Curvibasidium cygneicollum gen. nov., sp. nov.
and *Curvibasidium pallidicorallinum* sp. nov.,
novel taxa in the Microbotryomycetidae
(Urediniomycetes), and their relationship with
Rhodotorula fujisanensis and *Rhodotorula*
nothofagi

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Strains of *Rhodotorula fujisanensis* (Basidiomycota, Urediniomycetes, Microbotryomycetidae), including the type strain, are sexually compatible and produce clamped mycelium with teliospores. However, as teliospore germination had not been documented, the complete sexual cycle was not known. During the course of this work, the basidial stage of *R. fujisanensis* was characterized. In addition, mating studies employing isolates that were identified preliminarily as *Rhodotorula nothofagi*, a species that is related closely to *R. fujisanensis*, yielded mycelium with teliospores, which formed basidia and basidiospores. The new data were evaluated by using several criteria, including the available molecular phylogenetic framework for the Microbotryomycetidae.

Curvibasidium gen. nov. is described here, to accommodate two teleomorphs: *Curvibasidium cygneicollum* sp. nov. (CBS 4551^T), which is described as the sexual stage of *R. fujisanensis*, and *Curvibasidium pallidicorallinum* sp. nov. (CBS 9091^T), which is related closely to *R. nothofagi*, but does not represent its sexual stage.

The dimorphic basidiomycetes are distributed among several lineages of the Basidiomycota (Swann & Taylor, 1995; Sampaio & Fonseca, 2002; Scorzetti *et al.*, 2002; Sampaio & Bauer, 2003). They constitute a remarkably diverse group of fungi, based on DNA sequence divergence studies, ultrastructural markers, ecological adaptations and

metabolic properties. The yeast stage allows the colonization of a wide range of ecological niches and is also important for dispersion. The filamentous stage tends to be more resilient, particularly when specialized resting structures, such as teliospores, are formed. Hyphae are also important in the development of the sexual stage and in the establishment of mycoparasitism or phytoparasitism.

Many species of basidiomycetous yeasts are known only in their asexual phase and are classified in genera such as *Rhodotorula* Harrison or *Cryptococcus* Vuillemin. However, mating studies have allowed the disclosure of the sexual stage of species that were previously classified in these genera. Typically, the teleomorphic stage is initiated with conjugation between two compatible yeast cells, followed by production of mycelium. When environmental conditions are adequate, basidia and basidiospores are produced. The investigation of new sexual states allows a better

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Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the sequences described in this work are as follows: AY383746, AY383747 and AY383748 (complete ITS region sequences of *Curvibasidium pallidicorallinum* CBS 6231, VKM Y-2861 and VKM Y-1135, respectively); AY383749 (complete ITS region sequence of *Rhodotorula nothofagi* A45); and AY383750 and AY383751 (partial 26S rRNA gene sequences of *Rhodotorula ingensiosa* CBS 6728 and *Rhodotorula* sp. CBS 4858, respectively).

understanding of the evolutionary processes that underlie the present diversity of dimorphic basidiomycetes, including the phylogenetic relationships between and within anamorphic genera.

Sexual compatibility is known for certain strains of *Rhodotorula fujisanensis* (Soneda) Johnson & Phaff, and clamped mycelium with teliospores has been documented (Fell *et al.*, 1984; Barnett *et al.*, 1990; Boekhout & Fell, 1995). However, until now, teliospore germination had not been reported and, therefore, the complete sexual cycle was not known. In the present report, the basidial stage of *R. fujisanensis* is characterized. In addition, the study of another sexual stage, obtained by using isolates that were identified preliminarily as *R. nothofagi* Ramírez & González, a species related closely to *R. fujisanensis*, is also presented. The data were evaluated by using several criteria, including the molecular phylogenetic framework that is available for the Microbotryomycetidae. A new genus, *Curvibasidium* gen. nov., is described, to accommodate the two teleomorphs. The relationship of *Curvibasidium* with *R. fujisanensis* and *R. nothofagi* is discussed.

For microscopy, cultures were grown on MYP agar (malt extract, 0.7% w/v; yeast extract, 0.05% w/v; soytone peptone, 0.25% w/v; agar, 1.5% w/v) 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-day-old cultures were crossed on MYP agar, potato dextrose agar (PDA) and corn-meal agar (CMA), incubated at room temperature and examined regularly for the production of mycelium and teliospores. Teliospore germination required a prolonged resting stage (9–12 months). After this period, agar blocks containing the teliospores were transferred to 2% water agar and observed regularly under the microscope.

