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Additional Information

Cadophora sabaouae sp. nov. and *Phaeoacremonium* species associated with Petri disease on grapevine propagation material and young grapevines in Algeria

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24 **Abstract**

25 W. Aigoun-Mouhous, A. E. Mahamedi, M. León, C. Chaouia, A. Zitouni, K. Barankova, A.
26 Eichmeier*, J. Armengol, D. Gramaje and A. Berraf-Tebbal. 2020. *Cadophora sabaouae* sp.
27 nov. and *Phaeoacremonium* species associated with Petri disease on grapevine propagation
28 material and young grapevines in Algeria. Plant Dis. XX:XX-XX.

29

30 A field survey conducted on asymptomatic grapevine propagation material from nurseries and
31 symptomatic young grapevines throughout different regions of Algeria yielded a collection of
32 70 *Phaeoacremonium*-like isolates and three *Cadophora*-like isolates. Based on morphology
33 and DNA sequence data of β-tubulin (*tub2*) and actin (*act*), five *Phaeoacremonium* species
34 were identified including *Phaeoacremonium minimum* (22 isolates), *P. venezuelense* (19
35 isolates), *P. parasiticum* (17 isolates), *P. australiense* (8 isolates) and *P. iranianum* (4 isolates).
36 The latter two species (*P. australiense* and *P. iranianum*) were reported for the first time in
37 Algeria. Multi-locus phylogenetic analyses (ITS, *tub2*, *tef1*) and morphological features,
38 allowed the description of the three isolates belonging to the genus *Cadophora* (WAMC34,
39 WAMC117 and WAMC118) as a novel species, named *Cadophora sabaouae* sp. nov.
40 Pathogenicity tests were conducted on grapevine cuttings cv. Cardinal. All the identified species
41 were pathogenic on grapevine cuttings.

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47 ## Introduction

48 Grapevine Trunk Diseases (GTDs) have spread alarmingly over the last three decades
49 into grapevine growing regions, worldwide (Hofstetter 2012; Bertsch et al. 2013; Larignon
50 2016, Gramaje et al. 2018). At the same time, decline and dieback symptoms in young
51 vineyards also dramatically increased since the early 1990's, when the wine industry entered a
52 period of rapid expansion, in which growers were forced to replant sizeable vineyard areas.
53 This grapevine planting "boom" favored an increasing movement of potentially contaminated
54 planting material (Gramaje and Armengol 2011; Gramaje et al. 2018). Thus, special attention
55 has been given to the grapevine propagating process. To date, many research studies have been
56 conducted to determine the identity of fungal pathogens associated with GTDs in grapevine
57 nurseries and the sources of inoculum. This was done with the main goal to improve the
58 phytosanitary quality of planting material and to minimize their economic impact (Halleen et
59 al. 2003, 2004; Gramaje et al. 2009; Rego et al. 2009; Agustí-Brisach et al. 2011; Gramaje and
60 Armengol 2011; Cabral et al. 2012; Gramaje et al. 2018; Pintos et al. 2018). All these works
61 confirmed a decrease in the survival rates of grafted grapevines affected by GTDs, grown in
62 field nurseries and in young vineyards.

63 Premature decline and dieback of young grapevines are caused by several GTDs
64 pathogens including black-foot and Petri disease fungi (Halleen et al. 2004; Agustí-Brisach and
65 Armengol 2013), as well as Botryosphaeriaceae species (Úrbez-Torres 2011). Petri disease
66 causes significant economic losses due to yield and quality reductions, as well as vineyard
67 replanting (Scheck et al. 1998). Wounds made during the grafting process provide entry ports
68 for the fungal pathogens associated with Petri disease (Carlucci et al. 2017; Gramaje et al. 2018;
69 Pintos et al. 2018). The external symptoms of Petri disease include stunted growth, reduced
70 vigor, delayed or absent sprouting, shortened internodes, sparse and chlorotic foliage with

71 necrotic margins, bud mortality, failure of the graft unions and general decline. Internal
72 symptoms of Petri disease are characterized by the presence of dark-colored phenolic
73 compounds in xylem vessels of the trunk in response to the fungal species growing in and
74 around the xylem vessels (Gramaje and Armengol 2011; Gramaje et al. 2018; De la Fuente et
75 al. 2016). Indeed, several fungal species are associated with Petri disease including numerous
76 species of *Phaeoacremonium*, *Phaeomoniella chlamydospora*, *Pleurostoma richardsiae* and
77 species of *Cadophora* (Halleen et al. 2007; Gramaje and Armengol 2011; Travadon et al. 2015;
78 Araujo da Silva et al. 2017; Gramaje et al. 2018).

79 The genus *Phaeoacremonium* (*P.*) was established by Crous et al. (1996), and since
80 then, 61 species have been identified based on morphological and molecular characteristics
81 (Mostert et al. 2006; Gramaje et al. 2009; Gramaje et al. 2012; Gramaje et al. 2015; Spies et al.
82 2018). Species of the genus *Phaeoacremonium* have a worldwide distribution and a wide host
83 range, including woody plants, insect larvae, arthropods and humans (Mostert et al. 2006;
84 Mohammadi and Sharifi 2016; Hashemi et al. 2017; Spies et al. 2018). According to Gramaje
85 et al. (2015) and Spies et al. (2018), 29 *Phaeoacremonium* species are known only from
86 grapevine. Among them, *P. minimum* appears to be the most widely distributed and the most
87 common in grapevines (Mostert et al. 2006; Péros et al. 2008; Berraf-Tebbal et al. 2011);
88 followed by *P. parasiticum* which has been isolated in relatively high frequencies (Gramaje et
89 al. 2015; Spies et al. 2018).

90 The genus *Cadophora* was established by Lagerberg et al. (1927), with *C. fastigiata* as
91 the type species. Currently, this genus comprises 28 species isolated from plants, decaying
92 wood and soil (Nilsson 1973; Kerry 1990; Blanchette et al. 2004, 2010; Di Marco et al. 2004;
93 Hujslová et al. 2010; Gramaje et al. 2011; Agustí-Brisach et al. 2013; Crous et al. 2015;
94 Travadon et al. 2015; Walsh et al. 2018; Marin-Felix et al. 2019; Bien et al. 2020, Espargham
95 et al., 2020 ; Maciá-Vicente et al. 2020). *Cadophora* species isolated from grapevine include

96 *C. luteo-olivacea*, *C. malorum*, *C. melinii*, *C. novi-eboraci*, *C. orientoamericana*, *C. spadicis*
97 and *C. viticola*. The most prevalent species on grapevine is *C. luteo-olivacea,* which has been
98 isolated from both symptomatic and asymptomatic wood, in nursery and field plants showing
99 black vascular streaking (Halleen et al. 2007; Gramaje et al. 2011; Crous et al. 2015; Travadon
100 et al. 2015).

101 *Phaeomoniella chlamydospora* is considered one of the main causal agents of Petri
102 disease and esca (De la Fuente et al. 2016; Gramaje et al. 2018). This species has also been
103 isolated from symptomatic wood of olive trees (Úrbez-Torres et al. 2013), kiwifruit (Di Marco
104 et al. 2000) and from *Convolvulus arvensis* (Agustí-Brisach et al. 2011). Additionally,
105 *Pleurostoma richardsiae* has also been associated with Petri and esca diseases in California
106 (Eskalen et al. 2004; Rolshausen et al. 2010) and South Africa (Halleen et al. 2007).

107 The pathogens associated with GTDs, including the causal agents of Petri disease are
108 regularly isolated from young grapevines and grafted propagating material in nurseries
109 (Whitelaw-Weckert et al. 2013; Gramaje et al. 2018). Previous studies indicated that rootstock
110 cuttings are major sources of infections by GTD pathogens in young nursery vines (Halleen et
111 al. 2003; Retief et al. 2006; Aroca et al. 2010; Gramaje and Armengol 2011; Cardoso et al.
112 2013; Billones-Baaijens et al. 2013). Asymptomatic cuttings taken from infected mother vines
113 are frequent hosts of latent endophytic infections (Fourie and Halleen 2002; Halleen et al. 2003;
114 Aroca et al. 2010; Eichmeier et al. 2017). Infected propagation materials, particularly rootstock
115 material, has been indicated as a major means of spread of pathogens causing young vine
116 decline (Fourie and Halleen 2004; Aroca et al. 2010).

