*International Journal of Systematic and Evolutionary Microbiology* (2001), **51**, 221–229 Printed in Great Britain

# Taxonomic studies on the genus *Cystofilobasidium*: description of *Cystofilobasidium ferigula* sp. nov. and clarification of the status of *Cystofilobasidium lari-marini*

José Paulo Sampaio,[1] Mário Gadanho[1] and Robert Bauer[2]

Author for correspondence: José Paulo Sampaio. Tel: +351 21 2948300. Fax: +351 21 2948530. e-mail: jss@mail.fct.unl.pt

[1] Centro de Recursos Microbiológicos, Secção Autónoma de Biotecnologia, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2825-114 Caparica, Portugal

[2] Universität Tübingen, Institut für Biologie I, Lehrstuhl Spezielle Botanik und Mykologie, Auf der Morgenstelle, 1, D-72076 Tübingen, Germany

**A new species of the genus *Cystofilobasidium* is described as *Cystofilobasidium ferigula* sp. nov. The new taxon represents the teleomorphic stage of *Cryptococcus ferigula* and was obtained in mating experiments using three strains deposited in the Portuguese Yeast Culture Collection (mating types A1) and a recent isolate (mating type A2). *Cystofilobasidium ferigula* is characterized using an integrated approach encompassing morphological studies, investigation of the ultrastructure of the septal pore, a comparative study of physiological traits, determination of the DNA base composition, DNA reassociation experiments and PCR fingerprinting. During the course of this study, a close similarity of microsatellite-primed PCR fingerprints was detected between *Cystofilobasidium lari-marini* and *Cystofilobasidium capitatum*. DNA–DNA reassociation experiments gave high homology values, which indicates that *Cystofilobasidium lari-marini* must be regarded as a synonym of *Cystofilobasidium capitatum*.**

**Keywords:** heterobasidiomycetes, basidiomycetous yeasts, systematics, Cystofilobasidiales, *Cystofilobasidium ferigula* sp. nov.

## INTRODUCTION

The heterobasidiomycetous genus *Cystofilobasidium* Oberwinkler *et* Bandoni (Oberwinkler *et al*., 1983) was created to accommodate two teliospore-producing yeasts previously classified in *Rhodosporidium* Banno, namely *Cystofilobasidium bisporidii* (Fell, Hunter *et* Tallman) Oberwinkler *et* Bandoni and *Cystofilobasidium capitatum* (Fell, Hunter *et* Tallman) Oberwinkler *et* Bandoni. Members of the genus *Cystofilobasidium* produce *Filobasidium*-like basidia, i.e. holobasidia each having a narrow central region and a swollen apex (Oberwinkler *et al*., 1983), whereas in *Rhodosporidium* the basidia are tubular and transversely septate. At present, the genus *Cystofilobasidium* includes four species: three are pink-coloured and one is cream-coloured. The pigmented species are the two mentioned above and *Cystofilobasidium infirmo-miniatum* (Fell, Hunter *et* Tallman) Hamamoto, Sugiyama *et* Komagata (Hamamoto *et al*., 1988), also transferred from *Rhodosporidium*. More recently, *Cystofilobasidium lari-marini* (Saëz *et* Nguyen) Fell *et* Statzell-Tallman (cream-coloured) was transferred from *Leucosporidium* Fell, Statzell, Hunter *et* Phaff (Fell & Statzell-Tallman, 1992).

The order Cystofilobasidiales (Fell *et al*., 1999) was proposed on the basis of studies of 26S rDNA sequences. This order includes two teleomorphic genera, *Cystofilobasidium* and *Mrakia* Yamada *et* Komagata, the anamorphic genus *Udeniomyces* Nakase *et* Takematsu and three species of *Cryptococcus* Vuillemin, viz. *Cryptococcus aquaticus* (Jones *et* Slooff) Rodrigues de Miranda *et* Weijman, *Cryptococcus ferigula* (corrig.) Saëz *et* Rodrigues de Miranda and *Cryptococcus macerans* (Frederiksen) Phaff *et* Fell. According to Fell *et al*. (1999), the Cystofilobasidiales clade includes two subclades, the *Mrakia* subclade and the *Cystofilobasidium* subclade. The latter group includes all known species of *Cystofilobasidium* and also *Cryptococcus ferigula* and *Cryptococcus macerans*.

**Abbreviation:** CMA, corn meal agar; UPGMA, unweighted pair group method using arithmetic averages.

*Cryptococcus ferigula* is a pink yeast originally found in the oral cavity of several wild animals kept in captivity (Saëz & Rodrigues de Miranda, 1988). This report presents the description of the sexual state of *Cryptococcus ferigula* and the consequent proposal of a new species of the genus *Cystofilobasidium*. Our studies also revealed that *Cystofilobasidium lari-marini* should be considered as a synonym of *Cystofilobasidium capitatum*.

## METHODS

**Yeast cultures.** The list of strains used in this study, and relevant information, is shown in Table 1.

**Morphological and physiological characterization.** Physiological and biochemical characterization was performed 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 (1994), respectively. All assimilation tests were performed twice, in separate experiments. The results of the 78 tests selected by Sampaio & Fonseca (1995) were used to calculate the overall similarity of physiological and biochemical profiles, employing numerical taxonomy methods (the data matrix is available upon request). The similarity between strains was estimated using the simple-matching coefficient and cluster analysis was performed using the UPGMA (unweighted pair-group method using arithmetic averages) algorithm (Sneath & Sokal, 1973). These analyses were computed using the NTSYS-pc software package, version 2.0 (Rolf, 1998). For microscopy, cultures were grown at room temperature on YM agar (Yarrow, 1998) or on corn meal agar (CMA) and studied with phase-contrast optics.

**Ultrastructure.** For transmission electron microscopy, samples were fixed overnight in 2 % glutaraldehyde in 0·1 M sodium cacodylate buffer at pH 7·2. After 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, 50, 70 and 95 % (v/v), and three times in 100 % acetone. The material was embedded in Spurr's plastic (Spurr, 1969). Serial sections (65–75 nm) were cut with a Reichert–Jung Ultracut E (Leica) 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.

