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# Peculiar genomic traits in the stress-adapted cryptoendolithic endemic Antarctic fungus *Friedmanniomyces endolithicus*

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**Abstract**

*Friedmanniomyces endolithicus* is a highly melanized fungus endemic to the Antarctic, occurring exclusively associated with endolithic communities in the ice-free areas of the Victoria Land, including the McMurdo Dry Valleys, the coldest and most hyper-arid desert on Earth and accounted as the Martian analogue on our planet. *F. endolithicus* is highly successful in these inhospitable environments, and is the most widespread and commonly isolated species from these peculiar niches, indicating a high degree of adaptation. The nature of its extremo-tolerance has not been previously investigated. To support this, we sequenced the genome of *F. endolithicus* CCFEE 5311 to explore gene content and genomic patterns that could be attributed to its specialization. The predicted functional potential of the genes was assigned by similarity to InterPro and CAZy domains. The was compared to phylogenetically close relatives which are also melanized fungi occurring in extreme environments including *F. simplex, Acidomyces acidophilus, Hortaea thailandica* and *H. werneckii.* We tested if shared genomic traits existed among these species and the hyper-extremotolerant fungus *F. endolithicus*. We found that some characters for stress tolerance such as meristematic growth and cold tolerance are enriched in *F. endolithicus* that may be triggered by the exposure to Antarctic prohibitive conditions.

**Keywords:** Antarctica; black meristematic fungi; extremophiles; cryptoendolithic communities; comparative genomics; stress-tolerance.

## 1. Introduction

Highly melanized fungi are often described with the terms Black fungi, black yeasts and relatives, meristematic fungi, microcolonial fungi (MCF), and Rock Inhabiting Fungi (RIF). These fungi are an ecologically defined group of stress-specialists of the Fungal Kingdom that share morphologically similarity despite diverse phylogenetic placement and distance. The MCF are the most successful extremophiles and extremo-tolerant organisms and are distributed globally in harsh environments which prohibit the colonization by most life-forms. They are commonly isolated from saltpans (Plemenitaš and Gunde-Cimerman, 2005), acidic and hydrocarbon-contaminated sites (Seyedmousavi et al., 2011; Selbmann et al., 2012; Isola et al., 2013), exposed natural rocks (Ruibal et al., 2005) and stone monument surfaces (Sert et al., 2007), hot deserts (Staley et al., 1982), photocatalytic surfaces (Ruibal et al., 2018) and very cold icy habitats (Selbmann et al., 2005, 2008; Branda et al., 2010; Zalar et al., 2008; Brunner et al., 2011; Turchetti et al., 2018). Meristematic growth (de Hoog and Hermanides-Nijhof, 1977), i.e. conversion towards isodiametric expansion, is infrequent in the fungal kingdom and is a specific response to stress. It becomes a stable character for fungi living permanently in extreme conditions, as for some black fungi and, together with melanization and meristematic development, are primarily suited to cope with and adapt to highly diverse environmental stressors.

Black fungi are common in the endolithic microbial communities of the hyper-frozen and hyper arid ice-free areas of the Victoria Land, Antarctica (Coleine et al., 2018a, 2018b), which is considered a Martian analogue on Earth (Doran et al., 2010). There, life on rock surfaces is too challenging for Black Fungi and the endolithic environment offers a last chance of survival buffering temperatures by thermal inertia of the rock substratum (Friedmann, 1982). The genus *Friedmanniomyces* (Onofri et al., 1999) is endemic to the Antarctic Continent, to date includes two described species: *F. endolithicus* and *F. simplex* (Selbmann et al., 2005). They occur exclusively associated with endolithic microbial communities in the ice-free areas of the Victoria Land, comprising the McMurdo Dry Valleys characterized by high UV irradiation, low temperatures, and strict oligotrophy. Among the black meristematic fungi of these communities, the species *F. endolithicus* is the most widespread and frequently isolated (Selbmann et al., 2015), suggesting a high degree of adaptation to the prohibitive environmental conditions of this area. Yet, its responses and resistance to stress have been only scarcely investigated; proteomic studies highlighted that responses to sub-optimal temperature are related to a downregulation rather than a heat-shock protein over-expression (Tesei et al., 2012).

*F. endolithicus* has the ability to endure acute doses of gamma radiation (up to 400 Gy), and even increases its metabolic activity under radiation (Pacelli et al., 2018). Despite these advances, we have limited understanding of the suite of adaptations of this peculiar fungus. In this study, *F. endolithicus'* genome was sequenced and compared to sequences of relatives *F. simplex, Acidomyces acidophilus, Baudoinia panamericana, Hortaea thailandica* and *H. werneckii* as representatives of black fungi occurring in different extreme environments, to highlight the genomic traits of the hyper-adapted fungus *F. endolithicus*.

## 2. Material and methods

### *2.1. Fungal strains isolation*

The strain *Friedmanniomyces endolithicus* (strain CCFEE 5311) and *Friedmanniomyces simplex* (strain CCFEE 5184) were isolated from Antarctic cryptoendolithic communities collected in the Victoria Land (Continental Antarctica) at Ford Peak 75°43'S 160°27'E and Battleship Promontory 76°55'S 160°55'E (McMurdo Dry Valleys), respectively. *Hortaea thailandica* (strain CCFEE 6315) was isolated from colonized sandstone from Ricker Hills 71°25'S 163°00'E (Victoria Land) (data unpublished). Fungal isolation was performed by directly plating fragments of colonized rock on petri dishes containing 2% Malt Extract Agar (MEA) amended with 100 ppm Chloramphenicol (Fig. 1), according to Selbmann et al. (2005, 2008). Cultures analyzed in this study were kindly supplied by the Culture Collection of Fungi from Extreme Environments (CCFEE) of the Mycological Section of the Italian Antarctic National Museum (University of Tuscia, Italy).

### *2.2. DNA extraction and whole genome sequencing*

The pure cultures were grown on 2% MEA medium plates for 6 weeks at 10°C and DNA extracted from the total biomass following cetyltrimethylammonium bromide (CTAB) protocol (Fulton et al., 1995). Melanin was removed through two phenol-chloroform purification steps. Genomic DNA was sheared with Covaris S220 ultrasonic homogenizer and sequencing library constructed using a NeoPrep TruSeq Nano DNA sample prep kit (Illumina) in the University of California-Riverside Genomics Core, following the instructions of the manufacturers. Whole genome sequencing (2x300 bp paired-end) was carried out on Illumina Miseq platform.

