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1 ***Simplicillium pech-merlensis*, a new fungal species isolated from the air of the**
2 **Pech-Merle show cave**

3
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26 **Abstract**

27

28 An original fungal strain has been recovered during an aerobiological survey in the Pech-Merle
29 show cave (France). The use of multi-locus (ITS, LSU, SSU RPB1, RPB2 and TEF-1 α)
30 phylogenetic analysis of the strain by maximum likelihood and by Bayesian inference coupled with
31 a morphological characterization allowed us to place it in the *Simplicillium* genus as *Simplicillium*
32 *pech-merlensis* sp. nov. This new species seems morphologically close to *S. calcicola* and *S. album*,
33 which were also first isolated from a cave habitat. This paper discusses the phylogenetic place of *S.*
34 *pech-merlensis* and some other species in the genus *Simplicillium*.

35

36 **Keywords**

37

38 Cave, Cordycipitaceae, new species, *Simplicillium* *pech-merlensis*

39

40 **Introduction**

41

42 Caves are generally considered as a quasi-extreme environment for microbial life, characterized by
43 a lack of natural light, relatively low inputs of organic nutrients, high humidity and constant, usually
44 low temperatures (Bastian & Alabouvette 2009; Kuzmina *et al.* 2012; Northup & Lavoie 2001). The
45 environmental conditions of life in caves are usually affected by various factors such as air currents,
46 water movements, chemolithoautotrophy or human visitors (Barton & Jurado 2007; Kuzmina *et al.*
47 2012; Ogórek *et al.* 2013; Ortiz *et al.* 2014). However, a high microbial diversity is found in caves,
48 including a fungal diversity (Vanderwolf *et al.* 2013; Nováková 2009) that plays an important role
49 in cave ecosystem, for example in the biomineralization process (Barton & Northup 2007).
50 Although it had been confirmed that the extreme and specific environment of caves meant that they

51 contain a highly specific fungal diversity, Zhang *et al.* (2018) found that the divergence time of the
52 new fungal species described in caves was incompatible with a cave-borne speciation. These authors
53 therefore concluded that these fungi had travelled from other environments, although they had not
54 yet been reported in terrestrial ecosystems. Whatever the origin of these species, caves are a
55 remarkable source for the discovery of new fungal species (Zhang *et al.* 2017; Zhang *et al.* 2020b;
56 Jiang *et al.* 2017).

57 The Pech-Merle show cave (Occitanie, France) is famous for its Palaeolithic rupestrian
58 representations dating back between 25 and 15 ka (Pastors *et al.* 2017), and notably for its “dappled
59 horses” (Fig. 1). This paper focuses on a fungal species that was recovered from the air of this cave
60 during an aerobiological survey. The molecular identification of the strain through the use of the
61 nuclear ribosomal internal transcribed spacer (ITS) region was unsatisfactory, with the closest
62 species matching at a rate below 90 %. These species belonged to the genus *Beauveria* Vuill., the
63 morphological features of which bore no similarities to the isolated strain. Following this
64 misidentification, we decided to study the position of this new species among the Cordycipitaceae
65 family, of which *Beauveria* is a monophyletic group (Sung *et al.* 2007; Kepler *et al.* 2017). The
66 study of the new species in relation to the context of the Cordycipitaceae family leads us to consider
67 the *Simplicillium* Zare & W. Gams genus and related genera.

68 The genus *Simplicillium* was introduced by Zare & Gams (2001) to accommodate species
69 that are morphologically close to the genus *Lecanicillium* W. Gams & Zare, but lack branching
70 conidiophores and have mostly solitary phialides. These two genera were formerly placed in
71 *Verticillium* sect. *Prostrata* W. Gams, which was described by Gams (1971) for prostrate
72 conidiophore-producing species. *Simplicillium* was recognized as a true clade among
73 Cordycipitaceae, while *Lecanicillium* is known to be a paraphyletic clade (Zare & Gams 2001;
74 Kepler *et al.* 2017). Some species of the genus *Lecanicillium* were therefore transferred to the genus
75 *Akanthomyces* Lebert, but the name has been conserved for other species because of the difficulty

76 to place some of these species in other genera. Zare & Gams (2016) also underlined the molecular
77 closeness between *Simplicillium* genus and the new genus *Leptobacillium* Zare & W. Gams, which
78 is represented by its type species *L. leptobactrum* (W. Gams) Zare & W. Gams composed of strains
79 formerly identified as *Veticillium leptobactrum* W. Gams. Okane *et al.* (2020) therefore proposed
80 to move several *Simplicillium* species into the *Leptobacillium* genus.

81 The genus *Simplicillium* initially included four species: the type species *Simplicillium*
82 *lanosoniveum* (J.F.H. Beyma) Zare & W. Gams, *S. obclavatum* (W. Gams) Zare & W. Gams, *S.*
83 *lamellicola* (F.E.V. Sm.) Zare & W. Gams and *S. wallacei* H.C. Evans. *Simplicillium wallacei*
84 (teleomorph: *Torrubiella wallacei* H.C. Evans) was later transferred into the *Lecanicillium* genus
85 on the basis of molecular studies (Sung *et al.* 2007; Zare & Gams 2008). Today, the genus
86 *Simplicillium* includes nearly 20 species, including already accepted species and recently proposed
87 species (Wei *et al.* 2019; Chen *et al.* 2019; Kondo *et al.* 2020; Zhang *et al.* 2020b). The first species
88 described in *Simplicillium* genus were mainly fungicolous (Zare & Gams 2001), but other species
89 among the genus have since been isolated from various substrates including insects, plants, soil or
90 water (Gomes *et al.* 2018; Chen *et al.* 2019; Liu & Cai 2012; Nonaka *et al.* 2013). Several
91 *Simplicillium* species are known to be bioactive compound producers (Fukuda *et al.* 2014; Dong *et*
92 *al.* 2018; Liang *et al.* 2017). The genus therefore shows potential applications in medicine as an
93 producer of antibiotics against bacteria and yeasts (Uchida *et al.* 2019; Rukachaisirikul *et al.* 2019),
94 as a means to control fungal plant pathogens (Dai *et al.* 2018; Ward *et al.* 2012), and a tool in the
95 biocontrol of insects and nematodes (Zhao *et al.* 2020; Lim *et al.* 2014; Skaptsov *et al.* 2017).