117 In Algeria, surveys of GTDs on grapevine propagating materials or young vineyards
118 have never been conducted to date. In this country, *Pa. chlamydospora* and *Phaeoacremonium*
119 species have only been described on mature vines (Berraf and Peros 2005; Berraf-Tebbal et al.

120 2011). However, the identity and status of the known fungal trunk pathogens causing Petri
121 disease on this woody plant have not yet been investigated. Therefore, the purpose of this study
122 was to investigate and determine the incidence of *Phaeoacremonium* and *Cadophora* species
123 found associated with Petri disease in grapevine nurseries and young vineyards, as well as
124 evaluating their pathogenicity.

125

126 ## Materials and methods

127 **Sampling and fungal isolation.** From 2015 to 2017 a survey was conducted in commercial
128 nurseries and young vineyards from different regions of northern Algeria including Skikda,
129 Blida, Aïn Témouchent, Boumerdès, Algiers and Médéa. For this purpose, 190 one-year-old
130 apparently asymptomatic grapevine grafted plants including Muscat d'Alexandrie, Vitroblack,
131 Chasselat, Ora and rootstocks (SO4), were randomly collected and brought to the laboratory for
132 further analyses. Moreover, 100 young grafted grapevine plants, (aged between three to five
133 year-olds), exhibiting decline symptoms such as cankers and dieback were collected (Table 1).
134 Each plant was examined carefully by making transversal and longitudinal sections at three
135 areas; the grafting point, the basal part in the crown and the middle part between the grafting
136 point and the basal part in order to reveal internal symptoms of GTDs. Ten wood pieces from
137 each part of the plant were surface disinfected for 10 min in an 8 % sodium hypochlorite
138 solution and washed twice with sterile distilled water. Disinfected wood pieces were transferred
139 onto two Petri dishes containing potato dextrose agar (PDA, Biokar-Diagnostics, Zac de Ther,
140 France) amended with 0.5 g /l of streptomycin sulfate (Sigma-Aldrich, St. Louis, MO, USA)
141 (PDAS). Plates were incubated for two months at 25 °C in the dark. The plates were checked
142 every day, in order to transfer the fast-growing colonies into PDA and prevent the loss of slow
143 growing fungal pathogens, which were also transferred to this culture medium.

144

145 **Morphological description.** The slow growing colonies obtained were tentatively identified
146 according to colony appearance, culture characteristics, and microscopic structures.
147 *Phaeoacremonium* isolates were identified based on culture characters and pigments produced
148 on PDA, malt extract agar (MEA, Difco, France) and oatmeal agar (OA, Difco, France). The
149 microscopic structures including phialide type and shape, conidiophore morphology, hyphal
150 wart size and conidial shape and size from aerial mycelium were also used for the identification
151 of these fungal isolates (Crous et al. 1996; Di Marco et al. 2004; Mostert et al. 2006; Marin-
152 Felix et al. 2019).

153 The identification of *Cadophora* isolates was based on cultural and microscopic characteristics
154 of conidia, conidiophores, phialides, and collarettes (Travadon et al. 2015). Colony characters
155 and pigment production of these isolates were determined on MEA and PDA incubated at 25
156 ºC for 8 and 16 days. Colony colors were determined using taxonomic description color charts
157 of Rayner (1970). Cardinal temperatures for growth were determined by incubating MEA plates
158 in the dark at temperatures ranging from 5 to 40 ºC at 5 ºC intervals. Three replicate plates per
159 isolate were used and the experiment was conducted twice. Colony diameter was recorded after
160 eight days in two orthogonal directions. For each isolate, regression curves were fitted to the
161 values of radial growth in millimeters at the different temperatures. The optimum temperature
162 for radial growth and the maximum daily radial growth were calculated in the fitted equation
163 for each *Cadophora* isolate. Mycelial growth was adjusted to a third-degree polynomial model:
164 $Y = aT^3 + bT^2 + cT$, in which Y = mycelial growth (mm/day); a, b, and c are the regression
165 coefficients; and R^2 = coefficient of determination. Data of the optimum temperature for radial
166 growth and the maximum daily radial growth were analyzed using the Kruskal-Wallis test. Data
167 were analyzed using Statistix 9 (Analytical Software, Tallahassee, FL).

168

169 **DNA isolation, PCR and sequencing.** Mycelium and conidia of single-spored of
170 *Phaeoacremonium* and *Cadophora* isolates grown on PDA for two to four weeks at 25 °C in
171 the dark, were scraped and disrupted with four tungsten carbide beads of 3 mm diameter
172 (Qiagen, Hilden, Germany) using a Fast Prep-24TM5G (MP Biomedicals, California, USA) at
173 5 m/s for 20 s twice. Total DNA was extracted using the E.Z.N.A. Plant Miniprep Kit (Omega
174 Bio-tek, Doraville, USA) following manufacturer's instructions. All fungal species were
175 identified by amplifying the β-tubulin (*tub2*) region of DNA using the fungal universal primers
176 T1 (O'Donnell and Cigelnik 1997) and Bt2b (Glass et Donaldson, 1995) or BTCadF and
177 BTCadR (Travadon et al. 2015). Based on the results of *tub*2 sequence data, samples from each
178 *Phaeoacremonium* species were additionally sequenced for the actin (*act*) region using primers
179 ACT-512F and ACT-783R (Carbone and Kohn 1999). Whereas, a partial sequence of the
180 translation elongation factor genes (*tef1*) using the primer pairs EF1-728F/EF1-986R (Carbone
181 and Kohn 1999) and the internal transcribed spacer region (ITS) using primers pairs ITS1/ITS4
182 (White et al. 1990) were performed on *Cadophora* sp. to better resolve their phylogenetic
183 position. PCR amplifications were carried out in a final volume of 25 µl for one PCR reaction
184 constituted of 24 µl of mix solution [14.25 µl of ultrapure sterile H2O (Gibco), 2.5 µl of Buffer
185 B (10×), 2.5 µl of $MgCl_2$ (25 mM), 1µl of each primer (10 mM), 2.5 µl of dNTPs (8 mM), 0.25
186 µl of HotBegan™ Taq DNA Polymerase (Canvax Biotech SL, Córdoba, Spain) (5 U/ml)] and
187 1 µl of genomic DNA. The cycle conditions in a Peltier Thermal Cycler-200 (MJ Research) for
188 β-tubulin were: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at
189 94 °C for 30 s, annealing at 50 °C for 30 s, elongation at 72 °C for 45 s, and a final extension
190 at 72 °C for 10 min. For the actin, the cycle conditions are as described for beta-tubulin, but
191 annealing at 52 °C. The amplification conditions for ITS and *tef1* were as follow: initial
192 denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s,
193 annealing at 55 °C for 30 s and extension at 72 °C for 45 s and a final extension step at 72 °C

194 for 3 min. PCR products were visualized after electrophoresis on 1.5 % agarose gels stained
195 with ethidium bromide and was stored at −20 °C. After confirmation by agarose gel
196 electrophoresis, PCR products were sequenced in both directions using the same primer pairs
197 used for amplification by Macrogen Inc., Sequencing Center (The Netherlands, Europe). The
198 products were analyzed using Sequencer software v. 5.3 (Gene Codes Corporation, Ann Arbor,
199 MI, USA).