**Determination of DNA base composition and DNA reassociation experiments.** For DNA isolation, strains were

***Table 1.*** List of strains studied in this work

Culture collections are abbreviated as: IGC, Portuguese Yeast Culture Collection, FCT-UNL, Caparica, Portugal; CBS, Centraalbureau voor Schimmelcultures, Yeast Division, Delft, The Netherlands; FO, personal collection of F. Oberwinkler, University of Tübingen, Germany; ZP, personal collection of J. P. Sampaio.

| Species | Strain | Strain origin | Isolation source | Observations |
|---|---|---|---|---|
| *Cryptococcus macerans* | IGC 2869$^T$ | CBS 2206$^T$ | Dew-retted flax straw, Denmark | |
| *Cryptococcus macerans* | IGC 4482 | | Flower of *Cineraria* sp., Oeiras, Portugal | |
| *Cryptococcus macerans* | IGC 4737 | | Grasshopper, Sintra, Portugal | |
| *Cystofilobasidium bisporidii* | IGC 4415$^T$ | CBS 6346$^T$ | Seawater, Antarctic Ocean | Mating type A1B1 |
| *Cystofilobasidium capitatum* | IGC 4418$^T$ | CBS 6358$^T$ | Zooplankton, Antarctic Ocean | Self-sporulating |
| *Cystofilobasidium capitatum* | IGC 4309 | FO 31572.a | Gleba of *Phallus impudicus*, Tübingen, Germany | Self-sporulating |
| *Cystofilobasidium capitatum* | IGC 5626 | ZP 317 | *Trifolium* sp. leaf infected with rust, Sesimbra, Portugal | Self-sporulating |
| *Cystofilobasidium capitatum* | IGC 5627 | ZP 327 | Contaminant of MYP agar plate, Caparica, Portugal | Self-sporulating |
| *Cystofilobasidium ferigula* | IGC 5359$^T$ | CBS 7202$^T$ | Dead male *Papio papio* (baboon), Paris Zoological Gardens, France | Mating type A1 |
| *Cystofilobasidium ferigula* | IGC 4410 | CBS 7201 | *Rhea americana* (rhea), Paris Zoological Gardens, France | Mating type A1 |
| *Cystofilobasidium ferigula* | IGC 4410-I | | – | Colourless variant of IGC 4410 |
| *Cystofilobasidium ferigula* | IGC 5540 | CBS 6954 | Sawdust (locality of isolation unknown) | Mating type A1 |
| *Cystofilobasidium ferigula* | IGC 5628 | ZP 322 | Contamination of home-made sauerkraut, Caparica, Portugal | Mating type A2 |
| *Cystofilobasidium infirmominiatum* | IGC 3955$^T$ | CBS 323$^T$ | Air, Japan | Mating type A1 |
| *Cystofilobasidium infirmominiatum* | IGC 4414 | CBS 2204 | Deep-frozen vegetables, The Netherlands | Mating type A2 |
| *Cystofilobasidium lari-marini* | IGC 4530$^T$ | CBS 7420$^T$ | *Larus marinus* (Great black-backed gull), France | Self-sporulating |

***Table 2.*** Physiological characterization of strains of *Cystofilobasidium ferigula*

Growth is scored as: +, positive; −, negative; D, delayed; W, weak. All four strains gave positive results for utilization of the following compounds: D-glucose, L-sorbose, L-arabinose, L-rhamnose, cellobiose, salicin, arbutin, D-mannitol, inositol, glucono-$\delta$-lactone, 2-keto-D-gluconic acid, D-gluconic acid, D-glucuronic acid, saccharic acid and ethanol. All four strains gave negative results for utilization of the following compounds: D-galactose, D-glucosamine, sucrose, maltose, methyl $\alpha$-D-glucoside, melibiose, lactose, raffinose, melezitose, inulin, soluble starch, erythritol, galactitol, DL-lactic acid, succinic acid, L-tartaric acid, D-tartaric acid, *m*-tartaric acid, mucic acid, methanol, vanillyl alcohol, veratryl alcohol, vanillic acid, veratric acid, ferulic acid, syringic acid, sinapic acid, cinnamic acid, benzoic acid, *p*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, protocatechuic acid, catechol, gallic acid, salicylic acid, gentisic acid, *p*-coumaric acid, caffeic acid, phenol and guaiacol. All four strains gave the same results for the following nitrogen compounds (responses are in parentheses): potassium nitrate (+), sodium nitrite (+), ethylamine (−), L-lysine (D), cadaverine (−), creatine (−), creatinine (−) and D-glucosamine (−). All four strains gave the same results for the following additional tests (responses are in parentheses): growth in vitamin-free medium (−), growth with 0·1 % cycloheximide (−), growth at 30 °C (+), growth at 35 °C (−), formation of starch-like compounds (+), splitting of arbutin (+), hydrolysis of urea (+) and the colour reaction with diazonium blue B (+).

| Characteristic | IGC 5359$^T$ | IGC 4410 | IGC 5540 | IGC 5628 |
|---|---|---|---|---|
| Utilization of: | | | | |
| D-Ribose | DW | DW | − | DW |
| D-Xylose | + | D | D | + |
| D-Arabinose | + | + | − | − |
| $\alpha,\alpha$-Trehalose | + | DW | DW | + |
| Glycerol | − | −, DW | − | − |
| Ribitol | − | − | − | −, D |
| Xylitol | − | − | − | −, DW |
| D-Glucitol | + | D | D | + |
| Citric acid | DW | − | D | D |
| L-Malic acid | − | −, + | − | − |
| Growth with 0·01 % cycloheximide | − | − | − | D |

grown for 3–4 d at 22 °C in shaken YM broth (initial pH 8). Cells were ruptured using a hydraulic cell press and the DNA was purified using the method of Britten *et al*. (1970). The nuclear DNA base composition was determined by following the method of Marmur & Doty (1962) with a Gilford Response UV-VIS spectrophotometer and its Thermal Programming software with nDNA from *Candida parapsilosis* (Ashford) Langeron *et* Talice IGC 2545$^T$ (= CBS 604$^T$) (G+C content = 40·2 mol %) as a reference. For DNA–DNA reassociation experiments, the same instrument was used and the methods of Seidler & Mandel (1971), as modified by Kurtzman *et al*. (1980), were followed.