*2.3. Genome assembly and annotation and data collection*

De novo genome assembly was performed as previously described in Coleine et al. (2017, 2019). Briefly, quality of reads was checked with FastQC v0.11.3 (Andrews, 2010) followed by genome assembly with MaSuRCA v2.3.2 (Zimin et al., 2013), using default parameters (cgwErrorRate_0.15), including quality based read trimming and corrections. Trimmed reads averaged 198 bp. Genome scaffolds were filtered of vector contamination with Sequin v15.10 and redundant scaffolds eliminated if completely aligned with at least 95% identity to a longer contig using MUMmer v3.23 (Kurtz et al., 2004), using "funannotate clean" script from Funannotate v0.5.5 (Palmer and Stajich, 2017). Genome annotation was performed with funannotate and consensus gene models were produced by EVidenceModeler (EVM) (Haas et al., 2008) using *ab initio* predictions from AUGUSTUS v3.2.2 (Stanke et al., 2006) and GeneMark.hmm-ES v4.32 (Ter-Hovhannisyan et al., 2008) combined with protein-to-genome alignments from Exonerate v2.2.0 (Slater and Birney, 2005). Self-training for GeneMark.hmm-ES was performed using default parameters, AUGUSTUS was trained with alignments of the BUSCO ascomycota_odb9 data set v9 (Simão et al., 2015), and prediction parameters were archived in public repository (https://github.com/hyphaltip/fungi-gene-prediction-params). Structural and functional annotations of genes were performed according to various databases such as CAZymes (Carbohydrate-Active enZYmes Database) (Lombard et al., 2014; Huang et al., 2018) using HMMER v3.1b2 (Finn et al., 2011) and InterPro Protein Families Database (IPR) v5.20-59.0 (Jones et al., 2014) by BLASTP v2.5.0 (Altschul et al., 1997) searches. Using Funannotate additional comparative genomics was performed using "funannotate compare" script.

The annotated genomes were submitted to GenBank after processing with Genome Annotation Generator (Hall et al., 2014) associated with BioProject number PRJNA342238. The versions described in this paper are the first version, NAJP00000000.1 (*F. endolithicus* CCFEE 5311), NAJQ00000000.1 (*F. simplex* CCFEE 5184), and NAJL00000000.1 (*H. thailandica* CCFEE 6315). The *Friedmanniomyces* sequences were compared with *Hortaea werneckii* (EXF-2000) (Lenassi et al., 2013; Sinha et al., 2017), *Baudoinia panamericana* (UAMH 10762) (Ohm et al., 2012) and *Acidomyces acidophilus* (BFW) (Mosier et al., 2016). The genus *Acidomyces*, invalidly described by Baker et al. (2004), was later validated by Selbmann et al. (2008) and was found to be the synonymy of *A. richmondensis* and *Scytalidium acidophilum*, and the species described as *Acidomyces acidophilus* (Sigler & J.W. Carmich.) Selbmann, de Hoog & De Leo. In this study we, therefore, refer to strain

BFW as *Acidomyces acidophilus*. All the species were selected as representative of black fungi occurring in different extreme environments (Table 1).

### *2.4. Phylogenomics*

The genome-wide phylogenetic tree based on the genomes of the sequenced Antarctic strains, and additional available black fungal strains was constructed using PHYling (Stajich, 2018). Briefly, the tool identifies homologs of a set of previously identified single-copy genes in fungi to build a set of orthologous proteins which are each individually aligned followed by alignment trimming with trimAL (parameter -automated1) (Capella-Gutiérrez et al., 2009). The individual protein alignments were concatenated into a single super-matrix alignment. The phylogenetic tree was constructed from this alignment using FastTree v2.1.11 with parameters -gamma -wag (Price et al., 2010).

### *2.5. Annotation of orthologous gene clusters among multiple species*

The protein sequences from the annotated genomes were analyzed with the OrthoVenn2 web server (https://orthovenn2.bioinfotoolkits.net) for identification and comparison of orthologous clusters (Xu et al., 2019). Briefly, to identify orthologous groups, OrthoVenn2 employs the OrthoMCL (Li et al., 2003) clustering algorithm to annotate and compare ortholog groups. The OrthoMCL performs an all-against-all DIAMOND v0.9.24 alignment, identifies putative orthology and InParalogy relationships with the InParanoid algorithm (Östlund et al., 2010) and generates disjoint clusters of closely related proteins with the Markov Clustering Algorithm (MCL) (Dongen, 2000). The Gene Ontology (GO) terms for biological process, molecular function, and cellular component categories were assigned to the corresponding orthologous cluster by identifying similarity to sequences in the Uniprot (UniProt Consortium, 2018) database. The e-value cutoff for all-to-all protein similarity comparisons was 0.05 and the inflation value for the generation of orthologous clusters using the Markov Cluster Algorithm was 1.5.

## **3. Results**

### *3.1. Genome structure in Antarctic strains*

The assembled genome sizes varied among the species examined from 23.89 Mbp for *Hortaea thailandica* CCFEE 6315 to 37.79 Mbp for *Friedmanniomyces simplex* CCFEE 5184 and 46.75 for *Friedmanniomyces endolithicus* CCFEE 5311. The genome size of *Friedmanniomyces* genus is similar to the halotolerant *H. werneckii* (49.9) which has undergone a form of whole genome duplication or hybridization (Gostinčar et al., 2018). The *F. endolithicus* genome is larger than several other black fungal species such as *A. acidophilus* and *Baudoinia panamericana* which are 21.87 and 29.88 Mbp, respectively. The predicted gene counts in *F. endolithicus* (18,027) and 43 tRNA and *F. simplex* (13,766 protein coding genes) and 22 tRNAs were higher than most of the other black fungi genomes examined (Table 1). The assembled draft genome sequences of *F. endolithicus*, *F. simplex* and *H. thailandica* consist of 411, 2,885 and 148 contigs, respectively.

The ~40-45 Mb genome of the Antarctic *Friedmanniomyces* spp. was larger than those of the currently published black fungi genomes sequences (e.g. Teixeira et al. 2017; Moreno et al., 2019; Coleine et al., 2019), and also display a somewhat higher G+C content (56.5%) than ~50% on average observed other black fungi (Teixeira et al., 2017)

*3.2. Phylogenomics and comparative genomics*

To infer the phylogenomic relationship of the Antarctic strains, we used nearly 20 black fungal genomes in the class Dothideomycetes, using the Sordariomycetes *Neurospora crassa* and *Sordaria macrospora* as outgroups (Fig. 2). Consistent with previous analysis based on the sequence of the ITS-SSU regions, the whole-genome phylogeny showed that *F. endolithicus* and *F. simplex* are monophyletic Dothideomycetes and the Antarctic strain *H. thailandica* is sister to the halotolerant black fungus *H. werneckii* EXF-2000, supporting these named genera with whole genome comparisons.

Additionally, we determined the Transcription Factors composition and Carbohydrate Active Enzymes (CAZymes) distribution in *F. endolithicus* and compared this with other fungi such as *H. werneckii, B. panamericana* and *A. acidophilus* as representative of black yeasts isolated from different extreme environments.

