CBS 6566^{T}	= PYCC 4495	AF363640
Rhodosporidium sphaerocarpum	CBS 5939^{T}	= PYCC 4104	AF070425
Rhodosporidium toruloides	CBS 14	= PYCC 4416	AF207884
Rhodotorula araucariae	CBS 6031^{T}	= PYCC 4625	AF070427
Rhodotorula dairenensis	CBS 4406^{T}	= PYCC 4885	AF070429
Rhodotorula glutinis	CBS 20^{T}	= PYCC 4177	AF070430
Rhodotorula graminis	CBS 2826^{T}	= PYCC 4842	AF070431
Rhodotorula mucilaginosa	CBS 316^{T}	= PYCC 5166	AF070432
Sporidiobolus johnsonii	CBS 5470^{T}	= PYCC 3834	AF070435
Sporidiobolus microsporus	CBS 7041^{T}	= PYCC 5617	AF070436
Sporidiobolus pararoseus	CBS 491^{T}	= PYCC 5439	AF189977
Sporidiobolus ruineniae	CBS 5001^{T}	= PYCC 4201	AF070438
Sporidiobolus salmonicolor	CBS 490^{T}	= PYCC 4111	AF070439
Sporobolomyces alborubescens	CBS 482^{T}	= PYCC 5362	AF189961
Sporobolomyces blumae	JCM 10212^{T}		AY213010
Sporobolomyces marcilae	CBS 4217^{T}	= PYCC 5534	AF070440
Sporobolomyces nylandii	JCM 10213^{T}	= PYCC 5693	AF387123

Tab. 1. Continued

Taxon	Strain		GenBank
Sporobolomyces odoratus	PYCC 5694^{T}		AF387125
Sporobolomyces poonsookiae	JCM 10207^{T}	= PYCC 5699	AF387124
Sporobolomyces ruberrimus	CBS 7500^{AUT}	= PYCC 5678	AF070442
Sporobolomyces roseus	CBS 486^{LT}	= PYCC 4463	AF070441
Other taxa			
Bensingtonia yamatoana	CBS 7243^{T}	= PYCC 5346	AF189896
Camptobasidium hydrophilum	CCM 8060		AY212991
Colacogloea peniophorae	FO 22315	= PYCC 4285	AF189989
Heterogastridium pycnidioideum	CBS 591.93		AF189900
Kriegeria eriophori	DJM 463-SS6	= CBS 8387	AF189905
Leucosporidium antarcticum	CBS 5942^{T}	= PYCC 5541	AF189906
Leucosporidium fasciculatum	KGM 3696^{T}	= PYCC 5682	AY212993
Naohidea sebacea	CJC 1083		AF131061
Occultifur externus	PYCC 4817^{T}		AF131062
Rhodotorula auriculariae	CBS 6379^{T}	= PYCC 5049	AF189922
Rhodotorula bogoriensis	CBS 4101^{T}	= PYCC 3432	AF189923
Rhodotorula buffonii	CBS 7150^{T}	= PYCC 3050	AF189924
Rhodotorula cresolica	CBS 7998^{T}	= PYCC 5357	AF189926
Rhodotorula diffluens	CBS 5233^{T}	= PYCC 3670	AF075485
Rhodotorula ferulica	PYCC 4524^{T}	= CBS 7416	AF363653
*Rhodotorula ferulica**	PYCC 4504	= CBS 7402	AF189927
Rhodotorula foliorum	CBS 5234^{T}	= PYCC 3668	AF317804
Rhodotorula foliorum	CBS 6370		AF075499
Rhodotorula fujisanensis	CBS 4551^{T}	= PYCC 3116	AF189928
*Rhodotorula fujisanensis**	CBS 6371	= PYCC 4444	AF189929
*Rhodotorula fujisanensis**	CBS 8264		AF189930
Rhodotorula futronensis	CBS 8163^{T}	= PYCC 4841	AF189931
Rhodotorula hylophila	CBS 6226^{T}	= PYCC 4850	AF363645
Rhodotorula ingeniosa	CBS 4240^{T}	= PYCC 3188	AF189934
*Rhodotorula ingeniosa**	KCTC 17090		AF459707
Rhodotorula ingeniosa	KCTC 17089		AF459706
Rhodotorula javanica	CBS 5236^{T}	= PYCC 3669	AF189935
*Rhodotorula javanica**	CBS 4977		AY213007
Rhodotorula lignophila	CBS 7109^{T}	= PYCC 4522	AF189943
Rhodotorula minuta	CBS 319^{T}	= PYCC 4790	AF189945
Rhodotorula nothofagi	CBS 8166^{T}	= PYCC 4844	AF189950
Rhodotorula philyla	CBS 6272^{T}	= PYCC 4845	AF075471
Rhodotorula pilati	CBS 7039^{T}	= PYCC 4497	AF189963
Rhodotorula pustula	CBS 6527^{T}	= PYCC 4853	AF189964
Rhodotorula sonckii	CBS 6713^{T}	= PYCC 4848	AF189969
Rhodotorula sonckii	CBS 8115		AY213009
Rhodotorula vanillica	CBS 7404^{T}	= PYCC 4506	AF189970
Rhodotorula yarrowii	CBS 7417^{T}	= PYCC 4525	AF189971
Sakaguchia dacryoidea	CBS 6353^{T}	= PYCC 4491	AF189972
Sporobolomyces falcatus	CBS 7368^{T}	= PYCC 6838	AF075490
Sporobolomyces griseoflavus	CBS 7284^{T}	= PYCC 5421	AF189986
Sporobolomyces inositophilus	CBS 7310^{T}	= PYCC 5422	AF189987
Sporobolomyces singularis	CBS 5109^{T}	= PYCC 5371	AF189996
Sporobolomyces tsugae	CBS 5038^{T}	= PYCC 5424	AF189998

Acronyms of culture collections: CBS, Centraalbureau voor Schimmelcultures, Yeast Division, Utrecht, The Netherlands; CCM, Czech Collection of Microorganisms, Brno, Czech Republic; JCM, Japan Collection of Microorganisms, RIKEN, Japan; KCTC, Korean Collection for Type Cultures, Korea; KGM, Yeast Culture Collection of the Department of Soil Science, Moscow State University, Russia; PYCC, Portuguese Yeast Culture Collection, FCT-UNL, Portugal; VKM, All-Russian Collection of Microorganisms, Moscow, Russia. CJC, DJM, FO, GD, HUV, MP, RB: collections of Drs. C.-J. Chen, D.J. McLaughlin, F. Oberwinkler, G. Deml, K. Vánky, M. Piepenbring and R. Bauer, respectively.

^{AUT}, authentic strain; ^{LT}, lectotype; ^{T}, type strain.



Figs. 1-3. Line drawings of *Leucosporidium golubevii*. 1. Yeast cells grown on MYP agar at room temperature for 4-5 days. 2. Mycelium and teliospores of PYCC 5759T x PYCC 5760 grown on SG agar at room temperature for one week (hypha on the left) and three weeks (hyphae with teliospores). 3. Germinated teliospores, transversally septate basidia and sessile basidiospores of PYCC 5759T x PYCC 5761 (6 weeks old teliospores produced on SG agar at room temperature, soaked for 2 weeks at 4 °C, then transferred to 2% water agar and observed after 5 days). Bars = 10 μm (bar in 3 is the same for 2).