200 **Phylogenetic analyses.** ITS, *tef1* and *tub2* sequences of 42 taxa of *Cadophora* and two
201 outgroups (*Hyaloscypha finlandica* CBS 444.86 and IFM50530) were combined with the
202 *Cadophora* isolates obtained in this study. Also, sequences of the type and reference strains of
203 *Phaeoacremonium* species of each locus analysed (*tub2* and *act*) were retrieved from GenBank.
204 43 taxa including two outgroups (*Pl. richardsiae* CBS 270.33; *Pl. ochraceum* CBS 131321)
205 were combined with the newly generated sequences (Table 2). Alignments were checked and
206 manual adjustments were made using BioEdit Sequence Alignment Editor v.7.0.4.1 (Hall
207 1999). Then, sequences were aligned with MAFFT v.7 online version (Katoh et al. 2019) using
208 the default parameters. New generated sequences were deposited in GenBank (Table 2). The
209 phylogenetic approach was performed through Maximum Likelihood (ML) and Maximum
210 Parsimony (MP) analyses using MEGAX (Kumar et al. 2018) with the best fitting model
211 determined by the software. ML analysis was conducted on a Neighbour-Joining starting tree
212 automatically generated with the Nearest-Neighbour-Interchange (NNI) as the heuristic method
213 for tree inference. While for the MP analysis, the Tree-Bisection-Regrafting (TBR) algorithm
214 was applied and the initial trees were obtained by the random addition of sequences with 10
215 replicates. One thousand (1000) bootstrap replications were performed to evaluate robustness
216 of each of ML and MP trees.

217 **Pathogenicity tests.** Pathogenicity trials were conducted with 12 fungal isolates representative
218 of the *Cadophora* (WAMC34 and WAMC118) and *Phaeoacremonium* species, including *P.*

219 *minimum* (WAMC122, WAMC50), *P. venezuelense* (WAMC17, WAMC103), *P. parasiticum*
220 (WAMC100, WAMC43), *P. australiense* (WAMC107, WAMC44) and *P. iranianum*
221 (WAMC79, WAMC82), identified by phenotypical studies and phylogenetic analyses. These
222 species were selected to complete Koch's postulates on dormant grapevine cuttings (cv.
223 Cardinal). To prevent dehydration, the cuttings were immersed into clean tap water at ambient
224 temperature for two weeks. After that, cuttings were subjected to hot water treatment at 53 °C
225 for 30 min, to eliminate the presence of any fungal GTDs pathogens (Gramaje et al. 2009;
226 Carlucci et al. 2017; Aigoun-Mouhous et al. 2019). One hundred and twenty dormant cuttings
227 were cut into equal length (35 cm), containing 3–4 buds. Then, the cuttings were wounded
228 between two nodes with a scalpel and a 5 mm mycelial plug from a 10 days old colony of each
229 isolate grown on PDA was placed in the wound. Negative controls were inoculated with fresh,
230 non-colonized, PDA plugs. All inoculated cuttings were wrapped with wet sterile cotton and
231 Parafilm around the inoculation point to prevent desiccation. Ten replicates for each isolate
232 were used, with an equal number of control plants. After inoculation, plants were placed into
233 pots containing sterilized water as a growth substrate (10 cuttings per pot), which were
234 incubated in a phytotron at 25 °C in a completely randomized design and watered every three
235 days during three months. After this period, the cuttings were examined by removing the bark
236 and measuring the length of the wood lesions in both directions from the inoculation point.
237 Small pieces (0.2 to 0.5 cm) of necrotic tissue from the edge of each lesion were cut and placed
238 on PDAS to re-isolate and identify morphologically the inoculated fungi to complete Koch's
239 postulates.

240 **Statistical analysis.** Data of lesion lengths from pathogenicity trials was checked for normality
241 and differences in lesion lengths caused by the fungal species were subjected to a nonparametric
242 multiple comparisons of mean ranks using Kruskal-Wallis test. The function 'kruskal.test' in the

243 base R v.3.5.1 (Team 2013) was applied for this purpose. Statistical analysis were performed
244 for significance level $\alpha = 0.05$.

245

246 Results

247 Symptomatology and Morphological description

248 Internal wood necrosis, consisting of different brownish discolorations around the pith
249 more consistent at the basal part and less important at the medium part, were observed on cross
250 sections of the surveyed grapevine nursery and young grapevine plants. Seventy-three fungal
251 isolates characterized by slow-growing colonies were obtained from the samples. They were
252 tentatively arranged in two groups based on morphological features. The first group (70
253 isolates) was characterized by pale brown to brown, flat, slow-growing cultures on PDA and
254 MEA, abundant sporulation, aseptate and hyaline conidia. Septate hyphae were fasciculate or
255 single. The three types of phialides (type I, II and III phialides), variable in shape and size, were
256 observed in these fungal isolates. These morphological characters corresponded to the genus
257 *Phaeoacremonium* (Crous et al. 1996; Mostert et al. 2006). The second group (3 isolates)
258 formed white to pale yellow or vinaceous buff, felty, flat colonies on PDA. Conidia were
259 elongate or ellipsoid. Prominent flask-shaped phialides and collarettes were frequently
260 observed. Morphological and cultural characteristics of these isolates resembled those of
261 *Cadophora* sp. (Gramaje et al. 2011; Agustí-Brisach et al. 2013; Travadon et al. 2015). Species
262 of *Phaeoacremonium* (95.89% of the total isolates) were the prevalent fungi associated with
263 Petri disease symptoms from which isolations were made, whereas the species belonging to the
264 genus *Cadophora* represented only 4.10% of the fungi recovered in this study.

265 **Molecular identification and phylogenetic analyses.** The molecular identification of the
266 isolates was performed first using the primers Bt2b and T1. A PCR fragment of about 600 bp

267 was obtained for all of them. DNA sequence data showed high similarities (≥ 99%) with the
268 reference sequences deposited in the NCBI Genbank database (Table 2) and confirmed 70
269 isolates belonging to the genus *Phaeoacremonium*: *P. minimum* (22 isolates), *P. venezuelense*
270 (19 isolates), *P. parasiticum* (17 isolates), *P. australiense* (8 isolates) and *P. iranianum* (4
271 isolates) as well as three isolates belonging to the genus *Cadophora*. Results of ITS, *tef1* and
272 *tub2* genes for the isolates WAMC34, WAMC117 and WAMC118 showed similarity values of
273 95% when compared with *C. luteo-olivacea* sequences of *tef1* and *tub2* (seven nucleotide
274 differences for each gene region).

275 **Phylogeny of *Phaeoacremonium* species.** The combined *tub2* and *act* sequences alignment for
276 in-group and two outgroup isolates contained 843 characters composed of 610 for *tub2* and 233
277 for *act*. Of these, 315 were constant, 88 were variable and parsimony-uninformative and 418
278 were parsimony-informative. Five parsimonious trees were constructed through the heuristic
279 search of the 88 parsimony-informative characters resulted in 1000 equally parsimonious trees
280 after 1095 steps (CI = 0.56, RI = 0.88 and HI = 0.44). The ML tree is presented in Fig. 1. The
281 alignment and tree were deposited in TreeBASE under the study number 28052.

282

283 **Phylogeny of *Cadophora* species.** The combined ITS, *tef1* and *tub2* sequences alignment
284 consisted of 1489 characters of which, 768 were constant, 206 were variable and parsimony-
285 uninformative and 443 were parsimony-informative. The heuristic search of the parsimony-
286 informative characters resulted in 1000 equally parsimonious trees led to generate three
287 parsimonious trees through 1567 steps with CI = 0.61, RI = 0.85 and HI = 0.39. In the MP tree
288 (Fig. 2), *Cadophora* isolates obtained in this study formed a distinct clade comprised three
289 isolates with a high bootstrap support value (ML/MP = 100/100). The isolates were considered

290 to be newly described species named here as *Cadophora sabaouae* sp. nov. (Fig. 2). The
291 alignment and tree were deposited in TreeBASE under the study number 28046.

292 **Taxonomy.** Based on the morphological characters and phylogenetic analysis comparisons
293 coupled with the results of the combined three-gene dataset, the isolates WAMC34, WAMC117
294 and WAMC118 are identified as a strongly supported lineage for which no apparent species
295 name exists. Therefore, we propose the following new species name to properly circumscribe
296 this unique taxon (Fig. 3).

297

298 ***Cadophora sabaouae* sp. nov.** W. Aigoun-Mouhous, A. Berraf-Tebbal, J. Armengol & D.
299 Gramaje

300 MycoBank MB 837956; Fig. 3.

301 *Etymology*: Named after Professor Dr. Sabaou Nasserdine (1956–2019), outstanding Algerian
302 microbiologist and taxonomist.

