

1 **Geography and environmental pressure are predictive of class-specific**
2 **radioresistance in black fungi**

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19 **Originality statement**

20 Although previous studies showed the extraordinary ability of a few strains of black fungi to survive
21 ionizing radiation, the overall radioresistance of this group of organisms has not been defined yet.
22 Moreover, how and why radioresistance shifts across environmental gradients remain virtually
23 unknown. Here, we collected black fungi from locations across the globe and found that biogeography
24 shapes the responses of black fungi to environmental stress with UV light being significantly
25 correlated with radiotolerance. Our study provides a clear picture of the boundaries of life for black
26 fungi under ionizing radiation; further, we demonstrate, for the first time, that this ability in such
27 microorganisms, not only is related to taxonomy, but also may be a consequence of their adaptation
28 to various factors encountered in the environment where they live.

29

30 **Abstract**

31 Black fungi are among the most resistant organisms to ionizing radiation on Earth. However, our
32 current knowledge is based on studies on a few isolates, while the overall radioresistance limits across
33 this microbial group and the relationship with local environmental conditions remains largely
34 undetermined. To address this knowledge gap, we assessed the survival of 101 strains of black fungi
35 isolated across a worldwide spatial distribution to gamma radiation doses up to 100 kGy. We found
36 that intra and inter-specific taxonomy, UV radiation and precipitation levels primarily influence the
37 radioresistance in black fungi. Altogether, this study provides insights into the adaptive mechanisms
38 of black fungi to extreme environments and highlights the role of local adaptation in shaping the
39 survival capabilities of these extreme-tolerant organisms.

40

41 **Keywords:** Ionizing radiation, black fungi, survival, extremophiles, astrobiology, environmental
42 pressure

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48 Introduction

49 Radiation is one of the most dangerous hazards for life, among which gamma rays (i.e. high energy
50 photons having wavelengths less than 0.1 nm) represent a serious challenge for survival and integrity
51 of any life-forms (Dartnell, 2011). They may induce dramatic damage in biomolecules and cellular
52 structures by direct ionization or causing radiolysis of water and, consequently, oxidative stress
53 (Azzam et al., 2012). On Earth, there are three principal sources of gamma rays: (i) the natural
54 radioactive nuclides, mainly ^{40}K and the radionuclides from the ^{232}Th and ^{238}U series (Ichimiya et al.,
55 1998; Walencik-Łata, 2022); (ii) the cosmic rays colliding with the nuclei in the atmosphere (Share
56 et al., 2001; Wissmann et al. 2005); and (iii) human activities as in radioactive wastes, nuclear test
57 and nuclear accident sites (Yamamoto et al., 2008; Hu et al., 2010). Albeit radioactive environments
58 are limited only to restricted sites on our planet (Khan et al., 2020; Mousseau et al., 2020), ionizing
59 radiation is one of the major damaging factors encountered beyond Earth (Evans et al., 2003; Dartnell
60 et al., 2007; Roth et al., 2021); indeed, high gamma rays backgrounds are ubiquitous in interplanetary
61 space and on the surface of most of the planetary bodies.

62 Among terrestrial life-forms, a small number of microorganisms have been reported to survive
63 gamma radiation levels significantly higher than the background in the environments where they live
64 (Gabani et al., 2013; Kwang-Woo Jung et al., 2017; Coleine et al. 2022; Coleine & Delgado-
65 Baquerizo, 2022). Black meristematic fungi (thereafter black fungi) form an ascomycetous
66 polyphyletic group spanning two classes (*Dothideomycetes* and *Eurotiomycetes*, mainly represented
67 by the orders *Capnodiales* and *Chaetothyriales*, respectively) (Selbmann et al., 2020). Despite being
68 phylogenetically distant, they share morphological characteristics as convergent adaptation to the
69 extreme conditions where they live (Selbmann et al., 2005; Eisenman et al., 2012; Tesei, 2022). These
70 fungi are among the most extreme-tolerant organisms on our planet, encompassing a stunning ability
71 to survive and even flourish under prohibitive conditions, including radiation (Gorbushina et al. 2018;
72 Selbmann et al., 2018; Coleine et al., 2022). Indeed, it has been recently reported an extraordinary
73 resistance of these fungi to both acute and chronic exposure to a plethora of terrestrial and space
74 relevant radiation (e.g. UV-B, X-rays, gamma rays, and cosmic rays) (Selbmann et al., 2011; Pacelli
75 et al., 2018, 2020; Shuryak et al., 2019a; Aureli et al., 2020; Schultzhaus et al., 2020; Malo et al.,
76 2021). These microorganisms have been investigated as possible biological means for radioprotection
77 in contaminated environments and in manned space missions (Cordero et al., 2017; Aversch et al.,
78 2022). Additionally, the study of radioresistant microorganisms is of astrobiological significance to
79 unveil the survival and persistence capacity of life in highly irradiated extraterrestrial environments
80 (Dartnell, 2011, Moissl-Eichinger et al., 2016; Horne et al., 2022).

