



Genomic analysis of ant domatia-associated melanized fungi (Chaetothyriales, Ascomycota)

Leandro F. Moreno^{1,2,3} · Veronika Mayer⁴ · Hermann Voglmayr⁵ · Rumsais Blatrix⁶ · J. Benjamin Stielow³ · Marcus M. Teixeira^{7,8} · Vania A. Vicente³ · Sybren de Hoog^{1,2,3,9}

Received: 20 August 2018 / Revised: 16 December 2018 / Accepted: 19 December 2018
© The Author(s) 2019

Abstract

Several species of melanized (“black yeast-like”) fungi in the order Chaetothyriales live in symbiotic association with ants inhabiting plant cavities (domatia) or with ants that use carton-like material for the construction of nests and tunnels. To investigate the genetic basis and evolution of this lifestyle, genomes of a lineage of four chaetothyrialean strains were sequenced and the genes annotated. While many members of Chaetothyriales have a large ensemble of enzymes enhancing tolerance of extreme or toxic environmental conditions, such as soil polluted with benzene, toluene, ethylbenzene, and xylene (BTEX), members of the domatia symbiont clade experienced significant gene family contractions. This includes enzymes involved in detoxification processes such as cytochrome P450s, transporters, and alcohol dehydrogenases. Overall, the genomes of domatia-associated species are relatively small compared to other Chaetothyriales, with low numbers of protein-coding genes and with a high content of repetitive elements. Biosynthetic clusters involved in the production of secondary metabolites and with potential antimicrobial activities are overrepresented in the genomes of these fungi. We speculate that, despite the reduction of several protein families, members of the domatia-associated clade might tolerate, and perhaps even metabolize, toxic compounds produced from exocrine glands of the ants as defense against microbes. In addition, in this symbiotic association, the plant and the ants could benefit from the production of secondary metabolites by the Chaetothyriales that participate in this tripartite association. We consider a new ecological classification for Chaetothyriales based on genomic features: (i) derived species with high abundance of paralogs colonizing habitats rich in polyaromatic and (ii) potential producers of secondary metabolites with antimicrobial activities, beneficial for symbiotic interactions, occupying specific micro-habitats such as ant domatia.

Keywords Black yeast · Melanized fungi · Domatia fungi · Ant ecology · Chaetothyriales · Phylogeny · Genomics

Section Editor: Roland Kirschner

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11557-018-01467-x>) contains supplementary material, which is available to authorized users.

✉ Sybren de Hoog
s.hoog@westerdijkinstituut.nl

- ¹ Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands
- ² Institute of Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands
- ³ Department of Basic Pathology, Federal University of Paraná State, Curitiba, PR, Brazil
- ⁴ Division of Structural and Functional Botany, Department of Botany and Biodiversity Research, University of Vienna, Wien, Austria

- ⁵ Division of Systematic and Evolutionary Botany, Department of Botany and Biodiversity Research, University of Vienna, Wien, Austria
- ⁶ CEFE, CNRS, University of Montpellier, University Paul Valéry Montpellier 3, EPHE, IRD, Montpellier, France
- ⁷ Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, AZ, USA
- ⁸ Faculty of Medicine, University of Brasília, Brasília, Brazil
- ⁹ Center of Expertise in Mycology of Radboudumc / CWZ, Nijmegen, The Netherlands

Introduction

Black yeast-like fungi in the order Chaetothyriales are known for their preference for toxic (Seyedmousavi et al. 2014) or hostile climatic (Selbmann et al. 2015) conditions—lifestyles that may have enhanced their ability to cause infection in human hosts (Moreno et al. 2018d). In addition to less stressful habitats, such as soil and decaying plant material, several members of Chaetothyriales are polyextremophiles, colonizing a wide range of hostile environments. Some of the black yeast-like fungi living in symbiotic association with ants, however, seem to occupy restricted ecological niches and might be specialists (Fig. 1) (Voglmayr et al. 2011; Vasse et al. 2017).

Ant-associated Chaetothyriales can be classified in three ecological categories based on the nature of the fungal-ant interaction: (a) living in ant-occupied domatia in plants, (b) colonizing ant-made carton structures, and (c) living as parts of fungus-gardens of leaf-cutter ants (Vasse et al. 2017). Each type of ant-plant symbiosis seems to be associated with a

particular (series of) chaetothyrialean species. Domatia are mostly inhabited by only a single, or rarely up to three species (Voglmayr et al. 2011; Nepel et al. 2016). As hundreds of ant species are known to live in symbiosis with plants (Chomicki and Renner 2015), and as the ant species investigated thus far often live with black yeast-like fungi (Mayer et al. 2014), many new ant-plant associated Chaetothyriales remain to be discovered. In general, the domatia-associated Chaetothyriales are slow-growing and mesophilic. A recent phylogenetic classification revealed that many of the melanized ant-fungi cluster in a single clade, but some species are scattered across the phylogeny of the Chaetothyriales (Voglmayr et al. 2011; Vasse et al. 2017). Some of the generalist extremotolerant species, such as a fungus close to (99.4% ITS similarity) the hydrocarbon degrader *Exophiala oligosperma*, have also been found colonizing ant-plant domatia (Vasse et al. 2017), suggesting that hydrocarbon degradation may be an essential factor in symbiosis with ants.

Fungi of all three categories of ant-association described above experience pronounced environmental challenges. One