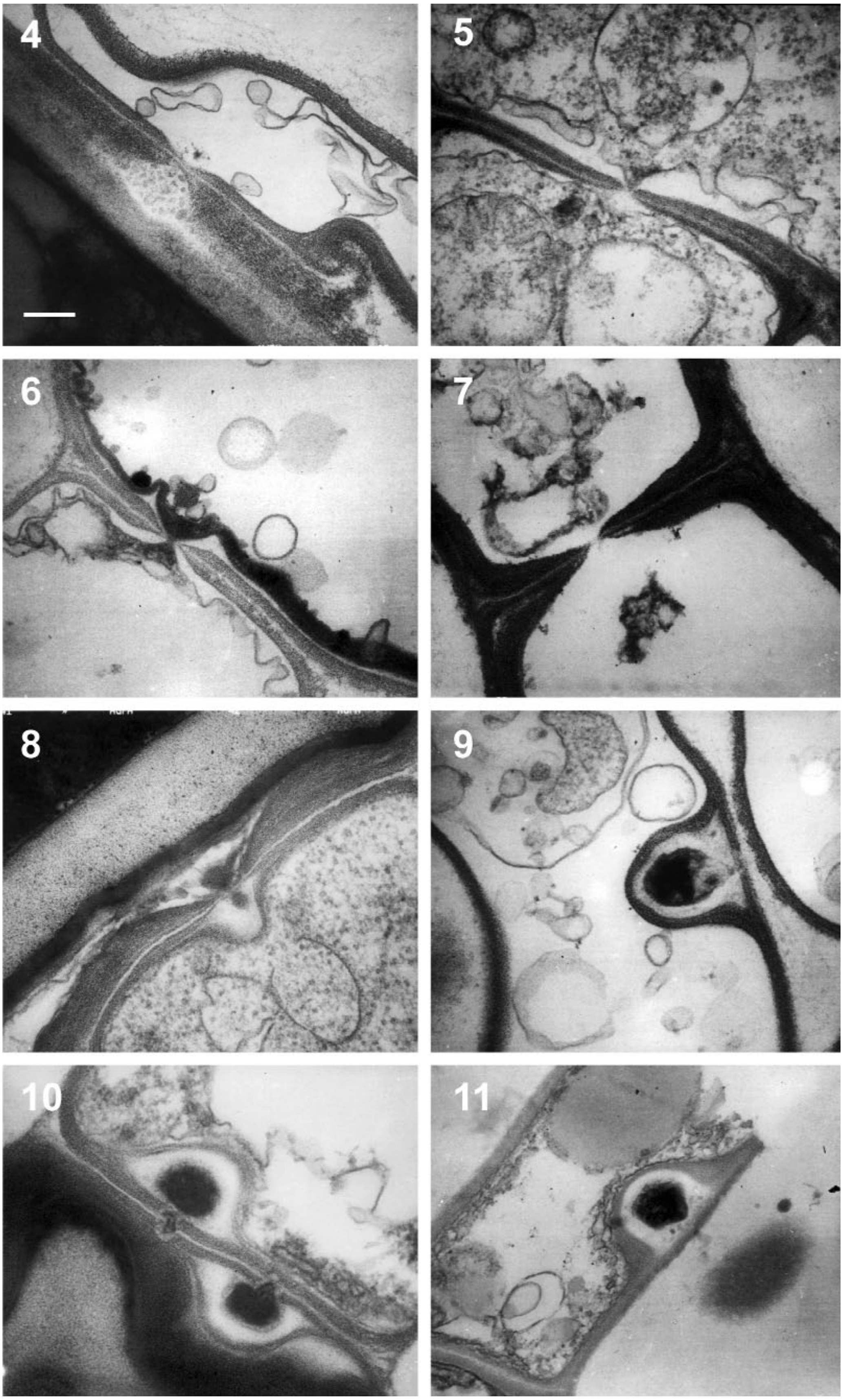
Results and Discussion

Leucosporidium golubevii Gadanho, Sampaio & Bauer sp. nov.

Cultura in striis post unum mensem ad 20–22 °C cremea, nitens, mucosa, laevis. Cellulae post 4 dies in malto-fermento-peptono-agaro (2) 3.5–4.5 x (4) 6–10 (11) μm. Mycelium 1–2 μm diametro, procreatum culturis sexualiter compatibilibus iunctis. Mycelium iuvene quasi efibulatum; mycelium maturum fibulis infrequentibus, plerumque basi teliosporarum. Teliosporae globosae, (8) 9–11 μm diametro, plerumque terminales. Basidia transversaliter septata, (2.5) 3–4 (4.5) x (30) 36–45 (56) μm, plerumque 4cellulata. Basidiosporae ovoideae, 1.5–2 x 4–5 μm. Pori septorum simplices. Colacosomata in mycelio praesentia. Fungus heterothallicus typis iungendi duobus cognitis. Cultura PYCC 5759 ad typum iungendi A1, culturae PYCC 5760 et PYCC 5761 ad typum iungendi A2 pertinent.

Streak culture after one month at 20–22 °C cream colored, shiny, mucilaginous, and surface smooth. Yeast cells after four days on MYP agar (2) 3.5–4.5 x (4) 6–10 (11) μm (Fig. 1). Mycelium 1–2 μm in diameter, formed after crossing of sexually compatible strains. Young mycelium almost devoid of clamp connections (Fig. 2). Mature mycelium has infrequent clamp connections, which normally are present at the base of teliospores (Fig. 2). Teliospores spherical, (8) 9–11 μm in diameter, frequently terminal. Basidia transversally septate, measuring (2.5) 3–4 (4.5) x (30) 36–45 (56) μm, normally four-celled (Fig. 3). Basidiospores ovoid, measuring 1.5–2 x 4–5 μm (Fig. 3). Septal pore simple (Fig. 8). Colacosomes present in the mycelium (Fig. 10). Heterothallic, two mating types known. The strain PYCC 5759 belongs to mating type A1 and the strains PYCC 5760 and PYCC 5761 belong to mating type A2. In the molecular phylogenetic analyses, the D1/D2 sequence of strain KCTC 17091 was retrieved from the GenBank database and found to be identical to those of the Portuguese isolates. Therefore, the strain from the Korean Collection of Type Cultures was considered as possible additional member of this species. However, since the culture was not available for study no definite conclusions could be drawn. The physiological and biochemical profile of *L. golubevii* is depicted in Table 2 and its phylogenetic placement is shown in Figs. 12 and 13.

Etymology. The specific epithet *golubevii* refers to Dr. W. I. Golubev and is a tribute to his numerous contributions to yeast systematics.



Figs. 4–11. Transmission electron micrographs of septal pores and colacosomes of several *Leucosporidium* species and of colacosomes of *Mastigobasidium intermedium*. Septal pores of *Leucosporidium antarcticum* PYCC 5541T (4), *L. fasciculatum* PYCC 5682T (5), *L. fellii* PYCC 4403T (6), *Rhodotorula fujisanensis* PYCC 3116T x PYCC 4444 (7) and *L. golubevii* PYCC 5759T x PYCC 5760 (8). Colacosomes of *L. fellii* PYCC 4403T (9), *L. golubevii* PYCC 5759T x PYCC 5760 (10) and *M. intermedium* PYCC 5340T x PYCC 5458 (11). Bar = 0.1 μm (same for all micrographs).

Origin, Type and Deposits. Strains PYCC 5759, PYCC 5760 and PYCC 5761 were isolated by M. Gadanho and J.P. Sampaio in February 2000 from a 300 ml water sample collected in the river Olo, Natural Park of Alvão, Northeast Portugal. Microscopic slides from the crossing of PYCC 5759 and PYCC 5761 showing mycelium, teliospores, basidia and basidiospores were deposited at the Portuguese Yeast Culture Collection under nº ZP-01-02 (holotype). Since the physiological and molecular characterization of a mixed culture presents obvious disadvantages, we propose that strain PYCC 5759 is designated the type strain of *L. golubevii*.

The life cycle of *L. golubevii* was investigated on SG agar. Conjugation occurred 24-72h after the crossing of sexually compatible strains. Scraping the yeast cells from the surface of the culture medium after approximately one week allowed the detection of localized zones with mycelium. In the initial stages of mycelial development, hyphae were devoid of clamp connections and wider than those that subsequently formed teliospores. Teliospores germinated directly on SG agar after approximately 6–10 weeks or, alternatively, after agar blocks of that age had been transferred to 2% water agar and incubated for 4–6 days at room temperature.

