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RESEARCH ARTICLE

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Moniliellomycetes and *Malasseziomycetes*, two new classes in *Ustilaginomycotina*

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Key words

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Abstract *Ustilaginomycotina* (*Basidiomycota*, *Fungi*) has been reclassified recently based on multiple gene sequence analyses. However, the phylogenetic placement of two yeast-like genera *Malassezia* and *Moniliella* in the subphylum remains unclear. Phylogenetic analyses using different algorithms based on the sequences of six genes, including the small subunit (18S) ribosomal DNA (rDNA), the large subunit (26S) rDNA D1/D2 domains, the internal transcribed spacer regions (ITS 1 and 2) including 5.8S rDNA, the two subunits of RNA polymerase II (*RPB1* and *RPB2*) and the translation elongation factor 1-α (*EF1-α*), were performed to address their phylogenetic positions. Our analyses indicated that *Malassezia* and *Moniliella* represented two deeply rooted lineages within *Ustilaginomycotina* and have a sister relationship to both *Ustilaginomycetes* and *Exobasidiomycetes*. Those clades are described here as new classes, namely *Moniliellomycetes* with order *Moniliellales*, family *Moniliellaceae*, and genus *Moniliella*; and *Malasseziomycetes* with order *Malasseziales*, family *Malasseziaceae*, and genus *Malassezia*. Phenotypic differences support this classification suggesting widely different life styles among the mainly plant pathogenic *Ustilaginomycotina*.

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INTRODUCTION

Basidiomycota (*Dikarya*, *Fungi*) contains three main phylogenetic domains, namely the subphyla *Agaricomycotina*, *Pucciniomycotina* and *Ustilaginomycotina* (Hibbett et al. 2007). Within *Ustilaginomycotina* three classes have been proposed, namely *Ustilaginomycetes*, *Exobasidiomycetes* and *Entorrhizomycetes* (Begerow et al. 1997, 2006, Bauer et al. 2001, 2006). Other researchers, however, questioned the status of *Entorrhizomycetes* and considered them *incertae sedis* among *Basidiomycota* (Matheny et al. 2006, Hibbett et al. 2007).

Ustilaginomycotina comprises plant pathogenic fungi (smuts), which are mostly dimorphic and present a yeast stage during the live cycle, and asexual fungi which are known only as yeasts or yeast-like species, e.g. *Acaromyces* spp., *Pseudozyma* spp., *Meira* spp., *Tilletiopsis* spp. and *Malassezia* spp. (Boekhout 1991, 1995, Boekhout et al. 1995, 2003, 2011, Begerow et al. 2000, 2006, Fell et al. 2000). *Pseudozyma* spp. belong to *Ustilaginomycetes* and the genera *Acaromyces*, *Meira* and *Tilletiopsis* to *Exobasidiomycetes* (Begerow et al. 2006, Hibbett et al. 2007, Boekhout et al. 2011). The taxonomic position of *Malassezia* spp., that is an important inhabitant of the human and animal skin microbiota (Guého-Kellermann et al. 2010, Sugita et al. 2010, Gaitanis et al. 2012, Findley et al. 2013), is not settled. It has been proposed that the genus belongs to *Ustilaginomycotina* (Begerow et al. 2000, Xu et al. 2007), but its final affiliation within this group remained problematic. The genus was treated to represent a distinct order *Malasseziales* in the *Exobasidiomycetes* based on molecular phylogenetic analyses of the nuclear ribosomal RNA genes alone or in combination with protein genes (Begerow et al. 2000, 2006, Bauer et al. 2001, Weiß et al. 2004). However, Matheny et al. (2006) suggested that the *Malasseziales* might be affiliated with *Ustilaginomycetes*. Therefore, Hibbett et al. (2007) excluded *Malasseziales* from *Exobasidiomycetes* and treated it as *incertae sedis* in *Ustilaginomycotina*.

The yeast-like genus *Moniliella* was described by Stolk & Dakin (1966). In the following year the genus *Trichosporonoides* was introduced by Haskins & Spencer (1967). Both have a basidiomycetous affinity because they can hydrolyse urea, show a positive Diazonium Blue B (DBB) staining and have multi-lamellar cell walls. The septal pores of the species studied so far (*M. oedocephala*, *M. spathulata*, *M. suaveolens*) show a diversity of septa. *Moniliella suaveolens* has typical dolipores with an arch of endoplasmic reticulum, *M. spathulata* has a micropore-like structure and *M. oedocephala* has both (Haskins 1975, Martínez 1979). Boekhout (1998) and de Hoog & Smith (1998a, b) indicated that *Trichosporonoides* was probably synonymous with *Moniliella*. Later Rosa et al. (2008) confirmed that both genera were indeed congeneric based on 26S rRNA D1/D2 domain sequence analysis and, consequently, transferred all species into the genus *Moniliella*. These authors were, however, not sure about the phylogenetic relationships with either *Ustilaginomycotina* or *Agaricomycotina*.