Physiological and biochemical characterization was carried out according to techniques described by Yarrow (1998). Additional assimilation tests were performed by using aldaric acids and aromatic compounds as described by Fonseca (1992) and Sampaio (1999), respectively.

For determination of the extent of DNA homology, total genomic DNA was extracted and purified by using procedures described previously (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 rRNA gene sequence analysis, total DNA was extracted by using the protocol of Sampaio *et al.* (2001) and amplified by using primers ITS5 (5'-GGAAGTAAAAGTCGTAAC-AAGG-3') and LR6 (5'-CGCCAGTTCTGCTTACC-3'). Cycle sequencing of the 600–650 bp region at the 5' end of the 26S rRNA gene D1/D2 domains employed forward primer NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and reverse primer NL4 (5'-GGTCCGTGTTTCAAGACGG-3').

The internal transcribed spacer (ITS) region was sequenced by using the forward primer ITS1 (5'-TCCGTAGGTGAA-CCTGCGG-3') and the reverse primer ITS4 (5'-TCCTCC-GCTTATTGATATGC-3'). Sequences were obtained with an Amersham Pharmacia ALF Express II automated sequencer by using 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 branch-swapping, starting from trees that were obtained by random addition of sequences, multrees option on, deepest descent option off) and was validated by using 1000 rounds of bootstrap analysis (Felsenstein, 1985). Maximum-parsimony and bootstrap calculations used PAUP* software (Swofford, 2001).

Latin diagnosis of *Curvibasidium* Sampaio et W. Golubev gen. nov.

Fungi dimorphi Microbotryomycetidarum. Mycelium poris simplicibus fibulatis. Colacosomata nulla. Teliosporae globosae, holobasidiis germinant. Basidiosporae ovoideae ad bacilliformes, sessiles, gemmis germinant. Ballistoconidia nulla. Fermentatio nulla. D-Glucuronatum nitratumque non assimilantur. Productio compositorum amylo similium nulla. Systema CoQ 9 dominans. Polysaccharida extracellularia fucosum rhamnosumque continent.

Description of *Curvibasidium* Sampaio & W. Golubev gen. nov.

Curvibasidium (Cur.vi.ba.si'di.um. N.L. neut. n. *Curvibasidium* referring to the curved shape of the basidium).

Dimorphic. Belongs to the subclass Microbotryomycetidae. Mycelium with clamp connections. Simple septal pore. Colacosomes are not produced. Spherical teliospores germinate, producing a holobasidium. Sessile, ovoid-to-bacilliform basidiospores are released passively and germinate by budding. Ballistoconidia are not formed. Fermentative ability is absent. No assimilation of D-glucuronate or nitrate occurs. Starch-like compounds are not formed. Dominant CoQ system is 9. Extracellular polysaccharides contain fucose and rhamnose. Phylogenetic placement, as deduced by analysis of the D1/D2 domains of 26S rRNA gene, is shown in Fig. 1. The type species is *Curvibasidium cygneicollum* Sampaio.

Latin diagnosis of *Curvibasidium cygneicollum* Sampaio sp. nov.

Fungus dimorphus. Hyphae 1.5–2.5 µm diametro, conjugatione culturalum compatibilium procreantur. Septa fibulata, colacosomata nulla. Teliosporae plerumque globosae, 12–18 (22) µm diametro, terminales vel intercalares. Basidia stipitata, curvata, matura retroflexa, 6–8 (10) × 15–22 (26) µm, aseptata, basidiosporas sessiles, ovoideas ad bacilliformes in appendicibus basidialibus brevibus procreant. Basidiosporae gemmis germinant. Cellulae zymosae longe ovoideae ad cylindraceae, (1.5) 2–3 × (5) 7–12 (15) µm.

Fig. 1. Phylogenetic tree of *Curvibasidium* and related taxa of the Microbotryomycetidae. Maximum-parsimony analysis of an alignment of the D1/D2 region of the 26S rRNA gene. The topology was rooted with *Naohidea sebacea*, *Occultifur externus*, *Rhodotorula minuta* and *Sakaguchia dacryoidea*. Numbers on branches are bootstrap values (1000 replicates; values below 50 % are not shown). GenBank accession numbers of the sequences are indicated after strain numbers.

Cultura in striis cremea ad aurantiaca. Characteres biochemici physiologicique Curvibasidii cygneicollum in http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm describuntur.

Description of *Curvibasidium cygneicollum* Sampaio sp. nov.