The new species was found after performing crossings of three cream-colored yeast strains isolated from fresh water. Evidence that the three strains were conspecific and distinct from other macroscopically similar strains isolated from the same water sample was obtained in DNA fingerprinting experiments using the microsatellite-primed PCR approach (MSP-PCR). This method was already employed by us for the detection of new yeast species, see for example GADANHO, SAMPAIO & SPENCER-MARTINS (2001), and in the present study primer $(GTG)_5$ was used (data not shown).

Phylogenetic placement of *Leucosporidium golubevii*

In order to determine the phylogenetic placement of the new species, the nucleotide sequences of the D1/D2 domains of the 26S rDNA of representative members of the Microbotryomycetidae were analyzed. The various molecular phylogenetic analyses yielded consistent results as shown in Figs. 12 and 13 (BIONJ and MCMC, respectively; MP and ML trees not shown). The closest teleomorphic relative of *Leucosporidium golubevii* was *L. scottii*, the type species of the genus. Considering also mitosporic taxa, *Rh. fragaria* (J.A. Barnett & Buhagiar) Rodrigues de Miranda & Weijman was the closest relative of *L. golubevii* with only three nucleotide substitutions in the D1/D2 domains and almost identical physiological profiles (Table 3). In order to elucidate in more detail the relationship between *L. golubevii* and its relatives, the complete ITS region, normally more variable, was investigated. Again, *Rh. fragaria* (CBS 6254, AF444530) was the closest relative of *L. golubevii* (PYCC 5759, AY212987) but eighteen nucleotide differences were recorded between them, which supports the proposal of *L. golubevii* as a distinct species. Another argument pointing to the concept of distinct species is that no mating reactions were observed between *Rh. fragaria* and *L. golubevii*. A phylogenetic tree based on ITS data is not presented because the large number of nucleotide substitutions observed for the Microbotryales, *Leucosporidium* and closely related *Rhodotorula* species originated unreliable sequence alignments.

The heterogeneity of *Leucosporidium*

The extensive molecular phylogenetic analyses shown in Figs. 12 and 13 indicate that *Leucosporidium* is polyphyletic. According to the BIONJ tree (Fig. 12), *Leucosporidium antarcticum* Fell, Statzell, Hunter & Phaff and the recently described *L. fasciculatum* Bab'eva & Lisichkina (BAB'EVA & LISICHKINA, 2000) are not related to *L. scottii, L. golubevii* and *L. fellii* Giménez-Jurado & van Uden. The *Leucosporidium* core group includes also *Mastigobasidium intermedium* Golubev and the anamorphic species *Rhodotorula creatinivora* Golubev, *Rh. fragaria*, *Rh. muscorum* (di Menna) von Arx & Weijman and *Rh. yakutica* Golubev. Also in the MCMC tree (Fig. 13), *L. antarcticum* and *L. fasciculatum* are separated from *L. scottii* and *L. golubevii*. The main discrepancies between the two trees are the positions of *L. fellii* and *M. intermedium*. Although in all analyses performed these two species remain closely related, in the BIONJ tree they appeared at the base of the *Leucosporidium* core group, whereas in the MCMC tree they were located at the base of the Microbotryales.

The heterogeneity of *Leucosporidium* with respect to sequence analysis correlates with the presence or absence of colacosomes. The species of the core group in the BIONJ tree (*L. scottii*, *L. fellii* and *L. golubevii*) possess these structures: colacosomes of *L. fellii* and *L. golubevii* are depicted in Figs. 9 and 10 respectively, and colacosomes of *L. scottii* have been reported by KREGER VAN RIJ & VEENHUIS 1971 and MOORE 1972. In *M. intermedium*, another teleomorphic species of the *Leucosporidium* core group, colacosomes were also found (Fig. 11). However, in *L. antarcticum* and *L. fasciculatum* we were unable to detect colacosomes. Interestingly, in the mycelial stage of *Rh. fujisanensis*, obtained by mating two sexually compatible strains of this species (CBS 4551^T and CBS 6371), we were also not able to detect colacosomes. In all phylogenetic analyses *Rh. fujisanensis* is a close relative of *L. fasciculatum*. The absence of colacosomes in *L. antarcticum* correlates with its phylogenetic placement inferred by molecular analyses. Its closest relatives are *Camptobasidium hydrophilum* Marvanová & Suberkropp and *Kriegeria eriophori* Bres., two species investigated by us and found to be devoid of colacosomes. The septal pores of *L. antarcticum, L. fasciculatum, L. fellii, L. golubevii* and *Rh. fujisanensis* were investigated during the current study. They correspond to the typical pore type observed within the Microbotryomycetidae and are depicted in Figs. 4-8. No differences were detected between this type of septal pore and the septal pore of *L. scottii*.

Tab. 2. Physiological characteristics of the strains of *Leucosporidium golubevii* (D, delayed; W, weak results).

	PYCC 5759	PYCC 5760	PYCC 5761
Carbon compounds			
D-Glucose	+	+	+
D-Galactose	D	+	+
L-Sorbose	+	+	+
D-Glucosamine	+	+	+
D-Ribose	+	+	+
D-Xylose	+	+	+
L-Arabinose	+	+	+
D-Arabinose	D	D	D
L-Rhamnose	–	–	–
Sucrose	+	+	+
Maltose	+	+	+
α,α-Trehalose	+	+	+
Methyl-α-D-glucoside	+	+	+
Cellobiose	+	+	+
Salicin	+	+	–
Melibiose	–	–	–
Lactose	+	+	+
Raffinose	+	+	+
Melezitose	+	+	+
Inulin	–	–	–
Soluble Starch	–	–	–
Glycerol	+	+	+
Erythritol	–	–	–
Ribitol	+	+	+
Xylitol	+	+	+
D-Glucitol	+	+	+
D-Mannitol	+	+	+
Galactitol	–	DW	DW
Inositol	–	–	–
Glucono-δ-lactone	+	+	+
D-Gluconic acid	+	+	+
D-Glucuronic acid	+	+	+
D,L-Lactic acid	+	+	+
Succinic acid	+	+	+
Citric acid	+	+	+
L-Malic acid	+	+	+
L-Tartaric acid	+	+	+
D-Tartaric acid	–	–	–
m-Tartaric acid	–	–	–
Saccharic acid	–	–	–

	PYCC 5759	PYCC 5760	PYCC 5761
Carbon compounds			
Mucic acid	–	–	–
Methanol	–	–	–
Ethanol	+	+	+
Vanillic acid	+	+	+
Veratric acid	–	–	–
Ferulic acid	+	+	+
p-Hydroxybenzoic acid	+	+	+
m-Hydroxybenzoic acid	+	+	+
Protocatechuic acid	+	+	+
Catechol	–	–	–
Gallic acid	–	–	–
Salicylic acid	–	–	+
Gentisic acid	+	+	+
Phenol	–	–	–
Nitrogen compounds			
Nitrate	+	+	+
Nitrite	+	+	+
Ethylamine	+	+	+
L-Lysine	+	+	+
Cadaverine	–	–	–
Creatine	–	–	–
Creatinine	–	–	–
Other tests			
Growth in vitamin-free medium	+	+	+
Growth with 0.01% cycloheximide	D	D	D
Growth with 0.1% cycloheximide	–	–	–
Growth at 25 °C	+	+	+
Growth at 30 °C	–	–	–
Formation of starch-like compounds	–	–	–
Splitting of arbutin	–	–	–
Hydrolysis of urea	+	+	+
Colour reaction with Diazonium Blue B	+	+	+