In order to clarify the phylogenetic affiliations of *Malassezia* and *Moniliella*, we performed phylogenetic analyses based on the six genes that were used to address the higher-level phylogeny of the *Fungi* (James et al. 2006, Hibbett et al. 2007). Our results demonstrated that *Malassezia* and *Moniliella* belong to *Ustilaginomycotina* where they form deep and well-supported lineages with a sister relationship to both *Ustilaginomycetes* and *Exobasidiomycetes*. We therefore propose two new classes, *Malasseziomycetes* and *Moniliellomycetes* for these lineages, with additional support from phenotypic characters.

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Table 1 Taxa sampled and sequence accession numbers employed (those in **bold** are determined in this study).

Species	Strain	D1D2	ITS	SSU	*RPB1*	*RPB2*	*EF1-α*
Ustilaginomycetes							
Cintractia limitata	HAJB 10488	DQ645506	DQ645508	DQ645507	DQ645510	DQ645509	DQ645511
Melanotaenium endogenum	AFTOL ID1918	DQ789979	DQ789981	DQ789980	–	–	–
Melanotaenium euphorbiae	voucher HUV17733	JN367314	JN367289	JN367342	–	–	JN367365
Rhodotorula acheniorum	AS 2.3198^T	AF190001	AB038128	AJ496256	**KF706499**	**KF706522**	**KF706474**
Schizonella melanogramma	CBS 174.42	DQ832210	DQ832212	DQ832211	DQ832214	DQ832213	DQ832215
Sporisorium reilianum	CBS 131460	**KF706430**	**KF706438**	**KF706441**	–	**KF706511**	**KF706472**
Urocystis colchici	AFTOL-ID1647	DQ838576	DQ839596	DQ839595	–	DQ839597	DQ839598
Urocystis eranthidis	voucher hmk292	JN367324	JN367299	JN367352	JN367428	–	JN367375
Ustanciosporium gigantosporum	HRK 023	JN367325	JN367300	JN367353	JN367429	–	JN367376
Ustilago hordei	CBS 131470	**KF706429**	**KF706437**	**KF706442**	**KF706498**	**KF706521**	**KF706473**
Ustilago maydis	CBS 504.76/MS 115	AF453938	AY854090	X62396	XM401478	AY485636	AY885160
Ustilago tritici	CBS 669.70	DQ094784	DQ846894	DQ846895	DQ846897	DQ846896	DQ846898
Exobasidiomycetes							
Exobasidium gracile	DSM 4460	DQ663699	DQ663700	DQ785786	DQ663702	DQ663701	DQ663703
Exobasidium rhododendri	CBS 101457	DQ667151	DQ667153	DQ667152	DQ667155	DQ667154	DQ667156
Jaminaea angkoriensis	C5b	EU587489	EU604147	EU604148	–	–	–
Microstroma juglandis	CBS 287.63	AF009867	DQ789988	DQ789987	DQ789990	DQ789989	DQ789991
Quambalaria cyanescens	CBS 876.73	DQ317616	DQ317623	**KF706440**	–	**KF706531**	**KF706485**
Rhamphospora nymphaeae	CBS 72.38	DQ831032	DQ831034	DQ831033	–	DQ831035	DQ831036
Tilletiaria anomala	CBS 436.72	AJ235284	DQ234558	AY803752	DQ234571	AY803750	DQ835991
	CBS 607.83^T	AJ235282	AB025704	**KF706451**	–	**KF706530**	**KF706483**
Tilletiopsis washingtonensis	CBS 544.50^T	AJ235278	DQ835994	AJ271382	–	DQ835995	DQ835996
Malasseziomycetes							
Malassezia caprae	CBS10434^T	AY743616	AY743656	**KF706456**	**KF706495**	**KF706513**	**KF706467**
Malassezia dermatis	CBS 9169^T	AB070365	AY390284	**KF706452**	**KF706490**	**KF706532**	**KF706461**
Malassezia equina	CBS 9969^T	AY743621	**KF706439**	**KF706454**	**KF706492**	**KF706515**	**KF706463**
Malassezia furfur	CBS 1878^T	AF063214	AY743634	**KF706457**	**KF706497**	**KF706516**	**KF706469**
Malassezia globosa	CBS 7966^T	AF064025	AY387132	–	**KF706493**	**KF706518**	**KF706465**
Malassezia japonica	CBS 9431^T	EF140672	EF140669	**KF706458**	–	**KF706514**	**KF706464**
Malassezia nana	CBS 9558^T	EF140673	EF140667	**KF706453**	**KF706491**	**KF706510**	**KF706462**