96 The species isolated in this study was characterized from its morphological and phylogenetic
97 features. The preliminary phylogenetic study in the Cordycipitaceae family placed the species
98 between *Torrubiella wallacei* and *Simplicillium* clade. The phylogenetic position of the species was
99 therefore closely studied in comparison to *Simplicillium* genus and its molecularly close

100 *Leptobacillum* genus after verifying that the species was closer to these two genera than to the
101 *Lecanicillium* complex.

102

103 **Materials & Methods**

104

105 *Collection and isolation*

106 The strain was collected on the 7th October 2016 during air samplings in the Palaeolithic show cave
107 of Pech-Merle (Cabrerets, France, 44° 30' 27" N, 1° 38' 40" E; Fig. 2). A Duo SAS Super 360 air
108 sampler (VWR-pbi, Milan, Italy) was used to detect cultivable microorganisms in cave air. 50 L of
109 air was collected using a 219-hole impactor containing appropriate culture media in 55 mm Petri
110 dishes. Fungi were isolated on malt extract agar (MEA; Merck KGaA, Darmstadt, Germany). The
111 Petri dishes containing impacted media were then taken to the laboratory for analysis. The plates
112 were incubated in a IPP55 incubators (Memmert GmbH + Co. KG, Büchenbach, Germany) for
113 seven days at 24 °C. Fungi were then isolated from each other using the same culture conditions.

114

115 *Morphological study*

116 The macroscopic features were assessed on Malt Extract Agar and on Potato Dextrose Agar (PDA,
117 VWR International, Radnor, USA) after a 10-day incubation at 5 °C, 25 °C or 30 °C. The
118 microscopic features were assessed on MEA after a 10-day incubation at 25 °C. Observations were
119 performed with a Jenavert optical microscope (Zeiss, Oberkochen, Germany) after treatment with
120 lactophenol cotton blue solution (Pro-Lab Diagnostics, Richmond Hill, Canada). Microscopic
121 characteristics were captured with an Lt365R digital camera (Teledyne Lumenera, Ottawa, Canada)
122 using the Archimed program (Microvision Instruments, Evry, France).

123

124

125 *Reference strains*

126 The strains used by Wei *et al.* (2019) and by Zare & Gams (2008) were chosen as reference for
127 *Simplicillium* and *Leptobacillium* genera respectively (Table 1). Additional strains and sequences
128 related to species not included in Wei *et al.* (2019) and in Zare & Gams (2008) were also included
129 by gathering information about these strains in the respective studies. For all these reference strains,
130 any sequences relative to DNA regions that were not studied in these original studies were also
131 added whenever they were available in the GeneBank. The names of the fungal species were updated
132 with the current names of the species, as they are defined in the Mycobank (Crous *et al.* 2004).

133

134 *DNA extraction, PCR amplification and sequencing*

135 Fungal DNA was extracted as described by Edel *et al.* (2001). Appropriate primers (Table 2) were
136 used to amplify the nuclear ribosomal internal transcribed spacer (ITS) region, the large subunit of
137 nuclear encoded ribosomal DNA (LSU), the small subunit of nuclear encoded ribosomal DNA
138 (SSU), the translation elongation factor 1-alpha gene (TEF1- α), the largest subunit of RNA
139 polymerase II (RPB1) and the second largest subunit of RNA polymerase II (RPB2). PCR was
140 performed in 25 μ l reactions, with 1 μ l of template DNA, 1 U of Taq DNA polymerase (Invitrogen,
141 Carlsbad, USA), 2.5 μ l of 10X Taq DNA polymerase buffer, 1 μ l of 2 mmol l⁻¹ dNTPs (Thermo
142 Fisher Scientific, Waltham, USA) and 1.5 μ l of each 10 μ mol l⁻¹ primer (Eurogentec, Seraing,
143 Belgium). Amplifications were performed on a PrimeG thermocycler (Bibby Scientific, Stone, UK)
144 using the following parameters: a 4-minute step at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30
145 s at the appropriate annealing temperature for each primer pair (Table 3), 40 s at 72 °C, and a final
146 10-minute extension step at 72 °C. PCR products were sequenced by Genoscreen (Lille, France)
147 using the same primer set. Generated sequences were submitted to the NCBI Genbank database
148 (Clark *et al.* 2016).

