RESEARCH ARTICLE

Kwoniella mangroviensis gen. nov., sp. nov. (*Tremellales*, *Basidiomycota*), a teleomorphic yeast from mangrove habitats in the Florida Everglades and Bahamas

Adele Statzell-Tallman[1], Carmela Belloch[2] & Jack W. Fell[1]

[1]Rosenstiel School of Marine and Atmospheric Science, University of Miami, Key Biscayne, FL, USA; and [2]Institute of Agrochemistry and Food Technology, Burjassot, Valencia, Spain

Correspondence: Jack W. Fell, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Key Biscayne, FL, USA. Tel.: +305 421 4603; fax: +305 421 4600; e-mail: jfell@rsmas.miami.edu

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Tremellales; *Kwoniella mangroviensis*; yeast; mangrove habitat.

Abstract

Mangrove forests inhabit the shoreline regions of tropical and subtropical marine habitats, where they are the basis of a multi-trophic level food web that drives the shellfish and fisheries industries. Yeasts, and other fungi, have significant roles in these ecosystems as they decompose plant organic material and serve as a food source for small invertebrates. Studies designed to examine yeast communities of mangrove regions of the Florida Everglades and the Bahamas demonstrated the repeated presence of an undescribed teleomorphic basidiomycetous yeast. The yeast is heterothallic, with a sexual cycle that can be observed on artificial media. Mating between compatible pairs produces polymorphic basidia. Some basidia are globose, ovoid or lageniform, with longitudinal to oblique and transverse septa, whereas other basidia are navicular with one to three transverse septa. Basidiocarps and ballistoconidia are absent. Molecular sequence analysis of a partial region (D1/D2 domains) of the large subunit rRNA demonstrated that the yeast is phylogenetically distinct from other teleomorphic *Tremellales* with close relationships to the anamorphic species *Cryptococcus dejecticola*, *Cryptococcus bestiolae* and *Bullera dendrophila*. The molecular and phenotypic data indicate that this teleomorph should be classified in a novel genus. Therefore, *Kwoniella mangroviensis* gen. nov., sp. nov. (Type strain CBS 8507), is proposed.

Introduction

Mangroves, and their associated mycobiota, are essential components of an ecologically and economically important food web in tropical and subtropical regions. Mangroves consist of a phylogenetic complexity of plants that includes 69 species in 26 genera and 20 families. The mangrove habitat, or mangal, occurs in 112 countries, with an estimated coverage of 10–24 million hectares with a global biomass of 8.7 Gton dry weight including 4.0 Gton of carbon (Twilley *et al.*, 1992; Kathiresan & Bingham, 2001). Fungi play a major role in the mangal through the decomposition of plant material. In the presence of exogenous nitrogen, fungi convert complex plant carbon compounds, mostly lignin and cellulose, to microbial protein (Newell *et al.*, 1984), which is a food source for marine invertebrates and fish in the mangrove food web (Hogarth, 1999). Fungi and fungal-like organisms are involved in two facets of the decomposition process: degradation of particulates and conversion of leachates. There has been considerable research on the mycobiota of mangrove wood (Schmidt & Shearer, 2004) and litter (Fell & Master, 1980; Tan & Pek, 1997).

The target of this research is the microbial community associated with the dissolved organic material (DOM) with a specific focus on the unicellular fungi, i.e. the yeasts. The study includes two geographical regions: the Shark River Slough in the Florida Everglades and mangrove habitats in the North and Central Bahamas. The Bahamian study consists of a survey of yeast communities in geographically different locations. In contrast, the Everglades study includes seasonal transects in the wet and dry seasons at six stations that progressed from the freshwater sawgrass communities to the mangrove marshes. Preliminary data from the Everglades study indicate a yeast diversity that consists of 55 species of ascomycetes and 58 species of basidiomycetes. Approximately 50% of the total number of these species represents undescribed taxa.

Among the undescribed species in the Florida Everglades and the Bahamas, we repeatedly isolated strains of a species whose phenotypic and molecular taxonomic characteristics classified the yeast among the *Tremellales*. The species appears to be associated with mangrove habitats, as the yeast was not found in freshwater regions of the Florida Everglades. The species is heterothallic and mating between compatible pairs of strains results in clamped hyphae with different types of basidia. Some basidia are globose with longitudinal and transverse septa, whereas other basidia are ovoid and lageniform with a single transverse septum or with transverse and longitudinal septa. Navicular basidia, with one to three transverse septa, may also occur. Not all of the mangrove strains are sexually compatible, although ribosomal DNA sequence analysis indicates that the strains belong to a single species. In addition to the mangrove-associated strains, two anamorphic strains, which were isolated from corkwood in Spain and from an Atlantic Ocean beach in Florida, are included in this study due to the similarities in their ribosomal DNA sequences (ITS and D1/D2 large subunit (LSU) domains) with the mangrove strains. The cork and beach strains may represent a separate genotype. A formal description of the species, a new genus and the available ecological data are presented.

Materials and methods

Sampling sites

Bahama Islands (Fig. 1)

(1) Mangrove Cay, Little Bahama Bank, is an overwash mangrove island with an interior 1–2 m deep creek. (2) Manjack Cay is an island in the Abaco chain with an interior mixed hardwood forest and a mangrove lined tidal creek. (3) Alan's Pensicola, Abacos, is an island with a mixed hardwood forest and a shoreline that is covered, in part, by mangroves. (4) Cross Key, Andros Island, is a mangrove-covered island off the NW coast of Andros Island. (5) Shroud Cay, Exumas, is a complex of small islands with multiple mangrove creeks. (6) Pipe Creek, Exumas, is a tidal swept region with a series of islands with mangroves, sandy beaches and coral rock shorelines. Collections were made during research cruises on the University of Miami (UM)

