Exploring the genomic diversity of black yeasts and relatives (Chaetothyriales, Ascomycota)

M.M. Teixeira 1,2,3, L.F. Moreno 3,11,14,21, B.J. Stielow 3, A. Muszewwska 4, M. Hainaut 5, L. Gonzaga 5, A. Abouelleil 7, J.S.L. Patané 6, M. Priest 8, R. Souza 8, S. Young 7, K.S. Ferreira 8, Q. Zeng 9, M.M.L. da Cunha 10, A. Gladki 8, B. Barker 7, V.A. Vicente 11, E.M. de Souza 11, S. Almeida 11, B. Henriissat 12, A.T.R. Vasconcelos 12, S. Deng 12, H. Voglmayr 13, T.A.A. Moussa 14,15,16, A. Gorbushina 19, M.S.S. Felipe 21, C.A. Cuomo 22, and G. Sybren de Hoog 22

1Division of Pathogen Genomics, Translational Genomics Research Institute (TGen), Flagstaff, AZ, USA; 2Department of Cell Biology, University of Brasilia, Brasilia, Brazil; 3Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; 4Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; 5Université Aix-Marseille (CNRS), Marseille, France; 6The National Laboratory for Scientific Computing (LNCC), Petropolis, Brazil; 7Broad Institute of MIT and Harvard, Cambridge, USA; 8Department of Biochemistry, University of São Paulo, Brazil; 9Department of Biological Sciences, Federal University of São Paulo, Diadema, SP, Brazil; 10Núcleo Multidisciplinar de Pesquisa em Biologia UFRJ-Xerém-NUMPEX-BIO, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; 11Department of Basic Pathology, Federal University of Paraná State, Curitiba, PR, Brazil; 12Department of Biochemistry and Molecular Biology, Federal University of Paraná, Curitiba, PR, Brazil; 13Department of Clinical and Toxicological Analysis, University of São Paulo, São Paulo, SP, Brazil; 14Institute of Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands; 15Shanghai Institute of Medical Mycology, Changzheng Hospital, Second Military Medical University, Shanghai, China; 16Department of Systematic and Evolutionary Botany, University of Vienna, Vienna, Austria; 17Biological Sciences Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia; 18Botany and Microbiology Department, Faculty of Science, Cairo University, Giza, Egypt; 19Federal Institute for Material Research and Testing (BAM), Berlin, Germany

*Correspondence: C.A. Cuomo, cuomo@broadinstitute.org; G. Sybren de Hoog, s.hoog@westerdijkinstitute.nl
21These authors contributed equally to the manuscript.

Abstract: The order Chaetothyriales (Pezizomycotina, Ascomycetes) harbours obligatorily melanised fungi and includes numerous etiologic agents of chromoblastomycosis, phaeohyphomycosis and other diseases of vertebrate hosts. Diseases range from mild cutaneous to fatal cerebral or disseminated infections and affect humans and cold-blooded animals globally. In addition, Chaetothyriales comprise species with aquatic, rock-inhabiting, ant-associated, and mycoparasitic life-styles, as well as species that tolerate toxic compounds, suggesting a high degree of versatility extremotolerance. To understand their biology and divergent niche occupation, we sequenced and annotated a set of 23 genomes of main human opportunists within the Chaetothyriales as well as related environmental species. Our analyses included fungi with diverse life-styles, namely opportunistic pathogens and closely related saprobes, to identify genomic adaptations related to pathogenesis. Furthermore, ecological preferences of Chaetothyriales were analysed, in conjuncture with the order-level phylogeny based on conserved ribosomal genes. General characteristics, phylogenomic relationships, transposable elements, sex-related genes, protein family evolution, genes related to protein degradation (MEROPS), carbohydrate-active enzymes (CAzymes), melarin synthesis and secondary metabolism were investigated and compared between species. Genome assemblies varied from 25.81 Mb (Capronia coronata) to 43.03 Mb (Cladophialaphora immunda). The bantiana-clade contained the highest number of predicted genes (12 817 on average) as well as larger genomes. We found a low content of mobile elements, with DNA transposons from Tc1Mariner superfamily being the most abundant across analysed species. Additionally, we identified a reduction of carbohydrate degrading enzymes, specifically many of the Glycosyl Hydrolase (GH) class, while most of the Pectin Lyase (PL) genes were lost in etiological agents of chromoblastomycosis and phaeohyphomycosis. An expansion was found in protein degrading peptidase enzyme families S12 (serine-type D-Ala-D-Ala carboxypeptidases) and M38 (isoaspartyl dipeptidases). Based on genomic information, a wide range of abilities of melanin biosynthesis was revealed; genes related to metabolically distinct DHN, DOPA and pyomelanin pathways were identified. The MAT (Mating Type) locus and other sex-related genes were recognized in all 23 black fungi. Members of the assexual genera Fonsecaea and Cladophialaphora appear to be heterothallic with a single copy of either MAT-1-1 or MAT-1-2 in each individual. All Capronia species are homothallic as both MAT1-1 and MAT1-2 genes were found in each single genome. The genomic synteny of the MAT locus flanking genes (SLA2-APN2-COX13) is not conserved in black fungi as is commonly observed in Eurotiomycetes, indicating a unique genomic context for MAT in those species. The heterokaryon (het) genes expansion associated with the low selective pressure at the MAT locus suggests that a parasexual cycle may play an important role in generating diversity among those fungi.

Key words: Black yeast, Comparative genomics, Chaetothyriales, Ecology, Evolution, Herpetrichiellaceae, Phylogeny.

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INTRODUCTION

The order Chaetothyriales (Pezizomycotina, Ascomycetes) harbour many melanised, non-lichenised fungi with a large morphological diversity. The order is included in the subclass Chaetothyriomycetidae along with the lichenised orders Verrucariales, Pyrenulales, and Clocothriales. Within the Chaetothyriales, at least five families are recognized: Chaetothyriaceae, Cyphellophoraceae, Epiphyllaceae, Herpetrichiellaceae, and Trichomeriaceae (Batista & Ciferni 1962, Réblöva et al. 2013), while some clades are as yet unassigned. The members of Chaetothyriales exhibit a complex ecological variation, and species are found in habitats characterised by extreme and adverse conditions, e.g. on rock surfaces in hot, arid climates, in toxic niches with hydrocarbons and heavy metals, and remarkably often occur in vertebrates as opportunistic pathogens (de Hoog 2014). Some species cause mutilating or even fatal infectious diseases, often in apparently healthy individuals. Recent studies sequenced rDNA from a large number of undescribed melanised fungi from ant colonies that clustered in various families of Chaetothyriales.
The asexual morphs of members of Chaetothyriales show large morphological diversity, whereas the sexual morph shows limited variation over the entire order. Some genera produce budding cells or are entirely yeast-like, and hence the order is often referred to as “black yeasts and relatives” (BY) (Fig. 1).

The family Chaetothyriaceae contains species that generally are epiphytes, growing on the surface of plant leaves, but it is still unclear whether those species are plant pathogens or symbionts. The mycelium resides on the surface of plant leaves without truly penetrating the host plant cuticle (Chomnunti et al. 2012b). Members of this family are mainly distributed in tropical regions and are characterised by producing a sooty melanised mycelium resembling a loose network of hyphae covering the substrate. Ascomata are formed below the mycelial web and are easily released from the plant cuticle. Asexual Chaetothyriaceae are only reported for genera Chaetothyrium (Merismella) and Ceramothyrium (Stanhugesia) (Hyde et al. 2011).

The family Herpotrichiellaceae harbours a vast diversity of polyphyletic asexual morphs, which include both saprobic species on plant debris and clinically important species (Fig. 1) (Untereiner & Naveau 1998). Among the latter are causative agents of chromoblastomycosis, phaeohyphomycosis, disseminated infections, and primary cebritis (McGinnis 1983, Garnica et al. 2009). Main asexual genera are Cladophialophora, Exophiala, Fonsecaea, Phialophora, and Rhinocladiella, which all include opportunistic pathogens that cause a wide array of clinical syndromes in cold- and warm-blooded vertebrates (Crous et al. 2007, Seyedmousavi et al. 2013). Most species reproduce asexually with conidia generated by a filamentous

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**Fig. 1.** Phylogenomic distribution of Chaetothyriales and related ascomycetes used for comparative genomics. The majority of species are placed in the families Cyphellophoraceae (C) and Herpotrichiellaceae (H). The main characteristics such as niche, isolation source (red boxes – anthropophilic pathogens, orange boxes, zoophilic pathogens and green boxes geophilic), anamorphs, teleomorphs and sexual locus organization are displayed for each compared species.
phase, while members of the genus *Exophiala* show yeast-like budding. Occasionally meristematic growth is observed (Fig. 1) (de Hoog et al. 2011). Muriform cell segmentation is the unique invasive form inside host tissue in chromoblastomycosis (*da Silva et al. 2002, 2008*). *Capronia* is the homothallic sexual genus covering all asexual members of *Herpotrichiellaceae*. Ascomata are setose containing 8–32-spored ascii; ascospores are pale to dark brown and are generally transversally septate or muriform (Untereiner 1995). Species of the family are generally found in nutrient-poor habitats such as showers and sinks in bathrooms or washing machines and dishwashers (Hamada & Abe 2010, Lian & de Hoog 2010, Zalar et al. 2011, Zupancic et al. 2016), while some thrive in extreme environments such as on rocks or in toxic niches (Badali et al. 2011, Seyedmousavi et al. 2011, de Hoog 2014). A significant number of ant-associated undescribed species from carton galleries is also affiliated with this family (Voglmayr et al. 2011, Nepel et al. 2014).

The family *Cyphellophoraceae* is a small monophyletic group of species which are known through their asexual morphs only (Réblóvá et al. 2013). Conidial heads may be hyaline and one-celled, but several species have pale brown, curved conidia with thin cross walls. Conidiogenous cells are inconspicuously phialidic and are cylindrical and intercalary, or swollen and lateral. This family includes mild opportunists on human skin and nails in *Cyphellophora* and *Philophora* (Fig. 1) (Feng et al. 2012, Gao et al. 2015).

The family *Trichomeriaceae* is composed by epiphytic species (Chomnunti et al. 2012a) and a large clade of rock-inhabiting species recently added (Isola et al. 2016). Remarkably also the genus *Arthrocladium* clusters in the family, known for a single strain causing a fatal disseminated human infection (Nascimento et al. 2016a). The single sexual morph in the family is the genus *Trichomerium*, which morphologically is very similar to *Capronia* above (Chomnunti et al. 2012a). *Trichomerium* was first placed within the *Chaetothyriaceae* on the basis of morphological similarities of sooty mould-like mycelium, but later a separate family was erected using improved phylogenetic analyses (Chomnunti et al. 2012a). Ascomata of the *Trichomerium* species are spherical, covered by long, scattered setae, and contain 8-spored ascii with septate, often brownish ascospores. Recently, phylogenetic studies also added some paraphyletic taxa, which morphologically are very deviant, such as the asexual species *Brycekendrickomyces acaciae* (Crous et al. 2009). Also, some simple morphology known in the *Herpotrichiellaceae* is recurrent in the *Trichomeriaceae* in *Cladophialophora modesta* and *Cl. protea* (Badali et al. 2008). Meristematic, non-sporulating species were classified in the genera *Kunzia* and *Lithophila*, a group of largely rock-inhabiting species (with the exception of the lichenicolous species *Knufia petitgeae*) within the *Trichomeriaceae* (Isola et al. 2016). Numerous undescribed species of ant-associated fungi characterised by sooty mould-like mycelium are also contained within this family (Voglmayr et al. 2011, Nepel et al. 2014).

A recently proposed family is *Epibryaceae* (Gueidan et al. 2014), covering the genus *Epibryon* and the asexual morph *Leptomeilola ptldii*, as well as some more simply structured asexual morphs that morphologically are classified as *Cladophialophora sylvestris*, *Cl. humicola* and *Cl. minutissima* (Badali et al. 2008, de Hoog et al. 2011, Gueidan et al. 2014). Several species are bryophilous fungi, but some have a rock-inhabiting life style, or occur in soil or on vascular plants. Ascomata are located superficially on or penetrating leaf tissue. Straight or curved dark setae cover globose to oval or pyriform, ostiolate, pale to dark brown to black ascomata, and the 8-spored ascii are oval, ellipsoidal or subcylindrical, without apical structures and containing transversely septate, ellipsoidal to fusiform ascospores (Döbbeler 1997, Gueidan et al. 2014).

Of the families of *Chaetothyriales*, the *Herpotrichiellaceae* species exhibit highly diversified life styles and show recurrent infection of a variety of vertebrate hosts (de Hoog 2014). Often opportunistic behaviour in human patients is partly explained by a saprobic behaviour combined with thermotolerance, as in *Mucorales* where resistance to high temperatures – often associated with other types of extremotolerance – is classically viewed as a prime virulence factor (Scholer et al. 1983). Opportunistic species often possess dynamic and versatile pathways to sequester carbon from a wide range of substrates in the environment. By chance, when an opportunistic pathogen colonises its host, the abundance and diversity of genes associated with acquiring energy from particular carbon sources might be an advantage. Thus, metabolic plasticity combined with tolerance of adverse conditions could be considered as virulence factors in opportunistic fungi.

In *Herpotrichiellaceae*, warm- as well as cold-blooded vertebrates with intact immunity are commonly affected (Seyedmousavi et al. 2014), suggesting the presence of intrinsic virulence factors that are independent from temperature. This led us to perform a comparative genome approach in order to comprehend the general background of ecology-driven traits, adaptation to harsh and toxic environments, and association with vertebrate hosts. The phylogeny of the family *Herpotrichiellaceae* has been intensively investigated for several years by multi-locus sequence analyses based on ITS, *TEF1*, *BT2*, and *ACT1*, and occasionally with other genes. de Hoog et al. (2011) recognised six approximate clades, which showed somewhat different ecological trends (Fig. 1). The europaee-clade located in the basal position has recently been upgraded to family level as *Cyphellophoraceae* (Réblóvá et al. 2013). The jeanselmei-clade is basal to the *Herpotrichiellaceae* s.s. and contains several clinically relevant species, next to species which were often derived from environments rich in toxic monoaromatic hydrocarbons (Zeng et al. 2013). The dermatitidis-clade contains thermophilic *Exophiala* species from hot, low-nutrient water systems, sometimes causing disseminated infections in humans (de Hoog et al. 2011). The salmonis-clade harbours mainly mesophilic water-borne *Exophiala* species, often infecting aquatic animals such as fish and amphibians, but rarely humans (de Hoog et al. 2011). The two remaining clades comprise the major agents of phaeohyphomycosis and chromoblastomycosis, but species can also be found in the environment on plant debris (Salgado et al. 2004, Vicente et al. 2008). The carrioni-clade harbours some species that consistently cause chromoblastomycosis and which may perhaps be regarded as primary human pathogens (de Hoog et al. 2007). The same pattern is observed in the bantiana-clade, which harbours *Fonsecaea* and *Cladophialophora*, with an abundance of species causing serious human diseases (de Hoog et al. 2011, Najafzadeh et al. 2011a, b, Sun et al. 2012) as well in *Rhinocladiella mackenziei*. The trends in all clades are approximate since pathogenic species are often flanked by free-living species. Also *herpotrichiellacean* asexual and sexual morph genera are polyphyletic, but as yet molecular phylogeny is too unstable to replace morphology-based taxonomy (Untereiner 1995, Untereiner & Naveau 1998, Haase et al. 1999, de Hoog et al. 2011).
The origin of Chaetothyriales is estimated at approximately 229 MYA during the Middle Triassic (Gueidan et al. 2011). It has been suggested that the Permian–Triassic (P–T) mass extinction, which deeply affected terrestrial and marine ecosystems, led to the development of a thermotolerant life-style on rock, possibly in association with toxin-producing lichens. After this, a rapid diversification of Chaetothyriales took place. In this vision, extreme- and thermotolerance, and an efficient metabolism of carbon sources are atavisms from this period (Gueidan et al. 2011). The five families proposed in Chaetothyriales all contain a number of basal rock-inhabiting species with epiphytic or epilithic growth, suggesting a common origin of these life styles (Gueidan et al. 2014).