149

150 *Sequence alignment and phylogenetic analyses*

151 The sequences generated in this study were verified and assembled with BioEdit v. 7.2.5 (Hall
152 1999). The sequences related to reference strains were downloaded from the NCBI GenBank
153 database. Multiple sequence alignments for each studied DNA region were performed using the
154 FFT-NS-i alignment strategy from the MAFFT v. 7 web server (Kato *et al.* 2019). The
155 uninformative gaps and ambiguous regions were removed using the Gblocks program v. 0.91.1
156 implemented in the NGPhylogeny.fr service, with the program set for a lightly stringent selection
157 (Minimum number of sequences for a conserved position: 50 % of the sequences + 1; Minimum
158 length of a block: 5; Allowed gap position: “with half”);(Talavera & Castresana 2007; Lemoine *et*
159 *al.* 2019). The maximum likelihood (ML) analyses of concatenated regions were performed using
160 the graphical interface of RAxML v. 8 set to 1000 bootstrap iterations and the GTRGAMMA
161 substitution model (Stamatakis 2014; Edler *et al.* 2019). Bayesian inference (BI) analyses of
162 concatenated regions were performed using MrBayes v. 3.2.6 implemented in PhyloSuite v. 1.2.1,
163 based on a Markov Monte Carlo Chain (MCMC) set to two simultaneously executed runs for
164 10,000,000 generations with the GTRGAMMA substitution model (Ronquist *et al.* 2012; Zhang *et*
165 *al.* 2020a). Trees were sampled every 1000 generations, with 25 % of obtained trees being burned.
166 Generated trees were edited using MEGA X (Kumar *et al.* 2018).

167

168 **Results**

169

170 The phylogenetic study of *Simplicillium pech-merlensis* among 85 taxa of *Simplicillium* and
171 *Leptobacillium* genera produced slight differences between the maximum likelihood and the
172 Bayesian inference studies (Fig. 3 and 4). In both phylogenetic strategies, *Simplicillium pech-*
173 *merlensis* was clearly separated of all other species known in *Simplicillium* and *Leptobacillium*
174 genera.

175 *Simplicillium pech-merlensis* was located between the *Leptobacillium* and the *Simplicillium*
176 clades in 84% of the trees generated by the maximum likelihood study (Fig. 3), the closest diverging
177 species after *S. pech-merlensis* being *S. formicidae* W.H. Chen, C. Liu, Y.F. Liang & Z.Q. Liang.
178 *Simplicillium chinense* F. Liu & L. Cai, *S. coffeaneum* A.A.M. Gomes & O.L. Pereira and *S.*
179 *filiforme* R.M.F. Silva, R.J.V. Oliveira, Souza-Motta, J.L. Bezerra & G.A. Silva, were located in the
180 *Leptobacillium* clade in this study.

181 The Bayesian inference study placed *Simplicillium pech-merlensis* in the *Simplicillium* clade
182 between *S. formicidae* and the other species of the clade (Fig. 4). This placement was moderately
183 supported by a posterior probability of 0.61. *Simplicillum chinense*, *S. coffeaneum* and *S. filiforme*
184 were also located in the *Leptobacillium* clade in the Bayesian inference study as in the study led
185 through maximum likelihood.

186

187 **Taxonomy**

188

189 *Simplicillium pech-merlensis* J. Leplat, *sp. nov.* (Fig. 5)

190 *CBS: 147188*

191 *GenBank:* MW031272 (ITS), MW031268 (LSU), MW031740 (SSU), MW033222 (RPB1),
192 MW033223 (RPB2), MW033224 (TEF1- α), MW033221 (Tub)

193 *Systematic position:* Fungi, Dikarya, Ascomycota, Pezizomycotina, Sordariomycetes,
194 Hypocreomycetidae, Hypocreales, Cordycipitaceae

195 **Holotype** —FRANCE. Cabrerets, 44° 30' 27" N, 1° 38' 40" E, 07 October 2016. CBS 147188.

196 Isolated from the air of Pech-Merle cave.

197 *Etymology:* —The epithet *pech-merlensis* refers to the place where the species was isolated, namely

198 Pech-Merle cave.

199 Colonies on PDA reaching 20–22 mm diam. after 10 days at 25 °C, cottony, low convex,
200 margin entire to slightly undulated, white. Reverse white to pale yellow. No growth at 5 °C nor at
201 30 °C.

202 Colonies on MEA reaching 21–25 mm diam. after 10 days at 25 °C, cottony, low convex to
203 raise with concave edge, margin entire, white. Reverse pale yellow to orangey. No growth at 5 °C
204 nor at 30 °C.

205 *Asexual morph:* Hyphae hyaline and smooth-walled, Phialides 16–31 × 0.9–1.2 μm, mostly
206 solitary, arising from prostrate hyphae, straight to slightly curved, tapering to the apex. *Conidia* 1-
207 celled, smooth-walled, variable in size and shape, Microconidia 1.8–3 × 0.9–1.5 μm, adhering in
208 globose slimy heads, subglobose to ellipsoidal. Macroconidia 5–8 × 1–1.6 μm, fusiform. Octahedral
209 crystals absent.

210 *Notes:* —*Simplicillium pech-merlensis* seems morphologically close to *S. calcicola* Z.F. Zhang, F.
211 Liu & L. Cai, *S. album* Z.F. Zhang & L. Cai and *S. lamellicola*. Furthermore, *S. pech-merlensis*, *S.*
212 *calcicola* and *S. album* were isolated from the same habitat, i.e. karst cave. While *S. pech-merlensis*
213 was isolated from the cave air, *S. calcicola* was isolated from the limestone (Zhang *et al.* 2017) and
214 *S. album* was isolated from the soil (Zhang *et al.* 2020b). Each species produces both microconidia
215 and macroconidia. *Simplicillium lamellicola* is distinguished from the three other species by the
216 production of octahedral crystals. *Simplicillium album* produces larger macroconidia (8.0–11.0 (–
217 13.0) × 2.0–3.5 μm) than *S. pech-merlensis* (5–8 × 1–1.6 μm) and *S. calcicola* (4.5–8.0 × 1.0–2.0
218 μm). Finally, *Simplicillium pech-merlensis* grows slower in culture media (20–22 mm diam after 10
219 days on PDA) than *S. calcicola* (34–38 mm diam after 10 days on PDA).