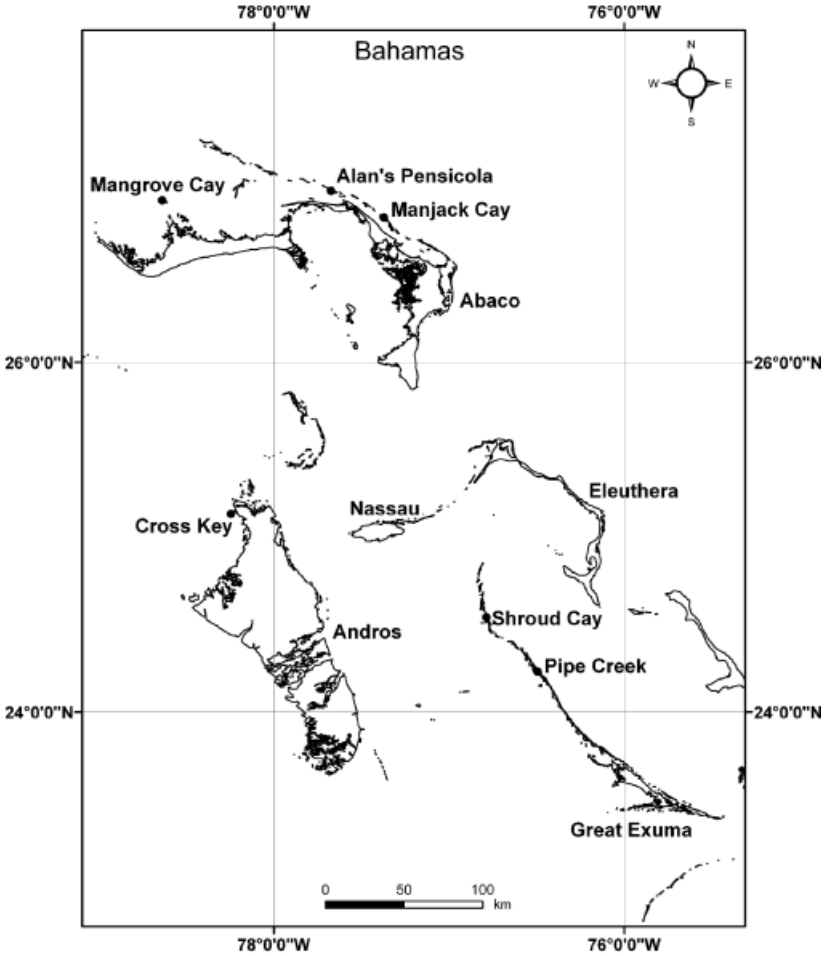

Fig. 1. Location of collection sites (Mangrove Cay, Alan's Pensicola, Manjack Cay, Cross Key, Shroud Cay and Pipe Creek) in the Bahama Islands.

Table 1. Origin of strains studied

Strain number	GenBank# D1/D2	GenBank# ITS 1 and 2	Collection Date	Salinity (p.p.t.)	Geographical location	Mating type
ML3810 (CBS8507)	*AF444742	†AF444646	10/16/1996	37	Mangrove Cay	α
ML3895	*EF174032	EF174039	7/12/1997	37	Mangrove Cay	n
ML3904 (CBS8886)	*EF174031	EF174038	5/19/1996	37	Mangrove Cay	n
ML3914	*	AF444646	5/19/1996	37	Mangrove Cay	n
ML3916	*		5/19/1996	37	Mangrove Cay	n
ML4091	*		5/22/1996	37	Mangrove Cay	n
ML4078	*	‡	5/25/1996	37	Manjack Cay	n
ML4089	*		5/24/1996	37	Manjack Cay	n
ML4135 (CBS10435)	*EF174033	EF174040	7/14/1997	37	Mangrove Cay	A
ML4136	*EF174034	‡EF174041	7/15/1997	37	Alan's Pensacola	α
ML4631	*	†	10/15/1999	38	Cross Key	α
ML4641	*		10/15/1999	40	Cross Key	n
ML4656	*	†	7/16/1999	38	Shroud Cay Creek	α
ML4657	*	†	7/16/1999	38	Shroud Cay Creek	α
ML4659	*	†	7/17/1999	40	Shroud Cay Creek	n
ML4671	*		7/18/1999	39	Pipe Creek	α
ML4673	*		7/18/1999	40	Pipe Creek	n
ML4674	*	†	7/18/1999	40	Pipe Creek	n
ML4688	*		7/17/1999	38	Shroud Cay	n
EY0352	§EF174035		2/18/2003	0	Everglades SRS 4	n
EY0369	EF174037		2/18/2003	0	Everglades SRS 4	n
EY0393	§		2/18/2003	0	Everglades SRS 4	n
EY0424	§		2/18/2003	0	Everglades SRS 4	n
EY0875	§		6/19/2003	2	Everglades SRS 4	n
EY0927	§		6/19/2003	8	Everglades SRS 5	n
EY0929	§		6/19/2003	8	Everglades SRS 5	n
EY8-116	§		9/1/2004	17	Everglades SRS 6	n
EY8-126	*		9/1/2004	17	Everglades SRS 6	n
EY9-095	*		3/9/2005	30	Everglades SRS 6	n
EY9-099	§		3/9/2005	30	Everglades SRS 6	n
CECT11955	AY167602		11/1/1999	0	Cork wood, Spain	n
CECT11979	AY296055	EF586207	11/2000	0	Cork wood, Spain	n
CV10-2	EF174036	EF172042	2001	37	Beach sand, Florida	n

*D1/D2 sequence identical to ML3810 (AF444742).
†ITS sequence identical to ML3810 (AF444646).
‡ITS sequence identical to ML4136 (EF174041).
§D1/D2 sequence identical to EY0352 (EF174035).
n, no mating reaction.

research ship R.V. Calanus in May and October 1996; July 1997; and July and October 1999 (Table 1).

Shark River Slough (SRS), Florida Everglades (Fig. 2)

The region is on the southwest corner of the Florida Everglades. The Shark River initiates in a freshwater marsh and terminates in a mangrove habitat in Florida Bay. Collections were made at six sites that are maintained and routinely sampled for physical and biological data by the Florida International University (FIU) National Science Foundation (NSF) Long Term Ecological Research (LTER) program. Detailed information for each site can be obtained at the Florida Coastal Everglades LTER website: http://fcelter.fiu.edu/.

