International Journal of Systematic and Evolutionary Microbiology (2008), **58**, 736–741 **DOI** 10.1099/ijs.0.65580-0

Reappraisal of the *Sporobolomyces roseus* species complex and description of *Sporidiobolus metaroseus* sp. nov.

Elisabete Valério, Mário Gadanho and José Paulo Sampaio

Centro de Recursos Microbiológicos, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

Correspondence
José Paulo Sampaio
jss@fct.unl.pt

Here, we investigate a group of red to pinkish ballistoconidia-forming yeasts that were preliminarily identified as *Sporobolomyces roseus* or *Sporidiobolus pararoseus*. Detailed molecular and micromorphological studies revealed that the sexual strains and several conspecific anamorphic isolates belonged to a novel teleomorph that represents the sexual stage of *Sporobolomyces roseus*. Consequently, a new taxon in the genus *Sporidiobolus* is here described as *Sporidiobolus metaroseus* sp. nov. (type strain CBS 7683^T). The main characteristics of *Sporidiobolus metaroseus* are presented and compared with those of the more closely related species. Our studies also led to the clarification of the life cycle of *Sporidiobolus pararoseus*. We confirm that the teliospores of this species germinate by forming short branches of hyphae, instead of basidia.

INTRODUCTION

Sporobolomyces roseus Kluyver & van Niel is one of the most common phylloplane yeasts (Derx, 1930; Tubaki, 1953; Last, 1955; Nakase, 2000). This species has also been found in other substrates such as air (Hirst, 1953), seawater (Hernandez-Saavedra *et al.*, 1992; Gadanho *et al.*, 2003) and freshwater (Libkind *et al.*, 2003). Molecular phylogenetic analyses have indicated that *Sporidiobolus pararoseus* Fell & Tallman is the closest teleomorphic relative of *Sporobolomyces roseus* (Fell *et al.*, 1998, 2000; Bai *et al.*, 2002). The nutritional profiles of the two species are similar (Boekhout, 1991; Boekhout & Nakase, 1998) but, whereas *Sporobolomyces roseus* is nitrate positive, *Sporidiobolus pararoseus* is nitrate negative. Most strains of *Sporidiobolus pararoseus* are heterothallic, and only a single isolate has been reported to be self-fertile (Boekhout, 1991; Statzell-Tallman & Fell, 1998). In the present study, a group of red to pinkish ballistoconidia-forming yeasts isolated in recent years, mostly from the phylloplane in Portugal and other European countries, was investigated. The majority of the isolates were anamorphic and corresponded phenotypically to the delimitation of *Sporobolomyces roseus*. Additionally, a few isolates were able to produce clamped mycelium with teliospores and, based on this and on their nutritional profiles, were preliminarily identified as self-fertile strains of *Sporidiobolus pararoseus*. More detailed studies revealed that the sexual strains and several conspecific anamorphic isolates belonged to a novel teleomorph that represents the sexual stage of *Sporobolomyces roseus* and is here described as *Sporidiobolus metaroseus* sp. nov. Characterization of the novel teleomorphic species also led to the clarification of the life cycle of *Sporidiobolus pararoseus*.

The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this study are EU003452–EU003469 (26S rDNA D1/D2) and EU003480–EU003482 (complete ITS region and 5.8S rDNA).

METHODS

Cultures, sexual compatibility experiments and microscopy. The cultures used in this study are detailed in Table 1. For the characterization of the sexual stage, the cultures were grown on cornmeal agar (Difco), incubated at room temperature (20–23 °C) and examined for the production of mycelium and teliospores after 1 week. For microscopy, an Olympus BX50 microscope equipped with phase-contrast optics was employed.

DNA–DNA reassociation experiments and rDNA sequence analyses. For determination of the extent of DNA relatedness, total genomic DNA was extracted and purified using the procedures described by Sampaio *et al.* (2001). For DNA–DNA reassociation experiments, a Gilford Response UV-VIS spectrophotometer and its thermal programming software were used and the methods of Kurtzman *et al.* (1980) were followed.