The dark colouration of chaetothyrialean mycelium is determined by the high production of melanin pigments, which was shown to contribute to the above discussed ecological niches as well contributing to resistance against host immune responses (Schnitzler et al. 1999, Zhang et al. 2013). The presence of melanin alone is not sufficient to explain pathogenicity as these polymers are known to be present in many Pezizomycotina, and additional factors discussed above may be involved to explain the pathogenic status of these fungi. The virulence of opportunistic black yeasts has been suggested to have evolved from adaptations to extreme environments, e.g. melanisation (Schnitzler et al. 1999, Feng et al. 2001), meristematic growth (Mendoza et al. 1993, Karuppayil & Szaniszlo 1997), and general extremotolerance (Liu et al. 2004). Application of concepts of “focused” virulence and “dual ecology” may be considered for chaetothyrialean fungi to explain their ability to infect vertebrate hosts (Casadevall et al. 2003). Although the source of many black fungal infections are plant-debris and occasionally living plants, their association as common degraders of plant biomass could be a misconception. In order to understand the basic biology of Herpotrichiellaceae, their phenominal adaptation to extreme environments, and mechanisms associated with infection of vertebrate hosts we sequenced the genomes of 23 type species and compared them to related pathogens in Eurotiiales and Onygenales (Fig. 1). The general genomic characteristics (i.e., genome size, synteny, gene content, repetitive elements), phylogenomic tree, transposable elements, sex-related genes, gene family expansions and contractions, evolution of protein- and carbohydrate-degrading genes, and secondary metabolism were deeply investigated in order to understand processes of adaptation of Chaetothyriales to multiple environments.

MATERIALS AND METHODS

rDNA LSU phylogeny

Phylogenetic assessment was carried out for all 172 black yeast fungal strains deposited at the CBS-KNAW Fungal Biodiversity Centre (CBS), Utrecht, The Netherlands (Table S1). LSU rDNA sequences were retrieved from GenBank and aligned by means of MAFFT v. 7.273 (Katoh & Standley 2013). Isolates and GenBank accession numbers are listed in Table S1. Phylogenetic analyses using Maximum Likelihood (ML) and a Neighbour-Joining (NJ) were performed by MEGA v. 6 (Tamura et al. 2013) with Kimura 2-parameter model and statistical bootstrapping procedure involving 500 replications.

Strains, DNA and RNA extraction

A set of 23 black fungal ex-type strains was obtained from CBS-KNAW Fungal Biodiversity Centre and cultivated in Malt Extract Broth (MEB) for 7 d with shaking at 150 rpm at 25 °C (Table S2). DNA extraction was performed via a cetyltrimethylammonium bromide (CTAB)-based method and phenol-chloroform/isooamyl alcohol purification (Möller et al. 1992). Total DNA was purified with Qiagen Genomic Buffer Set and the Qiagen Genomic-tip 100/G. Total RNA was isolated with RNEASY Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. The additional strain Cl. carnii KSF (dH 23894) DNA was obtained from 7-d-old mycelia cultured on Sabouraud Glucose Agar (BBL™) at 25 °C. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) according to manufacturer protocols.

Genome assembly and gene prediction and annotation

The genome of Cl. carnii KSF (dH 23894) was pyrosequenced using the platform 454 GS FLX (Roche). Shotgun and 3Kb paired-end libraries were sequenced using the GS FLX Titanium XLR70 chemistry (~450 bp reads). This genome was assembled using the NEWBLER software combining both paired-end and shotgun libraries (Margulies et al. 2005). The P. atae genome was sequenced and annotated as previously described (Mooro et al. 2015). For the other 21 species, the genomes were sequenced using Illumina technology. The genome of E. dermatitidis was previously described (Chen et al. 2014). For the 20 remaining species, genomic DNA was used to construct two libraries with approximate insert size of 180 bp and 3 kb; for F. multimorphosa only a 180 bases-insert library was constructed. Each library was sequenced on an Illumina HiSeq 2000 to generate 101 base paired-end reads. All sequence was assembled using Ailpaths (version R48559 for most assemblies); assemblies were inspected for regions of aberrant coverage, % GC, or sequence similarity using GAEMR (www.broadinstitute.org/software/gaemr) and contaminating sequence including was removed.

Genes were predicted and annotated by combining calls from multiple methods. A training set was generated using Genewise (Binney et al. 2004) and Genemark (Lomsadze et al. 2005), and then GlimmerHMM (Majoros et al. 2004), Snap (Korf 2004) and Augustus (Stanke & Waack 2003) was used to generate ab initio gene models. For seven species, strand-specific libraries were constructed from total RNA using the Illumina TruSeq RNA Library prep. For each species, paired 76 base reads were generated on an Illumina HiSeq 2000. RNA-Seq was assembled using Trinity (Grabherr et al. 2011) (version r20140413p1) in genome-guided mode (with parameters genome_guided_max_intron 10000 – SS_lib_type RF – trimmomatic – min_kmer_cov 2). All assembled transcripts were aligned to the genome using PASA (Haas et al. 2003) and used to update gene models, predict alternatively spliced transcripts, and add UTR predictions. In addition, any ORF present in the PASA transcripts that did not overlap a gene prediction was used to recover missed genes. The best gene model at a given locus was selected from these data sets using EVidenceModeler (EVM) (Haas et al. 2008); conserved genes missing in gene sets were identified using OrthoMCL (Li et al. 2003) and combined with the EVM set (Haas et al. 2008). All raw sequence data, assemblies,
and annotations were submitted to NCBI (Finn et al. 2010) (Table S2).

Annotation of transposons

In order to ensure a robust detection of repeat element, we used inverted repeat finder (IRF) (Warburton et al. 2004) and Repeat Modeler (http://www.repeatmasker.org/RepeatModeler.html). IRF was set to identify pairs of repeats within a given of 20 kb. False positives candidates were filtered using the reference Pfam profile (using pfam_scan.pl with E-value threshold 0.00001) and RPS-BLAST against CDD profiles (with E-value threshold 0.001) (Finn et al. 2010, Marchler-Bauer et al. 2011). Multiple overlapping hits, were removed by cd-hit (Fu et al. 2012) clustering with sequence similarity threshold set to 100 and query coverage set to 99 % of the shorter sequence. The resulting customized reference was merged with RepBase and used as input for Repeat Masker searches (Jurka et al. 2005). All resulting sequences were translated in six frames and searched against a fixed list of reference Pfam HMM (Hidden Markov Model) profiles (using pfam_scan.pl with E-value threshold 0.01) and RPS-BLAST against CDD profiles (with E-value threshold 0.001). Transposon classification was curated manually based on the encoded protein domains.

Annotation of CYP genes

Identification of Cytochrome p450 monoxygenases (CYPs) were carried out by HMMR v. 3.1 (Finn et al. 2011) which was used to perform sequence-profile HMM searches with the PFAM (Finn et al. 2010) profile PF00667 (downloaded from the PFAM protein families database, http://pfam.xfam.org/, last accessed September 16, 2014) against all 23 black yeast proteomes. Proteins that achieved the cut-off 1e−03 were submitted to BLASTP searches against the fungal p450 CYPs database (Nelson 2009) (http://blast.uthsc.edu). The predicted CYPs p450 were assigned to family and subfamily types based on their BLASTP sequence identity. As recommended by the International P450 Nomenclature Committee, the cut-off of sequence identity was set at 40 % for family and 55 % for subfamily levels. Partial CYP p450 sequences (BLASTP identity >40 % and coverage <40 %) were classified as potential pseudogenes.

Annotation of transporter genes

Transporter gene classification was achieved with best match BLASTP (E-value threshold 1e−05, and at least 50 % alignment-length coverage) to transporter sequences available at Transporter Classification Database (TCDB) (Saier et al. 2014).

Single-copy orthologue extraction and species tree inference

Clustering of single-copy orthologues across multiple fungal species was performed using ORTHOMCL (Li et al. 2003) version 1.4 with a Markov inflation index of 1.5 and a maximum e-value of 1 × 10−5. Individual amino-acid sequences were aligned with MUSCLE (Edgar 2004) and poorly aligned regions were automatically removed using TRIMAL (Capella-Gutierrez et al. 2009) under the “−automated1” setting. The sequences were concatenated with FASCONCAT (Kuck & Meusemann 2010) v. 1.0 and species trees were inferred by maximum likelihood RAXML (Stamatakis 2006) using PROTGAMMA-BLOSUM62 and 1000 bootstraps was used to infer branch support. Beyond the 23 herein analysed black yeast-like fungi, the following outgroups from the orders Eurotiales and Onygeales were applied: Trichophyton rubrum, Coccidioides immitis, Paracoccidioides brasiliensis, Aspergillus nidulans and A. fumigatus (Fig. 1).

Genome-scale chaetothyrialean phylogeny and divergence times

The phylogenomic position of Chaetothyriales was inferred based on 264 single-copy orthologous protein clusters identified among 53 fungal species as mentioned above. Concatenated amino-acid sequences were aligned using MUSCLE (Edgar 2004). In order to select the most-reliable positions in the alignment, TRIMAL (Capella-Gutierrez et al. 2009) was used to eliminate poorly aligned regions (-automated1 option) resulting in 124 693 amino acid positions in the final alignment. Phylogenetic tree and branch lengths were inferred by Maximum Likelihood via a stochastic algorithm implemented in IQ-TREE software (Nguyen et al. 2015). Best-fit amino acid model selection was assessed using an automatic model selection (MODELFINDER) and also considering the FREERATE model (-m TESTNEW option), which assesses the fit of multiple of multiple mixture GTR within the same model, in many cases having a better fit when compared to models that use a single parametric distribution (Soubrier et al. 2012). Phylogenetic branch support was inferred by the ultrafast bootstrap approximation approach (UFBOOT), a measure that is better correlated to the actual probability of existence of a branch than the usual bootstrap (Minh et al. 2013). Divergence times were inferred using the RELTIME method (Tamura et al. 2012) implemented in the MEGA 7 software (Kumar et al. 2016) using the LG model (Le & Gascuel 2008). Batrachochytrium dendrobatidis (Chytridiomycota) was used as outgroup and three calibration constraints were considered for divergence time estimations: (1) Basidiomycota/Ascomycota split: 390–1 490 MYA; (2) Pezizomycetes crown: 230–970 MYA; and (3) Sordariomycetes stem: 210–890 MYA. Those calibrations were based on conservative intervals considering both primary (fossil) and secondary calibrations discussed in Lucking et al. (2009). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories; +G, parameter = 0.6655).

Functional domain prediction gains and losses

To identify functional domain gains and losses, INTERPRO (Mitchell et al. 2015) domains were predicted using INTERPROSCAN (Jones et al. 2014b) in all 23 black yeast-like species and in the outgroups with previously released genomes: Trichophyton rubrum, Coccidioides immitis, Paracoccidioides brasiliensis (order Onygeales), Aspergillus nidulans and Aspergillus fumigatus (order Eurotiales). Gene family evolution was estimated with CAFÉ (De Bie et al. 2006) v. 3.0 using a significance family-wide p-value threshold of <0.05 and VITERBI p-values of <0.001. To search for birth (A) values we ran the program with the “−a” option. Two files were used as input in CAFÉ analyses: a table containing the organism’s number of
Fig. 2. Phylogenetic analysis of members of Chaetothyriales (Class Eurotiomycetes). The Maximum likelihood tree, based on 172 LSU sequences, was determined using MEGA v. 6 with Kimura 2-parameter model with default settings and statistical bootstrapping procedure involving 500 replicates. Bootstrap values above 70 % are shown at the nodes. Family boundaries are indicated with coloured blocks. The tree was rooted to Verrucula granulosaria AFTOL-ID 2304.
Fig. 2. (Continued).
copies of each INTERPRO domain and an ultrametric tree constructed based on the species tree using a custom R script.

RESULTS

Phylogeny

The aligned LSU dataset of 172 black fungal strains was used to determine a phylogenetic tree of the entire order Chaetothyriales; the LSU gene was sufficiently conserved to allow confident comparison over the entire dataset. Both Maximum Likelihood and Neighbour-joining analyses produced corresponding trees in which the same clades were supported (Fig. 2). Moreover, the tree topology was congruent with previously reported phylogenies of Chaetothyriales (Réblová et al. 2013, Gueidan et al. 2014), supporting the presence of five distinctive families: Chaetothyriaceae, Cyphellophoraceae, Epipharyngiaceae, Herpotrichiellaceae, and Trichomeriaceae. In the most represented family Herpotrichiellaceae, the species were resolved in six clades with different ecological preferences as reported by de Hoog et al. (2011). Overall, this family included several clinically relevant fungi as well as species isolated from a variety of environmental sources, especially sites contaminated with toxic monoaromatic hydrocarbons. Two sub-clades at family level resolution were identified within Herpotrichiellaceae: The upper clade harbour most of the Exophiala and Rhinocladiella asexual morphs while the lower clade is overrepresented by the genus Fonsecaea and Cladosphialophora. Similarly, the family Cyphellophoraceae, which forms a supported monophyletic group, harbours both saprobes and medically important species responsible for mild opportunistic infections in human and animals. In contrast, the majority of the isolates belonging to the family Trichomeriaceae have an inert surface-inhabiting life style, while several are epiphytic. Arthrocladium fulminans encoded proteins such as reverse transcriptase (RT domain -IPR00477). Despite the low incidence of repetitive elements in BY genomes, we detect several TEs in the bantiana- and jeanselmei-clade. As presented in our previous work (Teixeira et al. 2003), which could contribute to their thermotolerance (Nishio et al. 2003). This corresponds with low single dinucleotide repetitions found in BY genomes.

Using ORTHOMCL clustered proteins, we determined the protein core families that were conserved in all black yeasts under investigation and other related fungi. This resulted in 4031 genes per genome in the core set conserved in all species (Fig. 3). The KOG annotation for these amino-acid sequences revealed that proteins responsible for housekeeping functions, particularly for translation and RNA processing, were more represented in the core set (Fig. S2). We also assessed the proteins specific to each clade. We considered as clade-specific proteins those proteins that were present in orthologous groups found in a unique clade but were absent from all others. Non-core proteins may provide insight into specific processes and may be indicative of certain ecological preferences. For example, enzymes related to metabolism of carbohydrate (G) were found to be over-represented in the jeanselmei-clade (p-value = 1e−04; Fisher’s exact test). Similarly, enzymes associated to secondary metabolites (Q) were found to be enriched in the bantiana- (p-value = 3e−13; Fisher’s exact test), salmonis- (p-value < 1e−08; Fisher’s exact test) and jeanselmei-clades (p-value = 7e−08; Fisher’s exact test).

On the other hand, the dermatitidis-clade proteins were under-represented for these functions (G: p-value 2–e01; Q: p-value 9–e02; Fisher’s exact test) suggesting a reduced secondary metabolite producing capacity (Fig. S2).

Genome assembly and annotation

The assemblies were highly contiguous, with 12 consisting of 19 or fewer scaffolds, suggesting that many correspond to complete chromosomes. Genome assembly size varied from 25.8 Mbp for Capronia coronata CBS 617.96 to 43.3 Mbp for Cladosphialophora immunda (CBS 834.96; Table S2, Fig. S1). Repetitive element identification was considered particularly low (ranging from 0.03 % to 5.2 %; Table S3) compared to other fungal species (Galagan et al. 2003; Martinez et al. 2012; Teixeira et al. 2014). This suggests that repeat content might not play an important role in determining genome size in black yeast-like fungi.

