Class-wide genomic tendency throughout specific extremes in Black Fungi

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Abstract

The classes *Dothideomycetes* and *Eurotiomycetes* include constitutively melanized fungi adapted to extreme conditions and they are widely distributed in diverse hostile habitats worldwide. Yet, despite the growing interest in these fungi, there is a considerable gap of knowledge on their functionality. Their genomic analysis is still in its infancy and the possibility to understand their adaptive strategies and exploit their potentialities in bioremediation is very limited. Here, we supply a genome catalog of 118 black fungi, encompassing different ecologies, phylogenies and lifestyles, as a first example of a comparative genomic study at high level of diversity. Results indicate that, as a rule, *Dothideomycetes* show more variable genome size and that larger genomes are associated with harshest conditions; low temperature tolerance and DNA repair capacity are overrepresented in their genomes. In *Eurotiomycetes* high temperature tolerance and capacity to metabolize hydrocarbons are more frequently present and these abilities are positively correlated with the human presence. The genomic features are consistent with the prevalent ecologies in the two classes. Indeed, *Dothideomycetes* are more common in cold and dry environments with high capacity for DNA repair being consistent with the normally highly UV-impacted conditions in their habitats; in contrast, *Eurotiomycetes* spread mainly in hot human-impacted sites with industrial pollution. Mean annual temperature and isothermality are positively correlated with tolerance to high temperatures in *Dothideomycetes*, suggesting that, despite their preference for the cold, they are potentially equipped to survive even when temperatures rise due to the global warming.

Keywords

Black fungi; stress resistance; comparative genomics; extreme environments

Introduction

Black fungi compose a polyphyletic and morpho-ecological group within *Ascomycota*, mainly in the classes *Eurotiomycetes* and *Dothideomycetes*, and count among the most successful extreme-tolerant organisms on Earth. The group, as a whole, displays exceptional skills to exploit virtually all kinds of extremes, spanning from hypersaline and acidic sites to toxic, hydrocarbon-contaminated sites, glaciers, hot and cold deserts, high mountain peaks, solar panels, building roofs, and exposed rocks in Polar and Alpine regions (Gunde-Cimermana et al. 2000; Selbmann et al. 2014, 2015; Ruibal et al. 2018; Baron et al. 2021; Blasi et al. 2016; Gostinčar et al. 2012; Prenafeta-Boldú et al. 2018). Some black fungi are also common colonizers of artificial environments like dishwashers, steam baths and sauna facilities, while others have been isolated from silicone seals and occur planktonic in tap water (Matos et al. 2002; Seyedmousavi et al. 2011; Blasi et al. 2015; Gümral et al. 2016). In addition, a few species are opportunistic pathogens of humans and cold-blooded waterborne vertebrates and serve as important model organisms with respect to clinical mycology (Liu et al. 2019). We argue that traits present in phylogenetically unrelated fungal groups, such as preponderance of clonal propagation, synthesis of melanin-like pigments, thick cell walls, flexible morphology, and meristematic phenotypes, either after switching or as a stable character, may be expressions of convergent evolution. These adaptations facilitate persistence, which enables diversifying evolution under conditions on the edge of life, as well as biotope switches.

Although all black fungi can resist extreme conditions, there are evident differences between black fungi in the *Eurotiomycetes* and *Dothideomycetes.* For instance, different temperature relations and physiological parameters determine their distribution: *Eurotiomycetes* are, in fact, typically found in the urban environment under the influence of pollutants and traffic emissions, while *Dothideomycetes*are recurrent in natural, extremely cold and scarcely competitive habitats. Based on their consistent extremophilic tendency, it has been hypothesized that all black fungal lineages derived from a common rock-inhabiting or lichen-associated ancestor that has later evolved into other ecologies and lifestyles (Schlick-Steiner et al. 2008; Mayer and Voglmayr 2009; Voglmayr et al. 2011; Gueidan et al., 2007, 2008, 2011a; Quan et al. 2020; Muggia et al. 2021). Regardless of their phylogenetic position, indeed, many of them reside on or within bare rock, both natural outcrops and manmade artworks (Isola et al. 2016; Sterflinger 2010; Ruibal et al. 2005). Main areas of distribution of rock-associated species are in the Mediterranean basin, in hot and cold deserts, and on high mountain peaks and these are generally referred to as Rock-Inhabiting Fungi (RIF; Ruibal et al. 2009). Rocky niches impose a number of environmental challenges that these lithobionts have to cope with, and this has promoted an uncommon ability to thrive under stress factors including high solar and UV exposure and nutrient shortage, as well as an excellent capacity to resurrect from dry conditions (Gorbushina et al. 2008). For example, metabolically inactive, dry colonies of some black fungi can survive up to 120 °C for 30 minutes, ionizing radiation, alpha particles, and even conditions of outer space (Dadachova and Casadevall 2008a; Laura Selbmann et al. 2011a; Onofri et al. 2015, 2019; Sterflinger 1998). RIF are predominant in *Dothideomycetes*, and show diverse ecological trends in the two classes: those growing in extremely cold environments have mainly representatives in the class *Dothideomycetes*, namely the order *Capnodiales*. Conversely, RIF in *Eurotiomycetes*, from the order *Chaetothyriales*, are nearly exclusively found in hot, arid climates and are recurrent on marble monuments in the Mediterranean basin (Isola et al. 2016; Diakumaku et al. 1995).

For the growing evidence of their importance in many different fields of fundamental and applied science, black fungi are no longer a subject for few specialists but are supplying ever-expanding fields of study, spanning from microbial ecophysiology, evolution and adaptation to extremes, geomycology, as well as in applied research, such as human pathogenicity, bioremediation, biodeterioration of monuments and astrobiology. Despite this, to date, information on black fungi genomes and their potential functionality is still scant. In *Eurotiomycetes*, nearly all species with genomes belong to the single family *Herpotrichiellaceae*; in addition, numerous species have been described in older literature which have not been cultured (e.g. Chen et al. 2014; Quan et al. 2020) while also new habitats are being discovered and need exploration (Quan et al. 2021). Reference genomes are even less representative in *Dothideomycetes*, albeit if they comprise the majority of extremophilic black fungi, and this hampers our understanding of the evolution and adaptation strategies of fungi in the extremes.

To address this knowledge gap, we present a first comprehensive comparison of 118 black fungal genomes, including different life-styles, phylogeny and ecologies, to investigate the main traits explaining the differences and adaptability between the two classes. We focus on genomic traits associated with key metabolic competences for their extremophilic behavior such as ability to withstand low or high temperatures, UV radiation, efficiency in DNA repair and degradation of monoaromatic toxins and pollutants. Our results may provide a foundation to disentangle the processes that govern the evolution of extremophilic abilities in two of the most extended groups of eukaryotic organisms in terrestrial extreme environments.

Materials and Methods

Strain selection

For the study, 118 strains of black fungi belonging to *Dothideomycetes* and *Eurotiomycetes* were selected from two collections: i) the Culture Collection of Fungi from Extreme Environments (CCFEE) and ii) CCFEE of the Mycological Section of the Italian National Museum of Antarctica (MNA-CCFEE), Viterbo, University of Tuscia, Italy. The strains were chosen to represent various extreme habitats, with an emphasis on fungi isolated from rocks, montane environments, monuments, and human impacted/polluted sites (Figure 1).

Figure 1. Examples of extreme environments where black fungi have been isolated. **a)** Battleship Promontory, Southern Victoria Land, Antarctica (Photo credit : Italian National Antarctic Research Program; **b)** Gran Sasso, Italy; **c)** Mt. Aconcagua, Argentine; **d)** Glen Canyon, Utah, United States of America; **e)** marble monuments in the Bonaria cemetery, Sardinia, Italy; **f)** motor vehicle.

