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1 **Diversity of *Parengyodontium* spp. strains isolated from the cultural heritage**
2 **environment: phylogenetic diversity, phenotypical diversity and occurrence.**

3

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11 **Diversity of *Parengyodontium* spp. strains isolated from the cultural heritage**
12 **environment: phylogenetic diversity, phenotypical diversity and occurrence.**

13

14 **ABSTRACT**

15 *Parengyodontium album* is fungal species that frequently occurs in the cultural heritage
16 environment. Although three subclades were initially described in the species, no study has
17 sought to determine the occurrence of each subclade in the cultural heritage context. These
18 subclades are easily distinguishable phylogenetically, but their morphological identification is
19 more difficult. Eighteen strains isolated from different cultural sites and initially identified as
20 *P. album* were studied phylogenetically, morphologically and in terms of their susceptibility to
21 econazole nitrate 0.2 %, an antifungal product used as preservation treatment in cultural
22 heritage domain. The phylogenetic study revealed that all studied strains belonged to *P. album*
23 subclade 1 or *P. torokii* (*P. album* subclade 3), while none belonged to *P. album* subclade 2.
24 The morphological study revealed the best characteristics to differentiate the three
25 subclades/species, namely the ability of the strains to grow at 32 °C and 35 °C on PDA medium,
26 and the shape of conidia. Finally, the strains displayed variable susceptibilities to econazole
27 nitrate, with no apparent link to any particular subclade/species.

28

29 **KEY WORDS**

30 Morphology; *Parengyodontium album*; *Parengyodontium torokii*; Phylogeny; Population study;
31 Susceptibility to antifungal products

32

33

34 **INTRODUCTION**

35 The genus *Parengyodontium* was added to the Cordycipitaceae family by Tsang et al. (2016)
36 to accommodate the single species *Parengyodontium album* (Limber) C.C. Tsang, J.F.W. Chan,
37 W.M. Pong, J.H.K. Chen, A.H.Y. Ngan, M. Cheung, C.K.C. Lai, D.N.C. Tsang, S.K.P. Lau &
38 P.C.Y. Woo, which formerly belonged to the genus *Engyodontium* for the description of the
39 genus (De Hoog 1978). *Parengyodontium album*, which is characterized by conidiogenous cells
40 bearing zigzag-shaped terminal regions, was different from other species in the genus
41 *Engyodontium* in term of its phylogenetic and chemotaxonomic features. Today, the genus
42 *Parengyodontium* is composed of only three species: *P. album*, *P. americanum* M.M. Teixeira,
43 Wiederh. & B.M. Barker (Teixeira et al. 2020), and *P. torokii* N.K. Singh & K. Venkateswaran,
44 which was described very recently by Parker et al. (2022).

45 Tsang et al. (2016) identified three subclades among *P. album* species, which were difficult to
46 differentiate using solely phenotypical features. These authors noticed that the strains belonging
47 to subclade 2 were the only ones able to grow at temperatures over 31 °C. The *P. album* strains
48 also exhibited different reverse colors on Potato Dextrose Agar medium (PDA) according to
49 their subclade: most of the strains were orange-brown in subclade 1, all strains were dark brown
50 in subclade 2 and all strains were white in subclade 3. Tsang et al. (2016) therefore concluded
51 that further investigations would position subclades 2 and 3 at a species level. Parker et al.
52 (2022) very recently transferred the *P. album* subclade 3 to the new species *P. torokii* on the
53 basis of phylogenetic and morphological features, pointing out the different shape of conidia
54 produced by *P. torokii* in comparison with *P. album*. However, they did not mention any
55 difference in reverse color of the colonies on PDA medium, probably because they only studied
56 *P. album* strains belonging to subclade 3.

57 *Parengyodontium album* and *P. americanum* species have been described in the context of
58 medical studies. *Parengyodontium album* is regarded as an opportunistic human pathogen

59 (Augustinsky et al. 1990; Macêdo et al. 2007). Tsang et al. (2016) reported that only *P. album*
60 subclade 2 strains were involved in deep-seated infections, while subclade 1 and 3 strains were
61 only involved in superficial body sites. *Parengyodontium americanum* strains were recovered
62 from clinical samples of confirmed coccidiomycoses cases without determining if these strains
63 were sampling contaminants or the result of a co-infection with *Coccidioides* spp (Teixeira et
64 al. 2020). Regarding the susceptibility of *P. album* to different antifungal agents used in a
65 medical environment, susceptibilities differed not only between antifungals, but also between
66 *P. album* subclades and even among strains belonging to the same subclade (Tsang et al. 2016).
67 This result was particularly observed for azoles, one of the largest classes of antifungal agents
68 in clinical use (Odds et al. 2003).

69 While *Parengyodontium album* and *P. americanum* were initially described in a medical
70 context, *Parengyodontium album* is a species that is widely distributed in the environment. It
71 has been isolated from plants (Belfiori et al. 2021; Lucero et al. 2006; Wu et al. 2013), soil
72 (Kachuei et al. 2012; Ma et al. 2013), and especially marine environments including substrates
73 ranging from coastal plants (Liu et al. 2021) and marine sediments (Khusnullina et al. 2018) to
74 sea water (Pindi 2012). Incidentally, the *P. torokii* holotype was isolated from the Mars 2020
75 spacecraft assembly facility. One of the most original contexts in which *P. album* is frequently
76 encountered is in cultural heritage, often in monuments suffering from deterioration, but also
77 in other contexts (Leplat et al. 2020a). This fungal species has been recovered on monuments
78 (Gorbushina and Petersen 2000; Ponizovskaya et al. 2019) and on artworks conserved in
79 museums or libraries (Borrego and Molina 2019; Principi et al. 2011). Materials as various as
80 stone, wall paintings, plaster, brick, glass, wood or paper have been affected by the presence of
81 *P. album* (Jurado et al. 2021; Koziróg et al. 2014; Piotrowska et al. 2014; Principi et al. 2011;
82 Schabereiter-Gurtner et al. 2001; Trovão et al. 2019). In addition, *P. album* has also been
83 frequently isolated from the air of hypogean sites (Saarela et al. 2004), and notably in

84 Paleolithic decorated caves (Dominguez-Moñino et al. 2021; Leplat et al. 2019; Liñán et al.
85 2018). The similarity between these cultural sites, like in the marine environment, is the
86 presence of humid salty conditions that are favorable for the growth of these fungal species
87 (Leplat et al. 2020a). When found in relation to the deterioration process of monuments, the
88 abundance of *P. album* varied greatly from insignificant amounts to it being the most abundant
89 species recovered (Leplat et al. 2017; Nugari et al. 2009; Ponizovskaya et al. 2019). It is
90 therefore important to consider possible remediation methods to deal with these species.

91 This study aimed to characterize the diversity of *Parengyodontium* spp. strains isolated from
92 the cultural heritage environment by working on a set of strains recovered from several different
93 sites between 2004 and 2019. All these strains were identified as *P. album* based on the
94 knowledge of morphological and/or phylogenetic features before a description of *P. torokii*
95 became available. This set was a sample group of *Parengyodontium* spp. strains that were kept
96 in a collection. First, the study of the phylogenetic diversity of these strains was carried out by
97 analysis of additional DNA regions to those analyzed by Teixeira et al. (2020) and Tsang et al.
98 (2016). Teixeira et al. (2020) used the nuclear internal transcribed spacer region (ITS) to
99 describe *P. americanum*, while Tsang et al. (2016) used the ITS, the large subunit of nuclear
100 ribosomal region (LSU) and the protein coding gene beta-tubulin (TUB) to describe *P. album*.
101 This study also uses the small subunit of nuclear encoded ribosomal region (SSU), the protein
102 coding gene translation elongation factor 1-alpha (TEF) and the largest (RPB1) and second
103 largest (RPB2) subunits of RNA polymerase II. These phylogenetic markers are also often used
104 when studying the *Cordycipitaceae* family (Kepler et al. 2017), and were also studied by Parker
105 et al. (2022) for the description of *P. torokii*. Our phylogenetic study sought to identify which
106 of the *Parengyodontium* strains defined by Tsang et al. (2016) and Parker et al. (2022) can be
107 encountered in a cultural heritage environment. The subsequent study of the phenotypic
108 diversity of the strains should then confirm or disprove the morphological differences noticed

109 between the three subclades/species. Finally, the susceptibility of *Parengyodontium* spp. strains
110 to econazole nitrate was tested to check a possible variability in the effectiveness of remediation
111 methods used against fungal colonization in cultural heritage. Econazole nitrate is an widely
112 used antifungal agent in the medical field (Heel et al. 1978; Kumar et al. 2014), and is also
113 occasionally used for the same purpose in the cultural heritage field (Leplat et al. 2017).