Malassezia obtusa	CBS 7876^T	AB105197	AY387137	**KF706455**	–	**KF706519**	**KF706470**
Malassezia pachydermatis	CBS 1879^T	AY743605	AB118941	DQ457640	DQ785792	DQ408140	DQ028594
Malassezia restricta	CBS 7877^T	AF064026	AY743636	EU192367	**KF706496**	**KF706520**	**KF706471**
Malassezia slooffiae	CBS 7956^T	AJ249956	AY743633	**KF706459**	–	–	–
Malassezia sympodialis	CBS 7222^T	AF064024	AY743632	**KF706460**	–	–	–
Malassezia yamatoensis	CBS 9725^T	AB125263	AB125261	–	**KF706494**	**KF706512**	**KF706466**
Moniliellomycetes							
Moniliella acetoabutens	CBS 169.66^T	AF335523	EU252153	**KF706443**	**KF706500**	**KF706523**	**KF706476**
Moniliella madida	CBS 240.79^T	AF335522	–	**KF706447**	**KF706502**	**KF706525**	**KF706478**
Moniliella megachiliensis	CBS 190.92^T	EF137916	**KF706433**	**KF706448**	**KF706501**	**KF706524**	**KF706477**
Moniliella mellis	CBS 350.33^T	EU545185	–	**KF706446**	–	**KF706528**	**KF706481**
Moniliella nigrescens	CBS 269.81^T	AF335527	**KF706436**	–	**KF706504**	**KF706527**	**KF706480**
Moniliella oedocephalis	CBS 649.66^T	AF335521	**KF706435**	**KF706449**	–	–	**KF706484**
Moniliella pollinis	CBS 461.67^T	AF335525	**KF706434**	**KF706450**	**KF706505**	**KF706529**	**KF706482**
Moniliella spathulata	CBS 241.79^T	AF335526	**KF706432**	**KF706444**	**KF706503**	**KF706526**	**KF706479**
Moniliella suaveolens	CBS 126.42^T	AF335520	**KF706431**	**KF706445**	–	–	**KF706475**
Pucciniomycotina							
Bensingtonia ciliata	AS 2.1945^T	AF189887	AF444563	D38233	**KF706509**	**KF706536**	**KF706486**
Chrysomyxa arctostaphyli	CFB22246	AY700192	DQ200930	AY657009	–	DQ408138	DQ435789
Endocronartium harknessii	CFB22250	AY700193	DQ206982	AY665785	–	DQ234551	DQ234567
Erythrobasidium hasegawianum	AS 2.1923^T	AF189899	AF444522	D12803	**KF706506**	**KF706534**	**KF706488**
Naohidea sebacea	CBS 8477	DQ831020	DQ911616	–	**KF706508**	**KF706535**	**KF706487**
Platygloea disciformis	IFO32431	AY629314	DQ234556	DQ234563	–	DQ234554	DQ056288
Puccinia graminis	CRL75-36-700-3/ECS	AF522177	AF468044	AY125409	XM_003334476	XM_003321826	XM_003333024
Sporidiobolus salmonicolor	CBS 490^T	AF070439	AY015434	AB021697	**KF706507**	**KF706533**	**KF706489**
Agaricomycotina							
Auriculibuller fuscus	CBS 9648^T	AF444763	AF444669	KF036604	KF036314	KF036727	KF036999
Bulleromyces albus	CBS 501^T	AF075500	AF444368	X60179	KF036334	KF036745	KF037016
Boletellus projectellus	MB03-118	AY684158	AY789082	AY662660	AY788850	AY787218	AY879116
Dacryopinax spathularia	GEL 5052	AY701525	AY854070	AY771603	AY857981	AY786054	AY881020
Filobasidiella depauperata	CBS 7841^T	AF487884	EF211248	AJ568017	KF036417	KF036885	KF037150
***Wallemia* clade**							
Wallemia ichthyophaga	EXF994/EXF1059	DQ847516	AY302523	AY741382	DQ847522	DQ847519	DQ847525
Wallemia muriae	MZKI-B952/EXF1054	DQ847517	AY302534	AY741381	DQ847523	DQ847520	DQ847526
Wallemia sebi	EXF483	DQ847518	AY328917	AY741379	DQ847524	DQ847521	DQ847527
Ascomycota							
Aleuria aurantia	OSC 100018	AY544654	DQ491495	NG013139	DQ471120	DQ247785	DQ466085
Aspergillus nidulans	NRRL 2395/FGSC4	EF652445	AY373888	U77377	XM_653321	XM_677297	XM_656730
Neurospora crassa	NRRL 13141/ICMP 6360	AY681158	AY681193	AY046271	XM_959004	AF107789	AF402094
Schizosaccharomyces pombe	972H-	CU329672	CU329672	CU329672	D13337	X56564	NC_003421

