

# Phylogenetic placements of ustilaginomycetous anamorphs as deduced from nuclear LSU rDNA sequences\*

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In order to integrate ustilaginomycetous anamorphs into the general phylogenetic system of Ustilaginomycetes, partial nuclear large subunit ribosomal DNA sequences of 56 teleomorphic and 19 anamorphic species of the Ustilaginomycetes were analysed. Maximum parsimony and neighbour joining confirm the new suprageneric system of Ustilaginomycetes and indicate that (i) the species of *Pseudozyma* represent anamorphs of Ustilaginales parasitizing grasses, (ii) *Pseudozyma prolifica*, the type of *Pseudozyma*, is very closely related to *Ustilago maydis*, (iii) *Pseudozyma tsukubaensis* is probably synonymous with *Ustilago spermophora*, (iv) the species of *Malassezia* represent a group of its own within the Exobasidiomycetidae, (v) *Tilletiopsis cremea*, *T. lilacina* and *T. washingtonensis* belong to the Entylomatales and (vi) *T. flava*, *T. fulvescens* and *T. minor* are members of the Georgefischeriales. Like all *Tilletiopsis* species tested, *T. albescens* and *T. pallescens* are members of the Exobasidiomycetidae, but they cannot be ascribed to any of the known orders of this subclass. The description of the Malasseziales is emended.

Many basidiomycetes lack either a perfect state or it is currently unknown. Because the characters used for classifying basidiomycetes are predominantly derived from the process of sexual reproduction, these anamorphic fungi are segregated into artificial deuteromycetous taxa. In modern phylogenetic considerations, however, both teleomorphic and anamorphic fungi should be integrated where possible. Comparative morphological, ultrastructural, physiological, biochemical and molecular studies are useful for such an undertaking (e.g. Swann & Taylor, 1995).

This study was made to examine the phylogenetic relationships among a diversity of ustilaginomycetous species including both teleomorphic and anamorphic species. Partial nuclear large subunit rDNA sequences of 56 teleomorphic species of the Ustilaginomycetes were analysed together with sequences of seven species of *Pseudozyma* (Boekhout, 1995), eight species of *Tilletiopsis* (Boekhout, Fell & O'Donnell, 1995) and four species of *Malassezia* (Guillot & Guého, 1995). It is well known that the species of *Pseudozyma* and *Tilletiopsis* represent anamorphs of the Ustilaginomycetes. For example, species of *Ustilago* from morphologically similar colonies in the haploid state as the species of *Pseudozyma*, whereas species of *Entyloma*, for example, develop *Tilletiopsis*-like cultures in the haploid state (Boekhout, 1987, 1991, 1995). Yeast cells of species of *Pseudozyma* and *Tilletiopsis* are ellipsoidal, ovoidal to cylindrical and usually have polar budding. Their hyphae are

narrow and lack clamp connections. The species of *Pseudozyma* differ predominantly from those of *Tilletiopsis* by the lack of ballistoconidia. *Malassezia* comprises lipophilic yeasts morphologically characterized by small cells with unipolar, enteroblastic, and repetitive budding. In *Malassezia sympodialis*, however, proliferation may be sympodial (Yarrow & Ahearn, 1984; Guého, Midgley & Guillot, 1996). Fell, Boekhout & Freshwater (1995) and Guillot, Guého & Prévost (1995) indicated the ustilaginomycetous nature of *Malassezia* using ultrastructural and molecular characteristics.

Both maximum parsimony and neighbour joining algorithms were used to determine the phylogenetic positions of the test species within the new suprageneric system of the Ustilaginomycetes, consisting of three subclasses with ten orders. This system is based predominantly on ultrastructural analysis (Bauer, Oberwinkler & Vánky, 1997) and is well supported by molecular sequence data (Begerow, Bauer & Oberwinkler, 1997).

## MATERIALS AND METHODS

The analysed species are listed in Table 1. DNA was isolated from cultures or herbarium specimens using the SDS method as described previously (Begerow *et al.*, 1997). The 5' region of the nuclear large subunit of the ribosomal RNA gene was amplified using the polymerase chain reaction and the primers NL1 and NL4 (O'Donnell, 1993). The PCR product was purified using the QIAquick™ protocol (QIAGEN). This dsDNA was sequenced directly using the ABI PRISM™ Dye-

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Table 1. Species studied<sup>1</sup>