Curvibasidium cygneicollum (cyg.nei.col'lum. L. n. *cygnus* swan; L. n. *collum* neck; N.L. n. *cygneicollum* swan-neck,

referring to the typical shape of the stalked basidium, which resembles the neck of a swan).

Dimorphic. Hyphae (1.5–2.5 µm in diameter) are formed after mating of sexually compatible strains. Two mating types are known (see Table 1). Clamp connections are present, but colacosomes are lacking. Teliospores are usually spherical [12–18 (22) µm in diameter], terminal or intercalary (Fig. 2a). Basidia are produced after a prolonged resting stage of the teliospores. Basidia are stalked, curved and bent over the stalk when matured (Fig. 2b). Stalks

Table 1. Strain numbers, isolation source and sexuality of the two novel species of *Curvibasidium* described in this study and of *R. nothofagi*

Species/strain*	Isolation source	Sexuality†
<i>C. cygneicollum</i>		
CBS 4551 ^{T‡}	Hare faeces, Mount Fuji, Japan	MT A1
CBS 7950	Wood of <i>Quercus suber</i> , Arrábida, Portugal	MT A1
CBS 8056	Berry of <i>Vitis coignetiae</i> (wild grape), Japan	MT A1
PYCC 4187	Flower of <i>Acacia</i> sp., Portugal	MT A1
CBS 6371	Leaf, France	MT A2
PYCC 4694	Rotten wood, Arrábida, Portugal	MT A2
CBS 8163§	Rotten trunk of <i>Laurelia sempervirens</i> , Futrono, Chile	ANA
CBS 7949	Dry leaf, Arrábida, Portugal	SFII
CBS 7951	Tree root, Gerês, Portugal	ANA
CBS 8264	Leaf of <i>Acacia</i> sp., New Zealand	ANA
PYCC 4705	Caterpillar nest, Sintra, Portugal	ANA
PYCC 4445	Woodland soil, Lisbon, Portugal	ND
PYCC 4968	Tree trunk, Arrábida, Portugal	ANA
PYCC 4947	Rotten tree trunk, Lisbon, Portugal	ANA
VKM Y-2859	Herbaceous plants, Prioksko-terrasny Biosphere Reserve, Moscow region, Russia	MT A2
JI 06	Fruiting body of <i>Exidiopsis</i> sp., Caramulo, Portugal	ND
JI 21	Fruiting body of <i>Exidiopsis</i> sp., Caramulo, Portugal	ND
<i>C. pallidicorallinum</i>		
VKM Y-2284 ^T (=CBS 9091 ^T)	Gramineous plant, Moscow region, Russia	MT A1
CBS 6231	Frass of <i>Ruguloscolytus rugulosus</i>	MT A1
VKM Y-2861	Herbaceous plants, Prioksko-terrasny Biosphere Reserve, Moscow region, Russia	MT A2
VKM Y-1135	Air, Kishinev, Moldavia	MT A2
VKM Y-2860	Herbaceous plants, Prioksko-terrasny Biosphere Reserve, Moscow region, Russia	MT A1
PTZ 185	Herbaceous plants, Prioksko-terrasny Biosphere Reserve, Moscow region, Russia	MT A1
<i>R. nothofagi</i>		
CBS 8166 ^T	Rotten trunk of <i>Nothofagus obliqua</i> , Futrono, Chile	ANA
A45	Sea water off Faro, south of Portugal	ANA

*CBS, Centraalbureau voor Schimmelcultures, Yeast Division, Utrecht, The Netherlands; PYCC, Portuguese Yeast Culture Collection, FCT-UNL, Portugal; VKM, All-Russian Collection of Microorganisms, Pushchino, Russia; JI, PTZ personal collections of J. Inácio and W. I. Golubev, respectively; A, collection of yeasts isolated from aquatic environments by M. Gadanho and J. P. Sampaio.

†ANA, Anamorphic; MT, mating type; ND, not determined.

‡Type strain of *R. fujisanensis* (Soneda) Johnson & Phaff.