Strain originated from widely different geographical locations, with a bias towards isolates obtained from Antarctica, across a wide range of ecological and environmental (e.g. different altitudes) conditions and spanning 4 continents (Antarctica, Europe, America, Asia) (Table 1; Figure 2). The specimens were also selected to include phylogenetically distinct members of black fungi; 71 strains belong to the class *Dothideomycetes* (representing 4 families, 12 genera, 8 unidentified, all from rocks of Polar desert, mountains, monuments) and 47 to the class *Eurotiomycetes* (representing 2 families, 3 genera, 2 unidentified, from rocks of Polar desert, mountains, monuments and human impacted/polluted sites). The predominant genus of this class, according to the availability in the collections, was *Exophiala*, isolated from the entire environmental range but with a predominance of polluted sites (Supplementary Table S1).

Table 1. List of strains selected in this study. Complete metadata are reported in Supplementary Table S1.

Strain	Species	Environment	Country	Latitude	Longitude

Dothideomycetes					
MNA-CCFEE 515	*Cryomyces antarcticus*	rock	Antarctica	-75,214	164,003
MNA-CCFEE 524	*Friedmanniomyces endolithicus*	rock	Antarctica	-75,214	164,003
MNA-CCFEE 534	*Cryomyces antarcticus*	rock	Antarctica	-75,214	164,003
MNA-CCFEE 536	*Cryomyces antarcticus*	rock	Antarctica	-75,214	164,003
MNA-CCFEE 670	*Friedmanniomyces endolithicus*	rock	Antarctica	-75,214	164,003
MNA-CCFEE 690	*Friedmanniomyces endolithicus*	rock	Antarctica	-75,214	164,003
MNA-CCFEE 5001	*Friedmanniomyces endolithicus*	rock	Antarctica	-74,169	162,427
MNA-CCFEE 5018	*Rachicladosporium* sp.	rock	Antarctica	-77	160,9
MNA-CCFEE 5184	*Friedmanniomyces simplex*	rock	Antarctica	-77	160,9
MNA-CCFEE 5187	*Cryomyces minteri*	rock	Antarctica	-77	160,9
MNA-CCFEE 5193	*Friedmanniomyces endolithicus*	rock	Antarctica	-74,169	162,427
MNA-CCFEE 5195	*Friedmanniomyces endolithicus*	rock	Antarctica	-74,169	162,427
MNA-CCFEE 5199	*Friedmanniomyces endolithicus*	rock	Antarctica	-75,482	159,591
MNA-CCFEE 5200	*Friedmanniomyces endolithicus*	rock	Antarctica	-75,482	159,591
MNA-CCFEE 5208	*Friedmanniomyces endolithicus*	rock	Antarctica	-75,482	159,591
MNA-CCFEE 5264	*Recurvomyces mirabilis*	rock	Antarctica	-76,91	160,934
MNA-CCFEE 5269	*Friedmanniomyces endolithicus*	rock	Antarctica	-74,169	162,427
MNA-CCFEE 5273	*Friedmanniomyces endolithicus*	rock	Antarctica	-74,169	162,427
MNA-CCFEE 5275	*Friedmanniomyces endolithicus*	rock	Antarctica	-76,91	160,934
MNA-CCFEE 5277	*Friedmanniomyces endolithicus*	rock	Antarctica	-76,91	160,934
MNA-CCFEE 5281	*Friedmanniomyces endolithicus*	rock	Antarctica	-76,91	160,934
MNA-CCFEE 5283	*Friedmanniomyces endolithicus*	rock	Antarctica	-76,91	160,934
MNA-CCFEE 5305	*Friedmanniomyces endolithicus*	rock	Antarctica	-76,91	160,934

MNA-CCFEE 5307	*Friedmanniomyces endolithicus*	rock	Antarctica	-76,91	160,934
MNA-CCFEE 5311	*Friedmanniomyces endolithicus*	rock	Antarctica	-76,91	160,934
MNA-CCFEE 5312	*Extremus antarcticus*	rock	Antarctica	-76,91	160,934
MNA-CCFEE 5313	*Elasticomyces elasticus*	Fungi	Antarctica	-76,91	160,934
MNA-CCFEE 5316	*Elasticomyces elasticus*	Fungi	Antarctica	-74,883	163,716
MNA-CCFEE 5319	*Elasticomyces elasticus*	Fungi	Antarctica	-74,883	163,716
MNA-CCFEE 5320	*Elasticomyces elasticus*	Fungi	Antarctica	-74,883	163,716
MNA-CCFEE 5328	*Friedmanniomyces* sp.	rock	Antarctica	-76,901	160,91
CCFEE 5386	*Rachicladosporium monterosium*	rock	Italy	46	7,866
CCFEE 5401	*Meristemomyces frigidus*	rock	Italy	46	7,866
CCFEE 5457	*Meristemomyces frigidus*	rock	Italy	46	7,866
MNA-CCFEE 5474	*Elasticomyces elasticus*	rock	Antarctica	-76,941	161,078
MNA-CCFEE 5485	*Recurvomyces mirabilis*	rock	Antarctica	-76,911	160,909
MNA-CCFEE 5486	*Friedmanniomyces endolithicus*	rock	Antarctica	-76,91	160,934
CCFEE 5501	*Meristemomyces frigidus*	rock	Argentina	-32,669	-69,955
CCFEE 5506	*Elasticomyces elasticus*	rock	Argentina	-32,607	-69,957
CCFEE 5508	*Meristemomyces frigidus*	rock	Argentina	-32,669	-69,955
MNA-CCFEE 5521	*Capnodiales* sp.	rock	Antarctica	-69,5	65
MNA-CCFEE 5522	*Oleoguttula mirabilis*	rock	Antarctica	-69,5	65
MNA-CCFEE 5527	*Rachicladosporium antarcticum*	rock	Antarctica	-69,5	65
CCFEE 5536	*Recurvomyces mirabilis*	rock	Norway	62,777	11,121
CCFEE 5537	*Elasticomyces elasticus*	rock	India	32,893	77,194
CCFEE 5543	*Elasticomyces elasticus*	rock	India	32,893	77,194
CCFEE 5544	*Elasticomyces elasticus*	rock	India	32,893	77,194
CCFEE 5547	*Elasticomyces elasticus*	rock	Italy	44,713	6,18
CCFEE 5714	*Vermiconia calcicola*	rock	Italy	39,211	9,124

