

1 **Trunk sap rot of Japanese cedar (*Cryptomeria japonica*) caused by *Fomitiporella***  
2 ***sinica***

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23 **Abstract**

24 Trunk rot caused by the wood decay fungus *Fomitiporia torreyae* is a serious disease of  
25 Japanese cedar. The rot is characterized by white sap rot with brown zone lines and  
26 vertical depressions on the trunk. During our field investigations on this disease, we found  
27 another sap rot on Japanese cedar, characterized by white rot with brown zone lines, with  
28 or without less obvious depressions on the trunk. The results of phylogenetic analysis of  
29 the most frequently isolated fungus from the diseased trees and morphological  
30 observation of basidiocarps previously identified as *Fomitiporella umbrinella* showed  
31 that both represented *Fomitiporella sinica*. Our inoculation tests using Japanese cedar  
32 showed that *F. sinica* caused longer discoloration in sapwood compared with control  
33 treatment. Therefore, we concluded that *F. sinica* was the cause of trunk rot on Japanese  
34 cedar as reported for *F. torreyae*, but trunk depressions were not obvious in the trees  
35 infected with *F. sinica*. Moreover, branch scars were found around the middle of decaying  
36 parts of diseased trees infected with *F. sinica*, and infection with this fungus was assumed  
37 to occur via dead branches or pruning scars.

38

39 **Keywords:** decay disease, *Fomitiporella umbrinella*, *Fomitiporia torreyae*, wood decay

40

41 **Introduction**

42 Japanese cedar, *Cryptomeria japonica* (Thunb. ex L.f.) D. Don, is widely planted for  
43 timber production and is one of the most important silvicultural tree species in Japan.  
44 Decay diseases often result in economic losses of silvicultural living trees, but serious  
45 decay diseases in Japanese cedar plantations have not been reported except for trunk rot  
46 by *Fomitiporia torreyae* Y.C. Dai & B.K. Cui. This disease was first reported in southern  
47 Ibaraki Prefecture (Imazeki 1960) and was later found in Chiba Prefecture (Aoshima et  
48 al. 1964). More than 90% of the trees were occasionally affected by this disease at  
49 severely damaged stands (Matsubara et al. 2009). This trunk rot is characterized by white  
50 sap rot with brown zone lines and vertical depressions on the trunk (Imazeki 1960)  
51 (Figures 1A, B, C). In severely damaged trees, the depressions are more than 6 m in length  
52 and 5 cm in depth, and decayed wood below the depression is frequently exposed after  
53 debarking (Imazeki 1960). As branch scars are always found around the middle of the  
54 trunk depression, infection by the fungus causing the decay is assumed to occur via dead  
55 branches (Imazeki 1960; Aoshima et al. 1964). In addition, the depressions on the trunk  
56 may develop vertically because the causal fungus likely kills cambium along with vertical  
57 spread of the decay (Imazeki 1960).

58 This disease had long been found only in Chiba Prefecture and limited areas of the  
59 neighboring Ibaraki Prefecture (Miyuki et al. 2014), although *F. torreyae* was reported to  
60 cause dieback of Japanese umbrella pine, *Sciadopitys verticillata* (Thunb. Siebold &  
61 Zucc) (Hattori et al. 2012; Ota et al. 2014) and dwarfing of Japanese pear, *Pyrus pyrifolia*  
62 var. *culta* (Makino) Nakai in other prefectures (Shiota et al. 2008; Ota et al. 2014;  
63 Nakamura 2016). Recently, a similar trunk rot on Japanese cedars was found in Kyoto  
64 Prefecture, more than 350 km from Chiba and Ibaraki Prefectures, and the causal fungus

65 of the decay was also identified as *F. torreyae* based on morphological observations of  
66 the basidiocarps produced on diseased trees and phylogenetic analysis of isolates obtained  
67 from the decayed wood of diseased trees (Ota et al. 2016). In addition, Ichihara et al.  
68 (2021) detected another wood decay fungus, *Fomitiporella umbrinella* (Bres.) Murrill  
69 from trunk rot on Japanese cedar trees at the same site, but a relationship between the  
70 fungus and the rot had not been examined. During additional field investigations of the  
71 decay in this site, we found another serious sap rot on Japanese cedar, characterized by  
72 white rot with brown zone lines, with or without less obvious depressions on the  
73 trunk. Thus, another serious rot on Japanese cedar except for caused by *F. torreyae* would  
74 be present in this site.

