Genomic Insights into the Atopic Eczema-Associated Skin Commensal Yeast *Malassezia sympodialis*

Anastasia Gioti,a Björn Nystedt,b Wenjun Li,c Jun Xu,d Anna Andersson,a Anna F. Averette,c Karin Münch,f Xuying Wang,f Catharine Kappauf,g Joanne M. Kingsbury,c Bart Kraak,h Louise A. Walker,b Henrik J. Johansson,h Tina Holm,k Janne Lehtio,i Jason E. Stajich,j Piotr Mieczkowski,k Regine Kahmann,j John C. Kennell,j Maria E. Cardenas,g Joakim Lundeberg,f Charles W. Saunders,n Teun Boekhout,g,o Thomas L. Dawson,k Carol A. Munro,n Piet W. J. de Groot,p Geraldine Butler,n Joseph Heitman,n Annika Scheyniusn

Science for Life Laboratory, Translational Immunology Unit, Department of Medicine, Solna, Karolinska Institutet, Stockholm, Sweden; Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden; Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, USA; Procter & Gamble Co, Miami Valley Innovation Center, Cincinnati, Ohio, USA; Translational Immunology Unit, Department of Medicine, Solna, Karolinska Institutet, Stockholm, Sweden; Max Planck Institute for Terrestrial Microbiology, Marburg, Germany; CBS-Fungal Biodiversity Centre, Utrecht, The Netherlands; Institute of Medical Sciences, University of Aberdeen, Aberdeen, United Kingdom; Cancer Proteomics Mass Spectrometry, Science for Life Laboratory, Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden; Department of Plant Pathology & Microbiology and Institute for Integrative Genomic Biology, University of California, Riverside, California, USA; Department of Genetics, Carolina Center for Genome Science, School of Medicine, University of North Carolina, at Chapel Hill, Chapel Hill, North Carolina, USA; Department of Biology, Saint Louis University, St. Louis, Missouri, USA; Science for Life Laboratory School of Biotechnology, Division of Gene Technology, Royal Institute of Technology, Stockholm, Sweden; Department of Internal Medicine and Infectious Diseases, University Medical Center, Utrecht, The Netherlands; Regional Center for Biomedical Research, Albacete Science & Technology Park, University of Castilla-La Mancha, Albacete, Spain; and School of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland

ABSTRACT *Malassezia* commensal yeasts are associated with a number of skin disorders, such as atopic eczema/dermatitis and dandruff, and they also can cause systemic infections. Here we describe the 7.67-Mbp genome of *Malassezia sympodialis*, a species associated with atopic eczema, and compare its genome repertoire with that of *Malassezia globosa*, associated with dandruff, as well as those of other closely related fungi. Ninety percent of the predicted *M. sympodialis* protein coding genes were experimentally verified by mass spectrometry at the protein level. We identified a relatively limited number of genes related to lipid biosynthesis, and both species lack the fatty acid synthase gene, in line with the known requirement of these yeasts to assimilate lipids from the host. *Malassezia* species do not appear to have many cell wall-localized glycosylphosphatidylinositol (GPI) proteins and lack other cell wall proteins previously identified in other fungi. This is surprising given that in other fungi these proteins have been shown to mediate interactions (e.g., adhesion and biofilm formation) with the host. The genome revealed a complex evolutionary history for an allergen of unknown function, Mala s 7, shown to be encoded by a member of an amplified gene family of secreted proteins. Based on genetic and biochemical studies with the basidiomycete human fungal pathogen *Cryptococcus neoformans*, we characterized the allergen Mala s 6 as the cytoplasmic cyclophilin A. We further present evidence that *M. sympodialis* may have the capacity to undergo sexual reproduction and present a model for a pseudobipolar mating system that allows limited recombination between two linked MAT loci.

IMPORTANCE *Malassezia* commensal yeasts are associated with a number of skin disorders. The previously published genome of *M. globosa* provided some of the first insights into *Malassezia* biology and its involvement in dandruff. Here, we present the genome of *M. sympodialis*, frequently isolated from patients with atopic eczema and healthy individuals. We combined comparative genomics with sequencing and functional characterization of specific genes in a population of clinical isolates and in closely related model systems. Our analyses provide insights into the evolution of allergens related to atopic eczema and the evolutionary trajectory of the machinery for sexual reproduction and meiosis. We hypothesize that *M. sympodialis* may undergo sexual reproduction, which has important implications for the understanding of the life cycle and virulence potential of this medically important yeast. Our findings provide a foundation for the development of genetic and genomic tools to elucidate host-microbe interactions that occur on the skin and to identify potential therapeutic targets.

Received 6 December 2012 Accepted 11 December 2012 Published 22 January 2013

Citation Gioti A, et al. 2013. Genomic insights into the atopic eczema-associated skin commensal yeast *Malassezia sympodialis*. mBio 4(1):e00572-12. doi:10.1128/mBio.00572-12.

Editor Judith Berman, University of Minnesota

Copyright © 2013 Gioti et al. This is an open-access article distributed under the terms of the Creative Commons Attribution-Noncommercial-ShareAlike 3.0 Unported license, which permits unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

Address correspondence to Annika Scheynius, annika.scheynius@ki.se.
liculitis, dandruff, and seborrheic dermatitis and even with systemic infections (2, 3). Currently there are 14 recognized species of *Malassezia* that have been isolated from humans or other warm-blooded animals (4). Various studies show differences in the *Malassezia* species found on human skin (2), suggesting that there may be geographic variation in the commensal flora and also the species associated with disease. All *Malassezia* species except *M. pachydermatis* require exogenous lipids for growth. They are frequently associated with sebum-rich areas of the skin, where they obtain fatty acids to fulfill their lipid requirements. Because of their unique nutritional requirements, specialized media such as Dixon’s medium (or Leeming and Notman agar) are required for their in vitro growth (2). Another unique characteristic of *Malassezia* is the cell wall, which is very thick (~0.12 μm) compared to other yeasts and consists of ~70% sugars, ~10% protein, and 15 to 20% lipids (2). The cell wall is surrounded by a lipid-rich capsule-like structure (5), which may be involved in interactions of this yeast with its host. Thus far, no sexual cycle has been observed for *Malassezia*. A region corresponding to the mating-type locus (MAT) and genes encoding key proteins required for meiosis have, however, been identified in the genome of *Malassezia globosa* (6). Because infection of the plant host by the phylogenetically closely related species *U. maydis* is coupled to the sexual cycle (7), it will be of interest to explore in future studies whether a similar pathogenic mechanism may also operate in *Malassezia*.

Among the *Malassezia* species, *M. sympodialis* is one of the most frequently isolated from both atopic eczema patients and healthy individuals (2). Atopic eczema is a common chronic inflammatory skin disease, and the prevalence of this disorder has doubled or tripled in industrialized countries during the past three decades, with 15 to 30% of children and 2 to 10% of adults being afflicted (8). Approximately 50% of adult patients with atopic eczema are sensitized to *M. sympodialis*, as reflected by allergen-specific IgE and/or T cell reactivity to the yeast (9). Reactivity is rarely observed in other allergic diseases (10) indicating a specific link between atopic eczema and *Malassezia*. The pathogenesis of atopic eczema likely results from the combination of a disturbed skin barrier and genetic and environmental factors such as lifestyle, stress, allergens, and microbes (11). The altered skin barrier provides an environment that leads to elevated skin pH, which enhances the release of IgE-binding proteins (allergens) from *M. sympodialis* (12). Ten allergens have been identified in *M. sympodialis* so far (9). Interestingly, several of the identified allergens are homologous to host proteins, suggesting the possibility of cross-reactive immune responses, whereas others are proteins of unknown function with no sequence homology to characterized proteins (9).

In this study, we focused on *M. sympodialis* as a model to advance our understanding of how the normal skin microbiota interacts with the host and contributes to atopic eczema pathogenesis. We sequenced the genome of *M. sympodialis* to a high coverage with the aims of (i) exploring genomic features related to the biology of the yeast, (ii) investigating the function and molecular evolution of allergens related to atopic eczema, and (iii) elucidating the presence of a potential sexual cycle. The *M. sympodialis* genome was compared to the published genome of *M. globosa* (6), a species associated with pityriasis versicolor and dandruff, as well as to genomes of other fungi, such as those found on human skin and *U. maydis*, which is found on plants.

### RESULTS AND DISCUSSION

**The genome of *M. sympodialis*.** The draft high-coverage genome of *M. sympodialis* ATCC 42132 was assembled from a shotgun 454 data set and was extended and scaffolded using a 3-kb insert Illumina HiSeq mate-pair data set (Table 1) to a total of 65 scaffolds (*L*_50 = 511 kb), corresponding to a nuclear genome of 7.67 Mbp (Table 1). According to CEGMA (13) analysis, this assembly shows a high degree of completeness: 88.3 to 93.1%, comparable to 89.5 to 93.5% for the genome of the closely related species *M. globosa* (6). The estimated genome size of *M. sympodialis* is smaller than that of the *M. globosa* genome (8.96 Mbp), while both are in line with previously reported genome sizes from electro-photoret karyotyping experiments (14). The *Malassezia* genomes are among the smallest in the fungal kingdom, a feature probably related to their dependence on warm-blooded animals. Whole-genome alignments showed extensive synteny between the *M. sympodialis* and *M. globosa* scaffolds (Fig. 1A).

We predicted a total of 3,517 protein-coding genes in *M. sympodialis* using multiple lines of evidence (*ab initio* predictors,
pressed sequence tags (ESTs) from *M. globosa*, and protein and nucleotide alignments) and manual annotations for specific genes. We applied mass spectrometry (MS) proteomics using peptide isoelectric focusing (IEF) on a broad and a narrow pH range to achieve high proteome coverage of *M. sympodialis* protein extracts and confirmed 3,176 (90%) of the predicted proteins. Of the MS-identified proteins, 98% have 2 or more peptide spectrum matches (PSMs) supporting their identification and 90% have 2 or more unique peptides identified (Fig. 1B) with sequence coverage of 13.5% or more for 75% of the proteins (Fig. 1C). The completeness of the annotation of protein-coding genes was estimated using unique peptides identified by mass spectrometry from both predicted protein-coding genes and peptides generated by searching the 6-reading-frame (6RF) translation of *M. sympodialis*.