The heterogeneity of *Rhodotorula* and allied anamorphic genera

Currently, anamorphic species of the Microbotryomycetidae are classified in the genera *Rhodotorula* Harrison when ballistoconidia are absent, and in *Sporobolomyces* Kluyver & van Niel or, in a few cases, in *Bensingtonia* Ingold emend. Nakase & Boekhout, when ballistoconidia are produced. *Sporobolomyces* is characterized by the presence of CoQ 10 whereas *Bensingtonia* has CoQ 9. The taxonomic tools presently in use in yeast systematics, especially DNA sequence analyses, indicate that all these anamorphic genera are polyphyletic. We favor the concept of restriction of anamorphic genera to coherent and phylogenetically related groups of species since, among other advantages, such naturally defined assemblages can be classified in the system originally built exclusively for teleomorphic taxa. In the case of those three genera, we consider that *Rhodotorula* should be restricted to the type species – *Rh. glutinis* (Fresenius) Harrison – and closely related species; *Sporobolomyces* should encompass only *Sp. salmoni-*

Tab. 3. Salient physiological / biochemical differences between *Leucosporidium golubevii* and the other taxa of the Leucosporidiales (V, variable results)

	L-Rhamnose	Ribitol	Cadaverine	Gallic acid	Catechol	0.01% Cyclohex.
Leucosporidium golubevii	–	+	–	–	–	+
Leucosporidiella fragaria	–	+	–	–	–	–
Leucosporidium scottii	+	–	+	+	+	V
Leucosporidium fellii	+	+	–	–	+	+
Mastigobasidium intermedium	–	+	+	–	–	V
Leucosporidiella creatinivora	–	+	+			+
Leucosporidiella muscorum	–	+	+	–	+	+
Leucosporidiella yakutica	+	+	–			+

color (Fisher & Brebeck) Kluyver & van Niel (type species) and related taxa such as *Sp. roseus* Kluyver & van Niel; and, since *B. ciliata* Ingold, the type species of *Bensingtonia*, is not a member of the Microbotryomycetidae, this generic name should not be used for ballistoconidial CoQ 9 yeasts belonging to this subclass.

In a recent taxonomic revision of the realm of basidiomycetous yeasts, Boekhout et al. (1998) treated the anamorphic genera apart from the teleomorphic taxa in the families Cryptococcaceae Kützing emend. van der Walt and Sporobolomycetaceae Derx emend. van der Walt. However, Boekhout et al. (1998) remarked that these families are artificial and stated: „It can be expected that they will become superfluous when the taxonomy of the anamorphic basidiomycetous yeasts becomes integrated with the teleomorphic heterobasidiomycetes“. We consider that the time has come to implement the necessary changes in the classification scheme of dimorphic basidiomycetes in order to accommodate mitosporic and meiosporic taxa in a single system. The taxonomic proposals presented below take this view into consideration.

Taxonomic changes

Presently, *Leucosporidium* is classified in the order Sporidiales Moore (Moore 1980) and in the family Sporidiobolaceae Moore emend. Boekhout, Bandoni, Fell & Kwon-Chung, together with *Sporidiobolus* Nyland and *Rhodosporidium* Banno (Boekhout et al. 1998). In view of the available information based on sequence data (Figs. 12 and 13 and also Fell et al. 2000, 2001), grouping *Leucosporidium* with the other two genera mentioned above at the order or family level results in an unnatural classification scheme since the three genera do not form a monophylum but instead originate a paraphyletic taxon. Moreover, as we have previously discussed, *Leucosporidium* is itself heterogeneous, i.e., polyphyletic. In order to reflect in the classification system of the Microbotryomycetidae the phylogenetic relationships between the organisms, we propose the following taxonomic changes: (i) erection of the order Leucosporidiales for the species of *Leucosporidium* related to the type species, *Mastigobasidium* and related anamorphic species, (ii) transfer of the anamorphic species of the Leucosporidiales to the new genus *Leucosporidiella*, and (iii) erection of the order Sporidiobolales and validation of the family Sporidiobolaceae for *Sporidiobolus*, *Rhodosporidium* and related anamorphic species.

Leucosporidiales Sampaio, Weiss & Bauer, ord. nov.

Fungi Microbotryomycetidarum non-phytoparasitici, sexuales vel asexuales, in statu unicellulari coloniis cremeis. In statu sexuali mycelium colacosomatibus porisque septorum simplicibus, sine haustoriis, teliosporas procreans. Teliosporae basidia transversaliter septata procreando germinantes; basidiosporae non eiciuntur. In statu unicellulari praeter gemmas etiam ballistoconidia procreari possunt.

Asexual or sexual, non-phytoparasitic members of the Microbotryomycetidae having white to cream colored colonies. In the sexual stage, the mycelium is devoid of haustoria, has colacosomes and simple septal pores, and gives rise to teliospores. Teliospores germinate by producing transversally septate basidia and release basidiospores passively. In the unicellular state, besides budding yeast cells, ballistoconidia can be produced. Salient physiological traits are depicted in Fig. 19 and the circumscription of the order is shown in Figs. 12 and 13. Typus ordinis: Leucosporidiaceae Sampaio, Weiss & Bauer, opsum ipsum.

The salient morphological features of the teleomorphic taxa of the Leucosporidiales (other than *L. golubevii*) are presented in Figs. 14-18. Basidia of *L. scottii* can in some cases present a stalk (Fig. 14) measuring up to 75 μm, more frequently 45–60 μm. Teliospores are spherical (8–10 μm in diameter), basidia measure (3) 3.5–5 (6) x (18) 20–45 (50) μm and basidiospores are ovoid (2–3.5 x 5–7.5 μm). Stalks in *L. golubevii* were not observed (Fig. 3) and in *L. fellii* the stalk, when present, had a much shorter length. The bacilliform shape of the basidiospores (1.5–2 x 10–13 μm) is a salient feature of *L. fellii* (Fig. 15). Teliospores of *L. fellii* are spherical (10–12 μm in diameter), basidia measure 3.5–5 x (30) 40–65 (70) μm. According to the data presented by Golubev (1999), confirmed in the present study, the teliospores of *Mastigobasidium intermedium* are normally larger [(10) 13–15 (18) μm in diameter] than those of *Leucosporidium*, germinate after a rather