The SRS sites (Fig. 2) are located in a subtropical moist environment with distinct wet (June–November) and dry (December–May) seasons. The entire region is subject to seasonally driven freshwater sheet flow. In addition, Stations SRS 4, 5 and 6 have tidally driven oceanic inputs. Stations SRS 1a, 2 and 3 are located in freshwater wetlands dominated by sawgrass (*Cladium jamaicense*) interspersed with spike rushes (*Eleocharis cellulosa)* and maidencane (*Panicum hemitomon)*. Stations SRS 4, 5 and 6 are located in a tidal creek within a mangrove forest consisting of mainly red (*Rhizophora mangle*) and black (*Avicennia germinanus*) mangrove trees. At SRS 4, the trees are low in height with a

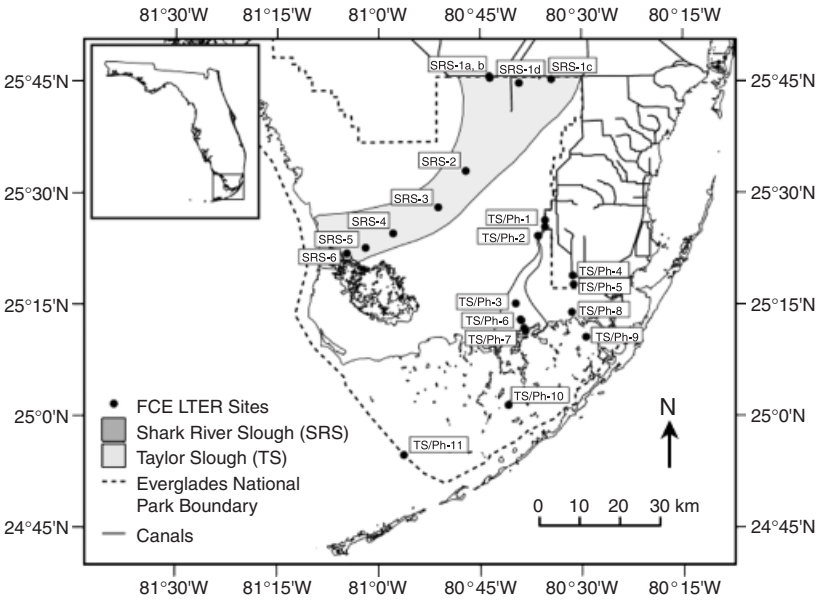

Fig. 2. Location of collection sites in the Florida Everglades, Shark River Slough Stations SRS 1a, 2, 3, 4, 5 and 6.

dwarf stature (*c.* 2–4 m high), at SRS 5 the trees have an intermediate stature (*c.* 6 m), whereas at SRS 6 the trees are large (*c.* 12 m).

Nine seasonal transects were taken at the six SRS stations: July and November 2002, February, June and October 2003, January, April and September 2004 and March 2005. At the times of collection, the salinities were: SRS 1a, 0 p.p.t., SRS 2 and SRS 3, 0–2 p.p.t., SRS 4, 0–17 p.p.t., SRS 5, 0–30 p.p.t., and SRS 6, 12–33 p.p.t.

Cork Oak Trees, Spain

In the province of Badajoz, Spain, cork is harvested from cork oak trees (*Quercus suber*) every 11 years. The harvest is stacked and stored for several years in fields before it is processed into cork. Yeasts were isolated in November 1999 and November 2000 during the manufacturing stages before the cork planks were pulverized to produce the stoppers. Collection, isolation and identification of cork isolates were performed at the Institute of Agrochemistry and Food Technology, Burjassot, Spain (Villa-Carvajal *et al.*, 2004).

Southeast Florida Beaches

Sand samples at recreational beaches in South Florida were studied for yeast populations from August 2001 to July 2002 (Vogel *et al.*, 2007). The researchers provided a D1/D2 sequence and DNA for ITS sequencing from their strain CV10-2.

Yeast isolation

Bahamas

Seawater was collected in sterile WhirlPak Bags (Nasco, Ft. Atkinson, WI) or autoclave-sterilized bottles. The samples were transported to the ship, which was usually within 2 km of the collection site. The seawater was vacuum-filtered through Millipore cellulose acetate membranes (0.45 μm pores). The water volumes ranged from 10 to 1000 mL based on previous knowledge of the yeast population densities in the specific sampling areas. The filters were placed on enrichment agar in 50 mm Millipore (Ballerica, Maryland) petri dishes. The enrichment medium (5.0 mL) consisted of 2% glucose, 1% peptone and 0.5% yeast extract agar, with 0.02% chloramphenicol to retard bacterial growth. The plates were incubated at 12 °C. The resulting colonies were counted and streaked onto enrichment agar for purity. All steps were performed on shipboard.

At the University of Miami (UM) laboratory, the isolates were grown in duplicate in 1 mL of glucose peptone yeast extract (GPY) broth in 2 mL microcentrifuge tubes on a roller drum for 24–48 h. Cells from one growth tube were stored in liquid nitrogen (vials of cell suspensions in 1500 μL of 2% GPY broth, 10% glycerine), whereas cells from the other tube were used for genomic DNA extraction or for direct amplification of the genes of interest.

Shark River Slough

Water from 20 cm below the surface was collected in sterile, 1-L Nalgene bottles. The samples were maintained at 5–12 °C until processed, usually within 24 h. Preliminary sampling results indicated widely variable total numbers of CFU at each station. Consequently, a series of aliquots (10, 25, 50 and 100 mL) was filtered from each sample. Three samples were collected at each station to increase the accuracy of the estimate of population variability. The

remaining process was the same as for the Bahama samples. All processing was in the UM laboratory.

Yeast identification

Phenotypic methods

The morphological descriptions and physiological tests followed the modified methods of Yarrow (1998). The carbon and nitrogen assimilation assays were tested in 2-mL microcentrifuge tubes with 1 mL of liquid media. The tubes were placed on a roller drum modified to accept the microcentrifuge tubes. Readings were taken after 3 days, 1, 2 and 3 weeks.

Molecular methods

DNA extraction followed the methods of Fell *et al.* (2000). Alternatively, DNA was directly amplified from a light cell suspension in water as the DNA template in 50 μL PCR reactions with a HotMaster mix (Eppendorf North America, Westbury, NY). Molecular sequence analysis of the D1/D2 domains of the LSU and the ITS1, 5.8S and ITS2 rRNA gene regions followed the procedures of Fell *et al.* (2000) and Scorzetti *et al.* (2002).