When inspecting the synteny of orthologous proteins shared between *M. globosa* and *M. sympodialis*, we observed a tendency for genes which are distinct in the *M. globosa* genome to be fused into the same open reading frame (ORF) in the *M. sympodialis* genome. These discrepancies, as well as the difference between the two species regarding the total number of genes, mainly reflect differences between annotation platforms, the lack of RNA evidence for gene predictions in *M. sympodialis*, and, to a smaller extent, errors in sequencing. The annotation of the genomes of *Malassezia* species is particularly challenging, given the limited
availability of transcript evidence (only 1,392 ESTs are available for *M. globosa*). Furthermore, the genomes of only a few species from this phylogenetic clade have been sequenced to date, such as *U. maydis* (15), *Ustilago hordei* (16), and *Sporisorium reilianum* (17). This renders the task of gene predictions via homology searches challenging, especially for fast-evolving and species- and genus-specific genes. For *M. sympodialis*, high-coverage sequencing of RNA extracted from distinct growth conditions is now under way to allow better annotation of the genome in a future update (A. Scheynius and T. L. Dawson, personal communication).

**The mitochondrial genome of *M. sympodialis***. The mitochondrial genome was assembled from the 454 data and maps as a circular fragment of 38,622 bp with an estimated GC content of 32% (Table 1). Overall it is syntenic with the mitochondrial genome of *M. globosa* (Fig. 2). The genome contains all 15 expected protein-coding genes, 2 rRNA genes, and 25 tRNAs representing all 20 amino acids, with Ser, Leu, and Arg in two copies and Met in three copies. This coding content is the same as that of the related species *U. maydis* (accession number NC_008368). Although there is no synteny between *Malassezia* and *Ustilago* regarding gene order, genes are present on both strands in each species. In contrast to the mitochondrial genome of *M. globosa*, that of *M. sympodialis* has several group I introns (8 in total, including 3 that encode putative homing endonucleases).

A conspicuous feature of the *M. sympodialis* mitochondrial DNA (mtDNA) is a large (5.9-kb) inverted repeat containing the ATP9 gene and tRNA genes for Met, Leu, and Arg (Fig. 2). The inverted repeat is also present in the *M. globosa* mitochondrial genome, although it is shorter and poorly conserved between the two species, apart from regions corresponding to ATP9 and tRNAs. Large inverted repeats are common in chloroplast genomes (18), yet they occur infrequently in fungal mtDNAs and have been reported for only a few genera. In *Candida* species, homologous recombination between large inverted repeats in mtDNAs of *Candida* species has been proposed to play a role in replication and is associated with genome rearrangements (19, 20). Strand invasion structures in the inverted repeats of *C. albicans* mtDNA support a recombination-driven mechanism of DNA initiation (20), while comparative studies of the mitochondrial genomes of *Candida* species indicate that large inverted repeats are involved in conversions between circular and linear forms of the genome, as well as the formation of multipartite linear forms (19). Comparative analysis of mtDNAs of additional *Malassezia* species and clinical isolates is necessary to assess the functional significance of the inverted repeats and to determine whether mitochondria play a role in virulence, as has been reported with other fungal pathogens (21–23).

**Differences between *Malassezia* species regarding metabolism**. We investigated the metabolic pathways for potential changes that could explain the *in vitro* nutritional requirements of *Malassezia* species. Comparing the genomes of *M. globosa* and *M. sympodialis* for genes involved in lipid metabolism, we found that both genomes encode a plethora of lipid-hydrolyzing enzymes, such as lipases, phospholipases C, and acid sphingomyelinases (Table 2). *M. sympodialis* has a slightly reduced number of lipases and phospholipases C compared to *M. globosa*. It is not clear whether this contributes to any physiological differences or if this pattern is simply due to the potentially incomplete predicted
proteome of *M. sympodialis*. No Δ2,3-enoyl coenzyme A isomerase gene was found in either genome, suggesting defects in utilizing unsaturated fatty acids. Notably, a Δ9 desaturase gene, absent in both the *M. globosa* and *Malassezia restricta* genomes, was found in the *M. sympodialis* genome (MSY001_2159). This suggests that *M. sympodialis*, in contrast to *M. globosa*, may be able to add a double bond to generate oleic acid if provided with stearic acid in the culture medium. However, since oleic acid is included in the m Dixon medium used to grow *Malassezia*, the difference between the two species regarding the Δ9 desaturase is unlikely to explain the reported better in vitro growth of *M. sympodialis* (24), unless oleic acid uptake is limiting in *M. globosa* and *M. restricta*.

A second explanation for the differences in growth between the two species could relate to genes involved in sugar assimilation, such as those encoding β-glucosidases. In contrast to *M. globosa*, *M. sympodialis* is positive in the cell wall, which is very thick compared to the cell walls of other yeasts (2). The *M. sympodialis* genome contains representatives of the major polysaccharide biosynthesis genes (Table 3). These include genes encoding six chitin synthases, proteins associated with β-1,6-glucan synthesis, and only one β-1,3-glucan synthase catalytic subunit. Other basidiomycetes also have one Fks-like β-1,3-glucan synthase catalytic subunit, whereas ascomycetes such as *S. cerevisiae* generally harbor a number of alternative Fks subunits (Table 3). There are six predicted chitin deacetylases, which is similar to the number found in other basidiomycetes but larger than the number generally seen in ascomycetes. This suggests that a large proportion of cell wall chitin may be converted to chitosan, the deacetylated form of chitin. The chromosomal location of this gene family suggests that there have been three separate gene duplication events leading to expansion of the chitin deacetylase family. Studies in *C. neoformans* have shown that chitosan helps to maintain cell wall integrity (26), suggesting that chitin deacetylases and the chitosan made by them may prove to be excellent antifungal targets. There is also an abundance of putative exoglucanases with similarity to *S. cerevisiae* Exgl (Table 3). The classical cell wall integrity or protein kinase C (PKC) pathway seems to be highly conserved in *M. sympodialis* (see Table S1 in the supplemental material). Putative enzymes involved in O-glycosylation are represented in the genome such as the O protein mannosyltransferase (Pmt) family, but there is little evidence of orthologs of N-glycosylation enzymes, in particular those that add sugars to the outer chains to N-glycan (see Table S1).

In yeasts such as *S. cerevisiae* and *Candida albicans*, the major class of cell wall-localized proteins comprises proteins that are modified by the addition of a glycosylphosphatidylinositol (GPI) anchor (27). The posttranslational addition of a GPI anchor targets proteins to the plasma membrane. Through a poorly understood mechanism, the GPI anchor of a subset of GPI proteins is cleaved and the proteins are translocated to the wall and covalently attached to β-1,6-glucan. Only ten *M. sympodialis* proteins (and 20 in *M. globosa*) were predicted to become GPI anchored.

### Table 2: Main lipid-hydrolyzing enzymes in *M. globosa* and *M. sympodialis*

| Gene family                      | No. of enzymes in: |
|----------------------------------|--------------------|
|                                  | *M. globosa*       | *M. sympodialis* |
| M. globosa LIP lipase like       | 6                  | 4                |
| M. globosa LIP1 lipase like      | 6                  | 5                |
| Acid sphingomyelinase            | 4                  | 4                |
| Phospholipase C                  | 6                  | 4                |

*From reference 6.*

### Table 3: Cell wall genes

| Gene class                      | No. of genes in: |
|---------------------------------|------------------|
|                                 | Msym  | Mglo  | Umay | Cneo | Scer |
| Chitin synthase                 | 6     | 7     | 8    | 9    | 3    |
| Chitin deacetylase              | 6     | 4     | 6    | 4    | 2    |
| Chitinase (class IV)            | 1     | 1     | 2    | 4    | 2    |
| Catalytic subunit of β-1,3-glucan synthase (FKS) | 1     | 1     | 1    | 1    | 3    |
| Exo-β-1,3-glucanase (EXG1)      | 8     | 6     | 8    | 8    | 3    |
| Transglucosylase (GH16, CRH)    | 1     | 1     | 2    | 2    | 3    |
| Transglucosylase (GH72, GAS)    | 0     | 0     | 1    | 1    | 5    |
| Mixed-linked glucanase (MLG1)   | 2d    | 2     | 4    | 5    | 0    |
| Putative β-1,6-glucan transglycosylase (KRE6) | 4     | 4     | 8    | 6    | 2    |
| ER chaperone involved in protein N- and O-glycosylation (ROT1) | 1     | 2     | 4    | 1    | 1    |
| Predicted GPI proteins          | 10    | 20    | 55e  | 63   | 59f/66g |

* Homologous gene or gene families in *S. cerevisiae* (except for MLG1, which is from *Cochliobolus carbonum*).

* Msym, *M. sympodialis*; Mglo, *M. globosa*; Umay, *U. maydis*; Cneo, *C. neoformans*; Scer, *S. cerevisiae*.

* One of the gene models seems incorrect and comprises two paralogous genes.

* Second paralog identified by TBLASTn but no gene model available.

* Data from reference 101.

* Data from reference 102.