114

115 MATERIALS AND METHODS

116 *Strains of Parengyodontium spp. studied*

117 Eighteen *Parengyodontium* spp. strains isolated between 2004 and 2019 in various cultural
118 heritage sites were considered in this study (Table 1). Fifteen of the strains were isolated from
119 five different Paleolithic decorated caves, two were isolated from a museum and one strain was
120 isolated from the crypt of a church. Twelve of the strains were isolated through air samplings,
121 and six were isolated through surface samplings made with sterile swabs. The airborne strains
122 were collected with a Duo SAS Super 360 air sampler (VWR-pbi, Milan, Italy). Fifty litres of
123 air were collected at each sampling point using a 219-hole impactor containing malt extract
124 agar (MEA; Merck KGaA, Darmstadt, Germany) culture media in 55 mm Petri dishes. The
125 Petri dishes containing impacted media were then taken to the laboratory for analysis. The
126 plates were incubated in an IPP55 incubator (Mettler GmbH + Co. KG, Büchenbach,
127 Germany) for seven days at 24 °C. Fungi were then isolated from each other using the same
128 culture conditions as those described before. The strain isolated through swabbing followed the
129 same laboratory process.

130 The strains were preserved on MEA under paraffin oil at 4 °C. Two of the eighteen strains
131 preserved in the collection did not grow back from the collection, but their DNA had already
132 been extracted. The phenotypic diversity of *Parengyodontium* spp. was therefore only studied

133 on sixteen strains, while the phylogenic study was performed on all eighteen strains. With
134 regard to the susceptibility test, one of the two undeveloped strains had already been tested in
135 the past (Leplat et al. 2017). All strains except one were therefore tested for susceptibility to
136 econazole nitrate 0.2%.

137

138 *Phylogenetic study*

139 *Reference strains*

140 The strains used by Tsang et al. (2016), Teixeira et al. (2020) and by Parker et al. (2022) were
141 chosen as reference strains for *Parengyodontium* and *Engyodontium* genera (Table 2). For all
142 these reference strains, any sequences related to DNA regions that were not studied in the
143 aforementioned studies were also added whenever they were available in the NCBI GenBank
144 database (Clark et al. 2016). The names of the fungal species were updated with the current
145 names of the species, as defined in the Mycobank (Crous et al. 2004).

146

147 *DNA extraction, PCR amplification and sequencing*

148 Fungal DNA was extracted as described by Edel et al. (2001). Appropriate primers (Table S1)
149 were used to amplify the nuclear ribosomal internal transcribed spacer (ITS) region, the large
150 subunit of nuclear encoded ribosomal DNA (LSU), the small subunit of nuclear encoded
151 ribosomal DNA (SSU), the translation elongation factor 1-alpha gene (TEF), the largest subunit
152 of RNA polymerase II (RPB1), the second largest subunit of RNA polymerase II (RPB2) and
153 the beta-tubulin (TUB). PCR was performed in 25 µl reactions, with 1 µl of template DNA, 1
154 U of Taq DNA polymerase (Invitrogen, California), 2.5 µl of 10X Taq DNA polymerase buffer,
155 1 µl of 2 mmol l⁻¹ dNTPs (Thermo Fisher Scientific, Massachusetts) and 1.5 µl of each 10 µmol
156 l⁻¹ primer (Eurogentec, Belgium). Amplifications were performed on a PrimeG thermocycler

157 (Antylia Scientific, Illinois) using the following parameters: a 5-minute step at 94 °C, followed
158 by 35 cycles of 1 min at 94 °C, 1 min at the appropriate annealing temperature for each primer
159 pair (Table S1), 1 min at 72 °C, and a final 10-minute extension step at 72 °C. PCR products
160 were sequenced by Genoscreen (France) using the same primer set. Generated sequences were
161 submitted to the NCBI Genbank database.

162

163 *Sequence alignment and phylogenetic analyses*

164 The sequences generated in this study were verified with BioEdit v. 7.2.5 (Hall 1999). The
165 sequences related to reference strains were downloaded from the NCBI GenBank database.
166 Multiple sequence alignments for each studied DNA region were performed using the FFT-NS-
167 i alignment strategy from the MAFFT v. 7 web server (Katoh et al. 2019). Uninformative gaps
168 and ambiguous regions were removed using the Gblocks program v. 0.91.1 implemented in the
169 NGPhylogeny.fr service, with the program set for a lightly stringent selection (minimum
170 number of sequences for a conserved position: 50 % of the sequences + 1; minimum length of
171 a block: 5; allowed gap position: “with half”; Lemoine et al. 2019; Talavera and Castresana
172 2007). The maximum likelihood (ML) analyses of concatenated regions were performed using
173 the graphical interface of RAxML v. 8 set to 1000 bootstrap iterations and the GTRGAMMA
174 substitution model (Edler et al. 2019; Stamatakis 2014). Bayesian inference (BI) analyses of
175 concatenated regions were performed using MrBayes v. 3.2.6 implemented in PhyloSuite v.
176 1.2.1, based on a Markov Monte Carlo Chain (MCMC) set to two simultaneously executed runs
177 for 10,000,000 generations with the GTRGAMMA substitution model (Ronquist et al. 2012;
178 Zhang et al. 2020). Trees were sampled every 1000 generations, the burning fraction being set
179 to 25 %. Generated trees were edited using MEGA X (Kumar et al. 2018).

180

181

182 ***Morphological study***

183 The macroscopic features were assessed on MEA and on Potato Dextrose Agar (PDA; VWR
184 International, Pennsylvania) after a 14-day incubation at 25 °C and 32 °C. The microscopic
185 features were assessed on MEA after a 14-day incubation at 25 °C. Observations were
186 performed with a Jenavert optical microscope (Zeiss, Germany) after treatment with
187 lactophenol cotton blue solution (Pro-Lab Diagnostics Canada). Microscopic features were
188 captured with an Lt365R digital camera (Teledyne Lumenera, Canada) using the Archimed
189 program (Microvision Instruments, France).

190

191 ***In vitro susceptibility testing to econazole nitrate***

192 The susceptibility of *Parengyodontium* spp. strains to econazole nitrate 0.2 % (CTS France,
193 France) was tested by disk diffusion method on MEA. The plates were inoculated with a fungal
194 suspension of the tested strains with one strain per plate. The fungal suspensions were prepared
195 from fresh cultures in sterile water supplemented with triton 0.005 % (Thermo Fisher Scientific,
196 Massachusetts). Then a cellulose disc (diameter = 6 mm, BioMerieux, France) soaked in
197 econazole nitrate 0.2 % was laid on each plate and the plates were incubated at 25 °C for seven
198 days. The diameter of the inhibition zones outside the disk was measured in millimetres.
199 Duplicate tests were carried out for all strains. A control was performed for all strains with a
200 cellulose disk soaked in sterile water.

201

202 ***Statistical analysis***

203 The growth rates of the strains on MEA and PDA media at 25 °C as well as the results of
204 susceptibility tests were analysed using analysis of variance (ANOVA) implemented in R 4.0.1
205 (package stats; function aov; Chambers 2017; R Core Team 2021). The growth rates were
206 subjected to a power transformation to ensure the normality and the variance equality of the

207 data (package car; function powerTransform; Weisberg 2013), while the results of the
 208 susceptibility tests were subjected to a logarithmic transformation. The strain species and the
 209 medium were used as quantitative variables in the first ANOVA model (1), with the growth
 210 rate used as the quantitative variable. The strain number and the strain species were used as
 211 qualitative variables in the two subsequent ANOVA models (2 and 3), where the size of the
 212 inhibition halo was the quantitative variable. The two qualitative variables could not therefore
 213 be used in the same model because the variables are redundant when studied together, each
 214 different strain belonging to just one species.

215

$$216 \text{ Growth rate} = \mu + \text{Strain species}_i + \text{Medium}_j + \text{Strain species}_i:\text{Medium}_j + \varepsilon_{ijk} \quad (1)$$

217

218 Where μ is a constant, *Strain species* is the effect of each species ($i = 1, 2$), *Medium* is the effect
 219 of each medium ($j=1, 2$), *Strain species:Medium* is the effect of the interaction between the two
 220 variables and ε is the residual error.

221

$$222 \text{ Inhibition halo size} = \mu + \text{Strain number}_i + \varepsilon_{ij} \quad (2)$$

223

224 Where μ is a constant, *Strain number* is the effect of each studied strain ($i = 1, 2, \dots, 17$) and ε
 225 is the residual error.

226

$$227 \text{ Inhibition halo size} = \mu + \text{Strain species}_i + \varepsilon_{ij} \quad (3)$$

228

229 Where μ is a constant, *Strain species* is the effect of each *P. album* subclade ($i = 1, 2$) and ε is
 230 the residual error.

231

232 The ANOVA tests were completed by *post hoc* Tukey's HSD analysis to rank the different
233 means (package multcomp; function cld; Piepho 2004). All the tests performed in this study
234 were carried out using $\alpha = 5 \%$.