CCFEE 5805	*Dothideomycetes* sp.	rock	Italy	42,471	13,563
CCFEE 5806	*Elasticomyces elasticus*	rock	Italy	42,471	13,563
CCFEE 5810	*Elasticomyces elasticus*	rock	Italy	42,471	13,563
CCFEE 5887	*Vermiconia calcicola*	rock	Italy	41,904	12,451
CCFEE 5935	*Saxophila tyrrhenica*	rock	Italy	39,223	9,121
CCFEE 5966	*Elasticomyces elasticus*	rock	Italy	46,417	10,183
MNA-CCFEE 6074	*Friedmanniomyces endolithicus*	rock	Antarctica	-73,49	163,912
MNA-CCFEE 6078	*Friedmanniomyces endolithicus*	rock	Antarctica	-74,178	162,514
MNA-CCFEE 6081	*Friedmanniomyces endolithicus*	rock	Antarctica	-75,756	161,062
MNA-CCFEE 6082	*Friedmanniomyces endolithicus*	rock	Antarctica	-75,858	159,973
MNA-CCFEE 6086	*Elasticomyces elasticus*	rock	Antarctica	-74,178	162,514
MNA-CCFEE 6096	*Friedmanniomyces endolithicus*	rock	Antarctica	-74,178	162,514
CCFEE 6128	*Elasticomyces elasticus*	rock	Italy	46,417	10,183
MNA-CCFEE 6249	*Friedmanniomyces endolithicus*	rock	Antarctica	-74,17	162,425
MNA-CCFEE 6250	*Friedmanniomyces endolithicus*	rock	Antarctica	-75,483	159,589
MNA-CCFEE 6253	*Teratosphaeriaceae* sp.	rock	Antarctica	-75,483	159,589
MNA-CCFEE 6256	*Teratosphaeriaceae* sp.	rock	Antarctica	-75,483	159,589
MNA-CCFEE 6315	*Hortaea thailandica*	rock	Antarctica	-76	159,227
MNA-CCFEE 6416	*Friedmanniomyces endolithicus*	rock	Antarctica	-75,859	159,974
MNA-CCFEE 6420	*Friedmanniomyces* sp.	rock	Antarctica	-74,178	162,514
MNA-CCFEE 6461	*Cryomyces antarcticus*	rock	Antarctica	-75,859	159,974
MNA-CCFEE 6464	*Friedmanniomyces endolithicus*	rock	Antarctica	-74,178	162,514
MNA-CCFEE 6590	*Recurvomyces mirabilis*	rock	Antarctica	-75,704	159,227
MNA-CCFEE 6595	*Dothideomycetes* sp.	rock	Antarctica	-77,75	160,745

Eurotiomycetes					
CCFEE 5649	*Exophiala xenobiotica*	rock	Austria	48,161	16,311
CCFEE 5737	*Coniosporium uncinatum*	rock	Italy	39,211	9,124
CCFEE 5748	*Exophiala sideris*	rock	Italy	38,028	14,144
CCFEE 5749	*Exophiala sideris*	rock	Italy	38,028	14,144
CCFEE 5784	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 5792	*Exophiala bonariae*	rock	Italy	39,211	9,123
CCFEE 5801	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 5811	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 5816	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 5819	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 5823	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 5874	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 5877	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 5882	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 5885	*Lithohypha guttulata*	rock	Italy	41,904	12,451
CCFEE 5907	*Lithohypha guttulata*	rock	Italy	41,904	12,451
CCFEE 5908	*Lithohypha guttulata*	rock	Italy	41,904	12,451
CCFEE 5910	*Lithohypha guttulata*	rock	Italy	41,904	12,451
CCFEE 5925	*Lithohypha guttulata*	rock	Italy	41,904	12,451
CCFEE 5928	*Lithohypha guttulata*	rock	Italy	41,904	12,451
CCFEE 5985	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6036	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6043	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6059	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6060	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6068	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6142	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6169	*Chaetothyriales* sp.	rock	USA	36,865	-111,588
CCFEE 6180	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6182	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6190	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6194	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6196	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264

CCFEE 6221	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6233	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6237	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6238	*Exophiala xenobiotica*	motor vehicle	Italy	40,625	14,38
MNA-CCFEE 6314	*Exophiala mesophila*	rock	Antarctica	-71,25	163
CCFEE 6327	*Exophiala oligosperma*	rock	Italy	39,225	9,122
CCFEE 6328	*Exophiala sideris*	rock	Spain	39,749	-3,004
CCFEE 6333	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6336	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6357	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6362	*Exophiala xenobiotica*	motor vehicle	Italy	42,383	12,264
CCFEE 6388	*Eurotiomycetes* sp.	motor vehicle	Italy	42,383	12,264

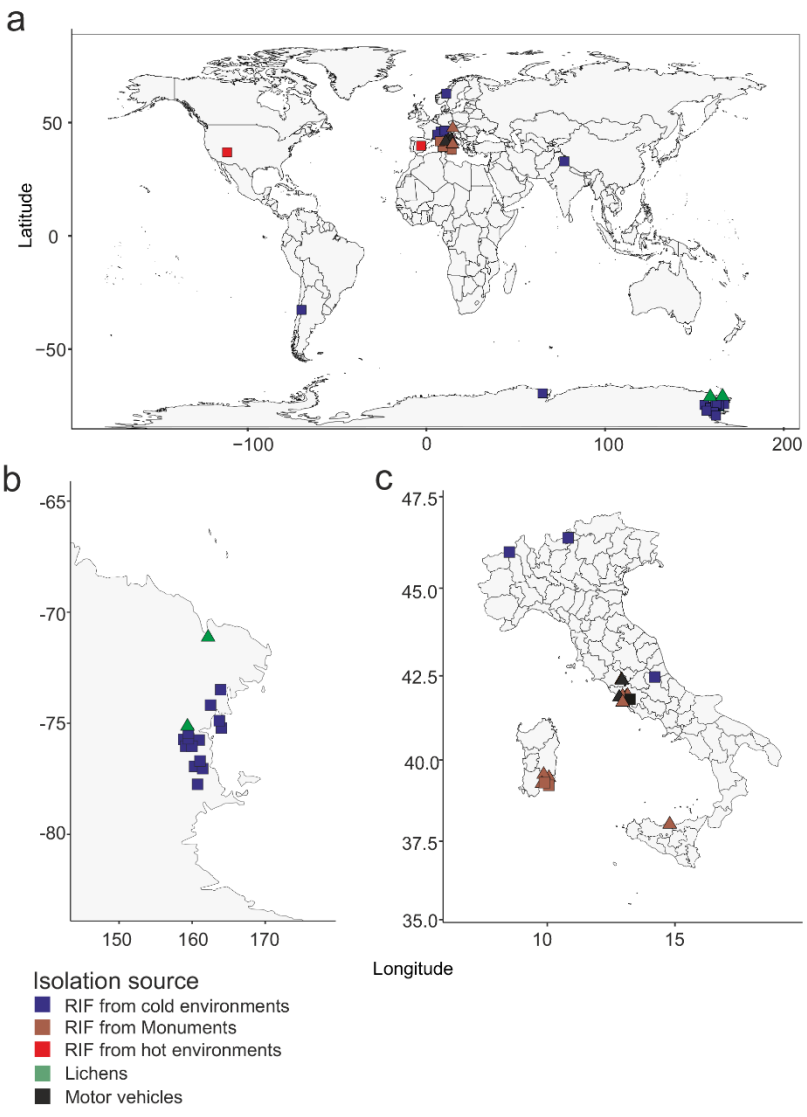

Figure 2. Study areas. a) Map of sampled localities with magnification of Victoria Land, Antarctica **(b)** and Italian Peninsula **(c).** A complete list of sampled sites is reported in the Supplementary Table S1.

DNA extraction and genome sequencing

The dothideomycetous pure cultures, isolated mostly from cold environments, were grown on 2 % Malt Extract Agar (MEA) medium plates for 8-10 weeks at 15 °C, while eurotiomycetous strains, mainly originating from mesophilic environments, were grown on MEA for 2-4 weeks at 20 °C. DNA was extracted from the total biomass following cetyltrimethylammonium bromide (CTAB) protocol, according to Coleine et al. (2019b). Melanin was removed through two phenol-chloroform purification steps. Genomic DNA was sheared with Covaris S220 ultrasonic homogenizer and sequencing library constructed using the Kapa HyperPlus kit coupled with the KAPA Unique Dual Index adapters (Roche), following the instructions of the manufacturers. Sequencing libraries were prepared using the Nextera DNA Flex Library Preparation Kit (Illumina, CA, USA), following the manufacturer's guidelines. Sequencing was performed on the Illumina NovaSeq 6000 platform following manufacturer's protocols.