* Data from reference 30.
Evidence of linkers) act by modulating the cell wall polysaccharides chitin and integrity. These proteins (transglucosidases and chitin-glucan cross-modified proteins also include carbohydrate-active enzymes with pathogens that cause systemic and bloodstream infections. GPI-catheters, and thereby also contribute to biofilm formation (27). Both adhesion and biofilm formation play a role in virulence in invading pathogens that cause systemic and bloodstream infections. GPI-modified proteins also include carbohydrate-active enzymes with important roles in cell wall construction and maintenance of cell wall integrity. These proteins (transglucosidases and chitin-glucan cross-linkers) act by modulating the cell wall polysaccharides chitin and β-1,3-glucan. Only one putative chitin-glucan cross-linker gene, a Utr2 homolog, was identified in the M. sympodialis genome. No Dfg5/Dcw1 family members were identified. These putative mannosidases are generally assumed to be involved in the cleavage of GPI anchors, consistent with the lack of GPI cell wall proteins. This prompted us to look for homologs of the proteins that synthesize the GPI anchor itself, and we found representatives of the enzymes that synthesize most steps in the pathway in S. cerevisiae (see Table S1 in the supplemental material).

From the above, we can surmise that there are β-1,3-glucan, β-1,6 glucan (probably β-1,3/β-1,6 glucan), chitin, and chitosan in the cell wall of M. sympodialis, and glycosylation may be limited primarily to O-glycosylation. Analysis of the genome provides no evidence of α-glucan synthesis and no or very few “classical” fungal cell wall proteins. These in silico results are in agreement with a previous cell wall carbohydrate analysis that revealed that the M. sympodialis cell wall is composed primarily of β-1,6 glucan, with trace amounts of branched β-1,6/β-1,3-glucan and mannann (29). We further performed high-pressure freezing–transmission electron microscopy (HPF-TEM) to corroborate our observations on the absence of cell wall proteins and proteins for outer N-mannosylation, which participate in forming a fibrillar layer. M. sympodialis indeed lacks the extensive outer fibrillar layer (Fig. 3) that is evident on the wall of S. cerevisiae and C. albicans (30, 31).

**Molecular evolution and function of allergens.** Genes coding for all 10 allergens previously cloned from M. sympodialis (Mala s 1 and s 5 to s 13) and for the three allergens from M. furlur (Mala f 2 to 4) (9) were identified in the M. sympodialis genome (Table 4). For some of the allergens, where the previously described sequence was incomplete (Mala s 13) or contained sequencing errors at the 5′ end (Mala s 11), the availability of the genome sequence and protein alignments allowed identification of the full coding sequence, including the start codon. Sequence identity between M. sympodialis and M. globosa at both the nucleotide and amino acid levels is generally high (Table 4). Despite the high degree of protein identity between putative orthologs, a molecular evolution analysis indicated high levels of nucleotide substitutions (dS > 1) for all of them (Table 4). A population of 56 clinical M. sympodialis isolates from healthy individuals and atopic eczema patients (see Table S2 in the supplemental material) was further analyzed for the presence of two major allergens, Mala s 1, an allergen of unknown function (32), and Mala s 12, showing sequence similarity to the GMC oxidoreductase family (33). The partial genes for Mala s 1 and Mala s 12 (encoding 81% and 45% of the mature proteins, respectively) were amplified (primers are listed in Table S3 in the supplemental material) in all clinical isolates and showed strong conservation. This finding suggests that the regions of the genes we examined are under high selective constraints, possibly reflecting the maintenance of essential roles in the interaction with the host.

The function of Mala s 1 is still an enigma despite the availability of its three-dimensional (3-D) structure. The 3-D structure indicates that Mala s 1 is a β-propeller-folded protein. This novel fold among allergens has structural similarity in the potential homologs Q4P4P8 and Tri 14, from the plant pathogens U. maydis and Gibberella zeae, respectively (34), suggesting that Mala s 1 and the plant pathogen proteins may have similar functions. Because gene deletion approaches have not been established for Malassezia, we investigated the role of the Mala s 1 ortholog in the related smut fungus U. maydis. Quantitative real-time PCR revealed that expression of the Mala s 1 ortholog in U. maydis (um04915), encoding a protein predicted to be secreted, is induced during colonization of maize seedlings (see Fig. S2A in the supplemental ma-

![FIG 3](mBio.asm.org) M. sympodialis cell wall architecture revealed by HPF-TEM (high-pressure freezing–transmission electron microscopy). M. sympodialis was grown on mDixon agar at 32°C for 4 days. (A) Transmission electron micrograph of a budding M. sympodialis yeast cell. (B) Ultrastructure of the M. sympodialis cell wall. Bars: 0.5 μm (magnification, ×25,000) (A) and 100 nm (magnification, ×130,000) (B).
terial). The gene um04915 was deleted from the genome of the solopathogenic strain SG200 (see the supplemental methods in the supplemental material). With respect to SG200, um04915 mutants were unaltered in growth sensitivity to various stressors, including H2O2, sorbitol, Calcofluor white, and Congo red, or inants were unaltered in growth sensitivity to various stressors, in- (see Fig. S2B in the supplemental material). Therefore, the gene is not directly related to Malassezia sympodialis genome Analysis

| Allergen | Accession no. (reference) | Predicted function | Prediction for secretion (both species) | M. sympodialis gene | M. globosa ortholog | % identity | Amino acid | Nucleotide |
|----------|--------------------------|--------------------|----------------------------------------|---------------------|---------------------|------------|------------|------------|
| Mala s 1 | X96486 (32)              | Unknown; similarity to G. zea Tri14 | Secreted                             | MSY001_0607         | MGL_1303            | 69         | 66         | 0.23       | 4.09       |
| Mala f 2 | AB011804 (103)           | Peroxisomal protein | No                                     | MSY001_2163         | MGL_4042            | 55         | 61         | 0.39       | 3.45       |
| Mala f 3 | AB011805 (103)           | Peroxisomal protein | No                                     | MSY001_2163         | MGL_4042            | 55         | 61         | 0.39       | 3.45       |
| Mala f 4 | AF084828 (104)           | Malate dehydrogenase | No                                     | MSY001_0149         | MGL_2703            | 84         | 77         | 0.10       | 2.74       |
| Mala s 5 | AJ011955 (43)            | Peroxisomal protein | No                                     | MSY001_2163         | MGL_4042            | 55         | 61         | 0.39       | 3.45       |
| Mala s 6 | AJ011956 (43)            | Cytoplasmic cyclophilin | No                                     | MSY001_1373         | MGL_3612            | 93         | 85         | 0.04       | 1.61       |
| Mala s 7 | AJ011957 (39)            | Unknown | Secreted                             | MSY001_3348         | MGL_0966d            | NA         | NA         | NA         | NA         |
| Mala s 8 | AJ011958 (39)            | Unknown | Secreted                             | MSY001_0606         | MGL_1304            | 71         | 68         | 0.22       | 4.52       |
| Mala s 9 | AJ011959 (39)            | Unknown | No                                     | MSY001_1912         | MGL_2179            | 77         | 74         | 0.19       | 1.95       |
| Mala s 10 | AJ428052 (105)          | Heat shock protein | No                                     | MSY001_0570         | MGL_0201            | 89         | 78         | 0.07       | 4.60       |
| Mala s 11 | AJ548421 (105)          | Manganese superoxide dismutase | No                                     | MSY001_2804         | MGL_3190            | 71         | 73         | 0.18       | 3.23       |
| Mala s 12 | AJ871960 (33)           | GMC oxidoreductase | Secreted                             | MSY001_2108         | MGL_0750            | 64         | 65         | 0.29       | 2.52       |
| Mala s 13 | AJ937746 (106)          | Thiorodoxin | No                                     | MSY001_0904         | MGL_1781            | 85         | 81         | 0.10       | 1.76       |

a Isolated from ATCC 42132 except for Mala f 2, 3, and 4, which come from isolate 2782 (Teikyo Institute for Medical Mycology, Tokyo, Japan).

b Evidence for no secretion is absence of signal peptides, transmembrane domains, and GPI-anchoring peptides.
c Single-copy orthologs between the two species were identified with a bidirectional best-hit BLASTP approach (E-value = 1E − 50).
d The gene shows weak similarity to the gene encoding Mala s 7, and due to gene family amplification [see the text], it cannot be safely assigned as its ortholog; therefore, dN/dS analysis between the two copies is not applicable (NA).

dN/dS values calculated by a codon-based method using the M. sympodialis Mala s 7 sequence as the outgroup. Values for Mala s 7b were not calculated.

dN/dS ratios ≥ 1.0 indicate positive selection and the potential for adaptive evolution.

The gene shows weak similarity to the gene encoding Mala s 7, and due to gene family amplification [see the text], it cannot be safely assigned as its ortholog; therefore, dN/dS analysis between the two copies is not applicable (NA).
chromosomes is required to resolve whether this is a segmental duplication or whether the duplicated fragments lie on distinct chromosomes. A segmental duplication event could also be at the origin of the other two Mala s 7 copies in M. sympodialis and M. globosa, as the respective genes in both species are located only 1.5 kb apart. These genes are Mala s 7c and 7d in M. sympodialis and MGL_0968 and a gene missed in previous annotations (accession number JX857443) in M. globosa. Gene conversion often occurs between copies lying in close proximity in the genome, and the species-based clustering of genes for Mala s 7-like proteins in the gene tree (see Fig. S3 in the supplemental material) is in line with this scenario. Another interesting observation is that the Mala s 7-like proteins in M. globosa (MGL_0968, MGL_2673, and JX857443) are shorter and have low similarity with their M. sympodialis counterparts at both the C and N termini, which could indicate that Mala s 7 in M. sympodialis comes from a fusion of two smaller Mala s 7-like proteins. A molecular evolution analysis using branch models in PAML (40, 41) did not further elucidate this family’s complex history, due to inconclusively high dS values. Overall, the genome sequence revealed a gene family amplification for the Mala s 7 allergen in M. sympodialis, which merits further investigation. Gene duplication is a major force driving evolution of new traits, including virulence, and thus it will be of interest to determine the roles of the secreted Mala s 7-like proteins in the interaction with the host and their evolutionary history.