235

236 RESULTS

237 *Phylogenetic study*

238 The phylogenetic studies of the eighteen strains isolated from cultural heritage environment
239 among forty-three taxa through both maximum likelihood and Bayesian inference confirmed
240 their affiliation to *Parengyodontium* spp. (Fig. 1). Eleven of the strains were classified as *P.*
241 *album* subclade 1, while the seven remaining strains were classified as *P. torokii*. None of the
242 strains were classified as *P. album* subclade 2. The simultaneous use of the seven phylogenetic
243 loci also highlighted some extent of variability inside *P. album* subclade 1 and *P. torokii*.

244 The sequencing of TEF locus failed for all the strains belonging to *P. torokii* clade because of
245 the production of multiple bands by the PCR reaction under the experimental conditions of the
246 study, while these conditions allowed a correct sequencing for all the strains belonging to *P.*
247 *album* subclade 1.

248 Regarding the distribution of *P. album* subclade 1 and *P. torokii*, the two species had been
249 isolated through both surface samplings and air samplings. The two species were isolated
250 several times from the same location on the same date: for example, LRMH C325 (belonging
251 to *P. torokii*) and LRMH C327 (belonging to *P. album*) were both isolated through surface
252 sampling in the Chauvet cave during the January 2019 campaign. Similarly, *P. album* subclade
253 1 and *P. torokii* were identified among the 9 strains isolated at the same time through air
254 sampling in the Cosquer cave. Nevertheless, *P. album* subclade 1 was dominant, representing
255 7 of the 9 strains isolated in the cave. Finally, all the strains that were not isolated from caves,

256 *i.e.* LRMH C120, isolated from a crypt, and LRMH C267 and LRMH C268, isolated from a
257 museum, belonged to *P. album* subclade 1.

258

259 ***Morphological study***

260 *Macroscopic features*

261 The study of the macroscopic features of strains revealed common and different characteristics
262 between *Parengyodontium album* subclade 1 and *P. torokii*, and also some intraspecific
263 variability inside each species (Table 3; Fig 2 and 3). The most significant difference between
264 the two species was the growth of all *P. album* strains except LRMH C268 at 32 °C on PDA,
265 while none of the *P. torokii* strains was able to develop at this temperature. This growth
266 remained slow, with the highest growth rate of 15.59 mm in two weeks obtained for LRMH
267 C358, while LRMH C327 only produced unmeasurable traces. The difference between the two
268 species was more difficult to evaluate on MEA medium, as four out of the nine studied strains
269 of *P. album* did not grow at 32 °C. *Parengyodontium torokii* strains also failed to grow at 32
270 °C on MEA. The second difference between the two species was the reverse color of the
271 colonies on MEA and PDA at 25 °C. *Parengyodontium album* and *P. torokii* reverses displayed
272 orange tints on MEA, but the color was generally intense for *P. album*, while the color was
273 much slighter for *P. torokii*. On PDA, the *P. album* strains displayed shades ranging from
274 slightly yellow to slightly brown, with some strains showing intensely yellow coloring, while
275 *P. torokii* were generally white to very slightly yellow.

276 Inversely, common points between both species are that they were both white in color, and did
277 not produce diffusible pigment on MEA and PDA media. The strains of both species were
278 velvety to floccose on the two media. All the studied strains displayed radial growth on MEA
279 and some strains did not on PDA. This is not related to the species but rather to the texture of

280 the colony, since the intensity of the radial growth seemed stronger on velvety strains than on
281 floccose strains. Similarly, some of the strains of *P. album* subclade 1 and *P. torokii* produced
282 colorless to yellow exudate on MEA and/or on PDA media. *Parengyodontium album* strains
283 had growth rates between 31.14 and 40.53 mm in two weeks on MEA and between 22.30 and
284 36.90 mm on PDA, while *P. torokii* strains had growth rates between 19.80 and 36.10 mm on
285 MEA and between 20.51 and 36.00 mm on PDA. Thus, *P. album* subclade 1 generally showed
286 a significantly higher growth rate than *P. torokii*, but some strains of the two species grew
287 rapidly, while other strains grew more slowly (Table S2; Fig.S1).

288

289 *Microscopic features*

290 The main microscopic difference between *P. album* subclade 1 and *P. torokii* was the shape of
291 the conidia (Table. 4; Fig 4). In the two species, the conidia were one-celled, smooth and
292 hyaline, but the conidia of *P. torokii* were clearly ellipsoidal to subcylindrical, while *P. album*
293 conidia were mostly globose to subglobose, with rare examples tending towards an ellipsoidal
294 shape. Some *P. torokii* strains were able to produce longer conidia than *P. album*:
295 *Parengyodontium torokii* conidia were comprised in a range of 2.0 – 4.1 μm x 1.2 – 1.7 μm ,
296 while those of *P. album* ranged from 1.5 – 2.7 x 1.3 – 1.9.

297 *Parengyodontium album* subclade 1 and *P. torokii* both produce characteristic conidiogenous
298 cells composed of an elongated basal portion and a terminal fertile zigzag-shaped portion
299 beared by smooth, hyaline and septate vegetative hyphae. The conidiogenous cells were mostly
300 solitary in the two species, but whorls of up to three conidiogenous cells were also observed to
301 various extents in the majority of the studied strains. Conidiogenous cells measured 13 – 50 μm
302 in *P. album* and 21 – 33 μm in *P. torokii*. Some *P. album* strains therefore produced longer
303 conidiogenous cells than *P. torokii*, but with a greater degree of variability in each species.

304

305 ***In vitro* susceptibility testing to econazole nitrate**

306 The inhibition zones during the susceptibility tests of *Parengyodontium* spp. strains to
307 econazole nitrate 0.2 % varied between 1.98 and 48.36 mm (Table S2; Fig. 5 and S2).
308 *Parengyodontium torokii* strains were statistically more susceptible to econazole nitrate than *P.*
309 *album* subclade 1, but there was more variability between strains when examined in closer
310 detail. The two species contained relatively susceptible and tolerant strains to the biocide
311 product, meaning that there was no specific link between susceptibility and species.

312

313 **DISCUSSION**314 ***Occurrence of Parengyodontium spp. strains in the cultural heritage environment***

315 *Parengyodontium album* is a fungal species of great interest in the domain of cultural heritage
316 preservation due to its isolation from various historic materials that are in some cases suffering
317 from biodeterioration (Leplat et al. 2020a). This species has recently been recovered at sites
318 ranging from the Florence cathedral in Italy to Paleolithic decorated show caves in Spain
319 (Dominguez-Moñino et al. 2021; Santo et al. 2021). Despite the presence of this species in
320 cultural heritage sites, no study to date has determined precisely which type of *P. album* strains
321 are associated to cultural heritage, although Tsang et al. (2016) identified three subclades when
322 they described *P. album* as the single species of *Parengyodontium* genus. Note that subclade 3
323 has recently been moved to the *Parengyodontium torokii* species (Parker et al. 2022). Our study
324 of eighteen strains that had been initially identified as *P. album* proved that at least *P. album*
325 subclade 1 and *P. torokii* can be recovered on cultural heritage sites. Moreover, both species
326 are able to coexist in the same place at the same time. The two species were also recovered
327 from both surface samplings and air sampling. These results suggest that the ecological

328 requirements of the two species are similar. *Parengyodontium album* is known to be well
329 adapted to humid and salty environments, since this species has been abundantly isolated from
330 marine environments (Khusnullina et al. 2018; Liu et al. 2021; Pindi 2012), and in particular in
331 the cultural heritage domain in monuments suffering from salt disorders (Ponizovskaya et al.
332 2019; Trovão et al. 2019). The majority of the strains we studied were isolated from Paleolithic
333 decorated cave environments, with only three of the eighteen strains isolated in another
334 environment, *i.e.* church crypt or museum. All of these three strains belonged to *P. album*
335 subclade 1, but additional data should be collected to understand whether this result could
336 reflect a slight difference in ecological niches between *P. album* subclade 1 and *P. torokii*. No
337 *P. album* subclade 2 strain was identified among the eighteen strains studied. Interestingly, this
338 was the only subclade involved in deep-seated infection among the panel of strains studied by
339 Tsang et al. (2016) during the description of the genus *Parengyodontium*, while the two other
340 subclades were only involved in infections at superficial body sites. These results could suggest
341 some kind of adaptation of this subclade as a human pathogen, as observed for other fungal taxa
342 (De Hoog et al. 1998; Rokas et al. 2020). Tsang et al. (2016) noted that only the strains
343 belonging to subclade 2 were able to grow at temperatures above 35 °C, while *P. album*
344 subclade 1 and *P. torokii* were not. In general, the fungi that cause systemic disease must be
345 able to grow and multiply at 37 °C (Kobayashi 1996).

