RESEARCH ARTICLE

Farysizyma gen. nov., an anamorphic genus in the *Ustilaginales* to accommodate three novel epiphytic basidiomycetous yeast species from America, Europe and Asia

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Received 10 August 2007; revised 15 January 2008; accepted 16 January 2008.
First published online 1 April 2008.

DOI:10.1111/j.1567-1364.2008.00377.x

Editor: Teun Boekhout

Keywords
phylloplane; basidiomycetous yeasts; Ustilaginomycotina; *Farysizyma*.

Abstract

Among many isolates that resulted from four independent surveys of yeasts associated with plants in Brazil, the USA, Portugal and Taiwan, we have characterized eighteen basidiomycetous strains, two of which were conspecific with the type strain of *Rhodotorula acheniorum*, whereas the remaining sixteen isolates appeared not to correspond to any previously described species. Microsatellite-PCR fingerprinting with primers M13 and $(GTG)_5$ confirmed that the latter strains formed three genetically distinct groups. Each group was considered to represent a distinct species based on nucleotide sequences of the D1/D2 domains of the 26S rRNA gene and the internal transcribed spacer (ITS) region. Phylogenetic analyses of sequence data placed the putative novel species in a clade with *R. acheniorum* and the dimorphic smut fungus *Farysia chardoniana*. A novel anamorphic genus, *Farysizyma*, is created to accommodate the three undescribed species, which were named *Farysizyma itapuensis*, *Farysizyma setubalensis* and *Farysizyma taiwaniana*. A new combination, *Farysizyma acheniorum*, is proposed for *R. acheniorum*, which may represent the yeast-phase anamorph of *Farysia thuemenii*.

Introduction

Basidiomycetous yeasts are found in three major lineages: Hymenomycetes, Urediniomycetes and Ustilaginomycetes (Fell *et al*., 2000; Scorzetti *et al*., 2002). Recently, Bauer *et al*. (2006) proposed a new classification scheme for basidiomycetous fungi in three subphyla: the Agaricomycotina, the Pucciniomycotina and the Ustilaginomycotina, which replaced the Hymenomycetes, Urediniomycetes and Ustilaginomycetes, respectively, *sensu* Swann & Taylor (1995). The Ustilaginomycotina comprise more than 1400, mainly phytoparasitic, fungal species (e.g. smuts and smut-allied fungi) that have a type B secondary structure of the 5S rRNA and a cell wall carbohydrate composition with dominance of glucose and absence of xylose (Bauer *et al*., 2006). The phylogeny and classification of Ustilaginomycotina have been recently revised in the light of multigene analyses by Begerow *et al*. (2006). A few yeast species are included in the Ustilaginomycotina (e.g. *Pseudozyma* spp., *Rhodotorula acheniorum* and *Rhodotorula bacarum*) and are usually found on plant surfaces. Some authors reported the isolation of ustilaginomycetous yeasts also from clinical samples (Sugita *et al*., 2003). Several other ustilaginomycetous species have biotechnological and/or agricultural importance (Grigorova *et al*., 1999; Avis & Bélanger, 2002; Martinez-Espinoza *et al*., 2002; Feldbrügge *et al*., 2004).

Among many isolates obtained in the course of four independent surveys of yeasts associated with plants in Brazil, the USA, Portugal and Taiwan, we have selected 18 ustilaginomycetous strains that displayed similar phenotypic and genotypic traits. This paper deals with characterization of those isolates and with the formal description of the new taxa created to accommodate the majority of those strains.

Materials and methods

Sample collection and yeast isolation

Leaf samples of the bromeliads *Dyckia* sp., *Tillandsia gardneri*, *Tillandsia geminiflora*, *Vrisea friburgensis* and *Vrisea procera* (*Bromeliaceae*) were aseptically collected from two locations in Itapuã Park (Pedreira Beach and Betânia Trail), near Porto Alegre, Rio Grande do Sul State, South of Brazil (approximate coordinates: 30°22′ S, 51°04′ W). Leaves of *Cistus albidus* (*Cistaceae*) were collected at two sites in the Arrábida Natural Park, a Mediterranean-type ecosystem, south of Lisbon, Portugal (approximate coordinates: 38°27′ N, 9°02′ W). Leaves of *Daphniphyllum glaucescens* ssp. *oldhamii* (*Daphniphyllaceae*) were collected at NanJen-Shan Natural Reserve, Taiwan (approximate coordinates: 22°40′ N, 120°29′ E). Yeast isolation was based on the plating of leaf washings onto solid culture media, with some differences among the Brazilian, Portuguese and Taiwanese surveys. Bromeliad leaves collected in Brazil were first gently washed with sterile distilled water for 10 min, which was then replaced by a 0.5% Tween 20 solution and vigorously agitated for an additional 30 min. Decimal dilutions of the resultant suspension were spread onto YM agar (1% glucose, 0.3% malt extract, 0.3% yeast extract, 0.5% peptone and 2% agar), supplemented with 0.04% chloramphenicol and acidified to pH 4.0. *Cistus albidus* leaves were cut into small fragments and washed vigorously in 10 mL of sterile Ringer's solution (NaCl 0.45%) for 1 min. Decimal dilutions were spread onto MYP agar (malt extract 0.7%, soytone 0.25%, yeast extract 0.05% and agar 1.5%), supplemented with 0.05% chloramphenicol and 0.004% Rose Bengal. *Daphniphyllum glaucescens* ssp. *oldhamii* leaves were washed and agitated for 30 min in sterile distilled water and aliquots were spread on acidified YM agar plates. Pooled samples of nectar from *Gelsemium rankinii* (*Gelsemiaceae*) were collected in a backyard garden in Valdosta, GA (approximate coordinates: 30°51′ N, 83°17′ W), and plated on YM agar plates with 0.05% chloramphenicol. Plates were incubated at room temperature and representative colonies of the different morphological types, including the ustilaginomycetous yeasts treated in this report, were purified and maintained on agar slants at 4 °C. More details about the Portuguese yeast survey can be found elsewhere (Inácio *et al.*, 2002). Some background on the significance of yeasts in the nectar of *Gelsemium* has been published (Manson *et al.*, 2007).