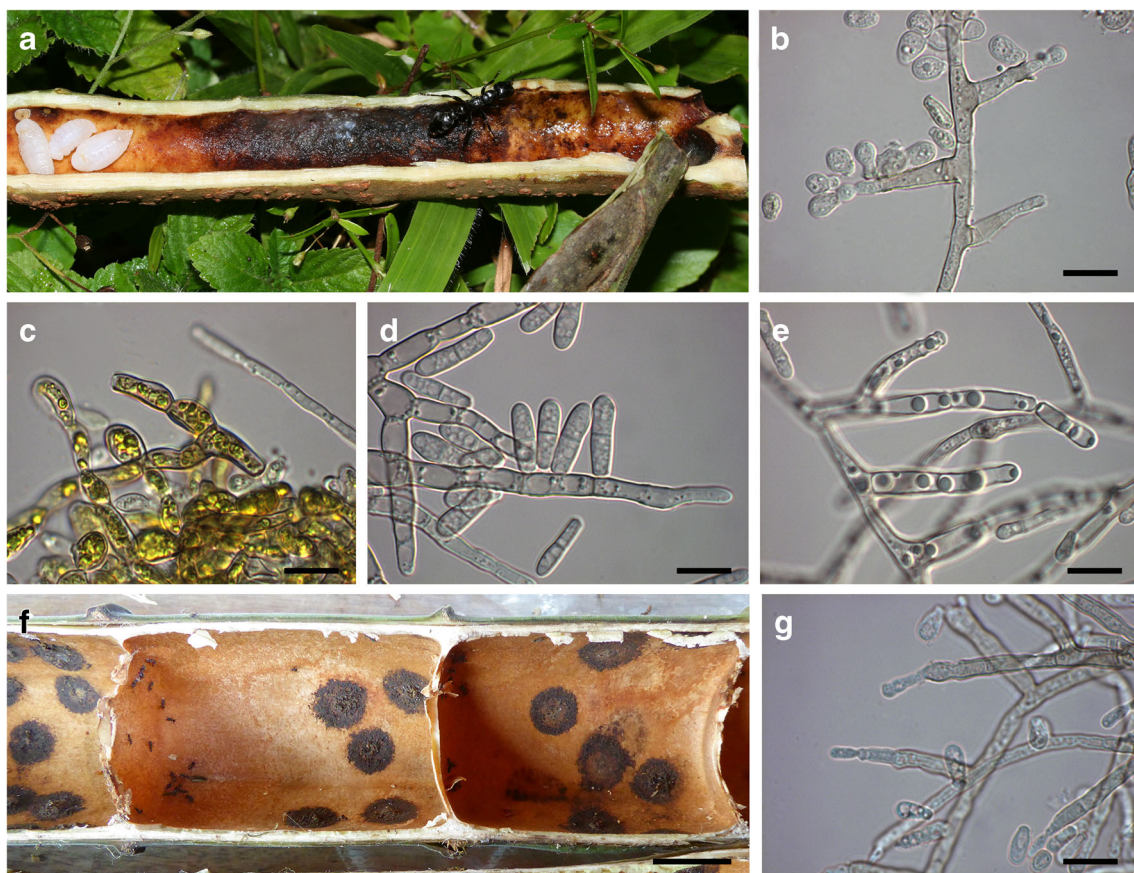


Fig. 1 Chaetothyrialean domatia symbionts of the *Barteria fistulosa*/*Tetraponera aethiops* (a–e) and *Cecropia obtusifolia*/*Azteca constructor* (f, g) ant/plant symbioses. **a** Opened stem of *Barteria fistulosa* with *Tetraponera aethiops*; black patch containing chaetothyrialean symbiont fungi. **b**, **c** Strain CBS 134920 on 2% MEA showing conidiophores with unicellular pyriform conidia (**b**) and surface mycelium from the colony

center with yellow granular contents (**c**). **d**, **e** Strain CBS 135597 on 2% MEA showing conidiophores with bicellular cylindrical conidia. **f** Opened stem of *Cecropia obtusifolia* with small *Azteca constructor* workers and ring-shaped fungal patches. **g** Strain CBS 132003, showing conidiophores and unicellular clavate conidia. Bar: **b**–**e**, **g** 10 μ m; **f** 2 cm

of these is mechanical stress due to chewing by the ants, and another is the presence of toxic exhaust to keep the nests free of pathogens (Defosse et al. 2009). On the other hand, plant domatia may confer a certain degree of protection against abiotic stress, preventing fungi from dehydration, UV radiation exposure, and temperature variation. The presence of aromatic acids, mostly having antimicrobial activity, could be a main intrinsic characteristic of this habitat. Ants possess numerous exocrine glands producing a large diversity of compounds (Hölldobler and Wilson 1990), and this characteristic has been proposed as a key selective agent responsible for dominance of Chaetothyriales in domatia (Voglmayr et al. 2011). Interestingly, the major class of chemicals in the metapleural gland secretion of some attine ants are phenylacetic acids (PAA). Some Chaetothyriales are able to metabolize PAA during the breakdown of xenobiotics, via homogentisic acid, possibly as an alternative nutrient source (Cox et al. 1993; Cox et al. 1996; Prenafeta-Boldu et al. 2006; Rustler et al. 2008; Moreno et al. 2018a).

In this context, determination of genome and gene content of Chaetothyriales living in association with ants may help to unravel the functional capacities of these fungi and how they emerged during the evolution of fungal symbiosis with ants. To address this question, we sequenced the whole genome of a selected number of domatia-inhabiting members of the order Chaetothyriales. Their genomic landscape was compared with 24 previously sequenced black yeasts of the chaetothyrialean families Herpotrichiellaceae, Cyphellophoraceae, and Trichomeriaceae. We analyzed key gene families (cytochrome P450, transporters, alcohol dehydrogenase, CAZymes) thought to be essential for the success of these organisms in colonizing extreme and toxic habitats, and which might also play a role in recurrent opportunism in the most derived family, Herpotrichiellaceae.

Materials and methods

Strains, growth conditions, and DNA extraction

For extracting the genomic DNA, four strains (CBS 134916, CBS 135597, CBS 132003, CBS 134920; Table 1) were obtained from the reference collection of Centraalbureau voor Schimmelcultures housed at Westerdijk Fungal Biodiversity Institute and cultivated in malt extract broth (MEB) for 7 days, with shaking at 150 rpm at 25 °C. The mycelia were separated using 0.2- μ m filters and frozen by liquid nitrogen followed by grinding with a mortar and pestle. DNA extraction was performed from the fine frozen powder via a cetyltrimethylammonium bromide (CTAB)-based method followed by phenol-chloroform/isoamyl alcohol purification (Moller et al. 1992).

Genome sequencing, assembly, and repetitive element identification

Library construction (180 bases-insert library) and genome sequencing (Illumina HiSeq platform) were performed at Eurofins Genomics (Ebersberg, Germany). The quality of the 101-bp Illumina reads was assessed using FastQC v0.11.3 (<http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc>), and low-quality sequences were removed by Trimmomatic (Bolger et al. 2014) and adaptors were removed by BBDuk from the BBMap package (<https://sourceforge.net/projects/bbmap/>). High-quality reads were assembled using SPAdes genome assembler v3.10.0 (Bankevich et al. 2012) with default parameters for Illumina paired-end reads. Repeated elements were identified and masked using RepeatMasker (<http://www.repeatmasker.org>) and to identify copies of de novo repeats identified by RepeatModeler (<http://www.repeatmasker.org/RepeatModeler.html>). The completeness of the genome assemblies was accessed by quantifying the presence of the Core Eukaryotic Genes (CEGs) via CEGMA (Parra et al. 2007).

Gene prediction

For gene prediction, the software Augustus (Stanke and Waack 2003) was initially used for generating training gene sets and therefore used as input the models of each organism produced for Genemark-ES v4.30 (Lomsadze et al. 2005). The trained models were used again by Augustus (Stanke and Waack 2003) for the gene prediction in the masked genomic sequences. In addition to protein-coding genes, tRNAs were predicted by tRNAscan-SE v1.23 (Lowe and Eddy 1997). The protein sequences were functionally annotated by InterProScan (database version 5.27–66.0) and BLAST against Swiss-Prot database.

Functional annotation

To predict CYP450s, we identified proteins carrying the PFAM domain PF00067 and compared, by using BLAST, their sequences against the CYP previously described by Teixeira et al. (2017). Putative CYP450 genes were classified into families and subfamilies as recommended by the International P450 Nomenclature Committee (Nelson 2006) using the BLAST identity cutoff of 35% for families and 55% for subfamilies. CYP proteins that could not be assigned to families or subfamilies based on the International P450 Nomenclature Committee were aligned and subjected to phylogenetic analyses, as described elsewhere (Chen et al. 2014a).

Carbohydrate-Active Enzymes (CAZymes) were identified using the dbCAN2 web server (Zhang et al. 2018). For

Table 1 Assembly and annotation statistics for domatia-associated species sequenced in this study

	CBS 134916	CBS 132003	CBS 134920	CBS 135597
Source	Domatium	Domatium	Domatium	Domatium
Ant host	<i>Cladomyrma petalae</i>	<i>Azteca constructor</i>	<i>Tetraponera aethiops</i>	<i>Tetraponera aethiops</i>
Plant host	<i>Saraca thaipingensis</i>	<i>Cecropia obtusifolia</i>	<i>Barteria fistulosa</i>	<i>Barteria fistulosa</i>
Continent	Asia	America	Africa	Africa
Genome size (Mb)	26.3	22.8	20.6	20.6
Percent of completeness	96	94	93	95
Contig N50	60,287	312,613	164,768	570,188
Number of contigs	1226	118	408	134
Number of ORFs	6719	5812	5988	5689
%G + C content	50.55	54.00	53.82	52.73
Repetitive elements	16.32%	4.09%	8.67%	8.43%
tRNAs	37	43	76	60
GenBank ID	QQXO00000000	QRBJ00000000	QQSL00000000	QQXN00000000

comparative studies, we repeated the same analyses using the 24 previously sequenced black yeasts (Teixeira et al. 2017).

Protein sequences corresponding to the *MAT1-1* or *MAT1-2* genes, as well as the flanking genes *APN2*, *SLA2*, *APC5*, and *COX13*, commonly found conserved among Eurotiomycetes, were obtained from Teixeira et al. (2017) and compared by BLAST searches against the newly sequenced genomes.