§Type strain of *Rhodotorula futronensis* (Ramírez & González) Roijmans *et al.*

||Originally this strain was self-fertile, but currently it lacks the ability to produce mycelium with teliospores.

measure 1.5–2.5 × 15–35 (50) µm. Basidia [6–8 (10) × 15–22 (26) µm] are devoid of septa and produce sessile, ovoid-to-bacilliform basidiospores [1.5–2 × 6–12 (20) µm] on short basidial appendages (Fig. 2b). Each basidium has two to four sites of basidiospore formation and multiple basidiospores are formed at each site. Basidiospores germinate by budding. Yeast cells are long and ovoid to cylindrical [(1.5) 2–3 × (5) 7–12 (15) µm] (Fig. 2a). Streak cultures are cream-coloured or pale orange, butyrous and semi-dull. Colony margins are entire, and fringed with pseudomycelium in some cases. Physiological and biochemical features of *C. cygneicollum* are available at http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm and tests that allow its differentiation from *Curvibasidium pallidicorallinum* are depicted in Table 2. The phylogenetic placement of *C. cygneicollum* is

shown in Fig. 1. *C. cygneicollum* is sensitive to the mycocins produced by *Rhodotorula glutinis* and *Rhodotorula mucilaginosa*, but insensitive to mycocins secreted by *Rhodotorula pallida* and by species of the genera *Cryptococcus*, *Cystofilobasidium*, *Filobasidium* and *Sporidiobolus*.

Microscopic slides from the crossing of CBS 4551^T and CBS 6371, showing mycelium, teliospores, basidia and basidiospores, were deposited in the Portuguese Yeast Culture Collection under number ZP-01-03 (holotype). As the physiological and molecular characterization of a mixed culture presents obvious difficulties, we propose that strain CBS 4551^T, isolated from hare droppings collected at Mount Fuji, Japan, should be designated as the type strain of *C. cygneicollum*. This strain is deposited in the Centraalbureau voor Schimmelcultures, Yeast Division,

Fig. 2. Line drawings of different developmental stages of *Curvibasidium cygneicollum* (a, b), *Curvibasidium pallidicorallinum* (c, d) and *Leucosporidium fasciculatum* (e, f). Yeast cells (after 4–6 days on MYP agar), mycelium and teliospores (after 1–2 weeks on PDA) (a, c, e) are shown; for *Curvibasidium pallidicorallinum*, the detail of the clamp connections is presented. Germinated teliospores, basidia and sessile basidiospores (b, d, f) are shown. Note the holobasidia of *Curvibasidium cygneicollum* and *Curvibasidium pallidicorallinum* and the phragmobasidia of *L. fasciculatum*. Bar, 10 μ m.

Utrecht, The Netherlands, and in the Portuguese Yeast Culture Collection, FCT-UNL, Portugal, as PYCC 3116^T.

Table 2. Physiological characteristics that allow differentiation between *C. cygneicollum*, *C. pallidicorallinum*, *R. nothofagi* and *L. fasciculatum*

Species: 1, *C. cygneicollum*; 2, *C. pallidicorallinum*; 3, *R. nothofagi*; 4, *L. fasciculatum*. D, Delayed results. The complete dataset of physiological and biochemical features is available at http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm.

Characteristic	1	2	3	4
Sucrose	–	–	–	+
Maltose	–	–	–	+
α,α -Trehalose	–	–	–	+
<i>m</i> -Hydroxybenzoic acid	–	–	–	+
Gallic acid	+	+	+	–
Catechol	–	–	–	+
DL-Lactic acid	–	D	+	–
Cadaverine	+	–	–	+
Growth at 30 °C	–	–	+	–

Latin diagnosis of *Curvibasidium pallidicorallinum* W. Golubev, Fell et N. Golubev sp. nov.

Fungus dimorphum. Hyphae 1.5–2.5 μ m diametro, conjugatione culturarum compatibilium procreantur, fibulatae. Colacosomata nulla. Teliosporae plerumque globosae (10–19 μ m diametro) interdum ovoideae, pyriformes (9–16 $\times$ 10–21 μ m), terminales vel intercalares. Basidia unicellulata, 3.5–5 (6.5) $\times$ 30–50 (70) μ m, curvata. Basidiosporae sessiles, bacilliformes, 2.5–3.5 $\times$ 6–15 (19) μ m, in appendicibus brevibus lateris convexi basidii procreantur, gemmis germinantes. Cellulae zymosae longe ovoideae ad cylindraceae, 2–5 $\times$ 5–11 μ m. Cultura in striis pallide corallina ad roseo-cremea. Characteres biochemici physiologici Curvibasidii pallidicorallini in http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm describuntur.

Description of *Curvibasidium pallidicorallinum* W. Golubev, Fell & N. Golubev sp. nov.

Curvibasidium pallidicorallinum (pal.lid.i.cor.al'li.num. L. adj. *pallidus* pale; L. n. *corallium* coral; L. adj. *pallidicorallinum* pale coral, referring to the pinkish-cream colour of cultures of this species).