Phenotypic identification of isolates

Phenotypic characterization and identification of the Brazilian, Portuguese and North American isolates were performed according to Yarrow (1998) and the keys presented in Kurtzman & Fell (1998), Barnett *et al.* (2000), and/or the computer program YEASTCOMPARE (Ciriello & Lachance, University of Western Ontario, London, ON). Some isolates were further characterized by determination of the assimilation profile of aldaric acids and aromatic compounds (Fonseca, 1992; Sampaio, 1994). The Taiwanese isolate was phenotypically characterized with the MicroLog™ (Biolog) and BCCM™/Allev 2.00 yeast identification systems (Robert *et al.*, 1997). Evaluation of eventual mating compatibility was performed by mixing pairs of cultures on corn meal agar.

Microsatellite-PCR fingerprinting

Genomic DNA isolation and microsatellite-PCR fingerprinting using the primers M13 (5′-GAG GGT GGC GGT TCT-3′) and $(GTG)_5$ followed the protocols described elsewhere (Sampaio *et al.*, 2001). Gel electrophoresis images were acquired with the KODAK DIGITAL SCIENCE ID IMAGE ANALYSIS software (Rochester, NY) and analyzed with GELCOMPAR (version 4.1, Applied Maths, Sint-Martens-Latem, Belgium) using the Pearson's correlation coefficient. Dendrograms were computed using the UPGMA clustering method.

DNA sequence analysis

The internal transcribed spacer (ITS) ribosomal DNA region, which includes the ITS1 and ITS2 spacers and the 5.8S rRNA gene, and the D1/D2 domains of the 26S (or LSU) rRNA gene were PCR amplified as described previously (Lachance *et al.*, 1999). The resulting amplicons were concentrated and cleaned with either Qiaquick PCR columns (Qiagen, Chatsworth, CA) or GFX Band Purification Kit (Amersham Biosciences, Piscataway, NJ). Cycle sequencing employed standard protocols with the following primers: NL1 (5′-GCA TAT CAA TAA GCG GAG GAA AAG-3′) and NL4 (5′-GGT CCG TGT TTC AAG ACG G-3′) for the D1/D2 domains; ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) for the ITS region. Primers used for amplification and sequencing of rRNA gene were developed by other authors (see http://www.biology.duke.edu/fungi/mycolab/primers.htm). Sequences were obtained with either an ALFexpress II DNA Analyser (Amersham Pharmacia Biotech, Uppsala, Sweden) or an ABI DNA Sequencer (Applied Biosystems), according to the manufacturer's instructions. Sequences were aligned with the CLUSTAL algorithm of MEGALIGN (DNASTAR Inc., Madison, WI). Phylogenetic trees were computed with PAUP version 4.0b8 (Sinauer Associates Inc., Sunderland, MA) using the neighbour-joining method and the Kimura two-parameter model for calculating distances. Gaps were treated as missing data. Nucleotide sequences were deposited in GenBank under the accession numbers given in Table 1. Additional sequences were retrieved from GenBank (accession numbers are indicated on the phylogenetic trees).

Results and discussion

Four independent surveys of yeasts associated with the phylloplane of several plants in Brazil, Portugal and Taiwan, and with nectar of flowers in the USA yielded many isolates, 18 of which are herein described (Table 1). Analysis of microsatellite-PCR DNA fingerprints with primers M13 and $(GTG)_5$ led to grouping of the majority of those strains in four distinct clusters (Fig. 1). Clusters A and B included two Portuguese isolates each, whereas the Taiwanese isolate TOH1-2 presented a unique microsatellite-PCR profile. The major cluster C included all eleven bromeliad isolates from Brazil, with strains BI238 and BI181 having slightly deviating microsatellite-PCR profiles. The North American isolates were not analyzed by microsatellite-PCR fingerprinting. The analysis of the sequences from the D1/D2 (Fig. 2) and ITS (data not shown) regions revealed that each microsatellite-PCR group corresponded to a distinct yeast species. All three species appeared to be phylogenetically related to *R. acheniorum*, in a clade within the *Ustilaginales* that also includes the smut fungus *Farysia chardoniana* ('*Farysia* clade', Fig. 2). Two of the isolates from *C. albidus* leaves appeared to represent *R. acheniorum* (3CVF5 and 5CVFe5, microsatellite-PCR cluster B), as they had D1/D2 and ITS sequences identical to those of the type strain of this species (CBS 6386). Isolates 5CSFe9 and 3CVF35 (microsatellite-PCR cluster A) seemed to represent an undescribed species, as they presented divergent D1/D2