MATERIALS AND METHODS

Taxon sampling

Almost all the recognised *Malassezia* and *Moniliella* species were employed (Table 1). Reference species representing *Pucciniomycotina*, *Agaricomycotina* and currently recognised classes of *Ustilaginomycotina* were selected based on sequence availability for the six genes used in the AFTOL1 project (http://aftol.org/data.php) (Table 1). Sequence data generated in this study or data retrieved from GenBank were mostly from type strains of the taxa compared.

Table 2 PCR and sequence primers used.

Locus	Primers (5'-3')
RPB1	RPB1-Af: GAR TGY CCD GGD CAY TTY GG RPB1-Cr: CCN GCD ATN TCR TTR TCC ATR TA
RPB2	fRPB2-5F: GAY GAY MGW GAT CAY TTY GG fRPB2-7cR: CCC ATR GCT TGY TTR CCC AT bRPB2-6F: TGG GGY ATG GTN TGY CCY GC gRPB2-6R: GCA GGR CAR ACC AWM CCC CA
EF1-α	EF1-983F: GCY CCY GGH CAY CGT GAY TTY AT EF1-2218R: AT GAC ACC RAC RGC RAC RGT YTG EF1-1577F: CAR GAY GN TAC AAG ATY GGT GG EF1-1567R: AC HGT RCC RAT ACC ACC RAT CTT

PCR and DNA sequencing

Genomic DNA was extracted from the living cultures grown on the Yeast Extract Peptone Dextrose (YPD) plate using the method as described by Bolano et al. (2001). A set of six genes were selected, including three protein-coding genes, namely *RPB1* (the largest subunit of RNA polymerase II), *RPB2* (the second largest subunit of RNA polymerase II) and *EF1-α* (translation elongation factor 1-α), and three rRNA genes namely small subunit (SSU or 18S) ribosomal DNA (rDNA) D1/D2 domains of the large subunit (LSU or 26S) rDNA, and the ITS 1+2 regions (including 5.8S rDNA) of the rDNA. PCR and sequencing of the rRNA genes were performed using the methods described previously (Fell et al. 2000, Scorzetti et al. 2002, Wang et al. 2003). PCR and sequencing primers for *RPB1*, *RPB2* and *EF1-α* are listed in Table 2. PCR parameters for amplifying *RPB1* and *RPB2* were as follows: an initial denaturation step at 94 °C for 4 min; 36 cycles of denaturation at 94 °C for 1 min, annealing at 50–52 °C for 1 min and extension at 72 °C for 1 min; and a final extension step of 8 min at 72 °C. Amplification of the *EF1-α* gene used a touchdown PCR: an initial denaturation at 94 °C for 4 min; an annealing temperature of 62 °C in the first cycle, successively reducing the Tm by 1 °C per cycle over the next nine cycles to reach a final Tm of 52 °C, which is used in the remaining 30 cycles and extension at 72 °C for 1 min; and a final extension step of 8 min at 72 °C. Cycle sequencing was performed using the ABI BigDye cycle sequencing kit (QIAGEN, Valencia, California). Electrophoresis was done on an ABI PRISM 3710 or 3730 DNA sequencer.

Molecular phylogenetic analyses

Sequences were aligned with the Clustal X program (Thompson et al. 1997). The alignment datasets were firstly analysed with Modeltest v. 3.04 (Posada & Crandall 1998) using the Akaike information criterion (AIC) to find the most appropriate model of DNA substitution. A general time-reversible model of DNA substitution additionally assuming a percentage of invariable sites and *Γ*-distributed substitution rates at the remaining sites (GTR+I+G) was selected for further analyses. Maximum likelihood (ML) analyses were conducted in MEGA v. 5 (Tamura et al. 2011). Maximum parsimony (MP) analysis was conducted in PAUP v. 4.0b10 (Swofford 2002) where the support of the branching topologies was derived from 1 000 replicates with 10 random additions. Three to four ascomycetous species were used as outgroups. Bayesian posterior probability analyses were conducted in MrBayes v. 3.2 (Ronquist et al. 2012) with parameters set to 1 000 000 generations, four runs and four chains. The chains were heated to 0.25 and a stop value of 0.01 was used. The alignment matrix was deposited in TreeBASE (www.treebase.org) with submission ID 14907.

RESULTS

A total of 108 new sequences were generated from 31 species in this study (Table 1). New sequences and those retrieved from GenBank generated from the same species were concatenated in different combinations to form three separate datasets as follows: 1) a rRNA gene dataset formed by SSU, LSU D1/D2 and ITS (including 5.8S rDNA); 2) a protein gene dataset consisted of *RPB1*, *RPB2* and *EF1-α*; and 3) a six-gene dataset formed by the combination of the former two datasets as used in James et al. (2006). All the datasets were subjected to ML, MP and Bayesian analyses respectively and the topologies of the trees obtained were visually examined for phylogenetic concordance.

In the majority of the trees, four distinct monophyletic clades, namely *Exobasidiomycetes*, *Malassezia*, *Moniliella* and *Ustilaginomycetes*, were consistently resolved within *Ustilaginomycotina* (Table 3, Fig. 1, 2). The *Malassezia*, *Moniliella* and *Ustilaginomycetes* clades each received 1.0 post probability and 99–100 % bootstrap support in the trees constructed using different algorithms based on the six-gene dataset. These three clades were also clearly resolved and strongly supported (1.0 post probability and 92–100 % bootstrap values) in the trees drawn from the rRNA gene and the protein gene datasets, except for the *Ustilaginomycetes* clade which received weak (51–65 %) bootstrap support in the ML and MP trees drawn from the rRNA gene dataset (Table 3, Fig. 2). The *Exobasidiomycetes* was resolved as a monophyletic clade in the trees constructed from the rRNA gene and the six-gene datasets, but only received strong support in the Bayesian and ML trees drawn from the rRNA gene dataset. This clade was shown to be non-monophyletic in all the trees drawn from the protein gene dataset (Table 3, Fig. 1, 2). In the Bayesian and ML trees (Fig. 2d, e), *Tilletiopsis fulvescens* and *Tilletiaria anomala* formed a

Table 3 Statistical support values for the major clades resolved in *Ustilaginomycotina*.