81 Despite these advances, a vast bulk of questions still remains unanswered. In particular, there are
82 three major sources of uncertainties that have precluded scientists to untangle how and why black
83 fungi manage to resist ionizing radiation. First, most efforts have focused on a few strains (i.e.
84 *Cryomyces*, *Friedmanniomyces* and *Exophiala* spp.) only, while a comprehensive study exploring
85 the potential diverse radioresistance abilities among phylogenetically and ecologically distinct
86 members, including those colonizing both natural and polluted anthropized niches, is still lacking.
87 Second, most isolates come from a narrow range of environmental conditions and from local regions,
88 while a more comprehensive study on black fungi across broader spatial distribution is lacking.
89 Finally, very little is known on how environmental stressors, including the solar radiation levels of

90 the regions from which these taxa are isolated, explain the actual levels of radioresistance in
91 individual taxa.

92 Here, we tested the resistance of 101 black fungal strains to acute exposure to gamma radiation to
93 relate these responses to the environmental conditions. The specimens were selected from
94 *Dothideomycetes* and *Eurotiomycetes* classes and had a worldwide spatial distribution to cover the
95 broadest range of different ecologies, life-styles, and geography (e.g. from Antarctica to temperate
96 regions and from anthropic impacted to polluted sites). We aimed to: i) define the radioresistance
97 limits of the broadest selection of black fungi tested so far; ii) identify new possible model organisms
98 for radioprotection and astrobiology studies; iii) evaluate the potential differences in radioresistance
99 between *Dothideomycetes* and *Eurotiomycetes*. Indeed, the first are mainly isolated from extreme dry
100 and cold natural environments, whereas the latter show a remarkable ability to colonize hot and
101 polluted anthropized environments (Selbmann et al., 2005; Isola et al., 2021; Tesei, 2022); iv) identify
102 the main environmental parameters driving radioresistance in these fungi. Altogether, this
103 information will provide new insights into the ability of black fungi to cope with high doses of
104 ionizing radiation, paving the way for further genomic and metabolic investigations and provide a
105 fundamental contribution to define the habitability of terrestrial environments contributing for
106 speculations on a potential terrestrial-like life in the Solar System and beyond.

107

108

109 **Materials and Methods**

110 *Strains selection*

111 For the study, 101 strains of black meristematic fungi were selected from the Culture Collection of
112 Fungi from Extreme Environments (CCFEE) and National Antarctic Museum Culture Collection of
113 Fungi from Extreme Environments (MNA-CCFEE), Mycological Section of the Italian National
114 Museum of Antarctica. The strains belong to 20 species of the order *Capnodiales* (class
115 *Dothideomycetes*) and 7 species of the order *Chaetothyriales* (*Eurotiomycetes*) (Table S1). The strain
116 selection was performed to study phylogenetically distinct members of meristematic black fungi and
117 to cover a range of different environmental conditions (Table S2).

118

119 *Exposure conditions*

120 Fungal colonies of the selected strains were incubated in triplicate on Malt Extract Agar (MEA)
121 medium in Petri dishes according to the temperatures recorded in the localities from which the strains
122 were isolated and the optimum growth temperature and growth rate recorded in representative strains
123 of the tested species in previous laboratory tests: 15 °C for 3 months for *Dothideomycetes* and 25 °C
124 for 1 week for *Eurotiomycetes* (Selbmann et al., 2005; Egidi et al., 2014). After the growth, the
125 colonies were retrieved from each replicate and separately desiccated under laminar flow in a sterile
126 cabinet. Dried colonies from each triplicate were exposed to gamma radiation under ambient
127 conditions using a ⁶⁰Co source at the Gamma Irradiation CALLIOPE Facility (ENEA Casaccia
128 Research Centre, Rome) (Baccaro et al., 2019). The irradiation was performed at doses of 0.1, 0.5, 1,
129 2, 3, 5, 15, 30, 50, and 100 kGy through different exposure times at a dose rate of 1.12 kGy(air)/h.

130 To obtain control samples, colonies from each desiccated replicate were not exposed to gamma
131 radiation but maintained under the same environmental conditions as the irradiated samples.

132

133 *Survival assessment*

134 The survival of microorganisms after exposure to each gamma radiation dose was measured using
135 the colony-forming unit (CFU) test. Three colonies from each replicate exposed to gamma radiation
136 were separately rehydrated in NaCl 0.9% solution for 72 h at 25 °C and 15 °C for *Eurotiomyces*
137 and *Dothideomyces*, respectively. After rehydration, 0.1 mL of each cellular suspension at a
138 concentration of 15,000 CFU/mL was plated in triplicate on MEA medium in Petri dishes. The dishes
139 inoculated with *Eurotiomyces* strains were incubated for 1 week at 25 °C, whereas dishes with
140 *Dothideomyces* strains for 3 months at 15 °C. The survival of microorganisms at each dose was
141 expressed as the ratio between the mean of colonies scored in the exposed replicates and the mean of
142 colonies scored in non-exposed samples. The decimal reduction dose (D10) value, defined as the
143 absorbed radiation dose required to inactivate 90% of the microbial population, was estimated for
144 each strain from its survival curve. To calculate D10 values, the best curves describing the survival
145 of each strain were determined by fitting the exponential function ($y = \exp^{-bx}$) to the observed data
146 through the Python SciPy package V.1.9.0. The curve fits were evaluated through the χ^2 test by using
147 the Python code.