Table 1. List of strains and DNA sequences used in this study

				GeneBank accession no.	
Species	Strain*	Other collections	Origin	D1/D2	ITS
Farysizyma acheniorum comb. nov.	3CVF5	CBS 10244	*Cistus albidus* leaves, Fonte do Veado (Arrábida Natural Park, Portugal)	EU002860	EU002887
	5CVFe5	CBS 10243	*Cistus albidus* leaves, Fonte do Veado (Arrábida Natural Park, Portugal)	EU002859	–
F. itapuensis sp. nov.	BI120^T	CBS 10428, NRRL Y-48116	*Vrisea friburgensis* leaves, Pedreira Beach (Itapuã Park, Brazil)	DQ767831	DQ767831
	BI52	–	*V. friburgensis* leaves, Pedreira Beach (Itapuã Park, Brazil)	–	–
	BI63	CBS 10425, NRRL Y-48114	*V. procera* leaves, Pedreira Beach (Itapuã Park, Brazil)	–	–
	BI77	CBS 10426, NRRL Y-48115	*Tillandsia gardneri* leaves, Pedreira Beach (Itapuã Park, Brazil)	–	–
	BI109	–	*V. friburgensis* leaves, Betânia Trail (Itapuã Park, Brazil)	–	–
	BI224	–	*T. geminiflora* leaves, Pedreira Beach (Itapuã Park, Brazil)	–	–
	BI229	–	*V. friburgensis* leaves, Betânia Trail (Itapuã Park, Brazil)	–	–
	BI154	–	*T. geminiflora* leaves, Betânia Trail (Itapuã Park, Brazil)	–	–
	BI235	CBS 10430, NRRL Y-48118	*V. friburgensis* leaves, Betânia Trail (Itapuã Park, Brazil)	DQ784567	–
	BI181	CBS 10429, NRRL Y-48117	*Dyckia* sp. leaves, Betânia Trail (Itapuã Park, Brazil)	DQ784568	DQ855949
	BI238	CBS 10431, NRRL Y-48119	*V. friburgensis* leaves, Betânia Trail (Itapuã Park, Brazil)	DQ784569	DQ855950
	UWOPS 07JM104.2	–	Nectar from *Gelsemium rankinii* (Georgia, USA)	EU024553	EU024553
	UWOPS 07JM104.3	–	Nectar from *Gelsemium rankinii* (Georgia, USA)	–	–
F. setubalensis sp. nov.	3CVF35^T	CBS 10241, PYCC 5952	*Cistus albidus* leaves, Fonte do Veado (Arrábida Natural Park, Portugal)	EU002857	EU002888
	5CSFe9	CBS 10242	*Cistus albidus* leaves, Mata do Solitário (Arrábida Natural Park, Portugal)	EU002858	EU002889
F. taiwaniana sp. nov.	TOH1-2 ^{T}	CBS 9927, BCRC 23028	*Daphniphyllum glaucescens* ssp. *oldhamii* leaves, NanJen-Shan Natural Reserve of Taiwan	AY551270	AY555071

*Type strain, T.

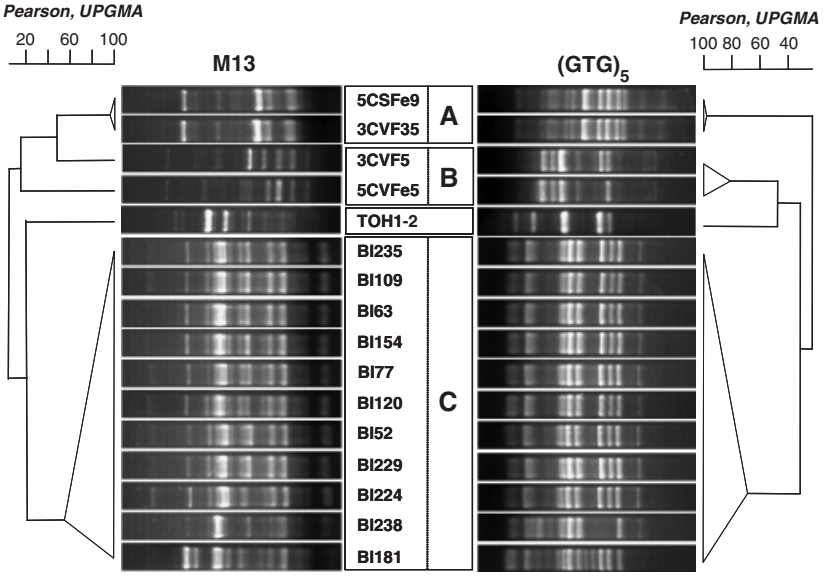

Fig. 1. MSP-PCR fingerprinting of ustilaginomycetous phylloplane isolates: DNA banding patterns obtained with primers M13 and $(GTG)_5$ and resulting dendrograms using Pearson's coefficient and the UPGMA clustering method.