346

347 ***Phenotypic differences and similarities between P. album and P. torokii***

348 The difference in the capability to grow above 35 °C between *P. album* subclade 2, *P. album*
349 subclade 1 and *P. torokii* raises the issue of the morphological differences between the three
350 subclades/species. Tsang et al. (2016) considered the three subclades of *P. album* and described
351 them as potential cryptic species given the significant phylogenetic differences obtained
352 through the study of ITS, LSU and TUB markers and the lack of morphological differences. In

353 our study, the additional use of SSU, TEF, RPB1 and RPB2 markers confirmed the differences
354 between the three subclades/species and highlighted some intraspecific variability within *P.*
355 *album* and *P. torokii*. However, this variability was not high enough to consider the description
356 of new species. Interestingly, the sequencing for TEF of all strains belonging to *P. torokii* failed
357 in our study because of the production of multiple parasitic bands through the PCR reaction,
358 while the sequencing of all the *P. album* was successful. The use of a hot-start Taq DNA
359 polymerase (Paul et al. 2010), as well as the use of touchdown PCR (Korbie and Mattick 2008),
360 did not resolve this issue. The fact that all sequencing of TEF failed for *P. torokii* and succeeded
361 for *P. album* was actually an indirect clue of a similar pattern in *P. torokii* strains that made
362 DNA amplification incompatible with the used primers. Despite the morphological similarity
363 between *P. album* subclades 1 and 2 and *P. torokii*, Tsang et al. (2016) pointed out some
364 possible differences, the first being the growth of *P. album* subclade 2 at temperatures above
365 35 °C, while *P. subclade* 1 and *P. torokii* did not grow at temperatures above 31 °C. The second
366 difference was the reverse color of the colonies on PDA medium: *P. album* subclade 1 strains
367 were in general orange-brown, *P. album* subclade 2 strains were dark brown and *P. torokii*
368 strains were white. Parker et al. (2022) also noticed that *P. torokii* conidia were subcylindrical
369 to ellipsoidal, while *P. album* conidia were globose. Unlike Tsang et al. (2016), eight out of the
370 nine *P. album* subclade 1 strains we inspected were able to slowly develop on PDA medium at
371 32 °C, while none of the seven *P. torokii* strains grew in the same conditions. Intraspecific
372 variability in fungal growth according to temperature is known in several fungal taxa (Möller
373 et al. 1996; Van Long et al. 2021). In some cases, this variability was linked to geographic
374 location and was also supported by phylogenetic variability (Möller et al. 1996), while in other
375 cases there was no clear relationship between growth parameters and ecological characteristics
376 or genetically distinct populations (Van Long et al. 2021). The difference between our results
377 and those of Tsang et al. (2016) cannot be explained by the geographic location of the strains,

378 as Tsang et al. (2016) studied *P. album* subclade 1 strains isolated from different parts of the
379 world. Similarly, no clear phylogenetic difference occurred between the strains. This
380 characteristic should therefore be studied in greater depth because it could be an easier way to
381 morphologically differentiate *P. album* subclade 1 from *P. torokii*. We also observed some color
382 differences between *P. album* and *P. torokii* on the reverse of the colonies grown on PDA
383 medium. As noticed by Tsang et al. (2016), *P. torokii* were white in general, with some tending
384 to be slightly yellow. The reverse colors of *P. album* strains were more marked on PDA medium
385 although brown coloring was not as intense as the coloring reported by Tsang et al. (2016). The
386 same phenomenon seemed to occur on MEA medium, where *P. album* showed more intense
387 orange shades than *P. torokii*. It is difficult to evaluate this characteristic as a means to clearly
388 differentiate between the two species. Finally, our observations confirmed those of Parker et al.
389 (2022) regarding the shape of conidia: *P. album* conidia were globose to subglobose while *P.*
390 *torokii* were ellipsoidal to subcylindrical; this is the easiest microscopic feature available to
391 distinguish *P. torokii* from *P. album*.

392

393 ***Susceptibility of Parengyodontium spp. to the biocide product used in the cultural heritage***
394 ***domain***

395 Econazole nitrate is a fungicide belonging to the imidazole family and is generally used in a
396 medical context (Firooz et al. 2015; Terrell 1999). It is also used as an antifungal agent in the
397 cultural heritage domain, and is mainly used in France (Billerbeck et al. 2002; Rakotonirainy
398 et al. 1999), with scarce references to its utilization in other parts of the world (Fabbri and
399 Pretelli 2014). This antifungal agent was tested because the strain LRMH C120, identified as
400 *P. album* subclade 1, had proved tolerant to this product in a previous study about the fungal
401 contamination of the crypt vault at Saint-Savin-sur-Gartempe Abbey church (Leplat et al.
402 2017). The susceptibility of the studied strains to econazole nitrate 0.2% was found to be highly

403 variable and was not species dependent; some strains were relatively tolerant and others
404 susceptible in both *P. album* and *P. torokii*. This result has already been observed in other fungal
405 taxa where strains of the same species expressed different susceptibilities toward azole products
406 in general and to econazole in particular (Choukri et al. 2014; Hammer et al. 2000; Hassan et
407 al. 2020). Tsang et al. (2016) tested the susceptibility of *P. album* to various azoles compounds
408 excluding econazole, and found different results according to the compound, the subclade and
409 even the strain considered. Strains that we had isolated at the same place and time had different
410 susceptibilities to econazole nitrate: one of the two *P. album* strains recovered from the Bastia
411 museum in December 2016 was strongly susceptible to econazole nitrate 0.2 %, while the other
412 was relatively tolerant. These results highlight the importance of checking the susceptibility of
413 fungal strains before applying a treatment, especially in a context such as cultural heritage
414 preservation. This verification is necessary to ensure the efficiency of the treatment, especially
415 since the use of such chemical products is increasingly questioned for environmental and health
416 reasons (Dresler et al. 2017; Kakakhel et al. 2019).

417

418 In conclusion, this study showed that the two species *Parengyodontium album* subclade 1 and
419 *P. torokii* can be recovered from cultural heritage sites and can occur in the same time in the
420 same site. The morphological study showed that most of the strain of *P. album* subclade 1 were
421 able to slowly grow at 32 °C, while none of the *P. torokii* strains were. The shape of the
422 microconidia was also different between the two species. The study of the susceptibility of the
423 recovered strains to econazole nitrate 0.2 % showed that some strains were relatively tolerant
424 and others relatively susceptible in the two species. These results highlight the importance of
425 checking the susceptibility of fungal strains before applying a treatment.

426

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432 **LITERATURE CITED**

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649

650 Table 1: Description of *Parengyodontium* spp. strains selected to study phylogeny, phenotypes
 651 and susceptibility to econazole nitrate.

Collection number	Species	Sampling place	Sampling date	Sampling method	Preceding study referring to the strain
LRMH C057	<i>Parengyodontium torokii</i>	Lascaux cave (Nouvelle-Aquitaine, France)	12/2004	Air sampling	(-)
LRMH C120*	<i>P. album</i>	Crypt of Saint-Savin-sur Gartempe Abbey church (Nouvelle-Aquitaine, France)	11/2015	Surface sampling	(Leplat et al. 2017)
LRMH C133	<i>P. torokii</i>	Chauvet cave (Auvergne-Rhône-Alpes, France)	03/2016	Surface sampling	(-)
LRMH C243	<i>P. torokii</i>	Pair-non-Pair cave (Nouvelle-Aquitaine, France)	10/2016	Air sampling	(-)
LRMH C267	<i>P. album</i>	Bastia Museum	12/2016	Surface sampling	(-)
LRMH C268	<i>P. album</i>	Bastia Museum	12/2016	Surface sampling	(-)
LRMH C325	<i>P. torokii</i>	Chauvet cave (Auvergne-Rhône-Alpes, France)	01/2019	Surface sampling	(-)
LRMH C327	<i>P. album</i>	Chauvet cave (Auvergne-Rhône-Alpes, France)	01/2019	Surface sampling	(-)
LRMH C343*	<i>P. album</i>	Cosquer cave (Provence-Alpes-Côte d'Azur, France)	07/2019	Air sampling	(-)
LRMH C345	<i>P. album</i>	Cosquer cave (Provence-Alpes-Côte d'Azur, France)	07/2019	Air sampling	(-)
LRMH C346	<i>P. album</i>	Cosquer cave (Provence-Alpes-Côte d'Azur, France)	07/2019	Air sampling	(-)
LRMH C349	<i>P. album</i>	Cosquer cave (Provence-Alpes-Côte d'Azur, France)	07/2019	Air sampling	(-)
LRMH C350	<i>P. album</i>	Cosquer cave (Provence-Alpes-Côte d'Azur, France)	07/2019	Air sampling	(-)
LRMH C355	<i>P. album</i>	Cosquer cave (Provence-Alpes-Côte d'Azur, France)	07/2019	Air sampling	(-)
LRMH C356	<i>P. torokii</i>	Cosquer cave (Provence-Alpes-Côte d'Azur, France)	07/2019	Air sampling	(-)
LRMH C357	<i>P. torokii</i>	Cosquer cave (Provence-Alpes-Côte d'Azur, France)	07/2019	Air sampling	(-)
LRMH C358	<i>P. album</i>	Cosquer cave (Provence-Alpes-Côte d'Azur, France)	07/2019	Air sampling	(-)
LRMH C364	<i>P. torokii</i>	Pigeonnier cave (Nouvelle-Aquitaine, France)	10/2016	Air sampling	(Leplat et al. 2020b)

652 * Strains only included in the phylogenetic study; the preserved strains did not grow back from the collection tubes and were therefore excluded
 653 from the phenotype study.