220

221

222

223

224 Discussion

225

226 The phylogenetic study of the species isolated in the air of the Pech-Merle cave placed it in the
227 *Simplicillium* genus as the new species *S. pech-merlensis*. The *Simplicillium* genus is characterized
228 by the absence of branching conidiophores and by mostly solitary phialides (Zare & Gams 2001),
229 features that are also expressed by *S. pech-merlensis*. While *S. pech-merlensis* matched with the
230 *Simplicillium* clade when the phylogeny was studied through Bayesian inference, the study through
231 maximum likelihood raised the possibility that this new species could belong to *Leptobacillum*
232 genus due to the switch of placement between *S. pech-merlensis* and *S. formicidae*, *S. pech-*
233 *merlensis* being placed between *S. formicidae* and the *Leptobacillum* clade. Different results
234 obtained with these two phylogenetic methods often stem from insufficient data in which case each
235 method treats ambiguities differently (Brooks *et al.* 2007). The DNA sequences available regarding
236 *S. formicidae* are ITS, RPB1, RPB2 while ITS, SSU, LSU, and TEF-1 α sequences are mainly used
237 for other species included in this study, RPB1 and RPB2 sequences being scarcely available. This
238 difference in available DNA sequences could be the cause of the uncertain placement of *S.*
239 *formicidae*. Every species described to date in the genus *Leptobacillum* is characterized by chains
240 of conidia (Okane *et al.* 2020; Zare & Gams 2016; Sun *et al.* 2019), and this does not correspond to
241 the features of *S. pech-merlensis*. The new species was therefore described as a *Simplicillium* genus
242 member.

243 The morphological study highlighted the proximity between *S. pech-merlensis*, *S. album* and
244 *S. calcicola* since each species produces both microconidia and macroconidia and is characterized
245 by an absence of octahedral crystals (Zhang *et al.* 2017; Zhang *et al.* 2020b). Furthermore, the three
246 species were first isolated from caves. However, the slower growth rate of *S. pech-merlensis*
247 compared to *S. calcicola*, its smaller macroconidia compared to *S. album* and the significant
248 differences noticed between the DNA sequences of these three species supported the description of

249 a new species. Since only one specimen of the new species *S. pech-merlensis* has been isolated to
250 date, further isolations of other specimens of the same species are necessary in the future.

251 Besides these three species first isolated from a cave habitat, other *Simplicillium* species have
252 also often been recovered in sediments, in bat guano, on bats or on insect cadavers in the cave
253 environment (Vanderwolf *et al.* 2016; Kubátová & Dvorák 2005; Mitova *et al.* 2017; Nováková
254 2009). *Simplicillium* species were first described as fungicolous fungi (Zare & Gams 2001), but
255 several species are known for their association with arthropods (Chen *et al.* 2019; Lim *et al.* 2014;
256 Wei *et al.* 2019). It is therefore not surprising to frequently isolate *Simplicillium* species from caves,
257 as arthropods are generally abundant in this type of environment (Jurado *et al.* 2008).

258 The number of new species proposed in the genus *Simplicillium* has rapidly increased over
259 the past years. Since the description of *S. calcicola* in 2017 (Zhang *et al.* 2017), *S. coffeaneum* and
260 *S. filiforme* were described in 2018 (Gomes *et al.* 2018; Crous *et al.* 2018), *S. cicadellidae* W.H.
261 Chen, C. Liu, Y.F. Han, J.D. Liang & Z.Q. Liang, *S. formicae* D.P. Wei & K.D. Hyde, *S. formicidae*
262 and *S. lepidopterum* W.H. Chen, C. Liu, Y.F. Han, J.D. Liang & Z.Q. Liang were described in 2019
263 (Chen *et al.* 2019; Wei *et al.* 2019), and *S. spumae* N. Kondo, H. Iwasaki & Nonaka, *S. album*, *S.*
264 *humicola* and very recently *S. yunnanense* H. Yu, Y.B. Wang, Y. Wang & Zhu L. Yang were
265 described in 2020 (Kondo *et al.* 2020; Zhang *et al.* 2020b; Wang *et al.* 2020). Unfortunately, the
266 description of this last species was subsequent to our phylogenetic study. However, the chains of
267 conidia observed in *S. yunnaense* are not formed in *S. pech-merlensis*, leading to the reasonable
268 conclusion that these two species are different.

269 Changes occur rapidly in *Simplicillium* genus: Okane *et al.* (2020) have already proposed to
270 move the recently described species *S. coffeaneum*, as well as *S. chinense*, to the *Leptobacillium*
271 genus with the respective new names *L. coffeaneum* and *L. chinense* following an ITS and LSU
272 sequencing study that placed these two species in the *Leptobacillium* clade. The results of our study
273 confirmed this place in the *Leptobacillium* clade and a similar result was found concerning *S.*

274 *filliforme*, which could therefore also be moved to the *Leptobacillium* genus as *L. filiforme comb.*
275 *nov.* As we noted previously, with the exception of *S. coffeaneum*, all of these species and other
276 species that have already been described as belonging to the genus *Leptobacillium* produce chains
277 of conidia, which could therefore be a good phylogenetic marker to differentiate *Leptobacillium*
278 from *Simplicillium*. It would also be interesting to examine the affiliation of the new species *S.*
279 *yunnanense* to *Leptobacillium* genus because this species also produces chains of conidia, and the
280 original study describing this species mainly focuses on the Cordycipitadeae family and did not
281 focus on *Simplicillium* and *Leptobacillium* genera.