For rDNA sequence analysis, total DNA was extracted using the protocol of Sampaio *et al.* (2001) and amplified using primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG) and LR6 (5′-CGCCAGTT-CTGCTTACC). Cycle sequencing of the 600–650 bp region at the 5′ end of the 26S rDNA D1/D2 domains employed forward primer NL1 (5′-GCATATCAATAAGCGGAGGAAAAG) and reverse primer NL4 (5′-GGTCCGTGTTTCAAGACGG). The internal transcribed spacer (ITS) region was sequenced using the forward primer ITS1 (5′-TCCGTAGGTGAACCTGCGG) and the reverse primer ITS4 (5′-TCCTCCGCTTATTGATATGC). Sequences were obtained with an Amersham Pharmacia ALF Express II automated sequencer using

Table 1. Strains and sequences used in this study

Abbreviations: ANA, anamorphic; MT, mating type; SF, self-fertile; ND, not determined.

Strain	GenBank accession no.		Isolation source	Sexuality
	D1/D2	ITS		
Sporidiobolus pararoseus				
CBS 491^T	AF189977	AY015429	Soil, Japan	MT A2
CBS 484	AF070437	AF417115	Air, USA	MT A1
CBS 499	EU003454		Air in dairy, USA	MT A1
CBS 2638	EU003455		*Fragaria* sp., Japan	ANA
CBS 4216	EU003453	AF444604	Barley, Japan	MT A2
CBS 5329	EU003456		Atlantic Ocean off the USA	MT A2
CBS 7716	EU003452		Soil, USSR	MT A2
KCTC 17092	AF459709		Unknown	ND
JCM 5350	AB030338		Leaves, Thailand	ND
NL 8208205	EU003457		Flower of *Hibiscus rosa-sinensis*, Taichung, Taiwan	ND
***Sporidiobolus metaroseus* sp. nov.**				
CBS 7683^T	EU003461	EU003482	Plant leaf, Portugal	SF
CBS 485	EU003466	AY069996	Mycotic skin lesion, Italy	ANA
CBS 486*	EU003462	AY015438	Unknown	ANA
CBS 993	EU003467	AY069997	Soil, Somalia	ANA
CBS 1014	EU003468		Sediment in bottle of Maidstone beer	ANA
CBS 2646	EU003469	AY069998	Madura foot, Austria	ANA
CBS 5541	EU003458	EU003480	Flower of *Fumaria* sp., France	SF
CBS 9035	AF406925		Flower of *Rhinantus alectorolophus*, Germany	ND
CBS 7684	EU003463		Leaf of *Aesculus hippocastanum*, UK	SF
CBS 7685	EU003464		Leaf of *Quercus robur*, UK	SF
CBS 7686	EU003465		Aquatic plant, UK	ANA
PYCC 4223	EU003470		Leaf of poplar, Portugal	ANA
PYCC 4354	EU003471		Unknown	ANA
PYCC 4386	EU003472		Flower of lilac, Portugal	ANA
PYCC 4906	EU003473		Leaf of *Quercus* sp., Arrábida, Portugal	ANA
99-12-01	EU003474		Fruiting body of *Exidiopsis* sp., Lagoa de Albufeira, Portugal	ANA
ZP 494	EU003459	EU003481	Fruiting body of *Dacrymyces* sp., Sesimbra, Portugal	ANA
ZP 495	EU003460		Fruiting body of *Dacrymyces* sp., Sesimbra, Portugal	ANA
A172	AF485999		Seawater, Algarve, Portugal	ND
CH 2.500	AY070018	AY070006	Wilting leaf of *Parthenocissus* sp., China	ND

*Type strain of *Sporobolomyces roseus*.

standard protocols. Alignments were made with MEGALIGN (DNASTAR) and corrected visually. Heuristic maximum-parsimony analysis was employed [100 rounds of heuristic search with tree bisection-reconnection (TBR) branch swapping, starting from trees obtained by random addition of sequences, multrees option on, deepest descent option off], and was validated using 1000 rounds of bootstrap analysis (Felsenstein, 1985). Maximum-parsimony and bootstrap calculations used the PAUP* software (Swofford, 2002).