Table 2: Fungal taxa used in the phylogenetic study.

Species	<i>Parengyodontium album</i> subclade	Collection number	ITS	SSU	LSU	TEF	RPB1	RPB2	TUB	Reference study
<i>Parengyodontium album</i>	Subclade 1	HKU48	LC092879	(-)	LC092898	(-)	(-)	(-)	LC092917	(Tsang et al. 2016)
<i>P. album</i>	Subclade 1	CBS 504.83	LC092880	(-)	LC092899	LC425535	(-)	(-)	LC092918	(Tsang et al. 2016)
<i>P. album</i>	Subclade 1	CBS 570.71	LC092881	(-)	LC092900	LC425551	(-)	(-)	LC092919	(Tsang et al. 2016)
<i>P. album</i>	Subclade 1	CBS 836.71	LC092882	(-)	LC092901	LC382178	(-)	(-)	LC092920	(Tsang et al. 2016)
<i>P. album</i>	Subclade 1	CBS 121919	LC092883	(-)	LC092902	LC425536	(-)	(-)	LC092921	(Tsang et al. 2016)
<i>P. album</i>	Subclade 1	UAMH 1441	LC092884	(-)	LC092903	LC425557	(-)	(-)	LC092922	(Tsang et al. 2016)
<i>P. album</i>	Subclade 1	UAMH 10043	LC092885	(-)	LC092904	LC425537	(-)	(-)	LC092923	(Tsang et al. 2016)
<i>P. album</i>	Subclade 1	NRRL 2312	LC092886	(-)	LC092905	(-)	(-)	(-)	LC092924	(Tsang et al. 2016)
<i>P. album</i>	Subclade 2	IHEM 4198	LC092887	(-)	LC092906	LC425561	(-)	(-)	LC092925	(Tsang et al. 2016)
<i>P. album</i>	Subclade 2	UAMH 4512	LC092888	(-)	LC092907	LC382182	(-)	(-)	LC092926	(Tsang et al. 2016)
<i>P. album</i>	Subclade 2	UAMH 8313	LC092889	(-)	LC092908	(-)	(-)	(-)	LC092927	(Tsang et al. 2016)
<i>P. album</i>	Subclade 2	UAMH 11234	LC092890	(-)	LC092909	LC425560	(-)	(-)	LC092928	(Tsang et al. 2016)
<i>P. album</i>	Subclade 1	LRMH C120	KX821728	OL913902	OL981475	OM201767	OL904974	OL904992	OM201750	This study
<i>P. album</i>	Subclade 1	LRMH C267	OL913885	OL913905	OL981478	OM201768	OL904977	OL904995	OM201753	This study
<i>P. album</i>	Subclade 1	LRMH C268	OL913886	OL913906	OL981479	OM201769	OL904978	OL904996	OM201754	This study
<i>P. album</i>	Subclade 1	LRMH C327	OL913888	OL913908	OL981481	OM201770	OL904980	OL904998	OM201756	This study
<i>P. album</i>	Subclade 1	LRMH C343	OL913889	OL913909	OL981482	OM201771	OL904981	OL904999	OM201757	This study
<i>P. album</i>	Subclade 1	LRMH C345	OL913890	OL913910	OL981483	OM201772	OL904982	OL905000	OM201758	This study
<i>P. album</i>	Subclade 1	LRMH C346	OL913891	OL913911	OL981484	OM201773	OL904983	OL905001	OM201759	This study
<i>P. album</i>	Subclade 1	LRMH C349	OL913892	OL913912	OL981485	OM201774	OL904984	OL905002	OM201760	This study
<i>P. album</i>	Subclade 1	LRMH C350	OL913893	OL913913	OL981486	OM201775	OL904985	OL905003	OM201761	This study
<i>P. album</i>	Subclade 1	LRMH C355	OL913894	OL913914	OL981487	OM201776	OL904986	OL905004	OM201762	This study
<i>P. album</i>	Subclade 1	LRMH C358	OL913897	OL913917	OL981490	OM201777	OL904989	OL905007	OM201765	This study
<i>Parengyodontium americanum</i>	(-)	AZ2	KY683770	(-)	(-)	(-)	(-)	(-)	(-)	(Teixeira et al. 2020)
<i>P. americanum</i>	(-)	CA11	KY683771	(-)	(-)	(-)	(-)	(-)	(-)	(Teixeira et al. 2020)
<i>P. americanum</i>	(-)	CA13	KY683772	(-)	(-)	(-)	(-)	(-)	(-)	(Teixeira et al. 2020)
<i>P. americanum</i>	(-)	CA19	KY683773	(-)	(-)	(-)	(-)	(-)	(-)	(Teixeira et al. 2020)
<i>P. americanum</i>	(-)	CA21	KY683774	(-)	(-)	(-)	(-)	(-)	(-)	(Teixeira et al. 2020)
<i>Parengyodontium torokii</i>	Subclade 3	NRRL 64203*	MT704894	(-)	(-)	(-)	(-)	(-)	(-)	(Parker et al. 2022)
<i>P. torokii</i>	Subclade 3	CBS 368.72	LC092891	(-)	LC092910	LC382183	(-)	(-)	LC092929	(Tsang et al. 2016)
<i>P. torokii</i>	Subclade 3	UAMH 9836	LC092892	(-)	LC092911	LC382184	(-)	(-)	LC092930	(Tsang et al. 2016)

<i>P. torokii</i>	Subclade 3	LRMH C057	OL913882	OL913901	OL981474	(-)	OL904973	OL904991	OM201749	This study
<i>P. torokii</i>	Subclade 3	LRMH C133	OL913883	OL913903	OL981476	(-)	OL904975	OL904993	OM201751	This study
<i>P. torokii</i>	Subclade 3	LRMH C243	OL913884	OL913904	OL981477	(-)	OL904976	OL904994	OM201752	This study
<i>P. torokii</i>	Subclade 3	LRMH C325	OL913887	OL913907	OL981480	(-)	OL904979	OL904997	OM201755	This study
<i>P. torokii</i>	Subclade 3	LRMH C356	OL913895	OL913915	OL981488	(-)	OL904987	OL905005	OM201763	This study
<i>P. torokii</i>	Subclade 3	LRMH C357	OL913896	OL913916	OL981489	(-)	OL904988	OL905006	OM201764	This study
<i>P. torokii</i>	Subclade 3	LRMH C364	OL913898	OL913918	OL981491	(-)	OL904990	OL905008	OM201766	This study
<i>Engyodontium rectidentatum</i>	(-)	CBS 206.74	LC092893	(-)	LC092912	LC425553	(-)	(-)	LC092931	(Tsang et al. 2016)
<i>E. rectidentatum</i>	(-)	CBS 547.82	LC092894	(-)	LC092913	LC425544	(-)	(-)	LC092932	(Tsang et al. 2016)
<i>E. rectidentatum</i>	(-)	CBS 641.74	LC092895	(-)	LC092914	LC425540	(-)	(-)	(-)	(Tsang et al. 2016)
<i>Engyodontium parvisporum</i>	(-)	IHEM 22910	LC092896	(-)	LC092915	LC425558	(-)	(-)	LC092933	(Tsang et al. 2016)
<i>Engyodontium aranearum</i>	(-)	CBS 658.80	LC092897	(-)	LC092916	(-)	(-)	(-)	LC092934	(Tsang et al. 2016)

*Although Parker et al. (2022) used each one of these seven phylogenetic markers, the ITS sequence is the only locus to be specifically available in the Genbank, the other loci being included in a Whole-Genome sequence composed of 30,430,964 bp. Therefore, these loci could not be used in this study.

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Table 3: Macroscopic features of the *Parangyodontium* spp. strains studied.

