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

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Abstract

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

Introduction

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

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

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

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

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

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

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

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

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

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

Materials and methods

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

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

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

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

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

Phylogenetic analyses. Resulted PCR amplicons of ITS, *tef1*, *tub2* and *act* were checked and manual adjustments were made using BioEdit Sequence Alignment Editor v.7.0.4.1 (Hall 1999). Then, sequences were aligned with MAFFT v.7 online version (Katoh et al. 2019) using the default parameters. New generated sequences were deposited in GenBank (Table 2). The phylogenetic approach was performed through Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses using MEGAX (Kumar et al. 2018) with the best fitting model determined by the software. ML analysis was conducted on a Neighbour-Joining starting tree automatically generated with the Nearest-Neighbour-Interchange (NNI) as the heuristic method for tree inference. While for the MP analysis, the Tree-Bisection-Regrafting (TBR) algorithm was applied and the initial trees were obtained by the random addition of sequences with 10 replicates. One thousand (1000) bootstrap replications were performed to evaluate robustness of each of ML and MP trees.

Pathogenicity tests. Pathogenicity trials were conducted with 11 fungal isolates representative of the *Cadophora* (WAMC34 and WAMC118) and *Phaeoacremonium* species (WAMC122, WAMC50, WAMC17, WAMC103, WAMC100, WAMC43, WAMC107, WAMC44, WAMC79, and WAMC82) determined by phenotypical studies and phylogenetic analyses. These species were selected to complete Koch's postulates on dormant grapevine cuttings (cv. Cardinal). To prevent dehydration, the cuttings were immersed into clean tap water at ambient temperature for two weeks. After that, cuttings were subjected to hot water treatment at 53 °C

for 30 min, to eliminate the presence of any fungal GTDs pathogens (Gramaje et al. 2009; Carlucci et al. 2017; Aigoun-Mouhous et al. 2019). One hundred and twenty dormant cuttings were cut into equal length (35 cm), containing 3–4 buds. Then, the cuttings were wounded between two nodes with a scalpel and a 5 mm mycelial plug from a 10 days old colony of each isolate grown on PDA was placed in the wound. Negative controls were inoculated with fresh, non-colonized, PDA plugs. All inoculated cuttings were wrapped with wet sterile cotton and Parafilm around the inoculation point to prevent desiccation. Ten replicates for each isolate were used, with an equal number of control plants. After inoculation, plants were placed into pots containing sterilized water as a growth substrate (10 cuttings per pot), which were incubated in a phytotron at 25 °C in a completely randomized design and watered every three days during three months. After this period, the cuttings were examined by removing the bark and measuring the length of the wood lesions in both directions from the inoculation point. Small pieces (0.2 to 0.5 cm) of necrotic tissue from the edge of each lesion were cut and placed on PDAS to re-isolate and identify morphologically the inoculated fungi to complete Koch's postulates.

Statistical analysis. Data of lesion lengths from pathogenicity trials was checked for normality and differences in lesion lengths caused by the tested isolates of different species was subjected to a one-way ANOVA analysis using 'anova' function of the base R v.3.5.1 (Team 2013). By using the function 'leveneTest' in the 'car' package v. 3.0-8, homogeneity of variance was verified according to Levene's test. When significant differences were detected, the corresponding LSD value were calculated at $P < 0.05$.

Results

Symptomatology and Morphological description

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

Molecular identification and phylogenetic analyses. The molecular identification of the isolates was performed first using the primers Bt2b and T1. A PCR fragment of about 600 bp was obtained for all of them. DNA sequence data showed high similarities (≥ 99%) with the reference sequences deposited in the NCBI Genbank database (Table 2) and confirmed 70 isolates belonging to the genus *Phaeoacremonium*: *P. minimum* (22 isolates), *P. venezuelense* (19 isolates), *P. parasiticum* (17 isolates), *P. australiense* (8 isolates) and *P. iranianum* (4 isolates) as well as three isolates belonging to the genus *Cadophora*. Results of ITS, *tef1* and *tub2* genes for the isolates WAMC34, WAMC117 and WAMC118 showed similarity values of

95% when compared with *C. luteo-olivacea* sequences of *tef1* and *tub2* (seven nucleotide differences for each gene region).