282

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284

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Accepted Version

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287

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Accepted Version

493 **TABLE 1.** Fungal taxa used in the study of the position of *Simplicillium pech-merlensis* among *Simplicillium* and *Leptobacillium* genera.

Species	Collection number	Référence						
		ITS	SSU	LSU	TEF1- α	RPB1	RPB2	
<i>Leptobacillium leptobactrum</i>	CBS 414.70	MH859773	EF641846	MH871535	-	-	-	(Zare & Gams 2008)
<i>Leptobacillium leptobactrum</i>	CBS 775.69	MH859422	-	MH871193	-	-	-	(Zare & Gams 2008)
<i>Leptobacillium leptobactrum</i>	CBS 305.93	EF641871	-	-	-	-	-	(Zare & Gams 2008)
<i>Leptobacillium leptobactrum</i>	CBS 266.94	EF641870	-	-	-	-	-	(Zare & Gams 2008)
<i>Leptobacillium leptobactrum</i>	CBS 116723	EF641869	-	-	-	-	-	(Zare & Gams 2008)
<i>Leptobacillium leptobactrum</i>	CBS 109351	EF641863	-	-	-	-	-	(Zare & Gams 2008)
<i>Leptobacillium leptobactrum</i>	JCM 39056	LC496868	LC496903	LC496888	LC496918	-	-	(Kondo <i>et al.</i> 2020)
<i>Leptobacillium leptobactrum</i> var. <i>calidius</i>	CBS 748.73	EF641867	EF641851	KU382227	-	-	-	(Zare & Gams 2008)
<i>Leptobacillium leptobactrum</i> var. <i>calidius</i>	CBS 703.86	EF641866	EF641850	KU382226	-	-	-	(Zare & Gams 2008)
<i>Leptobacillium leptobactrum</i> var. <i>calidius</i>	CBS 251.81	KU382173	-	-	-	-	-	(Zare & Gams 2008)
<i>Leptobacillium leptobactrum</i> var. <i>calidius</i>	CBS 160.94	KU382172	-	-	-	-	-	(Zare & Gams 2008)
<i>Leptobacillium leptobactrum</i> var. <i>leptobactrum</i>	CBS 774.69	MH859421	-	MH871192	-	-	-	(Zare & Gams 2008)
<i>Leptobacillium leptobactrum</i> var. <i>leptobactrum</i>	IRAN 1230	-	-	KU382225	-	-	-	(Zare & Gams 2016)
<i>Leptobacillium leptobactrum</i> var. <i>leptobactrum</i>	CBS 771.69	EF641868	EF641852	KU382224	-	-	-	(Zare & Gams 2008)
<i>Leptobacillium muralicola</i>	CGMCC 3.19014	MH379983	-	MH379997	-	-	-	(Sun <i>et al.</i> 2019)
<i>Leptobacillium symbioticum</i>	NBRC 104297	LC485674	-	AB378539	-	-	-	(Okane <i>et al.</i> 2020)
<i>Leptobacillium symbioticum</i>	NBRC 113865	LC485673	-	LC506046	-	-	-	(Okane <i>et al.</i> 2020)
<i>Leptobacillium symbioticum</i>	OPTF00168	LC485675	-	LC506047	-	-	-	(Okane <i>et al.</i> 2020)
<i>Simplicillium album</i>	CGMCC 3.19635	MK329133	-	MK329038	MK336068	-	-	(Zhang <i>et al.</i> 2020b)
<i>Simplicillium album</i>	LC 12543	MK329134	-	MK329039	MK336069	-	-	(Zhang <i>et al.</i> 2020b)
<i>Simplicillium album</i>	LC 12557	MK329135	-	MK329040	MK336070	-	-	(Zhang <i>et al.</i> 2020b)
<i>Simplicillium aogashimaense</i>	JCM 18167	AB604002	LC496889	LC496874	LC496904	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium aogashimaense</i>	JCM 18168	AB604004	LC496890	LC496875	LC496905	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium calcicola</i>	LC 5371	KU746705	KY883300	KU746751	KX855251	-	KY883258	(Wei <i>et al.</i> 2019)
<i>Simplicillium calcicola</i>	LC 5586	KU746706	KY883301	KU746752	KX855252	-	KY883257	(Wei <i>et al.</i> 2019)
<i>Simplicillium chinense</i>	LC 1342	JQ410323	-	JQ410321	-	-	-	(Wei <i>et al.</i> 2019)