Dimorphic. Hyphae (1.5–2.5 µm in diameter) are formed after mating of sexually compatible strains. Two mating types are known (see Table 1). Clamp connections are present and medallion-shaped (Fig. 2c). Colacosomes are absent. Teliospores are usually spherical (10–19 µm in diameter), sometimes ovoid or pear-shaped (9–16 × 10–21 µm), terminal or intercalary (Fig. 2d). Basidia are one-celled [3.5–5 (6.5) × 30–50 (70) µm], curved and produced after a prolonged resting stage of the teliospores (Fig. 2d). Sessile, bacilliform basidiospores [2.5–3.5 × 6–15 (19) µm] are produced on short basidial appendages that originate on the convex side of the basidium (Fig. 2d). Each basidium has one to four sites of basidiospore formation and multiple basidiospores are formed at each site. Basidiospores germinate by budding. Yeast cells are long and ovoid to cylindrical (2–5 × 5–11 µm) (Fig. 2c). Streak cultures are pale orange to pinkish-cream, flat, smooth, butyrous, semi-dull or glistening. Colony margins are entire. Physiological and biochemical features of *C. pallidicorallinum* are available at http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm and tests that allow its differentiation from *C. cygneicollum* and *R. nothofagi* are depicted in Table 2. The phylogenetic placement of *C. pallidicorallinum* is shown in Fig. 1. *C. pallidicorallinum* is sensitive to the mycocins produced by *R. glutinis* and *R. mucilaginosus*, but insensitive to mycocins secreted by *R. pallida* and by species of the genera *Cryptococcus*, *Cystofilobasidium*, *Filobasidium* and *Sporidiobolus*.

Microscopic slides from the crossing of VKM Y-2284^T and VKM Y-2861, including mycelium, teliospores, basidia and basidiospores, were deposited in the Portuguese Yeast Culture Collection under number ZP-02-03 (holotype). As the physiological and molecular characterization of a mixed culture presents obvious difficulties, we propose that strain VKM Y-2284^T, isolated from a gramineous plant collected in the Moscow region, Russia, should be designated the type strain of *C. pallidicorallinum*. This strain is deposited in the Russia Collection of Microorganisms (VKM), Institute for Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Russia. Two strains have also been deposited in the Centraalbureau voor Schimmelcultures (CBS), Yeast Division, Utrecht, The Netherlands: CBS 9091^T (= VKM Y-2284^T) and CBS 9642 (= VKM Y-2861).

The life cycles of *C. cygneicollum* and *C. pallidicorallinum* were investigated on MYP agar, CMA and PDA. Conjugation of opposite mating types resulted in the formation of true mycelium with clamp connections. In *C. pallidicorallinum*, clamp connections have a peculiar medallion shape (Fig. 2c). For both species, teliospores are abundant 1 week after inoculation, but their germination requires a prolonged resting stage. For *C. cygneicollum*, crossings made on PDA plates and incubated for 2 weeks at room temperature were sealed and maintained at 15 °C for 9 months. After that period, small agar blocks containing teliospores were transferred to 2% water agar and

incubated at room temperature. Teliospores germinated approximately 1 week after transfer to water agar. Although the same procedures were also successful for *C. pallidicorallinum*, the original observation of the complete life cycle of this species employed CMA. Agar pieces containing 1-month-old teliospores were soaked in distilled water and maintained at 5 °C for 1 year. Teliospore germination occurred on transfer of the agar blocks to 2% water agar and incubation for 1 week at room temperature. The type strain of *C. pallidicorallinum* has mycocinogenic activity (Golubev, 1992). Its killing patterns are unique, as they include not only urediniomycetous yeasts (*Rhodospiridium*, *Rhodotorula* and *Sporidiobolus*), but also hymenomycetous yeasts (*Cryptococcus*, *Cystofilobasidium*, *Filobasidium* and *Itersonilia*). This is the first report of mycocins that are active against yeasts of two different classes.