Assembly, gene prediction, and functional annotation

All genomes were *de novo* assembled with the AAFTF pipeline (v.0.2.3) (Palmer and Stajich 2022) which performs read quality control and filtering with BBTools bbduk (v.38.86) (Bushnell 2014), followed by SPAdes (v.3.15.2) (Bankevich et al. 2012) assembly using default parameters, followed by screening to remove short contigs < 200 bp and contamination using NCBI's VecScreen. The BUSCO ascomycota_odb10 database (Manni et al. 2021) was used to determine completeness. Genes in each near-complete genome assembly with Funannotate (v1.8.1) (Palmer and Stajich 2020). A masked genome was created by generating a library of sequence repeats with the RepeatModeler pipeline (Bankevich et al. 2012). These species-specific predicted repeats were combined with fungal repeats in the RepBase (Bao et al. 2015) to identify and mask repetitive regions in the genome assembly with RepeatMasker (v.4-1-1) (Smit 2004). To predict genes, *ab initio* gene predictors SNAP (v.2013_11_29) (Korf 2004) and AUGUSTUS (v.3.3.3) (Stanke et al. 2006) were used along with additional gene models by GeneMark.HMM-ES (v.4.62_lic) (Brůna et al. 2020), and GlimmerHMM (v.3.0.4) (Majoros et al. 2004) utilize a self-training procedure to optimize *ab initio* predictions. Additional exon evidence to provide hints to gene predictors was generated by DIAMOND BLASTX alignment of SwissprotDB proteins and polished by Exonerate (v.2.4.0) (Slater and Birney 2005). Finally, EvidenceModeler (v.1.1.1) (Haas et al. 2008) generated consensus gene models in Funannotate that were constructed using default evidence weights. Non-protein-coding tRNA genes were predicted by tRNAscan-SE (v.2.0.9) (Lowe and Chan 2016). Putative protein functions were assigned to genes based on sequence similarity to the Interpro database. Using InterProScan5 software (v.5.51-85.0) (Jones et al. 2014) like TIGRFAM, PANTHER, CDD, Prosite, and many others (InterProScan documentation at https://interproscan-docs.readthedocs.io/en/latest/). In this case, InterProScan was used with default parameters scanning the sequences to all databases available in November 2021. Complementary to InterProScan, EggNOG software (v.5) (Huerta-Cepas et al. 2017, 2019) was used to obtain and KEGG functional orthologs (Kanehisa et al. 2014). Both were executed as stand-alone software on a High Performance Computing cluster.

Environmental metadata

To determine the ecological preferences of the two studied classes, environmental parameters were included in the analysis. Climatic metadata were collected from the WorldClim database [(https:// www.world clim.org), ~1 km resolution (Fick and Hijmans 2017) and included a range of variables related to temperature and precipitation variability that are considered important drivers of fungal distribution at large scales – for instance, Köppen-Geiger climate classification subgroup (KG climate), mean annual temperature (MAT), precipitation seasonality (PSEA), temperature seasonality (TSEA), Mean Temperature of the Warmest Quarter (MTWAQ), Mean Temperature of the Coldest Quarter (MTCQ), Mean Annual Precipitation (MAP). Ultraviolet (UV) and solar radiation, isothermality, and Human Influence Index (HII) were obtained from the NEO (NASA Earth Observations) database (https://neo.gsfc.nasa.gov/). A complete list of metadata is available in Supplementary Table S1.

KEGG functional orthologs selection and statistical analyses

To explore the genomic composition of selected strains and relate it to the taxonomy, we ordinated the gathered genomes through nonmetric multidimensional scaling (NMDS). Bray–Curtis dissimilarity index was calculated on Hellinger-transformed KEGG orthologs abundances. Significance testing between *Dothideomycetes* and *Eurotiomycetes* gene composition for beta diversity was assessed using permutational multivariate analysis of variance (PERMANOVA) using the R 'vegan' (Dixon 2003) v.2.5-6. Further, for comparative purposes, we focused on KEGG functional orthologs related to specific metabolic competences; i.e., DNA repair, temperature and UV radiation tolerance and ability to degrade hydrocarbons. Pairwise comparison of gene composition between the two classes within each site was assessed by Wilcoxon test with Benjamini–Hochberg FDR multiple test correction. A complete list of KEGGs analyzed in this study is reported in the Supplementary Table S2.

We additionally used the Random Forest (RF) model as described in (Delgado-Baquerizo et al. 2018) to identify the major significant environmental predictors explaining the variation of metabolic competences in black fungi according to environmental variables (see Environmental metadata section). The importance of each predictor variable is determined by evaluating the decrease in prediction accuracy, i.e., increase in the mean square error between observations and OOB (out-of-bag) predictions, when the data for that predictor is randomly permuted. RF was implemented using the 'randomForest' package v.4.6–14 in the R environment. In addition, to exclude possible confounding effects due to spatial autocorrelation of environmental variables, we repeated the correlation analysis, while controlling for space (e.g. latitude and longitude).

We then used correlation (Spearman's rank) analyses and PERMANOVA ($P < 0.05$) to identify the most important environmental factors associated with the metabolic capabilities of selected strains using the 'ppcor' package. Spearman rank correlations measure the strength and direction of association between two ranked variables. They do not require normality of data, and linearity is not a strict assumption of these analyses. We used a false discovery rate approach to determine adjusted P values for all the correlations to control for spurious (false positives) correlations.

Statistical downstream analyses were performed using genomes with BUSCO completeness ≥ 87%.

In addition, to exclude possible confounding effects due to over-representation of individual species, we repeated the statistical analysis between, while controlling for this factor (e.g. number of strains from same species).

Results

Genome structure of black fungi

Genome sequences of 71 *Dothideomycetes* and 47 *Eurotiomycetes* black fungi colonizing extreme environments were determined by Illumina high-throughput sequencing and *de novo* assembled. For *Dothideomycetes*, the average genome size was very variable among the strains, ranging from 22.13 Mbp (*Meristemomyces frigidus* CCFEE 5401) to 121 Mbp (*Elasticomyces elasticus* CCFEE 5544), while the average number of predicted genes ranged from 8,844 (CCFEE 5410) up to 52,079 (CCFEE 5544) (Table 2). Overall, *Friedmanniomyces endolithicus* and *E. elasticus* encompass the highest genome sizes, up to 76.16 and 121.19 Mbp in *Friedmannyomyces endolithicus* MNA-CCFEE 524 and *Elasticomyces elasticus* CCFEE 5544, respectively. For *Eurotiomycetes*, the genome size of the sequenced strains were more homogeneous, the average was 32.4 Mbp, and ranged from 24.43 (*Lithohypha guttulata* CCFEE 5910) to 57.34 Mbp (*Exophiala xenobiotica*. CCFEE 6182 from gasoline dispenser) (Table 2). The numbers of predicted genes varied from 8,794 (*Lithohypha guttulata* CCFEE 5925) to 20,193 (*Exophiala xenobiotica* CCFEE 6182). The average GC content was 55 for *Dothideomycetes*, ranging from 50.94 to 59.49 and 51 for *Eurotiomycetes*, varying from 48.51 to 55.29 (Table 2). We assessed the completeness and evaluated our assemblies quantifying the content of Benchmarking Universal Single-Copy Orthologs (BUSCOs). This analysis revealed that the genomes sequenced in this study are highly complete, averaging 90.5 and 94.8 (*Dothideomycetes* and *Eurotiomycetes*, respectively).
The statistics of the genome sequencing, assembly and annotation are reported for each strain in Supporting Information Table S3.

Table 2. Statistics for the sequenced genomes. Complete data for each genome is available in the Supplementary Table S3. BUSCOs, Benchmarking Universal Single-Copy Orthologues.