Strain	Size (mm)*	Color	Reverse color	Texture	Radial growth	Exudate	Size (mm)**	Size (mm)*	Color	Reverse color	Texture	Radial growth	Exudate	Size (mm)**	
MEA							PDA								
25 °C							32 °C				25 °C				32 °C
<i>Parangyodontium album</i> subclade 1															
LRMH C267	34.35			Velvety	Strong		18.36	32.09		Yellow	Velvety to floccose	Slight		19.04	
LRMH C268	36.31			Floccose	Slight			29.20		Yellow to slightly brown	Floccose	(-)		(-)	
LRMH C327	31.52			Floccose	Slight		(-)	22.30		Yellow to slightly brown	Floccose	(-)	(-)	Traces	
LRMH C345	38.04			Floccose	Slight			35.19		Yellow to slightly brown	Velvety to floccose	Slight		13.58	
LRMH C346	30.08	White	Strongly orangey	Velvety	Strong	(-)		30.64	White	White to yellow	Velvety	Slight		8.55	
LRMH C349	31.14			Velvety	Strong		9.45	31.24		Yellow to slightly brown	Velvety to floccose	Slight	Slight, colorless to yellow	14.78	
LRMH C350	31.16			Velvety	Strong		6.12	29.76		Yellow to slightly brown	Velvety to floccose	Slight	Slight, colorless to yellow	14.53	
LRMH C355	31.69			Velvety	Strong		14.06	31.25		White to yellow	Velvety to floccose	Slight		14.50	
LRMH C358	40.53			Floccose	Slight	Yellow	13.57	36.9		White to slightly yellow	Floccose	(-)	(-)	15.59	
<i>Parangyodontium torkii</i> (<i>Parangyodontium album</i> subclade 3)															
LRMH C057	33.80			Floccose	Slight	(-)		36.00			Floccose	Slight	(-)		
LRMH C133	30.56			Floccose	Very slight	Yellow		34.44			Floccose	(-)	Colorless to yellow		
LRMH C243	26.13			Velvety to floccose	Strong			24.37			Floccose	Slight			
LRMH C325	20.60	White	Orangey	Floccose	Slight		(-)	20.84	White	White to slightly yellow	Floccose	(-)		(-)	
LRMH C356	33.00			Velvety	Strong	(-)		35.11			Velvety to floccose	Slight	(-)		
LRMH C357	36.10			Velvety	Strong			35.20			Velvety to floccose	Slight			
LRMH C364	19.80			Velvety	Strong			20.51			Velvety to floccose	Slight	Slight, colorless to yellow		

*The sizes at 25 °C were obtained by the mean of three replications on the same Petri dish.

**The sizes at 32 °C were obtained by the mean of the number of replications that grew (from 1 to 3) on the same Petri dish.

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660 Table 4: Microscopic features of the *Parangyodontium* spp. strains studied*.

Strain	Conidiogenous cells		Conidia	
	Organization	Limit sizes measured (μm)	Shape	Limit sizes measured (μm)
<i>Parangyodontium album</i> subclade 1				
LRMH C267	Mostly solitary, occasionally whorls of 2 cells	14 – 22	Globose to subglobose	2.0 – 2.2 x 1.3 – 1.9
LRMH C268	Solitary and in whorls of 2 to 3 cells	13 – 21	Globose to subglobose	1.5 – 2.1 x 1.5 – 1.9
LRMH C327	Solitary and in whorls of 2 to 3 cells	25 – 26	Subglobose to ellipsoidal	2.2 – 2.7 x 1.1 – 1.6
LRMH C345	Solitary and in whorls of 2 to 3 cells	28 – 34	Globose to subglobose	2.1 – 2.3 x 1.5 – 1.6
LRMH C346	Solitary and in whorls of 2 to 3 cells	37 – 50	Subglobose to ellipsoidal	2.0 – 2.9 x 1.7 – 1.8
LRMH C349	Solitary and in whorls of 2 cells	36 – 44	Globose to subglobose	2.1 – 2.3 x 1.5 – 1.6
LRMH C350	Solitary and in whorls of 2 to 3 cells	27 – 28	Globose to subglobose	1.9 – 2.7 x 1.3 – 1.4
LRMH C355	Solitary and in whorls of 2 cells	16 – 27	Subglobose to ellipsoidal	2.0 – 2.5 x 1.4 – 1.6
LRMH C358	Solitary and in whorls of 2 to 3 cells	33 – 40	Globose to subglobose	2.0 – 2.1 x 1.5 – 1.6
<i>Parangyodontium torkii</i> (<i>Parangyodontium album</i> subclade 3)				
LRMH C057	Mostly solitary, occasionally whorls of 2 cells	26 – 28	Ellipsoidal to subcylindrical	2.2 – 3.4 x 1.4 – 1.5
LRMH C133	Mostly solitary, occasionally whorls of 2 cells	24 – 30	Ellipsoidal to subcylindrical	2.0 – 2.7 x 1.4 – 1.6
LRMH C243	Solitary and in whorls of 2 cells	21 – 33	Ellipsoidal to subcylindrical	2.1 – 2.2 x 1.4 – 1.5
LRMH C325	Solitary and in whorls of 2 to 3 cells	23 – 28	Ellipsoidal to subcylindrical	2.3 – 2.4 x 1.4 – 1.5
LRMH C356	Solitary and in whorls of 2 cells	22 – 23	Ellipsoidal to subcylindrical	2.5 – 3.0 x 1.2 – 1.4
LRMH C357	Solitary and in whorls of 2 cells	25 – 27	Ellipsoidal to subcylindrical	2.5 – 2.7 x 1.4 – 1.5
LRMH C364	Solitary and in whorls of 2 cells	25 – 27	Ellipsoidal to subcylindrical	3.1 – 4.1 x 1.5 – 1.7

661 *Only features that introduce a degree of diversity between species and/or strains are listed in the table; the features common to all strains are
662 not included.

663 **LEGENDS**

664 Figure 1: Phylogenetic studies of *Parengyodontium* spp. strains isolated from a cultural heritage
665 environment through maximum likelihood and Bayesian inference.

666 The trees were obtained from a dataset including 43 taxa leading to a concatenated alignment
667 of 4677 bp from seven nuclear regions (ITS, LSU, SSU, RPB1, RPB2, TEF and TUB). The
668 trees were rooted with *Engyodontium* clade as an outgroup. Bootstrap values greater than or
669 equal to 70 % and Bayesian posterior probabilities greater than or equal to 0.70 are located
670 close to the corresponding node. The specific strains studied are marked in bold.

671

672 Figure 2: Macroscopic features of the *Parengyodontium album* subclade 1 strains studied.

673 For each strain, growth on MEA medium is displayed on the left and growth on PDA medium
674 on the right. For each Petri dish, the recto side is displayed on the left and the reverse side on
675 the right.

676

677 Figure 3: Macroscopic features of the *Parengyodontium torokii* (*P. album* subclade 3) strains
678 studied.

679 For each strain, growth on MEA medium is displayed on the left and growth on PDA medium
680 on the right. For each Petri dish, the recto side is displayed on the left and the reverse side on
681 the right.

682

683 Figure 4: Microscopic features of *Parengyodontium* spp. strains studied.

684 Shape of microconidia: Ellipsoidal to subcylindrical conidia of *P. torokii* (a) and globose to
685 subglobose conidia of *P. album* subclade 1 (b). Size of conidiogenous cells: short
686 conidiogenous cells (c) and long conidiogenous cells (d). Organization of conidiogenous cells:
687 solitary conidiogenous cells (e) and conidiogenous cells in whorls (f and g). Scale bars: 10 μm .

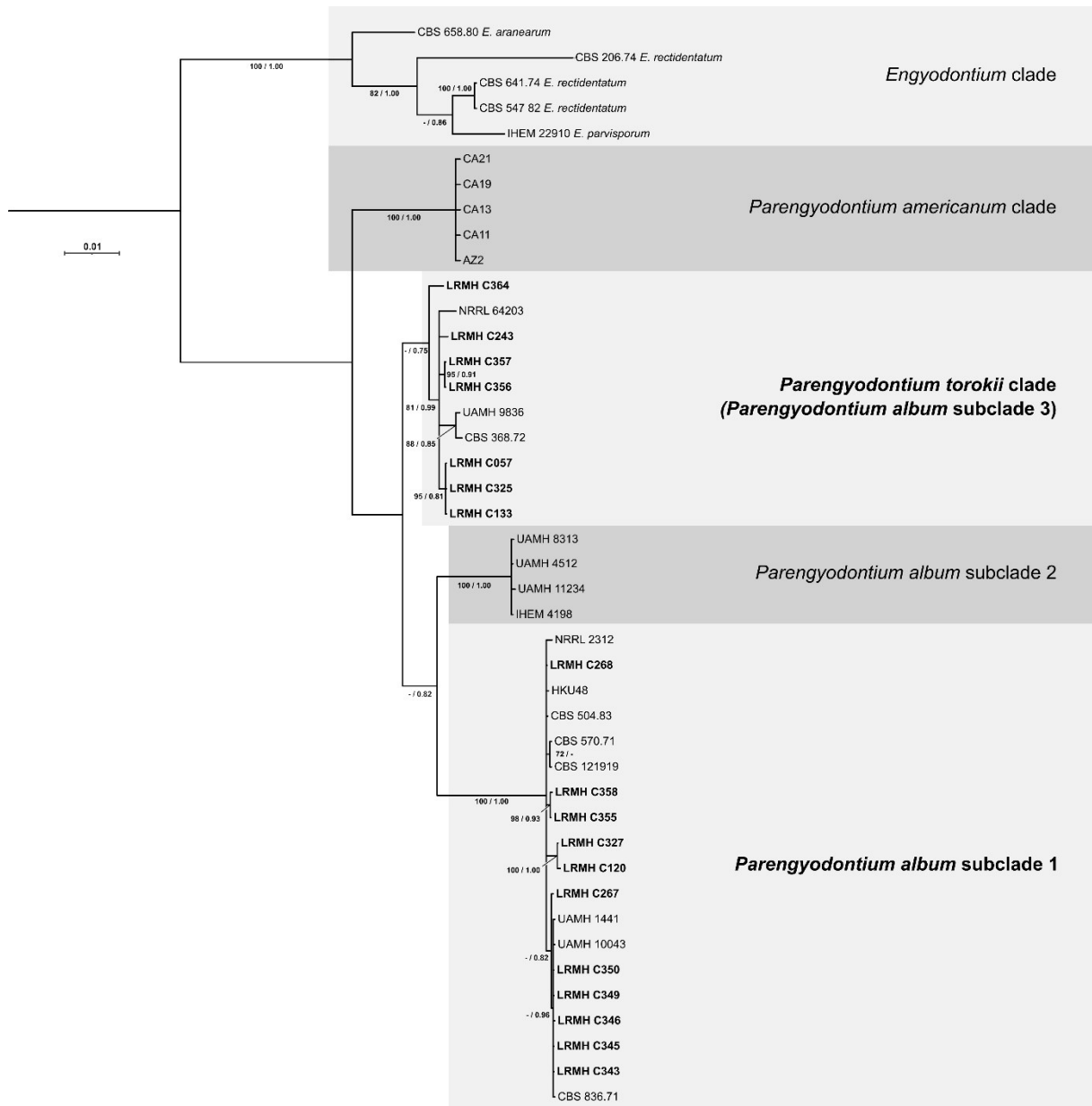
689 Figure 5: Susceptibility of *Parengyodontium* spp. strains to econazole nitrate 0.2 %.