Transcription factors (TFs) are critical for orchestrating the regulation of gene expression and the repertoire of TFs dictate the networks of gene regulation that exist in an organism (Shelest, 2008). The determination of the repertoire of TFs in a species is the first step to uncovering these regulatory networks. To annotate the TF genes, we identified genes with

InterPro domains (McDowall and Hunter, 2011; Mitchell et al., 2015), which have been identified as typically found in fungal TFs, and found a total of 6,302 potential TF genes in the genomes of the 6 black fungal species (Supplementary Table S1). Among the TFs identified, *F. endolithicus* and *H. werneckii* had the broadest collection of TF types and the highest overall copy number of TFs (i.e., IPR000232: Heat shock factor (HSF)-type; IPR000679: Zinc finger, GATA-type; IPR001005: DNA-binding; IPR001138: fungal transcriptional regulatory protein, N-terminal; IPR007219: Transcription factor domain (Fig. 3A).

Carbohydrate-active enzymes (CAZymes) are responsible for the degradation, modification, and biosynthesis of carbohydrates and glycoconjugates (Cantarel et al., 2009). The family classification system is based on amino-acid sequence and structure similarities to group CAZymes into five classes of enzymatic activities: glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), and auxiliary activities (AAs), and the associated module carbohydrate-binding modules (CBMs). A total of 126 CAZymes (Table S2) were identified in the predicted protein sets across the 6 species. The GH and GT were the most abundant families (64 and 29, respectively); while, the AA and CBM were found to be in lower abundance (9 and 8, respectively). Only 3 copies of enzymes belonging to polysaccharide lyases (PLs) were identified. Overall, *F. endolithicus* and *H. werneckii* had the highest copy numbers of domains (for example, the subfamilies GH 5.9 (exo-β-1,3-glucanase), GH 5.14 (β-glucosidase), GH 5.15 (β-1,6-glucanase), GH 5.45 (β-glucopyranosidase), GH 5.49 (endo-β-1,4-glucanase) and GT 22 (mannosyltransferase) subfamilies *(Fig. 3B)*.

*3.3. Stress-adaptation strategies*

Orthologous clustering of the predicted proteome of *F. endolithicus* along with the proteomes of *F. simplex*, *H. thailandica, H. werneckii, B. panamericana*, and *A. acidophilus* was performed to identify unique and/or shared gene families among them which could be linked to specific functional capabilities of these organisms. By comparing the orthologous proteins, we could infer potential differences in the allocation of cellular resources supporting cellular functions. OrthoVenn2 produced 11,291 clusters, of these 11,221 orthologous clusters contained at least two species. *F. endolithicus'* genome formed 8691 clusters and 2278 singletons, while *F. simplex* 9,325 and 2,883, *H. werneckii* 7,875 and 1,156, *H. thailandica*

7,703 and 881, *A. acidophilus* 7,705 and 2,146, and *B. panamericana* 7,763 and 2,556, respectively. The Venn diagram in Figure 4 show a total of 5,366 putative orthologous proteins shared among all species and 627 between the two Antarctic isolates *F. endolithicus* and *F. simplex*. These species have at least 300 singleton orthologous clusters which are unique to each species (Supplementary Table S3).

Comparison of the functional annotation based on Gene Ontology (GO), a total of 194 genes were assigned and shared across analyzed species. Of these genes, 18 were redundantly assigned into Cellular Component Ontology, 30 into Molecular Function Ontology and 146 into Biological Process Ontology (Table S4-S6). Most of the genes were annotated to oxidoreductase (80) activity in the Molecular Function Ontology. In the Biological Process the overrepresented functions were carbohydrate (167), nitrogen compound (830), and RNA (612) metabolic process and response to stimulus (335) and biological regulation (1,902).
We extracted Gene Ontology (GO) terms that are shared and significantly over- or under-represented in sets of genes within analyzed species and we found that genes involved in response to oxidative stress (oxido-reductase, GO:0016491) and UV irradiation (UV damage, GO:0034644) were enriched. GO terms associated with X-rays (GO:0010165), DNA damage (GO:0042772), and salt stress (GO:0009651) responses were shared in *Friedmanniomyces* spp. Instead, GO terms related to meristematic growth (GO:0010073) and cold adaptation (GO:0070417) were unique for *F. endolithicus* (Figure 5A, B).

## 4. Discussion

Black meristematic fungi, comprise an assemblage of lineages within the Pezizomycotina, mostly in the classes Dothideomycetes (Ruibal et al., 2009) and Eurotiomycetes (Teixeira et al. 2017); they are a recurrent presence in the Antarctic cryptoendolithic communities (Selbmann et al. 2005, 2008, 2015; Egidi et al., 2014) and are among the most extremotolerant fungi on Earth.
The endemic cryptoendolithic *Friedmanniomyces endolithicus*, is undoubtedly the most widespread in Antarctic deserts, indicating a high degree of adaptation to those harsh conditions.

To investigate the genomic basis of this exceptional extremophile adaptation, we sequenced the genome of this species and compared the assembly and annotation with other black fungi,

including *Hortaea werneckii, Acidomyces acidophilus* and *Baudoinia panamericana* and other two additional Antarctic strains analyzed in this study (i.e. *Friedmanniomyces simplex* and *Hortaea thailandica*).

Genome assembly and annotation showed variation in genome size (ranging from 21.87 Mbp in *A. acidophilus* to 49.9 in *H. werneckii.* The *F. endolithicus* genome is of 46.75 Mbp, making it much larger than its close and more distant relatives. The genomes of *H. werneckii* and *Friedmanniomyces* spp. strains were larger in size than the average size in black fungi; black yeasts' genomes ranged from 20 up to 50 Mbp; in Chaetothyriales (Eurotiomycetes) ranging from 25.8 Mb in *Capronia coronata* to 43 Mb in *Cladophialophora immunda* (Teixeira et al. 2017; Moreno et al. 2019), while *H. werneckii* genome assembly (~50 Mbp) is the largest in Dothideomycetes.

All BY genomes have, on average, high GC content (49-56.5%) (Teixeira et al. 2017; this study); these data could be peculiar of the extremes-associated ecology of black fungi; indeed, high GC content was already found as a common feature in extremophilic prokaryotes as it helps to stabilize after DNA damage (Gregory et al., 2007; Musto et al., 2006).

A large number of predicted proteins was found in two other Antarctic cryptoendolithic black fungi genomes which each contained around 18,000 genes (Coleine et al., 2017). The highly halotolerant black fungus *H. werneckii* (Capnodiales), frequently isolated from hypersaline environments as sea spray areas and salterns (Kogej et al., 2005; Lenassi et al., 2013; Marchetta et al., 2018; De Leo et al., 2019), comprises more than 15,000 protein- coding genes, underwent a recent Whole- Genome Duplication (WGD) due to hybridization triggered by the exposure to salt stress (Lenassi et al., 2013; Sinha et al., 2017).

The similar genome size between H. *werneckii* and the two *Friedmanniomyces* spp. strains, might suggest evolutionary advantages due to a large-scale genome duplication in the Antarctic species' genome to adapt and survive to the hostile conditions of the Antarctic ice-free areas, lethal for the most.