	Host substrate	Accession no. <sup>2</sup>	Collection no. <sup>3</sup>
1. Entorrhizomycetidae			
<i>Entorrhiza aschersoniana</i> (Magnus) Lagerh. <sup>B</sup>	<i>Juncus bufonius</i> L.	AF 009851	H.U.V. 15899
<i>E. casparyana</i> (Magnus) Lagerh. <sup>B</sup>	<i>J. articulatus</i> L.	AF 009852	H.U.V. 17623
2. Ustilaginomycetidae			
<i>Cintractia axicola</i> (Berk.) Cornu <sup>B</sup>	<i>Fimbristylis tetragona</i> R. Br.	AF 009847	H.U.V. 17460
<i>Farysia chardoniana</i> Zundel <sup>B</sup>	<i>Carex polystachya</i> Swartz ex Wahlenb.	AF 009859	M.P. 2062
<i>Heterotolyposporium piluliforme</i> (Berk.) Vánky <sup>B</sup>	<i>Juncus planifolius</i> R. Br.	AF 009871	H.U.V. 15732
<i>Melanopsichium pennsylvanicum</i> Hirschh. <sup>B</sup>	<i>Polygonum hispidum</i> H.B.K.	AF 009863	M.P. 801
<i>Melanotaenium endogenum</i> (Unger) de Bary <sup>BO</sup>	<i>Galium mollugo</i> L.	AJ 235294	CBS 481.91
<i>M. euphorbiae</i> (L. W. Lenz) M. D. Whitehead & Thirum. <sup>B</sup>	<i>Euphorbia geniculata</i> (Kl. & Garke) Ortega	AF 009865	H.U.V. 17733
<i>Moesziomyces bullatus</i> (J. Schröt.) Vánky <sup>B</sup>	<i>Paspalum distichum</i> L.	AF 009868	H.U.V. 15514
<i>Mundkurella kalopanaxis</i> Vánky <sup>B</sup>	<i>Kalopanax pictus</i> (Thumb.) Nakai	AF 009869	H.U.V. 16732
<i>Pseudozyma antarctica</i> (Goto, Sugiy. & Iizuka) Boekhout <sup>BO</sup>	Unpolished rice	AJ 235301	CBS 516.83
<i>P. aphidis</i> (W. Henninger & Windisch) Boekhout <sup>BO</sup>	Aphid secretion	AJ 235303	CBS 517.83
<i>P. flocculosa</i> (Traq., L. A. Shaw & Jarvis) Boekhout & Traq. <sup>BO</sup>	Clover infected with mildew	AJ 235299	CBS 167.88
<i>P. fusiformata</i> (Buhagiar) Boekhout <sup>BO</sup>	Cauliflower	AJ 235304	CBS 6951
<i>P. prolifica</i> Bandoni <sup>BO</sup>	Litter of <i>Scirpus microcarpus</i> Presl	AJ 235298	CBS 319.87
<i>P. rugulosa</i> (Traq., L. A. Shaw & Jarvis) Boekhout & Traq. <sup>BO</sup>	Maize infected with moulds	AJ 235300	CBS 170.88
<i>P. tsukubaensis</i> (Onishi) Boekhout <sup>BO</sup>	Flower	AJ 235297	CBS 6389
<i>Schizonella melanogramma</i> (DC.) J. Schröt. <sup>B</sup>	<i>Carex pilulifera</i> L.	AF 009870	F.O. 37174
<i>Sorosporium saponariae</i> Rudolphi*	<i>Silene alba</i> (Mill.) E. H. Krause	AF 133579	H.U.V. 12677
<i>Sporisorium culmiperdium</i> (J. Schröt.) Vánky*	<i>Andropogon gerardii</i> Vitm.	AF 133580	M.P. 2060
<i>S. sorghi</i> Ehrenb. ex Link <sup>B</sup>	<i>Sorghum bicolor</i> (L.) Moench	AF 009872	M.P. 2036a
<i>Thecaphora amarantii</i> (Hirschh.) Vánky <sup>B</sup>	<i>Amaranthus hybridus</i> L.	AF 009873	H.U.V. 15882
<i>T. seminis-convolvuli</i> (Desm.) S. Ito <sup>B</sup>	<i>Convolvulus arvensis</i> L.	AF 009874	G.D. 1391
<i>Tolyposporium junci</i> (J. Schröt.) Woronin ex J. Schröt. <sup>B</sup>	<i>Juncus bufonius</i> L.	AF 009876	H.U.V. 17169
<i>Trichocintractia utricularicola</i> (Henn.) M. Piepenbr. <sup>B</sup>	<i>Rhynchospora corymbosa</i> (L.) Britton	AF 009877	M.P. 2075
<i>Urocystis colchici</i> (Schldtl.) Rabenh. <sup>B</sup>	<i>Colchicum autumnale</i> L.	AF 009878	R.B. 2041
<i>U. ranunculi</i> (Lib.) Moesz <sup>B</sup>	<i>Ranunculus repens</i> L.	AF 009879	R.B. 609
<i>Ustacystis waldsteiniae</i> (Peck) Zundel <sup>B</sup>	<i>Waldsteinia geoides</i> Wild.	AF 009880	F.O. 38439
<i>Ustilago affinis</i> Ellis & Everh.*	<i>Stenotaphrum secundatum</i> (Walt.) Kuntze	AF 133581	M.P. 692
<i>U. avenae</i> (Pers.) Rostr.*	<i>Arrhenatherum elatius</i> (L.) J. & C. Presl	AJ 236140	R.B. 2043