148

149 *Intraspecific differences in radioresistance*

150 Among the selected species, *Exophiala xenobiotica*, *Recurvomyces mirabilis*, *Meristemomyces*
151 *frigidus*, *Elasticomyces elasticus*, *Extremus antarcticus*, *Friedmanniomyces endolithicus*, and
152 *Cryomyces antarcticus* species were represented by more than one strain. To quantify the
153 radioresistance variability in species including more than one strain, the coefficient of variation (CV)
154 was calculated through the Python SciPy package. The one-way ANOVA post hoc Tukey HSD test
155 and t-test were performed through Python bioinfokit package V.2.1.0. to assess the difference in D10
156 values between strains of *E. elasticus*, *M. frigidus*, *R. mirabilis* and *F. endolithicus* collected from
157 distinct localities and among the mean D10 values calculated in the resistance groups.

158

159 *Environmental metadata acquisition*

160 The ecological bases of the resistance to ionizing radiation was investigated by considering 27
161 environmental parameters obtained from the localities where the strains were isolated: Annual Mean
162 Temperature (AMT); Mean Temperature of the Warmest Quarter (MTWAQ); Mean Temperature of
163 the Coldest Quarter (MTCQ); Annual Precipitation (AP); Precipitation of the Wettest Month
164 (PWEM); Precipitation of the Driest Month (PDM); Precipitation Seasonality (Coefficient of
165 Variation) (PS); Precipitation of the Wettest Quarter (PWEQ); Precipitation of the Driest Quarter
166 (PDQ); Precipitation of the Warmest Quarter (PWAQ); Precipitation of the Coldest Quarter (PCQ);
167 Mean Diurnal Range (Mean of monthly (maximum temperature - minimum temperature)) (MDR);
168 Temperature Seasonality (standard deviation $\times 100$) (TS); Maximum Temperature of the Warmest
169 Month (MTWAM); Minimum Temperature of Coldest Month (MTCM); Temperature Annual Range

170 (calculated as MTWAM - MTCM) (TAR); Mean Temperature of the Wettest Quarter (MTWEQ);
171 Mean Temperature of the Driest Quarter (MTDQ); Isothermality (calculated as MDR/TAR) ($\times 100$)
172 (IT); solar radiation; UV index; biome type (biome); isolation source; country of isolation (country);
173 Köppen-Geiger climate classification subgroup (KG climate); class to which the strains belong
174 (Class); the coordinates of the localities where the samples were collected. The environmental data
175 were available for 86 of the 101 strains tested in the study, related to 36 different localities
176 (Supplementary Table 2, Fig. 2).

177

178 *Identification of the most important environmental parameters and statistical analyses*

179 To identify the environmental parameters that are linked to the radioresistance of the strains, a
180 classification predictive model was constructed through the eXtreme Gradient Boosting (XGBoost)
181 ensemble tree-based learning algorithm. The algorithm was implemented using the Python XGBoost
182 package V.1.6.2. Categorical data (biome, isolation source, country, KG climate, Class) were
183 converted through ordinal encoding pre-processing. Similarly, the D10 values were grouped into 9
184 categories and transformed into an ordinal variable. We then estimated the importance of each
185 environmental parameter by evaluating the mean decrease in the model score when the values of the
186 parameter were randomly shuffled in 1000 repeats through the permutation feature importance
187 approach by using the Python ELI5 package V.0.13.0. The XGBoost model and the permutation were
188 first performed considering all the strains with environmental data available. Successively, the
189 analyses on subgroups consisting of *Dothideomycetes* and *Eurotiomycetes* were performed to identify
190 predictors of radioresistance in strains from the two classes. Finally, the 24 strains of *F. endolithicus*
191 were considered in relation with environmental data to identify parameters that may influence
192 intraspecific differences in radioresistance. The *F. endolithicus* species was selected due to the
193 availability of environmental data observed in 9 distinct localities where the strains were collected.
194 Along with *F. endolithicus*, *C. antarcticus*, *E. elasticus* and *E. xenobiotica* were the most represented
195 species (8, 10 and 30 strains, respectively). However, the lack of environmental data related to part
196 of *C. antarcticus* and *E. elasticus* strains did not allow the construction of a model, whereas most of
197 *E. xenobiotica* strains were isolated from the same locality.

198 To examine the differences in mean decreases in the model score obtained for each environmental
199 variable, we performed the one-way ANOVA post hoc Tukey HSD test through the Python bioinfokit
200 package V.2.1.0. Spearman's rank correlation coefficient and p-value were calculated through the
201 Python SciPy package V.1.9.0.

202

203 **Results**

204 *Survival of fungal strains after gamma radiation exposure*

205 The ability to generate colonies after gamma radiation exposure revealed a non-linear effect of
206 radiation on black fungi cell survival, which was highly dependent on taxonomy. Indeed, three
207 distinct responses can be observed from the dose-survival curves of *Eurotiomycetes* and
208 *Dothideomycetes* strains, despite all exhibiting a similar trend that can be described by the exponential
209 curve $y = \exp^{-bx}$ (Fig. S1-S3). Specifically, in all *Eurotiomycetes*, a threshold in cell survival was

210 reported between 0.1 and 0.5 kGy, with the survival fraction reaching values below 50%. However,
211 a drastic change in the slopes occurred beyond 0.5 kGy, thus determining a slower decrease in cell
212 survival until reaching the maximum resistance doses of each strain (Fig. 3).

213 On the other hand, two different general responses to gamma radiation were shown among
214 *Dothideomycetes* strains. The first observed response was displayed by 33 strains. Although the
215 slopes of the curves among these strains were highly variable, they all showed a drop in cell survival
216 from the dose of 0.5 kGy followed by a slower decrease while approaching the maximum doses of
217 resistance. Instead, the remaining 37 strains, that included exclusively the totality of
218 *Friedmanniomyces* and *Cryomyces* strains, showed a general drop in the cell survival between 3 and
219 15 kGy, followed by a slight decline in survival fraction (Fig. 3).