In order to compare the protein content among the black yeasts, we built clusters of orthologs using the OrthoMCL pipeline v1.4 with a Markov index of 1.5 and maximum *e*-value of 1×10^{-5} . The protein sequences of 20 black yeasts included in this analysis were downloaded NCBI: *Rhinochlamydia mackenziei* (JYBU00000000), *Fonsecaea pedrosoi* (JYBS00000000), *Fonsecaea nubica* (LVCJ00000000), *Fonsecaea erecta* (LVYI00000000), *Fonsecaea monophora* (LVKK00000000), *Fonsecaea multimorphosa* (JYBV00000000), *Capronia coronata* (AMWN00000000), *Capronia epimyces* (AMGY00000000), *Capronia semiimmersa* (JYCC00000000), *Cladophialophora bantiana* (JYBT00000000), *Cladophialophora carrionii* (AOFF00000000), *Cladophialophora immunda* (JYBZ00000000), *Cladophialophora psammophila* (AMGX00000000), *Cladophialophora yegresii* (AMGW00000000), *Exophiala aquamarina* (AMGV00000000), *Exophiala mesophila* (JYBW00000000), *Exophiala oligosperma* (JYCA00000000), *Exophiala sideris* (JYBR00000000), *Exophiala spinifera* (JYBY00000000), *Exophiala xenobiotica* (JYCB00000000), and *Exophiala dermatitidis* (CM001238).

To assess phylogenetic relationships of the newly sequenced species, the amino acid sequences of each single-

copy orthologs (SCO) were aligned with MUSCLE (Edgar 2004) and poorly aligned regions were automatically removed using TRIMAL v1.2 (Capella-Gutierrez et al. 2009) by setting the “-automated1” option. The alignments were concatenated with FASCONCAT v1.0 (Kuck and Meusemann 2010), and the phylogenetic tree was inferred by maximum likelihood via RAXML (Stamatakis 2014) on the CIPRES Science Gateway web portal (http://www.phylo.org/sub_sections/portal/) employing the amino acid substitution model PROTCATWAG (Miller et al. 2010).

To identify and classify clusters of genes involved in the production of secondary metabolites, the genomes of the domatia-associated species were mined by the antiSMASH web server (Weber et al. 2015), fungal version 4.1.0 (<https://fungismash.secondarymetabolites.org/>).

Membrane transport proteins were identified and classified as described elsewhere (Teixeira et al. 2017). Briefly, protein sequences were compared using BLASTP (*E*-value threshold $1e-05$) against the transporter sequences available at Transporter Classification Database (TCDB) (Saier et al. 2014).

To predict changes in size of protein families, functional domains were counted and compiled for 21 Herpotrichiellaceae and in the domatia-associated species. Significant contractions of specific InterPro domain were determined by Fisher’s exact test using the Python library SciPy.

Mitochondrial genome extraction and annotation

Mitochondrial genomes were extracted by aligning the contigs generated from the whole genome assembly against the reference mitochondrial genome of *Exophiala dermatitidis* NIH/UT8656 (Genbank NW_008751656) using NUCmer (default

parameters) (Kurtz et al. 2004). Mitochondrial genome annotations were done using MFannot (<http://megasun.bch.umontreal.ca/cgi-bin/mfannot/mfannotInterface.pl>) and manually adjusted. Annotation of tRNA genes was improved using tRNAscan-SE (Lowe and Eddy 1997).

Code availability

Custom codes used in this paper as well as raw data produced by the prediction of CAZy and InterProScan are available in http://www.github.com/leandrofmorero/ant-associated_black_yeasts.

Results

Sequencing, assembly, and annotation

Genome sequences of four Chaetothyriales colonizing ant domatia were determined by Illumina high-throughput sequencing and de novo assembled. Assembly size varied from 20.6 Mbp, in the fungal species isolated from plant-ant domatium of *Barteria fistulosa-Tetraponera aethiops*, to 26.3 Mbp in the species associated with *Saraca thaipingensis-Cladomyrma petalae* (Table 1 and Fig. 2). The GC content ranged from 50.55 to 54%, being consistent with other black yeast species (Teixeira et al. 2017). The content of repetitive elements was considered high and unique among the order *Chaetothyriales*, varying from 4.09 to 16.32%. The majority of the transposons found in these species belonged to the subclasses of retrotransposons LINE and LTR. We assessed the completeness and evaluated our assemblies quantifying the presence of the Core Eukaryotic Genes (CEGs). This analysis revealed that the genomes sequenced in this study are highly complete including at least 93% of 248 CEGs (Table 1).

The protein-coding gene composition was determined by ab initio gene prediction method (without experimental evidence). We observed a phylogenetic correlation between genome size and total gene number across the order Chaetothyriales (Fig. 2). Highest counts of genes were found in the isolate CBS 134916 (6719 gene modes), although this number is lower than any other Chaetothyriales sequenced so far (Teixeira et al. 2017). Even more extreme values were found in the isolates CBS 134920, CBS 132003, and CBS 135597 (Table 1), in which compact proteomes containing less than 6000 proteins were estimated. It is worth noting that the genome assembly of CBS 134916 was more fragmented, which could lead to an increase in the number of gene counts caused by coding regions being split.

We compared 323,280 protein sequences from 21 Herpotrichiellaceae, 2 Cyphellophoraceae and 1 Trichomeriaceae in order to group protein homologs into

clusters. We obtained 29,551 orthologous clusters, being 679 clusters composed by single-copy orthologues (one copy of gene from each species). Maximum likelihood tree was constructed using concatenated alignments of all the single-copy orthologous identified (Fig. 2), revealing that the ant domatia-associated black fungi are placed at an intermediated position to Cyphellophoraceae and Trichomeriaceae.

Mitochondrial genomes of the four sequenced species were assembled into single contigs and are AT-rich with G + C content ranging between 23.8% in CBS 134916 to 26.4% in CBS 134920. The sizes of the mitochondrial genomes vary from 40,056 to 66,938 Mbp in CBS 132033 and CBS 134920, respectively. Genome size variation between the different species is correlated to the variable number of introns. All species analyzed in this study possess introns in the following protein-coding genes: *cob*, *nad5*, and *cox1*. Additionally, we observed that the strains CBS 134920 and CBS 134916 hold introns located in the gene coding for a cytochrome c oxidase (*cox13*).

Gene family expansions and contractions

By comparing the content of protein functional domains identified in the Chaetothyriales colonizing ant domatia against the family Herpotrichiellaceae, we verified 23 significant (Fisher's exact test, q value < 0.05) gene family contractions (Table 2). Such contractions are unique among the Chaetothyriales and provide specific genomic signatures of black domatia-associated fungi. Several contracted families belong to classes of enzymes thought to confer the metabolic plasticity previously described in Herpotrichiellaceae, including cytochrome P450s, transcription factors, alcohol dehydrogenases, and membrane transporters of the superfamily major facilitator, such as sugar transporter-like (Table 2). Protein domains associated with the metabolism of volatile organic compounds (VOC), such as the S-hydroxymethylglutathione (IPR006913), as well as flavin monooxygenase-like (IPR020946), which constitute a family of xenobiotic-metabolizing enzymes, were also found depleted, but not absent, in this group of fungi.