RESULTS

We started by reinvestigating the life cycle of *Sporidiobolus pararoseus* and studied several crosses of strains of this species. Germination of the teliospores did not yield basidia. Instead, short branches of hyphae were observed repeatedly developing from the teliospores (Fig. 1a). In most cases, these branches had limited growth and formed yeast cells occasionally. This unusual situation had been reported previously for this species by Fell & Tallman (1981) and Fell & Statzell-Tallman (1984), and a review of the literature confirmed that basidia have not been reported in *Sporidiobolus pararoseus*.

In contrast to what was observed for *Sporidiobolus pararoseus*, the teliospores of the self-fertile strains formed transversely septate basidia with four compartments (CBS 7683^T, CBS 7684 and CBS 7685; Fig. 1b) or two compartments (CBS 5541; Fig. 1c). Molecular phylogenetic analysis indicated that the self-fertile strains did not belong to *Sporidiobolus pararoseus* (10 nucleotide substitutions in the D1/D2 region and 33 substitutions in the complete ITS region) and that they were closely related to, and probably conspecific with, *Sporobolomyces roseus* (Fig. 2). The D1/D2 and ITS sequences of the self-fertile strains had almost

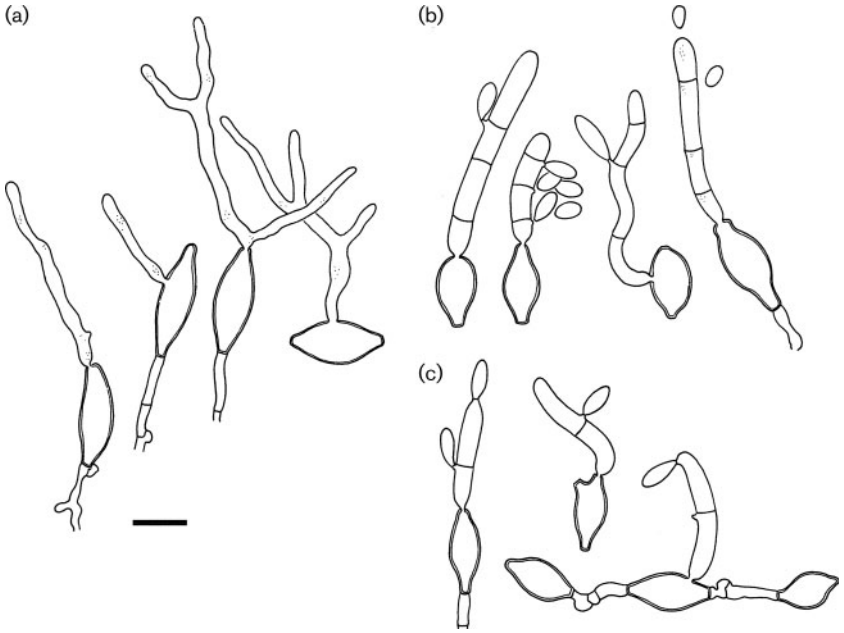

Fig. 1. Line drawings of teliospore germination in *Sporidiobolus pararoseus* and *Sporidiobolus metaroseus* sp. nov. (a) Germinated teliospores from crosses of strains CBS 491^T×CBS 484 and CBS 7716×CBS 484 of *Sporidiobolus pararoseus*; note that basidia are not formed but, instead, small stretches of hyphae develop from the teliospores. (b) Four-celled basidia observed in strains CBS 7683^T, CBS 7684 and CBS 7685 of *Sporidiobolus metaroseus*. (c) Two-celled basidia formed by *Sporidiobolus metaroseus* CBS 5541. Bar, 10 μm (same for all illustrations).