220 The D10 values obtained from the survival curves ranged from 0.30 (*Eurotiomyces* sp.
221 CCFEE6388) to 23.5 kGy (*C. minteri* MNA-CCFEE5187) (Table S1). Although marked differences
222 were observed in the survival among the studied strains, colonies from most samples were able to
223 grow after exposure at doses higher than 0.1 kGy. Furthermore, except for *E. xenobiotica*
224 CCFEE5985, all strains formed colonies at doses higher than 1 kGy, with overall higher survival
225 fractions observed in the *Dothideomycetes* strains (Fig. 4).

226 In *Eurotiomyces*, the mean D10 was 0.8 ± 0.3 kGy with a CV 0.45. The values of the group were
227 distributed between 0.3 and 1.4 kGy (Fig. 4), shown by *Eurotiomyces* sp. CCFEE6388,
228 *Chaetothyriales* sp. CCFEE6169, and *E. xenobiotica* CCFEE6180, respectively (Table S1).
229 Moreover, only the strains *E. xenobiotica* CCFEE5819, CCFEE6180, CCFEE6182, CCFEE6196 and

230 CCFEE6237 showed D10 above 1 kGy. The maximum resistance dose of 3 kGy was recorded for the
231 strain *E. xenobiotica* CCFEE5877, whereas 21 out of 31 strains showed the ability to grow at the
232 maximum dose of 1 kGy (Table S1).

233 In contrast, high variability in radioresistance was observed among *Dothideomycetes*, whose values
234 ranged between 0.4 and 23.5 kGy, observed in *M. frigidus* CCFEE5401 and *C. minteri* MNA-
235 CCFEE5187, respectively (Fig. 4). The D10 values showed a discontinuous distribution that defined
236 two groups of radioresistance in *Dothideomycetes*. The first group was represented by strains
237 exhibiting D10 below 4 kGy (mean D10, mean $1.5 \pm$ SD 0.7 kGy), whose values partially overlapped
238 the values found in *Eurotiomyces*. However, all the strains of the group survived at doses of 2 kGy
239 or higher, with 12 strains reaching the maximum survival dose at 5 kGy. This group consisted of all
240 tested species of *Dothideomycetes*, with the exception of species belonging to *Cryomyces* and
241 *Friedmanniomyces* genera. The D10 values of the second group ranged between 13.4 and 23.5 kGy,
242 making these strains the most resistant among the tested black fungi (mean D10 $17.6 \pm$ 2.9 kGy, CV
243 0.15). Furthermore, all the strains of the second group formed colonies at a dose of 15 kGy or higher,
244 with 10 strains reaching the maximum survival dose at 50 kGy (Supplementary Table S1).

245

246 *Relation between radioresistance and environmental parameters, taxonomy and*
247 *geographical distribution*

248 We further investigate the correlation between environmental and spatial conditions with the capacity
249 of black fungi to withstand radiation. To investigate the ecological bases of the radioresistance in
250 black fungi, we considered the importance of geography and a set of crucial environmental parameters

251 occurring in the localities from which the strains were isolated in predicting the D10 values. The
252 permutation feature importance scores obtained from the models indicated the spatial distribution (i.e.
253 the longitude and the distance from the equator) of the localities as the major predictors of the
254 radioresistance while considering the strains altogether and *Dothideomyces* separately, although the
255 relative importance of the two variable varies between them. Among the environmental variables, the
256 main predictors of the radioresistance considering both the strains altogether and *Dothideomyces*
257 only were those related to the solar radiation exposure (i.e. UV index and solar radiation) and
258 precipitation (i.e. precipitation in the driest month (PDQ) and precipitation seasonality (PS)) (Fig. 5a,
259 b).

260 Unlike *Dothideomyces*, strains of *Eurotiomyces* were collected from both environments directly
261 exposed to natural factors (e.g. surfaces of monuments) and anthropogenic and polluted environments
262 (i.e. fuel tanks). When considering only strains exposed to natural environmental factors, parameters
263 linked to temperature (the annual mean temperature (AMT) and the mean temperature of coldest
264 quarter (MTCQ)) and the distance from the equator were the most important variables, whereas the
265 solar radiation exposure and precipitation levels were shown not to play a pivotal role in
266 radioresistance prediction for this class (Fig. 5c).

267

268 *Intraspecific differences in radioresistance and their correlation to environmental factors*

269 In our study, 7 fungal species are represented by more than one strain (Table S1). Among them, the
270 highest intraspecies variation in the survival data was observed in the *E. elasticus* and *M. frigidus*
271 strains (CV 0.5 and 0.6, respectively), whereas *F. endolithicus* strains had a relatively lower CV (i.e.
272 0.1). In *E. elasticus*, we observed a significantly higher radioresistance (D10 above 2 kGy) in strains
273 isolated from Antarctica compared to those isolated from temperate regions (D10 below 1 kGy) (Fig.
274 6a). Similar responses were observed among the strains of *M. frigidus*, whose strains from
275 Argentinian Andes were observed to be significantly more resistant than the strains from Italy, with
276 a difference in D10 of about 1 kGy among the two groups (Fig. 6b). These data indicated that
277 geography and the local environmental conditions may influence the level of radioresistance in these
278 fungi.