<i>U. hordei</i> (Pers.) Lagerh. <sup>B,B</sup>	Host not cited	L 20286	D.M. 11.2C
<i>U. maydis</i> (DC.) Corda <sup>B,B</sup>	Host not cited	L 20287	R.H. 521
<i>U. nuda</i> (C. N. Jensen) Kellerm. & Swingle*	<i>Hordeum leporinum</i> Link	AJ 236139	H.U.V. 17782
<i>U. scitaminea</i> Syd.*	<i>Saccharum</i> sp. cult.	AJ 236138	M.P. 541
<i>U. spermophora</i> Berk. & M. A. Curtis ex. de Toni*	<i>Eragrostis ferruginea</i> Beauv.	AF 133585	H.U.V. 13634
<i>U. trichophora</i> (Link) Körn.*	<i>Echinochloa colonum</i> (L.) Link	AJ 236141	M.P. 1898
3. Exobasidiomycetidae			
<i>Conidiosporomyces ayresii</i> (Berk.) Vánky <sup>BO</sup>	<i>Paenicum</i> sp.	AJ 235308	CBS 482.41
<i>Doassansia epilobii</i> Farl. <sup>B</sup>	<i>Epilobium montanum</i> L.	AF 007523	F.O. 38252
<i>D. hydrophilae</i> Thirum. <sup>B</sup>	<i>Hydrophila spinosa</i> T. Anders	AF 007524	H.U.V. 15474
<i>Doassansiopsis deformans</i> (Setch.) Dietel <sup>B</sup>	<i>Sagittaria lanceolata</i> L.	AF 009849	M.P. 2066
<i>D. limnocharidis</i> (Cif.) Vánky <sup>B</sup>	<i>Limnocharis flava</i> (L.) Buchenau	AF 009850	H.U.V. 15198
<i>Doassinga callitrichis</i> (Liro) Vánky, R. Bauer & Begerow <sup>B</sup>	<i>Callitriche stagnalis</i> Scop.	AF 007525	R.B. 1079
<i>Entyloma calendulae</i> (Oudem.) de Bary <sup>BO</sup>	<i>Calendula officinalis</i> L.	AJ 235296	CBS 746.85
<i>E. dactylidis</i> (Pass.) Cif. <sup>B</sup>	<i>Agrostis stolonifera</i> L.	AF 009853	R.B. 915
<i>E. ficariae</i> A. A. Fisch. Waldh. <sup>BO</sup>	<i>Ficaria verna</i> Huds.	AJ 235295	CBS 480.91
<i>E. gaillardianum</i> Vánky <sup>V</sup>	<i>Gaillardia aristata</i> Pursh.	AF 133575	R.B. 2055
<i>E. holwayi</i> Syd. & P. Syd. <sup>B</sup>	<i>Cosmos caudatus</i> H.B.K.	AF 009854	M.P. 1769
<i>E. microsporum</i> (Unger) J. Schröt. <sup>B</sup>	<i>Ranunculus repens</i> L.	AF 007530	F.O. 37329
<i>E. polysporum</i> (Peck) Farl. <sup>B</sup>	<i>Ambrosia artemisiifolia</i> L.	AF 007529	H.U.V. 2960
<i>Erratomyces patelii</i> (Pavgi & Thirum.) M. Piepenbr. & R. Bauer <sup>B</sup>	<i>Phaseolus vulgaris</i> L.	AF 009855	M.P. 1991
R. Bauer <sup>B</sup>			
<i>Exobasidium rhododendri</i> (Fuckel) C. E. Cramer <sup>B</sup>	<i>Rhododendron ferrugineum</i> L.	AF 009856	R.B. 2050
<i>E. rostrupii</i> Nannf. <sup>B</sup>	<i>Vaccinium oxycoccus</i> L.	AF 009857	R.B. 949
<i>E. vaccinii</i> (Fuckel) Woronin <sup>B</sup>	<i>Vaccinium vitis-idaea</i> L.	AF 009858	R.B. 945
<i>Georgefischeria riveae</i> Thirum. & Naras. <sup>B</sup>	<i>Rivea hypocrateriformis</i> Chois	AF 009861	H.U.V. 15614
<i>Graphiola phoenicis</i> (Moug.) Poit. <sup>B</sup>	<i>Phoenix canariensis</i> Chaub.	AF 009862	F.O. 29350
<i>Ingoldiomyces hyalosporus</i> (Masse) Vánky*	<i>Nassella mexicana</i> (Hitchc.) Pohl	AF 133576	H.U.V. 16038
<i>Malassezia furfur</i> (C. P. Robin) Baill. <sup>G</sup>	Human pityriasis capitis	not submitted	CBS 1878
<i>M. pachydermatis</i> (Weidman) C. H. Dodge <sup>G</sup>	Dog, skin lesion	not submitted	J.G. 1
<i>Malassezia</i> sp.*	Human skin	AF 133577	H.K. 174
<i>M. sympodialis</i> R. B. Simmons & E. Guého <sup>G</sup>	Human normal skin	not submitted	CBS 7222
<i>Melanotaenium brachiariae</i> Viegas <sup>B</sup>	<i>Brachiaria distachya</i> (L.) Stapf	AF 009864	H.U.V. 17510
<i>Microstroma juglandis</i> (Berenger) Sacc. <sup>B</sup>	<i>Juglans regia</i> L.	AF 009867	F.O. 39211
<i>Nannfeldtiomyces sparganii</i> (Lagerh.) Vánky <sup>V</sup>	<i>Sparganium ramosum</i> Huds.	AF 133578	BBA 68271