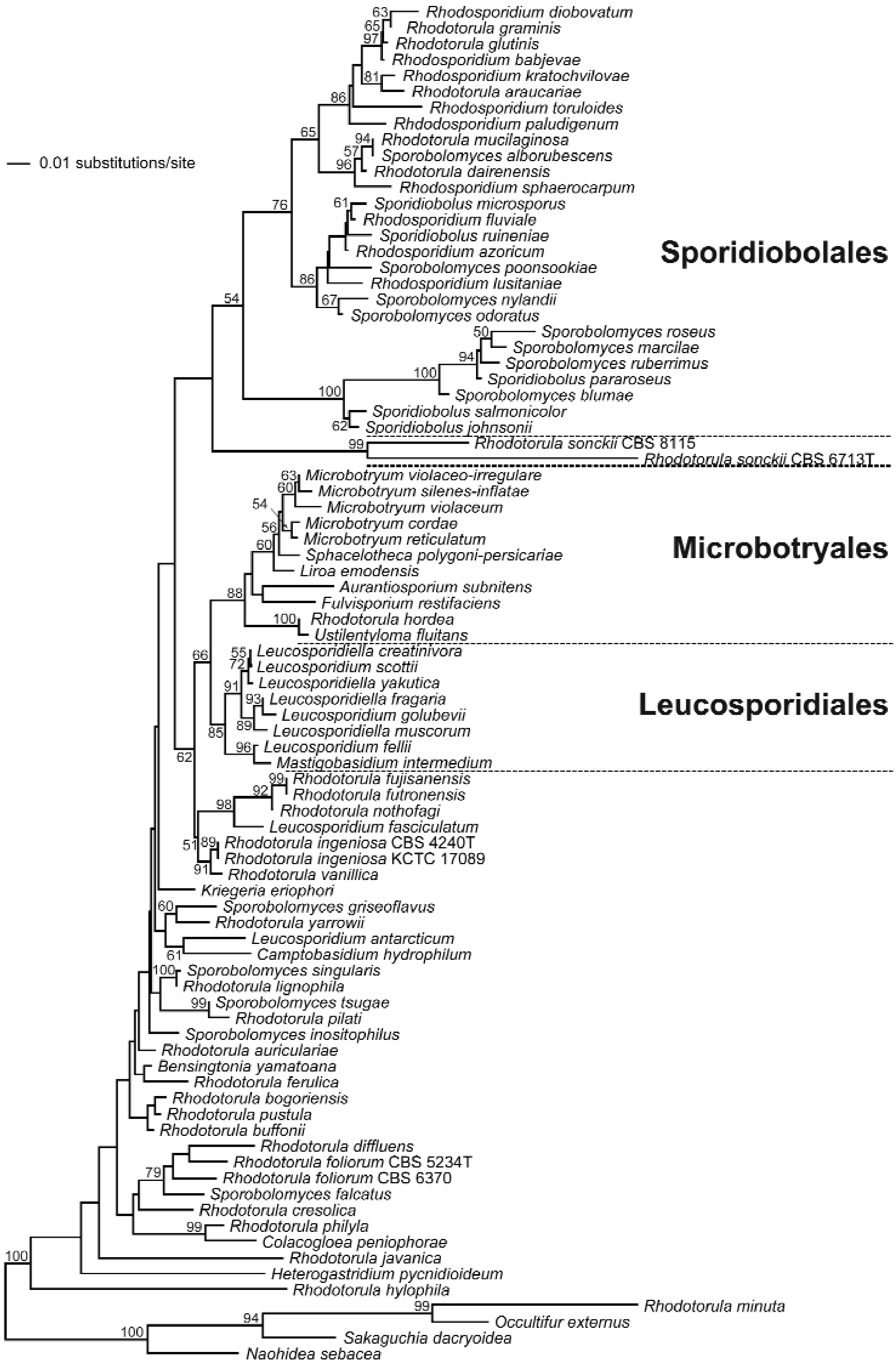


Fig. 12. Phylogenetic relationships of selected urediniomycetous taxa: Neighbor-joining analysis (BIONJ modification) of an alignment of nuclear DNA sequences from the D1/D2 region of the ribosomal large subunit using genetic distances derived from the TrN+I+G model of DNA substitution. Numbers on branches are bootstrap values (1000 replicates; values below 50 % not shown). The topology was rooted with *Rhodotorula minuta*, *Occultifur externus*, *Sakaguchia dacryoidea*, and *Naohidea sebacea*.

prolonged resting period, and are able to originate more that one basidium (Fig. 16). A stalk of up to 200 μm can be produced. Cylindrical, sometimes curved basidia are produced and measure 4–6 x (50) 60–80 (90) μm. Basidiospores of *M. intermedium* are sessile although ballistoconidia are produced in the yeast stage of this fungus. Besides subglobose to ovoid basidiospores measuring 2–4 x 3–6.5 μm, other larger and more irregular structures are produced on the basidia (Figs. 17, 18).

In the MCMC analysis (Fig. 13), *L. fellii* and *M. intermedium* together with the Microbotryales form a monophylum, supported by an *a posteriori* probability value of 80 %. On the contrary, in the BIONJ analysis (Fig. 12) these two species belong to the *Leucosporidium* core group, supported by a bootstrap value of 85 %. The association of *L. fellii* and *M. intermedium* with *Microbotryum* and allied genera in the MCMC tree contrasts with the absence of colacosomes and the phytoparasitic life strategy of the Microbotryales. According to the Bayesian analysis of Fig. 13, it is possible that the common ancestor of the Microbotryales and of *L. fellii* and *M. intermedium* was a saprophytic or mycoparasitic fungus with colacosomes and that these structures were lost in the lineage that evolved a phytoparasitic life cycle. With respect to other taxonomic criteria, whereas *L. fellii* and *M. intermedium* have CoQ 9 (Yamada & Nakagawa, 1992; Nakase & Suzuki, 1986), *Microbotryum* and *Sphacelotheca* have CoQ 10 (Prillinger et al. 1991) as most species of the Microbotryomycetidae studied to date. However, since for *Leucosporidium scottii* both CoQ 9 and CoQ 10 strains are known (Sugiyama et al. 1985), in this case the relevance of this trait is doubtful. Morphologically, *Mastigobasidium* stands apart because it is the only species of the *Microbotryum* / *Leucosporidium* group that forms ballistoconidia and that originates multiple basidia from a single teliospore. We analyzed the nutritional profiles of the Microbotryales and Leucosporidiales using numerical taxonomy methods. Interestingly, the dendrogram depicted in Fig. 19 revealed that each of the two orders has particular physiological properties and two main groups were formed. *Leucosporidium fellii* and *M. intermedium* were not assigned to any of these clusters and occupy an intermediate position between them. Because of the conflicting results obtained in the phylogenetic placement of *L. fellii* / *M. intermedium* and also because of the closer phenetic resemblance towards *Leucosporidium* rather than *Microbotryum,* we tentatively place these two species in the Leucosporidiales. We consider that the description of more species in this order will be essential to a better understanding of the evolutionary history of *L. fellii* and *M. intermedium* and to the refinement of the classification system proposed here. The present difficulties related with the classification of these two species led us to exclude them from the family Leucosporidiaceae.

Leucosporidiaceae Sampaio, Weiss & Bauer, fam. nov.

Descriptio analoga ordini Leucosporidialium sed excludens Leucosporidium fellii Mastigobasidiumque intermedium.

Typus familiae: Leucosporidium Fell, Statzell, Hunter & Phaff.

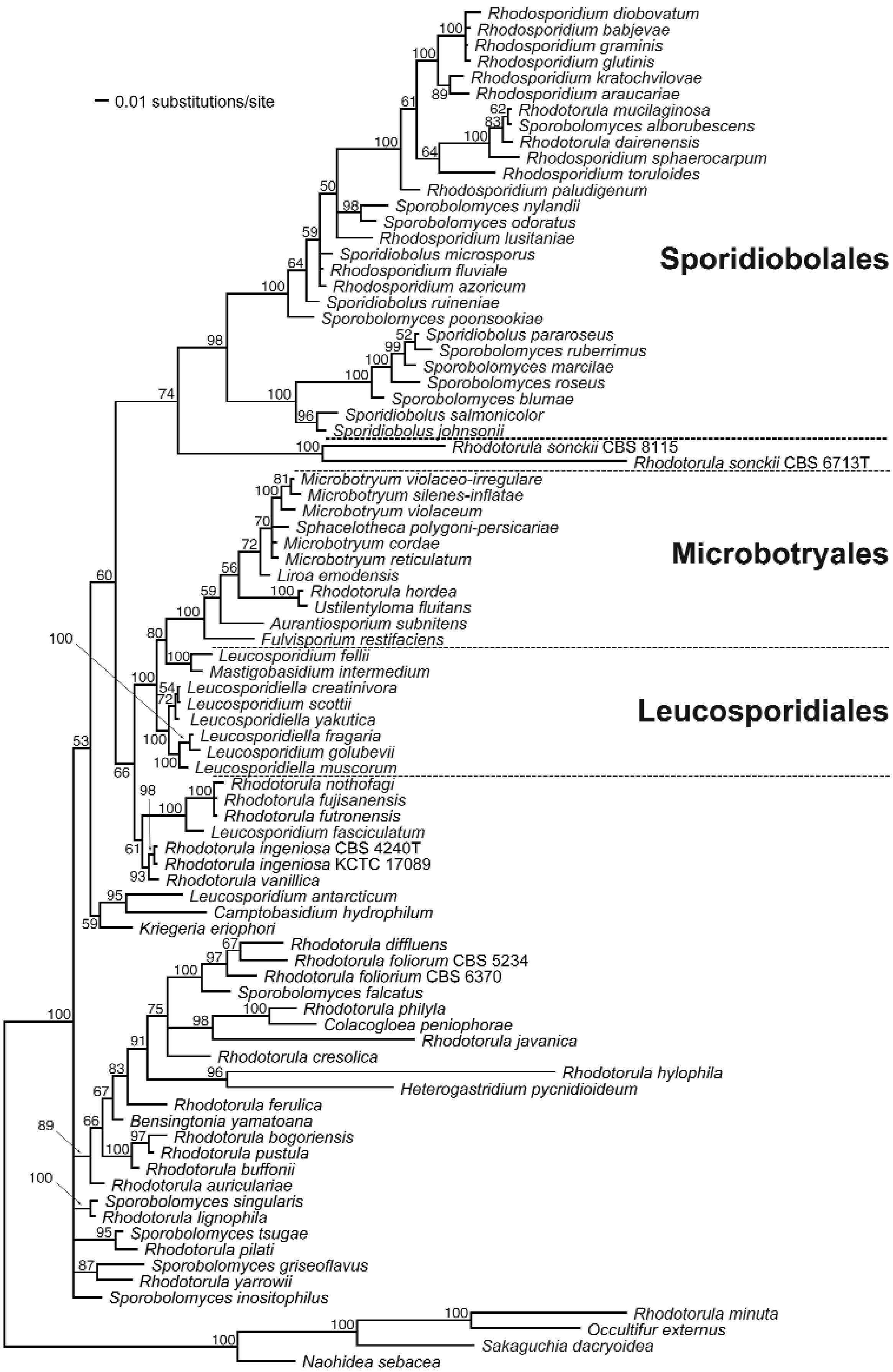
Leucosporidiella Sampaio, gen. nov.