WGDs have been inferred in many eukaryotic lineages; this is especially true for plants, where ancient WGDs are abundant, particularly in *Arabidopsis thaliana* (Vision et al., 2000; Bowers et al., 2003; Simillion et al., 2002). In fungi, it has also been proved that duplication events and/or WGD lead to the ability of this group to adapt to such a wide range of environmental extremes or contributing to the evolution of novel functions (e.g. human pathogen *Rhizopus oryzae* (Ma et al., 2009) and the dung fungus *Phycomyces blakesleeanus*

306 (Corrochano et al., 2016). Other experimental studies reported that adaptation of
307 *Saccharomyces cerevisiae* to UV radiation and salt stresses was associated with increases in
308 genome size (Lidzbarsky et al., 2009; Dhar et al., 2011). Duplication events may be an
309 important evolutionary stage allowing the highly melanized to adapt and exploit most
310 extreme niches, although, to date, *Hortaea* appears unusual and remains the only described
311 example among black yeasts.

312 In order to individuate characteristic genomic traits of highly extreme-tolerant *F.*
313 *endolithicus,* we compared its proteome with the other selected black fungal species. In
314 particular, we focused on TFs that may give clues on the possible existence or absence of
315 particular signaling pathways (Shelest et al., 2008) and on putative enzymes assigned to
316 CAZy. In our study, among the most abundant TFs, the top five InterPro protein domains
317 (IPRs) included IPR000232 (Heat shock factor), IPR000679 (Zinc finger, GATA-type),
318 IPR001005 (DNA-binding), IPR001138 (fungal transcriptional regulatory protein, N-
319 terminal), and IPR007219 (Transcription factor domain) were most frequent in the
320 halotolerant *H. werneckii* and the Antarctic *F. endolithicus*. InterPro entries IPR001138 and
321 IPR007219, frequently found in *S. cerevisiae* (Hashimoto et al., 1983) and *Aspergillus niger*
322 (van Peij et al., 1998), are TFs domains that commonly occur together, including proteins in a
323 wide variety of cellular and metabolic processes that might allow to keep metabolic systems
324 and enzymes that are still active at limiting conditions (e.g. low temperatures). The Heat
325 shock factor IPR000232 is TF-type DNA-binding domains typically found in all eukaryotic
326 lineages, including fungi and also found most abundant in Antarctic strains and *H. werneckii.*
327 In previous studies it was observed a downregulation of proteins expression in the
328 psychrophilic *F. endolithicus* without a consequent heat-shock proteins production, when
329 cultured at sub-optimal temperature of 28 °C (Tesei et al. 2012).

330 Likewise, the highest number of CAZymes were found in *F. endolithicus* and *H. werneckii*
331 and the variation observed between species was considered low, while the lowest number was
332 found in *A. acidophilus*. In particular, CAZyme families GH5 and GH 22 (glycoside
333 hydrolases) were found enriched in these two species, possibly reflecting their genome
334 complexity.
335 In total, 127 CAZymes families were identified in the predicted protein sets. Member of PL
336 (PL1, PL2, PL3) superfamily was detected in *Hortaea* genus only; indeed, depletions in the
337 pectinases PL was reported in most yeast-like fungi, including Onygenales (Desjardins et al.,

2011), while they are frequent in Eurotiales (Teixeira et al., 2014). The generalist lifestyle of some fungi is linked with the ability of degrading a diversity of polysaccharides, particularly those present in plant material (de Vries et al., 2017). The absence of such enzymes in the genome of Antarctic strains, remarkably, suggests that these organisms may have lost the ability to obtain nutrients from plant material, while proteins involved in oligotrophy and aridity stresses response may be evolved and enriched.

Orthologs are genes in different species that have evolved from a common ancestral gene via speciation and often retain the same function in the course of evolution. Comparing orthologs is important to identify events of gene gain or loss. As expected, in all analyzed black fungi, the majority of COG-annotated genes are involved in basic cellular functions such as oxidoreductase activity (GO:0016491) and repair after UV irradiation (GO:0034644). (Gostinčar and Gunde-Cimerman, 2018) demonstrated that the maximum tolerated salinity correlated with the number of genes encoding enzymes of the cellular oxidative stress response in the halophilic basidiomycete *Wallemia ichthyophaga*, and halotolerant ascomycetous black yeasts *Hortaea werneckii* and *Aureobasidium pullulans*. Their finding supported the possible link between the antioxidant capacity of cells and their halotolerance and the importance of cell wall pigmentation for extremotolerance, providing a barrier against oxidative damage. Indeed, it is well known that melanin plays an important role in the ability of melanized fungi to survive excessive heat or cold, extreme pH or osmotic conditions, simulated space and Martian conditions, and even UV-radiation (Gadd and de Rome, 1988; Gunde-Cimerman et al., 2000; Onofri et al., 2008).

On the other hand, some genomic features are unique for *Friedmanniomyces* spp. strains only, such as responses to x-rays radiation (GO:0010165), DNA damage (GO:0042772), and salt tolerance stress (GO:0009651).

The melanized 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, and have been found in nuclear reactors and reactor cooling water (Zhdanova et al., 2000; Dadachova et al., 2007; Dadachova and Casadevall, 2008).

Recently, it has been demonstrated that acute doses of gamma radiation (up to 400 Gy) did not significantly affect vitality and metabolic activity of endemic *F. endolithicus*; authors suggested the existence of a more radio-resistant sub-population of cells, or a tremendous

capability of fungus to perform DNA repair in the irradiated samples (Pacelli et al., 2018). We may suggest that this high resistance could be a consequence of an evolutionary adaptation to repair DNA damage induced by desiccation (Mattimore and Battista, 1996) as an advantage colonize arid and -hyper arid areas, that represent, promoting adaptive radiation and speciation, a reservoir for new radio-resistant taxa. Resistance in Antarctic black fungi has been extensively investigated in *Cryomyces antarcticus*, isolated from McMurdo Dry Valleys, that exhibit a stunning endurance after exposure to high doses of space-relevant gamma $^{60}$Co (up to 117.07 kGy), deuterons $^{2}$H (up 1,500 Gy) sparsely (X-rays up to 300 Gy) radiation, and even Martian-simulated and Space conditions (Pacelli et al., 2017; Onofri et al., 2018, 2019; Selbmann et al., 2018), representing, therefore, an astrobiological test organism for understanding the possible limits for life as well as evolution and adaptation to extreme conditions.

Genomic traits associated to meristematic growth (GO:0010073) and cold adaptation (GO:0070417) were unique for *F. endolithicus*. Meristematic growth is infrequent in the fungal kingdom but is accounted as a specific response to stress: the advantage of meristematic development lies in optimizing the volume/surface ratio minimizing exposition to external stressors (Wollenzien et al., 1995).