Results and discussion

Latin description: *Kwoniella* Statzell-Tallman & Fell gen. nov.

Status zymoticus apparet. Genus *Kwoniella* ad *Tremellales* pertinet. Fungus heterothallicus. Hyphae fibulatae formantur. Basidiocarpa absunt. Basidia tremelloidea, globosa aut ellipsoidea, uniseptata aut multiseptata. Ballistoconidia aut ballistosporae absunt. Fermentatio nulla. Materia amyloidea formatur. Inositolum acidum glucuronicumque assimilantur. Pigmentum fuscum aliquando formatur. Reactio Diazonii coerulei B positiva. Ureum finditur. Typus *Kwoniella mangroviensis*.

Standard description: *Kwoniella* Statzell-Tallman & Fell gen. nov.

Etymology: *Kwoniella* is named in recognition of Dr K.J. Kwon-Chung for her research contributions to the *Tremellales*, with specific acknowledgement of her significant advances in the systematics and biology of *Filobasidiella neoformans*.

Type species: *K. mangroviensis* Statzell-Tallman, Belloch & Fell.

Based on the life cycle and molecular phylogenetic sequence analysis of the rRNA gene (LSU D1/D2 domains, ITS1, 5.8S and ITS2), *Kwoniella* is a member of the *Tremellales*. Basidiocarps are absent. The fungus is dimorphic, with a yeast state and extensive dikaryotic hyphae with clamp connections that develop following conjugation of compatible pairs of strains. Basidia form laterally on branches, intercalarily or on the terminal hyphal cell. Basidia vary in shape from globose to subglobose, lageniform to ovoid with transverse and longitudinal to oblique septa. Navicular basidia have one to three transverse septa. The globose basidia occur singly, in pairs or in chains. Basidiospores are globose, ovoid to cylindrical and passively released. Neither ballistoconidia nor ballistospores are present. Fermentation is absent. Starch-like compounds are produced. Myo-inositol and D-glucuronate are utilized for growth. A brownish pigment is produced that diffuses into solid media (potato dextrose agar). Diazonium blue B and urease reactions are positive.

Latin description*: K. mangroviensis* Statzell-Tallman, Belloch & Fell sp. nov.

Morphologia: Post dies 3 ad 22 °C in medio liquido (ME) cellulae ovoideae, ellipsoideae ad globosae, singulae vel breviter catenatae. Post mensem unum sedimentum apparet at non pellicula, nec annulus. Post mensem unum ad 22 °C in medio ME cum agaro cultura alba, glabra, lucida, convexa butyrosaque, margine expresso. Hyphae raro formantur.

Post dies 7 ad 22 °C in medio CM cum agaro pseudomycelium non formatur, post dies 21 pseudomycelium sparsum formatur. ML 3895 solum verum mycelium generat. Post dies 14 in medio PD cum agaro, cultura alutacea pigmentum fuscum in medio diffundet.

Fungus heterothallicus. In medio CM cum agaro ad 22 °C post dies 5 hyphae fibulatae formantur. Post dies 10–14 pleomorpha basidia, proferentes basidiosporae globosae aut ovoideae, a latere vel terminaliter oriuntur. Post 7 dies fibulae formantur. Sexualis conjunctio bipolaris.

Fermentatio nulla.

Glucosum, galactosum, L-sorbosum, sucrosum (lente), raffinose (aliquando), maltosum, cellobiosum, trehalosum, lactosum, melezitosum, amylum solubile (lente), D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, ethanolum, glycerolum, erythritolum (lente), ribitolum, galactitolum, D-mannitolum, D-glucitolum, α-methyl-D-glucosidum (lente), salicinum (lente), acidum gluconicum, acidum succinicum, acidum citricum, inositolum, acidum glucuronicum, acidum saccharicum, acidum 2-keto-D-gluconicum, acidum 5-keto-D-gluconicum, assimilantur, ad non melibiosum, inulinum, D-glucosaminum, methanolum, acidum lacticum, nec hexadecanum. Kalii nitratum et D-glucosaminum non assimilantur. Maxima crescentiae temperatura: 30 °C. Reactio Diazonii coerulei B positiva. Ureum finditur.

Typus CBS 8507, isolatus in Mangrove Cay, Little Bahama Bank, Bahamas.

Standard description: *K. mangroviensis* Statzell-Tallman, Belloch & Fell sp. nov.