Cytochrome P450s (CYPs)

The plethora of CYPs was assessed and revealed that the domatia-associated species possess the smallest CYP repertoire among the Chaetothyriales analyzed so far, ranging from 36 to 46 genes in CBS 134926 and CBS 135597, respectively. The entire repertoire of CYPs of the four sequenced species contains 115 CYPs classified into 24 families and 36 subfamilies (Table S1). In addition, several CYPs are found in the domatia-colonizing species, with homologs in non-related fungi, but not other Chaetothyriales (Table S1). Four CYPs could not be assigned to any already defined CYP family or subfamily following the International P450 Nomenclature

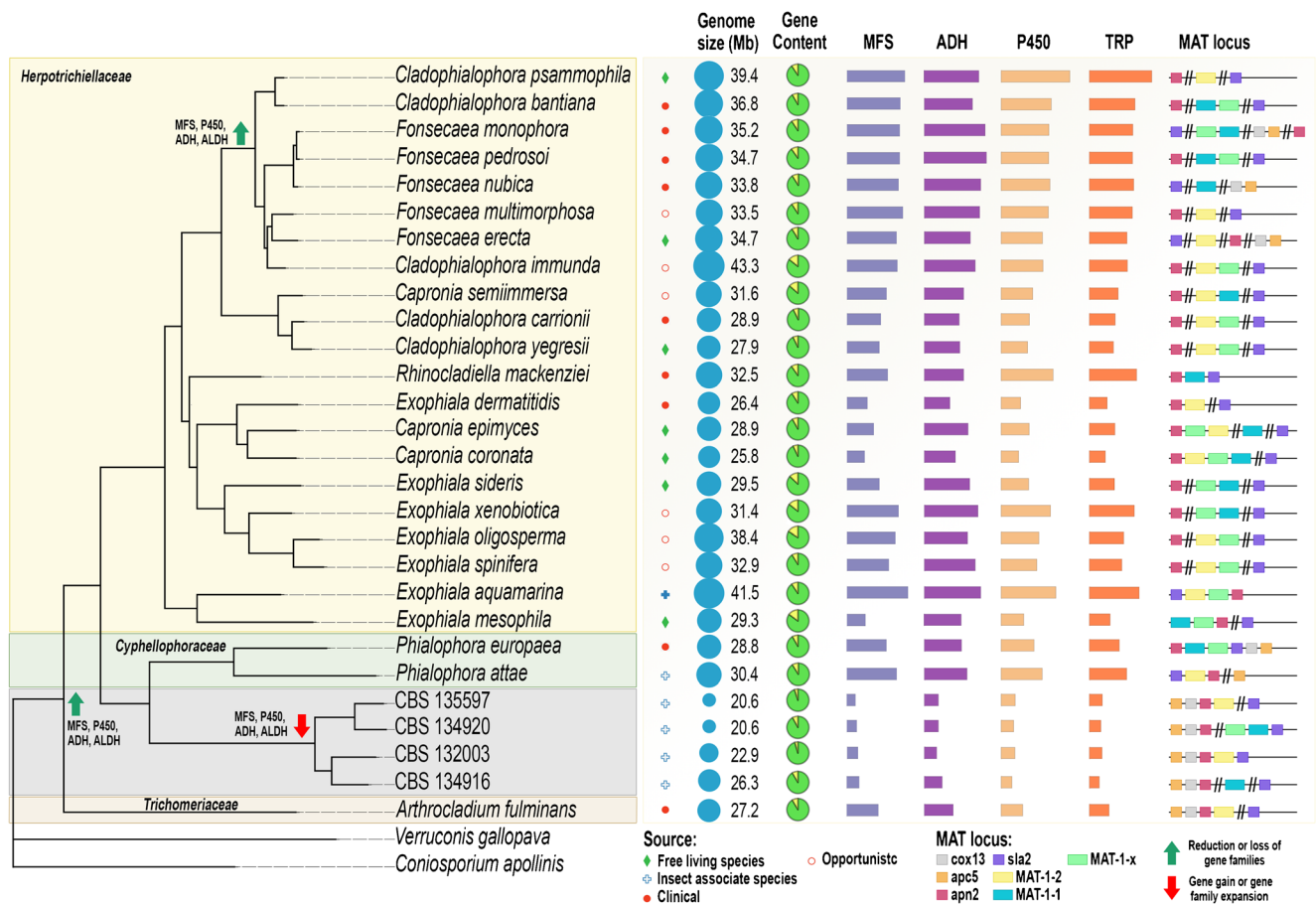


Fig. 2 Genomic landscape of the order Chaetothyriales. Phylogenomic tree was built based on the amino acid sequences of 679 single-copy orthologs shared by all the studied species. Arrows in the node of the phylogenetic tree represent gene family expansion (green) and contraction (red) events that occurred during evolution of black yeasts. The abundance of protein domains corresponding to the major facilitator

superfamily (MFS), alcohol dehydrogenase (ADH), cytochrome P450 (P450), and transcription factor (TRP) are represented in each species by bars colored in light purple, dark purple, light orange, and dark orange, respectively. Segments that are not clustered in the MAT locus are indicated by double slash

Committee. Non-classified CYPs were considered as species-specific CYPs (Table S1). Seven CYP families are shared by all four analyzed fungi: CYP617, CYP532, CYP505, CYP545, CYP504 (phenylacetate 2-hydroxylase), CYP628, and CYP53 (benzoate 4-hydroxylase).

Carbohydrate-active enzymes (CAZymes)

The number of CAZyme families is low compared to Chaetothyriales in other families, with the exception of the dermatitidis clade (Fig. 3). Species associated with the ant host *Tetraponera aethiops* (CBS 134920 and CBS 135597) possess the pectate lyase subfamilies 4 (PL1_4), g2833.t1 and g3705.t1, respectively, responsible for degradation and modification of plant-derived polysaccharides. The presence of pectate lyase in the order Chaetothyriales is exceptional, thus far having been reported in members of the family Cyphellophoraceae and in *Capronia coronata* only (Teixeira et al. 2017). Comparative analysis with distantly related

ascomycetes showed that highly conserved homologs of pectate lyase are shared with some important fungal plant pathogens, such as *Diaporthe helianthi* and *Grosmannia clavigera* (Fig. 3). Our analyses confirmed that other polysaccharide lyase families, such as PL3, PL4, PL7, PL9, and PL10, have been lost completely in black yeasts associated to ant domatia. Enzymes linked to degradation of cellulose, such as the glycoside hydrolase subfamilies GH5_5 and GH5_22, were completely absent in the domatia-associated species. Such enzymes were previously found among other black yeast-like fungi, but as yet their activity has not been tested (Table S2) (Teixeira et al. 2017). Similarly, enzymes involved in the breakdown of many polysaccharides are absent, such as the polymeric xylan (GH10, GH11, GH62, GH67, GH115, CE1, and CE15), galactomannan (GH5_7, GH26, GH27, GH36, and GH134), and xyloglucan (GH12, GH29, GH74, and GH95). While an apparent reduction was noted of enzymes related to the degradation of cellulose, the abundance of enzymes in chitin catabolism (chitinases and N-

Table 2 Protein domains depleted in black yeasts colonizing ant domatia

InterPro ID	Functional domain	Depletion: domatia associated Chaetothyriales × Herpotrichiellaceae (<i>p</i> value)
IPR001138	Zn(2)-C6 fungal-type DNA-binding	8.53E–37
IPR007219	Transcription factor domain, fungi	5.93E–32
IPR021858	Fungal transcription factor	2.47E–15
IPR005828	Major facilitator, sugar transporter-like	1.80E–14
IPR006913	Glutathione-dependent formaldehyde-activating enzyme	2.09E–12
IPR010573	Siderophore iron transporter 1/trichothecene efflux pump	9.05E–12
IPR002938	FAD-binding domain	1.60E–11
IPR002347	Short-chain dehydrogenase/reductase SDR	3.64E–11
IPR022124	Protein of unknown function DUF3659	5.27E–10
IPR020946	Flavin monooxygenase-like	1.21E–07
IPR005123	Oxoglutarate/iron-dependent dioxygenase	1.70E–06
IPR001128	Cytochrome P450	2.16E–06
IPR026992	Non-haem dioxygenase N-terminal domain	3.38E–05
IPR010730	Heterokaryon incompatibility	1.21E–04
IPR011234	Fumarylacetoacetase-like, C-terminal	7.14E–04
IPR013154	Alcohol dehydrogenase, N-terminal	8.95E–04
IPR004360	Glyoxalase/fosfomycin resistance/dioxygenase domain	1.33E–03
IPR011701	Major facilitator superfamily	2.01E–03
IPR000073	Alpha/beta hydrolase fold-1	3.61E–03
IPR013149	Alcohol dehydrogenase, C-terminal	8.91E–03
IPR001509	NAD-dependent epimerase/dehydratase	2.08E–03
IPR002018	Carboxylesterase, type B	2.53E–02
IPR025110	AMP-binding enzyme, C-terminal domain	4.83E–02

InterPro domain counts were compared against members of the family Herpotrichiellaceae and significant contraction in gene families were calculated by Fisher's exact test

acetylglucosaminidases) was consistent across black yeasts, with CAZy families GH18 (chitinases), GH20 (N-acetylglucosaminidases), GH75 (chitosanases), GH2 (glucosaminidase), and CE4 (chitin deacetylases) (Table S2).