Table 1. (cont.)

	Host substrate	Accession no. <sup>2</sup>	Collection no. <sup>3</sup>
<i>Rhizophora nymphaeae</i> D. D. Cunn. <sup>B</sup>	<i>Nymphaea alba</i> L.	AF 007526	R.B. 862
<i>Tilletia caries</i> (DC.) Tul. <sup>BO</sup>	<i>Triticum aestivum</i> L.	AJ 235308	CBS 160.85
<i>Tilletiaria anomala</i> Bandon & B. N. Johri <sup>BO</sup>	Decaying wood	AJ 235284	CBS 436.72
<i>Tilletiopsis albescens</i> Gokhale <sup>BO</sup>	Sewage	AJ 235289	CBS 608.83
<i>T. cremea</i> Tubaki <sup>BO</sup>	Leaves	AJ 235279	CBS 605.83
<i>T. flava</i> (Tubaki) Boekhout <sup>BO</sup>	Leaves of <i>Acer</i>	AJ 235285	CBS 401.84
<i>T. fulvescens</i> Gokhale strain A <sup>BO</sup>	Leaves of <i>Rhus</i>	AJ 235281	NB 244
<i>T. fulvescens</i> strain B <sup>BO</sup>	Leaf of <i>Forsythia</i>	AJ 235282	CBS 607.83
<i>T. lilacina</i> Tubaki <sup>BO</sup>	Leaves	AJ 235309	CBS 435.92
<i>T. minor</i> Nyland <sup>BO</sup>	Leaves	AJ 235286	CBS 346.33
<i>T. pallescens</i> Gokhale <sup>BO</sup>	Powdery mildew	AJ 235291	CBS 438.90
<i>T. washingtonensis</i> Nyland <sup>BO</sup>	Leaves	AJ 235278	CBS 544.50
<i>Tolyposporella brunckii</i> (Ellis & Galloway) G. P. Clinton <sup>B</sup>	<i>Andropogon saccharoides</i> Swartz	AF 009875	H.U.V. 17816

<sup>1</sup> Origin of sequences: B = Begerow, Bauer & Oberwinkler (1997), BE = Berres, Szabo & McLaughlin (1995), BO = Boekhout, Fell & O'Donnell (1995), G = Guillot & Guého (1995), V = Vánky, Bauer & Begerow (1998), \* = new sequences.

<sup>2</sup> Sequences submitted to GenBank.

<sup>3</sup> Abbreviations: BBA = Biologische Bundesanstalt, CBS = Centraalbureau voor Schimmelcultures, D.M. = D. Mills, F.O. = F. Oberwinkler, G.D. = G. Deml, H.K. = Hautklinik of the University of Tübingen, H.U.V. = K. Vánky, J.P. = J. Guillot, M.P. = M. Piepenbring, N.B. = T. Nakase, R.B. = R. Bauer, R.H. = R. Holliday.

Termination Cycle Sequencing Kit (Applied Biosystems) on an automated sequencer (ABI 373A, Applied Biosystems). An alignment of 540 bp was created using MEGALIGN of the Lasergene-package (DNASTAR, Inc. 1997) and reworked by hand. The PHYLIP package, version 3.572 (Felsenstein, 1995), was used to perform the following analyses: neighbour joining of a distance matrix (Kimura 2-parameter model, transition to transversion rate: 2.0) with 1000 bootstrap replicates and maximum parsimony (heuristic) with the jumble option turned on 10 replicates and 100 bootstrap replicates. The sequences are deposited in GenBank (see Table 1).

## RESULTS

### The system

Analyses of neighbour joining and maximum parsimony resulted in similar tree topologies (compare Fig. 1 and Fig. 2). Using the species of *Entorrhiza* as root, the species clustered into three groups. These groups were congruent to the Entorrhizomycetidae, Ustilaginomycetidae and Exobasidiomycetidae as identified by Bauer *et al.* (1997). In addition, the groups recognized by Bauer *et al.* (1997) as orders were also evident. They are the Urocystales and Ustilaginales (Ustilaginomycetidae), and the Entylomatales, Exobasidiales, Doassansiales, Georgefischeriales, Microstromatales and Tilletiales (Exobasidiomycetidae) (Figs 1–2). The two methods of analysis, however, showed some differences concerning the arrangement of the orders of the Exobasidiomycetidae (Figs 1–2).

### *Pseudozyma*

In both neighbour joining and maximum parsimony analyses all the anamorphic yeast species of *Pseudozyma* were located in the clade representing the Ustilaginales (Figs 1–2). In both analyses the Ustilaginales occurring on grasses, *Melano-*

*psichium pennsylvanicum* and *Pseudozyma* species, formed a group that is statistically supported with 77 and 75% respectively (Figs 1 and 2). The sequence of *P. tsukubaensis* was identical to that of *Ustilago spermophora*, and *P. prolifica*, the type species of *Pseudozyma*, appeared next to *U. maydis*.

### *Tilletiopsis*

The species tested of *Tilletiopsis* were scattered among the species representing the Exobasidiomycetidae (Figs 1–2), indicating a high degree of polyphyly for this asexual genus. Both analyses placed *T. cremea*, *T. lilacina* and *T. washingtonensis* on the clade representing the Entylomatales. They appeared as the sister-group of the *Entyloma* species.

*Tilletiopsis minor*, *T. fulvescens* and *T. flava* appeared within the clade representing the Georgefischeriales and the two strains of *T. fulvescens* appeared next to one another, although they differ in 12 bp. Within the Georgefischeriales, there were three clades, representing the Tilletiariaceae, Georgefischeriaceae and the *Entyloma oryzae*-group (Bauer *et al.*, 1997). *T. fulvescens* and *T. flava* were located on the clade representing the Tilletiariaceae. This group is well supported by a high bootstrap value (Figs 1–2).