Clade	Three rRNA genes			Three protein genes			Combined six genes		
	PP	MLBP	MPBP	PP	MLBP	MPBP	PP	MLBP	MPBP
Exobasidiomycetes	1.0	76	< 50	nm	nm	nm	1.0	51	< 50
Malassezia	1.0	100	100	1.0	100	100	1.0	100	100
Moniliella	1.0	100	100	1.0	100	100	1.0	100	100
Ustilaginomycetes	1.0	51	65	1.0	92	98	1.0	99	99

MLBP = bootstrap percentage from maximum likelihood analysis.
MPBP = bootstrap percentage from maximum parsimony analysis.
PP = posterior probability from Bayesian analysis.
nm = not monophyletic.

separate branch, while the remaining taxa of the *Exobasidiomycetes* formed an unsupported (0.6 post probability) group in the Bayesian tree (Fig. 2d) and two separate groups in the ML tree (Fig. 2e). In the MP tree (Fig. 2f), two *Exobasidium* species formed a separate branch from the other *Exobasidiomycetes* taxa, which in turn formed an unsupported group.

The phylogenetic relationships among the *Exobasidiomycetes*, *Malassezia*, *Moniliella* and *Ustilaginomycetes* clades were clearly resolved by Bayesian analysis of the six-gene dataset, showing that the *Exobasidiomycetes* clade was located basal to the other three clades; *Malassezia* and *Moniliella* were sister clades and *Ustilaginomycetes* was basal to them. Their relationships were all strongly supported by 1.0 post probability (Fig. 1, 2g). However, the relationships among the four clades within *Ustilaginomycotina* were largely unresolved in the other trees (Fig. 2).

DISCUSSION

Basidiomycetous species in the subphylum *Ustilaginomycotina* are usually dimorphic, producing a saprobic haploid yeast phase and a parasitic dikaryotic hyphal phase. A considerable number of cultivable ustilaginomycetous fungi are only known by asexual yeasts and yeast-like microbes and are classified mainly based on physiological, biochemical and molecular criteria commonly used for yeasts, forming a taxonomic system hitherto independent from that of filamentous fungi (Boekhout 1991, Boekhout et al. 2011). Molecular methods recently showed the affiliation of these yeasts with various filamentous smuts and merged the two groups into a unified taxonomic system (Bauer et al. 2001, Begerow et al. 2000, 2006, Weiß et al. 2004, Matheny et al. 2006, Boekhout et al. 2011). However, the fine phylogenetic positions of the yeast and yeast-like groups have been a matter of debate. Here we show that the *Malassezia* and *Moniliella* clades present two deeply rooted lineages within the smut fungi (*Ustilaginomycotina*, *Basidiomycota*, *Fungi*). They also possess unique phenotypic (morphological, ultrastructural, physiological and biochemical) characters distinct from those of *Ustilaginomycetes* and *Exobasidiomycetes*.

The genus *Moniliella* was originally classified in the order *Moniliales* of fungi imperfecti (Stolk & Dakin 1966). A sexual morph has not been observed (de Hoog 1979a) and all members of the genus have greyish to olivaceous black colonies, yeast-like growth, hyphae that disarticulate in arthroconidia (Martinez et al. 1979, de Hoog 1979b), and CoQ-9 as the major ubiquinone (de Hoog et al. 2011). All species, except *M. fonsecae*, can ferment glucose and some species also galactose, sucrose and/or raffinose (Martínez et al. 1979, de Hoog et al. 2011), which among *Basidiomycota* is a rare trait. Some species are considered xerophilic (Hocking & Pitt 1981, de Hoog et al. 2011)