Secondary metabolism

The genomes were screened for the prediction of clusters of genes involved in the production of secondary metabolites. On average, the species possess 19 biosynthetic clusters, with type I PKS (t1pks) being the most prevalent cluster type ranging from 10 copies in CBS 134916 to 14 copies in CBS 132003 (Table S3). Several of these genes were homologous with enzymes for the production of antimicrobial compounds (Table 3). Type III PKS cluster (t3pks), previously reported in Herpotrichiellaceae, Cyphellophoraceae, and Trichomeriaceae, was not observed in the domatia-associated species. The hybrid cluster III PKS/I PKS was found only in CBS 135597 and CBS 134920 that both lived in symbiotic relationship with the ant

host *Tetraponera aethiops* and the plant host *Barteria fistulosa* (Table S3).

Membrane transport proteins

Membrane transport proteins were identified by comparing the protein sequences of the domatia-associated lineages against the Transporter Classification Database (TCDB). We identified 3622 proteins of the transporters superfamily in all 4 strains. Possessing 926 transporters, CBS 134916 contains the highest number of this protein family, followed by CBS 132003, CBS 134920, and CBS 135597, containing 909, 900, and 887 transporters, respectively. Striking contractions in the transporter content among black yeasts were observed in the gene families nicotinate permease (2.A.1.14.11), trichothecene efflux pump (2.A.1.3.47), MFS transport (2.A.1.2.33), peroxisomal isopenicillin N importer (2.A.1.2.86), and multi-drug resistance transporter (2.A.1.3.73) (Fig. 4).

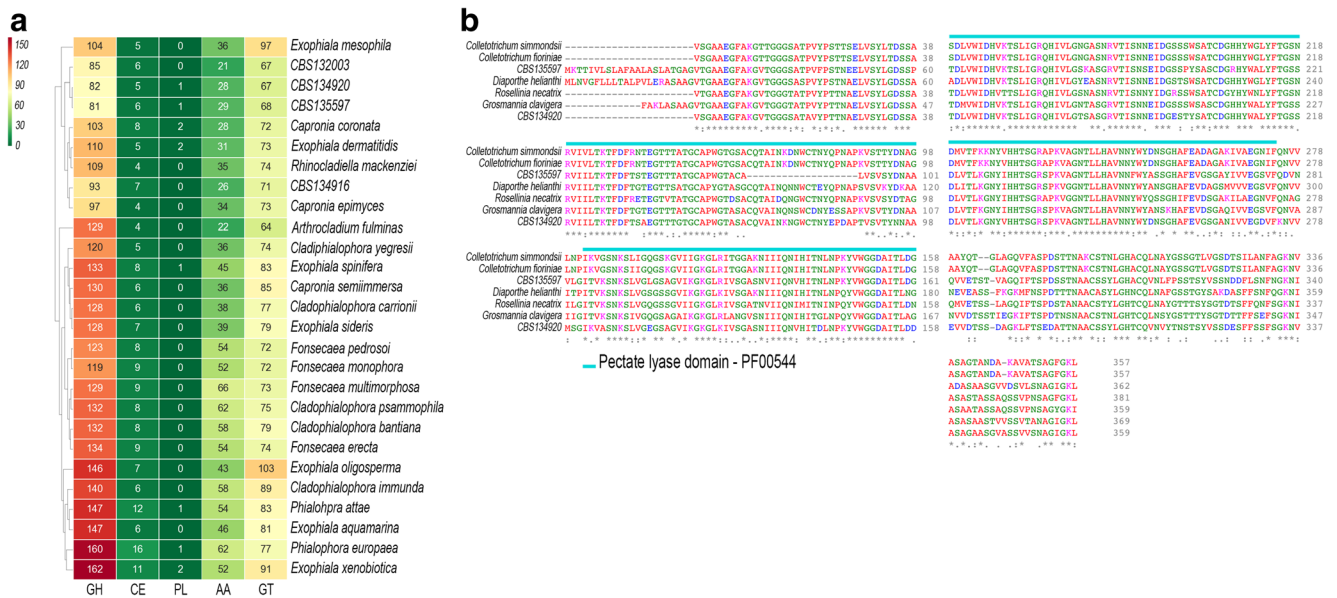


Fig. 3 Composition of CAZymes in Chaetothyriales. **a** Heatmap representing the abundance of CAZymes across the order Chaetothyriales. **b** Alignment of pectate lyase found in CBS 134920

Asexual and sexual reproduction

The sexual identity of the sequenced strains was investigated by homology with the mating-type locus (MAT) structure previously described in Chaetothyriales. Strains proved to be heterothallic, since either *MAT1-1* or *MAT1-2* genes were found in a single scaffold, in addition to the flanking genes *SLA2-APN2-COX13*. CBS 134916 and CBS 134920 had *MAT1-1* genes, while CBS 135597 and CBS 132003 harbored *MAT1-2* (Fig. 2). In CBS 134920 and CBS 132003 the *SLA2* gene was located within the MAT locus, while in CBS 134916 and CBS 135597 this gene was not clustered but was found in a separate scaffold. Regarding protein family domains

and CBS 135597 with fungal plant pathogens. The functional domain PF00544 corresponding to pectate lyase is represented by a blue line

associated with the parasexual cycle, the heterokaryon incompatibility protein (HET-PF06985) derived from 10 domains in the fungus CBS 134920 to 31 domains in CBS 134916 (Table S4).

Discussion

We fully sequenced the genome of four ant domatia-associated strains that represent as yet undescribed species, using high-throughput genome sequencing. We observed that the content of repetitive elements is inversely proportional to the genome size of these species, which have the smallest

Table 3 Clusters of secondary metabolite biosynthesis with antimicrobial activities in domatia associated black fungi

Secondary metabolite	Biosynthetic cluster ID	Species	Number of homologs	Average similarity (%ID/%cov)
Emericellin	BGC0001271_c1	CBS 134920	3/14	54/96
		CBS 135597	3/14	60/73
		CBS 132003	5/14	63/84
Viridicatumtoxin	BGC0000168_c1	CBS 134920	8/22	66/86
		CBS 135597	10/22	66/90
Acetylaranotin	BGC0000022_c1	CBS 135597	2/10	51/97
Asperfuranone	BGC0000121_c1	CBS 135597	6/11	57/69
		CBS 132003	3/11	51/86
Pestheic acid	BGC0000121_c1	CBS 134916	2/20	58/97
		CBS 132003	2/20	50/73
Yanuthone D	BGC0000170_c1	CBS 132003	6/10	59/79
Monacolin	BGC0000098_c1	CBS 134916	2/9	56/69
Brefeldin	BGC0001141_c1	CBS 134920	2/10	47/80