**PCR fingerprinting.** Cultures grown on MYP agar were suspended in 500 µl lysing buffer (50 mM Tris/HCl, 250 mM NaCl, 50 mM EDTA, 0·3 % SDS, pH 8) and glass beads (425–600 µm; Sigma) equivalent to a volume of 200 µl were added. After being vortexed for 3 min, the tubes were incubated for 1 h at 65 °C. The suspensions were then centrifuged for 30 min at 4 °C. Nucleic acids were precipitated during 24 h at −20 °C with a 0·1 vol 3 M sodium acetate and 2 vols absolute ethanol. Finally, the DNA was washed with ethanol (70 %), vacuum-dried and then resuspended in TE (100 mM Tris/HCl, pH 8, 100 mM EDTA).

The primer $(GTG)_5$ (Meyer *et al*., 1993), synthesized by Pharmacia Amersham Biotech, was used for the microsatellite-primed PCR experiments. PCR reactions were performed in 25-µl reaction volumes containing 1 × PCR buffer (Pharmacia Amersham Biotech), 2 mM each of dATP, dCTP, dGTP and dTTP (Promega), 0·8 µM primer, 10–15 ng genomic DNA and 1 U *Taq* DNA polymerase (Pharmacia Amersham Biotech). Amplification was performed in a Uno II Thermal Cycler (Biometra) and consisted of an initial denaturation step at 95 °C for 5 min, followed by 40 cycles of 45 s at 93 °C, 60 s at 50 °C and 60 s at 72 °C and then a final extension step of 6 min at 72 °C. A negative control, in which DNA was replaced by sterile distilled water, was also included. Amplified DNA fragments were separated by electrophoresis in 1·4 % agarose gel (Gibco), in 0·5 × TBE (Tris/borate/EDTA) buffer at 90 V for 3·5 h and stained with ethidium bromide. On each gel, a molecular size marker was used for reference ($\lambda$DNA cleaved with *Hin*dIII and ΦX174 DNA cleaved with *Hae*III; Pharmacia Amersham Biotech). DNA banding patterns were visualized under UV transillumination and images were obtained using a Kodak Digital Science EDA 120 system and the Kodak Digital Science 1D Image Analysis software. DNA banding patterns were analysed using the GELCOMPAR software package, version 4.1 (Applied Maths). Similarities among isolates were estimated using the Dice coefficient and the clustering was based on UPGMA.

## RESULTS AND DISCUSSION

A pink yeast strain (IGC 5628), isolated by us from home-made sauerkraut, was identified (on the basis of standard yeast identification tests) as *Cryptococcus ferigula*. Crossing experiments performed on CMA revealed that IGC 5628 was sexually compatible with the type strain of this species. The teleomorph of *Cryptococcus ferigula* is described as a new species of the genus *Cystofilobasidium*.

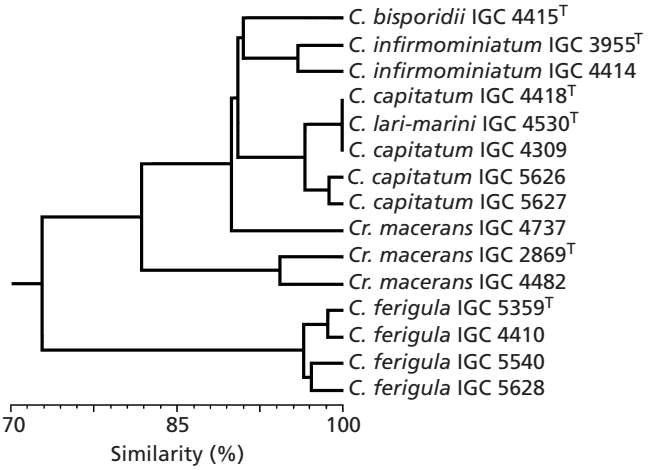


***Fig. 1.*** Phenogram of *Cystofilobasidium* (*C.*) and *Cryptococcus* (*Cr.*) *macerans* based on overall similarity (simple-matching coefficient) and cluster analysis (UPGMA) of 78 physiological tests.

### Characterization of *Cystofilobasidium ferigula* sp. nov.

A mycelium with clamp connections forms 2 d after inoculation, and large numbers of teliospores can be detected after 1 week at room temperature (20–22 °C). Germination of teliospores requires a resting period. Good results were obtained by harvesting the teliospores from 1-month-old cultures and soaking them in demineralized water for 6 weeks at 4 °C. The soaked agar cubes containing the teliospores were transferred to 2 % water agar and germination was observed after 2 d at room temperature.

Nutritional and biochemical profiles are given in Table 2. The nutritional characterization of the four strains of *Cystofilobasidium ferigula* corresponds to published data (Barnett *et al.*, 1990). However, three discrepant results were found. In our study, glycerol gave negative results with most strains, citric acid was utilized by three strains and L-lysine was utilized by all strains. Moreover, of the 76 assimilation tests performed, seven gave variable results. The utilization of L-malic acid was considered variable for IGC 4410, since, in four determinations, positive results were recorded twice and negative results were also recorded twice. In spite of the discrepant results, the four strains of *Cystofilobasidium ferigula* formed a distinct cluster in the overall comparison of physiological data (Fig. 1).