and ITS sequences relative to their nearest neighbors (e.g. 14 mismatches to *R. acheniorum* in D1/D2). The single Taiwanese strain also presented unique D1/D2 and ITS sequences and was thus deemed to represent another novel species (four and more than 30 mismatches to strain BI120 in D1/D2 and ITS region, respectively). Following the same criteria (microsatellite-PCR cluster C, Fig. 1; see also Fig. 2), the 11 Brazilian strains appeared to represent yet a different species. Strains BI238 and BI181 had one mismatch in the D1/D2 region in comparison to all the other Brazilian isolates (Fig. 2), but identical ITS sequences (data not shown). The North American isolates (represented by strain UWOPS 07JM104.2) had two nucleotide substitutions in D1/D2 and two others in ITS compared with strain BI120 (data not shown). However this was not considered enough to exclude them from the same new species, because the morphological and physiological/biochemical profiles were identical (Table 2). Mating reactions between multiple strains within each species were negative.

The genus *Rhodotorula* is polyphyletic, with most of its members, including the type species *Rhodotorula glutinis*, distributed among several lineages within the Pucciniomycotina (Fell & Statzell-Tallman, 1998; Bauer *et al.*, 2006). However, a few species are placed in the Ustilaginomycotina: *R. acheniorum* in the *Ustilaginales* and *R. bacarum* and *Rhodotorula phylloplana* in the *Microstromatales* (Sampaio, 2004). The gene sequences accumulated in public databases (mainly from rRNA gene regions) have provided the basis for molecular phylogenetic analyses that have clearly shown the polyphyletic nature of *Rhodotorula*, as well as of other anamorphic genera (e.g. Scorzetti *et al.*, 2002). Because holding on to the current circumscription of polyphyletic genera for the sake of taxonomic stability is no longer warranted (e.g. Sampaio, 2004; Okoli *et al.*, 2007), we decided not to include the novel species in *Rhodotorula*. Another option would be to accommodate them in the genus *Pseudozyma* that comprises yeast-like anamorphs closely related to species of *Sporisorium* and *Ustilago* (e.g. Boekhout & Fell, 1998; Sampaio, 2004). However, on the basis of molecular phylogenetic analyses (Fig. 2), our novel species, as well as *R. acheniorum* (see also Sampaio, 2004), are only distantly related to the species in the above-mentioned genera, which belong to the *Ustilaginaceae* (*sensu* Begerow *et al.*, 2006). In fact, members of the '*Farysia* clade' appear to belong to a sister lineage within the *Ustilaginales*, which contains smut genera such as *Schizonella* and *Stegocintractia*, and may correspond to a redefined circumscription of *Anthracoideaceae* according to Begerow *et al.* (2006). Moreover, unlike *Pseudozyma* species (e.g. Boekhout & Fell, 1998), the novel taxa do not produce abundant true hyphae or assimilate inositol. A third possibility would be to include the novel species in the genus *Farysia* Raciborski due to the observed phylogenetic proximity to *F. chardoniana* (Fig. 2). However, all of our isolates originated from apparently healthy leaves or flowers of plants totally unrelated to the known *Farysia* hosts, i.e. *Carex* spp. (Vánky, 2002). Because anamorph/teleomorph connections could not be established for the three novel species from the molecular analyses (Fig. 2), the anamorph genus *Farysizyma* gen. nov. is hereby created to accommodate them, a proposal allowed by article 59 of the International Code of Botanical Nomenclature (St Louis Code). Proposal of novel anamorphic genera for putative yeast states of dimorphic plant parasites is not unprecedented and a parallel may be drawn with the teleomorph/anamorph genus pairs *Ustilago*/*Pseudozyma* (Boekhout, 1995) and *Taphrina*/*Lalaria* (Inácio *et al.*,