75 This study was performed to identify the causal fungi of trunk rot on Japanese cedar other  
76 than *F. torreyae*. To clarify the taxonomic positions of isolates obtained from the rot, we  
77 conducted phylogenetic analysis of the isolates and morphological observation of  
78 basidiocarps previously identified as *F. umbrinella* collected in Japan. To characterize  
79 the decay, we examined the damage and compared the symptoms with those caused by  
80 *F. torreyae*. In addition, to evaluate the pathogenicity, these isolates were inoculated onto  
81 living Japanese cedar.

82

### 83 **Materials and methods**

#### 84 ***Sampling of diseased trees and observation of damage***

85 Sampling was conducted at the Momoyama Experimental Forest Site, Kansai Research  
86 Center, Forestry and Forest Product Research Institute (34°56'26"N, 135°46'21"E)  
87 located in Kyoto. We cut 21 diseased standing trees mainly with longitudinal depressions  
88 on the trunk between 2012 and 2020. The approximate ranges of age and diameter at

89 breast height of the examined trees were 7–40 years and 10–25 cm, respectively. Among  
90 the trees examined, three standing trees showed trunk sap rot and colonization of a decay  
91 fungus different from *F. torreyae*. These trees and cultures were examined for decay and  
92 to determine the associated fungus.

93 To characterize external symptoms, we examined the above three trees to determine  
94 whether there were depressions in the trunk. These trees were cut into logs 20-cm in  
95 length and were then cut vertically at the center of the decayed area for observation of  
96 longitudinal sections. To characterize internal symptoms, we examined the decay  
97 characteristics and looked for possible infection routes into the trees, including branch  
98 scars and trunk injury at and around the middle of the decayed areas. In addition, the  
99 vertical lengths and heights of the decayed parts were measured on photographs using  
100 ImageJ software (Schneider et al. 2012).

101

### 102 ***Isolation and identification***

103 To isolate wood decay fungi from diseased trees, several wood pieces of approximately  
104  $2 \times 2 \times 2$  mm were excised from fresh-cut surfaces of the decayed wood of each sampling  
105 tree using flame-sterilized knives. The wood pieces were placed on potato dextrose agar  
106 (PDA; Nissui Pharmaceutical, Tokyo, Japan) with or without 10 µg/L benomyl (Benlate;  
107 Sumitomo Chemical, Tokyo, Japan) and then incubated at 20°C for 2 weeks to 1 month.  
108 During the incubation period, hyphal tips emerging from the inoculated wood pieces were  
109 transferred to fresh PDA for pure culture. The isolated fungi were identified based on the  
110 DNA sequence of the internal transcribed spacer (ITS) region of rRNA gene.

111 For molecular identification, genomic DNA was extracted from 2–4-week-old cultures  
112 on PDA at 20°C using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA)

113 according to the manufacturer's instructions. The ITS region was amplified using the  
114 primers ITS1F (Garden and Bruns 1993) or ITS5 and ITS4 (White et al. 1990).  
115 Polymerase chain reaction (PCR) was performed using 25- $\mu$ L reaction mixtures  
116 containing GoTaq Master Mix (Promega, Madison, WI, USA) according to the  
117 manufacturer's instructions. PCR consisted of an initial denaturation step at 95°C for 2  
118 min, followed by 35 cycles of 95°C for 1 min, 56°C for 30 s, and 72°C for 1 min, with a  
119 final extension at 72°C for 5 min. The resulting PCR products were purified using  
120 ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA). Direct sequencing of the  
121 PCR products was performed using the same primers as described above with the Big  
122 Dye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).  
123 Purified products were analyzed on the ABI 3130xl Genetic Analyzer (Applied  
124 Biosystems). As some sequencing results included ambiguous base calls possibly because  
125 of heterozygosity, these PCR products were cloned using the pGEM-T Easy Vector  
126 System (Promega). At least three transformed clones per isolate were amplified and  
127 sequenced as described above. Sequences were assembled and edited using MEGA X  
128 (Kumar et al. 2018) and were deposited in the DDBJ with accession nos. LC662769–  
129 LC662790 (Table 1).

130 Based on the results of BLASTn search (Altschul et al. 1997) using the sequences  
131 analyzed in this study, most isolates of wood decay fungi obtained from the trunk rot were  
132 determined to be a *Fomitiporella* sp. Therefore, phylogenetic analysis was performed  
133 using select sequencing data from *Fomitiporella* spp. and their allied taxa based on  
134 Salvador-Montoya et al. (2020), as well as those from isolates from the trunk rot.  
135 Moreover, we also included newly analyzed sequences of isolates from basidiocarps  
136 previously identified as *F. umbrinella* (= *Phellinus umbrinellus* (Bres.) Ryv.) collected in

137 Japan, i.e. Kyoto, Kagoshima and Okinawa Prefectures and Bonin Islands (Table 1). In  
138 the analysis, *Inocutis dryophila* was used as an outgroup according to Salvador-Montoya  
139 et al. (2020). Sequences were aligned using the online version of MAFFT 7 with the E-  
140 INS-i options (Kato et al. 2019). The sequence alignment was deposited in TreeBASE  
141 (ID: S29135). Phylogenetic trees were inferred by maximum likelihood analysis using  
142 MEGA X. Alignment gaps and missing data were eliminated from the alignment data  
143 using the complete deletion option, and 1000 bootstrap replicates were performed. The  
144 most appropriate nucleotide substitution model was determined in MEGA X using the  
145 model selection option.