690 The bars display the inhibition halo size around the cellulose disc soaked in econazole nitrate.

691 The error bars were obtained by calculating the mean of two repetitions. The results regarding

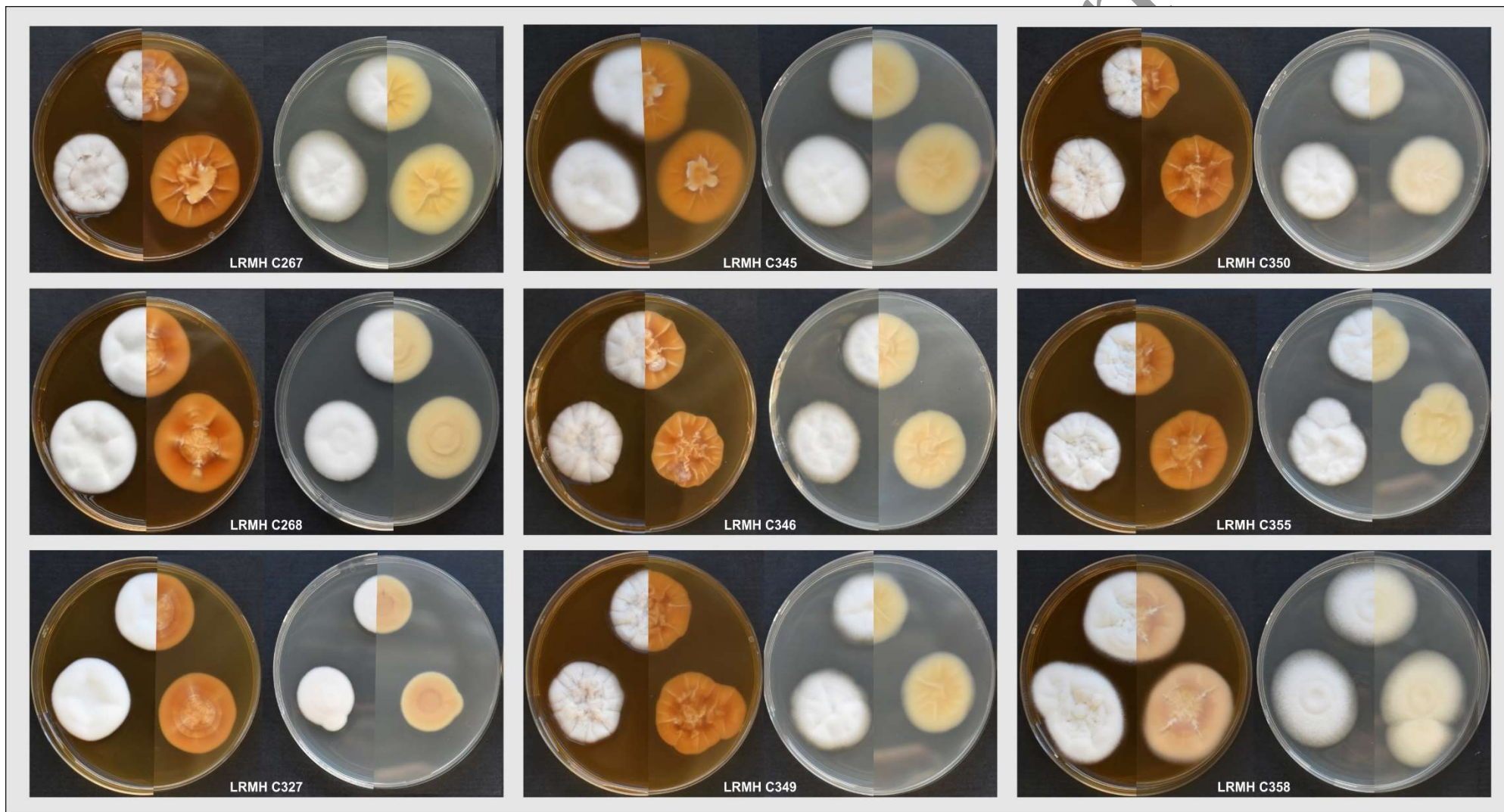
692 the strain LRMH C120 were obtained from (Leplat et al. 2017).

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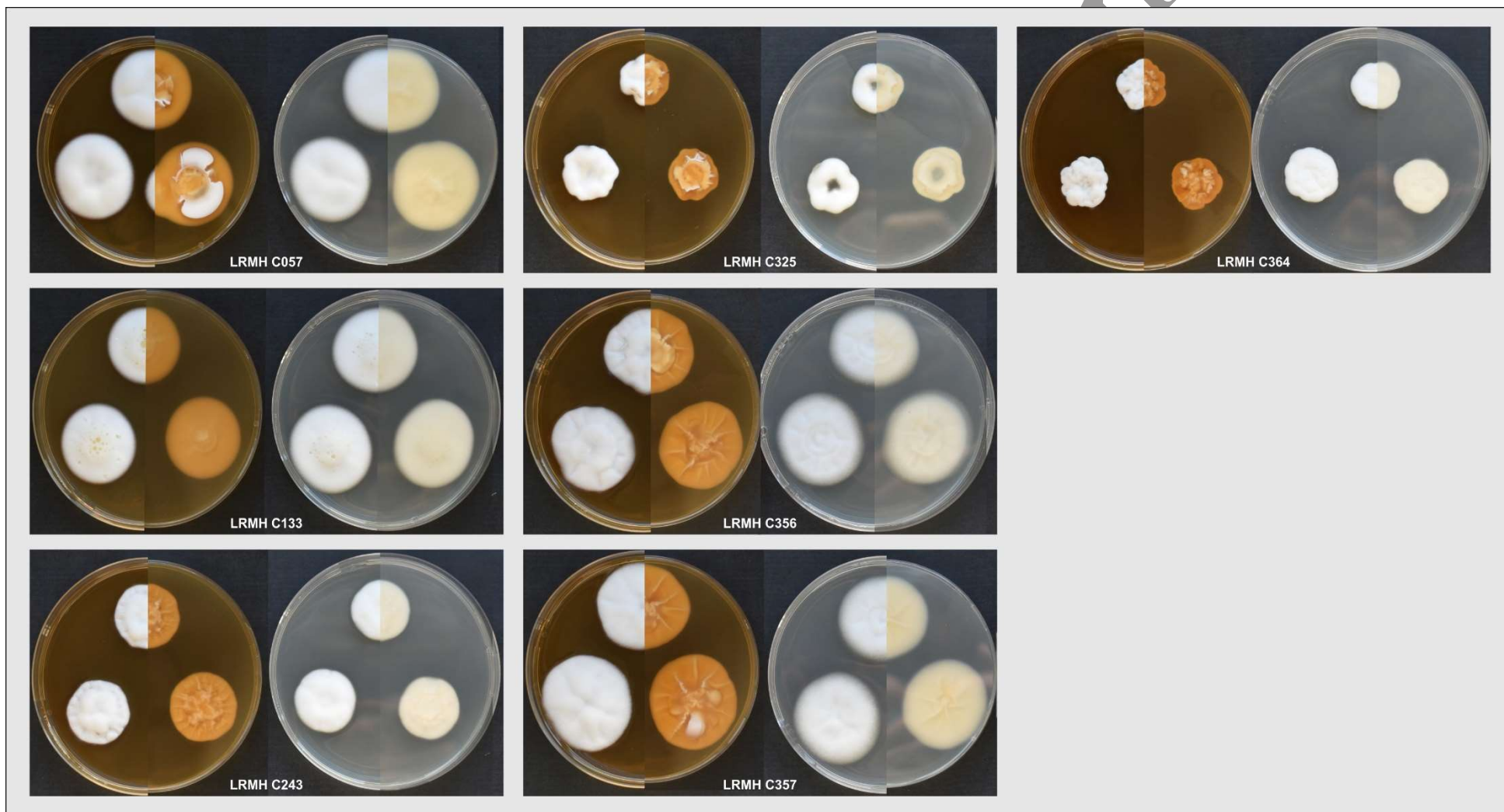
* corresponding author e-mail: johann.leplat@culture.gouv.fr