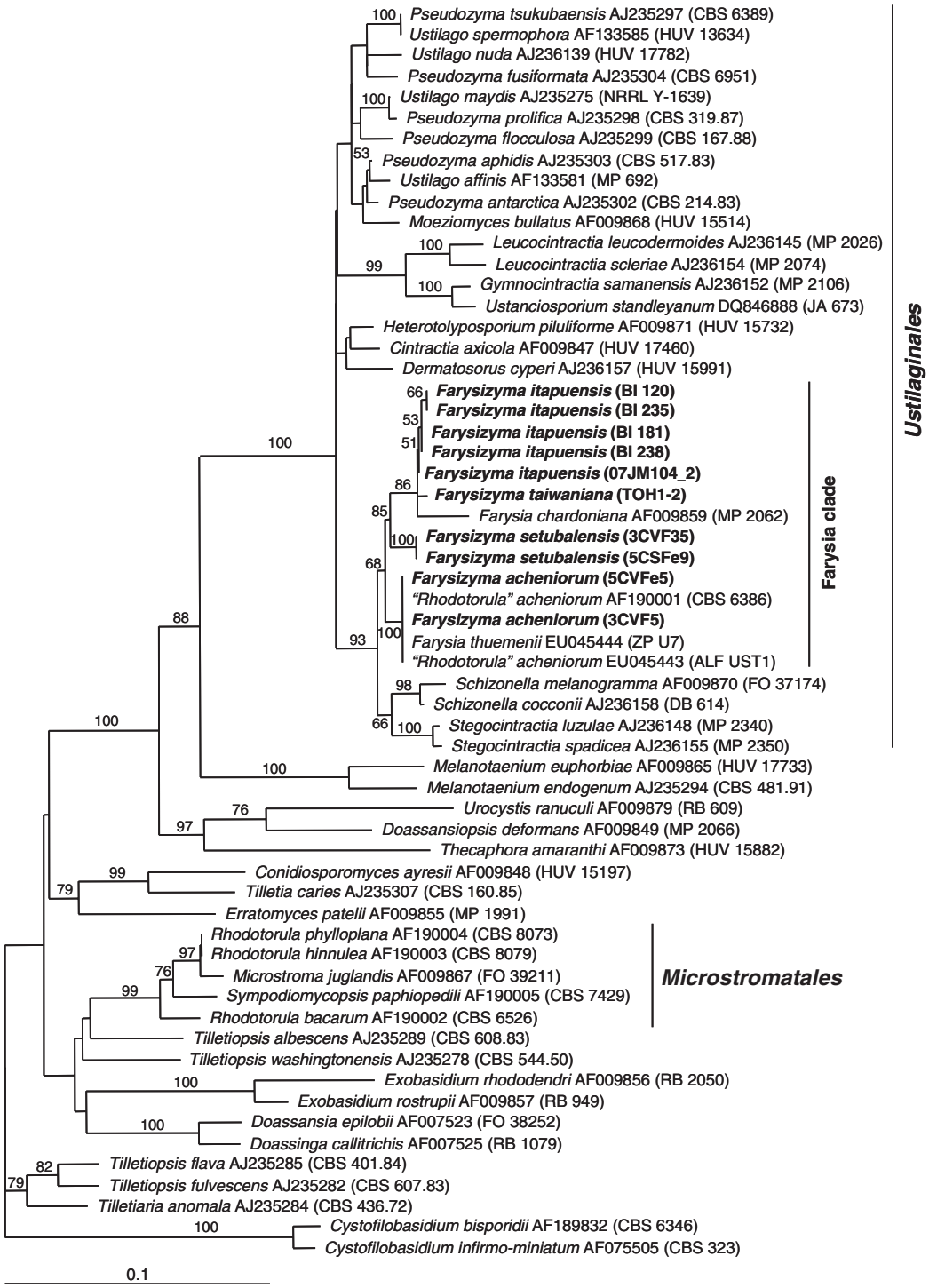

Fig. 2. Phylogenetic tree of representative phylloplane isolates and of selected species of the Ustilaginomycota obtained by neighbor-joining analysis of 26S rRNA gene (D1/D2 domains) sequences using PAUP 4.0b8. The numbers given on the branches are the frequencies (> 50%) with which a given branch appeared in 1000 bootstrap replications. *Cystofilobasidium* spp. were used as outgroup. Sequences determined by the authors of the present study are typed in boldface. Additional sequences were retrieved from GenBank (species names followed by the corresponding accession number and strain number between parentheses).

Table 2. Physiological/biochemical test responses of the newly proposed species and of *Farysizyma acheniorum*

Test responses*	*Farysizyma acheniorum*†	*Farysizyma setubalensis*‡	*Farysizyma taiwaniana*§	*Farysizyma itapuensis*¶
Glucose fermentation	−	−	−	−
Carbon sources				
D-Glucose	+	+	+	+
D-Galactose	+	+	+	+
L-Sorbose	−	−	−	−, W
D-Glucosamine	−	−	−	−, D
D-Ribose	+	D	W	+
D-Xylose	+	+	+	+
L-Arabinose	+	+, D	+	+
D-Arabinose	V	+, D	W	V
L-Rhamnose	−	−	−	−, W
Sucrose	+	+	+	+
Maltose	+	+, D	+	+
α,α Trehalose	+	+	+	V
Methyl-α-D-glucoside	D	−	−	ND
Cellobiose	+	D	W	V
Salicin	−	D, W	−	−, W
Melibiose	+	−	+	+, W
Lactose	V	D	W	+
Raffinose	+	+	+	+
Melezitose	+	+	+	+
Inulin	−	−	W	V
Soluble starch	−	−	ND	V
Glycerol	+	D	W	+
Erythritol	+	+, D	W	+
Ribitol	D	+	−	−, D
Xylitol	+	−	W	W, D
D-Glucitol	+	+	+	+, W
D-Mannitol	+	+	+	+, W
Galactitol	D, −	−	−	W, D
Inositol	−	−	−	−
Glucono-D-lactone	V	−	W	ND
D-Gluconic acid	−	D, −	W	+
D-Glucuronic acid	V	+	−	ND
DL-Lactic acid	+	+	W	V
Succinic acid	+	+	+	+
Citric acid	+	+	W	−, W
Methanol	−	−	−	−
Ethanol	+	+	−	−, W
L-Malic acid	+	+	ND	ND
L-Tartaric acid	−	−	ND	ND
N-sources				
Nitrate	+	+	−	V
Nitrite	+	+	ND	+
Ethylamine	+	+, D	ND	−
L-Lysine	V	+	ND	+
Cadaverine	+	+	ND	ND
Creatine	−	−	ND	−
Creatinine	−	−	ND	−
Other tests				
Growth in vitamin-free medium	−	−	ND	ND
Growth with 50% glucose	ND	ND	−	−
Growth with 0.01% cycloheximide	D, −	−, D	ND	ND
Growth with 0.1% cycloheximide	−	−	ND	ND
Splitting of arbutin	V	+	−	ND
Formation of starch-like compounds	−	−	−	−
Hydrolysis of urea	+	+	+	+

Table 2. Continued.