<i>Simplicillium chinense</i>	LC 1345	NR155782	-	JQ410322	-	-	-	(Chen <i>et al.</i> 2019)
<i>Simplicillium chinense</i>	EXF 8701	KP034998	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium chinense</i>	CGMCC 3.14970	JQ410324	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium cicadellidae</i>	GY11011	MN006243	-	-	MN022263	MN022271	-	(Chen <i>et al.</i> 2019)
<i>Simplicillium cicadellidae</i>	GY11012	MN006244	-	-	MN022264	MN022272	-	(Chen <i>et al.</i> 2019)
<i>Simplicillium coffeanum</i>	COAD 2057	MF066034	-	MF066032	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium coffeanum</i>	COAD 2061	MF066035	-	MF066033	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium cylindrosporium</i>	JCM 18169	AB603989	LC496891	LC496876	LC496906	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium cylindrosporium</i>	JCM 18170	AB603994	LC496892	LC496877	LC496907	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium cylindrosporium</i>	JCM 18171	AB603997	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium cylindrosporium</i>	JCM 18172	AB603998	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium cylindrosporium</i>	JCM 18173	AB603999	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium cylindrosporium</i>	JCM 18174	AB604005	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium cylindrosporium</i>	JCM 18175	AB604006	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium filiforme</i>	URM 7918	MH979338	-	MH979399	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium formicae</i>	MFLUCC 18-1379	MK766511	MK765046	MK766512	MK926451	MK882623	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium formicidae</i>	DL10041	MN006241	-	-	-	MN022269	MN022267	(Chen <i>et al.</i> 2019)
<i>Simplicillium formicidae</i>	DL10042	MN006242	-	-	-	MN022270	MN022268	(Chen <i>et al.</i> 2019)
<i>Simplicillium humicola</i>	CGMCC 3.19573	MK329136	-	MK329041	MK336071	-	-	(Zhang <i>et al.</i> 2020b)
<i>Simplicillium humicola</i>	LC 12494	MK329137	-	MK329042	MK336072	-	-	(Zhang <i>et al.</i> 2020b)
<i>Simplicillium lamellicola</i>	UAMH 2055	AF108471	-	-	-	-	-	(Chen <i>et al.</i> 2019)
<i>Simplicillium lamellicola</i>	UAMH 4785	AF108480	-	-	-	-	-	(Chen <i>et al.</i> 2019)
<i>Simplicillium lamellicola</i>	IMI 234410	AY555956	-	-	-	AY555900	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium lamellicola</i>	CBS 116.25	AJ292393	AF339601	AF339552	DQ522356	DQ522404	DQ522462	(Wei <i>et al.</i> 2019; Zare & Gams 2008)
<i>Simplicillium lamellicola</i>	KYK00006	AB378533	-	-	-	-	-	(Chen <i>et al.</i> 2019)
<i>Simplicillium lanosoniveum</i>	CBS 101267	AJ292395	AF339603	AF339554	DQ522357	DQ522405	DQ522463	(Zare & Gams 2008)
<i>Simplicillium lanosoniveum</i>	CBS 962.72	EF641862	-	-	-	-	-	(Wei <i>et al.</i> 2019; Zare & Gams 2008)
<i>Simplicillium lanosoniveum</i>	SSBG2	MG807436	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium lanosoniveum</i>	CBS 531.72	MH860557	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium lanosoniveum</i>	CBS 321.72	MH860488	MK463995	-	-	-	-	(Wei <i>et al.</i> 2019)

<i>Simplicillium lanosoniveum</i>	vecl-02	KM035982	-	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium lanosoniveum</i>	vecl-01	KM035981	-	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium lanosoniveum</i>	Cs0701	EU939525	-	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium lanosoniveum</i>	CHE CNRCB-373	KX686123	-	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium lanosoniveum</i>	CBS 123.42	MH856100	-	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium lanosoniveum</i>	2502	KT878334	-	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium lanosoniveum</i>	Tr3	MG026635	-	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium lanosoniveum</i>	YLAC-5	KY552635	-	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium lanosoniveum</i>	MFLUCC 18-1385	MK752683	MK752791	MK752849	MK926450	MK882622	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium lanosoniveum</i>	CBS 704.86	AJ292396	AF339602	AF339553	DQ522358	DQ522406	DQ522464	-	(Wei <i>et al.</i> 2019; Zare & Gams 2008)
<i>Simplicillium lanosoniveum</i> var. <i>tianjinensis</i>	DT06	HM989951	-	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium lepidopterorum</i>	GY29131	MN006246	-	-	MN022265	MN022273	-	-	(Chen <i>et al.</i> 2019)
<i>Simplicillium lepidopterorum</i>	GY29132	MN006245	-	-	MN022266	MN022274	-	-	(Chen <i>et al.</i> 2019)
<i>Simplicillium minatense</i>	JCM 18176	AB603992	LC496893	LC496878	LC496908	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium minatense</i>	JCM 18177	AB603991	-	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium minatense</i>	JCM 18178	AB603993	LC496894	LC496879	LC496909	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium obclavatum</i>	JCM 18179	AB604000	-	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium obclavatum</i>	CBS 311.74	AJ292394	AF339567	AF339517	EF468798	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium pech-merlensis</i>	CBS 147188	MW031272	MW031740	MW031268	MW033224	MW033222	MW033223	This study	
<i>Simplicillium spumae</i>	JCM 39050	LC496869	LC496898	LC496883	LC496913	-	-	-	(Kondo <i>et al.</i> 2020)
<i>Simplicillium spumae</i>	JCM 39051	LC496870	LC496899	LC496884	LC496914	-	-	-	(Kondo <i>et al.</i> 2020)
<i>Simplicillium spumae</i>	JCM 39054	LC496871	LC496902	LC496887	LC496917	-	-	-	(Kondo <i>et al.</i> 2020)
<i>Simplicillium subtropicum</i>	JCM 18180	AB603990	LC496895	LC496880	LC496910	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium subtropicum</i>	JCM 18181	AB603995	LC496896	LC496881	LC496911	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium subtropicum</i>	JCM 18182	AB603996	-	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium subtropicum</i>	JCM 18183	AB604001	-	-	-	-	-	-	(Wei <i>et al.</i> 2019)
<i>Simplicillium subtropicum</i>	JCM 39052	LC496872	LC496900	LC496885	LC496915	-	-	-	(Kondo <i>et al.</i> 2020)
<i>Simplicillium subtropicum</i>	JCM 39053	LC496873	LC496901	LC496886	LC496916	-	-	-	(Kondo <i>et al.</i> 2020)
<i>Simplicillium sympodiophorum</i>	JCM 18184	AB604003	LC496897	LC496882	LC496912	-	-	-	(Wei <i>et al.</i> 2019)

495 **TABLE 2.** Primers used to amplify *Simplicillium pech-merlensis* in this study.