279 *F. endolithicus* species was represented by 27 strains isolated from 11 different localities in Antarctica
280 (Fig. 1, Table S1). The analysis showed the geography, the precipitation parameters (AP and PDQ),
281 the UV index, the temperature (AMT and MTCQ) as the major predictors of the radioresistance in *F.*
282 *endolithicus* strains (Fig. 6c). The relationship between AP, PDQ and UV index and the radioresistance
283 was confirmed by the Spearman's correlation (Fig. 6d).

284

285 **Discussion**

286 Major unknown persist about how and why different black fungi taxonomies withstand radiation
287 across large environmental gradients worldwide. Here, the ability of 101 *Dothideomyces* and
288 *Eurotiomyces* black fungi to form colonies after acute exposure to gamma radiation was assessed
289 to define the radioresistance limits of the broadest range of these extreme-tolerant guilds tested so far.
290 Moreover, we correlate this radioresistance with environmental and spatial conditions supporting

291 black fungi across global environmental gradients. We showed that taxonomy is a critical component
292 when explaining the capacity of black fungi to resist radiation. Moreover, environmental conditions
293 were critical in explaining the variation in blackfungi radioresistance with UV light being an
294 important predictor. These results suggest that blackfungi radioresistance levels are in consistency
295 with the environment surrounding these organisms.

296
297 The amplitude of radioresistance capacity varied considerably among black fungi in our wide
298 selection; many of them were able to form colonies after gamma radiation exposure doses from 0.5
299 to 5 kGy. Yet, some others were still viable when exposed at the extraordinary value of 50 kGy. D10
300 also varied considerably among strains tested, ranging from 0 kGy up to above 23 kGy, and the
301 species falling into two distinct categories. In this study, we found a general higher aptitude to cope
302 with ionizing radiation in dothideomycetous black fungi; in fact, the capacity to grow after lethal dose
303 exposition over 5 kGy was an exclusive prerogative of few *Dothideomycetes*, indicating a class-
304 tendency throughout this ability. In addition, the highest radioresistance observed (up to 50 kGy) was
305 displayed specifically by black fungal *Dothideomycetes*, which have been isolated in the driest and
306 coldest desert on Earth only: the ice-free areas in continental Antarctica, including the the martian
307 analogue McMurdo Dry Valleys. Based on this evidence, we investigated in deeper detail the origin
308 of this difference studying the effect of various environmental factors in shaping radioresistance
309 tendency in black fungi, taking into consideration that dothideomycetous do prefer highly stressing
310 cold-dry natural environments, while eurotiomycetous spread preferentially in hot or anthropic
311 impacted or polluted environments.

312 Our study showed that biogeography, sun exposure, and the levels of precipitation were the major
313 predictors of D10 in strains exposed to natural parameters, indicating that the ability to cope with
314 ionizing radiation may be related to the adaptation to other environmental stressors (Shuryak et al.,
315 2019a). Indeed, factors encountered in the extreme environments such as UV exposure, drought, and
316 low and high temperatures can also cause oxidative stress, requiring the activation of cellular
317 mechanisms similar to those that mediate the responses to ionizing radiation (Leprince et al., 2010;
318 Lushchak, 2011; Kostadinova et al., 2012; Braga et al., 2015; Mejía-Barajas et al., 2017; Gostinčar
319 et al., 2018). The burden of skills for the success in such environments, therefore, may represent a
320 pre-adaptation for radioresistance in these fungi (Etemadifar et al., 2016; Sharma et al., 2017; Lim et
321 al., 2019; Coleine et al., 2022). In fact, although exhibiting overall high radioresistance, none of the
322 strains tested in our study were isolated from radioactive environments; either, the environmental
323 background radiation should not have represented an evolutionary driver for this ability.
324 In our study, the adaptation of black fungi to a broad range of different habitats may explain the large
325 diversity that we observed in their radioresistance. In addition to interspecific fluctuation in
326 radioresistance, the distinct localities may have driven the intraspecific variability in different species
327 by inducing the local adaptation to various environmental factors (Ellison et al., 2011; Gladieux et
328 al., 2014; Branco, 2019). Consistently with this hypothesis, the highest intraspecific variability
329 observed was exhibited by strains of *E. elasticus* and *M. frigidus*, whose distribution spans different
330 continents, which was highly correlated to increasing environmental pressure; indeed, the most
331 resistant strains were found in Antarctica and Argentinian Andes, respectively.