*T. minor* appeared as sister species of *Melanotaenium brachiariae*, which represents the *Entyloma oryzae*-group in this study. The two methods of analysis conflicted in phylogenetic placement (compare Fig. 1 and Fig. 2) of these two species, but neither of these positions is well supported by high bootstrap values.

In both analyses *T. albescens* and *T. pallescens* were not placed in any of the groups recognised by Bauer *et al.* (1997) as orders (Figs 1 and 2). In addition, there were differences in phylogenetic placements of *T. albescens* and *T. pallescens* between the two algorithms and there was no statistical significance for the positions of these two species illustrated in Figs 1 and 2.

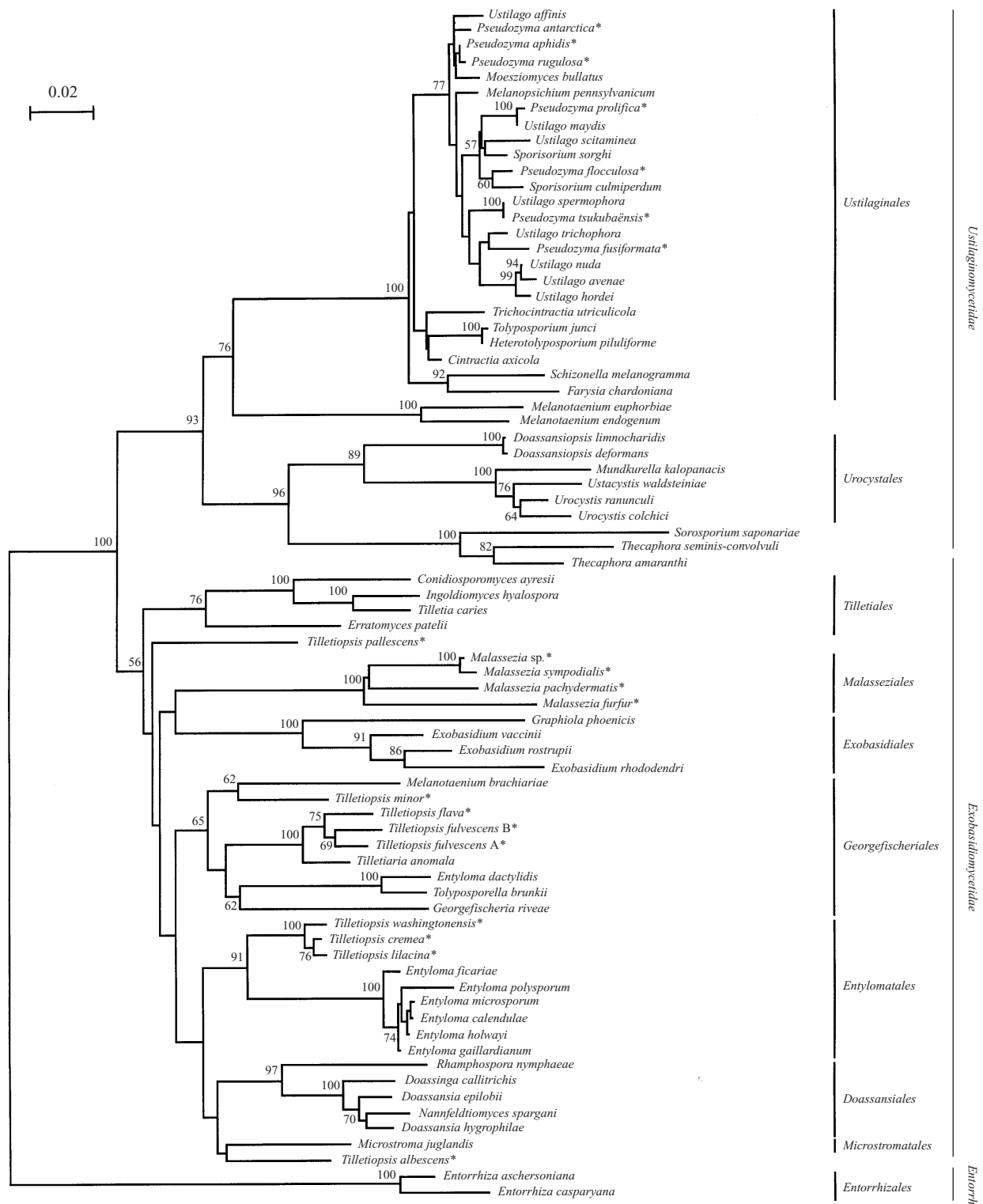


Fig. 1. Neighbor joining analysis rooted with *Entorrhiza*. Bootstrap values under 50% are not shown. Anamorphic species are marked by asterisks.