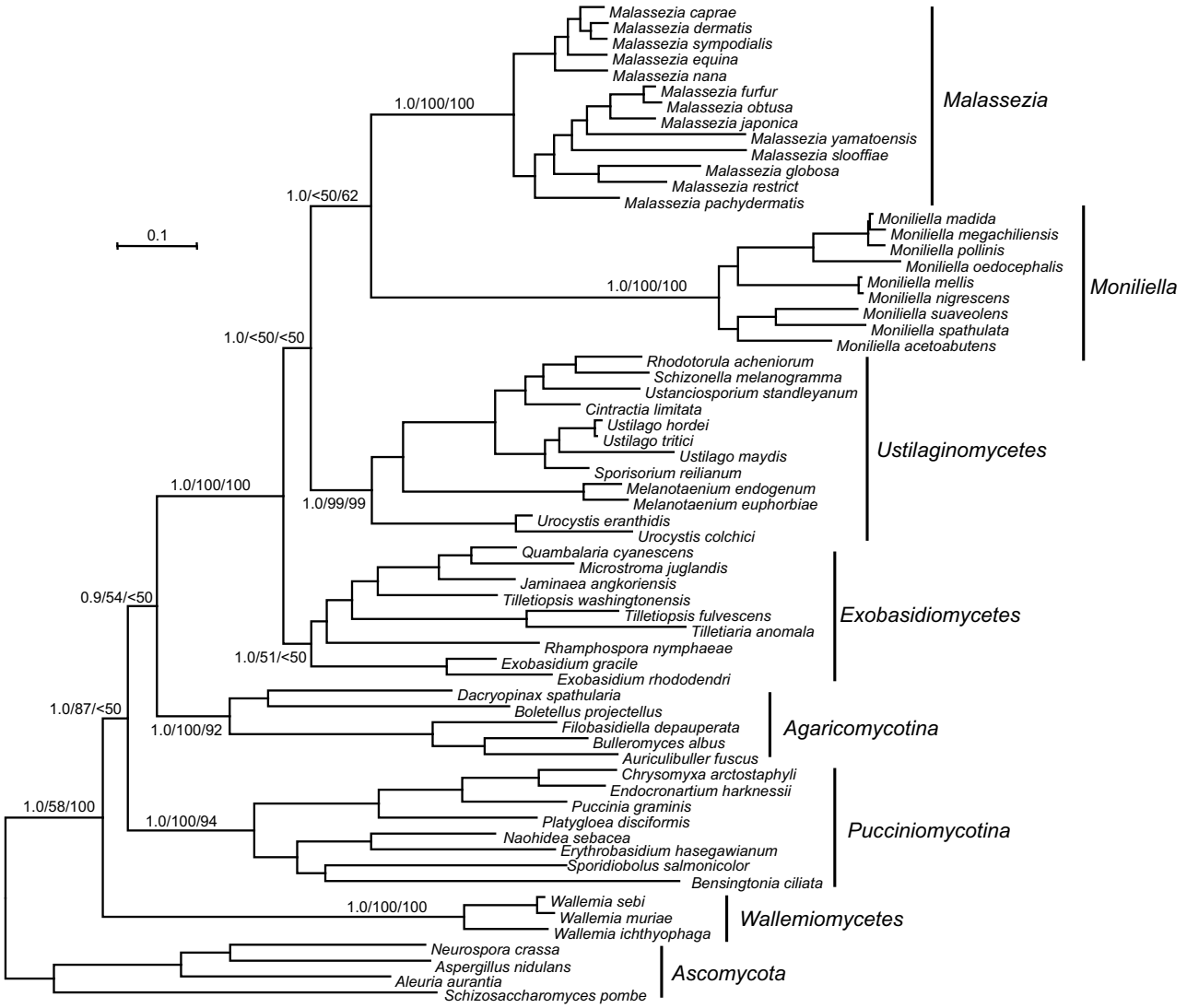

Fig. 1 Phylogenetic tree constructed using Bayesian analysis of the combined sequences of 18S rDNA, 26S rDNA D1/D2 domains, ITS regions (including 5.8S rDNA), *RPB1*, *RPB2* and *EF1-α* depicting the phylogenetic placements of genera *Moniliella* and *Malassezia* within the *Ustilaginomycotina*. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site. Bayesian posterior probabilities and bootstrap percentages from 1 000 replicates of maximum likelihood and maximum parsimony analyses are shown respectively from left to right on the deep and major branches resolved.