Many black yeasts may shift to meristematic growth when stressed but, for species living under permanent stress, it may become a stable character. *F. endolithicus* shows exclusively a meristematic development and the enrichment of this genomic trait coupled to the cold adaptation found in this study, make it particularly adapted and suited to succeed in the prohibitive conditions of ice-free areas of Victoria Land; this fungal species is, in fact, the most widespread and frequently retrieved in over 20-years of Italian Antarctic Campaigns, reaching until 3300 m asl and 96 km of sea distance (Selbmann et al., 2015; Coleine et al., 2018a).

## 5. Conclusions and future perspectives

This study represents the first release on the genomic traits of the endemic Antarctic cryptoendolithic black fungi *Friedmanniomyces endolithicus,* the most widespread and frequent species in the rocks of the desert areas in Victoria Land. Our study identified genomic traits in response to salt, X-rays, cold and DNA damage stresses confirming its

401 exceptional poly-extremotolerance enabling the fungus to survive across a wide variety of
402 stresses. The genome and the number of predicted proteins are among the larger observed for
403 black fungi and therefore, it may be considered as a good candidate for Whole Genome
404 Duplication.
405 To date, only almost 60 black yeast genomes are available in different databases; ongoing
406 efforts through the Joint Genome Institute's Department of Energy's Community Sequencing
407 Project "Shed light in the dark lineages of the Fungal Tree Of Life (STRES)" will see to
408 generate more genomic sequence information from hundreds of strains and nearly 100
409 species. This and other studies will provide a backbone to facilitate comparative analyses to
410 better trace the evolutionary history and adaptive processes of this intriguing group of fungi.

411
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**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

438 **Figure captions**

439 **Figure 1. 1a)** Map of Antarctica, the Victoria Land is marked with a red circle; **1b)** example
440 of sandstone colonized by cryptoendolithic communities; **1c)** *Friedmanniomyces endolithicus*
441 CCFEE 5311.

442 **Figure 2.** Phylogenomic tree constructed with black fungal genomes available online, using
443 FastTree v2.1.11. Branches have bootstrap values of 100%.
444 Antarctic endolithic black fungi genomes, available on NCBI/Genbank are marked in red.

445 **Figure 3. 3a)** Heatmap of transcription factors expression abundance across the analyzed
446 genomes. The abundance value of each gene is used to plot the heatmap. White = absent, dark
447 blue = abundant. 3b) Distribution of the most abundant carbohydrate-active enzymes across
448 the analyzed genomes. GH glycoside hydrolase, GT glycosyltransferase, CE carbohydrate
449 esterases. White = absent, dark red = abundant.

450 **Figure 4.** Venn diagram, calculated with OrthoVenn2, show predict unique and shared
451 clusters orthologous among these species.

452 **Figure 5.** Functional categories unique for *Friedmanniomyces endolithicus* CCFEE 5311,
453 associated to **a)** meristematic growth and **b)** cold-adaptation, were taken from the Gene
454 Ontology classification, using AmiGo tool (Carbon et al. 2009).

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**Table 1**

| Species | Strain | Habitat and sampling site | Reference |
|---|---|---|---|
| *Friedmanniomyces endolithicus* | CCFEE 5311 | Cryptoendolithic communities, Antarctica | This study |
| *Friedmanniomyces simplex* | CCFEE 5184 | Cryptoendolithic communities, Antarctica | This study |
| *Hortaea thailandica* | CCFEE 6315 | Cryptoendolithic communities, Antarctica | This study |
| *Hortaea werneckii* | EXF-2000 | Marine solar salterns, Slovenia | Lenassi et al. 2013; Sinha et al. 2017 |
| *Baudoinia panamericana* | UAMH 10762 | Ethanol vapor | Ohm et al. 2012 |
| *Acidomyces acidophilus* | BFW | Richmond Mine, California | Mosier et al. 2016 |

**Table 1**. Metadata of strains compared in this study.

**Table 2.** Genome content of the strains analyzed in this study.

| Species | Strain | Genome size | GC (%) | Protein | tRNA | Gene |
|---|---|---|---|---|---|---|
| *Friedmanniomyces endolithicus* | CCFEE 5311 | 46.75 | 56.5 | 18,027 | 43 | 18,070 |
| *Friedmanniomyces simplex* | CCFEE 5184 | 37.79 | 56.6 | 13,766 | 22 | 13,788 |
| *Hortaea thailandica* | CCFEE 6315 | 23.89 | 55.5 | 8,778 | 23 | 8,801 |
| *Hortaea werneckii* | EXF-2000 | 49.89 | 53.5 | 15,987 | 28 | 15,649 |
| *Baudoinia panamericana* | UAMH 10762 | 29.88 | 49.5 | 10,757 | N/A | N/A |
| *Acidomyces acidophilus* | BFW | 21.87 | 54.8 | 10,508 | 41 | 10,549 |