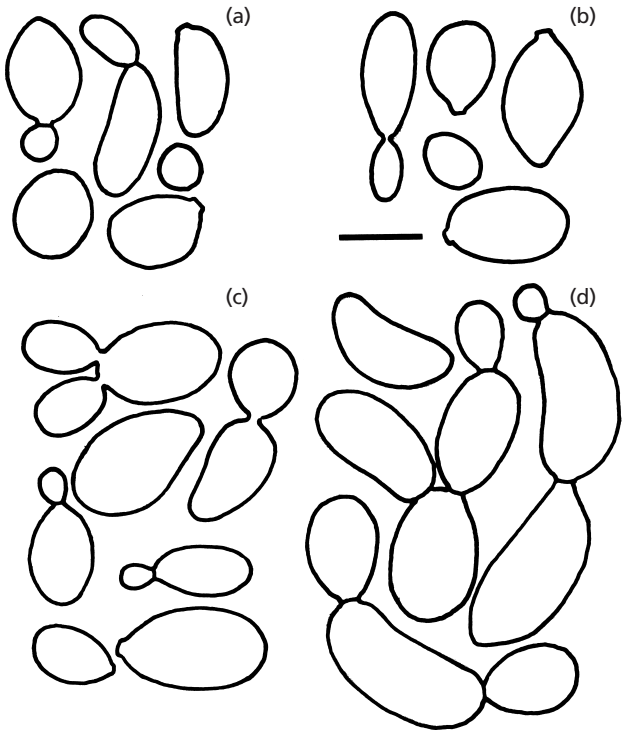


***Fig. 2.*** Line drawings of yeast cells of *Cystofilobasidium ferigula* strains IGC 5359T (a), IGC 4410 (b), IGC 5540 (c) and IGC 5628 (d) grown on YM agar for 1 week at 25 °C. Bar, 5 μm.

Physiological differences relevant to the other species in the *Cystofilobasidium* subclade are shown in Table 3.

Micromorphological differences were found between the four *Cystofilobasidium ferigula* isolates (Fig. 2).

***Table 3.*** Relevant physiological differences between the species of the subclade *Cystofilobasidium*

*C.*, *Cystofilobasidium*.

| Characteristic | *C. ferigula* | *C. bisporidii* | *C. infirmominiatum* | *C. capitatum* | *C. lari-marini* | *Cryptococcus macerans* |
|---|---|---|---|---|---|---|
| Utilization of: | | | | | | |
| Erythritol | − | − | − | − | − | + |
| L-Malic acid | −* | + | + | + | + | + |
| L-Tartaric acid | − | − | + | − | − | − |
| Ethylamine | − | + | + | + | + | + |
| Cadaverine | − | + | + | + | + | + |
| Growth at 30 °C | + | − | − | − | − | − |

* Variable for strain IGC 4410.

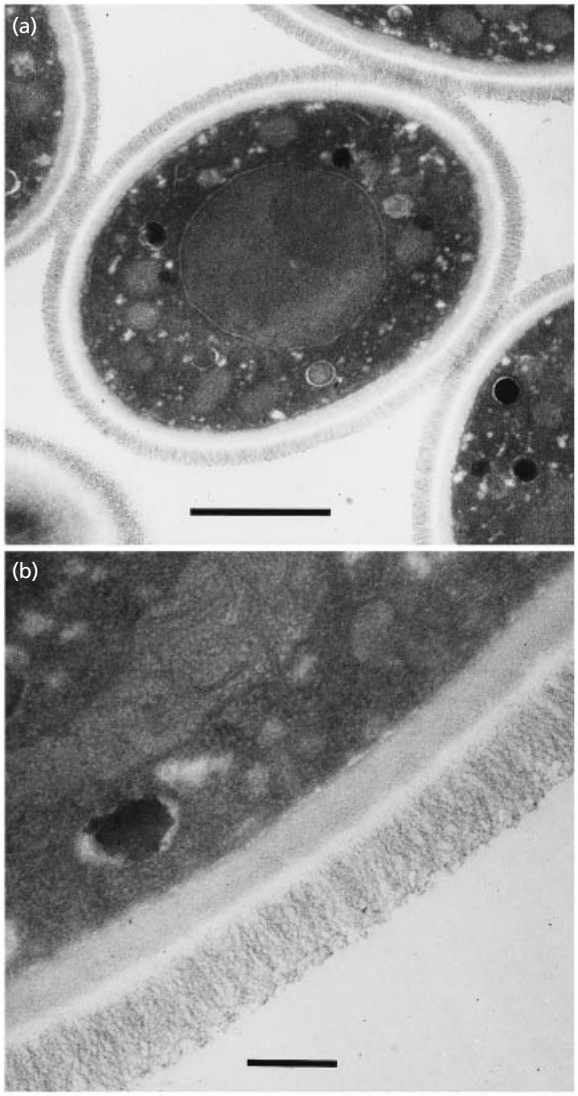


***Fig. 3.*** Transmission electron micrographs of *Cystofilobasidium ferigula* (IGC 5359$^T$ × IGC 5628) grown for 1 month on CMA. Note that the yeast cells have a layer of fine fibrils at the surface. Bars: 1 μm (a) and 0·2 μm (b).