146

#### 147 ***Morphological observations***

148 To clarify the taxonomic position of the *Fomitiporella* sp., morphological characteristics  
149 of the type specimen of *Fomitiporella sinica* Y.C. Dai, X.H. Ji & Vlasák were compared  
150 with specimens previously identified as *F. umbrinella* collected in Japan. All Japanese  
151 specimens were deposited at the Mycological Herbarium of the Forestry and Forest  
152 Products Research Institute (TFM), Japan (Table 1). Microscopic examination was  
153 conducted on freehand sections of specimens mounted in water, 5% (w/v) KOH solution,  
154 1% (w/v) phloxine solution, Melzer's reagent, and Cotton Blue. Spore measurements ( $n$   
155 = 40 per specimen) were made in KOH. The following abbreviations were used: IKI-,  
156 neither amyloid nor dextrinoid stained with Melzer's reagent; CB(+), cyanophilic after  
157 12 h of staining with Cotton Blue;  $Q$ , spore length/width ratio;  $Q_m$ , mean  $Q$ ;  $L_m$ , mean  
158 spore length;  $W_m$ , mean spore width.

159

#### 160 ***Evaluation of pathogenicity***

161 We conducted inoculation tests to evaluate the pathogenicity of the *Fomitiporella* sp.  
162 isolated from the decay on Japanese cedar. Two isolates (FFPRI421038 and  
163 FFPRI421039) (Table 1) were inoculated onto three Japanese cedar trees (ca. 20 years  
164 old, 9.5 m in height, 12 cm in diameter at breast height) at the nursery of the Forestry and  
165 Forest Product Research Institute (36°00'30"N, 140°07'54"E) on 30 Jul 2020. In addition,  
166 one *F. torreyae* isolate (FFPRI421024), which was isolated from decayed wood of  
167 Japanese cedar in Ibaraki Prefecture, was also used for the inoculation test. Japanese  
168 beech (*Fagus crenata*) sapwood dowels, 18 mm in length and 9 mm in diameter, were  
169 sterilized in distilled water at 121°C for 15 min. The dowels were then co-incubated with  
170 each isolate for 3 months on sawdust rice bran medium, which consisted of 25 g Japanese  
171 beech sawdust, 5 g rice bran, and 70 g distilled water. Incubated dowels were used as  
172 inocula for the inoculation test. We bored four holes, 18 mm in depth and 10 mm in  
173 diameter, on the stem of each test tree at a height of approximately 150 cm above the  
174 ground using a surface-sterilized hand drill. Inocula of the three isolates and a sterilized  
175 dowel as a control were inserted into the holes on each test tree. Thus, four treatments,  
176 i.e., inoculation of the three isolates and control, were conducted on each tree.  
177 Inoculated trees were cut down on 30 Jun 2021, approximately 48 weeks after inoculation.  
178 Logs approximately 40 cm in length were collected from approximately 20 cm above and  
179 below the inoculation points and cut vertically at each inoculation point. Each  
180 longitudinal section was photographed, and the lengths of discolored sapwood above and  
181 below the inoculation points were measured on the photographs using ImageJ. The  
182 lengths were defined as the distances from the upper or lower tips of the discolored areas  
183 to the edges of the inoculation holes. The average length of the upper and lower sides  
184 from each hole was used for evaluation. Fungal re-isolation was conducted from

185 discolored sapwood near each inoculation point in the same manner as described above  
186 for isolation from diseased trees.

187 Pairwise comparisons of the lengths between each isolate and the control were conducted  
188 by the Mann–Whitney U-test. Statistical analyses were performed using R v. 4.0.4 (R  
189 Core Team 2021).