332

333 The importance of environmental factors for radioresistance was also confirmed by our results on the
334 Antarctic endemic species *F. endolithicus* strains, living in the driest, coldest highly solar and UV
335 radiation impacted environment and here resulted the far most radioresistant guild, together with
336 *Cryomyces* strains living in the same desert (i.e. Antarctica). The relation occurring between the
337 radioresistance and the adaptation to other environmental stressors may be mediated by both
338 morphological and physiological properties of the fungal cells. Previous studies have indicated
339 morphological characteristics of black fungi as possible determinants against environmental stressors
340 (Kogej et al., 2006). Furthermore, the melanized cell wall was shown to play a key role in mediating
341 the resistance of pigmented fungi to ionizing radiation (Dadachova et al., 2008; Shuryak et al., 2014).
342 Recent advances showed various possible trends, represented by both linear and non-linear threshold
343 correlations (Pacelli et al., 2018b; Schultzhaus et al., 2020). In our study, all the tested strains showed
344 a non-linear exponential relationship between the gamma rays doses and the survival fraction,
345 suggesting that radioresistance may be mediated by additional physiological and molecular processes
346 differentially expressed in distinct species of black fungi (Robertson et al., 2012; Sharma et al., 2017;
347 Romsdahl et al., 2020; Kreusch et al., 2021; Das et al., 2022; Kanekar & Kanekar, 2022). This may
348 indicate how the response of microorganisms to ionizing radiation depends not only on the tested
349 organisms, but also on the exposure conditions. In this perspective, future studies focusing on the
350 genetic and molecular mechanisms mediating the radioresistance in black fungi should be performed.
351 Our study confirms black fungi as excellent models for the study of the mechanisms used by
352 eukaryotic cells to cope with ionizing radiation (Selbmann et al., 2018). We observed a general
353 stunning radioresistance of most of the black fungi if compared to other microorganisms and some of
354 them exceed the survival limits of the most radioresistant prokaryotic and eukaryotic forms of life
355 (e.g. Cox et al., 2005; Daly, 2009; Sukhi et al., 2009; Singh et al., 2013; Krisko, 2013; Shuryak,
356 2019b). However, it should be highlighted that the response to ionizing radiation in microorganisms
357 can vary markedly with the cellular state and exposure conditions (Hafer et al., 2010; Schultzhaus et
358 al., 2020; Couceiro et al., 2021). Considering the dose rates recorded in the most radioactive
359 environments on Earth, our results show how part of black fungi may hypothetically survive there
360 even after thousands of years exposure to ionizing radiation in a silent desiccated state (Kashparov et
361 al., 2018; Matsuo et al., 2019). Similarly, the radiation levels recorded in the interplanetary space and
362 on the surface of celestial bodies that could host forms of life, such as Mars, are remarkably lower
363 than those tested in our experiment (Zeitlin et al., 2013; Hassler et al., 2014; Inozemtsev et al., 2015).

364 Taken together, by simultaneously testing a broad set of ecologically and phylogenetically distinct
365 fungal strains, the present study supplies the first characterization of the radioresistance in black
366 fungi. Along with ecological information on the strains, these results allowed us to display how the
367 radioresistance in black fungi may be the result of their adaptation to various environmental variables.
368 From an evolutionary perspective, our findings demonstrate how the pressure exerted by a few
369 stressors may induce mechanisms that enable the microorganisms to cope with a broader range of
370 possible damaging factors. In the overall, this work may give new insights into the prediction of
371 evolutionary responses to extreme environments and into the ability of life to adapt and persist in
372 harsh terrestrial and extraterrestrial environments (e.g. Mars). This information represents the
373 baseline for untangling the genomic and metabolomic traits underlying the radioresistance of black
374 fungi, which may pave the way to untangle the limits of life in extreme radioactive environments.

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387

388 **Authors contributions**

389 L.A., C.C., and L.S., designed the study and interpreted the results; L.A. performed molecular and
390 statistical analysis and led the writing; all authors revised the manuscript and approved the final
391 version.

392

393 **Data availability statement**

394 All data generated or analyzed during this study are included in this published article and its
395 supplementary information files.

396

397 **Conflict of interest**

398 The authors declare no competing financial interests.

399

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602

603 **Supporting information captions**

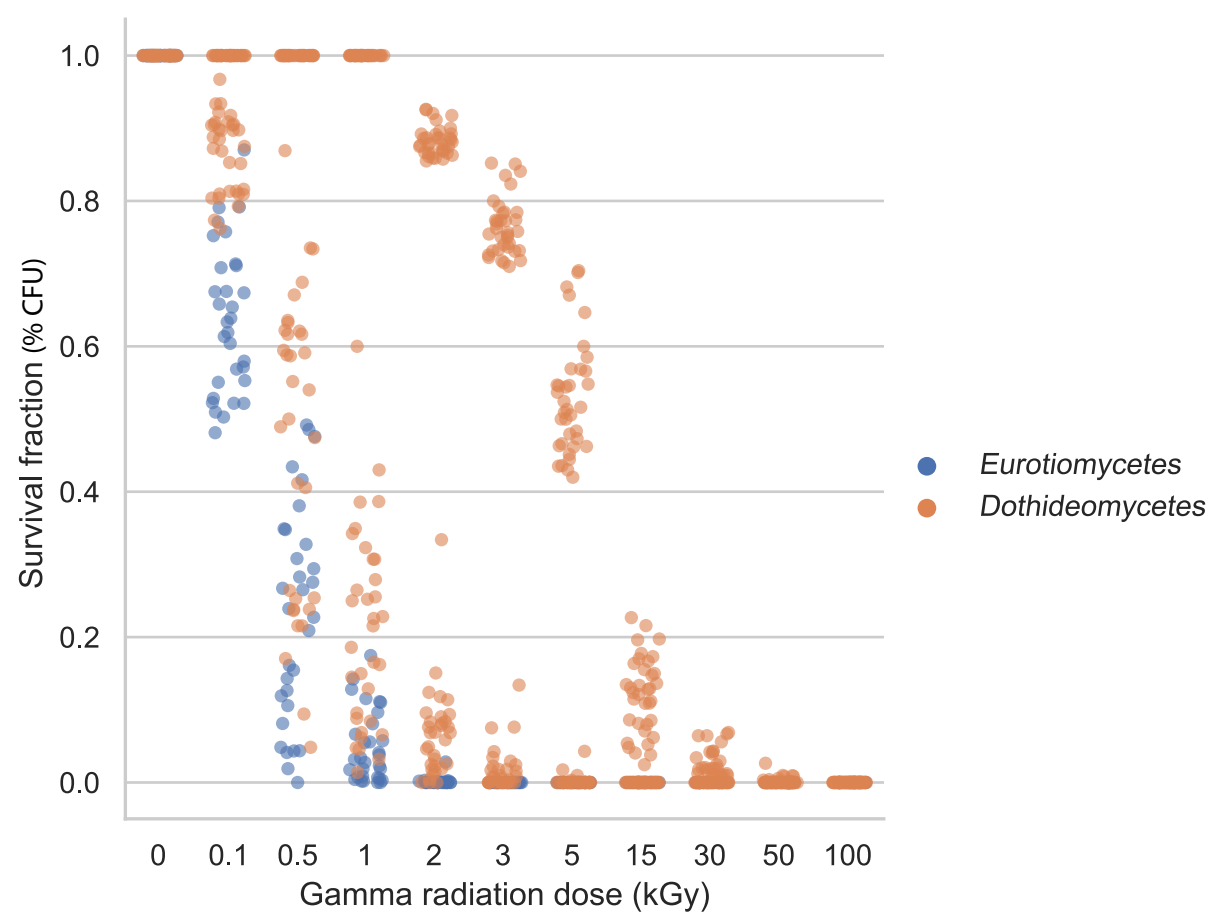
604 **Figure S1.** Survival curves and calculated D10 values in strains of *Eurotomyces*.