100 % identity to those of the type strain of *Sporobolomyces roseus*. D1/D2 and complete ITS sequences were also obtained and analysed for approximately 20 additional strains that were asexual (absence of teliospores in the homothallic condition and after mating) and related phenotypically to *Sporobolomyces roseus*. All of these isolates had high sequence identity to the sequences of the self-fertile strains. When the sequences of the entire group were considered, two polymorphic sites (one in the D1/D2 region and one in the ITS region) were detected. The sequence differences allowed the formation of two subgroups. The type strain of *Sporobolomyces roseus* showed greater resemblance to the group that included the self-fertile isolates CBS 7683^T, CBS 7684 and CBS 7685

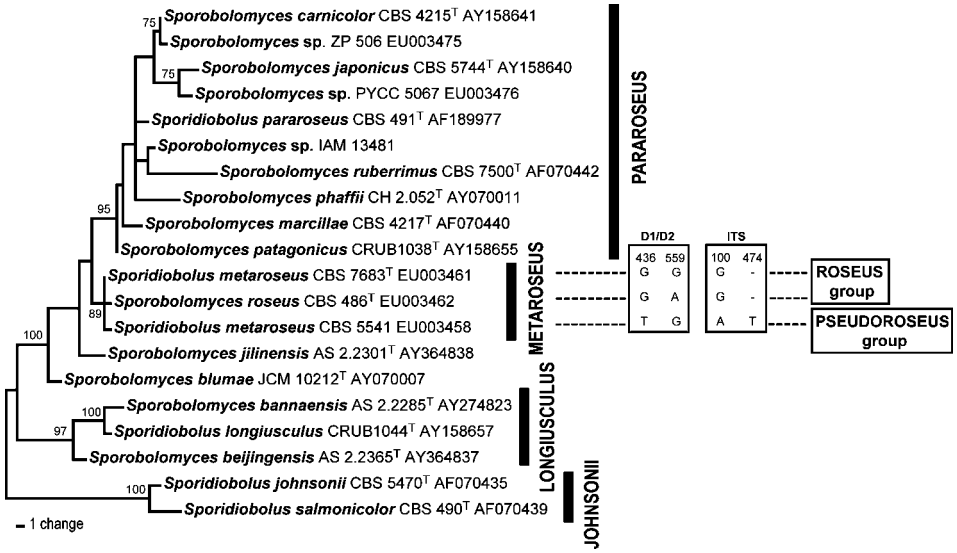

Fig. 2. One of 220 most-parsimonious phylogenetic trees of *Sporidiobolus metaroseus* sp. nov. and related species based on an alignment of partial large-subunit rDNA sequences (D1/D2 domains). The topology was rooted with *Sporidiobolus johnsonii* CBS 5470^T and *Sporidiobolus salmonicolor* CBS 490^T. Numbers on branches are percentage bootstrap values (1000 replicates; values below 50 % are not shown). GenBank accession numbers are indicated after strain names. The salient nucleotide differences between the Roseus and Pseudoroseus groups of *Sporidiobolus metaroseus* are depicted (numerals indicate positions in the D1/D2 and ITS sequence alignments and refer to the sequences of CBS 7683^T).

than to the group that included the self-fertile strain CBS 5541. However, the type strain of *Sporobolomyces roseus* had a unique sequence substitution in the D1/D2 region (confirmed by resequencing) that was not shared by any other strain. A summary of sequence comparisons is depicted in Fig. 2.