Statistics	Minimum	Maximum	Mean
Dothideomycetes			
Genome assembly size (Mb)	22.13	121.194	45.66
Number of contigs	90	71,270	9,248
Genes count	8,844	52,079	18,241
GC content (%)	50,94	59,49	55
Complete BUSCOs (%)	38	98.1	90
Single-copy BUSCOs (%)	4.3	97.8	47
Duplicated BUSCOs (%)	0.1	92.8	43
Fragmented BUSCOs (%)	0.3	22.1	3
Missing BUSCOs (%)	1.5	42.2	7
Eurotiomycetes			
Genome assembly size (Mb)	24.43	57.34	32.43
Number of contigs	51	39,292	2,405
Genes count	8,794	20,193	11,891
GC content (%)	48.51	55.29	51.20
Complete BUSCOs (%)	33.9	98.6	93.37
Single-copy BUSCOs (%)	26.6	98.1	97.74

Statistics	Minimum	Maximum	Mean
Duplicated BUSCOs (%)	0.5	7.3	0.63
Fragmented BUSCOs (%)	0.3	15.3	1.27
Missing BUSCOs (%)	0.9	50.8	5.22

Genomic signatures of diverse metabolic competences in black fungi

To determine whether functional and taxonomic correlations exist among the sequenced strains, non-metric multidimensional scaling (NMDS) and PERMANOVA analyses were performed (Figure 3a). In the NMDS ordination plot, KEGG orthologs datasets splitted *Dothideomycetes* and of *Eurotiomycetes* in two clusters ($P < 0.05$), with a few strains scattered at isolated positions. On the other hand, while clustering of some strains could be related to habitat or geography, this was not absolute, some strains from similar habitats and/or locations were found in remote positions.

We analyzed the predicted proteins (primarily their copy numbers) annotated on the KEGG orthologs that are known to be involved in stress tolerance and biodegradation of hydrocarbons. The search for metabolic competences in the predicted proteomes of black fungi (Figure 3b) led to the identification of many predicted KEGGs involved in DNA repair, resistance to high and low temperatures, tolerance of UV radiation and ability to degrade hydrocarbons (a complete list of KEGG terms is reported in Supplementary Table S2). The major global differences between *Dothideomycetes* and *Eurotiomycetes* were found in KEGG genes related to DNA repair, tolerance of low temperature and hydrocarbon catabolism (Figure 3b). Specifically, we found that the KEGG abundance, together with DNA repair and cold tolerance were significantly increased in *Dothideomycetes*. This difference did not reach statistical significance when analyzing terms related to high temperature and UV radiation tolerance. On the other hand, *Eurotiomycetes* were significantly enriched with members of the KEGGs related to hydrocarbon catabolism.

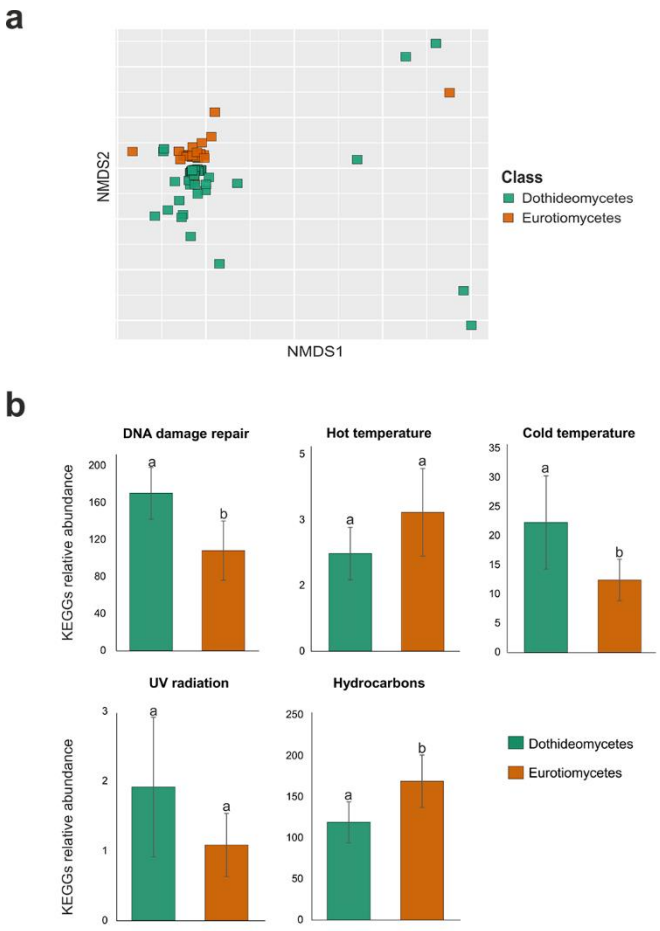

Figure 3. KEGG functional ortholog clustering and composition. a) Non-metric multidimensional scaling of entire KEGG functional orthologs datasets by comparing *Dothideomycetes* and *Eurotiomycetes*. The two classes are represented by colors, the shape of which corresponds to the habitat. **b)** Relative abundance of KEGG functional orthologs for five selected metabolic competences in the studied fungi. T-tests were used to test the significance of the differences between the abundances of these genes for the following pairs of species groups: *Dothideomycetes versus Eurotiomycetes*. Significant differences ($P < 0.05$) are indicated with different letters.

Results were confirmed when comparing the most abundant KEGGs involved in the above-mentioned metabolisms (Figure 4). Albeit the differences in KEGGs abundance related to high temperature tolerance between *Dothideomycetes* and *Eurotiomycetes* did not reach statistical significance, *Eurotiomycetes* were mostly isolated in hot/temperate climates and presented a higher abundance of KEGG K17867, suggesting a diffused ability in the class to cope with this stress. This specific ortholog was consistently represented throughout the selected genomes, even in fungi originating from very cold environments as for *Exophiala mesophila* MNA-CCFEE6314 isolated from Antarctic endolithic communities. *Eurotiomycetes* were, however, poorly competent in coping with stress of low temperature or UV radiation: KEGGs K00324, K01993, K02386, K03704, K05934, K12741, K00658, K01934, K02959, K03522, K06681, K07151, K00627, K14798 and KEGGs K04485, K14055, K21249, as relevant genes for the two stresses, respectively, were absent or present in low frequency. Black fungal *Eurotiomycetes* are also recurrent in anthropogenic or industrially polluted environments and this ecology is widely represented in our selection. The ability to degrade hydrocarbons (KEGGs pathways map00621, map00622, map00630, map00640, map00642) also

characterizes this class, even if at a much lower extent than high temperature tolerance. Surprisingly the most competent strains did not originate from polluted sites, such as motor vehicles, but from exposed marble artworks. This holds true for *Lithohypha guttulata* CCFEE5910 and *Exophiala bonariae* CCFEE5792 both isolated from the monumental Bonaria cemetery.

Differently, *Dothideomycetes* were enriched in DNA repair orthologs (KEGGs K04485, K014055, K021249), particularly *Friedmanniomyces endolithicus* MNA-CCFEE5208, *Vermiconidia calcicola* CCFEE5714 and *Elasticomyces elasticus* CCFEE5544, which were isolated from Antarctic rock, monument in the Mediterranean area and Indian Ladakh range, respectively. Consistently, these are also the strains showing the highest KEGGs orthologs abundance for UV radiation tolerance, despite this ability being quite widespread in the class (K21249). The capacity to cope with low temperatures, practically absent among tested *Eurotiomycetes* regardless their provenience from hot or cold environments, is present in *Dothideomycetes*, particularly in *Friedmanniomyces endolithicus* MNA-CCFEE5208, from Antarctica, and *Elasticomyces elasticus* CCFEE5544, and *Meristemomyces frigidus* CCFEE5457 from high altitude in the Alps. KEGGSs orthologs for high temperature resistance are less present and abundant in this class; surprisingly the most tolerant strains to this stress were *Friedmanniomyces endolithicus* MNA-CCFEE6074 and *Cryomyces minteri* MNA-CCFEE5187, two endemic species of the Antarctic desert.