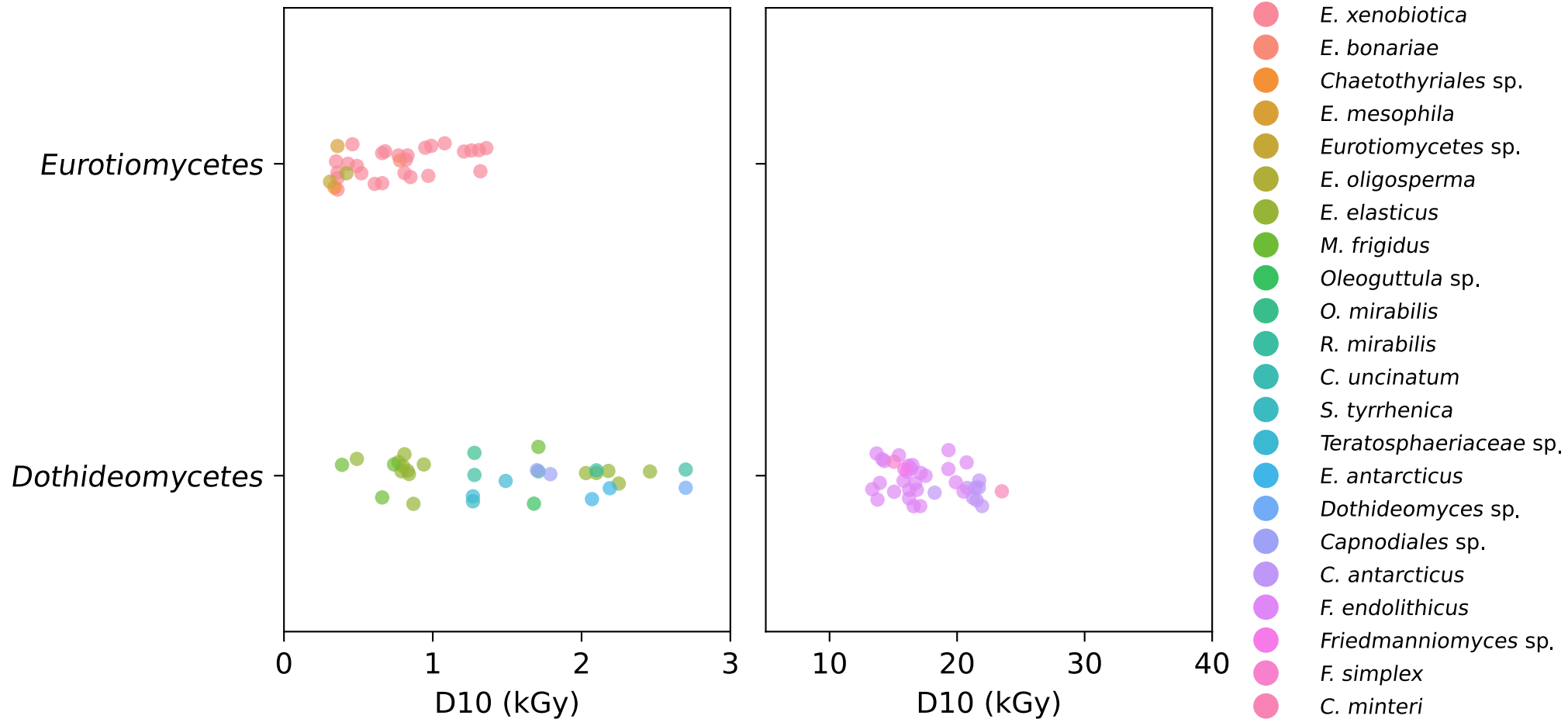
605 **Figure S2.** Survival curves and calculated D10 values in strains of lowly resistant *Dothideomyces*