Genes were predicted combining de novo reconstruction of transcriptomes from RNA-seq data for some species and with ab initio and sequence homology based gene models. Corresponding with genome assembly sizes, high gene counts were found in Capronia coronata (9231 predicted genes) and Cladosphialophora immunda (14033 predicted genes) (Table S2, Fig. S1). However, we did not observe a phylogenetic correlation between genome size and total gene number in the species examined (Fig. S1). Species of the jeanselmei- and bantiana-clades mostly experienced an increase in genome size as well as in predicted Open Reading Frames (ORFs) compared to ancestral populations (Fig. S1). Exceptions were E. aquamarina CBS 119918 with 41.7 Mb, while E. sideris CBS 121828 had a size of 29.5 Mb, as small as members of the carrionii-clade and similar to those of the dermatitidis-clade. In contrast, members of the dermatitidis-clade experienced a notable decrease in genome size and gene content (Fig. S1). Within Ascomycota, BY genomes had the highest percentages of G+C content reported to date, i.e., varying from 49 % in E. aquamarina to 54.3 % in Cl. carrionii, which could contribute to their thermotolerance (Nishio et al. 2003). This corresponds with low single dinucleotide repetitions found in BY genomes.

Transposable elements

The members of the families Herpotrichiellaceae and Cyphellophoraceae have low content of transposable elements (Fig. S3, Table S3). Prevention of accumulation of transposable elements in BY genomes might be driven by the hyper-mutation process of repeat-induced point mutation (RIP). The scarcity of transposable elements results in decreased abundance of transposon encoded proteins such as reverse transcriptase (RT domain -IPR00477). Despite the low incidence of repetitive elements in BY genomes, we detect several TEs in the bantiana- and jeanselmei-clades, especially of the DNA Transposons LINE and theLTRsretrotransposons when compared to remaining clades (Fig S3). Rhinocladiella mackenziei, not assigned to any clade, also contained a higher number of elements with some specific expansions, such as the Helitron class (Table S3). The E. aquamarina genome presented the highest number of TEs (5.2 %), possibly reflecting its relatively significant genome expansion compared to other BY genomes (Fig. S3). It has been described previously that eukaryotic genomes of moderate sizes tend to have a linear correlation between complexity and genome size (Metcalfe & Casane 2013). Black yeast moderate genome sizes correlate well with the scarcity of repeats.
Within the Onygenales, which are generally related to animal hosts either as saprophytes or pathogens, there are organisms with small compact genomes and others with expanded complex genomes. The transposon-rich Ajellomycetaceae (Blastomyces, Histoplasma, and Paracoccidioides) and Onygenaceae (Coccidioides) compared to dermatophyte Arthrodermataceae (Trichosporon, Arthroderma, Microsporum), which have streamlined genomes with single repeats. The opportunistic Onygenales seem to have a more diverse TE landscape whereas specialised dermatophytes reduced their genomes. Blastomyces transposons have expanded up to 63% of the genomes in a low GC genomic environment (Muñoz et al. 2015). Lower GC is expected to favour mobile element integration (Wicker et al. 2007). Additionally during genome duplication often mobile elements proliferate (Ma et al. 2009).

Vma1-a inteins reveal a new evolutionary trend

We detected the presence of self-cleaving parasite proteins of the MEROPS N09 family, nested within Asparagine Peptide Lyases among some of the BY genomes. The N09 domain is commonly found within intein-containing V-type proton ATPase catalytic subunit A in several species of yeasts and genera of Archaea, i.e. Thermoplasma and Pyrococcus (Perler 2002) (vma-1a and vma-1b inteins, respectively) (Perler et al. 1994, Liu 2000). This mobile element is spliced out from host protein sequences (or exteins) after its translation through an autocatalytic process. This parasite domain, which was suggested to have been acquired by a process of horizontal gene transfer (HGT), has a high sequence similarity with Archaea/Bacteria because of its convergent evolution along the fungal tree of life (Goddard & Burt 1999, Poulter et al. 2007, Swithers et al. 2013). We detected the presence of vma-1a class intein in the R. mackenziei (Z518_00231) and F. pedrosoi (Z517_06303) genomes, suggesting a broader distribution within Ascomycetes (Fig. S4). We extended our analysis throughout other Pezizomycotina using INBASE (Perler 2002), and the vma-1a intein was also found in members of Sordariomycotina, i.e. Sporothrix schenckii, S. brasiliensis, and Stachybotrys chartarum genomes, bringing the total number of non-yeast species with
inteins herein to five. In contrast, this self-cleaving protein is widely distributed among Saccharomycotina. Those five Pezizomycotina species are nested in a monophyletic clade apart from remaining species of Saccharomyces, which may represent a new class of this element. The Host V-type proton ATPase protein, splicing and DOD homing endonuclease motifs were all identified and conserved with Candida glabrata vma 1-a (Fig. S4). However, the DOD homing endonuclease motif blocks D and E do not seem to be conserved with vma 1-a in Saccharomycetales. On the other hand, motif blocks D and E appear to be highly conserved with those present in the PRP8 intein among Pezizomycotina (data not shown). Here we report on additional vma-1a intein, showing that they are found in more diverse fungal species within Pezizomycotina. V-ATPases are in general responsible for acidification of a variety of intracellular compartments, especially the vacuolar membrane vesicles of Eukaryotes. These mobile genetic elements are self-spliced due stress adaptation (Senejani et al. 2001, Topilina et al. 2015, Novikova et al. 2016) and may play an important role in the regulation of extremotolerance of many BYs.

Origin of black yeast species and divergence times

A multigene phylogenetic species tree for a broad panel of 53 fungal species was generated using 264 single-copy orthologues. Representatives of chaetothyrialean families other than Herpotrichiellaceae and Cyphellophoraceae are still missing. With this limitation, two groups are sufficiently remote to conclude that as yet the order Chaetothyriales harbours two monophyletic families, Herpotrichiellaceae and Cyphellophoraceae which ancestry is found around 130 MYA during the cretaceous period (Fig. 4). Based on morphological and molecular methods with conserved genes, Coniosporium apollinis has previously been placed early in the Chaetothyriales. However, using a genome-scale phylogenetic tree, we demonstrated that this fungus is more closely related to the order Botryosphaeriales in the Dothideomycetes (Fig. 4). The Chaetothyriales are close to Verrucariales and Phaeomoniellales. These orders are displayed as paraphyletic branches and compose, along with Onygenales and Eurotiales, the subphylum Eurotiomycetes. Judging from the calibrated phylogenetic tree, the early and major BY lineages of Herpotrichiellaceae and Cyphellophoraceae are contemporaneous and emerged around 75–50 MYA during/after the Cretaceous-Paleogene (K-Pg) extinction event (Fig. 4). It is worth noting that the radiation of Chaetothyriales took place more recently than that of Onygenales.

Gene family evolution in black yeast

INTERPROSCAN was used to identify protein domains in all 23 black yeasts and in related fungi in Eurotiomycetes, including Trichophyton rubrum, Coccidioides immitis, Paracoccidioides brasiliensis (order Onygenales), and Aspergillus nidulans and Aspergillus fumigatus (order Eurotiales). The species tree was inferred by maximum likelihood RAxML (Stamatakis 2006) based on the concatenated amino acid sequences of 4 031 single-copy orthologous genes shared by all 23 species. Domain gain events (expansions) and domain loss events (contractions) were estimated with CAFÉ (De Bie et al. 2006) in each black yeast and ancestral node of the species tree. The dynamic evolution of protein domain families in black yeast is shown in Fig. 5A.

Overall, we observed 46 genomic novelties associated with protein domain expansions and contractions, which arose early in the evolution of these fungi and that were present in the common ancestor of Chaetothyriales examined (Table S4). We speculate these expanded domain families have provided selective advantage and extremotolerance for adaptation to ecological niches that are subjected to environmental stress. Black yeasts are known for their extremotolerance and are able to grow and thrive in hostile habitats, such as those containing toxic compounds, high and low temperature, scarcity of nutrients, or conditions of dryness (Gostincar et al. 2010). We assessed a correlation between the seven domain family expansions and the ecological preferences in herpotrichiellaceous black yeast. These functional domains are likely to be involved in metabolic processes with oxidoreductase activity. Among them, four domains are related to Aldehyde dehydrogenases (ALDHs), which catalyse the oxidation of different aldehydes to their corresponding carboxylic acids (Perozich et al. 1999). Since several aldehydes are toxic at low levels, this vast repertory of ALDHs present in BYs is likely to play a role in diverse reactions supporting extremotolerance. Three domains are related to zinc-containing alcohol dehydrogenase (Adh), which catalyse the oxidation of alcohols to their corresponding acetaldehyde or ketone. The IPR013154 and IPR013149 correspond, respectively, to the N-terminal and C-terminal portions of this enzyme and IPR011032 represents an oligomeric molecular chaperone associated with the N-terminal region involved in the folding protein process (Walter 2002). Adh are thought to be proteins prone to evolutionary changes following gene duplication due to their ability to assume new functions as consequence of their broad spectrum of substrates (Piskur et al. 2006, Conant & Wolfe 2008). Furthermore, a physiological role of Adh has been reported in many biochemical pathways including stress tolerance, pathogenicity, detoxification, and substrate specificity (Piskur et al. 2006, Grahl et al. 2011). As expected for a fungal family with many members tolerating extreme conditions, the expansion of alcohol dehydrogenase domains in the black yeast from a common ancestor may have determined the diversification of these organisms in a range of ecological niches. Another important domain expansion verified in the common ancestor of all black yeasts analysed was the trichothecene efflux pump (IPR010573), which might have been important in black yeast to colonize sites contaminated with this class of compounds.

Analysis focussing on individual clades revealed that the bantiana- and carrioni-clades, which have pronounced trends in vertebrate infection, evolved in opposite directions (Fig. 5B). Several domains expanded in the bantiana-clade appeared to be reduced in the carrioni-clade. This would suggest that specific expansions in the bantiana-clade are attributed to ecological preferences in these organisms. However, the clades contain Fonsecaea pedrosoi and Cladophialophora carrioni, respectively, which cause the same disease, chromoblastomycosis, with the same invasive form, the muriform cell, and which thus do not share specific domains.

We did not observe expansions exclusive to the dermatidios-clade. Previously domain expansions attributed to Exophiala dermatitidis (Chen et al. 2014), such as IPR002656 and IPR020843, were also found expanded in members of the jeanesmei- and salmonis-clades. Unlike truly pathogenic fungi possessing a specialized thermosensitive tissue phase
Fig. 4. Genome-scale of chaetothyrialean phylogeny and divergence times. Calibration points are highlighted in blue and were used to infer the divergence times for Chaetothyriales (upper panel). The red node displays the divergence dates of Chaetothyriales and the red asterisk bolded area highlights a common era for both Cyphellophoraceae and Herpotrichiellaceae. The bottom scale presents the main geological and periods and eras.

Fig. 5. Dynamic evolution of protein families. (A) Phylogenetic tree showing the relationship between species and altered protein families. Pie diagrams and numbers at the nodes represent the abundance of contractions (red) expansion (blue) and No change (black) of 1771 protein families during evolution of black yeasts. (B) Heatmap showing expansion and contractions of protein families found in species belonging to the bantiana- and carrionii-clades, respectively. Domains are grouped by category similarity. All domains shown are significantly changed, and were identified using CAFE with cut-off of family p-values <0.05 and Viterbi p-values <0.01.
Cytochrome p450 expansion and diversification

Cytochrome p450 genes (CYPs) play a fundamental role in primary, secondary, and xenobiotic metabolism (van den Brink et al. 1998). Due to their participation in a large number of detoxification reactions as well as in the metabolism of specific xenobiotics which may be co-assimilated as carbon source, CYPs are thought to be critical for the colonization of new ecological niches (Moktali et al. 2012). In fungi, point mutation and overexpression of CYP family-specific genes have been found to be responsible for drug resistance (Lamb et al. 1997, Lupetti et al. 2002, Ma et al. 2006). The evolution of fungal pathogenesis is thought to be associated with CYP family expansion and diversification through gene duplication. Our CYP prediction analysis revealed an extraordinary p450 repertoire in black yeast-like fungi ranging from 231 predicted CYPs in Cladosiphialophora psammophila to 60 predicted CYPs in Capronia coronata (Table 1). Notably, Cl. psammophila was found in a hydrocarbon-polluted environment (Badali et al. 2011), while Ca. coronata is a coloniser of decorticated wood in nature (Müller et al. 1987). A comparison of the predicted number of CYPs to those of other species in the Fungal Cytochrome P450 Database (FCPD) (Park et al. 2008) showed that some black yeasts are among the Ascomycota species with the highest number of CYPs (Fig. 6).

A total of 2740 CYP sequences were clustered in 131 families (Table S5) and 175 subfamilies according to their amino acid sequences (BLASTP coverage >40 %) were classified as potential pseudogenes due to the occurrence of premature stop codons or presence of frameshifts (Table S6). These sequences are shorter than their homologues in other fungi. Potential pseudogenes were not included in downstream analysis. Comparative analyses revealed striking differences and expansions across the black yeast-like fungi in a range of CYP p450 families. We observed notorious CYP family expansions, mainly, but not exclusively, in species belonging to the bantiana-clade (CYP530, CYP682, CYP504, and CYP52) (Table S5). These CYP families potentially affect the metabolism of phenolic compounds and aromatic hydrocarbons (Olivera et al. 1994, Cox et al. 1996, Lin et al. 2011, Moktali et al. 2012, Zhang et al. 2012). Our findings are consistent, to some extent, with previous studies showing that some black yeasts appear to have adapted to grow in environments polluted with aromatic hydrocarbons (Woertz et al. 2001, Prenafeta-Boldú et al. 2002, 2006, Zhao et al. 2010a).

Particularly important due to its abundance in some black yeast species, CYP530 is thought to participate in the degradation of several fatty acids and hydrocarbons (Moktali et al. 2012). This CYP was found ranging from 13 copies in Cladosiphialophora psammophila and Fonsecaea pedrosoi to complete loss in Cl. yegresi (Table S7). The phylogenetic tree of CYP530 revealed multiple recent duplications and expansions. In addition, we observed two monophyletic clades likely correspond to distinctive subfamilies of CYP530 (Fig. S5). This gene redundancy observed might have been used to guard the above described critical functions as was shown in other fungi (Skamnioti et al. 2008). To the best of our knowledge, the 13 copies is the highest rate of CYP530 reported in the fungal Kingdom (Table S7). Since Cladophialophora yegresi was only isolated from thorns of living cactus and was able to grow as an endophyte in cactus tissue (Zeppenfeldt et al. 1994, de Hoog et al. 2007), it might be speculated that the absence of genes involved in secondary metabolism, such as CYP530, may implicate a biotrophic lifestyle where the organism obtains essential nutrients from its host.

At the subfamily level, we verified that the housekeeping genes CYP51F (encoding lanosterol 14α-demethylase) and CYP61A (encoding sterol delta22-desaturase), which are implicated in sterol biosynthesis (Yoshida & Aoyama 1984, Podust et al. 2001, Lepesheva et al. 2008, Park et al. 2011) comprise one of the most conserved subfamilies across black yeast-like fungi. Azole antifungal agents interacting with CYP51 lead to growth inhibition and the death of fungal cells due to an ineffective conversion of lanosterol to ergosterol (Yoshida 1988, Kelly et al. 1997). It has been demonstrated that additional copies of, as well as point mutations in, the CYP51 gene may lead to acquisition of resistance in fungi (Sanglard et al. 1998, Jones et al. 2014a). Our analyses revealed that most species have two CYP51F copies, whereas members of the dermatitidis-clade, Rhinocladiella mackenziei and the outgroups Coniosporium apollinis and Verrucosis gallopava, have a unique CYP51F gene (Fig. S6). The important Y136F mutation (Mullins et al. 2011, Jones et al. 2014a) associated with CYP51 copy number variation and involved in azole resistance was not identified in these genes. This suggests that the tolerant allele, responsible for the azole resistance, is acquired only in the presence of azole fungicides. CYP61A was found in a single copy in all genomes studied.