190

## 191 **Results**

### 192 *Molecular phylogenetic analysis*

193 Based on the results of BLASTn search to infer the closest taxa, *Fomitiporella* sp. was  
194 the species most frequently isolated from the decayed wood of all sampled trees, and  
195 another wood decay fungus, *Amylostereum orientale* S.H. He & Hai J. Li, was  
196 occasionally isolated from one sampled tree (data not shown). From the newly analyzed  
197 sequences in this study, two representative sequences were chosen for phylogenetic  
198 analysis. Our isolates obtained from both decayed wood of Japanese cedar and  
199 *Fomitiporella* basidiocarps collected in Japan formed a strongly supported clade with  
200 sequences of *F. sinica* described in China (Figure 2). In addition, three phylogenetic  
201 lineages within *Fomitiporella* species described by Salvador-Montoya et al. (2020), i.e.  
202 *Fomitiporella* s.s., Clade I and II, were resolved. Consequently, we concluded that *F.*  
203 *sinica* is the correct name to represent the fungus precisely identified as *F. umbrinella* (as  
204 *P. umbrinellus*) in Japan.

205

### 206 *Taxonomy*

207 *Fomitiporella sinica* Y.C. Dai, X.H. Ji & Vlasák, Mycologia 109: 317. 2017

208 Basidiocarps perennial, resupinate. Poroid surface rust-brown to dull brown, more or less  
209 glancing and occasionally cracked when dry. Pores angular, 5–8 per mm. Tubes stratified,  
210 each layer up to 2.5 mm deep. Hyphal system dimitic. Generative hyphae hyaline, thin-  
211 to thick-walled, occasionally branched, frequently septate. Skeletal hyphae dominant,  
212 thick-walled, unbranched, aseptate. Setae absent. Cystidia and cystidioles absent.  
213 Rhomboid crystals variably present according to specimen, sparse or abundant.  
214 Basidiospores broadly ellipsoid, thick-walled, smooth, yellowish-brown in water,  
215 becoming chestnut to ferruginous in KOH, IKI–, CB(+),  $3.5\text{--}4.5 \times 2.5\text{--}3.5 \mu\text{m}$  based on  
216 seven specimens ( $n = 280$ ) including the holotype, range of  $L_m \times W_m = 4.0\text{--}4.3 \times 2.8\text{--}3.0$   
217  $\mu\text{m}$ ,  $L_m = 4.1 \mu\text{m}$ ,  $W_m = 2.9 \mu\text{m}$ ,  $Q = 1.18\text{--}1.70$ ,  $Q_m = 1.41$ ,  $Q_m$  range =  $1.35\text{--}1.53$ .

218 **Type of rot:** White rot.

219 **Distribution and substrate:** Known from warm temperate and subtropical areas of  
220 China and Japan. Basidiocarps found mainly on hardwood species (*Casuarina* sp.,  
221 *Clethra barbinervis* Siebold & Zucc., *Melia* spp., *Rhaphiolepis indica* (L.) Lindl.,  
222 *Rhododendron* sp.) but also on the conifer *C. japonica* in Japan (specimens not referred  
223 here). Based on this and previous phylogenetic analyses (Salvador-Montoya et al. 2020),  
224 this fungus also grows on *P. pyrifolia* var. *culta* and the roots of *Erythrorchis altissima*  
225 (Blume) Blume.

226 **Specimens examined:** CHINA, Jiangxi Prov., Guangzhou, Fenyi County, Dagang  
227 Mountain, on dead *Rhododendron* sp., 18 Sep 2008, coll. Y.C. Dai (holotype, BJFC  
228 004710); JAPAN, Kyoto Pref., Kyoto City, Mt. Kodaiji, on hardwood, 25 Oct 2012, Coll.  
229 T. Hattori (TH; TFM F-30082); Kyoto City, Matsugasaki, on dead standing *Clethra*  
230 *barbinervis*, 7 Mar 2021, Coll. Y. Ichihara (TFM F-30084); Okinawa Pref., Iriomote Is.,  
231 Ohmija, on hardwood, 20 Oct 2010, Coll. TH (TFM F-29139); Okinawa Is., Kunigami-

232 gun, Motobu, on living *Melia azedarach*, 11 Dec 2020, Coll. S. Tsujimoto (TFM F-  
233 30083); Tokyo Metro., Bonin Islands, Hahajima Is., Nakanotaira, on *Rhaphiolepis indica*,  
234 5 Nov 2012, Coll. TH, Y. Ota (YO) and H. Masuya (HM; TFM F-28658); Bonin Islands,  
235 Chichijima Is., Kuwanokiyama, on hardwood, 10 Nov 2013, Coll. TH, YO and HM (TFM  
236 F-28811).

237

### 238 ***Disease symptoms***

239 We found sap rot with or without less obvious depressions on the trunks of living Japanese  
240 cedars at the site (Figures 1D, E), and all of the associated decay was white rot with brown  
241 zone lines (Figure 1F). In addition, we observed branch scars around the middle of the  
242 decaying parts, and the scars were often more severely decayed than the surrounding  
243 sapwood (Figure 1G). The heights of the scars ranged from 2.4 to 7.3 m above the ground.  
244 The vertical lengths of the decaying parts of the three sampled trees ranged from 0.6 to  
245 2.4 m.