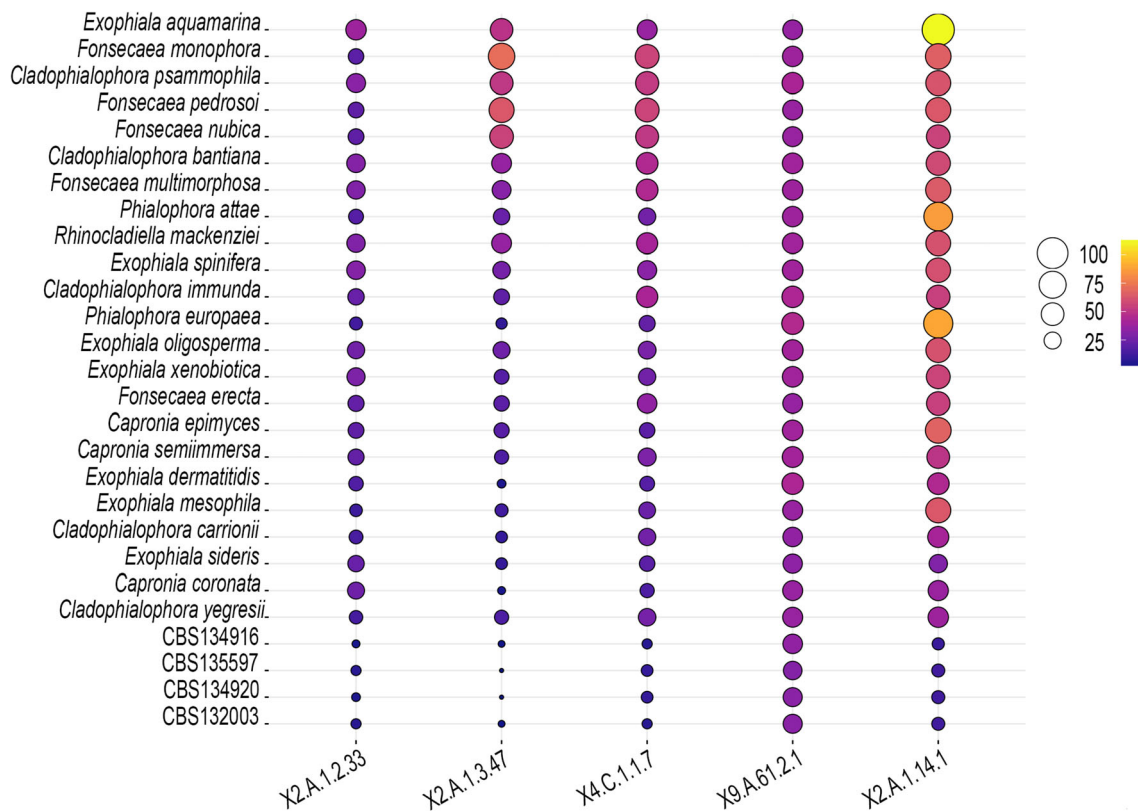


Fig. 4 Balloon plot showing the distribution of common membrane protein transporters in melanized Chaetothyriales. The families represented according to the TCDB database classification: nicotinate

permease = 2.A.1.14.11, trichothecene efflux pump = 2.A.1.3.47, MFS transport = 2.A.1.2.33, peroxisomal isopenicillin N importer = 2.A.1.2.86, multidrug resistance transporter = 2.A.1.3.73

genomes reported in Chaetothyriales so far (Teixeira et al. 2017). Despite their reduced genome size, these organisms possess the highest repetitive element content in the order Chaetothyriales (Table 1). Similarly to what has been published in *Exophiala dermatitidis* and *Rhinoctadiella mackenziei*, the mitochondrial genomes of black yeasts colonizing ant domatia are AT-rich and their sizes correlate with the variable number of introns found on mitochondrial genes (Moreno et al. 2015; Teixeira et al. 2017; Vicente et al. 2017; Moreno et al. 2018c).

We compared the protein domains of the four black yeasts colonizing ant domatia against members of Herpotrichiellaceae, a derived family in Chaetothyriales, to examine differences in functional content, using hypergeometric test. We observed that the domatia-associated species underwent contraction in gene families previously described to be expanded in other Chaetothyriales (Chen et al. 2014b; Teixeira et al. 2017; Vicente et al. 2017; Moreno et al. 2018a). At a gross level, the reduced genome size of these species might be explained by a large number of gene family contractions (Fig. 2). Depletion of gene families in this group of fungi seems to be an extremely rare evolutionary event, having been described in the fungus *Rhinoctadiella mackenziei* only (Chen et al. 2014b; Teixeira et al. 2017;

Vicente et al. 2017; Moreno et al. 2018a). Among the significantly contracted functional domains, CYPs, transporters, and alcohol dehydrogenases are thought to play an important role for the survival of black yeasts under hostile conditions, conferring, e.g., tolerance to toxic xenobiotics. Expansion/contraction of transcription factors in Chaetothyriales has not yet been discussed in detail, although these are essential regulators of gene expression. Their expansion correlates with the number of protein-coding genes and seems to be linked to gene duplication (Shelest 2017). This suggests that the increased number of duplicated genes in derived Chaetothyriales are still functional and under fine-tuned regulation. The expansion of gene families has been associated with the success of free-living black yeasts in colonizing extreme habitats. Although the environment of the domatium occupied by black yeasts can be harsh and toxic because of the antimicrobial chemicals produced by ants (Fernández-Marín et al. 2006, 2015; Penick et al. 2018), the chemical stress induced is predictable and the number of compounds relatively low. This could explain the lower number of isoforms for specific gene families in Chaetothyriales specialized in colonizing ant-occupied domatia. As the nature of chemical compounds produced by ants is species-specific (Attygalle and Morgan 1984; Hölldobler and Wilson 1990), we may

expect that gene family contraction is correlated with ant-host specialization of Chaetothyriales. Further studies determining the exact chemical nature and content of antimicrobial chemicals within domatia will shed light upon the origin of resistance to toxic compounds in black yeasts.

Due to the abundance and high diversity of CYPs in Chaetothyriales (Teixeira et al. 2017; Vicente et al. 2017), this family of enzymes has been suggested to play an essential role in the adaptation to extreme environments. For example, in the hydrocarbon degrading fungus *Cladophialora immunda*, CYPs are among the most overrepresented protein domains and are upregulated when the fungus was grown in the presence of toluene (Blasi et al. 2017). Indeed, recent studies have concluded that some families of CYPs are associated with the initial oxidation of the methyl group of toluene being one of the key reactions for the degradation of pollutants (Luykx et al. 2003; Blasi et al. 2017). CYPs are essential for the metabolism of polycyclic aromatic hydrocarbons from multiple anthropogenic sources, such as oil spills and incomplete combustion of organic materials (e.g., coal, oil, petrol, and wood) (Abdel-Shafy and Mansour 2016). It is noteworthy that, despite the observed reductions, frequent CYP families, involved in the degradation of xenobiotics, were found in all analyzed genomes, from ant-associated fungi to human opportunists in Herpotrichiellaceae. A remarkable example is CYP504 (coding for phenylacetate 2-hydroxylase), which is highly duplicated across the black yeasts (Teixeira et al. 2017) but was found as a single copy in the domatia-associated species. This enzyme is overexpressed in fungi when they are grown in the presence of phenylacetic acid (PAA), and it is responsible for the conversion of this chemical into 2-hydroxyphenylacetate (Harris et al. 2009). Moreover, PAA is the most abundant active compound secreted by the metapleural gland of attine ants to avoid pathogens entering the fungus gardens (Do Nascimento et al. 1996; Fernandez-Marin et al. 2015). The presence of CYP504 in ant-associated species and its conservation during evolution of the black yeasts suggests that the breakdown of chemicals via PAA by phenylacetate 2-hydroxylase may represent one of the ancient mechanisms for degrading xenobiotics in this group of fungi.