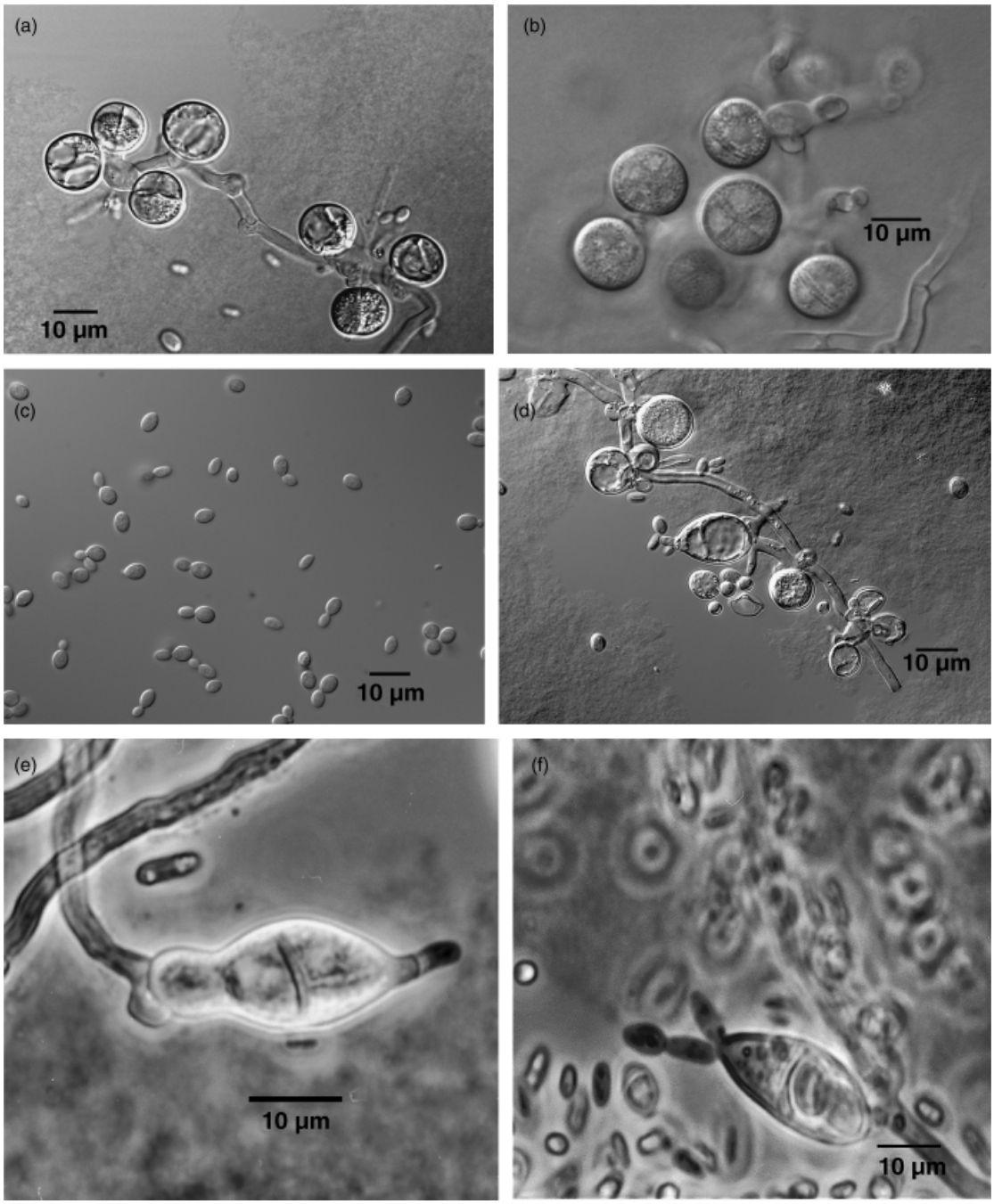

Fig. 3. *Kwoniella mangrovensis*. (a) Globose basidia formed on clamped hyphae, 2 weeks at 22 °C after mating pair was mixed on CMA. Olympus differential interference contrast. (c) Vegetative cells of CBS 8507 grown at 22 °C for 3 days in 5% malt extract broth. Olympus differential interference contrast. (e) A lageniform basidium with an inflated base, 1 month at 22 °C after mating pair was mixed on CMA. A transverse septum is visible. Wild-phase contrast. (b) Globose basidia six days at 22 °C after mating pair was mixed on CMA. Olympus differential interference contrast. (d) A lageniform basidium on a clamped hypha, 1 month at 22 °C after mating pair was mixed on CMA. Olympus differential interference contrast. (f) Basidiospores, one budding, remain attached to the apex of a lageniform basidium. One month at 22 °C after mating pair was mixed on CMA. Wild-phase contrast.

Etymology: Named for the type habitat of the species – the mangroves, which occur in tropical and subtropical marine intertidal environments.

Morphology: After 3 days at 22 °C in 5% malt extract broth (MEB), the cells (Fig. 3) are ovoid, ellipsoid to globose, 3–8 µm × 3–6 µm, and they may be single or with

one to two attached multilateral buds and in short chains. Sediment is present after 1 month. Neither a ring nor a pellicle forms on the surface of the broth.

After 1 month on 5% malt extract agar at 22 °C, the colony is tannish-white, smooth, glistening and raised. The texture is butyrous. The margins are entire and hyphae are rarely formed. After 1 week at 22 °C on a corn meal agar (CMA) Dalmau plate, pseudohyphae are not formed; after 21 days, sparse rudimentary pseudohyphae can occur. True hyphae with false clamp connections are present in one strain (ML 3895). After 2 weeks on potato dextrose agar, the colony is light tan and a brown pigment has diffused into the solid medium.

Sexual reproduction (Fig. 3): Pairs of compatible strains, when mixed on CMA at 22 °C for 5 days, develop sparse hyphae with one or more clamps at the septa. Phragmobasidia form within 5–14 days on the apical hyphal cell and intercalary or laterally on the hyphae. Some hyphae grow at an angle after basidial formation, which results in an angular growth pattern. The basidia are polymorphic (Fig. 3): lageniform (7 μm × 27 μm) with longitudinal and transverse septa; navicular (10–13 μm × 19–21 μm) with one to three transverse septa; four-celled globose (13–19 μm) and ovoid with longitudinal to oblique and transverse septa. The globose basidia occur singly, in pairs and in chains. Germination usually occurs within 1 week when 1-month-old basidia in agar blocks are transferred to 2% water agar. Basidiospores are globose (4–11.3 μm) or ovoid to cylindrical (6–10 μm × 7.5–11.3 μm). The basidiospores are passively released and may bud to form colonies in the agar. Basidiospores may also bud while attached to the basidium. The mating system appears to be unifactorial biallelic, as represented by one strain of mating type A and five strains of mating type α. Mating reactions were not obtained with 13 strains (Table 1), which are considered to belong to the species based on sequence analyses. These nonmating strains could represent a separate sexual incompatibility factor as part of a multiple allelic system. The mating reaction, on Bacto CMA with additional 2% Bacto agar, results in more extensive hyphal development with masses of yeast cells, which appear to be the result of basidiospore replication.

Fermentation of carbohydrates is absent.

Carbon compounds that support growth: glucose, galactose, L-sorbose (slow), sucrose, maltose, cellobiose, trehalose, lactose, melezitose, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, ethanol, glycerol, ribitol, galactitol, D-mannitol, D-glucitol, D-methyl-α-glucoside (slow), salicin (slow), D-gluconate, succinate, citrate, inositol, D-glucuronate, saccharate, 2-keto-D-gluconate and 5-keto-D-gluconate.

Growth is not supported with melibiose, inulin, D-glucosamine, methanol, DL-lactate and hexadecane.

Growth is variable with raffinose, erythritol, *N*-acetyl-D-glucosamine and soluble starch.

Growth on nitrogen compounds: nitrate is negative, nitrite is positive, and creatinine is negative.

Gelatin liquefaction is negative.