Phylogeny of *Phaeoacremonium* species. The alignment of *tub2* and *act* sequences included 54 ingroup isolates belonging to 25 species of *Phaeoacremonium* and two outgroup taxa (*Pl. richardsiae* CBS 270.33; *Pl. ochraceum* CBS 131321). The alignment consisted of 843 characters composed of 610 for *tub2* and 233 for *act*. Of these, 315 were constant, 88 were variable and parsimony-uninformative and 418 were parsimony-informative. Five parsimonious trees were constructed through the heuristic search of the 88 parsimony-informative characters resulted in 1000 equally parsimonious trees after 1095 steps (CI = 0.56, RI = 0.88 and HI = 0.44). The ML tree is presented in Fig. 1. (we are waiting for the treebase ID)

Phylogeny of *Cadophora* species. The combined ITS, *tef1* and *tub2* sequences comprised 33 ingroup isolates belonging to 28 species of *Cadophora* and one outgroup taxon (*Hyaloscypha finlandica* CBS 444.86). The sequences alignment consisted of 1489 characters of which, 768 were constant, 206 were variable and parsimony-uninformative and 443 were parsimony-informative. The heuristic search of the parsimony-informative characters resulted in 1000 equally parsimonious trees led to generate three parsimonious trees through 1567 steps with CI = 0.61, RI = 0.85 and HI = 0.39. In the MP tree (Fig. 2), *Cadophora* isolates obtained in this study formed a distinct clade comprised three isolates with a high bootstrap support value (ML/MP = 100/100). The isolates were considered to be newly described species named here as *Cadophora sabaouae* sp. nov. (Fig. 2). The alignment and tree were deposited in TreeBASE under the study number 28046.

Taxonomy. Based on the morphological characters and phylogenetic analysis comparisons coupled with the results of the combined three-gene dataset, the isolates WAMC34, WAMC117

and WAMC118 are identified as a strongly supported lineage for which no apparent species name exists. Therefore, we propose the following new species name to properly circumscribe this unique taxon (Fig. 3).

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

MycoBank MB 837956; Fig. 3.

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

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

Culture characteristics: Colonies reaching 22.5–25.5 mm diam after 8 d at 25 ºC. The minimum and maximum temperature for growth were 10 ºC and 35 ºC, respectively. Significant differences were found in the optimal temperature between *Cadophora sabaouae* isolates (WAMC34: 20.0 ºC; WAMC117: 24.6 ºC; WAMC118: 25.0 ºC). According to the Kruskal-Wallis test, maximum growth rates of isolates did not differ significantly ($P > 0.05$) (WAMC34: 2.8 mm/day; WAMC117: 3.2 mm/day; WAMC118: 3.0 mm/day). Colonies on MEA flat, felty,

315 with even margins after 16 d, white to greenish-olivaceous close to the center. Colonies on PDA
316 flat, felty and cottony in the middle, with even margins after 16 d, white to grey-olivaceous.
317 Colonies on OA were flat, felty and cottony in the middle, with an even edge and varying in
318 color from buff to olivaceous-buff.

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

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

325 Notes: *Cadophora sabaouae* is phylogenetically related to *C. luteo-olivacea*. It differs from *C.*
326 *luteo-olivacea* in its faster colony growth (*C. sabaouae*: av. 3 mm/day; *C. luteo-olivacea* av.
327 2.1 mm/day) and the minimum temperature for growth (*C. sabaouae*: 10 ºC; *C. luteo-olivacea*:
328 5 ºC) (Gramaje et al. 2011). A total of 14 polymorphisms can distinguish *C. sabaouae* from *C.*
329 *luteo-olivacea*: seven bp in *tub2* positions 93 (T/A), 102 (T/A), 109 (A/T), 137 (T/A), 141
330 (C/T), 152 (A/C) and 153 (C/G); seven pb in *tef1* locus 191 (A/-), 192 (T/C), 194 (A/C), 198
331 (C/G), 242 (A/G), 246 (C/T) and 424 (G/T). No difference was found in ITS region.