Aromatic compound metabolism

Comparative analyses revealed that the genes PHA and HGD are organized in a syntenic cluster with at least six additional conserved genes (Fig. S7). We verified that this gene cluster organisation was retained by natural selection in most Herpotrichiellaceae. Besides the PHA and HGD genes, this cluster includes a variable number of genes coding for hypothetical proteins, an MFS transporter, a trehalose-6-phosphate hydrolase (T6P-hydrolase), and a fumarylacetoacetase (Fig. S7). T6P has been linked to diverse roles, such as energy source, protectant against stress of heat, freezing, starvation, dehydrogenation, and desiccation (Wiemken 1990, Iturriaga et al. 2009), and is important in fungal pathogenicity (van Dijck et al. 2002, Petzold et al. 2006, Ngamskulrungroj et al. 2009). The presence of PHA, HGD, and fumarylacetocetase in this cluster overlaps the styrene degradation pathway, which might support the involvement of these genes in the degradation of aromatic compounds (Fig. S7). The MFS transporter may be involved in energy production transporting simple sugars across the mitochondrial membrane. As the synteny of these genes is highly conserved in several black yeast-like fungi, we hypothesize that the cluster configuration was probably acquired by their common ancestor, and subsequent gene rearrangement resulted in the current gene order and orientation in the extant species.

Secondary metabolism

Fungal secondary metabolites (SMs) are natural products important for the colonization of specific ecological niches.
Despite their wide variation, all secondary metabolites are produced by a few common biosynthetic pathways and classified according to the enzyme involved in their biosynthesis: polyketides (PKS), non-ribosomal peptides (NRPS), terpenes and indole alkaloids (Keller et al. 2005). We identified a large number of potential gene clusters for secondary metabolite present in black yeast (Table 2). The majority of these biosynthetic clusters correspond to PKS I/III (101 clusters), terpene (91 clusters) and NRPS (61 clusters), although it was verified that some species possess hybrid clusters (Table 2). In addition, the PKS III cluster was found only in Chaetothyriales since Coniosporium apollinis and Verruconis gallopava lack such gene cluster.

**Table 1. Overview of Cytochrome p450 in black yeasts.**

| Clade                | Species                  | Strain      | # CYP | # Family | # Subfamily | # CYP not assigned |
|----------------------|--------------------------|-------------|-------|----------|-------------|--------------------|
| jeanselmei-clade     | *Exophiala xenobiotica*  | CBS 118157  | 164   | 62       | 39          | 41                 |
|                      | *E. spinifera*           | CBS 89968   | 122   | 56       | 28          | 30                 |
|                      | *E. oligosperma*         | CBS 725.88  | 131   | 52       | 30          | 30                 |
|                      | *E. sideris*             | CBS 121828  | 97    | 40       | 23          | 25                 |
| dermatitidis-clade   | *E. dermatitidis*        | CBS 525.76  | 62    | 24       | 27          | 9                  |
|                      | *Capronia epimyces*      | CBS 656.96  | 99    | 40       | 32          | 15                 |
|                      | *C. coronata*            | CBS 617.96  | 60    | 25       | 19          | 16                 |
| Rhinocadiella mackenziei-clade | *Rhinocadiella mackenziei* | CBS 650.93 | 161   | 56       | 46          | 44                 |
| carnionii-clade      | *Cladophialophora carnionii* | CBS 160.54 | 101   | 37       | 31          | 29                 |
|                      | *C. yegresi*             | CBS 114405  | 88    | 34       | 26          | 26                 |
|                      | *C. seminissima*         | CBS 27337   | 109   | 44       | 28          | 36                 |
| bantiana-clade       | *Fonsecaea pedrosoi*     | CBS 271.37  | 164   | 70       | 38          | 38                 |
|                      | *F. multiformosa*        | CBS 102226  | 165   | 67       | 44          | 40                 |
|                      | *C. immunda*             | CBS 834.96  | 144   | 51       | 38          | 37                 |
|                      | *C. bantiana*            | CBS 173.52  | 175   | 68       | 42          | 48                 |
| salmonis-clade       | *E. aquamarina*          | CBS 119918  | 179   | 68       | 36          | 51                 |
|                      | *E. mesophila*           | CBS 402.95  | 75    | 39       | 19          | 19                 |
| Cyphellophoraceae     | *Philophora europaea*    | CBS 101466  | 117   | 49       | 28          | 33                 |
|                      | *P. atro*               | CBS 131968  | 135   | 59       | 32          | 37                 |
|                      | *Coniosporum apollinis*  | CBS 100218  | 77    | 37       | 25          | 8                  |
| Outgroup             | *Verruconis gallopava*   | CBS 437.64  | 84    | 39       | 23          | 14                 |

![Fig. 6. Distribution of CYP p450 genes in Ascomycota. TOP 10 fungal species with highest CYP p450 numbers in ascomycetous genomes, based on search against the Fungal Cytochrome P450 Database (FCPD).](image)

**Melanin synthesis**

Fungi may produce melanin via distinct pathways: the eumelanin via the DHN and DOPA pathways, and the pyomelanins via L-tyrosine degradation pathway (Langfelder et al. 2003). Recently, homologues of these three pathways have been identified in the pathogenic black yeast *Exophiala dermatitidis* and in other filamentous fungi (Youngchim et al. 2004, Chen et al. 2014). Similarly, we found that members of Herpotrichiellaceae possess several melanin-associated genes, suggesting they would be able to produce melamins using all different pathways, as was also suggested for *Fonsecaea monophora* (Li et al. 2016). Unlike
other filamentous fungi, where the melanin genes are frequently encoded in biosynthetic gene clusters (Kimura & Tsuge 1993, Woo et al. 2010), we did not verify this organisation in black yeast-like fungi.

The dark polymer 1,8-dihydroxynaphthalene (DHN) melanin is produced via the DHN-melanin pathway and believed to be the best characterised fungal melanin biosynthetic pathway. Comparative analyses between previously released melanin-associated genes (Chen et al. 2014) and our dataset revealed that many, but not all black yeasts possess homologues for the production of melanin by the DHN pathway (Table S8). The equally dark-pigmented outgroup Verruconis gallopava and Coniosporium apollinis outside of or basal to the Chaetothyriales showed the highest number of missing genes, including the known multicopper oxidases (MCOs) required for melanin biosynthesis. This suggests that the DHN-melanin pathway has been better conserved among the Herpotrichiellaceae and Cyphellophoraceae. However, MCOs with low similarity to known and well-characterised enzymes have been reported in fungi (Tamayo-Ramos et al. 2012) and additional knowledge about their enzymatic properties is required to elucidate the DHN-melanin pathway in these species.

Similar to the DHN pathway, DOPA-melanin pathway homologues were identified across all black yeast-like species. Of particular interest was the high number of tyrosinases and laccases found in Herpotrichiellaceae, but not in the outgroups Verruconis gallopava and Coniosporium apollinis (Table S8). The presence of multiple laccases only in Herpotrichiellaceae supports a diversification of this enzyme that occurred late in the evolution of black yeasts. A possible explanation for the presence of multiple laccase genes would be the various functions that have been attributed to this enzyme other than pigmentation, such as degradation of organic pollutants and lignin, and stress tolerance (Baldrian 2006, Rodriguez Couto & Toca Herrera 2006).

Protein degradation

The overall counts of the main MEROPS (Rawlings 2010, Rawlings et al. 2014) peptidase families revealed the abundance of serine- (S) and metallo- (M) peptidase families in Chaetothyriales (Table S9). With the exception of the dermatididi-clade, members of both Herpotrichiellaceae and Cyphellophoraceae presented specific and significant number of S09 (S09X sub-family), S33, and M38 families according to CAFÉ analysis (Fig. S8). S09X and S33 families appear to be significantly depleted in Eurotiales and Onygenales, while these proteins might play an important role in the ecology of Chaetothyriales (Muszewska et al. 2011). Cluster analysis of sequences from the S09X family revealed that most BY protein expansions were found in two different clusters (Fig. S8). Protein sequence classification showed that S09X corresponds to alpha/beta hydrolase fold-3 (IPR013094/ PF07859) and proteins containing a carboxylesterase type B (IPR002018/PF00135) domain (Fig. S9). Gene tree reconstruction showed that main gene duplication events were at the basis of the M38 (IPR002018/PF00135) domain expansion in BY, while several losses in Eurotiales and Onygenales explain the relative accumulation of IPR013094/PF07859 proteins in BY (Fig. S9). According to several authors, the expansion of metalloproteases M35 and M36 could be associated with mammal-host association (Sharpton et al. 2009, Martinez et al. 2012, Whiston & Taylor 2015). According to MEROPS classification, the M35 and M36 protein families are depleted in Cyphellophoraceae and absent among Herpotrichiellaceae (Fig. S9). On the other hand, we detected an expansion of M36 proteins in BY, which may be associated with β-aspartyl dipeptidase acting in the release of iso-aspartate residues from peptides as characterised for bacteria (Borek et al. 2004). Cluster analysis of the M38 family revealed that most of the BY protein expansions were found to be enriched in the highlighted three clusters (Fig. S10). Protein sequence classification of those three clusters revealed that M38 BY enrichment corresponds to an amidohydrolase/metal-dependent hydrolase (IPR011059, IPR006680/PF01979) domain containing proteins. In cluster III, beyond these domains, we detected the presence of a tryptophan synthase (IPR001926) domain expanded in Herpotrichiellaceae and Cyphellophoraceae (Fig. S10).

Carbohydrate-active enzymes

Carbohydrate-active enzymes (CAZymes) are responsible for the degradation, modification, and biosynthesis of carbohydrates and glycoconjugates (Cantarel et al. 2009). The family classification system based on amino-acid sequence and structure similarities has been used to group the CAZymes into five classes of enzyme activities and one associated module: glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), auxiliary activities (AAAs), and the associated module carbohydrate-binding modules (CBMs) (Cantarel et al. 2009). In this study we determined the CAZymes composition distributed across the black yeasts and compared this with other ascomycetes. In total, 154 CAZymes families were identified in the predicted protein sets. Generally, the highest and the lowest number of CAZymes were found in members of the jeanselmei- and dermatididi-clades, respectively, although the variation observed between species within clades was considered low. Some CAZymes appeared to be clade-specific. For example, the GH62 family was only found in the euroaeca-clade (Cyphellophoraceae). On the other hand, several CAZymes were identified in all species examined (Table S10).

Some striking depletions were verified in CAZyme families involved in the degradation of plant material. Plant cell wall polysaccharides are subdivided into three categories: cellulose, hemicellulose (including xylan, xyloglucan, glucogalactomannan, galactan, and respective side chains), and pectin (composed of galacturonan, rhamnogalacturonan, and respective side chains) (Amselem et al. 2011). Most black yeast-like fungi lack the pectinases PL1, PL3, PL4, PL7, PL9, and PL10 (Table S10). Comparable depletions have been reported in species of Onygenales (Desjardins et al. 2011), and Sporothrix (Teixeira et al. 2014) while they are present in Eurotiales. The β-1,4-glycosidase hydrolase family 28 (GH28) is another family linked to the breakdown of pectins. This enzyme is absent in the dermatididi-clade, jeanselmei-clade, and salmonis-clade, but present in the bantiana- and euroaeca-clades. Similarly, pectin methyltransferase family CE8 and pectin acetyltransferase family CE12 are absent in Herpotrichiellaceae. Comparable patterns are found in the xylan-associated enzyme family GH11 (endo-β-1,4-xylanase), present only in the carnionii- and euroaeca clades, as well as in Eurotiales, and CE 1 (acetyl xylan esterase) missing in Onygenales and in all black yeasts examined.
| Species                   | Terpene | III PKS | I PKS | NRPS | Terpene/Indole/ PKs | NRPS + terpene | Phosphonate | Lantipeptide | I pks/terpene | I PKS/NRPS | NRPS + indole | Indole |
|---------------------------|---------|---------|-------|------|---------------------|----------------|-------------|-------------|---------------|------------|---------------|--------|
| Capronia coronata         | 4       | 1       | 2     | 4    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| Exophiala dermatitidis    | 4       | 1       | 2     | 5    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| C. epimyces               | 6       | 1       | 6     | 2    | 0                   | 0              | 1           | 0           | 0             | 0          | 0             | 0      |
| Cladophialaphora psammophila | 3   | 1       | 2     | 2    | 1                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| E. aquamaria              | 6       | 1       | 6     | 4    | 0                   | 0              | 1           | 0           | 0             | 0          | 0             | 0      |
| C. carrionii              | 5       | 1       | 4     | 3    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| Phialophora atae          | 4       | 1       | 3     | 1    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| P. europaea               | 4       | 0       | 2     | 2    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| C. semimmera              | 4       | 0       | 4     | 4    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| E. xenobiotica            | 4       | 1       | 6     | 2    | 0                   | 0              | 1           | 0           | 0             | 0          | 0             | 0      |
| E. olgosperma             | 4       | 1       | 3     | 4    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| E. spinifera              | 3       | 1       | 4     | 3    | 0                   | 0              | 1           | 0           | 0             | 0          | 0             | 0      |
| Verrucosis gallopava       | 3       | 0       | 5     | 2    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| E. mesophila              | 4       | 1       | 1     | 2    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| E. sideris                | 4       | 2       | 2     | 2    | 0                   | 0              | 2           | 1           | 0             | 0          | 0             | 0      |
| Coniosporium apollinis     | 5       | 0       | 3     | 1    | 0                   | 1              | 0           | 0           | 0             | 0          | 0             | 0      |
| Fonsecaea pedrosoi        | 4       | 1       | 3     | 3    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| Rhinocladiella mackenziei | 4       | 1       | 9     | 5    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| C. bantiana               | 3       | 1       | 2     | 2    | 1                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| F. multimorphosa          | 4       | 1       | 5     | 2    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| C. immunda                | 4       | 2       | 5     | 2    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
| C. yegresii               | 5       | 1       | 2     | 3    | 0                   | 0              | 0           | 0           | 0             | 0          | 0             | 0      |
Depletions were also verified in chitin-related enzymes, a critical component of the fungal cell wall (Latgé 2007). Black yeasts on average have 5 members of the chitinase family GH18 per species. In contrast, Onygenales and Eurotiinales have 10 and 21 members per species on average, respectively (Table S10). Moreover, the Carbohydrate-Binding Module Family 18, which is often found attached to a number of chitinase catalytic domains, is depleted in black yeasts. These comparisons suggest that the breakdown of chitin is likely reduced compared to other filamentous fungi.

The family AA4 contains vanillyl-alcohol oxidase (VAO), which is missing in several other ascomycete fungi and is well represented in BY. VAOs catalyse the oxidation of a wide range of phenolic compounds and are abundant in black yeast genomes ranging from 10 copies in Cladophilaphora psammophila to two copies in Capronia coronata. This finding is consistent with the ability of many black yeasts to degrade aromatic compounds (Isola et al. 2013).

Cell-wall biosynthesis

The cell wall is an essential structure involved in protective functions against osmotic pressure and environmental stress (Bowman & Free 2006). The three major fungal cell wall constituents are chitin, mannan, and β-glucan. These components have been implicated in fungal virulence and represent targets for immune surveillance mechanisms (Bulawa et al. 1995). In agreement with previously published data (Chen et al. 2014), BY genomes encode an arsenal of genes involved in chitin synthesis. All 7 proposed classes of chitin synthase genes (CHS) previously described in fungi (Roncero 2002) were present in BY, except Class VI, which is missing in Rhinocladiella mackenziei. This species is recognised as an important causative agent of cerebral phaeohyphomycosis (Li & de Hoog 2009); mutants in CHS-VI are viable and less virulent (Bulawa et al. 1995). Proteins linked to the regulation and exportation of chitin synthase are conserved in BYs (Table S11). In contrast, comparative analysis of chitin degradation genes showed that black yeasts lack chitosanase, which is conserved in Saccharomyces cerevisiae and Schizosaccharomyces pombe (Table S11). Additionally, BYs have fewer chitinase proteins belonging to the family GH18 compared to other filamentous fungi, as described above (Table S11). Chitin deacetylases, which are believed to be secreted exclusively during modification of chitin in the cell wall (Zhao et al. 2010b), are missing in the carrionii-clade and in R. mackenziei.

Investigation of the genes related to synthesis and processing of 1,3-α-glucan revealed they are altered significantly in the species analysed. The Ags family of 1,3-α-glucan synthase is absent only in Exophiala dermatitidis, but the Agn family of 1,3-α-glucanases is absent from the dermatiditidis-, jeanselmei-, and salmonis-clades, and in Rhinocladiella mackenziei, even though these families are present with multiple copies in Aspergillus. Furthermore, BYs possess a putative α-amylase believed to be involved in the formation and/or modification of α-glucans (Table S11).