303 Mycelium composed of branched, septate hyphae occurring singly or in bundles of up to 6;
304 hyphae tuberculate with warts up to 3 µm diam, verruculose to smooth, olivaceous brown, 2.5–
305 3.0 µm diam. Conidiophores were mostly short, usually unbranched, arising from aerial or
306 submerged hyphae, erect to flexuous, up to 5-septate, pale brown, (10–) 11.5–41(–46) (av. =
307 27) µm long and 2–3.5 (av. = 2.5) µm wide. Phialides terminal or lateral, mostly monophialidic,
308 smooth, hyaline, with 2–3 µm long, 2–2.5 µm wide, mostly cylindrical collarettes, some
309 elongate-ampulliform, attenuated at the base or navicular, (3.5–)9–19.5(–25) × 1.5–3(–3.5) (av.
310 = 6 × 2.5) µm. Conidia hyaline, ovoid or oblong ellipsoidal, (3–)3.5–6.5 × 2.5–3 (av. = 4.5 ×
311 2.5) µm.

312 *Culture characteristics*: Colonies reaching 22.5–25.5 mm diam after 8 d at 25 ºC. The minimum
313 and maximum temperature for growth were 10 ºC and 35 ºC, respectively. Significant

314 differences were found in the optimal temperature between *Cadophora sabaouae* isolates
315 (WAMC34: 20.0 ºC; WAMC117: 24.6 ºC; WAMC118: 25.0 ºC). According to the Kruskal-
316 Wallis test, maximum growth rates of isolates did not differ significantly ($P > 0.05$) (WAMC34:
317 2.8 mm/day; WAMC117: 3.2 mm/day; WAMC118: 3.0 mm/day). Colonies on MEA flat, felty,
318 with even margins after 16 d, white to greenish-olivaceous close to the center. Colonies on PDA
319 flat, felty and cottony in the middle, with even margins after 16 d, white to grey-olivaceous.
320 Colonies on OA were flat, felty and cottony in the middle, with an even edge and varying in
321 color from buff to olivaceous-buff.

322 *Typification*: Algeria: Blida (WAMC34), isolated from the basal part of rootstock SO4 in a one-
323 year-old nursery plant (cv. Vitroblack grafted on SO4) and Aïn Témouchent (WAMC117;
324 WAMC118), isolated from the apical part of rootstock SO4 in a one-year-old nursery plant,
325 May 2017. W. Aigoun-Mouhous (CBS H-24563 – holotype; CBS 147192 = WAMC34
326 WAMC117, WAMC118 – ex-type culture).

327 *Known distribution*: Northern Algeria, Blida and Aïn Témouchent.

328 Notes: *Cadophora sabaouae* is phylogenetically related to *C. luteo-olivacea*. It differs from *C.*
329 *luteo-olivacea* in its faster colony growth (*C. sabaouae*: av. 3 mm/day; *C. luteo-olivacea* av.
330 2.1 mm/day) and the minimum temperature for growth (*C. sabaouae*: 10 ºC; *C. luteo-olivacea*:
331 5 ºC) (Gramaje et al. 2011). A total of 14 polymorphisms can distinguish *C. sabaouae* from *C.*
332 *luteo-olivacea*: seven bp in *tub2* positions 93 (T/A), 102 (T/A), 109 (A/T), 137 (T/A), 141
333 (C/T), 152 (A/C) and 153 (C/G); seven pb in *tef1* locus 191 (A/-), 192 (T/C), 194 (A/C), 198
334 (C/G), 242 (A/G), 246 (C/T) and 424 (G/T). No difference was found in ITS region.

335 **Frequency and localization of the species.** A total of 73 isolates were obtained by sampling
336 from commercial grapevine nurseries and young vineyards. *Phaeoacremonium minimum* with
337 an incidence of 30.2 % (22 isolates) was the most prevalent species. It was isolated from all the

338 prospected regions: Aïn Témouchent (6 isolates), Algiers (3 isolates), Blida (4 isolates), Médéa
339 (2 isolates) and Skikda (7 isolates). The second most isolated species was *P. venezuelense* with
340 26 % (19 isolates), sampled from four of the five regions, including Algiers (2 isolates), Blida
341 (14 isolates), Médéa (1 isolate) and Skikda (2 isolates). *Phaeoacremonium parasiticum* with
342 23.3 % (17 isolates) was recovered from Aïn Témouchent (3 isolates), Blida (7 isolates),
343 Boumerdès (1 isolate), Médéa (3 isolates) and Skikda (3 isolates). *Phaeoacremonium*
344 *australiense* with 10.95 % (8 isolates) was found in three sampled regions: Algiers (1 isolate),
345 Blida (3 isolates) and Skikda (4 isolates); while *P. iranianum* with 5.5% (4 isolates) was the
346 least frequent species of *Phaeoacremonium*, isolated from Algiers, Blida, Boumerdès and
347 Médéa with one isolate from each region. Lastly, *C. sabaouae* with 4.1% (3 isolates) was
348 isolated from two regions, including Aïn Témouchent (2 isolates) and Blida (1 isolate).
349
350 **Pathogenicity tests.** All the *Phaeoacremonium* and *Cadophora* isolates evaluated were
351 pathogenic to grapevine cuttings cv. Cardinal. Ninety days after inoculation, irregular black to
352 brown necrosis developed on the wood tissue, under the bark, starting from the point of
353 inoculation. External discoloration and internal lesions developed on both ends of the
354 inoculation points. No symptoms were observed on the negative control plants, which led to
355 this null result being excluded from the statistical analysis. The percentage recovery of the
356 pathogens from the inoculated cuttings was more than 95%, and the reisolated species were
357 confirmed morphologically to be identical to the previously inoculated ones. No fungal isolates
358 were obtained from the negative control.
359 The most aggressive species was *C. sabaouae* sp. nov. with a lesion length of 8.48 ±
360 0.56 cm for WAMC34 and 8.16 ± 0.79 cm for WAMC118. However, all the five
361 *Phaeoacremonium* species developed lesion length ranging from 1.58 ± 0.47 (*P. iranianum*) to
362 3.84 ± 1.36 cm (*P. minimum*). Variation in aggressiveness has been noticed between isolates of

363 the same species and between different species as well (Fig. 4). Significant difference in lesion
364 lengths were detected through the ANOVA test ($F = 65.517$; $P < 0.0001$) with an assigned LSD
365 value of 0.853. According to the nonparametric Kruskal–Wallis test, mean ranks of lesion
366 length values calculated for the tested species were significantly different ($H = 77.11$, $P <$
367 0.0001) at $\alpha = 0.05$.

368

369 Discussion

370 This study is part of a large investigation aiming to identify the fungal trunk pathogens
371 associated with Petri disease in Algeria and it confirms the presence of *Phaeoacremonium* spp.
372 and the new species *C. sabaouae* on young and nursery grapevine plants. Thus, this is the first
373 report of Petri disease and its associated fungal pathogens in Algerian young grapevines and
374 commercial nurseries.

375 The combination of morphological characters and DNA sequence data allowed the
376 identification of six species belonging to the genera *Phaeoacremonium* and *Cadophora.* They
377 were isolated from internal xylem necrosis from the grapevine grafted plants and rootstocks
378 surveyed.

379 In this investigation, among the 29 *Phaeoacremonium* species already reported on
380 grapevine growing regions worldwide (Gramaje et al. 2018; Spies et al. 2018), the following
381 species were hosted in the sampled plants: *P. minimum*, *P. parasiticum*, *P. venezuelense, P.*
382 *australiense* and *P. iranianum*. These last two species represent new records for Algeria.
383 Among the *Phaeoacremonium* species previously described in Algeria, *P. minimum*, *P.*
384 *parasiticum*, *P. hispanicum* and *P. venezuelense* were reported from mature grapevine (Berraf
385 and Péros 2005; Berraf-Tebbal et al. 2011), while *P. inflatipes* was found in the intestinal

386 contents of old of *Platypus cylindrus* larvae living in a cork oak forest of the coastal north-
387 western Algeria (Belhoucine et al. 2012).