**Figure 1**

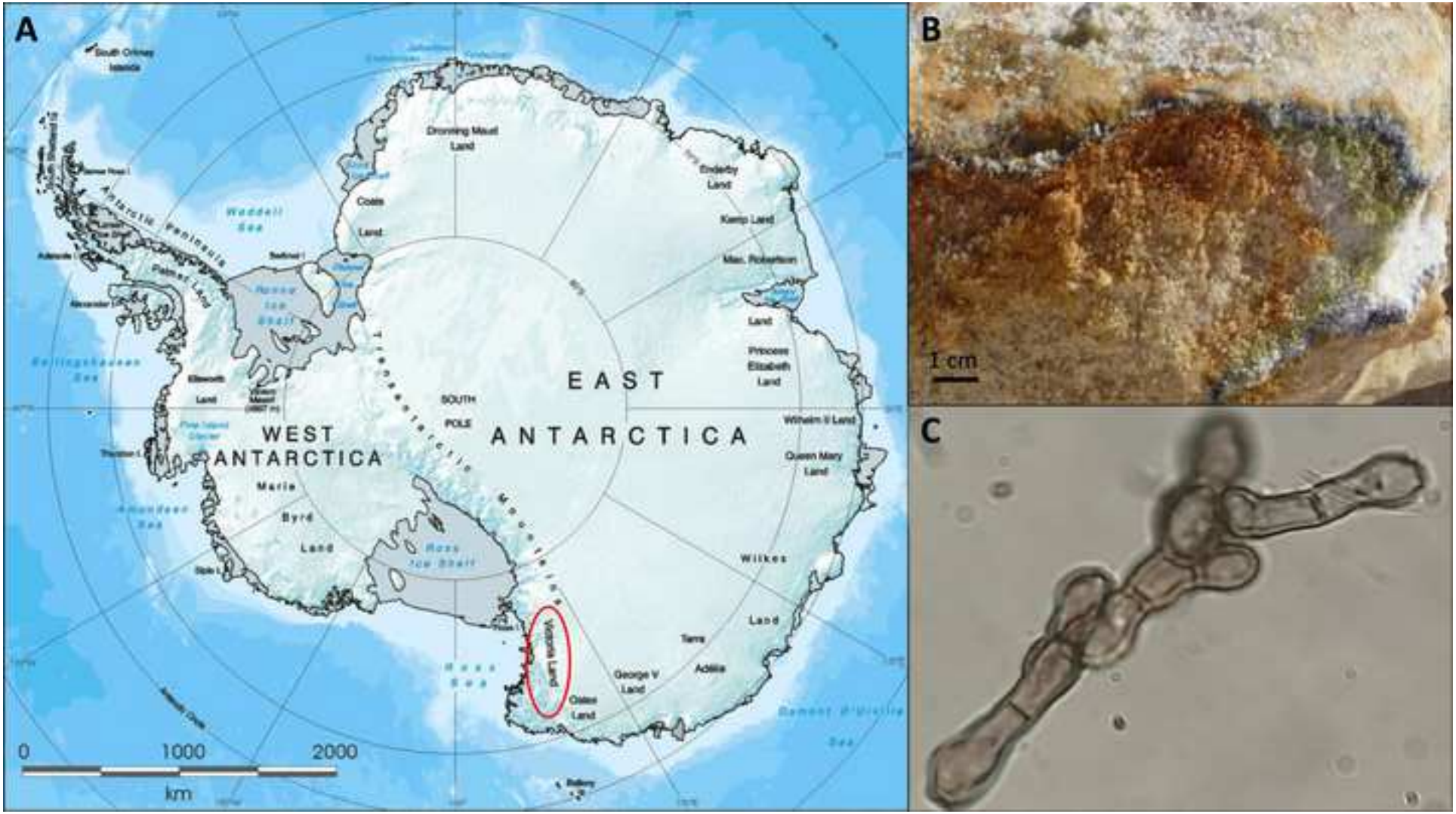

A
B
C
1 cm
EAST
ANTARCTICA
WEST
ANTARCTICA
SOUTH
POLE
Victoria Land
Dronning Maud Land
Enderby Land
Kemp Land
Mac. Robertson Land
Princess Elizabeth Land
Wilhelm II Land
Queen Mary Land
Wilkes Land
Terre Adélie
George V Land
Oates Land
Marie Byrd Land
Ellsworth Land
Palmer Land
Antarctic Peninsula
Coats Land
Weddell Sea
Ross Sea
Ross Ice Shelf
Ronne Ice Shelf
Amundsen Sea
Bellingshausen Sea
Somov Abyssal Plain
0
1000
2000
km

Figure 2

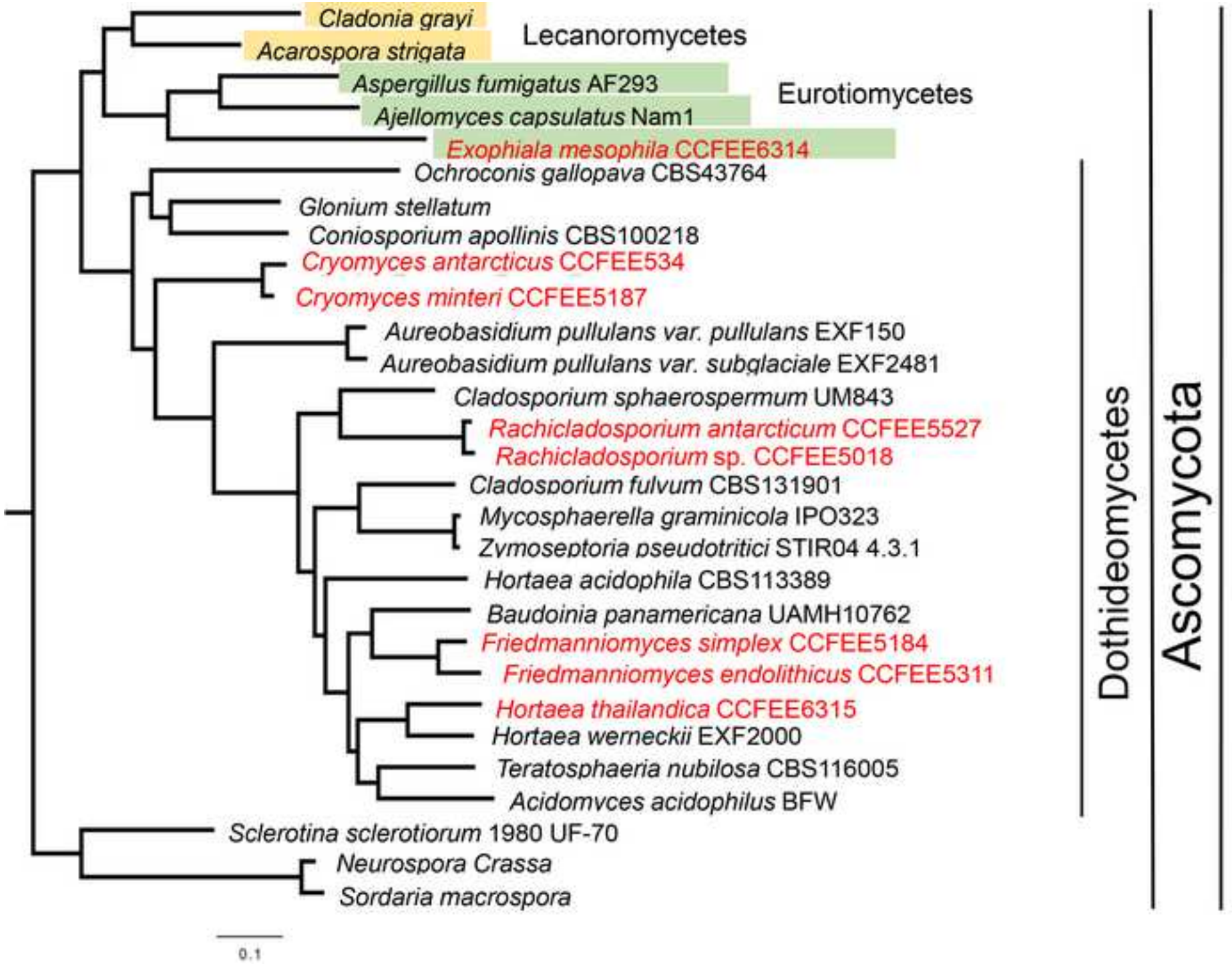

Cladonia grayi
Acarospora strigata
Lecanoromycetes
Aspergillus fumigatus AF293
Ajellomyces capsulatus Nam1
Eurotiomycetes
Exophiala mesophila CCFEE6314
Ochroconis gallopava CBS43764
Glonium stellatum
Coniosporium apollinis CBS100218
Cryomyces antarcticus CCFEE534
Cryomyces minteri CCFEE5187
Aureobasidium pullulans var. pullulans EXF150
Aureobasidium pullulans var. subglaciale EXF2481
Cladosporium sphaerospermum UM843
Rachicladosporium antarcticum CCFEE5527
Rachicladosporium sp. CCFEE5018
Cladosporium fulvum CBS131901
Mycosphaerella graminicola IPO323
Zymoseptoria pseudotritici STIR04 4.3.1
Hortaea acidophila CBS113389
Baudoinia panamericana UAMH10762
Friedmanniomyces simplex CCFEE5184
Friedmanniomyces endolithicus CCFEE5311
Hortaea thailandica CCFEE6315
Hortaea werneckii EXF2000
Teratosphaeria nubilosa CBS116005
Acidomyces acidophilus BFW
Sclerotina sclerotiorum 1980 UF-70
Neurospora Crassa
Sordaria macrospora
0.1
Dothideomycetes
Ascomycota