Test responses*	*Farysizyma acheniorum*†	*Farysizyma setubalensis*‡	*Farysizyma taiwaniana*§	*Farysizyma itapuensis*¶
Colour reaction with Diazonium Blue B	+	+	+	+
Growth at 25 °C	+	+	+	+
Growth at 30 °C	−	−	ND	−
Growth at 37 °C	ND	ND	−	−

*Test results: +, positive; D, delayed positive; W, weak; −, negative; V, variable; ND, not determined.

†Responses for the following strains: CBS 6386^T, 3CVF5 and 5CVFe5.

‡Responses for the following strains: 3CVF35 and 5CSFe9.

§Responses for the following strain: TOH1-2.

¶Responses for the following strains: BI120, BI63, BI77, BI235, BI181, BI238, BI52, BI109, BI154, BI224, BI229, UWOPS 07JM104.2 and UWOPS 07JM104.3.

2004). In those cases the teleomorphs are also dimorphic plant parasites that have a plant pathogenic mycelial phase and a saprobic yeast phase, and the anamorphs are phylogenetically related yeasts that are found as epiphytes on plants other than the teleomorph hosts. The species name *Farysizyma setubalensis* sp. nov. is proposed for the Portuguese strains (5CSFe9 and 3CVF35), *F. taiwaniana* sp. nov. for the single Taiwanese strain (TOH1-2) and *F. itapuensis* sp. nov. for the Brazilian and the North American strains (Table 1). Another observation that suggests a connection between the novel taxa and *Farysia* is the fact that strain ZP U7, isolated from teliospores of *Farysia thuemenii* (J.P. Sampaio, pers. commun.), had a D1/D2 sequence identical to that of the type strain of *R. acheniorum* (Fig. 2), thus suggesting that *R. acheniorum* could represent the yeast-phase anamorph of *F. thuemenii*. However, inoculation experiments would be required to verify this hypothesis and the remaining strains identified as *R. acheniorum* originated from plants (viz. *C. albidus* and *Fragaria* spp.; Table 1; see also below) unrelated to the hosts of *F. thuemenii* (*Carex* spp.). *Rhodotorula acheniorum* is thus transferred to the newly proposed genus under a new combination, *F. acheniorum* comb. nov. In spite of the relatively low bootstrap value for the '*Farysia* clade' in the phylogenetic analyses depicted in Fig. 2, we found that all the sequenced strains of *Farysia* and *Farysizyma* species had a signature sequence in the D1 region (ATGCA, starting at the homologous position 255 of the *Saccharomyces cerevisiae* LSU rRNA gene; GenBank no. J01355), which constitutes a synapomorphy for members of the clade.

The physiological profiles of the three novel species as well as that of *F. acheniorum* are very similar (Table 2). *Farysizyma setubalensis* can be differentiated from *F. acheniorum*, *F. taiwaniana* and *F. itapuensis* by the inability to assimilate melibiose and xylitol as carbon sources. *Farysizyma acheniorum* is unable to assimilate D-gluconic acid, in contrast to *F. taiwaniana* and *F. itapuensis*. The latter two species can be differentiated by the ability to assimilate galactitol.

Species of *Farysizyma* appear to occur as epiphytes on different plants, often as phylloplane colonists (Table 1), where they may represent common inhabitants. Regarding *F. acheniorum* (formerly *R. acheniorum*), strains deposited at the CBS collection, CBS 6384 and CBS 6386, were also isolated from plant sources (fruits of *Fragaria vesca*, wild strawberries; UK). A third strain isolated from leaves of *Callistemon viminalis* (*Myrtaceae*) in Australia (CBS 8078), was previously considered a representative of *R. acheniorum* (Barnett *et al.*, 2000), but a D1/D2 sequence available in the CBS yeast database (www.cbs.knaw.nl/yeast/BioloMICS.aspx) suggests that it does not belong to *F. acheniorum* (data not shown). A fourth presumptive *F. acheniorum* strain, ALF UST1 (D1/D2 sequence available in GenBank; see Fig. 2) was also isolated from garden strawberries (*Fragaria* × *anassa*) in Portugal (J.P. Sampaio, pers. commun.).

Latin diagnosis of *Farysizyma* Á. Fonseca gen. nov.

Genus epiphyticorum et anamorphicorum fungorum Ustilaginalium Farysiae speciebus affinium. Cellulae zymoidea enteroblastice gemmantes. Hyphae septatae non formantur. Ballistoconidia nulla. Inositolum non assimilatur. Productio compositorum amylosimilium nulla. Assimilatio kalii nitrati variabilis. Typus *Farysizyma itapuensis* M. F. Landell et P. Valente sp. nov.