Growth on 50% (w/w) glucose yeast extract agar is negative.

Growth in vitamin-free medium is variable.

Urease activity is present.

The color reaction with Diazonium Blue B (DBB) is positive.

Growth on 10% NaCl/5% glucose is positive and delayed.

Growth at 25 °C is positive.

Growth at 30 °C is positive.

Growth at 37 °C is negative.

Starch formation (< pH 5.0) is positive.

Type strain: ML 3810 (CBS 8507, NRRL Y-48353), designated mating type α, was isolated from the tidal creek of Mangrove Cay, Little Bahama Bank, Bahamas, on 16 Oct 1996. The complementary mating type A (ML 4135, CBS 10435) was collected on 14 July 1997 on the outgoing tide from waters surrounding Mangrove Cay.

Additional strains. A total of 30 strains (Table 1) were isolated from mangrove habitats in the North and Central Bahamas and in the Shark River Slough of the Florida Everglades. Two strains (CECT 11955 and CECT 11979) were isolated from the bark of cork oak trees during the manufacturing process of cork stoppers in Extremadura, Spain (Villa-Carvajal *et al*., 2004). Another strain (CV 10-2) was isolated from an Atlantic Ocean beach at John U Lloyd Beach State Park, Hollywood, FL (Vogel *et al*., 2007). The strain has been lost (C. Vogel, pers. commun.).

Ecology. Strains were isolated from marine waters in mangrove habitats at six locations at geographically dispersed sites in the Bahamas Islands (Fig. 1) in 1996, 1997 and 1999 (Table 1). The salinity at the Bahamian locales ranged from 37 to 0 p.p.t. In the Florida Everglades Shark River Slough, strains were isolated in 2003, 2004 and 2005 from waters within mangrove habitats at Stations SRS 4, 5 and 6. Salinities at the time of collection ranged from 0 to 17 p.p.t. (Table 1). While the levels of salinity do not appear to be significant, strains were not isolated from freshwater sawgrass habitats (SRS 1a, 2 and 3) of the Florida Everglades, suggesting that the Everglades and Bahamian strains are associated with the mangrove forests rather than fresh water habitats.

Based on the multiple isolations, *K. mangroviensis*, has a widespread occurrence in mangrove habitats of the Bahamas and Shark River Slough. Numerically, the total number of cells of yeasts (ascomycetes and basidiomycetes) in the samples associated with *K. mangroviensis* from the Bahamas ranged from four to 280 cells (average of 72 CFU L^{-1} for 15 collections). In the Shark River Slough, the range was 70–1300 CFU L^{-1} (average 463 CFU L^{-1} for seven collections). The differences in the abundance of yeast cells may

be attributed to the organic contents of the seawater habitats. The Everglades is a large land mass system with a heavy nutrient load, as indicated by dark, tannin-colored waters. In contrast, the Bahamian islands, where the authors collected, are geographically isolated from extensive terrestrial run-off. As a result, the surrounding waters are characteristically 'crystal clear'. There is an abundant yeast biodiversity in these mangrove habitats. For example, the preliminary SRS data demonstrate a diversity that includes 55 species of ascomycetes and 58 species of basidiomycetes, of which *c.* 50% are undescribed species. The diversity within the basidiomycetous yeasts includes Agaricomycotina (= *Hymenomycetes*), Pucciniomycotina (= *Uredinomycetes*) and Ustilaginomycotina (= *Ustilaginomycetes*) (Aime *et al.*, 2006; Begerow *et al.*, 2006; Hibbett, 2006). The specifics of this yeast diversity in the mangroves will be the topic of a future publication.

Yeasts, in mangrove habitats, presumably contribute to the microbial food web via decomposition of organic materials and as a food source for filter-feeding invertebrates. However, the specific ecological role of *Kwoniella* in mangrove habitats is unknown. The focal point of activity could be in the water or on land based on substrates such as wood or leaves, with conidia and basidiospores washed into the water by tides and rain. Investigations need to be undertaken to determine whether *K. mangroviensis* is free-living or a mycoparasite as observed with other members of the *Tremellales* (Boekhout *et al.*, 1998).

Physiology/Biochemistry. *Kwoniella* shares phenotypic characteristics with other members of the *Tremellales*, specifically the ability to utilize myo-inositol, D-glucuronate and the production of extra-cellular starch-like compounds. *Kwoniella* produces a diffusible light brown pigment, when grown on potato dextrose agar. Other members of the *Tremellales*, such as *Bullera japonica, Auriculibuller fuscus*, *Filobasidiella neoformans, Cryptotrichosporon anacardii* and *Cryptococcus podzolicus* (Petter *et al.*, 2001; Sampaio *et al.*, 2004; Okoli *et al.*, 2007), produce tan and dark brown pigments that may be related to melanin. However, Boekhout (unpublished observation) did not observe melanin on L-DOPA medium (Yarrow, 1998) with strains of *K. mangroviensis*.

Phylogeny. The *Tremellales* is a large, complex and weakly structured group of basidiomycetes as can be seen in Fig. 4, which is a synoptic view constructed from a limited number of gene analyses and a partial representation of the species that occur in nature (Sampaio *et al.*, 2002; Scorzetti *et al.*, 2002). The recent move to polyphasic systematics (phenotypic characteristics, life cycles and molecular analyses) is resulting in the description of new species and genera, and an expanded understanding of the phylogenetic relationships within the *Tremellales* (Chen, 1998). The genus *Tremella* includes at least 120 valid species, which are usually parasitic on other fungi or lichens (Bandoni, 1995). The species have dimorphic life cycles with basidocarps and basidia, which are subglobose to longitudinally pyriform or obliquely septate (Bandoni & Boekhout, 1998; Sampaio *et al.*, 2004).