332 **Frequency and localization of the species.** A total of 73 isolates were obtained by sampling
333 from commercial grapevine nurseries and young vineyards. *Phaeoacremonium minimum* with
334 an incidence of 30.2 % (22 isolates) was the most prevalent species. It was isolated from all the
335 prospected regions: Aïn Témouchent (6 isolates), Algiers (3 isolates), Blida (4 isolates), Médéa
336 (2 isolates) and Skikda (7 isolates). The second most isolated species was *P. venezuelense* with
337 26 % (19 isolates), sampled from four of the five regions, including Algiers (2 isolates), Blida
338 (14 isolates), Médéa (1 isolate) and Skikda (2 isolates). *Phaeoacremonium parasiticum* with

23.3 % (17 isolates) was recovered from Aïn Témouchent (3 isolates), Blida (7 isolates), Boumerdès (1 isolate), Médéa (3 isolates) and Skikda (3 isolates). *Phaeoacremonium australiense* with 10.95 % (8 isolates) was found in three sampled regions: Algiers (1 isolate), Blida (3 isolates) and Skikda (4 isolates); while *P. iranianum* with 5.5% (4 isolates) was the least frequent species of *Phaeoacremonium*, isolated from Algiers, Blida, Boumerdès and Médéa with one isolate from each region. Lastly, *C. sabaouae* with 4.1% (3 isolates) was isolated from two regions, including Aïn Témouchent (2 isolates) and Blida (1 isolate).

Pathogenicity tests. All the *Phaeoacremonium* and *Cadophora* isolates evaluated were pathogenic to grapevine cuttings cv. Cardinal. Ninety days after inoculation, irregular black to brown necrosis developed on the wood tissue, under the bark, starting from the point of inoculation. External discoloration and internal lesions developed on both ends of the inoculation points. No symptoms were observed on the negative control plants, which led to this null result being excluded from the statistical analysis. The percentage recovery of the pathogens from the inoculated cuttings was more than 95%, and the reisolated species were confirmed morphologically to be identical to the previously inoculated ones. No fungal isolates were obtained from the negative control.

The most aggressive species was *C. sabaouae* sp. nov. with a lesion length of 8.48 ± 0.56 cm for WAMC34 and 8.16 ± 0.79 cm for WAMC118. However, all the five *Phaeoacremonium* species developed lesion length ranging from 1.58 ± 0.47 (*P. iranianum*) to 3.84 ± 1.36 cm (*P. minimum*). Variation in aggressiveness has been noticed between isolates of the same species and between different species as well (Fig. 4). Significant difference in lesion lengths were detected through the ANOVA test ($F = 65.517$; $P < 0.0001$) with an assigned LSD value of 0.853. According to Levene's test of homogeneity, an equality of variances was detected between both tested isolates of each of *C. sabaouae*, *P. australiense*, *P. iranianum*, and *P. venezuelense* at $\alpha = 0.05$.

Discussion

This study is part of a large investigation aiming to identify the fungal trunk pathogens associated with Petri disease in Algeria and it confirms the presence of *Phaeoacremonium* spp. and the new species *C. sabaouae* on young and nursery grapevine plants. Thus, this is the first

report of Petri disease and its associated fungal pathogens in Algerian young grapevines and commercial nurseries.

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

In this investigation, among the 29 *Phaeoacremonium* species already reported on grapevine growing regions worldwide (Gramaje et al. 2018; Spies et al. 2018), the following species were hosted in the sampled plants: *P. minimum, P. parasiticum, P. venezuelense, P. australiense* and *P. iranianum*. These last two species represent new records for Algeria. Among the *Phaeoacremonium* species previously described in Algeria, *P. minimum, P. parasiticum, P. hispanicum* and *P. venezuelense* were reported from mature grapevine (Berraf and Péros 2005; Berraf-Tebbal et al. 2011), while *P. inflatipes* was found in the intestinal contents of old of *Platypus cylindrus* larvae living in a cork oak forest of the coastal north-western Algeria (Belhoucine et al. 2012).

Throughout this survey, *P. minimum* was the most frequent species, isolated from both young and nursery grapevines. It was also, the most prevalent, collected from the five prospected regions. This result was expected, since this species is considered to be the main pathogen associated with Petri disease, and the most aggressive *Phaeoacremonium* species on mature grapevines worldwide (Mugnai et al. 1999; Mostert et al., 2006; Berraf-Tebbal et al. 2011; Mohammadi et al. 2013; Úrbez-Torres et al. 2014; Gramaje et al. 2016). Moreover, *P. minimum* has been reported from a wide range of woody hosts and cause damages on several economically important crops such as *Prunus* sp., *Malus* sp., *Punica granatum*, *Salix* sp.,

almond, pistachio and walnut and *Citrus* spp. (Kazemzadeh Chakusary et al. 2017; Spies et al. 2018; Espargham et al. 2020; Sohrabi et al. 2020).