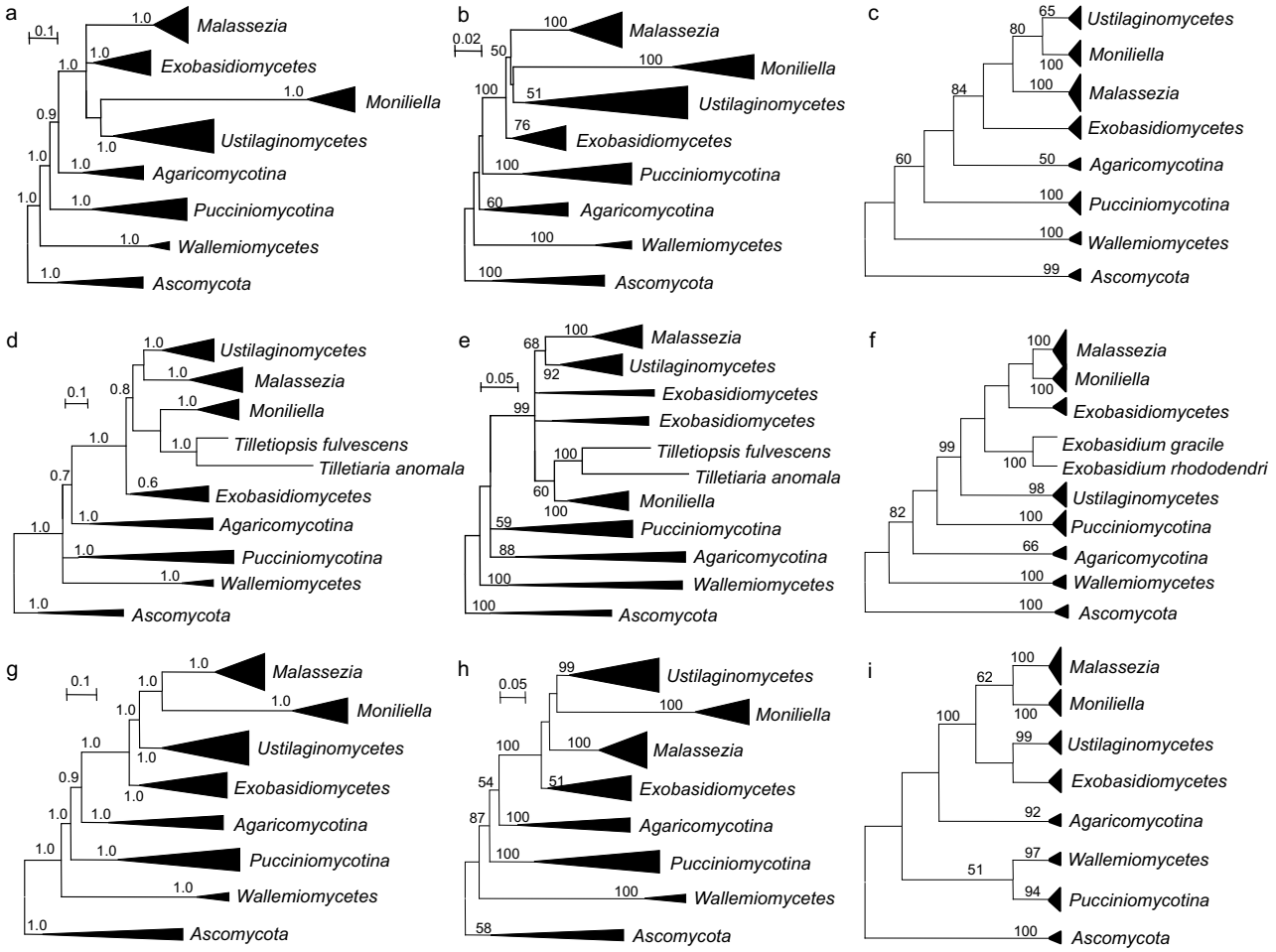

Fig. 2 Phylogenetic trees based on datasets of rRNA genes including 18S, D1/D2 domains of 26S and ITS-5.8S (a, b and c); protein genes including *RPB1*, *RPB2* and *EF1-α* (d, e and f) and the combined six genes (g, h and i) using Bayesian (a, d and g), maximum likelihood (b, e and h) and maximum parsimony (c, f and i) analyses, showing the major clades resolved within the *Ustilaginomycotina*. Bayesian posterior probabilities above 0.7 or bootstrap percentages over 50 % from 1 000 replicates are shown.

and most are known from industrial settings, food stuffs, fats, oils and acids or substrates with low water activity or were isolated from flowers in tropical forest ecosystems (de Hoog et al. 2011). Thus their origin and ecology also differs from the vast majority of *Ustilaginomycotina* that typically are plant pathogens (Begerow et al. 2006). Glucose, galactose and mannose (low) were found to be present in the cell walls of *Moniliella* species, whereas xylose and fucose were absent (Weijman 1979), which led this author to conclude that the genus may not be related to the *Agaricomycotina*. *Ustilaginomycotina*, as analysed thus far, have glucose as the dominant cell wall sugar, with some galactose and mannose, but without xylose (Dörfler 1990, Boekhout et al. 1992, Bauer et al. 1997, 2001, Prillinger et al. 2011, van der Klei et al. 2011), thus agreeing with the cell wall composition as known from *Moniliella* species.

The septal ultrastructure of the *Moniliella* species as investigated so far revealed a rather complex pattern with dolipores in *M. suaveolens*, micro-pore-like structures in *M. spathulata* and both types occurring in *M. oedocephala*. The dolipores resemble those of *Agaricomycetes* but with an arch of endoplasmic reticulum instead of parenthesomes (Haskins 1975, Martinez 1979, Bauer et al. 2006, van Driel et al. 2009). These differences in pore structures may be growth stage dependent, and differ between yeast-like, hyphal and arthroconidial stages, but they may also be caused by the fixation protocols used. The dolipore structure is quite different from the simple pore with membrane caps observed in the *Ustilaginomycotina* and the simple pore without membrane apparatus typical for the *Pucciniomycotina* (Boekhout et al. 1992, Bauer et al. 1997, 2001, 2006, van der Klei et al. 2011). The biochemical and ultrastructural characters made the taxonomic and phylogenetic positions of *Moniliella* elusive for a long time. However, our results clearly show that the *Moniliella* species belong to the *Ustilaginomycotina* with strong support (Fig. 1, 2).

Our analyses showed that the species of *Malassezia* formed a highly supported deep lineage in the *Ustilaginomycotina* that we rank at the class level. This is further sustained by the unique monopolar budding, the thick helicoidal cell wall, the lipid dependency of most *Malassezia* species and the lipophily of *M. pachydermatis* (Guého-Kellermann et al. 2011). *Malassezia* species occur commonly on human and animal skin, but metagenomic data also revealed the presence of the species in some unexpected habits, such as forest soil, nematodes or corals (Guého-Kellermann et al. 2010, Gaitanis et al. 2012). The genome comparison between *Ustilago maydis* and *M. globosa* revealed some major differences in the enzyme armamentarium used by human inhabiting *Malassezia* yeasts that differs from that of the plant pathogen *U. maydis* (Xu et al. 2007, Sun et al. 2013).