We next addressed the function of Mala s 6, which is a member of the cyclophilin panallergen family (42). The sequence of the M. sympodialis Mala s 6 protein exhibited highest similarity to the cytoplasmic form of cyclophilin A, which is in agreement with the predicted absence of a secretion signal peptide (Table 4). Mala s 6 was found to be the most conserved protein in pairwise comparisons between M. globosa and M. sympodialis (Table 4), in line with its reported conservation across the tree of life. Using the C. neoformans model system, we investigated the functions of Mala s 6 using immunological, enzymatic, and drug inhibition assays. Recombinant Mala s 6 (43) reacted with a Cpa1-specific antiserum (Fig. 4A) that successfully recognizes both the Cpa1 and Cpa2 cyclophilin A proteins in C. neoformans (44). Using a chymotrypsin-coupled assay that measures the cis-to-trans isomerization of a synthetic peptide (45), we found that the Mala s 6 recombinant protein shows robust cis-trans peptidyl-prolyl isomerase activity (Fig. 4B). Furthermore, the recombinant Mala s 6 protein is sensitive to inhibition by cyclosporine A (Fig. 4B), an effective immunosuppressive natural product that targets cyclophilin A and calcineurin (44). Cyclosporine A shows beneficial effects in atopic eczema, but due to side effects, its use is limited to patients with severe refractory disease (11). In conclusion, we demonstrated that Mala s 6 is a bona fide cyclophilin A and targeted by cyclosporine A.

The mating-type (MAT) locus of M. sympodialis corresponds to a pseudobipolar mating system. We identified in the M. sympodialis genome assembly two scaffolds corresponding to the A (or pheromone/receptor [P/R]) and B (or homeodomain [HD]) mating type (MAT) loci based on sequence similarity shared with MAT genes of the closely related species M. globosa (6). The A and B mating-type loci in M. sympodialis were not linked in our assemblies; however, aligning them to the M. globosa MAT locus, where the two alleles are linked (6), identified three additional scaffolds which may lie between these loci and which could define a contiguous mating-type locus in M. sympodialis (Fig. 5A). Alignments of raw Illumina reads onto these scaffolds and PCR experiments (see the supplemental methods) provided further evidence for this contiguous assembly. Thus, similar to M. globosa (6), the M. sympodialis A and B loci appear to be physically linked and lie about ~141 kb apart.

In basidiomycetes, linkage of the A and B loci, along with strict biallelism, indicative of absence of recombination between these alleles, commonly defines bipolar mating systems, while tetrapolar mating systems contain unlinked and multiallelic A and B loci (46). However, in contrast to expectations for bipolar species, both the A and B MAT locus alleles of M. sympodialis showed extended flanking synteny with the corresponding M. globosa MAT locus regions (Fig. 5A). In comparison, in the bipolar species U. hordei, where the ancestral A and B MAT regions lie 430 to 500 kb apart, separated by a region that is highly rearranged between the two mating types, there is synteny on the 5’ end flanking the B locus and on the 3’ end flanking the A locus but not on their other flanks (47). Sequencing of the M. sympodialis A and B MAT alleles in a population sample of isolates (see Table S2 and the supplemental methods in the supplemental material) further indicated that the mating system of this species might not fit either of the traditional bipolar and tetrapolar systems, similar to what was previously reported for the pseudobipolar species Sporidiobolus salmonicolor (48). Below we present evidence suggesting that M. sympodialis has an intermediate mating system, termed the pseudobipolar mating system, where multiallelism might occur despite physical linkage (for a comparison of mating systems, see Fig. S4 in the supplemental material).

The A locus present in the genome of ATCC 42132 encodes a pheromone and a pheromone receptor arranged as two adjacent
divergently oriented genes (Fig. 5A) and was designated \( a1 \). A MAT A locus allele that was distinct in terms of both sequence conservation and orientation of the genes was identified by sequencing the same region in a collection of isolates (see Table S2 and supplemental methods in the supplemental material) and designated \( a2 \). Both \( a1 \) and \( a2 \) are embedded in highly conserved, syntenic regions (Fig. 5B). In contrast to the \( a \) locus in the closely related species \( U. maydis \), which is biallelic, the \( a1 \) and \( a2 \) MAT alleles from \( M. sympodialis \) do not contain a pheromone pseudo-gene, which in \( U. maydis \) is thought to descend from a tri-allelic
P/R mating type system shared with the last common ancestor with S. reilianum, which is also tri-allelic (49–51). Moreover, the M. sympodialis A MAT locus alleles both lack the lga2 and rga2 genes that are present in the U. maydis a2 and S. reilianum a2 alleles, and which are involved in governing uniparental mitochondrial inheritance in those species (52, 53).

Similarly, the M. sympodialis B MAT locus of the reference strain ATCC 42132 (Fig. 5A) was designated allele b1 and contains divergently oriented genes encoding the homeodomain transcription factors bE and BW (by analogy with U. maydis). Again, PCR with flanking primers and sequence analysis revealed the presence of two additional alleles, b2 and b3, distinguished from b1 by a series of substitutions, many of which lie in the N-terminal region or in the protein–protein interaction domains of the two homeodomain factors and are nonsynonymous (Fig. 5B; also, see Fig. S5 in the supplemental material). The differences observed in the M. sympodialis B locus alleles may be sufficient to represent different mating types based on two lines of reasoning (54, 55). First, comparison to similar amino acid changes that are naturally occurring in U. maydis B locus alleles of different mating types reveals a similar degree of substitution and in similar regions of the proteins. Second, amino acid substitutions found in U. maydis mutants that exhibit compatibility with different-partner homeodomain proteins also exhibit a similar pattern to the changes observed between the b1, b2, and b3 mating type alleles of M. sympodialis. The finding of biallelism for the A locus and triallelism for the B MAT locus suggests that recombination could occur between these regions. Further evidence for this is based on an allele compatibility test that revealed five of the six possible allelic configurations predicted for two unlinked MAT loci (a1b1, a1b2, a2b1, a2b2, and a2b3) in a collection of M. sympodialis isolates (see Table S2 in the supplemental material). In contrast, if the three B alleles observed were simply the result of drift, we would have expected linkage between the A and B alleles (a1b1 and a2b2), but not the recombinant a1b2 or a2b1 combinations. These results suggest that the ~141 kb region separating the A and B MAT loci does not suppress recombination as in the biallelic MAT locus of U. hordei (56).

Overall, our comparative genomic and polymorphism analyses are consistent with a pseudobipolar mating system for M. sympodialis, distinct from tetrapolar mating systems such as the one observed in U. maydis and from strict biallelic bipolar systems, such as in the species U. hordei (47, 48, 54, 56–59). The pseudobipolar mating system of M. sympodialis may have arisen recently from a tetrapolar ancestor, so that large-scale rearrangements between the two linked loci that erase flanking synteny have not yet occurred, in contrast to other derived bipolar species, such as U. hordei. Multiple independent transitions from an ancestral tetrapolar state to a bipolar state and possibly also a pseudobipolar derived state in the Basidiomyota have frequently been reported (47, 60). In this view, M. globosa could also represent a pseudobipolar state derived from a tetrapolar ancestor; however, evidence for multiallelism in isolates of this species is needed to confirm this hypothesis.

Genes involved in mating and meiosis. Pheromone response during mating in fungi is regulated through a MAP kinase cascade, coupled to a heterotrimeric G protein consisting of α, β, and γ subunits. The genes of the MAP kinase module (e.g., FUS3, STE7, STE11) and the receptor for a-factor pheromone (STE5) are generally conserved in Malassezia (Table 5; for a full list, see Table S4 in the supplemental material). In contrast, the G protein subunits show more diverged profiles: of the three (or four in U. maydis [61] and S. reilianum) Ga subunits present in filamentous ascomycetes and many basidiomycetes (GPA1–4) (62), only one is present in Malassezia species (Table 5). This protein is most closely related to Gpa3 from U. maydis, required for mating (61). The Gβ subunit, associated with mating in many fungi (for examples, see reference 63), is an ortholog of Ste4 of S. cerevisiae (64); homologs of this protein are present in U. maydis, S. reilianum, and M. globosa but not in the M. sympodialis genome (Table 5). However, mating is regulated in U. maydis by a WD-40 protein related to Gβ subunits, Rak1 (65); Rak1 is conserved in all the Ustilagomycotina, including Malassezia species (see Table S4). It is possible that this protein interacts with the single Gα (Gpa3) in Malassezia as part of the mating signaling response. We could not identify a Gγ subunit (Ste18) ortholog in either of the Malassezia species. However, Gγ proteins are small and poorly conserved and can be difficult to find in genomic sequences.

Of the 29 genes defined as “core” for meiosis in eukaryotes (66, 67), only 19 are unequivocally present in the Malassezia genomes (Table 5). However, many losses are not specific to these species. For example, in most organisms, strand invasion is promoted following formation of double-strand breaks (DSB) by the activity of two proteins that arose from a gene duplication event that preceded the evolution of eukaryotes: Rad51, required during recombination in meiosis and for repairing DSBs in somatic cells, and Dmc1, which functions only in meiosis (68). Similarly to the genomes of other Ustilagomycotina species (M. globosa, U. maydis, and S. reilianum), the yeasts Candida guilliermondii and Candida lusitaniae (28, 69), the microsporidian species Encephalitozoon cuniculi, Caenorhabditis elegans, and Drosophila melanogaster, the M. sympodialis genome contains only a Rad51 homolog, no Dmc1. The loss of the DMC1 gene is correlated with the absence of genes (see Table S4 in the supplemental material) coding for the assembly factors Sae3 and Mei5 (70, 71). It is therefore possible that Rad51 alone is required for recombination in Malassezia, as previously proposed for C. elegans and D. melanogaster (72). Initiation of meiotic recombination in eukaryotes requires the formation of double-strand breaks in DNA by a complex containing Spo11. Spo11 orthologs are conserved in almost all eukaryotes, apart from one lineage of protists (73). It was initially difficult to identify the Spo11 gene in M. sympodialis (MSY001_2221). However, preliminary RNA-Seq data (T. Holm and A. Scheynius, unpublished data) provided evidence for a gene structure that contains six introns, one of which has a noncanonical splice site. SPO11 is expressed at a low level in two-day cultures of M. sympodialis, suggesting that the organism has retained the capacity to undergo meiosis. A putative multi-intron SPO11 candidate is also found in the M. globosa genome (J. Xu and C. W. Saunders, unpublished data).