Figure 3

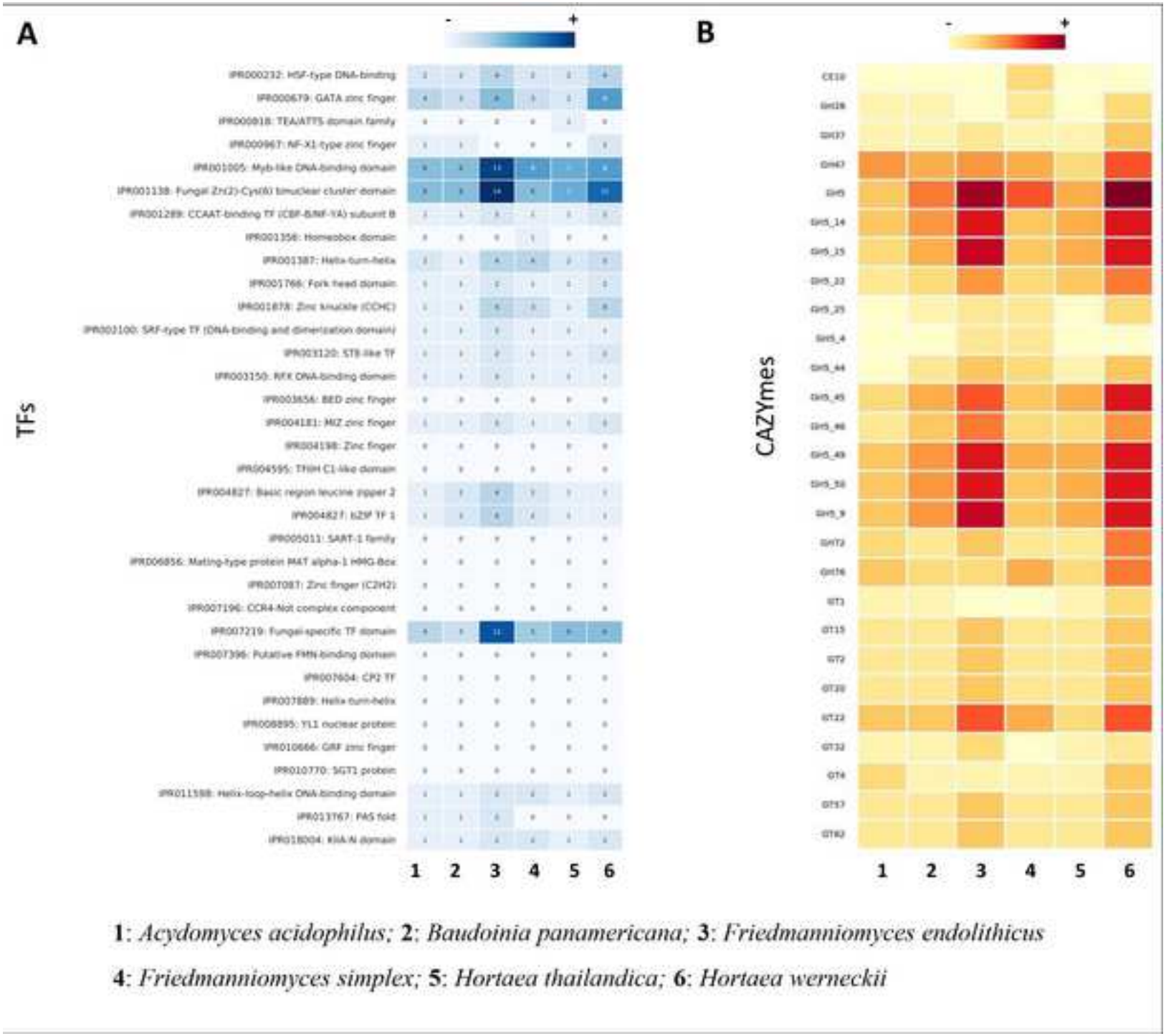
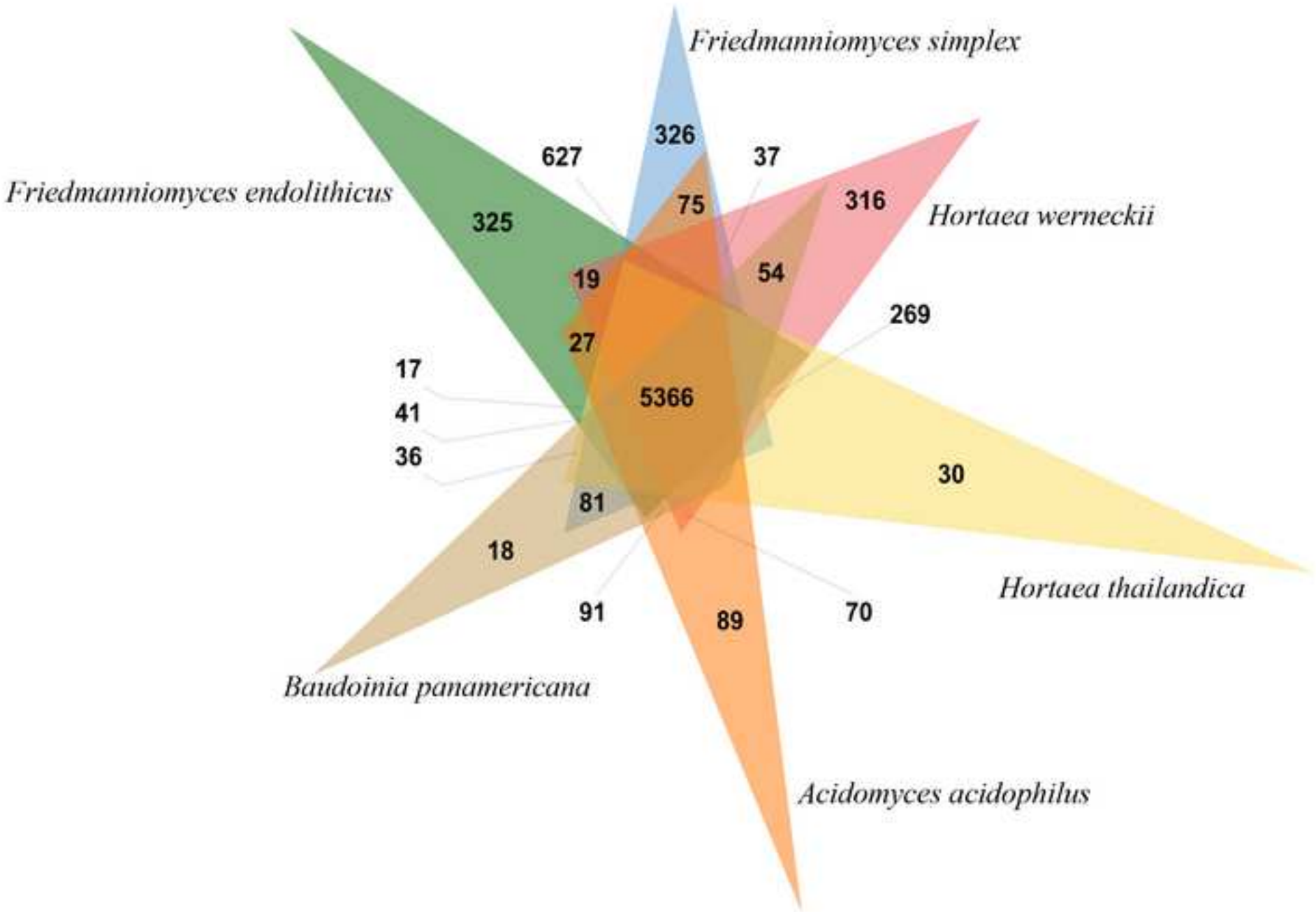