Target gene	Primer	Primer DNA sequence	Annealing temperature	Reference
ITS	ITS1-F	CTTGGTCATTTAGAGGAAGTAA	50 °C	(Gardes & Bruns 1993) (White <i>et al.</i> 1990)
	ITS4	TCCTCCGCTTATTGATATGC		
LSU	LR0R	GTACCCGCTGAACTTAAGC	56 °C	(Vilgalys & Hester 1990)
	LR5	ATCCTGAGGGAAACTTC		
SSU	NS1	GTAGTCATATGCTTGCTC	55 °C	(White <i>et al.</i> 1990)
	NS4	CTCCGTCAATTCCTTTAAG		
TEF-1 α	983F	GCYCCYGGHCAYCGTGAYTTYAT	55 °C	(Rehner & Buckley 2005)
	2218R	ATGACACCRACRGCRCRGTGTG		
RPB1	CRPB1	CCWGGYTTYATCAAGAARGT	55 °C	(Castlebury <i>et al.</i> 2004) (Matheny <i>et al.</i> 2002)
	RPB1Cr	CCNGCDATNCRTRTCCATRTA		
RPB2	fRPB2-5f	GAYGAYMGWGATCAYTTYGG	58 °C	(Liu <i>et al.</i> 1999)
	fRPB2-7cR	CCCATRGTGTYTRCCCAT		

496

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497 **FIGURE 1.** Pointed-horses panel in the Pech-Merle show cave

498 The panel represents the paintings of dappled horses and negative hands for which the Pech-
499 Merle cave is known. The representations were mainly drawn with black pigments composed
500 of manganese and barium oxides as well as charcoal (Lorblanchet 2018). The drawing features
501 the use of red pigments composed of red ochre.

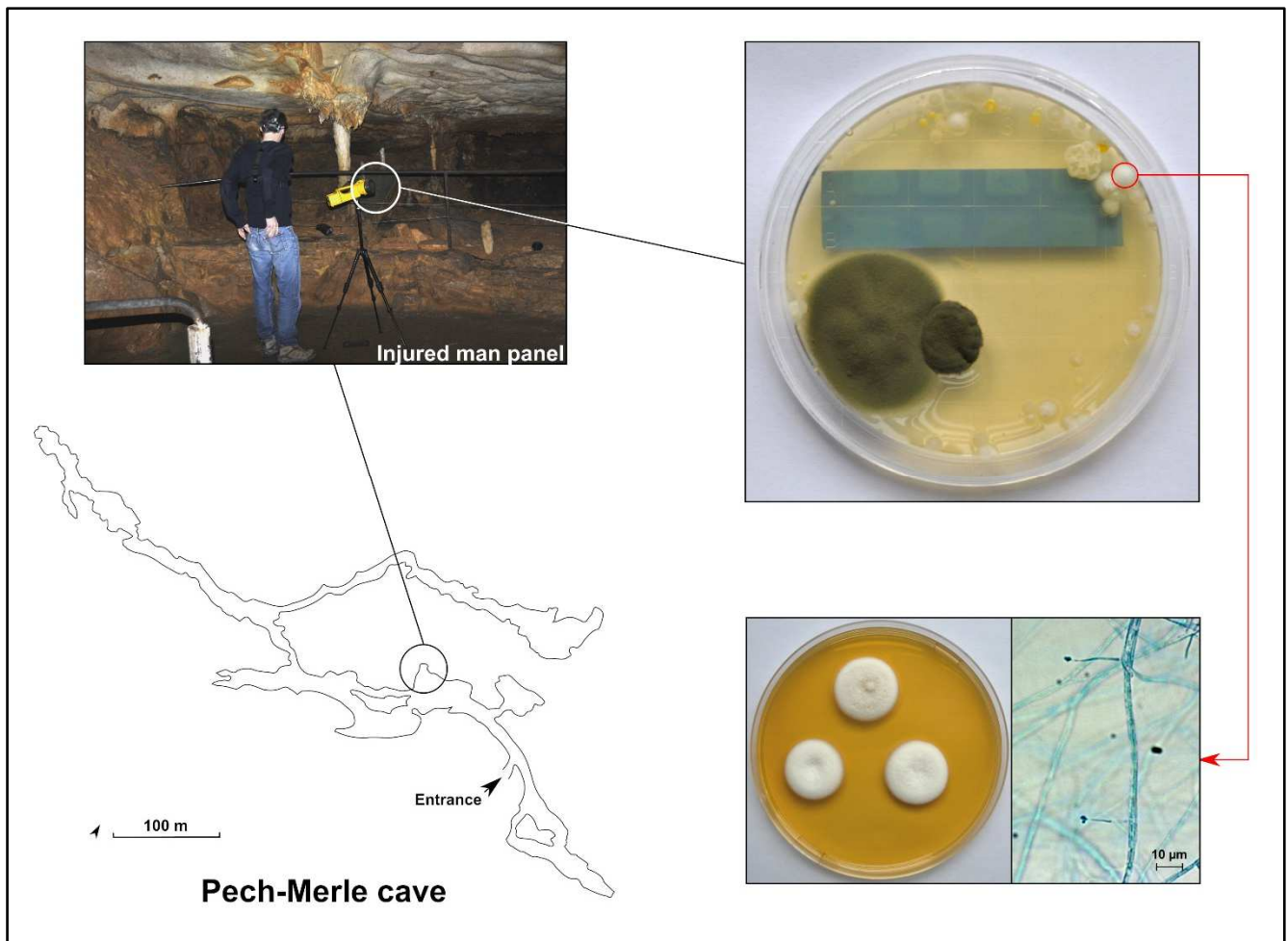


Acc

502 **FIGURE 2.** Isolation of *Simplicillium pech-merlensis*.