Most eukaryotic genomes contain two paralogs of the essential cohesion complex, namely, REC8 and RAD21; the corresponding proteins are required for cohesion of sister chromatids during mitosis and meiosis (66). Rec8 is a meiosis-specific component (74). Surprisingly, the Malassezia genomes contain only one paralog, and this is more closely related to RAD21 than to REC8 (see Fig. S6 in the supplemental material). Both paralogs are present in the other Ustilagomycotina (U. maydis and S. reilianum) and in other basidiomycetes (e.g., C. neoformans) (see Table S4 in the supplemental material). The loss of Rec8 is unusual in fungi; how-
ever, this protein is also missing from the protists (66). It is possible that Rad21, which plays a role in meiosis in mammals (75, 76), may substitute for Rec8. Other meiotic subunits of the cohesion complex (Smc1, Smc3, Scc3, and Pds5) are present in Malassezia and related basidiomycetes (see Table S4 in the supplemental material).

Overall, the Malassezia species have lost a substantial number of genes associated with mating and meiosis (Table 5; also, see Table S4 in the supplemental material). However, few of the gene losses are unique; rather, most are shared with other species with an extant sexual cycle. Examples include genes encoding proteins involved in formation of the synaptonemal complex (SC) (HOP1, ZIP2, ZIP3, and RED1). These genes are absent not only in *Malassezia* spp. (see Table S4) but also in *U. maydis* (77, 78), a species in which sexual reproduction is well documented, which may suggest that the *Ustilagomycotina* do not form SCs at all, similar to *Schizosaccharomyces pombe* and some *Candida* species (28, 79). The genomic evidence is consistent with the possibility that there may be a sexual cycle in Malassezia resembling that of other *Ustilagomycotina*.

**Sexual reproduction in Malassezia?** In this study, we identified independent lines of evidence for sexual reproduction in the Malassezia genus: (i) the presence of a MAT locus with apparently intact MAT alleles in the genomes of three *Malassezia* species (Fig. 5A) (6); (ii) evidence for recombination in a population of isolates of *M. sympodialis*, as shown by the discovery of segregating poly-

---

**Table 5** Genes involved in mating signaling and in basidiomycetes

| Process                                | Gene<sup>a</sup> | Presence<sup>b</sup> of gene in: |
|----------------------------------------|------------------|---------------------------------|
|                                        |                  | Msym | Mglo | Umay | Sreil | Cneo |
| Mating signaling                       | ASC1             | 1    | 1    | 1    | 1     | 1    |
|                                        | FUS3             | 1    | 1    | 1    | 1     | 1    |
|                                        | GPA1             | 0    | 0    | 1    | 1     | 1    |
|                                        | GPA2             | 0    | 0    | 1    | 1     | 1    |
|                                        | GPA3             | 1    | 1    | 1    | 1     | 1    |
|                                        | GPA4             | 0    | 0    | 1    | 1     | 0    |
|                                        | STE3             | 1    | 1    | 1    | 1     | 1    |
|                                        | STE4             | 0    | 1    | 1    | 1     | 1    |
|                                        | STE7             | 1    | 1    | 1    | 1     | 1    |
|                                        | STE11            | 1    | 1    | 1    | 1     | 1    |
|                                        | STE18<sup>d</sup> | ?    | ?    | 1    | 1     | 1    |
| Recombination and crossing over        | DMC1             | 0    | 0    | 0    | 0     | 1    |
|                                        | HOP1             | 0    | 0    | 0    | 0     | 1    |
|                                        | HOP2             | 0    | 0    | 0    | 0     | 1    |
|                                        | MND1             | 0    | 0    | 0    | 0     | 1    |
|                                        | MRE11            | 1    | 1    | 1    | 1     | 1    |
|                                        | RAD50<sup>e</sup> | 1    | 1    | 1    | 1     | 1    |
|                                        | RAD51<sup>e</sup> | 1    | 1    | 1    | 1     | 1    |
|                                        | RAD52<sup>e</sup> | 1    | 1    | 1    | 1     | 1    |
|                                        | SPO11            | 1    | 1    | 1    | 1     | 1    |
| Crossover resolution                   | MER3             | 0    | 0    | 1    | 1     | 1    |
|                                        | MSH2             | 1    | 1    | 1    | 1     | 1    |
|                                        | MSH4             | 0    | 0    | 1    | 1     | 1    |
|                                        | MSH5             | 0    | 0    | 1    | 1     | 1    |
|                                        | MSH6             | 1    | 1    | 1    | 1     | 1    |
|                                        | RAD1<sup>f</sup> | 1    | 1    | 1    | 1     | 1    |
| Cohesin complex                        | PDS5<sup>f</sup> | 1    | 1    | 1    | 1     | 1    |
|                                        | RAD21            | 1    | 1    | 1    | 1     | 1    |
|                                        | REC8             | 0    | 0    | 1    | 1     | 1    |
|                                        | SCC3             | 1    | 1    | 1    | 1     | 1    |
|                                        | SMC1             | 1    | 1    | 1    | 1     | 1    |
|                                        | SMC2             | 1    | 1    | 1    | 1     | 1    |
|                                        | SMC3             | 1    | 1    | 1    | 1     | 1    |
|                                        | SMC4             | 1    | 1    | 1    | 1     | 1    |
|                                        | SMC5             | 1    | 1    | 1    | 1     | 1    |
|                                        | SMC6             | 1    | 1    | 1    | 1     | 1    |
| Mismatch repair                        | MLH1             | 1    | 1    | 1    | 1     | 1    |
|                                        | MLH2             | 0    | 0    | 0    | 0     | 0    |
|                                        | MLH3             | 0    | 0    | 0    | 0     | 1    |
|                                        | PMS1<sup>g</sup> | 1    | 1    | 1    | 1     | 1    |

<sup>a</sup> Core meiosis genes are in bold.

<sup>b</sup> 1, presence; 0, absence. Msym, *M. sympodialis*; Mglo, *M. globoza*; Umay, *U. maydis*; Sreil, *S. reilianum*; Cneo, *C. neoformans*.

<sup>c</sup> The gene is present in the genome based on tBLASTn, but there is no model available.

<sup>d</sup> STE18 homologs were not found, but this might be due to the fact that they are small and poorly conserved.

<sup>e</sup> The RAD50 homolog in *M. globoza* is incorrectly split into two genes, MGL_0431 and MGL_0432.

<sup>f</sup> The PDS5 homolog is split into MGL_3630 and MGL_3631.

<sup>g</sup> The *M. sympodialis* gene model (MSY001_1319) might be an incorrect fusion of two genes, corresponding to *M. globoza* MGL_0016 and MGL_0017.
morphisms in the A and B regions of the \textit{MAT} locus (Fig. 5B; also, see Fig. S3 in the supplemental material); and (iii) conservation of genes required for meiosis and signaling in the mating process in both \textit{M. globosa} and \textit{M. sympodialis} (Table 5; also, see Table S4 in the supplemental material). Although some of the core meiotic genes are absent, these are not consistently defined as “core” genes in the literature (for example, see reference 72), and their absence does not necessarily suggest an absence of sex (80). For example, genes absent from \textit{Malassezia} but present in the other \textit{Ustilagomycotina} include \textit{MSH4}, \textit{MSH5}, and \textit{MER3}, required for the resolution of Holliday junctions during recombination in \textit{S. cerevisiae}. All three are also missing from sexual fungi such as \textit{C. guilliermondii}, \textit{C. lusitaniae} (28), and \textit{S. pombe} and from other eukaryotes with intact sexual cycles, including \textit{Plasmodium} and \textit{Drosophila} (66).

One way to confirm sexual reproduction and establish the role of \textit{MAT} alleles in this process would be to conduct mating assays for fertility. In an attempt to detect an extant sexual cycle, a collection of \textit{M. sympodialis} isolates (see the supplemental methods and Table S2 in the supplemental material) have been cocultured in pairwise and more complex mixtures under a variety of different media and environmental conditions (see the supplemental methods). However, despite these efforts, to date none of the morphological features associated with basidiomycete sexual reproduction (e.g., dikaryotic hyphae, clamp connections, basidia, and basidiospores) have been observed. Thus, the right combination of strains or conditions to detect an extant sexual cycle, if one exists, has not yet been established. The \textit{Malassezia} sexual cycle might differ morphologically from that of other fungi and could require genetic approaches with marked strains to detect responses to pheromones, cell-cell fusion, or genetic recombination. No sexual cycle is known for any \textit{Malassezia} species, but previous studies provide evidence for \textit{M. furfur} hybrids that may result from mating between varieties or cryptic species (81; T. Boekhout, personal communication). Furthermore, recombination was observed in allozyme studies of the species \textit{P. pachydermatis} (82).

\textbf{Concluding remarks.} In summary, the genome of \textit{M. sympodialis} reported here, combined with previous studies of the \textit{M. restricta} and \textit{M. globosa} genomes, provides a rich foundation for future studies to elucidate the unique features of these ubiquitous commensals of human skin associated with multiple disease states. Moreover, apparent transitions in the mating type locus suggest that much remains to be learned about how, when, and where sexual reproduction might occur. Sexual reproduction may occur on human skin, with implications for antigens presented by the yeast, which might provoke immune reactions, leading to disease. This study provides insight into a number of hypotheses previously proposed for \textit{Malassezia} species to become specialized to live on the skin as commensals but also to provoke disease.