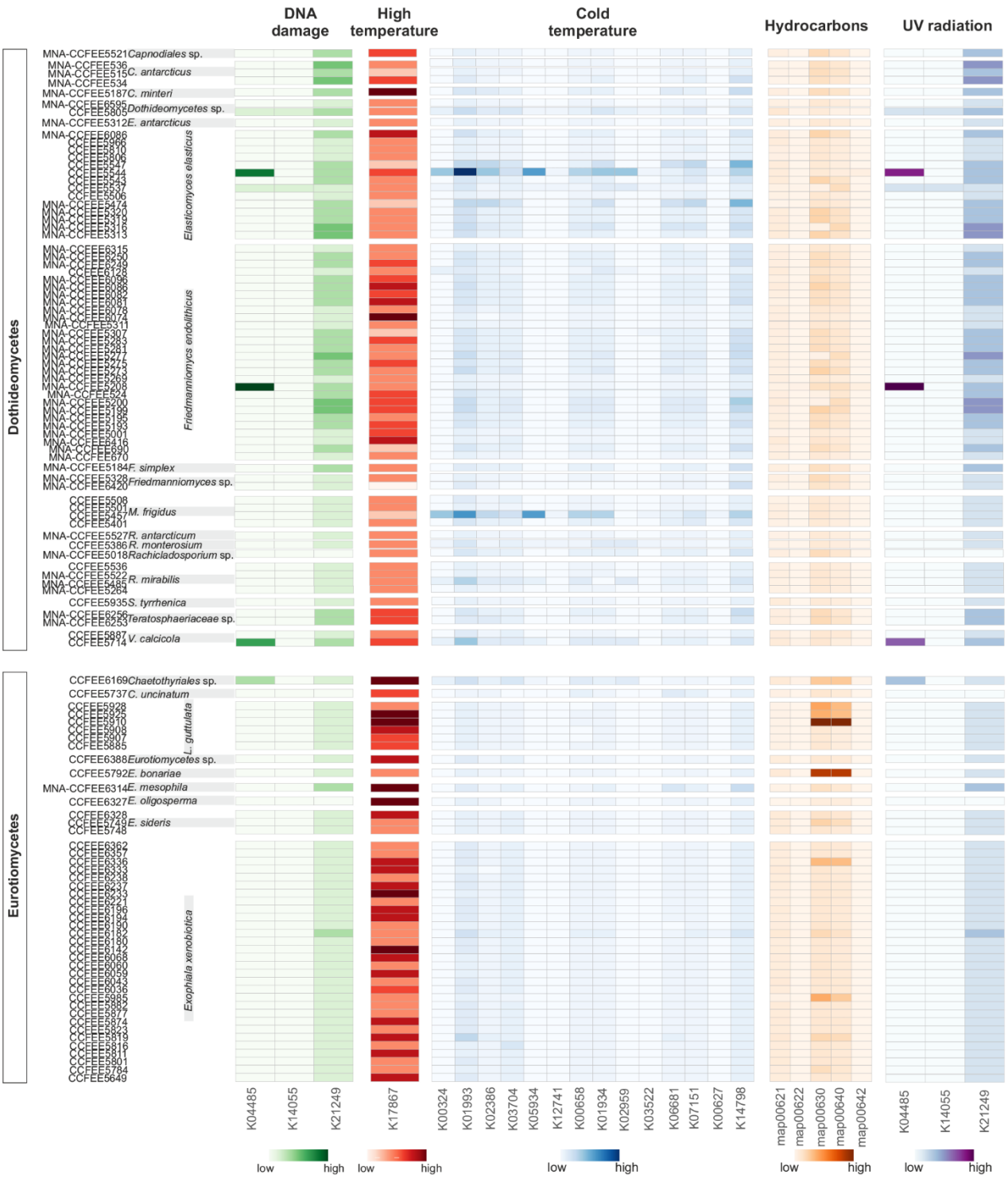

Figure 4. Metabolic potential of black fungi. Heatmap showing the relative abundance of KEGG functional orthologs estimated to encode five particular metabolic competences.

Environmental factors driving genomic and metabolic competences of black fungi

To investigate the main environmental factors associated with metabolic competences of black fungi, we performed a Random Forest (RF) model (Figure 5a). Globally, analyzing all *Dothideomycetes* and *Eurotiomycetes* genomes, the RF model provided evidence that taxonomic affiliation, human influence, climate, and UV index were the most important universal predictors ($P < 0.05$).

Isothermality was relevant in all metabolisms analyzed, except for hydrocarbon degradation, where, instead, human influence, temperature seasonality (TSEA) and precipitation (PSEA) were most strongly associated. Significant associations ($P < 0.05$) were also obtained when controlling for spatial autocorrelation (i.e. using latitude and longitude as controlling matrix). At more detailed level, by implementing non-parametric correlation analyses (Figure 5b), we found that in *Dothideomycetes* mean annual temperature (MAT) and isothermality are positively correlated with tolerance to high temperatures and latitude and mean diurnal range (MDR) are instead negatively correlated with this competence; differently, these factors did not have particular significance in *Eurotiomycetes*. MAT is negatively correlated with this last group when low temperature tolerance is considered. Human influence is found positively correlated with the capacity of *Eurotiomycetes* to degrade hydrocarbons and resist high temperature. A positive association with hydrocarbon degradation metabolism was also reported between *Eurotiomycetes* and TSEA. In addition, as the Worldclim database may be not suitable to precisely detect MAT under car hood, to avoid misinterpretation of results we repeated correlation analysis excluding *Eurotiomycetes* strains isolated from motor vehicles. Results were highly consistent (Spearman correlation, p value <0.05) and confirmed our previous analysis.

Lastly, we searched for eventual correlations between genomes (i.e. genome size, genes count, and GC content) and environmental factors. We found that genome size positively correlated with gene counts and GC content (Spearman correlation 0.91 and 0.21 respectively, p value <0.05). Yet, gene count was both negatively influenced by the HII (Spearman correlation -0.21, p value 0.03) and positively correlated with UV index (Spearman correlation 0.18, p value 0.05).

Significant associations ($P < 0.05$) were also obtained when controlling for over-representation of individual species.