We have mined the genomes of the black yeasts living in symbiotic association with ants in order to identify clusters of genes coding for enzymes related to the production of secondary metabolites. Remarkably, these species harbor the highest number of biosynthetic clusters predicted in Chaetothyriales so far (Table S3). While the average of clusters in the families Herpotrichiellaceae and Cyphellophoraceae was predicted to be 11 and 9, respectively (Teixeira et al. 2017), domatia-associated species possess on average 19 clusters. In particular, the I PKS cluster is well represented in all strains isolated from domatia. The gene composition of the I PKS clusters identified in these species have homology with other biosynthetic pathways already described in several microorganisms,

for example, the antibiotic/antiviral viridicatumtoxin/brefeldin biosynthetic gene cluster, identified in CBS 134920 and CBS 135597, and the pesthelic acid biosynthetic gene cluster found in CBS 134916 and CBS 132003. We speculate that, in addition to the production of compounds with antimicrobial activity by the ants (Do Nascimento et al. 1996; Voglmayr et al. 2011), the antibiotic-producing black yeasts have an important role in keeping the domatia free of pathogens. Therefore, black yeasts colonizing ant domatia might benefit from the abundance of aromatic hydrocarbons produced by the ants, which could be used as carbon source via PAA degradation, while helping in the protection of the ant nests by producing antibiotics.

The composition of CAZyme families in fungi seems to be responsible, to some extent, for increased metabolic plasticity with respect to carbon utilization (Benoit et al. 2015). For example, the generalist lifestyle of some fungi is linked with the ability of degrading a diversity of polysaccharides, particularly those present in plant material. Therefore, species possessing a large arsenal of these enzymes seems to be more capable of thriving in a wide range of substrates, being not restricted to a specific ecological niche (de Vries et al. 2017). Comparative genomic analyses revealed that the black yeasts and relatives own a complex set of CAZymes, but experienced important gene losses in the families associated with the breakdown of pectin. The absence of such enzymes in the genome of Chaetothyriales suggests that these organisms, including the domatia-colonizing species, might have limited ability to obtain nutrients from plant material. The composition of CAZymes found in the black yeasts associated to ant domatia suggests that these organisms are unlikely to be efficient polysaccharide decomposers; this contrasts with the classic view of mutualistic fungus-ant relationship, where the fungal partner degrades plant material incorporated by the ants (De Fine Licht et al. 2010).

Upregulation of membrane transporters and increased gene dosage of this family of proteins are well-known mechanisms to acquire resistance against a variety of stressors (Sanglard et al. 2009; Morschhauser 2010; Yew et al. 2016). In the genomes of fungi colonizing ant domatia, the number of membrane proteins is reduced compared to other Chaetothyriales (Blasi et al. 2017; Teixeira et al. 2017). This finding is consistent with the contraction of the major facilitator, sugar transporter-like domain IPR005828 represented in Fig. 2. Despite this reduction, members of the investigated lineage have most of the conserved transporters reported previously in the order Chaetothyriales; these are likely involved in niche adaptation to hostile environments, although with limited gene dosage. For example, the toxin efflux pumps of the class trichothecene (TC 2.A.1.3.47) are highly abundant in most of the Chaetothyriales (i.e., 65 gene copies in *Cladophialophora immunda*) (Blasi et al. 2017; Teixeira et al. 2017), while the domatia-associated lineages harbor 9

to 10 gene copies coding for this transporter. These data suggest that, similar to CYPs and alcohol dehydrogenases, transporters are vastly duplicated in the derived Chaetothyriales which are not associated with ant domatia; this may be related to their lifestyle in environments where nutrient access requires complex metabolic plasticity, such as soil polluted with benzene, toluene, ethylbenzene, and xylene (BTEX) and shells of the babassu coconut palm tree containing a rich diversity of terpenoids and fatty acids (Badali et al. 2011; Nascimento et al. 2017).

By assessing the MAT locus of the sequenced strains, we propose heterothallism as prevalent reproductive mode, because a single mating type idiomorph (either MAT1–1 or MAT1–2) was found per haploid genome. In contrast to the MAT locus in the family Herpotrichiellaceae, in the ant-associated Chaetothyriales, the syntenic flanking genes are organized in way that resembles that of black fungi in the families Cyphellophoraceae and Trichomeriaceae, and of other Eurotiomycetes: *APC5-COX13-APN2* (Fig. 2) (Coppin et al. 1997; Fraser et al. 2007; Teixeira et al. 2017; Moreno et al. 2018b). An interesting pattern within the genomes of several previously sequenced black yeasts was the high abundance of hypothetical protein coding genes and HET protein domains (Teixeira et al. 2017), which was not observed in CBS 134916, CBS 134920, CBS 135597, and CBS 132003. The parasexual cycle is an important source of genetic diversity in fungi, in which the HET locus confers non-self-discrimination during vegetative growth, and has been proposed to function as defense systems against competitors and pathogens (Glass and Dementhon 2006). Another incompatibility system characterized in fungi includes the participation of the WD-repeat domain (Espagne et al. 2002; Paoletti and Saupé 2009). Contrary to the abundance of HET domains that vary considerably across the black yeasts, the number of WD-repeat domains seems to be constant in Chaetothyriales.

Overall, our findings suggest that Chaetothyriales living in domatia together with ants possess more compact genomes, and despite their lower diversity of enzymes involved in the metabolism of toxic compounds, this lineage is expected to tolerate and eventually utilize xenobiotics as carbon source.

Funding Acquisition of fungal genomic data was partially supported through funds from the Faculty of Life Sciences, University of Vienna, to VM.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

- Abdel-Shafy HI, Mansour MSM (2016) A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. *Egypt J Pet* 25:101–123
- Attygalle AB, Morgan ED (1984) Chemicals from the glands of ants. *Chem Soc Rev* 13:245–278
- Badali H, Prenafeta-Boldu FX, Guarro J et al (2011) *Cladophialophora psammophila*, a novel species of Chaetothyriales with a potential use in the bioremediation of volatile aromatic hydrocarbons. *Fungal Biol* 115:1019–1029
- Bankevich A, Nurk S, Antipov D et al (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477
- Benoit I, Culleton H, Zhou M et al (2015) Closely related fungi employ diverse enzymatic strategies to degrade plant biomass. *Biotechnol Biofuels* 8:107
- Blasi B, Tafer H, Kustor C et al (2017) Genomic and transcriptomic analysis of the toluene degrading black yeast *Cladophialophora immunda*. *Sci Rep* 7:11436
- Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120
- Capella-Gutierrez S, Silla-Martinez JM, Gabaldon T (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25:1972–1973
- Chen W, Lee MK, Jefcoate C et al (2014a) Fungal cytochrome p450 monooxygenases: their distribution, structure, functions, family expansion, and evolutionary origin. *Genome Biol Evol* 6:1620–1634
- Chen Z, Martinez DA, Gujja S et al (2014b) Comparative genomic and transcriptomic analysis of *Wangiella dermatitidis*, a major cause of phaeohyphomycosis and a model black yeast human pathogen. *G3 (Bethesda)* 4:561–578
- Chomicki G, Renner SS (2015) Phylogenetics and molecular clocks reveal the repeated evolution of ant-plants after the late Miocene in Africa and the early Miocene in Australasia and the Neotropics. *New Phytol* 207:411–424
- Coppin E, Debuchy R, Arnaise S et al (1997) Mating types and sexual development in filamentous ascomycetes. *Microbiol Mol Biol Rev* 61:411–428
- Cox HHJ, Houtman JHM, Doddema HJ et al (1993) Growth of the black yeast *Exophiala jeanselmei* on styrene and styrene-related compounds. *Appl Microbiol Biotechnol* 39:372–376
- Cox HH, Faber BW, Van Heiningen WN et al (1996) Styrene metabolism in *Exophiala jeanselmei* and involvement of a cytochrome P-450-dependent styrene monooxygenase. *Appl Environ Microbiol* 62:1471–1474
- De Fine Licht HH, Schiott M, Mueller UG et al (2010) Evolutionary transitions in enzyme activity of ant fungus gardens. *Evolution* 64:2055–2069
- de Vries RP, Riley R, Wiebenga A et al (2017) Comparative genomics reveals high biological diversity and specific adaptations in the industrially and medically important fungal genus *Aspergillus*. *Genome Biol* 18:28
- Defossez E, Selosse MA, Dubois MP et al (2009) Ant-plants and fungi: a new three-way symbiosis. *New Phytol* 182:942–949
- Do Nascimento RR, Schoeters E, Morgan ED et al (1996) Chemistry of metapleural gland secretions of three attine ants, *Atta sexdens rubropilosa*, *Atta cephalotes*, and *Acromyrmex octospinosus* (Hymenoptera: Formicidae). *J Chem Ecol* 22:987–1000
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797
- Espagne E, Balhadere P, Penin ML et al (2002) HET-E and HET-D belong to a new subfamily of WD40 proteins involved in vegetative incompatibility specificity in the fungus *Podospora anserina*. *Genetics* 161:71–81