Several teleomorphic genera have been described in the past few years. Four of these genera (*Cuniculitrema*, *Bulleromyces*, *Papiliotrema* and *Auriculibuller*) are phylogenetically located in the only strongly supported clade in the *Tremellales* (Fig. 4). *Cuniculitrema*, which is vectored by bark beetles, has longitudinally septate basidia and yeast cells that bud from stalks similar to the budding mechanisms found in *Fellomyces* (Kirschner *et al.*, 2001). *Bulleromyces* (Boekhout *et al.*, 1991) is a phylloplane yeast with subglobose, clavate or ovoid basidia, which are longitudinally, obliquely or transversely septate with two to four cells. Basidiospores are both sessile and forcibly ejected (Boekhout, 1998). *Auriculibuller* is a leaf-inhabiting yeast with cylindrical, two-celled, transversely septate basidia, which produce ballistospores. Ballistoconidia are formed in the yeast phase (Sampaio *et al.*, 2004). *Papiliotrema*, in contrast to the other three genera, produces minute basidiocarps, which are associated with pyrenomycetous ascomycetes on grass. The basidia, which are transversely septate, have only been observed in nature. Ballistospores are formed (Sampaio *et al.*, 2002).

Fibulobasidium is phylogenetically separated from the *Cuniculitrema* cluster of genera. *Fibulobasidium* is distinguished by a basidiocarp, which occurs as a slimy mass on the surface of a decomposing tree branch (Sampaio *et al.*, 2002). The basidia are two- to four-celled, globose to cylindrical and sometimes irregular in shape. Ballistospores are produced, although ballistoconidia are not formed. Another genus, *Bulleribasidium*, appears with *Fibulobasidium* in a cluster that lacks bootstrap support (Fig. 4). The basidia formed by *Bulleribasidium* are morphologically variable: they are globose or elongate, two- or three-celled with transverse and longitudinal septa. Basidiocarps are lacking, and ballistoconidia are present.

Kwoniella was introduced to the literature (Scorzetti *et al.*, 2002) as *Cryptococcus* sp. CBS 8507, where the strain was recognized through sequence analysis as a member of the *Tremellales*. Formation of basidia by compatible mating types established the presence of a teleomorphic state. Based on the phylogenetic architecture depicted by the D1/D2 tree, *Kwoniella* (Fig. 5) resides in a cluster, which is separate from other teleomorphic yeasts. *Kwoniella* shares characteristics with the other genera; however, *Kwoniella* is distinct by the combination of characteristics: lack of ballistospores, ballistoconidia and an observed basidiocarp, and the presence of morphologically variant basidia, which are globose, ovoid or lageniform with longitudinal to oblique and transverse septa and navicular with transverse septa. Both types of *Kwoniella*

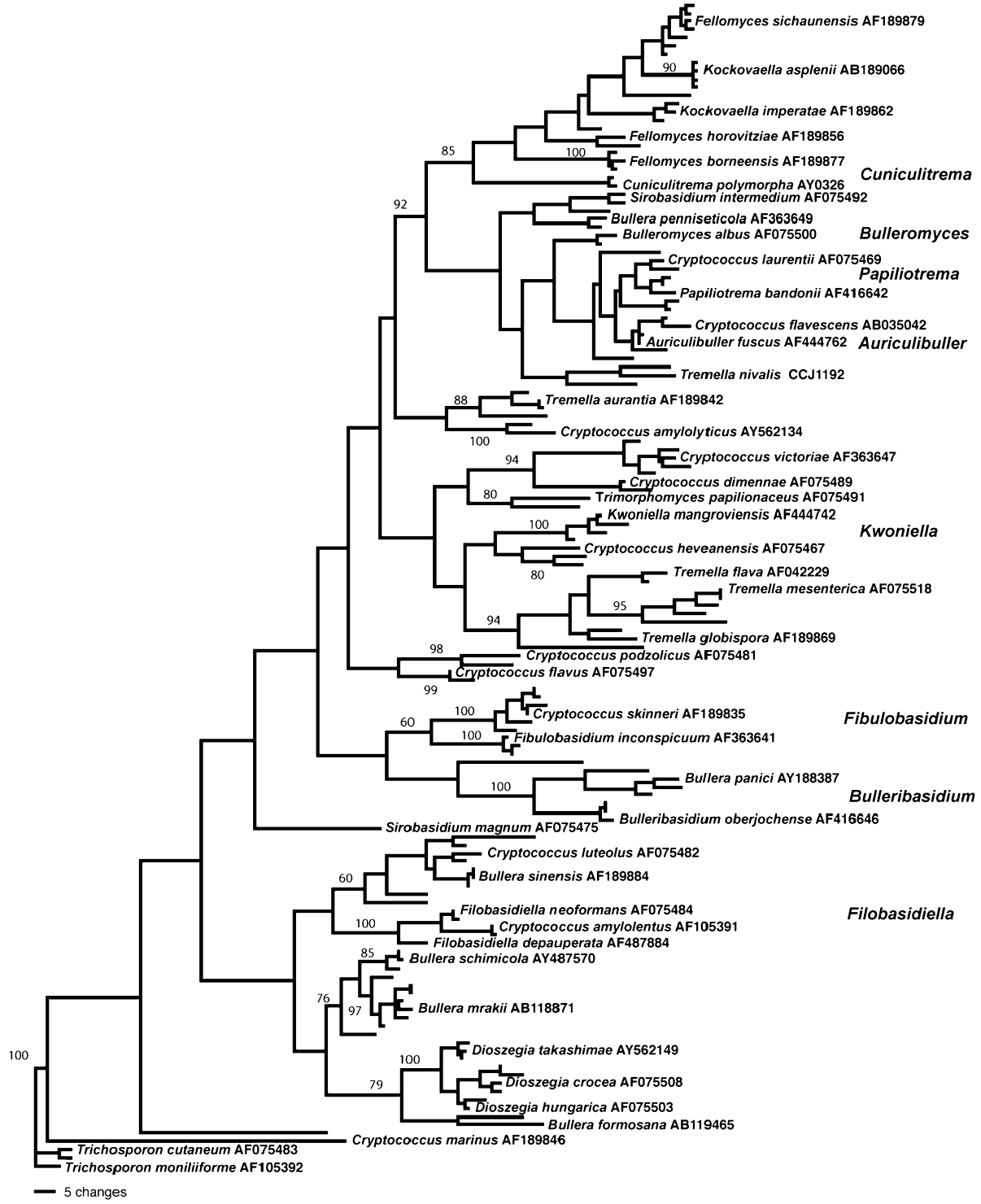

Fig. 4. Phylogenetic stick tree of the *Tremellales*, with cluster representatives, to demonstrate the relationship of the teleomorphic genera. The tree is based on a parsimony analysis, heurisitic search (PAUP 4.0b10) of the D1/D2 LSU rRNA gene. One of 100 equally parsimonious trees. Numbers on the branches represent boostrap percentages (> 50%) from 100 full-heuristic replications.