Transporters

Black yeasts like other filamentous Ascomycota possess a large proportion of genes associated with transporter activity. Our InterProScan analysis revealed that the most abundant protein domain verified in several BYs contains several families of transporters, particularly the Major Facilitator Superfamily (MFS). To better understand the transporter mechanisms in BYs, we annotated transporter subfamilies across all 21 species based on their best match to the curated transporter database TCDB (Saier et al. 2006). Overall, black yeasts possess more MFS transporters than species of Onygenales and Eurotiinales.

The most abundant transporter subfamily found in BYs is a potential nicotinate permease. It has 27 candidate genes in the outgroups Venturina macrosiphon and Coniosporium apollinus, but up to 93 candidate genes in Exophiala aquamarina. This transporter belongs to the family of the Anion:Cat ion Symporter (ACS) (TC 2.A.1.14.11) of the major facilitator superfamily. Another MFS subfamily with a remarkably high number of predicted members is the triothecene efflux pump (TC 2.A.1.3.47) of the Sugar Porter (SP) family. Since toxin efflux pumps are responsible for mediating both intrinsic and acquired resistance to toxic compounds, this result provides genomic insight into the known extremetolerance of black yeast-like fungi. Moreover, this finding is consistent with the expansion of the triothecene efflux pump protein domain (IPR010573), as described above.

At family level, the Sugar Porter (SP) Family, the Anion:Cat ion Symporter (ACS) Family, and the Drug:1+ Antporter-1 (12 Spanner) (DHA1) Family are the most abundant in BYs. Interestingly, among the family DHA1 we verified that the subfamily 2.A.1.2.77, which confers phenylacetate resistance, is well-represented in the majority of the species examined. Other families verified in all BYs analysed comprise the Ferroporin (Fpn) Family (TC 2.A.100), the Proton-dependent Oligopeptide Transporter (POT/ PTR) Family, and the Equilibrative Nucleoside Transporter (ENT) Family.

Sexual and parasexual reproduction

Fungi exhibit a wide diversity of reproductive modes, including sexual, asexual, and parasexual cycles. Recombination is an important and needed process in any fungal life-cycle, and may alter virulence traits, increase fitness in new ecological niches, and eliminate deleterious mutations (Heitman 2006, Lee et al. 2010, Ni et al. 2011). We used models of sexual and parasexual cycles of Aspergillus nidulans and Neurospora for BY comparisons (Glass et al. 1990, Paolletti et al. 2007, Debuchy et al. 2010, Zhao et al. 2015). We first identified the mating-type idiomorph within each assembled genome. Homothalism of Capronia coronata and Ca. epimyces was confirmed by identifying both MAT1-1 and MAT1-2 Aspergillus homologues closely clustered in a single assembled scaffold (Fig. 1). With the exception of outgroup species Verruconis gallopava (Venturinales), the remaining analysed 20 genomes of assexual species harboured a single mating type idiomorph (either MAT1-1 or MAT1-2) within each assembly, confirming that these fungi are heterothallic (Fig. 1).

We analysed the MAT locus organisation within the main groups of Herpotrichiellaceae and Cyphellophoraceae using genomic information from the MAT flanking genes. Among Eurotomyces, the flanking genomic regions of the MAT locus harbours APN2, SLA2, APC5, and COX13 genes, which are conserved and organized in synteny (Coppin et al. 1997, Fraser et al. 2007, Paolletti et al. 2007). We first aligned and compared
the gene models from both dothideaceous species \textit{V. gallopava} and \textit{Coniosporium apollinis}. The \textit{APN2}, \textit{COX13}, \textit{APC5}, and \textit{CIA30} genes appear to be conserved in synteny and preserved in the right \textit{MAT} flanking region (Fig. 7). However, the \textit{SLA2} gene was not found in the \textit{MAT} locus in these species, but is not genomic linked. \textit{Coniosporium apollinis} is inferred to be a heterothallic species since it harbours a single copy of the \textit{MAT1-1} gene in its genome. In the left flanking site of the \textit{MAT} locus we found a homologous protein that was syntenically conserved between the two dothideaceous species (PV09_01802/ W97_06799); in the genomic alignments it is adjacent to the \textit{MAT1-1} genes of \textit{Coniosporium apollinis} W97_06800 and W97_06801 (Fig. 7). Thus, the \textit{MAT} locus of \textit{V. gallopava} harbours two different ORFs: PV09_01800 and PV09_01801, which are not present in the \textit{MAT} locus of the \textit{Coniosporium apollinis} genome. According to the protein classifications and annotation, the ORF PV09_01801 encodes a homeodomain-like (HD) protein that carries a DNA-binding homeodomain motif (Fig. 7). This domain is found within the mating types 1 and 2 genes (\textit{MAT}/\textit{MTL}2, \textit{Pi} and \textit{MAT}/\textit{MTL}1) in yeasts of \textit{Saccharomycotina} and \textit{Taphrinomycotina} in \textit{Ascomycota}, as well in \textit{Basidiomycota} (Martin et al. 2010). According to Lee et al. (2010), this domain was lost during speciation of \textit{Pezizomycotina}, but our analysis of additional species revealed that the HD domain was recognized as a potential mating regulator in \textit{Venturia} (Fig. 7). On the other hand, we confirmed the lack of the HD in \textit{Eurotiomycetes} (including BYs) once an \alpha-box and HMG were found in the \textit{MAT} locus.

We detected the \textit{MAT1-1} (\alpha-box) and \textit{MAT1-1-5} genes within the mating type 1 locus and/or the \textit{MAT1-2} (HMG) gene in \textit{Chaetothyriales} (Fig. 1). The function of \textit{MAT1-1-5} in mating is not well established, and appears poorly conserved with \textit{MAT1-1-4} gene among \textit{Onygenales} (Mandel et al. 2007, Burmester et al. 2011). As reported previously for some ascomycete species (Yun et al. 1999, Tsui et al. 2013), we obtained indirect evidence of a truncated version of the \textit{MAT1-1} gene within the \textit{MAT1-2} idiomorph, potentially driven by unequal recombination at the \textit{MAT} locus in an ancestor of \textit{Chaetothyriales} (Figs 8–12). The loss of a functional \alpha-box domain suggests that the truncated \textit{MAT} gene might have diverged under low selective pressure after unequal recombination, or was silenced due to interference if both HMG and \alpha-box domains were present. The \textit{COX13} gene appears not to be conserved among \textit{Chaetothyriales} in the flanking regions of the \textit{MAT} locus as usually observed in \textit{Eurotiomycetes} (Coppin et al. 1997, Debuchy et al. 2010, Lee et al. 2010).

The \textit{Cyphellophoraceae} species \textit{Cyphellophora europaea} and \textit{Phialophora attae} presented a rather conserved \textit{MAT} locus structure compared to other \textit{Eurotiomycetes}. The right \textit{MAT} flanking domain harbouring the genes \textit{SLA2}, \textit{APC5}, and \textit{SAICARS} appeared to be conserved, and at the opposite side in the left flanking area of both species the \textit{APN2} and other hypothetical proteins were organized in synteny (Fig. 7). Gene content within the \textit{MAT} locus diverges in the \textit{Cyphellophoraceae}: \textit{Cyphellophora europaea} has \textit{MAT1-1} and \textit{MAT1-5} configuration, while \textit{Phialophora attae} harbours the \textit{MAT1-2} gene.

The structure of the \textit{MAT} locus of \textit{Hetrophellhiaceae} deviates from that of most other members of \textit{Eurotiomycetes}. We observed an expansion or collapse of the canonical \textit{MAT} structure compared to model species in, for example, \textit{Aspergillus}. The flanking site of the \textit{MAT} genes of some BY was inflated with the accumulation of novel genes or was even unrelated to the \textit{MAT} locus in other ascomycetes, which suggests a low selective pressure in this important genomic region within the family (Fig. 7). \textit{Exophiala aquamarina} in the salinis-clade had a heterothallic \textit{MAT} locus structure with the \textit{MAT1-1} gene, as well as flanking genes \textit{SLA2}, \textit{VPS13}, and \textit{APN2} conserved in synteny with other \textit{Eurotiomycetes}. The heterothallic \textit{MAT} locus structure of \textit{E. mesophilia} lacked this structure. The right flanking area of \textit{E. mesophilia} showed homology and structural conservation with \textit{SLA2} and \textit{VPS13} genes of \textit{E. aquamarina}, but lacked synteny in the left flanking region of the \textit{MAT} locus (Fig. 7). No homology at the left flank of the \textit{MAT} locus was detected between the two species. In addition, the \textit{APN2} gene was located in another scaffold of \textit{E. mesophilia}, unrelated to the \textit{MAT} locus. Within the \textit{dermatidis-clade} we detected an expansion of the \textit{MAT} locus, which followed a speciation process of the three members of this clade. \textit{Exophiala dermatitidis} is placed as the basal taxon of the \textit{dermatidis-clade} and presents a well-conserved, heterothallic \textit{MAT} structure with other \textit{Eurotiomycetes} (Fig. 8). On the other hand, we detected a chromosomal expansion at the right flanking site of both \textit{Capronia homothallic MAT} loci, which was followed by gene inflation at this locus. This locus has some peculiar features. First, we identified a novel \textit{MAT} gene that is found within the \textit{MAT} locus only in those three species (HMPREF1120_08861/A103_06090/A101_07968). Second, within the canonical \textit{SLA2–APN2} \textit{MAT} locus structure, unique genes were detected within each of the three species, with the highest frequency in \textit{Ca. epimyces}, since this has a larger \textit{SLA2–APN2} genomic range. We also detected an expansion of the \textit{MAT} locus that is followed by a speciation process with acquisition and inflation of genes in the \textit{Exophiala} species of the jeanselmei-clade, \textit{E. xenobiotaica}, \textit{E. spinifera}, and \textit{E. oligosperma} (Fig. 9). Frequent appearance of new and family-specific genes is observed throughout the \textit{Heterothriceliaceae} along the mating type genes, which might be a source of adaptive novelties of mating regulators.

\textit{Exophiala sideris} is the most basal species in the \textit{jeanselmei-clade} and its \textit{MAT} locus structure followed the classical \textit{SLA2–APN2} configuration. However, we detected a fused \textit{MAT1-1}/\textit{MAT1-2} gene configuration in this species (Fig. S11). Protein classification analysis revealed that both \alpha-box and HMG domains are present within a single mating regulator gene, leading us to hypothesize this as an unusual gene fusion event potentially giving a homothallic status to this species. The protein was blasted against the Conserved Domain Database (CDD) (Marchler-Bauer et al. 2011) in order to achieve \textit{MAT} gene configuration common to fungi, as found e.g. in the homothallic ascomycetes \textit{Curvularia homomorpha}, \textit{Bipolaris luttrellii}, and \textit{Penicillium rubens} (Fig. S11). The disposition of both \alpha-box and HMG domains varies across the species panel analysed, either being separated along the gene or fused, where mostly HMG binding sites are found within the \alpha-box domain (Fig. S11). The latter case led us to speculate that HMG insertions could be an atavism from an ancient homothallic state, since the majority of the gene sequence is related to \textit{MAT1-1}, or it could be a product of gene fusion and unequal crossing over of two opposite mating type strains. This last scenario confirms earlier reports where cryptic homothallism was proven to occur in \textit{Curvularia homomorpha} and \textit{Bipolaris luttrellii} (Yun et al. 1999). Possibly the fused \textit{MAT1-1}/\textit{MAT1-2} also plays a role in cryptic homothallism of our species under study. Homothallism as an ancestral state was demonstrated in the carnionii-clade, which
differed from what was observed in the jeanselmei- and dermatitidis-clades (Fig. 10): *Capronia semiimmersa* harbours both MAT1-1 and MAT1-2 genes in a single haploid genome. In addition, these species exhibits an apparent expansion of the MAT locus in that the classical SLA2-APN2 configuration was not found, while new genes were detected down/upstream the MAT genes (Fig. 10). On the other hand, the heterothallic species *Cl. carrionii* and *Cl. yegresii* displayed the SLA2-APN2 structure, and new *carrionii-clade-specific* genes were detected along with the MAT genes. In addition, we detected a *Cl. carrionii*-specific gene acquisition within the MAT locus as represented by the yellow boxes in Fig. 10.

The most degenerated MAT locus structure within the *Herpotrichiellaceae* was found within the bantiana-clade. *Cladophialophora bantiana* (MAT1-1) and *Cl. psammophila* (MAT1-2) shared a large degree of synteny at the left side of the MAT genes extending to the SLA2 gene (Fig. 11). We also detected unique and shared genes between this specific *Herpotrichiellaceae* clade, represented by yellow boxes in Fig. 11. At the right flank of the MAT genes, APN2 are poorly conserved between these two species. The APN2 gene is assembled in a scaffold different from that of the MAT genes. The remaining species of the bantiana-clade, *Fonsecaea pedrosoi*, *F. multimorphosa*, and *Cladophialophora immunda* did not share any synteny at the flanking regions of the MAT genes.

Overall, we detected a low selective pressure within the MAT locus structure of *Chaetothyriales*, compared to other Eurotiomycetes (Coppin et al. 1997, Debuchy et al. 2010, Lee et al. 2010, Burmester et al. 2011). The species-specific, non-characterised genes and gene duplications near the MAT genes are...
unique to *Chaetothyriales*. Despite the presence of components of the mating/pheromone-response pathway and their respective domains among *Chaetothyriales*, we hypothesize that due to the low selection of the MAT locus, those domains could represent an alternative system for generating diversity via parasexuality. The parasexual cycle has not been explored or characterised in any species of *Chaetothyriales*. Parasexuality is a process triggered by cell-cell fusion and ploidy reduction through random chromosome loss and has been reported in various filamentous fungi including *Aspergillus nidulans*, *Neurospora crassa*, and *Podospora anserina* (Pontecorvo 1956, De Serres 1962, Labarere & Bernet 1977). Undifferentiated vegetative cells
undergo haploid cell fusion and produce heterokaryons, as with aberrant ploidy due to mitotic crossing-over (Tolmsoff 1983). Cell compatibility is dependent on a combination of loci known as heterokaryon (het) incompatibility loci (allorecognition loci) (Saupe & Glass 1997, Zhao et al. 2015). When incompatible, the vegetative cells undergo genetically programmed cell death (Glass et al. 2000). There is strong evidence that recombination can be triggered by a parasexual cycle in some of the consistently asexual BYs: (1) Based on gene family evolution analysis of BY, we identified a dramatic increase of het-containing
with ancestral and derived families in the correct position. Phylogenomic data are thus far available for Herpotrichiellaceae and Cyphellophoraceae only.