\textbf{MATERIALS AND METHODS}

\textbf{DNA extraction.} DNA was isolated from \textit{M. sympodialis ATCC 42132 cultured on Dixon agar} (24) modified to contain 1% (vol/vol) Tween 60, 1% (wt/vol) agar, and no oleic acid (mDixon) at 32°C for 4 days using the QiAamp DNA minikit (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instructions with small modifications. Briefly, glass beads were added to the cell suspension, which was vortexed for 4 min prior to the lysing incubation at 56°C for 3 h with additional vortexing during the incubation period.

\textbf{Genome sequencing, assembly, and CEGMA analysis.} Pyrosequencing was performed using 454 Titanium chemistries (Roche/454 Life Sciences, Branford, CT). Illumina libraries were made with the Illumina mate pair kit (3-kb insert) according to the manufacturer’s instructions, followed by 50-bp paired-end sequencing on one lane of an Illumina HiSeq instrument. Genome assembly of the 454 data was accomplished with the GS De Novo assembler, v. 2.3 (Roche Diagnostics, Basel, Switzerland). Contig extension and scaffolding were based on the Illumina data using SPACE, v. 1.0 (83). We noted that a large fraction (~50%) of the Illumina mate-pair data in reality represented standard noncircularized paired ends, but no attempts to confirm or extend the scaffold structure by long-range PCRs were done at this point. The mitochondrial assembly was performed with Newbler 2.6, using a random subset of 40,000 reads from the 454 shotgun sequencing, representing ~25× coverage of the mitochondrial genome. Scaffolding and identification of the inverted repeat were aided by mapping of the Illumina 3-kb jumping library on the mitochondrial contigs. Finally, the genome was fully completed by manual identification and addition of 454 reads spanning the assembly gaps flanking the inverted repeat. CEGMA analysis was run using a set of 248 core eukaryotic genes (CEGs) as queries against the assemblies of \textit{M. sympodialis} and \textit{M. globosa}, with the completeness reported as a percentage reflecting the number of CEGs found as complete or partial genes, respectively.

\textbf{Gene predictions.} The genome of \textit{M. sympodialis} was annotated using the program MAKER versions 2.10 and 2.25 (84, 85). As evidence for gene annotations we used (i) protein alignments to a set of 67,086 publicly available proteins derived from the species \textit{M. globosa}, \textit{U. maydis}, \textit{C. neoformans}, \textit{Fusarium graminearum}, \textit{Magnaporthe grisea}, \textit{Neurospora discreta}, \textit{Neurospora tetrasperma}, and \textit{Sordaria macrospora} and clustered using Cd-hit (86) version 4.02 and a protein identity threshold of 90%; (ii) nucleotide alignments to 1,392 EST sequences coming from an \textit{M. globosa} sequenced library (accession number LIBEST_028020) previously used for gene predictions in \textit{M. globosa} (6); and (iii) the \textit{ab initio} predictors Augustus (87), using a \textit{U. maydis} model, GenenMark-E (88), trained with the \textit{M. sympodialis} scaffolds, and SNAP (89), trained within MAKER as follows. MAKER was run four consecutive times, and each annotation output file from MAKER (genome gift file) was converted into a model using the instructions from the SNAP documentation and provided as an input model in the next run. We complemented the predicted genes from the fourth MAKER run with (i) a set of 542 models, identified by protein alignments of the \textit{M. globosa} proteome against \textit{ab initio} models not retained by MAKER using the “pass-through” method implemented in MAKER version 2.25, and (ii) a few genes manually retrieved (e.g., \textit{SP011}, genes for \textit{M. globosa} 7-like proteins, and a pheromone gene) using tBLASTn and manual curation. The gene models from the above analyses have not been further curated. Mitochondrial assembly was performed with Newbler 2.6, using a random subset of 40,000 reads from the 454 shotgun sequencing, representing ~25× coverage of the mitochondrial genome. Scaffolding and identification of the inverted repeat were aided by mapping of the Illumina 3-kb jumping library on the mitochondrial contigs. The genome was completed by manual identification and addition of 454 reads spanning the assembly gaps flanking the inverted repeat. The mitochondrial DNA was annotated following steps described in reference 90; the mitochondrial map was generated using Geneious Pro, v.5.6.4 (Biomatters, Auckland, New Zealand). Details on identification of specific genes presented in tables are found in the supplemental methods.

\textbf{Mass spectrometry.} Four replicates of \textit{M. sympodialis} (ATCC 42132) were cultured on mDixon agar (see above) at 32°C for 2 and 15 days. Cells were harvested and washed twice with PBS (phosphate-buffered saline) by centrifugation at 1,200 g for 5 min. Pellets were frozen at ~80°C. To extract proteins for mass spectrometry analyses, 30 mg of every pellet was dissolved in 200 μl PBS and transferred to tubes containing 200 μl 425 to
600 μm acid-washed glass beads (Sigma-Aldrich, Sweden). The cells were disrupted by homogenization in a Preccells 24 tissue homogenizer (Bertin Technologies, France). Approximately 100 μl cell suspension from every sample was removed and transferred to a new tube and a 1:1 volume of lysis buffer was added to obtain a final concentration of 4% (wt/vol) SDS, 1 mM DTT (dithiothreitol), 25 mM HEPEs (pH 7.6). The samples were heated at 95°C for 5 min followed by sonication twice for 30 s each time. Protein concentration was determined with a DC protein assay (Bio-Rad, Sweden). Samples were subsequently reduced by dithiothreitol and alkylated by iodoacetamide followed by overnight trypsinization (Promega). The four biological replicates of each sample from the 2- and 15-day cultures were pooled and separated by immobilized pH gradient isoelectric focusing (IPG-IEF) on a narrow-range pH 3.7 to 4.9 strip and a 3- to 10-ml strip as described previously (91). Extracted fractions from the IPG-IEF were separated using an Agilent 1200 Nano-LC system coupled to a Thermo Scientific LTQ Orbitrap Velos. Proteome discoverer 1.3 with Sequest-Percolator (Thermo Scientific) was used to search predicted proteins or a 6-reading frame translation of the M. sympodialis genome merged with the Bos taurus database (UniProt canonical sequences, 120,727) for protein identification, limited to a false discovery rate of <1%. Peptide matches to the B. taurus database were considered remnants from the culture medium and removed.

**High-pressure freezing (HPF)-transmission electron microscopy (TEM).** HPF of Malassezia isolates was carried out as described previously (31). Briefly, samples were prepared by high-pressure freezing with an EMPACT2 high-pressure freezer and rapid transport system (Leica Microsystems Ltd., Milton Keynes, United Kingdom). After freezing, cells were freeze-substituted in substitution reagent (1% [wt/vol] OsO₄ in acetone) with a Leica EMAFS2. Samples were then embedded in Spurr resin and additional infiltration was provided under a vacuum at 60°C before etone) with a Leica EMAFS2. Samples were then embedded in Spurr resin parallel with a protein extract from M. sympodialis ATCC 42132 by PCR amplification using the polymerase noted above and primers listed in Table S3 in the supplemental material. The following cycling conditions were used: an initial denaturation at 98°C for 1 min followed by 35 cycles of 15 s at 98°C, 15 s at 64°C, and 2.5 min at 72°C and a final elongation step at 72°C for 7 min. The PCR products were purified using the QIAquick PCR purification kit (Qiagen) and sequenced at a verified core facility (KIGene, Karolinska Institutet, Stockholm, Sweden) using the same primers as the PCR amplification. Sequencing reactions were performed on an ABI 3730 Prism DNA analyzer (Applied Biosystems, Foster City, CA) using the BigDye terminator, v3.1 (Applied Biosystems). The retrieved forward and reverse sequences were aligned using Geneious software, version 5.5.7 (Biomatters Ltd.), and the resulting consensus sequences were used for further analysis. The copies of Malassezia s 7a and 7b were confirmed in M. sympodialis ATCC 42132 by PCR amplification using the polymerase noted above and primers listed in Table S3 in the supplemental material. The following cycling conditions were used: an initial denaturation at 98°C for 1 min followed by 35 cycles of 15 s at 98°C, 15 s at 64°C, and 2.5 min at 72°C and a final elongation step at 72°C for 10 min. The product was analyzed by electrophoresis in a 1% (wt/vol) agarose gel (Invitrogen, Groningen, Netherlands) with SYBR Safe DNA gel staining (Invitrogen) in 1× TAE (Tris-acetate-EDTA).

**Western blotting and biochemical assays of Malassezia s 6.** Total proteins (50 μg) from C. neoformans strains, including wild-type H99 and cpa1 and cpa1 cpa2 mutants (44), were fractionated by 18% (wt/vol) SDS-PAGE in parallel with a protein extract from M. sympodialis ATCC 42132 (97) and recombinant Malassezia s 6 (43). Proteins were transferred to PVDF (polyvinylidene difluoride) membranes (Bio-Rad) and incubated with an anti-Mala s 6 antibody (43). Membranes were developed using the enhanced chemiluminescence (ECL) advanced detection kit (Amersham). Pepsidylprolyl isomerization activity of Malassezia s 6 was assayed by an improved method described previously (45). Briefly, 1 ml reaction buffer containing 0.5 mg/ml chymotrypsin (Sigma) and 500 ng of the recombinant Malassezia s 6 protein (43) or 500 ng of the C. albicans Cyp1 cyclophilin A (Y. L. Chen and M. E. Cardenas-Corona, unpublished data) was pre-equilibrated to 10°C and then rapidly mixed in chilled cuvettes containing 10 μl of the substrate peptide (from a stock solution of 0.5 mM N-succinyl-Ala-Ala-Pro-Phe-nitroanilide [Sigma] dissolved in trifluoroethanol containing 470 mM LiCl). The cuvettes were immediately placed in the spectrophotometer, and the release of p-nitroanilide was monitored at 395 nm and 10°C with a Beckman DU-600 spectrophotometer. Cyclosporine A (LC Laboratories) from a 100 mM stock solution in methanol was added to 1-ml reaction mixtures at a final concentration of 1 μM prior to the addition of substrate peptide to test for inhibition of the enzyme activity.