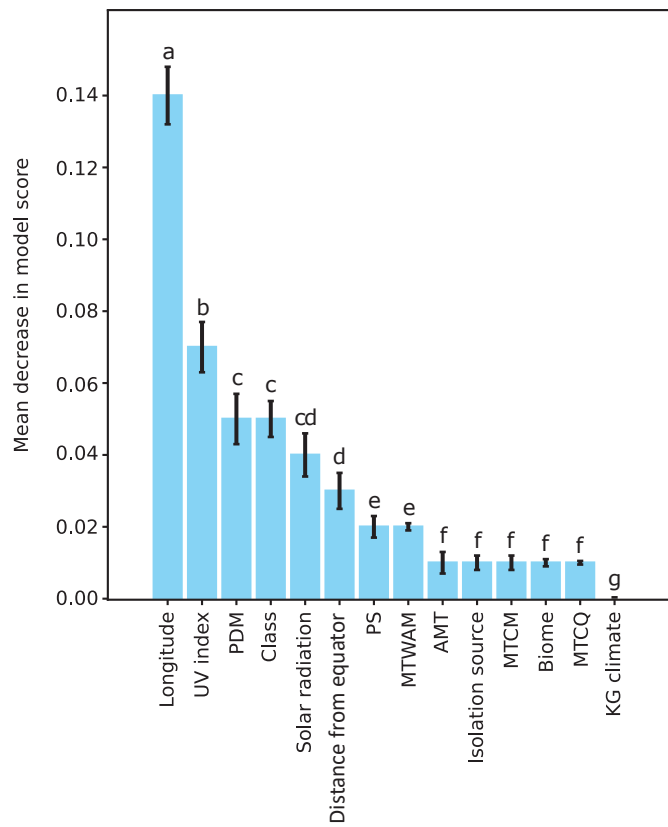
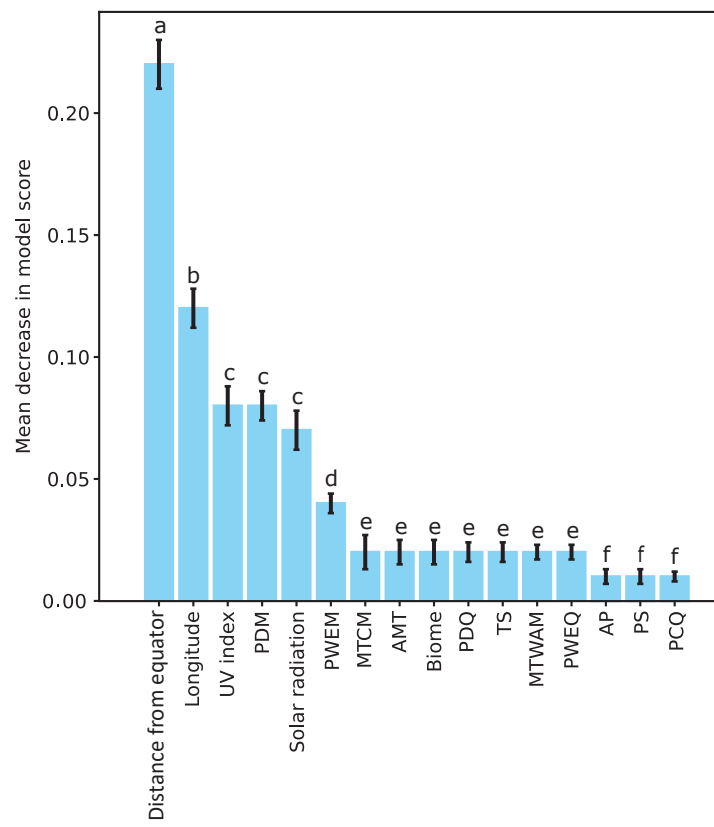
606 **Figure S3.** Survival curves and calculated D10 values in strains of highly resistant *Dothideomyces*.

607 **Supplementary Table S1.** Fungal strains tested in the study and their response to radiation exposure
608 in terms of D10 and the maximum survival dose.

609 **Supplementary Table S2.** Fungal strains and the localities from where they were collected and the
610 related environmental metadata. AMT: Annual Mean Temperature; MTWAQ: Mean Temperature of
611 the Warmest Quarter; MTCQ: Mean Temperature of the Coldest Quarter; AP: Annual Precipitation;
612 PWEM: Precipitation of the Wettest Month; PDM: Precipitation of the Driest Month; PS:
613 Precipitation Seasonality (Coefficient of Variation); PWEQ: Precipitation of the Wettest Quarter;
614 PDQ: Precipitation of the Driest Quarter; PWAQ: Precipitation of the Warmest Quarter; PCQ:
615 Precipitation of the Coldest Quarter; MDR: Mean Diurnal Range (Mean of monthly (maximum
616 temperature - minimum temperature)); TS: Temperature Seasonality (standard deviation ×100);
617 MTWAM: Maximum Temperature of the Warmest Month; MTCM: Minimum Temperature of
618 Coldest Month; TAR: Temperature Annual Range (calculated as MTWAM - MTCM); MTWEQ:
619 Mean Temperature of the Wettest Quarter; MTDQ: Mean Temperature of the Driest Quarter; IT:
620 Isothermality (calculated as MDR/TAR) (×100); biome: biome type; country: country of isolation;
621 KG climate: Köppen-Geiger climate classification subgroup.





(a) All strains**(b) *Dothideomycetes*****(c) *Eurotiomycetes***