Etymology: the genus name refers to the fact that the species included therein are either yeast-phase anamorphs of the smut genus *Farysia* or closely related epiphytic yeasts, as suggested by the molecular phylogenetic evidence.

Description of *Farysizyma* Á. Fonseca gen. nov.

Genus of epiphytic and anamorphic fungi that belongs to the *Ustilaginales* and is closely related to species of the genus *Farysia*. Yeast cells reproduce by enteroblastic budding. Septate hyphae are not formed. Ballistoconidia are absent. Inositol is not assimilated. Starch-like compounds are not

formed. Nitrate assimilation is variable. Type species: *Farysizyma itapuensis* M.F. Landell & P. Valente sp. nov.

Farysizyma acheniorum (Buhagiar & J.A. Barnett) Á. Fonseca, comb. nov.

Basionym: *Sterigmatomyces acheniorum* Buhagiar R.W.M. & J.A. Barnett; in *J Gen Microbiol* 77 : 78 (1973); type strain: CBS 6386. Synonym: *R. acheniorum* (Buhagiar & J.A. Barnett) Rodrigues de Miranda, in von Arx *et al.*, *Stud Mycol* 14 : 28 (1977). Novel strains: CBS 10244 (3CVF5), CBS 10244 (5CVFe5; see Table 1 and Fig. 2); ALF UST1 (from strawberries, see Fig. 2).

Latin diagnosis of *Farysizyma itapuensis* M.F. Landell & P. Valente sp. nov.

Status teleomorphosis incognitus. In liquido YM, post dies 3 ad 25 °C, cellulae vegetativae ovoideae, ellipsoideae vel elongatae, 2.3–7.7 μm × 1.5–2.3 μm, singulae aut binae. Pellicula formatur post dies 14. Hyphae et pseudohyphae non formatur. Ballistoconidia nulla. Fermentatio nulla. In tabula (Table 2) characteres biochemices physiologicesque declarates sunt. Characteres moleculares (culturae typi): sequentia acidi nucleici 'rDNA ITS' et '26S (D1/D2)', DQ767831, in collectione sequentiarum acidi nucleici NCBI (GenBank) deposita est. Typus: BI120 (= CBS 10428^T; NRRL Y-48116^T) isolatus ex *V. friburgensis* in Brasilia.

Description of *F. itapuensis* M.F. Landell & P. Valente sp. nov.

Etymology: *Farysizyma itapuensis* – this Latin-derived epithet refers to the fact that the type strain was isolated in Itapuã Park (Rio Grande do Sul, Brazil).

Teleomorphic state unknown. In YM broth after 3 days at 25 °C, the vegetative cells are ovoid, ellipsoidal to elongate 2.3–7.7 μm × 1.5–2.3 μm, occur singly, in parent–bud pairs. A pellicle is formed after 14 days. On GYP agar, after 7 days at 25 °C, the streak culture is butyrous, cream, plain, undulate and with regular margin, brilliant. After 14 days the colonies dry up and become yellow dark. In Dalmau plate cultures on corn meal agar after 2 weeks true hyphae are not formed. Ballistoconidia are not formed. Glucose fermentation is absent. Physiological and biochemical characteristics are presented in Table 2. Results of additional physiological tests for strains UWOPS 07JM104.2 and UWOPS 07JM104.3: growth in *N*-acetyl-D-glucosamine; weak growth in methyl-α-D-glucoside and 2-keto-D-gluconate; delayed growth in glucono D-lactone and D-glucuronic acid; no growth in hexadecane; growth in cadaverine; delayed growth in 0.01% cycloheximide; no growth in 0.1% cycloheximide; weak growth in vitamin-free medium; growth without aminoacids; no growth in 1% acetic acid or 16% NaCl; no acid production or lipase activity; negative or delayed gelatin liquefaction. Molecular characteristics (type strain): sequence of the ITS region and D1/D2 domains of the rRNA gene 26S deposited in NCBI (GenBank accession DQ767831). Type strain: BI120 (= CBS 10428^T; NRRL Y-48116^T). The type strain BI120 as well as strains BI52, BI63, BI77, BI109, BI154, BI181, BI224, BI229, BI235 and BI238 were isolated from leaves of the bromeliads in Itapuã Park, Rio Grande do Sul/Brazil and have been deposited in the Centraalbureau voor Schimmecultures and ARS Yeast Culture Collections (Table 1). Strains UWOPS 07JM104.2 and UWOPS 07JM104.3 were isolated from nectar of *Gelsemium rankinii* in GA, USA (Table 1).

Latin diagnosis of *Farysizyma taiwaniana* P.-H. Wang, Y.-T. Wang & S.-H. Yang sp. nov.