**Identification of the Malassezia mating type (MAT) locus and sequencing of the region.** The genomic scaffolds containing the MAT locus were retrieved using BLASTn using as queries the M. globosa genes pral (MGL_0964), a putative pheromone gene (MGL_0963), bW1 (MGL_0883), and bE1 (MGL_0884). Whole-genome alignments between M. globosa and M. sympodialis genomes with Mummer (98) allowed identification of the three additional scaffolds located between the A (P/R) and B (HD) loci in M. globosa, which were not linked in the original M. sympodialis assembly. To further characterize the M. sympodialis MAT locus, we designed primers (see Table S3 in the supplemental material) to amplify the A and B regions from M. sympodialis isolates (see Table S2) cultured on modified Dixon agar (24) to containing 1% (wt/vol) peptone, 1% (wt/vol) desiccated ox bile, 1% (wt/vol) Tween 60, 2% (wt/vol) agar, and no oleic acid at 30°C for 4 days. Primers were designed based on alignments with M. globosa using Primer3 (99). The PCR products (~3 kb for the A locus and ~4 kb for the B locus) were purified on a 1% (wt/vol)
agarse gel using a Qiaquick gel DNA extraction kit and used for direct DNA sequencing by primer walking. Sequencing reactions were carried out at the Genome Sequencing & Analysis Core Facility at the Duke Institute for Genome Sciences & Policy (IGSP), at Eton BioScience (Research Triangle Park, NC) and at CBS Fungal Biodiversity Centre, Utrecht, The Netherlands. For details on linkage analysis of MAT loci, mating assays, and mating type identification, see the supplemental methods.

**Nucleotide sequence accession numbers.** The nuclear and mitochondrial genomes of *M. sympodialis* have been deposited in the EMBL database and were assigned accession numbers HE999549-HE999613 and HF558646, respectively. The EST data from *M. globosa* are accessible under accession number LIBEST_028020. ITS (internal transcribed spacer) and MAT locus sequences of different *M. sympodialis* isolates were deposited in GenBank under accession numbers JX964840 to JX964847, JX964880 to JX964802, and JX964848 to JX964850 (see Table S2 in the supplemental material).

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00572-12/-/DCSupplemental.

Text S1, PDF file, 0.6 MB.
Figure S1, PDF file, 0.1 MB.
Figure S2, PDF file, 0.3 MB.
Figure S3, PDF file, 0.1 MB.
Figure S4, PDF file, 0.2 MB.
Table S1, XLSX file, 0.1 MB.
Table S2, XLSX file, 0.1 MB.
Table S3, XLSX file, 0.1 MB.
Table S4, XLSX file, 0.1 MB.
Text S1, PDF file, 0.6 MB.

**ACKNOWLEDGMENTS**

This work was supported by the Swedish Research Council, the Center for Allergy Research Karolinska Institutet, the Cancer and Allergy Association, through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and the Karolinska Institutet, and by funds from Procter and Gamble and R01 grant AI50113 and AI50113 and R37 grant AI39115 from the NIH/NIAID to J.H. We acknowledge Science for Life Laboratory, Swedish National Infrastructure for large-Scale DNA Sequencing (SNISS), and the Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) for providing massive parallel sequencing and computational infrastructure. We are also grateful for the computational resources made available through the University of California, Riverside Bioinformatics Core in the Institute for Integrative Genome Biology.

We thank Gustav Wilkberg, Karolinska University Hospital, Stockholm, and Julie Segre and Keisha Findley, National Institutes of Health, for providing clinical samples of *Malassezia*, Christine Ribbing, Karolinska Institutet, for technical support with DNA extraction, Mario Heitman, Duke University, for technical assistance with DNA extraction, mating assays, and medium preparation, Gillian Milne, University of Aberdeen, for help with EM, Bart Theelen, CBS, for help in the identification of *M. sympodialis* isolates and their mating types, Eveline Gueho and the *Malassezia* Research Consortium (MRC) for useful insights, and Marco Coelho, Fred Dietrich, Michael Lorenz, and Ted White for helpful discussions.

We have no conflicts of interest. J.X., C.W.S., and T.L.D. are employed by Procter & Gamble.

**REFERENCES**

1. Nagata R, et al. 2012. Transmission of the major skin microbiota, *Malassezia*, from mother to neonate. Pediatr. Int. 54:350–355.
2. Ashbee HR, Scheynius A. 2010. *Malassezia*, p 209–230. In Ashbee HR, Bignell EM (ed), Pathogenic yeasts. The yeast handbook. Springer-Verlag, Berlin, Germany.
3. Saunders CW, Scheynius A, Heitman J. 2012. *Malassezia* fungi are specialized to live on skin and associated with dandruff, eczema, and other skin diseases. PLoS Pathog. 8:e1002701. http://dx.doi.org/10.1371/journal.ppat.1002701.
4. Hort W, Maysper P. 2011. *Malassezia* virulence determinants. Curr. Opin. Infect. Dis. 24:100–105.
5. Mittag H. 1995. Fine-structural investigation of *Malassezia furfur*. II. The envelope of the yeast cells. Mycoses 38:13–21.
6. Xu J, et al. 2007. Dandruff-associated *Malassezia* genomes reveal convergent and divergent virulence traits shared with plant and human fungal pathogens. Proc. Natl. Acad. Sci. U. S. A. 104:18730–18735.
7. Feldbrügge M, Kämper J, Steinberg G, Kammann R. 2004. Regulation of mating and pathogenic development in *Ustilago maydis*. Curr. Opin. Microbiol. 7:666–670.
8. Tiber T. 2008. Mechanisms of disease: atopic dermatitis. N. Engl. J. Med. 358:1483–1494.
9. Scheynius A, Cramer R. 2010. *Malassezia* in atopic eczema/dermatitis, p 212–228. In Boekhout T, Guého-Kellerman E, Maysper P, Velegraki A (ed), *Malassezia* and the skin. Springer-Verlag, Berlin, Germany.
10. Casagrande BF, et al. 2006. Sensitization to the yeast *Malassezia sympo- dialis* is specific for extrinsic and intrinsic atopic eczema. J. Invest. Dermatol. 126:2414–2421.
11. Akdis CA, et al. 2006. Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/ PRACTALL Consensus report. J. Allergy Clin. Immunol. 118:152–169.
12. Selander C, Zargari A, Möllby R, Rasool O, Scheynius A. 2006. Higher pH level, corresponding to that on the skin of patients with atopic eczema, stimulates the release of *Malassezia sympodialis* allergens. Allergy 61:1002–1008.
13. Parra G, Bradnam K, Korf I. 2007. CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. Bioinformatics 23:1061–1067.
14. Sugita T, et al. 2010. Epidemiology of *Malassezia*-related skin diseases, p 65–19. In Boekhout T, Guého-Kellerman E, Maysper P, Velegraki A (ed), *Malassezia* and the skin. Springer-Verlag, Berlin, Germany.
15. Kämper J, et al. 2006. Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. Nature 444:97–101.
16. Laurie JD, et al. 2012. Genome comparison of barley and maize smut fungi reveals targeted loss of RNA silencing components and species-specific presence of transposable elements. Plant Cell 24:1733–1745.
17. Schirawski J, et al. 2010. Pathogenicity determinants in smut fungi revealed by genome comparison. Science 330:1546–1548.
18. Bock R. 2007. Structure, function, and inheritance of plastid genomes, p 29–63. In Bock R (ed), Cell and molecular biology of plastids. Springer-Verlag, Berlin, Germany.
19. Valach M, et al. 2011. Evolution of linear chromosomes and multipartite genomes in yeast mitochondria. Nucleic Acids Res. 39:4202–4219.
20. Gerhold JM, Aun A, Sedman T, Jöers P, Sedman J. 2010. Strand invasion structures in the inverted repeat of *Candida albicans* mitochondrial DNA reveal a role for homologous recombination in replication. Mol. Cell 39:851–861.
21. Ma H, et al. 2009. The fatal fungal outbreak on Vancouver Island is characterized by enhanced intracellular parasitism driven by mitochondrial regulation. Proc. Natl. Acad. Sci. U. S. A. 106:12980–12985.
22. Olson A, Stenlid J. 2001. Plant pathogens: mitochondrial control of fungal hybrid virulence. Nature 418:438.
23. Xue J, et al. 2012. Mitochondrial sorting and assembly machinery subunit Sam37 in *Candida albicans* insight into the roles of mitochondria in fitness, cell wall integrity, and virulence. Eukaryot. Cell 11:532–544.
24. Gueho E, Midgley G, Guillot J. 1996. The genus *Malassezia* with description of four new species. Antonie Van Leeuwenhoek 69:337–355.
25. Gueho-Kellerman E, Boekhout T, Begerow D. 2010. Biodiversity, phylogeny and ultrastructure, p 17–76. In Boekhout T, Gueho-Kellerman E, Maysper P, Velegraki A (ed), *Malassezia* and the skin. Springer-Verlag, Berlin, Germany.
26. Baker LG, Specht CA, Donlin MJ, Lodge JK. 2007. Chitosan, the deacetylated form of chitin, is necessary for cell wall integrity in *Cryp- tococcus neoformans*. Eukaryot. Cell 6:855–867.
27. De Groot PW, Ram AF, Klis FM. 2005. Features and functions of covalently linked proteins in fungal cell walls. Fungal Genet. Biol. 42:657–675.
35. Vilhelmsson M, et al. 2013. Zargari A, et al. 2009. The a2 mating-type locus gene lga2 of Ustilago maydis interferes with mitochondrial dynamics and fusion, partially in dependence on a Dnm1-like fission component. J. Cell Sci. 122(14):2402–2412.