- Fernández-Marín H, Zimmerman JK, Rehner SA, Weislo WT (2006) Active use of the metapleural glands by ants in controlling fungal infection. *Proc R Soc B Biol Sci* 273:1689–1695
- Fernández-Marín H, Nash DR, Higginbotham S et al (2015) Functional role of phenylacetic acid from metapleural gland secretions in controlling fungal pathogens in evolutionarily derived leaf-cutting ants. *Proc R Soc B Biol Sci* 282:20150212
- 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*. *Eukaryot Cell* 6:622–629
- Glass NL, Dementhon K (2006) Non-self recognition and programmed cell death in filamentous fungi. *Curr Opin Microbiol* 9:553–558
- Harris DM, van der Krogt ZA, Klaassen P et al (2009) Exploring and dissecting genome-wide gene expression responses of *Penicillium chrysogenum* to phenylacetic acid consumption and penicillinG production. *BMC Genomics* 10:75
- Hölldobler B, Wilson EO (1990) *The ants*. Harvard University Press
- Kuck P, Meusemann K (2010) FASconCAT: convenient handling of data matrices. *Mol Phylogenet Evol* 56:1115–1118
- Kurtz S, Phillippy A, Delcher AL et al (2004) Versatile and open software for comparing large genomes. *Genome Biol* 5:R12
- Lomsadze A, Ter-Hovhannisyan V, Chernoff YO et al (2005) Gene identification in novel eukaryotic genomes by self-training algorithm. *Nucleic Acids Res* 33:6494–6506
- Lowe TM, Eddy SR (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 25:955–964
- Luykx DM, Prenafeta-Boldu FX, de Bont JA (2003) Toluene monooxygenase from the fungus *Cladosporium sphaerospermum*. *Biochem Biophys Res Commun* 312:373–379
- Mayer VE, Frederickson ME, McKey D et al (2014) Current issues in the evolutionary ecology of ant-plant symbioses. *New Phytol* 202:749–764
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*:1–8
- Moller EM, Bahnweg G, Sandermann H et al (1992) A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Res* 20:6115–6116
- Moreno LF, Stielow JB, de Vries M et al (2015) Draft genome sequence of the ant-associated fungus *Phialophora attae* (CBS 131958). *Genome Announc* 3:e01099–e01015
- Moreno LF, Ahmed AAO, Brankovics B et al (2018a) Genomic understanding of an infectious brain disease from the desert. *G3 (Bethesda)* 8:909–922
- Moreno LF, Costa F, Vicente VA et al (2018b) Draft genome sequence of the human opportunistic fungus *Arthrocladium fulminans* (CBS 136243). *Genome Biol Evol* **in press**
- Moreno LF, Vicente VA, de Hoog S (2018d) Black yeasts in the omics era: achievements and challenges. *Med Mycol* 56:32–41
- Morschhauser J (2010) Regulation of multidrug resistance in pathogenic fungi. *Fungal Genet Biol* 47:94–106
- Nascimento MMF, Vicente VA, Bittencourt JVM et al (2017) Diversity of opportunistic black fungi on babassu coconut shells, a rich source of esters and hydrocarbons. *Fungal Biol* 121:488–500
- Nelson DR (2006) Cytochrome P450 nomenclature, 2004. *Methods Mol Biol* 320:1–10
- Nepel M, Voglmayr H, Blatrix R, Longino JT, Fiedler K, Schönerberger J, Mayer VE (2016) Ant-cultivated Chaetothyriales in hollow stems of myrmecophytic *Cecropia* sp. trees – diversity and patterns. *Fungal Ecol* 23:131–140
- Paoletti M, Saupe SJ (2009) Fungal incompatibility: evolutionary origin in pathogen defense? *Bioessays* 31:1201–1210
- Parra G, Bradnam K, Korf I (2007) CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. *Bioinformatics* 23:1061–1067
- Penick CA, Halawani O, Pearson B, Mathews S, López-Urbe MM, Dunn RR, Smith AA (2018) External immunity in ant societies: sociality and colony size do not predict investment in antimicrobials. *R Soc Open Sci* 5(2). <https://doi.org/10.1098/rsos.171332>
- Prenafeta-Boldu FX, Summerbell R, Sybren de Hoog G (2006) Fungi growing on aromatic hydrocarbons: biotechnology's unexpected encounter with biohazard? *FEMS Microbiol Rev* 30:109–130
- Rustler S, Chmura A, Sheldon RA et al (2008) Characterisation of the substrate specificity of the nitrile hydrolyzing system of the acidotolerant black yeast *Exophiala oligosperma* R1. *Stud Mycol* 61:165–174
- Saier MH Jr, Reddy VS, Tamang DG et al (2014) The transporter classification database. *Nucleic Acids Res* 42:D251–D258
- Sanglard D, Coste A, Ferrari S (2009) Antifungal drug resistance mechanisms in fungal pathogens from the perspective of transcriptional gene regulation. *FEMS Yeast Res* 9:1029–1050
- Selbmann L, Zucconi L, Isola D et al (2015) Rock black fungi: excellence in the extremes, from the Antarctic to space. *Current Genet* 61:335–345
- Seyedmousavi S, Netea MG, Mouton JW et al (2014) Black yeasts and their filamentous relatives: principles of pathogenesis and host defense. *Clin Microbiol Rev* 27:527–542
- Shelest E (2017) Transcription factors in Fungi: TFome dynamics, three major families, and dual-specificity TFs. *Front Genet* 8:53
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313
- Stanke M, Waack S (2003) Gene prediction with a hidden Markov model and a new intron submodel. In: *Bioinformatics* 19 Suppl 2: ii215–225
- Teixeira MM, Moreno LF, Stielow BJ et al (2017) Exploring the genomic diversity of black yeasts and relatives (Chaetothyriales, Ascomycota). *Stud Mycol* 86:1–28
- Vasse M, Voglmayr H, Mayer V et al (2017) A phylogenetic perspective on the association between ants (Hymenoptera: Formicidae) and black yeasts (Ascomycota: Chaetothyriales). *Proc R Soc B Biol Sci* 284:20162519
- Vicente VA, Weiss VA, Bombassaro A et al (2017) Comparative genomics of sibling species of *Fonsecaea* associated with human Chromoblastomycosis. *Front Microbiol* 8:1924
- 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 Biol* 115:1077–1091
- Weber T, Blin K, Duddela S et al (2015) antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. *Nucleic Acids Res* 43:W237–W243
- Yew SM, Chan CL, Ngeow YF et al (2016) Insight into different environmental niches adaptation and allergenicity from the *Cladosporium sphaerospermum* genome, a common human allergy-eliciting Dothideomycetes. *Sci Rep* 6:27008
- Zhang H, Yohe T, Huang L et al. (2018) dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. *Nucleic Acids Res*