246

### 247 ***Pathogenicity***

248 Inoculated fungi were not re-isolated from one inoculated part with FFPRI421038 (*F.*  
249 *sinica*) and one with FFPRI421024 (*F. torreyae*). These data were excluded from  
250 subsequent analyses. In addition, another wood decay fungus, *Peniophora* sp., was  
251 isolated from one inoculated part in the control treatment, and the discoloration length  
252 was 60 mm. Although we have no information about the pathogenicity of *Peniophora* sp.  
253 on Japanese cedar, the data were excluded from the analyses to ensure reliable results.  
254 We did not find decayed areas on all of the inoculated trees but did observe discoloration

255 around inoculation holes on all trees. The lengths of discoloration caused by both of the  
256 inoculated fungi were significantly longer than those of the control ( $p < 0.05$ ) (Figure 3).

257

## 258 **Discussion**

259 The fungus examined here was first reported as *Poria inermis* Ellis & Everh. (= *Phellinus*  
260 *inermis* (Ellis & Everh.) G. Cunn., *Fomitiporella inermis* (Ellis & Everh.) Murrill) in  
261 Japan (Yasuda 1918). However, *P. inermis* has spores measuring  $5\text{--}6 \times 4\text{--}4.5 \mu\text{m}$  and  
262 strong host preference for Aquifoliaceae (Gilbertson and Ryvarden 1987), while Yasuda  
263 (1918) described the fungus as having smaller spores ( $4\text{--}5 \times 3\text{--}4 \mu\text{m}$ ), and host preference  
264 was not recognized in Japan. Therefore, *Phellinus umbrinellus* (= *Fomitiporella*  
265 *umbrinella*) has long been applied for the Japanese fungus (e.g., Hattori and Hongo 1990;  
266 Neda and Hattori 1991; Núñez and Ryvarden 2000). This fungus is often associated with  
267 living Japanese pear (Fukuta et al. 2017). Salvador-Montoya et al. (2020) concluded that  
268 *F. umbrinella* from Japanese pear as well as one of the mycorrhizal fungi on the climbing  
269 orchid *E. altissima* examined by Ogura-Tsujita et al. (2018) represent *F. sinica*, recently  
270 described from China, based on comparison of the donated sequencing data. The present  
271 study also confirmed that both the isolates from trunk sap rot of living Japanese cedar and  
272 several specimens identified as *F. umbrinella* in Japan actually represent *F. sinica*.

273 *Fomitiporella sinica* was described based on materials from dead and living angiosperm  
274 trees in China, but its pathogenicity in living trees was not noted in the original description  
275 (Ji et al. 2017). This fungus is often associated with Japanese pear trees damaged by  
276 Japanese pear dwarf disease (Fukuta et al. 2017). Imai and Inubushi (2019), however,  
277 concluded that *F. sinica* is not the causal fungus of this disease but may cause distinctive

278 trunk rot on living Japanese pear because, on inoculation tests, its decay length was three  
279 times that caused by *F. torreyae*, the causative fungus of Japanese pear dwarf disease.  
280 *Fomitiporella sinica* was isolated from most of the decayed parts of all diseased trees  
281 examined here. *Amylostereum orientale* (previously reported as *Amylostereum*  
282 *laevigatum* (Fr.) Boidin in Japan) was another decay fungus occasionally isolated from  
283 one of the diseased trees, but it causes wood discoloration on Japanese cedar and is not a  
284 cause of distinctive white rot with brown zone lines and trunk depressions (Tabata and  
285 Abe 1997, 1999a). In addition, *A. orientale* (as *A. laevigatum*) has limited decay ability  
286 on Japanese cedar (Tabata and Abe 1999b). Therefore, we concluded that *F. sinica* was  
287 the cause of the trunk rot on living Japanese cedar examined in this study. In our  
288 inoculation tests, the length of the discoloration caused by *F. sinica* isolates was longer  
289 than or almost identical to that caused by a *F. torreyae* isolate. Aoshima et al. (1964)  
290 reported that the length of depressions on trunks caused by *F. torreyae* was up to 6.0 m,  
291 suggesting that the decay length caused by this fungus can be nearly 6.0 m in some cases.  
292 Meanwhile, the decay length caused by *F. sinica* in this study was up to 2.5 m, although  
293 the number of samples examined in this study was limited. *Fomitiporella sinica* can be  
294 another serious trunk rot pathogen on Japanese cedar as reported for *F. torreyae*.  
295 The decay symptoms on Japanese cedar caused by *F. sinica* were partly similar to those  
296 caused by *F. torreyae*, sharing white rot with brown zone lines on the trunk sapwood  
297 (Figures 1A, F) and decay length up to several meters. However, trunk depression was  
298 not obvious in the trees infected by *F. sinica* examined in this study, while distinct stem  
299 depression above the decayed area is one of the characteristic symptoms caused by *F.*  
300 *torreyae*. Stem depression by *F. torreyae* is considered to be due to cambium necrosis  
301 following trunk rot below the depression (Imazeki 1960). In the present study, no or only