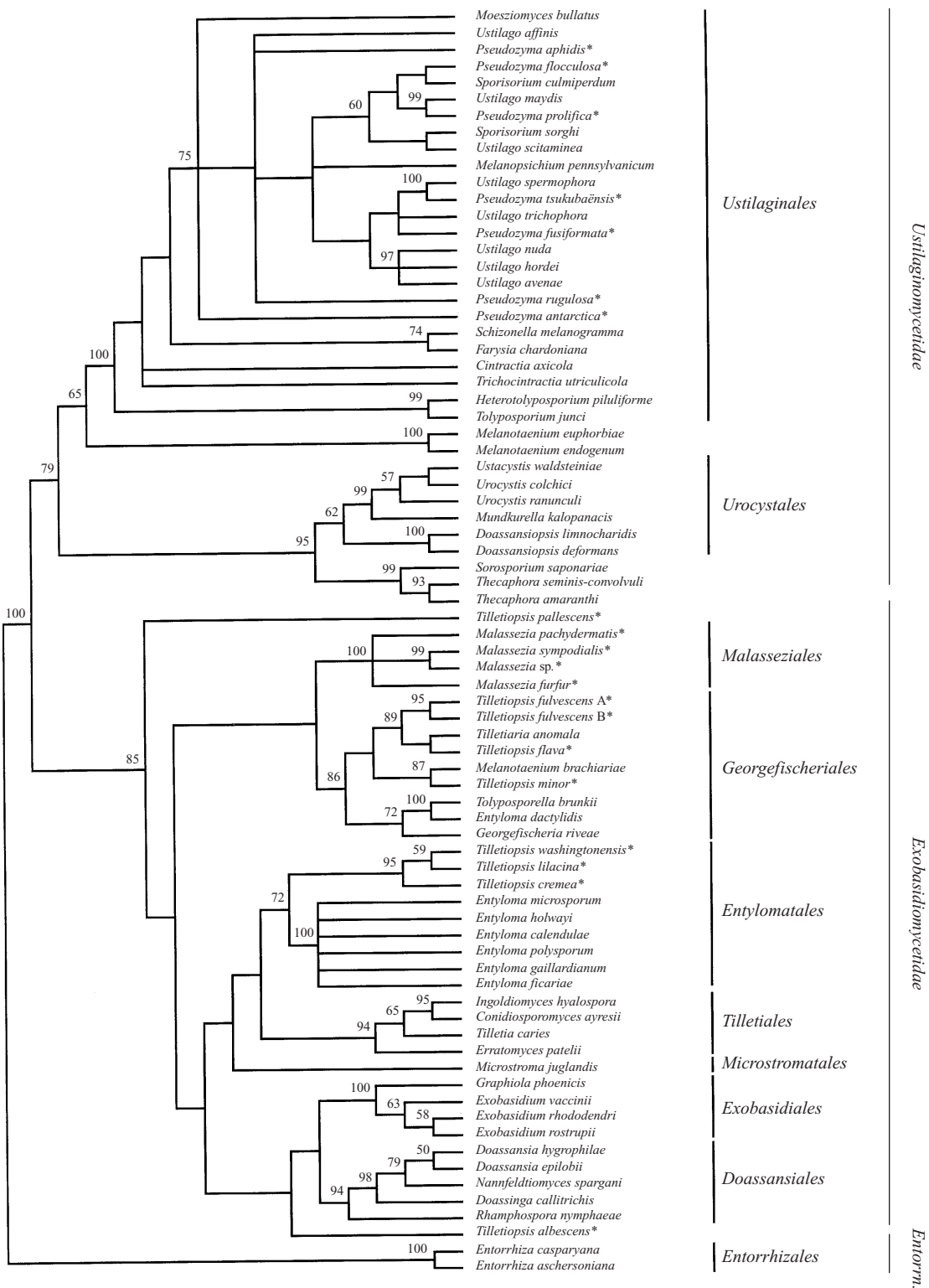
### *Malessezia*

Both methods of analysis placed the four *Malessezia* species investigated together on a common well-supported branch, but the phylogenetic position within the Exobasidiomycetidae differs between the two analyses and is not statistically supported.