Species of Chaetothyriales can be found on phanerogam leaf litter and plant debris, but in contrast to common litter fungi such as Alternaria and Cladosporium, decomposed, tannin-rich material is mostly preferred. Species selectively grow with the presence of aromates and etheric oils, e.g., on babassu coconut shells, which contain a remarkable diversity of black yeasts (Nascimento et al. 2016b). Many species even seem to prefer artificial, human-made habitats, such as gasoline tanks (Isola et al. 2013) and toxic mine waste rich in heavy metals (Seyedmousavi et al. 2011). Several studies have indicated that herpotrichiellaceous black yeasts and their filamentous counterparts are potent degraders of toxic monoaromatics and are frequently found inhabiting industrial bio-filters (Middelhoven et al. 1989, Middelhoven 1993, Cox et al. 1997, Prenafeta-Boldú et al. 2006). This property is observed in members of nearly all clades (Woertz et al. 2001, de Hoog et al. 2006, Badal et al. 2011, Seyedmousavi et al. 2011). Phenolic and indolic compounds are substrate units for melanin formation, and the production of this pigment ultimately results from their oxidative polymerization (Jacobson 2000, Plonka & Grabacka 2006, Vávricka et al. 2010). Numerous additional studies have reported the presence of clinical and non-clinical species in oil-related environments (Phillips et al. 1998, Prenafeta-Boldú et al. 2001, 2006, Sterflinger & Píllinger 2001), and noted preference of creosoted wood over untreated wood (Seyedmousavi et al. 2011, Dögen et al. 2013a, b, Gümrü et al. 2014). The fungi become nearly the sole colonizers when the material contains toxic hydrocarbons, e.g., on creosoted telephone poles and railway sleepers (Gümrü et al. 2014). Several possible routes of aromatic hydrocarbon metabolism have become known in black yeast-like fungi (Cox et al. 1996). Among these the degradation of benzene derivatives via phenylacetate and homogentisate seems to be one of the most important pathways in the family Herpotrichiellaceae (Gunsch et al. 2005). Overexpression of the genes 2-hydroxyphenylacetate (PHA) and homogentisate 1,2-dioxygenase (HGD) when the fungus is grown in the presence of ethylbenzene, supports the involvement of these enzymes in organic compound degradation (Gunsch et al. 2005). It has also been demonstrated that genes of catabolic pathways, which may enhance survival under different environmental conditions, are physically clustered preserving gene order and orientation (Keller & Hohn 1997). This organization might favour the co-inheritance and the co-expression of multiple enzymes, which handle toxic intermediate compounds (Takos & Rook 2012, McGary et al. 2013) and suggests an essential role for this ability in Herpotrichiellaceae. Regarding the phenolic compound catabolism, homologues of the styrene pathway were found present in the species studied, except in Capronia coronata: this species lacks the genes coding to 4-hydroxyphenylpyruvate dioxygenase and maleylacetatoic isomerase. This suggests that across the analysed black yeasts, only Capronia coronata would not be able to synthetize pyomelanin via the accumulation of homogentisate. Remarkably, the same species described above can also be found in clear water environments that are very poor in nutrients. For example, Exophiala dermatitidis on the one hand occurs on creosoted wood (Dögen et al. 2013b) and in gasoline (Isola et al.

DISCUSSION

Black yeasts and similar fungi in the order Chaetothyriales are known for their morphological plasticity, asexual diversity, and divergent habitat choice. To date (01-01-2016), 23 species of the order have had their genomes sequenced (Fig. 1). The order contains four or five families according to Réblóvá et al. (2013) who upgraded the “euroea-clade” to family level (Fig. 1). Fig. 2 shows a phylogenetic tree of all available species based on LSU-sequences, which is as yet the only parameter alignable in BYs exceeds the number of allorecognition loci in N. crassa or Aspergillus species (Table S12). (3) Random gene duplication across BY genomes, which could be linked to impaired mitotic chromosomal reduction via aneuploidy.

With few exceptions, het-containing proteins are expanded in most BY genomes (Table S12). While species of Eurotiales harbour 2–12 het-containing proteins, in the Chaetothyriales the numbers range from a single copy in E. dermatitidis and Cyphellophora euroea to 134 copies in C. psammophila. Clustering analysis via CLANS of het-containing proteins (PF06985/IPR010730 – Pfam/InterPro) of 23 black yeasts, Onygenales, Eurotiales, and N. crassa did not show any significant sub-cluster within PF06985/IPR010730 containing genes. Alignment of the 1439 het-containing proteins in order to access the phylogenetic distribution did not return well-conserved alignment blocks due to high sequence dissimilarity. In the attraction graphs of the PF06985/IPR010730 family (Fig. 12), four clusters within het-containing proteins were visually defined in order to narrow down alignment discrepancies. We extracted the sequences related to each of the four identified groups and protein alignment was performed for phylogenetic analysis. All predicted het containing proteins of N. crassa (Zhao et al. 2015) were included, among which were three het loci, viz. tol, het-6, and pin-c. Phylogenetic analysis shows that most het-expanded BY harbour orthologues of well-characterised Neurospora tol, het-6, and pin-c (Fig. S12). We also identified a cluster of proteins that were related to the HET-E/D/R loci in Podospora anserina (Fig. S12). Beyond the het domain, this particular group of proteins display a GTP binding site followed by WD40 repeats, which play an important role in specificity of vegetative incompatibility in the P. anserina parasexual cycle (Espagne et al. 2002). BY genomes display a vast repertoire of heterokaryon incompatibility proteins and mechanisms of sexual or parasexual recombination, which needs further investigation.

REFERENCES

[References are not provided in the text, but the sections and figures mentioned suggest a comprehensive study involving various species and families within the order Chaetothyriales].

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COMPARATIVE GENOMICS OF CHAETOTHYRIALES
black fungi from ant domatia and cartons which appeared to be (Fig. 2) and their affinities to Chaetothyriales, including their E. dermatitidis classification (Crous et al. 2012), are known biotrophs. However, ancestral clades in the Eurotiales, with e.g. Cladophialophora hostae (Crous et al. 2007), Exophiala eucalyptorum (Crous et al. 2007), or Metulocladosporiella musae (Crous et al. 2006). CAZyme families, involved in the degradation of plant material, do not seem to play a major role in the black yeast-like fungi, as most of them lack the pectinases PL1, PL3, PL4, PL7, PL9, and PL10 (Table S10). Comparable depletions have been reported in species of Onygenales, which contain numerous obligate pathogens of humans and other vertebrates, while they are present in Eurotiales, with e.g. Aspergillus fumigatus essentially being a degrader of plant debris. Only the Epibryaceae, which have recently been associated with Chaetothyriales (Gueidan et al. 2014) after they had been regarded as members of Dothideomycetidae for decades (Stenroos et al. 2010), are known biotrophs. However, ancestral clades in Chaetothyriales including Epibryaceae show long branches (Fig. 2) and their affiliation with the order may be due to incomplete taxon sampling.

Voglmayr et al. (2011) isolated a large number of undescribed black fungi from ant domatia and cartons which appeared to be closely related to the Chaetothyriales. Ants and fungi have close symbiotic relationships. The ecological roles of chaetothyrialean symbionts appear to be different between domatia and carton associations (Voglmayr et al. 2011). In the domatia they play important roles such as in recycling ant waste, and there is also evidence that the ants feed on the fungi, contributing to rapid recycling of nitrogen in the tripartite symbiosis of ant, plant and fungi (Little & Currie 2008, Defossez et al. 2011, Blatix et al. 2012). In the carton association, ant tunnels or nests are largely made of black fungal material. It has been hypothesized that the fungus serves as building material (Lauth et al. 2011) and that the fungal layer on the carton walls could act as mechanical protection against radiation, humidity and microbial decomposition (Mayer & Voglmayr 2009, Zakharova et al. 2013), enhancing the durability. Commonly several black species co-occur on the same carton, forming complex associations, which rely on constant maintenance and care by the ants. Most carton species lack conidiation. In domatia a rather specific association with the host may be observed, the fungi producing a dense mat inside the domatium. These species are hyaline to light brown and show conidiation. Ants possess a great arsenal of exocrine glands (Möglich et al. 1974, Attygalle et al. 1989, Poulsen et al. 2002, Fernandez-Marín et al. 2006) secreting organic compounds that are effective against fungi and bacteria (Schlüs & Crozier 2009). Some species of leaf-cutting ants exude antimicrobial flavonoid and tannin-like compounds and communicate by aliphatic hydrocarbons (Brandt et al. 2009). Chaetothyriales have been shown to use toxic compounds such as aromatic hydrocarbons as unique nutritional carbon sources (Prenafeta-Boldú et al. 2006, Zhao et al. 2010a), which thus act as key selective agents promoting the dominance of Chaetothyriales in ant nests. The evolutionary origin of Formicinae dates back to Cretaceous times, around 100 MYA. Given the large extant diversity of Formicinae, as well as of associated Chaetothyriales, it seems possible that ants and black yeast-like fungi have diversified in concert. We estimated that the common ancestor of Herpotrichiellaceae–Cyphellaphoraceae emerged around 75–50 MYA, shortly after the Cretaceous–Paleogene (K-Pg) extinction event. In a similar study, Gueidan et al. (2011) calculated the ancestral groups of Chaetothyriales with a rock-inhabiting life-style and likened Verrucariales around 250 MYA. Verrucariales might have been the first to colonise barren rock after the meteor impact that marked the transition from Perm to Trias. Early Chaetothyriales were thought to be hyper-parasites on lichens (Gostincar et al. 2012) lacking an algal component. Lichens produce large amounts of toxic metabolites, and thus the pathogens must have been able to cope with harsh climatic conditions as well as with life in toxic habitats. This may have been the period where early Chaetothyriales developed stress tolerance, nutritional oligotrophism, and physiological versatility to survive the wide array of toxic secondary metabolites produced by the lichens (Gostincar et al. 2010).

With these ecological and evolutionary speculations, two factors are of particular interest: melanin and action of the cytochrome p450 enzymes. The presence of eumelanin produced via the DHN and DOPA pathways, and pyromelansins via L-tyrosine degradation pathway (Alviano et al. 1991, Sun et al. 2011, Eisenman & Casadevall 2012, Li et al. 2016) are fundamental to the obligatory melanisation of the cell wall to enhance stress tolerance. Melanised fungi are resistant to environmental challenges found particularly in extreme habitats, including irradiation, nutrient depletions, and high temperature (Rosas & Casadevall 1997); this matches well with the conditions of a rock-inhabiting lifestyle. The melanised fungi are even able to survive in radioactive environments. Fungi growing on surfaces with direct sunlight exposure are highly adapted to cope with ionizing radiation via the constitutive presence of melanin, which acts as energy transduction molecules (Dadachova & Casadevall 2008). Melanised Exophiala dermatitidis cells exposed to ionizing radiation grow faster than non-exposed cells, suggesting a critical role of irradiated melanin and its conversion as a source of energy in herpotrichiaceous fungi (Dadachova et al. 2007). Their energy transduction is multifunctional, also playing an essential role in resistance to oxidative burst of vertebrate phagocytes (Henson et al. 1999, Jacobson 2000, Eisenman & Casadevall 2012).

Melanins can also act as scavengers of free and oxidative radicals, are cross-linked to fungal cell wall carbohydrates, and also interact with surrounding molecules. These black pigments may therefore have a protective function against natural predators, such as amoebae (Nosanchuk & Casadevall 1997); this matches well with the conditions of a rock-inhabiting lifestyle. The melanised fungi are even able to survive in radioactive environments. Fungi growing on surfaces with direct sunlight exposure are highly adapted to cope with ionizing radiation via the constitutive presence of melanin, which acts as energy transduction molecules (Dadachova & Casadevall 2008). Melanised Exophiala dermatitidis cells exposed to ionizing radiation grow faster than non-exposed cells, suggesting a critical role of irradiated melanin and its conversion as a source of energy in herpotrichiaceous fungi (Dadachova et al. 2007). Their energy transduction is multifunctional, also playing an essential role in resistance to oxidative burst of vertebrate phagocytes (Henson et al. 1999, Jacobson 2000, Eisenman & Casadevall 2012).
(Little & Currie 2008, Defossez et al. 2011, Blatix et al. 2012). Melanins thus have become a potential virulence factor. Indeed, we witness the largest number of systemic opportunistic species on humans and cold-blooded animals in the Herpotrichiellaceae, while this behaviour is nearly absent from other families, with Arthrocladium fulminans in the Trichomeriaceae as the only exception (Nascimento et al. 2016a).

Very little is known about the cell wall organization in BY and its dynamic composition. Loss of cell wall alpha-glucan synthase appears to be specific to Exophiala dermatitidis and the two outgroups Verruconis gallopava and Coniosporium apollinis. Other enzymes involved in 1,3-alpha-glucan processing are missing from remaining black yeast species studied. In contrast, the presence of the alpha-amylases, believed to be involved in the formation and/or modification of alpha-glucans (van der Kaaij et al. 2007), suggests that BY carry a cell wall that deviates from that of other filamentous fungi. Importantly, some recent studies have shown that the chronicity observed in chromoblastomycosis is due to a failure of pattern recognition receptor (PRR) costimation (van der Kaaij et al. 2007). For example, innate recognition of F. pedrosi is mediated by C-type lectin receptors (CLRs), but not by Toll-like receptors (TLRs), triggering an inadequate protective inflammatory response and leading to chronic infection (van der Kaaij et al. 2007). TLRs can recognize pathogen-associated molecular patterns (PAMPs), such as alpha-glucans in fungi (Takeda et al. 2003). We therefore speculate that BY genetic variation associated with cell wall composition, the main source of PAMPs, might affect the pattern of recognition of invading pathogens by the host. Indeed, enzymatic treatment to modify alpha-glucans from the conidial surface has been reported to decrease the phagocytic index in other ascomycetes (Bittencourt et al. 2006). Further studies will provide a better understanding of the role of alpha-glucans in the induction of innate immune response as well as its structural organization in black yeasts. No specific virulence differences were found between closely related, pathogenic versus environmental siblings, e.g. between Cladophilaphora bantiana/Cf. psammaphila, and between Cladophilaphora carrionii/Cf. yegesi. It may be concluded that pathogenicity of black fungi is primarily of opportunistic nature, enhanced by the combination of extemtolerance and assimilative abilities of aromatic compounds, similar to pathogenicity in Cryptococcus.

Genetic diversity plays an important role in the adaptation of fungal populations to changing environments (Milgroom 1996, Heitman 2006, Giraud et al. 2010). Increased genotypic variation allows some individuals in a given population to inherit variations of alleles that are more suitable for a specific condition such as virulence (Engering et al. 2013). Genetic diversity is favoured by recombination that positively eliminates deleterious alleles that have accumulated during clonal development (Heitman 2006, Barton 2010). The two main sources of recombination in ascomycetes are sexual and parasexual reproduction (Calo et al. 2013). Sexual reproduction in those fungi is orchestrated by the mating type locus (MAT1-1 and/or MAT1-2), which codify for transcription factors (alpha-box and HMG, respectively) that regulate genes required for mating and meiosis (Coppin et al. 1997). Two mating recognition systems are well described in Ascomycota: homothallic (self-fertile) species carry both mating-type alleles (MAT1-1/MAT1-2 genes) in a single haploid genome, and sexual reproduction takes place in a single individual. Heterothallic (self-sterile) fungi are species that carry a single allele (MAT1-1 or MAT1-2) per haploid genome and need an opposite mating-type for sexual reproduction (Debuchy et al. 2010, Lee et al. 2010, Ni et al. 2011). The other main source of recombination in fungi is the parasexual cycle, where meiosis and development of sexual structures remain absent (Pontecorvo 1956). This non-sexual mechanism is governed and controlled by the het locus (heterokaryon incompatibility) that confers vegetative recognition of some filamentous ascomycetes (Glass et al. 2000). This process is initiated by cytoplasmic fusion in which different nuclei and cytoplasmic organelles co-occupy the same cellular space. Nuclear fusion takes place producing a diploid nucleus (karyogamy), which is, however, unstable and produces segregants by recombination, evoking the mitotic crossing-over followed by haploidisation.

There are two direct indications that recombination takes place in BY. First, the Capronia sexual morph is a homothallic (self-fertile) genus and is polyphyletic, distributed over Herpotrichiellaceae and Cyphellophoraceae (Unterreiner 1995, Unterreiner & Naveau 1998, Unterreiner, 2000), and the Trichomeriaceae have, morphologically, a very similar sexual morph in Trichomerium (Chomnunti et al. 2012a). Second, by in silico methods, recombination has been reported to occur in Cladophilaphora carrionii (Deng et al. 2015). However, as most species of Chaetothyriales lack a known sexual morph, only asexual morphs have been recognised. This leads us to hypothesize the following options: (1) species may lack a genomic apparatus for sexual reproduction, (2) species may be purely heterothallic but with cryptic sex taking place under specific conditions, or (3) species may have genomic signatures for a parasexual cycle under het locus control. In addition, we have identified a potential fused MAT1-1/MAT1-2 gene configuration in Exophiala sidersis disposing both alpha-box and HMG domains (Fig. 9, Fig. S11). Cryptic homothallism was proven to occur in Curvularia homomorpha and Bipolaris lutrellii, which are ascomycete species presenting similar configurations as herein reported for Exophiala sidersis (Fig. S11). Another remarkable finding was the occurrence of a mating regulator that codifies for a transcription factor carrying a DNA binding homeodomain motif in V. gallopava (Fig. 7). This domain is largely found in yeasts in Ascomycota and Basidiomycota (Martin et al. 2010) and, according to (Lee et al. 2010) this domain was lost during speciation of Pezizomycotina. We herein hypothesize that the HD domain is a potential mating regulator in Venturiales (Fig. 7) and suggest this domain was maintained more recently in Eurotiomycetes speciation.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.simyco.2017.01.001.