302 limited cambium necrosis was observed, and stem depression may not have been  
303 produced or may have been limited on the trees infected by *F. sinica*. However, our  
304 survey was conducted on limited trees within a single study site, and it is necessary to  
305 observe more diseased trees at various sites to define the exact symptoms caused by *F.*  
306 *sinica*.

307 Trunk rot caused by *F. sinica* may be confused with that caused by *F. torreyae*, especially  
308 during the early stages, and may be present in diseased stands infected with the latter  
309 because of the similar decay symptoms as indicated above. To evaluate the disease impact  
310 by *F. sinica* against timber production of Japanese cedar, it is necessary to re-examine the  
311 causal fungus at diseased areas previously identified as *F. torreyae*. Trunk rot of Japanese  
312 cedar by *F. torreyae* is often identified not only by the symptoms but also by  
313 morphological characteristics of the basidiocarps made on diseased trees and molecular  
314 characteristics of the isolates obtained from decayed wood. Identification by the latter  
315 two methods can be more accurate, but basidiocarps are produced infrequently on the  
316 infected trees, and molecular identification is time consuming. With regard to Japanese  
317 pear dwarf disease, selective primers and a multiplex loop-mediated isothermal  
318 amplification method were developed for specific detection of *F. torreyae* and *F. sinica*  
319 (as *F. umbrinellus*) (Suzuki et al. 2015; Fukuta et al. 2017). Use of this technique may  
320 enable accurate and rapid diagnosis of these trunk rots of Japanese cedar.

321 Branch scars were found around the middle of the decaying parts of diseased trees  
322 infected with *F. sinica*. Therefore, infection by the fungus is assumed to occur via dead  
323 branches or pruning scars. As our study site was an experimental stand and was not  
324 managed regularly for timber production, dead branches would have been more likely  
325 routes of infection than pruning scars. In the decay disease caused by *F. torreyae*, the

326 infection was also assumed to occur via dead branches (Imazeki 1960; Aoshima et al.  
327 1964). In fact, disease occurrence was lower in stands where pruning was performed to  
328 prevent and remove dead branches than in stands without pruning (Nakagawa 2000;  
329 Matsubara et al. 2009). Therefore, exclusion of possible infection routes can reduce the  
330 likelihood of disease occurrence. In this study, the heights of branch scars assumed to be  
331 infection routes by *F. sinica* ranged from 2.4 m to 7.3 m above the ground, suggesting  
332 that *F. sinica* may spread via basidiospores from infected trees to other trees. Therefore,  
333 basidiocarp formation may play an important role in disease spread within stands and to  
334 adjacent areas. *Fomitiporella sinica* appears on various hardwoods (Fukuta et al. 2017; Ji  
335 et al. 2017), and the basidiocarps in hardwood stands around Japanese cedar stands may  
336 act as infection sources. Basidiocarps of *F. torreyae* are rarely found on living Japanese  
337 cedar trees but are often observed on the cut and thinned wood residues of diseased trees  
338 unattended within stands (Iwasawa and Miyuki 2011). These residues would increase the  
339 inoculum mass of *F. torreyae* and the likelihood of disease occurrence. Although we do  
340 not have information about the effects of surrounding hardwood stands as sources of  
341 basidiospores to cause *Fomitiporella* stem rot on Japanese cedar, stand management to  
342 control this disease should include adequate pruning and removal of the diseased wood  
343 residues outside the stands.