## DISCUSSION

### The system

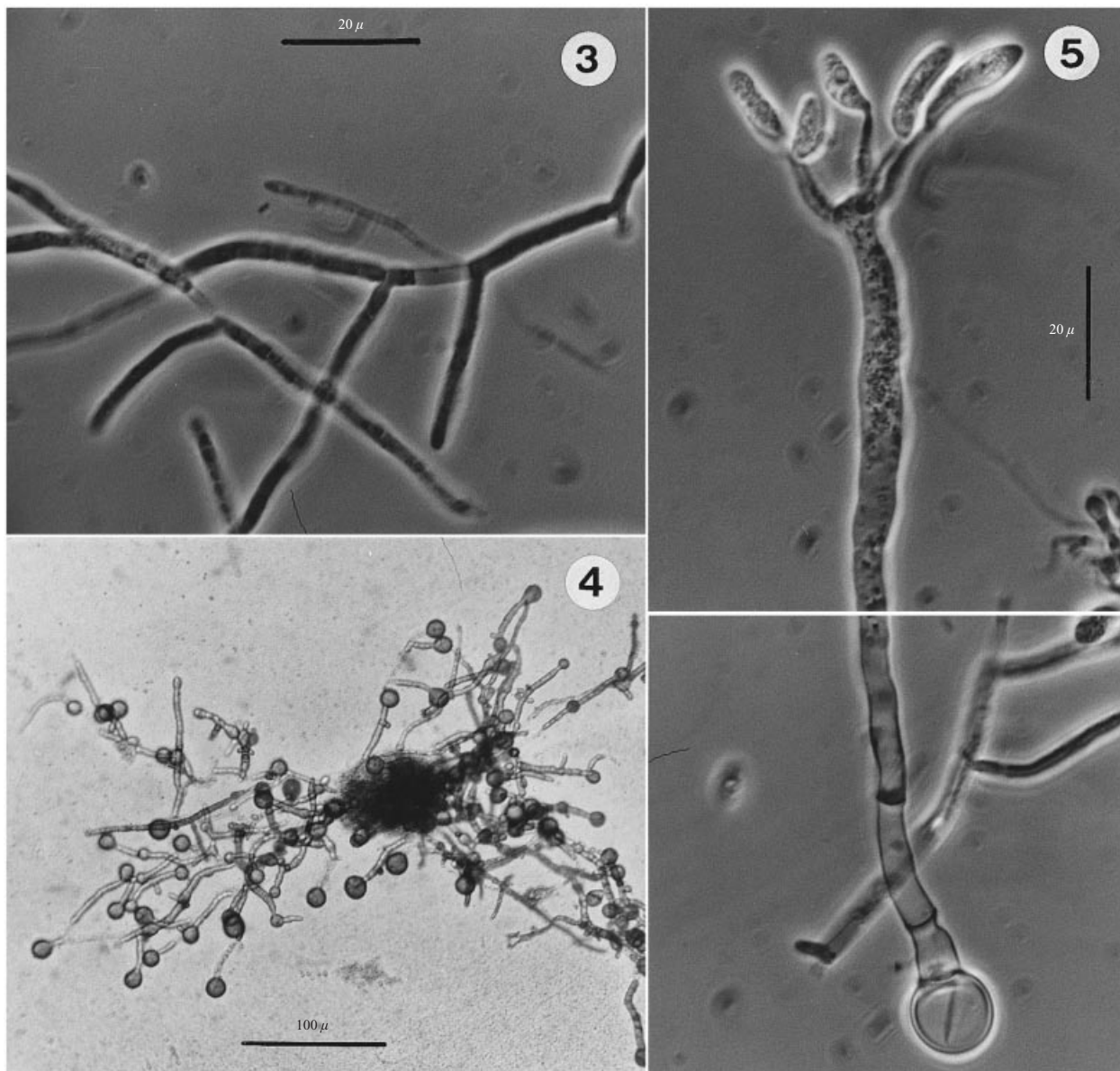
The groups of Ustilaginomycetes reported by Bauer *et al.* (1997) and Begerow *et al.* (1997) are also evident in this molecular study with an expanded set of species. These are the Entorrhizomycetidae with the Entorrhizales, the Ustilagino-



**Fig. 2.** Strict consensus tree of 71 most parsimonious trees (1516 steps) found by heuristic analysis, rooted with *Entorrhiza*. Bootstrap values under 50% are now shown. Anomorph species are marked by asterisks.

mycetidae with the Urocystales and Ustilaginales and the Exobasidiomycetidae with the Georgerfischeriales, Tilletiales, Entylomatales, Microstromatales, Doassansiales and Exobasidiales. Interestingly, the Exobasidiomycetidae are better supported by bootstrap values in the recent study than in the study of Begerow *et al.* (1997), possibly due to the larger

number of taxa in this study. In contrast with the sequence studies, however, the ultrastructural analysis resolves the relationship among three orders of the Exobasidiomycetidae (Bauer *et al.*, 1997). The unclear taxonomic situation of Melanotaeniaceae and Glomosporiaceae is discussed in detail in Begerow *et al.* (1997).



**Figs 3–5.** *Tilletiopsis* sp. FO 37346. **Fig. 3.** *Tilletiopsis*-like growth. Scale bar = 20 µm. **Fig. 4.** Teliosporogenesis. Scale bar = 100 µm. **Fig. 5.** Teliospore germination. Note the orientation of the basidiospores. Scale bar = 20 µm.

### *Pseudozyma*

Species of *Pseudozyma* have been isolated from quite different substrates (see Table 1). Our results indicate that they are anamorphs of species of Ustilaginales that parasitize grasses. These species form with *Melanopsichium pennsylvanicum* a statistically well supported subgroup of the Ustilaginales. *Pseudozyma prolifica* is most closely related to *Ustilago maydis*, as also observed by Boekhout *et al.* (1995). *P. tsukubaënsis* may represent the yeast-form of *U. spermophora*. Certainly, the yeasts of the Ustilaginales occurring on grasses fit the concept of the anamorph genus *Pseudozyma* (Boekhout, 1987, 1995). We are not surprised by the association between *Pseudozyma* and the grass-infecting species of the Ustilaginales. About 800 from ca 1400 known species of Ustilaginomycetes are living on grasses. Most of them are members of the Ustilaginales. Furthermore, Poaceae cover about a third of the land surface

with extensive populations, so yeasts of Ustilaginales occurring on grasses might be very abundant in nature.