A
B
TFs
CAZYmes
1
2
3
4
5
6
1: Acydomyces acidophilus; 2: Baudoinia panamericana; 3: Friedmanniomyces endolithicus
4: Friedmanniomyces simplex; 5: Hortaea thailandica; 6: Hortaea werneckii

Figure 4


Friedmanniomyces simplex
Friedmanniomyces endolithicus
Hortaea werneckii
Hortaea thailandica
Baudoinia panamericana
Acidomyces acidophilus
326
627
37
325
75
316
19
54
269
27
17
5366
41
36
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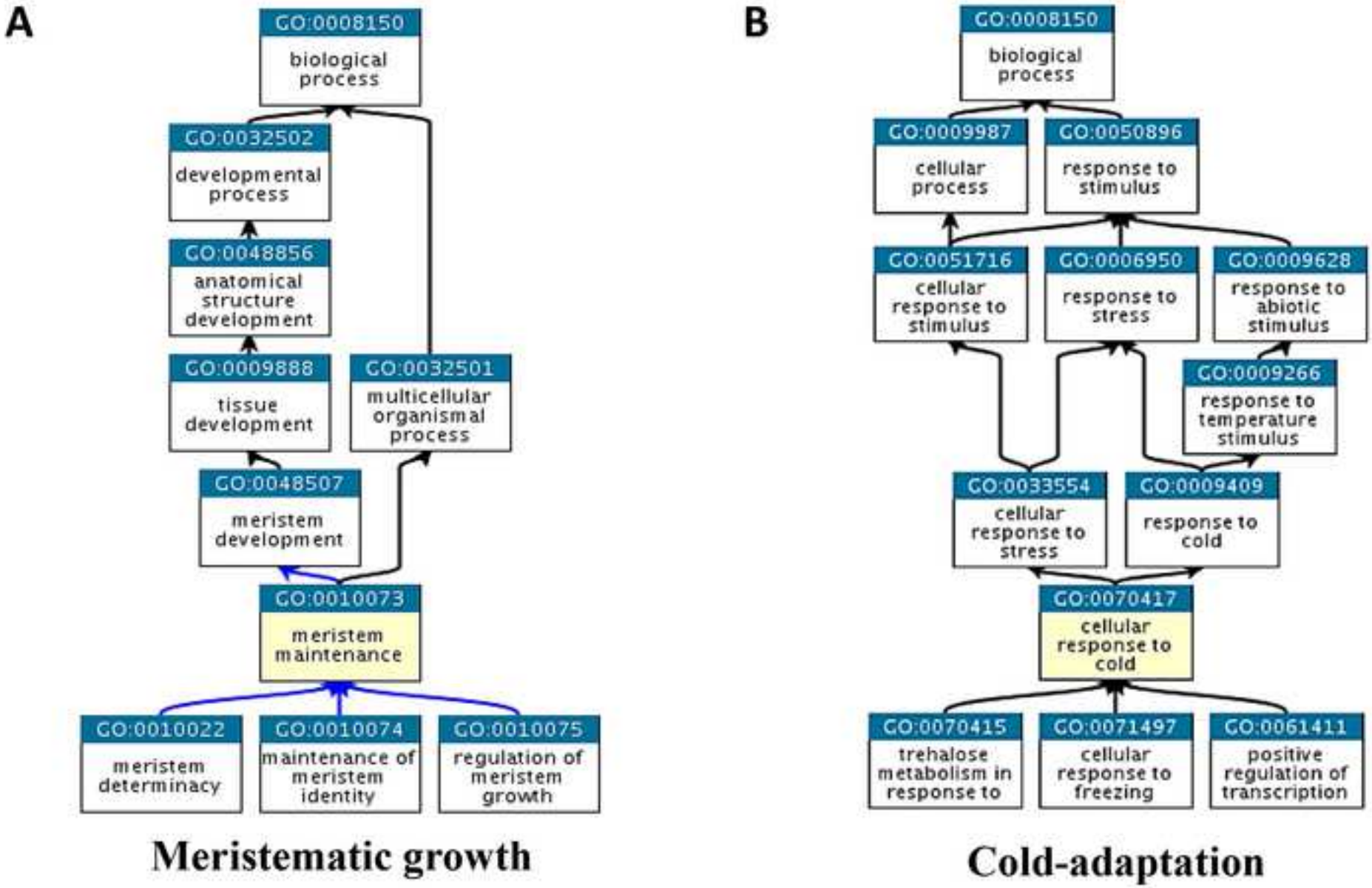
81
18
91
89
70

Figure 5


A
GO:0008150
biological process
GO:0032502
developmental process
GO:0048856
anatomical structure development
GO:0009888
tissue development
GO:0032501
multicellular organismal process
GO:0048507
meristem development
GO:0010073
meristem maintenance
GO:0010022
meristem determinacy
GO:0010074
maintenance of meristem identity
GO:0010075
regulation of meristem growth
Meristematic growth
B
GO:0008150
biological process
GO:0009987
cellular process
GO:0050896
response to stimulus
GO:0051716
cellular response to stimulus
GO:0006950
response to stress
GO:0009628
response to abiotic stimulus
GO:0009266
response to temperature stimulus
GO:0033554
cellular response to stress
GO:0009409
response to cold
GO:0070417
cellular response to cold
GO:0070415
trehalose metabolism in response to
GO:0071497
cellular response to freezing
GO:0061411
positive regulation of transcription
Cold-adaptation