503 The species was isolated near the “injured man” panel in the Pech-Merle show cave, via air

504 samplings with impaction on Malt Extract Agar (MEA).



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505 **FIGURE 3.** Phylogenetic placement of *Simplicillium pech-merlensis* within *Simplicillium*
506 genus and *Simplicillium* related genera assessed through maximum likelihood.
507 Unrooted tree obtained from a dataset including 85 taxa, leading to a concatenated alignment
508 of 4725 bp from seven nuclear regions (ITS, LSU, SSU, RPB1, RPB2 and TEF1- α). Bootstrap
509 values greater than or equal to 50 % are located close to the corresponding node. The newly
510 described species is indicated in red.

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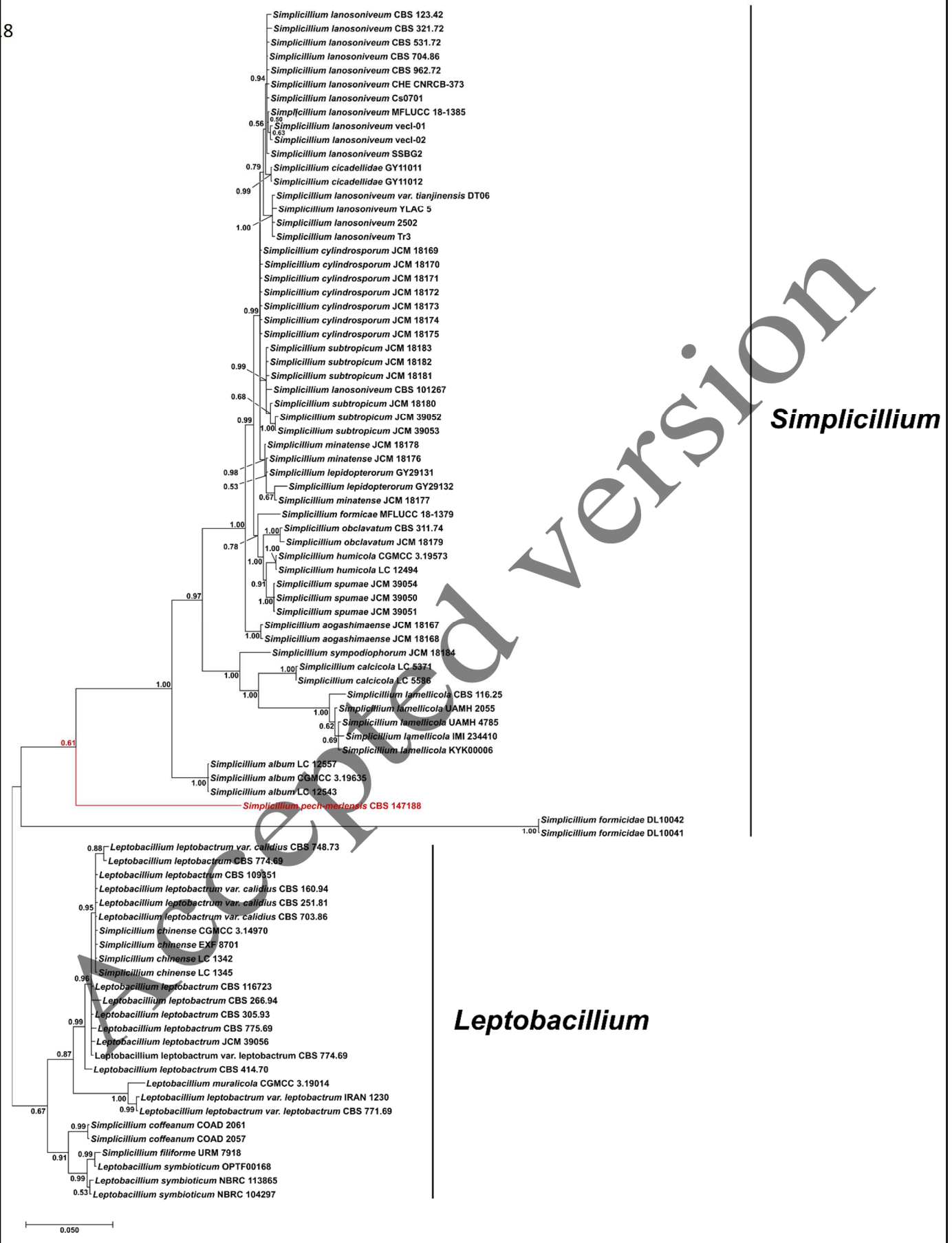


Simplicillium

Leptobacillum

512 **FIGURE 4.** Phylogenetic placement of *Simplicillium pech-merlensis* within *Simplicillium* and
513 *Leptobacillium* genera assessed through Bayesian inference.
514 Unrooted tree obtained from a dataset including 186 taxa, leading to a concatenated alignment
515 of 4863 bp from seven nuclear regions (ITS, LSU, SSU, RPB1, RPB2 and TEF1- α). Bayesian
516 posterior probabilities greater than or equal to 0.50 are located close to the corresponding node.
517 The newly described species is indicated in red.

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519 **FIGURE 5.** *Simplicillium pech-merlensis*

520 a–b. Upper and reverse views of the culture on MEA (10 d at 25 °C). c–d. Upper and reverse
521 views of the culture on PDA (10 d at 25 °C). e–f. Solitary phialides and microconidia that adhere
522 to each other to form globose slimy heads. g. Microconidia and macroconidia. h. Solitary
523 phialide and macroconidia. Scale bars: e–h = 10 µm.

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