Phylogenetic placement

The phylogenetic position of *Curvibasidium* was inferred by comparing the D1/D2 domains of the 26S rRNA gene sequences of the two species with representative members of the Microbotryomycetidae (Fig. 1). *C. cygneicollum* differed from *C. pallidicorallinum* by three substitutions. The sequences of the two species showed no intraspecific variability. The closest relative of *Curvibasidium* was *Leucosporidium fasciculatum* Bab'eva & Lisichkina, with 19 mismatches with respect to *C. cygneicollum* and 22 mismatches with respect to *C. pallidicorallinum*. The association of *L. fasciculatum* with *Curvibasidium* received strong statistical support (Fig. 1). As *L. fasciculatum* is not related to *Leucosporidium scottii* Fell, Statzell, Hunter & Phaff, this species was excluded from the circumscription of the order Leucosporidiales (Sampaio *et al.*, 2003). We re-examined the micromorphological features of *L. fasciculatum* (Fig. 2e, f) and confirmed the observations made by Bab'eva & Lisichkina (2000). The mycelium of *L. fasciculatum*, in contrast to that of *Curvibasidium*, lacks clamp connections (Fig. 2e) and the basidia are septate (phragmobasidia) (Fig. 2f). As the holobasidium of *Curvibasidium* is a unique trait in the Microbotryomycetidae, this feature is an important diagnostic property for the new genus and is probably a derived characteristic. The absence of this characteristic in *L. fasciculatum* prevented us from transferring this species to *Curvibasidium*. Moreover, *Curvibasidium* has CoQ 9 (Goto & Oguri, 1983), which is an uncommon feature in the Microbotryomycetidae. However, this chemotaxonomic marker has not yet been determined for *L. fasciculatum*. From nutritional and physiological perspectives, *L. fasciculatum* is also well-separated from *Curvibasidium* (Table 2). We consider that a study of additional teleomorphic species related to *Curvibasidium* and *L. fasciculatum* is needed before a change in the scope of *Curvibasidium*, or the creation of a new genus for *L. fasciculatum*, is made.

Another important feature of *Curvibasidium* is the absence of colacosomes (also referred to as lenticular bodies). Among members of the Microbotryomycetidae that are

depicted in Fig. 1, the plant parasites of the Microbotryales and *Kriegeria eriophori* Bresadola lack colacosomes, as do the non-plant parasites *Camptobasidium hydrophilum* Marvanová & Suberkropp, *Leucosporidium antarcticum* Fell, Statzell, Hunter & Phaff and *L. fasciculatum*. The presence of colacosomes has been related to a mycoparasitic life strategy (Bauer & Oberwinkler, 1991). These subcellular structures have only been reported for certain dimorphic basidiomycetes classified in the Microbotryomycetidae, such as *Colacogloea* Oberwinkler & Bandoni, the Sporidiobolales (*Rhodospiridium* Banno and *Sporidiobolus* Nyland), the Leucosporidiales (*L. scottii*, *Leucosporidium fellii* Giménez-Jurado & van Uden and *Mastigobasidium* Golubev) and *Heterogastridium* Oberwinkler & Bauer, which lacks a yeast stage (Kreger-van Rij & Veenhuis, 1971; Sampaio *et al.*, 2003).

Anamorph–teleomorph connections

As the type strain of *R. fujisanensis* is a mating strain of *C. cygneicollum*, it is evident that *C. cygneicollum* is the sexual stage of *R. fujisanensis*. A similar connection was suggested for *C. pallidicorallinum* and *R. nothofagi* on the basis of phylogenetic analysis of the D1/D2 domains (Fig. 1). However, several attempts to mate the type strain of *R. nothofagi* with the available sexually compatible strains of *C. pallidicorallinum* invariably gave negative results. The relationships of *R. nothofagi*, *C. pallidicorallinum* and *C. cygneicollum* were investigated further by analysing the complete ITS region (ITS1 + 5.8S rRNA gene + ITS2). No variability was detected for *C. cygneicollum* in the ITS region, whereas for *C. pallidicorallinum*–*R. nothofagi*, four sites were variable (a phylogenetic tree is available at http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm). In the analysis of the ITS data, the four sexually compatible strains of *C. pallidicorallinum* were separated from the two asexual strains (the type strain of *R. nothofagi* and strain A45). Nuclear DNA–DNA reassociation experiments also supported this separation, as homology between the type strains of *C. pallidicorallinum* and *R. nothofagi* was not high and ranged from 40 to 46 % in three independent essays. Therefore, on the basis of the lack of sexual compatibility and the ITS and DNA reassociation data, we consider that *R. nothofagi* is not the asexual state of *C. pallidicorallinum*. *R. nothofagi* can be distinguished from *C. pallidicorallinum* on the basis of the ITS sequences, the absence of mating and the ability to grow at 30 °C. In both *C. cygneicollum* and *C. pallidicorallinum*, sexual compatibility seems to be biallelic, as crossing experiments suggested the presence of only two mating types. Six strains of *C. cygneicollum* (Table 1) are regarded as anamorphic, as they did not react sexually with the two mating types of this species.

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