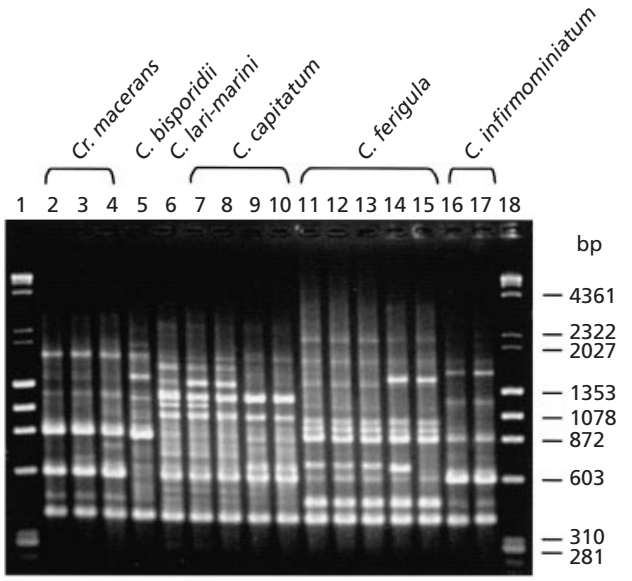


***Fig. 4.*** Microsatellite-primed PCR fingerprints of *Cystofilobasidium* (*C.*) and *Cryptococcus* (*Cr.*) *macerans* obtained with primer $(GTG)_5$. Lanes: 1 and 18, λDNA cleaved with *Hin*dIII/ΦX174 DNA cleaved with *Hae*III marker; 2–4, *Cryptococcus macerans* IGC 2869$^T$, IGC 4482 and IGC 4737; 5, *Cystofilobasidium bisporidii* IGC 4415$^T$; 6, *Cystofilobasidium lari-marini* IGC 4530$^T$; 7–10, *Cystofilobasidium capitatum* IGC 4418$^T$, IGC 4309; IGC 5626 and IGC 5627; 11–15, *Cystofilobasidium ferigula* IGC 5359$^T$, IGC 4410, IGC 4410-I, IGC 5540 and IGC 5628; 16–17, *Cystofilobasidium infirmominiatum* IGC 3955$^T$ and IGC 4414.

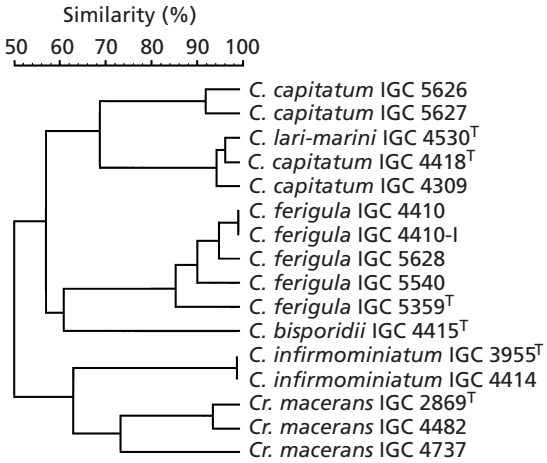


***Fig. 5.*** Dendrogram of *Cystofilobasidium* species and *Cryptococcus macerans* based on the analysis of the PCR fingerprints obtained with primer $(GTG)_5$, the Dice coefficient and the UPGMA cluster method.

Cells of strains belonging to mating type A1 were normally smaller than those of strain IGC 5628 (mating type A2). Moreover, cells of IGC 5628 were slightly curved. Transmission electron microscopy revealed that the yeast cells had a layer of fine fibrils at the surface (Fig. 3).

The G+C content of the nuclear DNA of strain IGC 5359$^T$ was 67·0±0·40 mol% (six determinations) and that of strain IGC 5628 was 66·4±0·12 mol% (six determinations). The homology value determined in DNA–DNA reassociation experiments with the two strains ranged between 87 and 95% (three determinations).

A method of PCR fingerprinting based on the microsatellite DNA primer $(GTG)_5$ was used to evaluate the relationships among *Cystofilobasidium ferigula* strains. Other strains belonging to the genus *Cystofilobasidium* and to *Cryptococcus macerans* were also tested using this approach. The PCR patterns obtained are depicted in Fig. 4, and their respective clustering, based on the Dice coefficient, is shown in Fig. 5.

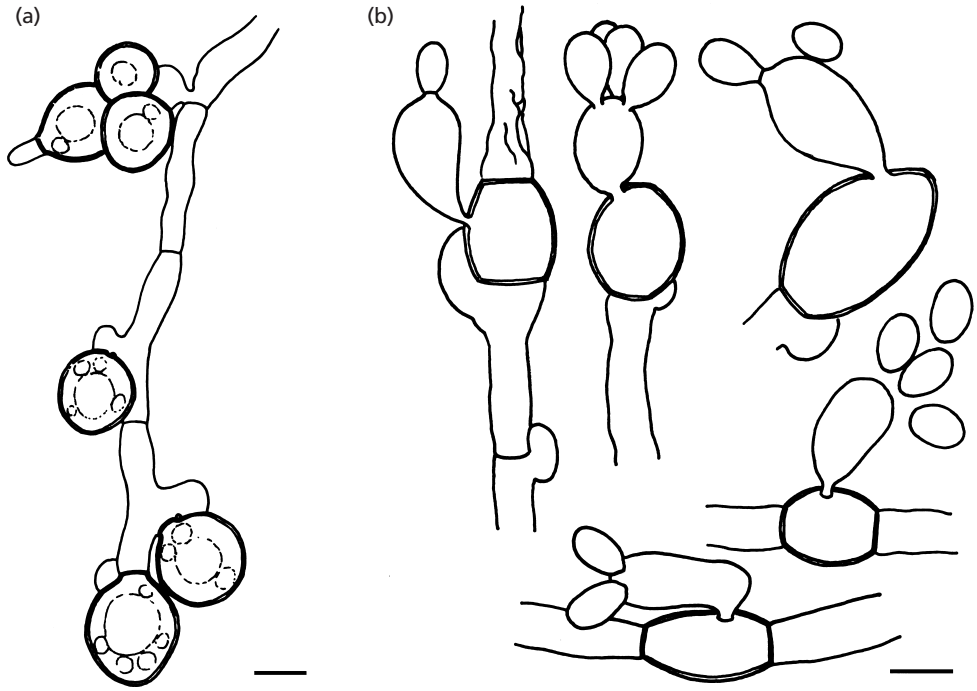


***Fig. 6.*** Line drawings of *Cystofilobasidium ferigula* showing (a) a hypha with lateral and terminal teliospores (IGC 5359$^T$ × IGC 5628) after 1 month on CMA and (b) germinated teliospores with basidia and basidiospores (IGC 5359$^T$ × IGC 5628 and IGC 4410 × IGC 5628). Bars, 5 μm.