388 Throughout this survey, *P. minimum* was the most frequent species, isolated from both
389 young and nursery grapevines. It was also, the most prevalent, collected from the five
390 prospected regions. This result was expected, since this species is considered to be the main
391 pathogen associated with Petri disease, and the most aggressive *Phaeoacremonium* species on
392 mature grapevines worldwide (Mugnai et al. 1999; Mostert et al., 2006; Berraf-Tebbal et al.
393 2011; Mohammadi et al. 2013; Úrbez-Torres et al. 2014; Gramaje et al. 2016). Moreover, *P.*
394 *minimum* has been reported from a wide range of woody hosts and cause damages on several
395 economically important crops such as *Prunus* sp., *Malus* sp., *Punica granatum*, *Salix* sp.,
396 almond, pistachio and walnut and *Citrus* spp. (Kazemzadeh Chakusary et al. 2017; Spies et al.
397 2018; Espargham et al. 2020; Sohrabi et al. 2020).

398 Interestingly, the second most prevalent pathogen isolated in this study was *P.*
399 *venezuelense* with 19 isolates, which represents 26% of the total isolates. This species was
400 reported in Algeria in 2011, where it was isolated from mature vines showing esca and eutypa
401 dieback symptoms (Berraf-Tebbal et al. 2011). *Phaeoacremonium venezuelense* was found first
402 on a mycetoma infected human foot in Venezuela (Mostert et al. 2005), and was also reported
403 from other tree crops, such as *Prunus armeniaca,* in Spain (Olmo et al. 2014), *Santalum album*
404 in Australia (Gramaje et al. 2014) *Rosa* sp. in South Africa (Spies et al. 2018) and *Azadirachta*
405 *indica* in Iran (Ghasemi-Sardareh and Mohammadi 2020). However, in the present study, *P.*
406 *venezuelense* was found in almost all the sampled regions; this fact is in contrast with the
407 previous reports, where it was isolated in a very low frequency (Mostert et al. 2005; Gramaje
408 et al. 2015).

409 *Phaeoacremonium parasiticum*, the type species of the genus, was the third most
410 abundant species occurring on asymptomatic grafted plants and rootstocks as well as on young
411 plants exhibiting decline symptoms. It was recovered from the five sampling sites, which
412 matches the findings of previous studies indicating its cosmopolitan nature. This species is
413 known from Algeria (Berraf-Tebbal et al. 2011), Argentina (Gatica et al. 2000; 2001; Dupont
414 et al. 2002), Australia (Mostert et al. 2005), Brazil (Correia et al. 2013), Chile (Auger et al.
415 2005), Iran (Arabnezhad and Mohammadi 2012; Mohammadi et al. 2013), Italy (Essakhi et al.
416 2008), Peru (Romero-Rivas et al. 2009; Álvarez et al. 2012), Spain (Aroca et al. 2006; Gramaje
417 et al. 2010), South Africa (Mostert et al. 2005, 2006; White et al. 2011) and USA (Rolshausen
418 et al. 2010). In addition to its occurrence on grapevine, *P. parasiticum* has been recorded from
419 more than ten different hosts, worldwide, including *A. chinensis, Prunus armeniaca*, *Olea*
420 *europa*ea, *Malus* (*M.*) *domestica*, *Pyrus communis, Punica* (*P.*) *granatum, Cydonia* (*Cy.*)
421 *oblonga, Ficus carica* and *Citrus* sp., *Azadirachta indica* (Ghasemi-Sardareh and Mohammadi
422 2020) and has also been isolated from soil (Dupont et al. 2002; Damm et al. 2008; Agustí-
423 Brisach et al. 2013; Sami et al. 2014; Gramaje et al. 2015; Spies et al. 2018; Espargham et al.
424 2020).

425 In the current study, eight isolates belonging to the species *P. australiense* were obtained
426 from grafted and rootstocks plants and also from young grapevine plants. It was detected in all
427 the sampled sites. To date, this species has only been reported in Australia, South Africa and
428 Uruguay. This study expands its known geographical range and adds Algeria to the list.
429 *Phaeoacremonium australiense* was first described by Mostert et al. (2005) in Australia, then
430 in Uruguay (Abreo et al. 2011) on grapevine. It was then reported in South Africa on *Prunus*
431 species by Damm et al. (2008) and other woody hosts by Spies et al. (2018) namely *Ps. guajava,*
432 *Cy. oblonga, P. granatum, F. carica, Eriobotrya japonica, V. vinifera, Rosa* sp. and *M.*
433 *domestica*.

434 The less frequent *Phaeoacremonium* species found in this study was *P. iranianum*. This
435 species was described for the first time by Mostert et al. (2006) in Iran and Italy from *Vitis* sp.
436 and *A. chinensis*. It was also reported in studies from other countries namely Canada (Úrbez-
437 Torres et al. 2014), Italy (Essakhi et al. 2008), South Africa (White et al. 2011), Spain (Gramaje
438 et al. 2009) and Iran (Espargham et al. 2020).

439 Moreover, a new species belonging to the genus *Cadophora* (*Cadophora sabaouae.* sp.
440 nov.) was described based on morphological characters and analysis of partial sequences of β-
441 tubulin genes, ITS and *tef1* sequence data. The type specimen was then described and deposited
442 in publicly-available collections. This species was isolated only from grapevine nursery plants
443 and absent in young grapevines. Most *Cadophora* species are primarily isolated from soil and
444 plants or interacting as plant pathogens, root colonizers, or saprobes (Travadon et al. 2015). In
445 grapevine, the colonization of *Cadophora* spp. into the xylem of young grapevines at the
446 nursery or newly established vineyards through root or basal end of the rootstock infections
447 from the soil is still unclear. Recently, the presence of *Cadophora* species in vineyard soils has
448 been confirmed using ITS high-throughput amplicon sequencing (HTAS) approach by
449 Martínez-Diz et al. (2019). However, the species *C. luteo-olivacea* was barely detected from
450 vineyard soils using a droplet digital PCR approach (Maldonado-González et al. 2020) or using
451 traditional isolation methods from symptomless vascular tissues of weeds (Agustí-Brisach et
452 al. 2011) or bait plants (Agustí-Brisach et al. 2013). Nevertheless, and even its absence in this
453 study, *C. luteo-olivacea* is still reported as the most frequent *Cadophora* species isolated from
454 both asymptomatic (Halleen et al. 2007; Casieri et al. 2009; Eichmeier et al. 2018) and
455 symptomatic grapevine wood, in nursery (Navarrete et al. 2011) and field plants (Rooney-
456 Latham 2005; Úrbez-Torres et al. 2014), as well as, from contaminated nursery stock or soil-
457 borne inoculum (Halleen et al. 2007; Gramaje et al. 2011; Agusti-Brisach et al. 2013).

458 In the pathogenicity tests, all *Phaeoacremonium* and *Cadophora sabaouae* isolates were 459 able to infect, colonize, and produce lesions on grapevine cuttings, confirming their 460 pathogenicity and their status as Petri disease pathogens. The most aggressive species was *C.* 461 *sabaouae* sp. nov. with a lesion length of 8.48 ± 0.56 cm, developed in 12 weeks. In other 462 studies, *C. luteo-olivacea* produced lesions of up to 9.2 cm in grapevine rootstock cuttings after 463 14 weeks (Gramaje et al. 2011). A recent study showed that *Cadophora* spp. were considerably 464 aggressive in English walnut in the Czech Republic, with 11.1 cm lesion length after 24 weeks 465 of incubation (Eichmeier et al. 2019). The five *Phaeoacremonium* species developed lesions 466 ranging from 1.58 ± 0.47 to 3.84 ± 1.36 cm in length. These findings confirm also previous 467 studies, in which severe disease symptoms were reproduced by inoculating *Phaeoacremonium* 468 species onto several hosts such as grapevine, *Prunus* spp., kiwi fruit and oak (Gramaje et al. 469 2015; Baloyi et al. 2018). Adding to this, in similar studies achieved by Mostert et al. (2006), 470 Halleen et al. (2007), Aroca and Raposo (2009) and Úrbez-Torres et al. (2014), isolates of 471 *Phaeoacremonium* species inoculated on detached grapevine shoots were able to cause lesions.