Our analyses of the three rRNA genes and the combined six genes indicated that *Exobasidiomycetes* seem to be monophyletic when the *Malassezia* clade is excluded, but it remains polyphyletic in the analysis of the three protein-coding genes. Begerow et al. (1997, 2000) and Bauer et al. (2001) showed that the *Exobasidiomycetes* were weakly supported or with 56–85 % bootstrap support values based on the LSU rDNA

analysis. Begerow et al. (2006) indicated that *Exobasidiomycetes* were paraphyletic using a combined analysis of SSU, D1/D2, ITS, atp6 and β-tubulin sequences, but this lineage was found to be monophyletic when the SSU data were excluded, but with weak bootstrap support. Matheny et al. (2006) indicated that the taxa within *Exobasidiomycetes* except the *Malassezia* clustered together with or without statistical support based on the different gene combination datasets. Thus, for a better understanding of the phylogeny of *Exobasidiomycetes* further analyses using more molecular data or genomic data are needed.

In addition to the Clustal X, we also used the MAFFT program (Katoh & Standley 2013) to align the sequences and the Gblocks program (Talavera & Castresana 2007) to remove ambiguously aligned blocks from the alignments. The alignments produced by the MAFFT and those treated by the Gblocks were all subjected to Bayesian, ML and MP analyses. The consensus was that the *Malassezia*, *Moniliella* and *Ustilaginomycetes* taxa were respectively resolved as strongly supported monophyletic clades in all the trees obtained, while the *Exobasidiomycetes* was monophyletic without statistic support in the trees drawn from the six-gene dataset but polyphyletic in the trees drawn from the rRNA gene and the protein gene datasets (data not shown). The polyphyletic nature of the *Exobasidiomycetes* was magnified by using the new programs, implying that this group may not represent a single class.

In conclusion, multiple gene phylogenetic analyses and phenotypic comparisons suggest that the species of *Malassezia* and *Moniliella* form two independent deep lineages representing sister groups to the recognised classes *Ustilaginomycetes* and *Exobasidiomycetes* within *Ustilaginomycotina*. Therefore, we propose two new classes to accommodate these fungi.

TAXONOMY

Moniliellomycetes Q.M. Wang, F.Y. Bai & Boekhout, *class. nov.* — MycoBank MB805229

Type order. *Moniliellales.*

Etymology. The nomenclature of the class is derived from the generic name of *Moniliella* Stolk & Dakin, Antonie van Leeuwenhoek 32: 399. 1966.

Member of *Ustilaginomycotina*. *Sexual morph* unknown. *Colonies* are smooth or velvety, greyish to olivaceous black. *Budding cells* are ellipsoidal and form terminally on true hyphae that disarticulate with artroconidia. *Pseudohyphae* and *chlamydospores* may be present. *Cell walls* are multi-lamellar. *Hyphal septa* typically possess dolipores with an arch of endoplasmic reticulum, but 'micropore'-like structures may also be present. Sugars are fermented by most species. Nitrate is assimilated. Urease and diazonium blue B (DBB) reactions are positive. Coenzyme Q-9 is present. Xylose and fucose are absent from whole-cell hydrolysates.

Moniliellales Q.M. Wang, F.Y. Bai & Boekhout, *ord. nov.* — MycoBank MB805230

Type family. *Moniliellaceae.*

The diagnosis of the order *Moniliellales* is based on the description of the class *Moniliellomycetes*. The nomenclature of the order is based on the genus *Moniliella* Stolk & Dakin, Antonie van Leeuwenhoek 32: 399. 1966.

Moniliellaceae Q.M. Wang, F.Y. Bai & Boekhout, *fam. nov*. — MycoBank MB805231

Type genus. *Moniliella* Stolk & Dakin, Antonie van Leeuwenhoek 32: 399. 1966.

The diagnosis of the family *Moniliellaceae* is based on the description of the order *Moniliellales*. The nomenclature of the family is based on the genus *Moniliella* Stolk & Dakin, Antonie van Leeuwenhoek 32: 399. 1966.

Malasseziomycetes Boekhout, Q.M. Wang & F.Y. Bai, *class. nov*. — MycoBank MB805514

Type order. *Malasseziales* R.T. Moore with family *Malasseziaceae* Denchev & R.T. Moore, Mycotaxon 110: 379. 2009, and genus *Malassezia* Baillon (1889).

Etymology. The nomenclature of the class is derived from the order name of *Malasseziales* R.T. Moore, Bot. Mar. 23: 371. 1980, emend. Begerow et al., Mycol. Res. 104: 59. 2000.

Member of *Ustilaginomycotina*. *Sexual morph* unknown. *Cells* are globose, ovoid or cylindrical. *Budding* is typically monopolar on a more or less broad base, enteroblastic and percurrent. The cell wall is multi-lamellate, and the inner layer of the cell wall is corrugated with a groove spiralling from the bud site. The species are lipid dependent or lipophilic. Sugars are not fermented. Urease and diazonium blue B (DBB) reactions are positive. Coenzyme Q-9 is formed. Xylose is absent in whole-cell hydrolysates.

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