Two types of basidial morphology can be distinguished in *Cystofilobasidium*. In *Cystofilobasidium capitatum*, *Cystofilobasidium bisporidii* and *Cystofilobasidium lari-marini*, the basidia are elongated. The capitate apical swelling is separated from the teliospore by a tubular structure measuring up to 80 μm in *Cystofilobasidium capitatum* (Fell *et al*., 1973; Oberwinkler *et al*., 1983), up to 140 μm in *Cystofilobasidium bisporidii* (Fell *et al*., 1973) and up to 106 μm in *Cystofilobasidium lari-marini* (Fell & Statzell-Tallman, 1992). The other basidial type is present in *Cystofilobasidium infirmo-miniatum* and in the new species, *Cystofilobasidium ferigula*. In *Cystofilobasidium infirmominiatum*, the basidia are usually pyriform (Fig. 18 of Fell *et al*., 1973), with the large base attached to the teliospore, and basidial length does not exceed 14 μm (Fell *et al*., 1973). In *Cystofilobasidium ferigula*, basidia are normally cylindrical and their maximum length is 12 μm (Fig. 6). It has been reported that the species belonging to the first group occasionally produce short basidia, which can resemble those of the second group (Fig. 12 of Fell *et al*., 1973). However, the occurrence of long basidia has never been observed in *Cysto-filobasidium infirmominiatum* or *Cystofilobasidium ferigula*. The short basidial type of *Cystofilo-basidium* is similar to the basidia produced by species of *Mrakia*, both in shape and in size (Fig. 12 of Fell *et al*., 1969 and Fig. 336 of Fell & Statzell-Tallman, 1998).

In addition to basidial morphology, other traits support the assignment of the new species to *Cystofilobasidium*. *Cystofilobasidium ferigula* and the other species in the genus assimilate inositol, glucuronate, nitrate and nitrite, possess a CoQ 8 system (Fell *et al*., 1999) and have xylose in the cell walls (Fell *et al*., 1999). Moreover, *Cystofilobasidium ferigula* does not utilize any of the aromatic compounds tested (Table 2). The incapacity to as-similate aromatic compounds was observed for the vast majority of species of the *Cystofilobasidiales* (Sampaio, 1999). The three known exceptions are *Cystofilobasidium capitatum*, *Cystofilobasidium lari-marini* and *Cryptococcus huempii* (Ramírez *et* González) Roeijmans, van Eijk *et* Yarrow, which were able to assimilate caffeic acid (Sampaio, 1999). Fur-thermore, the hyphal septa of *Cystofilobasidium ferigula* have dolipores without parenthesomes (Fig. 7). This septal pore type is normally interpreted as a retained primitive characteristic and is also present in *Cystofilobasidium capitatum* (Oberwinkler *et al*., 1983), *Cystofilobasidium lari-marini* (Suh & Sugiyama, 1993), *Cystofilobasidium infirmominiatum* and *Mrakia frigida* (Suh *et al*., 1993). In *Cystofilobasidium ferigula*, cisternae of the endoplasmic reticulum were sometimes found in the vicinity of the septal pore (Fig. 7). In contrast to the parenthesomes, these cisternae do not have an exact position in relation to the septal pore and lack an additional internal layer.

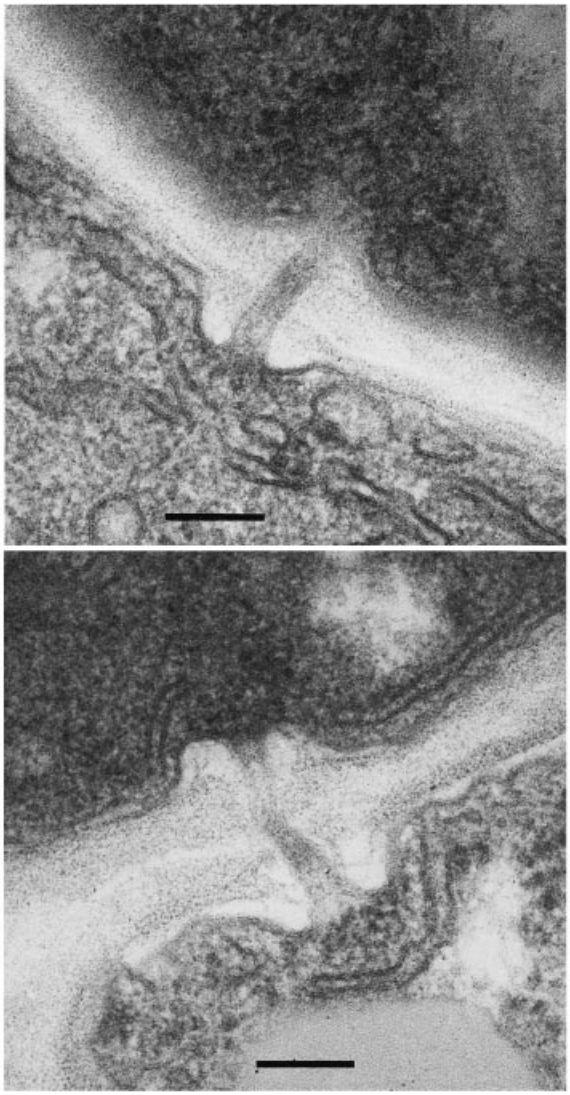

***Fig. 7.*** Transmission electron micrographs of *Cystofilobasidium ferigula* (IGC 5359$^T$ × IGC 5628) grown for 1 month on CMA, showing dolipores without parenthesomes. Note that cisternae of endoplasmic reticulum are located in the neighbouring areas of the pores. Bars, 0·1 μm.