### *Tilletiopsis*

Species of *Tilletiopsis* are frequently found as epiphytes on leaves, especially those infected with powdery mildew or rust fungi (Boekhout, 1991; Urquhart & Punja, 1997). We found *Tilletiopsis* in nearly all sporulations of *Exobasidium* spp. In fact, because of the frequent association with *Tilletiopsis* it is difficult to get a pure culture of species of *Exobasidium* Woronin (compare also Boekhout *et al.*, 1995). Among the Ustilaginomycetes, only the Melanotaeniaceae of the Ustilaginomycetidae, the Georgerfischeriaceae and Tilletariaceae of the Georgerfischeriales and the Entylomatales form *Tilletiopsis*-like pseudohyphal anamorphs that

produce ballistoconidia (Boekhout, 1987, 1991; Ingold, 1988; Bauer *et al.*, 1997; Begerow *et al.*, 1997). It is, therefore, not surprising that most of the species of *Tilletiopsis* tested belong either to the Georgefischeriales or Entylomatales. Finally, our results are in agreement with other molecular analyses (Boekhout *et al.*, 1995; Takashima & Nakase, 1996).

*Tilletiopsis cremea*, *T. lilacina* and *T. washingtonensis* are members of the Entylomatales. In terms of molecular analysis, however, these three asexual fungi are well apart from the species of *Entyloma* tested. They are, probably, representatives of a second family of the Entylomatales, which is possibly characterised by the loss of a phytoparasitic phase.

Our sequence analyses demonstrate that *Tilletiopsis flava* and *T. fulvescens* belong to the Tilletiariaceae *sensu* Bauer *et al.* (1997) of the Georgefischeriales. *Tilletiaria anomala* forms ballistospore phragmobasidia and is known only from cultures (Bandoni & Johri, 1972). Except for *Georgefischeria*, the other sexual species of the Georgefischeriales parasitize grasses (Bauer *et al.*, 1997). The systematic position of *T. minor* within the Georgefischeriales is not clear. We favour a position as the sister-group of the *Entyloma oryzae*-group, because the species of that group, represented here by *Melanotaenium brachiariae*, do not form *Tilletiopsis*-like anamorphs (Bauer *et al.*, 1997). We are surprised that no species of *Tilletiopsis* clusters with species representing that Georgefischeriaceae. *Entyloma dactylidis*, for example, a member of this family with a *Tilletiopsis*-like anamorph is very abundant in nature, infecting nearly all individuals in a grass population in some years.

*T. albescens* and *T. pallescens* are members of the Exobasidiomycetidae, but they cannot be ascribed to any of the known groups of this subclass. We are convinced that the 'chlamydospore' germination of these two species as drawn by Boekhout (1987, fig. 1A; 1991, fig. 52e) represent basidia of the exobasidiaceous type (Oberwinkler, 1978). For example, we have isolated a *Tilletiopsis*-like fungus (Fig. 3) from *Calendula officinalis* L. It developed teliospores (Fig. 4) after some months on malt-yeast-peptone-agar (Bandoni, 1972). After transferring the teliospores on water agar, they germinated with basidia of the exobasidiaceous type (hilar appendices of the basidiospores oriented abaxially, Fig. 5). These basidia are very similar to the 'chlamydospore' termination of *T. albescens* and *T. pallescens* drawn by Boekhout (1987, 1991). Species with this basidial type occur in the Georgefischeriaceae, Tilletiales and Doassansiales (Bauer *et al.*, 1997). Neither *T. albescens* nor *T. pallescens* cluster with representatives of these groups. It is possible, therefore, that these species are representatives of two hitherto unknown higher groups of the Exobasidiomycetidae.

### *Malassezia*

*Malassezia* comprises lipophilic yeasts that constitute part of the mycota of the skin of warm-blooded animals (Guého *et al.*, 1998). They have also been found associated with a variety of pathological conditions in humans including pityriasis versicolor, seborrheic dermatitis, folliculitis and systemic infections (see Guillot & Guého, 1995; Guého *et al.*, 1996; and the references therein). The cell wall of the *Malassezia* yeasts is thick, multilamellate and reveals a unique substructure with an

electron-opaque, helicoidal band that corresponds to a helicoidal evagination of the plasma membrane (Takeo & Nakai, 1986; Guillot *et al.*, 1995; Guého *et al.*, 1996). Obviously, the band is formed at the top of the evagination of the plasma membrane.

Molecular analyses indicate that the species of *Malassezia* are members of the Exobasidiomycetidae, representing a group of its own – the Malasseziales (Moore, 1980). As discussed above, the separate position of the *Malassezia* is in agreement with morphological ultrastructural, and physiological characteristics. To accommodate the Malasseziales in the Exobasidiomycetidae (Ustilaginomycetes) the description is emended as follows as follows:

### **Malasseziales** R. T. Moore 1980

Zoophilic members of the Exobasidiomycetidae (Ustilaginomycetes) with a monopolar budding yeast phase showing percurrent or sympodial proliferation of the buds. The yeasts are lipid-dependent or lipophilic, have a multilayered cell wall and a helicoidal evagination of the plasma membrane.

The sexual phase of *Malassezia* is unknown. Are the species of *Malassezia* phytoparasitic in the dikaryophase, or did they originate from plant parasites? We do not know the answer. On one hand, among the numerous ustilaginomycetous anamorphs we have investigated ultrastructurally, there was no yeast with the *Malassezia* ultrastructure. On the other hand, Ustilaginomycetes have different strategies during different phases of their life-cycle, and this may be true for *Malassezia* as well.

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