Status teleomorphosis incognitus. In medio liquido YM post 3 dies ad 26 °C, cellulae ovoideae vel ellipsoidae 3–4 × 1–2 μm, singulae aut binae. Hyphae et pseudohyphae non formatur. Fermentatio nulla. In tabula (Table 2) characteres biochemices physiologicesque declarates sunt. Characteres moleculares (culturae typi): sequentiae acidi nucleici 'rDNA 26S (D1/D2)', AY551270, et 'rDNA ITS', AY555071, in collectione sequentiarum acidi nucleici NCBI (GenBank) depositae sunt. Typus: TOH1-2 (= CBS 9927^T, BCRC 23028^T) isolatus ex *D. glaucescens* in Formosa.

Description of *F. taiwaniana* P.-H. Wang, Y.-T. Wang & S.-H. Yang sp. nov.

Etymology: *Farysizyma taiwaniana* – this Latin-derived epithet refers to the fact that the species was isolated in Taiwan (NanJen-Shan Natural Reserve).

Teleomorphic state unknown. In YM broth after 3 days growth at 26 °C, the cells are ovoid to ellipsoid, occur singly or in pairs and measure 3–4 μm × 1–2 μm. Budding is unipolar, bipolar or multilateral. TOH1-2 give rise to convex, circular, mot, smooth cream butyrous colony, presence of carotenoid pigments, no fermentation, no ballistospores. No sexual state is observed from pure culture plated on YM agar or malt agar. Under these conditions, no hyphae or pseudohyphae are formed. After 7 days Dalmau plate culture on corn meal agar, neither pseudomycelium nor true mycelium was produced. Physiological and biochemical characteristics are presented in Table 2. Results of additional physiological tests: growth in 2-keto-D-gluconate and propane-1,2-diol; weak growth in L-arabinitol; no growth in D-galacturonate, 5-keto-D-gluconate and butane-2,3-diol. Molecular characteristics (type strain): sequences of the D1/D2 domains of the rRNA gene 26S and of the ITS region deposited in NCBI (GenBank accessions: AY551270 and AY555071, respectively). Type strain: TOH1-2 (= CBS 9927^T, BCRC 23028^T). The type

strain was isolated from the phylloplane of *D. glaucescens* ssp. *oldhamii* in NanJen-Shan Natural Reserve of Taiwan.

Latin diagnosis of *Farysizyma setubalensis* Á. Fonseca & J. Inácio sp. nov

Status teleomorphosis incognitus. In medio liquido YM post 3 dies ad 25 °C, cellulae elongatae vel ellipsoidae 3.7–4.5 × 1.1–1.5 μm. Hyphae et pseudohyphae non formatur. Fermentatio nulla. In tabula (Table 2) characteres biochemices physiologicesque declarates sunt. Characteres moleculares (culturae typi): sequentiae acidi nucleici 'rDNA 26S (D1/D2)', EU002857, et 'rDNA ITS', EU002888, in collectione sequentiarum acidi nucleici NCBI (GenBank) depositae sunt. Typus: 3CVF35 (= CBS 10241^T, PYCC 5952) isolatus ex *C. albidus* in Lusitania.

Description of *F. setubalensis* Á. Fonseca & J. Inácio sp. nov

Etymology: *Farysizyma setubalensis* – this Latin-derived epithet refers to the fact that the species was isolated in the Setúbal region (Portugal).

Teleomorphic state unknown. After 3 days at 25 °C in YM broth the cells are ellipsoidal or elongate, 3.7–4.5 μm × 1.1–1.5 μm. After 5–7 days at 25 °C in MYP agar, the cultures are pale to dark brownish cream. No hyphae or pseudohyphae were observed. Physiological and biochemical characteristics are presented in Table 2. Molecular characteristics (type strain): sequences of the D1/D2 domains of the rRNA gene 26S and of the ITS region deposited in NCBI (GenBank accessions: EU002857 and EU002888, respectively). Type strain: 3CVF35 (= CBS 10241^T, PYCC 5952). The type strain as well as strain 5CSFe9 (= CBS 10242) were isolated from the phylloplane of *C. albidus* in the Arrábida Natural Park, Portugal (Table 1).

Acknowledgements

M.F.L. and P.V. acknowledge financial support from the Fundação de Amparo a Pesquisa do Rio Grande do Sul (Fapergs – process no. 02/1754-0) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – process no. 477528/2003-1). M.F.L. received a fellowship from CNPq. J.I. holds a Post-doc fellowship (SFRH/BPD/18453/2004) from Fundação para a Ciência e a Tecnologia, Portugal. The Portuguese yeast survey was partially supported by project contract no. Praxis XXI/2/2.1/BIA/413/94 (Fundação para a Ciência e a Tecnologia, Portugal). P.H.W. was funded by the National Science Council, Taiwan (NSC 92-2621-B-031-001). The North American work was funded by grants from the Natural Sciences and Engineering Research Council of Canada to M.-A.L. and J.D. Thompson. We are also grateful to Pierre Evrard at BCCM/MUCL Industrial Fungi & Yeasts Collection, Belgium, for assisting with the Allev analysis, and to José Paulo Sampaio at Centro de Recursos Microbiológicos, Portugal, for depositing D1/D2 sequences of strains ZPU7 and ALFUST1 in GenBank and sharing unpublished observations on strain ZPU7. We thank Michael Weiss (University of Tübingen, Germany) for correcting the Latin diagnosis of the novel genus. J.S.M. thanks J. Pascarella and H. Coiner for field assistance.

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