344

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353

#### 354 **Disclosure statement**

355 The authors declare no conflicts of interest.

356

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449

450 Table 1. Isolates examined in this study.

Isolate no.	Location	Substrate	Specimen accession no. (TFM F)	Accession no. of ITS sequence
Isolates obtained from diseased trees				
FFPRI421036	Momoyama, Kyoto	decayed wood of <i>Cryptomeria japonica</i> 1	-	LC662775, LC662776
FFPRI421038	Momoyama, Kyoto	decayed wood of <i>Cryptomeria japonica</i> 2	-	LC662779, LC662780
FFPRI421039	Momoyama, Kyoto	decayed wood of <i>Cryptomeria japonica</i> 2	-	LC662781, LC662782
FFPRI421042	Momoyama, Kyoto	decayed wood of <i>Cryptomeria japonica</i> 3	-	LC662787, LC662788
Isolates previously identified as <i>Fomitiporella umbrinella</i>				
FFPRI421032	Ohmijya, Iriomote Is., Okinawa	basidiocarp on hardwood	29139	LC662769, LC662770
FFPRI421033	Ohsumi, Kagoshima	basidiocarp on hardwood	-	LC662771
FFPRI421034	Mt. Kodaiji, Kyoto	basidiocarp on hardwood	30082	LC662772, LC662773
FFPRI421035	Nakanotaira, Hahajima Is., Tokyo	basidiocarp on <i>Rhaphiolepis indica</i>	28658	LC662774
FFPRI421037	Kuwanokiyama, Chichijima Is., Tokyo	basidiocarp on hardwood	28811	LC662777, LC662778
FFPRI421040	Motobu, Okinawa	basidiocarp on <i>Melia azedarach</i>	30083	LC662783, LC662784
FFPRI421041	Matsugasaki, Kyoto	basidiocarp on <i>Clethra barbinervis</i>	30084	LC662785, LC662786

451

452 **Legends for figures**

453 Figure 1. Representative images of diseased Japanese cedar trees infected with  
454 *Fomitiporia torreyae* and *Fomitiporella sinica*. A: White rot with brown zone lines  
455 caused by *F. torreyae*. B: Trunk depression caused by *F. torreyae*. C: Trunk depressions  
456 on cross-sections of diseased trees by *F. torreyae*. D: Decay without obvious depression  
457 on cross-sections of diseased trees infected with *F. sinica*. E: Decay with less depression  
458 on cross-sections of diseased trees infected with *F. sinica*. F: White rot with brown zone  
459 lines caused by *F. sinica*. G: Branch scar on a longitudinal section around the middle of  
460 decaying parts due to *F. sinica*. dw: decayed wood; bs: branch scar. *Bars*: 3 cm.

461

462 Figure 2. Maximum likelihood tree generated from DNA sequences of the ITS region.  
463 Bootstrap values (1000 replicates) > 60% are indicated at the branch nodes. Sequences  
464 newly analyzed in this study are shown in **bold**.

465

466 Figure 3. Longitudinal lengths of discolored sapwood after inoculation with  
467 *Fomitiporella sinica* and *Fomitiporia torreyae* isolates. Values are means  $\pm$  SD.  $N = 4$  for  
468 FFPRI421038, FFPRI421024, and control;  $n = 5$  for FFPRI421039. FFPRI421038 and  
469 FFPRI421039 are *F. sinica*, and FFPRI421024 is *F. torreyae*.