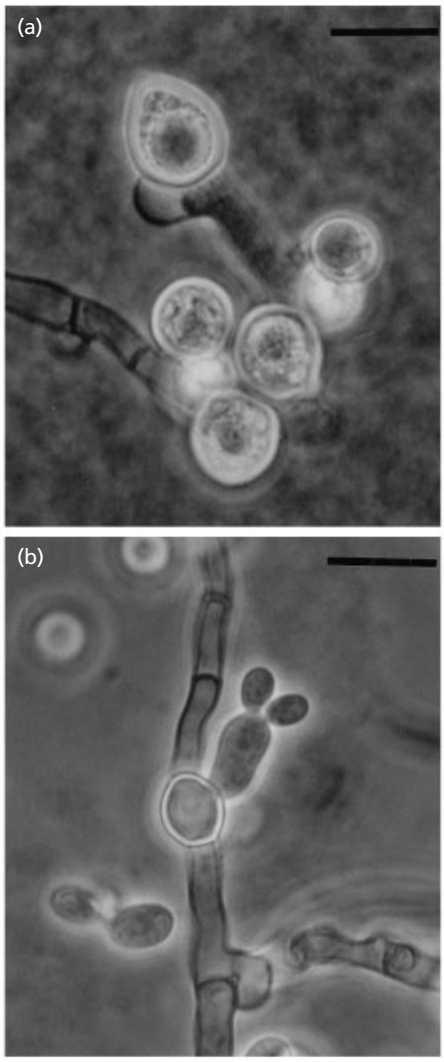


***Fig. 8.*** Micrographs of *Cystofilobasidium ferigula* showing (a) 2-week-old teliospores on CMA (IGC 5359$^T$ × IGC 5628) and (b) a germinated teliospore with a basidium and two basidiospores (IGC 4410 × IGC 5628). Bars, 10 μm.

At the molecular level, phylogenetic studies using 26S rDNA sequences indicate a close relationship between *Cystofilobasidium ferigula* (*Cryptococcus ferigula*) and the remaining species of *Cystofilobasidium* (Fell *et al*., 1999).

The microsatellite-primed PCR fingerprinting method using primer $(GTG)_5$ proved to be a useful approach for differentiating most species of *Cystofilobasidium* (Figs 4 and 5). Moreover, the three strains of *Cryptococcus macerans* were grouped in one cluster (Fig. 5), which contradicts the heterogeneity of the species suggested in the analysis of phenotypic data (Fig. 1). During this study, a non-pigmented variant of strain *Cystofilobasidium ferigula* IGC 4410 was detected, purified and maintained as IGC 4410-I. The PCR-fingerprinting banding profiles validated the origin of IGC 4410-I as a non-pigmented variant of IGC 4410 (Fig. 4). Moreover, this non-pigmented variant was also sexually compatible with IGC 5628. The exception to the good species separation was *Cystofilobasidium lari-marini*, which clustered among the strains of *Cystofilobasidium capitatum* (Fig. 5). In addition, the banding profile of *Cystofilobasidium lari-marini* was identical to the pattern obtained for the type strain of *Cystofilobasidium capitatum* (Fig. 4). These results suggested conspecificity between the two species; in order to test this hypothesis, the G+C content of the nuclear DNA was determined and DNA–DNA reassociation experiments were carried out using both type strains. *Cystofilobasidium capitatum* had a G+C content of 60·0±0·61 mol% (five determinations) and *Cystofilobasidium lari-marini* had a value of 60·2± 0·33 mol% (five determinations). The homology

values obtained ranged between 85 and 100% (three determinations), which confirms that *Cystofilobasidium lari-marini* must be regarded as a synonym of *Cystofilobasidium capitatum*.

In spite of different culture pigmentation, a close relationship had been observed between *Cystofilobasidium capitatum* and *Cystofilobasidium lari-marini* in molecular phylogenetic studies using 26S rDNA sequences (Fell *et al*., 1999). Moreover, *Cystofilobasidium capitatum* and *Cystofilobasidium lari-marini* are also very similar with respect to basidial morphology (Fell & Statzell-Tallman, 1992), septal pore ultrastructure (Suh & Sugiyama, 1993) and nutritional profiles (Table 3 and Fig. 1). Culture pigmentation must therefore be considered to be variable in *Cystofilobasidium capitatum*, since typical isolates of this species are orange in colour, whereas IGC 4530 has cream-coloured colonies. Pigmented yeast species can sometimes give rise to colourless variants, as was reported for *Rhodosporidium toruloides* Banno (Joo *et al*., 1988) and as observed in the present study with strain IGC 4410-I of *Cystofilobasidium ferigula*.

### Latin diagnosis of *Cystofilobasidium ferigula* Sampaio, Gadanho *et* Bauer sp. nov.

*Hyphae hyalinae* (3–3·5 μm *in diametro*), *septis fibulatis*. *Teliosporae laterales*, *terminales vel intercalares*, *fibulatae*, *in culturis duorum mensium subglobosae ad plus minusve dacryoideae*, 7–11 × 7–12 μm *si terminales vel laterales*, *elongatiores* (6–8 × 8–13 μm) *si intercalares*, *aggregatae demum cinnamomeae*, *basidiis ovoideis ad cylindraceis* (4–7 × 7–12 μm) *germinant*. *Basidiosporae sessiles*, *plerumque in parte apicali basidii oriuntur*, *gemmis germinant*.

*Typus depositus in collectionis* Portuguese Yeast Culture Collection, FCT-UNL, Caparica, *Lusitania* (IGC 5359$^T$).

### Description of *Cystofilobasidium ferigula* Sampaio, Gadanho *et* Bauer sp. nov.

Anamorphic stage: *Cryptococcus ferigula* (nom. corrig.) Saëz *et* Rodrigues de Miranda 1988 (spelled incorrectly in the original publication as *Cryptococcus feraegula*).

Hyphae hyaline (3–3·5 μm in diameter), with clamp connections. Teliospores lateral, terminal or intercalary on the mycelium and subtended by a clamp connection. Teliospores in 1-month-old cultures are subglobose to slightly dacryoid (Figs 6a and 8a), 7–11 × 7–12 μm when terminal or lateral, more elongated (6–8 × 8–13 μm) when intercalary, formed in large groups that become orange-brown in colour. Upon germination, teliospores originate ovoidal to cylindrical basidia (4–7 × 7–12 μm) (Figs 6b and 8b). One to four sessile subglobose basidiospores (3–3·5 × 4–5 μm) are formed, normally on the apical portion of the basidia (Fig. 6). Basidiospores germinate by budding.