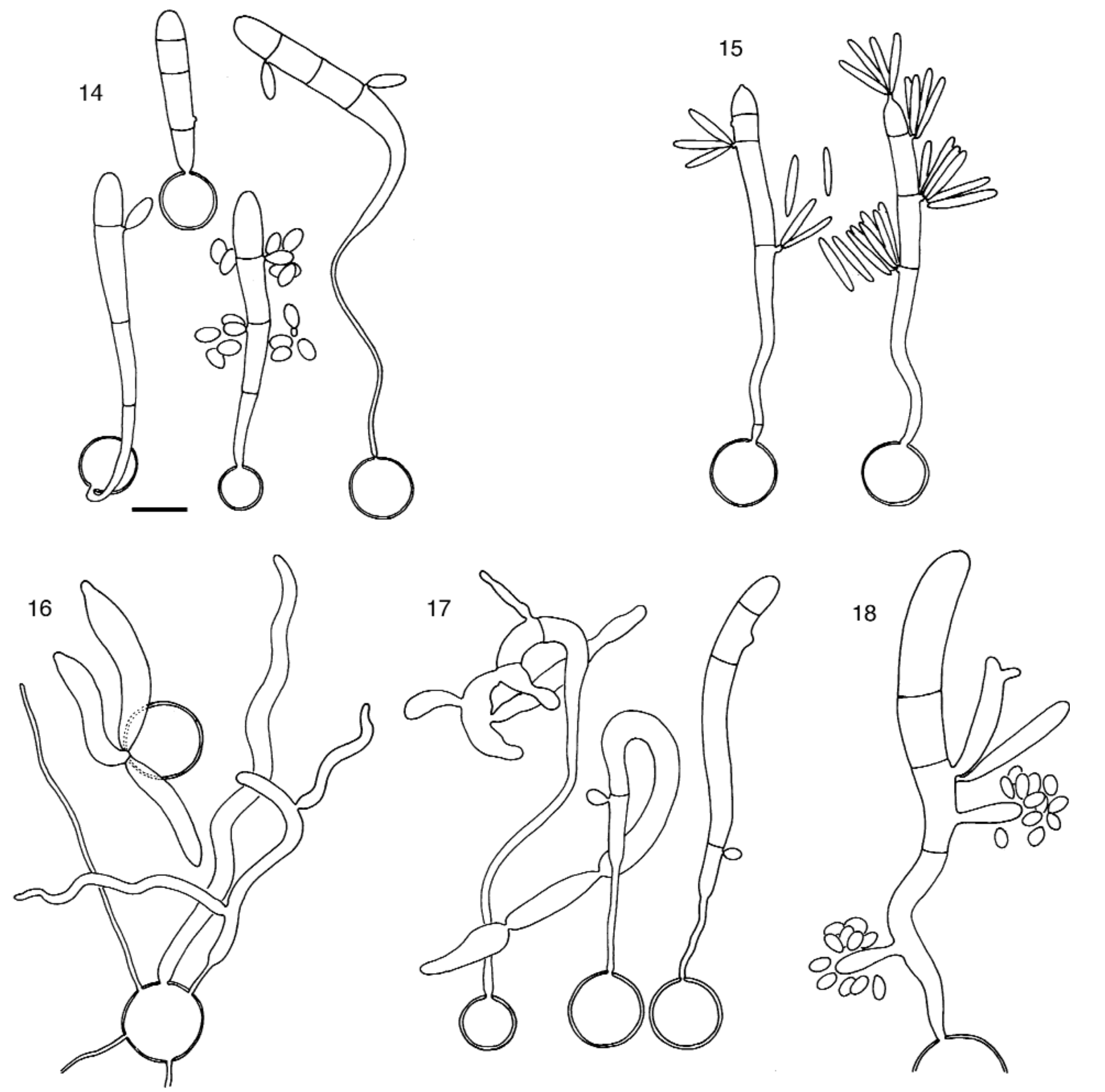
Fungi Leucosporidialium asexuales. Mycelium verum creari potest. Culturae plerumque cremeae, mucosae. CoQ systemata 9 vel 10 dominantia. Assimilatio inositolei procreatioque compositorum amylo similium nulla, assimilatio nitrati ut unica origo nitrogeni assimilatioque compositorum aromaticorum acidi protocatechuici, acidi vanillici acidique ferulici adsunt.

Asexual members of the Leucosporidiales. True mycelium can be produced. Cultures are normally cream colored and mucoid. Dominant CoQ systems are 9 or 10. Assimilation of inositol and production of amyloid compounds are negative, utilization of nitrate as sole source of nitrogen is positive as well as the utilization of D- glucuronate and the aromatic compounds protocatechuic, vanillic and ferulic acids.
Typus generis: *Leucosporidiella muscorum* (di Menna) Sampaio.

The genus *Rhodotorula* Harrison was originally created to accommodate asexual pink or red pigmented yeasts (Harrison 1928). When the distinction between ascomycetous and basidiomycetous mitosporic yeasts became possible due to the utilization of such methodologies as analysis of ultrastructure and of chemical composition of cell wall, determination of coenzyme Q type, determination of mol. % G+C, and DBB and urea hydrolysis tests, the genus *Candida* Berkhout was rendered more homogeneous by the transfer of its basidiomycetous species either to *Cryptococcus* Vuillemin (species normally producing starch-like compounds and able to grow with inositol) or to *Rhodotorula* (starch-like compounds not produced and inositol not assimilated) (von Arx & Weijman 1979; Weijman, Rodrigues de Miranda & van der Walt 1988; Roeijmans, van Eijk & Yarrow 1989). As a consequence of those taxonomic changes, non-pigmented species were added to *Rhodotorula* and the heterogeneity of the genus increased.