REFERENCES

Alviano CS, Farbizar SR, De Souza W, et al. (1991). Characterization of Fonsecaea pedrosoi melanin. Journal of General Microbiology 137: 837–844.

Amselem J, Cuomo CA, van Kan JA, et al. (2011). Genomic analysis of the necrotrophic fungal pathogens Sclerotinia sclerotiorum and Botrytis cinerea. PLoS Genetics 7: e1002230.

Aptroot A, Giederich P, Sérusiaux E, et al. (1997). Lichens and lichenicolous fungi. Bibliotheca Lichenologica 64: 1–220.

Attygalle AB, Siegel B, Vostrowsky O, et al. (1989). Chemical composition and function of metapleural gland secretion of the ant, Crematogaster desformis smith (Hymenoptera: Myrmicinae). Journal of Chemical Ecology 15: 317–328.

Badal H, Guedian C, Najafzadeh MJ, et al. (2008). Biodiversity of the genus Cladophialaphora. Studies in Mycology 61: 175–191.

Badal H, Prenafeta-Boldú FX,Guarro J, et al. (2011). Cladophialaphora psammophila, a novel species of Chaetothyriales with a potential use in the bioremediation of volatile aromatic hydrocarbons. Fungal Biology 115: 1019–1029.

Baldrin P (2008). Fungal laccases – occurrence and properties. FEMS Microbiology Reviews 30: 215–242.

Barr ME (1972). Preliminary studies on the Dotheiales in temperate North America. University of Michigan Herbarium, United States of America: 525–664.

Barton NH (2010). Mutation and the evolution of recombination. Philosophical Transactions of the Royal Society 365: 1281–1294.

Batista AC, Ciferri R (1962). The Chaetothyriales. Beihefte zur Sydowia 3: 1–129.

Birney E, Clamp M, Durbin R (2004). GeneWise and Genomewise. Genome Research 14: 988–995.

Bittencourt VC, Figueiredo RT, da Silva RB, et al. (2006). An alpha-glucan of Pseudallescheria boydii is involved in fungal phagocytosis and Toll-like receptor activation. The Journal of Biological Chemistry 281: 22614–22623.

Blatnić R, Djieto-Lordon C, Mondolot L, et al. (2012). Plant-ants use symbiotic fungi as a food source: new insight into the nutritional ecology of ant-plant interactions. Proceedings of the Royal Society of London B 279: 3940–3947.

Borek D, Michałska K, Brzeziński K, et al. (2004). Expression, purification and catalytic activity of Lupinus luteus asparagine beta-amidohydrolase and its Escherichia coli homolog. European Journal of Biochemistry 271: 3215–3226.

Bowman SM, Free SJ (2006). The structure and synthesis of the fungal cell wall. Bioessays 28: 790–808.

Brandt M, van Wilgenburg E, Sulc R, et al. (2009). The scent of supercolonies: the discovery, synthesis and behavioural verification of ant colony recognition cues. BMC Biology 7: 71.

Builawa CE, Miller DW, Henry LR, et al. (1995). Attenuated virulence of chin- deficient mutants of Candida albicans. Proceedings of the National Academy of Sciences of the United States of America 92: 10570–10574.

Burmester A, Shelest E, Glocker G, et al. (2011). Comparative and functional genomics provide insights into the pathogenicity of dermatophytic fungi. Genome Biology 12: R7.

Cai S, Bilymyre RB, Heitman J (2009). TrimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25: 1972–1973.

Casadevall A, Steenbergen JN, Nosanchuk JD (2003). ‘Ready made’ virulence and ‘dual use’ virulence factors in pathogenic environmental fungi – the Cryptococcus neoformans paradigm. Current Opinion in Microbiology 6: 332–337.

Chen Z, Martinez DA, Gujia S, et al. (2014). Comparative genomic and transcriptomic analysis of Wangiella dermatitidis, a major cause of phaeohyphomycosis and a model black yeast human pathogen. G3: Genes, Genomes, Genetics 4: 561–578.

Chomnunti P, Bhat DJ, Jones EBG, et al. (2012). Trichromatia, a new sooty mould family of Chaetothyriales. Fungal Diversity 56: 63–76.

Chomnunti P, Ko TW, Chukeatrote E, et al. (2012). Phylogeny of Chaetothyriales in northern Thailand including three new species. Mycologia 104: 382–395.

Conant GC, Wolfe KH (2008). Turning a hobby into a job: how duplicated genes find new functions. Nature Reviews Genetics 9: 938–950.

Coppin E, Debuchy R, Maïsse S, et al. (1997). Mating types and sexual development in filamentous ascomycetes. Microbiology and Molecular Biology Reviews 61: 411–428.

Cox HH, Faber BW, van Heiningen WN, et al. (1996). Steroye metabolism in Exophiala jeaneselmei and involvement of a cytochrome P-450-dependent steroye monooxygenase. Applied and Environmental Microbiology 62: 1471–1474.

Cox HH, Moerman RE, van Baalen S, et al. (1997). Performance of a styrene-degrading biotofler containing the yeast Exophiala jeaneselmei. Biotechnology and Bioengineering 53: 259–266.

Crous PW, Braun U, Wingfield MJ, et al. (2009). Phylology and taxonomy of obscure genera of microfungi. Persoonia 22: 139–161.

Crous PW, Schroers HJ, Groenewald JZ, et al. (2006). Melolonthodispora gen. nov. for the causal organism of Cladosporespere disease of banana. Mycological Research 110: 241–275.

Crous PW, Schubert K, Braun U, et al. (2007). Opportunistic, human-pathogenic species in the Herpotrichiellaceae are phenotypically similar to saprobic or phytopathogenic species in the Venturinaceae. Studies in Mycology 58: 185–217.

Dadachova E, Bryan RA, Huang X, et al. (2007). Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. PLoS One 2: e457.

Dadachova E, Casadevall A (2008). Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. Current Opinion in Microbiology 11: 525–531.

da Silva JP, Alviano DS, Alviano CS, et al. (2002). Comparison of Fonsecaea pedrosoi scerotic cells obtained in vivo and in vitro: ultrastructure and antigenicity. FEMS Immunology and Medical Microbiology 33: 63–69.

da Silva MB, da Silva JP, Yamano SSP, et al. (2008). Development of natural culture media for rapid induction of Fonsecaea pedrosoi scerotic cells in vitro. Journal of Clinical Microbiology 46: 3839–3841.

de Almeida-Paes R, Nosanchuk JD, Zanconó-Oliveira RM (2012). Fungal melanins: biosynthesis and biological functions. Nova Science Publishers: 77–107.

De Bie T, Cristiani N, Demuth JP, et al. (2006). CAFE: a computational tool for the study of gene family evolution. Bioinformatics 22: 1269–1271.

Debuchy R, Berteaux-Lecellier V, Silar P (2010). Mating systems and sexual morphogenesis in ascomycetes. Cellular and Molecular Biology of Fila- mentous Fungi, 1–2.

Defossez R, Djieto-Lordon C, McKay D, et al. (2011). Plant-ants feed their host plant, but above all a fungal symbiont to recycle nitrogen. Proceedings of the Royal Society of London B 278: 1419–1426.

de Hoog GS (1993). Evolution of black yeasts: possible adaptation to the human host. Antonie van Leeuwenhoek 63: 105–109.

de Hoog GS (2014). Ecology and phylology of black yeast-like fungi: diversity in unexplored habitats. Fungal Diversity 65: 1–2.

de Hoog GS, Nishikaku AS, Fernandez-Zeppefiedt G, et al. (2007). Mo- lecular analysis and pathogenicity of the Cladophialaphora carrioni complex, with the description of a novel species. Studies in Mycology 58: 219–234.

de Hoog GS, Vicente VA, Najafzadeh MJ, et al. (2011). Waterborne Exophiala species causing disease in cold-blooded animals. Persoonia 27: 46–72.

de Hoog GS, Zeng JS, Harrak MJ, et al. (2006). Exophiala xenobiotica sp. nov., an opportunistic black yeast inhabiting environments rich in hydrocarbons. Antonie van Leeuwenhoek 90: 257–258.

Deng S, Tsui CK, Gerrits van den Ende AHG, et al. (2015). Global spread of human chromoblastomycosis is driven by recombinten Cladophialaphora carrioni and predominantly clonal Fonsecaea species. PLoS Neglected Tropical Diseases 9: e0044004.

de Serres FJ (1962). Heterokaryon-incompatibility factor interaction in tests between Neurospora mutants. Science 138: 1342–1343.

Desgardins CA, Champion MD, Holder JW, et al. (2011). Comparative genomic analysis of human fungal pathogens causing paracoccidioidomycosis. PLoS Genetics 7: e1002345.
Döbbeler P (1997). Biodiversity of bryophilous ascomycetes. Biodiversity & Conservation 6: 721–738.

Dögen A, Ikiti M, de Hoog GS (2013a). Black yeast habitat choices and species spectrum on high altitude creosole-treated railway ties. Fungal Biology 117: 692–696.

Dögen A, Kaplan E, Ikiti M, et al. (2013b). Massive contamination of Exophiala dermatitidis gene transforms in railway stations in subtropical Turkey. Mycopathologies 175: 381–386.

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

Eisenman HC, Casadevall A (2012). Synthesis and assembly of fungal melanin. Journal of Clinical Microbiology 93: 931–940.

Engering A, Hogerwerf L, Slingenbergh J (2013). Pathogen-host-environment interplay and disease emergence. Emerging Microbes & Infections 2: e5.

Espagne E, Balhadere P, Penin ML, et al. (2009). Differentiation and high throughput. Nucleic Acids Research 39: W93–W97.

Feng B, Wang X, Hauser M, et al. (2015). Three new species of Cyphellophora and its relatives in Phialophora: biodiversity and possible role in human infection. Fungal Diversity 65: 17–45.

Fernandez-Marín H, Zimmerman JK, Rehner SA, et al. (2006). Active use of the metapleural glands by ants in controlling fungal infection. Proceedings of the Royal Society of London B 273: 1689–1695.

Finn RD, Clements J, Eddy SR (2011). HMmer web server: interactive sequence similarity searching. Nucleic Acids Research 39: W29–W37.

Finn RD, Mistry J, Tate J, et al. (2010). The Pfam protein families database. Nucleic Acids Research 38: D211–D222.

Fraser JA, Stajich JE, Tarcha EJ, et al. (2007). Evolution of the mating type locus: insights gained from the dimorphic primary fungal pathogens Histoplasma capsulatum, Coccidioides immitis, and Coccidioides posadasii. Eukaryotic Cell 6: 622–629.

Fu L, Niu B, Zhu Z, et al. (2012). CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28: 3150–3152.

Galagan JE, Calvo SE, Borkovich KA, et al. (2003). The genome sequence of the filamentous fungus Neurospora crassa. Nature 422: 859–868.

Gao L, Ma Y, Zhao W, et al. (2015). New subfamily of WD40 proteins involved in vegetative incompatibility and high throughput. Nucleic Acids Research 39: 5654–5666.

Gao L, Ma Y, Zhao W, et al. (2008). Automated eukaryotic gene structure annotation using EVidenceModeler and the Program to Assemble Spliced Alignments. Genome Biology 9: R7.

Gao L, Ma Y, Zhao W, et al. (2008). Automated eukaryotic gene structure annotation using EVidenceModeler and the Program to Assemble Spliced Alignments. Genome Biology 9: R7.

Gandara P, Delcher AL, Mount SM, et al. (2003). Improving the Arabidopsis genome annotation using maximal transcript alignment assemblies. Nucleic Acids Research 31: 5654–5666.

Hansen JM, Butler MJ, Day AW (1999). The dark side of the mycelium: melanins of phytotrophic fungi. Annual Review of Phytopathology 37: 447–471.

Henson JM, McKenzie EHC, KoKo TW (2011). Towards incorporating anamorphic fungi in a natural classification – checklist and notes for 2010. Mycosphere 2: 38.

Isola D, Selbmann L, de Hoog GS, et al. (2013). Isolation and screening of black fungi as degraders of volatile aromatic hydrocarbons. Mycopathologia 175: 369–379.

Isola D, Zuccini L, Onofri S, et al. (2016). Extreme tolerant root inhabiting black fungi from Italian monumental sites. Fungal Diversity 76: 75–96.

Ituriaga G, Suarez R, Nova-Franco B (2009). Trehalose metabolism: from osmoprotection to signaling. International Journal of Molecular Sciences 10: 3793–3810.

Jacobson ES (2000). Pathogenic roles for fungal melanins. Clinical Microbiology Reviews 13: 708–717.

Jones L, Riaz S, Morales-Cruz A, et al. (2014a). Adaptive genomic structural variation in the grape powdery mildew pathogen, Erysiphe necator. BMC Genomics 15: 1081.

Jones P, Binns D, Chang HY, et al. (2014b). InterProScan 5: genome-scale protein function classification. Bioinformatics 30: 1236–1240.

Jurka J, Kapitonov VV, Pavlicek A, et al. (2001). Koonin P, Meusemann K (2010). FASconCAT: convenient handling of data sets. Current Opinion in Insect Science 1: 386–393.

Katoh K, Standley DM (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 702–706.

Keller NP, Hohn TM (1997). Metabolic pathway gene clusters in Chaetothyriomycetes. Fungal Diversity 31: 386–393.

Keller NP, Turner G, Bennett JW (2005). Fungal secondary metabolism and its function interplay and disease emergence. Current Opinion in Insect Science 1: 386–393.

Kuck P, Meusemann K (2010). FASconCAT: convenient handling of data sets. Current Opinion in Insect Science 1: 386–393.
Labarere J, Bernet J (1977). Protoplasmic incompatibility and cell lysis in Podospora anserina. I. Genetic investigations on mutations of a novel modifier gene that suppresses cell destruction. Genetics 87: 249–257.

Lamp DC, Kelly DE, Schunck WH, et al. (1997). The mutation T315A in Candida albicans sterol 14alpha-demethylase causes reduced enzyme activity and fluconazole resistance through reduced affin. Journal of Biological Chemistry 272: 5682–5689.

Langleieder K, Streibel M, Jahn B, et al. (2003). Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genetics and Biology 38: 143–158.

Lateg JP (2007). The cell wall: a carboxylic armour for the fungal cell. Molecular Microbiology 66: 279–290.

Lauth J, Ruiz-Gonzalez MX, Overell J (2011). New findings in insect fungiculture: have ants evolved hands-free, agricultural products? Communicative & Integrative Biology 4: 72–73.

Le SQ, Gascuel O (2008). An improved general amino acid replacement matrix. Molecular Biology and Evolution 25: 1307–1320.

Lee SC, Ni M, Li W, et al. (2010). The evolution of sex: a perspective from the fungal kingdom. Microbiology and Molecular Biology Reviews 74: 298–340.

Lepesheva GI, Hargrove TY, Kleshchenko Y, et al. (2011). New–Li DM, de Hoog GS (2009). Cerebral phaeohyphomycosis cutaneous infection. Metarhizium robertsii demethylase target gene (CYP51) mediates fungicide resistance in Podospora anserina. BMC Genomics 12: e20973.

Liu XQ (2000). Protein-splicing intein: genetic mobility, origin, and evolution. Annual Review of Genetics 34: 61–76.

Lonsdale J, de Hoog GS (2010). Indoor wet cells harbour melanized agents of human pathogenic fungi. Fungal Genetics and Biology 38: 622–628.

Liu F, Wang W, Liao X, et al. (2011). The McYP52 cytochrome P450 monooxygenase gene of Metarhizium robertsii is important for utilizing insect epicuticular hydrocarbons. PLoS One 6: e28984.

Little AE, Currie CR (2008). Black yeast symbionts compromise the efficiency of antibiotic defenses in fungus-growing ants. Ecology 89: 1216–1222.