In view of these results, we describe here a novel teleomorphic species, *Sporidiobolus metaroseus* sp. nov., typified by CBS 7683^T and including both sexual and asexual representatives. In order to take a closer look at the relationship between the two groups of *Sporidiobolus metaroseus* and at the relationship between these groups and *Sporobolomyces roseus*, we performed a set of nuclear DNA–DNA reassociation experiments. As shown in Table 2, two groups were detected. One group included the type strain of *Sporobolomyces roseus*, the self-fertile isolate CBS 7863^T and the asexual strain 99-12-01. The other group encompassed the self-fertile strain CBS 5541 and the asexual strain ZP 494. By combining these results with those from DNA sequencing analyses, we resolved all the strains that were studied into two groups that are designated the Roseus group (the first group) and the Pseudoroseus group (the second one).

The two groups of *Sporidiobolus metaroseus* could not be differentiated on the basis of phenotypic markers (micromorphological features or physiological features). Besides the sequence divergence already reported for the type strain of *Sporobolomyces roseus* (CBS 486^T), we also observed a number of nutritional discrepancies of this strain towards the physiological profile of *Sporidiobolus metaroseus* (see below).

Latin diagnosis of *Sporidiobolus metaroseus* Sampaio & Valério sp. nov.

Fungus dimorphus Sporidiobolalium (Microbotryomycetes, Pucciniomycota). *In statu zymogeno cellulae ovoideae ad ellipsiodeae*, 2–5 × 8–12 μm. *Ballistoconidia reniformia. Mycelium* 1.5–2 μm *diametro, septis fibulatis. Teliosporae ellipsoideae*, 8–10 × 15–20 μm, *terminales vel intercalares. Basidia* 2–4 × 28–50 μm, *transversalitre septata*, 2- *ad* 4-*cellularia. Basidiosporae ovoideae*, 3–4 × 6–8 μm. *Positio phylogenetica in* Fig. 2 *depicta. Cultura typica* CBS 7683^T *in* Centraalbureau voor Schimmelcultures (CBS), *Ultrajecti, Hollandia, conservatur.*

Table 2. DNA–DNA reassociation values (%) within *Sporidiobolus metaroseus*

Strain	Roseus group		Pseudoroseus group	
	CBS 486	99-12-01	CBS 5541	ZP 494
Roseus group				
CBS 7683^T	71–73	95–99	27–33	20–27
CBS 486	ND	ND	ND	21–24
99-12-01	79–87	ND	ND	12–17
Pseudoroseus group				
CBS 5541	27–28	ND	ND	72–85

ND, Not determined.

Description of *Sporidiobolus metaroseus* Sampaio & Valério sp. nov.

Sporidiobolus metaroseus [me.ta.ro.se′us. Gr. pref. *meta-* (from Gr. prep. *meta*) after, changed, altered, beyond, etc.; L. adj. *roseus* rose-coloured, rosy; N.L. masc. adj. *metaroseus* changed *roseus*, referring to the close relationship to *Sporidiobolus pararoseus*].