Fell & Statzell-Tallman (1998) recognized 34 species of *Rhodotorula*. More recent additions to the genus were *Rh. vanillica* Sampaio (Sampaio 1994), *Rh. cresolica* Middelhoven & Spaaij (Middelhoven & Spaaij 1997), *Rh. creatinivora* Golubev and *Rh. yakutica* Golubev (Golubev 1998), *Rh. yarrowii* (Fonseca & van Uden) Boekhout, Fell, Fonseca, Prillinger & Roeijmans (Boekhout et al. 2000), *Rh. lamellibrachii* Nagahama, Hamamoto, Nakase & Horikoshi (Nagahama et al. 2001), *Rh. dairenensis* (Hasegawa & Banno) Fell, Sampaio & Gadanho (Gadanho & Sampaio 2002). Moreover, former synonyms of *Rh. minuta* (Saito) Harrison, namely *Rh. laryngis* Reiersöl, *Rh. marina* Phaff, Mrak & Williams, *Rh. pallida* Lodder and *Rh. slooffiae* Novák & Vörös-Felkai were found to represent distinct species (Fell et al. 2000). The type species group includes 5 species and all of them are pink colored. A second group contains 25 species, all non-pigmented except for *Rh. fujisanensis* (Soneda) Johnson & Phaff and *Rh. nothofagi* (Ramírez & González) Roeijmans, van Eijk & Yarrow that may include light pink colored strains. The species in the second group are not closely related to the type


0.01 substitutions/site
Sporidiobolales
Microbotryales
Leucosporidiales
Rhodosporidium diobovatum
Rhodosporidium babjevae
Rhodosporidium graminis
Rhodosporidium glutinis
Rhodosporidium kratochvilovae
Rhodosporidium araucariae
Rhodotorula mucilaginosa
Sporobolomyces alborubescens
Rhodotorula dairenensis
Rhodosporidium sphaerocarpum
Rhodosporidium toruloides
Rhodosporidium paludigenum
Sporobolomyces nylandii
Sporobolomyces odoratus
Rhodosporidium lusitaniae
Sporidiobolus microsporus
Rhodosporidium fluviale
Rhodosporidium azoricum
Sporidiobolus ruineniae
Sporobolomyces poonsookiae
Sporidiobolus pararoseus
Sporobolomyces ruberrimus
Sporobolomyces marcilae
Sporobolomyces roseus
Sporobolomyces blumae
Sporidiobolus salmonicolor
Sporidiobolus johnsonii
Rhodotorula sonckii CBS 8115
Rhodotorula sonckii CBS 6713T
Microbotryum violaceo-irregulare
Microbotryum silenes-inflatae
Microbotryum violaceum
Sphacelotheca polygoni-persicariae
Microbotryum cordae
Microbotryum reticulatum
Liroa emodensis
Rhodotorula hordea
Ustilentyloma fluitans
Aurantiosporium subnitens
Fulvisporium restifaciens
Leucosporidium fellii
Mastigobasidium intermedium
Leucosporidiella creatinivora
Leucosporidium scottii
Leucosporidiella yakutica
Leucosporidiella fragaria
Leucosporidium golubevii
Leucosporidiella muscorum
Rhodotorula nothofagi
Rhodotorula fujisanensis
Rhodotorula futronensis
Leucosporidium fasciculatum
Rhodotorula ingeniosa CBS 4240T
Rhodotorula ingeniosa KCTC 17089
Rhodotorula vanillica
Leucosporidium antarcticum
Camptobasidium hydrophilum
Kriegeria eriophori
Rhodotorula diffluens
Rhodotorula foliorum CBS 5234
Rhodotorula foliorium CBS 6370
Sporobolomyces falcatus
Rhodotorula philyla
Colacogloea peniophorae
Rhodotorula javanica
Rhodotorula cresolica
Rhodotorula hylophila
Heterogastridium pycnidioideum
Rhodotorula ferulica
Bensingtonia yamatoana
Rhodotorula bogoriensis
Rhodotorula pustula
Rhodotorula buffonii
Rhodotorula auriculariae
Sporobolomyces singularis
Rhodotorula lignophila
Sporobolomyces tsugae
Rhodotorula pilati
Sporobolomyces griseoflavus
Rhodotorula yarrowii
Sporobolomyces inositophilus
Rhodotorula minuta
Occultifur externus
Sakaguchia dacryoidea
Naohidea sebacea



Figs. 14-18. Line drawings of teliospores, basidia and basidiospores of *Leucosporidium scottii*, *L. fellii* and *Mastigobasidium intermedium*. 14. *Leucosporidium scotti* PYCC 4405T x PYCC 4696 (3 germinated teliospores on the lower part) and PYCC 4347 (self-fertile, germinated teliospore on the left upper part), grown on SG agar at room temperature for two months, then transferred to 2% water agar and observed after 4 days. 15. PYCC 4403T grown on MYP agar at room temperature for 2 weeks, soaked in demineralized water at 4 °C for 5 months, then transferred to 2% water agar and observed after 4 days. 16-18 *Mastigobasidium intermedium* PYCC 5340T x PYCC 5458 grown on MYP agar at room temperature for 2 months, soaked in demineralized water at 4 °C for 8 months, then transferred to 2% water agar and observed after 5 days. 16. Initial stages of teliospore germination (note multiple, still immature basidia in both teliospores). 17-18. Germinated teliospores with mature basidia (note, for some basidia, the curved shape and the irregular pattern of germination). Bar = 10 μm (same for all illustrations).

Fig. 13. Phylogenetic relationships of selected urediniomycetous taxa: Bayesian Markov chain Monte Carlo analysis of an alignment of nuclear DNA sequences from the D1/D2 region of the ribosomal large subunit using the GTR+G model of DNA substitution with random starting trees, default starting parameters of the substitution model, and four incrementally heated simultanous Markov chains. 50 % majority rule consensus tree from 16000 trees that were sampled after the Markov chains had reached stationarity (trees were sampled every 100 generations). Numbers on branches are estimates for *a posteriori* probabilities, i.e., probabilities that the respective groups are monophyletic given the alignment. Branch lengths were averaged over the sampled trees. The topology was rooted with *Rhodotorula minuta*, *Occultifur externus*, *Sakaguchia dacryoidea*, and *Naohidea sebacea*.

species group but both assemblages are classified in the Microbotryomycetidae. A third group, composed by 9 pigmented species, encompasses *Rh. minuta* and allied taxa and has, as their closest teleomorphic relatives, *Occultifur* Oberwinkler, *Naohidea* Oberwinkler and *Sakaguchia* Yamada, Maeda & Mikata. A fourth group includes 4 species and belongs to the Ustilaginomycetidae and a fifth group with a single species belongs to the Agaricostilbomycetidae. The following new combinations correspond to the transfer to *Leucosporidiella* of four *Rhodotorula* species of the second group, which are closely related to *Leucosporidium scottii* and therefore should also be classified in the Leucosporidiales. These taxonomic changes aim at rendering the anamorphic basidiomycetous genera phylogenetically coherent.

Leucosporidiella creatinivora (Golubev) Sampaio, comb. nov.

= *Rhodotorula creatinivora* Golubev – Mikologiya i Fitopatologiya 32: 8, 1998 (basionym, spelled incorrectly in the original publication as *Rhodotorula creatinovora*).

Leucosporidiella fragaria (J.A. Barnett & Buhagiar) Sampaio, comb. nov.

= *Rhodotorula fragaria* (J.A. Barnett & Buhagiar) Rodrigues de Miranda & Weijman
= *Candida fragariorum* (J.A. Barnett & Buhagiar) S.A. Meyer & Yarrow
= *Torulopsis fragaria* J.A. Barnett & Buhagiar – J. Gen. Microbiol. 67: 237-238, 1971 (basionym).

Leucosporidiella muscorum (di Menna) Sampaio, comb. nov.

= *Rhodotorula muscorum* (di Menna) von Arx & Weijman
= *Azymocandida muscorum* (di Menna) E.K. Novák & Zsolt
= *Candida muscorum* di Menna – J. Gen. Microbiol. 18: 269, 1957 (basionym).