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

Phaeoacremonium parasiticum, the type species of the genus, was the third most abundant species occurring on asymptomatic grafted plants and rootstocks as well as on young plants exhibiting decline symptoms. It was recovered from the five sampling sites, which matches the findings of previous studies indicating its cosmopolitan nature. This species is known from Algeria (Berraf-Tebbal et al. 2011), Argentina (Gatica et al. 2000; 2001; Dupont et al. 2002), Australia (Mostert et al. 2005), Brazil (Correia et al. 2013), Chile (Auger et al. 2005), Iran (Arabnezhad and Mohammadi 2012; Mohammadi et al. 2013), Italy (Essakhi et al. 2008), Peru (Romero-Rivas et al. 2009; Álvarez et al. 2012), Spain (Aroca et al. 2006; Gramaje et al. 2010), South Africa (Mostert et al. 2005, 2006; White et al. 2011) and USA (Rolshausen et al. 2010). In addition to its occurrence on grapevine, *P. parasiticum* has been recorded from more than ten different hosts, worldwide, including *A. chinensis*, *Prunus armeniaca*, *Olea europa*ea, *Malus* (*M.*) *domestica*, *Pyrus communis*, *Punica* (*P.*) *granatum*, *Cydonia* (*Cy.*)

oblonga, *Ficus carica* and *Citrus* sp., *Azadirachta indica* (Ghasemi-Sardareh and Mohammadi 2020) and has also been isolated from soil (Dupont et al. 2002; Damm et al. 2008; Agustí-Brisach et al. 2013; Sami et al. 2014; Gramaje et al. 2015; Spies et al. 2018; Espargham et al. 2020).

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

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

Moreover, a new species belonging to the genus *Cadophora* (*Cadophora sabaouae.* sp. nov.) was described based on morphological characters and analysis of partial sequences of β-tubulin genes, ITS and *tef1* sequence data. The type specimen was then described and deposited in publicly-available collections. This species was isolated only from grapevine nursery plants and absent in young grapevines. Most *Cadophora* species are primarily isolated from soil and plants or interacting as plant pathogens, root colonizers, or saprobes (Travadon et al. 2015). In

grapevine, the colonization of *Cadophora* spp. into the xylem of young grapevines at the nursery or newly established vineyards through root or basal end of the rootstock infections from the soil is still unclear. Recently, the presence of *Cadophora* species in vineyard soils has been confirmed using ITS high-throughput amplicon sequencing (HTAS) approach by Martínez-Diz et al. (2019). However, the species *C. luteo-olivacea* was barely detected from vineyard soils using a droplet digital PCR approach (Maldonado-González et al. 2020) or using traditional isolation methods from symptomless vascular tissues of weeds (Agustí-Brisach et al. 2011) or bait plants (Agustí-Brisach et al. 2013). Nevertheless, and even its absence in this study, *C. luteo-olivacea* is still reported as the most frequent *Cadophora* species isolated from both asymptomatic (Halleen et al. 2007; Casieri et al. 2009; Eichmeier et al. 2018) and symptomatic grapevine wood, in nursery (Navarrete et al. 2011) and field plants (Rooney-Latham 2005; Úrbez-Torres et al. 2014), as well as, from contaminated nursery stock or soil-borne inoculum (Halleen et al. 2007; Gramaje et al. 2011; Agusti-Brisach et al. 2013).

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

Halleen et al. (2007), Aroca and Raposo (2009) and Úrbez-Torres et al. (2014), isolates of *Phaeoacremonium* species inoculated on detached grapevine shoots were able to cause lesions.

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

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

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

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

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Figure captions

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

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

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

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

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

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

				GenBank accession numbers			
Species	**Isolate number**	**Origin**	**Host**	**ITS**	***tub2***	***act***	***tef1***
Phaeoacremonium minimum	CBS 246.91	South Africa	*Prunus salicina*	-	AF246811	AY735497	-
P. 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	**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.