472 It is important to emphasize that the mycelium plug, which was used as the inoculum in 473 this study, provided a high inoculum pressure, which is somewhat different from real situations. 474 In nature, spores are the most probable inoculum that may infect natural wounds of roots and 475 wounds made in planting material through the propagation process in grapevine nurseries. 476 Different inoculation methods may produce different results in length wood discoloration. In 477 the case of *Cadophora* spp., different inoculation methods, such as insertion of mycelial plugs 478 (Halleen et al. 2007; Gramaje et al. 2011; Gramaje et al. 2014) or conidial suspensions (Halleen 479 et al. 2007; Travadon et al. 2015) into side wounds or cut ends of the grapevine stems, and 480 vacuum-inoculation of conidial suspensions throughout the vascular system of rootstock 481 cuttings (Gramaje et al. 2010) have been used in pathogenicity tests. Further work is necessary

482 to disentangle the effects of the inoculation method on differential wood responses to fungal
483 infection.

484 This study confirms the presence of *Phaeoacremonium* and *Cadophora* species as
485 causal agents of internal wood necrosis of grafted grapevine and rootstocks currently associated
486 with Petri disease in Algeria and adds a new species to the genus *Cadophora*. Our results are
487 in agreement with those obtained by Gramaje and Armengol (2011) which reported that the
488 infected propagation material is considered one of the main sources of *Phaeoacremonium*
489 inoculum in vineyards. Waite et al. (2018) reported that latent infections by GTD pathogens in
490 rootstock cuttings are a major source of the pathogens in the grapevine nurseries and the newly
491 established vineyards and also pointed out that mother vines with unprotected pruning wounds
492 are typically heavily infected, particularly if they are not trellised.

493 Healthy grapevine planting material is essential to the longevity and productivity of
494 vineyards. Moreover, propagating new mother vines under improved phytosanitary conditions
495 is essential to maintain a good health status in cuttings from well managed mother vines (Waite
496 et al. 2018). Therefore, pruning wound protection is an extremely important preventative
497 treatment (Gramaje et al. 2018). Several preventive treatments were tested such as hot water
498 treatments (HWT) of dormant cuttings and young dormant vines (Crous et al. 2001; Gramaje
499 et al. 2009; Eichmeier et al. 2018), fungicide treatments and biological control agents (Álvarez-
500 Pérez et al. 2017; Daraignes et al. 2018; Mondello et al. 2018; Andreolli et al. 2019; Del Frari
501 et al. 2019; Mondello et al. 2019; Trotel-Aziz et al. 2019; Berbegal et al., 2020; Niem et al.
502 2020; Martínez-Diz et al. 2020), as well as the well managed harvesting operations in mother
503 vine blocks, which appeared to be critical to the maintenance of cutting quality (Gramaje and
504 Di Marco 2015).

505 In conclusion, further studies are needed to evaluate the epidemiology, pathogenicity,
506 the role and impact of *Phaeoacremonium* and *Cadophora* species in the Algerian grapevines.
507 Pathogenicity studies under field conditions are also suggested to assess the real potential
508 impact of these fungi in young and nursery grapevine decline.

509

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829 **Figure captions**

830 **Fig. 1.** Maximum likelihood tree generated from the combined analysis *tub2* and *act* sequence
831 data of *Phaeoacremonium* species. ML/MP bootstrap values are given at the nodes. Bootstrap
832 values less than 50 % are not shown. The tree was rooted to *Pleurostoma richardsiae* and *Pl.*
833 *ochraceum.*

834 **Fig. 2.** Maximum parsimony tree generated from the combined analysis of ITS, *tef1* and *tub2*
835 sequences data of *Cadophora* species. ML/MP bootstrap values are given at the nodes.
836 Bootstrap values less than 50 % are not shown. The tree was rooted to *Hyaloscypha finlandica.*

837 **Fig. 3.** *Cadophora sabaouae* sp. nov. A-T, aerial structures on MEA; A-C, phialides; D-F,
838 conidiophores; G, hyphal swellings; H, conidia. Scale bars: A, F and H = 5 um; scale bar for A
839 applies to B-E; scale bar for F applies to G.

840 **Fig. 4.** Mean lesion lengths (cm) caused by the five *Phaeoacremonium* species and *Cadophora*
841 *sabaouae* associated with grapevine nurseries and young grapevine decline at three months
842 after inoculation. Each column represents an individual tested isolate and vertical error bars
843 indicate the corresponding standard deviation.

844

845 **Table 1.** Grapevine sampling regions and number of plants collected.

Sampling	Region	Age of plants (year)	Number of plants
Nurseries	Aïn Témouchent	1	30
	Blida	1	70
	Skikda	1	90
Young vineyards	Aïn Témouchent	3-5	20
	Algiers	3-5	20
	Boumerdès	3-5	20
	Médéa	3-5	20
	Skikda	3-5	20
Total			290

846

847 **Table 2.** *Phaeacremonium* and *Cadophora* species included in the phylogenetic analysis.

				GenBank accession numbers			
Species	**Isolate number**	**Origin**	**Host**	**ITS**	***tub2***	***act***	***tef1***
Pheoacremonium alvesii	CBS 110034	Brazil	*Homo sapiens*	**-**	AY579234	AY579301	**-**
P. alvesii	CBS 408.78	USA	Human	**-**	AY579303	AY579236	**-**
P. amstelodamense	CBS 110627	The Netherlands	*H. sapiens*	**-**	AY579295	AY579228	**-**
P. angustius	CBS 114991	USA	*Vitis vinifera*	**-**	DQ173104	DQ173127	**-**
P. angustius	CBS 114992	USA	*V. vinifera*	**-**	DQ173104	DQ173127	**-**
P. australiense	CBS 113589	Australia	*V. vinifera*	**-**	AY579296	AY579229	**-**
P. australiense	CBS 113592	Australia	*V. vinifera*	**-**	AY579297	AY579230	**-**
P. australiense	**WAMC08**	**Algeria**	***V. vinifera***	**-**	**MT598107**	**MT598120**	**-**
P. australiense	**WAMC10**	**Algeria**	***V. vinifera***	**-**	**MT598108**	**MT598121**	**-**
P. cinereum	Pm5	Iran	*V. vinifera*	**-**	FJ517161	FJ517153	**-**
P. cinereum	Pm4	Iran	*V. vinifera*	**-**	FJ517160	FJ517152	**-**
P. croatiense	113Pal	Croatia	*V. vinifera*	**-**	EU863482	EU863514	**-**
P. fraxinopennsylvanicum	CBS 110212	USA	*Fraxinus pensylvanica*	**-**	DQ173109	DQ173136	**-**
P. fraxinopennsylvanicum	CBS 101585	USA	*V. vinifera*	**-**	KF764684	DQ173137	**-**
P. inflatipes	CBS 391.71	USA	*Quercus virginiana*	**-**	AF246805	AY579259	**-**
P. inflatipes	CBS 113273	USA	*H. truncatum*	**-**	AY579323	AY579260	**-**