Liu H, Kauffman S, Becker JM, et al. (2004). Wangiella (Exophiala) dermatitidis WoCl3Sp, a class V chitin synthase, is essential for sustained cell growth at temperature of infection. Eukaryotic Cell 3: 40–51.

Liu XQ (2000). Protein-splicing intein: genetic mobility, origin, and evolution. Annual Review of Genetics 34: 61–76.

Lomsadze A, Ter-Hovhannisyan V, Chernoff YO, et al. (2005). Gene identifica
cion in novel eukaryotic genomes by self-training algorithm. Nucleic Acids Research 33: 6494–6506.

Lucking R, Huhndorf S, P

Mayer VE, Voglmayr H (2009). Mycological carton galleries of Azteca brevis (Formicidae) as a multi-species network. Proceedings of the Royal Society of London B 276: 3285–3273.

McGary KL, Slot JC, Rakas A (2013). Physical linkage of metabolic genes in fungi is an adaptation against the accumulation of toxic intermediate compounds. Proceedings of the National Academy of Sciences of the United States of America 110: 11481–11486.

McGinnis MR (1983). Chromoblastomyces and phaeohyphomycosis: new concepts, diagnosis, and mycology. Journal of the American Academy of Dermatology 8: 1–16.

Mendoza L, Karuppaiyil SM, Szanszio PJ (1993). Calcium regulates in vitro dimorphism in chromoblastomycotic fungi. Mycoses 36: 157–164.

Metaffee CJ, Casane D (2013). Accommodating the load: the transposable element content of very large genomes. Mobile Genetic Elements 3: e242775.

Middelhoven WJ (1993). Catabolism of benzene compounds by ascomycetous and basidiomycetous yeasts and yeast-like fungi. A literature review and an experimental approach. Antonie van Leeuwenhoek 63: 125–144.

Middelhoven WJ, de Hoog GS, Notermans S (1989). Carbon assimilation and extracellular antigens of some yeast-like fungi. Antonie van Leeuwenhoek 55: 165–175.

Milgroom MG (1996). Recombination and the multilocus structure of fungal populations. Annual Review of Phytopathology 34: 457–477.

Minh BQ, Nguyen MA, van Haezele A (2013). Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution 30: 1186–1195.

Mitchell A, Chang HY, Daugherty L, et al. (2015). The InterPro protein families database: the classification resource after 15 years. Nucleic Acids Research 43: D213–D221.

Moglich M, Maschwitz U, Holdoiber B (1974). Tandem calling: a new key of signal in ant communication. Science 186: 1046–1047.

Moktari P, Park J, Fedorova-Abrahms ND, et al. (2012). Systematic and searchable classification of cytochrome P450 proteins encoded by fungal and oomycete genomes. BMC Genomics 13: 525.

Möller EM, Bahnweg G, Sandermann H, et al. (2013). Tracing the origin of the fungal neurotrope Exophiala dermatitidis. BMC Genomics 14: 5688.

Molecular Biology and Evolution 0: 1–38. (2011). Independent subtilases and close relative Emmonsia. PLoS Genetics 11: e1005493.

Mszewuska A, Taylor JW, Szczesny P, et al. (2011). Independent subtilases expansions in fungi associated with animals. Molecular Biology and Evolution 28: 3395–3404.

Najafzadeh MJ, Sun J, Vicente VA, et al. (2011a). Molecular epidemiology of Fonsecaea species. Emerging Infectious Diseases 17: 464–469.

Najafzadeh MJ, Vicente VA, Sun J, et al. (2011b). Fonsecaea multimorphosa sp. nov., a new species of Chaetothyriales isolated from a feline cerebral abscess. Fungal Biology 115: 1066–1076.

Nascimento MM, Selbmann L, Shafyiai S, et al. (2016a). Arthrocladium, an unexpected human opportunist in Trichomycetes (Chaetothyriales). Fungal Biology 120: 207–218.

Nascimento MM, Vicente VA, Bittencourt JYM, et al. (2016b). Diversity of opportunistic black fungi on Babassu coconut shells, a rich source of esters and hydrocarbons. Fungal Biology: In press.

Nelson DR (2009). The cytochrome p450 homepage. Fungal Genomics 4: 59–65.

Nepal M, Voglmayr H, Schonenberger J, et al. (2014). High diversity and low specificity of chaetothyrialean fungi in carrot galleries in a neotropical ant-plant association. PLoS One 9: e112736.

Nögelakufungring P, Himmelreich U, Breger JA, et al. (2009). The trehalose synthesis pathway is an integral part of the virulence composite for Cryptococcus gattii. Infection and Immunity 77: 4584–4596.
Nguyen LT, Schmidt HA, von Haeseler A, et al. (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268–274.

Ni M, Feretaki M, Sun S, et al. (2011). Sex in fungi. Annual Review of Genetics 45: 405–430.

Nishio Y, Nakamura Y, Kawarabayasi Y, et al. (2003). Comparative complete genome sequence analysis of the amino acid replacements responsible for the thermotolerance of Corynebacterium efficiens. Genome Research 13: 1572–1579.

Nosanchuk JD, Casadevall A (2003). The contribution of melanin to microbial pathogenesis. Cell Microbiology 5: 203–223.

Novikova O, Jayachandran P, Kelley DS, et al. (2011). Heterologous expression and characterization of the sterol 14alpha-demethylase CYP51F1 from Candida albicans. Archives of Biochemistry and Biophysics 509: 9–15.

Park J, Lee S, Choi J, et al. (2012). Melanization affects susceptibility of Cryptococcus neoformans to heat and cold. FEMS Microbiology Letters 153: 265–272.

Saier Jr MH, Reddy VS, Tamang DG, et al. (2014). The transporter classification database. Nucleic Acids Research 42: D251–D258.

Saier Jr MH, Tran CV, Barabote RD (2006). TCDB: the transporter classification database for membrane transport protein analyses and information. Nucleic Acids Research 34: D161–D168.

Sanglard D, Ischer F, Koymans L, et al. (1998). Amino acid substitutions in the cytochrome P-450 14anthera-demethylase (CYP51A1) from azole-resistant Candida albicans clinical isolates contribute to resistance to azole antifungal agents. Antimicrobial Agents and Chemotherapy 42: 241–253.

Sapaj S, Glass NL (1997). Allelic specificity at the het-c heterokaryon incompatibility locus of Neurospora crassa is determined by a highly variable domain. Genetics 146: 1299–1309.

Schlüns H, Crozier RH (2009). Molecular and chemical immune defenses in ants (Hymenoptera: Formicidae). Mymecological News 12: 14.

Schnitzler N, Pel trore-Llac sahuanga H, Bestier N, et al. (1999). Effect of melanin and carotenoids of Exophiala (Wangiella) dermatitidis on phagocytosis, oxidative burst, and killing by human neutrophils. Infection and Immunity 67: 94–101.

Scholer HJ, Muller E, Schipper MAA (1983). Mucorales. In: Fungi pathogenic for humans and animals (Howard DH, ed). Marcel Dekker, New York: 9–59.

Senejani AG, Hilario E, Gogarten JP (2001). The intein of the thermoplasma A-form of the formylglycinamidine reductase from the chlamydial C2 domain. Protein Science 10: 1115–1127.

Serkiz J, Rozpedowska E, Polakova S, et al. (2001). Crystal structure of cytochrome P450 14alpha-sterol demethylase (CYP51) from Candida albicans in complex with azole inhibitors. Proceedings of the National Academy of Sciences of the United States of America 98: 3068–3073.

Sfranish P, Lysyak MY, Vervoort J, et al. (2001). Functional metabolism of toluene: monitoring of fluorinated analogs by (19)F nuclear magnetic resonance spectroscopy. Applied and Environmental Microbiology 67: 1030–1034.

Sharpton TJ, Stalpich JE, Roosnies SD, et al. (2009). Comparative genomic analyses of the human fungal pathogens Coccidioides and their relatives. Genome Research 19: 1722–1731.

Stenroos S, Laukka T, Huhtinen S, et al. (2015). Intein clustering suggests bias in acquired horizontal gene transfer. Molecular Biology and Evolution 32: 203–214.

Sudhaham D, Prakitsin S, Sivichai S, et al. (2008). The neurotropic black yeast Exophiala dermatitidis has a possible origin in the tropical rain forest. Studies in Mycology 61: 145–155.

Sun J, Najafzadeh MJ, Gerrits van den Ende AHG, et al. (2012). Molecular characterization of pathogenic members of the genus Fonsecaea using multilocus analysis. PLoS One 7: e41512.

Sung J, Zhang J, Najafzadeh MJ, et al. (2011). Melanization of a meristematic mutant of Fonsecaea monophora increases tolerance to stress factors.
while no effects on antifungal susceptibility. Mycopathologia 172: 373–380.

Switters KS, Soucy SM, Lasek-Nesselquist E, et al. (2013). Distribution and evolution of the mobile vma-1b intein. Molecular Biology and Evolution 30: 2676–2687.

Takeda K, Kaisho T, Akira S (2003). Toll-like receptors. Annual Review of Immunology 21: 335–376.

Takos AM, Rock F (2012). Why biosynthetic genes for chemical defense compounds cluster. Trends in Plant Science 17: 383–388.

Tamayo-Ramos JA, van Berkel WJ, de Graaff LH (2012). Biocatalytic potential of laccase-like multicopper oxidases from Aspergillus niger. Microbial Cell Factories 11: 165.

Tamura K, Stecher G, Peterson D, et al. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729.

Teixeira MM, de Almeida LG, Kubitschek-Barreira P, et al. (2014). Comparative genomics of the major fungal agents of human and animal sporotrichosis. Sporothrix schenckii and Sporothrix brasiliensis. BMC Genomics 15: 943.

Tolmsoff WJ (1983). Heterothallism as a mechanism of variability among fungi. Annual Review of Phytopathology 21: 317–340.

Topolina NI, Novikova O, Stanger M, et al. (2015). Post-translational environmental switch of RdaA activity by extein-intein interactions in protein splicing. Nucleic Acids Research 43: 6631–6648.

Tsui CK, DiGuisti S, Wang Y, et al. (2013). Unequal recombination and evolution of the mating-type (MAT) loci in the pathogenic fungus Grommannia clavigera and relatives. G3: Genes, Genomes, Genetics 3: 465–480.

Untereiner WA (1995). Fruitin studies in species of Capronia (Herpotrichiellaceae). Antonie van Leeuwenhoek 68: 3–17.

Untereiner WA (2000). Capronia and its anamorph: exploring the value of morphological and molecular characters in the systematics of the Herpotrichiellaceae. Studies in Mycology 45: 141–149.

Untereiner WA, Naveau FA (1998). Molecular systematics of the Herpotrichiellaceae with an assessment of the phylogenetic positions of Exophiala dermatitidis and Phialophora americana. Mycologia 91: 67–83.

van den Brink HM, van Gorcom RJF, van den Hondel CAJ, et al. (1998). Cytochrome P450 enzyme systems in fungi. Fungal Genetics and Biology 23: 1–17.

van der Kaaij RM, Janecek S, van der Maarel MJ, et al. (2002). Distribution and biochemical characterization of a novel cluster of intracellular fungal alpha-amylase enzymes. Antonie van Leeuwenhoek 69: 335–376.

van der Maarel MJ, van Dijck P, de Rop L, Szlufcik K, van den Brink HM, van Gorcom RF, van den Hondel CAJ, Untereiner WA (1995). Fruiting studies in species of dermatitidis trichiellaceae. Studies in Mycology 28: 177–182.

Vavricka CJ, Christensen BM, Li J (2010). Melanization in living organisms: a perspective of species evolution. Protein Cell 1: 830–841.

Vicente VA, Attili-Angelis D, Pie MR, et al. (2008). Environmental isolation of black yeast-like fungi involved in human infection. Studies in Mycology 61: 137–144.

Voglmayr H, Mayer V, Maschwitz U, et al. (2011). The diversity of ant-associated black yeasts: insights into a newly discovered world of symbiotic interactions. Fungal Biology 115: 1077–1091.

Voglmayr H, Mayer V, Maschwitz U, et al. (2011). The diversity of ant-associated black yeasts: insights into a newly discovered world of symbiotic interactions. Fungal Biology 115: 1077–1091.

Warburton PE, Giordano J, Cheung F, et al. (2004). Inverted repeat structure of the human genome: the X-chromosome contains a preponderance of large, highly homologous inverted repeats that contain testes genes. Genome Research 14: 1861–1869.

Whiston E, Taylor JW (2015). Comparative phylogenomics of pathogenic and nonpathogenic species. G3: Genes, Genomes, Genetics 6: 235–244.

Wicker T, Sabot F, Hua-Van A, et al. (2007). A unified classification system for eukaryotic transposable elements. Nature Reviews Genetics 8: 973–982.

Wiemken A (1990). Trehalose in yeast, stress protectant rather than reserve carbohydrate. Antonie van Leeuwenhoek 58: 209–217.

Woo PC, Tam EW, Chong KT, et al. (2010). High diversity of polyketide synthase genes and the melanin biosynthesis gene cluster in Penicillium marneffei. FEBS Journal 277: 3750–3758.

Yoshida Y (1988). Cytochrome P450 of fungi: primary target for azole antifungal agents. Current Topics in Medical Mycology 2: 388–418.

Yoshida Y, Aoyama Y (1984). Yeast cytochrome P-450 catalyzing lanosterol 14 alpha-demethylabolysis. I. Purification and spectral properties. The Journal of Biological Chemistry 259: 1655–1660.

Youngchim S, Morris-Jones R, Hay RJ, et al. (2004). Production of melanin by Aspergillus fumigatus. Journal of Medical Microbiology 53: 175–181.

Yun SH, Berbee ML, Yoder OC, et al. (1999). Evolution of the fungal self-fertile reproductive life style from self-sterile ancestors. Proceedings of the National Academy of Sciences of the United States of America 96: 5592–5597.

Zakharova K, Tesei D, Marzban G, et al. (2013). Microcolonial fungi on rocks: a life in constant drought? Mycopathologia 175: 537–547.

Zalar P, Novak M, de Hoog GS, et al. (2011). Dishwashers – a man-made ecological niche accommodating human opportunistic fungal pathogens. Fungal Biology 115: 987–1007.

Zeng J, Feng P, Gerrits van den Ende AHG, et al. (2013). Multilocus analysis of the Exophiala jeanesienei clade containing black yeasts involved in opportunistic disease in humans. Fungal Diversity 65: 3–16.

Zeppenfeldt G, Richard-Yeges N, Yeges F (1994). Cladosporium carinii: hongo dimórfico en cactáceas de la zona endémica para la cromomicosis en Venezuela. Revista Iberoamericana de Micología 11: 61–63.

Zhang J, Wang L, Xi L, et al. (2013). Melanin in a meristematic mutant of Fonsecaea monophora inhibits the production of nitric oxide and Th1 cytokines of murine macrophages. Mycopathology 175: 515–522.

Zhang S, Widemann E, Bernard G, et al. (2012). CYP52X1, representing new polyketide synthase genes and the melanin biosynthesis gene cluster in Exophiala lecanii-corni. Biotechnology and Bioengineering 75: 550–558.

Zhao Y, Park RD, Muzzarelli RA (2010b). Chitin deacetylases: properties and applications. G3: Genes, Genomes, Genetics 5: 373–388.

Zhou J, Gladeux F, Hutchison E, et al. (2015). Identification of allorereognition loci in Neurospora crassa by genomics and evolutionary approaches. Molecular Biology and Evolution 32: 2417–2432.

Zhao J, Zeng J, de Hoog GS, et al. (2010a). Isolation and identification of black yeasts by enrichment on atmospheres of monoaromatic hydrocarbons. Microbial Ecology 60: 149–156.

Zhou Y, Park RD, Muzzarelli RA (2010b). Chitin deacetylases: properties and applications. Marine Drugs 8: 24–46.

Zupancic J, Novak Babcic M, Zalar P, et al. (2016). The black yeast Exophiala dermatitidis and other selected opportunistic human fungal pathogens spread from dishwashers to kitchens. PLoS One 11: e0148166.