The type strain of *Cystofilobasidium ferigula*, strain IGC 5359$^T$ (mating type A1), has been deposited in the Portuguese Yeast Culture Collection, FCT-UNL, Caparica, Portugal.

## ACKNOWLEDGEMENTS

This work was supported financially by grant PRAXIS/PCNA/C/BIA/174/96 (FCT, Portugal) and by the Deutsche Forschungsgemeinschaft. M. G. was supported by grant PRAXIS/BTI/16804/98. The authors are indebted to Professors Isabel Spencer-Martins and Jack Fell, for critical reading of the manuscript, and to Dr Michael Weiß for preparing the Latin diagnosis.

## REFERENCES

**Barnett, J. A., Payne, R. W. & Yarrow, D. (1990).** *Yeasts: Characteristics and Identification*, 2nd edn. Cambridge: Cambridge University Press.

**Britten, R. J., Pavich, M. & Smith, J. (1970).** A new method of DNA purification. *Carnegie Inst Wash Year Book* **68**, 400–402.

**Fell, J. W. & Statzell-Tallman, A. (1992).** Systematic placement of the basidiomycetous yeast *Cystofilobasidium lari-marini* comb. nov. as predicted by rRNA nucleotide sequence analysis. *Antonie Leeuwenhoek* **62**, 209–213.

**Fell, J. W. & Statzell-Tallman, A. (1998).** *Mrakia* Y. Yamada & Komagata. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 676–677. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.

**Fell, J. W., Statzell, A. C., Hunter, I. L. & Phaff, H. J. (1969).** *Leucosporidium* gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. *Antonie Leeuwenhoek* **35**, 433–462.

**Fell, J. W., Hunter, I. L. & Tallman, A. S. (1973).** Marine basidiomycetous yeasts (*Rhodosporidium* spp. n.) with tetrapolar and multiple allelic bipolar mating systems. *Can J Microbiol* **19**, 643–657.

**Fell, J. W., Roeijmans, H. & Boekhout, T. (1999).** Cystofilobasidiales, a new order of basidiomycetous yeasts. *Int J Syst Bacteriol* **49**, 907–913.

**Fonseca, A. (1992).** Utilization of tartaric acid and related compounds by yeasts: taxonomic implications. *Can J Microbiol* **38**, 1242–1251.

**Hamamoto, M., Sugiyama, J. & Komagata, K. (1988).** Transfer of *Rhodosporidium infirmominiatum* to the genus *Cystofilobasidium* as *Cystofilobasidium infirmominiatum* comb. nov. *J Gen Appl Microbiol* **34**, 271–278.

**Joo, W. H., Sugiyama, J. & Komagata, K. (1988).** A colorless variant derived from *Rhodosporidium toruloides* strain YK 212 (IAM 13509, IFO 1236) and its taxonomic implication. *J Gen Appl Microbiol* **34**, 387–390.

**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 mississippiensis*: characterization by hybridization and deoxyribonucleic acid reassociation. *Int J Syst Bacteriol* **30**, 208–216.

**Marmur, J. & Doty, P. (1962).** Determination of the base composition of DNA from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

**Meyer, W., Mitchell, T. G., Freedman, E. Z. & Vilgalys, R. (1993).** Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to

distinguish strains of *Cryptococcus neoformans*. *J Clin Microbiol* **31**, 2274–2280.

**Oberwinkler, F., Bandoni, R., Blanz, P. & Kisimova-Horovitz, L. (1983).** *Cystofilobasidium*: a new genus in the Filobasidiaceae. *Syst Appl Microbiol* **4**, 114–122.

**Reynolds, E. S. (1963).** The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J Cell Biol* **17**, 208–212.

**Rolf, F. J. (1998).** NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System, version 2.0. New York: Applied Biostatistics.

**Saëz, H. & Rodrigues de Miranda, L. (1988).** Deux nouvelles espèces de levure, d'origine animale, isolées au Parc Zoologique de Paris: *Cryptococcus feraegula* et *Candida nanaspora*. *Bull Soc Mycol France* **104**, 213–215.

**Sampaio, J. P. (1994).** Utilization of low molecular weight lignin-related aromatic compounds for the selective isolation of yeasts: *Rhodotorula vanillica*, a new basidiomycetous yeast species. *Syst Appl Microbiol* **17**, 613–619.

**Sampaio, J. P. (1999).** Utilization of low molecular weight aromatic compounds by heterobasidiomycetous yeasts: taxonomic implications. *Can J Microbiol* **45**, 491–512.

**Sampaio, J. P. & Fonseca, A. (1995).** Physiological aspects in the systematics of heterobasidiomycetous yeasts. *Stud Mycol* **38**, 29–46.

**Seidler, R. J. & Mandel, M. (1971).** Quantitative aspects of deoxyribonucleic acid renaturation: base composition, site of chromosome replication, and polynucleotide homologies. *J Bacteriol* **106**, 608–614.

**Sneath, P. H. A. & Sokal, R. R. (1973).** *Numerical Taxonomy*. San Francisco: W. H. Freeman.

**Spurr, A. R. (1969).** A low-viscosity epoxy resin embedding medium for electron microscopy. *J Ultrastruct Res* **26**, 31–43.

**Suh, S.-O. & Sugiyama, J. (1993).** Septal pore ultrastructure of *Leucosporidium lari-marini*, a basidiomycetous yeast, and its taxonomic implications. *J Gen Appl Microbiol* **39**, 257–260.

**Suh, S.-O., Hirata, A., Sugiyama, J. & Komagata, K. (1993).** Septal ultrastructure of basidiomycetous yeasts and their taxonomic implications with observations on the ultrastructure of *Erythrobasidium hasegawianum* and *Sympodiomycopsis paphiopedili*. *Mycologia* **85**, 30–37.

**Yarrow, D. (1998).** Methods for the isolation, maintenance and identification of yeasts. In *The Yeasts*, *a Taxonomic Study*, 4th edn, pp. 77–100. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.