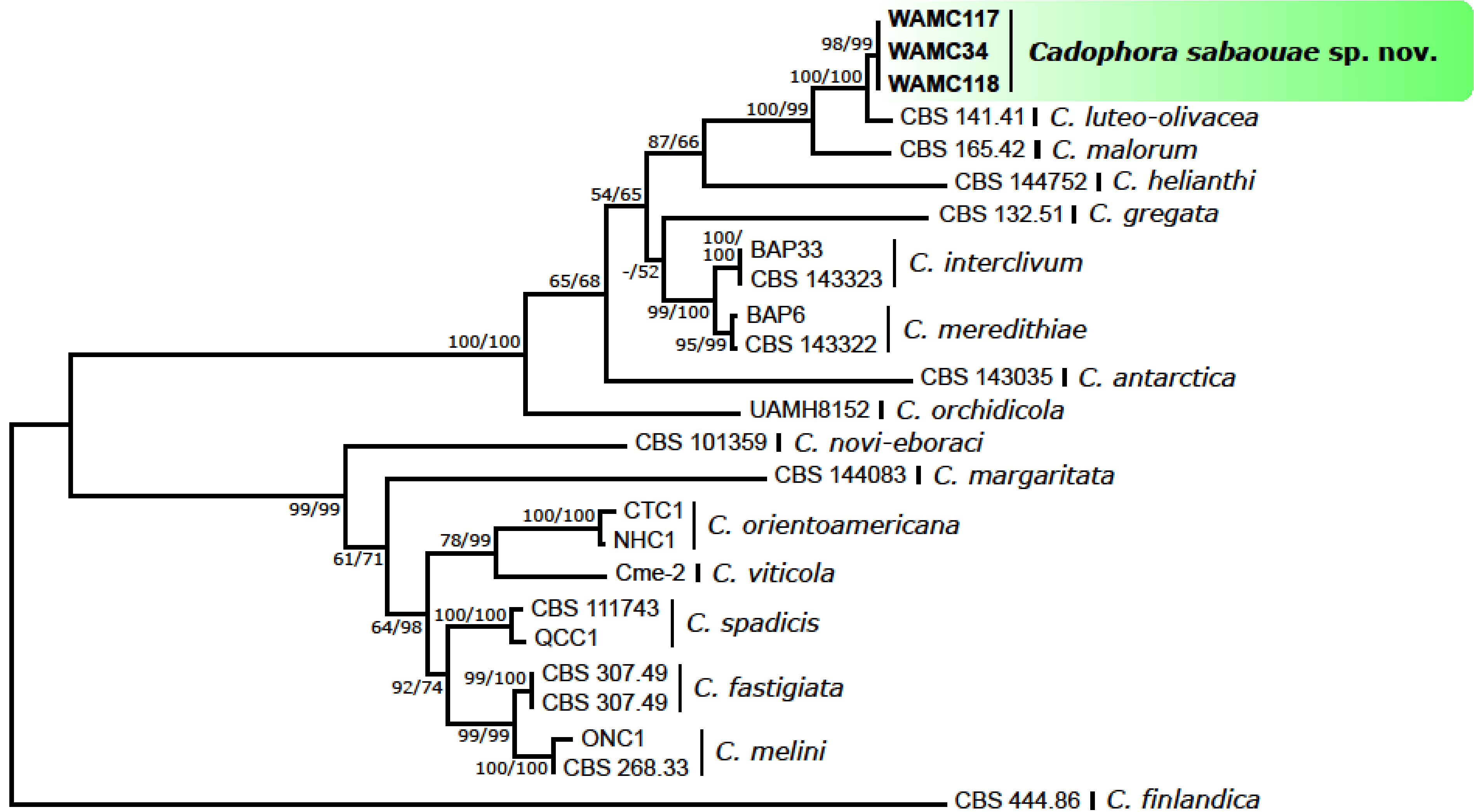
P. iranianum	CBS 101357	Italy	*Actinidia chinensis*	**-**	DQ173097	DQ173120	**-**
P. iranianum	CBS 117114	Iran	*V. vinifera*	**-**	DQ173098	DQ173121	**-**
P. iranianum	**WAMC62**	**Algeria**	***V. vinifera***	**-**	**MT598109**	**MT598122**	**-**
P. iranianum	**WAMC79**	**Algeria**	***V. vinifera***	**-**	**MT598110**	**MT598123**	**-**
P. italicum	CSN206	South Africa	*Ficus* (*F.*) *carica*	**-**	KY906697	KY906696	**-**
P. italicum	CSN277	South Africa	*Prunus persica*	**-**	KY906711	KY906710	**-**
P. longicollarum	CBS 142699	South Africa	*P. armeniaca*	**-**	KY906689	KY906688	**-**
P. longicollarum	CBS 142700	South Africa	*Psidium* (Ps.) *guajava*	**-**	KY906879	KY906878	**-**
P. luteum	A16	Australia	*Santalum album*	**-**	KF823800	KF835406	**-**
P. luteum	A34	Australia	*S. album*	**-**	KJ533541	KJ533543	**-**
P. minimum	CBS 110703	South Africa	*V. vinifera*	**-**	DQ173094	DQ173115	**-**
P. minimum	STEU 6986	South Africa	*V. vinifera*	**-**	JQ038909	JQ038920	**-**
P. minimum	CBS 246.91	South Africa	*Prunus salicina*	**-**	AF246811	AY735497	**-**
P. minimum	**WAMC06**	**Algeria**	***V. vinifera***	**-**	**MT598111**	**MT598124**	**-**
P. minimum	**WAMC122**	**Algeria**	***V. vinifera***	**-**	**MT598113**	**MT598126**	**-**
P. minimum	**WAMC12**	**Algeria**	***V. vinifera***	**-**	**MT598114**	**MT598127**	**-**
P. minimum	**WAMC68**	**Algeria**	***V. vinifera***	**-**	**MT598112**	**MT598125**	**-**
P. occidentale	ICMP:17037	New Zealand	*V. vinifera*	**-**	EU596524	EU595464	**-**
P. pallidum	STEU 6104	South Africa	*P. armeniaca*	**-**	EU128103	EU128144	**-**
P. parasiticum	CBS 514.82	USA	Human	**-**	AY579306	AY579240	**-**
P. parasiticum	CBS 860.73	USA	Human	**-**	AF246803	AY579253	**-**
P. parasiticum	**WAMC102**	**Algeria**	***V. vinifera***	**-**	**MT598116**	**MT598129**	**-**
P. parasiticum	**WAMC14**	**Algeria**	***V. vinifera***	**-**	**MT598115**	**MT598128**	**-**
P. paululum	CBS 142705	-	*Ps. guajava*	**-**	KY906880	KY906881	**-**

P. rubrigenum	CBS 112046	USA	*H. sapiens*	**-**	AY579305	AY579239	**-**
P. rubrigenum	CBS 498.94	USA	Human	**-**	AF246802	AY579238	**-**
P. Santali	A37	Australia	*S. album*	**-**	KJ533534	KJ533538	**-**
P. Santali	A4	Australia	*S. album*	**-**	KF823791	KF835397	**-**
P. scolyti	CBS 112585	Czech Republic	*Scolytus intricatus*	**-**	AY579292	AY579223	**-**
P. tuscanicum	1Pal	Italy	*V. vinifera*	**-**	EU863458	EU863490	**-**
P. venezuelense	CBS 65185	Venezuela	*H. sapiens*	**-**	AY579320	AY579256	**-**
P. venezuelense	CBS 113595	Canada	Human	**-**	AY579319	AY579255	**-**
P. venezuelense	**WAMC07**	**Algeria**	***V. vinifera***	**-**	**MT598117**	**MT598130**	**-**
P. venezuelense	**WAMC17**	**Algeria**	***V. vinifera***	**-**	**MT598118**	**MT598131**	**-**
P. venezuelense	**WAMC32**	**Algeria**	***V. vinifera***	**-**	**MT598119**	**MT598132**	**-**
P. viticola	CBS 113065	South Africa	*V. vinifera*	**-**	DQ173105	DQ173128	**-**
P. viticola	CBS 428.95	Germany	*Sorbus intermedia*	**-**	DQ173107	DQ173133	**-**
Pleurostoma ochraceum	CBS 131321	Sudan	*Homo sapiens*	**-**	JX073271	JX073275	**-**
Pl. richardsiae	CBS 270.33	Sweden	Herb	**-**	AY579334	AY579271	**-**
Cadophora africana	CBS 120890	South Africa	*Prunus salicina*	MN232936	MN232967	-	MN232988
C. antarctica	CBS 143035	Antarctica	Soil	NR_156381	MK993426	-	MK993427
C. bubakii	CBS 198.30	Czech Republic	*Margarine*	MH855111	-	-	MN232989
C. constrictospora	CBS 146371	Bulgaria	*Microthlaspi sp.*	KT269023	-	-	MN325874
C. echinata	CBS 146383	Spain	*M. perfoliatum*	KT270239	-	-	MN325932
C. fascicularis	CBS 146382	Germany	*M. erraticum*	KT269992	-	-	MN325918
C. fastigiata	CBS 307.49	Sweden	Pine wood	AY249073	KM497131	-	KM497087
C. fastigiata	CBS 869.69	Germany	-	MH859469	-	-	-