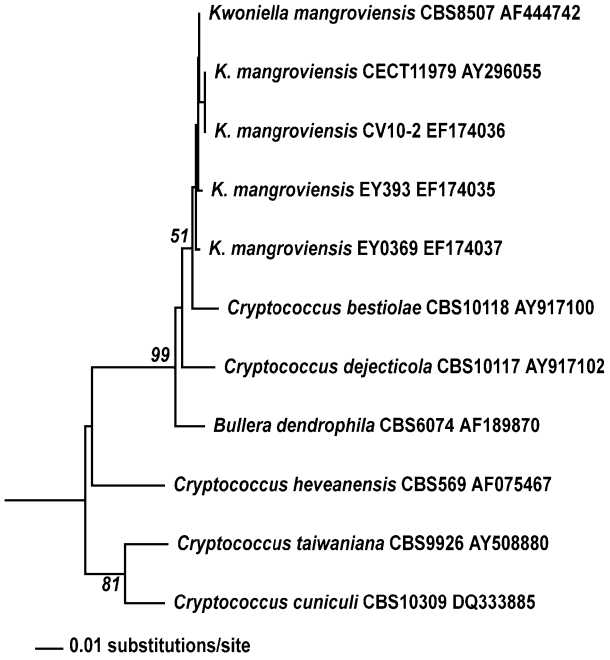

Fig. 5. Neighbor-joining analysis of the D1/D2 LSU rRNA gene to illustrate strain variability in *Kwoniella* and depict the closely related species from Fig. 4. Numbers on the branches represent bootstrap percentages (> 50%) from 1000 full-heuristic replications.

basidia can be observed on a single hyphal strand, often in an angular formation. *Bulleribasidium*, for example, has morphologically variable basidia and lacks basidiocarps, but produces ballistoconidia.

Thanh *et al.* (2006), based on sequence analysis, demonstrated that CBS 8507 is closely related to *Cryptococcus dejecticola, Cryptococcus bestiolae* and *Bullera dendrophila* and did not mate with any of those species. Their comparison of phenotypic characteristics demonstrated a similarity between species, with distinguishing characteristics based on inulin (*C. bestiolae* and *C. dejecticola* positive, CBS 8507 and *B. dendrophila* negative) and raffinose (*B. dendrophila* negative, CBS 8507, *C. bestiolae* and *C. dejecticola* positive). Raffinose, however, was found to be positive with other strains of *K. mangroviensis*.

In contrast to the D1/D2 results, the ITS data of Scorzetti *et al.* (2002) and Thanh *et al.* (2006) indicate that *Kwoniella* is related to the human pathogens *Filobasidiella neoformans* and *Filobasidiella bacillispora*. *Filobasidiella* has a distinctive slender, elongate holobasidium, which expands abruptly into an apex that bears four chains of basidiospores (Kwon-Chung, 1998). The phylogenetic relationship between *Filobasidiella* and *Kwoniella* is not obvious based on life cycles. Because the D1/D2 and ITS data do not agree, the phylogenic relationship of these genera might benefit from a multi-gene approach.

Strain variability within *K. mangroviensis* is present in the D1/D2 region. The cork strains (CECT 11955 and CECT 11979) and the beach strain (CV 10-2) differ from all other strains in the D1 region (at position 192 from the forward primer: TAAGCGGAGGAAAAG) by GATG, rather than the consensus GACG. Several of the Everglades strains (EY352, 393, 424, 875, 9–27, 9–99) have a single D2 nucleotide position difference at position 530. The sequence of these strains is represented by GTTG, whereas the consensus is GTCG.

The ITS region also demonstrates strain variability (Fig. 6), most of which consists of scattered single nucleotide differences that are irrespective of mating type. Significantly, the cork and beach strains, as noted in the D1/D2 region, are distinct from other strains of *K. mangroviensis*. Differences from the mangroves strains include seven positions for the cork isolates and six for the beach strain. These results, in combination with the ecological information, suggest the presence of distinct genotypes. Further genetic and mating studies with additional strains will be required to test this observation.

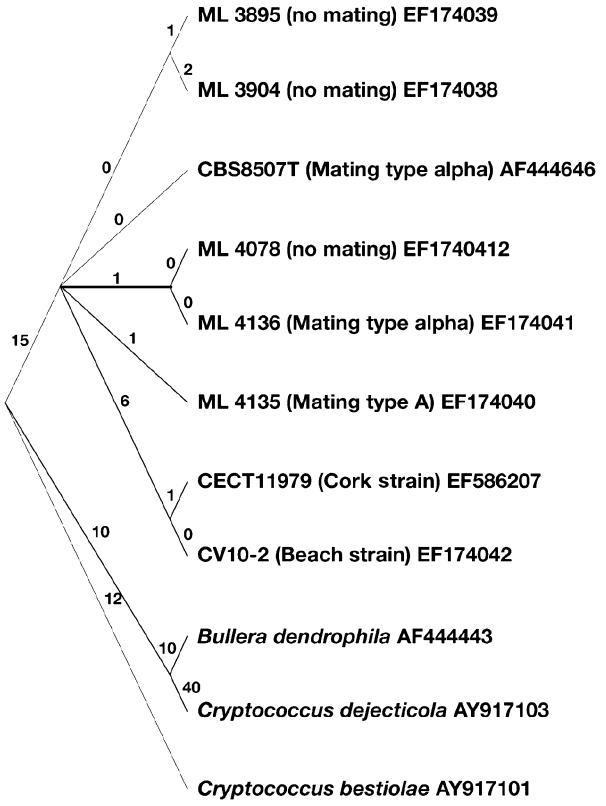

Fig. 6. *Kwoniella* strain variability within the ITS1 & 2 and the relationship with *Bullera dendrophila*, *Cryptococcus bestiolae* and *Cryptococcus dejecticola*. Parsimony analysis, heuristic search, one of three equally parsimonious trees (PAUP 4.0b10). Numbers on branches represent branch lengths.

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