Dimorphic. Belonging to the subphylum Pucciniomycotina, class Microbotryomycetes, order Sporidiobolales (Bauer *et al.*, 2006). Yeast cells after 1 week on MYP agar are ovoidal to ellipsoidal, 2–5 × 8–12 μm. Ballistoconidia are reniform. After 1 month at room temperature, the streak culture is pink, semi-dull, butyrous and smooth. Hyphae are 1.5–2 μm in diameter and are formed only by self-fertile strains. Clamp connections are present. Teliospores are ellipsoidal (8–10 × 15–20 μm), terminal or intercalary. Basidia (2–4 × 28–50 μm) are transversely septate, not stalked, four- or two-celled (Fig. 1b, c). Basidiospores are ovoidal (3–4 × 6–8 μm) (Fig. 1b, c). The phylogenetic placement of *Sporidiobolus metaroseus* is shown in Fig. 2. Positive for the assimilation of the carbon compounds D-glucose, D-galactose, L-sorbose*, D-ribose, D-arabinose, sucrose, maltose, α,α-trehalose, cellobiose, raffinose, melezitose, soluble starch, D-glucitol*, D-mannitol*, glucono-δ-lactone*, D-gluconic acid, DL-lactic acid*, L-malic acid, ethanol, *p*-hydroxybenzoic acid and protocatechuic acid and the nitrogen compounds nitrate*, nitrite*, ethylamine* and L-lysine* (for the compounds marked by an asterisk, CBS 486^T, the type strain of *Sporobolomyces roseus*, gave negative results). Positive or weak results are obtained for salicin, arbutin, succinic acid and citric acid. Positive or delayed results are obtained for glycerol and ribitol. Positive results are also obtained for growth in vitamin-free medium, growth with 0.01 % cycloheximide, splitting of arbutin, hydrolysis of urea and colour reaction with diazonium blue B. Negative for the assimilation of the carbon compounds D-glucosamine, L-arabinose, L-rhamnose, melibiose, lactose, inulin, erythritol, xylitol, galactitol, inositol, D-glucuronic acid, L-tartaric acid, D-tartaric acid, *m*-tartaric acid, saccharic acid, mucic acid, methanol, veratric acid, catechol, gallic acid, salicylic acid, gentisic acid and phenol and the nitrogen compounds creatine and creatinine. Negative results are also obtained for growth with 0.1 % cycloheximide, growth at 35 °C and formation of starch-like compounds. Variable results are obtained for the assimilation of the carbon compounds D-xylose, methyl α-D-glucoside, vanillic acid, ferulic acid and *m*-hydroxybenzoic acid, assimilation of the nitrogen compound cadaverine and for growth at 30 °C.

The type strain, CBS 7683^T, is deposited at the Centraalbureau voor Schimmelcultures, Utrecht, The

Netherlands. This strain was isolated from a plant leaf in Portugal.

DISCUSSION

In this study, we propose a novel species in the sexual genus *Sporidiobolus*. This species represents the sexual stage of *Sporobolomyces roseus*, which seems to be a common yeast in the phylloplane and other habitats in several geographical regions located on the temperate zone but not in the tropics (Nakase, 2000). To our knowledge, all the isolates that have been molecularly identified as *Sporobolomyces roseus* using D1/D2 sequences show at least one sequence mismatch towards CBS 486^T, the type strain of this species. This discrepancy is maintained in the comparison between the novel species, *Sporidiobolus metaroseus*, and CBS 486^T. In this analysis, we used the three D1/D2 sequences available in GenBank for CBS 486^T (=PYCC 4463^T), GenBank accession numbers DQ832234, AF070441 and EU003462 (since AF070441 differed in two positions from the other two sequences, and from all the other *Sporobolomyces roseus* sequences analysed, these two discrepancies in AF070441 were considered to be artefacts). In spite of this difference, we consider that our data justify the merger of *Sporobolomyces roseus* with *Sporidiobolus metaroseus* because no additional sequence differences were detected in the D1/D2 and complete ITS sequences and because CBS 486^T shows high DNA–DNA reassociation values when compared with the type strain of *Sporidiobolus metaroseus*. Another salient aspect of CBS 486^T is the nine physiological tests that are discrepant towards all other strains (approx. 20) of *Sporidiobolus metaroseus* that were studied. In the phylogenetic tree of Fig. 2, the sequences of some undescribed *Sporobolomyces* species that are phenotypically very close to *Sporobolomyces metaroseus* are included for comparison. The sequence of *Sporobolomyces* sp. IAM 13481, incorrectly designated *Sporobolomyces roseus*, is also included because the complete sequence of the genome of this strain has been released recently by the Joint Genome Institute (http://www.jgi.doe.gov).