C. ferruginea	CBS 146363	Spain	*M. perfoliatum*	KT268618	-	-	MN325861
C. gamsii	CBS 146379	France	*M. erraticum*	KT269668	-	-	MN325899
C. gregata	CBS 132.51	-	Soybean root	U66731	MF677920	-	MF979586
C. helianthi	CBS 144752	Ukraine	*Helianthus annuus*	MF962601	MH733391	-	MH719029
C. interclivum	CBS 143323	Canada	*Carex sprengelii*	MF979577	MF677917	-	MF979583
C. interclivum	BAP33	Canada	*Picea glauca*	MF979578	MF677918	-	MF979584
C. lacrimiformis	MFLU 16-1486	Russia	*Brassicaceae*	NR_163787	-	-	-
C. luteo-olivacea	CBS 141.41	Sweden	-	AY249066	KM497133	-	KM497089
C. luteo-olivacea	CBS 357.51	Italy	*Malus domestica*	GU128589	KF764682	-	KF764611
C. malorum	CBS 165.42	The Netherlands	*Amblystoma mexicanum*	AY249059	KM407134	-	KM497090
C. malorum	CBS 266.31	-	-	MH855209	-	-	-
C. margaritata	CBS 144083	Turkey	*Populus tremula*	KJ702027	MH327786	-	-
C. melinii	CBS 268.33	Sweden	-	AY249072	KM497132	-	KM497088
C. melinii	ONC1	Canada	*V. vinifera*	KM497033	KM497114	-	KM497070
C. meredithiae	CBS 143322	Canada	*Carex sprengelii*	MF979574	MF677914	-	MF979580
C. meredithiae	BAP6	Canada	*Picea glauca*	MF979575	-	-	-
P. microspore	MFLU 18-2672	UK	*Apiaceae* sp.	MK584939	-	-	-
C. novi-eboraci	CBS 101359	Italy	*Actinidia chinensis*	DQ404350	KM407135	-	KM497092
C. obovata	CBS 146374	Germany	*M. erraticum*	KT269230	-	-	MN325888
C. obscura	CBS 269.33	Sweden	Fresh water	MN232948	-	-	MN232996
C. orchidicola	UAMH8152	Canada	Green orchid	AF214576	MF677921	-	MF979587

C. orientoamericana	CTC1	USA	*V. vinifera*	KM497012	KM497093	-	KM497049
C. orientoamericana	NHC1	USA	*Vitis* hybrid	KM497018	KM497099	-	KM497055
C. prunicola	CBS 120891	South Africa	*Prunus salicina*	MN232949	MN232979	-	MN232997
C. prunicola	GLMC 276	Germany	*P. cerasus*	MN232951	MN232980	-	MN232998
C. ramose	CBS 111743	Italy	*A. chinensis*	DQ404351	KM497091	-	KM497136
C. ramose	QCC1	USA	*V. vinifera*	KM497031	KM497112	-	KM497068
C. variabilis	CBS 146360	Croatia	*M. perfoliatum*	KT268493	-	-	MK550890
C. viticola	Cme-1	Spain	*V. vinifera*	HQ661097	-	-	HQ661082
C. sabaouae	**WAMC34= CBS 147192**	**Algeria**	***V. vinifera***	**MT644187**	**MT646749**	**-**	**MT646746**
C. sabaouae	**WAMC117**	**Algeria**	***V. vinifera***	**MT524745**	**MT646750**	**-**	**MT646747**
C. sabaouae	**WAMC118**	**Algeria**	***V. vinifera***	**MT524744**	**MT646751**	**-**	**MT646748**
Hyaloscypha finlandica	CBS 444.86	Finland	-	NR_121279	KM497130	-	KM497086

* **Abbreviations**: ***act*** : actin gene; **CBS**: CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; **GLMC**: Culture collection of Senckenberg Museum of Natural History Görlitz, Görlitz, Germany; **ICMP:** International Collection of Micro-organisms from Plants, Lincoln, New Zealand; **ITS** : internal transcribed spacer and intervening 5.8S gene region; **STEU**: University of Stellenbosch, Stellenbosch, South Africa; ***tef1***: translation elongation factor 1-α; ***tub2*** : partial regions of the β-tubulin; **UAMH:** University of Alberta Microfungus Collection and Herbarium, Canada; **WAMC**: Personal culture collection of W. Aigoun-Mouhous. **In bold face:** the newly obtained isolates.



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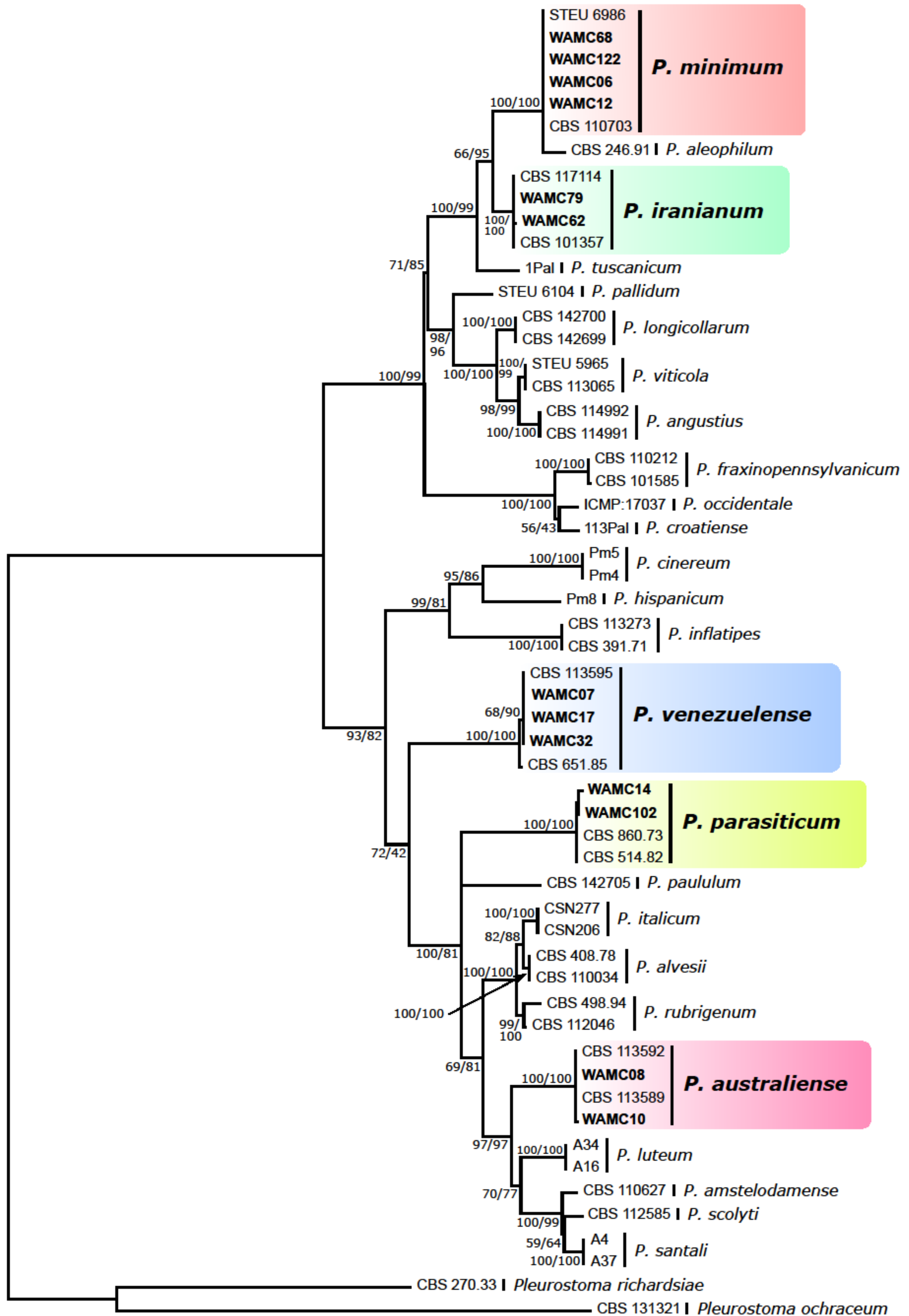

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