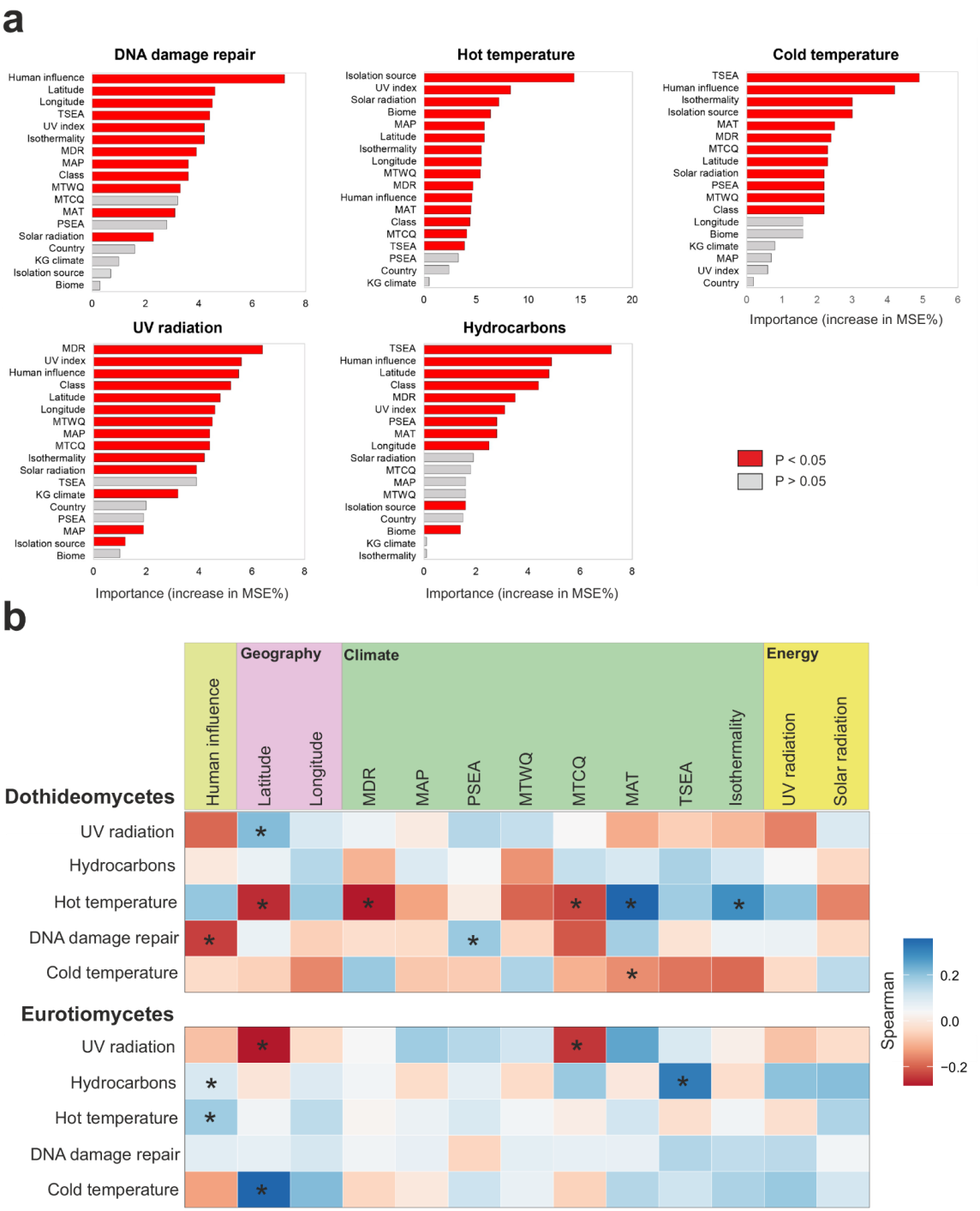

Figure 5. Influence of environmental parameters on metabolic competences of black fungi. a) Random forest (RF) analyses identifying the importance of potential predictors of black fungi metabolic competences (proportion of KEGGs associated with those metabolisms, see Methods). RF Importance = Increase in % mean square error. Coloured and white columns represent $P < 0.05$ and $P > 0.05$, respectively. **b)** Heatmap showing Spearman correlation between KEGG functional orthologs estimated to encode five particular metabolic competence and environmental parameters. Significant Spearman correlation coefficients (**$P < 0.05$**) are shown with *.

Abbreviations are as follows: MDR (mean diurnal range), mean annual temperature (MAT), precipitation seasonality (PSEA), temperature seasonality (TSEA), Mean Temperature of the Warmest Quarter (MTWAQ), Mean Temperature of the Coldest Quarter (MTCQ), Mean Annual Precipitation (MAP). Ultraviolet (UV) and solar radiation, isothermality, and Human Influence Index (HII) were obtained from the NEO (NASA Earth Observations).

Discussion

Despite the increasing interest in the intriguing group of black fungi, there is a considerable gap of knowledge of their functionality and their ability to adapt to extreme conditions, with the genomic analysis still in its infancy. In the current study, a genome catalog of 118 black fungi was generated, significantly increasing the repertoire of genomic data for several taxa and, to date, representing the first example of comparative genomics of black fungi at large scale. Compared with the previous studies on black fungi genomes, this dataset was constructed using a diversified selection of strains from *Eurotiomycetes*, but also from the hitherto much less uncovered *Dothideomycetes*. Available genomic information, indeed, covering ca. 50 black fungal species, suggests that the genomic amplitude of these guilds would be relatively small, ranging from 20 to 50 Mbp, regardless of the source: human opportunists, environmental species and ant association (Teixeira et al. 2017; Moreno et al. 2018, 2019; Coleine et al. 2019a; Quan et al. 2022). Our study highlights that, despite the arithmetic means for both *Dothideomycetes* and *Eurotiomycetes* falling within this range, the genome size variability is wider in *Dothideomycetes.* In fact, it ranges from 22.13 to 121.194 Mbp (*Meristemomyces frigidus* CCFEE 5401 and *Elasticomyces elasticus* CCFEE 5544, respectively). High variability was also observed within a single species: for example, genome sizes varied from 23.49 to 76.16 in *F. endolithicus* strains MNA-CCFEE 5001 and MNA-CCFEE 524, respectively, and from 30 to 121.19 in *E. elasticus* strains CCFEE 5810 and CCFEE 5844, respectively. Conversely, genome size in *Eurotiomycetes* never exceeded 57.34 Mbp (*Exophiala xenobiotica* CCFEE 6182 from gasoline dispenser).

Large genomes (as for *F. endolithicus* and *E. elasticus*) are mostly encountered in species from very harsh natural environments such as the Antarctic deserts or highest mountain peaks. The largest genome was found in *E. elasticus* CCFEE 5544, a fungus from high altitude of Indian Ladak, where annual mean temperature does not exceed -10 °C. Interestingly, genome size is positively correlated with UV index (Spearman correlation 0.18, p value 0.05), and the above-mentioned environments are all highly UV impacted. We also found that the wider the genomes are the higher is the GC content, which may increase the stability of DNA chains in organisms living in highly exposed environments. Larger genomes also give higher counts of genes, and the highest were found in the species *F. endolithicus* MNA-CCFEE 524 and *E. elasticus* CCFEE 5544, isolated from the most extreme environments analyzed in our dataset, in which proteomes contain more than 30,000 and 50,000 predicted proteins, respectively. Conversely, genome size is negatively influenced by human impact (HII, Spearman correlation -0.21, p value 0.03); typically, eurotiomycetous black fungi in our selection, originating from miscellaneous anthropogenic sources such as monuments, urban and polluted environments, showed smaller genomes than most *Dothideomycetes*.

Studies on extremotolerant/extremophilic dothideomycetous black fungi by Gunde-Cimerman and collaborators recently reported that the level of recombination in the black fungus *Aureobasidium pullulans* is higher than in most fungi (e.g. Gostinčar et al. 2019, 2022). They also reported inbreeding and hybridization events, analyzing ca. 100 genomes of black fungal strains belonging to *Hortaea werneckii* and *Aureobasidium melanogenum*, where the average assembly size was 26.52 Mbp (±1.47 SD) for haploid and 49.30 Mbp (±1.74 SD) for diploid genomes. Lenassi et al. (Lenassi et al. 2013) reported the genome size of *Hortaea werneckii* as 51.6 Mbp, larger than most phylogenetically related fungi and coding for almost twice the usual number of predicted genes (23k), due to a possible

relatively recent whole-genome duplication or hybridization. Gene duplication events might have enabled a rapid evolution of proteins and consequently enhanced metabolic plasticity, increasing the fitness during the colonization of hostile ecological niches. Genome duplication have been also inferred in other eukaryotic lineages such as plants; for example, in *Arabidopsis thaliana*, whole genome duplication (WGD) influences a stress response evolution enhancing tolerance to drought stress (Bowers et al. 2003; Simillion et al. 2002).