Another matter that merits some consideration is the variability observed in the set of strains of *Sporidiobolus metaroseus* that we studied and that led to the formation of two groups, named Roseus and Pseudoroseus. Initially, we considered the possibility of describing two novel *Sporidiobolus* species, *Sporidiobolus metaroseus* and '*Sporidiobolus pseudoroseus*'. The arguments in favour of such an option are the distinct basidial morphology, the few (but consistent among a large dataset of strains) sequence differences and the intermediate to low DNA–DNA reassociation values among the representatives of the two 'species'. However, we also weighed the contrary arguments, i.e. the absence of a sexual state in most of the strains that were studied (which are therefore phenotypically undistinguishable based on morphology or physiology), the low level of sequence divergence, the impossibility of using DNA–DNA reassociation on a routine basis and the peculiar phylogenetic position of the type strain of *Sporobolomyces roseus* (which has a unique sequence mismatch in the D1/D2 sequence towards all the other strains that were considered). Consequently, we opted to describe a single sexual species, *Sporidiobolus metaroseus*, encompassing all the self-fertile strains and the phylogenetically related asexual strains, including the type strain of *Sporobolomyces roseus*. In the future, if our DNA–DNA reassociation results are corroborated by new sequence data, the recognition of the strains of the Pseudoroseus group as a distinct species might have to be considered.

The third matter that justifies some attention is the prevalence of asexual strains in *Sporidiobolus metaroseus*. In fact, we could observe the sexual stage in only four of more than 20 strains that were studied. Initially, we reasoned that, besides the self-fertile strains, heterothallic (self-sterile) strains would be found after mating experiments were carried out, as happened before for *Rhodosporidium kratochvilovae* and *Sporidiobolus ruineniae* (Sampaio *et al.*, 2001; Valério *et al.*, 2002). We performed crosses on cornmeal agar on several occasions but failed to observe the development of mycelium with teliospores. At present, we do not have an explanation for the apparent lack of sexual behaviour of the majority of the strains. In *Sporidiobolus*, apart from *Sporidiobolus microsporus* and *Sporidiobolus ruineniae*, phylogenetically more related to *Rhodosporidium fluviale* than to the other species of *Sporidiobolus*, only *Sporidiobolus longiusculus* and *Sporidiobolus salmonicolor* seem to have a typical sexual life cycle, since neither *Sporidiobolus johnsonii* (Valério *et al.*, 2008) nor *Sporidiobolus pararoseus* (this study) seems to produce basidia or basidiospores. It is not possible to differentiate *Sporidiobolus metaroseus* from its closest relatives using phenotypic criteria. The sexual and asexual species depicted in the Longiusculus, Metaroseus and Pararoseus clusters (Fig. 2) are difficult or impossible to distinguish in the absence of molecular data. Salient physiological characteristics of *Sporidiobolus metaroseus* are the abilities to grow on lactate and nitrate, which are absent in *Sporidiobolus longiusculus* and *Sporidiobolus pararoseus*.

ACKNOWLEDGEMENTS

The authors are indebted to Dr Vincent Robert (CBS, The Netherlands) for providing valuable cultures and to Dr Michael Weiß (University of Tübingen, Germany) for preparing the Latin diagnosis.

REFERENCES

Bai, F.-Y., Zhao, J.-J., Takashima, M., Jia, J.-H., Boekhout, T. & Nakase, T. (2002). Reclassification of the *Sporobolomyces roseus* and *Sporidiobolus pararoseus* complexes, with the description of *Sporobolomyces phaffii* sp. nov. *Int J Syst Evol Microbiol* **52**, 2309–2314.

Bauer, R., Begerow, D., Sampaio, J. P., Weiß, M. & Oberwinkler, F. (2006). The simple-septate basidiomycetes: a synopsis. *Mycol Prog* **5**, 41–66.

Boekhout, T. (1991). A revision of ballistoconidia-forming yeasts and fungi. *Stud Mycol* **33**, 1–194.

Boekhout, T. & Nakase, T. (1998). *Sporobolomyces* Kluyver & van Niel. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 828–843. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.

Derx, H. G. (1930). Etude sur les Sporobolomycetes. *Ann Mycol* **28**, 1–23 (in French).