Fig. 1

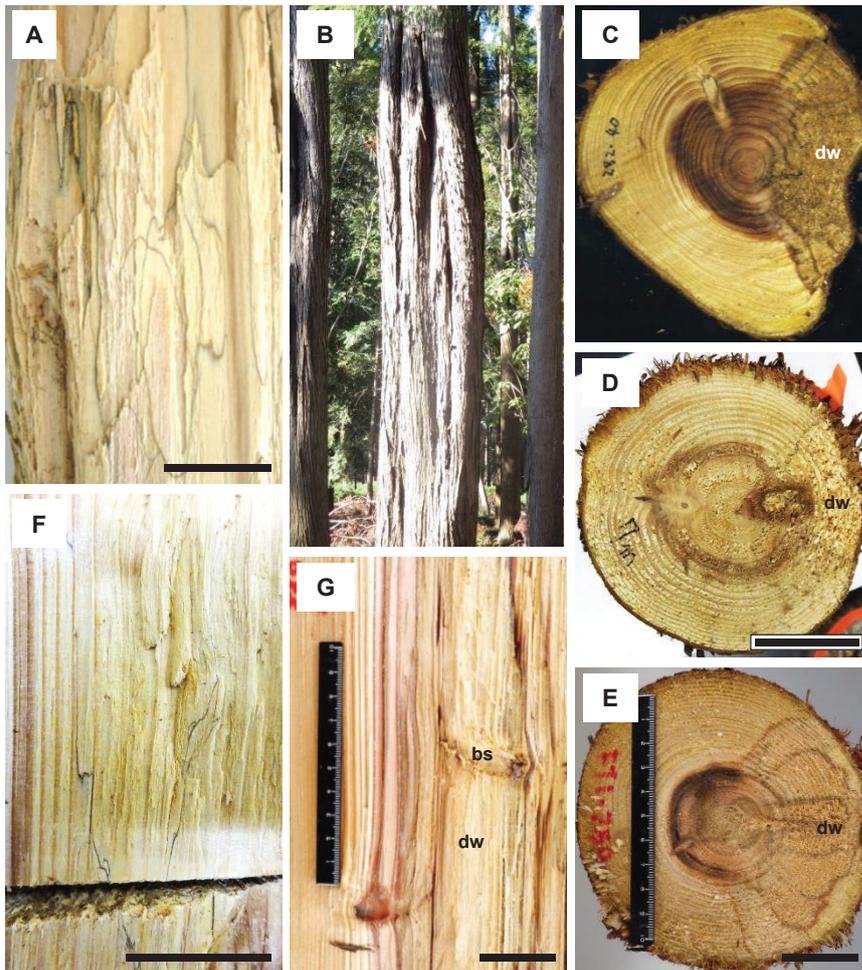


Fig. 2

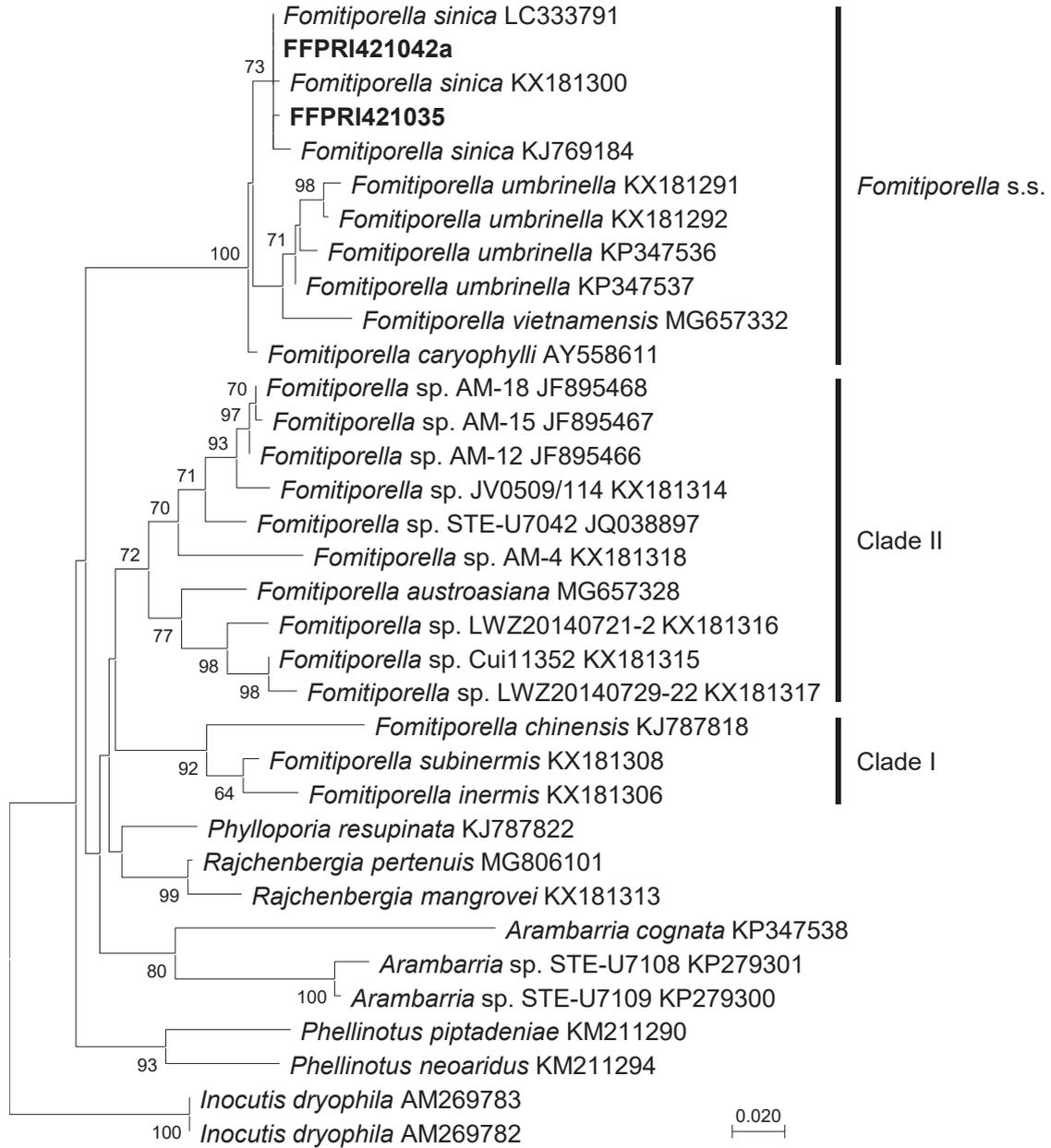


Fig. 3