36. Schulz B, et al. 1990. The b alleles of U. maydis, whose combinations program pathogenic development, code for polypeptides containing a homeodomains-related motif. Cell 60:295–306.

37. Kämper J, Reichmann M, Romeis T, Bolker M, Kahmann R. 1995. Multiallelic recognition: nonself-dependent dimerization of the bE and bW homeodomain proteins in Ustilago maydis. Cell 81:73–83.

38. Butler G, et al. 2013. Allergen characterization of Malassezia sympodialis allergen Mala s 12 is a major allergen in patients with atopic eczema and has sequence similarities to the GMC oxidoreductase family. Allergy 62:695–703.

39. Vilhelmsson M, et al. 2007. Crystal structure of the major Malassezia sympodialis allergen Mala s 1 reveals a beta-propeller fold: a novel fold among allergens. J. Mol. Biol. 369:1079–1086.

40. Skibbe DS, Doehlemann G, Fernandes J, Walbot V. 2010. Maize tumors caused by Ustilago maydis require organ-specific genes in host and pathogen. Science 328:89–92.

41. Zargari A, Emilson A, Halldén G, Johansson S, Scheynius A. 1997. Cell surface expression of two major yeast allergens in the pityrosporum genus. Clin. Exp. Allergy 27:584–592.

42. Zargari A, Emilson A, Halldén G, Johansson S, Scheynius A. 1997. Cell surface expression of two major yeast allergens in the pityrosporum genus. Clin. Exp. Allergy 27:584–592.

43. Dyer RB, Plattner RD, Kendra DF, Brown DW. 2000. Cloning, characterization and expression of com- mon allergens from the skin of Malassezia furfur. J. Invest. Dermatol. 113:156–161.

44. Wang P, Cardenas ME, Cox GM, Perfect JR, Heitman J. 2000. Two unipolar, bipolar, and tetrapolar patterns of sexuality, p 97–160. PLoS Genet. 7:e1001052. http://dx.doi.org/10.1371/journal.pgen.0001025.

45. Kellner R, Vollmeister E, Feldbrügge M, Begerow D. Interspecific sex in grass Smuts and the genetic diversity of their pheromone-receptor system. PLoS Genet. 7:e1001052. http://dx.doi.org/10.1371/journal.pgen.1001052.

46. Schön C, Cossart P. 2009. The a2 mating-type locus gene Mal a 2 of Ustilago maydis: an essential gene with multiple roles in filamentous fungi. Annu. Rev. Microbiol. 63:467–477.

47. Steiner K, Heitman J, Koller A, Cardenas ME, Hall MN. 2008. An expanded inventory of conserved meiotic genes provides evidence for sex in Trichomonas vaginalis. PLoS One 3:e2879. http://dx.doi.org/10.1371/journal.pone.0002879.

48. Masson JY, West SC. 2001. The Rad51 and Dmc1 recombinases: a non-identical twin relationship. Trends Biochem. Sci. 26:131–136.

49. Riad L, Floyd AM, Heitman J. 2007. Mechanistic plasticity of sexual reproduction and meiosis in the Candida pathogenic species complex. Curr. Biol. 17:2827–2841.

50. Takayama K, Hayashi M, Miyazawa K, Kojima T, Takeuchi K. 2004. Orchestration of sexual reproduction and virulence by the fungal mating-type locus. Curr. Opin. Microbiol. 11:517–524.

51. Regenfelder E, et al. 1997. G proteins in Ustilago maydis: transmission of multiple signals? EMBO J. 16:1934–1942.

52. Li L, Wright SJ, Krystofova S, Park G, Borkovich KA. 2007. Hetero-trimeric G protein signaling in filamentous fungi. Annu. Rev. Microbiol. 61:423–452.

53. Stacey P, Wilting AW, Stefanaki LM, Schurko AM, Logsdon JM, Jr. 2008. An expanded inventory of conserved meiotic genes provides evidence for sex in Trichomonas vaginalis. PLoS One 3:e2879. http://dx.doi.org/10.1371/journal.pone.0002879.

54. Masson JY, West SC. 2001. The Rad51 and Dmc1 recombinases: a non-identical twin relationship. Trends Biochem. Sci. 26:131–136.

55. Reedly JL, Floyd AM, Heitman J. 2007. Mechanistic plasticity of sexual reproduction and meiosis in the Candida pathogenic species complex. Curr. Biol. 17:2827–2841.

56. Hayase A, et al. 2004. A protein complex containing Mei5 and Sae3 promotes the assembly of the meiosis–specific RecA homolog Dmc1. Cell 119:927–940.

57. Tsubouchi H, Roeder GS. 2004. The budding yeast Mei5 and Sae3 proteins act together with Dmc1 during meiotic recombination. Genetics 168:1219–1230.

58. Villeneuve AM, Hillers KJ. 2001. Whence meiosis? Cell 106:647–650.

59. Malik SB, Ramesh MA, Hulstrand AM, Logsdon JM, Jr. 2007. Protist homology of the meiotic Spo11 gene and topoisoisomerase VI reveal an evolutionary history of gene duplication and lineage-specific loss. Mol. Biol. Evol. 24:2827–2841.

50. Buonomo SB, et al. 2000. Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell 103:387–398.

51. Xu H, et al. 2004. A new role for the mitotic RAD21/SCC1 cohesin in meiotic chromosome cohesion and segregation in the mouse. EMBO Rep. 5:378–384.

52. Prieto I, et al. 2002. STAG2 and Rad21 mammalian mitotic cohesins are implicated in meiosis. EMBO Rep. 3:543–550.

53. Donaldson ME, Saville BJ. 2008. Bioinformatic identification of Ustilago maydis meiosis genes. Fungal Genet. Biol. 45(Suppl 1):S57–S53.

54. Holloman WK, Schirawski J, Holliday R. 2008. The homologous re-
combination system of *Ustilago maydis*. Fungal Genet. Biol. 45(Suppl 1):S31–S39.

79. Loidl J. 2006. *S. pombe* linear elements: the modest cousins of synaptonemal complexes. Chromosom 115:260–271.

80. Ramesh MA, Malik SB, Logsdon JM. 2005. A phylogenomic inventory of meiotic genes: evidence for sex in *Giardia* and an early eukaryotic origin of meiosis. Curr. Biol. 15:185–191.

81. Theelen B, Silvestri M, Guého E, van Belkum A, Boekhout T. 2001. Identification and typing of *Malassezia* yeasts using amplified fragment length polymorphism (AFLPTm), random amplified polymorphic DNA (RAPD) and denaturing gradient gel electrophoresis (DGGE). FEMS Yeast Res. 1:79–86.

82. Midreuil F, et al. 1999. Genetic diversity in the yeast species *Malassezia pachydermatis* analysed by multilocus enzyme electrophoresis. Int. J. Syst. Bacteriol. 578–579.

83. Cantarel BL, et al. 2004. Gene finding in novel fungal genomes using an ab initio algorithm with unsupervised training. Genome Res. 14:215–225.

84. Li W, Godzik A. 2004. MUSCLE: a fast program for clustering and comparison of large sets of protein or nucleotide sequences. Bioinformatics 20:1276–1283.

85. Larkin MA, et al. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948.

86. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797.

87. Yang Z, Nielsen R. 2000. Estimating synonymous and nonsynonymous substitution rates under realistic evolutionary models. Mol. Biol. Evol. 17:32–43.

88. Guindon S, et al. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59:307–321.

89. Leeming JP, Notman FH. 1987. Improved methods for isolation and enumeration of *Malassezia furfur* from human skin. J. Clin. Microbiol. 25:2017–2019.

90. Zargari A, Midgley G, Bäck O, Johansson SG, Scheynius A. 2003. IgE-reactivity to seven *Malassezia* species. Allergy 58:306–311.

91. Kurtz S, et al. 2004. Versatile and open software for comparing large genomes. Genome Biol. 5:R12.

92. Rozen S, Skaletsky H. 2000. Primer3 on the WWW for general users and for biologist programmers. Methods Mol. Biol. 132:363–386.

93. Carver TJ, et al. 2005. ACT: the Artemis comparison tool. Bioinformatics 21:3422–3423.

94. Ruiz-Herrera J, Ortiz-Castellanos L, Martinez AI, León-Ramírez C, Sentandreu R. 2008. Analysis of the proteins involved in the structure and synthesis of the cell wall of *Ustilago maydis*. Fungal Genet. Biol. 45(Suppl 1):S71–S76.

95. Guindon S, et al. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies. Mol. Biol. Evol. 27:578–579.

96. Kurtz S, et al. 2004. Versatile and open software for comparing large genomes. Genome Biol. 5:R12.

97. Stanke M, Waack S. 2003. Gene prediction with a hidden Markov model and a new intron submodel. Bioinformatics 19(Suppl 2):ii215–ii225.

98. Ter-Hovhannisyan V, Lomsadze A, Chernoff YO, Borodovsky M. 2008. Gene Finding in Novel Fungal Genomes Using an ab Initio Algorithm with Unsupervised Training. Genome Res. 18:188–196.

99. Holt C, Yandell M. 2011. MAKER2: an annotation pipeline and genome-database management tool for second-generation genome projects. BMC Bioinformatics 12:491.

100. Li W, Godzik A. 2006. Cd-hit: a fast program for clustering and comparison of large sets of protein or nucleotide sequences. Bioinformatics 22:1658–1659.

101. Stanke M, Waack S. 2003. Gene prediction with a hidden Markov model and a new intron submodel. Bioinformatics 19(Suppl 2):ii215–ii225.

102. Eriksson H, et al. 2008. Quantitative membrane proteomics applying narrow range peptide isoelectric focusing for studies of small cell lung cancer resistance mechanisms. Proteomics 8:3008–3018.

103. Larkin MA, et al. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948.