Leucosporidiella yakutica (Golubev) Sampaio, comb. nov.

= *Rhodotorula yakutica* Golubev – Mikologiya i Fitopatologiya 32: 9, 1998 (basionym).

MOORE (1980, p. 365) introduced the names Sporidiales, Sporidiaceae and Sporidiobolaceae without diagnosis and typification. Moreover, the concept of the order was based on a supposed close phylogenetic relationship between *Leucosporidium* and *Rhodosporidium*, which is not supported by the available molecular sequence data. Later, BOEKHOUT et al. (1998) emended the Sporidiobolaceae in order to include in this family *Sporidiobolus*, *Rhodosporidium* and *Leucosporidium*. Sporidiaceae was regarded as a synonym of Sporidiobolaceae. We consider that the concepts of the Sporidiales sensu Moore and of the emended Sporidiobolaceae are not consistent with our present knowledge on the evolution of these yeasts. The main disadvantages are their paraphyletic nature and their restriction to teleomorphic taxa. Therefore, we propose the following classification system for *Sporidiobolus*, *Rhodosporidium* and related anamorphic taxa.

Sporidiobolales Sampaio, Weiss & Bauer, ord. nov.

Fungi Microbotryomycetidarum non-phytoparasitici culturis roseis. In statu sexuali mycelium colacosomatibus (praeter *Rhodosporidium sphaerocarpum* Newell & Fell), poris septorum simplicibus, sine haustoriis, teliosporas procreans. Teliosporae basidia transversaliter septata procreando germinantes; basidiosporae non eiciuntur. In statu unicellulari praeter gemmas etiam ballistoconidia procreari possunt. Assimilatio D-glucuronati inositoleique deest.

Sexual or asexual, non-phytoparasitic members of the Microbotryomycetidae having pink colored cultures. In the sexual stage, the mycelium is devoid of haustoria, has colacosomes (except for *Rhodosporidium sphaerocarpum* Newell & Fell) and simple septal pores, and gives rise to teliospores. Teliospores germinate by producing transversally septate basidia and release basidiospores passively. In the unicellular state, besides budding yeast cells, ballistoconidia can be produced. Salient physiological traits are the incapacity to utilize D- glucuronate, and inositol.
Typus ordinis: Sporidiobolaceae Moore emend. Sampaio, Weiss & Bauer, opus ipsum.

Sporidiobolaceae Moore emend. Sampaio, Weiss & Bauer

Descriptio analoga ordini *Sporidiobolalium*.
Typus familiae: *Sporidiobolus* Nyland.

The concept of the *Sporidiobolales* is consistent with the phylogenetic analyses performed in the present study. Interestingly, in both analyses, the two available strains of *Rhodotorula sonckii* (Hopsu-Havu, Tunnela & Yarrow) Rodrigues de Miranda & Weijman, a non-pigmented species, cluster at the base of the Sporidiobolales. Within the Sporidiobolales two major groups can be detected in the molecular analyses shown in Figs. 12 and 13. In both trees the two groups are statistically well supported. The larger group includes the type species of *Rhodosporidium* – *R. toruloides* Banno – and *Rhodotorula* Harrison – *Rh. glutinis* (Fresenius) Harrison –, whereas the smaller group comprises the type species of *Sporidiobolus* (*S. johnsonii* Nyland). The first group includes all non-ballistoconidiogenic species of the Sporidiobolales and a few species of *Sporobolomyces* and *Sporidiobolus*. The second group is exclusively ballistoconidiogenic. No relevant discrepancies could be found between the ballistoconidiogenic species of the two groups of the Sporidiobolales and therefore no additional taxonomic proposals within the order will be made at this time.

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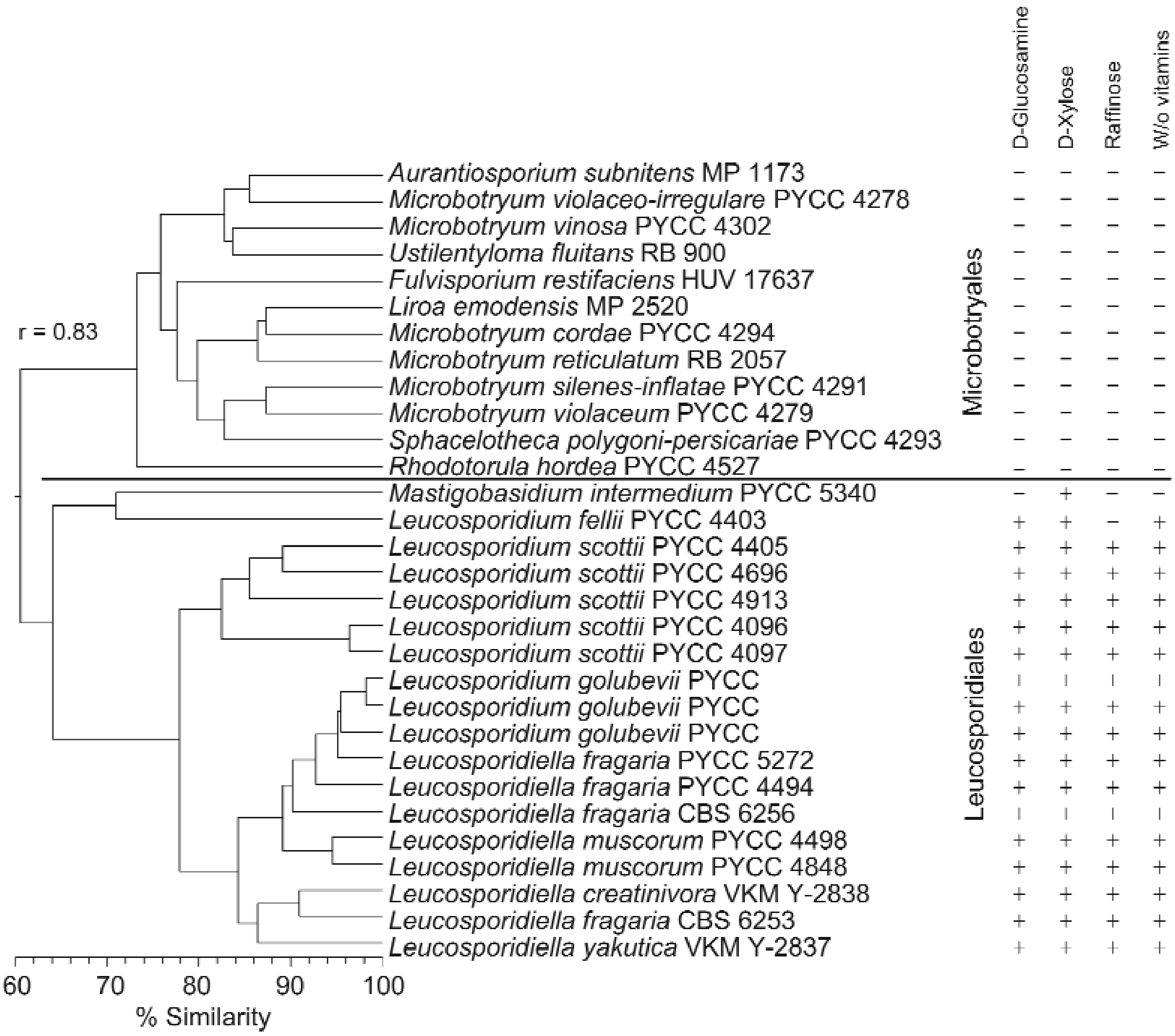

Fig. 19. Phenogram of species of Microbotryales and Leucosporidales based on overall similarity (simple matching coefficient) and cluster analysis (UPGMA) of 68 physiological tests (r = co-phenetic correlation coefficient). The results of a selected group of relevant tests (assimilation of D-Glucosamine as sole carbon source, D-xylose and raffinose, and growth in the absence of vitamins) are indicated on the right side.

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