Based on these observations, we envisage that genomes analyzed in this study, reflecting a large diversity of genome sizes, phylogenetic allocation, lifestyles and ecologies, may be an attractive model to unravel the role of clonality and ploidy in the evolution of extreme-tolerant fungi. We can surmise that partial or whole genome duplication may be a strategy to adapt to the harshest habitats on Earth, e.g. cold and hot deserts. In fungi, it has been already proven that duplication events can lead to the ability to adapt to such a wide range of environmental extremes or contributing to the evolution of novel functions (Lidzbarsky et al. 2009).

Analyzing both whole predicted proteomes and specific metabolic competences, we also found that taxonomy is related to functional diversity. Genes associated with low temperature tolerance were significantly enriched in *Dothideomycetes* strains; they were also particularly enriched in genes involved in DNA repair, supporting the hypothesis that this may reflect an evolutionary adaptation to repair DNA after damage induced by desiccation (Mattimore and Battista 1996; Turnbull et al. 2009) as an advantage to colonize arid and -hyper arid regions. Although UV radiation reaches high levels in those regions where most *Dothideomyetes* were isolated and this parameter has been recently proposed as one the most important environmental factors driving black fungi diversity in global natural environments (Coleine et al. 2022), overall we did not observe a class-wide tendency. Instead, when strains were analyzed separately, most of them were enriched in genes related to UV radiation tolerance, confirming the observations of Selbmann and collaborators. In fact, *Cryomyces antarcticus*, an endemic RIF of the Antarctic desert, easily survives increasing UV-B (280–360 nm) irradiation doses (Selbmann et al. 2011b); this corresponds to over five to eight times the Antarctic terrestrial UV-B irradiance, which is substantially higher than elsewhere on Earth (50%–130% more UV radiation reaching the Earth's surface) (Madronich et al. 1998). Highly melanized fungi, in general, are skilled to survive also radioactive environments (Dadachova et al. 2007; Dadachova and Casadevall 2008b; Robertson et al. 2012) and those isolated from Antarctic endolithic communities in particular represent notable examples of radio-resistant organisms. To name a few, *F. endolithicus* MNA-CCFEE 5208, here also incidentally the richest in genes associated with UV radiation tolerance, was proven resistant to acute doses of gamma radiation (up to 400 Gy), accompanied by increase in metabolic activity (Coleine and Selbmann 2021a).

On the other hand, several genomic traits were instead significantly associated with *Eurotiomycetes*. Chaetothyrialean fungi are well known for the ability to colonize toxic niches contaminated with hydrocarbons and heavy metals (hydrocarbonoclastic activity) (Coleine and Selbmann 2021b; Isola et al. 2013; Baron et al. 2021). For example, the genera *Exophiala* and *Cladophialophora* have been isolated from various hydrocarbon-polluted environments such as industrial spills, car gasoline tanks and air biofilters, but the genus also contains opportunistic human pathogens able to cause neurotropic infections (Zhao et al. 2010; Tesei 2022). Transcriptomic analysis of *Cladophialophora immunda*, grown in the presence of toluene as sole carbon source, revealed the identification of five clusters of

genes involved in toluene degradation into CO_2, with 65% of the C-toluene being recovered as C-CO_2 (Blasi et al. 2017). However, despite these recent observations, a genome comparison at class-wide level largely remained unexplored. We herein provide, for the first time, clear evidence that all *Eurotiomycetes* analyzed, primarily isolated from monuments in Mediterranean historical sites characterized under high temperature and air pollution and from diesel car tanks, are enriched in genes involved in hydrocarbon degradation.

The evolutionary and adaptive tendency throughout differently stress-impacted environments in the two classes of black fungi may be related to their evolutionary history. Dothideomycetous black fungi diversified in the Silurian–Devonian era in a period of 386–498 Mya under drier and colder conditions than those occurring today. Conversely, chaetothyrialean lineages are estimated to have diverged from rock-inhabiting lichen order *Verrucariales* in the middle Triassic, about 229 (186–277) million years ago (MYA) when global temperatures were much higher. The epilithic-lichen-association may have become an evolutionary hint to acquire the ability to cope and metabolize toxicants stored in the thallus (i.e. lichenic acids), coupled with a higher thermotolerance capacity (Gueidan et al. 2011b; Quan et al. 2020). Therefore, tolerance to low temperatures *versus* association to high temperature and capability to metabolize toxic substances may represent pre-adaptations within black fungi in the two taxonomic groups. Indeed dothideomycetous black fungal species of our selection are recurrent in rocks of the Antarctic desert, high mountain peaks or other cold-natural environments. Conversely, the ability distributed in eurotiomycetous black fungi to metabolize hydrocarbons, particularly alkylbenzenes, may explain their success in colonizing anthropogenic habitats with industrial pollution.

Our conclusion was also corroborated by random forest modeling and correlation analysis that we implemented to identify the most important predictors of metabolic competences in black fungi. Our results, indeed, showed that the abilities to tolerate hot temperature and degrade hydrocarbons are positively correlated with the HII, which covers human population pressure (population density), human land use and infrastructure, and human access (coastlines, roads, railroads, navigable rivers). Notably, the positive association between climate (i.e. MAT and isothermality) and high temperatures competence suggests that *Dothideomycetes* are potentially equipped to survive even when warmer temperatures will be established (e.g. global warming); this association has not been observed in *Eurotiomycetes*.

It is worth considering that these speculations are based on results obtained analyzing a large but still not complete dataset. The selection of black fungi here studied comprised strains available in two collections, MNA-CCFEE and CCFEE, which do not cover the entire ecological amplitude of black fungi, particularly in the class *Eurotiomycetes*, for which some key ecologies such as ant-associated or bryophilic species are absent or under-represented. A significant, further, contribution pushing ahead our acquaintance with this intriguing group of fungi is expected from the large-scale community "Shed LighT in the daRk lineagES of the Fungal tree of life" (STRES) project (www.stresblackfungi.org), funded by the U.S. Department of Energy (DOE) (Selbmann et al. 2020). The main aim of this project is to better clarify the relationship between stress response, ecology and phylogeny, by sequencing 92 species as reference genomes and > 550 as population genomic resequencing and tracking transcripts and metabolites expressed genes under different stress conditions (i.e. salinity, dryness, UV radiation, and oligotrophy). The STRES consortium includes mycologists, molecular biologists and bioinformaticians from nineteen universities and research

institutions mainly from Europe and the US as well as private or public fungal culture collections worldwide.

Taken together, this work fills a major knowledge gap in the understanding of black fungal biology, ecology and functioning, successfully identifying class-genomic traits linked to diverse life-styles. It will serve as a reference and foundation for untangling how such fungi adapt and succeed in the extremes and for predicting the fate of these guilds in a global warming scenario. This will also inform on their possible applications in pollutant treatment as well as possible preventative measures for material protection (e.g. cultural heritage and solar panel).

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Competing interest

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Claudia Coleine, Sybren de Hoog, and Laura Selbmann conceived the study. Claudia Coleine, Nicola Segata, Jason E. Stajich e Claudio Donati produced the sequencing data. Tania Kurbessoian, Giulia Calia, Jason E. Stajich, Alessandro Cestaro, and Claudia Coleine assembled and annotated genomes. Manuel Delgado-Baquerizo has provided environmental metadata. Statistical analyses and environmental modeling were done by Claudia Coleine. The manuscript was written by Claudia

Coleine, Sybren de Hoog, and Laura Selbmann with contributions from all authors. All authors read and approved the final manuscript.

Data Availability

The genome assemblies and annotation datasets are available on Zenodo repository (10.5281/zenodo.7764743, https://doi.org/10.5281/zenodo.7764743).

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