Fell, J. W. & Statzell-Tallman, A. (1984). *Sporidiobolus* Nyland. In *The Yeasts, a Taxonomic Study*, 3rd edn, pp. 532–540. Edited by N. J. W. Kreger-van Rij. Amsterdam: Elsevier.

Fell, J. W. & Tallman, A. S. (1981). Heterothallism in the basidiomycetous yeast genus *Sporidiobolus* Nyland. *Curr Microbiol* **5**, 77–82.

Fell, J. W., Blatt, G. M. & Statzell-Tallman, A. (1998). Validation of the basidiomycetous yeast, *Sporidiobolus microsporus* sp. nov., based on phenotypic and molecular analyses. *Antonie van Leeuwenhoek* **74**, 265–270.

Fell, J. W., Boekhout, T., Fonseca, A., Scorzetti, G. & Statzell-Tallman, A. (2000). Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. *Int J Syst Evol Microbiol* **50**, 1351–1371.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Gadanho, M., Almeida, J. M. F. & Sampaio, J. P. (2003). Assessment of yeast diversity in a marine environment in the south of Portugal by microsatellite-primed PCR. *Antonie van Leeuwenhoek* **84**, 217–227.

Hernandez-Saavedra, N. Y., Hernandez-Saavedra, D. & Ochoa, J. L. (1992). Distribution of *Sporobolomyces* (Kluyver et van Niel) genus in the western coast of Baja California Sur, Mexico. *Syst Appl Microbiol* **15**, 319–322.

Hirst, J. M. (1953). Changes in atmospheric spore content: diurnal periodicity and the effects of water. *Trans Br Mycol Soc* **36**, 375–393.

Kurtzman, C. P., Smiley, M. J., Johnson, C. J., Wickerham, L. J. & Fuson, G. B. (1980). Two new and closely related heterothallic species, *Pichia amylophila* and *Pichia mississipiensis*: characterization by hybridization and deoxyribonucleic acid reassociation. *Int J Syst Bacteriol* **30**, 208–216.

Last, F. T. (1955). Seasonal incidence of *Sporobolomyces* on cereal leaves. *Trans Br Mycol Soc* **38**, 221–239.

Libkind, D., Brizzio, S., Ruffini, A., Gadanho, M., van Broock, M. & Sampaio, J. P. (2003). Molecular characterization of carotenogenic yeasts from aquatic environments in Patagonia, Argentina. *Antonie van Leeuwenhoek* **84**, 313–322.

Nakase, T. (2000). Expanding world of ballistosporous yeasts: distribution in the phyllosphere, systematics and phylogeny. *J Gen Appl Microbiol* **46**, 189–216.

Sampaio, J. P., Gadanho, M., Santos, S., Duarte, F. L., Pais, C., Fonseca, A. & Fell, J. W. (2001). Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodosporidium*: *Rhodosporidium kratochvilovae* and related anamorphic species. *Int J Syst Evol Microbiol* **51**, 687–697.

Statzell-Tallman, A. & Fell, J. W. (1998). *Sporidiobolus* Nyland. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 693–699. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.

Swofford, D. L. (2002). PAUP*: phylogenetic analysis using parsimony (and other methods), version 4. Sunderland, MA: Sinauer Associates.

Tubaki, K. (1953). Studies on the Sporobolomycetaceae in Japan. III. On *Sporobolomyces* and *Bullera*. *Nagaoa* **3**, 12–21.

Valério, E., Gadanho, M. & Sampaio, J. P. (2002). *Sporobolomyces odoratus* sp. nov., a new species in the *Sporidiobolus ruineniae* clade. *FEMS Yeast Res* **2**, 9–16.

Valério, E., Gadanho, M. & Sampaio, J. P. (2008). *Sporidiobolus johnsonii* and *Sporidiobolus salmonicolor* revisited. *Mycol Prog* (in press). Published online ahead of print 6 September 2007. doi:10.1007/s11557-007-0547-8.