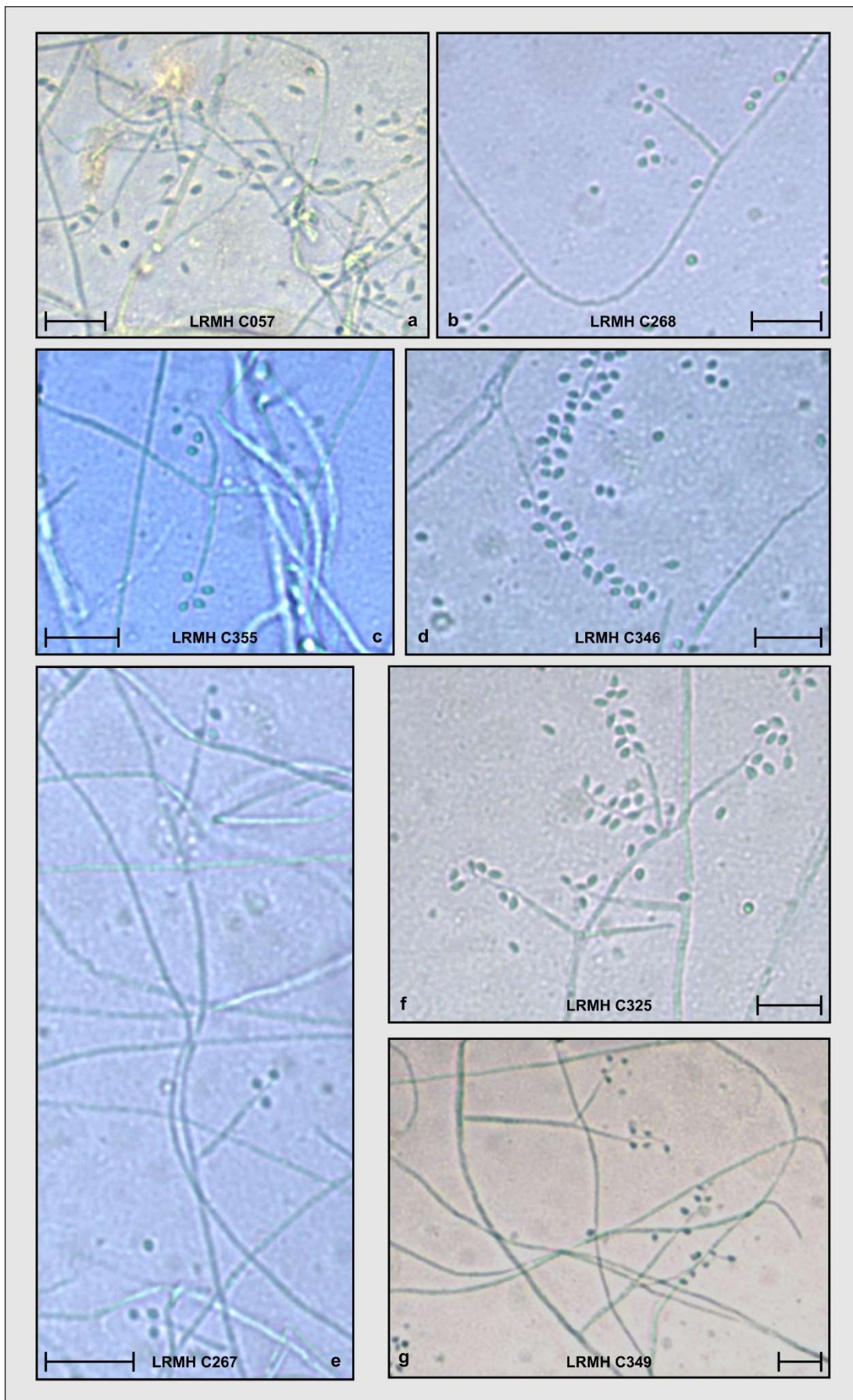
* corresponding author e-mail: johann.leplat@culture.gouv.fr

697 Figure 3

698



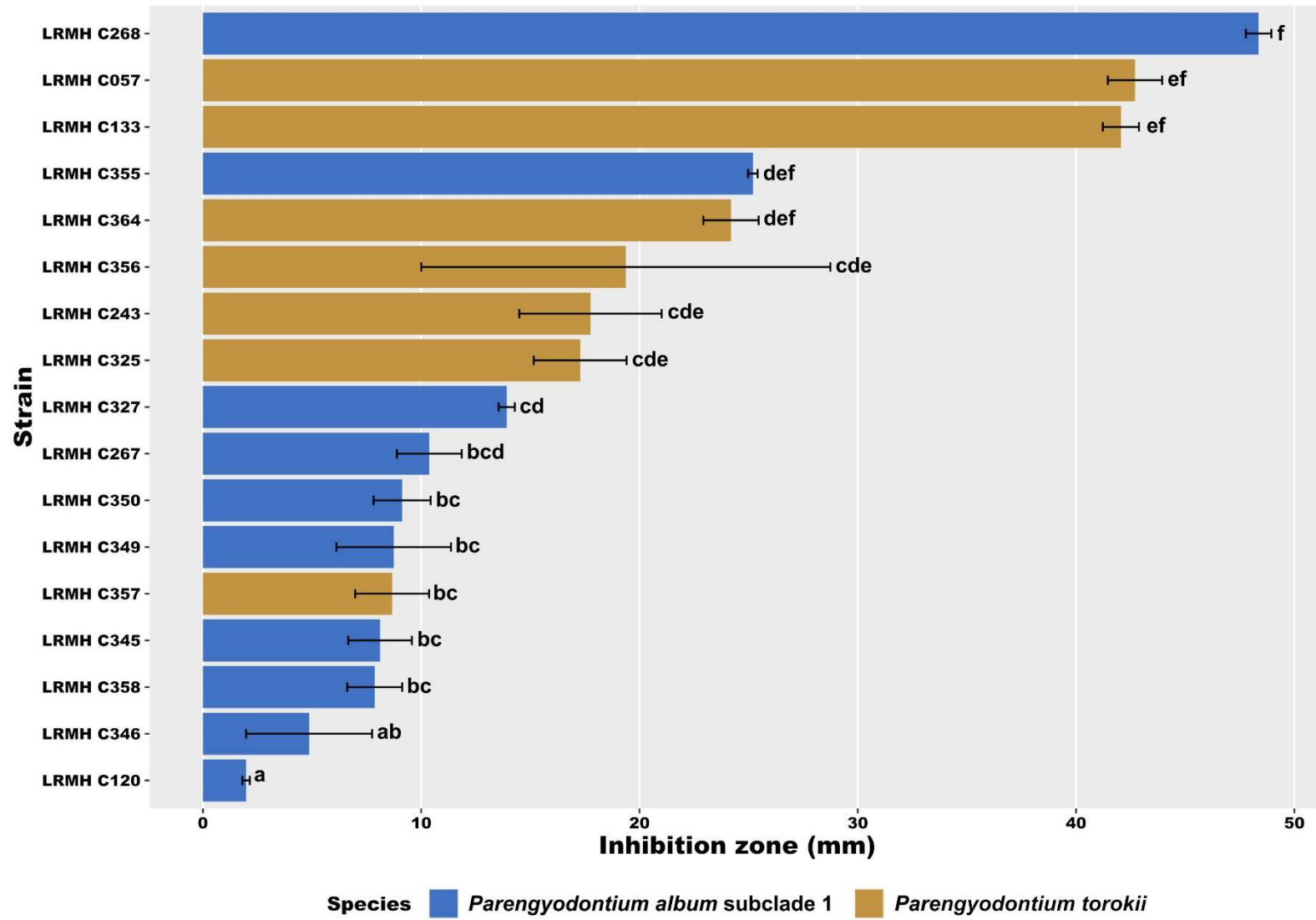
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701 Figure 5

702



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